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Darcyribeirine, a novel pentacyclic indole alkaloid from *Rauvolfia grandiflora* Mart.

Náuvia Maria Cancelieri, Ivo José Curcino Vieira,* Jan Schripsema, Leda Mathias and Raimundo Braz-Filho

Setor de Química de Produtos Naturais-LCQUI-CCT, Universidade Estadual do Norte Fluminense, 28015-620, Campos dos Goytacazes, Rio de Janeiro, Brazil

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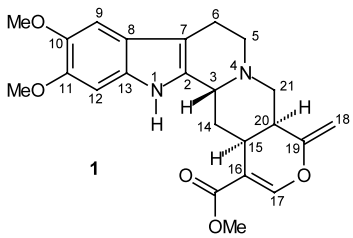
Abstract—Three pentacyclic indole alkaloids, one novel named darcyribeirine (**1**) and the known isoreserpiline (**2**) and isoreserpine (**3**), were isolated from the root bark of the *Rauvolfia grandiflora* Mart. The structures were established by spectroscopic data.

Rauvolfia genus, family Apocynaceae, continues to be fascinating as it produces a number of indole alkaloids with novel skeletons, which are interesting from the biosynthetic point of view as well as for their medicinal aspect[1] and spectroscopic analysis. *Rauvolfia grandiflora* Mart., which are bushes of 2–5 m, are commonly known as 'Saco-de-Gambá' in the Atlantic forest in the northern part of Rio de Janeiro State. In the present paper, results of an investigation of *R. grandiflora* Mart. root bark are reported describing the chemical characterization of a new pentacyclic indole alkaloid with the unique feature of a C-18–C-19 double bond in an ajmalan skeleton, which was named darcyribeirine (**1**) and two known indole alkaloids isoreserpiline (**2**)[2,3] and isoreserpine (**3**).[3]



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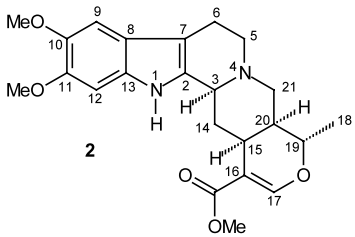

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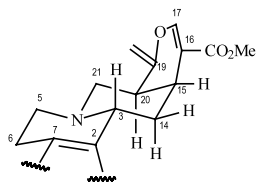
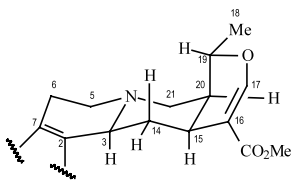


1a



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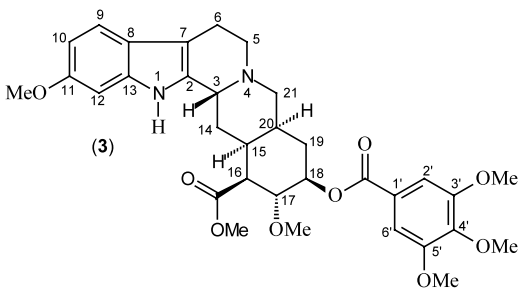

(3)

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* Corresponding author. Tel.: 55-24-27261597; fax: 55-24-27261519; e-mail: curcino@uenf.br

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The known isoreserpiline (**2**) and isoreserpine (**3**) were identified by spectral data, involving mainly ^{1}H and ^{13}C NMR spectra and comparison with literature values.[2,3] The 1D and 2D NMR spectra of **2** were used to assign unambiguously the ^{1}H and ^{13}C chemical shifts (Tables 1 and 2).

Dried and powdered root bark (1.67 kg) from *Rauvolfia grandiflora* Mart. was extracted at room temperature using CH_2Cl_2 furnishing, after solvent evaporation, 40.52 g of the extract.

The 50% CH_2Cl_2 extract was chromatographed on a silica gel column with a gradient of MeOH in $CHCl_3$ supplying seven fractions. The first fraction (3.40 g) it was rechromatographed on silica gel column with gradient of MeOH in $CHCl_3$ supplying four fractions. The compounds **1** (154 mg) and **2** (180 mg) were purified from the fraction one (0.40 g) of the second column after having been rechromatographed in column with silica gel and eluted with hexane:ethyl acetate (8:2). The compound **3** (5 mg) were purified from the five fraction (0.7 g) of the first column with the same conditions.

