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Molecular Structure of Nicotine As Studied by Gas Electron Diffraction Combined with Theoretical Calculations

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The molecular structure of nicotine was determined by means of gas electron diffraction. The nozzle temperature was about 116 °C. The results of RHF, MP2, and DFT calculations were used as supporting information. The electron diffraction data were well reproduced by assuming the mixture of two conformers where both the methyl group and the pyridine ring are in the equatorial positions of the pyrrolidine ring. The determined structural parameters (r_g and $\angle_\alpha$) are as follows: $\langle r(\mathrm{C-N})_{\mathrm{pyrrol}}\rangle = 1.462(4)$ Å; $\langle r(\mathrm{C-C})_{\mathrm{pyrrol}}\rangle = 1.541(4)$ Å; $\langle r(\mathrm{C-N})_{\mathrm{pyrid}}\rangle = 1.345(2)$ Å; $\langle r(\mathrm{C-C})_{\mathrm{pyrid}}\rangle = 1.397$ Å (d.p.); $r(\mathrm{C_{pyrrol}-C_{pyrid}}) = 1.502$ Å (d.p.); $\langle r(\mathrm{C-H})_{\mathrm{pyrrol}}\rangle = 1.116(4)$ Å; $\langle r(\mathrm{C-H})_{\mathrm{pyrid}}\rangle = 1.106$ Å (d.p.); $\angle \mathrm{C-N-C_{pyrrol}} = 108.4(15)°$; $\langle \angle \mathrm{N-C-C_{pyrrol}}\rangle = 102.4°$ (d.p.); $\langle \angle \mathrm{C-C-C_{pyrrol}}\rangle = 108.2(33)°$; $\angle \mathrm{C-N-C_{pyrid}} = 116.8(2)°$; $\langle \angle \mathrm{N-C-C_{pyrid}}\rangle = 124.1°$ (d.p.); $\langle \angle \mathrm{C-C-C_{pyrid}}\rangle = 118.3°$ (d.p.); $\angle \mathrm{N_{pyrrol}-C_{pyrrol}-C_{pyrid}} = 116.8(10)°$; $\angle \mathrm{C_{pyrrol}-C_{pyrrol}-C_{pyrid}} = 115.8(20)°$; $\langle \angle \mathrm{H-C-H}\rangle = 111.9(41)°$; $\alpha = 45.7(25)°$; $\beta = 32.9(38)°$; $\phi = -87.7(74)°$. Angle brackets denote averaged values, values in parentheses are 3 times the estimated standard errors referring to the last significant digit, and d.p. denotes dependent parameters. Angle α is the methyl out-of-plane angle, and β is the puckering angle of the pyrrolidine ring. Angle ϕ is the dihedral angle between the planes of pyrrolidine and pyridine rings. The distances between the two N atoms are determined to be 4.885 ± 0.006 and 4.275 ± 0.007 Å for the two conformers. By comparing these values with the distance between the N and carbonyl O atoms of acetylcholine, a strong nicotinic agonist, it is concluded that among the two conformers of nicotine, only the one with the longer N···N distance has the nicotinic activity.

Introduction

After successful structure determination of typical mesogens, MBBA (4-methoxybenzylidene-4′-*n*-butylaniline)[1] and PAA (*p*-azoxyanisole),[2] by gas electron diffraction, we became more confident that this experimental technique can be applied to larger and more complicated molecules than what had so far been studied by this method. Therefore, we have decided to make the investigation of geometrical structures of some bioactive compounds our next project. As the first target of this line, we have chosen the geometrical structure and conformation of nicotine (Figure 1), a strong agonist of the nicotinic acetylcholine receptor (nAChR).

Making a reliable model for nAChR is quite important for drug design, and its future success partly relies on the knowledge of the precise geometrical structure of the agonists such as nicotine, cytisine, and epibatidine. So there have been many structural investigations of nicotine. Among many structural parameters, the most interesting is the interatomic distance of two N atoms because it has long been pointed out that the physiological resemblance between nicotine and acetylcholine (Figure 1) comes from the fact that the two N atoms in a nicotine molecule play the same role in binding with the receptor as the N and carbonyl O atoms of acetylcholine (see, for example, ref 3).

In case of nicotine, the most flexible part of geometrical structure is the torsion around the C_4-C_7 bond connecting pyrrolidine and pyridine rings, and this torsional angle is the most important parameter for determining the N···N distance precisely. Pitner et al. found that the two rings of nicotine are perpendicular to each other from the analyses of their ^{1}H and ^{2}H NMR spectra measured in a mixed solvent of $CDCl_3$ and $CFCl_3$,[4] but they did not report a specific value for the torsional angle. The solvent used in their study is quite different from that of biological environment. In addition, the structural determination from NMR spectrum in isotropic solvent is, more or less, indirect and is subject to some amount of ambiguity. On the other hand, Barlow et al. determined the crystal structure of nicotine−hydroiodide salt.[5] The obtained dihedral angle, $\phi(C_6-C_4-C_7-C_8)$, of monoprotonated form of nicotine was $-123.8° \pm 0.3°$, and the N···N distance was 4.740 ± 0.005 Å. One of the recent theoretical studies for the structure of nicotine is that by Elmore and Dougherty.[6] They carried out RHF/6-31G** ab initio calculations of nicotine as well as its protonated species and found two conformers, the energies of which are much lower than those of others. The calculated N···N distances for the two conformers are 4.793 and 4.247 Å, the former of which seems to correspond to the conformer reported by Barlow et al.

