Alkaloids of the Flowers of *Hippeastrum vittatum*

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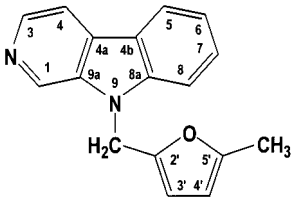
Investigation of an ethanolic extract of the fresh flowers of *Hippeastrum vittatum* yielded the new alkaloids vittacarboline (**1**) and *O*-methylismine (**3**), together with the known compound ismine (**2**). The structures of **1**−**3** were established by spectroscopic methods including one- and two-dimensional NMR and mass spectrometry.

The Amaryllidaceae alkaloids have been found to possess a wide range of pharmacological properties, particularly antiviral[1,2] and antitumor activities.[3−5] In the course of our ongoing investigation of the constituents of the Egyptian Amaryllidaceae plants,[6−8] we have investigated the flowers of *Hippeastrum vittatum* Herbert. Previous studies on the bulbs of the plant have led to the isolation of a variety of alkaloids belonging to different classes.[9−17] In a continuation of our efforts, extensive column chromatography of the alkaloidal fraction of a defatted ethanolic extract of the fresh flowers led to the isolation of two new alkaloids, vittacarboline (**1**) and *O*-methylismine (**3**), together with the known alkaloid ismine (**2**).[18] The structures of the alkaloids were unambiguously established on the basis of their 1D and 2D NMR and mass spectra. Application of the selective INEPT NMR experiment allowed the unambiguous assignments of quaternary carbons in **3**.



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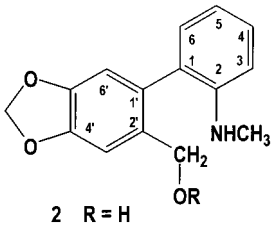
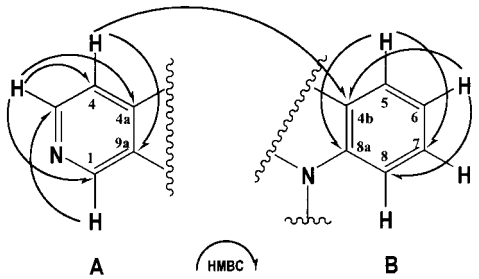
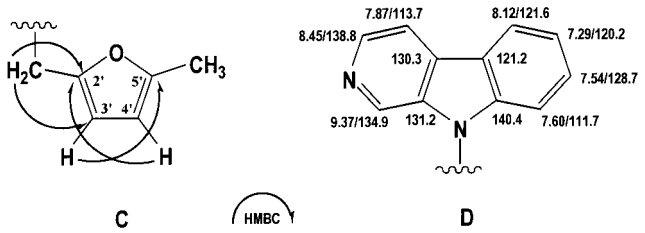


2 R = H
3 R = CH_3

Vittacarboline (**1**) was isolated as yellow needles with a molecular formula of $C_{17}H_{14}N_2O$ as determined by HREIMS. The ^{1}H NMR spectrum displayed signals integrating for 14 protons, of which nine were in the aromatic region ranging from 6.45 to 9.37 ppm and five were accounted for by signals for a methylene and a methyl group at δ 4.85 and 1.38, respectively. Concerted interpretation of the 2D NMR spectra (COSY, HMQC, and HMBC) of **1** led to the assembly of three structural fragments, the first composed of four contiguous protonated carbon resonances (**B**), and the others of two two-spin systems (**A** and



A HMBC **B**



C HMBC **D**

Figure 1. HMBC correlations of **1**.

