A Novel Alkaloid from *Aspidosperma pyricollum* (Apocynaceae) and Complete ^{1}H and ^{13}C Chemical Shift Assignments

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A novel natural product indole, alkaloid, named *rel*-pyricolluminol (**1**), was isolated from *Aspidosperma pyricollum* MÜLL.ARG. together with six known metabolites sitsirikine (**2**), aparicin (**3**), ulein (**4**), stemmadenine (**5**), lupeol (**6**), and (3*β*)-sitoster-3-yl *β*-D-glucopyranoside (**7**). These compounds were characterized on the basis of their spectral data, mainly 1D- (^{1}H,^{13}C-DEPTQ) and 2D-NMR (^{1}H,^{1}H-COSY, NOESY, HSQC, and HMBC), and mass spectra (EI-MS and HR-ES-MS), involving also comparison with data from the literature.

Introduction. – The *Aspidosperma* (Apocynaceae) genus is endemic to the Americas and found mainly in regions between Mexico and Argentina [1]. The *Aspidosperma* genus continues to be a fascinating and 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 pyricollum* MÜLL.ARG. commonly known as 'Guatambu' in Atlantic forest in the North of Espírito Santo State, appears as a tree of 3–10 m. Previous findings concerning the isolation and structure elucidation of eleven alkaloids from an *A. pyricollum* species collected in Brazil were reported [1]. In the present article, we describe the isolation and characterization of one novel indole alkaloid, named as *rel*-pyricolluminol (**1**), along with six known compounds sitsirikine (**2**), aparicin (**3**), ulein (**4**), stemmadenine (**5**), lupeol (**6**), and (3*β*)-sitoster-3-yl *β*-D-glucopyranoside (**7**). Their structures (*Fig.*) were established by spectrometric techniques, mainly 1D- and 2D-NMR, and HR-ESI-MS, involving comparison with data described in the literature.

Results and Discussion. – Elaboration of the MeOH extract of the stem bark and seeds from *A. pyricollum* by classical chromatographic methods resulted in the isolation of seven compounds **1**–**7**, whose structures are shown in the *Figure.* The new natural product *rel*-pyricolluminol (**1**), along with six known compounds, sitsirikine (**2**) [3], aparicin (**3**) [4][5], ulein (**4**) [6][7], stemmadenine (**5**) [5][8], lupeol (**6**) [9], and (3*β*)-sitoster-3-yl *β*-D-glucopyranoside (**7**) [10], were characterized on the basis of ^{1}H- and ^{13}C-NMR spectral data, especially 2D-NMR, and mass spectral data involving comparison with values described in the cited literature.