Darcyribeirine (**1**) was obtained as an amorphous solid $[\alpha]_D^{20}$ −36° ($CHCl_3$, *c* 0.05). The UV spectrum showed absorption at λ_{max} 226 and 299 nm (log ε 4.53 and 4.01, respectively) typical of a substituted indole chromophore,[4] while the IR spectrum revealed bands at ν_{max} 3366 (N–H), 1701 (conjugated carbonyl ester group stretching), 2932–2833 (C–H stretching), 1570 (characteristic of a methoxyl indole) and 906–761 cm^{-1} (C–H bending of substituted benzene ring).[4] The EIMS showed a molecular peak at *m/z* 412 daltons ($[M]^{\bullet+}$) which together with ^{1}H and ^{13}C NMR spectral data (Table 1) allowed to deduce the molecular formula $C_{23}H_{28}N_2O_5$ (twelve degrees of unsaturation) compatible with an ajmalan skeleton.[5,6]

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR for darcyribeirine (**1**) and isoreserpiline (**2**), in DMSO-d_6 (**1**) and $CDCl_3$ (**2**) as solvents and residual DMSO-d_6 and $CDCl_3$ used as internal references (δ_H 2.50 and δ_C 39.50; 7.24 and 70.00, respectively). Chemical shifts (δ, ppm) and coupling constants (*J*, Hz, in parentheses)[a]

	1		**2**	
	δ_C	δ_H	δ_C	δ_H
C				
2	132.73	–	133.14	–
7	106.22	–	107.82	–
8	119.98	–	119.94	–
10	144.28	–	144.78	–
11	145.94	–	146.41	–
13	130.57	–	130.13	–
16	108.44	–	109.49	–
19	155.61		–	–
22	165.70	–	168.03	–
CH				
3	54.18	3.36 (dddd, 9.4, 3.1, 2.4, 1.5), H-3ax	59.91	3.32 (m)
9	101.59	6.88 (s)	100.35	6.89 (s)
12	96.64	6.94 (s)	94.82	6.81 (s)
15	28.42	2.80 (ddd, 5.1, 4.5, 4.3)	31.29	2.75 (dt, 4.6, 11.9)
17	151.03	7.44 (s)	155.75	7.56 (s)
19	–	–	72.47	4.49 (dq, 6.2, 10.4)
20	35.95	2.90 (ddd, 8.9, 4.6, 4.5), H-20ax	38.41	1.69 (m)
CH_2				
5	51.47	3.02 (ddd, 11.6, 5.8, 1.5), H-5eq 2.61 (ddd, 11.6, 11.2, 4.5), H-5ax	53.67	2.95 (dd, 5.9, 9.9) 2.54 (dt, 9.9, 5.3)
6	19.82	2.80 (dddd, 14.8, 11.2, 5.8, 2.4), H-6ax 2.51 (dddd, 14.8, 4.5, 1.5, 1.5), H-6 eq	21.82	2.88 (m) 2.64 (m)
14	29.52	2.69 (ddd, 13.6, 5.1, 3.1), H-14eq 1.77 (ddd, 13.6, 9.4, 4.3), H-14ax	34.26	2.48 (ddd, 3.2, 4.0, 11.9) 1.55 (q, 11.9)
18	93.19	4.69 (d, 1.5) 4.56 (d, 1.5)	–	–
21	51.71	2.84 (dd, 11.4, 4.6), H-21eq 2.62 (dd, 11.4, 8.9), H-21ax	56.25	3.09 (dd, 12.3, 2.0) 2.72 (dd, 12.3, 3.5)
CH_3				
18	–	–	18.49	1.40 (d, 6.2)
MeO-10	56.41	3.77 (s)	56.25	3.89 (s)
MeO-11	56.10	3.76 (s)	56.41	3.91 (s)
MeO-22	50.56	3.74 (s)	51.10	3.74 (s)
HN-1		10.12 (s)		7.72 (s)

[a] Number of hydrogens bound to carbon atoms deduced by comparative analysis of HBBD- and DEPT-^{13}C NMR spectra. Chemical shifts and coupling constants (*J*) obtained from 1D ^{1}H NMR spectrum. Superimposed ^{1}H signals are described without multiplicity and chemical shifts deduced by HMQC, HMBC (Table 2) and ^{1}H–^{1}H COSY spectra.

The main peaks observed in the EIMS spectrum are in agreement with the proposed fragmentation mechanisms summarized in Scheme 1.

