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Molecular structure of cotinine studied by gas electron diffraction combined with theoretical calculations

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Abstract

The molecular structure of cotinine ((*S*)-1-methyl-5-(3-pyridinyl)-2-pyrrolidinone), the major metabolite of nicotine, has been determined at about 182˚C by gas electron diffraction combined with MP2 and DFT calculations. The diffraction data are consistent with the existence of the (ax, *sc*), (ax, *ap*), (eq, *sp*) and (eq, *ap*) conformers, where ax and eq indicate the configuration of the pyrrolidinone ring by means of the position (axial and equatorial) of the pyridine ring, and *sc*, *sp* and *ap* distinguish the isomers arising from the internal rotation around the bond connecting the two rings. The $(\mathrm{CH_3})\mathrm{NCCC(N)}$ dihedral angles, ϕ, of the (ax, *sc*) and (eq, *sp*) conformers were determined independently to be 158(12)° and 129(13)°, respectively, where the numbers in parentheses are three times the standard errors, 3σ. According to the MP2 calculations, the corresponding dihedral angles for the (ax, *ap*) and (eq, *ap*) conformers were assumed to differ by 180° from their *syn* counterparts. The ratios x(ax, *sc*) / x(ax, *ap*) and x(eq, *sp*) / x(eq, *ap*) were taken from the theoretically estimated free energy differences, ΔG, where x is the abundance of the conformer. The resultant abundances of (ax, *sc*), (ax, *ap*), (eq, *sp*) and (eq, *ap*) conformers are 34(6)%, 21% (d.p.), 28% (d.p.), and 17% (d.p.), respectively, where d.p. represents dependent parameters.

The determined structural parameters (r_g(Å) and $\angle_\alpha$(°)) of the most abundant conformer, (ax, *sc*), are as follows: $r(\mathrm{N{-}C})_\mathrm{pyrrol}$ = 1.463(5); $r(\mathrm{N{-}C_{methyl}})$ = 1.457(←); $r(\mathrm{N{-}C({=}O)})$= 1.384(12); $r(\mathrm{C{=}O})$= 1.219(5); $<r(\mathrm{C{-}C})_\mathrm{pyrrol}>$ = 1.541(3); $r(\mathrm{C_{pyrrol}{-}C_{pyrid}})$ = 1.521(←); $<r(\mathrm{C{-}C})_\mathrm{pyrid}>$= 1.396(2); $<r(\mathrm{C{-}N})_\mathrm{pyrid}>$ = 1.343(←); $\angle(\mathrm{CNC})_\mathrm{pyrrol}$= 113.9(11); $\angle\mathrm{CCC_{pyrrol}(-C_{pyrid})}$ = 103.6(←); $\angle$NCO = 124.1(13); $\angle\mathrm{NC_{pyrrol}C_{pyrid}}$ = 113.1(12); $\angle\mathrm{C_{pyrrol}C_{pyrrol}C_{pyrid}}$ = 113.3(←); $\angle(\mathrm{CNC})_\mathrm{pyrid}$ = 117.1(2); $<\angle(\mathrm{NCC})_\mathrm{pyrid}>$ = 124.4(←); $\angle\mathrm{C_{methyl}NC({=}O)}$ = $\angle\mathrm{C_{methyl}NC(-C_{pyrid})}$ = 122.8(d.p.); $\angle\mathrm{NC({=}O)C}$ = 107.1(d.p.); $\angle\mathrm{NC_{pyrrol}(-C_{pyrid})C_{pyrrol}}$ = 103.0(d.p.) and $\angle\mathrm{CCC({=}O)}$ =105.2(d.p.), where ← in the parentheses means that the parameter is bound to the preceding one and < > denote average values. The puckering angle, α, of the pyrrolidinone ring is 26(3)°.

The N···N distances of the (ax, *sc*) and (eq, *sp*) conformers, which are 4.844(5) and 4.740(5) Å, respectively, are close to that of the most stable conformer of nicotine, 4.885(6) Å and the corresponding one of arecoline, 4.832(13) Å. It is concluded that the weak nicotinic activity of cotinine cannot be ascribed to such a small difference in the N···N distances.

Keywords: Cotinine, Gas electron diffraction, Molecular structure, Theoretical calculations, Nicotinic activity

1. Introduction

It has long been stated that the distances between the active sites of agonistic substance of the nicotinic acetylcholine receptor (nAChR) are close to that between the N and carbonyl O atoms of acetylcholine [1]. Recently we have determined the molecular structures and conformation of typical nicotinic agonists, nicotine and arecoline, by gas electron diffraction (GED) [2, 3]. In those studies, it was found that at least one of the stable conformers of nicotine and arecoline has indeed the N···N or N···$O_{carbonyl}$ distance close to the N···$O_{carbonyl}$ distance of acetylcholine.

There is another factor ruling the nicotinic activity according to Yamamoto and co-workers who studied the relationship between the activity of nicotine and a number of related compounds and the charge distribution of their N atoms by means of ^{15}N NMR [4]. They stated that the agonist binds strongly with the nAChR of vertebrates if the sp^3 N atom such as that in the five membered ring of nicotine has one unit of positive charge because of protonation. On the other hand, if the N atom is bonded to the electron withdrawing group, it becomes partially positive but cannot be protonated, and consequently, the interaction with the nAChR of vertebrates becomes weak. On the other hand, no discussion is given on the relationship between the active site distance and the nicotinic activity in Ref. [4].

In the present study, the molecular structure and conformation of cotinine ((*S*)-1-methyl-5-(3-pyridinyl)-2-pyrrolidinone, see Fig. 1) is investigated by GED. Cotinine is the major metabolite of nicotine and is also a nicotinic agonist with much lower affinity for the receptor than most of known agonists [5]. This compound is simultaneously present with nicotine *in vivo* environment. Although cotinine is not included in the compounds investigated in Ref. [4], it is clearly classified as the weak agonist of the nAChR of vertebrates according to Yamamoto and co-workers [4]. This is because cotinine has the electron withdrawing C=O group adjacent to the N atom and hence the charge is weaker than the monoprotonated N atom. Therefore it is expected that the structural determination of cotinine will give some information on the factor of the nicotinic activity.

If the N···N distance of cotinine is considerably different from those of the corresponding distances of nicotine and arecoline, the weak nicotinic activity is not necessarily ascribed to the difference in the charge of the N atoms. On the other hand, if the N···N distance of cotinine is close to the corresponding distances of nicotine and arecoline, this may support the conclusion by Yamamoto et al.

In the present study, the MP2 and DFT theoretical calculations are performed to assist the data analysis of GED especially in determining the population of conformers with low relative abundances. Determined structural parameters are compared with those of nicotine and arecoline.

2. Experimental

The sample with a purity of better than 98% was purchased from Sigma Chemical Co. and was used without further purification. Electron diffraction patterns were recorded on 8 × 8 in. Kodak projector slide plates with an apparatus equipped with an r^3–sector [6].

Two camera distances were used to cover the s range of 2.2 to 33.4 Å^{-1}. Because cotinine has very low vapor pressure at room temperature, the nozzle was heated up to about 182 ˚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 optical densities of the plates were measured as written elsewhere [7]. The experimental intensities leveled by theoretical backgrounds are listed in the Table S1 of Supplementary Material. The scale factor was calibrated to the r_a (C=S) distance of CS_2 (1.5570 Å) [8].

Elastic atomic scattering factors were calculated as described in Ref. [9], while inelastic ones were taken from Ref. [10]. The experimental molecular scattering intensities are shown in Fig. 2 with the final fitting curves. 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 region while it was reduced in the small and large s regions by using two gaussian functions [11].

3. Theoretical calculations

3.1. Possible conformers

The conformation of cotinine is classified by the following two factors. First, as the five-membered (pyrrolidinone) ring has an sp^2 atom, it performs the puckering motion like a four-membered ring to make two nonequivalent envelope forms, which are distinguished by means of the position (axial and equatorial) of the pyridine ring. Second, the internal rotation around the C–C bond connecting the rings may cause the rotational isomers, *sp* (or *sc*) and *ap*, whose dihedral angles $(CH_3)NCCC(N)$ differ by about 180° from each other. Consequently, the overall conformation of cotinine is expressed as (ax, *sc*), (ax, *ap*), (eq, *sp*) and (eq, *ap*). These conformers are shown in Fig.

3, where the rotational isomers, *sc*, *sp* and *ap*, are defined by using the H_{18}– C_4– C_7–C_8 dihedral angle.

3.2 Geometrical calculations

GAUSSIAN 98 program [12] was used in all the following calculations. At first, geometries of (ax, *sc*), (ax, *ap*), (eq, *sp*) and (eq, *ap*) conformers were optimized by using the RHF method with the 6-31G** basis set. The geometries of them were further optimized by using the MP2 (frozen core) method with the 6-31G** basis set in order to obtain more reliable structural parameters. The conformational energy differences obtained by the RHF and MP2 calculations are compared in Table 2.

Clearly, the additivity of the energy differences between the (ax)- and (eq)-conformers and those between the *sc* (or *sp*) and *ap* conformers holds. First, on going from the (ax) to (eq)-conformers, energy decreases only slightly in the MP2 calculations, while the difference is much larger in the RHF calculations. Second, the energy differences between *sc* (or *sp*) and *ap* conformers are about 2.4 kJ mol^{-1} according to the MP2 calculations. The potential functions of the internal rotation around the C–C bond connecting the rings were obtained for the (ax) and (eq) conformers by a series of MP2/6-31G** calculations in which the C–C torsional angle was fixed at an interval of 30°. The obtained potential functions (not shown) revealed that no minimum exists other than those corresponding to the above mentioned four conformers.

The structural parameters obtained from the MP2 calculations are listed in Table 3. This table shows that the *sc* (or *sp*) and *ap* conformers have essentially the same structural parameters except for the difference in the dihedral angles, X– C_4– C_7–Y, that represent the internal rotation of the pyridine ring by 180°. The obtained dihedral angles suggest that the N_1, C_3, C_4, C_5 and O_{13} atoms are nearly on the same plane. This can be explained as a result of the sp^2 character of the N_1 and C_3 atoms that forces co-planarity for these atoms. On the other hand, the C_6 atom is positioned out of the plane to reduce the eclipsing of the C–H bonds.

