Spruceanumines A and B, Novel Plumeran Indole Alkaloids from *Aspidosperma spruceanum* (Apocynaceae)

Vilma B. Oliveira,* Ivo J. Cercino Vieira,* R. Braz-Filho,*b Leda Mathias,* Norberto P. Lopes,* Antonio E. M. Crotti and Daniel E. de A. Uchôa*

*Laboratório de Ciências Químicas (LCQUI)-CCT, Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), 28013-602 Campos dos Goytacazes-RJ, Brazil

*Pesquisador Visitante Emérito-FAPERJ, Laboratório de Ciências Químicas (LCQUI)-CCT-UENF/PPGQO-DEQUIM-UFRRJ, 28013-602 Campos dos Goytacazes-RJ, Brazil

Faculdade de Ciências Farmacêuticas de Ribeirão Preto-USP, 14040-903 Ribeirão Preto-SP, Brazil

Núcleo de Pesquisas em Ciências Exatas e Tecnológicas, Universidade de Franca, 14404-600 Franca-SP, Brazil

Centro Nordestino de Aplicação e Uso da Ressonância Magnética Nuclear, Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, 60021-97 Fortaleza-CE, Brazil

Dois novos alcalóides indólicos com esqueleto plumerano, spruceanumines A (1) e B (2), e oito alcalóides indólicos conhecidos, aspidospermidina (3), desmetoxipalosina (4), aspidocarpina (5), aspidolimina (6), fendlerina (7), aspidolimidina (8), obscurinervida (9) e obscurinervina (10), foram isolados do extrato metanólico das cascas do caule e sementes de *Aspidosperma spruceanum*. As estruturas dos compostos foram elucidadas com base na análise de dados espectroscópicos, principalmente os obtidos por espectros de RMN $^1$H e $^{13}$C (1D e 2D) e por espectrometria de massas.

Two novel indole alkaloids with plumeran skeleton, spruceanumines A (1) and B (2), and eight known indole alkaloids, aspidospermidine (3), demethoxypalosine (4), aspidocarpine (5), aspidolimine (6), fendlerine (7), aspidolimidine (8), obscurinervida (9) and obscurinervine (10) were isolated from stem bark and seeds methanolic extracts of *Aspidosperma spruceanum*. Compounds structures were elucidated on the basis of spectroscopic data, mainly those obtained by $^1$H and $^{13}$C NMR (1D and 2D) and mass spectrometry.

**Keywords:*** Aspidosperma spruceanum, Apocynaceae, plumeran indole alkaloids

**Introduction**

The *Aspidosperma* (Apocynaceae) genus is endemic to Americas and is found mainly in regions between Mexico and Argentina.¹ *Aspidosperma* genus continues to be fascinating as an expressive source of indole alkaloids with novel skeletons, which are interesting from a biosynthetic perspective and reported biological properties. Several species of *Aspidosperma* are broadly used in popular medicine as potential antimalarial agents, leishmaniose treatment, uterus and ovary inflammation, as contraceptive, in diabetes, in stomach problems, against cancer, fever and rheumatism.²

*Aspidosperma spruceanum* (*A. spruceanum*), commonly known as “Paratudo-Branco” in Atlantic forest in the North of Espírito Santo State, appears as a tree of 5-20 m. The isolation and structure elucidation of two alkaloids from stem bark of *A. spruceanum* collected in Rio de Janeiro State, Brazil, were reported.³

In the present paper, we describe the isolation and characterization of two novel plumeran indole alkaloids named as spruceanumines A (1) and B (2), along with known indole alkaloids: aspidospermidine (3),⁴,⁵ demethoxypalosine (4),⁷,⁹ aspidocarpine (5),⁸,¹⁰,¹⁴ aspidolimine (6),⁸,¹⁴ fendlerine (7),¹⁵,¹⁶ aspidolimidine (8),⁸,¹³,¹⁵ obscurinervida (9)¹⁴,¹⁷ and obscurinervine (10).¹⁴,¹⁷ Their structures were established by spectrometric techniques, mainly one- and two-dimensional nuclear
magnetic resonance (NMR), as well as high resolution electron spray ionization mass spectra (HRESIMS).

**Results and Discussion**

Elaboration of stem bark and seeds methanol extract of *A. spruceanum* by classical chromatographic methods resulted in the isolation of ten plumeran indole alkaloids (1-10), whose structures are shown in Figure 1. The well-known plumeran indole alkaloids, aspidospermidine (3), demethoxypalosine (4), aspidocarpine (5), aspidolimine (6), fendlerine (7), aspidolimidine (8), obscurinervidine (9) and obscurinervine (10) were identified on the basis of δ and 13C NMR spectral data, including 1H-1H correlation spectroscopy (COSY), 1H-1H nuclear overhauser effect spectroscopy (NOESY), heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC) NMR experiments,18 which were also used to complete unambiguous δH and 13C chemical shift assignments of 1 and 2.

