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^{1}H AND ^{13}C NMR ANALYSIS OF LYCORINE AND α-DIHYDROLYCORINE*

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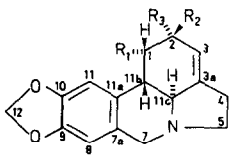
Key Word Index—*Sternbergia lutea*; Amaryllidaceae; alkaloids; lycorine; α-dihydrolycorine; α-dihydrolycorinelactam; ^{1}H and ^{13}C NMR spectra.

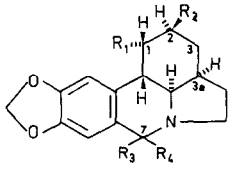
Abstract—The ^{1}H and ^{13}C NMR spectra of lycorine and its α-dihydro derivative have been studied. The employment of nuclear magnetic double resonance, nuclear Overhauser effect and acetylated derivatives, allows the assignment of all proton resonances. The assignments of the carbon shifts have been obtained by means of proton noise decoupled, single frequency off-resonance decoupled, single frequency selective decoupling, time dependence nuclear Overhauser effect and by comparison with reference compounds.

INTRODUCTION†

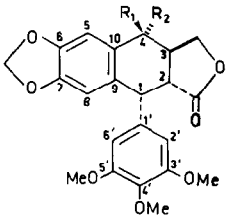
Lycorine **1** [1], an alkaloid extracted from various Amaryllidaceae [2], is a powerful inhibitor of growth and cell division [3] in higher plants, algae and yeasts. At very low concentrations (10^{-6} M) **1** inhibits the biosynthesis of ascorbic acid (AA) [4]. Thus, it prevents the development of cyanide-insensitive respiration [5]. These effects are accounted for by the need of AA in the biosynthetic control of hydroxyproline-containing proteins [6].



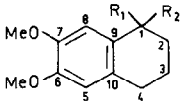
1 $R_1=R_2=OH$, $R_3=H$
4 $R_1=R_2=OAc$, $R_3=H$
5 $R_1=OAc$, $R_2=OH$, $R_3=H$
6 $R_1=OH$, $R_2=OAc$, $R_3=H$
8 $R_1=OH$, $R_2+R_3=O$



2 $R_1=R_2=OH$, $R_3=R_4=H$
3 $R_1=R_2=OH$, $R_3+R_4=O$
7 $R_1=R_2=OAc$, $R_3=R_4=H$



9 $R_1=H$, $R_2=OH$
10 $R_1+R_2=O$



11 $R_1=H$, $R_2=OH$
12 $R_1+R_2=O$

A study of the interaction between lycorine and receptor(s) binding site is instrumental in clarifying the mechanism of action of the alkaloid. An investigation of the NMR of lycorine has been undertaken as a first approach toward this goal. In this paper we report the assignment of the chemical shifts of all protons and carbons in the ^{1}H and ^{13}C NMR spectra of lycorine and α-dihydrolycorine, **2** [7], which also shows the same activity on AA biosynthesis [8].

RESULTS AND DISCUSSION

Lycorine is insoluble in the common organic solvents and is unstable in CF_3COOH. In the spectra of **1** carried out in aqueous acid solution (1% D_2SO_4) double signals were observed. This could be explained by slow interconversion of two conformers, probably due to protonation of the nitrogen atom. This phenomenon is absent in the spectra of **2**, the α-reduced form of **1**, at $\Delta^{3,3a}$. One likely explanation can be attributed to a larger conformational freedom of **2** as compared to **1** and, therefore, a lowering of the energy barrier between the two different conformers. The best resolution conditions of ^{1}H and ^{13}C NMR spectra were obtained by using CD_3OD–CD_3COOD (3:1) as solvent.

Table 1 lists the proton shifts of **1** and **2** assigned by integration, multiplicity and nuclear magnetic double resonance. Moreover, other support was obtained by nuclear Overhauser effect (NOE) measurements and by spectral analysis of some derivatives: α-dihydrolycorinelactam, **3** [9]; 1,2-*O*,*O*-diacetyl-lycorine, **4** [7]; 1-*O*-acetyl-lycorine, **5** [7]; 2-*O*-acetyl-lycorine, **6** [1]; 1,2-*O*,*O*-diacetyl-α-dihydrolycorine, **7** [10], and lycorine-2-one, **8** [8]. Proton resonances of **4** and **7** are partially reported by other authors [11]. The signals at δ4.58, double doublet, and at 4.26, multiplet, were assigned to H-1 and H-2, respectively, on the basis of the following results. Acetylation of HOC-1 (**1** → **4**, **1** → **5**) caused a downfield shift (Δδ1.24 and 1.18) of the signal at δ4.58; the same deshielding (Δδ1.03 and 1.06) was observed for the signal at 4.26 when HOC-2 was acetylated (**1** → **4**, **1** → **6**). The signal at δ4.26 was absent in the spectra of **8**.