C) (Figure 1). The carbon correlating in HMQC to the isolated singlet at δ_H 9.37 (δ_C 134.9) exhibited a long-range ^{1}H−^{1}H COSY coupling to the signal at δ_H 8.45 (δ_C 138.8), which coupled vicinally to the proton resonating at δ_H 7.87 (δ_C 113.7). These resonances were consistent with a 3,4-disubstituted pyridine in a β-carboline nucleus as shown in **A**.[19]

The ^{1}H−^{1}H COSY spectrum showed correlations within the four-spin system (H-8/H-7/H-6/H-5) of fragment **B**; these protons correlated in the HMBC spectrum to resonating signals at δ 111.7, 128.7, 120.2, and 121.6, respectively (Table 1). These partial ^{1}H and ^{13}C NMR data are comparable with those reported for an *ortho*-disubstituted benzene ring in a β-carboline system.[19−21]

The third spin-coupling system showed two doublets at δ 6.45 and 7.22 ($J_{3',4'}$ = 3.5 Hz) assigned as a 2,5-disubstituted furan moiety.[22,23] These signals are correlated in the HMQC spectrum with carbons at δ_C 110.8 and 109.5. In addition, the COSY spectrum showed ^{1}H−^{1}H long-range correlations between the signal at δ 1.38 (3H) and 7.22, and between δ 4.85 (2H) and 6.45, positioning the methyl and the methylene moieties at C-5′ and C-2′ of fragment **C**, respectively.

Unequivocal assignment of all signals within **A**, **B**, and **C** were based on long-range HMBC correlations (Figure 1

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Table 1. NMR Assignments for **1**−**3** ($CDCl_3$)

	1				**2**			**3**		
position	δ_C (ppm)	δ_H (mult., J_{Hz})	HMBC C → H	position	δ_C (ppm)	δ_H (mult., J_{Hz})	HMBC C → H	δ_C (ppm)	δ_H (mult., J_{Hz})	HMBC C → H
1	134.9	9.37 (br s)		1	127.2		3, 5	129.8[b]		
3	138.8	8.45 (d, 5.2)	4	2	146.5		6, NCH_3	141.1		NCH_3
4	113.7	7.87 (d, 5.2)	3	3	110.7	6.71 (dd, 8.0, 1.0)	5	117.0	7.21 (dd, 8.0, 1.0)	
4a	130.3		3	4	128.9	7.27 (ddd, 8.0, 7.5, 1.7)	6	129.3	7.36 (ddd, 8.0, 7.5, 1.5)	6
4b	121.2		4, 6	5	117.9	6.79 (ddd, 7.5, 7.5, 1.7)	3	123.7	7.13 (ddd, 7.5, 7.5, 1.5)	3
5	121.6	8.12 (br d, 8.0)		6	129.8	6.97 (7.5, 1.7)	4	131.0	7.09 (dd, 7.5, 1.5)	3, 4
6	120.2	7.29 (ddd, 8.8, 8.0, 2.0)		1′	130.9		3′, CH_2	129.8[b]		3′, CH_2
7	128.7	7.54 (ddd, 8.0, 8.0, 1.2)	5, 6	2′	133.8		6′, CH_2	132.8		6′, CH_2
8	111.7	7.60 (br d, 8.0)	6	3′	109.7	6.99 (s)	CH_2	110.4	6.99 (s)	CH_2
8a	140.4		5	4′	147.5		6′	147.9		OCH_2O, 6′
9a	131.2		4	5′	147.4		3′	147.7		OCH_2O, 3′
CH_2	57.8	4.85 (s)		6′	110.2	6.65 (s)		110.2	6.65 (s)	
2′	154.4[a]		4′, CH_2	NH					6.10 (br s)	
3′	110.8	6.45 (d, 3.5)	CH_2	NCH_3	30.7	2.70 (s)		33.7	2.70 (s)	
4′	109.5	7.22 (d, 3.5)		OCH_3				54.7	3.62 (s)	
5′	154.4[a]		3′	CH_2	63.5	4.24 (d, 12.0), 4.18 (d, 12.0)	3′	63.3	4.26 (d, 12.0), 4.12 (d, 12.0)	3′
5′-CH_3	29.7	1.38 (s)		OCH_2O	101.2	5.97 (s)		101.4	5.98 (s)	

