Bioorganic Chemistry **30,** 3–15 (2002)
doi:10.1006/bioo.2001.1222, available online at http://www.idealibrary.com on IDEAL®

Symmetry of O-H-O and N-H-N Hydrogen Bonds in 6-Hydroxy-2-formylfulvene and 6-Aminofulvene-2-aldimines[1]

Charles L. Perrin[2] and Brian K. Ohta

Department of Chemistry and Biochemistry, University of California at San Diego, La Jolla, California 92093-0358

Received June 5, 2001

The symmetry of the hydrogen bonds in 6-hydroxy-2-formylfulvene and two *N,N′*-diaryl-6-aminofulvene-2-aldimines is probed by the NMR technique of isotopic perturbation. Observed deuterium-induced ^{13}C NMR isotope shifts at several positions can be attributed to a combination of an intrinsic shift and the perturbation of a tautomeric equilibrium. The most dramatic are at the aldehydic or aldiminic carbon signals, where the observed isotope shift for the unlabeled carbon is +376 or +223 ppb. This large downfield shift is contrary to the small upfield shift expected for a four-bond intrinsic shift and can be attributed only to a perturbation shift. Therefore these intramolecular hydrogen bonds are asymmetric, the proton resides in a double-minimum potential surface, and each molecule exists as a pair of rapidly interconverting tautomers, regardless of solvent. The symmetry of the hydrogen bond is not governed only by the O-O or N-N distance. It is proposed that symmetric hydrogen bonds can be observed in crystalline phases but not as yet in solution because the disorder of the solvation environment induces an asymmetry of the hydrogen bond, whereas a crystal can guarantee a symmetric environment. These results provide no insight into the source of the stabilization attributed to low-barrier hydrogen bonds if they lack the special feature of symmetry.

Key Words: symmetry; hydrogen bonds; NMR; isotopic perturbation; LBHBs.

INTRODUCTION

Hydrogen bonds are a key feature of molecular structure and the focus of an enormous number of theoretical and experimental studies (*1*). Usually the motion of the hydrogen-bonded hydrogen is described by a double-well potential, but in symmetric hydrogen bonds it is a single well. Such hydrogen bonds require donor atoms of equal basicity and an unusually short O-O distance of $\leq$2.5 Å (*2*). These hydrogen bonds are unusually strong, perhaps because resonance energy is maximized when two resonance forms are of equal energy, or because of covalent character (*3*). These have been called short, strong hydrogen bonds or low-barrier hydrogen bonds (LBHBs) or centered or symmetric hydrogen bonds, depending on the observational criterion.

[1] This paper is dedicated to F. H. Westheimer, on the occasion of his 90th birthday.

[2] To whom correspondence and reprint requests should be addressed. Fax: (858) 822-0386. E-mail: cperrin@ucsd.edu.

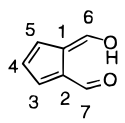
0045-2068/02 $35.00

Recently LBHBs have been proposed to play a role in enzymatic catalysis (*4*). An extra stabilization is claimed to reduce the activation barrier for the formation of high-energy intermediates or transition states. Yet hydrogen-bond strengths are often considerably lower than the 10–20 kcal/mol suggested (*5*). Consequently this proposal has generated considerable controversy (*6*), and there are some enzymic reactions where LBHBs are not required (*7*). Authoritative recent reviews are available (*8*). The potential significance of symmetric hydrogen bonds has prompted us to search for examples in solution.

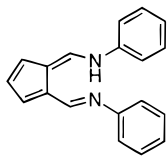
The question is whether the proton in the hydrogen bond resides in a single- or a double-minimum potential surface. Alternatively, the crucial distinction is between a single symmetric structure and a pair of rapidly interconverting asymmetric tautomers.

Our previous studies addressed the intramolecular O-H-O hydrogen bonds in 3-hydroxy-2-phenylpropenal (*9*) and in dicarboxylate monoanions (*10*), as well as the N-H-N hydrogen bonds in protonated 1,8-bis(dimethylamino)naphthalenes (*11*). We now extend those studies to the O-H-O and N-H-N hydrogen bonds of neutrals 6-hydroxy-2-formylfulvene (**1**), *N*,*N*′-diphenyl-6-aminofulvene-2-aldimine (**2**), and *N*,*N*′-bis(3,5-dimethylphenyl)-6-aminofulvene-2-aldimine (**3**).

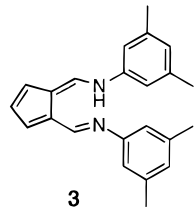
These molecules are good candidates for symmetric hydrogen bonding. The short O-O and N-N distances, 2.51 and 2.79 Å, respectively (*12*), favor transformation of a double-well potential into a single-well one. The donor and acceptor basicities are necessarily matched. An unusually strong bond is indicated by ^{15}N-^{15}N and $^{15}N \cdots H$ scalar couplings across the N-H-N hydrogen bond in a desymmetrized analog (*13*). Moreover, aromaticity in the cyclopentadienyl ring may provide additional stabilization to the symmetric structure.



1



2



3

There is considerable evidence against symmetric hydrogen bonds in these molecules, from X-ray and neutron-diffraction data (*12*), quadrupole coupling constants (*14*), UV-visible absorption (*15*), X-ray photoelectron spectroscopy (*16*), and microwave studies (*17*). A 6-31G**/MP2 calculation indicates that the symmetric structure of **1** is 1.8 kcal/mol less stable than the asymmetric one, but the stabilities reverse, by 0.5 kcal/mol, when zero-point energy is included (*18*). Although N-H-N hydrogen bonds are generally longer and thus weaker than O-H-O, the O-O distance in a fully planar 1,2-dicarboxylate monoanion (a seven-membered ring, including H) would be shorter than optimum (*19*), so that the N-N distance in **2** or **3** may be more favorable for a strong, symmetric hydrogen bond.

