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ALKALOIDS FROM *CRINUM FIRMIFOLIUM* VAR. *HYGROPHILUM*

JUDITH RAZAFIMBELO, MARTA ANDRIANTSIFERANA,* GENEVIEVE BAUDOUIN and FRANÇOIS TILLEQUIN†

Laboratoire de Pharmacognosie de l'Université René Descartes, U.A. au C.N.R.S. no 1310, Faculté de Pharmacie, 4, Avenue de l'Observatoire, F-75006 Paris, France; *Département de Chimie Organique, Etablissement d'Enseignement Supérieur des Sciences, Université d'Antananarivo, Antananarivo, Madagascar.

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Key Word Index—*Crinum firmifolium* var. *hygrophilum*; Amaryllidaceae; alkaloids; criwelline; trisphaeridine; 3-hydroxy-8,9-methylenedioxyphenanthridine.

Abstract—Eight alkaloids, lycorine, criwelline, crinamine, 6-hydroxycrinamine, hamayne, ismine and trisphaeridine, and the novel 3-hydroxy-8,9-methylenedioxyphenanthridine have been isolated from whole plants of *Crinum firmifolium* var. *hygrophilum*. The structure of the new phenanthridine alkaloid has been established by spectroscopic methods and confirmed by total synthesis using 6-chloropiperonal and 3-benzyloxyaniline as starting materials.

INTRODUCTION

The genus *Crinum* belongs to the tribe Amaryllideae and includes some 120 to 130 species [1, 2]. *Crinum firmifolium* Baker is a widespread native of Madagascar (vernacular names: kingatsy, vahondrano) where the bulb is commonly employed in external use for the treatment of various parasitic skin diseases [3]. This species has been divided into three varieties, *hygrophilum* H. Perr., *geophilum* H. Perr. and *xerophilum* H. Perr. [4, 5]. The only previous chemical work on this plant describes the isolation of lycorine from bulbs [6]. As part of our studies on the chemical constituents of the Amaryllidaceae [7], we report here the isolation from dried whole plants of *C. firmifolium* var. *hygrophilum* of eight alkaloids. One of them, 3-hydroxy-8,9-methylenedioxyphenantridine (**1**) is a new compound and its structure, deduced from its spectral data has been confirmed by total synthesis. In addition to the alkaloids, the well-known tyramine and the neutral α-ionone, vomifoliol [8], have also been obtained from this plant source.

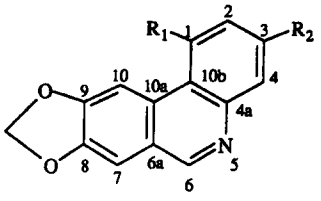
RESULTS AND DISCUSSION

Fractionation of the crude alkaloid extract of *C. firmifolium* var. *hygrophilum* yielded eight alkaloids. Comparison of their physical and spectroscopic characteristics with the litterature data led to the identification of seven of them: lycorine [9–11], criwelline (**2**) [12, 13], crinamine [10, 14], 6-hydroxycrinamine [15, 16], hamayne [16–18], ismine [19–21] and trisphaeridine (= 8,9-methylenedioxyphenanthridine = [1,3]dioxolo-[4,5-j]phenanthridine) (**3**) [20, 22, 23]. The high resolution ^{1}H NMR and ^{13}C NMR data of compound **2**, which have not been previously published, are summarized in Table 1.

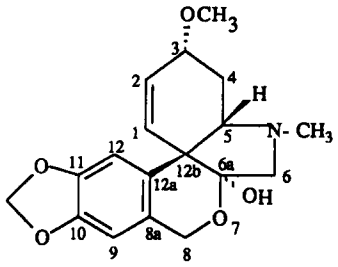
Compound **1** recrystallized as colourless needles from methanol and high resolution analysis of the $[M]^+$ *m/z* 239 ion in the EI mass spectrum gave the empirical formula $C_{14}H_9NO_3$. The UV spectrum showing maxima at 257, 287, 301 (sh.), 310 (sh.), 330 (sh.), 353 (sh.) and 370 (sh.) nm was typical of a phenanthridine derivative [24, 25]. Changes in this spectrum in an alkaline medium suggested the presence of a phenolic group. The IR spectrum exhibited characteristic bands at 3450 and 940 cm^{-1}, associated with aromatic hydroxyl and methylenedioxy groups, respectively. The ^{1}H NMR spectrum revealed one methylenedioxy group and a 6-H system characteristic of a 3,8,9-trisubstituted phenanthridine nucleus (Table 2). This evidence allowed the determination of the structure of **1** as 3-hydroxy-8,9-methylenedioxyphenanthridine (= 3-hydroxy[1,3]dioxolo[4,5-j]phenanthridine), in perfect agreement with the ^{13}C NMR spectrum (Table 3), whose main features were the signals of C-2 (δ117.7), C-4 (δ110.6), C-7 (δ105.0), and C-10 (δ99.0) strongly shielded by the effects of the hydroxyl and methylenedioxy groups, when compared with data previously published for phenanthridine itself [26].

