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

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Dois novos alcalóides indólicos com esqueleto plumerano, spruceanuminas A (1) e B (2), e oito alcalóides indólicos conhecidos, aspidospermidina (3), desmetoxipalosina (4), aspidocarpina (5), aspidolimina (6), fendlerina (7), aspidolimidina (8), obscurinervidina (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 H e 13C (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), obscurinervidina (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 1H and 13C 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),4,7 demethoxypalosine (4),7,9 aspidocarpine (5),8,10,14 aspidolimine (6),8,14 fendlerine (7),15,16 aspidolimidine (8),8,13,15 obscurinervidina (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), obscurinervide (9) and obscurinervine (10) were identified on the basis of 1H 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, which were also used to complete unambiguous 1H and 13C chemical shift assignments of 1 and 2.

Spruceanamines A (1) and B (2), were obtained as a mixture of amorphous form, [α]D 12 = -101.7 (CHCl₃, c 0.61). Infrared (IR) spectrum showed bands at ν_max 3100-2890 (C-H stretching), 1755 (stretching of the γ-lactone carbonyl group) in addition to other bands at ν_max 1624, 1606 and 1497 (C=O stretching of the benzene ring), and 887 and 739 cm⁻¹ (C-H bending of substituted benzene ring).

Comparative analysis of the 1H- and distortionless enhancement by polarization transfer (DEPT) 135°- (C-H bending of substituted benzene ring), (C=C stretching of the benzene ring), and 887 and 739 cm⁻¹ (one aromatic at δ_C 101.78/ δ_H 6.63 (s) and two olefinic at δ_C 123.31/δ_H 5.81 (ddd) and 130.79/δ_H 5.37 (brd)), seven (1) and eight (2) sp³ methylene [(CH₂), or (CH₃)], including one linked to oxygen atom at δ_C 72.26 (1) and 70.20 (2, revealing γ-effect of the methyl group CH₃-4')] and three methyl [δ(CH₃): δ_C 15.10/δ_H 1.12 (d, J = 6.2 Hz), 1: δ_C 9.39/δ_H 0.98 (t, J = 7.5 Hz), 2; and (MeO)], represented by signals at δ_C 56.49/δ_H 3.70 (s) and 61.18/δ_H 3.81 (s), 1: δ_C 56.97/δ_H 3.74 (s) and 61.18/δ_H 3.86 (s), 2] carbon atoms, allowing to deduce the expanded molecular formulae (C₉)(C=O)(N-C-O)(CH₂)(O-CH₂)(CH₂)(O-CH₂)(O-CH₂)(CH₂)(O-CH₂)(CH₂)(O-CH₂)(MeO) for 1 and 2, respectively. This later contains additional methylene group CH₂ (δ_C 22.56/δ_H 1.69 (m) and 1.46 (m) coupled to the hydrogens of an adjacent methyl group (δ_C 9.39/δ_H 0.98 (t, J = 7.5 Hz).

The high resolution electro-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 (C₂₅H₂₅N₂O₅ = m/z 425.2076, Δ_m/z 0.0094) and 439.2332 of 2 (C₂₅H₂₅N₂O₅ = m/z 439.2233, Δ_m/z 0.0099) Daltons, which

![Figure 1](image-url)
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together with the NMR \(^{13}\text{C}\) (125 MHz) NMR 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\(_{25}\)H\(_{30}\)N\(_2\)O\(_5\) = H\(_2\)), which is consistent with the structure of alkaloids containing the nucleus of 21-oxo-aspidospermidine\(^{20}\) (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

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**Table 1.** \(^{1}\text{H}\) (500 MHz) and \(^{13}\text{C}\) (125 MHz) NMR data of mixture spruceamine A (1) and B (2), in CDCl\(_{3}\), as solvent and TMS used as internal reference. Chemical shifts (\(\delta\), ppm) and coupling constants (\(J\), Hz, in parenthesis)*

