[Chem. Pharm. Bull. 24(10)2521—2524(1976)]

UDC 547.834.2.02.03 : 543.543.422.25

Constituents of *Rhizoma Nupharis*. XXVI.[1] Carbon-13 Nuclear Magnetic Resonance Spectra of Nupharamine, Anhydronupharamine, Nuphamine, and Related Compounds

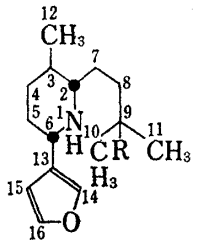
Yoshitaka Itatani, Shingo Yasuda, Miyoji Hanaoka, and Yoshio Arata

Faculty of Pharmaceutical Sciences, Kanazawa University[2]

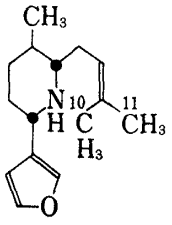
(Received February 2, 1976)

The ^{13}C nuclear magnetic resonance (^{13}C-NMR) spectra of the piperidine-type Nuphar alkaloids and their derivatives (I—V) were recorded with complete assignments. Two methyl groups of C-10 and C-11 in I and II were shown to be magnetically nonequivalent. The ^{13}C-NMR spectroscopy was demonstrated to be more useful than ^{1}H-NMR spectroscopy in studies on magnetic nonequivalence of dimethyl groups associated with molecular asymmetry. The shift values for some carbons caused by the introduction of the double bond, the hydroxyl group, and the acetyl group were examined.

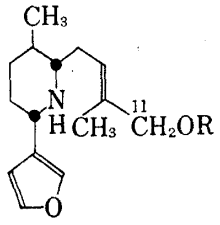
The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy has been developed explosively in organic chemistry[3] and the spectral data on a number of alkaloids have been also reported.[4] The recent report[5] on the ^{13}C-NMR spectra of the quinolizidine-type Nuphar alkaloids prompted us to publish those of the piperidine-type Nuphar alkaloids, nupharamine (I),[6,7] anhydronupharamine (III),[7,8] and nuphamine (IV),[7,9] and their derivatives, deoxynupharamine (II)[9] and O-acetylnuphamine (V).



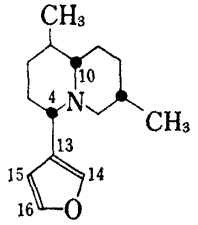
I: R=OH nupharamine
II: R=H deoxynupharamine



III: anhydronupharamine



IV: R=H nuphamine
V: R=$COCH_3$



VI: deoxynupharidine

Chart 1

The ^{13}C-signal assignments are based on the chemical shifts, on the multiplicities shown in the off-resonance decoupling and the single frequency off-resonance decoupling (SFORD)[3]

1) Part XXV: Y. Arata, S. Yasuda, and K. Yamanouchi, *Chem. Pharm. Bull.* (Tokyo), **16**, 2074 (1968).
2) Location: *Takara-machi, Kanazawa, 920, Japan.*
3) J.B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, 1972; G.C. Levy and G.L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists," Wiley-Interscience, New York, 1972.
4) E. Wenkert, J.S. Bindra, Ch. J. Chang, D.W. Cochran, and F.M. Schell, *Accounts Chem. Res.*, **7**, 46 (1974).
5) R.T. LaLonde, T.N. Donvito, and A.I.-M. Tsai, *Can. J. Chem.*, **53**, 1714 (1975).
6) T. Ohashi, *Yakugaku Zasshi*, **79**, 729 (1959); *idem, ibid.*, **79**, 734 (1959).
7) The originally reported absolute stereochemistry, 2*R*; 3*S*; 6*R*, of I, III, and IV should be revised to 2*S*; 3*R*; 6*S* as shown in Chart 1.
8) Y. Arata, T. Ohashi, M. Yonemitsu, and S. Yasuda, *Yakugaku Zasshi*, **87**, 1094 (1967).
9) Y. Arata and T. Ohashi, *Chem. Pharm. Bull.* (Tokyo), **13**, 1247 (1965); *idem, ibid.*, **13**, 1365 (1965).

spectra, on the partially relaxed Fourier transform (PRFT) spectra,[10] and on the spin-lattice relaxation time (T_1).[3,11] The results are listed in Table I.

