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Article

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 ^{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**), obscurinervidine (**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**),[4-7] demethoxypalosine (**4**),[7-9] aspidocarpine (**5**),[8,10,14] aspidolimine (**6**),[8,14] fendlerine (**7**),[15,16] aspidolimidine (**8**),[8,13,15] obscurinervidine (**9**)[14,17] and obscurinervine (**10**).[14,17] Their structures were established by spectrometric techniques, mainly one- and two-dimensional nuclear

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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 ^{1}H and ^{13}C NMR spectral data, including ^{1}H-^{1}H correlation spectroscopy (COSY), ^{1}H-^{1}H 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 ^{1}H and ^{13}C chemical shift assignments of **1** and **2**.

Spruceanumines A (**1**) and B (**2**), were obtained as a mixture of amorphous form, $[\alpha]_D^{23}$ = -101.7 ($CHCl_3$, *c* 0.61). Infrared (IR) spectrum showed bands at ν_{max} 3100-2890 (C-H stretching), ν_{max} 1755 (stretching of the γ-lactone carbonyl group) in addition to other bands at ν_{max} 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 {^{1}H}- and distortionless enhancement by polarization transfer (DEPT) 135°- ^{13}C 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 [$(C)_9$: three sp^3 (including one bounded to nitrogen and oxygen atoms at δ_C 106.79), six sp^2 (including one carbonyl group at δ_C 175.10 and five sp^2 attributed to aromatic ring], five methine [$(CH)_5$: two sp^3 linked to nitrogen atom (δ_C 68.91/δ_H 3.50 and δ_C 44.73/δ_H 3.27 correlated in the HSQC spectrum with ^{1}H chemical shifts at δ_H 3.50 and 3.27, respectively, as indicated also in the direct subsequent correlations, $^{1}J_{CH}$) and three sp^2 (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**) sp^3 methylene [$(CH_2)_7$ or $(CH_2)_8$, including one linked to oxygen atom at δ_C 72.26 (**1**) and 70.20 (**2**, revealing γ-effect of the methyl group CH_3-4')] and three methyl [(CH_3): 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 $(MeO)_2$ 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 $(C)_7(C{=}O)(N\text{-}C\text{-}O)(CH)_5(O\text{-}CH_2)(CH_2)_6(CH_3)(MeO)_2$ and $(C)_7(C{=}O)(N\text{-}C\text{-}O)(CH)_5(O\text{-}CH_2)(CH_2)_7(CH_3)(MeO)_2$ for **1** and **2**, respectively. This later contains additional metylene group CH_2 (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** ($C_{24}H_{29}N_2O_5$ = *m/z* 425.2076, $\Delta_{m/z}$ 0.0094) and 439.2332 of **2** ($C_{25}H_{31}N_2O_5$ = *m/z* 439.2233, $\Delta_{m/z}$ 0.0099) Daltons, which

