Alkaloids of *Perriera madagascariensis*

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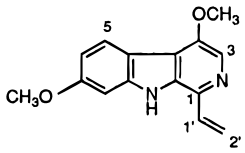
Two major alkaloids were isolated from root bark of *Perriera madagascariensis* Courchet (Simaroubaceae). The first alkaloid was identified as 4,7-dimethoxy-1-vinyl-β-carboline (**1**), previously isolated from stems of the same plant. The second alkaloid, to which the trivial name kirondrine was given, was new, and its structure (**2**) was established largely by means of 1D and 2D NMR techniques as a dimeric alkaloid biogenetically related to compound **1**. Compound **2** might correspond to the dimeric alkaloid formerly isolated from stems of *P. madagascariensis*.

Perriera madagascariensis Courchet (Simaroubaceae), commonly known under the vernacular name "*Kirondro*", is a tree growing in the west part of Madagascar.[1] Stem bark is used in the indigenous medicine as a bitter tonic and febrifuge, while roots are reported to be toxic.[2] In an initial study in 1964, two quassinoids were isolated from the fruits of this plant and identified as glaucarubinone and glaucarubin.[3] In a later investigation, stems and stem bark were found to contain two new indole alkaloids and three quassinoids, of which one was new.[4] The structure of the first alkaloid was established as 4,7-dimethoxy-1-vinyl-β-carboline (**1**) largely by interpretation of its 60 MHz ^{1}H-NMR spectrum followed by chemical methods, while the second alkaloid, obtained in a comparatively minor quantity, was tentatively assigned as a dimeric indole alkaloid. No further report to resolve the structure of the uncharacterized alkaloid has appeared subsequently in the literature. The present paper deals with the isolation and structure elucidation of two indole alkaloids isolated from roots of the same plant.



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Si gel column chromatography of an EtOH extract of *P. madagascariensis* root bark using CH_2Cl_2 with increasing amounts of MeOH as eluents led to the isolation of two major alkaloids in crystalline form. The first alkaloid was routinely identified as 4,7-dimethoxy-1-vinyl-β-carboline (**1**) on the basis of spectral data, because no authentic compound was available for comparison purposes. Because this compound was first characterized from stems of *P. madagascariensis* before the routine availability of the highfield ^{1}H and ^{13}C NMR, we report in Table 1 unambiguous assignments of its ^{1}H- and ^{13}C-NMR data. Assignments were assisted by the performance of COSY, HETCOR, and COLOC experiments.

Table 1. ^{13}C- and ^{1}H-NMR Chemical Shifts (ppm), Multiplicity (m), Coupling Constants (*J*), and ^{1}H−^{13}C Long-Range Correlations for 4,7-Dimethoxy-1-vinyl-β-carboline (**1**)

numbering (C,H)	δ ^{13}C	δ ^{1}H, m, *J*(Hz)	HMBC
1	135.0		H-3, H-1′, N-H
3	121.4	8.02, s	
4	150.8		4-OMe
4a	118.9		H-3, H-5, N-H
4b	114.8		H-6, H-8
5	124.8	8.18, d (J = 9.5)	
6	109.6	6.88, dd (J = 9.5, 2.0)	H-8
7	160.1		H-5, 7-OMe
8	94.6	6.85, d (J = 2.0)	H-6
8a	141.4		H-5
8b	133.6		
1′	132.6	7.17, dd (J = 17.2, 11.0)	
2′	126.7	a:5.46, dd (J = 11.0, 1.5)	
		b: 6.23, dd (J = 17.6, 1.5)	
O-CH_3	56.0	4.08, s	
O-CH_3	54.4	3.80, s	
NH		9.92, s	

The molecular weight of kirondrine (**2**) was determined as *m/z* 508 by chemical ionization mass spectrum, which was in agreement with the composition $C_{30}H_{28}O_4N_4$ obtained by HRMS. The UV spectrum of the hydrochloride derivative, λ_{max} (log ϵ) 247 (4.16), 302 (3.55), 336 (3.76), and 367 (3.65) was indicative of noninteracting β-carboline and β-carbolinium chromophores, as observed for the dimeric alkaloids picrasidine F, G, and S isolated from *Picrasma quassioides* Bennet.[5,6] These preliminary observations, together with the comparison of the ^{1}H NMR of compound **1** as free base to that of kirondrine (**2**) suggested that 4,7-dimethoxy-1-vinyl-β-carboline (**1**) was a monomeric precursor of kirondrine. On the basis of this hypothesis, two distinct AMX substitution patterns of two 1,3,4-tri-*O*,C,*N*-substituted benzene rings were respectively identified as follows: one doublet at δ 8.13 (J = 8.8 Hz), one broad doublet at δ 7.04 (J = 8.8 Hz), and one broad