[a,b] Overlapped signals.

and Table 1). For example, $^3J_{CH}$ correlations in the HMBC were observed between H-3 and C-1, H-1 and C-3, together with $^2J_{CH}$ correlation between H-3 and C-4 as shown by **A**. In a similar fashion, the carbon at δ 130.3 showed $^3J_{CH}$ correlation to H-5, positioning this carbon *para* to the proposed pyridine nitrogen, at C-4a. The quaternary carbon resonating at δ 131.2 (C-9a) showed $^3J_{CH}$ coupling to the proton doublet of H-4 at δ 7.87. The quaternary carbon resonating at δ 121.2 (C-4b in **B**) showed HMBC correlations to the proton resonating at δ 7.87 (H-4 in **A**) and to the proton resonating at δ 7.29 (H-6 in **B**), thereby positioning it in the correct context as shown by **D**. Finally, the quaternary carbon resonating at δ 140.4 (C-8a) showed HMBC correlation to the proton at δ 8.12 (H-5), completing the assembly of the β-carboline subunit. Finally, the remaining bonds from C-9a and C-8a were linked to N-9 to satisfy valence requirements. The absence of absorbance for NH (above 3300 cm^{-1}) in the IR spectrum of **1** supported the tertiary nature of N-9 and the attachment of the substituted furan moiety to it. N-9 was bonded in turn, to a methylene group, which linked directly to C-2′ of the furan moiety **C**.

Also, the 2,5-disubstituted furan subunit was elucidated from a combination of the results from the COSY, HMQC, and HMBC spectra. The HMBC spectrum showed correlation between the quaternary carbon resonating at δ 154.4 and the protons resonating at δ 6.45 ($^3J_{H-3',C-5'}$) and δ 7.22 ($^3J_{H-4',C-2'}$) and to the two-proton singlet at δ 4.84 ($^2J_{CH2,C-2'}$), suggesting the unequivocal assignment of the two overlapped resonances of both C-2′ and C-5′ at δ 154.4. Accordingly, vittacarboline (**1**) was assigned the structure N9-(5-methyl-2-furyl)methyl-β-carboline. Vittacarboline with its substitution pattern represents a new structural type within the family Amaryllidaceae. β-Carboline alkaloids are not common in the members of Amaryllidaceae. The first example belonging to this group is perlolyrine and was reported in *Crinum augustum*.[24]

Ismine (**2**) was purified as colorless prisms, with a molecular formula of $C_{15}H_{15}NO_3$ as deduced from HREIMS. This compound was identified as the known compound ismine,[18] which was identified through its ^1H NMR data.[18] However, this is the first report of its ^{13}C NMR data. The NMR data of **2** are presented in Table 1 and include unequivocal assignments of all quaternary carbons.

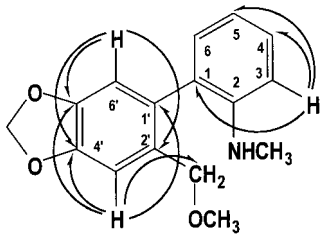

Figure 2. Selective INEPT enhancements for **3**.

O-Methylismine (**3**) was isolated as colorless oil with a molecular formula of $C_{16}H_{17}NO_3$, as established by HREIMS. Its NMR data were similar to those of ismine **2**, with the exception of the appearance of a new signal at δ_H 3.62 (δ_C 54.7) for a methoxy group (Table 1). Accordingly, **3** was assigned as *O*-methylismine. The selective INEPT NMR technique was used to assign the ^{13}C NMR chemical shifts of the quaternary carbons in **3** (Figure 2).

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. NMR experiments were recorded at 300 MHz for ^1H and at 75 MHz for ^{13}C NMR. Mass spectra were determined on a Finnigan MAT-312 at 70 eV. IR spectra were recorded on a Perkin-Elmer 1310/84 spectrometer. TLC was performed on precoated silica gel 60 F_{254} and RP-18 F_{254S} (Merck) plates.

