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Variations in Average Electronic Excitation Energy as the Cause of ^{13}C Nuclear Magnetic Resonance Protonation Shifts of 4-Aminoazobenzene[1]

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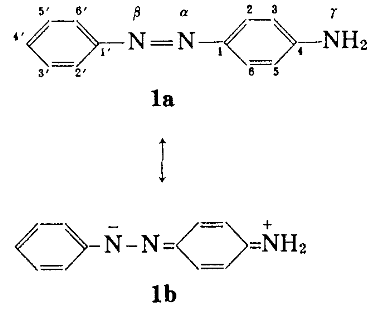
Correlations between ^{13}C nuclear magnetic resonance protonation shifts and protonation-induced charge densities on the carbon atoms as calculated by the INDO-MO method have been examined for mono- and dicationic species of 4-aminoazobenzene. The paramagnetic shielding constants have been calculated with the average excitation energy approximation. It is concluded that the deviation from the correlation between ^{13}C protonation shifts and protonation-induced charge densities on some carbon atoms (C1, C3, 5 *etc.*) is mainly due to variations in the average excitation energy term which are attributable to delocalization of lone-pair electrons assisted by resonance structure which makes the molecule planar.

Keywords——average electronic excitation energy; ^{13}C NMR; protonation shift; lone-pair electron; 4-aminoazobenzene; *trans*-azobenzene; *cis*-azobenzene; benzo[*c*]-cinnoline

Introduction

The correlation between ^{13}C chemical shifts and electronic charges (σ or $\sigma+\pi$) as calculated by molecular orbital methods has been extensively studied in a variety of compounds.[2] This is because if there is a good correlation between them, information can be obtained on the electronic structures of molecules by simply observing their ^{13}C nuclear magnetic resonance (NMR) spectra. It is now well established that ^{13}C chemical shifts and electronic charges correlate well in special cases where other factors, that is, average excitation energy (ΔE) and bond order (Q_{AB}) terms, remain constant throughout the carbons in the molecule.[3] In some cases, however, pronounced variations in these two terms are seen, usually in carbons belonging to nitrogen heteroaromatics.[2,4] In the nitrogen heteroaromatics, since the molecules are almost planar, nitrogen lone-pair electrons can easily migrate, more or less affecting the Q_{AB} and ΔE terms of each carbon. This lone-pair delocalization can be confirmed experimentally by observing ^{13}C NMR spectra of the corresponding protonated molecules in acid solutions. For example, in pyridine the carbon alpha to nitrogen shifts upfield upon protonation, whereas a downfield shift would be predicted on the basis of electronic charges.[4] This decisive discrepancy shows that the lone-pair electrons play an important role in the ^{13}C chemical shifts of the alpha carbons in the pyridine–pyridinium ion system.

Previously, in the course of our studies on the protonation site of 4-aminoazobenzene **1**, we found good correlations between ^{13}C protonation shifts and protonation-induced charge densities on the carbon atoms of amino (N_γ)-protonated 4-aminoazobenzene and of diprotonated (N_α and N_γ) 4-amino-



1a

1b

Chart 1

azobenzene, except for C1 of the latter.[5] The C1 signal of diprotonated species shifted upfield upon protonation, whereas charge density considerations predicted a downfield shift. This situation resembles that of pyridine and suggests that delocalization of lone-pair electrons belonging to azo nitrogens may be responsible for the discrepancy. We can easily envisage the delocalization of lone-pair electrons in nitrogen heteroaromatics, but not in the substituted benzenes. It is desirable, therefore, to investigate in more detail the correlation between ^{13}C chemical shifts and charges of 4-aminoazobenzene.