*Number of hydrogens bound to carbon atoms deduced by comparative analysis of \(^{1}\text{H}\)- and DEPT-\(^{13}\text{C}\) NMR spectra. Chemical shifts and coupling constants (\(J\)) were obtained of 1D \(^{1}\text{H}\) NMR spectrum. \(^{1}\text{H}\)-\(^{1}\text{H}\)-COSY and \(^{1}\text{H}\)-\(^{1}\text{H}\)-NOESY experiments were also used to these assignments. Superimposed \(^{1}\text{H}\) signals are described without multiplicity and chemical shifts deduced by HSQC and HMBC spectra.*
involving the N-substituent and the oxygen atom sustained by carbon atom C-12, justifying the presence of OCH\(_2\) (I: \(\delta\_c = 72.26/\delta\_h = 4.27\) and 3.90; 2: \(\delta\_c = 70.20/\delta\_h = 4.35\) and 4.00, revealing shielding induced by \(\gamma\)-effect of the methyl 3H-4'), methyl group represented by a doublet signal \((J = 6.2\) Hz) at \(\delta\_h = 1.12\) (3H-3' correlated in the HSQC spectrum with \(\delta\_c = 15.10\)) coupled hydrogen linked to nitrogenated carbon atom \((\delta\_h = 3.27\), \(m\), H-2' correlated with \(\delta\_c = 44.73\), CH-2') in the alkaloid 1 and by a triplet signal \((J = 7.5\) Hz) at \(\delta\_h = 0.98\) (3H-4') coupled to hydrogen atoms of the additional methylene of 2 \((\delta\_h = 1.69\) and 1.46 correlated in the HSQC with \(\delta\_c = 22.56\)). The lower field \(\delta\_c = 44.73\) in compound 2 when to that of 1 \((\delta\_c = 44.73\) is indicative of a \(\beta\)-effect induced by the methyl group CH-4', as shown in Table 1.

The identity of the six-membered heterocyclic ring containing and oxygen, was supported by \(\gamma\)-CH HMBC correlations between C-12 \([\delta\_c = 136.24\) and 1) and 2H-1' \(\delta\_h = 4.27\) and 3.90 (1); \(\delta\_h = 4.35\) and 4.00 (2) (Table 1), as well as by \(\gamma\)-H-H-COSY cross-peaks displayed by H-1'b \((\delta\_h = 4.27\) in 1; 4.35 in 2), H-1'a \((\delta\_h = 3.90\) in 1; 4.00 in 2), H-2' \((\delta\_h = 3.27\) in 1; 3.13 in 2).

The \(\gamma\)-H-COSY spectrum (Table 1) showed coupling of methylenic hydrogens at \(\delta\_h = 4.27\) \([\delta d, J = 10.7\) and 2.7 Hz, H-1'b (1) and \(\delta\_h = 3.90\) \([\delta d, J = 10.7\) and 8.8 Hz, H-1'a (1)] with the methinic hydrogen at \(\delta\_h = 3.27\) (m, H-2', 1) and at \(\delta\_h = 4.35\) \([\delta d, J = 10.8\) and 2.6 Hz, H-1'b (2)] and \(\delta\_h = 4.00\) \([\delta d, J = 10.8\) and 8.6 Hz, H-1'a (2)] correlated with the signal at \(\delta\_h = 3.13\) \([m, H-2', 2]\), in agreement with the presence six-membered ring formation.

The assignment of a methyl group at C-2' was confirmed by its \(\gamma\)-H-H-COSY and \(\gamma\)-CH, HMBC correlations with H-2' \((\delta\_h = 3.27\) and 2H-1' \((\delta\_h = 4.35\) and 4.00), respectively.

In spruceanumine B (2), the presence of an ethyl group at C-2 was confirmed by the coupling of the methylenic hydrogens CH-2' \((\delta\_h = 1.69\) and 1.46) with the vicinal methyl group \((\delta\_h = 0.98\) and H-2' \((\delta\_h = 3.13\).

The \(\gamma\) NMR spectrum of mixture showed signals at \(\delta\_h = 3.70\) (1), 3.74 (2) and \(\delta\_h = 3.81\) (1), 3.86 (2), which are characteristics of methoxyl groups linked to the benzene ring.\(^9\) These signals showed heteronuclear interaction via one bond \((\gamma\)-CH) with the signals at \(\delta\_c = 56.49\) (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 \((\gamma\)-CH, n=2 and 3) observed in the HMBC spectrum, as summarized in Table 1. The signal at \(\delta\_c = 61.18\) (Table 1) observed in the \(\gamma\)-C 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 \(\gamma\)-C NMR spectrum (Table 1) revealed the presence of a \(\gamma\)-lactone covering the carbon atoms C-20 e CH-21 by the signal at \(\delta\_c = 175.10\) (C-18), consistent with carbonyl carbon lactone of five members,\(^20\) that was also confirmed by long-range coupling of C-18 \((\delta\_c = 175.10\) with both hydrogen atoms 2H-19 represented by the signals at \(\delta\_h = 2.50\) (H-19b) and \(\delta\_h = 2.12\) (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-aspidobalbide (18-oxo by actual numeration utilized in the literature), previously isolated from Aspidosperma exalatum\(^10\), and they are also in agreement with the presence of 18,21-olide function in 1 and 2, as suggested by signals at \(\delta\_c = 175.10\) (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 \(\delta\_c = 123.51\) (CH-14, 1 and 2), 130.79 (CH-15, 1) and 130.66 (CH-15, 2), with olefinic hydrogens at \(\delta\_h = 5.81\) (H-14), and \(\delta\_h = 5.37\) (H-15). The vicinal coupling between these hydrogen atoms was confirmed in the \(\gamma\)-H-H-COSY spectrum.\(^18,21\)

