Polish J. Chem., **77**, 1693–1702 (2003)

Low-Barrier Hydrogen Bonds in Pyridine-Dichloroacetic Acid Complexes*

by C.L. Perrin, J.S. Lau and B.K. Ohta**

Department of Chemistry, University of California – San Diego, La Jolla, CA 92093-0358, USA
E-mail: cperrin@ucsd.edu

(Received June 3rd, 2003; revised manuscript July 23rd, 2003)

A mixture of $Cl_2CHCOOH$-$^{16}O_2$ and $Cl_2CHCOOH$-$^{18}O_2$ was combined with an excess of a pyridine and studied by NMR at 23 °C, –43°C, and –81°C. The 1:1 complexes were analyzed by isotopic perturbation of equilibrium. Plots of OH chemical shift and of ^{13}C isotope shift *vs.* pK_a of the pyridinium ion both exhibit maxima ~ 5, when the basicities of the hydrogen-bond acceptors become matched, the hydrogen bond is strongest, and the equilibrium is maximally perturbed by the isotope. In contrast, for the complexes of pyridine and 3-picoline the isotopic perturbation seems to disappear, consistent with a low-barrier hydrogen bond in a single structure, rather than a hydrogen that can take either of two positions in a mixture of two tautomers.

Key words: pyridines H-bonded complexes, NMR, isotope effects, low-barrier H-bond

"Low-barrier" hydrogen bonds. Hydrogen bonds are important structural elements that play a major role in physics, chemistry, and the life sciences [1]. They typically consist of a donor–acceptor system of the form A–H···B, where A and B are N, O, or halogen. Hydrogen bonds have been studied by X-ray crystallography, NMR and IR spectroscopy, and thermochemical measurements. They are usually described by a double-well potential-energy surface, corresponding to a tautomeric equilibrium between **1a** and **1b**.

$$\underset{\textbf{1a}}{\text{A–H---B}} \rightleftarrows \underset{\textbf{1b}}{\text{A---H–B}} \qquad \underset{\textbf{2}}{\text{A---H---B}}$$

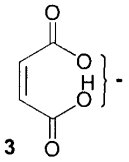
An active field of research is the investigation of exceptionally strong hydrogen bonds [2]. These are unusually short, and the hydrogen is centered with respect to the two heavy atoms, as in **2**. The barrier to hydrogen transfer has decreased to less than the zero-point energy. Such a hydrogen bond is accordingly described by a single-well potential. When A is identical to B, **2** can be called "symmetric". The key distinction though is between a mixture of two species in equilibrium, *versus* a single structure where the hydrogen is shared between the two donor atoms. The contribution of resonance forms of equal energy, or a "covalent character", may then explain their exceptional

---

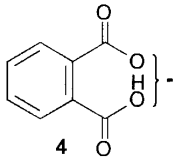
* Dedicated to Prof. M. Szafran on the occasion of his 70th birthday.
**Author for correspondence.

strength [3]. It is not necessary that A and B be identical [4]. It has been proposed that such "short", "strong", "low-barrier" hydrogen bonds are involved in some enzyme-catalyzed reactions, and evidence for them has been sought [5].

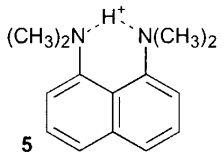
Although dicarboxylate monoanions such as maleate (**3**) and phthalate (**4**) show symmetric hydrogen bonds in crystals, they are a mixture of two tautomers in solution, with the hydrogen closer to one oxygen than to the other and jumping rapidly between the two [6]. Protonated 1,8-bis(dimethylamino)naphthalenes (**5**) are also a mixture [7]. The difference between crystals and solution has been attributed to the disorder of the solvation environment, and computations support this proposal [8].



3



4



5

The role of solvation may be especially important for ions, such as **3–5**. If so, symmetric hydrogen bonds may be more likely for neutrals. Even though some neutrals are not symmetric, this could be due to too long an N–N distance [9]. The question here is whether it is possible to find a centered hydrogen bond (**2**) in a complex between a neutral acid and a neutral base. Although hydrogen transfer produces an ion pair, this too is overall neutral. Of course, since A and B are different, such a hydrogen bond, even if centered, cannot be symmetric. Another potential advantage is that the interaction is intermolecular, so that there are no geometric constraints imposed on the A–B distance, and the hydrogen bond can adjust to its most stable configuration.