In this work, we have newly observed ^{13}C chemical shifts of azo (N_β)-protonated species of 4-aminoazobenzene and also calculated the paramagnetic shielding constants (σ_p) for variously protonated models in order to estimate the extents of contribution from the Q_{AB} and ΔE terms. By comparing the calculated and observed ^{13}C protonation shifts, it was found that the resonance structure **1b** of 4-aminoazobenzene plays an important role in the lone-pair delocalization, which may lead to a delocalized excitation of low-lying n-π^* transitions. This view was also supported by the results of an examination of the correlations between ^{13}C protonation shifts and protonation-induced charge densities on the carbon atoms of *trans*- and *cis*-azobenzene and benzo[*c*]cinnoline, for which no resonance structure such as **1b** can be expected.

Experimental

Materials——The samples of 4-amino-^{15}N-azobenzene-α,β-$^{15}N_2$ (AAB-$^{15}N_3$), 4-aminoazobenzene-α-^{15}N (AAB-$^{15}N_1$), and *cis*-azobenzene were taken from the same batches of preparations as those used in the previous work.[5,6]

Measurements——The ^{13}C NMR spectra were recorded on a JEOL FX-200 FT NMR spectrometer operating at 50.18 MHz for AAB-$^{15}N_3$ and AAB-$^{15}N_1$ or on a Varian CFT-20 FT NMR spectrometer operating at 20.0 MHz for *cis*-azobenzene. The measurements were made with complete proton noise decoupling and in 15 mm (FX-200) or 5 mm (CFT-20) spinning sample tubes. The normal operating conditions were 30—45° pulses, 0.0—1.0 s pulse delay, 1.2 Hz (FX-200) or 1.1 Hz (CFT-20) spectral resolution. The chemical shifts were referred to internal DMSO-d_6 (39.6 ppm) for AAB-$^{15}N_3$ and AAB-$^{15}N_1$ and to external dioxane (67.4 ppm) for *cis*-azobenzene. The visible absorption spectra were recorded on a JASCO UVIDEC 505 spectrophotometer. Solutions were prepared in 95% H_2SO_4–DMSO-d_6 (3: 7, v/v, *ca.* 15 mM) for AAB-$^{15}N_3$ and AAB-$^{15}N_1$ and in 22N D_2SO_4–EtOH (7: 1, v/v, 0.5—0.6 M) for *cis*-azobenzene. This acidic condition employed for 4-aminoazobenzene was selected so as to give a maximum absorption intensity at about 500 nm (which is due to N_β-protonated species) while maintaining the concentration at more than 10 mM. The ratio of absorbances between the bands at about 500 nm and at about 320 nm, A(500)/A(320) was 2.5/1. As was described previously,[6] *cis*-azobenzene isomerizes rapidly to the corresponding *trans*-form in strong acid solution. Therefore, several trials with a short number of transients (*ca.* 1000) for freshly prepared *cis*-form materials were necessary to obtain all the ^{13}C chemical shifts of *cis*-azobenzene. On the other hand, on account of the poor solubility of 4-aminoazobenzene (maximum *ca.* 15 mM) under the presently employed acidic condition, a large sample tube (15 mm diameter) and many accumulations (70000—170000 transients) were required to obtain a moderate signal-to-noise ratio. ^{15}N-enriched 4-aminoazobenzenes were used to assist in assigning the ^{13}C resonances from ^{13}C-^{15}N splittings, but no well resolved spin-couplings could be obtained owing to line broadening.

Results and Discussion

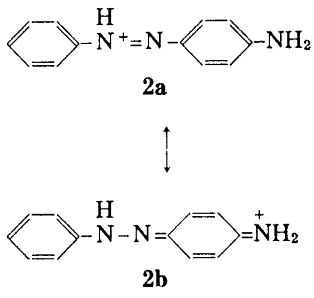
^{13}C protonation shifts of 4-aminoazobenzene observed in 95% H_2SO_4–DMSO-d_6 (3: 7, v/v, solvent I) are shown in the second column of Table I. Our previous experimental data observed in 20% HCl–DMSO-d_6 (1: 10, v/v, solvent II)[5] and the predicted changes in chemical shift upon protonation which were obtained by INDO–MO calculations are also shown in Table I. The ^{13}C protonation shift is the difference between the ^{13}C chemical shift observed in acid solution and that observed in DMSO-d_6; a positive value corresponds to a downfield shift on protonation. Assignments for ^{13}C signals observed in solvent I were determined by taking into account our previous results on the acidity dependence of ^{13}C chemical shifts of 4-aminoazobenzene,[5] multiplicity determination by J-modulated spin-echo,[7] and signal broadening due to ^{13}C–^{15}N spin-couplings.

