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Anisotropic Reorientation of Nicotine in Solution. A Carbon-13 Spin-Lattice Relaxation Time Study of Molecular Conformation

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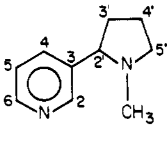
Abstract: The ^{13}C NMR spin-lattice relaxation times of nicotine (**1**) are analyzed in terms of anisotropic rotational diffusion constants. The data agree best with an inter-ring orientation in which the H(2′)-C(2′)-C(3)-C(2) dihedral angle is approximately 0°.

Introduction

The hope that a knowledge of the conformation of nicotine (**1**)



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will lead to a fuller understanding of its pharmacological activity mechanism has prompted several studies directed toward describing its conformation.[1-12] We have previously examined the solution conformation of nicotine and some of its geometrical isomers by ^{1}H and ^{2}H NMR spectroscopy. The principal features of the predominant solution conformation are shown in Figure 1: the pyrrolidine ring is an envelope[11] with the *N*′-methyl and pyridine substituents equatorial;[10] the two rings are perpendicular to one another, the C(2′)-H(2′) bond being coplanar with the pyridine ring.[11] However, we were not able to determine whether the inter-ring orientation shown ($\theta = 0°$; θ is the H(2′)-C(2′)-C(3)-C(2) dihedral angle) is preferred or the one in which the pyridine ring is rotated by 180° ($\theta = 180°$). The goal of the present study is to answer this question using the conformational dependence of ^{13}C T_1's in anisotropically tumbling molecules.

Experimental Section

^{13}C NMR T_1's (20.1 MHz) were obtained with a Bruker WP-80 spectrometer employing the 180°-τ-90° method. A three-parameter exponential fit was utilized to calculate T_1's from the relaxation data in order to minimize systematic errors.[13] Care was taken to ensure that the sample was contained entirely within the rf coil. For T_1 and NOE (nuclear Overhauser effect)[14] measurements, **1** was dissolved in CDCl$_3$ (Merck and Co., Inc.) in a 10-mm NMR sample tube (Wilmad Glass Co.). Oxygen was removed by bubbling nitrogen through the solution. A low concentration of **1** (0.2 *m*) was chosen to preclude the possibility of intermolecular interaction between nicotine molecules.

Theoretical Considerations

As a molecule tumbles in an external magnetic field, the ^{13}C-^{1}H dipolar couplings of the nuclei in the molecule are modulated by the field, generating rf frequency components whose distribution is described by the spectral density function $J(\omega)$. If $J(\omega)$ contains components at the Larmor frequency, the tumbling provides one mechanism to allow the nuclear spins to approach a Boltzmann equilibrium.[15] The time constant for this equilibration process, the dipolar spin-lattic relaxation time ($T_{1\mathrm{DD}}$), is linked to the spectral density functions via the folowing relationship[16] which describes the contribution of each ^{13}C-^{1}H interaction to the ^{13}C relaxation rate ($1/T_{1\mathrm{DD}}$).

$$\frac{1}{T_{1\mathrm{DD}}} = \frac{1}{20}\frac{\gamma_{\mathrm{H}}^2\gamma_{\mathrm{C}}^2\hbar^2}{r_{\mathrm{CH}}^6}[J(\omega_{\mathrm{H}} - \omega_{\mathrm{C}}) + 3J(\omega_{\mathrm{C}}) + 6J(\omega_{\mathrm{H}} + \omega_{\mathrm{C}})] \quad (1)$$

The γ's are magnetogyric ratios; ω's, Larmor frequencies; and r_{CH}, the carbon-proton distance. Measurement of $T_{1\mathrm{DD}}$ thus allows access to information on molecular dynamics through the components of $J(\omega)$ generated by the dynamic processes.