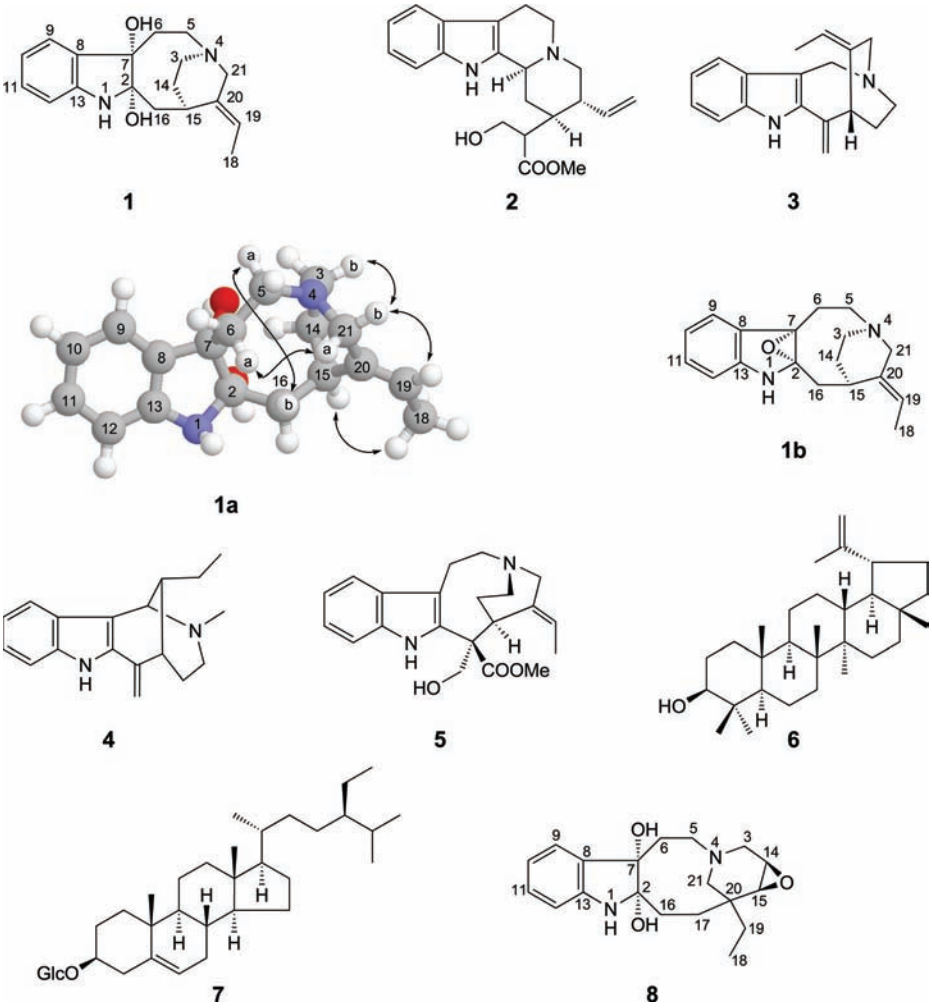


Figure. *Structures of compounds* **1**–**7**, *isolated from* Aspidosperma pyricollum, *and NOESY correlations* (H↔H) *of* **1**

Alkaloid **1** was isolated as yellow oil. The IR spectrum showed bands at ν_{max} 3100 (O–H stretching), in addition to other bands at ν_{max} 1600 and 1490 (C=C stretching of the benzene ring), and 750 cm^{-1} (C–H bending of benzene ring) [11].

The ^{13}C-DEPTQ NMR spectra (*Table*) revealed signals corresponding to 18 C-atoms, including five quaternary C (three sp^2 and two sp^3), six CH (one sp^3 and five sp^2 (including four aromatic), and one vinylic C (δ(C) 118.3)), six CH_2 (including three ones linked to a N-atom at δ(C) 56.0, 58.6, and 52.1), and one Me (δ(C) 11.4).

The EI-MS spectrum of **1** showed no peak corresponding to the molecular ion (M^+) at m/z 300 Daltons, but revealed peaks at m/z 283 (30.9%, $[M-OH]^+$) and 282 (28.6%, $[M-H_2O]^+$), compatible with the formation of fragments suggesting the loss

Table. *^1H-* (500 MHz) *and ^{13}C-NMR* (125 MHz) *Data of Alkaloid* **1** *in CD_3OD and* of *Alkaloid* **8** *in $(D_6)DMSO$.* δ in ppm, J in Hz.