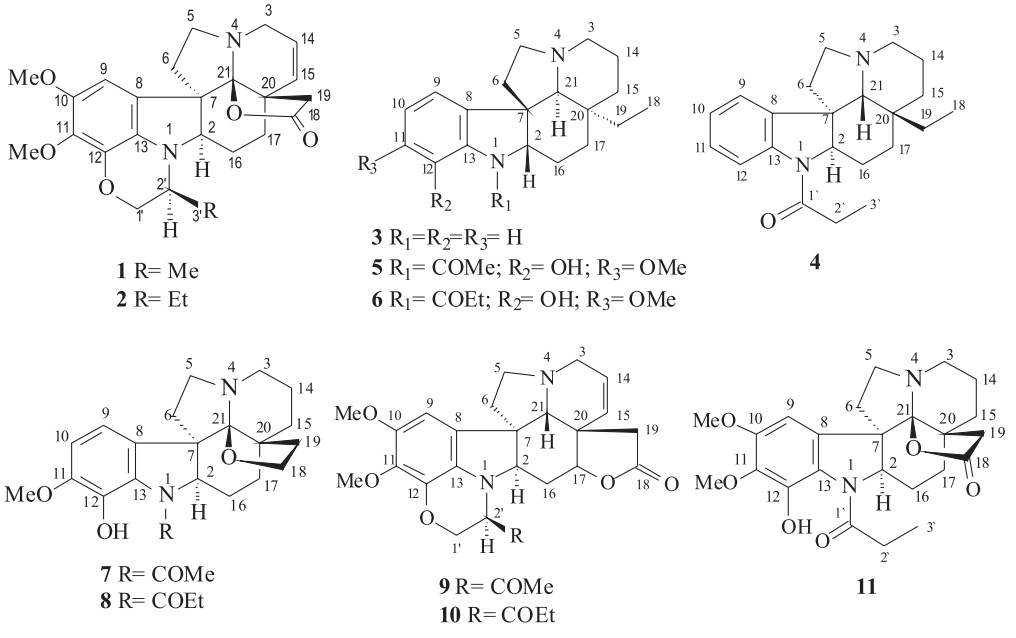


Figure 1. Structure of the plumeran indole alkaloids isolated from *A. spruceanum*.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of mixture spruceanumines A (**1**) and B (**2**), in $CDCl_3$ as solvent and TMS used as internal reference. Chemical shifts (δ, ppm) and coupling constants (J, Hz, in parenthesis)*

	1 HSQC		**2** HSQC		**1 + 2** HMBC	
	δ_C	δ_H			$^{2}J_{CH}$	$^{3}J_{CH}$
C						
7	60.21	-	60.21	-	H-2; 2H-6	H-5a; H-9; 2H-16
8	124.90	-	124.76	-	H-9	H-6b
10	147.45	-	147.27	-	H-9	MeO-10
11	136.53	-	136.53	-		H-9; MeO-11
12	136.24	-	136.24	-		2H-1'
13	131.03	-	131.06	-		H-9
18	175.10	-	175.10	-	2H-19	
20	43.90	-	43.90	-	H-15; 2H-27	H-14; 2H-16
21	106.79	-	106.79	-		
CH						
2	68.91	3.50 (*m*)	68.91	3.50 (*m*)		H-1'; 2H-6; 2H-17
9	101.78	6.63 (*s*)	101.85	6.63 (*s*)		
14	123.51	5.81 (*ddd*, 9.9, 3.7, 1.7)	123.51	5.81 (*ddd*, 9.9, 3.7, 1.7)	H-3	
15	130.79	5.37 (*brd*, 9.9)	130.66	5.37 (*brd*, 9.9)		H-3; 2H-17; 2H-19
2'	44.73	3.27 (*m*)	50.43	3.13 (*m*)	2H-1'; 2H-3'	3H-4'
CH_2						
3	45.00	3.60-3.40 (*m*)	45.88	3.60-3.40 (*m*)	H-14	H-15
5	50.09	3.34 (*m*), 3.15 (*m*)	50.43	3.34 (*m*), 3.15 (*m*)	2H-6	
6	33.76	2.55 (*m*), 2.03 (*m*)	33.89	2.55 (*m*), 2.03 (*m*)	2H-5	H-2
16	18.96	1.77 (*m*), 1.47 (*m*)	19.11	1.77 (*m*), 1.47 (*m*)	2H-17	
17	28.82	1.75 (*m*), 1.58 (*m*)	28.82	1.75 (*m*), 1.58 (*m*)	2H-16	H-19a
19	40.53	2.50 (*d*, 16.4) 2.12 (*d*, 16.4)	40.53	2.50 (*d*, 16.4) 2.12 (*d*, 16.4)		H-15; 2H-17
1'	72.26	4.27 (*dd*, 10.7, 2.7) 3.90 (*dd*, 10.7, 8.8)	70.20	4.35 (*dd*, 10.8, 2.6) 4.00 (*dd*, 10.8, 8.6)	H-2'	2H-3'
3'	-	-	22.56	1.69 (*m*), 1.46 (*m*)	H-2'; 3H-4'	2H-1'
CH_3						
3'	15.10	1.12 (*d*, 6.2)	-	-		
4'	-	-	9.39	0.98 (*t*, 7.5)	2H-3`	H-2`
MeO						
10	56.49	3.70 (*s*)	56.97	3.74 (*s*)		
11	61.18	3.81 (*s*)	61.18	3.86 (*s*)		