The ^{13}C-NMR spectra of all the compounds examined were divided in three well defined regions. The low-field region, >100 ppm, contained the sp^2 carbons, the middle-field region, 50—70 ppm, contained the sp^3 carbons adjacent to nitrogen or oxygen, and the remaining sp^3 carbons were located in the high-field region, 10—40 ppm.

TABLE I. ^{13}C Chemical Shifts and Splitting of Compounds I, II, III, IV, and V[a,b]

Carbon No.	Compound I	II	III	IV	V
2	62.95 (d)	63.73 (d)	63.83 (d)	63.73 (d)	63.28 (d)
3	34.22 (d)	35.87 (d)	35.72 (d)	35.48 (d)	35.73 (d)
4	33.59[c] (t)	34.02[c] (t)	34.07[c] (t)	33.83[c] (t)	33.85[c] (t)
5	33.93[c] (t)	34.51[c] (t)	34.46[c] (t)	34.32[c] (t)	34.34[c] (t)
6	53.10 (d)	53.68 (d)	53.63 (d)	53.63 (d)	53.51 (d)
7	28.39 (t)	31.31 (t)	32.23 (t)	31.60 (t)	32.03 (t)
8	39.65 (t)	34.85 (t)	121.30 (d)	121.83 (d)	126.50 (d)
9	68.92 (s)	28.44 (d)	134.50 (s)	137.90 (s)	132.87 (s)
10	29.27 (q)	22.33 (q)	18.10 (q)	14.08 (q)	14.32 (q)
11	30.24 (q)	23.01 (q)	25.97 (q)	68.20 (t)	70.07 (t)
12	18.54 (q)	18.44 (q)	18.40 (q)	18.44 (q)	18.38 (q)
13	128.77 (s)	129.60 (s)	129.64 (s)	129.16 (s)	129.41 (s)
14	138.43 (d)	138.33 (d)	138.23 (d)	138.43 (d)	138.27 (d)
15	109.21 (d)	109.16 (d)	109.11 (d)	109.16 (d)	109.09 (d)
16	142.99 (d)	142.65 (d)	142.65 (d)	142.75 (d)	142.70 (d)
17	—	—	—	—	170.80 (s)
18	—	—	—	—	20.93 (q)

a) in ppm from TMS
b) s=singlet, d=doublet, t=triplet, q=quartet
c) Assignments may be reversed.

Low-field Region

The sp^2 carbons belong to the furan ring were assigned according to the data reported on deoxynupharidine (VI) and related Nuphar alkaloids.[12] Identification of the signals arising from the remaining sp^2 carbons, C-8 and C-9, in the spectra of III, IV, and V were straightforward on the basis of their splittings in the off-resonance decoupling spectra. The most deshielded signal at 170.80 ppm in the spectrum of V was due to the carbonyl carbon.

Middle-field Region

Assignments of the signals due to C-9 in I and C-11 in IV and V were unambiguous on the ground of their chemical shifts and splittings in the off-resonance decoupling spectra. The signals at 62.95 and 53.10 ppm in the spectrum of I would be assigned to C-2 and C-6, respectively, from the comparison of their chemical shifts with those reported on VI.[13] Corroboration for these assignments were obtained by measurement of the SFORD spectra; irradiation at H-6 (3.58 ppm) or H-2 (2.35 ppm) resulted in the selective coalescence of the doublet at 53.10 or 62.95 ppm. Accordingly the signals at *ca.* 63 and 53.5 ppm should be assigned to C-2 and C-6, respectively, in the spectra of II—V.