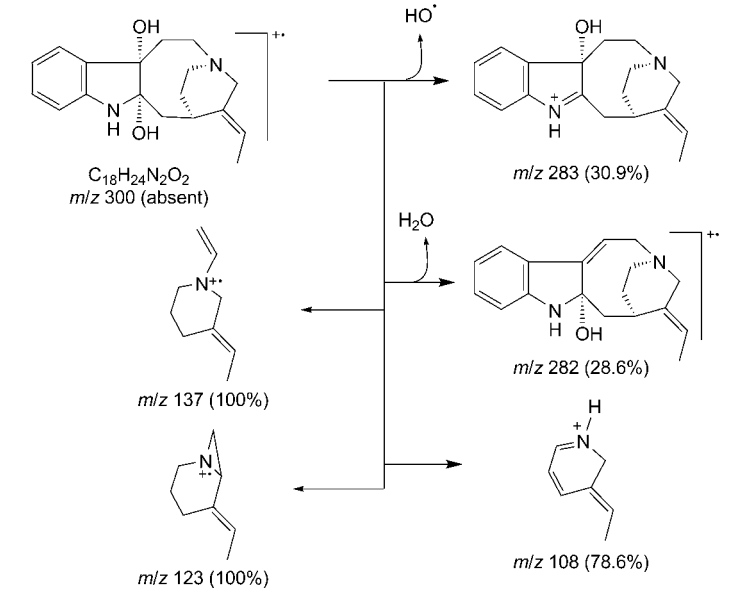
Position	**1**				**8**
	HSQC (H → C)		HMBC (H → C)		
	δ(H)	δ(C)	^2J	^3J	δ(C)
2	–	99.4	CH_2(16)	H_b-6, H-15	**101.6**
3	3.77 (*t*, *J* = 11.3), 2.52–2.48 (*m*)	52.1	–	–	–
5	3.47–3.46 (*m*), 3.40–3.30 (*m*)	56.0	H_a-6		62.2
6	2.65 (*dt*, *J* = 13.9, 7.8), 2.51 (*dd*, *J* = 13.9, 7.2)	41.6	H_b-5		24.5
7	–	86.7	H_a-6	H_a-5, H-9	**85.2**
8	–	132.0		CH_2(6), H-10, H-12	132.6
9	7.31 (*d*, *J* = 7.4)	122.4		H-11	122.3
10	6.94 (*d*, *J* = 7.4)	121.0		H-12	119.9
11	7.21 (*t*, *J* = 7.4)	130.0		H-9	129.2
12	6.78 (*d*, *J* = 7.4)	110.5		H-10	110.5
13	–	145.6		H-9, H-11	145.1
14	2.45–2.40 (*m*), 2.01–1.98 (*m*)	22.9		H_a-16	47.9
15	3.50–3.45 (*m*)	26.1		H-9, H_b-21	57.4
16	2.73 (*d*, *J* = 14.7), 2.10 (*dd*, *J* = 14.7, 4.1)	33.0			40.3
17	–	–	–	–	24.3
18	1.75 (*dd*, *J* = 6.8, 1.1)	11.4			7.2
19	5.52 (*q*, *J* = 6.8)	118.3	Me(18)	H_b-21	30.2
20	–	130.1	H_a-21	CH_2(16), Me(18)	31.7
21	4.57 (br. *d*, *J* = 14.9), 3.98 (*d*, *J* = 14.9)	58.6		H-15, H-19	56.6

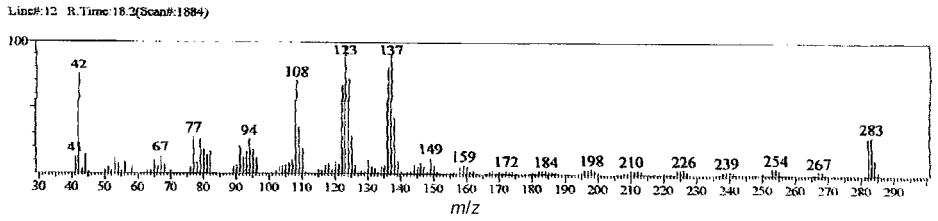
of OH groups (*Scheme*). Together with the ^{13}C-DEPTQ NMR spectrum, the molecular formula was proposed to be $C_{18}H_{24}N_2O_2$, indicating eight degrees of unsaturation (five belonging to the indole moiety, one C=C bond, and two additional rings). The above peaks suggested the 19,20-dihydro-2α,7α-dihydroxyvoaphylline (**8**) skeleton [12]. The principal fragments observed in the mass spectrum were interpreted as summarized in the *Scheme* (*Part A*). Surprisingly, the HR-ES-MS spectrum of **1** also showed no peak corresponding to the molecular ion (M^+) at m/z 300.1838 ($C_{18}H_{24}N_2O_2$) Daltons, but revealed peaks at m/z 283.1808 ($[M - H_2O + H]^+$, $C_{18}H_{23}N_2O^+$; calc. 283.1810, $\Delta_{m/z}$ 0.0002 Daltons) and 265.1693 ($[M - H_2O + H - H_2O]^+$, $C_{18}H_{21}N_2$, calc. 265.1705, $\Delta_{m/z}$ 0.0012 Daltons), as summarized in the *Scheme* (*Part B*). The structural alternative **1b** involving an epoxy function located at C(2) and C(7) (*Fig.*) was immediately eliminated by its chemical shifts at δ(C) 99.4 (C(2)) and 86.7 (C(7)), compared with the values revealed by the epoxy C-atoms in the alkaloid **8** (*Table*).