*Number of hydrogens bound to carbon atoms deduced by comparative analysis of {^{1}H}- and DEPT-^{13}C NMR spectra. Chemical shifts and coupling constants (*J*) were obtained of 1D ^{1}H NMR spectrum. ^{1}H-^{1}H-COSY and ^{1}H-^{1}H-NOESY experiments were also used to these assignments. Superimposed ^{1}H signals are described without multiplicity and chemical shifts deduced by HSQC and HMBC spectra.

together with the NMR ^{13}C spectrum enable to propose molecular formulas $C_{24}H_{28}N_2O_5$ (**1**) and $C_{25}H_{30}N_2O_5$ (**2**), respectively, containing twelve degrees of unsaturation ($C_{24}H_{52}N_2O_5$ - $C_{24}H_{28}N_2O_5 = H_{24}$ or $C_{25}H_{54}N_2O_5$ -$C_{25}H_{30}N_2O_5$ = H_{24}), which is consistent with the structure of alkaloids containing the nucleus of 21-oxo-aspidoalbidine[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

involving the N-substituent and the oxygen atom sustained by carbon atom C-12, justifying the presence of OCH_2 (**1**: δ_C 72.26/δ_H 4.27 and 3.90; **2**: δ_C 70.20/δ_H 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 δ_H 1.12 (3H-3' correlated in the HSQC spectrum with ^{13}C chemical shift at δ_C 15.10) coupled hydrogen linked to nitrogenated carbon atom (δ_H 3.27 , *m*, H-2' correlated with ^{13}C signal at δ_C 44.73, CH-2') in the alkaloid **1** and by a triplet signal (J = 7.5 Hz) at δ_H 0.98 (3H-4') coupled to hydrogen atoms of the additional methylene of **2** (δ_H 1.69 and 1.46 correlated in the HSQC with ^{13}C chemical shift at δ_C 22.56). The lower field ^{13}C chemical shift CH-2' (δ_C 50.43) in compound **2** when to that of **1** (δ_C 44.73) is indicative of a β-effect induced by the methyl group CH_3-4', as shown in Table 1.

The identity of the six-membered heterocyclic ring containing and oxygen, was supported by $^3J_{CH}$ HMBC correlations between C-12 [(δ_C 136.24, **1** and **2**)] and 2H-1' [δ_H 4.27 and 3.90 (**1**); δ_H 4.35 and 4.00 (**2**) (Table 1), as well as by ^{1}H-^{1}H-COSY cross-peaks displayed by H-1`b ($\delta_H$ 4.27 in **1**; 4.35 in **2**), H-1`a (δ_H 3.90 in **1**; 4.00 in **2**), H-2` (δ_H 3.27 in 1; 3.13 in **2**).

The ^{1}H-^{1}H-COSY spectrum (Table 1) showed coupling of methylenic hydrogens at δ_H 4.27 [(*dd*, J = 10.7 and 2.7 Hz, H-1'b (**1**)] and δ_H 3.90 [(*dd*, J = 10.7 and 8.8 Hz, H-1'a (**1**)] with the methinic hydrogen at δ_H 3.27 (m, H-2', **1**) and at δ_H 4.35 [(*dd*, J = 10.8 and 2.6 Hz, H-1'b (**2**)] and δ_H 4.00 [(*dd*, J = 10.8 and 8.6 Hz, H-1'a (**2**)] correlated with the signal at δ_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 ^{1}H-^{1}H-COSY and $^3J_{CH}$ HMBC correlations with H-2' (δ_H 3.27) and 2H-1' (δ_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_2-3' (δ_H 1.69 and 1.46) with the vicinal methyl group (δ_H 0.98) and H-2' (δ_H 3.13).