Complexes between carboxylic acids and pyridine. A suitable system is the 1:1 complexes (**6**) between pyridines and carboxylic acids. These are known to form strong hydrogen bonds, and dichloroacetic acid (R = $CHCl_2$) has been extensively studied [10]. One of the necessities for formation of single-well hydrogen bonds (**2**) is a matching between the basicities of A and B. In known examples such as crystalline **3** or **4** this is an automatic consequence of the symmetry. With **6** a range of pyridine basicities may be scanned by attaching electron-donating or electron-withdrawing substituents X.

Figure 1 presents this approach. The potential-energy surface for a pyridine–dicarboxylic acid complex (**6a**, **6b**, R = $CHCl_2$) changes with the basicity of the pyridine. As basicity increases, the energy minimum moves to a longer O–H distance, and the equilibrium shifts from **6a** toward **6b**. At some intermediate stage, the basicity matches that of the carboxylate, and the double-well potential-energy surface becomes effectively symmetric, even though nitrogen and oxygen are not the same. At this point a transition to the single-well potential of a short, strong, low-barrier hydrogen bond becomes possible.

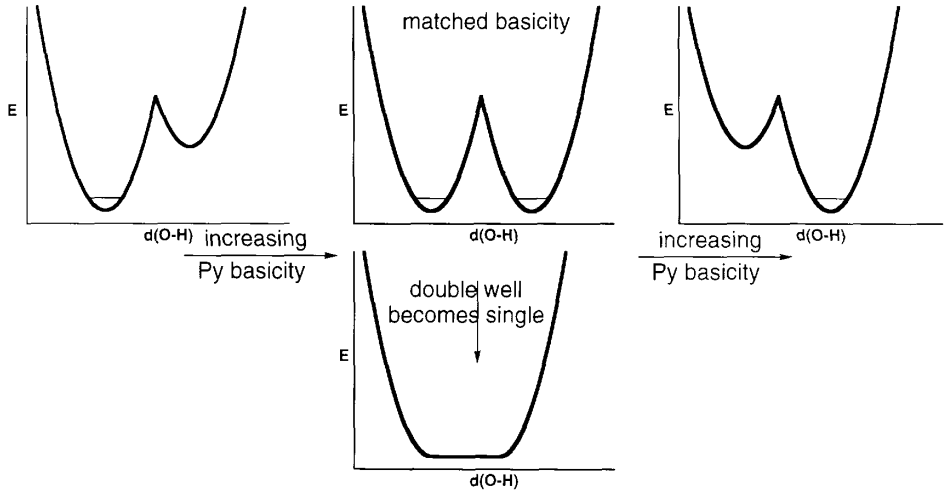


Figure 1. Variation of potential-energy surface for pyridine–dicarboxylic acid complex with increasing basicity of pyridine, and possible transition to a single-well potential when basicities are matched.

This approach is justified by computations on the NHO hydrogen bonds in a keto-hydrazone-azoenol system [11]. Also, experimental results on complexes of pyridine-^{15}N with a series of carboxylic acids show such a variation with basicity, but the proper study of hydrogen bonding in these systems requires low temperatures and aprotic solvents [12]. We therefore have used CD_2Cl_2 at 23°C, –43°C, and –81°C.

As a measure of the basicity of the pyridines, we rely on their tabulated pK_a values in water [13]. Since the pK_a of dichloroacetic acid is 1.29, a pyridine whose conjugate acid has this pK_a would be matched, but only in aqueous solution. In a less polar solvent the pK_a of dichloroacetic acid is higher, so a more basic pyridine is necessary [14]. It is not predictable how much more basic a pyridine is needed, especially since acidities are further modified by the formation of an ion pair. Instead we take an empirical approach, which is well-established [15]. Matched basicity is recognizable through the associated strengthening of the hydrogen bond, which is manifested in the chemical shift of the OH. Therefore, we can rely on the most downfield OH shift as indicating a regime where the equilibrium between **6a** and **6b** is balanced. The question then is whether the central potential barrier disappears and the potential becomes single-well.