Spruceamine A (1) and B (2) were obtained as a mixture of amorphous form, [α]D2 = -101.7 (CHCl3, c 0.61). Infrared (IR) spectrum showed bands at νmax 3100-2890 (C-H stretching), νmax 1755 (stretching of the γ-lactone carbonyl group) in addition to other bands at νmax 1624, 1606 and 1497 (C=C stretching of the benzene ring), and 887 and 739 cm⁻¹ (C-H bending of substituted benzene ring).19

Comparative analysis of the [1H]- and distortionless enhancement by polarization transfer (DEPT) 135°-13C NMR spectra (Table 1) revealed signals corresponding to 24 (1) or 25 (2) carbon atoms, allowing to recognize the presence of signals corresponding to nine nonhydrogenated [(C)n; three sp3 (including one bounded to nitrogen and oxygen atoms at δc 106.79), six sp2 (including one carbonyl group at δc 175.10 and five sp2 attributed to aromatic ring), five methine [(CH)2; two sp3 linked to nitrogen atom (δc 68.91/δh 3.50 and δc 44.73/δh 3.27 correlated in the HSQC spectrum with 1H chemical shifts at δh 3.50 and 3.27, respectively, as indicated also in the direct subsequent correlations, 1H-1H)), and three sp2 (one aromatic at δc 101.78/δh 6.63 (s) and two olefinic at δc 123.31/δh 5.81 (ddt) and 130.79/δh 5.37 (brd)), seven (1) and eight (2) sp3 methylene [(CH2)2, or (CH2)3, including one linked to oxygen atom at δc 72.26 (1) and 70.20 (2, revealing γ-effect of the methyl group CH3-C2)] and three methyl [(CH3), δc 15.10/δh 0.98 (t, J = 7.5 Hz), 2; and (MeO), represented by signals at δc 56.49/δh 3.70 (s) and 61.18/δh 3.81 (s)], 1; 2] carbon atoms, allowing to deduce the expanded molecular formulae (C)n(C=O)(N-C-O)(CH2)(O-CH2)(CH2)2(CH2)(CH2)2(CH2)(MeO), and (C)n(C=O)(N-C-O)(CH2)(O-CH2)(CH2)2(CH2)2(CH2)(MeO), for 1 and 2, respectively. This later contains additional methylene group CH2 (δc 22.56/δh 1.69 (m) and 1.46 (m) coupled to the hydrogens of an adjacent methyl group (δc 9.39/δh 0.98 (t, J = 7.5 Hz)).

The high resolution electron-spray ionization mass spectrum (ESI-MS) of 1 and 2 showed peaks corresponding to the protonated molecules [M+H]+ at m/z 425.2170 of 1 (C24H22N2O5 = m/z 425.2076, Δmcalc 0.0094) and 439.2322 of 2 (C25H31N2O5 = m/z 439.2233, Δmcalc 0.0099) Daltons, which

![Figure 1](image-url)
together with the NMR $^{13}$C spectrum enable to propose molecular formulas C$_{24}$H$_{28}$N$_2$O$_5$ (1) and C$_{25}$H$_{30}$N$_2$O$_5$ (2), respectively, containing twelve degrees of unsaturation (C$_{24}$H$_{28}$N$_2$O$_5$ - C$_{24}$H$_{28}$N$_2$O$_5$ = H$_2$ or C$_{25}$H$_{30}$N$_2$O$_5$ - C$_{25}$H$_{30}$N$_2$O$_5$ = H$_3$), which is consistent with the structure of alkaloids containing the nucleus of 21-oxo-aspidobildine (11, aspidospermidin-18,21-olide, using actual numeration) as basic structure (eleven degrees of unsaturation = four corresponding to aromatic ring, two to carbonyl lactone group and additional pentacyclic moiety), which after the location of one 1,2-disubstituted double bond between the carbon atoms CH-14 and CH-15 and of one heterocyclic
involving the N-substituent and the oxygen atom sustained by carbon atom C-12, justifying the presence of OCH₃ (1: δᵣ 72.26/δ₃ 4.27 and 3.90; 2: δᵣ 70.20/δ₃ 4.35 and 4.00, revealing shielding induced by γ-effect of the methyl 3H-4’), methyl group represented by a doublet signal (J = 6.2 Hz) at δᵣ 1.12 (3H-3’ correlated in the HSQC spectrum with ¹³C chemical shift at δᵣ 15.10) coupled hydrogen linked to nitrogenated carbon atom (δᵣ 3.27 , m, H-2’ correlated with ¹³C signal at δᵣ 44.73, CH-2’) in the alkaloid 1 and by a triplet signal (J = 7.5 Hz) at δᵣ 0.98 (3H-4’) coupled to hydrogen atoms of the additional methylene of 2 (δᵣ 1.69 and 1.46 correlated in the HSQC with ¹³C chemical shift at δᵣ 22.56). The lower field ¹³C chemical shift CH-2’ (δᵣ 50.43) in compound 2 when to that of 1 (δᵣ 44.73) is indicative of a β-effect induced by the methyl group CH₃-4’, as shown in Table 1.