This study also addresses the influence of solvation on the symmetry of the hydrogen bond. Because solvation stabilizes localized charges more than delocalized ones (*20*), the hydrogen bonds in the ions studied previously may suffer from a bias toward asymmetry that is not operative with neutrals. Although previous work showed the

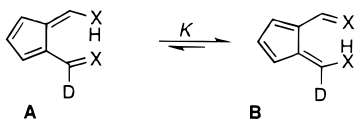
O-H-O hydrogen bond of uncharged 3-hydroxy-2-phenylpropenal to be asymmetric (*9*), this may simply be a consequence of too long an O-O distance. Besides, oxygens possess additional lone pairs that can hydrogen bond to solvent. We now study N-H-N systems that are neutral and that lack lone pairs or NH that can hydrogen bond to solvent, as well as a comparison O-H-O system.

METHODOLOGY OF ISOTOPE SHIFTS

Isotopic perturbation of equilibrium is a powerful and widely applicable NMR technique for distinguishing a single symmetric structure from a pair of asymmetric tautomers (*21*). It succeeds even when interconversion is so rapid that separate signals from individual tautomers are not seen. It relies on measuring the isotope shift ${}^{n}\Delta_{obs}$, the difference (Eq. (1)) between the ^{13}C chemical shifts in molecules with and without deuterium (*22*). This includes an intrinsic contribution ${}^{n}\Delta_{0}$, which is usually <0 (upfield) and falls off rapidly with n, the number of bonds between the ^{13}C and the D.

$$ {}^{n}\Delta_{obs} = \delta_{C(D)} - \delta_{C(H)} \tag{1} $$

The isotopolog of **1, 2**, or **3** with exactly one deuterium at C6,7 can be synthesized by known procedures. If the hydrogen bond is asymmetric, there is an additional contribution to Δ_{obs}, arising from perturbation of the equilibrium between the two tautomers **A** and **B** (X = O, NAr). Because an enol or enamine has a higher C-H stretching frequency than an aldehyde or imine, tautomer **A** has a higher zero-point energy. Therefore the equilibrium favors the other tautomer **B**, and the time-averaged chemical shifts are displaced from those in d_0, where the two tautomers are of identical stability. This is seen as a perturbation (of equilibrium) isotope shift given by Eq. (2), where $D = \delta_{XH} - \delta_{=X}$, the chemical-shift difference between exchange-related carbons proximal and distal to the OH or NH in a static tautomer.



$$ {}^{n}\Delta_{e} = \frac{K - 1}{2(K + 1)} D \tag{2} $$

The perturbation shifts can be estimated. By analogy to 3-hydroxy-2-phenylpropenal, ${}^{4}\Delta_{e}$ at the aldehydic carbons of **1** is expected to be ca. +0.76 ppm (*9*). For **2** or **3** at 25°C the equilibrium constant K is ca. 1.09, from the difference of 18.3 cm^{-1} in CH vs CD zero-point energies between enamine (ν = 2990 cm^{-1}) and aldimine (ν = 2865 cm^{-1}) (*23*). The chemical shifts can be estimated (δ = 130, 160 ppm) from these same models (*24*). Then, according to Eq. (2), the aldiminic CH is expected to exhibit a ${}^{4}\Delta_{e}$ of +0.66 ppm, and the CD a ${}^{4}\Delta_{e}$ of −0.66 ppm, but shifted further upfield by ${}^{1}\Delta_{0}$ and observable only with ^{2}H decoupling. The perturbation shift ${}^{4}\Delta_{e}$ at C4 is automatically zero by symmetry. The chemical shifts for the fulvene carbons of a frozen tautomer of **1** or **2** can be estimated from the acetyl and trimethylsilyl

derivatives, and the chemical shifts for phenyl carbons in a frozen tautomer of **2** or **3** can be estimated by using *N*-benzylaniline and *N*-benzylideneaniline as models (*25*). Table 1 lists the estimated pertubation isotope shifts, all but one of which are positive (downfield for the carbon nearer H).

Isotope shifts are now used to determine the shape of the potential surface of the O-H-O hydrogen bond of **1** and the N-H-N hydrogen bonds of **2** and **3**. If the hydrogen bond is symmetric, the labeled compound will exhibit only intrinsic shifts. Instead, a combination of an intrinsic isotope shift and a shift due to perturbation of a tautomeric equilibrium is seen at several carbons. Thus each of these species exists as a pair of rapidly interconverting tautomers, and the hydrogen bond is characterized by a double-well potential.

MATERIALS AND METHODS

6-Dimethylamino-2-(N,N-dimethylformiminium)fulvene perchlorate (26). This precursor was prepared from 6-(dimethylamino)fulvene (*27*) and dimethylformamide-$POCl_3$ complex (*28*) in THF, precipitated with $NaClO_4$ in methanol, and recrystallized: 17% yield, mp. 229–233°C (lit. 235–237°C). ^{13}C NMR ([$^2H_6$]DMSO) δ 48.5, 118.4, 125.3, 128.5, 154.3.