For rigid nonsymmetrical molecules undergoing rotational diffusion, $J(\omega)$ is of the form[17]

$$J(\omega) = \frac{C_+\tau_+}{(1 + \omega^2\tau_+^2)} + \frac{C_-\tau_-}{(1 + \omega^2\tau_-^2)} + \frac{C_1\tau_1}{(1 + \omega^2\tau_1^2)} + \frac{C_2\tau_2}{(1 + \omega^2\tau_2^2)} + \frac{C_3\tau_3}{(1 + \omega^2\tau_3^2)} \quad (2)$$

where $1/\tau_\pm = 6[R \pm (R^2 - L^2)]$; $1/\tau_1 = 4R_1 + R_2 + R_3$; $1/\tau_2 = 4R_2 + R_1 + R_3$; $1/\tau_3 = 4R_3 + R_1 + R_2$; $R = {}^1/_3(R_1 + R_2 + R_3)$; $L^2 = {}^1/_3(R_1R_2 + R_1R_3 + R_2R_3)$. The C's are geometrical constants defined in ref 17, which describe the orientation of the ^{13}C-^{1}H internuclear vector relative to the principal axes of diffusion. The R's are the diffusion constants of rotation about each of the three principal axes. In general, these diffusion constants are not equal for nonsymmetrical molecules, so that the spectral density resulting from a given ^{13}C-^{1}H interaction will depend on the placement of that ^{13}C nucleus within the molecule. This, in turn, will manifest itself in the T_1 measured for that nucleus. Therefore, for molecules which could exist in several well-defined conformations, each of which places the ^{13}C nuclei in different locations within the molecular framework, it is possible to determine the predominant conformation. It should be noted that eq 2 contains a singularity, so that it is convenient to transform

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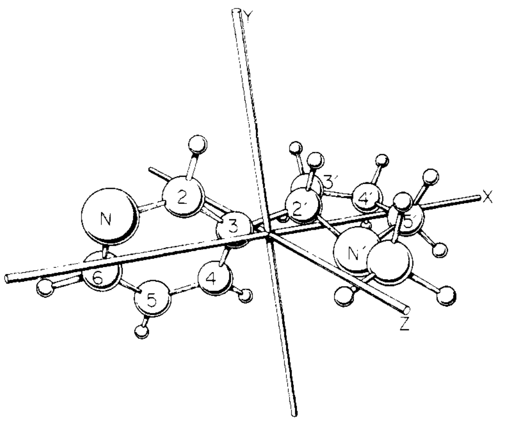

Figure 1. The most probable solution conformation of nicotine. The carbons are numbered for reference.

Table I. Observed and Calculated ^{13}C T_1's, Diffusion Constants, and Principal Moments of Inertia for Nicotine

carbon[a]	$T_{1\,\mathrm{obsd}}$	(SD)	NOE	$T_{1\,\mathrm{DD}}$	$T_{1\,\mathrm{calcd}}$
2	5.58	(0.10)	2.6_8	6.6_4	6.71
4	5.10	(0.05)	2.6_9	6.0_3	5.97
5	5.21	(0.05)	2.6_5	6.3_1	6.26
6	3.91	(0.05)	2.6_7	4.6_8	4.70
2'	5.98	(0.05)	2.7_4	6.8_7	6.89
3'	3.73	(0.05)	2.8_7	3.9_9	3.40
4'	3.79	(0.08)	2.7_9	4.2_3	3.24
5'	3.07	(0.05)	2.8_1	3.3_9	3.09
CH_3	2.56	(0.05)	2.8_0	2.8_4	1.98
$R_x = 4.8 \pm 0.5 \times 10^{10}$ rad s^{-1}				$I_x = 246$ amu Å^2	
$R_y = 3.4 \pm 0.8 \times 10^{10}$ rad s^{-1}				$I_y = 942$ amu Å^2	
$R_z = 0.5 \pm 0.5 \times 10^{10}$ rad s^{-1}				$I_z = 864$ amu Å^2	

[a] For assignments see ref 21.

it in a manner such as demonstrated in ref 18 to eliminate that singularity.

