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A NEW GROUP OF ISOQUINOLINE DIMERS FROM *HERNANDIA VOYRONII*

Philippe Rasoanaivo, Suzanne Ratsimamanga-Urverg
Institut Malgache de Recherches Appliquées, B.P. 3833, 101 Antananarivo, Madagascar

Corrado Galeffi
Laboratorio di Chimica del Farmaco, Istituto Superiore di Sanità, Viale Regina Elena 229, 00161 Roma, Italy

Marcello Nicoletti
Dipartimento di Biologia Vegetale, Università "La Sapienza", 00185 Roma, Italy

François Frappier* and Marie-Thérèse Martin
Laboratoire de Chimie, URA 401 CNRS, Muséum National d'Histoire Naturelle, 63 rue Buffon, 75231 Paris Cedex 05, France

Abstract: As guided by bioassay to reverse chloroquine resistance in strains of *Plasmodium falciparum*, three bisisoquinoline alkaloids pertaining to a novel group, the pavine-benzyltetrahydroisoquinoline dimers, were isolated from stem barks of *Hernandia voyronii* Jumelle. Their chemical structures were determined by conventional spectral methods assisted by the performance of the 2D NMR techniques, namely COSY LR, HMQC and HMBC.

In our research program directed towards the discovery of drugs that could complement chloroquine action, we have investigated the stem bark of *Hernandia voyronii* Jumelle = *Hazomalania voyronii* R. Capuron[1] (Hernandiaceae) used as adjuvant to chloroquine in the Malagasy herbal practice[2]. Because of its particular resistance to water, the trunk is also widely used to manufacture dugouts and coffins. This has led to the alarming disappearence of the plant which is now ranged among the most endangered species in Madagascar[3]. Previous studies showed that stem bark contains 2.9 % of fatty oils, 0.5 % of crude alkaloids and a camphor-like oil later identified as (+) perillaldehyde[4]. Some of us recently demonstrated that the chloroquine resistance-reversing constituents were localized in the alkaloid fraction[5]. Further fractionation using counter-current distribution (CCD) technique led to the isolation of three constituents belonging to a new group of isoquinoline dimers, the pavine-benzyltetrahydroisoquinolines, together with the known alkaloids norpredicentrine[6] and ocobotrine[7]. In this paper, we report the structure elucidation of the three new alkaloids named herveline A, **1**, herveline B, **2**, and herveline C, **3** (Fig. 1).

Herveline A, **1** showed the molecular ion at m/z 652.3140 corresponding to molecular formula $C_{39}H_{44}N_2O_7$. As evident from the ^1H NMR, the structure of **1** includes two N-Me groups, five methoxy groups, one para-disubstituted aromatic ring and five aromatic singlet protons. These observations and the UV absorption at 282 and 319 nm were indicative of a bisbenzylisoquinoline like structure.

Assignments of the ^1H and ^{13}C NMR were assisted by the performance of the 2D NMR techniques, namely COSY, COSY LR, HMQC and HMBC (Table 1 and 2).

Three CH-CH_2 and one CH_2-CH_2 units were routinely identified from the COSY data. The two N-Me signals appearing respectively at δ 2.50 and 2.48 were differenciated by the analysis of HMBC spectrum in which the latter was correlated to a tertiary and a secondary carbon at δ 65.2 and 48.3, respectively.

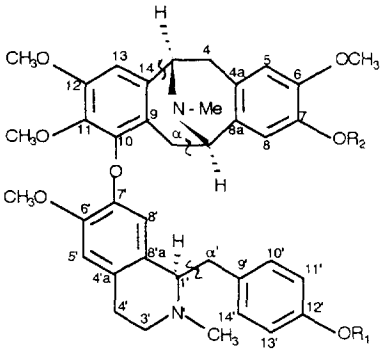
These assignments and the presence in the eims of complementary peaks at *m/z* 545 (100%) and 107 (52%)[8], indicated the presence of a hydroxybenzyl moiety whose aromatic proton signals were assigned by. long range connectivities obtained from HMBC spectrum. The two signals at δ 6.58 and δ 5.64 were assigned to H-5' and H-8', respectively. Furthermore the signal at δ 3.83 which showed ^1H-^1H long range connectivity with H-5' was attributed to 6'-OMe.