Isotopic perturbation. The key method used in this investigation is isotopic perturbation of equilibrium [16]. It succeeds in distinguishing a single species (**2**) from a tautomeric mixture (**1a** plus **1b**) even if interconversion is rapid, as must be the case

for hydrogen motion. The basis of this method is that substitution with a heavier isotope lowers the zero-point energy of a vibration. This can stabilize either **1a** or **1b** and thereby change the position of the equilibrium between them. For the specific case of **6a** and **6b** the substitution is here a double one, with two ^{18}Os in the carboxyl group. Their effect is to reduce the acidity of the carboxylic acid. Then, since NMR chemical shifts are quite sensitive to the state of carboxyl protonation, the shift of the equilibrium can be detected. Of course it must be acknowledged that isotope effects due to ^{18}O substitution are much smaller than those due to the deuterium substitution that is most often used for studies of hydrogen bonding [17].

The isotope shift is defined as the chemical shift of the carboxyl carbon of the $^{18}O_2$ acid, relative to that of the $^{16}O_2$ acid (eq 1) [18]. The observed isotope shift is composed of an intrinsic shift Δ_0 and a shift Δ_{pert} due to perturbation of an equilibrium between the two tautomers (eq 2). This latter can be shown to be given by eq 3, where δ_{OH} and δ_{O^-} are the chemical shifts of the carboxyl carbons in tautomers **6a** and **6b**, respectively, K_e is the equilibrium constant [**6b**]/[**6a**], taken as the geometric average over ^{16}O and ^{18}O acids, and K is the ratio of K_e for the ^{16}O acid to that for the ^{18}O acid. According to ^{18}O isotope effects on acidity constants of carboxylic acids [19], K is expected to be ~1.02 at 25°C, although this may be modified by the hydrogen bonding. However, it is not necessary to know this value.

$$\Delta_{obs} = \delta_{C^{18}O_2} - \delta_{C^{16}O_2} \tag{1}$$

$$\Delta_{obs} = \Delta_0 + \Delta_{pert} \tag{2}$$

$$\Delta_{pert} = (\delta_{OH} - \delta_{O^-}) \frac{K - 1}{1 + K + K^{1/2}K_e + K^{1/2}/K_e} \tag{3}$$

This Δ_{pert} has the proper limiting behavior, approaching zero for large or small K_e and reaching a maximum when $K_e = 1$. That maximum arises because the isotopic substitution exerts its greatest perturbation on the equilibrium between **6a** and **6b** when that equilibrium is balanced. However, if the symmetric double-well potential of Figure 1 is transformed into a single-well potential, then there will be only a single species, with no equilibrium. If so, Δ_{pert} will disappear, and Δ_{obs} will revert to the intrinsic Δ_0. This is thus a characteristic signature of a low-barrier hydrogen bond. It is a more sensitive test than that in an NDN hydrogen bond where distances are consistent with a low barrier [20]. In that case the characteristic signature is a zero primary isotope shift, but since that quantity changes sign as the ^{15}N chemical shift increases, it also must necessarily be zero merely by the principle of continuity. We now report a Δ_{pert} for a series of complexes (**6**) between pyridines and carboxylic acids, but not for pyridine or 3-picoline, where Δ_{pert} seems to disappear.

EXPERIMENTAL

General. 3-Fluoropyridine, 3-methoxypyridine, and 3,5-lutidine were dried with K_2CO_3 and fractionally distilled. 2-Phenylpyridine was dried with sodium and distilled under reduced pressure. Pyridine and 2,5- and 2,6-lutidine were dried with sodium and fractionally distilled. 3-Picoline was dried with $LiAlH_4$ and distilled. Pyridines were stored over 4A molecular sieves under dry N_2. Dichloroacetic acid was purified by fractional distillation and stored under dry N_2. Dichloroacetyl chloride and dichloroacetic anhydride were used as provided. Water-^{18}O, 94.1%, was obtained from ICON Services, Inc. Dichloromethane-d_2 in sealed ampules was obtained from Cambridge Isotope Laboratories.

