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The Solution Conformation of Nicotine. A ^1H and ^2H Nuclear Magnetic Resonance Investigation

T. Phil Pitner,* William B. Edwards, III,* Ronald L. Bassfield,* and Jerry F. Whidby*

Contribution from the Philip Morris U.S.A. Research Center, P.O. Box 26583, Richmond, Virginia 23261. *Received June 29, 1977*

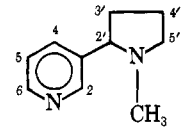
Abstract: The ^1H NMR spectrum of nicotine (**1**) is analyzed in detail. Selectively deuterated nicotine analogues afford considerable simplification of the pyrrolidine ^1H resonances by allowing partition of this seven-spin system into three-, four- and five-spin systems. In addition, the ^2H NMR chemical shifts of these analogues provide a means of assigning ^1H NMR chemical shifts to specific protons unambiguously. The vicinal coupling constants of the pyrrolidine ring protons suggest an envelope conformation for the five-membered ring, in which the methyl and pyridine moieties assume equatorial positions. A perpendicular spatial orientation of the pyridine and pyrrolidine rings is supported by two observations: a small long-range coupling constant (<0.05 Hz) between H(2′) and H(6), and nuclear Overhauser enhancements of 9 ± 2 and 5 ± 2% for the H(2′) resonances upon saturation of H(2) and H(4), respectively.

Introduction

Interest in describing the interactions between nicotine (**1**) and its receptors at the molecular level has prompted numerous studies concerned with facets of the conformation of nicotine.[1-9] Analogies have been drawn[4,6] between the conformational and electronic properties of acetylcholine, the naturally occurring compound which allows communication between nerve cells, and cholinergic agonists such as nicotine which also interact with nicotinic receptors. It is therefore desirable to obtain a more detailed conformational picture of nicotine to aid in the interpretation of its physiological activity in terms of structure–function relationships.

Conformational investigations of nicotine are centered on three basic aspects:

(1) Pyramidal inversion of the pyrrolidine nitrogen results in cis–trans isomerism of the methyl and pyridine moieties about the N(1′)–C(2′) bond. An earlier report[8] which suggested that the cis isomer predominated in solution was disproved in a recent study,[9] which concludes that the trans isomer is favored by 10:1. Molecular orbital calculations[6] on nicotine



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as well as x-ray crystallographic studies[1] on the dihydriodide also indicate a preference for the trans isomer.

(2) The rotational disposition of the pyridine and pyrrolidine rings about the C(2′)–C(3) bond has been the subject of x-ray studies, molecular orbital calculations,[3,6] and circular dichroism (CD) measurements.[7] These investigations suggest that the conformation is most favored in which the C(2′)–H(2′) bond and pyridine ring are coplanar, making the two rings perpendicular. Although the molecular orbital calculations and CD measurements indicate little preference for the rotamer with H(2′) and H(2) either syn or anti, nicotine dihydriodide crystallizes in the former conformation.

(3) A detailed analysis of the pyrrolidine ring geometry in solution has not as yet been presented. NMR paramagnetic

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shift measurements[5] have suggested an envelope conformation for the pyrrolidine ring when nicotine is complexed with shift reagents.

It is on the latter two conformational aspects that the present study is focused. The high-resolution ^1H NMR spectrum of nicotine is analyzed in detail, and the spectral parameters thus obtained are interpreted in terms of conformational preferences.

Experimental Section

General. ^1H (100 MHz) and ^2H (15.4 MHz) NMR spectra were obtained with a Varian XL-100 equipped with the Gyrocode Observe option and operating in the pulse-Fourier transform mode. The spectrometer utilizes a Digilab data system and pulse programmer. Sample concentrations were 0.206 (±0.2%) *m* (5-mm tubes) in a solvent mixture composed of 2 parts $CDCl_3$ and 1 part $CFCl_3$ by volume; the solvent was chosen to allow maximum internal lock flexibility for observing both ^1H and ^2H (50 transients) NMR spectra on the same sample. ^1H NMR spectra are referenced to internal tetramethylsilane. The deuterium resonance of $CDCl_3$ serves as a secondary chemical shift reference in ^2H spectra; its shift relative to natural abundance Me_4Si (7.21 ppm) was determined in a separate measurement. Samples used for nuclear Overhauser effect[10] measurements were degassed by five freeze–pump–thaw cycles.

Spectra were analyzed with the LAOCOON-3 computer program. The maximum standard deviations in the chemical shifts and coupling constants of fitted spectra were 0.06 Hz for the pyrrolidine protons and 0.04 Hz for the pyridine protons.