The structure of **1** was confirmed by total synthesis. The key step of our approach was a benzyne-mediated cyclization of a conveniently substituted *N*-(2-halobenzyl)-aniline [27, 28], using lithium diisopropylamide (LDA) in THF, since this method had previously given good results for the synthesis of benzo[c]phenanthridine alkaloids bearing methylenedioxy groups, such as nitidine [29]. Condensation of 6-chloropiperonal (**4**) with 3-benzyloxyaniline (**5**) yielded *N*-(2-chloro-4,5-methylenedioxybenzylidene)-3-benzyloxyaniline (**6**). Reduction of **6** with sodium borohydride gave *N*-(2-chloro-4,5-

†Author to whom correspondence should be addressed.



	R_1	R_2
1	H	OH
3	H	O
8	H	$OCH_2C_6H_5$
9	$OCH_2C_6H_5$	H



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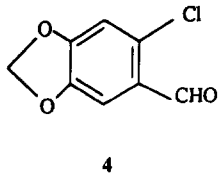

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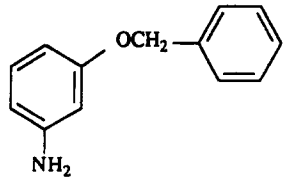

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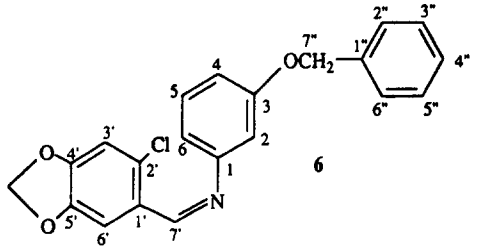

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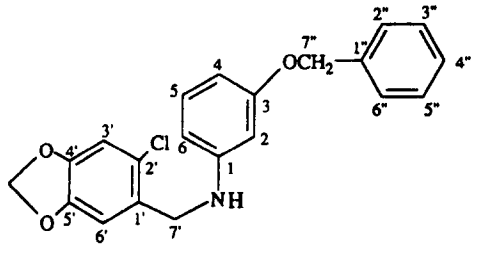

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methylenedioxybenzyl)-3-benzyloxyaniline (**7**). Cyclization of this latter by LDA in THF at −78° [21, 22] occurred smoothly and was followed by spontaneous air-oxidation of the unstable 5,6-dihydrophenanthridine

Table 1. ^1H and ^{13}C NMR data of criwelline (**2**) ($CDCl_3$, 300 and 75 MHz, respectively, δppm, J in Hz)

C	δ_H	δ_C
1	5.78 *d*, $J = 10$	130.1
2	6.20 *dd*, $J = 10, 3.5$	128.9
3	3.89 *ddd*, $J = 7, 4, 3.5$	72.1
4	1.93 *ddd*, $J = 15, 7, 3$	25.4
	2.09 *ddd*, $J = 15, 4, 3$	
5	2.95 *t*, $J = 3$	68.2
6	2.83 *d*, $J = 10.5$	64.5
	3.30 *d*, $J = 10.5$	
6a	—	102.6
8	4.68 *d*, $J = 15$	62.6
	4.94 *d*, $J = 15$	
8a		126.2
9	6.55 *s*	108.5
10	—	146.6*
11	—	146.2*
12	6.52 *s*	104.2
12a	—	130.9
12b	—	50.0
O–CH_3	3.45 *s*	56.7
N–CH_3	2.38 *s*	40.6
O–CH_2–O	5.92 *s*	100.9

*Assignments may be reversed.