The distances between the O_{13} atom and the H atom in the methyl group (H_{20}) of the four conformers are in the range of 2.40 – 2.41 Å, suggesting the hydrogen bonding between them.

3.3 Vibrational calculations

The geometry optimizations and vibrational calculations for these conformers were carried out with the 6-31G* basis set by using the B3LYP, DFT method, that is more suitable to obtain reliable force constants than the MP2 method. The Cartesian force constants obtained for the (ax, *sc*), (ax, *ap*), (eq, *sp*) and (eq, *ap*) conformers by the B3LYP/6-31G* calculations were transformed into the internal force constants, f_{ij}. The f_{ij}'s for each conformer were then modified by using the linear scaling formula f_{ij} (scaled) = $c\ f_{ij}$ (unscaled) [13]. The scale factor, c, of 0.9243, which is the square of the scaling factor of 0.9614 for the B3LYP/6-31G* frequency recommended by Scott and Radom [14] was used. The definitions of internal coordinates and the scaled force constants are available as Supplementary Material. The B3LYP/6-31G* energy differences between the four conformers are listed in Table 2.

Free energy differences, ΔG, at 455 K were estimated by using the energy differences, ΔE, and the rotational constants obtained from the MP2/6-31G** calculations, combined with the vibrational frequencies calculated by the above mentioned scaled DFT force constants. The obtained ΔG values and the derived abundances are listed in Table 2.

All these calculations using the results of Gaussian 98 were carried out by a series of computer programs made in our laboratory.

4. Analysis of electron diffraction data

For the analysis of electron diffraction data the following assumptions were adopted on the basis of the results of the MP2 *ab initio* calculations: (1) the ratio of the abundances of the (ax, *sc*) and (ax, *ap*), 0.608, and the ratio of the abundances of (eq, *sp*) and (eq, *ap*), 0.584, were adopted from the above mentioned theoretical ΔG values; (2) the dihedral angle, N_1–C_4–C_7–C_8, of the *ap* conformer differs from that of the *sc* (or *sp*) conformer by 180°; (3) the dihedral angle, C_4–N_1–C_3–C_5 is 0° and the O_{13} atom is assumed to be on the $C_4N_1C_3C_5$ plane; (4) the dihedral angle, C_2–N_1–C_3–C_5 is equal to the MP2/6-31G** value; (5) the absolute values of the ring puckering angle α of all the four conformers are equal (see Fig. 4 for the definition of α); (6) the C_2–N_1–C_3 and C_2–N_1–C_4 angles are equal; (7) each H–C–H angle in the pyrrolidinone ring bisects the C–C–C angle; (8) the pyridine ring is planar and each C–H bond in it bisects the N–C–C or C–C–C angle; (9) the C_4–C_7 bond bisects the C_8–C_7–C_{12} angle; (10) three N_1–C_2–H angles are equal to 109.5°, which is the average of the corresponding angles obtained by the MP2/6-31G** calculations; (11) all the C–H bond lengths in the pyrrolidinone ring and the methyl group are equal; (12) the C–H bond lengths in the pyridine ring are equal, and they are smaller than those in the *N*-methylpyrrolidinone group by 0.007 Å; (13) the differences between the similar structural parameters of intra- and inter-conformers are equal to the MP2/6-31G** values. The free energy differences, ΔG, between *sc* (or *sp*) and *ap* conformers had to be fixed (assumption (1)) because it is almost impossible to distinguish two conformers caused by the 180° internal rotation of the pyridine ring precisely [15]. In addition, Table 3 shows that the MP2/6-31G** calculations justify assumption (2). Assumption (3) was adopted because the theoretical dihedral angles, C_4–N_1–C_3–C_5, are zero within ±7° (see Table 3). Assumption (5) was adopted because the absolute values of the theoretical puckering angle, α, are equal to each other within 1.1°. Table 4 summarizes the adjustable parameters and the constraints.

Mean amplitudes, l, and shrinkage corrections [16], $r_a - r_\alpha$, were calculated from the scaled force constants by using a program made in our laboratory. They were fixed at the calculated ones in the data analysis. The model of small amplitude vibrations was adopted. Table S4 of Supplementary Material lists the mean amplitudes with the corresponding r_a distances. The anharmonicity parameters, κ, for bonded atom pairs were estimated as described in Ref. [17]. The adjustable parameters listed in Table 4 were determined by a least-squares calculation on the molecular scattering intensities.

5. Results and discussion

The results obtained by GED are listed in Tables 2, 5 and 6. The radial distribution curve is shown in Fig. 5. The absolute values of correlation coefficients among the independent parameters are less than 0.8. Table 2 shows that the determined populations of four conformers are consistent with those derived from the ΔG values estimated from the MP2 calculations and the scaled force constants of the B3LYP calculations. Table 5 lists the experimental structural parameters (r_g and $\angle_\alpha$ structure) of the most abundant conformer, (ax, *sc*), of cotinine and the corresponding ones given by the MP2 calculation (r_e structure). The differences between the r_g and r_e structures can be estimated using the diatomic approximation [16], $r_g - r_e \approx (3/2)al^2$ and assuming the Morse parameter, a, to be 2.0 Å^{-1}. This term is estimated to be about 0.018 Å for the C–H bonds, 0.007 – 0.010 Å for other single bonds, 0.006-0.007 Å for the bonds in the pyridine ring and 0.004 Å for the C=O bond. Therefore the bond lengths given by the MP2/6-31G** calculations for the single bonds are in agreement with the experimental ones within 3σ. The calculated C=O bond and the bonds in the pyridine ring are longer than the experimental ones by 9σ. The theoretical bond angles and dihedral angles are in moderate agreement with the experimental ones. The determined dihedral angles ϕ (N_1-C_4-C_7-C_8) of the (ax, *sc*) and (eq, *sp*) conformers are 158(12)° and 129(13)°, respectively. This difference is due to the difference in the axial and equatorial positions of the pyridine ring that influences the steric repulsion in the non-bonded H atom pairs such like $H_{18}\cdots H_{22}$ and $H_{21}\cdots H_{25}$.

Table 5 lists the structural parameters of the most abundant conformer of nicotine [2]. The $r_g(N_1\text{-}C_3)$ of cotinine is about 0.08 Å shorter than that of nicotine. The short N-C distance is common to the amide group. The considerable differences in the bond angles between the five-membered ring of cotinine and that of nicotine are due to different conformations of the rings (see Fig. 4).

As written in [1], the N···N distance in a nicotinic agonist is related to its affinity for the receptor (nAChR). The N···N distances of cotinine and nicotine are compared in Table 6. Also the $N\cdots O_{carbonyl}$ distances of arecoline are listed in this table. The N···N distances of cotinine have been determined experimentally for the first time in the present study. In nicotine the N···N distance of the most stable conformer is 4.885 (6) Å, while that of the next stable conformer is 4.275 (7) Å. The $N\cdots O_{carbonyl}$ distances of the Eq-s-*cis* and Eq-s-*trans* conformers of arecoline are 4.832(13) and 4.265(9) Å, respectively [3], and it was concluded that the major conformer with the longer $N\cdots O_{carbonyl}$ distance (Eq-s-*cis*) has the nicotinic activity. In cotinine the N···N distance of the most stable and the next stable conformers were found to be 4.844 (5) and 4.740 (5) Å, respectively, suggesting that the both of these conformers are those with the nicotinic activity.

Although these three molecules have conformers with the N···N or N···O atom pairs whose distances fall in the range of 4.7 – 4.9 Å, only cotinine has weaker nicotinic activity than other two. Therefore, our conclusion is that the weak nicotinic activity of cotinine cannot be attributed to the difference in the N···N or N···O distance. As mentioned in the Introduction, other explanation for the nicotinic activity by means of the difference in the charge on the sp^3 N atom has been proposed [4]. In order to investigate the relationship between the charge distribution and the nicotinic activity, the natural population analyses [18] for cotinine and protonated nicotine were carried out. They provided a negative charge on the N_1 atom of these species with that of cotinine slightly more negative (-0.49 *e* for cotinine and –0.44 *e* for protonated nicotine). This result seems consistent with what Yamamoto et al. have suggested in Ref. [4] only qualitatively, and it cannot be concluded if such a small difference in the atomic charge explains the difference in the nicotinic activity. Therefore, other explanation such as one by means of a formation of hydrogen bond between the carbonyl O and methyl H atoms cannot be ruled out at this stage.

6. Supplementary material

Tables of the leveled total intensities and the backgrounds, the local symmetry coordinates, the scaled force constants, mean amplitudes and r_a distances of the (ax, *sc*) conformer, the correlation matrix are documented.

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Table 1

Experimental conditions for gas electron diffraction for cotinine

	Short	Long
Camera distance (mm)	244.3	485.6
Nozzle temperature (K)	455	459
Electron wavelength (Å)	0.06334	0.06380
Uncertainty in the scale factor (3σ) (%)	0.06	0.06
Background pressure during exposure (10^{-6} Torr)	2.5	2.9
Beam current (μA)	1.4	1.4
Exposure time (s)	90 – 123	47 – 58
Number of plates used	3	4
Range of s value (Å^{-1})	6.1 – 33.4	2.2 – 17.4

Table 2

Relative energies ΔE and ΔG and estimated abundances x for the possible conformers of cotinine obtained from RHF/6-31G**, MP2/6-31G** and B3LYP/6-31G* calculations and GED

Conf. [a]	ΔE / kJ mol^{-1}			ΔG / kJ mol^{-1} [b]	x / %	
	RHF/6-31G**	MP2/6-31G**	B3LYP/6-31G*		theor. [c]	GED [d]
(ax *sc*)	2.17	0.53	2.51	0.00	34.6	34 (6)
(ax *ap*)	4.44	2.95	4.67	1.89	21.0	21 [e]
(eq, *sp*)	0.00 [f]	0.00 [g]	0.00 [h]	0.79	28.0	28 [e]
(eq, *ap*)	2.26	2.30	2.03	2.83	16.4	17 [e]

[a] See Fig. 3 for the definitions of the conformers.

