Asymmetry of Hydrogen Bonds in Solutions of Monoanions of Dicarboxylic Acids

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Abstract: Is a hydrogen bond symmetric (single-well potential) or asymmetric (double-well potential)? The NMR method of isotopic perturbation of equilibrium was used to answer this question for the monoanions of a wide variety of ^{18}O-labeled dicarboxylic acids. The observed ^{18}O-induced isotope shifts, especially at the ipso carbons, demonstrate that these exist as a pair of equilibrating tautomers in both aqueous and organic solvents. This conclusion for organic solvents is opposite a previous one based on similar data. As a further test, primary isotope shifts, which had been diagnostic for a single-well potential, were reinvestigated. The monoanions of 1,2-cyclopentenedicarboxylic, 3,4-furandicarboxylic, and 3,4,5,6-tetrahydrophthalic acids have negative primary isotope shifts and are confirmed as having asymmetric hydrogen bonds. Although hydrogen phthalate has a positive primary isotope shift, it too is judged to have an asymmetric hydrogen bond, according to ^{18}O-induced isotope shifts, which are considered more reliable.

Introduction

Symmetry of Hydrogen Bonds. Hydrogen bonding is a fundamental aspect of chemical structure and reactivity.[1] It is a key to understanding the structure and properties of water, proteins, and DNA, and it is currently of interest for designing systems that exhibit molecular recognition.[2]

Hydrogen bonds are usually thought to arise from electrostatic attraction between an O−H (or N−H or H−F) dipole and the electron density on a nearby O (or N or F). Hydrogen bonds may have extra stabilization, often viewed as arising from a covalent character, if the two contributing resonance forms, O−H···O$^-$ and O$^-$···H−O, are of equal or nearly equal energy.[3] This is more likely if the two donor atoms have the same basicity (matched pK_a values) and if the hydrogen is centered between them. Indeed, symmetric hydrogen bonds seem to be stronger than asymmetric ones.[4]

Thus, a key geometric aspect of a hydrogen bond is symmetry. If the hydrogen is localized in one well of a double-well potential, there are two distinct tautomers, in equilibrium, and the hydrogen bond is asymmetric. As the distance between the donors decreases, the barrier height is reduced until it disappears, generally at an O−O distance between 2.4 and 2.5 Å. A double-well potential thus becomes a single-well one, with a centered hydrogen. This is quite an unusual structure, since the O−H distance must be extended to ≥1.2 Å.

The geometry of hydrogen bonds has been extensively studied by X-ray and neutron diffraction.[1] The monoanions of dicarboxylic acids such as maleate (**1**) and phthalate (**2**) are classic examples of symmetric hydrogen bonds. Actually few crystals show centered hydrogens,[5] and most are noncentered, perhaps because of dissimilar environments surrounding the two carboxyls.[6] In all these monoanions the hydrogen bond is quite short, with an O−O distance of 2.4−2.5 Å.[7]

As the barrier to hydrogen transfer decreases, characteristic features often appear, such as ^{1}H NMR signals far downfield, toward 20 ppm,[8] and fractionation factors <1 for selectivity of deuterium over protium.[9] Such hydrogen bonds are called low-barrier hydrogen bonds (LBHBs). To the extent that these features are associated with a short O−O distance and an increased strength, these may also be called short, strong hydrogen bonds (SSHBs). The two terms have sometimes been used interchangeably, but they differ in the criteria used to recognize them. SSHBs have attracted renewed attention because of their possible role in some enzyme-catalyzed reactions. Gerlt and Gassman proposed that they reduce the activation energy needed to create a high-energy enolate intermediate.[10] According to Cleland and Kreevoy, that inter-

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mediate gains 10−20 kcal/mol of stabilization by formation of an SSHB, with matched pK_a values, in the enzyme−intermediate complex.[11]

The SSHB proposal has been criticized. Warshel et al. concluded that electrostatic effects alone can account for enzyme catalysis.[12] Schwartz and Drueckhammer have estimated that the strength of the intramolecular hydrogen bond in maleate monoanion is only 4−5 kcal/mol.[13] Guthrie claimed that hydrogen bond energies in solution are never sufficient to provide the stabilization needed.[14] For a series of substituted phthalate monoanions Herschlag and co-workers found a linear correlation between the energy of the hydrogen bond and the ΔpK_a between the donor and acceptor, but there is no large increase in energy as pK_a values become matched.[15] A response to these criticisms has been published,[16] and an impartial review is available.[17]

The connection between LBHBs and SSHBs is tenuous. There is no doubt that LBHBs exist, with their characteristic features. What is uncertain is the energetic consequence. Symmetric hydrogen bonds are the link, since one with a vanishing barrier is certainly a LBHB, and one with an O−O distance of 2.4 Å must have some special stabilization to compensate for stretching the O−H distance to 1.2 Å. Yet most examples of symmetric hydrogen bonds are in crystals, and calculations are inconclusive.[18] Therefore, evidence for them in solution would help to substantiate the SSHB proposal. Furthermore, they are of intrinsic interest as an important aspect of molecular structure.

