STRUCTURE OF LILIDINE

N. D. Abdullaev, K. Samikov, T. P. Antsupova, M. R. Yagudaev, and S. Yu. Yunusov

UDC 547.944/945+543.422+541.67

The new alkaloid lilidine, isolated for the first time from the epigeal part of *Lilium martagon* has been studied by special methods. It has the composition $B_5H_6NO_2$, mp 118-110°C, $[\alpha]_D$ −26.3°. The ^1H and ^{13}C NMR spectra were studied in detail. The values of the direct and long-range spin-spin coupling constants between the ^{13}C carbon nuclei and the ^1H nuclei of the alkaloid molecule were measured with the aid of $^{13}C-\{^1H\}$ selective heteronuclear double resonance. The structure of 5-hydroxy-3-methyl-3-pyrrolin-2-one is suggested for lilidine.

Plants of the section Martagon Wilson (family Liliaceae), including 12 species, grow predominantly in wooded meadows, in coniferous and mixed forests, and on the rocky hills and slopes of the Caucasus and of Western and Eastern Siberia, and in the European part of the USSR. The demands of the population for fresh and dried bulbs of *Lilium martagon* as a drug (vesicant, diuretic, healing) [1] has aroused the interests of specialists in this plant.

The chemical composition of *Lilium martagon* has not previously been studied. We have investigated the epigeal part of this plant collected in the environs of the town of Babushkin, Buryat ASSR, in the flowering period on July 11, 1983 for the presence of alkaloids [7].

From an aqueous ethanolic extract we isolated a base with the composition $C_5H_7NO_2$ mp 118-119°C, $[\alpha]_D$ −26.3°, M^+ 113 (by high-resolution mass spectrometry), which we have called lilidine. Its IR spectrum has absorption bands at (cm^{-1}) 1690 (amide carbonyl) and 1645 (double bond), and a broad band with its center at 3300 of hydroxy and amide NH groups.

In the PMR spectrum of lilidine recorded in CD_3OD with the addition of a very small amount of CH_3COOD there were two multiplets of 1 H each at 5.24 and 6.55 ppm and a triplet of 3 H at 1.77 ppm. In the spectrum taken with the use of deuterated pyridine as solvent, in addition to multiplets at 5.73 and 6.62 ppm and a triplet at 1.74 ppm, another two one-proton signals appeared — a doublet at 6.79 ppm with ^3J = 8.0 Hz and a broadened singlet at 9.31 ppm. When the temperature of the sample under investigation was raised, these signals underwent a diamagnetic shift, thereby showing a dependence of the chemical shifts on the temperature. These facts indicated that in the molecule of the compound under investigation two protons were mobile. The same conclusion followed from the mass spectrum of the base previously deuterated under mild conditions, in which the mass of the molecular ion had increased by two units and amounted to 115.

The ^{13}C NMR spectrum of lilidine consisted of five signals. Under the conditions of incomplete suppression of spin-spin coupling with protons, they appeared in the form of a quartet at 10.4 ppm (—CH_3), two doublets at 79.8 ppm (>CH—O) and 142.9 ppm (—CH=), and two singlets at 136.7 ppm (>C=) and 175.2 ppm. The assignments shown in parentheses of four of the signals followed from their multiplicities and the values of their chemical shifts. The remaining, unassigned, second singlet may belong to a carbonyl of an amide or of an ester group conjugated with a double bond in a five-membered ring [2-4]. However, in view of the presence in the PMR spectrum of a broadened singlet at 9.29 ppm (NH) and of absorption bands in the IR spectrum at 1690 cm^{-1}, it may be concluded that the line of carbon resonance at 175.2 ppm corresponds to the signal of the carbon of the carbonyl of an amide group.

When the PMR spectrum of lilidine was recorded under conditions of double proton-proton resonance, it was found that in the presence of an additional radiofrequency field with ν = 572 Hz the doublet at 6.79 ppm from a mobile proton fused into a singlet, thereby revealing that it belonged to a secondary hydroxy group. Consequently, the signals in the PMR spectrum

Institute of the Chemistry of Plant Substances of the Uzbek SSR Academy of Sciences, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 692-696, September-October, 1987. Original article submitted March 27, 1987.