*6-Hydroxy-2-formylfulvene (**1**) (26).* The perchlorate salt above was stirred with chloroform and aqueous NaOH. The organic material was collected, washed with water, and evaporated. The residue was stirred with aqueous NaOH at 50°C under N_2, then acidified with aqueous HCl, extracted with CH_2Cl_2, and evaporated carefully: 64% yield.

*N,N′-Diphenyl-6-aminofulvene-2-aldimine (**2**) (15).* A solution of perchlorate salt in ethanol was refluxed with aniline and cooled, and the resulting precipitate was recrystallized: 53% yield, mp 98–100°C (lit. 100°C).

*N,N′-Bis(3,5-dimethylphenyl)-6-aminofulvene-2-aldimine (**3**).* Perchlorate salt was refluxed in ethanol with 3,5-dimethylaniline and cooled, and the resulting precipitate was recrystallized: 54% yield, mp. 155–158°C. ^1H NMR ($CDCl_3$) δ 2.36 (s, 12H), 6.46 (t, J = 3.75 Hz, 1H), 6.85 (s, 2H), 6.94 (s, 4H), 7.06 (d, J = 3.5 Hz, 2H), 8.30 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 21.3, 117.1, 120.8, 122.2, 126.8, 134.3, 139.4, 145.3, 150.5.

Preparation of deuterated compounds. Labeled compounds were synthesized using

TABLE 1

Estimated Perturbation Isotope Shifts at CHX, Fulvene, and Phenyl Carbons of **1** or **2**

Carbon	Δ_{e1}, ppm	Δ_{e2}, ppm
C6,7	0.76	0.66
C1,2	0.47	0.07
C3,5	0.89	0.12
Ipso	—	0.09
Ortho	—	0.18
Meta	—	−0.01
Para	—	0.19

TABLE 2

^{1}H-Coupled ^{13}C NMR Data of **2** in [$^2H_4$]Methanol

Carbon	δ, ppm	Multiplicity	J, Hz
Ortho	120.1	ddd	157, 7.3, 6.0
4	121.9	dt	164, 4.5
1,2	124.0	m	
Para	126.0	dt	157, 8.4
Meta	130.9	ddd	152, 8.3, 2.3
3,5	136.7	dm	163
Ipso	147.1	m	
6,7	151.8	dd	160, 2.3

[$^2H_7$]dimethylformamide-$POCl_3$ complex as the source of the second formyl group. This results in exactly one deuterium and one protium on the two aldehydic or aldiminic carbons. The multiplicities and integration of the ^{1}H NMR spectra were in agreement with the expected product.

NMR Spectra and sample preparation. Spectra were recorded on a Varian Unity 500 spectrometer operating at a ^{13}C resonance frequency of 125 MHz and a ^{1}H resonance frequency of 500 MHz. Spectra are referenced to the solvent. Samples for measurement of isotope shifts were prepared by mixing equal weights of d_1 and d_0 compounds, except for 6-hydroxy-2-formylfulvene, which was synthesized as a mixture of d_1 and d_0. Decoupling of both ^{1}H and ^{2}H was achieved by sending an additional 76.85-MHz signal through the lock channel.

RESULTS

NMR signal assignments. Partially assigned NMR spectra of **2** had been reported (*14*). The full ^{1}H spectrum could be assigned on the basis of signal integrations and splitting patterns. The full ^{13}C spectrum was assigned on the basis of ^{1}H-couplings and a 2D HMQC spectrum that correlates ^{1}H and ^{13}C chemical shifts. Table 2 lists the carbon multiplicities and C-H coupling constants. Assignments for **3** were made by analogy to **2**.

6-Hydroxy-2-formylfulvene. The ^{13}C NMR data for the mixture of isotopologs of **1** in $CDCl_3$ are listed in Table 3. The second and third columns list n and n', the number of bonds between the label and the nearer or farther, respectively, of each

TABLE 3

^{13}C NMR Data from Mixture of **1** and 6-[^{2}]**1** in $CDCl_3$

Carbon	n	n'	δ_0, ppm	Δ_{down}, ppb	Δ_{up}, ppb
6,7	1	4	176.0	+376	—
1,2	2	3	126.5	+200	−279
3,5	3	4	141.2	+151	−195
4	4	4	125.2	<5	<5

set of carbons. The fourth column lists the NMR shifts for unlabeled **1**. The fifth and sixth columns list the observed downfield and upfield isotope shifts of 6-[^{2}H]**1** relative to **1**.

Without ^{2}H decoupling the only signal observed from C6,7 of 6-[^{2}H]**1** is the CH, since the CD is split into a triplet and lacks a nuclear Overhauser enhancement. The CH shows a large downfield shift of 376 ppb. For both C1,2 and C3,5 one signal of 6-[^{2}H]**1** is shifted upfield and the other downfield. The signal for C4 is a singlet, since it is too far from the label to show a resolvable $^4\Delta_0$ and since it is located symmetrically relative to the deuterated and undeuterated carbons and therefore cannot show a Δ_e.

N,N′-Diphenyl-6-aminofulvene-2-aldimine. Figure 1 shows individual NMR signals of cyclopentadienyl and CHN carbons from a 1:1 mixture of **2** and 6-[^{2}H]**2**. The spectra are aligned along the signal of unlabeled **2** and are plotted on the same scale. The signal for C4 is a singlet. Three signals are observed from some carbons, owing to isotope shifts. Because of the stoichiometry the two smaller signals can be assigned to 6-[^{2}H]**2**. For C1,2 and C3,5 of 6-[^{2}H]**1** one signal is shifted upfield and the other downfield, by unequal amounts. For ipso and ortho carbons (not shown) the signals are shifted by equal or nearly equal amounts. For meta carbons, the signals are singlets, with unresolvable isotope shifts. The para carbon shows a high-field shoulder, corresponding to a small intrinsic isotope shift.

