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NOBILISINE, A NEW ALKALOID FROM *CLIVIA NOBILIS*

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ABSTRACT.—A new alkaloid named nobilisine (5α-hydroxy-3α,5a-*epi*-masan-7-one) [**1**] was isolated from *Clivia nobilis* cultivated in Egypt. Its structure was established from spectroscopic data utilizing mass spectral and two-dimensional ^{1}H-nmr methods. Clivatine and lycorine were also isolated and identified as congeners of **1** in *C. nobilis*.

Clivia miniata and *Clivia nobilis* Regel (Amaryllidaceae), are the only *Clivia* species cultivated in Egypt. *C. miniata* was the subject of several previous studies in which a number of alkaloids were isolated (1–15). There are no reports on the alkaloids of *C. nobilis*, except an early investigation (1937) on the exotic plant which mentioned the presence of cliviine (lycorine) and clivianine (16). *Clivia* species have been the major source of alkaloids of a small subgroup represented by the 3a,4-dihydrolactone-[2]-benzopyrano [3,4-*g*] indole ring system (17). Further references to the origin of the alkaloids of this subclass may be found in the series "The Alkaloids" (18).

Compounds belonging to this class contain four chiral centers at the ring junction positions C-3a, 5a, 11b, and 11c, and one additional chiral center through the presence of an oxygen substituent at C-5. All alkaloids isolated to date belong to a single enantiomeric series in which the absolute stereochemistry at C-11b and C-11c positions occurs as *S* and *R*, respectively, with variations occurring at the C-3a and C-5a centers (17). In principle, by evaluation of the magnitude of *J*-couplings between the protons at the ring junctions, it is possible to distinguish four stereoisomeric classes I–IV (Figures 1–4). The difficulty with this approach is that a full analysis of the relevant multispin system is often not attainable, usually because of overlap of signals which often occurs in the conventional 1D spectra, even at high field. The issue is further compounded in certain compounds, especially those involving *cis*-ring fusions, by the need to understand the dynamics of the conformational characteristics in flexible systems to permit an

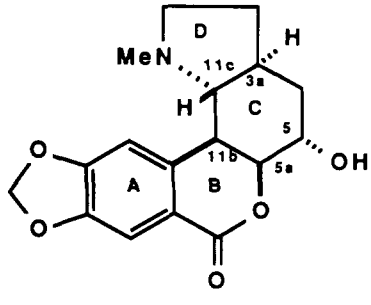

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FIGURE 1. Stereochemical class IV; *trans* B/C *anti*, *trans* C/D.

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unambiguous assignment of the configurational and conformational properties from coupling constant information.

In this report we describe the isolation and characterization of nobilisine [**1**], a new member of the 3a,4-dihydro lactone alkaloid series which is shown by mass spectral and 2D nmr methods to represent a new stereochemical variant IV (Figure 1) of the masa-nan-ring system.

RESULTS AND DISCUSSION

The alkaline $CHCl_3$ fraction of the EtOH extract of *C. nobilis* afforded three crystalline alkaloids (clivatine, lycorine, and **1**), when chromatographed over neutral alumina column. Clivatine and lycorine were identified by mp, chemical evidence, and spectroscopic analyses (uv, ir, ms, and ^{1}H nmr) and comparing the results with those of the reported data (3,11).

The structure of the new alkaloid **1** was deduced largely from ^{1}H-nmr and ms data. The mass spectral fragmentation pattern indicates that the alkaloid belongs to the dihydrolactone benzopyrano [3,4-*g*] indole alkaloids [the ion peaks at *m/z* 83 (98.1%, C_5H_9N) and *m/z* 96 (100%, $C_6H_{10}N$) indicating that this alkaloid is of the clivonine type (7,11)]. The ir spectrum revealed bands at 1700 cm^{-1} (C=O) and bands at 1480 and 920 cm^{-1}, assignable to methylenedioxy attached to an aromatic system. The ^{1}H-nmr spectrum (Table 1) confirmed this interpretation. The spectrum indicates the presence of a 9,10-methylenedioxy group (2H singlet at 5.99 ppm) and a three-proton *N*-methyl resonance at 2.34 ppm. The position of the methylenedioxy group and the lack of further aromatic substitution are revealed by two single-proton aromatic resonances (7.05 and 7.06 ppm).