Melting points (Thomas-Hoover apparatus) are uncorrected. GLC was carried out using a Bendix Model 2300 instrument with 5 ft × 0.25 in. stainless steel columns packed with 5% SE-30 on Chromosorb G-HP (80–100 mesh) with He carrier gas at 60 mL/min flow rate. Low-resolution MS were obtained on a CEC 21-104 at 70 eV, 10 μA, 2000 V ion accelerating voltage and a source temperature of 250 °C.

General Syntheses. The deuterated analogues were found to have >90 atom % enrichment by NMR and MS, and purity of >98% by GLC. The following deuterated analogues were prepared by established methods.[11] The prerequisite starting alkaloids were available from other studies.[11b] The nicotine-*2'*-d_1 (90%) and -*3',3'*-d_2 (86%) were prepared by HCO_2H/H_2CO methylation of the corresponding nornicotine-*2'*-d_1 and -*3',3'*-d_2. The former was synthesized (50%) by $NaBD_4$ reduction of myosmine. The latter was obtained (49%) by $NaBH_4$ reduction of myosmine-*3',3'*-d_2 (available from myosime (98%) by CF_3CO_2D-catalyzed exchange[11b]). The reduction of cotinine and cotinine-*4',4'*-d_2 (obtained (90%) from cotinine via base-catalyzed exchange) with $LiAlD_4$ and $LiAlH_4$, respectively, afforded nicotine-*5',5'*-d_2 (28%) and -*4',4'*-d_2 (20%). The myosmine-*3',3'*-d_2 and cotinine-*4',4'*-d_2, as initially isolated, were of sufficient purity for subsequent reaction. No purification of the nicotine-*2'*-d_1 and -*3',3'*-d_2 was necessary. The remaining analogues were purified by the preparative TLC procedure given below.

Nicotine-*N'*-d_3. To a cooled (ca. 5 °C), stirring solution of 1.55 g (3.2 mmol) of DCO_2D and 1.71 g (1.6 mmol) of 30% D_2CO in 2 mL of D_2O under a N_2 atmosphere in heat gun dried glassware was added 0.95 g (0.64 mmol) of nornicotine in 1 mL of D_2O. The reaction solution was heated under reflux for 16 h, basified (pH 11) at ca. 5 °C with 50% NaOH, and extracted with Et_2O (4 × 3 mL). The Et_2O was dried and removed [20 °C (15 mm)] to afford 1.02 g of a crude oil; GLC showed ca. 94% product. Preparative TLC gave 0.39 g (37%) of spectroscopically pure nicotine-*N'*-d_3,[11a] GLC purity >99%.

Cotinine-*2'*-d_1.[12] To a rapidly stirring solution of 2.6 g (1.6 mmol) of nicotine-*2'*-d_1 in 15 mL of 80% aqueous CH_3CO_2H (v/v) under a N_2 atmosphere was added over ca. 0.4 h 18.1 g (11.3 mmol) of Br_2 in 20 mL of 80% aqueous CH_3CO_2H. The resulting dark red, two-phase mixture was heated with rapid stirring until the phases became miscible (ca. 80 °C), and allowed to cool slowly to room temperature without stirring and to stand overnight. During this period, an oil separated which crystallized on standing. The reddish-orange crystals were collected by filtration. The majority of the solvent was removed by aspiration and the solid was air dried overnight to yield 7.62 g (83%) of 4',4'-dibromocotinine-*2'*-d_1 hydrobromide perbromide, mp 134–140 °C.

To a rapidly stirring slurry of 7.5 g (1.3 mmol) of 4',4'-dibromocotinine-*2'*-d_1 hydrobromide perbromide in a solution of 2.5 mL of concentrated HCl and 25 mL of 50% aqueous acetic acid (v/v) under a N_2 atmosphere was added 2.5 g (11.5 mmol) of Zn dust in portions over ca. 0.4 h. After stirring for 1 h, the mixture was filtered through a pad of Celite to remove the excess Zn and the Zn/Celite was washed with 40–60 mL of H_2O. The combined aqueous filtrates were basified at ca. 10 °C with concentrated NH_4OH to ph 8–9. The solution was extracted with $CHCl_3$ (4 × 50 mL). The $CHCl_3$ was dried (Na_2SO_4) and removed under reduced pressure to yield 2.28 g (99%) of cotinine-*2'*-d_1 as a yellowish oil, GLC purity >98%.

Nicotine-*2',5',5'*-d_3[13] was synthesized in 23% yield by reduction of cotinine-*2'*-d_1 with $LiAlD_4$, using an established procedure,[11b] GLC purity >98%.