The identity of the six-membered heterocyclic ring containing and oxygen, was supported by ¹J_CH HMBC correlations between C-12 [(δᵣ 136.24, 1 and 2)] and 2H-1’ [δᵣ 4.27 and 3.90 (1); δᵣ 4.35 and 4.00 (2) (Table 1), as well as by ¹H-¹H-COSY cross-peaks displayed by H-1’b (δᵣ 4.27 in 1; 4.35 in 2), H-1’a (δᵣ 3.90 in 1; 4.00 in 2), H-2’ (δᵣ 3.27 in 1; 3.13 in 2).

The ¹H-¹H-COSY spectrum (Table 1) showed coupling of methylic hydogen signals at δᵣ 4.27 [(dd, J = 10.7 and 2.7 Hz, H-1’b (1)) and δᵣ 3.90 [(dd, J = 10.7 and 8.8 Hz, H-1’a (1)] with the methinic hydrogen at δᵣ 3.27 (m, H-2’, 1) and at δᵣ 4.35 [(dd, J = 10.8 and 2.6 Hz, H-1’b (2)] and δᵣ 4.00 [(dd, J = 10.8 and 8.6 Hz, H-1’a (2)] correlated with the signal at δᵣ 3.13 [(m, H-2’, 2)], in agreement with the presence six-membered ring formation.

The assignment of a methyl group at C-2’ was confirmed by its ¹H-¹H-COSY and ¹J_COSY HMBC correlations with H-2’ (δᵣ 3.27) and 2H-1’ (δᵣ 4.35 and 4.00), respectively.

In spruceamine B (2), the presence of an ethyl group at C-2 was confirmed by the coupling of the methylene hydrogens CH₃-3’ (δᵣ 1.69 and 1.46) with the vicinal methyl group (δᵣ 0.98) and H-2’ (δᵣ 3.13).

The ¹H NMR spectrum of mixture showed signals at δᵣ 3.70 (1), 3.74 (2) and δᵣ 3.81 (1), 3.86 (2), which are characteristics of methoxyl groups linked to the benzene ring.¹⁹ These signals showed heteronuclear interaction via one bond (¹J_C) with the signals at δᵣ 56.49 (1), 56.97 (2) and 61.18 (1 e 2) observed in the HSQC spectrum, suggesting the presence of two methoxyl groups linked to the ring A. This, was confirmed by long range heteronuclear coupling (¹J_CH, n= 2 and 3) observed in the HMBC spectrum, as summarized in Table 1. The signal at δᵣ 61.18 (1) observed in the ¹³C NMR of 1 and 2 is a typical value corresponding to signal of methoxyl groups located at forbidden position (MeO-11), as also observed in the aromatic ring of 11 (MeO-11). These data allowed to and postulate the same substitution for 1 and 2, as indicated in Figure 1.

The ¹³C NMR spectrum (Table 1) revealed the presence of a γ-lactone covering the carbon atoms C-20 e CH-21 by the signal at δᵣ 175.10 (C-18), consistent with carbonyl carbon lactone of five members,²⁰ that was also confirmed by long-range coupling of C-18 (δᵣ 175.10) with both hydrogen atoms 2H-19 represented by the signals at δᵣ 2.50 (H-19b) and δᵣ 2.12 (H-19a). Additional heteronuclear long-range couplings are summarized in Table 1.

The main ions fragments observed in the ESI-MS/MS spectrum (low resolution) of 1 and 2 are summarized in Scheme 1. These fragmentation pattern are compatible with that of plumeran alkaloids, as 21-oxo-aspidodialbine (18-oxo by actual numeration utilized in the literature), previously isolated from Aspidosperma exalatum, and they are also in agreement with the presence of 18,21-oxide function in 1 and 2, as suggested by signals at δᵣ 175.10 (C-18) and 106.79 (C-21).