$Cl_2CHCOOH$-$^{18}O_2$. Dichloroacetyl chloride (0.5 mL, 5 mmol) was stirred in an ice bath with 0.3 mL $H_2{}^{18}O$ (16 mmol) for 1 hr, then warmed to room temperature and stirred for two days. Water was then evaporated under reduced pressure (< 1 mm Hg) for 48 hours. Incorporation of label was confirmed by ^{13}C NMR of a 1:2 mixture with unlabeled dichloroacetic acid in $CDCl_3$, and the center signal, due to $Cl_2CHCOOH$-^{18}O, represented only ~35% of the $Cl_2CHCOOH$-$^{18}O_2$ content.

Sample preparation. Samples were prepared under dry N_2 using syringe transfer techniques. Pyridine or a substituted pyridine (1.2 mmol) was added to dichloromethane-d_2 (1 g). Dichloroacetic acid-$^{18}O_2$ (5 μL, 0.06 mmol) and dichloroacetic acid (5 μL, 0.06 mmol) were added to the solution. The excess of pyridine guarantees that there are only 1:1 pyridine-carboxylic acid complexes, and no acid dimers [21]. To scavenge any adventitious water, dichloroacetic anhydride (9 μL, 0.06 mmol) was added and allowed to react at room temperature for 30 minutes. This caused some scrambling of the label, but the NMR signal of $Cl_2CHCOOH$-^{18}O was still resolvable. Samples were transferred by positive N_2 pressure to vacuum-evacuated NMR tubes fitted with J Young valves. The same sample was used at all temperatures.

NMR spectra. Spectra were recorded on a Varian Unity 500 spectrometer operating at a ^{13}C frequency of 125.823 MHz. Default parameters were used, except the ^{1}H sweep width was expanded to include the downfield OH signal, and the ^{13}C spectra were taken with a 4-fold augmentation of data points, to improve digital resolution. Low-temperature spectra were obtained using liquid N_2 coolant and a variable-temperature controller. Samples were equilibrated until the chemical shift of the exchangeable OH remained constant within ± 0.001 ppm. Temperatures were calibrated from the chemical shifts of a methanol sample.

RESULTS AND DISCUSSION

NMR chemical shifts and isotope shifts. The data at 23°C, –43°C, and –81°C are summarized in Table 1. The first two columns list the substituent on the pyridine ring and the aqueous pK_a of that pyridinium ion. The following columns list either the ^{1}H chemical shift of the hydrogen-bonded proton (δ) or the ^{13}C NMR isotope shift (Δ) of the carboxyl carbon, tabulated as $-\Delta$. The isotope shifts are small but measurable if care is taken to ensure high-resolution spectra. All isotope shifts are negative, since the effect of a heavier isotope is almost always a greater shielding [18].

Chemical shifts for the OH are very far downfield, well within the range associated with strong hydrogen bonds. The only exception is 3-fluoropyridine.

Figure 2 shows how the OH chemical shifts of these 1:1 complexes vary with the basicity of the pyridine. The smooth curves are parabolas, arbitrarily chosen to fit the data by a linear least-squares routine. The maxima at 23°C, –43°C, and –81°C are at pK_a 5.79, 5.46, and 5.17, respectively.

Table 1. ^{1}H chemical shifts (ppm) of OH and ^{13}C isotope shifts (ppb) of carboxyl carbon in pyridine-dichloroacetic acid complexes **6**.

X	pK_a [a]	δ_H [b]	δ_H [c]	$-\Delta_C$ [c]	δ_H [d]	$-\Delta_C$ [d]
3-F	2.97	13.0	14.2	56	17.2	56
2-Ph	4.48	18.8	19.9	60	20.2	60
3-CH_3O	4.88	19.5	20.4	60	20.5	60
H	5.17	19.8	20.6	52	20.6	55
3-CH_3	5.68	20.0	20.4	54	20.3	54
3,5-$(CH_3)_2$	6.14	20.1	20.2	57	19.9	56
2,5-$(CH_3)_2$	6.47	19.9	19.7	58	19.4	56
2,6-$(CH_3)_2$	6.60	19.6	19.4	52	19.1	52

[a]From Ref. 13. [b]23°C. [c]–43°C. [d]–81°C.