Occurrence of a one-proton multiplet at 4.02 δ and a one-proton doublet of doublets at 3.80 δ (J = 2.56, 10.5) was attributable to the H-5 and H-5a signals, respectively, and was clearly indicative of a *trans* B/C ring fusion by virtue of the large H-5a, H-11b J-coupling in the latter signal. As a consequence of the *trans* B/C ring fusion, the small H-5a, H-5 J-coupling can be interpreted unambiguously as indicating that the 5-hydroxy has the α configuration.

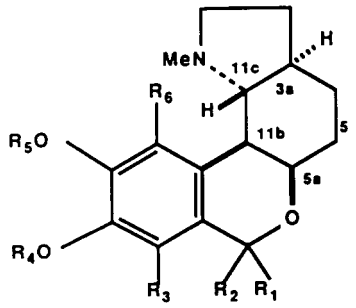
Although most of the remaining individual proton resonances are clearly resolved, overlap in the signals from 2β,3a and 3β,4α in the high field region of the spectrum

TABLE 1. Chemical Shifts and Coupling Constants of Nobilisine [**1**] in CD_3OD-Pyridine-d_5 (3:1).

Proton	Chemical Shift (ppm)	Coupling Constant (Hz)
H-2α	3.68	$J_{2\alpha,2\beta} = -11.3, J_{2\alpha,3\alpha} = 5.7, J_{2\alpha,3\beta} = 5.2$
H-2β	2.72	$J_{2\beta,3\alpha} = 12.6, J_{2\beta,3\beta} = 5.2$
H-3α	2.63[a]	$J_{3\alpha,3\beta} = -12.7, J_{3\alpha,3a} = 12.6$
H-3β	1.98[a]	[b]$J_{3\beta,3a} = 4.4$
H-3a	2.53	[b]$J_{3a,4\alpha} = 3.2$, [b]$J_{3a,4\beta} = 8.5$, [b]$J_{3a,11c} = 8.0$
H-4α	2.12	$J_{4\alpha,5} = 3.2$
H-4β	1.95	$J_{4\beta,4} = 2.8$
H-5	4.07	$J_{5,5a} = 2.8$
H-5a	3.91	$J_{5a,11b} = 11.0$
H-8	7.06	—
H-11	7.05	—
H-11b	4.10	$J_{11b,11c} = 11.45$
H-11c	3.44	—
9,10-OCH_2O	5.99	—
N-Me	2.34	

[a]Assignments could be exchanged.

[b]May not be accurate because of strong coupling.

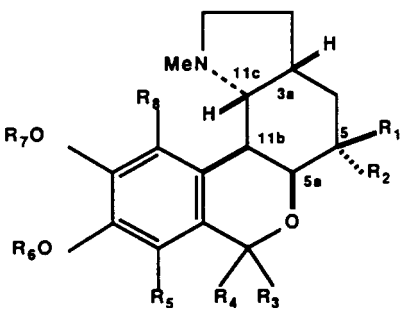


2 $R_1=R_2=R_3=R_6=H$; $R_4=R_5=Me$
3 $R_1,R_2=$ $=O$; $R_3=R_6=H$; $R_4=R_5=Me$
4 $R_1=R_2=R_6=H$; $R_3=OMe$; $R_4=R_5=Me$
5 $R_1=R_2=R_3=H$; $R_4,R_5=$-CH_2-; $R_6=OMe$
6 $R_1,R_2=$ $=O$; $R_3=R_6=H$; $R_5,R_4=$-CH_2-

FIGURE 2. Stereochemical class I; *cis* B/C *anti*, *trans* C/D.

precluded a complete analysis of the *J*-couplings from the 1D spectra. From a 2D ^1H COSY experiment it was possible to confirm the assignments of most of the signals in the spectrum of **1**, except those showing severe overlap. We attempted to use 2D *J*-spectroscopy to analyze the regions that were severely overlapped, but since the spins involved represented strongly coupled systems, as previously observed (19) in other strongly coupled networks, the results were not clearly interpretable.