[b] Theoretical values. Estimated by using the energy differences, ΔE, and the rotational constants obtained from the MP2/6-31G** calculations, combined with the vibrational frequencies calculated by the scaled B3LYP/6-31G* force constants.

[c] Estimated from the theoretical ΔG values at 455 K.

[d] Number in parentheses is three times the standard error (3σ) referring to the last significant digit.

[e] Dependent parameters.

[f] Absolute energy is –569.4719683 E_h.

[g] Absolute energy is –571.3202672 E_h.

[h] Absolute energy is –573.0331144 E_h.

Table 3

Geometrical parameters of the (ax, *sc*), (ax, *ap*), (eq, *sp*) and (eq, *ap*) conformers of cotinine obtained from the MP2(frozen core)/6-31G** calculations [a]

Parameters	(ax, *sc*)	(ax, *ap*)	(eq, *sp*)	(eq, *ap*)
Bond lengths (Å)				
$N_1–C_2$	1.446	1.446	1.448	1.448
$N_1–C_3$	1.376	1.377	1.380	1.380
$N_1–C_4$	1.451	1.450	1.456	1.455
$C_3–C_5$	1.523	1.523	1.522	1.522
$C_4–C_6$	1.546	1.546	1.542	1.542
$C_5–C_6$	1.527	1.527	1.526	1.526
$C_3{=}O_{13}$	1.228	1.228	1.227	1.227
$C_4–C_7$	1.513	1.513	1.503	1.504
$C_8–N_9$	1.343	1.345	1.344	1.343
$N_9–C_{10}$	1.345	1.344	1.344	1.346
$C_7–C_8$	1.400	1.400	1.398	1.401
$C_{10}–C_{11}$	1.395	1.396	1.397	1.395
$C_{11}–C_{12}$	1.393	1.392	1.391	1.394
$C_{12}–C_7$	1.398	1.398	1.399	1.396
$C_2–H_{19}$	1.090	1.090	1.093	1.093
$C_2–H_{20}$	1.087	1.087	1.087	1.087
$C_2–H_{21}$	1.092	1.092	1.089	1.089
$C_4–H_{18}$	1.095	1.096	1.101	1.101
$C_5–H_{14}$	1.088	1.088	1.092	1.092
$C_5–H_{15}$	1.092	1.092	1.088	1.088
$C_6–H_{16}$	1.090	1.091	1.089	1.089

C_6–H_{17}	1.089	1.089	1.091	1.090
C_8–H_{22}	1.087	1.085	1.086	1.085
C_{10}–H_{23}	1.084	1.084	1.084	1.084
C_{11}–H_{24}	1.082	1.082	1.082	1.082
C_{12}–H_{25}	1.084	1.085	1.084	1.084
$N_1 \cdots N_9$	4.859	4.224	4.795	4.243
Bond angles (degree)				
C_2–N_1–C_3	122.3	122.3	121.2	121.1
C_2–N_1–C_4	122.4	122.5	121.7	121.8
C_3–N_1–C_4	114.0	114.1	113.4	113.3
N_1–C_3–C_5	107.0	106.9	107.1	107.2
N_1–C_3=O_{13}	125.6	125.6	125.5	125.5
C_5–C_3=O_{13}	127.4	127.5	127.3	127.3
N_1–C_4–C_6	101.9	101.9	102.0	102.0
N_1–C_4–C_7	112.6	113.1	112.3	112.6
C_6–C_4–C_7	112.9	112.8	113.4	113.4
C_3–C_5–C_6	104.1	104.0	104.1	104.1
C_5–C_6–C_4	103.7	103.7	103.9	103.8
C_4–C_7–C_8	120.0	121.9	121.0	120.6
C_4–C_7–C_{12}	122.2	120.5	121.1	121.5
C_8–C_7–C_{12}	117.7	117.6	117.9	117.9
C_7–C_8–N_9	124.5	124.1	124.3	124.1
C_8–N_9–C_{10}	116.7	117.0	116.7	116.9
N_9–C_{10}–C_{11}	123.5	123.5	123.6	123.5
C_{10}–C_{11}–C_{12}	118.9	118.6	118.8	118.7
C_{11}–C_{12}–C_7	118.7	119.2	118.7	118.9
N_1–C_2–H_{19}	110.3	110.4	110.9	110.8

N_1–C_2–H_{20}	107.2	107.2	107.2	107.1
N_1–C_2–H_{21}	111.1	111.1	110.6	110.6
H_{19}–C_2–H_{20}	109.8	109.8	109.4	109.4
H_{19}–C_2–H_{21}	108.9	109.0	108.9	108.9
H_{20}–C_2–H_{21}	109.5	109.5	109.9	109.9
N_1–C_4–H_{18}	109.8	109.7	110.1	110.0
C_6–C_4–H_{18}	110.8	110.8	110.2	110.1
C_7–C_4–H_{18}	108.7	108.5	108.7	108.5
C_3–C_5–H_{14}	110.4	110.4	107.5	107.5
C_3–C_5–H_{15}	108.1	108.0	110.8	110.7
H_{14}–C_5–H_{15}	107.7	107.8	107.7	107.8
C_6–C_5–H_{14}	114.3	114.3	111.9	111.9
C_6–C_5–H_{15}	112.1	112.1	114.6	114.6
C_5–C_6–H_{16}	110.0	110.0	114.3	114.3
C_5–C_6–H_{17}	114.1	114.0	110.4	110.4
H_{16}–C_6–H_{17}	108.5	108.5	108.1	108.2
C_4–C_6–H_{16}	108.7	108.8	111.5	111.5
C_4–C_6–H_{17}	111.7	111.7	108.5	108.5
C_7–C_8–H_{22}	119.8	120.2	119.8	119.7
N_9–C_8–H_{22}	115.7	115.7	115.9	116.2
N_9–C_{10}–H_{23}	115.9	115.9	115.9	115.9
C_{11}–C_{10}–H_{23}	120.6	120.6	120.5	120.6
C_{10}–C_{11}–H_{24}	120.1	120.3	120.1	120.2
C_{12}–C_{11}–H_{24}	120.9	121.1	121.1	121.1
C_{11}–C_{12}–H_{25}	120.7	120.7	121.4	120.9
C_7–C_{12}–H_{25}	120.5	120.1	119.9	120.2
α^{b}	28.2	28.9	–27.9	–27.8

Dihedral angles (degree)				
C_5–C_3–N_1–C_4	5.5	4.2	–6.7	–7.0
C_6–C_5–C_3–N_1	14.4	15.6	–13.4	–13.2
C_4–C_6–C_5–C_3	–27.2	–27.9	26.9	26.9
N_1–C_4–C_6–C_5	29.7	29.7	–30.1	–30.3
C_3–N_1–C_4–C_6	–22.6	–21.7	23.5	23.8
C_2–N_1–C_4–C_6	170.6	170.3	–178.0	–177.7
C_5–C_3–N_1–C_2	172.4	172.3	–165.3	–165.6
O_{13}=C_3–N_1–C_2	–6.3	–6.4	13.3	12.9
O_{13}=C_3–N_1–C_4	–173.2	–174.5	171.9	171.5
C_6–C_5–C_3=O_{13}	–166.9	–165.8	168.0	168.3
C_3–N_1–C_4–C_7	98.7	99.7	145.3	145.7
C_5–C_6–C_4–C_7	–91.3	–91.9	–151.1	–151.7
N_1–C_4–C_7–C_8	151.9	–29.0	137.0	–43.7
C_6–C_4–C_7–C_8	–93.3	86.0	–108.0	71.5
N_1–C_4–C_7–C_{12}	–30.6	153.7	–45.0	138.6
C_6–C_4–C_7–C_{12}	84.2	–91.3	70.1	–106.2
H_{18}–C_4–C_7–C_8	30.1	–150.9	14.9	–165.8

[a] See Fig. 3 for the atom numbering and the definitions of the conformers.

[b] See Fig. 4 for the definition.

Table 4

Structural parameters and constraints for the (ax, *sc*), (ax, *ap*), (eq, *sp*) and (eq, *ap*) conformers of cotinine [a]

Parameter	(ax, *sc*)	(ax, *ap*)	(eq, *sp*)	(eq, *ap*)
Bond lengths (Å)				
N_1–C_2	r_1	r_1	r_1 + 0.002	r_1 + 0.002
N_1–C_4	r_1 + 0.005	r_1 + 0.005	r_1 + 0.010	r_1 + 0.010
N_1–C_3	r_2	r_2 + 0.001	r_2 + 0.004	r_2 + 0.004
C_3–C_5	r_3	r_3	r_3 – 0.001	r_3 – 0.001
C_4–C_6	r_3 + 0.023	r_3 + 0.023	r_3 + 0.019	r_3 + 0.019
C_5–C_6	r_3 + 0.004	r_3 + 0.004	r_3 + 0.003	r_3 + 0.003
C_4–C_7	r_3 – 0.011	r_3 – 0.010	r_3 – 0.020	r_3 – 0.019
C_3=O_{13}	r_4	r_4 – 0.001	r_4 – 0.001	r_4 – 0.001
C_8–N_9	r_5	r_5 + 0.002	r_5 + 0.001	r_5
N_9–C_{10}	r_5 + 0.003	r_5 + 0.001	r_5 + 0.001	r_5 + 0.003
C_7–C_8	r_5 + 0.057	r_5 + 0.058	r_5 + 0.055	r_5 + 0.059
C_{10}–C_{11}	r_5 + 0.052	r_5 + 0.053	r_5 + 0.054	r_5 + 0.052
C_{11}–C_{12}	r_5 + 0.051	r_5 + 0.049	r_5 + 0.049	r_5 + 0.051
C_7–C_{12}	r_5 + 0.055	r_5 + 0.055	r_5 + 0.056	r_5 + 0.053
C–H_{pyrro}	r_6	r_6	r_6	r_6
C–H_{pyrid}	r_6 – 0.007	r_6 – 0.007	r_6 - 0.007	r_6 – 0.007
Bond angles (degree)				