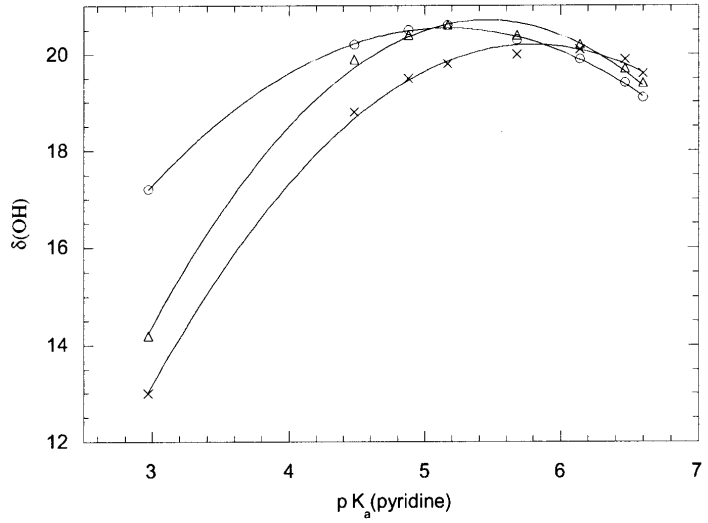


Figure 2. ^{1}H chemical shift δ_{OH} *vs*. pyridine pK_a: (x) at 23°C, (Δ) at –43°C, (o) at –81°C, fitted as parabolas.

At 23°C the carboxyl peaks were often too broad for ^{13}C isotope shifts to be resolved, perhaps owing to paramagnetic impurities, and no pattern could be detected for their basicity dependence. At –43°C and –81°C there are larger isotope shifts for the complexes of 2-phenyl-, 3-methoxy-, 3,5-dimethyl-, and 2,5-dimethyl-pyridine. Along with pyridine and 3-picoline these are the pyridines whose complexes show a maximum ^{1}H chemical shift.

Figure 3 shows how the magnitudes of the ^{13}C NMR isotope shifts vary with the basicity of the pyridine. At the extremities of low and high pK_a Δ_{obs} is simply the intrinsic Δ_0, but the magnitude of Δ_{obs} rises in the middle. The smooth curves are the fit to eqs 2 and 3, excluding pyridine and 3-picoline. They were obtained by a two-para-

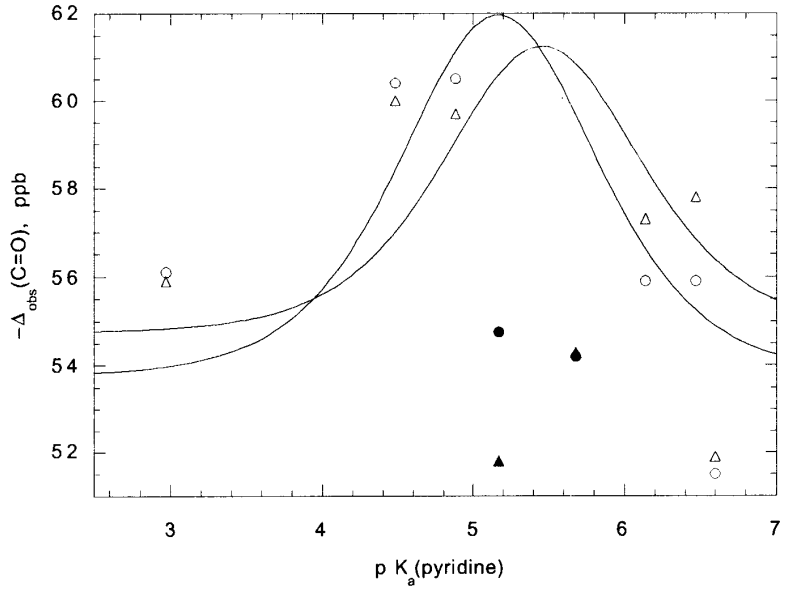


Figure 3. ^{13}C isotope shift Δ *vs.* pyridine pK_a: (Δ) at –43°C, (o) at –81 °C (pyridine and 3-picoline in solid symbols) , fitted to eq 3.

meter nonlinear least-squares routine that fixed pK_e as pK_{max} – pK_a, where pK_{max} is 5.46 or 5.17, the maxima in Figure 2. At both –43°C and –81°C Δ_0 is –54 ± 2 ppb.

The complexes of pyridine and 3-picoline do not fit on these curves. Instead their isotope shifts are quite close to the intrinsic Δ_0. The deviation is small, only a few ppb, but the precision in measuring isotope shifts is better than ± 1 ppb, which is adequate to conclude that their magnitude is lower than for the other pyridines of similar basicity.