An examination was made using solvent effects in an attempt to overcome problems of signal overlap. The best results were obtained using CD_3OD (0.3 ml) and pyridine-d_5 (0.1 ml). Selected *J* values were extracted from this spectrum by using a new pulse sequence, PE COSY, which is capable of providing the line positions within spin multiplets with high accuracy (20). Although we did not attempt to carry out a complete analysis of the 11 spins represented by the protons on the C/D rings, determination of the *J* values of the protons at the ring juncture positions 3a, 5a, 11b, and 11c indicated

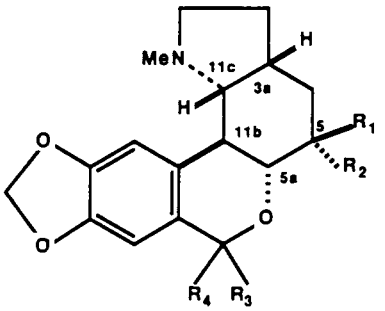


7 $R_1=R_2=R_3=R_4=R_5=R_8=H$; $R_6=R_7=Me$
8 $R_1=R_2=R_3=R_4=R_5=R_8=H$; $R_6,R_7=$-CH_2-
9 $R_1=R_2=R_3=R_4=R_8=H$; $R_5=OMe$; $R_6=R_7=Me$
10 $R_1=R_2=R_5=R_8=H$; $R_3,R_4=$ $=O$; $R_6=R_7=Me$
11 $R_1=R_2=R_3=R_4=R_5=H$; $R_6,R_7=$-CH_2-; $R_8=OMe$
12 $R_1=R_5=R_8=H$; $R_2=OH$; $R_3,R_4=$ $=O$; $R_6,R_7=$-CH_2-
13 $R_1=R_5=R_8=H$; $R_2=OAc$; $R_3,R_4=$ $=O$; $R_6,R_7=$-CH_2-
14 $R_1=R_5=H$; $R_2=OH$; $R_3,R_4=$ $=O$; $R_6,R_7=$-CH_2-; $R_8=OMe$
15 $R_1=OH$; $R_2=R_3=R_4=R_5=R_8=H$; $R_6,R_7=$-CH_2-
16 $R_1=OH$; $R_2=R_5=R_8=H$; $R_3,R_4=$ $=O$; $R_6,R_7=$-CH_2-
17 $R_1=OH$; $R_2=R_8=H$; $R_3,R_4=$ $=O$; $R_5=OMe$; $R_6,R_7=$-CH_2-

FIGURE 3. Stereochemical class II; *cis* B/C *anti*, *cis* C/D.

that each contained at least one coupling greater than 11.0 Hz. This finding is clearly indicative of the axial nature of these hydrogens and defines the relative stereochemistry of the B/C:C/D ring fusion and *trans, anti, trans*. If this compound belongs to the same enantiomeric series as its congeners, clivatine and lycorine, its structure may be represented as **1**. Nobilisine is the first representative of a lactone alkaloid of the 3a,4-dihydrobenzpyrano [3,4-*g*] indole series based on the *trans, anti, trans* B/C:C/D variation of the masanan-ring system.

EMPIRICAL CORRELATIONS OF *N*-METHYL CHEMICAL SHIFTS WITH STEREOCHEMICAL ASSIGNMENTS.—With the accumulation of ^{1}H-nmr data on Amaryllidaceae alkaloids of the 3a,4-dihydrobenzopyrano [3,4-*g*] indole series, a compilation of chemical shifts has suggested a useful correlation for the *N*-methyl signals which may be used to assign alkaloids of this subclass to one of the three major stereochemical variants I–III (Figures 2–4) encountered.



18 $R_1=R_2=R_3=R_4=H$
19 $R_1=Cl$; $R_2=R_3=R_4=H$
20 $R_1=Cl$; $R_2=H$; $R_3,R_4= =O$
21 $R_1=H$; $R_2=OH$; $R_3,R_4= =O$
22 $R_1=H$; $R_2=OAc$; $R_3,R_4= =O$

FIGURE 4. Stereochemical class III; *trans* B/C *anti, cis* C/D.