Preparative TLC Purification Procedure. Preparative TLC was carried out on 20 × 20 cm Analtech, Inc., 1000-μ silica gel GF plates. Streaking was done with a Kontes' Chromaflex streaker (K-416430) in CH_2Cl_2. The approximate loading levels were 200 mg per plate. Plates were developed (ca. 3 h) using $CHCl_3$–EtOH–NH_4OH (85:14:1) in Kontes' Chromaflex developing tanks (K-416150) with no more than two plates per tank. Plates were removed, the solvent was allowed to evaporate in air (ca. 1 h), and the silica gel band containing the desired compound was scraped off. The silica gel was treated with 10% HCl (ca. 10 mL per plate used) and stirred for 1–2 h. The silica gel was removed by filtration and washed twice with 10% HCl. The combined acid filtrates were cooled (<10 °C), basified with 50% NaOH (pH 11), and extracted with Et_2O. The Et_2O was dried and removed to give the desired compound. Traces of solvent were removed by pumping under vacuum (0.1 mm) for 3–6 h (nornicotines) and 0.5–1.5 h (nicotines).

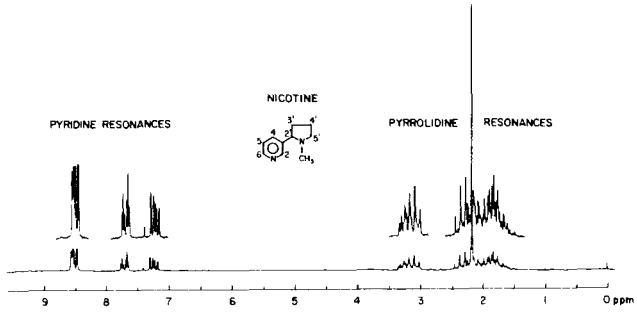


Figure 1. ^1H NMR spectrum of nicotine.

Table I. Pyridine Chemical Shifts and Coupling Constants of Nicotine

Chemical shifts, ppm			Coupling constants, Hz			
			2	4	5	6
2	8.540	2'	−0.51	−0.45	0.37	<0.05
4	7.683	2	*a*	2.27	0.89	0.0
5	7.218	4	*a*	*a*	7.86	1.70
6	8.475	5	*a*	*a*	*a*	4.79

[a] Indicates table redundancy or same proton.

Results

The ^1H NMR spectrum of nicotine is shown in Figure 1. Assignment of the pyridine resonances to specific protons has been accomplished previously.[14] Because of the first-order appearance of the aromatic resonances, it is straightforward to obtain a set of starting chemical shifts and coupling constants for the LAOCOON spectral analysis. Since H(2') couples significantly to some of the pyridine protons, it is included in the analysis of this region of the spectrum. The resonances of H(6) are broadened slightly (line width 0.7 Hz) owing to scalar coupling with the rapidly relaxing pyridine ^{14}N nucleus. In spite of this broadening, $J_{2',6}$ can be measured as less than 0.05 Hz, since only a slight narrowing (<0.05 Hz) of the H(6) peaks occurs when H(2') is decoupled. Final iterated parameters are given in Table I.

Assignment of the pyrrolidine resonances is not so straightforward; extreme spectral overlap in this seven-spin