The location of a double bond at CH-14, CH-15 was deduced from the HMBC correlations of carbons resonating at δᵣ 123.51 (CH-14, 1 and 2), 130.79 (CH-15, 1) and 130.66 (CH-15, 2), with olefinic hydrogens at δᵣ 5.81 (H-14), and δᵣ 5.37 (H-15). The vicinal coupling between these hydrogen atoms was confirmed in the ¹H-¹H-COSY spectrum.²¹

The relative stereochemistry of spruceamine A (1) and B (2) was suggested from the nuclear Overhauser effect (nOe) interactions displayed in the NOE spectrum, as summarized in Figure 2.

Figure 2. Selected NOESY correlations and relative stereochemistry for spruceamine A (1) and B (2). Arrows denote the main NOESY correlations.
\(^1\)H-\(^1\)H-NOESY correlations of H-2 and H-2' of 1 and 2 indicated both α-orientations; of H-2 with one hydrogen H-6 of the methylene group CH\(_2\)-6 of 1 and 2 was also used to establish the relative configuration 7(S); of H-2 with both H-2' and 2H-3 of the methylene group CH\(_2\)-3' of 2 revealed α-orientation of H-2; of H-16β with methyl group CH\(_3\)-3' of 1 and with methylene group CH\(_2\)-3' of 2 are consistent with β orientation of this hydrogen atom H-16; spatial interaction of the of the H-15 with both H-19 and H-17 indicated to these hydrogen atoms α and β-orientation, respectively, as shown in Figure 2.

The relative intensity of \(^1\)H NMR signals from the methyl groups CH\(_3\)-3' (1, \(\delta_H\) 1.12) and CH\(_3\)-4' (2, \(\delta_H\) 0.98) was used to deduce the approximated percentage of the 32.9% and 67.1% to spruceanumine A (1) and, spruceanumine B (2) in the mixture, respectively.

**Experimental**

**General Procedures**

Measures of optic rotation were obtained on a Perkin Elmer 343 digital polarimeter. Melting points were obtained on a Microquímica MQRPF and were uncorrected. Fourier transform infrared spectroscopy (FTIR) spectra were recorded on a FTIR-8300 Shimadzu spectrometer using KBr disk. ESI-MS (high resolution) and ESI-MS/MS (low resolution) mass spectra were obtained on a
MICROMASS UlrOTOF-Q (Bruker Daltonics, Billerica, MA) mass spectrometer, using the positive ion mode of analysis. Chromatographic purifications were carried out over silica gel (70-230 mesh). Silica gel 60F254 was used in thin layer chromatography analysis.

1H and 13C NMR spectra were measured on a Bruker DRX500 spectrometer, equipped with inverse probes and field gradient, operating at 500 (1H) and 125 (13C) MHz. CDCl3 was used as solvent and tetramethylsilane (TMS) as internal reference. Chemical shifts are given in ppm based on δ scale (ppm) and coupling constants J in Hz. One dimensional (1D) 1H and 13C NMR spectra were acquired under standard conditions by using a direct detection 5 mm 1H/13C 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 and seeds of A. spruceanum Benth ex. Mull. Arg. were collected in November 2004 at Reserva Florestal de Linhares, Linhares, Espírito Santo State, Brazil. A voucher specimen (CVRD-273) is deposited at the Reserva Florestal herbarium, Cia. Vale do Rio Doce, Linhares, Espírito Santo State.

Extraction and isolation

Dried and powdered stem bark (3.09 kg) and seeds (530.1 g) from A. spruceanum Benth ex. Mull Arg were extracted with methanol at room temperature, furnishing, after solvent evaporation, 63.7 g and 18.5 g of crude methanol extracts, respectively.

The methanol extract (63.7 g) from stem bark was successively partitioned with CH2Cl2/H2O. The CH2Cl2 fraction (7.7 g) was chromatographed over silica gel column with a gradient of hexane/ethyl acetate to afford ten fractions. Fraction 8 (475.8 mg) was rechromatographed over a silica gel column with a gradient of MeOH in CH2Cl2 supplying five fractions. Fraction 3.2 (20.6 mg) yielded the fendlerine (7) and aspidolimidine (8) alkaloids mixture, and fraction 3.2.4 (68.2 mg) afforded a mixture of obscurneridine (9) and obscurinervine (10).