C_3–N_1–C_4	θ_1	$\theta_1 + 0.1$	$\theta_1 - 0.6$	$\theta_1 - 0.6$
C_4–C_6–C_5	$\theta_1 - 10.2$	$\theta_1 - 10.3$	$\theta_1 - 10.1$	$\theta_1 - 10.2$
α[b]	θ_2	θ_2	$-\theta_2$	$-\theta_2$
C–C–H_{methyl}	109.5	109.5	109.5	109.5
H–C–$H_{\mathrm{methylene}}$	θ_3	θ_3	θ_3	θ_3
N_1–C_4–C_7	θ_4	$\theta_4 + 0.4$	$\theta_4 - 0.3$	θ_4
C_6–C_4–C_7	$\theta_4 + 0.3$	$\theta_4 + 0.2$	$\theta_4 + 0.8$	$\theta_4 + 0.7$
C_8–N_9–C_{10}	θ_5	$\theta_5 + 0.3$	$\theta_5 + 0.1$	$\theta_5 + 0.2$
C_7–C_8–N_9	$\theta_5 + 7.8$	$\theta_5 + 7.4$	$\theta_5 + 7.6$	$\theta_5 + 7.4$
N_9–C_{10}–C_{11}	$\theta_5 + 6.8$	$\theta_5 + 6.8$	$\theta_5 + 6.9$	$\theta_5 + 6.9$
N_1–C_3=O_{13}	θ_6	θ_6	$\theta_6 - 0.1$	θ_6
Dihedral angles (degree)				
N_1–C_4–C_7–C_8	ϕ_1	$\phi_1 + 180.0$	ϕ_2	$\phi_2 + 180.0$
C_2–N_1–C_3–C_5	172.4	172.3	– 165.3	– 165.6

[a] See Fig. 3 for the atom numbering and the definitions of the conformers.

[b] See Fig. 4 for the definition.

Table 5

Molecular structures of cotinine and nicotine

Parameters[a]	Cotinine[b] (ax, *sc*)			Nicotine[c] (*eq*, *eq*, *syn*)	
	ED (r_g and $\angle_\alpha$) [d]		MP2/6-31G**(r_e)	ED (r_g and $\angle_\alpha$) [d]	
Bond lengths (Å)					
N_1–C_2	1.457	(5)	1.446	1.456	(3)
N_1–C_4	1.463		1.451	1.467	
N_1–C_3	1.384	(12)	1.376	1.464	
C_3–C_5	1.532	(3)	1.523	1.534	(4)
C_4–C_6	1.555		1.546	1.541	
C_5–C_6	1.536		1.527	1.547	
C_4–C_7	1.521		1.513	1.502	
C_3=O_{13}	1.219	(5)	1.228		
C_8–N_9	1.342	(2)	1.343	1.354	(3)
C_{10}–N_9	1.344		1.345	1.344	
C_7–C_8	1.399		1.400	1.398	
C_{10}–C_{11}	1.394		1.395	1.397	
C_{11}–C_{12}	1.393		1.393	1.392	
C_7–C_{12}	1.397		1.398	1.399	
C–H_{pyrrol}	1.109	(5)	1.091 [e]	1.116	(3)
C–H_{pyrid}	1.102		1.084 [e]	1.116	
Bond angles and dihedral angles (degree)					
C_3–N_1–C_4	113.9	(11)	114.0	108.4 (15)	
C_4–C_6–C_5	103.6		103.7	108.6 [f]	
C_2–N_1–C_3	122.8 [f]		122.3	114.1 [f]	

C_2–N_1–C_4	122.8 [f]		122.4	114.1 [f]	
N_1–C_3–C_5	107.1 [f]		107.0	103.4 [f]	
N_1–C_3=O_{13}	124.1	(13)	125.6		
N_1–C_4–C_6	103.0 [f]		101.9	101.3 [f]	
N_1–C_4–C_7	113.1	(12)	112.6	116.8	(10)
C_6–C_4–C_7	113.3		112.9	115.8	(20)
C_3–C_5–C_6	105.2 [f]		104.1	107.7 [f]	
C_8–N_9–C_{10}	117.1	(2)	116.7	116.8	(2)
C_7–C_8–N_9	124.9		124.5	124.5	
N_9–C_{10}–C_{11}	123.9		123.5	123.7	
C_8–C_7–C_{12}	115.8 [f]		117.7	117.1 [f]	
C_{10}–C_{11}–C_{12}	117.4 [f]		118.9	118.3 [f]	
C_7–C_{12}–C_{11}	120.9 [f]		118.7	119.5 [f]	
H–C–$H_{methylene}$	108.4	(57)	108.1 [e]	111.9	(41)
N_1–C_2–H	109.5	(fix)	109.5 [e]	113.5	(fix)
α [g]	26.4	(33)	28.2	32.9	(38)
C_6–C_4–C_7–C_8 [h]	–86 [f]		–93.3	–87.7	(74)
N_1–C_4–C_7–C_8 [h]	158	(12)	151.9	153.1 [f]	
C_6–C_4–C_7–C_8 [i]	–114 [f]		–108.0		
N_1–C_4–C_7–C_8 [i]	129	(13)	137.0		
Indices of resolution [j]					
k (long)	0.941	(12)			
k (short)	0.901	(22)			

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[a] See Fig. 3 for the atom numberings. Angle brackets denote average values.

[b] Present work. See Fig. 3 for the definitions of the conformers. [c] See Ref. [2].

[d] Numbers in parentheses are three times the standard errors (3σ) referring to the last significant digit. [e] Average values [f] Dependent parameters. [g] Defined as the dihedral angle between the $C_4C_3C_5$ and $C_4C_6C_5$ planes (see Fig. 4). [h] (ax, *sc*) conformer. [i] (eq, *sp*) conformer. [j] The index of resolution, k, is defined as $sM(s)^{obs} = ksM(s)^{calc}$.

Table 6

The N$\cdots$N or N$\cdots$O$_{\mathrm{carbonyl}}$ distance and the abundance x of each conformer of cotinine, nicotine and arecoline.

Cotinine [a]			Nicotine [b]			Arecoline [c]		
Conf.[d]	N$\cdots$N /Å	x / %	Conf.	N$\cdots$N /Å	x /%	Conf.	N$\cdots$O /Å [e]	x / %
(ax, *sc*)	4.844(5)	34(6)	(*eq*, *eq*, *syn*)	4.885(6)	67 [f]	Eq-s-*cis*	4.832(13)	46(16)
(ax, *ap*)	4.198(5)	21 [g]	(*eq*, *eq*, *anti*)	4.275(7)	33 [f]	Eq-s-*trans*	4.265(9)	41 [g]
(eq, *sp*)	4.740(5)	28 [g]				Ax-s-*cis*	4.874(16)	7 [f]
(eq, *ap*)	4.299(5)	17 [g]				Ax-s-*trans*	4.289(12)	6 [f]

Numbers in parentheses are three times the standard errors (3σ) referring to the last significant digit. [a] The present study. [b] Ref. [2]. [c] Ref. [3]. [d] See Fig. 3 for the definitions of the conformers. [e] N$\cdots$O$_{\mathrm{carbonyl}}$ distances. [f] Assumed. [g] Dependent parameters.

Figure captions

Fig. 1. Chemical structures of cotinine and nicotine.

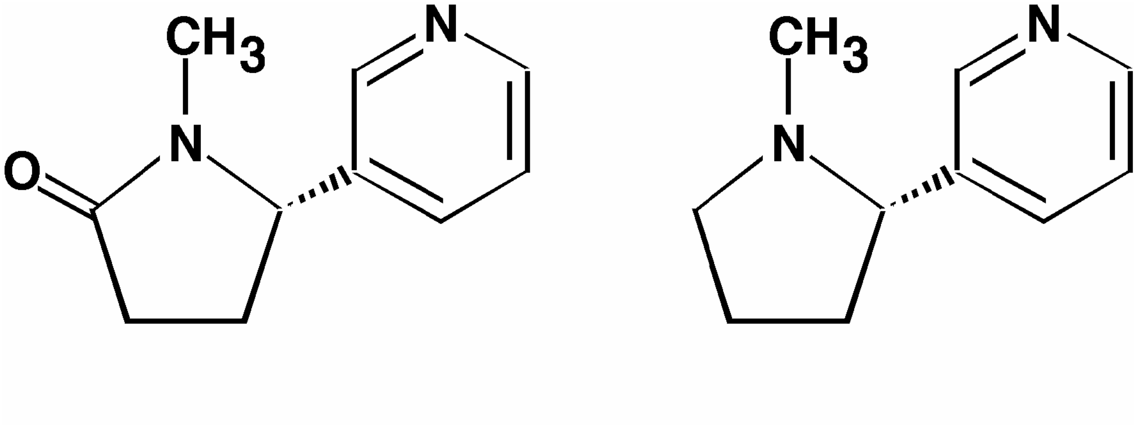
Fig. 2. Experimental (dots) and theoretical (solid curve) molecular scattering intensities of cotinine; $\Delta sM(s) = sM(s)^{\mathrm{obs}} - sM(s)^{\mathrm{calc}}$. The theoretical curves were calculated from the best fitting parameters.

Fig. 3. Molecular models and atom numbering for the four possible conformers of cotinine. The abbreviations of the conformer, such as (ax, *sc*) represent the positions of the pyridine ring and the H_{18}–C_4–C_7–C_8 dihedral angle, respectively.

Fig. 4. The ring puckering angle α of cotinine and nicotine. The angle α of cotinine is defined as the dihedral angle between the $C_4C_3C_5$ and $C_4C_6C_5$ planes. The line a-b is on the $C_4C_3C_5$ plane and perpendicular to the C_4-C_5 bond. For nicotine, α is the dihedral angle between the $C_3C_5C_6C_4$ and $C_3N_1C_4$ planes.

Fig. 5. Experimental radial distribution curve of cotinine; $\Delta f(r) = f(r)^{\mathrm{obs}} - f(r)^{\mathrm{calc}}$. The conformational composition of (ax, *sc*), (ax, *ap*), (eq, *sp*) and (eq, *ap*) used was 34%, 21%, 28% and 17%, respectively. Distance distributions are indicated by vertical bars. Those for nonbonded C···H, N···H and H···H pairs are not shown for simplicity.