Isotope Shifts. The NMR chemical shift δ of a reporter nucleus X can change with isotopic substitution *n* bonds away.[19] The difference is called an isotope shift, ${}^n\Delta$ (eq 1). A heavier isotope usually shifts X upfield, corresponding to $\Delta < 0$.

$$ {}^n\Delta = \delta(\mathrm{X_{heavier}}) - \delta(\mathrm{X_{lighter}}) \qquad (1) $$

These shifts can distinguish whether a species exists as a single symmetric structure or as two asymmetric tautomers in rapid equilibrium.[20] If the tautomers are degenerate, then any chemical shift is a 50:50 average. With isotopic substitution one tautomer can be favored. This isotopic perturbation introduces an equilibrium isotope shift, in addition to the intrinsic isotope shift, which is designated ${}^n\Delta_o$. Since ^{13}C NMR is quite sensitive to environment, small changes can be detected and taken as evidence for an equilibrium between two tautomers.

This method can be applied to the monoanion of a mono-^{18}O-labeled dicarboxylic acid.[21] For a single symmetric structure only an ^{18}O-induced intrinsic isotope shift should be observed. For a rapidly equilibrating mixture the ^{13}C NMR signal is averaged over the two tautomers, which differ by whether the proton is on the ^{16}O-labeled carboxyl or the ^{18}O one. (Strictly, there are four tautomers, and the proton can be on either O of a mono-^{18}O-labeled carboxyl. This arbitrariness is calculated to reduce by half the effect possible from double labeling.) The equilibrium constant between the two tautomers, K_T, or the ratio of ^{16}O and ^{18}O acidity constants, is >1,[22] owing to differences in zero-point energies. The favored ^{18}O-protonated tautomer is weighted more heavily in the averaging, shifting the ^{13}C^{16}O signal slightly downfield and the ^{13}C^{18}O slightly upfield. The isotope shift is related to K_T by eq 2,[19d,21] where the number of bonds (*n*) is omitted and where *D* is the difference between chemical shifts of carboxyl and carboxylate carbons of the monoanion (eq 3). Although these two signals cannot be observed separately, *D* can be estimated from the chemical shifts of the diacid and the dianion (eq 4). Regardless of this approximation, the qualitative conclusion is that this method can distinguish symmetric hydrogen bonds from asymmetric ones.

$$ \Delta = \Delta_o + \frac{K_T - 1}{K_T + 1} D \qquad (2) $$

$$ D = \delta_{\mathrm{COOH}} - \delta_{\mathrm{CO_2^-}} \qquad (3) $$

$$ D \approx \delta_{\mathrm{diacid}} - \delta_{\mathrm{dianion}} \qquad (4) $$

Perrin and Thoburn examined succinic, maleic, and phthalic acids.[21,23] The carboxyl carbons of the diacid or dianion show a small ^{18}O-induced intrinsic isotope shift of −26 ppb, regardless of solvent and temperature, and none of the other ^{13}C signals exhibits any resolvable intrinsic shift (≤4 ppb). Yet in aqueous solution the isotope shifts of the monoanions are larger, at both carboxyl and ipso carbons. This was firm evidence, supported by four control experiments (effect of D_2O, temperature dependence, comparison of mono-^{18}O- and di-^{18}O-labeled carboxyls, isotope shifts at distant carbons), for a tautomeric equilibrium and for asymmetric hydrogen bonds. The results on hydrogen maleate and phthalate were especially surprising since they show symmetric hydrogen bonds in crystals.

Yet in organic solvents such as CD_3CN, DMSO-d_6, and THF-d_8 hydrogen maleate and succinate appeared to have only an intrinsic isotope shift. It was concluded that these monoanions have a symmetric hydrogen bond in organic solvents. This conclusion was generally accepted, since it agreed with previous results in crystals and nonpolar solvents.[5,24] For hydrogen succinate the symmetric hydrogen bond contrasted with the absence of any intramolecular hydrogen bond in water,[25] but this difference could be rationalized.