Table 2. ^1H NMR data of phenanthridines **1**, **8** and **9** ($CDCl_3$, 300 MHz, δ relative to TMS, J in Hz)

H	**1**	**8**	**9**
1	8.05 *d*, $J = 9$	8.27 *d*, $J = 9$	—
2	7.01 *dd*, $J = 9, 2$	7.35 *dd*, $J = 9, 2$	7.17 *dd*, $J = 9, 1$
3	—	—	7.59 *t*, $J = 9$
4	7.13 *d*, $J = 2$	7.62 *d*, $J = 2$	7.82 *dd*, $J = 9, 1$
6	8.68 *s*	9.04 *s*	9.07 *s*
7	7.09 *s*	7.29 *s*	7.34 *s*
10	7.60 *s*	7.80 *s*	8.99 *s*
O–CH_2–O	5.97 *s*	6.13 *s*	6.12 *s*
2′, 6′	—	7.52 *dd*, $J = 8, 1$	7.57 *dd*, $J = 8, 1$
3′, 5′	—	7.43 *t*, $J = 8$	7.45 *m*
4′	—	7.39 *tt*, $J = 8, 1$	7.45 *m*
CH_2-7′	—	5.25 *s*	5.38 *s*

intermediates during the work-up. Thus, 3-benzyloxy-8,9-methylenedioxy phenanthridine (**8**) was obtained as major reaction product, accompanied by trace amounts of its isomer 1-benzyloxy-8,9-methylenedioxyphenanthridine (**9**). ^1H and ^{13}C NMR data of compounds **8** and **9** are summarized in Tables 2 and 3. Finally, hydrogenolysis of **8** was readily achieved using palladium on charcoal as catalyst, yielding 3-hydroxy-8,9-methylenedioxy-phenanthridine (**1**) identical in all respects with the natural product.

From a biosynthetic point of view, the co-occurrence in *C. firmifolium* var. *hygrophilum* of major alkaloids possessing a crinane skeleton (crinamine and 6-hydroxycrinamine) and of trace amounts of alkaloids deriving

Table 3. ^{13}C NMR data of phenanthridines **1**, **8** and **9** ($CDCl_3$, 75 MHz, δ relative to TMS)

C	1	8	9
1	123.2	123.2	156.7
2	117.7	118.5	108.8
3	157.1	158.6	127.3
4	110.6	110.5	123.0
4a	144.1	145.5	151.0*
6	151.2	152.1	152.5
6a	121.2	122.1	136.4
7	105.0	105.3	105.3
8	147.2	147.4	147.1
9	151.8	151.6	152.5
10	99.0	99.4	106.3
10a	130.8	130.5	146.3*
10b	117.2	118.7	115.7
O–CH_2–O	101.6	101.8	101.7
1′	—	136.6	136.4
2′, 6′	—	127.7	127.7
3′, 5′	—	128.6	128.7
4′	—	128.1	128.3
7′	—	70.2	71.2

*Assignments may be reversed.

from a simple phenanthridine nucleus (trisphaeridine and 3-hydroxy-8,9-methylenedioxyphenanthridine) is interesting since the latter can be considered as catabolic products formed through cleavage of the 'ethano'-bridge of crinane precursors. Indeed, a similar type of catabolic process has been demonstrated for the biogenesis of ismine [30, 31] and more recently postulated for related oxophenanthridine and phenanthridinium alkaloids [20].

EXPERIMENTAL

General. Mps: uncorr. UV and optical rotations were obtained in MeOH. IR were recorded as KBr pellets. ^{1}H and ^{13}C NMR were recorded at 300 and 75 MHz, respectively. Chemical shifts are given in δ (ppm) with TMS as int. standard. ^{1}H-^{1}H (COSY) and ^{1}H-^{13}C (HETCOR) correlations were performed for **1** and **2** using Bruker standard microprograms. EI and HREI-MS were obtained at 70 eV (direct inlet). CI-MS were obtained using NH_3 as reagent gas.

Plant material. Whole plants of *C. firmifolium* var. *hygrophilum* were collected by one of us (J.R.) at Mahatsara (Onibe, Toamasina province, Madagascar) in October 1992, at the end of the flowering period. Voucher specimens are kept in the Herbarium of the Musée de Matière Médicale, Faculté de Pharmacie de Paris, France.