Fig. 1



Cotinine

Nicotine

Fig. 2

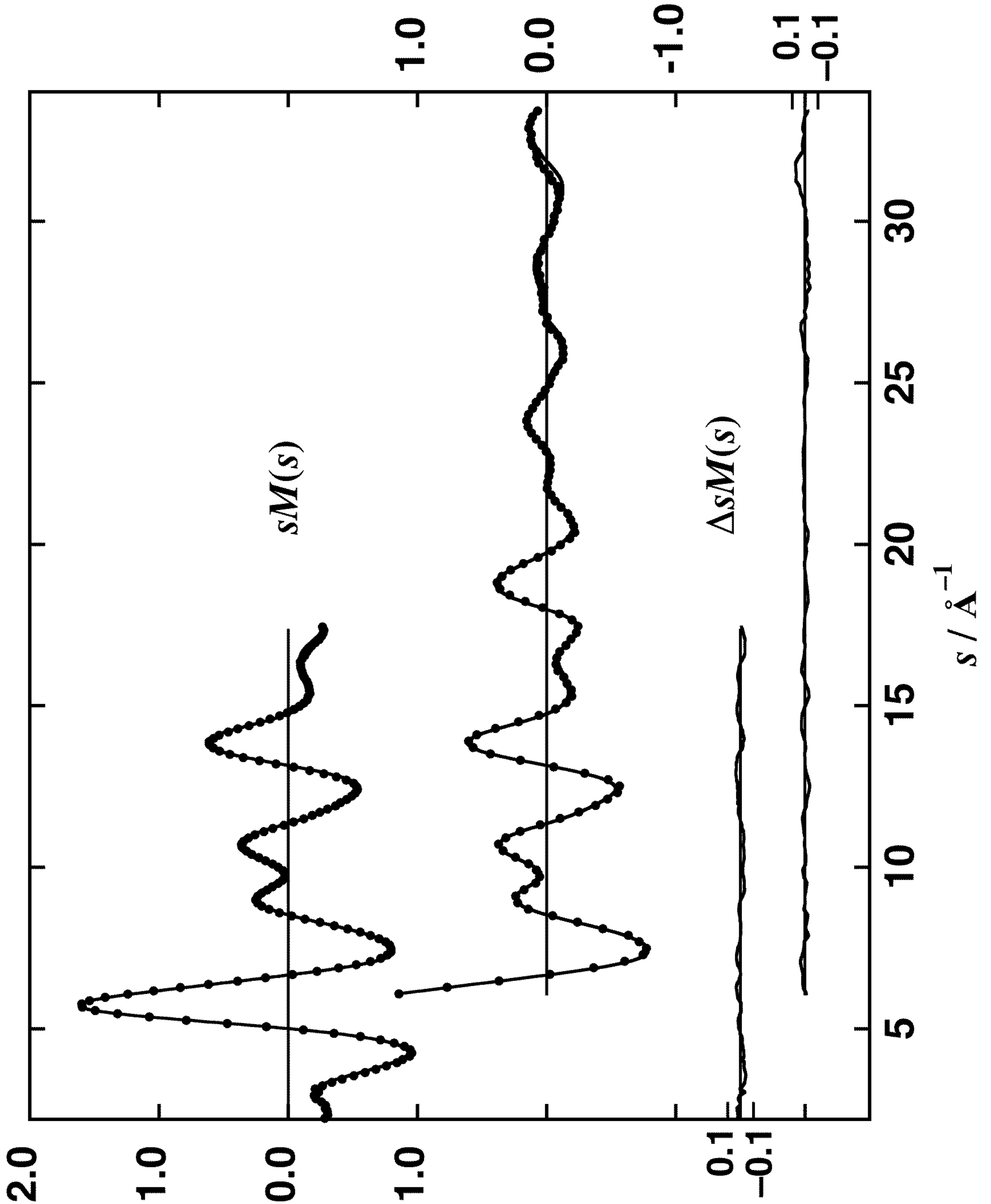
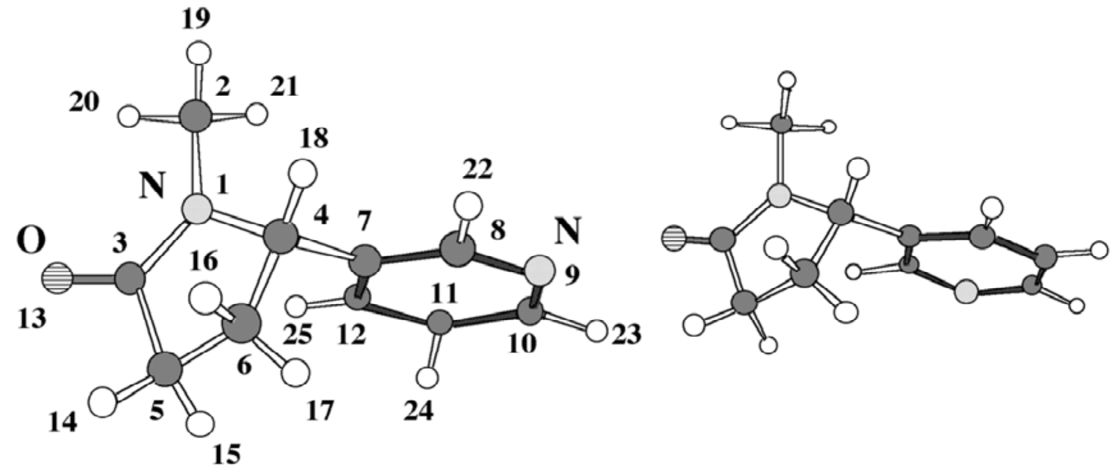
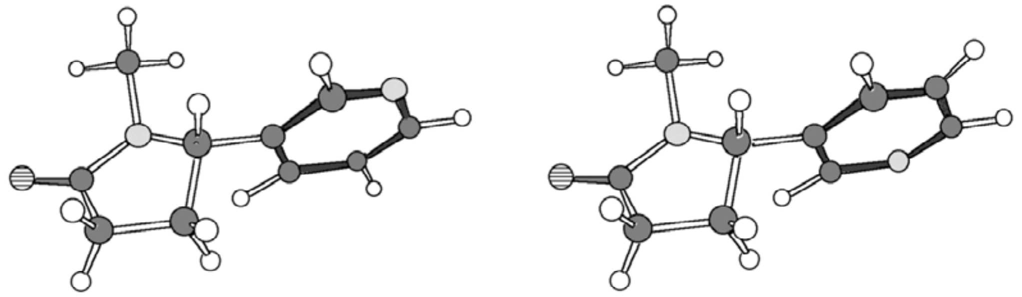


Fig. 3



(ax, *sc*)

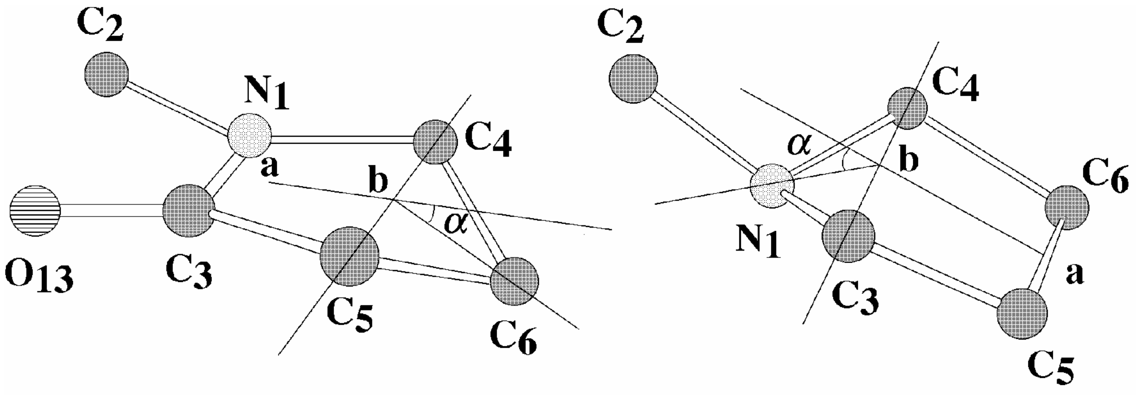
(ax, *ap*)



(eq, *sp*)

(eq, *ap*)