Therefore **1** appears to be formed of a benzyltetrahydroisoquinoline unit probably bonded to a second moiety through an ether bridge . The N-Me signal at δ 2.50 was correlated with two tertiary carbons, C-1 (δ 55.8) and C-3 (δ 56.4) and the resulting CH-N(CH_3)-CH fragment ruled out the presence of a second benzyltetrahydroisoquinoline moiety in **1**. However the following spectral data gave evidence of a pavine structure for the second moiety containing two aromatic rings. The observation of a three-bond connectivity between the proton signal at δ 6.38 and C-4 (δ 31.9) allowed the unambiguous assignment of H-5 which also coupled to C-8a (δ 128.5) and C-7 (δ 147.7). On its side H-8 coupled to C-1 (δ 55.8), C-4a (δ 122.7) and C-6 (δ 148.0). These assignments were substantiated by the observation of ^1H-^1H long range coupling between H-5 (δ 6.38) and 6-OMe (δ 3.71), and between H-8 (δ 6.47) and 7-OMe (δ 3.75). The observation of a three-bond connectivity between the proton signal at δ 6.57 and C-3 (δ 56.4) allowed the assignment of H-13 which also coupled to C-11 (δ 140.4) and C-9 (δ 118.8). Consequently the proton chemical shift of 12-OMe was assigned as δ 3.85 on account of its ^1H-^1H long range coupling with H-13. Finally two- and three-bond connectivities between H-3 and the neighbouring carbons strongly supported the above-mentioned assignments.

Furthermore, the protons H-3 and H-4 correlated to quaternary aromatic carbons C-14 (δ 133.8) and C-4a (δ 122.7) respectively. In addition H_2-α showed correlation to C-9 (δ 118.8) and two bond correlation to C-1 (δ 55.8). At last, from the HMBC spectrum, the signal at δ 3.43 was attributed to 11-OMe. For this singlet and the singlet at δ 5.64 corresponding to H-8' no ^1H-^1H long range correlation was observed.

Therefore the benzyltetrahydroisoquinoline and pavine moieties are bonded through an ether bridge which connects the C-10 and C-7' carbons. This structure was confirmed by the MS ions at *m/z* 204 (49 %) and 341 (12 %) (b and a respectively, Fig. 1) which resulted from the cleavage of bonds which were α–β to the nitrogen

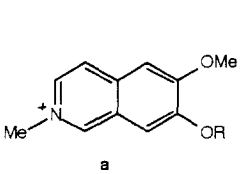
atom of pavine[9]. From all these data, structure **1** was unambiguously attributed to herveline A.



Herveline A **1** : $R_1 = H$; $R_2 = CH_3$
Herveline B **2** : $R_1 = CH_3$; $R_2 = H$
Herveline C **3** : $R_1 = R_2 = CH_3$

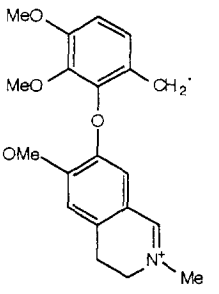
Figure 1 : Structures of herveline A, herveline B and herveline C.
The waving lines indicate the mass fragmentation.

For herveline B **2**, molecular formula $C_{39}H_{44}N_2O_7$ was assigned on the basis of the M+1 ion at m/z 653 . The analysis of the ^{1}H-NMR, ^{13}C-NMR and MS data of **2** suggested that its chemical structure was closely related to that one of **1**. Thus, when the MS spectrum of **2** was compared to that of **1**, the fragment peak at *m/z* 107 shifted to *m/z* 121, while the base peak at *m/z* 545 shifted to *m/z* 531. This was consistent with the presence of OMe group at C-12' in compound **2**. Correspondingly, the fragment ion at *m/z* 204 in compound **1** shifted to *m/z* 190 in compound **2**, clearly indicating that a methyl group was missing in fragment **a**. Apart from these divergences, the observation of peak b at *m/z* 341 provided evidence for a structural similarity of its skeleton to that of compound **1**. These data suggested that herveline B **2** was an isomer of **1**, the only difference being the substitution pattern of C-7 and C-12'. ^{1}H and ^{13}C NMR data of **2** (table 1) were assigned on the basis of comparison with those of **1**, combined with the interpretation of its HMQC and HMBC spectra.