The symmetry of the hydrogen bonds was suggested to be determined by the local environment.[23,26] In the disordered aqueous environment it is improbable that the two carboxyl groups are identically solvated, thus leading to an asymmetric hydrogen bond. In organic solvents that do not hydrogen bond

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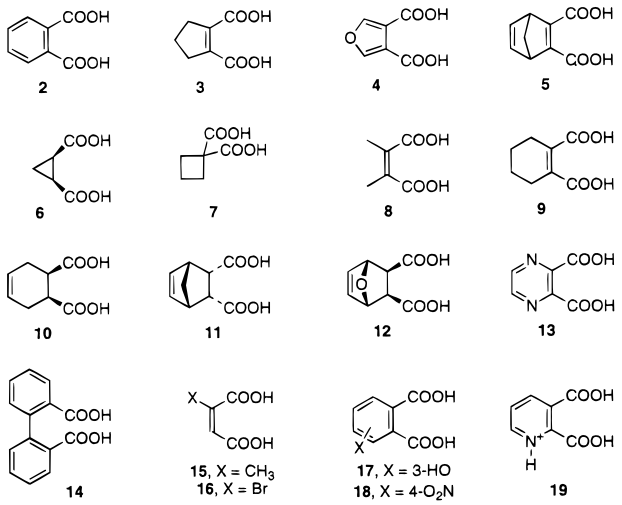
Chart 1



to the anion a symmetric hydrogen bond would allow the negative charge to be delocalized over the two carboxyl groups.

Proposed Experiments. The symmetry of the hydrogen bonds in monoanions of dicarboxylic acids is further explored. Among the aspects to investigate are the geometry of the anion, the influence of electronic asymmetry, and the role of the environment. For reasons described below we also reinvestigated primary isotope shifts.

Since symmetric hydrogen bonds are seen when the O−O distance d_{OO} is <2.5 Å, hydrogen bonding might become stronger at shorter d_{OO}. However, d_{OO} is actually too short in planar maleate (**1**) and phthalate (**2**), which distort but thereby weaken the hydrogen bond. McCoy assessed the optimal geometry for hydrogen bonding in monoanions of dicarboxylic acids.[27] More likely prospects are 1,2-cyclopentenedicarboxylic (**3**), 3,4-furandicarboxylic (**4**), and bicyclo[2.2.1]-heptadiene-2,3-dicarboxylic (**5**) acid monoanions. Other possibilities are *cis*-1,2-cyclopropanedicarboxylic (**6**), 1,1-cyclobutanedicarboxylic (**7**), dimethylmaleic (**8**), 3,4,5,6-tetrahydrophthalic (**9**), *cis*-1,2,3,6-tetrahydrophthalic (**10**), 5-norbornene-*endo*-2,3-dicarboxylic (**11**), *exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalic (**12**), pyrazine-2,3-dicarboxylic (**13**), and diphenic (**14**). (See Chart 1).

What is the effect of electronic asymmetry? Neutron diffraction of chloromaleate monoanion shows a centered hydrogen,[5c] even though its two carboxylates differ in basicity. If this difference is not too extreme, both tautomers of the monoanion will be present, in an equilibrium perturbable by isotopic substitution. The tautomeric equilibrium constant $K_{T\alpha}$ or $K_{T\beta}$ of α- or β-^{18}O-labeled monoanion is related to K_T, the pure isotope effect on acidity ($^{16}K_\alpha/^{18}K_\alpha$ or $^{16}K_\beta/^{18}K_\beta$), by eq 5, where R is $^{16}K_{\beta1}/^{16}K_{\alpha1}$, the ratio of microscopic acidity constants, and where D is as in eq 3. By comparing the chemical shifts in the various species this K_T can be related to the isotope shift by eq 6,[28] which properly simplifies to eq 2 when $R = 1$. The

$$K_T = K_{T\alpha}/R = RK_{T\beta} \tag{5}$$

$$\Delta = \Delta_o + \frac{(K_T{}^2 - 1)R}{(K_T + R)(K_TR + 1)}D \tag{6}$$

maximum difference between pK values can then be estimated as 1.3 by assuming $D = -6$ ppm, $K_T = 1.01$, and $\Delta - \Delta_o =$ 5 ppb, as observed previously for phthalate. According to model propenoic and benzoic acids,[29] this criterion is met by citraconic (**15**), bromomaleic (**16**), 3-hydroxyphthalic (**17**), and 4-nitrophthalic (**18**) acids, as well as quinolinic (**19**), whose conjugate base is a zwitterion with a 2.4 Å O−O distance but noncentered H.[30] (See Chart 1).

