Development and Applications for the Simulater of Intermolecular Potentials. II.† Hydration Effects on the Conformation of Tyramine

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Conformational energy maps of the tyramine monocation and its hydrated species were calculated using the SIMP system. The populations of the trans conformer were calculated to be 18% and 53% for the isolated monocation and the hydrated one respectively. The computational results was supported by the results of conformational analysis based on ^{1}H NMR spectroscopy.

Recently, the conformations of compounds which possess physiological activity have been studied widely in order to estimate the structural requirements for the active compounds and to reveal the chemical environments around the active sites of receptors.[2,3] For example, Pullman et al.[2] reported PCILO calculations in connection with conformational studies of the derivatives of phenethylamine, which is a basic skeleton of biological and medicinal interest. They concluded that the phenethylamines exist as a mixture of two nearly equally populated conformers in vacuo. Chuman[3] also reported, from a computational analysis of molecular mechanics, that the active form of enkephaline was similar to that proposed by Hudson et al.[4] However, these analyses have a common defect that the compounds are treated in vacuo and not in solution. Indeed, Chuman's active conformer of enkephaline was calculated to be located 25 kcal mol^{-1} above the global minimum. He claimed that such an energy difference was understandable if the computational accuracy, the conformational change by an induced fitting, and the differences in surroundings were considered (the virtual molecules for these calculations are in vacuo, whereas practical molecules are dissolved in solution). To overcome this defect, Pullman et al.[5] attempted conformational analyses of some hydrated models, which resemble actual states more closely in surroundings than do the molecules in vacuo, using the semiempirical PCILO and ab initio MO methods. Nevertheless, the PCILO method was not accurate enough with respect to the interaction energy, and the CPU time was not sufficient for a full ab initio MO calculation.

In a previous report,[1] we ourselves have developed the SIMP (Simulater of Intermolecular Potentials) system for the calculation of intermolecular interactions with sufficient accuracy and within a reasonably short CPU time. In the present study, we have estimated the preferred conformations of hydrated tyramine by this method and have compared them with those from NMR measurements. Tyramine has the ability to displace noradrenaline from a cytoplasmic mobile pool in adrenergic nerve terminals, leading to sympathomimetic effects. A similar displacement can also occur from granular storage sites. Since the structural specificity of this displacement is quite distinct[6] from that reported for the α-and β-receptors,[7] the preferred conformations of tyramine in an aqueous solution have attracted much attention.

We also explored the applicability of SIMP to the estimation of solvent effects on the conformational energies of this molecule.

Experimental

D_2O, $CDCl_3$, and CD_2Cl_2 (Uvasol reagents from E. Merck) were used as received. Tyramine (a special grade reagent) was used without any special purification. The saturated solutions of tyramine in the above solvents were used for the NMR measurements. The ^{1}H NMR spectra were observed with a HITACHI R-900M FT NMR spectrometer operating at 90 MHz and at 35 °C using 16k data points. Satisfactory signal-to-noise ratios were obtained after the accumulation of 128 transients for the D_2O solution, 5036 transients for the $CDCl_3$ solution, and 1024 transients for the CD_2Cl_2 solution. Programs for the SIMP and MNDO MO[8] calculations were the same as those in previous reports.[1,9] The MOPAC program system,[10] as updated by us for the SIMP calculations, was also used. The ^{1}H NMR spectra were analyzed by the use of the LAOCN5 program.[11] The numerical computations were done with the NEAC S1000 plus SX-1 system at the Computation Center Osaka University, with the M-780 plus VP-200 system at the Data Processing Center Kyoto University, and with the M680H plus S810/10 system at the Computer Center of the Institute for Molecular Science.

Results and Discussion

Since tyramine is known to take a monocation form in the neighbourhood of pH7, we have made computations with the monocation form. The structural parameters used for the MNDO MO calculations were taken from the X-ray crystallographic structures,[12] except for those relating to the hydrogen atoms. The distance of O-H, the angle of C-C-H, etc. were optimized using the MNDO MO method. The numbering of atoms in the tyramine monocation and the structural parameters are shown in Fig. 1 and Table 1 respectively. Tyramine has three flexible dihedral angles, as is defined in Fig. 1. Since the dihedral angles of θ_1 and

† See Ref. 1 for I.

