The Hydrogen Bond Studied by Nitrogen-14 Nuclear Magnetic Resonance. IV. Nitrogen-14 Chemical Shifts of Five- and Six-Membered N-Heterocycles Determined by Heteronuclear Magnetic Double Resonance with the Aid of Two- and Three-Bond N–H Spin Couplings

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Abstract: ^{14}N shifts of five- and six-membered N-heterocycles have been determined by the $^{1}H-\{^{14}N\}$ heteronuclear double resonance method with the aid of two- and three-bond N–H spin couplings. Applicability of this technique depends considerably upon properties of solvents which are responsible for an extent of the quadrupole relaxation effect. The amount of sharpening of the C–H proton peak by irradiation of ^{14}N nuclei was observed to be 10–70%. For the determination of the ^{14}N shift of five-membered N-heterocycles, in which coalescence of ^{14}N signals between two kinds of nitrogen atoms (>N and N–H) occurs by the rapid proton exchange, *N*-methyl derivatives were also employed for comparison. By comparison of ^{14}N shifts between a parent molecule and its *N*-methyl derivative, tautomeric forms in five-membered N-heterocycles are discussed. Further ^{14}N shifts were investigated both in the presence of proton acceptors and proton donors. It is found that proton donors make ^{14}N resonances shift upfield with respect to the proton acceptors. These are interpreted in terms of hydrogen-bond formation.

Although measurements of ^{14}N chemical shifts in N-heterocycles are important in connection with the elucidation of the biological activity, a relatively few data[2] have been accumulated since the first systematic study by Herbison-Evans and Richards.[3] This may be partly because the sensitivity of the ^{14}N nucleus is considerably low and the accurate measurement of the chemical shift is hampered by the quadrupole relaxation effect due to the nitrogen nucleus. Recently ^{15}N resonance,[4] which is free from the latter effect, has been employed in samples either enriched or of natural abundance. For the study of the conformation of biopolymers, however, the problems of the sensitivity of ^{14}N or ^{15}N resonance will be serious because of their low solubility and high molecular weight. On the other hand, the indirect method of ^{14}N or ^{15}N measurement[4d,5-17] by heteronuclear magnetic double resonance is promising as a potential means to probe the microstructure, since the measurement is feasible at the sensitivity of proton resonance. We have studied[12-13] displacement of ^{14}N shifts in the hydrogen-bond system by this method.

This paper is a continuation of this series and describes a method to determine ^{14}N shifts of biologically important N-heterocycles by decoupling the two- and three-bond N–H couplings. It is found that the success of this method mainly depends upon the solvents used, because the quadrupole relaxation effect, which particularly hampers the measurements of ^{14}N shifts from the decoupling of the smaller magnitude of the N–H coupling, varies with the interaction with the solvent molecule. ^{14}N shifts of five- and six-membered N-heterocycles are discussed in terms of the hydrogen-bond interaction, the paramagnetic shielding effect, and the effect of proton exchange. Since we have completed this research, Witanowski and coworkers[18] have published a paper dealing with similar compounds. Because of the lower sensitivity of the direct method, however, these measurements failed to detect the solvent effect of ^{14}N, especially the hydrogen-bond shift.

Experimental Section

Measurements of ^{14}N chemical shifts were performed by the $^{1}H-\{^{14}N\}$ double resonance method.[4d,5-17,19] A Varian HA-100 spectrometer equipped with a NMR Specialties HD-60B spin decoupler was used to irradiate the ^{14}N nucleus and to monitor the peak intensity of proton signals coupled with the nitrogen atom. The peak intensity was recorded by an external Varian G-14 recorder. The molecules studied in this paper lack one-bond N–H spin coupling or are in the equilibrium of rapid exchange except for pyrazole and 1,2,4-triazole in DMSO solution. Thus we explored the method to obtain ^{14}N shifts by decoupling two- or

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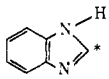
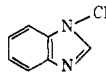
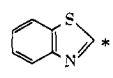
Table I. ^{14}N Shifts of Six-Membered N-Heterocycles[a]

Solute[b]	Solvents: Acetone: This work	Acetone: Lit. value[c]	H_2O	Difference[d]
	−299.0 ± 1 (−295.5 ± 1)[e]	−296.5 ± 2 (−292 ± 10)[e,f]	−276 ± 6 (−285.5 ± 3)[g]	+23 ± 7 (8.8 ± 4)[h]
	−273.9 ± 1	−275.5 ± 2	−265.5 ± 2	+8 ± 3
	−313.6 ± 1	−312.5 ± 2		
	−259.8 ± 1		−253.1 ± 10	+6.7 ± 11
	−292.8 ± 1		*i*	

[a] ^{14}N shift referred to the NH_4^+ ion (ppm). [b] C–H proton asterisked was employed as the monitor of the ^{14}N irradiation. [c] Reference 3. [d] Hydrogen-bond shift referred to acetone solution. [e] Neat liquid. [f] Reference 6. [g] Methanol solution (50 mol %), see reference 30. [h] Hydrogen-bond shift referred to neat liquid. [i] Insoluble to this solvent.