The ^{1}H chemical shift data indicate a maximum downfield shift that corresponds to an aqueous pyridinium pK_a near 5.77 at 23°C, 5.46 at –43°C, and 5.17 at –81 °C (Figure 2). These values represent the apparent pK_a of dichloroacetic acid in the dichloromethane solvent. They are higher than its tabulated pK_a of 1.29 because dichloromethane is less polar than water. A pyridine with that higher pK_a then has a basicity that matches that of dichloroacetate.

This pK_a decreases with decreasing temperature. This reflects the well-known, but somewhat counterintuitive fact, that solvent polarity, as measured by dielectric constant, increases with decreasing temperature. Such a temperature dependence of the condition for matching basicities has been noted previously [22].

The magnitude of the isotope shift of the carboxyl carbon increases in some complexes of substituted pyridines with dichloroacetic acid. At –43°C and –81°C these are 2-phenyl-, 3-methoxy-, 3,5-dimethyl-, and 2,5-dimethyl-pyridine. These are the pyridines whose aqueous pK_as are in the range of 5–6. They are among the ones that form the strongest hydrogen bonds, as judged by the maximum downfield OH chemical shifts. Thus the maxima of Figure 3 were fixed to correspond to those of Figure 2.

This increase in isotope shift is due to the contribution of Δ_{pert} (eq 3), arising from isotopic perturbation of equilibrium. This contribution is maximum when the basicities of the pyridine and dichloroacetate become matched. According to the fit to eq 3, that maximum corresponds to a Δ_{pert} of -6.5 ± 5 ppb at –43°C and -8 ± 3 ppb at –81°C. The magnitude is expected to be larger at lower temperature because K, the ^{18}O isotope effect on acidity, is larger at lower temperature. However, it must be recognized that the values are uncertain, especially since two complexes were excluded from the fitting.

The operation of isotopic perturbation requires the existence of an equilibrium between two tautomers, **6a** and **6b**. This behavior is consistent with the description of the hydrogen bond in these complexes as a double-well potential, in which the hydrogen is either on the oxygen or the nitrogen, and rapidly moving from one to the other. The equilibrium is closely balanced, so that the hydrogen can be found in either position.

The complexes of pyridine and 3-picoline are exceptions. They do not fit on these curves. Instead their isotope shifts appear to have reverted to the intrinsic Δ_0. The disappearance of Δ_{pert} is inconsistent with a tautomeric mixture. It is consistent with a single structure, with a low-barrier hydrogen bond. The neutral nature of this complex, and its intermolecularity, may be reasons why this hydrogen bond can adopt a geometry that permits the hydrogen to be shared between the two donor atoms.

There is considerable scatter in Figure 3. In part this is because of experimental error in measurement of isotope shifts, which is quite sensitive to problems of resolution. Another source is the imperfect transferability of pK_a values. These reflect the effect of substituents on the tendency of a pyridine to accept a proton from water. This is not necessarily the same as the effect of those substituents on the position of the equilibrium between **6a** and **6b**, involving a neutral hydrogen-bonded complex in a nonpolar solvent. To the extent that the potential-energy surface (Figure 1) is more sensitive than chemical shift to the details of pK_a matching, Figure 3 will show more scatter than Figure 2.

CONCLUSIONS AND UNCERTAINTIES

At –43°C and –81°C the magnitudes of the ^{18}O-induced ^{13}C NMR isotope shift of the carboxyl carbons in 1:1 complexes of dichloroacetic acid with pyridines show maxima. These are the same pyridines for which the chemical shift of the OH is maximum, corresponding to the strongest OHN hydrogen bonds. The increased isotope shift is due to perturbation of a closely balanced tautomeric equilibrium between **6a** and **6b**.

The maximum $-\Delta_{pert}$ of 6.5 ppb at –43°C or 8 ppb at –81°C is smaller than the ~20 ppb seen in monoanions of dicarboxylic acids at room temperature [6]. The diminution is not due to the factor of 1/2 that enters when the comparison is intermolecular, since this is compensated for by the double ^{18}O substitution. The discrepancy may be due to the hydrogen bonding to the pyridines. Nevertheless, the firm conclusion is

that each of these complexes is a mixture of two tautomers, not a single structure with a single-well-potential hydrogen bond.