Table 1. Bond Lengths, Bond Angles, and Dihedral Angles of Tyramine Used in This Work

Bond length/Å		Bond angle/°		Dihedral angle/°	
1-2	1.4246	1-2-3	120.00	1-b-a-H	120.00
4-O	1.3672	3-4-O	120.00	N-a-b-H	120.00
1-b	1.5272	2-1-b	120.00	3-4-O-H	90.00
b-a	1.5272	1-b-a	113.54		
a-N	1.5500	b-a-N	115.88		
2-H	1.0887	1-2-H	120.00		
O-H	0.9489	4-O-H	111.07		
b-H	1.1132	1-b-H	110.90		
a-H	1.1132	b-a-H	110.90		
N-H	1.0258	a-N-H	111.42		

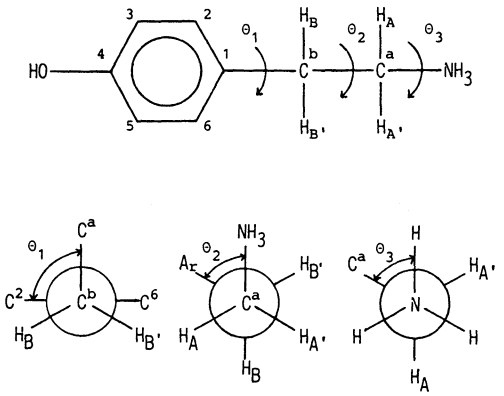

Fig. 1. The numbering of atoms and the definition of dihedral angles for tyramine.

θ_2 are considered to be more significant than that of θ_3 with respect to the overall molecular shape, we varied θ_1 and θ_2 for the computation of the conformational energy maps, while θ_3 was optimized in order to minimize the conformational energy for each set of θ_1 and θ_2 values. The other structural parameters were kept unchanged from the monomer values throughout the computations. Here, we will define the two regions of the conformations of tyramine. One is a gauche region which is defined by θ_2 being equal to 0°—120° or 240°—360°. The other is a trans region defined by θ_2=120°—240°, which is considered to be a structure similar to that of noradrenaline in an aqueous solution.[13]

The conformational energy map for the tyramine monocation obtained is shown in Fig. 2. Although the 'bulk repulsive concept,'[14] which is the basis of organic stereochemistry, would lead to a trans conformer as the preferred one, this map shows that the gauche conformer is more preferable than the trans one. In fact, the most stable values of θ_1 and θ_2 were calculated to be in a gauche region (75° and 57° respectively), though the conformational energy for the most stable conformation in the trans region was higher than the global minimum by only 3.9 kJ mol^{-1}. This profile is qualitatively consistent with that of the conformational energy map obtained by Pullman et al.[2] We have previously reported[15] that alkyl/aryl interac-

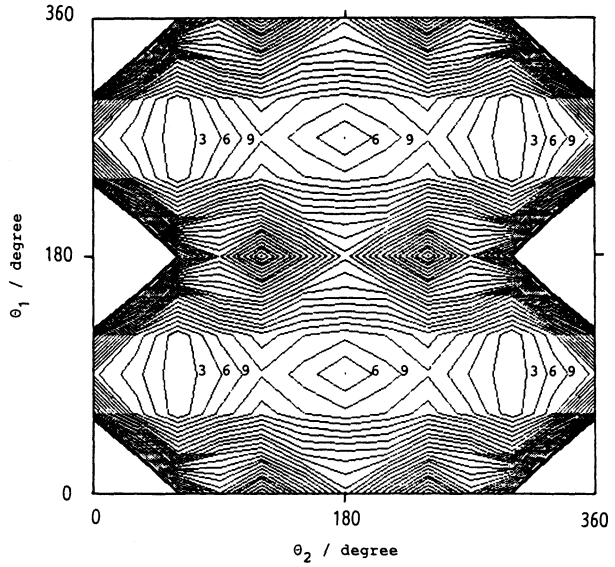

Fig. 2. The conformational energy map of tyramine monocation (kJ mol^{-1} with respect to the global minimum).