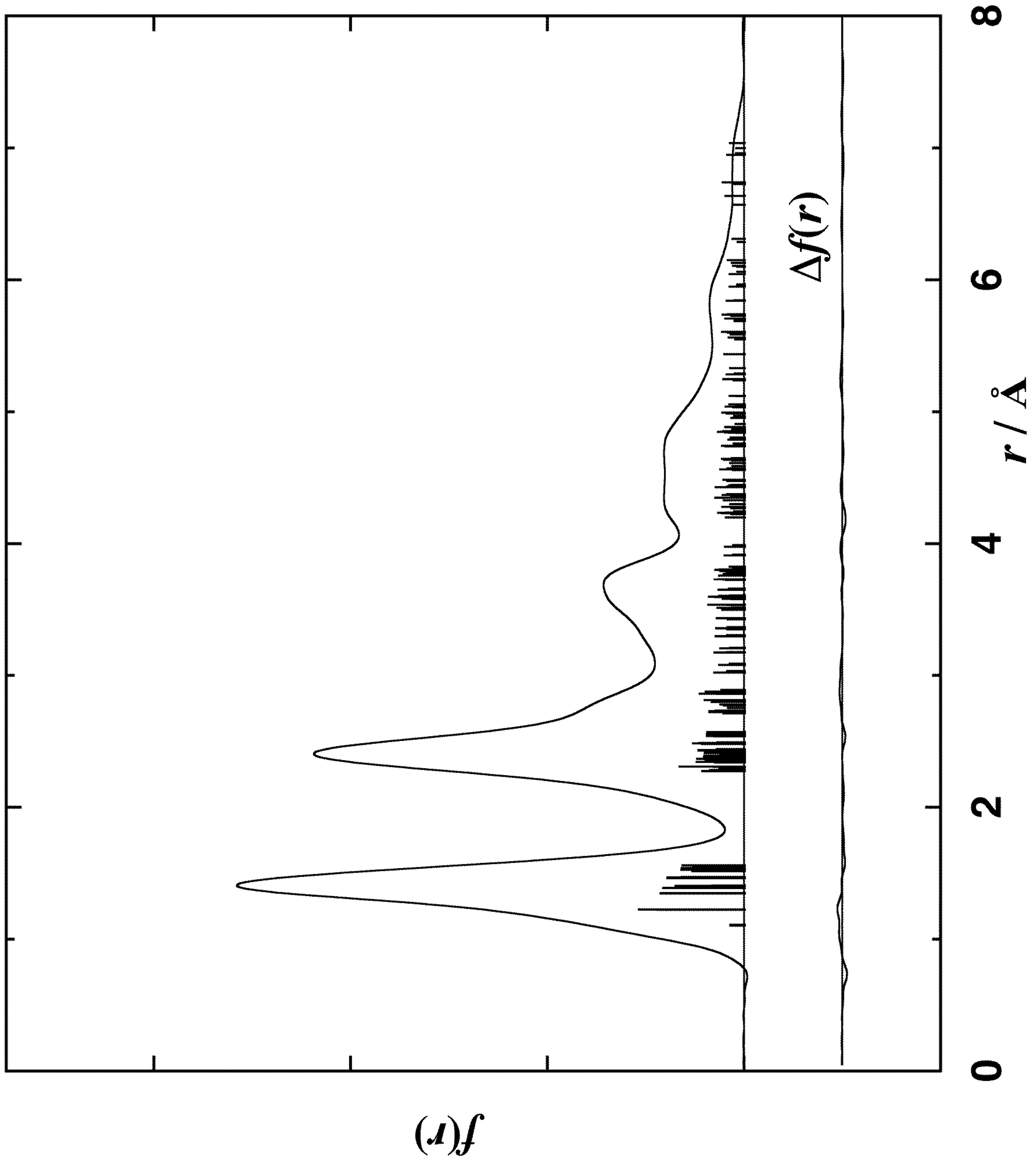
Fig. 4



Cotinine

Nicotine

Fig. 5



Molecular structure of cotinine studied by gas electron diffraction combined with theoretical calculations

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Supplementary Material

Table S1	The leveled total intensities (I_T) and the backgrounds (I_B) for cotinine.
Table S2	Definitions of internal coordinates of cotinine.
Table S3	The scaled harmonic force constants of the (eq, *sp*) conformer of cotinine.
Table S4	r_a Distances and mean amplitudes of the (ax, *sc*) conformer of cotinine (Å).
Table S5	The correlation matrix for cotinine.

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Table S1: The leveled total intensities (I_T) and the backgrounds (I_B) for cotinine [a]

Short camera distance

s	I_T	I_B	s	I_T	I_B	s	I_T	I_B
6.081	1.319	1.111	15.684	0.983	0.993	24.964	1.015	1.016
6.283	1.241	1.106	15.881	0.984	0.992	25.153	1.016	1.017
6.485	1.159	1.097	16.078	0.986	0.992	25.341	1.016	1.019
6.687	1.082	1.086	16.275	0.987	0.991	25.529	1.017	1.020
6.889	1.020	1.077	16.472	0.986	0.991	25.717	1.017	1.022
7.091	0.978	1.069	16.668	0.984	0.990	25.905	1.018	1.023
7.293	0.955	1.063	16.865	0.981	0.990	26.093	1.020	1.025
7.494	0.950	1.059	17.061	0.979	0.990	26.280	1.022	1.026
7.696	0.957	1.055	17.258	0.976	0.990	26.468	1.025	1.028
7.897	0.967	1.051	17.454	0.976	0.989	26.655	1.028	1.029
8.099	0.992	1.048	17.650	0.978	0.989	26.841	1.031	1.031
8.300	1.015	1.045	17.845	0.984	0.989	27.028	1.033	1.033
8.502	1.036	1.042	18.041	0.991	0.989	27.214	1.036	1.034
8.703	1.056	1.039	18.236	0.998	0.989	27.400	1.037	1.036
8.904	1.063	1.037	18.432	1.005	0.989	27.586	1.039	1.038
9.105	1.062	1.035	18.627	1.009	0.990	27.772	1.041	1.040
9.306	1.052	1.033	18.822	1.010	0.990	27.957	1.042	1.041
9.507	1.041	1.031	19.016	1.008	0.990	28.142	1.045	1.043
9.708	1.035	1.030	19.211	1.005	0.990	28.327	1.047	1.045
9.909	1.036	1.028	19.405	1.000	0.991	28.512	1.050	1.047
10.109	1.041	1.026	19.600	0.994	0.991	28.697	1.051	1.049
10.310	1.049	1.025	19.794	0.989	0.991	28.881	1.053	1.051
10.511	1.056	1.023	19.988	0.986	0.992	29.065	1.054	1.053
10.711	1.057	1.022	20.181	0.983	0.992	29.249	1.056	1.055
10.911	1.050	1.020	20.375	0.982	0.993	29.432	1.057	1.057
11.111	1.038	1.019	20.568	0.983	0.993	29.616	1.058	1.059
11.311	1.022	1.017	20.762	0.985	0.994	29.799	1.059	1.061
11.511	1.007	1.016	20.955	0.987	0.994	29.981	1.061	1.063
11.711	0.993	1.015	21.147	0.990	0.995	30.164	1.063	1.065
11.911	0.981	1.013	21.340	0.993	0.996	30.346	1.064	1.067
12.111	0.972	1.012	21.533	0.995	0.996	30.529	1.067	1.069
12.310	0.965	1.010	21.725	0.997	0.997	30.710	1.068	1.072
12.510	0.964	1.009	21.917	0.998	0.998	30.892	1.071	1.074
12.709	0.970	1.008	22.109	0.998	0.999	31.074	1.073	1.076
12.908	0.983	1.006	22.301	0.998	1.000	31.255	1.077	1.078
13.107	1.001	1.005	22.492	0.999	1.001	31.436	1.080	1.081
13.306	1.019	1.004	22.683	1.000	1.001	31.616	1.084	1.083
13.505	1.035	1.003	22.875	1.003	1.002	31.797	1.087	1.086
13.704	1.043	1.002	23.066	1.005	1.003	31.977	1.090	1.088
13.902	1.044	1.000	23.256	1.008	1.005	32.157	1.093	1.090
14.101	1.038	0.999	23.447	1.011	1.006	32.336	1.097	1.093
14.299	1.026	0.998	23.637	1.013	1.007	32.516	1.099	1.096
14.497	1.012	0.997	23.827	1.015	1.008	32.695	1.102	1.098
14.695	1.000	0.996	24.017	1.015	1.009	32.874	1.105	1.101
14.893	0.991	0.996	24.207	1.015	1.010	33.053	1.108	1.104
15.091	0.985	0.995	24.397	1.015	1.012	33.231	1.110	1.107
15.289	0.981	0.994	24.586	1.015	1.013	33.409	1.112	1.110
15.486	0.981	0.993	24.775	1.015	1.014			

Long camera distance

s	I_T	I_B	s	I_T	I_B	s	I_T	I_B
2.230	0.759	0.869	7.386	0.912	1.022	12.497	0.938	0.980
2.332	0.760	0.874	7.487	0.912	1.021	12.597	0.940	0.980
2.433	0.771	0.880	7.588	0.914	1.019	12.696	0.944	0.979
2.534	0.787	0.887	7.689	0.918	1.018	12.796	0.950	0.978
2.636	0.797	0.895	7.789	0.925	1.017	12.895	0.957	0.978
2.737	0.819	0.904	7.890	0.934	1.016	12.995	0.965	0.977
2.838	0.844	0.913	7.991	0.944	1.015	13.094	0.973	0.977
2.940	0.859	0.922	8.091	0.956	1.013	13.194	0.983	0.976
3.041	0.859	0.931	8.192	0.968	1.012	13.293	0.992	0.975
3.142	0.878	0.941	8.293	0.982	1.011	13.392	1.001	0.975
3.243	0.872	0.950	8.393	0.995	1.010	13.492	1.008	0.974
3.345	0.862	0.959	8.494	1.006	1.009	13.591	1.013	0.974
3.446	0.851	0.968	8.594	1.016	1.008	13.690	1.015	0.973
3.547	0.836	0.976	8.695	1.025	1.007	13.789	1.017	0.973
3.648	0.823	0.984	8.795	1.030	1.007	13.888	1.016	0.972
3.750	0.812	0.991	8.896	1.033	1.006	13.988	1.013	0.972
3.851	0.801	0.998	8.996	1.033	1.005	14.087	1.009	0.971
3.952	0.791	1.005	9.097	1.030	1.004	14.186	1.003	0.971
4.053	0.790	1.010	9.197	1.026	1.003	14.285	0.998	0.970
4.155	0.786	1.016	9.297	1.020	1.002	14.384	0.991	0.970
4.256	0.791	1.021	9.398	1.015	1.002	14.483	0.984	0.970
4.357	0.803	1.025	9.498	1.009	1.001	14.582	0.979	0.969
4.458	0.821	1.029	9.599	1.005	1.000	14.681	0.973	0.969
4.559	0.846	1.032	9.699	1.002	0.999	14.779	0.969	0.969
4.660	0.876	1.035	9.799	1.002	0.999	14.878	0.965	0.968
4.762	0.915	1.037	9.899	1.002	0.998	14.977	0.962	0.968
4.863	0.964	1.039	10.000	1.005	0.997	15.076	0.960	0.968
4.964	1.016	1.041	10.100	1.008	0.997	15.175	0.959	0.968
5.065	1.077	1.042	10.200	1.013	0.996	15.273	0.958	0.968
5.166	1.138	1.043	10.300	1.017	0.995	15.372	0.957	0.968
5.267	1.200	1.044	10.400	1.021	0.994	15.471	0.958	0.968
5.368	1.253	1.044	10.500	1.024	0.994	15.569	0.958	0.968
5.469	1.296	1.044	10.601	1.026	0.993	15.668	0.959	0.969
5.570	1.324	1.044	10.701	1.026	0.992	15.766	0.960	0.969
5.671	1.336	1.043	10.801	1.023	0.991	15.865	0.962	0.970
5.772	1.332	1.043	10.901	1.019	0.991	15.963	0.963	0.970
5.873	1.315	1.042	11.001	1.013	0.990	16.062	0.964	0.971
5.974	1.288	1.041	11.101	1.006	0.989	16.160	0.965	0.971
6.075	1.253	1.040	11.201	0.999	0.989	16.258	0.966	0.972
6.176	1.214	1.039	11.301	0.991	0.988	16.357	0.967	0.973
6.277	1.176	1.038	11.400	0.984	0.987	16.455	0.967	0.974
6.378	1.137	1.036	11.500	0.976	0.987	16.553	0.967	0.974
6.479	1.097	1.035	11.600	0.970	0.986	16.651	0.967	0.975
6.580	1.061	1.033	11.700	0.965	0.985	16.749	0.966	0.976
6.681	1.027	1.032	11.800	0.959	0.985	16.847	0.966	0.976
6.782	0.997	1.031	11.899	0.954	0.984	16.946	0.965	0.977
6.882	0.971	1.029	11.999	0.950	0.983	17.044	0.964	0.978
6.983	0.950	1.028	12.099	0.946	0.983	17.142	0.963	0.978
7.084	0.934	1.026	12.199	0.942	0.982	17.240	0.963	0.979
7.185	0.923	1.025	12.298	0.940	0.982	17.337	0.964	0.980
7.286	0.916	1.023	12.398	0.938	0.981	17.435	0.965	0.980