The complexes of pyridine and 3-picoline are exceptions. Their isotope shifts seem to revert to the intrinsic Δ_0. Such a reversion had been anticipated if the symmetric double-well potential undergoes a transition to the single-well potential of a short, strong, low-barrier hydrogen bond, as in Figure 1. An isotope shift equal to the intrinsic Δ_0 is not consistent with the perturbation of a tautomeric equilibrium between **6a** and **6b**. We therefore conclude that each of these complexes is a single structure, with its hydrogen shared between nitrogen and oxygen. However, this conclusion is tentative, because it is based on a limited number of experimental data.

Such hydrogen bonds seem to be quite elusive in solution, according to the criterion of the disappearance of isotopic perturbation. Our earliest studies had been of intramolecular hydrogen bonds in ions, where the disorder of solvation may be especially important and where geometric constraints may not permit the optimum distance. With these neutrals, which are formed by intermolecular association, the strong hydrogen bond can become one where the hydrogen is shared between the two donor atoms. However, this occurs only for the complexes with pyridine and 3-picoline, and not with substituted pyridines of similar basicity. It may be that the basicities of those others do not match closely enough to permit such a hydrogen bond.

It might be appropriate to scan basicities at a finer mesh, in order to find other matches. It would also be reassuring to measure isotope shifts in complexes with additional pyridines and at additional temperatures in order to evaluate the maximum Δ_{pert} more reliably. The question remains as to whether these hydrogen bonds occur over only a narrow range of basicities that include pyridine and 3-picoline. It may also be that the range is broader at lower temperature.

It also seems that hydrogen bonds with a shared hydrogen are only a low-temperature phenomenon. If so, we would again assert that it is the disorder of solvation that disrupts the favorable local environment that is a prerequisite for such a hydrogen bond.

Acknowledgments

This research was supported by NSF Grants CHE94-20739 and CHE99-82103 and by Instrumentation Grant CHE97-09183.

REFERENCES

1. Jeffrey G.A., An Introduction to Hydrogen Bonding, Oxford, 1997; Scheiner S., Hydrogen Bonding: A Theoretical Perspective, Oxford, 1997.
2. Emsley J., *Chem. Soc. Revs*., **9**, 91 (1980); Hibbert F. and Emsley J., *Adv. Phys. Org. Chem*., **28**, 255 (1990); Dega-Szafran Z. and Szafran M., *Heterocycles*, **37**, 627 (1994).
3. Gilli P., Bertolasi V., Ferretti V. and Gilli G., *J. Am. Chem. Soc*., **116**, 909 (1994).
4. Gilli P., Bertolasi V., Ferretti V. and Gilli G., *J. Am. Chem. Soc*., **122**, 10405 (2000).
5. Frey P.A., *Magn. Reson. Chem.*, **39**, S190 (2001); Westler W.M., Frey P.A., Lin J., Wemmer D.E., Morimoto H., Williams P.G. and Markley J.L., *J. Am. Chem. Soc*., **124**, 4196 (2002).