The theoretical structure of nicotine used in the modeling of nAChR is usually that in the isolated state, i.e., gas phase, while no experimental data are available for the structure or conformation of this molecule in the gas phase. It is obviously because nicotine is a too large system to be studied by diffraction or spectroscopic method. However, recent progress of computational resources has made it practical to carry out sophisticated calculations on molecules of this size to provide reliable supporting information for the analysis of electron diffraction

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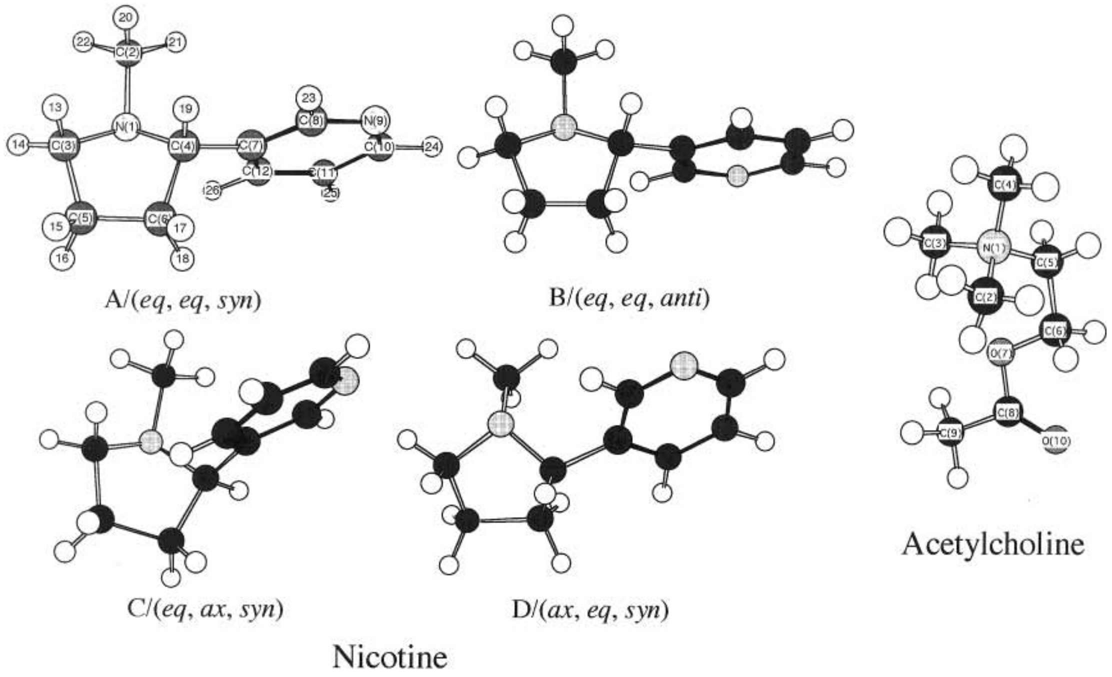

Figure 1. Molecular models and atom numbering for some possible conformers of nicotine and one conformer of acetylcholine. The abbreviations of the nicotine conformers such as A/(*eq*, *eq*, syn) correspond to the position of the methyl group, the position of the pyridine ring and the $H_{19}-C_4-C_7-C_8$ dihedral angle, respectively, preceded by prefix letters, A/, B/, C/, etc. given in the order of the RHF energy.

TABLE 1: Experimental Conditions for Gas Electron Diffraction Experiments of Nicotine

	short	long
camera distance/mm	244.5	489.5
nozzle temperature/K	388	389
electron wavelength/Å	0.06344	0.06346
uncertainty in the scale factor/%	0.05	0.04
background pressure during exposure/10^{-6} Torr	1.8−1.9	2.1−2.9
beam current/μA	2.0	2.6
exposure/s	26−39	25−29
number of plates used	4	4
range of s value/$Å^{-1}$	6.1−33.7	2.1−17.4

data. The purpose of the present study is to determine the precise geometrical structure in the gas phase of nicotine by means of electron diffraction combined with theoretical calculations.

Experimental Section

The sample of nicotine with purity of better than 98% was purchased from Sigma Chemical Co. and was used without further purification. Care was taken to treat the sample because nicotine is quite hygroscopic and sensitive to light and oxygen. Electron diffraction patterns were recorded on 8 × 8 in. Kodak projector slide plates with an apparatus equipped with an r^3-sector.[7] Two camera distances were used to cover s rage (2.1−33.7 $Å^{-1}$) sufficient for the molecule of this size. To get enough sample pressure, a high-temperature nozzle[2] was used with the nozzle tip temperature of 116 °C. The accelerating voltage of incident electrons was about 37 kV. Other experimental conditions are summarized in Table 1. The photographic plates were developed for 4.5 min in Dektol developer diluted 1:1. The photometry process was described in details elsewhere.[1] The experimental intensities and backgrounds are available as Supporting Information (Table S1). The electron wavelength was calibrated to the r_a (C═S) distance of CS_2 (1.5570 Å).[8]

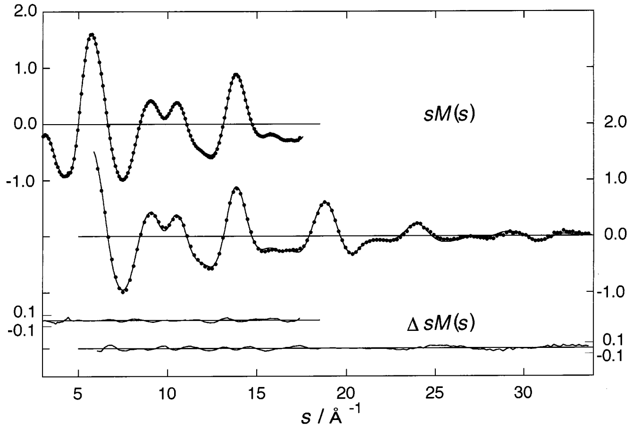

Figure 2. Experimental (dots) and theoretical (solid curves) molecular scattering intensities of nicotine; $\Delta sM(s) = sM(s)^{obs} - sM(s)^{calc}$. The theoretical curves were calculated from the best-fit parameters.