Table II. ^{14}N Shifts of Five-Membered N-Heterocycles[a,b] (ppm)

Solute		Solvents: Proton donor: Acetone	DMSO	Proton acceptor: H_2O	MeOH	CF_3COOH (H_2SO_4)[g]	CCl_4	Neat
		−127.5 ± 0.4	−134.8 ± 0.4	−132.8 ± 1.4			−120.7 ± 0.4	−125.7 ± 0.4
		−127.2 ± 1		*d*			−123.4 ± 2	
		−186.2 ± 1	−189.3 ± 1	−180.8 ± 1	−180.2 ± 2	−147.4 ± 2[f] (−145.2 ± 2)[g]	*d*	
	N_1 N_3	−140.5 ± 1 −233.9 ± 1	*c* *c*	−146.9 ± 1 −222.6 ± 5	*c* *c*	−153.5 ± 4 *c*	−138.6 ± 1 −247.8 ± 3	−143.9 ± 3 *c*
		−172.5 ± 1.5	−120.0 ± 4[f]	*d*	*c*		*d*	
	N_1 N_3	−126.2 ± 1.3 −222.7 ± 1		*d* *d*		*c* *c*	−120 ± 6 −230 ± 6	
		−228.5 ± 2	−180.6 ± 1[f]	−222.6 ± 1		*c*		
	N_1 N_2	−183.2 ± 2 −285.4 ± 2	*c* *c*	−179.0 ± 2 −264.4 ± 3			−178.0 ± 2 −289.3 ± 2	
		−222.3 ± 1	−224.7 ± 3 (−182.7 ± 1)[f]	−214.9 ± 2			*d*	
	N_1 N_2 N_4	−205.2 ± 2 −306.4 ± 3 −238.0 ± 2		−197.3 ± 4 −310.9 ± 3 −216.7 ± 2		−151.1 ± 2 −307.6 ± 2 −151.5 ± 2	−187.3 ± 2 −314.9 ± 2 −244.3 ± 2	
		−302.1 ± 1	−304.3 ± 2	−295.4 ± 4		−179.0 ± 2		−301.2 ± 1
		−296.2 ± 1	*c*	*d*				*c*

[a] ^{14}N shift referred to the NH_4^+ ion. [b] Intensity of the proton peak asterisked was used to determine ^{14}N shifts. [c] ^{14}N shift was not determined by the ^{14}N indor method. [d] Insoluble to this solvent. [e] Reference 6. [f] ^{14}N shift of the N–H group determined by the decoupling from one-bond N–H coupling. [g] H_2SO_4 solution.

Table III. Comparison of ^{14}N Shifts of N-Heterocycles with Their *N*-Methyl Derivatives[a]

	^{14}N shift			
	$\delta(\geqslant N)$	$\delta(NCH_3)$	$\frac{1}{2}\{\delta(NH) + \delta(\geqslant N)\}$	$\delta(\geqslant N) - \delta(NCH_3)$
N-Methylpyrazole	-285.4 ± 1.2	-183.2 ± 1.2	$-234.3\ (-228.5 \pm 1.5)^b$	102.2 ± 3
N-Methylimidazole	-233.9 ± 1	-140.5 ± 1	$-187.2\ (-186.2 \pm 1)^b$	93.4 ± 2
N-Methylbenzimidazole	-222.7 ± 0.8	-126.2 ± 1.3	$-174.5\ (-172.5 \pm 1.5)^b$	96.5 ± 2

[a] Parts per million in acetone solution. [b] ^{14}N shift of parent molecule.

three-bond N–H spin couplings. The ^{14}N shift was determined from a maximum point of the C–H proton signal in the plot of peak intensities against the ^{14}N irradiation frequencies swept manually. To prevent errors caused by a drift of baseline and field inhomogeneity, the same experiments were repeated at least three times. The irradiation frequency (7.22 MHz) was monitored by a Takeda-Riken TR-3977 radiofrequency counter. All measurements were carried out in frequency-sweep mode with a small amount of TMS dissolved into the samples to stabilize the field frequency. Measurements of ^{14}N shifts were performed in 1 mol % solution. ^{14}N chemical shifts were expressed from those of the ammonium ion.