6. Perrin C.L. and Thoburn J.D., *J. Am. Chem. Soc*., **111**, 8010 (1989); Perrin C.L. and Thoburn J.D., *J. Am. Chem. Soc*., **114**, 8559 (1992); Perrin C.L., *Science*, **266**, 1665 (1994); Perrin C.L. and Nielson J.B., *J. Am. Chem. Soc*., **119**, 12734 (1997).
7. Perrin C.L. and Ohta B.K., *J. Am. Chem. Soc*., **123**, 6520 (2001).
8. Garcia-Viloca M., Gonzalez-Lafont A. and Lluch J.M., *J. Am. Chem. Soc*., **121**, 9198 (1999); Bach R.D., Dmitrenko O. and Glukhovtsev M.N., *J. Am. Chem. Soc*., **123**, 7134 (2001).
9. Perrin C.L. and Ohta B.K., *Bioorg. Chem*., **30**, 3 (2002); Perrin C.L. and Ohta B.K., J. *Mol. Struc*t., **644**, 1 (2003).
10. Dega-Szafran Z. and Dulewicz E., *J. Chem. Soc. Perkin Trans. 2*, 345 (1983).
11. Gilli P., Bertolasi V., Pretto L., Lycka A. and Gilli G., *J. Am. Chem. Soc*., **124**, 13554 (2002).
12. Smirnov S.N., Golubev N.S., Denisov G.S., Benedict H., Schah-Mohammedi P. and Limbach H.H., *J. Am. Chem. Soc*., **118**, 4094 (1996); Smirnov S.N., Benedict H., Golubev N.S., Denisov G.S., Kreevoy M.M., Schowen R.L. and Limbach H.-H., *Canad. J. Chem*., **77**, 943 (1999); Shenderovich I.G., Burtsev A.P., Denisov G.S., Golubev N.S. and Limbach H.-H., *Magn. Reson. Chem*., **39**, S91-S99 (2001); Shenderovich I.G., Limbach H.-H., Smirnov S.N., Tolstoy P.M., Denisov G.S. and Golubev N.S., *Phys. Chem. Chem. Phy*s., **4**, 5488 (2002).
13. Jencks W.P. and Regenstein J., in Handbook of Biochemistry, 2 ed., Chemical Rubber Co., Cleveland, OH, 1970, p. J–216f.
14. Denisov G.S., Gindin V.A., Golubev N.S., Ligay S.S., Shchepkin D.N. and Smirnov S.N., *J. Mol. Liq*., **67**, 217 (1995).
15. Sobczyk L. and Pawelka Z., *J. Chem. Soc., Farad. Trans. 1*, **70**, 832 (1974); Brycki B., Dega-Szafran Z. and Szafran M., *Adv. Mol. Relax. Int. Proc*., **15**, 71 (1979); Dega-Szafran Z., Szafran M., Stefaniak L., Brevard C. and Bourdonneau M., *Magn. Reson. Chem*., **24**, 424 (1986).
16. Saunders M., Telkowski L. and Kates M.R., *J. Am. Chem. Soc*., **99**, 8070 (1977); Siehl H.-U., *Adv. Phys. Org. Chem*., **23**, 63 (1987); Forsyth D.A., In Isotopes in Organic Chemistry; Eds. Buncel E. and Lee C.C., Elsevier, Amsterdam, 1987, Vol. 6, Chapter 1.
17. Schah-Mohammedi P., Shenderovich I.G., Detering C., Limbach H.-H., Tolstoy P.M., Smirnov S.N., Denisov G.S. and Golubev N.S., *J. Am. Chem. Soc*., **122**, 12878 (2000); Dziembowska T., Rozwadowski Z., Filarowski A. and Hansen, P.E., *Magn. Reson. Chem*., **39**, S67 (2001); Grech E., Klimkiewicz J., Nowicka-Scheibe J., Pietrzak M., Schilf W., Pozharski A.F., Ozeryanski V.A., Bolvig S., Abildgaard J. and Hansen P.E., *J. Mol. Struct*., **615**, 121 (2002).
18. Batiz-Hernandez H. and Bernheim R.A., *Prog. Nucl. Magn. Reson. Spectrosc*., **3**, 63 (1967); Hansen P.E., *Annu. Rep. NMR Spectrosc*., **15**, 105 (1983); Hansen P.E., *Prog. Nucl. Magn. Reson. Spectrosc*., **20**, 207 (1988); Hansen P.E., *Magn. Reson. Chem*., **38**, 1 (2000).
19. Ellison S.L.R. and Robinson M.J.T., *J. Chem. Soc., Chem. Commun*., 745 (1983); Tanaka N. and Araki M., *J. Am. Chem. Soc*., **107**, 7780 (1985).
20. Benedict H., Limbach H.-H., Wehlan M., Fehlhammer W.-P., Golubev N.S. and Janoschek R., *J. Am. Chem. Soc*., **120**, 2939 (1998).
21. Barczynski P., Dega-Szafran Z. and Szafran M., *J. Chem. Soc., Perkin Trans. 2*, 901 (1987); Dega-Szafran Z., Dulewicz E. and Szafran M., *J. Mol. Struct*., **177**, 317 (1988).
22. Rospenk M., Sobczyk L., Schah-Mohammedi P., Limbach H.-H., Golubev N.S. and Melikova S.M., *Magn. Reson. Chem*., **39**, S81 (2001).