Comparison of chemical shifts of H, D, and T can also be used to probe the potential energy surface for a hydrogen bond.[24] The primary isotope shift is as in eq 1 with $n = 0$, but it is customarily designated $^P\Delta$, rather than $^0\Delta$. Equation 7 relates this to the shielding constant $\sigma(r)$ and to the vibrational wave functions ψ for H and D. Higher shielding corresponds to an upfield signal. The integrals can be expanded around the average OH or OD distance, $\langle r_{OH}\rangle$ or $\langle r_{OD}\rangle$, leading to eq 8.

$$^P\Delta = \int \sigma(r)\, \psi^2(r_{OH})\, dr - \int \sigma(r)\, \psi^2(r_{OD})\, dr \tag{7}$$

When σ is calculated,[31] it is usually found that $d\sigma/dr < 0$.[32]

$$^P\Delta \cong \frac{d\sigma}{dr}(\langle r_{OH}\rangle - \langle r_{OD}\rangle) + {}^1/_2\frac{d^2\sigma}{dr^2}[\langle (r_{OH}\text{-}\langle r_{OH}\rangle)^2\rangle - \langle (r_{OD}\text{-}\langle r_{OD}\rangle)^2\rangle] \tag{8}$$

Then the anharmonicity and the higher zero-point energy of H lead to $\langle r_{OH}\rangle$ greater than $\langle r_{OD}\rangle$. As a result, the D isotopologue is upfield, so that $^P\Delta$ is < 0. For ordinary hydrogen bonds $|^P\Delta|$ is small, <0.02 ppm.[19] A large negative value is consistent with a highly anharmonic potential, as in enols of β-diketones. However, if the hydrogen is centered, the first term in eq 8 vanishes, but the rms deviation of H motion is greater than that of D, and $d^2\sigma/dr^2$ is calculated to be >0. Then $^P\Delta > 0$, as reported for maleate and phthalate monoanions, consistent with symmetric hydrogen bonds.[24] Since this contradicts some of the conclusions obtained here, we reinvestigated primary isotope shifts in four monoanions.

Experimental Section

Materials. Phthalic anhydride (Aldrich) was recrystallized twice from chloroform. Bicyclo[2.2.1]-2,5-heptadiene-2,3-dicarboxylic and 3,4-furandicarboxylic acids (Aldrich) were recrystallized from ethyl acetate. Other anhydrides (Aldrich) were used without further purification. Tetrabutylammonium hydroxide (Aldrich, 40 wt %) was standardized by titration. Isotec and Cambridge Isotope Labs supplied $H_2{}^{18}O$ in 1 mL ampoules (97%, 95−98% ^{18}O). NMR solvents D_2O, $CDCl_3$, CD_2Cl_2, DMSO-d_6, and THF-d_8 were purchased from Cambridge Isotope Labs.

General Synthesis. The isotopically labeled dicarboxylic acids were prepared by stirring 0.10−0.20 mmol of anhydride with 10−20 μL of $H_2{}^{18}O$ in a 3 mL reaction vessel. For anhydrides that are only slightly soluble in water 50−100 μL of dry THF was added. Conditions for optimal mono-^{18}O-labeling were found by monitoring the reaction by thin layer chromatography. For phthalic acid these were ∼8 h at room temperature. With longer reaction times more than one ^{18}O could be incorporated. Tetra-^{18}O-labeled phthalate was prepared by heating 5 days with excess $H_2{}^{18}O$ and 5 mol % HCl. Unlabeled diacids were made similarly from distilled, deionized water. If the anhydride was unavailable, the diacid was labeled with 1 mol % HCl as catalyst, leading to a statistical mixture.

Preparation and Titration of Aqueous NMR Samples. Samples were prepared with 0.1−0.2 M dicarboxylic acid and 10−20% D_2O for spectrometer lock. Traces of paramagnetic metal ions were chelated

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with trace Na_2EDTA. Samples were deoxygenated with N_2. 1,4-Dioxane served as internal standard (δ 66.5).

A large-scale titration of unlabeled acid served to measure ^{13}C chemical shifts of diacid, monoanion, and dianion. Then for each labeled acid the ^{13}C chemical shifts and the ^{18}O-induced isotope shifts were measured after addition of each 5 μL aliquot of 5.0 M KOH. Since an acid is partially dissociated, the final sample was reacidified with 12 N HCl to obtain the diacid shifts.