TABLE 2: Relative Energies and Estimated Abundances for the Possible Conformers of Nicotine Obtained from the RHF/6-31G* ab Initio Calculations

conformers[a]	ΔE/kcal mol^{-1} [b]	abundance/%[c]
A/(eq, eq, syn)	0.00	66.3
B/(eq, eq, anti)	0.53	33.4
C/(eq, ax, syn)	4.54	0.2
D/(eq, ax, anti)	4.71	0.1
E/(ax, eq, syn)	7.24	0.0
F/(ax, eq, anti)	6.92	0.0
G/(ax, ax, syn)	*d*	
H/(ax, ax, anti)	*e*	

[a] See Figure 1 for the definitions of the conformers. [b] Absolute value of the energy is $-495.719520E_h$ for the A/(eq, eq, syn) conformer. [c] Estimated from the energy difference, ΔE, assuming the Boltzmann distribution at 389 K. [d] Converged to the A/(eq, eq, syn) conformer. [e] Converged to the B/(eq, eq, anti) conformer.

Elastic atomic scattering factors were calculated as described in ref 9, and inelastic ones were taken from ref 10. The experimental molecular scattering intensities are shown in Figure 2 with the final calculated ones. A diagonal weight matrix was used in the least-squares analysis on the molecular scattering intensities. The weight function was set unity in the medium s

TABLE 3: Geometrical Parameters and Relative Energies for the A/(eq, eq, syn) and B/(eq, eq, anti) Conformers of Nicotine Obtained from the RHF/6-31G* and MP2(Frozen Core)/6-31G ab Initio Calculations** [a]