Most of the materials used in this study are from commercial sources. *N*-Methylpyrazole,[20] *N*-methylbenzimidazole,[21] and 1-methyl-1,2,4-triazole[22] were prepared from N-methylation of parent molecules by methyl iodide.

Results

Although the accurate ^{14}N shift is available from the decoupling of one-bond N–H spin coupling ($^1J_{NH}$),[13-15] in many cases $^1J_{NH}$ is completely decoupled by the rapid proton exchange except in solvents such as trifluoroacetic acid and DMSO which are capable of reducing the rate of this phenomenon considerably.

Since the proton exchange rate in DMSO solutions is suppressed, N–H proton peaks of benzimidazole, pyrazole, and 1,2,4-triazole can be seen. At the same time, two kinds of C–H proton signals, one adjacent to the $\geqslant N$ and the other to the N–H group, are resolved in their compounds. The ^{14}N chemical shift determined with the use of this N–H proton signal as a monitor yields exclusively a ^{14}N shift of the N–H group in contrast with the observation based on decouplings of two- or three-bond N–H couplings, as described below. If a trace of water is involved in DMSO, this catalyzes the proton exchange which renders the determination of the ^{14}N shift by the use of one-bond N–H coupling impossible. The ^{14}N shift is also available from the decoupling of two- or three-bond couplings ($^2J_{NH}$ or $^3J_{NH}$). Irradiation of the ^{14}N nucleus causes sharpening of the C–H proton signal which is spin-coupled to the nitrogen nucleus by two- or three-bond N–H couplings. A plot of the intensity of the C–H proton peak against the ^{14}N irradiation frequency yields the resonance frequency of the ^{14}N nucleus. The ^{14}N shifts thus obtained are summarized in Tables I and II[23] for six- and five-membered N-heterocycles, respectively.

Because of the considerably smaller magnitude of these coupling constants in comparison with $^1J_{NH}$, however, applicability of this method depends mainly upon the extent of the quadrupole relaxation effect which tends to wash out this spin coupling. Kitzinger and Lehn[24] pointed out that lower temperature, higher viscosity of the solution and hydrogen bonding to solvent lead to washing of the N–H spin couplings. In many cases, for this reason the ^{14}N shift could not be obtained in DMSO, methanol, and trifluoroacetic acid solution (Table II). It is concluded that acetone is the most suitable solvent to observe the ^{14}N shift with this method. The amount of peak sharpening by decoupling N–H spin couplings is found to be about 10–70%. For nitrogen nuclei such as $\geqslant N$ and tautomeric type, the irradiation causes sharpening of C–H proton signals spin-coupled to nitrogen nuclei with $^2J_{NH}$. A significantly larger sharpening of peaks appears in the C–H proton signals of *N*-methylpyrrole and *N*-methylpyrazole coupled to $N-CH_3$ with $^3J_{NH}$. This result is in accord with measurements on ^{15}N-enriched N-heterocycles[25] that the magnitude of $^3J_{NH}$ is larger than that of $^2J_{NH}$. As a supplemental means to understanding the displacement of the ^{14}N shift, ^{13}C shifts of *N*-methylimidazole in various solvents are recorded as given in Table IV.

Discussion

^{14}N shifts determined in this work are in good agreement with those by Herbison-Evans and Richards[3] in the same solvent within the range of experimental error (Table I). This method, however, is superior to the terms of direct method in sensitivity. The former allows the measurements at concentration as low as 1 mol %. Accuracy of this method was improved by the procedure described above over the value first obtained by Baldeschwieler and Randall.[6]