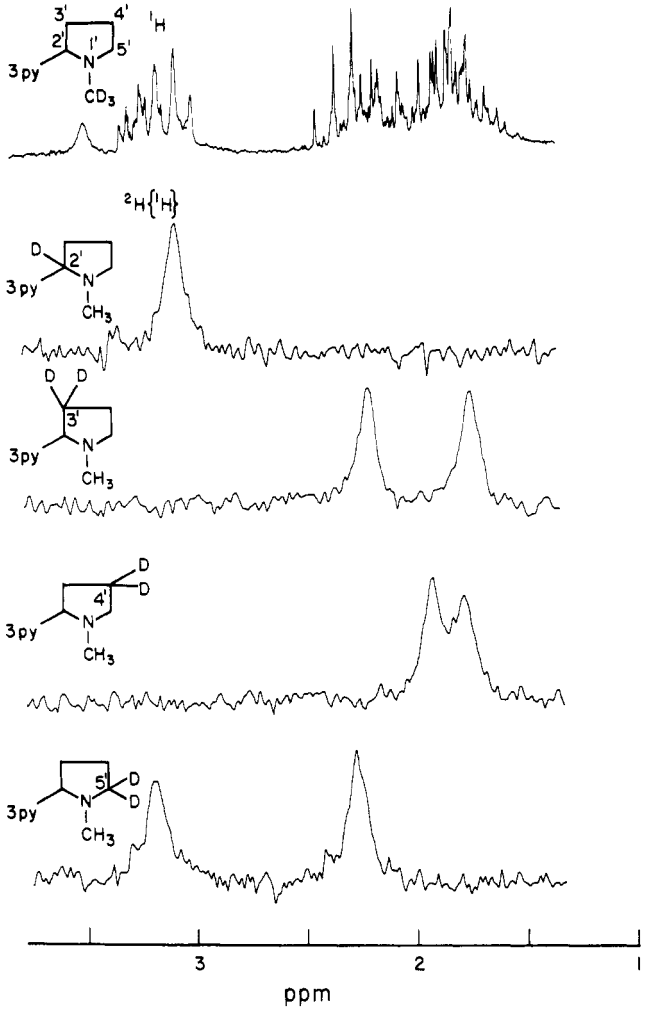


Figure 2. ^{1}H pyrrolidine resonances of nicotine-*N'*-d_3. ^{2}H {^{1}H} NMR spectrum of nicotine-*2'*-d_1, nicotine-*3',3'*-d_2, nicotine-*4',5'*-d_2, nicotine-*5',5'*-d_2. 3-py indicates a pyridine ring substituted at the 3 position.

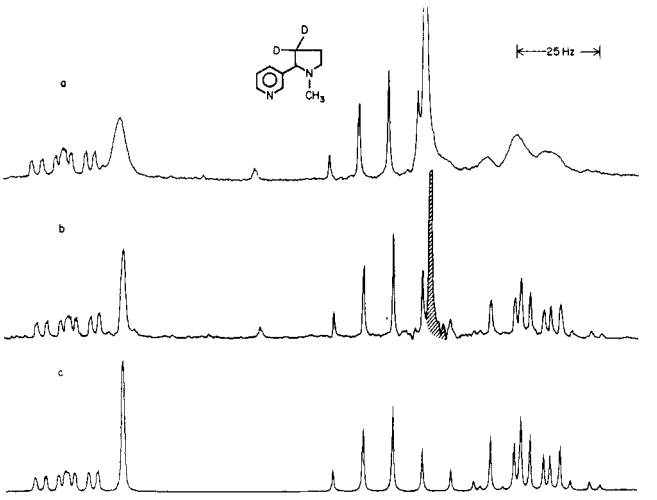


Figure 3. (a) ^{1}H NMR spectrum of the pyrrolidine resonances of nicotine-*3',3'*-d_2. (b) ^{1}H {^{2}H} spectrum. (c) Computer-fitted spectrum.

Table II. Pyrrolidine Chemical Shifts (ppm) of Nicotine

		^{1}H		
	^{2}H (±0.02)	Nicotine-*3',3'*-d_2	Nicotine-*4',4'*-d_2	Nicotine-*2',5',5'*-d_3
2′	3.07	3.084	3.095	
3′$_a$[a]	1.73		1.719	1.723
3′$_b$	2.21		2.189	2.194
4′$_a$	1.80	1.806		1.804
4′$_b$	1.95	1.952		1.955
5′$_a$	2.31	2.316	2.307	
5′$_b$	3.25	3.248	3.247	

[a] Subscripts a and b refer respectively to high-field and low-field chemical shifts of protons attached to a specific carbon.

system makes decoupling experiments difficult and chemical shift measurements nearly impossible. To simplify the complex pyrrolidine spectrum, selectively deuterated nicotine analogues were synthesized. The proton-decoupled ^{2}H NMR[15] spectra (Figure 2, Table II) of these analogues afford excellent estimates of corresponding proton chemical shifts, since the isotope effect on ^{2}H NMR shifts should be very small.[16] In addition, these analogues allow considerable simplification of the ^{1}H resonances; with the aid of ^{2}H decoupling, a complex seven-spin system is reduced to simpler three-, four-, and five-spin systems. Figure 3 illustrates this for nicotine-*3',3'*-d_2; broadening due to ^{2}H coupling (Figure 3a) is removed by irradiating the deuterium resonances (Figure 3b). Figure 3c shows the spectrum generated using the computer fit parameters shown in Tables II and III. In addition to providing starting values for the ^{1}H iterative procedure, the ^{2}H chemical shifts also allow unambiguous assignment of ^{1}H resonances to protons on specific carbons.

A similar approach is taken for nicotine-*4',4'*-d_2 and for nicotine-*2',5',5'*-d_3 (Figures 4 and 5 and Tables II and III). Small differences in chemical shift for corresponding protons in these three compounds probably result, at least in part, from isotope effects due to deuterium substitution on adjacent carbons. Presented in Figure 6 is the observed spectrum of nicotine-*N'*-d_3 together with a computer-generated spectrum, calculated using average chemical shifts from Table II and coupling constants from Table III. Excellent agreement is obtained between observed and calculated spectra. The additional broadening of H(2′) resonances results from coupling with pyridine protons discussed earlier.