	A/(eq, eq, syn)		B/(eq, eq, anti)	
parameters	RHF	MP2	RHF	MP2
Bond Lengths (Å)				
N_1-C_2	1.444	1.454	1.444	1.453
N_1-C_3	1.451	1.461	1.451	1.461
N_1-C_4	1.456	1.464	1.455	1.463
C_3-C_5	1.532	1.532	1.532	1.532
C_4-C_6	1.545	1.539	1.545	1.540
C_5-C_6	1.545	1.545	1.545	1.545
C_4-C_7	1.513	1.501	1.514	1.502
C_7-C_8	1.386	1.398	1.394	1.402
C_8-N_9	1.323	1.345	1.318	1.344
N_9-C_{10}	1.318	1.344	1.323	1.346
$C_{10}-C_{11}$	1.387	1.397	1.381	1.395
$C_{11}-C_{12}$	1.381	1.392	1.386	1.394
$C_{12}-C_7$	1.390	1.399	1.384	1.396
$N_1\cdots N_9$	4.793	4.812	4.251	4.223
C_2-H_{20}	1.094	1.101	1.094	1.101
C_2-H_{21}	1.082	1.089	1.082	1.089
C_2-H_{22}	1.084	1.089	1.084	1.089
C_3-H_{13}	1.095	1.103	1.095	1.104
C_3-H_{14}	1.084	1.091	1.084	1.091
C_4-H_{19}	1.095	1.107	1.096	1.108
C_5-H_{15}	1.084	1.089	1.084	1.089
C_5-H_{16}	1.083	1.089	1.083	1.089
C_6-H_{17}	1.084	1.090	1.084	1.090
C_6-H_{18}	1.083	1.090	1.083	1.089
C_8-H_{23}	1.077	1.086	1.075	1.084
$C_{10}-H_{24}$	1.076	1.084	1.076	1.084
$C_{11}-H_{25}$	1.075	1.082	1.074	1.082
$C_{12}-H_{26}$	1.075	1.083	1.077	1.084
Bond Angles (deg)				
$C_2-N_1-C_3$	113.6	112.7	113.7	112.8
$C_2-N_1-C_4$	115.0	112.9	115.1	113.0
$C_3-N_1-C_4$	105.6	103.9	105.7	103.9
$N_1-C_3-C_5$	104.1	103.6	104.1	103.6
$N_1-C_4-C_6$	103.2	102.6	103.2	102.6
$N_1-C_4-C_7$	113.2	112.1	113.4	112.4
$C_6-C_4-C_7$	113.9	113.9	113.9	113.9
$C_3-C_5-C_6$	104.1	104.1	104.1	104.1
$C_5-C_6-C_4$	104.9	104.4	104.9	104.4
$C_4-C_7-C_8$	121.1	121.3	121.4	120.4
$C_4-C_7-C_{12}$	121.9	120.8	121.7	121.8
$C_8-C_7-C_{12}$	116.9	117.9	116.9	117.8
$C_7-C_8-N_9$	124.4	124.4	124.1	124.1
$C_8-N_9-C_{10}$	117.8	116.7	118.0	116.9
$N_9-C_{10}-C_{11}$	123.2	123.6	123.2	123.6
$C_{10}-C_{11}-C_{12}$	118.4	118.9	118.2	118.7
$C_{11}-C_{12}-C_7$	119.3	118.7	119.6	119.0
Bond Angles (deg)				
$N_1-C_2-H_{20}$	112.5	112.2	112.5	112.2
$N_1-C_2-H_{21}$	110.3	109.7	110.3	109.7
$N_1-C_2-H_{22}$	109.5	109.3	109.5	109.3
$H_{20}-C_2-H_{21}$	108.2	108.6	108.3	108.6
$H_{21}-C_2-H_{22}$	108.0	108.5	107.9	108.5
$H_{22}-C_2-H_{20}$	108.1	108.5	108.1	108.5
$N_1-C_3-H_{13}$	111.1	110.8	111.1	110.8
$N_1-C_3-H_{14}$	110.5	110.5	110.5	110.5
$H_{13}-C_3-H_{14}$	107.5	107.8	107.5	107.8
$C_5-C_3-H_{13}$	110.4	110.3	110.4	110.3
$C_5-C_3-H_{14}$	113.3	113.7	113.3	113.7
$N_1-C_4-H_{19}$	110.1	110.3	110.0	110.3
$C_6-C_4-H_{19}$	108.5	109.0	108.5	108.9
$C_7-C_4-H_{19}$	107.9	108.7	107.7	108.6
$C_3-C_5-H_{15}$	111.7	111.5	111.7	111.5
$C_3-C_5-H_{16}$	110.5	110.6	110.5	110.6
$H_{15}-C_5-H_{16}$	107.4	107.6	107.4	107.6
$C_6-C_5-H_{15}$	112.7	112.6	112.7	112.6
$C_6-C_5-H_{16}$	110.5	110.5	110.4	110.5
$C_5-C_6-H_{17}$	112.9	113.3	112.9	113.3
$C_5-C_6-H_{18}$	110.7	111.0	110.7	111.0
$H_{17}-C_6-H_{18}$	107.2	107.7	107.2	107.7
$C_4-C_6-H_{17}$	110.9	111.0	111.0	111.1
$C_4-C_6-H_{18}$	110.2	109.3	110.2	109.3
$C_7-C_8-H_{23}$	119.9	119.8	119.5	119.2
$H_{23}-C_8-N_9$	115.7	115.9	116.4	116.7
$N_9-C_{10}-H_{24}$	116.4	115.9	116.2	115.9
$H_{24}-C_{10}-C_{11}$	120.5	120.5	120.6	120.5
$C_{10}-C_{11}-H_{25}$	120.3	120.1	120.5	120.2
$H_{25}-C_{11}-C_{12}$	121.2	121.1	121.3	121.1
$C_{11}-C_{12}-H_{26}$	121.0	121.9	120.2	120.9
$H_{26}-C_{12}-C_7$	119.7	119.4	120.2	120.1
Dihedral Angles (deg)				
$C_2-N_1-C_3-C_5$	−169.1	−167.0	−169.3	−167.2
$C_2-N_1-C_4-C_6$	167.3	167.7	167.6	167.9
$N_1-C_3-C_5-C_6$	25.2	25.4	25.2	25.4
$N_1-C_4-C_6-C_5$	−23.8	−28.0	−23.8	−28.1
$C_3-C_5-C_6-C_4$	−0.7	1.7	−0.7	1.7
$N_1-C_4-C_7-C_8$	138.1	140.3	−43.1	−40.8
$C_6-C_4-C_7-C_8$	−104.4	−103.7	74.5	75.4
$N_1-C_4-C_7-C_{12}$	−43.4	−41.8	138.6	141.7
$C_6-C_4-C_7-C_{12}$	74.0	74.2	−103.7	−102.2
$H_{19}-C_4-C_7-C_8$	16.1	18.0	−165.1	−163.7
ΔE/kcal mol^{-1} [b]	0.0	0.0	0.53	0.56
abundance (at 389 K)/% [c]		67.4		32.6

[a] See Figure 1 for the atom numberings and the definitions of the conformers. [b] Absolute values of the energy of the A/(eq, eq, syn) conformer given by the RHF and MP2 methods are $-495.719520E_h$ and $-497.439473E_h$, respectively. [c] Estimated from the theoretical ΔE values.

region while it was reduced in the small and large *s* regions by using two Gaussian functions.[11]

Theoretical Calculations

Conformational Expression. At first sight, nicotine seems to have many stable conformers arising from the combination of the following sources of flexibility: pseudorotation of the pyrrolidine ring that is responsible for the envelope and twisted forms of the ring and the equatorial and axial positions of the pyridine ring; inversion around the N atom in the pyrrolidine ring that is responsible for the equatorial and axial positions of the methyl group; internal rotation around the C−C bond between the rings. According to the gas electron diffraction studies of pyrrolidine[12] and *N*-methylpyrrolidine,[13] the pyrrolidine ring has an envelope conformation, with the C atoms forming a plane and the N atom being out of the plane. Therefore, it was assumed that the pyrrolidine ring in nicotine also has an envelope form and its N atom is out of the plane formed by the C atoms. This assumption still leaves the alternative of the axial or equatorial position for each of the methyl group and the pyridine ring and the freedom of internal rotation for the pyridine ring. As for the internal rotation, RHF/6-31G* ab initio calculations revealed two potential minima separated by about 180°. The dihedral angle, $H_{19}-C_4-C_7-C_8$, corresponding to one of the minima is in the region around 0° or around 180°. Thus, this dihedral angle is used to define the internal rotation angle as in ref 6, and hence the stable conformers are expressed as syn ($H_{19}-C_4-C_7-C_8 \approx 0°$) and anti ($H_{19}-C_4-C_7-C_8 \approx 180°$). Consequently, the overall conformation of the nicotine molecule is expressed in this study by using abbreviations in the order of the position of the methyl group, the position of the pyridine ring, and the $H_{19}-C_4-C_7-C_8$ dihedral angle such as A/(eq, eq, syn), C/(eq, ax, anti), and so on. The prefix letters A/, B/, C/, etc., are given in the order of the energy given by RHF calculations except for E/(ax, eq, syn) and F/(ax, eq, anti) (see Table 2). Note that the syn and anti in the present paper are labeled A and B in ref 6, respectively, and the conformers transA, transB, cisA, and cisB appearing in ref 6 correspond to our A/(eq, eq, syn), B/(eq, eq, anti), C/(eq, ax, syn), and D/(eq, ax, anti), respectively. Some of these conformers are shown in Figure 1. The following calculations were performed employing GAUSSIAN 94[14a] and GAUSSIAN 98[14b] programs.