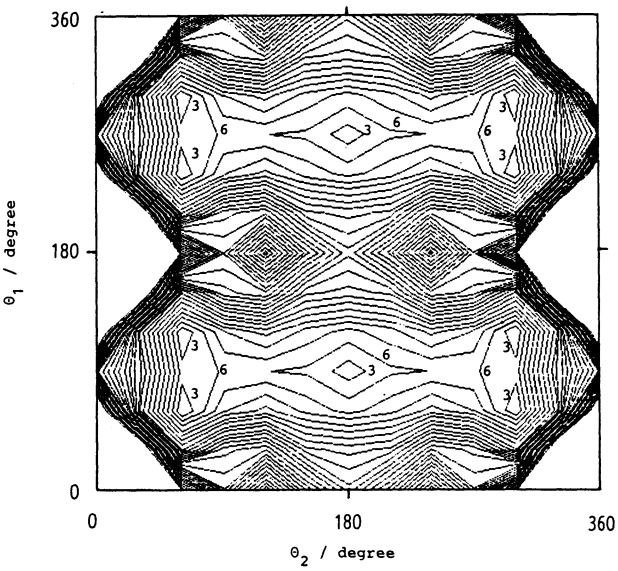

Fig. 3. The conformational energy map of hydrated tyramine monocation (kJ mol^{-1} with respect to the global minimum).

tions, called CH/π interactions by Nishio et al.,[16] can dominate the conformation of molecules, leading to a gauche conformer with respect to the alkyl and aryl groups. We have also reported, on the basis of the results of the energy decomposition analysis proposed by Kitaura et al.,[17] that the CH/π intramolecular interactions resemble the weak hydrogen bondings, as exemplified by the OH/π interactions. Since the most strong intramolecular interaction for the tyramine monocation in nature is an NH/π interaction, which is thought to be much stronger than the CH/π interaction, the preference of the gauche conformer may be reasonable.

In order to explore the hydration effects on the conformational energy map, four H_2O molecules were placed around the two polar sites of OH and NH_3. The angle of θ_3 and the distances between the polar sites and the H_2O molecules were optimized. The resulting conformational energy map of hydrated tyramine is shown in Fig. 3. Although the total profile of the energy map for the hydrated system was similar to that for the isolated system, the difference in energies was sufficiently large to change the population of each conformer. The difference in energies of the most stable conformations in the gauche and trans regions was calculated to be 2.4 kJ mol^{-1}, about half of that for the isolated system calculated above. In addition, the conformational energy increases around the regions of the global minimum more abruptly for the hydrated system than for the isolated system. In order to compare the population of trans and gauche conformers in detail, the populations were calculated by defining the trans conformer as θ_2=120°—240°, and the gauche one, as θ_2=0°—120° and θ_2=240°—360°. Under these conditions, the following equations hold:

$$P_t = \frac{1}{P_0}\int_{\frac{2}{3}\pi}^{\frac{4}{3}\pi}\int_0^{2\pi} \exp\{-\Delta E(\theta_1, \theta_2)/RT\}\,d\theta_1\,d\theta_2 \quad (1)$$

$$P_g = 1 - P_t \quad (2)$$

$$P_0 = \int_0^{2\pi}\int_0^{2\pi} \exp\{-\Delta E(\theta_1, \theta_2)/RT\}\,d\theta_1\,d\theta_2 \quad (3)$$

where P_t is the population of the trans conformer and P_g is that of the gauche conformer. The double integrations and the necessary interpolation of ΔE (θ_1, θ_2) were carried out using spline functions. The resultant populations at 35 °C are shown in Table 2. From these results, the trans conformer of tyramine is estimated to be more preferable in an aqueous solution than in vacuo. The reason for this populational change should be as follows: the NH/π interactions, which are considered to be the main origin of the preference of the gauche conformer, are replaced by the stronger interactions of the NH/H_2O interactions.