Table I. Comparison of the Observed and INDO-MO Predicted Changes in ^{13}C Chemical Shift upon Protonation (ppm)

	N_β protonation				N_γ protonation			
	Obsd.[a]	Corrected[c]	Predicted[d]	Δ[e]	Obsd.[b]	Corrected[c]	Predicted[d]	Δ[e]
C1	6.8	7.2	−6.1	−13.3	6.1	5.8	13.2	7.4
C2, 6	5.9	9.5	7.7	−1.8	−0.3	−3.1	−2.4	0.7
C3, 5	11.1	12.1	0.2	−11.9	9.3	8.5	12.9	4.4
C4	−1.3	6.1	9.3	3.2	−14.0	−19.7	−21.9	−2.2
C1′	−5.7	−8.1	−7.2	0.9	−1.5	0.4	−1.7	−2.1
C2′, 6′	4.1	6.2	4.5	−1.7	0.5	−1.1	2.5	3.6
C3′, 5′	4.1	6.2	2.8	−3.4	0.4	−1.3	0.2	1.5
C4′	5.0	6.7	6.9	0.2	2.0	0.6	3.2	2.6

a) 95% H_2SO_4-DMSO-d_6 (3: 7, v/v) was used as the acidic solvent. Accuracy, ±0.1 ppm.
b) 20% HCl-DMSO-d_6 (1: 10, v/v) was used as the acidic solvent. Accuracy, ±0.1 ppm.
c) Corrected by taking in to account the absorption intensities at about 320 and 500 nm. See the text.
d) Predicted value from INDO-MO charge. Conversion factor is 181.8 ppm/e.
e) Δ = predicted − corrected.

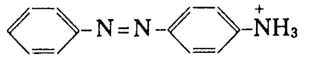
It is well-known that in weak acid solution 4-aminoazobenzene exists as a tautomeric equilibrium mixture of the N_β-protonated (*i.e.*, azonium) 2 and the N_γ-protonated (*i.e.*, ammonium) 3 cations; the former is responsible for the absorption maximum at around 500 nm and the latter for that at around 320 nm.[8] Therefore, strictly speaking, the experimental ^{13}C protonation shifts observed in solvent I and solvent II are not those of a single species. As suggested by Sawicki,[9] the ratio of the absorbances A(500)/A(320) gives an approximate measure of the concentration ratio [2]/[3]. Thus, in order to determine the correlation between "intrinsic" ^{13}C protonation shifts of each single species and the corresponding changes in charge densities, we calculated the ^{13}C protonation shifts of each single species by taking into account the ratios A(500)/A(320) obtained in solvent I (2.5/1) and in solvent II (1/3.5). The corrected ^{13}C protonation shifts thus calculated are shown in the third and the seventh columns of Table I.



2a

2b

Chart 2



3

Chart 3

Examination of Table I indicates that the observed ^{13}C protonation shifts and the corresponding corrected values do not differ greatly from one another, except for C4 of the N_β-protonated species. This finding means that solvents I and II gave almost pure N_β- and N_γ-protonated species, respectively. Overall agreement between the corrected and the predicted values is good for N_γ-protonated species, but not so good for N_β-protonated species, especially at the C1 and C3, 5 positions. Interestingly, the corrected value for C4 of the N_β-protonated species agrees better than the observed value. Furthermore, it is of special interest that C1 shifts downfield whereas C1′ shifts upfield upon N_β-protonation. As reported previously, as for the dicationic species **4** in which N_α and N_γ nitrogens are protonated, both C1 and C1′ showed upfield shifts, the former being much smaller than the latter.[5] For both these cations, the calculated charge densities followed well the large upfield shift of C1′ but not the large downfield shift or the small upfield shift of C1. Therefore, we can find no simple rules as to the