Spruceanumine A (1)

Amorphous solid, mp 195°C; [α]D20 = -101.7º (CHCl3, c 0.61); IR (KBr disk) νmax/cm-1: 3100-2890 (C-H stretching), 1755 (C=O); 1624, 1606, 1479 (benzene ring), 887, 739 (benzene ring). HRESI-MS ([M+H]+) Found: m/z 425.2170. Calc. for C18H12N2O4+: 425.2071 (see Scheme 1); 1H and 13C NMR: see Table 1.

Spruceanumine B (2)

Amorphous solid, mp 195°C; [α]D20 = -101.7º (CHCl3, c 0.61); IR (KBr disk) νmax/cm-1: 3100-2890 (C-H stretching), 1755 (C=O); 1624, 1606, 1479 (benzene ring), 887, 739 (benzene ring). HRESI-MS ([M+H]+) Found: m/z 439.2227. Calc. for C24H14N2O4+: 439.2227 (see Scheme 1); 1H and 13C NMR: see Table 1.

Acknowledgements

The authors thank to Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ-RJ-Brazil) for a visitant research fellowship and grants, to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-Brazil) for a research fellowship and grants and to Centro Nordestino de Aplicação e Uso da Ressonância Magnética Nuclear (CENAUREMN), Universidade Federal do Ceará (UFC), Fortaleza, Ceará, for the facilities in the obtention of the NMR (1D and 2D) spectra.

Supplementary Information

Available free of charge at http://jbcs.org.br, as PDF file.

References


*Received: November 17, 2008*  
*Web Release Date: April 30, 2009*

**FAPESP helped in meeting the publication costs of this article.**
Spruceanumines A and B, Novel Plumeran Indole Alkaloids from *Aspidosperma spruceanum* (Apocynaceae)

Vilma B. Oliveira,* Ivo J. Currino Vieira,* R. Braz-Filho,*b Leda Mathias,* Norberto P. Lopes,* Antonio E. M. Crotti, and Daniel E. de A. Uchoa

*Laboratório de Ciências Químicas (LCQUI)-CCT, Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), 28013-602 Campos dos Goytacazes-RJ, Brazil

*Pesquisador Visitante Emérito-FAPERJ, Laboratório de Ciências Químicas (LCQUI)-CCT-UENF/PPGQO-DEQUIM-UFRRJ, 28013-602 Campos dos Goytacazes-RJ, Brazil

Faculdade de Ciências Farmacêuticas de Ribeirão Preto-USP, 14040-903 Ribeirão Preto-SP, Brazil

Núcleo de Pesquisas em Ciências Exatas e Tecnológicas, Universidade de Franca, 14404-600 Franca-SP, Brazil

Centro Nordestino de Aplicação e Uso da Ressonância Magnética Nuclear, Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, 60021-970 Fortaleza-CE, Brazil

IR, ESI-MS, ESI-MS/MS, 1H NMR, 13C NMR, DEPT 13C NMR, 1H-1H-COSY, 1H-1H-NOESY, HSQC (JCH) and HMBC (JCH n=2 and 3) spectra of 1 and 2 are available free of charge at http://jbcs.sbq.org.br, as PDF file.

Figure S1. IR of the mixture alkaloids 1 and 2

*e-mail: braz@uenf.br
Spruceanumines A and B, Novel Ptumeran Indole Alkaloids from *Aspidosperma spruceanum*.

**Figure S2.** ESI-MS/MS of alkaloid 1.

**Figure S3.** ESI-MS/MS of alkaloid 2.
Figure S4. $^1$H NMR (500MHz) in CDCl$_3$ of mixture alkaloids 1 and 2.

Figure S5. $^{13}$C NMR (125 MHz) in CDCl$_3$ of mixture alkaloids 1 and 2.
Figure S6. $^{13}$C NMR-DEPT 135 (125 MHz) in CDCl$_3$ of mixture alkaloids 1 and 2.

Figure S7. Homonuclear correlation $^1$H-$^1$H COSY in CDCl$_3$ of mixture alkaloids 1 and 2.
Figure S8. Heteronuclear correlation HSQC in CDCl₃ of mixture alkaloids 1 and 2.

Figure S9. Homonuclear correlation 'H-'H-NOESY in CDCl₃ of mixture alkaloids 1 and 2.
Spruceanumines A and B, Novel Plumeran Indole Alkaloids from *Aspidosperma spruceanum*.

**Figure S10.** Heteronuclear correlation HMBC in CDCl3 of mixture of alkaloids 1 and 2.

**Figure S11.** Selected NOESY correlations and relative stereochemistry for spruceanumines A (1) and B (2). Arrows denote the main NOESY correlations.