Since dimethylmaleic (**8**) and diphenic (**14**) acids and anhydrides are not soluble in water, 1 equiv of KOH was added prior to titrating to the dianion. Upon reacidification precipitation occurs, so that the spectral data of the diacid are inaccessible. For quinolinic acid (**19**) the intrinsic isotope shifts were obtained from the dianion.

Sample Preparation in Organic Solvents. The labeled monoanions were prepared by mixing the ^{18}O diacid with 1 equiv of Bu_4NOH, NaOH, or KOH. Residual water was removed at 1 mmHg, or under high vacuum for 24 h for Bu_4N salts. Samples were prepared in a drybox and degassed on a vacuum line by the freeze−pump−thaw technique. Usually the NMR solvents from ampules were used without further drying, since drying DMSO-d_6 or THF-d_8 over CaH_2 made no difference. Salt concentrations were 0.1−0.2 M. Integration of the monoanion ^1H signals relative to the butyl signals was used to confirm that the sample was the monoanion. For every monoanion, except for quinolinic acid (**19**), a ^1H signal near 20 ppm was seen, representative of a strongly hydrogen-bonded proton.

For studies of the primary isotope shift sufficient D_2O was used in the preparation to produce a 50:50 H/D mixture. Samples were then dried overnight on a Schlenk line in a J. Young-valve NMR tube with Teflon adapters. Often a dry cosolvent such as THF or $CHCl_3$ was added later to help pull off residual water. DMSO-d_6 was dried over activated molecular sieves and transferred in a drybox. Other NMR solvents were dried over CaH_2 and line-transferred to NMR tubes.

Spectroscopy. ^{18}O-Induced isotope shifts were measured on a Varian Unity-500 FT-NMR spectrometer (500.0 MHz, ^1H; 125.7 MHz, ^{13}C) at 25 °C. The ^{13}C spectra were obtained using a heteronuclear broad band probe and with ^1H decoupling. Initial spectra used a spectral window of 25 000 Hz. Smaller spectral widths of 300−600 Hz were used with 4K to 8K data points and zero-filled to a final resolution of 14 points/Hz. The signal-to-noise ratio was maximized by pulsing at the Ernst angle (∼45°). Typically only 32−64 transients were needed. Line broadening of 0.2 Hz was added. The chemical and isotope shifts were recorded from the digital printout of peaks. For some isotope shifts only an upper limit can be assigned, governed by the line width of broadened signals. By comparing unlabeled and tetra-^{18}O-labeled hydrogen phthalate the intrinsic isotope shifts, per ^{18}O, at carboxyl and ipso carbons of the monoanion at 25 °C were found to be 26.5 and 1 ppb, respectively (29 and ≤2.5 ppb at −25 °C).

Primary isotope shifts were measured on a Bruker Avance DRX600 FT-NMR spectrometer (600.0 MHz, ^1H; 92.0 MHz, ^2H) with a heteronuclear broad band probe. For the ^2H spectra the lock was turned off and the irradiating ^2H frequency was sent through the lock cable. These spectra were obtained with 32−300 transients in a spectral width of 4000 Hz and with line broadening of 5 Hz. For each monoanion a ^1H NMR spectrum was taken and the chemical shift of the hydrogen-bonded proton was referenced to the residual ^1H of solvent: $CDCl_3$ (δ 7.26), CD_2Cl_2 (δ 5.32), DMSO-d_6 (δ 2.49), THF-d_8 (δ 3.58). Any sample showing extraneous signals was not used. A ^2H spectrum was next acquired and referenced to the solvent signal, whose primary isotope shift is negligible.[19] Low temperatures were equilibrated for 10−15 min before acquisition.

Asymmetric acids have the further complication of two carboxyl signals. Their intensities are unequal because the two carbonyls of the anhydride incorporate ^{18}O at different rates, but labeling was sufficient to observe the minor signal. Signals were assigned from proton-coupled ^{13}C NMR spectra and by comparison with model spectra. The assignment of the upfield signal to the heavier isotope, as generally observed,[19] was confirmed for **15** and **16** by addition of unlabeled acid.

Results

Aqueous Monoanions. Table 1 lists chemical shifts for all the dicarboxylic acids in H_2O. Not only carboxyl but also ipso and α signals generally shift downfield upon titration, by an average of 5.7 and 3 ppm, respectively, between diacid and dianion. In contrast, the olefinic α carbons of **15** and **16** shift downfield along the first half of the titration but upfield along the second, except for the CH of **16**, where these directions are reversed.