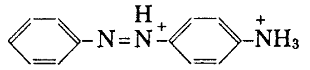


Chart 4

relative direction of the ^{13}C protonation shifts of C1 and C1′, nor any correlation between ^{13}C protonation shifts and protonation-induced charge densities at the C1 position. These findings tell us that other factors, that is, the Q_{AB} and/or ΔE terms, may be affecting the C1 carbon's chemical shift. Moreover, since carbons that deviate largely from the correlation usually belong to the aminobenzene ring (for example, C1 and C3, 5 of N_β- and N_γ-protonated species and C1 and C4 of dicationic species), there seems to be some relation with the resonance structure **1b** of the neutral molecule, in which the electronic structure of the aminobenzene moiety is highly perturbed, in contrast to that of the other benzene ring.

To obtain more explicit or quantitative information on the contribution of the Q_{AB} and ΔE terms to the ^{13}C protonation shifts and also on the relationship of these terms with the resonance structure **1b**, we calculated the paramagnetic shifts σ_p of neutral and protonated 4-aminoazobenzene using Pople's theory based on the average excitation energy approximation.[3,10] The MO method used was the INDO approximation and the necessary parameters were the standard ones of Pople *et al.*[11] Geometrical parameters of neutral and protonated 4-aminoazobenzene were the same as those employed previously.[5] The results are shown in Table II as the difference ($-\Delta\sigma_p$) of paramagnetic shift between protonated and neutral species; a positive value indicates a downfield shift upon protonation. The $-\Delta Q_{AB}$ and $-$ $-\Delta q_A$ columns represent the contributions from the bond order and the charge density terms, respectively. The method of calculation is rather simple in that it substitutes the average excitation energy ΔE for summing over all the excited states. Therefore, the absolute magnitude of σ_p itself is strongly dependent on the choice of ΔE. However, since we use the difference of σ_p but not its absolute magnitude, an appropriate value of ΔE may not introduce much error. In addition, since we have no means to estimate *a priori* the changes in ΔE upon protonation, the ΔE values of neutral and protonated molecules were assumed to be 8 eV as a matter of convenience. Thus, if the calculated and experimental values

TABLE II. Calculated and Experimental Changes in ^{13}C Chemical Shift of 4-Aminoazobenzene on Protonation (ppm)

Atom	$-\Delta Q_{AB}$[a]	$-\Delta q_A$[b]	$-\Delta\sigma_p$[c]	Exptl.	Δ[d]	ΔE[e]
		Monocation (N_β–H)				
C1	2.4	−3.8	−1.4	7.2	8.6	Down
C2, 6	−1.1	4.5	3.4	9.5	6.1	Down
C3, 5	0.0	0.1	0.1	12.1	12.0	Down
C4	1.1	5.9	7.0	6.1		
C1′	−3.2	−4.5	−7.7	−8.1		
		Monocation (N_γ–H)				
C1	0.0	8.3	8.3	5.8	−2.5	Up
C2, 6	0.1	−1.4	−1.3	−3.1		
C3, 5	−0.6	7.5	6.9	8.5		
C4	−2.5	−13.7	−16.2	−19.7	−3.5	Up
C1′	0.1	−1.1	−1.0	0.4		
		Dication (N_α–H and N_γ–H)				
C1	−3.3	4.8	1.5	−1.2	−2.7	Up
C2, 6	−0.3	1.6	1.3	2.4		
C3, 5	−0.3	8.2	7.9	11.9	4.0	Down
C4	−2.5	−8.1	−10.6	−18.9	−8.3	Up
C1′	2.6	−4.9	−2.3	−8.7	−6.4	Up

a) Changes in bond order term upon protonation.
b) Changes in charge density term upon protonation.
c) Changes in paramagnetic shift upon protonation.
$-\Delta\sigma_p = -\Delta Q_{AB} - \Delta q_A$. Positive value of $-\Delta\sigma_p$ corresponds to a downfield shift upon protonation.
d) The difference between exptl. and $-\Delta\sigma_p$; only values of more than 2 ppm are shown.
e) See the text.