The ^{1}H NMR spectrum of mixture showed signals at δ_H 3.70 (**1**), 3.74 (**2**) and δ_H 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 ($^1J_{CH}$) with the signals at δ_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 ($^nJ_{CH}$, n= 2 and 3) observed in the HMBC spectrum, as summarized in Table 1. The signal at δ_C 61.18 (Table 1) observed in the ^{13}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 ^{13}C NMR spectrum (Table 1) revealed the presence of a γ-lactone covering the carbon atoms C-20 e CH-21 by the signal at δ_C 175.10 (C-18), consistent with carbonyl carbon lactone of five members,[20-21] that was also confirmed by long-range coupling of C-18 (δ_C 175.10) with both hydrogen atoms 2H-19 represented by the signals at δ_H 2.50 (H-19b) and δ_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-aspidoalbidine (18-oxo by actual numeration utilized in the literature), previously isolated from *Aspidosperma exalatum*[20], and they are also in agreement with the presence of 18,21-olide function in **1** and **2**, as suggested by signals at δ_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 δ_C 123.51 (CH-14, **1** and **2**), 130.79 (CH-15, **1**) and 130.66 (CH-15, **2**), with olefinic hydrogens at δ_H 5.81 (H-14), and δ_H 5.37 (H-15). The vicinal coupling between these hydrogen atoms was confirmed in the ^{1}H-^{1}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.

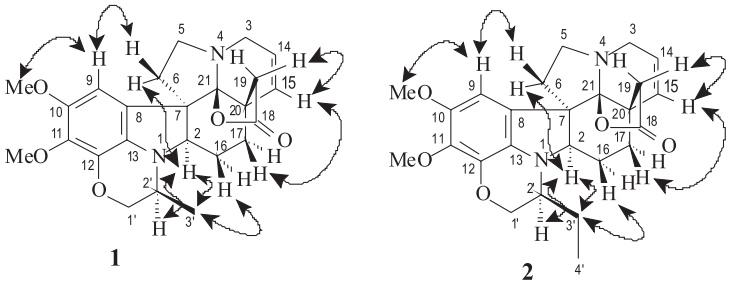
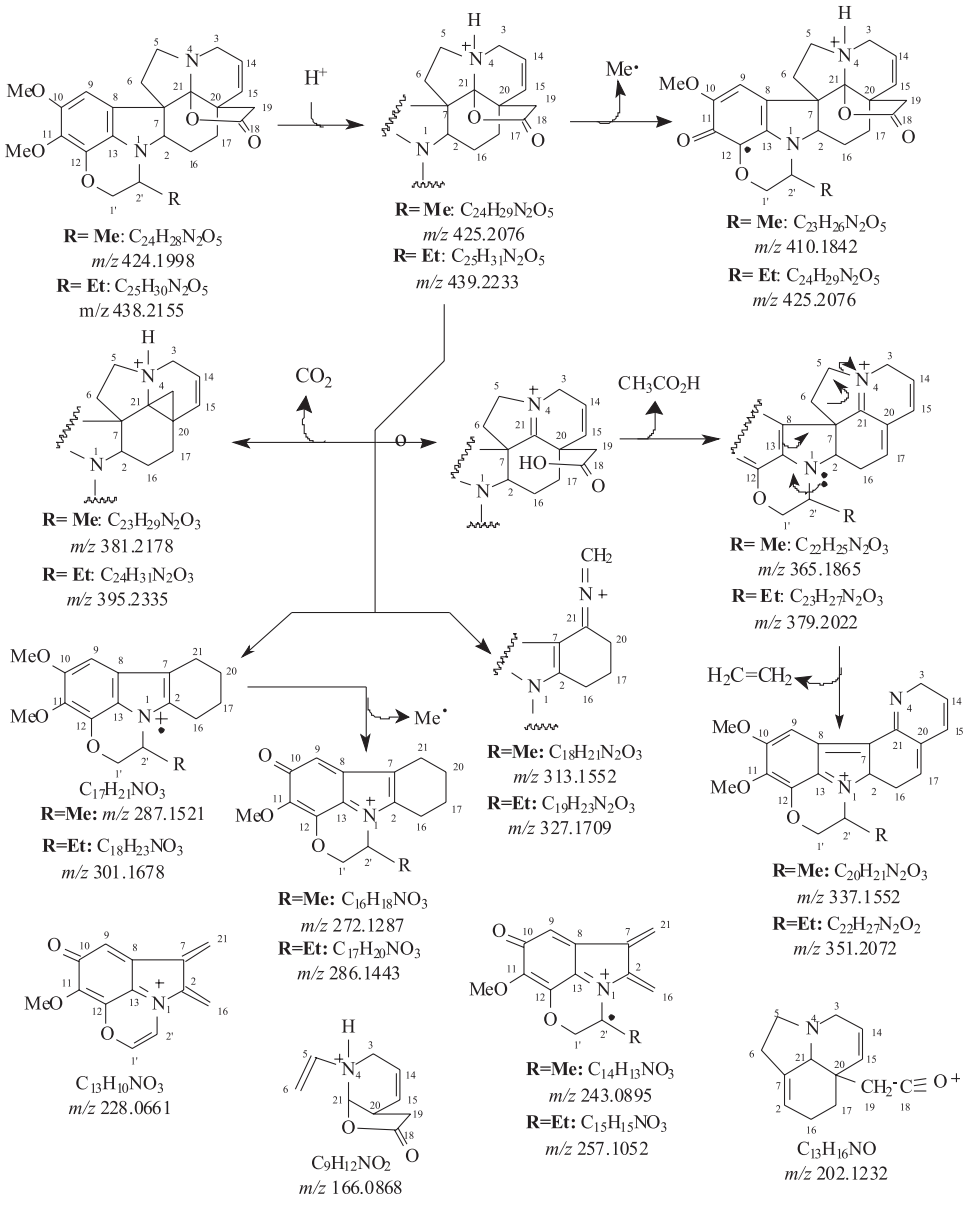