[a] The s-values ($s = (4\pi/\lambda)\sin(\theta/2)$) are in Å^{-1} units.

Table S2: Definitions of internal coordinates of cotinine

Coordinates	Definitions [a]
s_1	r(N1–C2)
s_2	r(N1–C3)
s_3	r(N1–C4)
s_4	r(C3–C5)
s_5	r(C4–C6)
s_6	r(C5–C6)
s_7	r(C3=O13)
s_8	r(C2–H19)+r(C2–H20)+r(C2–H21)
s_9	2r(C2–H19)–r(C2–H20)–r(C2–H21)
s_{10}	r(C2–H20)–r(C2–H21)
s_{11}	r(C5–H14)+r(C5–H15)
s_{12}	r(C5–H14)–r(C5–H15)
s_{13}	r(C6–H16)+r(C6–H17)
s_{14}	r(C6–H16)-r(C6–H17)
s_{15}	r(C4–C7)
s_{16}	r(C4–H18)
s_{17}	r(C8–N9)+r(N9–C10)
s_{18}	r(C8–N9)-r(N9–C10)
s_{19}	r(C7–C8)+r(C10–C11)
s_{20}	r(C7–C8)–r(C10–C11)
s_{21}	r(C7–C12)+r(C11–C12)
s_{22}	r(C7–C12)–r(C11–C12)
s_{23}	r(C8–H22)+r(C10–H23)
s_{24}	r(C8–H22)–r(C10–H23)
s_{25}	r(C11–H24)
s_{26}	r(C12–H25)
s_{27}	∠(C2–N1–C3)–∠(C2–N1–C4)
s_{28}	∠(N1–C3=O13)–∠(C5–C3=O13)
s_{29}	∠(C3–C5–H14)+∠(C3–C5–H15)–∠(C6–C5–H14)–∠(C6–C5–H15)

Table S2 continued

s_{30}	∠(C3–C5–H14)–∠(C3–C5–H15)–∠(C6–C5–H14)+∠(C6–C5–H15)
s_{31}	∠(C3–C5–H14)–∠(C3–C5–H15)+∠(C6–C5–H14)–∠(C6–C5–H15)
s_{32}	–∠(C3–C5–H14)–∠(C3–C5–H15)–∠(C6–C5–H14)–∠(C6–C5–H15)+4∠(H14–C5–H15)
s_{33}	∠(C5–C6–H16)+∠(C5–C6–H17)–∠(C4–C6–H16)–∠(C4–C6–H17)
s_{34}	∠(C5–C6–H16)–∠(C5–C6–H17)–∠(C4–C6–H16)+ ∠(C4–C6–H17)
s_{35}	∠(C5–C6–H16)–∠(C5–C6–H17)+∠(C4–C6–H16)– ∠(C4–C6–H17)
s_{36}	–∠(C5–C6–H16)–∠(C5–C6–H17)–∠(C4–C6–H16)– ∠(C4–C6–H17)+4∠(H16–C6–H17)
s_{37}	2∠(C7–C4–H18)–∠(C1–C4–H18)–∠(C6–C4–H18)
s_{38}	∠(C1–C4–H18)–∠(C6–C4–H18)
s_{39}	2∠(C7–C4–N1)–∠(C7–C4–C6)–∠(C7–C4–H18)
s_{40}	∠(C7–C4–C6)–∠(C7–C4–H18)
s_{41}	∠(N1–C2–H19)+∠(N1–C2–H20)+∠(N1–C2–H21)– ∠(H19–C2–H20)–∠(H19–C2–H21)–∠(H20–C2–H21)
s_{42}	2∠(N1–C2–H19)–∠(N1–C2–H20)–∠(N1–C2–H21)
s_{43}	∠(N1–C2–H20)–∠(N1–C2–H21)
s_{44}	–∠(H19–C2–H20)–∠(H19–C2–H21)+2∠(H20–C2–H21)
s_{45}	∠(H19–C2–H20)–∠(H19–C2–H21)
s_{46}	4∠(C3–N1–C4)–2∠(C2–N1–C3)–2∠(C2–N1–C4)–4∠(N1–C3–C5)+2∠(N1–C3=O13)+2∠(C5–C3=O13)–10∠(N1–C4–C6)+ 2∠(C7–C4–C6)+2∠(C7–C4–N1)+2∠(N1–C4–H18)+2∠(C6–C4–H18)+2∠(C7–C4–H18)+5∠(C3–C5–C6)–∠(C3–C5–H14)– ∠(C3–C5–H15)–∠(C6–C5–H14)–∠(C6–C5–H15)–∠(H14–C5–H15)+5∠(C5–C6–C4)–∠(C5–C6–H16)–∠(C5–C6–H17)– ∠(C4–C6–H16)–∠(C4–C6–H17)–∠(C16–C6–H17)

Table S2 continued

s_{47}	2∠(N1–C3–C5)–∠(N1–C3=O13)–∠(C5–C3=O13)–5∠(N1–C4–C6)+∠(C7–C4–C6)+∠(C7–C4–N1)+∠(N1–C4–H18)+∠(C6–C4–H18)+∠(C7–C4–H18)–5∠(C3–C5–C6)+∠(C3–C5–H14)+∠(C3–C5–H15)+∠(C6–C5–H14)+∠(C6–C5–H15)+∠(H14–C5–H15)+5∠(C5–C6–C4)–∠(C5–C6–H16)–∠(C5–C6–H17)–∠(C4–C6–H16)–∠(C4–C6–H17)–∠(C16–C6–H17)
s_{48}	∠(N9–C8–H22)–∠(C7–C8–H22)+∠(N9–C10–H23)–∠(C11–C10–H23)
s_{49}	∠(N9–C8–H22)–∠(C7–C8–H22)–∠(N9–C10–H23)+∠(C11–C10–H23)
s_{50}	∠(C4–C7–C8)–∠(C4–C7–C12)
s_{51}	∠(C10–C11–H24)–∠(C12–C11–H24)
s_{52}	∠(C11–C12–H25)–∠(C7–C12–H25)
s_{53}	–2∠(C8–C7–C12)+∠(C4–C7–C8)+∠(C4–C7–C12)–2∠(C7–C8–N9)+∠(C7–C8–H22)+∠(N9–C8–H22)+2∠(C8–N9–C10)–2∠(N9–C10–C11)+∠(N9–C10–H23)+∠(C11–C10–H23)–2∠(C10–C11–C12)+∠(C10–C11–H24)+∠(C12–C11–H24)+4∠(C7–C12–C11)–2∠(C7–C12–H25)–2∠(C11–C12–H25)
s_{54}	2∠(C8–C7–C12)–∠(C4–C7–C8)–∠(C4–C7–C12)–2∠(C7–C8–N9)+∠(C7–C8–H22)+∠(N9–C8–H22)+2∠(C8–N9–C10)–2∠(N9–C10–C11)+∠(N9–C10–H23)+∠(C11–C10–H23)+2∠(C10–C11–C12)–∠(C10–C11–H24)–∠(C12–C11–H24)–4∠(C7–C12–C11)+2∠(C7–C12–H25)+2∠(C11–C12–H25)
s_{55}	2∠(C8–C7–C12)–∠(C4–C7–C8)–∠(C4–C7–C12)–2∠(C7–C8–N9)+∠(C7–C8–H22)+∠(N9–C8–H22)+2∠(N9–C10–C11)–∠(N9–C10–H23)–∠(C11–C10–H23)–2∠(C10–C11–C12)+∠(C10–C11–H24)+∠(C12–C11–H24)
s_{56}	τ(N1–C2)
s_{57}	τ(N1–C3)–τ(C3–C5)+τ(C4–C6)–τ(N1–C4)
s_{58}	τ(N1–C3)–2τ(C3–C5)+2τ(C5–C6)–2τ(C4–C6)+τ(N1–C4)
s_{59}	τ(C4–C7)

Table S2 continued

s_{60}	$-\tau$(C8–N9)+2τ(C7–C8)–τ(C7–C12)–τ(C12–C11) +2τ(C10–C11)–τ(N9–C10)
s_{61}	τ(C8–N9)–τ(C7–C8)+τ(C7–C12)–τ(C12–C11) +τ(C10–C11)–τ(N9–C10)
s_{62}	τ(C8–N9)–τ(C7–C12)+τ(C12–C11)–τ(N9–C10)
s_{63}	ω(C3–N1–C4 : C2)
s_{64}	ω(N1–C3–C5 : O13)
s_{65}	ω(C7–C8–N9 : H22)
s_{66}	ω(N9–C10–C11 : H23)
s_{67}	ω(C8–C7–C12 : C4)
s_{68}	ω(C10–C11–C12 : H24)
s_{69}	ω(C7–C12–C11 : H25)

[a] Abbreviations used: r, stretching; $\angle$, bending; ω, out of plane bending; τ, torsion. See Fig. 3 for the atom numbering.