a

R = H : *m/z* 190
R = Me : *m/z* 204



b

m/z 341

Figure 2 : Mass spectral fragmentation of hervelines

The third alkaloid obtained in this investigation, herveline C, **3**, was shown also to be structurally related to compounds **1** and **2** on the basis of its spectral parameters. The molecular weight determined as 666, corresponding to molecular formula $C_{40}H_{46}N_2O_7$, and the observation of fragment ions at *m/z* 121, 174, 204, 341, and 545 in its MS suggested that compound **3** was a methyl derivative of herveline A or B. This was substantiated by the interpretation of its 1D conventional ^1H and ^{13}C-NMR data (table 1). Further confirmation was given by diazomethane methylation of compounds **1** and **2**, which yielded the same compound **3**, thereby strongly supporting the close structural relationships between the three isoquinoline dimers investigated.

In order to establish the absolute configuration of the pavine and benzyltetrahydroisoquinoline moieties of **1-3**, the aromatic chirality method[10] was applied. However, on account of the overlapping effects of the two units, the configuration assignments are tentative.

The first CD curve aplitting of herveline C(Θ_{290} = - 2.3 x 10^3 and Θ_{276} = + 6.4 x 10^3) around the UV band at 282 nm (logε 4.04) originated from the A -->^1L_b transition of the dimethoxybenzene showed negative exciton chirality (left-handed screw of the benzoate groups) as another pavine, (-) argemonine[11]. The second pair of Cotton effects (Θ_{235} = -34 x 10^3 and Θ_{220} = + 8.4 x 10^3) around the UV band at 227 nm (logε 4.63) originated from the A-->^1L_a transition leads to the same absolute configuration 1S, 3S. The strong exciton chirality (observed also in the A-->B transition, first extremum at 204 nm, [Θ] = - 111 x 10^3) suggested the same left-hand screw relationship between the two more distant benzoates of the benzyltetrahydroisoquinoline moiety and therefore the R configuration for C(1').

Herveline A, B, and C are the first examples of a novel group of isoquinoline dimers, the pavine-benzyltetrahydroisoquinoline. They may act as the biogenetic precursors of the pavine-aporphine dimers[9]. Moreover, compounds **1**, **2**, and **3** were shown to reverse chloroquine resistance in chloroquine-resistant strains of *Plasmodium falciparum*, and this has led to useful structure-activity relationships in the isoquinoline alkaloids[12].

EXPERIMENTAL

A Craig Post apparatus (200 stages, 10:10 ml, upper and lower phase) was used for CCD separations. Circular dichroism (CD) curves (MeOH) were registered with a Jasco J-40 apparatus and mass spectra with a Finnigan MAT 5100 spectrometer (e.i. 70 ev).

NMR spectra were recorded in $CDCl_3$ employing either a Bruker AC 300 spectrometer at 28°C, observing ^1H and ^{13}C at 300.13 and 75.47 MHz respectively or a Bruker AM 500 at 30°C, observing ^1H and ^{13}C at 500.13 and 125.77 MHz, respectively. The ^1H and ^{13}C chemical shifts are expressed in ppm relative to TMS, but were measured against the central solvent peak at δ 7.24 and 77.00 ppm respectively. The homonuclear ^1H-^1H shift correlated two-dimensional diagrams were obtained using the COSY-45 pulse sequence. HMQC experiments were monitored using 380 ms evolution delay for sequence BIRD and HMBC 70 ms for CH long range coupling.

PLANT MATERIAL

The stem barks of *Hernandia voyronii* were collected in Morondava, south-eastern region of Madagascar, in September 1991 and September 1992. The voucher specimens are kept at the Institut Malgache de Recherches Appliquées.