Table 1. ^{13}C Chemical Shifts and ^{18}O-Induced Isotope Shifts of Dicarboxylic Acids in Water

acid	signal	δ_{H_2A}, ppm	δ_{HA^-}, ppm	$\delta_{A^{2-}}$, ppm	$-\Delta_{HA^-}$, ppb	$\Delta_o - \Delta_{HA^-}$,[a] ppb
3	C=O	169.7	170.8	176.3	>31	>6
3	C1,2	143.4	145.5	141.4	>29	>25
4	C=O	167.9	169.6	171.2	57	31
4	ipso	117.9	121.4	124.3	>17	>13
5	C=O	168.8	169.8	175.4	>31	>5
5	C2,3	156.3	157.5	151.7	>32	>28
6	C=O	174.9	179.3	179.9	30	4
7	C=O	176.2	179.9	182.8	44	18
8	C=O	*b*	176.4	179.7	48	22
9	C=O	173.0	176.0	179.5	62	36
9	C1,2	135.7	135.9	135.0	126	122
10	C=O	178.2	181.4	183.4	37	11
11	C=O	177.1	180.8	182.3	34	8
11	Cα	48.2	49.6	51.5	24	20
12	C=O	176.1	178.6	180.8	>32	>8
13	C=O	167.5	169.9	172.4	40	14
13	ipso	144.2	146.6	149.2	52	48
14	C=O	*b*	176.6	179.5	>34	>8
14	ipso	*b*	135.8	140.0	>14	>10
15	α-C=O	174.6	175.9	180.8	42	16
15	*β*-C=O	169.5	171.2	175.3	>37	>12
15	Cα	148.6	151.0	148.8	<4	<4
15	C*β*	121.4	123.0	121.5	<4	<4
16	α-C=O	168.5	170.7	172.7	39	13
16	*β*-C=O	167.5	168.3	172.7	37	12
16	Cα	128.4	136.8	129.0	<4	<4
16	C*β*	128.2	125.8	129.2	<4	<4
17	1-C=O	173.1	173.6	174.5	>35	>9
17	2-C=O	173.5	176.6	179.4	>37	>11
17	ipso1	117.4	115.9	117.1	>33	>29
17	ipso2	134.0	138.6	142.4	>40	>36
18	1-C=O	168.4	170.6	174.1	47	20
18	2-C=O	170.3	173.3	176.0	47	21
18	ipso1	131.8	133.6	137.9	51	47
18	ipso2	138.1	142.1	144.9	51	47
19	**2**-C=O	163.3[c]	167.4[d]	176.1	37	13
19	**3**-C=O	167.8[c]	172.0[d]	175.2	36	12

[a] Intrinsic shift at carboxyl carbon, 26 ppb; at ipso, <4 ppb. [b] Insoluble. [c] Cation. [d] Zwitterion.

Table 1 also lists isotope shifts. An intrinsic isotope shift Δ_o of 25−26 ppb (not tabulated) for the carboxyl carbons is observed at the diacid and dianion end points. No resolvable isotope shift (<4 ppb) is seen for the ipso carbon of any diacid or dianion. All the acids show increased carboxyl separations along the titration, and most show even larger ones at the ipso carbons. These rise to a maximum in the monoanion. The difference $\Delta_o - \Delta_{HA^-}$ is tabulated in Table 1. The α carbon of 3,4,5,6-tetrahydrophthalic acid (**9**) shows an especially large value, but none is seen for **15** or **16**.

Monoanions in Organic Solvents. Table 2 lists chemical shifts and isotope shifts for diacids in DMSO-d_6. Although the carboxyl shift of a monoanion might appear to be the same, within experimental error, as the intrinsic shift Δ_o, the average difference $\Delta_o - \Delta_{HA^-}$ is 1.5 ppb and no value is <0. The magnitude of the ipso shift is uniformly >13 ppb, significantly greater than the intrinsic shift of ≤4 ppb seen in the diacid or the 1 ppb measured for phthalate (**2**) monoanion, which shows the same ipso isotope shift regardless of counterion. For the monoanion of 3,4,5,6-tetrahydrophthalic acid (**9**) the large increase in isotope shift is seen only above 50% water.