The ^{13}C NMR chemical shifts and isotope shifts of a mixture of **2** and 6-[^{2}H]**2** in [$^2H_6$]DMSO are listed in Table 4. The results in $CDCl_3$ in Table 5 are quite similar. Table 6 lists the data in [$^2H_4$]methanol. The assignment of C6,7 was facilitated by simultaneously decoupling not only ^{1}H but also ^{2}H, since otherwise the CD signal is invisible, as demonstrated by comparing Fig. 1a with Fig. 2, which shows C6,7 in the ^{1}H- and ^{2}H-decoupled ^{13}C NMR spectrum of **2**.

N,N′-Bis(3,5-dimethylphenyl)-6-aminofulvene-2-aldimine. The assignment of C6,7 signals in 6-[^{2}H]**3** was made from a ^{1}H-coupled ^{13}C NMR spectrum. The special feature of this derivative is that it permits the assignment of the ipso carbon signals of 6-[^{2}H]**3**, and by extension that of 6-[^{2}H]**2**. The ^{1}H-coupled spectrum is simplified by 3,5-dimethyl substitution, which eliminates the $^3J_{CH}$ between ipso carbon and meta CH of **2**. Consequently the ipso carbon of 6-[^{2}H]**3** shows a well-resolved downfield 6.6-Hz doublet, due to $^3J_{CH}$ to the aldiminic CH, which must be distal. In contrast, the other ipso carbon is an upfield singlet, broadened by an unresolvable $^3J_{CD}$, so it must be proximal to the label. The ^{13}C NMR data for the mixture of isotopologs of **3** in [$^2H_6$]DMSO are listed in Table 7.

DISCUSSION

6-Hydroxy-2-formylfulvene. The isotope shifts in Table 3 are not purely intrinsic. The signal from C6,7 of 6-[^{2}H]**1** that is readily observed, without ^{2}H decoupling, is the CH. Its large downfield shift of 376 ppb, relative to **1**, is entirely contrary to the small upfield shift expected from a $^4\Delta_0$ (*22*). This must be a perturbation shift. Likewise, the isotope shifts for both C1,2 and C3,5 in 6-[^{2}H]**1** are too large to be intrinsic. The inequality of upfield and downfield isotope shifts for each of these carbons means that intrinsic isotope shifts are not negligible. The combination of intrinsic and perturbation shifts could not be separated, but the data are consistent

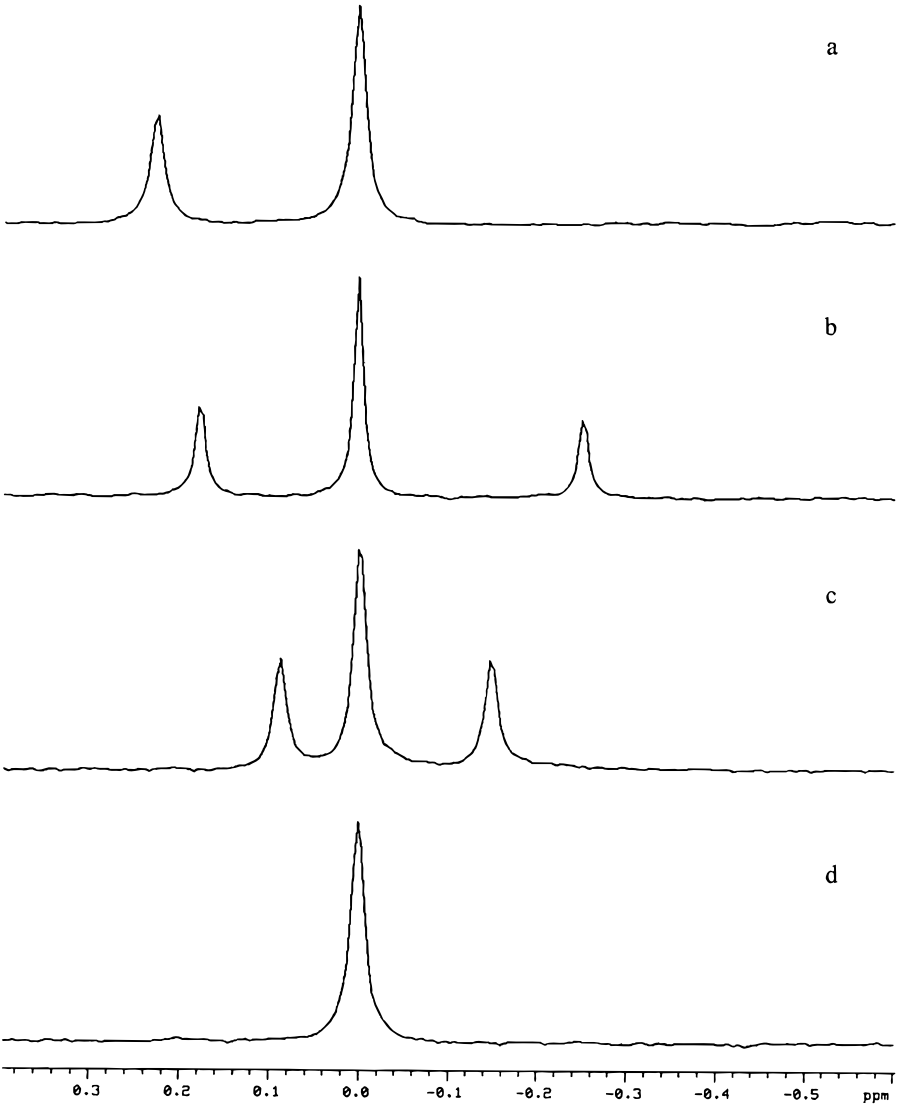


FIG. 1. ^{13}C NMR spectra of **2** and 6-[^{2}H]**2** in [$^{2}H_6$]DMSO expanded about (a) C6,7, (b) C1,2, (c) C3,5, and (d) C4.

with perturbation shifts of ± 200 and ± 151 ppb at C1,2 and C3,5, respectively, and intrinsic shifts $^{2}\Delta_0$ and $^{3}\Delta_0$ of -79 and -44 ppb.