RHF Calculations. At first, geometrical optimizations for various conformations were tried by using the RHF method with a 6-31G* basis set. The obtained relative energies are listed in Table 2. The optimizations starting at the G/(ax, ax, syn) and H/(ax, ax, anti) conformations that have both of the methyl group and the pyridine ring at the axial positions converged to the A/(eq, eq, syn) and B/(eq, eq, anti) conformations, respectively. As shown in this Table, the energies of A/(eq, eq, syn) and B/(eq, eq, anti) conformers are much lower than those of the others and only these two conformers are expected to have significant abundance assuming a Boltzmann distribution. This result is essentially the same as that of ref 6. At this stage, only A/(eq, eq, syn) and B/(eq, eq, anti) survived as the candidates for the stable conformers.

MP2 and B3LYP Calculations. According to the above-mentioned results, the geometries of A/(eq, eq, syn) and B/(eq, eq, anti) conformers were further optimized by using the MP2 (frozen core) method with a 6-31G** basis set in order to obtain more reliable structural parameters. The results are listed in Table 3 along with those of the RHF calculations. It should be noted that the RHF and MP2 methods provided the consistent energy differences between the two conformers. The ratio of the abundances estimated from the energy difference is about 2:1. Table 3 shows that these two conformers have essentially identical structural parameters except for the 180° differecne in the orientation of the pyridine rings. Therefore, their energy difference (0.56 kcal mol^{-1}) is ascribed to the difference in the total dipole moments, which are calculated to be 2.5 and 3.2 D for A/(eq, eq, syn) and B/(eq, eq, anti) conformers, respectively. As described below, these results were used for the structural constraints in the analysis. In addition, the vibrational calculations for these conformers were carried out with a 6-31G* basis set by using the B3LYP, DFT method, which is more suitable to obtain reliable force constants than the MP2 method.

Analyses

Normal Vibration Analysis. The Cartesian force constants obtained for the A/(eq, eq, syn) and B/(eq, eq, anti) conformers by B3LYP/6-31G* calculations were transformed into the internal force constants, f_{ij}. As the A/(eq, eq, syn) conformer was predicted to be more abundant by the theoretical calculations, f_{ij}'s for this conformer were then modified by the scaling method so as to reproduce the experimental vibrational wavenumbers reported by Aslanian et al.[15] The linear scaling formula[16] f_{ij} (scaled) $= (c_i c_j)^{1/2} f_{ij}$ (unscaled) was used, where c_i is a scale factor. The definitions of internal coordinates with the resultant scale factors are listed in Table S2 of Supporting Information. The scaled force constants and calculated vibrational wavenumbers are listed in Tables S3 and S4 of Supporting Information, respectively.

Analysis of Electron Diffraction Data. For the analysis of electron diffraction data of a complex molecule like this, the selection of adjustable parameters is important. To reduce the number of adjustable parameters to an appropriate level, the following assumptions were adopted on the basis of the results of the RHF and MP2 ab initio calculations: (1) only the A/(eq, eq, syn) and B/(eq, eq, anti) conformers exist with the abundances of 67.4% and 32.6%, respectively; (2) the dihedral angle $\phi(C_6-C_4-C_7-C_8)$ of the B/(eq, eq, anti) conformer differs from that of the A/(eq, eq, syn) conformer by 180°; (3) the $\phi(C_3-C_5-C_6-C_4)$ of the pyrrolidine ring is 0°; (4) the pyridine ring is planar; (5) the H−C−H angles in the pyrrolidine ring are equal, and they bisect the N−C−C or C−C−C angle and vice versa; (6) the C−H bonds in the pyridine ring bisect the N−C−C or C−C−C angle; (7) the three N_1-C_2-H angles are equal to that of *N*-methylpyrrolidine (113.5°);[13] (8) the C−H bond lengths in the *N*-methylpyrrolidine group are equal; (9) the C−H bond lengths in the pyridine ring are equal, and they differ from those in the *N*-methylpyrrolidine group by −0.010 Å; (10) the differences between the similar structural parameters of intra- and inter-conformers are equal to the MP2/6-31G** values. The relative abundance of the two conformers had to be fixed (assumption 1) because it is almost impossible to distinguish two conformers caused by a 180° internal rotation of a pyridine ring with a good precision.[17] Exclusion of the higher-energy conformers such as C/(eq, ax, syn) is justified by preliminary analyses in which each of the C/(eq, ax, syn) or E/(ax, eq, syn) conformers was assumed with 100% abundance (see Results and Discussion section). In addition, Table 3 shows that assumption 2 is a good approximation. Assumption 3 was adopted because the theoretical dihedral angles, $C_3-C_5-C_6-C_4$, are nearly equal to 0 within ±2° (see Table 3). The independent parameters and the constraints are summarized in Table 4.