Plant Material. Fresh flowers of *H. vittatum* were collected from plants propagated on the campus of Suez Canal University during the flowering period in July 1998; the species was identified by Dr. David Gardner at the Royal Botanic Gardens, Kew, Richmond, U.K. A voucher specimen (HV1) has been deposited at the Herbarium of the Faculty of Pharmacy, Suez Canal University.

Extraction and Isolation. Fresh flowers (3.65 kg) were chopped into small pieces and macerated in 10 L of EtOH (3 × 72 h) at room temperature. The combined crude extracts

were concentrated in vacuo and defatted with *n*-hexane (5 × 250 mL) (soluble in hexane = fraction A, insoluble in hexane = fraction B). Fraction B was dissolved in 2 L of 4% HCl, filtered, and shaken with $CHCl_3$ (5 × 250 mL). The combined $CHCl_3$ extracts gave fraction C. The residue was basified with concentrated NH_3 to pH 10 and extracted with $CHCl_3$ (15 × 250 mL). After evaporation, the combined $CHCl_3$ extracts yielded fraction D (2.7 g). Fraction D was flash chromatographed on a silica gel column using *n*-hexane, *n*-hexane−$CHCl_3$ mixtures, $CHCl_3$, $CHCl_3$−MeOH mixtures of increasing polarity, and pure MeOH. The collected fractions (150 mL each) were examined by TLC (Si gel) and visualized by Dragendorff's reagent. Fractions of similar composition were combined. Fractions 9−12 (*n*-hexane−$CHCl_3$, 9:1, 30 mg) were separated by MPLC (LiChroprep Si 60, Merck) using $CHCl_3$−acetone (92:8) as eluting system to give 8 mg of **1**. Fractions 23−34 ($CHCl_3$−MeOH, 95:5−9:1, 78 mg) were also subjected to chromatography by MPLC (LiChroprep RP-18, Merck) using MeOH−H_2O (85:15) as eluent, to afford **2** (17 mg) and **3** (9 mg).

Vittacarboline (1): yellow needles; mp 157−159 °C; UV λ_{max} (MeOH) (log ϵ) 240 (4.20), 256 (4.70), 268 (4.26), 291 (3.76), 305 (4.05), 350 (3.45), 3.70 (3.88), 385 (3.25) nm; IR (KBr) ν_{cm} 2900, 1625, 1490, 1295 cm^{-1}; NMR data, see Table 1; HREIMS *m*/*z* 262.1137 $[M]^+$ (98) (calcd for $C_{17}H_{14}N_2O$; 262.1106), 233 (35), 205 (100), 140 (15), 114 (23).

Ismine (2): colorless prisms; mp 100−101 °C (lit.[18] 98 °C); UV λ_{max} (MeOH) (log ϵ) 243 (4.25), 294 (3.75) nm; IR (KBr) ν_{cm} 3440, 3360, 1600, 1480, 1240, 1045, 930 cm^{-1}; NMR data, see Table 1; HREIMS *m*/*z* 257.1162 $[M]^+$ (45) (calcd for $C_{15}H_{15}NO_3$, 257.1052), 238 (100), 226 (7), 211 (7), 196 (14), 168 (15), 19 (11).

***O*-Methylismine (3):** colorless oil; UV λ_{max} (MeOH) (log ϵ) 245 (4.16), 290 (4.65) nm; IR (film) ν_{cm} 3375, 1605, 1485, 1245, 1050, 930 cm^{-1}; NMR data, see Table 1; HREIMS *m*/*z* 271.1306 $[M]^+$ (100) (calcd for $C_{16}H_{17}NO_3$, 271.1317), 196 (9), 180 (12), 152 (7), 19 (9).

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Supporting Information Available: ^1H, ^{13}C, COSY, HMQC, and HMBC NMR spectra of **1**−**3**, as well as selective 1D INEPT NMR experiments for **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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