N,N′-Diphenyl-6-aminofulvene-2-aldimine. At C4 and at the meta carbons of **2** no isotope shifts can be resolved, neither intrinsic nor perturbation. The para carbon shows a very small $^{6}\Delta_0$ of -16 ppb in DMSO and -24 ppb in methanol. Nevertheless, as for **1**, the isotope shifts for all the other carbons in Tables 4–6 are not purely intrinsic. A downfield shift of 223 ppb is observed from C6,7. This must be the CH

TABLE 4

^{13}C NMR Data from Mixture of **2** and **2**-*d* in [$^2H_6$]DMSO

Carbon	*n*	*n′*	δ_0, ppm	Δ_{down}, ppb	Δ_{up}, ppb
6,7	1	4	151.5	+223	−542[a]
1,2	2	3	122.2	+178	−252
3,5	3	4	135.6	+87	−147
4	4	4	120.8	<5	<5
Ipso	3	6	145.1	+194	−211
Ortho	4	7	119.2	+66	−66
Meta	5	8	130.0	<5	<5
Para	6	9	125.1	<5	−16

[a] Upfield carbon is proximal to deuterium.

of 6-[^{2}H]**2**, since it is seen without ^{2}H decoupling. This is much too large and even of the wrong sign to be a four-bond intrinsic shift, so it must be a perturbation shift. With ^{2}H decoupling the CD signal of 6-[^{2}H]**2** becomes visible 765 ppb upfield of the CH. This represents an isotope shift of −542 ppb, which equals the sum of $-\Delta_e$ and $^1\Delta_0$. If $^4\Delta_0$ is negligible at the CH, as at C4, the isotope shifts can be separated into perturbation shifts of ±223 ppb and an intrinsic shift of −319 ppb for the CD, which is consistent with other $^1\Delta_0$ (*22*).

The data in Tables 4–6 and the spectra in Fig. 1 show isotope shifts that decrease as the distance to the label (number of intervening bonds) increases. The difference between the upfield and downfield shift at C1,2 or C3,5 again implies substantial intrinsic isotope shifts. However, each observed isotope shift is the sum of an intrinsic shift and a perturbation shift, which could not be separated. The data in [$^2H_6$]DMSO are consistent with perturbation shifts of ±178 and ±87 ppb at C1,2 and C3,5, respectively. The similar magnitudes of the upfield and downfield shifts at phenyl carbons indicate that intrinsic shifts are small, as might be expected from the large number of intervening bonds. Instead the large downfield isotope shifts at ipso and ortho carbons do represent perturbation shifts.

N,N′-Bis(3,5-dimethylphenyl)-6-aminofulvene-2-aldimine. As with **2** the ^{13}C NMR

TABLE 5

^{13}C NMR Data from Mixture of **2** and 6-[^{2}H]**2** in $CDCl_3$

Carbon	*n*	*n′*	δ_0, ppm	Δ_{down}, ppb	Δ_{up}, ppb
6,7	1	4	150.9	+218	—
1,2	2	3	122.3	+176	−249
3,5	3	4	134.9	+92	−157
4	4	4	121.2	<5	<5
Ipso	3	6	145.5	182	−189
Ortho	4	7	119.3	+59	−57
Meta	5	8	130.8	<5	<5
Para	6	9	125.0	<5	−19

TABLE 6

^{13}C NMR Data from Mixture of **2** and 6-[^{2}H]**2** in [$^2H_4$]Methanol

Carbon	*n*	*n′*	δ_0, ppm	Δ_{down}, ppb	Δ_{up}, ppb
6,7	1	4	152.0	+267	—
1,2	2	3	124.1	+194	−273
3,5	3	4	136.8	+97	−170
4	4	4	122.1	<5	<5
Ipso	3	6	147.2	+213	−239
Ortho	4	7	120.3	+79	−85
Meta	5	8	131.0	<5	<5
Para	6	9	126.1	<5	−24

spectrum of **3** exhibits isotope shifts inconsistent with purely intrinsic shifts. The spectra are quite similar, except for the detectability of a $^3J_{CH}$ between the aldiminic proton and the ipso carbon. Since it is the downfield ipso carbon of 6-[^{2}H]**3** that appears as a doublet in a ^{1}H-coupled spectrum, this must be proximal to the H and distal to the D. Therefore we can conclude that $^3\Delta_e = +193$ ppb, not -205 ppb. This agrees with the sign predicted in Table 1 and supports those predictions. By inference the downfield ipso carbon of 6-[^{2}H]**2** is also proximal to H, so that $^3\Delta_e$ is +194 ppb in DMSO and +213 ppb in methanol, not −211 or −239 ppb.