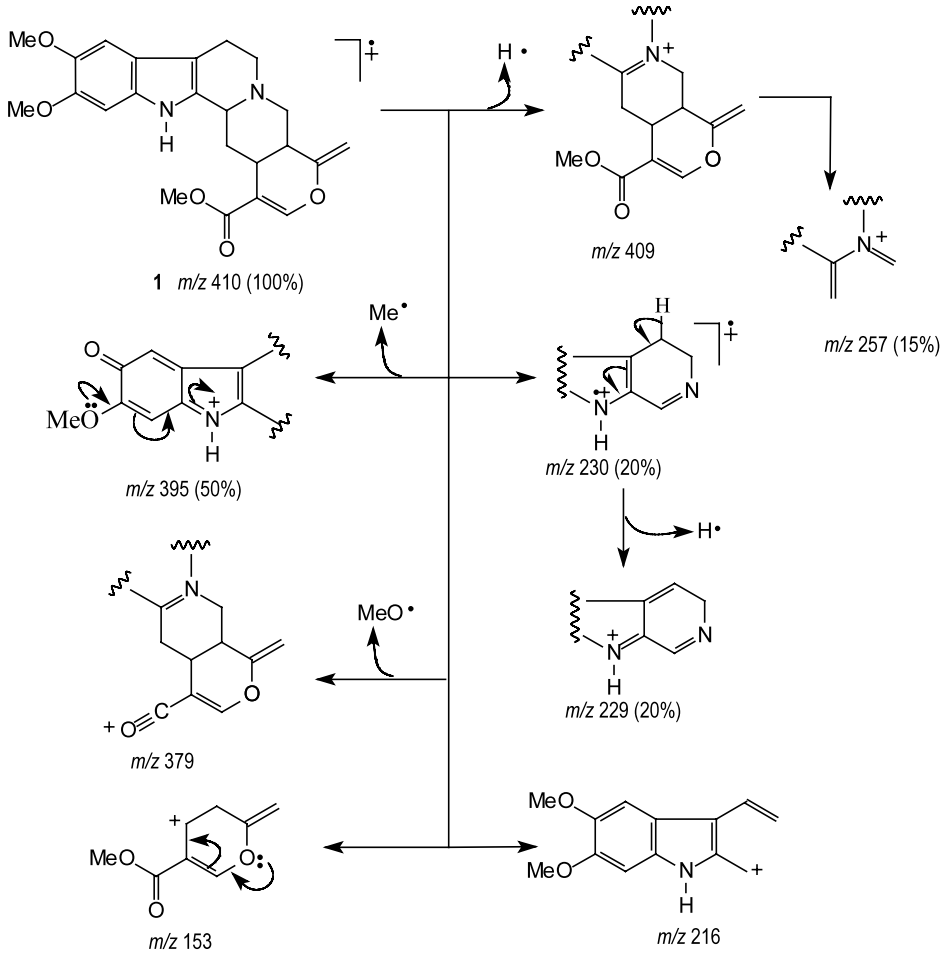
The ^{1}H and ^{13}C NMR spectra of **1**, together with ^{1}H–^{13}C COSY–$^nJ_{CH}$ ($n=1$, HMQC; $n=2$ and 3, HMBC) and comparison with ^{1}H and ^{13}C NMR spectral data of **2** (Tables 1 and 2), are in agreement with a pentacyclic indole alkaloid containing carbomethoxy group [**1**: C=O (δ_C 165.70) and MeO (δ_C 50.56 and δ_H 3.74, s); **2**: C=O (δ_C 168.03) and MeO (δ_C 51.19 and δ_H 3.74, s)] and indole moiety 10,11-dimethoxylated (Tables 1 and 2), revealing as a major difference from **2**, the presence of signals corresponding to an exocyclic double bond located between carbon atoms C-19 (δ_C 155.61) and CH_2-20 [δ_C 93.19 (CH_2-18); δ_H 4.69 (d, $J=1.5$ Hz, H-18a and 4.56 (d, 1.5 Hz, H-18b)]. The location of a double bond at C-20 was unequivocally deduced by heteronuclear spin–spin interaction between carbon C-19 (δ_C 155.61) and hydrogens 2H-18 (δ_H 4.69 and 4.56, $^2J_{CH}$), H-20 (δ_H 2.90, $^2J_{CH}$), H-15 (δ_H 2.80, $^3J_{CH}$) and H-17 (δ_H 7.44, $^3J_{CH}$) revealed by cross-peaks observed in the HMBC spectrum of **1**. Additional heteronuclear long-range couplings are summarized in Table 2.