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†Nomenclature: lycorine, 2,4,5,7,12b,12c-hexahydro-1*H*-(1,3)-dioxolo(4,5-*j*)pyrrolo(3,2,1-de)phenanthridine-1,2-diol.

Table 1. Proton shifts of compounds **1** and **2**

	1		**2**	
H-1	4.58 *dd**	J_{1-2} = 1.1 Hz J_{1-11b} = 2.2 Hz	4.53 *dd**	J_{1-2} = 2.0 Hz J_{1-11b} = 2.0 Hz
H-2	4.26 *m*	J_{1-2} = 1.1 Hz	4.07 *m*	J_{1-2} = 2.0 Hz
H-3	5.77 *br s*	—	2.27 *ddd** } AB 1.93 *ddd** } AB	J_{AB} = 15.3 Hz J_{3A-3a} = 6.7 Hz, J_{3A-2} = 3.9 Hz J_{3B-3a} = 3.9 Hz, J_{3B-2} = 2.0 Hz
H-3a	—	—	2.59 *m*	—
2H-4	2.88 *m*		2.59 *m* } AX 2.10 *m* } AX	J_{AX} = 14.0 Hz
2H-5	3.75 *m* } AX 3.49 *m* } AX	J_{AX} = 10.0 Hz	3.63 *m* } AB 3.40 *m* } AB	J_{AB} = 11.7 Hz
2H-7	4.48 *d* } AB 4.19 *d* } AB	J_{AB} = 14.0 Hz	4.60 *d* } AX 4.04 *d* } AX	J_{AX} = 14.0 Hz
H-8	6.80 *s*	—	6.85 *s*	—
2H-12	5.95 *s*	—	5.98 *s*	—
H-11	6.98 *s*	—	7.08 *s*	—
H-11b	2.99 *dd** } AM	J_{AM} = 11.8 Hz J_{1-11b} = 2.2 Hz	3.24 *dd** } AM	J_{AM} = 11.7 Hz J_{1-11b} = 2.0 Hz
H-11c	3.95 *d* } AM	J_{AM} = 11.8 Hz	3.65 *d* } AM	J_{AM} = 11.7 Hz

Chemical shifts in δ-values (ppm) from TMS (MeOH, δ 3.31, was used as int. standard).
* These multiplicities were observed in the ^{1}H resolution enhanced spectrum.

Such assignments were confirmed also by double resonance experiments. Irradiation of the double doublet (J_{1-11b} = 2.2 Hz, J_{1-2} = 1.1 Hz) at δ 4.58, due to H-1 (X part), simplified the double doublet centred at 2.99, due to H-11b, into a doublet (J_{AM} = 11.8 Hz). This latter signal was the simple M part of an AM system. The A part was assigned to the doublet (J_{AM} = 11.8 Hz) centred at δ 3.95, due to H-11c. Irradiation of H-1 also simplified the signal of H-2, multiplet, while irradiation of H-11b converted the double doublet of H-1 into a doublet (J_{1-2} = 1.1 Hz).

A clear distinction between the resonances of the aromatic protons, which showed two singlets at δ 6.98 and 6.80, was achieved by the following experiments. The acetylation of HOC-1 (**1** → **4**, **1** → **5**) induced shielding ($\Delta\delta$ 0.27) of the signal at δ 6.98, while acetylation of HOC-2 (**1** → **6**), did not elicit any effect. The analysis of NOE difference spectra, obtained by homonuclear selective decoupling, showed a clear effect at δ 6.98 by irradiation at 4.58 (H-l) and vice versa. When the signal at δ 6.80 was irradiated, no effect was observed. Therefore, the singlet at δ 6.98 must be attributed to H-11 and the singlet at 6.80 to H-8.

The olefinic proton appeared as a broad singlet at δ 5.77, but was absent in the spectrum of **2**. In the NOE difference spectrum, obtained by irradiation at δ 5.77 (H-3), a clear enhancement was observed at 4.26 (H-2) and vice versa.