Tautomers in Five-Membered N-Heterocycles. In five-membered N-heterocycles, there exist two kinds of nitrogen atoms, *i.e.*, $\geqslant N$ and N–H which are convertible to each other by the rapid proton exchange phenomenon. Although an appreciable difference of ^{14}N chemical shift should be noticed between them, only a single ^{14}N resonance peak is observed in solutions of imidazole, benzimidazole, pyrazole, and 1,2,4-triazole on the basis of the coalescence of ^{14}N peaks by the rapid N–H proton exchange. On the other hand, well-resolved signals are recorded in H–{^{14}N}double resonance spectra for *N*-methyl derivatives of imidazole, benzimidazole, and pyrazole. The difference of ^{14}N shifts between $\geqslant N$ and $N-CH_3$ is determined to be 93–102 ppm, as listed in Table III. This separation mainly arises from a contribution of $n-\pi$ transition in the paramagnetic shielding tensor of the nitrogen atom.[26] The mean ^{14}N frequencies of $\geqslant N$ and NCH_3 signals of the *N*-methyl derivatives are in good agreement with the ^{14}N shifts of parent molecules which are tautomeric

(20) G. Dedichen, *Ber.*, **39**, 1831 (1906). We thank Mr. Hosoi of this laboratory for his collaboration in preparing this compound.

(21) K. Hoffman, "Imidazole and Its Derivatives, Part I," Interscience, New York, N. Y., 1953, p 247.

(22) M. R. Atkins and J. B. Polya, *J. Chem. Soc.*, 141 (1954).

(23) In contrast to the results by Witanowski, *et al.*,[18] solvent effects on ^{14}N shifts are clearly noticed in our results, since measurements have been done in low concentrations such as 1 mol %.

(24) J. P. Kitzinger and J. M. Lehn, *Mol. Phys.*, **14**, 133 (1968).

(25) Reference 4h and references cited therein.

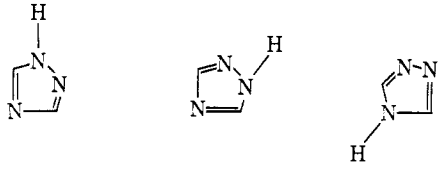
(26) Gil and Murell ascribed the upfield ^{14}N shift of pyridinium ion with respect to neat pyridine (97 ppm) to the paramagnetic contribution based on calculation by the theory of Pople;[27] V. M. S. Gil and J. N. Murrell, *Trans. Faraday Soc.*, **60**, 248 (1964).

(27) J. A. Pople, *J. Chem. Phys.*, **37**, 53 (1962).

because of the rapid proton exchange as might be expected. This result demonstrates that the effect of substitution of *N*-methyl in place of the N–H group is quite small in acetone solution. A similar trend is realized in comparison of ^{14}N shifts between pyrrole and *N*-methyl pyrrole of acetone solution. Furthermore, the ^{14}N shifts of the N–H group determined from the one-bond N–H coupling in DMSO are well in agreement with those of $N-CH_3$ deduced from *N*-methyl derivatives.

Similarly 1-methyl-1,2,4-triazole exhibits three kinds of ^{14}N signals, namely N_1, N_2, and N_4. The highest ^{14}N shift is straightforwardly assigned to N_1-CH_3 according to the discussion described above. In comparison with the ^{14}N shifts of *N*-methylpyrazole and *N*-methylimidazole, the middle and the lowest ^{14}N signals are assigned to N_4 and N_2, respectively. The difference of ^{14}N shifts between the symmetrical pair N_1-CH_3 and N_2 is 101.2 ppm which is in agreement with the results shown in Table III. Because of the asymmetrical position of N_2 with respect to the pair N_1-CH_3 and N_4, on the other hand, the above relation is no longer retained in this case (33 ppm).

In contrast to diazoles discussed above, it is not straightforward to correlate the ^{14}N shift of 1,2,4-triazole with the tautomeric forms I–III. Although



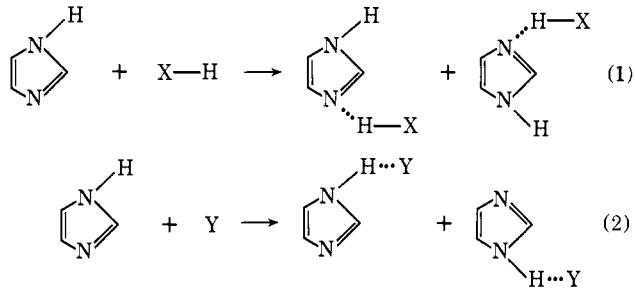
I II III

tautomers I and II are believed[28] to be the more stable in the solid phase at −155°, and also have been confirmed[29] to exist predominantly in the vapor phase by the microwave spectrum, the presence of III in solution cannot be ruled out. Actually, if I and II are the only tautomers present in the solutions studied in this paper, the ^{14}N shift in this case is estimated as −251 ppm from the average of the N_1 and N_2 signals of 1-methyl-1,2,4-triazole. This value is somewhat lower than the experimental results, $-223 \sim -225$ ppm (Table II).