Discussion

Pyrrolidine Ring Conformation. When one is dealing with a molecule in which there is significant internal motional freedom such as nicotine, it is important to bear in mind that NMR spectral parameters reflect a weighted time average of all conformers present. However, in many cases, steric constraints can cause the distribution of conformers to be weighted strongly in favor of energetically more stable orientations. The approach taken here is to identify these more stable conformations of nicotine.

The functional dependence of vicinal proton coupling constants (J_{vic}) on the H-C-C-H dihedral angle (ϕ) as described by the Karplus relationship[17,18] is well documented[19-21] and has been used widely in conformational studies.

$$J_{vic} = A_1 \cos^2 \phi + B \quad (0° < \phi < 90°)$$

$$J_{vic} = A_2 \cos^2 \phi + B \quad (90° < \phi < 180°)$$

Although exact values of the Karplus parameters A_1, A_2, and B depend on the detailed structure of the compound under investigation, and may vary depending on the specific H-C-C-H fragment being considered, an empirical set of parameters for all fragments can be determined if acceptable error limits are placed on the dihedral or torsional angles to be calculated. Such an approach has been taken for the five-

Table III. Pyrrolidine Coupling Constants (Hz) of Nicotine

	$3'_a$	$3'_b$	$4'_a$	$4'_b$	$5'_a$	$5'_b$
2′	8.99	7.96	*a*	*a*	*a*	0.50[c]
$3'_a$	*b*	−12.75[d]	5.68	10.92	*a*	*a*
		−12.79[e]				
$3'_b$	*b*	*b*	9.67	5.43	*a*	*a*
$4'_a$	*b*	*b*	*b*	−12.40[c]	8.30	2.19
				−12.55[e]		
$4'_b$	*b*	*b*	*b*	*b*	9.68	7.93
$5'_a$	*b*	*b*	*b*	*b*	*b*	−9.17[c]
						−9.14[d]

[a] Indicates long-range coupling constants not resolvable. [b] Indicates table redundancy or same proton. [c] Measured for nicotine-3′,3′-d_2. [d] Measured for nicotine-4′,4′-d_2. [e] Measured for nicotine-2′,5′,5′-d_3.

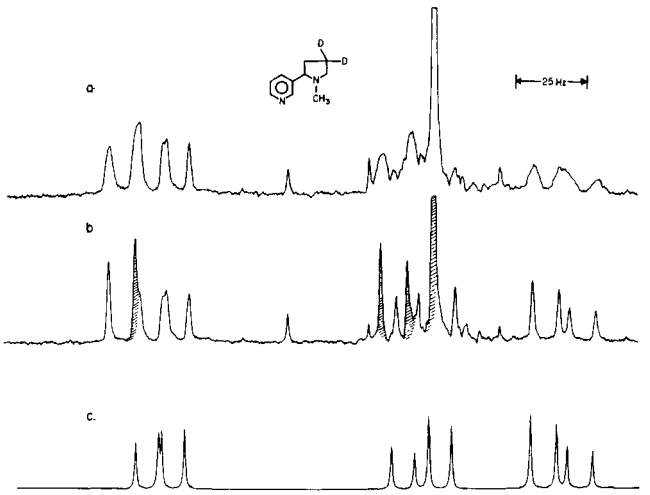


Figure 4. (a) ^1H NMR spectrum of the pyrrolidine resonances of nicotine-4′,4′-d_2. (b) ^1H $\{^2H\}$ spectrum. (c) Computer-fitted spectrum.

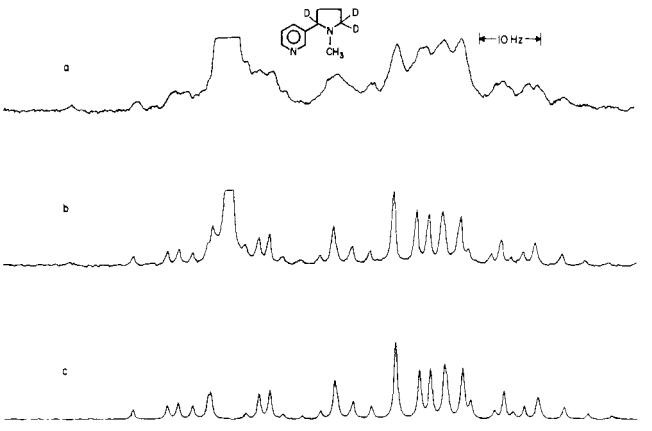


Figure 5. (a) ^1H NMR spectrum of the pyrrolidine resonances of nicotine-2′,5′,5′-d_3. (b) ^1H $\{^2H\}$ spectrum. (c) Computer-fitted spectrum.