disagree substantially, this can be ascribed to the fact that we have employed the same value of ΔE for both the neutral molecule and the corresponding protonated molecule.[12] The experimental and calculated shifts are shown in the last column of Table II, where down (up) means that ΔE of the protonated molecule should be reduced (increased) compared to that of the neutral molecule in order to obtain better agreement with the experimental value. Examination of these results clearly shows that the ΔE values of the N_γ-protonated monocation and those of the dication should increase upon protonation, whereas the ΔE values of the N_β-protonated monocation should decrease upon protonation. The former trend seems straightforward, but not the latter, since protonation removes the low-lying n-π^* transition which results in increase of ΔE. In fact, all the ^{13}C protonation shifts of nitrogen heteroaromatics so far reported are upfield, reflecting well the increase of ΔE.[4] For this rather puzzling situation, we can consider the following explanation in connection with the resonance structure of 4-aminoazobenzene. As can be seen in **2b**, this resonance structure of the N_β-protonated species is the same as that of the neutral species **1b**, leaving lone-pair electrons on the N_α atom. The ΔE values of aminobenzene ring carbons would then be expected to be smaller than those of the neutral molecule since lone-pair delocalization *via* the σ pathway becomes easier owing to the stabilized planar structure of the aminobenzene ring moiety, just as in the cases of nitrogen heteroaromatics. On the contrary, both N_γ- and N_α-protonations suppress such resonance stabilization as **2b** and thus result in increased ΔE compared to that of the neutral molecule.

It would naturally be expected that if we choose azo compounds having no such resonance structure as **1b**, the ^{13}C protonation shifts and protonation-induced charge densities would correlate better than in the case of 4-aminoazobenzene. This is in fact observed, and is shown in Fig. 1, where we show the correlations for *trans*- and *cis*-azobenzene and benzo[*c*]cinnoline. The ^{13}C chemical shifts of *cis*-azobenzene in strong acid media were observed in the present work and the other data were taken from our previous paper.[6] The charge densities were calculated using the same geometries as described previously.[6] The protonation-induced charge densities were converted into the chemical shift scale by using the conversion factor of 181.8 ppm/e.[13] Moreover, since the protonation can be considered to occur at both azo nitrogens of these molecules, the calculated charge densities (or the converted shifts) are the mean values of the two benzene rings for each compound. The good correlations shown in Fig. 1, especially at the C1 positions of *trans*- and *cis*-azobenzene lend further support to our interpretation that lone-pair delocalization promoted by the resonance structure **1b** greatly affects the ΔE values of 4-aminoazobenzene. Large discrepancies observed at C1 and C2 of benzo[*c*]cinnoline may be due to variations in ΔE analogous to those seen in the cases of nitrogen heteroaromatics or 4-aminoazobenzene. Finally, it is interesting to note that the C6 position of this molecule