Hydrogen-Bond Shift and Protonation Shift. Tables I and II show that ^{14}N shifts of six- and five-membered N-heterocycles vary with properties of solvents. Most of these displacements are explained in terms of the hydrogen bond with the solvent used. As we have reported previously,[30] displacements of ^{14}N shifts of $\geqslant N$ and N–H groups are upfield and downfield by hydrogen-bond formation with proton donor (X–H) and proton acceptor (Y), respectively, with respect to the free state. As expected from the above result, ^{14}N shifts of six-membered N-heterocycles in aqueous solution occur upfield with respect to those in neat or acetone solutions,[31] the latter being considered as the free state (Table I). In pyridine the largest upfield shift (23 ± 5 ppm) is obtained, and approximately one-half of the value of pyridine is observed in the aqueous solution of pyrimidine. Assuming that a 1:1 hydrogen bond is formed with the water molecule, one-half and one-third of the upfield shift of pyridine are expected for pyrimidine and 1,3,5-triazine, which have two and three nitrogen nuclei, respectively, in a molecule. This expectation is well in accord with the experimental result, although no appreciable displacement is observed in the latter compound because of the large amount of error included. For the hydrogen bond of pyridine with methanol (50 mol %), the upfield shift of 8.8 ± 4 ppm is in good agreement with the value previously reported with the direct method, 9.5 ± 3 ppm,[32] as shown in parentheses of Table I. In this case the upfield ^{14}N shift, the amount of which is much smaller than that in aqueous solution, is caused by the presence of a considerable amount of free solute in the concentration of 50 mol %. An explanation was made previously of the upfield ^{14}N shift on hydrogen bond formation which demonstrates the decrease of the paramagnetic shielding effect caused by the increase of $n-\pi$ excitation energy.

The similar upfield shifts of ^{14}N shifts are denoted for hydrogen bonding $\geqslant N\cdots H_2O$ in *N*-methyl derivatives of five-membered N-heterocycles. The upfield ^{14}N shifts are 11.3 ± 6 ppm and 21.0 ± 5 ppm with respect to the shift in acetone solution for *N*-methylimidazole and *N*-methylpyrazole, respectively. The upfield ^{14}N shift of 1-methyl-1,2,4-triazole in aqueous solution is denoted exclusively at the N_4 nucleus (21.3 ± 4 ppm). This result indicates that hydrogen bonding with water molecule occurs at the N_4 nucleus which bears a larger electron density in comparison with N_2 as expected from the ^{14}N shifts.

In contrast to the *N*-methyl derivatives described above, it is not permissible to assume the ^{14}N shift in acetone solution as the free state for the unsubstituted molecules, since the ^{14}N shift of the N–H group is known to shift downfield in this solvent. However, it is convenient to consider separately the hydrogen bond system in the following two cases. When X–H and Y stand for solvents acting as proton donor and acceptor, respectively, the hydrogen bondings with imidazole, for instance, are expressed as shown in eq 1 and 2. Two



(1)

(2)

tautomeric forms on the right-hand side represent the tautomer convertible to each other by the rapid proton exchange. The hydrogen bond (eq 1 and 2) make the ^{14}N signal of the imidazole shift upfield and downfield,

(28) P. Goldstein, J. Ladell, and G. Abowitz, *Acta Crystallogr., Sect. B*, **25**, 135 (1969).

(29) K. Bolton, R. D. Brown, F. R. Burden, and A. Mishra, *Chem. Commun.*, 873 (1971).

(30) H. Saitô, K. Nukada, H. Kato, T. Yonezawa, and K. Fukui, *Tetrahedron Lett.*, 111 (1965).

(31) The error of measurements increases considerably in aqueous solution and is extended over the displacement of the ^{14}N shift in 1,3,5-triazine. This is ascribable to further broadening of the ^{14}N signal by the quadrupole relaxation. According to the extensive work by Kitzinger and Lehn[24] the main contribution to the quadrupole relaxation in this case is a longer correlation time caused by hydrogen bonding with water.

(32) Recently a similar result was reported by Lichter and Roberts[4h] with the ^{15}N resonance of pyridine-^{15}N (7.6 ppm upfield shift).