For nonsymmetrical molecules, the orientation of the principal axes of diffusion in the molecular framework is not in general known. It is common to assume that intermolecular interactions are weak, so that the diffusion rates are determined by the distribution of mass within the molecule. This means that the diffusional axes coincide with the principal axes of the moment of inertia tensor. This approach is taken in the present study. The three principal moments of inertia corresponding to the three axes in Figure 1 are calculated by diagonalizing the moment of inertia tensor using a molecular geometry computed from standard bond lengths and bond angles and from torsional angles obtained by ^{1}H NMR[11]. Rotational diffusion constants, R_x, R_y, and R_z (R_1, R_2, and R_3 in eq 2), are determined iteratively by minimizing the standard deviation of the difference between observed $T_{1\mathrm{DD}}$'s and $T_{1\mathrm{DD}}$'s calculated from eq 1.

Results and Discussion

$T_{1\mathrm{DD}}$'s as computed from measured T_1's and NOEs[14] are shown in Table I. For an isotropically tumbling molecule the $nT_{1\mathrm{DD}}$ values of carbons bearing directly attached protons (n is the number of protons) should all be approximately equal except for differences due to bond length. The nonequality of the $T_{1\mathrm{DD}}$'s of carbons bearing one proton is consistent with the reorientational anisotropy expected for a nonsymmetric molecule such as **1**.

The 10% difference between T_1C(2) and T_1C(4) indicates that inter-ring rotation is slower than overall molecular tumbling; if inter-ring rotation were rapid enough to dominate relaxation, the difference in C(2)–H(2) and C(4)–H(4) bond lengths would only account for a 4% difference in T_1's. This leads us to consider the rings as fixed relative to one another, insofar as motion influencing ^{13}C relaxation is concerned. The internal motion of the pyrrolidine ring, however, is not a priori insignificant compared to overall tumbling, and the C(3′), C(4′), and C(5′) T_1's will likely contain contributions due to pseudorotation. Internal motion (rotation) might also be expected to contribute to relaxation of the methyl carbon. We therefore use only the T_1's of C(2), C(4), C(5), C(6), and C(2′) and the interaction of each of these carbons with all protons to fit eq 1 to the three diffusion constants. The use of these carbons, whose relaxation is dominated by a single directly attached proton, also serves to minimize multispin effects.[19] The fitting procedure treats nicotine as a rigid tumbling ellipsoid. Although pseudorotation in the pyrrolidine ring is anticipated as well as some slight torsional motion between the two rings, these motions are assumed to have minimal effects on the measured relaxation times for C(2), C(4), C(5), C(6), and C(2′).

The fitted diffusion constants and corresponding calculated T_1's assuming a fixed geometry and $\theta = 0°$ are shown in Table I. The agreement between calculated and observed T_1's is very good for C(2), C(4), C(5), C(6), and C(2′). The measured T_1's of C(3′), C(4′), and C(5′) are all longer than calculated, consistent with torsional motion in the five-membered ring. C(4′) exhibits the greatest deviation from the calculated value, probably since its motion is less restricted than C(3′) and C(5′), which are directly adjacent to the points of pyridyl and methyl substitution, respectively. Although rotation is most rapid about the axis having the lowest moment of inertia (Table I), the lack of an inverse square relationship between all of the moments of inertia and diffusion constants is consistent with motion not being inertial but rather diffusional, as would be predicted for a molecule as large as **1**. It is interesting to note that the CH_3 T_1 reflects some restriction to free rotation of the methyl group; $T_{1\mathrm{DD}}$ of the methyl carbon would be nine times $T_{1\mathrm{calcd}}$ for a freely rotating methyl group attached to an axially symmetric ellipsoid.

Equation 1 was fitted to diffusion constants for θ values 180 through −180°. Similar standard deviations are obtained for $-20° \leq \theta \leq 20°$; however, as θ is increased above 20° or decreased below −20°, the fit becomes progressively worse until at $\theta \simeq 180°$ the fit will not converge. Also, if T_1's are calculated using the diffusion constants of Table I and $\theta = 180°$, the T_1's for C(2) and C(4) are predicted to be approximately equal, so that, within the limits of the model proposed here, the most probable ring–ring orientation is that shown in Figure 1 ($\theta = 0°$).

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