Table 2. ^{13}C Chemical Shifts and ^{18}O-Induced Isotope Shifts of Dicarboxylic Acid Monoanions in DMSO-d_6, with Bu_4N Counterion (Except As Noted)

acid	signal	δ_{H_2A}, ppm	δ_{HA^-}, ppm	$-\Delta_{HA^-}$, ppb	$\Delta_o - \Delta_{HA^-}$,[a] ppb
2	C=O		167.6	27	1
2	ipso		134.7	16	11
2[c]	C=O	169.0	169.7	29	3
2[c]	ipso	133.9	136.1	16	11
3	C=O		166.1	27	2
3	C1,2		143.1	20	16
4	C=O	163.7	164.0	26	6
4	ipso	117.8	121.6	>13	>9
5	C=O		165.6	26	0
5	C2,3		155.2	46	42
6	C=O		174.3	26	0
8[b]	C=O		169.3	29	3
9	C=O		168.8	29	3
9	C1,2		138.3	31	27
15	α-C=O	171.1	169.3	25	1
15	*β*-C=O	167.2	168.2	26	1
16	α-C=O	164.3	161.4	25	0
16	*β*-C=O	164.2	164.0	26	2
19	2-C=O	168.0	166.4	28	3
19	3-C=O	166.6	166.9	28	1

[a] Intrinsic shift at carboxyl carbon, 20−27 ppb; at ipso, >4 ppb. [b] Na counterion. [c] K counterion.

Table 3. ^{13}C Chemical Shifts and ^{18}O-Induced Isotope Shifts of Dicarboxylate Monoanions in THF-d_8

acid	signal	δ_{HA^-}, ppm	$-\Delta_{HA^-}$, ppb	$\Delta_o - \Delta_{HA^-}$,[a] ppb
2	C=O	169.3	27	1
2	ipso	137.4	14	13
3	C=O	168.0	27	2
3	C1,2	144.7	21	17
4	C=O	165.8	26	0
4	ipso	123.8	>12	>8
9	C=O	170.5	28	2
9	C1,2	140.0	28	24
15	α-C=O	169.1	27	3
15	*β*-C=O	168.6	26	1
16	α-C=O	162.9	24	−1
16	*β*-C=O	165.4	26	2

[a] Intrinsic shift at carboxyl carbon, 24−26 ppb; at ipso, <4 ppb.

Table 4. ^{18}O-Induced ^{13}C Isotope Shifts of Hydrogen Phthalate (**2**) in Organic Solvents

solvent	$-\Delta^{CO}$, ppb	$\Delta_o - \Delta$,[a] ppb	$-\Delta^{ipso}$, ppb	$\Delta_o - \Delta$,[b] ppb
DMSO-d_6	27	1	16	15
THF-d_8	27−28[c]	1−2	14−16[c]	13−15
CD_2Cl_2	29	3	13	12
CD_2Cl_2[d]	30	4	15	14
$CDCl_3$	27	1	24	23

[a] Intrinsic shift at carboxyl carbon, 26 ppb. [b] Intrinsic shift at ipso carbon, 1 ppb per ^{18}O. [c] Variation with drying procedure. [d] −55 °C.

Chemical shifts and isotope shifts for some of these diacids in THF-d_8 are reported in Table 3. Some of these results were presented at the International Discussion Meeting, "Hydrogen Transfer: Experiment and Theory", Freie Universität Berlin, September 1997.[33] The results are similar to those in DMSO-d_6. Again the differences $\Delta_o - \Delta_{HA^-}$ at carboxyl carbon are small but rarely <0, whereas the ipso differences are significantly greater. (For **4** in both DMSO-d_6 and THF-d_8 separate ipso signals are not resolvable, but the line width is distinctly greater than that of the other aromatic carbon.)

Hydrogen phthalate (**2**) was examined in DMSO-d_6 and THF-d_8 and also in $CDCl_3$ and CD_2Cl_2. Table 4 summarizes the data. The carboxyl and especially the ipso isotope shifts are all larger in magnitude than the intrinsic shift. In CD_2Cl_2 at −55 °C the values of $\Delta_o - \Delta$ increase, as expected for the temperature effect on an equilibrium.

(33) Perrin, C. L.; Nielson, J. B.; Kim, Y.-J., submitted to *Ber. Bunseng. Phys. Chem.*

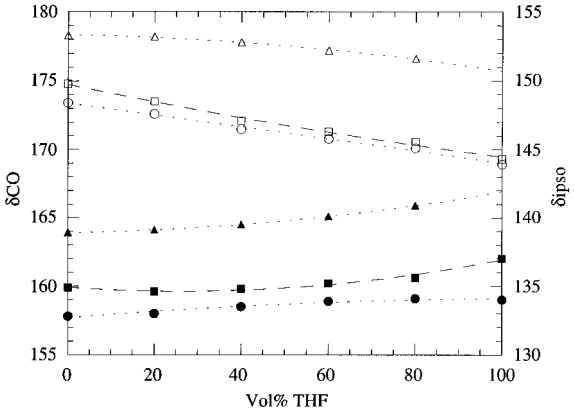

Figure 1. Carboxyl (open symbols) and ipso (filled symbols) ^{13}C chemical shifts of phthalic (**2**) acid (○), monoanion (□), and dianion (△) in THF−water mixtures. Dashed lines are parabolic fits, permitting extrapolation of dianion shifts to 100% THF.