Extraction and isolation. Dried and powdered whole plants (8.5 kg) were moistened with 50% NH_4OH and percolated exhaustively with CH_2Cl_2. The percolate was extracted with 1*N* HCl until a Mayer's test was negative. The acid layer was sepd, basified with NH_4OH and extracted with CH_2Cl_2. The CH_2Cl_2 soln was washed with H_2O, dried (Na_2SO_4) and evapd *in vacuo* to give 17.5 g of crude alkaloid extract (AE) (yield 0·21%). Crude AE was dissolved in MeOH (150 ml) and kept overnight at room temp. Lycorine (1.20 g) recrystallized as colourless prisms, mp 256–258°, $[\alpha]_D^{20} = -62°$ (*c* 0.1, EtOH) and was identified by direct comparison with an authentic sample. The MeOH mother liquor was evapd, dissolved in CH_2Cl_2–MeOH (2:1) (200 ml) and kept overnight at 0° to give crystals of 6-hydroxycrinamine (7.3 g), mp 210°, $[\alpha]_D^{20} = +45°$ (*c* 1.0, $CHCl_3$). The mother liquor was evapd and an aliquot (1.20 g) fractionated by CC on a silica gel 60 H column using CH_2Cl_2 and mixts of CH_2Cl_2–MeOH of increasing polarity to afford trisphaeridine (15 mg), ismine (10 mg), 3-hydroxy-8,9-methylenedioxyphenanthridine (5 mg), crinamine (520 mg), vomifoliol (10 mg), tyramine (15 mg), criwelline (10 mg) and hamayne (15 mg).

3-Hydroxy-8,9-methylenedioxyphenanthridine (**1**). Needles, mp 215–218° (dec.) UV λ MeOH max. nm (log.ε): 257 (4.69), 287 (4.07), (4.07), 301 (sh.), 310 (sh.), 330 (sh.), 353 (sh.), 370 (sh.); UV λ MeOH + NaOH max. nm: 269, 298, 312, 380. IR ν KBr max. cm^{-1}: 3450, 1610, 1460, 1270, 1195, 1040, 940, 860, 815, 755. EI-MS, *m/z* (rel. int.): 239 $[M]^+$ (100), 238 (15), 181 (10), 154 (12), 126 (9), 119 (10). HR-MS: $[M]^+$ found: 239.0586; $C_{14}H_9NO_3$ requires: 239.0582. ^{1}H NMR: see Table 2. ^{13}C NMR: see Table 3.

N-(2-chloro-4,5-methylenedioxybenzylidene)-3-benzyloxyaniline (**6**). A soln of 6-chloropiperonal (**4**) (923 mg, 5 mmol) and 3-benzyloxyaniline (**5**) (997 mg, 5 mmol) in EtOH (140 ml) was refluxed for 8 hr. Concn *in vacuo* furnished pure *N*-(2-chloro-4,5-methylenedioxybenzylidene)-3-benzyloxyaniline as a syrup (1.6 g, 87.5%). IR ν NaCl max cm^{-1}: 3035, 3020, 2905, 1580, 1470, 1245, 1030, 935, 880, 845, 770, 735. CI-MS, *m/z* 368 $[M + H]^+$, 366 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.83 (*s*, H-7′), 7.72 (*s*, *H*-6′), 7.50–6.80 (*m*, 9 Ar-H), 6.88 (*s*, H-3′), 6.07 (*s*, O–CH_2–O), 5.11 (*s*, CH_2-7″). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.5 (C-3), 156.6 (C-7′), 153.2 (C-1), 150.8 (C-4′), 147.3 (C-5′), 136.9 (C-1″), 129.9 (C-5), 128.6 (C-3″, C-5″), 128.5 (C-1′), 128.0 (C-4″), 127.5 (C-2″, C-6″), 127.2 (C-2′), 113.6 (C-4), 112.7 (C-3′), 109.8 (C-6), 107.7 (C-6′), 107.1 (C-2), 102.3 (O-CH_2-O), 70.1 (C-7″) (Found: C, 69.05; H, 4.53; Cl, 9.63; N, 3.81. $C_{21}H_{16}ClNO_3$ requires: C, 68.95; H, 4.40; Cl, 9.68; N, 3.82).