Environmental effects. The ^{13}C NMR spectrum of **2** does not change significantly

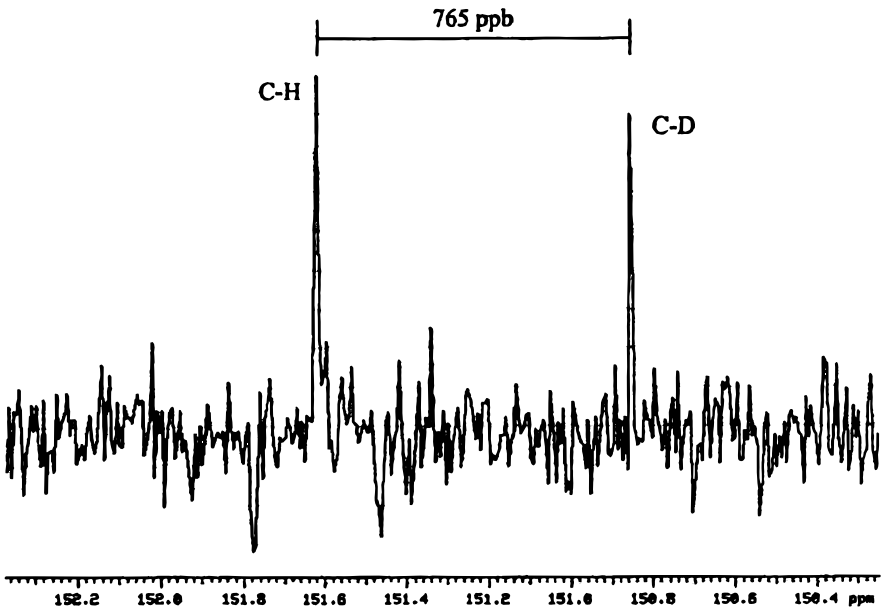


FIG. 2. ^{1}H- and ^{2}H-decoupled ^{13}C NMR spectrum of **2** and 6-[^{2}H]**2** in DMSO-d_6 expanded about C6,7.

TABLE 7

^{13}C NMR Data from Mixture of **3** and 6-[^{2}H]**3** in [$^2H_6$]DMSO

Carbon	n	n'	δ_0, ppm	Δ_{down}, ppb	Δ_{up}, ppb
6,7	1	4	150.9	214	—
1,2	2	3	122.1	178	−254
3,5	3	4	126.6	85	−145
4	4	4	120.4	<5	<5
Ipso	3	6	144.7	193	−205[a]
Ortho	4	7	116.8	67	−65
Meta	5	8	139.1	<5	<5
Para	6	9	134.3	<5	<5
CH_3	6	9	20.9	<5	<5

[a] Upfield carbon is proximal to the deuterium.

in different solvents (Tables 4–6). The small differences in the observed isotope shifts are probably due to changes in the perturbation shifts, through unpredictable variation of the chemical shift difference D in Eq. (2). The fact that observed shifts in [$^2H_4$]methanol are of larger magnitude is likely to be due to a greater K in Eq. (2), arising from the different vibrational frequencies with N-D-N hydrogen bonds. Such an increase was detected in *O*-[^{2}H]phthalate monoanion (*10*).

A purpose of this study was to probe the influence of solvation on the symmetry of hydrogen bonds. Previous examples of asymmetric hydrogen bonds involved ions, for which solvation favors charge localization (*20*). Here we study the hydrogen bonds in some nonionic species, to see if they might become symmetric in an appropriate solvent. Nevertheless, the observation of perturbation isotope shifts in **1, 2**, and **3** indicates that their hydrogen bonds are asymmetric too.

Although the net charge in **1, 2**, or **3** is zero, these molecules may have some local ionic character. There are substantial contributions from zwitterionic resonance forms with a cyclopentadienide anion and an oxenium or iminium cation. That cationic character may make these molecules sensitive to solvation, just as ions seem to be, and therefore unfavorable for symmetric hydrogen bonds.

Magnitude and sign of isotope shifts. The intrinsic shifts are uniformly negative, as is nearly universal (*22*), and they fall off with distance from the isotope. The observed (upfield) shift of −542 ppb for the CD in **1** is dominated by a $^1\Delta_0$ of ca. −319 ppb. The intrinsic shifts at C1,2, C3,5, and ipso carbons of **2** are also negative. The assignment of the upfield ipso carbon of 6-[^{2}H]**3** as proximal to CD shows that $^3\Delta_0 + {}^6\Delta_0$ must be negative. By inference this conclusion also holds for 6-[^{2}H]**2**.

The data show that perturbation isotope shifts also generally decrease with increasing number of intervening bonds from the label, but the decrease is not uniform. The largest is at the aldehydic or aldiminic carbon, and none is detectable at meta and para carbons. The perturbation shifts in the phenyl seem to be somewhat larger than in the fulvene. No $^4\Delta_e$ is seen at C4, since this must be zero by symmetry.

According to Eqs. (1–2) or Table 1, the estimated perturbation shift at the aldehydic or aldiminic CH of **1** or **2** is 760 or 660 ppb. The observed shifts in Tables 3 and 4

are only 376 and 223 ppb. Such a reduction was also seen for 3-hydroxy-2-phenylpropenal and was attributed to imperfections of the models used to estimate perturbation shifts (*9*). The key result is that the isotope shift is positive for the unlabeled carbon, as predicted. The labeled carbon in **2** has a perturbation shift of -223 ppb, as well as a $^1\Delta_0$ of -319 ppb, summing to the observed shift of -542 ppb.