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Table 2. ^{13}C- and ^{1}H-NMR Chemical Shifts (δ ppm), Multiplicity, Coupling Constants (Hz), of Kirondrine (**2**)

atom	δ ^{13}C	δ ^{1}H	HMBC	NOESY
1	24.0	H_a: 3.56, ddd (−17.0, 9.0, 8.0)	C-12a, C-12b	H_e-1, H_e-2, H_a-3
		H_e: 3.74, dd (−17.0, 4.2)		H_a-2,H_e-2
2	13.3	H_a: 1.89, m		H_e-2, H_e-3
		H_e: 2.01, m		H_e-3
3	26.7	H_a: 2.66, m		H_e-3, H-4
		Hb: 2.64, m		H-4
4	64.4	6.95, t		NH−9′
6	117.4	8.15, s	C-4, C-12b, C-7a	7-OMe
7	149.5			
7a	120.2			
7b	112.6			
8	124.9	8.13, d, J = 8.8	C-7a, C-7b, C-10, C-11a	H-9
9	112.4	7.04, br d, J = 8.8	C-7b, C-10, C-11	10-OMe
10	161.7			
11	94.6	7.25, br s	C-7b, C-9, C-10, C-11a	10-OMe
11a	144.3			
12a	133.7			
12b	136.7			
1′	134.4			
3′	120.5	7.80, s	C1′, C-4′, C-4′a, C-8′b	4′-OMe
4′	150.4			
4′a	117.8			
4′b	113.6			
5′	123.8	8.06, d, J = 8.6	C-4′a, C-7′, C-8′a	H-6′
6′	109.3	6.92, br d, J = 8.6	C-4′b, C-7′,C-8′	7′-OMe
7′	159.7			
8′	94.8	7.17, br s	C-4′b, C-6′, C-7′, C-8′a	7′-OMe, NH-9′
8′a	141.5			
9′a	132.9			
7-OMe	57.1	3.97	C-7	
10-OMe	55.4	3.95	C-10	
4′-OMe	55.8	4.02	C-4′	
7′-OMe	55.2	3.90	C-7′	
NH-9′		12.22	C-4′a, C-4′b, C-8′a, C-9′a	

singlet at δ 7.25 for the first benzene ring; one doublet at δ 8.06 (J = 8.6 Hz), one broad doublet at δ 6.92 (J = 8.6), and one broad singlet at δ 7.17 for the second benzene ring. As also evident from the ^{1}H-NMR spectrum, signals of four methoxyl groups appeared, respectively, at δ 3.90, 3.95, 3.97, and 4.02, while two singlets at δ 7.80 and 8.15 were indiscriminately assigned to the unspecified protons H-6 and H-3′. Further interpretation of the proton−proton correlations derived from a COSY experiment clearly indicated the presence of a $-CH_2-CH_2-CH_2-CH-$ sequence whose proton chemical shifts were reminiscent of those of picrasidine F, G, and S.[5,6] All these data strongly suggested that kirondrine (**2**) had a picrasidine-F-based skeleton; its structure differed only from the parent compound by the substitution pattern of the benzene rings. Confirmation of the proposed structure of **2** was given by HMQC, HMBC, and NOESY experiments. Thus, the observation of a long-range correlation between the proton at δ 8.15 and the carbon at δ 64.4 was of pivotal importance in unequivocally assigning the ^{1}H- and ^{13}C-NMR spectra of kirondrine. The carbon signal was unambiguously attributed to C-4, while the proton signal was assigned to H-6, which also showed three-bond connectivites to C-12b (δ 136.7) and C-7a (δ 120.2). The observation of long-range connectivities between the proton signal at δ 8.13 attributed to H-8 and C-7a (δ 120.2), C-7b (δ 112.6), C-11a (δ 144.3), and C-10 (δ 161.7), together with the existence of long-range couplings between H-1 (δ 3.56 and δ 3.74) and the two carbons C-12b (δ 136.7) and C-12a (δ 133.7) allowed the assembly of the four cyclic components of kirondrine. Regarding the second β-carboline unit, long-range connectivities observed between the four protons H-3′ (δ 7.80), H-5′ (δ 8.06), H-6′ (δ 6.92), and H-8′ (δ 7.17) and the carbons of this