The simple AB system, with a coupling constant (J_{AB} = 14.0 Hz) typical of geminal protons, was attributed to 2H-7; indeed, it was absent in the spectrum of **3**. The 2H-5 showed a more complex spin system produced by further coupling of their simple AX system (J_{AX} = 10.0 Hz) with 2H-4, an unresolved multiplet at δ 2.88. The X part of this AX system appeared as an eight-line multiplet centred at δ 3.75 and the A part appeared as a quartet centred at 3.49. All these assignments were in agreement with experimental evidence obtained by single frequency selective decoupled spectra. The assignment of the signals in the spectrum of **2** was based on similar considerations and by comparison with the absorptions of compound **7**. Analysis of aliphatic proton resonances showed a further AB system due to 2H-3 (J_{AB} = 15.3 Hz). The A part appeared as an eight-line multiplet centred at δ 2.27 due to further coupling with H-3a (J_{3A-3a} = 6.7 Hz) and H-2 (J_{3A-2} = 3.9 Hz); the B part at δ 1.93, appeared as a doublet of doublet of doublets (J_{3B-3a} = 3.9 Hz, J_{3B-2} = 2.0 Hz). These assignment were confirmed by double resonance experiments, as well as resolution enhancement, which also provided useful information to attribute the resonances of H-3a and H-4X, both centred as a multiplet at δ 2.59, and the resonance of H-4A, multiplet centred at 2.10 (J_{4X-4A} = 14.0 Hz).

Table 2 shows the carbon shifts of **1**–**3** assigned by proton noise decoupled, and single frequency off-resonance decoupled spectra, and on the basis of information obtained by single frequency selective decoupled spectra, comparison with reference compounds [12] and by experiments of time dependence NOE.

In the spectrum of **1**, the assignment of the signals at δ 149.7 and 148.1, due to the non-protonated oxygenated aromatic carbons C-9 and C-10, and of the signals at 130.6 and 125.7, due to C-7a and C-11a was crucial. Measurements of long range coupling (J^3_{C-H}), ^{13}C NOE and selective NOE, the latter obtained by irradiation of absorption frequencies of H-8 and H-11, were not sufficient to distinguish the signals of each pair. We have resolved this problem by comparing data obtained from the spectra of **3** with those of reference compounds [12], and performing two independent experiments of time

Table 2. Carbon shifts of compounds **1–3**

	1	**2**	**3**
C-1	70.1 *d*	69.6 *d*	70.4 *d*
C-2	71.9 *d*	70.5 *d*	73.9 *d*
C-3	122.9 *d*	27.8 *t*	30.8 *t*
C-3a	137.9 *s*	36.6 *d*	39.4 *d*
C-4	30.3 *t*	30.5 *t*	32.9 *t*
C-5	55.1 *t*	56.5 *t*	46.8 *t*
C-7	54.2 *t*	53.8 *t*	165.1 *s*
C-7a	130.6 *s*	133.0 *s*	126.0 *s*
C-8	108.8 *d*	108.6 *d*	108.9 *d*
C-9	149.7 *s*	149.7 *s*	147.8 *s*
C-10	148.1 *s*	147.7 *s*	152.3 *s*
C-11	106.4 *d*	106.9 *d*	106.4 *d*
C-11a	125.7 *s*	124.5 *s*	137.3 *s*
C-11b	38.2 *d*	33.2 *d*	36.1 *d*
C-11c	61.8 *d*	64.3 *d*	57.3 *d*
C-12	102.8 *t*	102.6 *t*	103.0 *t*

Chemical shifts in δ-values (ppm) from TMS (MeOH, δ 49.0, was used as int. standard).

dependence NOE [13, 14]. These latter were carried out by specific irradiation at δ6.80 and 6.98, the absorption frequencies of H-8 and H-11. The change of enhancement of the quaternary aromatic carbons was measured and plotted against proton decoupling time (Figs. 1 and 2). This type of experiment is the only one which allowed the

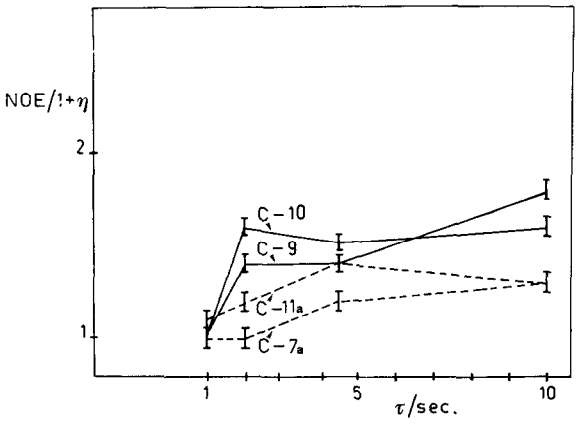

Fig. 1. Selective irradiation of H-11 and time dependence NOE for C-10, C-9, C-11a and C-7a. As shown, C-10 and C-11a were affected before the others; spin diffusion then affected all carbon resonances.