Typically, the ^{13}C-DEPTQ NMR of **1** revealed two signals of an exocyclic C=C bond at δ(C) 130.1 (*s*, C(20)) and 118.3 (*d*, C(19)). The location of this C=C bond was established by analysis of the HMBC spectrum, which revealed cross-peaks

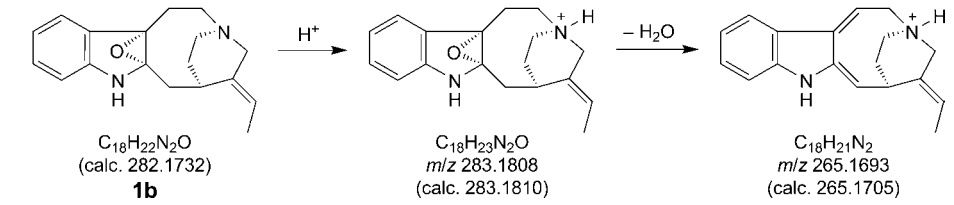
Scheme. *Fragments Proposed to Justify the Main Peaks Observed in the EI-MS of* **1**

Part A





Part B



corresponding to heteronuclear spin-spin couplings of C(20) (δ(C) 130.1) *via* three (^3J(H → C)) bonds with both the Me group (δ(H) 1.75, Me(18)) and the CH_2 group (δ(H) 2.73 and 2.10) and *via* two (^2J(H → C) with H–C(19) (δ(H) 5.52).

The presence of the two carbinolic C-atoms at positions C(2) and C(7) was also corroborated by analysis of the HMBC spectrum (*Table*), which revealed cross-peaks corresponding to heteronuclear spin-spin couplings of the signal of C(2) (δ(C) 99.4) with CH_2(16) (δ(H) 2.73 and 2.10, ^2J(H → C)) and H_b–C(6) (δ(H) 2.65, ^2J(H → C))

and H–C(15) (δ(H) 3.50–3.45, ^3J(H → C)). The signal of C(7) (δ(C) 86.7) revealed couplings with H_a–C(6) (δ(H) 2.51, ^3J(H → C)), H_a–C(5) (δ(H) 3.30–3.40, ^2J(H → C) and H–C(9) (δ(H) 7.31, ^3J(H → C)).

The complete analysis of the HMBC spectrum confirmed the presence of a similar basic skeleton to that of the indole alkaloid 19,20-dihydro-2α,7α-dihydroxyvoaphylline (**8**) skeleton [12]. This significantly contributed to the complete ^{1}H and ^{13}C chemical shift assignments (*Table*).

The relative configuration of **1** was determined predominantly by a NOESY spectrum that showed cross-peaks assigned to dipolar interaction (spatial proximity) of H_β–C(6) (δ(H) 2.65) with H_β–C(21) (δ(H) 4.57), H_β–C(5) (δ(H) 3.47–3.46) with H_α–C(16) (δ(H) 2.10), H–C(15) (δ(H) 3.50–3.45) with Me(18) (δ(H) 1.75), and H_α–C(21) with both H_α–C(3) (δ(H) 2.52–2.48) and H–C(19) (δ(H) 5.52), consistent with the relative configuration shown in **1** and **1a**.