In order to confirm these computational results, we have analyzed the ^{1}H NMR spectra of tyramine in an aqueous solution as well as in several organic solvents. Examples of the calculated and observed NMR spectra of tyramine are shown in Fig. 4. The shift values and the vicinal coupling constants obtained using the LAOCN5 program are listed in Table 3. The rms errors between the observed and calculated coupling constants for tyramine in H_2O, CD_2Cl_2, and $CDCl_3$ are 0.11, 0.14, and 0.11 Hz respectively. As is shown in Fig. 5, the observed coupling constants, J_{AB} and $J_{AB'}$, may be represented by the following equations:

$$J_{AB} = P_t J_t + P_g J_g + \varepsilon \quad (4)$$

$$J_{AB'} = P_t J_g + \frac{1}{2} P_g (J_g + J_t) + \varepsilon' \quad (5)$$

$$P_t + P_g = 1 \quad (6)$$

where J_g and J_t are the ideal coupling constants for the gauche conformer and the trans conformer respectively, and where ε and ε' are the error terms. The values of J_g and J_t were assumed to be 2.0 and 13.0 Hz

Table 2. Rotamer Populations of Tyramine at 35 °C as Calculated by SIMP

System	Trans/%	Gauche/%
Isolated	18	82
Hydrated	53	47

Table 3. ^{1}H NMR Parameters for the AA'BB' Part of Tyramine

Solvent	$\nu_A - \nu_B$/Hz	J_{AB}/Hz	$J_{AB'}$/Hz
CD_2Cl_2	21.12	6.70	6.68
$CDCl_3$	22.61	6.80	6.53
D_2O	23.40	9.17	2.84

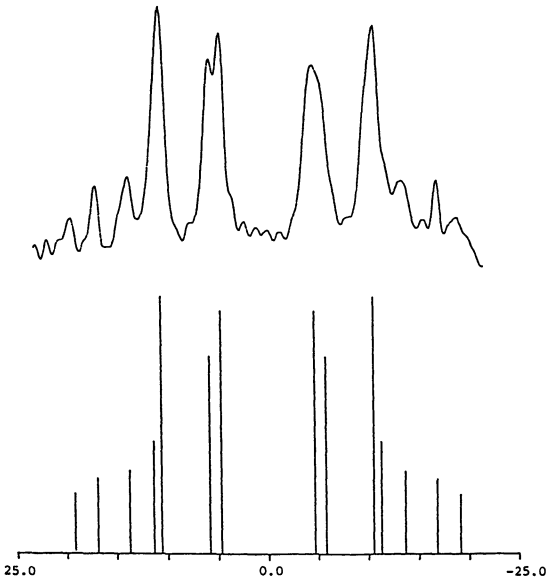

Fig. 4. The observed (upper) and the calculated (lower) NMR spectra of tyramine in CD_2Cl_2. The AA'BB' part from the alkyl side chain is shown. Lines at the higher field side are broadened by the small couplings with the aromatic protons.

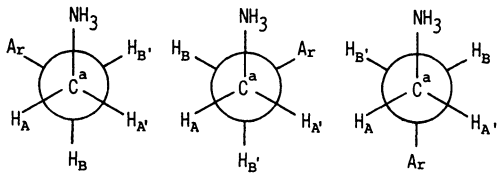

Fig. 5. Three possible conformations relating to the dihedral angle of θ_2.

Table 4. Rotamer Populations of Tyramine in Various Solvents

Solvent	Trans/%	Gauche/%
CD_2Cl_2	37	63
$CDCl_3$	38	62
D_2O	69	31

respectively, these values were cited from the literature.[18] When the value of $\varepsilon^2+\varepsilon'^2$ was minimized, P_t and P_g are determined experimentally as follows:

$$P_t = \frac{4J_{AB}-2J_{AB'}+J_t-3J_g}{5(J_t-J_g)} \quad (7)$$

$$P_g = 1 - P_t \quad (8)$$

Note from the definition (Fig. 5) that J_{AB} is larger than $J_{AB'}$ in Eq. 7. The resulting populations are listed in Table 4, which indicates that the trans conformer is preferable in D_2O, this is in agreement with the above SIMP calculations. In this way, our SIMP system is expected to offer a powerful method for determining hydration effects on the conformations of biologically significant molecules.

In Table 4, populations are also determined experimentally in CD_2Cl_2 and $CDCl_3$. The preference for the gauche conformer, which is indicated by the calculation for the isolated system in Table 2, is supported by the experiments.

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