Figure 2. Selected NOESY correlations and relative stereochemistry for spruceanumines A (**1**) and B (**2**). Arrows denote the main NOESY correlations.



Scheme 1. Proposed fragmentation mechanisms of **1** and **2** by MS/MS of the peaks at *m/z* **425.2183** ([M+H]$^+$, **1**, $C_{24}H_{29}N_2O_5$ = *m/z* 425.2076, $\Delta_{m/z}$ 0.0107) and **439.2332** ([M+H]$^+$, **2**, $C_{25}H_{31}N_2O_5$ = *m/z* 439.2332, $\Delta_{m/z}$ 0.0099), only peaks classified as principals.

^{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**, δ_H 1.12) and CH_3-4' (**2**, δ_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

MICROMASSUltrOTOF-Q (Brüker 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 $60F_{254}$ was used in thin layer chromatography analysis.

^1H and ^{13}C NMR spectra were measured on a Brüker DRX500 spectrometer, equipped with inverse probes and field gradient, operating at 500 (^1H) and 125 (^{13}C) MHz. $CDCl_3$ was used as solvent and tetramethylsilane (TMS) as internal reference. Chemical shifts are given in the δ scale (ppm) and coupling constants *J* in Hz. One dimensional (1D) ^1H and ^{13}C NMR spectra were acquired under standard conditions by using a direct detection 5 mm $^1H/^{13}C$ 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 CH_2Cl_2/H_2O. The CH_2Cl_2 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 CH_2Cl_2 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 CH_2Cl_2 supplying aspidocarpine (**5**, 97.9 mg) and aspidospermidine (**3**, 19.1 mg) alkaloids.

The methanol extract (18.5 g) from seeds was partitioned with CH_2Cl_2/H_2O. CH_2Cl_2 fraction (7.4 g) was chromatographed over silica gel column with a gradient of CH_2Cl_2/methanol supplying six fractions. Fraction 3 (3.9 g) was rechromatographed over a silica gel column with a gradient of MeOH in CH_2Cl_2 supplying four fractions. Fraction 3.1 (74.6 mg) provided the spruceanumines 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 CH_2Cl_2 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**).

Spruceanumine A (**1**)

Amorphous solid, mp 195°C; $[\alpha]_D^{23}$ $[\alpha]_D^{23}$ = -101.7° ($CHCl_3$, *c* 0.61); IR (KBr disk) ν_{max}/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 $C_{24}H_{29}N_2O_5^+$: 425.2071 (see Scheme 1); ^1H and ^{13}C NMR: see Table 1.

Spruceanumine B (**2**)

Amorphous solid, mp 195°C; $[\alpha]_D^{23}$ = -101.7° ($CHCl_3$, *c* 0.61); IR (KBr disk) ν_{max}/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 $C_{25}H_{31}N_2O_5^+$: 439.2227 (see Scheme 1); ^1H and ^{13}C NMR: see Table 1.

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Supplementary Information

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

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