TABLE 4: Structural Parameters and Constraints Based on MP2/6-31G Calculations for the A/(eq, eq, syn) and B/(eq, eq, anti) Conformers of Nicotine**[a]

parameter	A/(eq, eq, syn)	B/(eq, eq, anti)
	Bond Lengths (Å)	
N_1-C_2	r_1	$r_1 - 0.001$
N_1-C_3	$r_1 + 0.007$	$r_1 + 0.007$
N_1-C_4	$r_1 + 0.010$	$r_1 + 0.009$
C_3-C_5	r_2	r_2
C_4-C_6	$r_2 + 0.007$	$r_2 + 0.008$
C_5-C_6	$r_2 + 0.013$	$r_2 + 0.013$
C_4-C_7	$r_2 - 0.031$	$r_2 - 0.030$
$\langle C-H \rangle_{\mathrm{pyrrol}}$	r_3	r_3
$\langle C-H \rangle_{\mathrm{pyrid}}$	$r_3 - 0.010$	$r_3 - 0.010$
C_8-N_9	r_4	$r_4 - 0.001$
N_9-C_{10}	$r_4 - 0.001$	$r_4 + 0.001$
C_7-C_8	$r_4 + 0.053$	$r_4 + 0.057$
$C_{10}-C_{11}$	$r_4 + 0.052$	$r_4 + 0.050$
$C_{11}-C_{12}$	$r_4 + 0.047$	$r_4 + 0.049$
C_7-C_{12}	$r_4 + 0.054$	$r_4 + 0.051$
	Bond Angles (deg)	
$C_3-N_1-C_4$	θ_1	θ_1
$C_3-C_5-C_6$	θ_2	θ_2
β	θ_3	θ_3
α	θ_4	θ_4
$\langle C-C-H \rangle_{\mathrm{methyl}}$	113.5	113.5
$\langle H-C-H \rangle$	θ_5	θ_5
$N_1-C_4-C_7$	θ_6	$\theta_6 + 0.3$
$C_6-C_4-C_7$	θ_7	θ_7
$C_8-N_9-C_{10}$	θ_8	$\theta_8 + 0.2$
$C_7-C_8-N_9$	$\theta_8 + 7.7$	$\theta_8 + 7.4$
$N_9-C_{10}-C_{11}$	$\theta_8 + 6.9$	$\theta_8 + 6.9$
	Dihedral Angle (deg)	
$C_6-C_4-C_7-C_8$	ϕ	$\phi + 180.0$

[a] See Figure 1 for the atom numbering and the definitions of the conformers.

Mean amplitudes, *l*, and shrinkage corrections,[18] $r_a - r_\alpha$, were calculated from the above-mentioned scaled force constants. Those of the second stable conformer, B/(eq, eq, anti), were

TABLE 5: Molecular Structures of Nicotine and Relater Molecules[a]

	nicotine A/(eq, eq, syn)[c]		*N*-methylpyrrolidine[d]	pyridine[e]
parameters[b]	ED (r_g and $\angle_\alpha$)	MP2/6-31G**(r_e)	ED (r_a)	ED (r_g and $\angle_\alpha$)
	Bond Lengths (Å)			
N_1-C_2	1.456	1.454	1.446	
N_1-C_3	1.464 (4)	1.461	1.455 (3)	
N_1-C_4	1.467	1.464		
C_3-C_5	1.534	1.532	1.542 (4)	
C_4-C_6	1.541 (4)	1.539		
C_5-C_6	1.547	1.545	1.555	
C_4-C_7	1.502	1.501		
C_8-N_9	1.345	1.345		1.344
$C_{10}-N_9$	1.344	1.344		
C_7-C_8	1.398 (2)	1.398		1.399 (1)
$C_{10}-C_{11}$	1.397	1.397		
$C_{11}-C_{12}$	1.392	1.392		1.398
C_7-C_{12}	1.399	1.399		
$\langle C-H\rangle_{pyrrol}$	1.116 (4)	1.094	1.110 (3)	
$\langle C-H\rangle_{pyrid}$	1.106	1.084		1.094 (5)
	Bond Angles and Dihedral Angles (deg)			
$C_3-N_1-C_4$	108.4 (15)	103.9	107.4 (17)	
$N_1-C_3-C_5$	103.4[f]	103.6		
$N_1-C_4-C_6$	101.3[f]	102.6		
$C_3-C_5-C_6$	107.7 (33)	104.1		
$C_4-C_6-C_5$	108.6[f]	104.4		
$N_1-C_4-C_7$	116.8 (10)	112.1		
$C_6-C_4-C_7$	115.8 (20)	113.9		
$C_8-N_9-C_{10}$	116.8	116.7		116.1
$C_7-C_8-N_9$	124.5 (2)	124.4		124.6 (1)
$N_9-C_{10}-C_{11}$	123.7	123.6		
$C_8-C_7-C_{12}$	117.1[f]	117.9		117.8[f]
$C_{10}-C_{11}-C_{12}$	118.3[f]	118.9		
$C_7-C_{12}-C_{11}$	119.5[f]	118.7		119.1[f]
$\langle H-C-H\rangle$	111.9 (41)	107.7	113.8 (27)	
$\langle N_1-C_2-H\rangle$	113.5 (fix)	110.4	113.5 (24)	
α[g]	45.7 (25)	51.1	49.1[f]	
β[g]	32.9 (38)	45.7	41.7 (22)	
$C_6-C_4-C_7-C_8$	−87.7 (74)	−103.7		
$N_1-C_4-C_7-C_8$	153.1[f]	140.3		