N-(2-chloro-4,5-methylenedioxybenzyl)-3-benzyloxyaniline (**7**). $NaBH_4$ (2 g, 53 mmol) was added to a soln of **6** (1.3 g, 3.55 mmol.) in EtOH (150 ml) and the mixt. was stirred for 2 hr at 20°. The solvent was evapd and the residue decomposed with H_2O and extracted with EtOAc. The EtOAc soln was dried (Na_2SO_4) and evapd to dryness. CC (silica gel 60 H; cyclohexane–EtOAc, 4:1) afforded **7** (1.07 g, 82%) as colourless needles from EtOAc, mp. 66°. IR ν KBr max. cm^{-1}: 3430, 3030, 2910, 1600, 1470, 1245, 1195, 1035, 930, 870, 835, 755, 730. CI-MS, *m/z* 370 $[M + H]^+$, 368 $[M + H]^+$. ^{1}H NMR (300 NHz, $CDCl_3$) δ: 7.50–7.30 (*m*, 5 Ar-H), 7.12 (*t*, $J = 8$ Hz, H-5), 6.90 (*s*, H-6′), 6.88 (*s*, H-3′), 6.40 (*dt*, $J = 8$, 1 Hz, H-6), 6.28 (*dt*, $J = 8$, 1 Hz, H-4), 6.26 (*t*, $J = 1$ Hz,

H-2), 5.96 (*s*, O–CH_2–O), 5.04 (*s*, CH_2-7″), 4.32 (*s*, CH_2-7′), 4.17 (*br s*., D_2O exch., NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.0 (C-3), 149.0 (C-1), 147.1 (C-5′), 146.8 (C-4′), 137.2 (C-1″), 130.0 (C-5), 129.0 (C-1′), 128.5 (C-3″, C-5″), 127.8 (C-4″), 127.5 (C-2″, C-6″), 124.5 (C-2′), 109.9 (C-6′), 108.7 (C-3′), 106.3 (C-6), 103.7 (C-4), 101.6 (O–CH_2–O), 99.7 (C-2), 69.7 (C-7″), 45.6 (C-7′). (Found: C, 68.45; H, 5.02; Cl, 9.71; N, 3.74. $C_{21}H_{18}ClNO_3$ requires: C, 68.57; H, 4.93; Cl, 9.63; N, 3.80).

3-Benzyloxy-8,9-methylenedioxyphenanthridine (**8**) and *1-Benzyloxy-8,9-methylenedioxyphenanthridine* (**9**). A 2M lithium diisopropylamide soln in heptane-THF-ethylbenzene (1.5 ml, 3 mmol) was added at − 78° to a soln of **7** (368 mg, 1 mmol) in THF (10 ml). The reaction mixt. was stirred at − 78° for 3 hr and allowed to warm to room temp. within 12 hr. The resulting suspension was dild with CH_2Cl_2 (30 ml) and washed with H_2O (20 ml). The organic layer was dried (Na_2SO_4) and evapd to dryness. CC (silica gel 60 H, cyclohexane–EtOAc, 4:1) afforded successively **9** (20 mg, 6%) and **8** (72 mg, 22%) as amorphous solids. *3-Benzyloxy-8,9-methylenedioxyphenanthridine* (**8**). IR ν KBr max. cm^{-1}: 3050, 2920, 2880, 1590, 1260, 1155, 1025, 850, 775, 755. CI-MS, *m/z* 330 $[M + H]^+$. ^{1}H NMR: see Table 2. ^{13}C NMR see Table 3. *1-Benzyloxy-8,9-methylenedioxyphenanthridine* (**9**). IR ν KBr max. cm^{-1}: 3050, 2920, 2880, 1600, 1250, 1025, 855, 775, 750. CI-MS, *m/z* 330 $[M + H]^+$. ^{1}H NMR: see Table 2. ^{13}C NMR: see Table 3.

3-Hydroxy-8,9-methylenedioxyphenanthridine (**1**). A suspension of **8** (33 mg, 0.1 mmol) and 10% Pd/C (10 mg) in MeOH (25 ml) was stirred at 20° for 3 hr under 1 Atm. of H_2. The mixt. was filtered through a short column of Celite to remove catalyst and the solvent removed *in vacuo* CC (silica gel 60 H, CH_2Cl_2–MeOH, 24:1) afforded **1** (17 mg, 71%), as needles from MeOH. Physical and spectral data were identical with those of the natural product (mp, TLC, UV, IR, MS, ^{1}H and ^{13}C NMR).

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