The other carbon for which absolute isotope shifts could be unequivocally assigned is the ipso carbon of **3**. Since the upfield carbon is proximal to CD, requiring $^3\Delta_0 + {}^6\Delta_0$ to be negative, we can conclude that $^3\Delta_e$ of the other ipso carbon is $+193$ ppb. This is larger than the estimate in Table 1, but of the correct sign.

The magnitude of the isotope shifts at the aromatic carbons of **2** and **3** falls off with distance. None could be detected at the meta carbons, as expected from the estimates in Table 1. However, none was detected at para either, despite the estimates. Again this is likely to be due to an imperfection of the models.

The O-H-O hydrogen bond of **1** is qualitatively similar to the N-H-N hydrogen bond of **2** or **3**. The magnitudes of the observed isotope shifts in the former are slightly larger at each carbon. It is likely that the differences are due to a greater *K* and/or *D* (Eq. (2)) for **1**, but this is minor compared to the similarity that demonstrates that all of these have asymmetric hydrogen bonds.

CONCLUSIONS

Observed isotope shifts of labeled **1, 2**, and **3** can be attributed to a combination of intrinsic and perturbation shifts. The key result is that the perturbation isotope shifts establish the existence of a tautomeric equilibrium. Therefore these intramolecular hydrogen bonds are asymmetric, the proton resides in a double-minimum potential surface, and each molecule exists as a pair of rapidly interconverting tautomers. Changing the solvent does not qualitatively change the observed spectra or the isotope shifts for these uncharged species.

A significant conclusion from these studies is that the symmetry of the hydrogen bond is not determined simply by the O-O or N-N distance. Even though that distance is short here and could have been shorter in these seven-membered (including H) rings, it does not lead to a symmetric hydrogen bond. Yet crucial questions persist. If LBHBs are responsible for stabilization in some enzymatic catalysis, where does that stabilization come from if the hydrogen bonds do not have the special feature of symmetry? Why are symmetric hydrogen bonds observed in crystalline phases but not as yet in solution? Perhaps the disorder of the solvation environment induces an asymmetry of the hydrogen bond, whereas a crystal can guarantee a symmetric environment. If so, the unusual strength of such hydrogen bonds cannot be due to a maximum resonance stabilization associated with symmetry, since solvation is sufficient to disrupt the symmetry. It has been suggested that relief of strain may be responsible for the apparent strength of some hydrogen bonds (*18*).

ACKNOWLEDGMENTS

This research was supported by NSF Grants CHE94-20739 and CHE99-82103 and NIH Grant GM44527. Purchase of the 500-MHz NMR spectrometer was made possible by grants from NIH and NSF.