10) A. Allerhand and D. Doddrell, *J. Am. Chem. Soc.*, **93**, 2777 (1971).
11) G.C. Levy, *Accounts Chem. Res.*, **6**, 161 (1973).
12) The reported chemical shifts of C-13, C-14, C-15, and C-16 in VI are 130.40, 139.64, 109.93, and 143.08 ppm, respectively.[5]
13) The reported chemical shifts of C-10 and C-4 in VI are 69.75 and 60.33 ppm, respectively.[5]

High-field Region

Of the three quartets in the spectrum of I, two (due to C-10 and C-11) of them appeared at lower field, 29.27 and 30.24 ppm, than the other due to C-12 (18.54 ppm), because of the hydroxyl substitution effect.[3] Consequently the quartets resonated at *ca.* 18.5 ppm should be assigned to C-12 in the spectra of II—V.

The chemical shift nonequivalence of the two methyl carbons, C-10 and C-11, in I can be reasonably explained from the effect of molecular asymmetry.[14,15] This phenomenon was also observed in the ^{1}H-NMR spectrum of I; two methyl groups resonated at 1.17 and 1.19 ppm as two singlets. On the other hand, two methyl carbons of C-10 and C-11 resonated at 22.33 and 23.01 ppm in the ^{13}C-NMR spectrum of II, however, their magnetic nonequivalence could not be detected in the ^{1}H-NMR spectrum of II; all three methyl signals including C-12 appeared at 0.90 ppm as a doublet (J=6 Hz). Thus, these results demonstrated the utility of the ^{13}C-NMR spectroscopy in comparison with the ^{1}H-NMR spectroscopy in studies on magnetic nonequivalence of methyl groups associated with molecular asymmetry.

It is obvious to assign the doublet at 34.22 ppm to C-3 in the spectrum of I. Among the four methylene resonances the most deshielded signal (39.65 ppm) and shielded signal (28.39 ppm) were attributed to C-8 and C-7, respectively, on account of the β- and γ-effect of the hydroxyl group.[3] The signals at 33.93 and 33.59 ppm could be assigned to C-5 and C-4, respectively, by considering the β-effect of the amino group coupled with the data reported on the piperidine derivatives,[3,4,16] but the assignments may be reversed owing to their similar chemical shifts.

Two doublets at 35.87 and 28.44 ppm were easily identified to C-3 and C-9, respectively, in the spectrum of II. Measurement of T_1 and the PRFT spectra of II led to distinguish the triplet due to C-8 from other three triplets; the signal at 34.85 ppm was shown to have longer T_1[10] (2.88 sec) than the remaining triplets (*ca.* 1.5 sec). The similarity in the chemical shift between C-4 and C-5 as observed in I permitted the assignments of the signals at 34.02, 34.51, and 31.31 ppm to C-4, C-5, and C-7, respectively, in the spectrum of II.

A great difference in the chemical shift between the methyl carbons of C-10 and C-11 in the spectrum of III can be easily accounted for by their *cis-trans* geometry.[3] The *cis* methyl carbon of C-10 was further confirmed from its long T_1[17] (7.21 sec) relative to that for C-11 or C-12 (2.88 or 2.16 sec). The shielding effects of the olefinic bond observed for C-7, C-10, and C-11 on transforming II to III were +0.92, −4.58,[18] and +3.30 ppm,[18] respectively, which are in good accordance with the shift values reported on trisubstituted olefins.[19]

The shift values for C-8, C-9, C-10, and C-11 caused by the introduction of the hydroxyl group at C-11 in III were +0.53, +3.40, −4.02, and +42.23 ppm, respectively. On the other hand, the acetylation shifts observed for C-8, C-9, C-10, and C-11 were found to be +4.67, −5.03, +0.24, and +1.87 ppm, respectively, on transforming IV to V. The γ-effect of the hydroxyl substituent on sp^3 carbon was shown to be more pronounced than that on sp^2 carbon in this trisubstituted olefin, on the contrary, acetylation exerted much more γ-effect on sp^2 carbon than that on sp^3 carbon.

Thus, the ^{13}C-NMR spectra of the piperidine-type Nuphar alkaloids and their derivatives (I—V) were completely assigned. These results will provide the valuable data for the biosynthetic studies on Nuphar alkaloids using the ^{13}C-labelling method and further for the structural studies on the related alkaloids and terpenes.