The chemical shifts of the *N*-methyl group represent a weighted average between two rapidly interconverting invertomers. A compilation of *N*-methyl chemical shifts in Table 2 shows considerable variation from the *N*-methyl chemical shift of *N*-methylpyrollidine, which in $CDCl_3$ occurs at 2.36 δ. The differences in chemical shift for each series are undoubtedly due to a number of factors, and although no attempt will be made to analyze these in detail, one obviously important effect is the different anisotropic shielding of the *N*-methyl group that results from a different orientation of the proximate aromatic ring in each stereochemical series. The most highly shielded *N*-methyl shifts are displayed by those compounds with *cis* B/C *anti, cis* C/D ring fusion, as represented in I (Figure 2). Models show that the *N*-methyl β invertomer, which is more stable than its α analog (by reasons of unfavorable non-bonded interactions of the latter with H-11), is situated over the π system of the aromatic ring. In comparison, the *cis* B/C *anti, trans* C/D series, cf. II (Figure 3), have their more stable *N*-methyl β invertomer at a more distant position from the aromatic ring in approximately the same orientation as the class I series. On the other hand, compounds in the *trans* B/C *anti, cis* C/D, class III series (Figure 4) have their *N*-methyl group oriented more nearly in the plane of the aromatic ring. This may account for the slight deshielding effects observed for *N*-methyl chemical shifts of compounds in series III compared to *N*-methylpyrollidine. Since nobilisine is the only member of the class IV stereochemical series (Figure

TABLE 2. *N*-Methyl Chemical Shifts of 3a,4-Dihydro-[2] Benzopyrano [3,4-*g*] Indole Compounds.[a]

	Compound	*N*-Methyl Chemical Shift (ppm)
I.	*cis* B/C *anti*, *trans* C/D	
	3a-*epi*-Homolycorane [**2**]	2.00
	3a-*epi*-Homolycoran-7-one [**3**]	1.92
	8-Methoxy-3a-*epi*-homolycorane [**4**]	2.00
	11-Methoxy-3a-*epi*-masanane [**5**]	2.00
	3a-*epi*-Masan-7-one [**6**]	1.90
II.	*cis* B/C *anti*, *cis* C/D	
	Homolycorane [**7**]	2.21
	Masanane [**8**]	2.22
	8-Methoxyhomolycorane [**9**]	2.20
	Homolycoran-7-one [**10**]	2.16
	11-Methoxymasanane [**11**]	2.15
	5α-Hydroxymasan-7-one [**12**]	2.29
	5α-Acetoxymasan-7-one [**13**]	2.31
	5α-Hydroxy-11-methoxymasan-7-one [**14**]	2.32
	5β-Hydroxymasan-7-one [**15**]	2.18
	5β-Hydroxymasanane [**16**]	2.26
	5β-Hydroxy-8-methoxymasan-7-one [**17**]	2.22
III.	*trans* B/C *anti*, *cis* C/D	
	5a-*epi*-Masanane [**18**]	2.49
	5β-Chloro-5a-*epi*-masanane [**19**]	2.48
	5β-Chloro-5a-*epi*-masan-7-one [**20**]	2.55
	Clivonine [**21**]	2.52
	5α-Acetoxy-5a-*epi*-masan-7-one [**22**]	2.50
IV.	*trans* B/C *anti*, *trans* C/D	
	Nobilisine [**1**]	2.34

[a]All values reported as ppm relative to TMS as an internal standard in $CDCl_3$.
[b]Stereochemical class.

1), no conclusions can be drawn regarding *N*-methyl shifts in this series. However, the value of 2.34 δ represents an essentially unperturbed value in comparison to that of *N*-methylpyrollidine and is in agreement with that predicted from the model.