EXTRACTION AND FRACTIONATION

1.0 kg of air-dried and ground plant material was exhaustively extracted with 2 % aqueous acetic acid. The combined acid solutions were made alcaline with $NaHCO_3$ and extracted three times with chloroform to afford 6.3 g of crude alkaloids. This extract was then submitted to repeated CCD separations using chloroform as the stationary phase and Na_2HPO_4-citric acid buffer solution at discontinuously decreasing pH as the mobile phase. The separation was monitored by TLC on silica gel F_{254} using $CHCl_3$-MeOH (9/1) and n-BuOH-acetic acid-water (4/1/5) (upper phase) as eluents, and UV light (254 and 366 nm) and Dragendorff's spray reagent for detection.

At pH 5.4 norpredicentrine ($K_rK_b = 8 \times 10^{-10}$), herveline A ($K_rK_b = 5 \times 10^{-10}$) and herveline B ($K_rK_b = 2.2 \times 10^{-10}$) were separated in the order. Ocobotrine ($K_rK_b = 1.3 \times 10^{-10}$) was purified at pH 4.6 whereas herveline C ($K_rK_b = 2.5 \times 10^{-11}$) was purified at pH 4.0.

Herveline A, 1 : M.p. 114-116°C (from *n*-hexane), $[\alpha]_{20}^{D}$ = -2.0 (c = 0.02, CH_2Cl_2) ; UV (MeOH) λ_{max} (log ε) : 282 (3.94), 319 (3.67) nm ; CD [Θ] (bm) : + 8.6 x 10^3 (275), - 7.8 x 10^3 (251). MS, *m/z* (% intensity) : 653 (M + 1) (9) ; 545 (100), 341 (12), 204 (49), 174 (26), 107 (52). HRMS : Found 652.3140, $C_{39}H_{44}N_2O_7$, requires 652.3148.

Herveline B, 2 : M.p. 102-104°C (from *n*-hexane), $[\alpha]_{20}^{D}$ = + 2.4 (c = 0.3, CH_2Cl_2) ; UV (MeOH), λ_{max} (log ε) : 282 (4.05), 302 (2.91) nm ; CD : [Θ] (nm) : + 7.0 x 10^3 (277), - 5.8 x 10^3 (253). MS, *m/z* (% intensity) : 653 (M + 1) (8) ($C_{39}H_{44}N_2O_7$), 531 (100), 341 (9), 190 (35), 174 (20), 121 (52).

Herveline C, 3 : M.p. 85-86°C (from *n*-hexane), $[\alpha]_{20}^{D}$ = - 24.0 (c = 0.3, CH_2Cl_2) ; UV (MeOH), λ_{max} (log ε) : 227 (4.63), 282 (4.04), 320 (2.75) nm ; CD : [Θ](nm) : - 2.3x 10^3 (290), + 6.4 x 10^3 (276), - 34 x 10^3 (235), + 8.4 x 10^3 (220) - 111 x 10^3 (204). MS, *m/z* (% intensity) : 667 (M + 1) (6) ($C_{40}H_{46}N_2O_7$), 545 (100), 341 (10), 204 (32), 174 (20), 121 (49).

Methylation of 1 and 2 : Herveline A and herveline B dissolved in MeOH were separately methylated with an ethereal solution of diazomethane overnight. The derivatives were shown to be identical with herveline C by TLC and ^{1}H-NMR.

Table 1 : ^{13}C and ^{1}H NMR chemical shifts (ppm) and coupling constants (Hz) for herveline A, **1**, B, **2** and C, **3**