 0009-3130/87/2305-0576$12.50

TABLE 1. Values of the Chemical Shifts (δ, ppm) and Spin-Spin Coupling Constants (J, Hz) of the Protons and the ^{13}C Carbon Atoms of (I)

Proton	C_5D_5N, 0– HMDS	C atom	CD_3OD, 0– TMS
H-4	6,62 m, $^4J_{4,1}=1,5$; $^4J_{4,6}=1,8$; $^3J_{4,5}=1,7$	2	175,2 s
		3	136,7 s
		4	142,9 d
H-5	5,73 m, $^3J_{5,1}=1,4$; $^3J_{5,4}=1,7$; $^5J_{5,6}=1,2$; $^3J_{5,OH}=8,0$		$^1J_{4,H-4}=171,5$
		5	79,8 d
H-6	1,74 t, $^4J_{6,4}=1,8$; $^5J_{6,5}=1,2$; $^5J_{6,2}=0,3$		$^1J_{5,H-5}=159,0$; $^2J_{5,H-4}=6,1$; $^4J_{5,H-6}=0$
NH	9,31 br.s	6	10,4 q
			$^1J_{6,H-6}=127,0$;
OH	6,79 d, $^3J_{HO,3}=8,0$		$^3J_{6,H-4}=2,7$

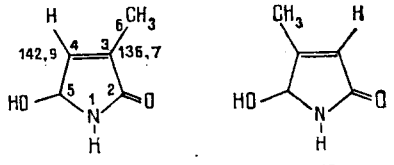
Symbols: m – multiplet; t –triplet; br. s. – broadened singlet; d – doublet; q – quartet.

of lilidine at 1.73, 5.73, and 6.79 ppm can be assigned to the protons of a methyl group at a double bond, to a methylenic proton at a secondary hydroxy group, and to an olefinic proton.

In spite of the simplicity of the overall pattern of the PMR spectrum of the base, the complete analysis of the chain of spin-spin interactions of the protons become possible only with the use of the procedure of homonuclear selective double resonance. As a result, it was found that each hydrogen atom of the lilidine molecule enters into vicinal, allyl, or homoallyl spin-spin interaction with protons in three different positions. The values of the corresponding ^nJ constants are given in Table 1.

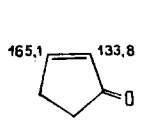
Thus, on the basis of all that has been said above, we found that the lilidine molecule contains the following groupings: $-CH=\overset{|}{C}-CH_3$, $-\overset{|}{C}H-OH$ and $-NH-C=O$. With the given elementary composition it is possible to form from these fragments only a five-membered ring in which a double bond may occupy the α,β-position to a carbonyl group or to an amide NH.

In the UV spectrum of lilidine in ethanol, there is an absorption maximum at 235 nm (log ε 3.1) which is characteristic of a carbonyl chromophore conjugated with a double bond [5]. On this basis, of the four alternative structures for the lilidine molecule, we considered (I) and (II) to be probable.

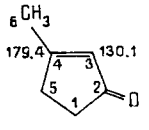


I

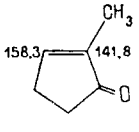
II



III



IV



V

To choose between them let us turn to the characteristics of the carbon magnetic resonance. It is known from the literature that the ^{13}C chemical shifts of olefinic carbon atoms in cyclic α,β-unsaturated ketones and, in particular, cyclopentenones, change in a peculiar way as a function of the position of a newly introduced methyl constituent [2, 6]. For example, in a comparison of the chemical shifts attached to the corresponding carbon atoms of compounds (III)-(V) we see that the magnitude of the α-contribution of the methyl group when it is introduced at C-4 (IV) is practically twice as great (+14.3 ppm) as in the case of its location at C-3 (+8.0 ppm, V). As a result, in the case of (IV) a considerable paramagnetic

change in the chemical shift to 179.4 is observed for the quaternary C-4 carbon atom. The chemical shifts of the proton-bearing olefinic carbon atoms in compounds (IV) and (V) also differ substantially.