The value of *N*-methyl chemical shift correlations in this series will be to differentiate class I from II, which at present relies either on chemical correlations or very detailed nmr analysis to assign the stereochemistry at the C-3a position. A similar use in designating the stereochemistry at C-3a to differentiate class III from class IV would also be valuable. The latter must await accumulation of additional data to determine whether this will be possible.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler hot stage microscope, uv spectra were measured on a Sp8-100 uv/vis Pye Unicam spectrophotometer, and the ir spectra were obtained on a Beckman model 4210 spectrometer. ^{1}H-nmr spectra were recorded on a Nicolet NT-360 wide bore spectrometer operating at 360 MHz under the control of a Nicolet 1180E/293C data analysis or on a JEOL GX 500 under conditions which have been previously described. Eims and cims data were obtained on a Finegan 4000 mass spectrometer using CH_4 as the reagent gas for the ci spectra.

PLANT MATERIAL.—*C. nobilis* used in this study was collected in Alexandria, Egypt, from fresh flowering cultivated plants. The plant was identified by Dr. Hosny Kamel, El-Orman Garden, Cairo, Egypt. A voucher specimen of the plant is kept at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Alexandria, Egypt.

EXTRACTION AND ISOLATION PROCEDURES.—Fresh whole plant (2 kg) was extracted by cold percolation with 95% EtOH. The combined extracts were concentrated under reduced pressure and then acidified with 1% HCl (pH 2). The aqueous acidic solution was washed with Et_2O, then rendered alkaline with NH_4OH solution and extracted with $CHCl_3$ (6 × 100 ml) and $CHCl_3$-MeOH (3:1) (3 × 50 ml). The $CHCl_3$ extracts were combined, washed with distilled H_2O, and dried over Na_2SO_4. The solution was concentrated to a small volume, and at this stage lycorine precipitated (350 mg). The mother liquor was freed from the solvent leaving 1.89 g of crude alkaloid fraction which was fractionated over a column of neutral alumina (activity II, 80 g). Elution was achieved using C_6H_6, $C_6H_6/CHCl_3$, $CHCl_3$, $CHCl_3$/MeOH mixtures of increasing polarity until pure MeOH was used. Fractions (50 ml each) were collected and analyzed using Si gel G chromatoplates and $CHCl_3$-MeOH (9:1 and 8:2). Chromatographic separation resulted in the isolation of clivatine (50 mg), lycorine (50 mg), and nobilisine [**1**] (250 mg).

Clivatine [**1**]. Colorless prisms from MeOH, mp 167–168° [lit. (12) 166–168°]; uv λ max (MeOH) 307, 268, 226 nm; ir ν max (cm^{-1}) 3520 (OH), 1725, 1700 (C=O); 1490, 925 (-O-CH_2-O-); cims *m/z* $[M+M]^+$ 404.3 (100%), 359.6 (3), 318.2 (9), 317.0 (1), 300.1 (17), 126.0 (0.5), 114.9 (3.84), 96.1 (6.41), 83.1 (15.38), 82 (32.05); ^1H nmr ($CDCl_3$) δ 7.75 (1H, s, H-11), 7.46 (1H, s, H-8), 6.05 (2H, s, -O-CH_2-O-), 5.4 (1H, m, H-5), 4.18 (1H, dd, H-5a, $J_{5a\text{-}11b}$ = 12 Hz, $J_{5a\text{-}5}$ = 3 Hz), 3.27 (1H, m, H-11b), 2.71 (1H, m, H-11c), 2.03 (3H, s, N-Me), 1.26 (3H, d, J = 9.5 Hz, -Me).

Lycorine [**2**]. Its identity was confirmed by the mp of its picrate, 195–197° (21), its diacetate, 220–222° (22), and direct co-chromatographic comparison and mmp with authentic lycorine.

Nobilisine [**1**]. Colorless plates from MeOH, mp 275–277°; uv λ max (MeOH) 292, 254, 224 nm; ir ν max (cm^{-1}) 3520 (OH), 1710 (conjugated δ-lactone), 1480, 940 (-O-CH_2-O-); cims *m/z* $[M+M]^+$ 318.1 (100%), 300.1 (4.3), 191.0 (1.07), 126 (1.0), 96 (2.74), 83 (5.25), $[M]^+$ 317 (5.9%), 191 (0.38), 190 (0.18), 162 (1.25), 126 (2.6), 96 (100), 83 (98.1), 82 (26.8).

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