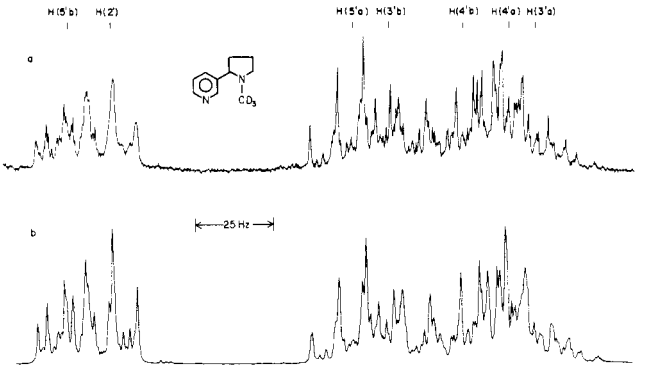
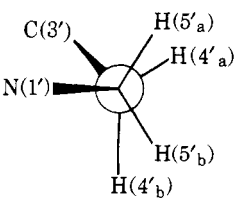


Figure 6. (a) ^1H NMR spectrum of the pyrrolidine resonances of nicotine-N′-d_3 showing the resonance position of each pyrrolidine proton. (b) Computer-simulated spectrum.

membered rings in poly(L-proline) II[22] and in specific proline derivatives.[23,24] For nicotine the set of empirically determined parameters, $A_1 = 7.8$, A_2, 10.5, and $B = 2$, yields consistent results for all coupling constants except one that is anomalously large. These values are similar to those obtained in the studies of proline derivatives ($A_1 = 8.5$, $A_2 = 10.5$, $B = 1.4$). Employing the appropriate set of parameters, the Karplus equation can now be used to describe the pyrrolidine ring conformation in terms of torsional angles (χ) calculated from vicinal coupling constants. (χ is defined as the angle of rotation about a carbon–carbon bond viewed end on with the lower numbered ring carbon nearest the viewer and the hydrogens eclipsed; a clockwise rotation of the carbon farthest away corresponding to a positive χ.)

As a starting point in the conformational analysis, consider the orientation of the C(5′) and C(4′) hydrogens (refer to Figure 7). The high-field 5′ resonance ($5'_a$) is assigned to the proton trans to the nitrogen lone pair, because of the trans shielding effect[25-28] of this lone pair. The proton cis to the long pair ($5'_b$) experiences couplings of 2.19 and 7.93 Hz to the vicinal 4′ protons. The Karplus equation yields dihedral angles of 98° (81° if $\phi < 90°$) for the $4'_a5'_b$ sequence and 29° for the $4'_b5'_b$ sequence. The fact that these two angles do not add exactly to 120° reflects the inability of the empirical Karplus parameters to predict angles exactly. In addition, the presence of minor isomers in rapid equilibrium (such as the 9% isomer with methyl and pyridine groups cis) may introduce further uncertainty in the calculated dihedral angles; determination of the conformation of minor isomers present at this level is precluded by the approximate nature of the Karplus parameters. However, it is clear that a significant fraction of the gauche isomer must be present in which $J_{5'b4'a}$ is small ($\phi \sim$ 90°), so that a small average coupling constant of 2.19 Hz can be obtained. This line of reasoning is also supported by the $5'_a$



coupling constants $J_{5'a4'a} = 8.30$ and $J_{5'a4'b} = 9.68$ Hz. Neither of these couplings would be so large if significant conformations were present in which either dihedral angle were near 90°. A direct consequence of the axial orientation of the $5'_a$ proton is that the adjacent cis N′-methyl group is equatorial as might be expected. In addition, the coupling constants to $5'_b$ allow assignment of the 4′ protons as indicated in Figure 7. The four

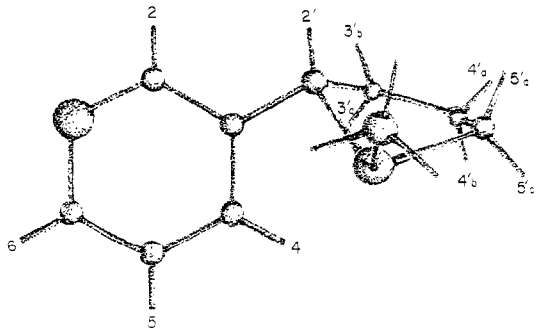


Figure 7. The conformation of nicotine in which H(2') and H(2) are syn.

vicinal couplings between the 4' and 5' protons allow an average $\chi_{4'5'}$ of $-27 \pm 3°$ to be calculated.