Table IV. ^{13}C and ^{14}N Shifts of *N*-Methylimidazole in Various Solvents

	^{13}C chemical shifts[a]				^{14}N chemical shifts	
	C-2	C-4	C-5	CH_3	N_1	N_3
CCl_4	−137.10	−119.29	−129.13	−32.66	−138.6 ± 1	−247.8 ± 3
Neat	−138.61	−121.19	−129.17	−33.17	−143.9 ± 3.3	
Acetone	−138.49	−120.79	−129.52	−33.14	−140.5 ± 1	−233.9 ± 1
D_2O	−140.63	−123.57	−130.12	−35.32	−146.9 ± 1	

[a] ^{13}C shift referred to TMS.

respectively.[33] The effect of self-association is probably negligible, since the concentration of the solute is low (1 mol %) in polar solvents. Then it is found that the ^{14}N shifts of imidazole in acetone and DMSO (proton acceptors) are shifted downfield with respect to methanol, water, and trifluoroacetic acid (proton donors). The difference of ^{14}N shifts is 6–9 ppm over the range of the experimental error. Similarly upfield ^{14}N shifts of 6 ± 3 ppm and 7.4 ± 3–9.8 ± 5 ppm are observed in pyrazole and 1,2,4-triazole, respectively. These values are recognized as about half of the corresponding values for *N*-methyl derivatives, taking into account the argument described above.

Sometimes difficulty arises in observing ^{14}N shifts in protonated species, because a longer correlation time due to protonation tends to broaden nmr lines and make the two- and three-bond N–H couplings unobservable. Protonation shifts on imidazole and thiazole are obtained by the decoupling of one-bond N–H couplings. The upfield ^{14}N shifts are 41 ± 3 ppm and 123.1 ± 3 ppm for imidazole and thiazole, respectively. The latter is in good agreement with the similar upfield shift of protonated pyridine, 123 ± 11 ppm.[6] The ^{14}N shift of protonation on imidazole by one nucleus is 82 ppm which is twice the value 41 ppm. This is too small compared with protonated pyridine, probably because the inductive effect by protonation to one nucleus causes another shift downfield.

Inductive Effect. It should be noted that there exists another cause of displacement of the ^{14}N shift in addition to the hydrogen bond formation mentioned above. A relatively larger downfield shift (−8.3 ± 2 ppm) is denoted in aqueous solution for the ^{14}N shift of the $N-CH_3$ group of *N*-methylimidazole with respect to carbon tetrachloride solution. On the basis of molecular structure, it is not ascribed to the hydrogen bond as discussed above. One possible explanation of this is the inductive effect by the hydrogen-bond formation to tertiary nitrogen atom with water. In order to confirm this postulate, measurements of ^{13}C resonance, the shifts of which being more accurately determined than ^{14}N resonance, are performed in several solvents. The parallel relationship among displacements of ^{13}C shifts of methyl, C-3 and C-4 and the ^{14}N shift of NMe shows the presence of this mechanism (Table IV). Polarization of molecules in polar medium[34] is also responsible for the downfield ^{13}C and ^{14}N shifts, though the displacement of the former is smaller than the latter.

(33) Strictly speaking it is too straightforward to conclude simply from the results of monofunctional compounds. If a hydrogen bond is formed between ≫N and XH, as in eq 1, the ^{14}N shift of the N–H group is also influenced by the inductive effect.

(34) H. Saitô, Y. Tanaka, S. Nagata, and K. Nukada, *Can. J. Chem.*, in press; H. Saitô and Y. Tanaka, in preparation.

Determination of the Tautomeric Form of the Imidazole Ring of L-Histidine in Basic Solution by Carbon-13 Magnetic Resonance Spectroscopy

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Abstract: Comparison of ^{13}C chemical shift–pH profiles for imidazole, L-histidine, and 1-methyl- and 3-methylhistidine provides conclusive evidence that the 1-H tautomer is the predominant tautomeric form of the imidazole ring of histidine in basic solutions. This viewpoint is supported by theoretical calculations of ^{13}C chemical shifts based on the average energy approximation and electron densities determined by CNDO/2 MO calculations. The characteristic titration shifts for histidine and the methylhistidines are used to determine the tautomeric equilibrium of the imidazole ring in several derivatives of histidine and in polypeptides containing a histidyl residue.

The amino acid L-histidine is trifunctional since in addition to the α-amino and α-carboxyl functions, the imidazole side chain is a protonation site (p*K* = 6.0).[2] The protonation step for the imidazole ring involves transition from a neutral species to a cationic

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(2) H. A. Sober, "Handbook of Biochemistry," 2nd ed, Chemical Rubber Publishing Co., Cleveland, Ohio, 1970, p J-198.