14) W.B. Jennings, *Chem. Rev.*, **75**, 307 (1975).
15) J.I. Kroschwitz, M. Winokur, H.J. Reich, and J.D. Roberts, *J. Am. Chem. Soc.*, **91**, 5927 (1969).
16) F. Bohlmann and R. Zeisberg, *Chem. Ber.*, **108**, 1043 (1975); G. Ellis and R.G. Jones, *J. Chem. Soc. Perkin Trans.* II, **1972**, 437; A.J. Jones and M.M.A. Hassan, *J. Org. Chem.*, **37**, 2332 (1972).
17) G.C. Levy and G.L. Nelson, *J. Am. Chem. Soc.*, **94**, 4897 (1972).
18) Calculated from the average value, 22.67 ppm, for C-10 and C-11 in II.
19) The corresponding shift values were reported to be +1.1, −4.6, and +3.4 ppm: D.E. Dorman, M. Jantelat, and J.D. Roberts, *J. Org. Chem.*, **36**, 2757 (1971).

Experimental

Melting point was measured with a Yanagimoto Micro Melting Point Apparatus. Melting point and boiling point are uncorrected. IR spectrum was measured with a JASCO-IR-G, ^{1}H-NMR spectra in $CDCl_3$ with a JEOL-PS-100, using tetramethylsilane (TMS) as an internal standard, mass spectrum with a JEOL-JMS-01SG.

Materials——The compounds (I—IV) were available from the previous works[6-9] and freshly purified. The ^{1}H-NMR spectra of I and II were as follows; I δ: 0.90 (3H, d, J=6 Hz, $C\underline{H}_3CH$), 1.17, 1.19 (each 3H, s, $(C\underline{H}_3)_2COH$), 2.35 (1H, W_H=16 Hz, C_2–H), 3.58 (1H, d–d, J=10; 2 Hz, C_6–H); II δ: 0.90 (3×3H, d, J=6 Hz, $3\times C\underline{H}_3CH$), 2.24 (1H, W_H=16 Hz, C_2–H), 3.62 (1H, d–d, J=11; 2 Hz, C_6–H).

O-Acetylnuphamine (V)——A solution of IV (770 mg) in acetic acid (10 ml) was heated at 115—120° for 3 hr and evaporated *in vacuo*. The residue was made alkaline with aq. K_2CO_3 solution and extracted with $CHCl_3$. The extract was washed with H_2O, dried over Na_2SO_4, and evaporated. The residue was purified with column chromatography (alumina, Brockmann grade II—III, Merck, $CHCl_3$) to give V (317 mg, 35.2%) as a pale yellow oil, bp 145—150° (bath temp.)/0.07 mmHg. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725 (C=O), 870 (furan). NMR δ: 0.92 (3H, d, J=6 Hz, $C\underline{H}_3CH$), 1.70 (3H, s, CH_3–C=), 2.05 (3H, s, CH_3–CO), 3.60 (1H, d–d, J=10; 2.5 Hz, C_6–H), 4.46 (2H, s, $AcOCH_2$). Mass Spectrum m/e: 291 (M^+), 164 (base peak). Picrolonate: yellow needles, mp 179—180° (EtOH). *Anal.* Calcd. for $C_{27}H_{33}O_8N_5$: C, 58.37; H, 5.99; N, 12.61. Found: C, 58.20; H, 5.85; N, 12.38.

^{13}C-NMR Spectra——The spectra were measured with a JEOL PS-100/PFT-100/EC-100 FT-NMR spectrometer at 25.15 MHz at 23°. The samples were 0.3—0.5 M solutions in $CDCl_3$ (1.5 ml) in 10 mm o.d. tubes. The chemical shifts were expressed in ppm downfield from TMS as an internal standard and are accurate to $\pm$0.1 ppm. The FT-NMR measurement conditions were as follows; spectral width: 5000 Hz, pulse width: 24 μsec (90° pulse) and 12 μsec (45° pulse), acquisition time: 3 sec, number of time-domain data points: 8191, and number of transients; 1500—2000. T_1's were obtained using the inversion-recovery method with the pulse sequence, $(180°-\tau-90°)_n$.

Acknowledgement The authors are grateful to Miss H. Hyuga of this Faculty for mass spectral measurement.