Thus, the new indole alkaloid, isolated from *Aspidosperma pyricollum* MÜLL.ARG., was characterized as *rel*-pyricolluminol (**1**).

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Experimental Part

General. Thin layer chromatography (TLC): silica gel *60 F_{254}* (SiO_2). Column chromatography (CC): SiO_2 *60* (70–230 mesh). IR Spectra: *Shimadzu 8300* FT-IR spectrometer using KBr disk; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker DRX-500* spectrometer, equipped with inverse probes and field gradient, operating at 500 (^{1}H) and 125 (^{13}C) MHz; in $CDCl_3$; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. One dimensional (1D) ^{1}H and ^{13}C NMR spectra were acquired under standard conditions by using a direct detection 5 mm ^{1}H/^{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. EI-MS: *Shimadzu QP5050A* mass spectrometer; in *m*/*z* (rel. %).

Plant Material. The stem bark of *Aspidosperma pyricollum* MÜLL.ARG. was collected in February 2010 at Cia Vale, Linhares City, Brazil, and identified by Botanist *Domingos A. Folly.* A voucher specimen (CVRD 293) was deposited with the Vale Cia Herbarium, Espírito Santo State, Brazil.

Extraction and Isolation. Dried and powdered stem bark (1.48 kg) from *Aspidosperma pyricollum* MÜLL.ARG. was extracted with MeOH at r.t., furnishing, after solvent evaporation, 128.7 g of the crude MeOH extract. Part of the MeOH extract (69.4 g) was chromatographed on a SiO_2 column with a gradient of MeOH/CH_2Cl_2, to afford five fractions, *Frs. 1–5*. *Fr. 1* (6.6 g) was rechromatographed with a gradient of hexane/AcOEt furnishing 18 fractions, *Frs. 1.1–1.18*. *Fr. 1.5* (472 mg) was rechromatographed on a SiO_2 column with a gradient of AcOEt/hexane to afford triterpene **6** (93.0 mg). *Fr. 3* (16.4 g) was chromatographed on a SiO_2 column with a gradient of MeOH/CH_2Cl_2 supplying twelve fractions, *Frs. 3.1–3.12*. *Fr. 3.5* (296.6 mg) was chromatographed on a SiO_2 column with a gradient of MeOH/CH_2Cl_2 to yield alkaloid **3** (10.0 mg). *Fr. 3.7* (578.6 mg) was chromatographed on a SiO_2 column with a gradient of MeOH/CH_2Cl_2 to give two alkaloids **1** (34.1 mg), **4** (13.6 mg), and steroid **7** (14.0 mg). *Fr. 3.9* (2.5 g) was chromatographed on a neutral alumina column with a gradient of MeOH/CH_2Cl_2 to yield alkaloid **2** (6.2 mg).

Dried and powdered seeds (1.3 kg) from *Aspidosperma pyricollum* MÜLL.ARG. were extracted with MeOH at r.t., furnishing, after solvent evaporation, 783 g of crude MeOH extract. Part of the MeOH extract (33.7 g) was chromatographed on a SiO_2 column with a gradient of MeOH/CH_2Cl_2 to afford eleven fractions, *Frs. 1–11*. *Fr. 5* (643 mg) was chromatographed on a SiO_2 column with a gradient of

MeOH/CH_2Cl_2 to yield alkaloid **3** (5.1 mg). *Fr. 6* (606.7 mg) was crystallized from CH_2Cl_2 to afford alkaloid **5** (49 mg).

rel-*Pyricolluminol* (= rel-(*5E,6R,7aS,12bS*)*-5-Ethylidene-4,5,6,7-tetrahydro-2*H*-3,6-ethanoazonino[5,4-*b*]indole-7a,12b(1*H*,8*H*)-diol*; **1**). Yellow oil. IR: 3100 (O–H stretching), 1600 and 1490 (C=C stretching of the benzene ring), and 750 (C–H bending of benzene ring). ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: see the *Scheme*.

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