Consider next the coupling interactions for the 3' and 4' protons. Each of these protons exhibits a similar pair of coupling constants to protons on the other carbon, one near 5.5 Hz and the other near 10 Hz. This symmetry suggests an eclipsed orientation about the C(3')-C(4') bond. Significant fractions of the gauche isomers would prohibit large J values. It is these large coupling constants, consistent with dihedral angles near 0°, that indicate the assignments shown in Figure 7 for the 3' protons. Although the $3'_a4'_b$ coupling constant of 10.92 Hz is too large to permit calculation of dihedral angles with the present set of Karplus parameters, the other three dihedral angles yield an average $\chi_{3'4'}$ of $0 \pm 7°$. The inequality of $J_{3'a4'b}$ and $J_{3'b4'a}$ (10.92 vs. 9.68 Hz), despite the fact that the corresponding dihedral angles would be equal for sp^3-hybridized carbons, underscores the approximate nature of the Karplus relationship.

The large values of $J_{2'3'a}$ and $J_{2'3'b}$ suggest the axial orientation shown in Figure 7 for the 2' proton. This in turn predicts an equatorial position for the pyridine ring as expected from steric constraints. The average value of $\chi_{2'3'}$ calculated from these coupling constants is $27 \pm 3°$.

Spatial Relationship of Pyridine and Pyrrolidine Rings. The conformational dependence of long-range coupling of exocyclic protons to aromatic protons has been calculated theoretically.[29,30] Coupling constants of the aromatic protons of toluene to a methyl proton are shown at several dihedral angles in Table IV. The numbering system is chosen for analogy with the pyridine ring and C(2')-H(2') fragment of nicotine; the dihedral angle θ refers to the H(2')-C(2')-C(3)-C(2) fragment. Included in Table IV are corresponding coupling constants measured for nicotine. Although exact values calculated for the pyridine ring could be different than those for the phenyl ring, the same general trends should be observed.[31] It is clear from Wasylishen and Schaefer's data abstracted in Table IV that the best agreement is obtained at approximately 0 and 180°. If the calculated coupling constants for these two isomers are averaged, as would occur with rapid interconversion due to ring rotation, then the agreement with observed coupling constants becomes even better. Especially diagnostic of the dihedral angle is the 2',6 coupling. In this case the principal mechanism for scalar coupling is provided by the π electron system, so that the analogy with toluene is closest (the σ contribution to long-range coupling is modified by insertion of the heteroatom, whereas the π contribution remains mainly unchanged[30,32]). At $\theta = 0$ and 180°, H(2') lies in a nodal plane of the π orbital, and the coupling interaction is a minimum. Thus the $J_{2',6}$ value of <0.05 Hz gives strong support for coplanarity of the C(2')-H(2') bond and the pyridine ring.

Nuclear Overhauser effect measurements confirm these orientations. Saturation of the H(2) and H(4) resonances of nicotine results in enhancements of 9 ± 2 and $5 \pm 2\%$, respectively, for the H(2') resonance indicating that H(2) and H(4) contribute significantly to the dipolar relaxation of H(2'). These measurements, when repeated in $CDCl_3$ solution, yield corresponding enhancements of 18 ± 3 and $7 \pm 2\%$, suggesting that the Overhauser effects measured in the mixed solvent may be attenuated as a result of intermolecular dipolar relaxation of H(2') by the ^{19}F nuclei of $CFCl_3$. Because of the inverse sixth-power dependence of dipolar relaxation on internuclear distance,[10] H(2) and H(4) must spend a large fraction of time adjacent to H(2'), otherwise relaxation of H(2') would be dominated by proximate protons on the pyrrolidine ring.

From these results it cannot be determined whether the isomer shown in Figure 7 or the one with $\theta \simeq 180°$ predominates, because slight deviations from the perpendicular arrangement of the two rings can change the relative extent of relaxation of H(2') by H(2) or H(4), thereby causing an ambiguous biasing of the Overhauser enhancements. Further, the general difficulty in interpreting NOE results obtained from conformationally mobile systems[10] precludes detailed interpretation of these data.

Table IV. Comparison of Calculated and Observed Long-Range Coupling Constants (Hz)

Coupling constant	Calcd[a] θ	0°	90°	180°	Obsd[b]
2',2[c]		−0.34	−1.47	−0.58	−0.51
2',4		−0.58	−1.47	−0.34	−0.45
2',5		0.83	0.89	0.23	0.37
2',6		−0.08	−1.22	−0.08	<0.05

[a] Calculated for a methyl proton in toluene.[29] [b] Observed for nicotine. [c] Numbering system chosen for analogy with nicotine.