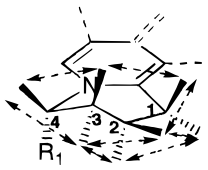
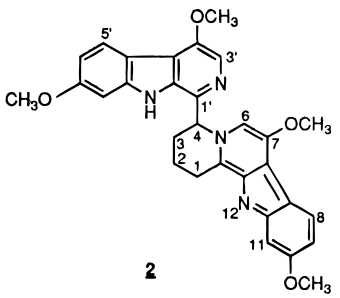


Figure 1.

unit permitted the assignments of quaternary carbons. This finding was further evidenced by the observation of two- and three-bond connectivities between the proton N′−H and C-8′a (δ 141.5), C-9′a (δ 132.9), C-4′a (δ 117.8), and C-4′b (δ 113.6). The signals and the exact positions of the four methoxyl groups were routinely differentiated by the analysis of HMQC and HMBC spectra. Further evidence for the proposed structure of **2** was given by interpretation of the NOESY spectrum, in which cross peaks among H-4, H-3, H-2, and H-1 were consistent with a half-chair conformation of the piperidine ring and permitted differentiation of the relative orientation of the geminal methylene protons (Figure 1). Furthermore, the position of the methoxyl groups was supported by the NOE contours observed between OMe and the ortho protons (see Table 2).



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Kirondrine is thus assigned as 7,10-dimethoxy-4(4′,7′-dimethoxy-9′H-pyrido[3,4-*b*]indol-1-yl)-1,2,3,4-tetrahydroindolo[2,3a]quinolizine and might be the uncharacterized dimeric alkaloid previously isolated from stem bark of *P. madagascariensis* as a minor constituent.[4]

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. UV spectra were obtained on a Unikon 930 spectrometer, and the IR spectra were measured on a Nicolet Impact 400D (KBr) interferometer. MS were recorded on a Nermag R10-10 apparatus. NMR spectroscopy: ^{1}H- and ^{13}C-NMR spectra of compound **1** were recorded at 300.13 and 75.47 MHz, respectively, on a Bruker AM-300 spectrometer by using standard programs. ^{1}H- and ^{13}C-NMR spectra of compound **2** were recorded at 400.13 and 100.6 MHz, respectively, on a Bruker AM-400 spectrometer. The ^{1}H and ^{13}C chemical shifts are expressed in parts per million relative to TMS, but were measured against the solvent peak ($CDCl_3$) set at 7.24 and 77.00 ppm, respectively. 2D NMR NOESY, HMQC, and HMBC experiments were carried out a previously described.[7]

Plant Material. Roots of *P. madagascariensis* were collected in Morondava, in western Madagascar, in March 1995. Authentication was achieved by comparison with specimens at the Parc Botanique et Zoologique de Tsimbazaza, Antananarivo. A voucher specimen has been deposited at the Institut Malgache de Recherches Appliquées.

Extraction and Isolation. Dried and powdered stem roots (1.2 kg) were exhaustively extracted by repeated maceration with EtOH. The combined alcoholic extracts were evaporated to dryness under reduced pressure to afford 74 g of a crude extract. The major portion (72 g) of this extract was submitted to Si gel column chromatography using $CHCl_3$ and increasing amounts of MeOH as eluents. The separation was monitored by TLC using a UV lamp and Dragendorff spray reagent. 4,7-Dimethoxy-1-vinyl-β-carboline (**1**, 112 mg) was eluted with $CHCl_3$−MeOH, 97:3, and crystallized from MeOH−Et_2O, while kirondrine (**2**, 132 mg) was eluted with $CHCl_3$−MeOH, 95:5, and crystallized from EtOAc.

Compound 1: mp 145 °C (lit.[4] mp 145−147 °C); $[\alpha]_D$ = 0 (*c* 1.5, MeOH); UV λ_{max} (log ϵ) 226 (4.37), 255 (4.40), 284 (4.36), 311 (4.10), 351 (3.93); EIMS *m*/*z* [M] 254 (100), 239 (40), 211 (57), 196 (18), 168 (30).

Kirondrine 2: mp 267 °C; $[\alpha]_D$ −15.6° (hydrochloride, *c* 0.48, MeOH); UV (hydrochloride, MeOH), λ_{max} (log ϵ) 210 (3.77), 247 (4.16), 300 (3.55), 336 (3.76) and 367 (3.65); IR ν_{max} 3427, 2940, 1630, 1500, 1460, 1269, 1202, 1156, 1130 cm^{-1}; CIMS *m*/*z* [M + 1] 509 (100), 495 (30), 267 (20); HREIMS *m*/*z* 508.2114 (calcd for $C_{30}H_{28}N_4O_4$, 508.2110).

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