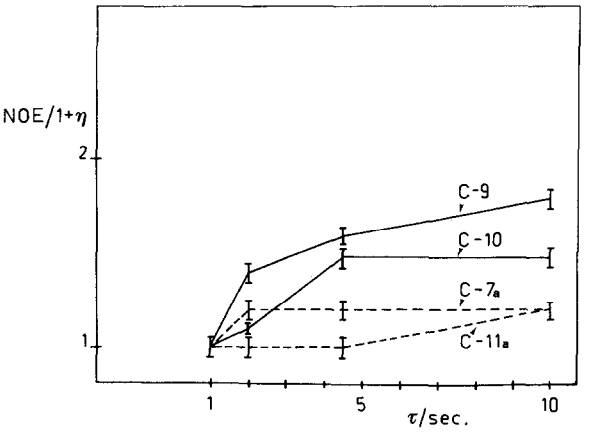

Fig. 2. Selective irradiation of H-8 and time dependence NOE for C-10, C-9, C-11a and C-7a. As shown, C-9 and C-7a were affected before the others; spin diffusion then affected all carbon resonances.

assignment of signals at δ 149.7 and 148.1 to C-9 and C-10 and those at 130.6 and 125.7 to C-7a and C-11a, respectively. These resonances were confirmed by evidence obtained by analysis of the data reported in Table 3. The mesomeric effect of the conjugated carbonyl group in **3** induced the shielding of the C-11a (Δδ 11.6) and of the C-10 (Δδ 4.2), as shown also from the spectral data of the reference compounds podophyllotoxin, **9**, podophyllotoxone, **10**, 1-hydroxy-6,7-dimethoxy-2,3,4-tetrahydronaphthalene, **11**, and 1-keto-6,7-dimethoxy-2,3,4-tetrahydronaphthalene, **12** [12].

The resonances at δ 137.9 and 122.9 were attributed to the olefinic carbons C-3a and C-3, respectively; these attributions were confirmed by their absence in the ^{13}C NMR spectrum of **2**.

In order to attribute the aromatic protonated and hydroxylated carbons, single frequency selective decoupled spectra were measured by irradiation at the absorption frequencies of their protons. On the basis of the clear collapse of the doublet into a singlet, the signals at δ 108.8 and 106.4 were assigned to C-8 and C-11, respectively, and those at 70.1 and 71.9 to C-1 and C-2, respectively. The same experiments were performed to attribute the resonances at δ 61.8 and 38.2 to C-11c and C-11b, respectively, and those at 55.1 and 54.2 to C-5 and C-7, respectively. This latter assignment was confirmed by the upfield shift (Δδ 111.3) induced by oxidation of 2HC-7 to O=C-7 (**2** → **3**). The assignments of the carbon shifts in **2** and **3** were based on similar arguments.

Table 3. Comparison of the chemical shifts of the quaternary aromatic carbons of **1–3** with those of reference compounds **9–12**

	1*	**2***	**3***		**9†**	**10†**		**11†**	**12†**
C-7a	130.6	133.0	126.0	C-10	133.1	128.0	C-9	130.3	125.0
C-8	108.8	108.6	108.9	C-5	106.2	105.5	C-8	111.1	107.7
C-9	149.7	149.7	147.8	C-6	147.2	147.8	C-7	147.1	147.1
C-10	148.1	147.7	152.3	C-7	147.2	152.9	C-6	148.0	152.7
C-11	106.4	106.9	106.4	C-8	109.3	110.2	C-5	111.1	109.5
C-11a	125.7	124.5	137.3	C-9	130.6	141.3	C-10	129.0	138.6

*CD_3OD–CD_3COOD (3:1).
†$CDCl_3$.

The accurate analysis of ^1H and ^{13}C NMR reported in this paper constitutes the basis for a study of the conformational aspects of lycorine and α-dihydrolycorine in solution as a starting point in the investigation of the interaction between lycorine and binding site.

EXPERIMENTAL

The samples were contained in vacuum sealed NMR tubes. ^1H NMR spectra were recorded at 270 MHz in CD_3OD–CD_3COOD (3:1) using CD_3OD (δ 3.31) as int. standard, at a 45° probe temp. FT-NMR conditions were as follows: 3.2 kHz spectral width, 16 000 data points, 0.51 sec pulse repeat time, 45° pulse flipping angle (90° in the NOE expts). ^{13}C NMR spectra were recorded at 67.8 MHz in CD_3OD–CD_3COOD (3:1) with CD_3OD (δ 49.0) as int. standard and using micro-cells for the spectrum of **3**. FT-NMR conditions were as follows: 15.0 kHz spectral width, 16 000 data points, 2.1 sec pulse repeat time, 45° pulse flipping angle (90° in the NOE expts). Lycorine was obtained from dried bulbs of *Sternbergia lutea* Ker-Gawl by extraction with 1% H_2SO_4 soln [8]. The derivatives of lycorine **2** and **8** were prepared according to an unpublished procedure.

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