Conclusion

The conformational features of the predominant isomer of nicotine in solution as determined by NMR are illustrated in Figure 7. Although exact torsional and dihedral angles for the pyrrolidine ring cannot be calculated unless Karplus parameters are available for each vicinal pair of spins, the general conformational aspects shown are supported strongly by the NMR data. The vicinal coupling constants require axial orientations for the 2' and 5'a protons and eclipsed orientations for the 3' and 4' protons. Taken together, these constraints indicate an envelope conformation for the pyrrolidine ring similar to that observed for nicotine dihydriodide by x-ray crystallography.[1] The long-range coupling constants and Overhauser enhancements require an approximately perpendicular spatial orientation of the pyridine and pyrrolidine rings, consistent with x-ray crystallographic studies,[1] molecular orbital calculations,[6] and circular dichroism measurements;[7] however, other methods will be needed to determine which of the ring orientations, $\theta \simeq 0$ or 180°, is preferred. It is the latter orientation with which an analogy has been drawn with acetylcholine and with which physiological activity has been associated. Since the nicotinium ion rather than the free base is implicated in the cholinergic activity of nicotine, further study is needed to determine the effect of protonating the pyrrolidine nitrogen on the conformational details of nicotine. The selectively deuterated nicotine analogues should aid greatly in such investigations since, as has been demonstrated here, selectively labeled compounds allow extraction of conformational data for molecules of biological interest, such as nicotine, whose NMR spectra would be hopelessly complex otherwise.

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An Electron Spin Resonance Investigation on Intermediates and Products in the Basic Oxidation of Nitrodiphenylethylenes

M. Barzaghi, P. L. Beltrame, A. Gamba,[1] and M. Simonetta*

Contribution from the C.N.R. Center for the Study of Structure/Reactivity Relations and Institute of Physical Chemistry, University of Milan, 20133 Milan, Italy.

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Abstract: The anion radicals of 1,1-di(*p*-nitrophenyl)ethylene, 2-ethoxy-1,1-di(*p*-nitrophenyl)ethylene, 1-*p*-nitrophenyl-1-phenylethylene, *cis*- and *trans*-2-bromo-1-*p*-nitrophenyl-1-phenylethylene, 4,4′-dinitrobiphenyl, *p*-*tert*-butoxynitrobenzene, *p*-nitrobenzophenone, *p*-nitrosobenzophenone, *p*-azobenzophenone, 4,4′-dinitrobenzophenone, and *p*-nitrobenzoic acid have been studied by ESR spectroscopy. These radicals are intermediates and products in the basic oxidation of 1,1-di(*p*-nitrophenyl)ethylene and/or 1-*p*-nitrophenyl-1-phenylethylene. Hyperfine splitting constants have been assigned on the basis of MO theories. Intramolecular exchange and relaxation broadenings of ESR lines have been investigated to get information on solution dynamics of these compounds.

Introduction

In a previous work[2] the anion radicals of a series of mononitro derivatives of 1,1-diphenylethylene have been studied by ESR spectroscopy. These radicals were obtained by electrolytic reduction in dimethyl sulfoxide (Me_2SO) and acetonitrile (ACN). Information on the spin densities and the conformation of each radical has been derived through the interpretation of the ESR spectra combined with quantum mechanical calculations based on MO theory.

In this paper the investigation has been extended to anion radicals of 1,1-di(*p*-nitrophenyl)ethylenes electrolytically generated in the same solvents. The anion radicals of 1-*p*-nitrophenyl-1-phenylethylene and 1,1-di(*p*-nitrophenyl)ethylene have been also obtained by reduction with alkali metal alkoxides in Me_2SO. Information on their reactivity has been obtained by means of a detailed study of the paramagnetic intermediates of their basic cleavage and oxidation by traces of molecular oxygen.

In order to get a better understanding of the role of the solvent on reaction mechanism, the investigation was extended to ESR line shapes, which are affected by the interaction between the radical and the medium. The proton and cation transfers were treated as intramolecular chemical exchanges.[3] Rotational diffusion of the paramagnetic species was invoked to explain the relaxation broadening of the ESR lines.[4] Line shapes were calculated by the density matrix method,[5,6] either in its whole formalism[7] or by using some suitable approximation.[8,9]

Calculations

Measured hyperfine coupling constants have been assigned on the ground of the theoretical spin densities calculated through the McLachlan method.[10] The starting HMO's have been evaluated adopting the set of parameters[11-14] collected in Table I. The cases in which the observed spin distribution appears localized on a molecular fragment and does not reflect the molecular symmetry have been interpreted according to Gutch, Waters, and Symons.[15] These authors suggested that a low-lying excited electronic state, when added to the ground state, can give two equivalent asymmetric structures. The

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