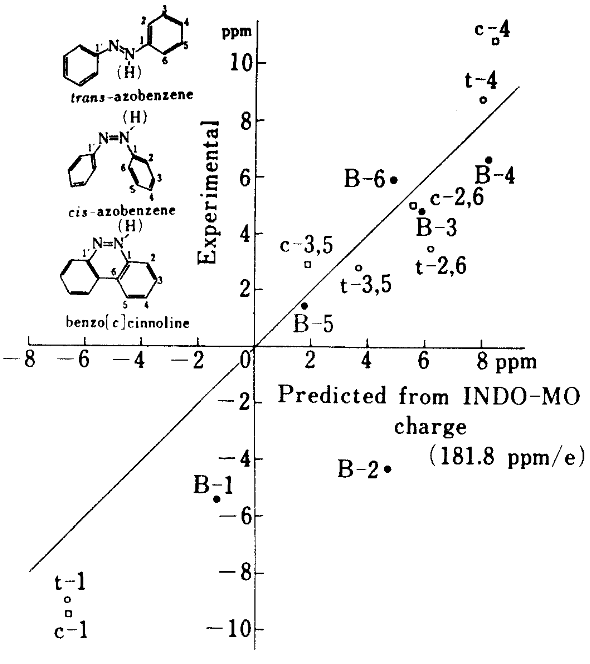


Fig. 1. Changes in ^{13}C Chemical Shifts upon Protonation predicted from INDO-MO Charge *vs.* Expeimental Values

t, *trans*-azobenzene; c, *cis*-azobenzene; B, benzo[*c*]cinnoline.

shows a good correlation, suggesting that lone-pair electrons migrate in the direction of N–C1–C2 but not N–C1–C6 as the preferred delocalization pathway.

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References and Notes

1) This work was presented at the 31st Meeting of the Kinki Branch of the Pharmaceutical Society of Japan, Kobe, November, 1981.
2) *a*) G.L. Nelson and E.A. Williams, *Prog. Phys. Org. Chem.*, **12**, 229 (1976); *b*) J.B. Stothers, "^{13}C NMR Spectroscopy," Academic Press, New York, 1972, p. 239.
3) *a*) J.A. Pople, *J. Chem. Phys.*, **37**, 53 (1962); *b*) M. Karplus and J.A. Pople, *J. Chem. Phys.*, **38**, 2803 (1963).
4) *a*) R.J. Pugmire and D.M. Grant, *J. Am. Chem. Soc.*, **90**, 697 (1968); *b*) T. Tokuhiro and G. Fraenkel, *J. Am. Chem. Soc.*, **91**, 5005 (1969); *c*) J.E. Bloor and D.L. Breen, *J. Am. Chem. Soc.*, **89**, 6835 (1967); *d*) T. Tokuhiro, N.K. Wilson, and G. Fraenkel, *J. Am. Chem. Soc.*, **90**, 3622 (1968); *e*) A. Mathias and V.M.S. Gil, *Tetrahedron Lett.*, 1965, 3163.
5) Y. Kuroda, H. Lee, and A. Kuwae, *J. Phys. Chem.*, **84**, 3417 (1980).
6) Y. Kuroda and Y. Fujiwara, *J. Phys. Chem.*, **85**, 2655 (1981).
7) C. Le Cocq and J.-Y. Lallemand, *J. Chem. Soc., Chem. Commun.*, **1981**, 150.
8) *a*) P.J. Krueger, "The Chemistry of the Hydrazo, Azo and Azoxy Groups," ed. by S. Patai, Wiley-Interscience, New York, 1975, p. 167; *b*) R.A. Cox and E. Buncel, *ibid.*, p. 844; *c*) I. Ya. Bershtein and O.F. Ginzburg, *Russ. Chem. Rev.* (*Engl. Transl.*), **41**, 97 (1972).
9) E. Sawicki, *J. Org. Chem.*, **22**, 621 (1957).
10) J.A. Pople, *Mol. Phys.*, **7**, 301 (1964).
11) J.A. Pople and D.L. Beveridge, "Approximate Molecular Orbital Theory," McGraw-Hill, New York, 1970, p. 80.
12) The small disagreement can be ascribed to other factors, *e.g.*, solvent effects on the observed ^{13}C chemical shifts, molecular geometries assumed in the present calculation, diamagnetic contribution, the neighbor anisotropy effect on the ^{13}C shifts, *etc.* However, the neighbor anisotropy effect may be cancelled out since the ^{13}C protonation shift is the difference between the neutral and protonated chemical shifts. The diamagnetic contribution to the ^{13}C protonation shifts is less than 0.5 ppm according to a calculation using the equation given in Ref. 4*c*.
13) G.L. Nelson, G.C. Levy, and J.D. Cargioli, *J. Am. Chem. Soc.*, **94**, 3089 (1972).