	1			**2**			**3**		
Carbon	δ,C	δ,H	J	δ,C	δ,H	J	δ,C	δ,H	J
1	55.8	3.93 dd	5.6, 1.5	56.0	3.85 d	5.8	56.0	3.91 dd	6.0, 1.0
3	56.4	4.12 d	5.8	56.5	3.97 d	5.8	56.5	3.99 d	5.3
4	31.9	3.38 dd 2.68 d	- 16.2, 5.8 - 16.2	34.1	3.38 dd 2.61 d	- 16.0, 5.8 - 16.0, 0	33.7	3.38 dd 2.60 d	- 16.0, 5.3 - 16.0
4a	122.7			122.5			123.2		
5	111.3	6.38 s		110.5	6.31 s		111.2	6.36 s	
6	148.0			145.9			147.7		
7	147.7			143.9			147.4		
8	110.4	6.47 s		112.9	6.46 s		110.0	6.44 s	
8a	128.5			130.6			129.7		
α	30.3	2.81 dd 2.74 dd	- 16.9, 5.6 - 16.9, 1.5	27.8	2.97 dd 2.47 dd	- 16.9, 5.8 - 16.9, 0	28.0	2.98 dd 2.58 dd	- 16.3, 6.0 -16.3, 1.0
9	118.8			119.1			119.0		
10	148.1			145.6			146.2		
11	140.4			140.7			140.7		
12	152.2			151.7			151.8		
13	107.0	6.57 s		107.6	6.56 s		107.6	6.56 s	
14	133.8			133.4			133.5		
1'	65.2	3.46 dd	8.0, 4.2	64.2	3.42 dd	5.3, 4.4	64.3	3.44 dd	6.2, 5.8
3'	48.3	3.04 m 2.63 m		47.0	3.10 m 2.70 m		47.2	3.04 m 2.70 m	
4'	26.4	2.67 m		24.6	2.70 m 2.55 m		25.3	2.70 m	
4'a	128.5			127.0			127.5		
5'	111.7	6.58 s		112.1	6.59 s		112.1	6.58 s	
6'	148.3			147.0			147.0		
7'	145.5			145.4			145.4		
8'	116.2	5.64 s		113.1	5.95 s		113.3	5.99 s	
8'a	129.7			130.2			130.3		
α'	39.9	3.01 dd 2.36 dd	- 13.5, 4.2 - 13.5, 8.0	40.3	2.68 dd 2.30 dd	- 14.7, 4.4 - 14.7, 5.3	40.2	2.70 dd 2.40 dd	- 14.2, 5.8 - 14.2, 6.2
9'	131.7			132.0			132.1		
10'	130,1	6.68 m		129.9	6.76 m		130.0	6.77 m	
11'	117.2	6.43 m		113.3	6.63 m		113.3	6.63 m	
12'	154.3			157.4			157.4		
13'	117.2	6.43 m		113.3	6.63 m		113.3	6.63 m	
14'	130.1	6.68 m		129.9	6.76 m		130.0	6.77 m	
N-Me	40.3	2.50 s		40.6	2.41 s		40.7	2.43 s	
N'-Me	42.4	2.48 s		42.2	2.33 s		42.3	2.36 s	
6-OMe	55.7	3.71 s		56.0	3.86 s		55.6	3.64 s	
7-OMe	55.6	3.75 s					55.6	3.69 s	
11-OMe	60.5	3.43 s		60.8	3.61 s		60.8	3.61 s	
12-OMe	56.2	3.85 s		56.1	3.86 s		56.0	3.85 s	
6'-OMe	55.9	3.83 s		55.5	3.57 s		55.6	3.85 s	
12'-OMe				55.1	3.74 s		55.1	3.74 s	

Table 2 : Correlation peaks used for the structure determination of herveline A, **1** and herveline B, **2**.

H	1	2 (CH_3)	3	4	5	6 (CH_3)	7 (CH_3)	8	α	11 (CH_3)	12 (CH_3)	13		1'	2' (CH_3)	5'	6' (CH_3)	8'	α'	10'	11'	12' (CH_3)
C-1		1, 2		2				1, 2	1				C-1'		1, 2			1, 2	1, 2			
C-2			1										C-3'		1, 2							
C-3		1, 2										1, 2	C-4'			1, 2						
C-4					1, 2								C-4'a					1, 2				
C-4a			1, 2	1, 2				1, 2					C-6'				1, 2	1, 2				
C-5				2									C-7'			1, 2						
C-6						1. 2		1, 2														
C-7					1, 2		1						C-8'a			1, 2						
C-8	1, 2												C-α'	1						1, 2		
C-8a				1, 2	1, 2				2				C-9'								1, 2	
C-9			2						1, 2			1, 2	C-10'							1, 2		
C-11										1, 2		1, 2	C-11'								1, 2	
C-12											1, 2		C-12'							1, 2		2
C-13			1, 2																			
C-14			1, 2	1, 2																		

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