[a] Numbers in parentheses are 3 times the estimated standard errors referring to the last significant digit. [b] See Figure 1 for the atom numberings. Angle brackets denote averaged values. [c] Present work. See Figure 1 for the definitions of the conformers. [d] Ref 13. [e] Ref 22. [f] Dependent parameters. [g] See Figure 3 for the definitions.

obtained from the results of the B3LYP/6-31G* calculations and were scaled with the same scale factor c_i as that of the most stable conformer, A/(eq, eq, syn). The model of small amplitude vibrations was adopted. The mean amplitudes of the C−H bonds were adjusted in a group and those of the C−C and C−N bonds were adjusted in another group. The differences among mean amplitudes in each group were fixed at the calculated values. The mean amplitudes of nonbonded pairs were also fixed at their calculated values. Table S5 of Supporting Information lists the mean amplitudes with the corresponding r_a distances. The anharmonicity parameters,[19] κ, for bonded atom pairs were estimated in a diatomic approximation,[20] $\kappa = (a/6)\cdot l^4$, where the Morse parameter, a, was assumed to be 2.0 Å^{-1}. Those for nonbonded atom pairs were assumed to be zero.

Results and Discussion

Table 5 lists the obtained structural parameters of the A/(eq, eq, syn) conformer. Figure 3 illustrates the definitions of the methyl out-of-plane angle α, and the puckering angle β, of the pyrrolidine ring. An experimental radial distribution curve with residuals is shown in Figure 4. The R-factor[21] of the analysis is 0.046. The change in the relative abundance of the A/(eq, eq, syn) and B/(eq, eq, anti) conformers did not change the fitting quality significantly. On the other hand, preliminary analyses showed that the change in one of the ring positions from eq to ax worsens the fitting significantly (see the lower traces of Figure 4). These results confirm the assumptions for the conformational composition and the structural constraints experimentally, except for the syn versus anti abundance. The correlation matrix in the least-squares fitting is given in Table S6 of Supporting Information. The strongest correlation was found between β and $\angle C_6-C_4-C_7$ with the correlation coefficient of −0.73. The indices of resolution, k, of the molecular scattering intensities were determined to be 1.00 ± 0.01 and 0.98 ± 0.03, for the long and short camera distances, respectively.

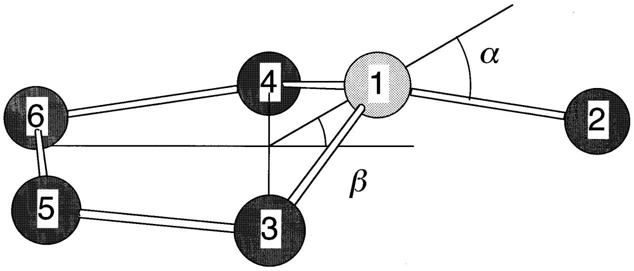

Figure 3. Definitions of the methyl out-of-plane angle α and the ring-puckering angle β of nicotine.

In Table 5, the structural parameters obtained from the MP2/6-31G** calculation and experimental structures of the related compounds, *N*-methylpyrrolidine[13] and pyridine[22] are also listed

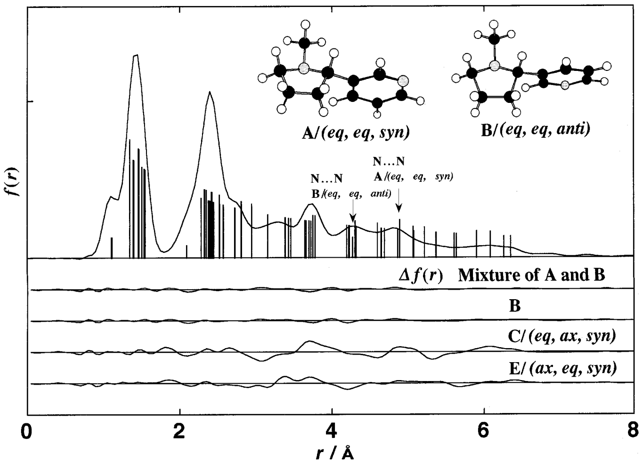

Figure 4. Experimental radial distribution curves of A/(eq, eq, syn) 67.4% and B/(eq, eq, anti) 32.6% mixture of nicotine; $\Delta f(r) = f(r)^{\mathrm{obs}} - f(r)^{\mathrm{calc}}$. Distance distributions for the A/(eq, eq, syn) conformer are indicated by vertical bars. Only the N···N distance is shown for the B/(eq, eq, anti) conformer. Those for the nonbonded C···H, N···H and H···H pairs are not shown for the simplicity. The lower traces are the residuals for preliminary analyses in which only the B/(eq, eq, anti), C/(eq, ax, syn) or E/(ax, eq, syn) conformers were assumed to exist.

for comparison. Agreement between the experimental and MP2/6-31G** structures is satisfactory for the bond lengths and is moderate for the bond angles. However, the MP2 calculation failed in predicting the angles to characterize the conformation, namely, α, *β*, and *ϕ* ($C_6-C_4-C_7-C_8$), with a good accuracy. There is no significant difference between the geometrical parameters of nicotine and the corresponding parameters of the related molecules with the exception that the pyrrolidine ring of nicotine is more nearly planar than that of *N*-methylpyrrolidine.