Table S3: The scaled harmonic force constants of the (eq, *sp*) conformer of cotinine [a]

i	*j*	*F*(*ij*)	*i*	*j*	*F*(*ij*)	*i*	*j*	*F*(*ij*)	*i*	*j*	*F*(*ij*)	*i*	*j*	*F*(*ij*)	*i*	*j*	*F*(*ij*)
1	1	5.000	1	2	0.278	2	2	6.109	1	3	0.182	2	3	0.311	3	3	4.686
2	4	0.350	4	4	3.682	3	5	0.271	5	5	3.658	1	6	-0.139	2	6	-0.108
3	6	-0.148	4	6	0.206	6	6	4.239	2	7	1.273	3	7	-0.161	4	7	0.657
5	7	-0.120	7	7	11.776	1	8	0.291	8	8	4.990	9	9	4.795	10	10	4.912
11	11	4.948	12	12	4.850	6	13	0.104	13	13	4.963	4	14	4.869	3	15	0.374
5	15	0.263	15	15	4.011	3	16	0.180	16	16	4.680	17	17	7.768	18	18	6.417
15	19	0.130	17	19	0.414	19	19	6.694	15	20	0.203	18	20	1.355	19	20	-0.101
20	20	6.063	15	21	0.208	19	21	0.162	21	21	7.274	15	22	0.209	18	22	-0.882
20	22	1.083	21	22	-0.116	22	22	5.905	17	23	0.263	19	23	0.106	23	23	5.090
18	24	0.286	20	24	0.125	24	24	5.085	25	25	5.214	21	26	0.123	26	26	5.191
2	27	0.222	3	27	-0.106	27	27	0.751	2	28	0.403	7	28	0.121	28	28	1.001
4	29	0.155	6	29	-0.256	29	29	0.600	30	30	0.592	12	31	0.151	31	31	0.660
4	32	-0.107	11	32	0.108	32	32	0.549	5	33	-0.279	6	33	0.273	33	33	0.652
34	34	0.647	14	35	0.124	35	35	0.727	5	36	-0.117	6	36	-0.106	13	36	0.113
36	36	0.563	15	37	0.490	37	37	1.605	3	38	0.265	5	38	-0.175	38	38	0.704
3	39	0.344	5	39	-0.151	15	39	0.257	37	39	0.764	39	39	1.413	5	40	0.162
15	40	0.272	16	40	-0.140	37	40	1.082	39	40	0.487	40	40	1.541	1	41	0.460
41	41	0.617	42	42	0.777	10	43	0.119	43	43	0.785	9	44	-0.144	44	44	0.560
10	45	0.141	45	45	0.552	1	46	-0.286	2	46	-0.164	3	46	-0.271	5	46	-0.351
6	46	0.576	7	46	0.331	15	46	0.105	28	46	0.120	46	46	1.697	2	47	0.136
3	47	-0.116	4	47	-0.113	5	47	0.192	6	47	-0.111	7	47	-0.252	46	47	-0.485
47	47	1.285	17	48	0.329	19	48	-0.172	48	48	0.581	18	49	0.273	20	49	-0.120
49	49	0.564	19	50	0.147	20	50	0.131	21	50	-0.164	22	50	-0.160	50	50	0.817
19	51	0.113	21	51	-0.127	22	51	0.110	51	51	0.488	22	52	-0.215	52	52	0.502
17	53	0.402	19	53	-0.290	21	53	0.313	53	53	1.138	15	54	-0.137	17	54	0.384
23	54	0.117	53	54	0.515	54	54	1.129	15	55	-0.171	18	55	-0.292	22	55	0.274
24	55	0.100	55	55	0.919	56	56	0.102	57	57	0.207	60	60	0.248	61	61	0.344
62	62	0.275	63	63	0.125	64	64	0.636	65	65	0.325	66	66	0.331	67	67	0.388
68	68	0.305	69	69	0.335												

[a] Units for energy, stretching and bending coordinates are aJ, Å and radians, respectively. The force constants with the absolute values greater than 0.1 in these units are listed although all the force constants were used in normal coordinate calculations. All the force constants are available from the authors upon request.

Table S4: r_a Distances and mean amplitudes of the (ax, *sc*) conformer of cotinine (Å)

atom pair [a]	r_a	l	atom pair	r_a	l
C–H_{pyridine}	1.097	0.077	C2···C7	3.166	0.159
C–H_{alkyl}	1.103	0.078	C2···C12	3.288	0.280
C3-O13	1.218	0.038	C5···C7	3.290	0.194
C8-N9	1.340	0.045	C6···C8	3.348	0.228
N9-C10	1.343	0.045	C3···C7	3.351	0.147
N1-C3	1.382	0.047	C6···C12	3.425	0.201
C11-C12	1.391	0.047	C3···C12	3.505	0.249
C10-C11	1.393	0.047	C4···O13	3.528	0.062
C7-C12	1.396	0.047	C6···O13	3.589	0.075
C7-C8	1.397	0.047	C5···C12	3.642	0.268
N1-C2	1.456	0.050	N1···C8	3.721	0.125
N1-C4	1.461	0.051	C2···C5	3.724	0.070
C4-C7	1.519	0.054	C2···C6	3.753	0.081
C3-C5	1.530	0.055	C4···N9	3.796	0.074
C5-C6	1.534	0.054	C4···C11	3.799	0.072
C4-C6	1.553	0.057	C4···C10	4.225	0.074
C8···C10	2.268	0.056	N1···C11	4.228	0.142
N1···O13	2.301	0.056	C2···C8	4.273	0.325
N1···C5	2.336	0.059	C12···O13	4.343	0.343
N1···C6	2.360	0.067	C5···C8	4.363	0.279
C3···C4	2.373	0.060	C7···O13	4.422	0.181
C10···C12	2.376	0.057	C2···C11	4.479	0.276
C8···C12	2.385	0.058	C6···N9	4.557	0.223
C7···C11	2.404	0.058	C3···C8	4.630	0.126
C4···C5	2.414	0.072	C6···C11	4.631	0.179
C3···C6	2.420	0.067	C3···C11	4.837	0.265
C7···N9	2.424	0.057	N1···N9	4.841	0.123
N9···C11	2.426	0.057	C5···C11	4.879	0.298
C2···C3	2.476	0.071	N1···C10	4.987	0.117
C5···O13	2.477	0.067	C6···C10	5.033	0.163
N1···C7	2.480	0.075	C2···N9	5.240	0.345
C4···C12	2.533	0.072	C2···C10	5.278	0.285
C4···C8	2.543	0.075	C5···N9	5.430	0.320
C2···C4	2.551	0.078	C5···C10	5.594	0.312
C6···C7	2.563	0.087	C11···O13	5.600	0.383
C7···C10	2.715	0.064	C3···N9	5.701	0.163
C8···C11	2.721	0.064	C8···O13	5.729	0.150
N9···C12	2.806	0.067	C3···C10	5.733	0.222
C2···O13	2.854	0.108	C10···O13	6.629	0.311
N1···C12	2.869	0.156	N9···O13	6.732	0.213

[a] See Fig. 3 for the atom numbering. Non-bonded C···H, N···H, O···H and H···H pairs are not listed although they were included in the data analysis.

Table S5: The correlation matrix for cotinine [a]

	k_1	k_2	x	r_1	r_2	r_3	r_4	r_5	r_6	θ_1	θ_2	θ_3	θ_4
k_1	1.00												
k_2	0.29	1.00											
x	0.01	-0.03	1.00										
r_1	0.15	0.24	0.20	1.00									
r_2	-0.03	-0.01	-0.22	-0.43	1.00								
r_3	0.28	0.25	-0.25	-0.38	0.22	1.00							
r_4	-0.22	-0.03	-0.07	-0.06	-0.05	0.23	1.00						
r_5	-0.12	-0.12	0.15	0.15	-0.75	0.06	0.40	1.00					
r_6	-0.30	-0.08	0.08	0.00	0.05	-0.02	0.30	0.22	1.00				
θ_1	0.04	0.08	0.44	0.09	-0.23	0.16	-0.04	0.25	0.02	1.00			
θ_2	-0.11	-0.08	0.18	0.19	-0.35	-0.55	-0.39	-0.06	-0.08	0.02	1.00		
θ_3	0.36	0.13	0.10	0.08	-0.03	0.21	0.01	0.07	0.14	0.11	-0.28	1.00	
θ_4	-0.05	-0.04	0.45	0.13	-0.51	-0.22	-0.04	0.44	0.06	0.22	0.05	-0.07	1.00
θ_5	0.05	0.00	-0.02	-0.04	0.18	-0.06	-0.13	-0.23	-0.01	0.10	0.33	0.01	-0.34
θ_6	0.03	-0.08	0.55	0.34	-0.54	-0.23	-0.15	0.38	0.04	0.51	0.25	0.23	0.54
ϕ_1	-0.17	-0.10	0.17	-0.03	0.00	-0.15	-0.01	0.01	0.00	0.05	0.03	-0.32	0.10
ϕ_2	-0.01	0.00	0.01	0.06	-0.04	-0.09	-0.07	-0.05	-0.02	0.09	0.19	-0.08	0.03

	θ_5	θ_6	ϕ_1	ϕ_2
θ_5	1.00			
θ_6	0.27	1.00		
ϕ_1	-0.23	-0.03	1.00	
ϕ_2	0.13	0.13	-0.04	1.00

[a] See Table 4 for the definitions of the structural parameters. k_1 and k_2 are the indices of resolution for the long and short camera distances, respectively. x is the molar fraction of the (ax, *sc*) conformer.