The facts given above may be considered a sufficient basis, with the obvious difference in the degrees of conjugation of the keto group and with the amide carbonyl, for giving preference to structure (I) for lilidine, to the olefinic carbon atoms of which correspond resonance lines with chemical shifts of 142.9 and 136.7 ppm in the ^{13}C NMR spectrum.

The values of the direct spin-spin coupling constants $^1J_{C-H}$ with the corresponding protons for the carbon atoms C-5 (159.0 Hz), C-4 (171.5 Hz), and C-6 (127.0 Hz) were determined on the basis of the results of a study of the high-resolution ^{13}C NMR spectrum of lilidine. It follows from the fine structure of the splitting of the signals of these nuclei that they also interact with other protons. By making use of the method of selective heteronuclear $^{13}C-\{^1H\}$ double resonance we established that H-4 interacts with C-5 with ^2J = 6.1 Hz and with C-6 with ^3J = 2.7 Hz. No spin-spin coupling was detected between C-5 and H-6 or between C-6 and H-5.

Thus, for lilidine the structure of 5-hydroxy-3-methyl-3-pyrrolin-2-one (I) is suggested.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded on a Tesla BS-567 A spectrometer at ν_0 = 100 and 24.142 MHz, respectively, and a Varian CFT-20 spectrometer; IR spectra on a UR-20 spectrometer (KBr), UV spectra on a Hitachi, EPS-3T instrument; and mass spectra on a MKh-1310 spectrometer.

Isolation of Lilidine. The dry epigeal part of (1.7 kg) of *L. martagon* was extracted with 80% ethanol. The ethanolic extract was evaporated in vacuum to a semiviscous state and the residue was dissolved in 50% sulfuric acid. The acid solution, after being washed with ether, was made alkaline with 10% caustic potash solution and was extracted with chloroform. This gave 0.45% of combined alkaloid (on the dry weight of the raw material). The combined alkaloids (0.487 g) were separated chromatographically on a column of alumina which was washed with chloroform and with chloroform–methanol (10:0.1; 10:0.3; 10:0.5; 10:1; and 10:3). The chloroform–methanol (10:0.5 and 10:1) eluates yielded 0.038 g of lilidine with the composition $C_5H_7NO_2$. mp 118-119°C (acetone), $[\alpha]_D$ −26.3° (c 0.34; methanol), R_f 0.27 (TLC, Al_2O_3, chloroform–methanol (10:1)). Found, %: C 53.12; H 7.17; N 12.59. Calculated, %: C 53.09; H 6.19; N 12.39.

CONCLUSIONS

The new alkaloid lilidine, which has been isolated for the first time from the epigeal part of *Lilium martagon*, has been studied by spectral methods. The structure of 5-hydroxy-3-methyl-3-pyrrolin-2-one has been suggested for it.

LITERATURE CITED

1. Flora of the USSR [in Russian], Moscow–Leningrad, Vol. IV, (1935), p. 288.
2. G. Levy and G. Nelson, Carbon-13 NMR for Chemists (1st ed.), Wiley, New York (1972).
3. K. Tori, H. Ishii, Z. Wolkowski, C. Chachaty, M. Sangare, F. Piriou, and G. Lukacs, Tetrahedron Lett., 1077 (1973).
4. L. F. Johnson and W. C. Jankowski, Carbon-13 NMR Spectra, Wiley-Interscience, New York (1972).
5. L. A. Kazitsina and N. B. Kupletskaya, The Use of UV, IR, and NMR Spectroscopy in Organic Chemistry [in Russian], Moscow (1979).
6. D. H. Marr and J. B. Stothers, Can. J. Chem., 43, 596 (1965).
7. T. P. Antsupova and K. Samikov, The Study of Alkaloids of *Lilium martagon* [in Russian], Dep. TsBNTI, Med. Prom-st, No. 200, MP-84, dep.,(1986).