It was found that the pyridine ring of nicotine is nearly perpendicular to the $C_3-C_5-C_6-C_4$ plane of the pyrrolidine ring. This can be regarded as a consequence of the repulsion among various H atoms. As a result of the relative position of two rings, the interatomic distances of two N atoms in nicotine were determined to be 4.885 ± 0.006 and 4.275 ± 0.007 Å, for the A/(eq, eq, syn) and B/(eq, eq, anti) conformers, respectively. These values should be compared with the corresponding distance between the binding sites of acetylcholine (N and carbonyl O) when it is docking with nAChR. Early theoretical calculation predicted this distance to be 4.93 Å.[3] A more sophisticated calculation based on DFT method was carried out recently; however, no detailed structural parameters were reported to derive the $N\cdots O_{\mathrm{carbonyl}}$ distance.[23] On the other hand, there has been no experimental structure of acetylcholine reported other than the crystal structures of some salts of it.[24-26] To obtain a reliable $N\cdots O_{\mathrm{carbonyl}}$ distance of the "nicotinic" conformer of acetylcholine, geometrical optimizations of acetylcholine were carried out by RHF/6-31G** and MP2(frozen core)/6-31G* calculations starting with various initial structures. From each of the RHF and MP2 calculation, several stable conformers were obtained, among which the one shown in Figure 1 is believed to have the nicotinic activity.[27,3] The determined geometrical parameters of this conformer are listed in Table 6. This conformer is also the same as those found in the crystal of acetylcholine chloride.[24] The $N\cdots O_{\mathrm{carbonyl}}$ distances of this conformer obtained from the RHF and MP2 calculations are 4.97 and 4.96 Å, respectively, which are in good agreement with the early prediction (4.93 Å).[3] Considering all these values, the most reliable guess of the $N\cdots O_{\mathrm{carbonyl}}$ distance of acetylcholine with nicotinic activity is about 4.96 Å. Therefore, it is likely that only the A/(eq, eq, syn) conformer of nicotine with the N···N distance of 4.885 ± 0.006 Å shows the physiological activity.

TABLE 6: Geometrical Parameters of the Nicotinic Conformer of Acetylcholine Obtained from the RHF/6-31G and MP2(frozen core)/6-31G* ab Initio Calculations**

parameters [a]	RHF/6-31G**	MP2/6-31G*
Bond Lengths (Å)		
N_1-C_2	1.495	1.500
N_1-C_3	1.501	1.507
N_1-C_4	1.498	1.503
N_1-C_5	1.516	1.522
C_5-C_6	1.517	1.513
C_6-O_7	1.409	1.434
O_7-C_8	1.355	1.396
C_8-C_9	1.499	1.498
$C_8{=}O_{10}$	1.181	1.211
$N_1\cdots O_{10}$	4.969	4.957
Bond Angles (deg)		
$C_2-N_1-C_3$	109.3	109.3
$C_2-N_1-C_4$	108.8	109.1
$C_3-N_1-C_4$	108.2	108.3
$C_2-N_1-C_5$	111.7	111.4
$C_3-N_1-C_5$	111.2	110.8
$C_4-N_1-C_5$	107.6	107.9
$N_1-C_5-C_6$	117.5	116.2
$C_5-C_6-C_7$	109.9	108.9
$C_6-C_7-C_8$	115.6	112.9
$C_7-C_8-C_9$	111.9	110.7
$C_7-C_8{=}O_{10}$	121.2	121.2
$C_9-C_8{=}O_{10}$	126.9	128.2
Dihedral Angles (deg)		
$C_2-N_1-C_5-C_6$	44.1	40.4
$C_3-N_1-C_5-C_6$	−78.3	−81.5
$C_4-N_1-C_5-C_6$	163.4	160.1
$N_1-C_5-C_6-O_7$	62.5	58.7
$C_5-C_6-O_7-C_8$	170.9	170.6
$C_6-O_7-C_8-C_9$	−178.2	−178.8
$C_6-O_7-C_8{=}O_{10}$	1.9	1.1

[a] See Figure 1 for the atom numberings. Those related for the C−H bonds are omitted.

Although the *ϕ*($C_6-C_4-C_7-C_8$) dihedral angle has been determined to be −88° with somewhat larger uncertainty (±7° as 3 times *σ*) in the present study, it can still be said that the gas-phase structure is significantly more perpendicular than that of the crystal structure of nicotine-hydroiodide salt (*ϕ*($C_6-C_4-C_7-C_8$) = −123.8°),[5] indicating the serious packing effect of the latter. On the other hand, other structural parameters are found to have almost the same values in the gas and crystal phases with the exception of N−C distances in the *N*-methylpyrrolidine ring. This change in the dihedral angle between the gas and crystal phases brings about the change in the N···N distances (4.885 in the gas and 4.740 Å in the crystal[5]).

To provide a reliable template to construct a nAChR model, it should be the best to determine the in vivo structures of agonists. However, there is no practical experimental method to obtain molecular structures in aqueous solutions, and it can be said that the gas-phase structures provide the best substitutes for them. This is because the gas-phase structures are free from the packing effect of solids that sometimes changes the molecular structure significantly.

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Supporting Information Available: Tables of the leveled total intensities and the backgrounds, definitions of the internal coordinates and the corresponding scale factors, the scaled harmonic force constants, vibrational wavenumbers, mean amplitudes, and the correlation matrix. This material is available free of charge via the Internet at http://pubs.acs.org.

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