The relative stereochemistry of **1** was determined from the coupling constants of relevant hydrogens and from the observed ^{1}H–^{1}H NOESY. The values corresponding to *vicinal* interaction ($^3J_{H,H}$) between the hydrogens H-3 and H-14ax ($J=9.4$ Hz, axial–axial interaction with H-3ax), H-15 and both 2H-14 ($J=5.1$ and 4.3 Hz) and H-20 ($J=4.5$ Hz) and H-20 ($J=8.9$ Hz, axial–axial interaction with H-21ax) are consistent with the relative configuration shown in **1** and **1a**, since the H-15 signal (δ_H 2.80, ddd, $J=5.1$, 4.5, 4.3 Hz) reveals no axial–axial couplings (Table 1). Consistent with these observations, the NOESY spectrum of **1** at 70°C showed cross-peaks assigned to dipolar interaction (spatial proximity, Fig. 1) of HN-1 (δ_H 10.12) with H-12 (δ_H 6.94) and H-14α (δ_H 1.77); H-3 (δ_H 3.36) with H-21β (δ_H 2.62); H-14α (δ_H 1.77) with H-15 (δ_H 2.80) and H-20 (δ_H 2.90); H-18b (δ_H 4.56) with H-21α (δ_H 2.69) and H-20 (δ_H 2.90); H-9 (δ_H 6.88) with H-6b (δ_H 2.51) and MeO-10 (δ_H 3.76); H-12 H-12 (δ_H 6.94) with MeO-11 (δ_H 3.77).

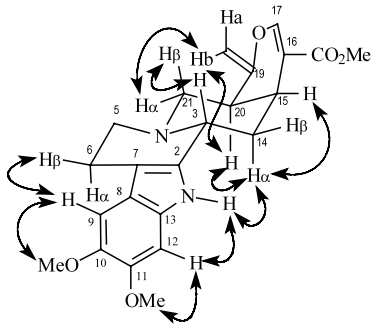
Table 2. Long-range couplings of hydrogen and carbon atoms observed in the HMBC ($^nJ_{CH}$, $n=2$ and 3) spectra of darcyribeirine (**1**) and isoreserpiline (**2**), in DMSO-d_6 (**1**) and $CDCl_3$ (**2**) as solvents and residual DMSO-d_6 and $CDCl_3$ used as internal references (δ_H 2.50 and δ_C 39.50; 7.24 and 70.00, respectively). Chemical shifts (δ, ppm) and coupling constants (J, Hz, in parentheses)[a]

	1			**2**		
	δ_C	$^2J_{CH}$	$^3J_{CH}$	δ_C	$^2J_{CH}$	$^3J_{CH}$
C						
2	132.73	HN-1	H-3, 2H-6	133.14	H-3, HN-1, H-12	H-14b, H-9, H-6b
7	106.22	2H-6	HN-1, H-9, H-5ax	107.82	2H-6	HN-1, H-9, 2H-5
8	119.98		H-12, HN-1	119.94		H-12, HN-1
10	144.28	H-9	H-12, MeO-10	144.78		H-12, MeO-10
11	145.94	H-12	H-9, MeO-11	146.41		H-9, MeO-11
13	130.57	H-12	H-9	130.13	HN-1, H-12	H-9
16	108.44	H-17		109.49	H-17, H-15	H-14b
19	155.61	2H-18, H-20	H-17, H-15	–		
22	165.70		H-17, MeO-22	168.03		H-15, H-17, MeO-22
CH						
3	54.18	H-14eq	2H-5, 2H-21	59.91	H-14b	2H-5, 2H-21
9	101.59			100.35		
12	96.64			94.82		
15	28.42	2H-14	H-17, H-21ax, H-19	31.29	2H-14	H-17, H-21a, H-19
17	151.03		H-15	155.75		H-15
19	–	–	–	72.47	3H-18	H-17, 2H-21
20	35.95	2H-21	H-14ax, 3H-18	38.41	2H-21	H-14a, 3H-18
CH_2						
5	51.47	2H-6	H-3	53.67		H-21a
6	19.82	H-5ax		21.82	H-5a	
14	29.52			34.26		
18	93.19			–	–	–
21	51.71			56.25		
CH_3						
18	–	–	–	18.49		
MeO-10	56.41			56.25		
MeO-11	56.10			56.41		
MeO-22	50.56			51.10		

[a] Number of hydrogens bound to carbon atoms deduced by comparative analysis of HBBD- and DEPT-^{13}C NMR spectra. Chemical shifts and coupling constants (J) obtained from 1D ^{1}H NMR spectrum. Superimposed ^{1}H signals are described without multiplicity and chemical shifts deduced by HMQC (Table 1) and ^{1}H–^{1}H COSY spectra.



Scheme 1. Proposed fragmentation mechanisms of **1** (only peaks classified as principals).



1a

Figure 1. Important ^{1}H–^{1}H NOESY correlations for darcyribeirine (**1**).

Thus, the structure of the new pentacyclic indole alkaloid isolated from *R. grandiflora* Mart. was established as *rel*-$\Delta^{19(18)}$-reserpiline (**1**), named darcyribeirine.

The results of the extensive application of 1D and 2D NMR spectral techniques were also used to confirm the structure and to establish the ^{1}H and ^{13}C resonance assignments of **1** and **2** (Tables 1 and 2).

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