The relative stereochemistry of spruceanumine 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 spruceanumes A (1) and B (2). Arrows denote the main NOESY correlations.
\[ H-1^H-N_{\text{NOESY}} \text{ correlations of } H-2 \text{ and } H-2' \text{ of } 1 \text{ and } 2 \text{ indicated both } \alpha-\text{orientations; of } H-2 \text{ with one hydrogen} \]

\[ H-6 \text{ of the methylene group } \text{CH}_2-6 \text{ of } 1 \text{ and } 2 \text{ was also used to establish the relative configuration } 7(S); \]

\[ \text{of } H-2 \text{ with both } H-2' \text{ and } 2H-3 \text{ of the methylene group } \text{CH}_2-3' \text{ of } 2 \text{ revealed } \alpha-\text{orientation of } H-2; \text{ of } H-16β \text{ with methyl group } \text{CH}_3-3' \text{ of } 1 \text{ and with methylene group } \text{CH}_2-3' \text{ of } 2 \text{ are consistent with } \beta \text{ orientation of this hydrogen atom } H-16; \text{ spatial interaction of the of the } H-15 \text{ with both } H-19 \text{ and } H-17 \text{ indicated to these hydrogen atoms } \alpha \text{ and } \beta \text{-orientation, respectively, as shown in Figure 2.} \]

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

\textbf{Experimental}

\textit{General Procedures}

Measurements 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 scheme 1. Proposed fragmentation mechanisms of 1 and 2 by MS/MS of the peaks at } m/z 425.2183 ([M+H]\^+), 1, C_{24}H_{29}N_{2}O_{5} = m/z 425.2076, Δ_{m/z} 0.0107) \text{ and 439.2332 ([M+H]\^+), 2, C_{25}H_{31}N_{2}O_{5} = m/z 439.2332, Δ_{m/z} 0.0099), only peaks classified as principals.\]
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 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 yielding aspidolimine (6, 15.9 mg) and demethoxypalosine (4, 34.7 mg). Fraction 10 (364.5 mg) was rechromatographed over a silica gel column with a gradient of MeOH in CH2Cl2 supplying aspidocarpine (5, 97.9 mg) and aspidospermidine (3, 19.1 mg) alkaloids.

The methanol extract (18.5 g) from seeds was partitioned with CH2Cl2/H2O. CH2Cl2 fraction (7.4 g) was chromatographed over silica gel column with a gradient of CH2Cl2/methanol supplying six fractions. Fraction 3 (3.9 g) was rechromatographed over a silica gel column with a gradient of MeOH in CH2Cl2 supplying four fractions. Fraction 3.1 (74.6 mg) provided the spruceanamines A-B (1-2) alkaloids mixture. Fraction 3.2 (103.2 mg) was rechromatographed over a silica gel column with a gradient of MeOH in CH2Cl2 supplying five fractions. Fraction 3.2.2 (20.6 mg) yielded the fendlerine (7) and aspidolimidine (8) alkaloids mixture, and fraction 3.2.4 (68.2 mg) afforded a mixture of obscurinervidine (9) and obscurinervine (10).

Spruceanamine A (1)

Amorphous solid, mp 195°C; [α]D25 [α]D23 = -101.7° (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 C20H20N2O2: 425.2071 (see Scheme 1); 1H and 13C NMR: see Table 1.

Spruceanamine B (2)

Amorphous solid, mp 195°C; [α]D25 [α]D23 = -101.7° (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 439.2233. Calc. for C20H19N2O2: 439.2227 (see Scheme 1); 1H and 13C NMR: see Table 1.

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Supplementary Information

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

References


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