REFERENCES

1. Pimentel, G. C., and McClellan, A. L. (1960) The Hydrogen Bond, Freeman, San Francisco. Schuster, P., Zundel, G., and Sandorfy C. (Eds.) (1976) The Hydrogen Bond: Recent Developments in Theory and Experiments, North-Holland, Amsterdam. Jeffrey, G. A., and Saenger, W. (1991) Hydrogen Bonding in Biological Structures, Springer, Berlin. Scheiner, S. (1997) Hydrogen Bonding: A Theoretical Perspective, Oxford, New York.
2. Emsley, J. (1980) *Chem. Soc. Revs.* **9,** 91. Hibbert, F., and Emsley, J. (1991) *Adv. Phys. Org. Chem.* **26,** 255.
3. Gilli, P., Bertolasi, V., Ferretti, V., and Gilli, G. (1994) *J. Am. Chem. Soc.* **116,** 909.
4. Gerlt, J. A., Kreevoy, M. M., Cleland, W. W., and Frey, P. A. (1997) *Chem. Biol.* **4,** 259. Cleland, W. W., and Kreevoy, M. M. (1994) *Science* **264,** 1887. Gerlt, J. A., and Gassman, P. G. (1993) *J. Am. Chem. Soc.* **115,** 11552. Frey, P. A., Whitt, S. A., and Tobin, J. B. (1994) *Science* **264,** 1927. Frey, P. A. (1995) *Science* **269,** 104. Cleland, W. W., and Kreevoy, M. M. (1995) *Science* **269,** 104. Hur, O., Leja, C., and Dunn, M. F. (1996) *Biochemistry* **35,** 7378. Ash, E. L., Sudmeier, J. L., De Fabo, E. C., and Bachovchin, W. W. (1997) *Science* **278,** 1128. Cleland, W. W., Frey, P. A., and Gerlt, J. A. (1998) *J. Biol. Chem.* **273,** 25529. Harris, T. K., and Mildvan, A. S. (1999) *Proteins: Struct. Funct. Genet.* **35,** 275.
5. Mock, W. L., and Chua, D. C. Y. (1995) *J. Chem. Soc., Perkin Trans. 2* 2069. Scheiner, S., and Kar, T. (1995) *J. Am. Chem. Soc.* **117,** 6970. Schwartz, B., and Drueckhammer, D. G. (1995) *J. Am. Chem. Soc.* **117,** 11902. Kato, Y., Toledo, L. M., and Rebek, J., Jr. (1996) *J. Am. Chem. Soc.* **118,** 8575. Warshel, A., and Papazyan, A. (1996) *Proc. Natl. Acad. Sci.* **93,** 13665. Shan, S., Loh, S., and Herschlag, D. (1996) *Science* **272,** 97.
6. Guthrie, J. P., and Kluger, R. (1993) *J. Am. Chem. Soc.* **115,** 11569. Warshel, A., Papazyan, A., and Kollman, P. A. (1995) *Science* **269,** 102. Alagona, G., Ghio, C., and Kollman, P. A. (1995) *J. Am. Chem. Soc.* **117,** 9855. Guthrie, J. P. (1996) *Chem. Biol.* **3,** 163.
7. Mulholland, A. J., Lyne, P. D., and Karplus, M. (2000) *J. Am. Chem. Soc.* **122,** 534. Mohr, M., and Zipse, H. (1999) *Chem. Eur. J.* **5,** 3046.
8. Perrin, C. L., and Nielson, J. B. (1997) *Annu. Rev. Phys. Chem.* **48,** 511. Frey, P. A., and Cleland, W. W. (1998) *Bioorg. Chem.* **26,** 175. Cleland, W. W. (2000) *Arch. Biochem. Biophys.* **382,** 1.
9. Perrin, C. L., and Kim, Y. J. (1998) *J. Am. Chem. Soc.* **120,** 12641. Perrin, C. L., Nielson, J. B., and Kim, Y.-J. (1998) *Ber. Bunsen-Ges. Phys. Chem.*, **102,** 403.
10. Perrin, C. L., and Thoburn, J. D. (1989) *J. Am. Chem. Soc.* **111,** 8010. Perrin, C. L., and Thoburn, J. D. (1992) *J. Am. Chem. Soc.* **114,** 8559. Perrin, C. L. (1994) *Science* **266,** 1665. Perrin, C. L., and Nielson, J. B. (1997) *J. Am. Chem. Soc.* **119,** 12734.
11. Perrin, C. L., and Ohta, B. K., (2001) *J. Am. Chem. Soc.* **123,** 6520.
12. Ammon, H. L., and Mueller-Westerhoff, U. (1974) *Tetrahedron* **30,** 1437. Fuess, H., and Linder, H. J. (1975) *Chem. Ber.* **108,** 3096.
13. Claramunt, R. M., Sanz, D., Alarcón, S. H., Torralba, M. P., Elguero, J., Foces-Foces, C., Pietrzak, M., Langer, U., and Limbach, H.-H. (2001) *Angew. Chem. Int. Ed.* **40,** 420.
14. Jackman, L. M., Trewella, J. C., and Haddon, R. C. (1980) *J. Am. Chem. Soc.* **102,** 2519.
15. Mueller-Westerhoff, U. (1970) *J. Am. Chem. Soc.* **92,** 4849.
16. Brown, R. S., Tse, A., Nakashima, T., and Haddon, R. C. (1979) *J. Am. Chem. Soc.* **101,** 3157.
17. Pickett, H. M. (1973) *J. Am. Chem. Soc.* **95,** 1770.
18. Morpurgo, S., Bossa, M., and Morpurgo, G. O. (1998) *J. Mol. Struct. (THEOCHEM)* **429,** 71.
19. McCoy, L. L. (1967) *J. Am. Chem. Soc.* **89,** 1673.
20. Pan, Y. P., and McAllister, M. A. (1997) *J. Am. Chem. Soc.* **119,** 7561. Chen, J. G., McAllister, M. A., Lee, J. K., and Houk, K. N. (1998) *J. Org. Chem.* **63,** 4611. Pan, Y. P., and McAllister, M. A. (1998) *J. Am. Chem. Soc*. **120,** 166. Garcia-Viloca, M., Gonzalez-Lafont, A., and Lluch, J. M. (1999) *J. Am. Chem. Soc.* **121,** 9198.
21. Saunders, M., Telkowski, L., and Kates, M. R. (1977) *J. Am. Chem. Soc.* **99,** 8070. Siehl, H.-U. (1987) *Adv. Phys. Org. Chem.* **23,** 63. Forsyth, D. A. (1987) *in* Isotopes in Organic Chemistry, (Buncel, E., and Lee, C. C., Eds.), Vol. 6, Chap. 1, Elsevier, Amsterdam.

22. Batiz-Hernandez, H., and Bernheim, R. A. (1967) *Prog. Nucl. Magn. Reson. Spectrosc.* **3,** 63. Hansen, P. E. (1983) *Annu. Rep. NMR Spectr.* **15,** 105. Jameson, C. J., and Osten, H. J. (1986) *Ann. Rep. NMR Spectrosc.* **17,** 1. Hansen, P. E. (1988) *Progr. Nucl. Magn. Reson. Spectrosc.* **20,** 207. Hansen, P. E. (2000) *Magn. Reson. Chem.* **38,** 1.
23. Muller, K., Previdoli, F., and Desilvestro, H. (1981) *Helv. Chim. Acta* **64,** 2497. Lewis, J. W., and Sandorfy, C. (1982) *Can. J. Chem.* **60,** 1720.
24. Bureau, R., Mortier, J., and Joucla, M. (1993) *Bull. Soc. Chim. Fr.* 584. Fraser, R. R., and Chuaqui-Offermanns, N. (1981) *Can. J. Chem.* 3007.
25. Hartke, K., Kohl, A., and Kaempchen, T. (1983) *Chem. Ber.* **116,** 2653. Sadtler Standard Spectra (1969) spectra 7807C and 2016C, Sadtler Research Laboratories, Philadelphia.
26. Hafner, K., Vopel, K. H., Ploss, G., and Konig, C. (1963) *Justus. Liebigs. Ann. Chem.* **661,** 52.
27. Hafner, K., Vopel, K. H., Ploss, G., and Konig, C. (1967) *Org. Syn.* **47,** 52.
28. Bredereck, H., Gompper, R., Klemm, K., and Rempfer, H. (1959) *Chem. Ber.* **92,** 837.