Table 5. Chemical Shifts and Primary Isotope Shifts of Hydrogen Phthalate (**2**) at 25 °C

sample	solvent	$\delta(^1H)$, ppm	$^P\Delta$, ppm
1	CD_2Cl_2	20.58	−0.07
2		20.59	−0.14
3		20.66	0.00
4		20.72	0.04
5		20.72	0.04
6		20.73	0.05
7	$CDCl_3$	20.70	−0.10
8		20.74	0.00
9	DMSO-d_6	20.70	−0.75
10		20.71	−0.82
11	THF-d_8	20.59	−0.14
12		20.79	−0.03
13		20.88	0.08
14		20.90	0.12

All the previous results in organic solvents had relied upon the carboxyl isotope shifts. The ipso carbons of phthalates are also informative. The only such data previously observed were for phthalate (**2**) monoanion in DMSO-d_6, which shows a 16 ppb isotope shift, indicative of an asymmetric hydrogen bond. It was suggested that this was due to residual water. The monoanion of phthalic acid was examined in THF-d_8 to test the role of water. The results are included in Table 4. Two samples were prepared, one in THF-d_8 directly from 1 mL ampules and one in THF-d_8 dried over CaH_2 and transferred on a Schlenk line. The isotope shifts in the drier THF-d_8 are not significantly smaller. In both cases, although the carboxyl isotope shift is hardly larger than the intrinsic value, the ipso isotope shift is still 16 ppb, as in DMSO.

Figure 1 shows the variations of chemical shifts of phthalic (**2**) acid, monoanion, and dianion in D_2O−THF-d_8 mixtures. The dianion value at 100% THF is an extrapolation, owing to insolubility. The data in aqueous DMSO-d_6 are similar. As the water content decreases, the monoanion's carboxyl chemical shifts, and the ipso shifts to a lesser degree, diverge increasingly from the average of the diacid and dianion shifts.

Primary Isotope Shifts. The ^1H NMR chemical shifts and the primary isotope shifts of the monoanions of phthalic (**2**), 1,2-cyclopentenedicarboxylic (**3**), 3,4-furandicarboxylic (**4**), and 3,4,5,6-tetrahydrophthalic (**9**) acids in four organic solvents are reported in Tables 5 and 6. The primary isotope shifts for hydrogen phthalate (**2**) are negative, zero, or positive, depending

Table 6. Chemical Shifts and Primary Isotope Shifts of Monoanions (**3**, **4**, **9**) at 25 °C

	cyclopentene-dicarboxylate		furandicarboxylate		tetrahydrophthalate	
solvent	$\delta(^1H)$, ppm	$^p\Delta$, ppm	$\delta(^1H)$, ppm	$^p\Delta$, ppm	$\delta(^1H)$, ppm	$^p\Delta$, ppm
CD_2Cl_2	20.29	0.01	20.08	−0.11	19.94	−0.13
$CDCl_3$	20.32	−0.04	20.07	−0.14	19.80	−0.23
DMSO-d_6	20.25	−0.01	20.14	−0.12	19.85	−0.12
THF-d_8	20.40	−0.01	20.06	−0.08	19.93	−0.16

Table 7. Temperature Variation of Chemical and Primary Isotope Shifts of Hydrogen Phthalate (**2**) and 3,4-Furandicarboxylate (**4**) in CD_2Cl_2

acid	*T*, °C	$\delta(^1H)$	$^p\Delta$, ppm
phthalic no. 1	25	20.72	0.04
	10	20.77	0.05
	−25	20.88	0.11
	−55	20.95	0.18
phthalic no. 1[a]	25	20.66	0.00
	−55	20.94	0.15
phthalic no. 2	25	20.58	−0.07
	10	20.66	−0.03
	−25	20.77	0.02
	−55	20.89	0.04
phthalic no. 2[b]	10	20.63	−0.08
	−55	20.88	−0.02
3,4-furandicarboxylic	25	20.08	−0.12
	10	20.12	−0.10
	−55	20.29	−0.05

[a] Next day. [b] Next day, after additional drying overnight at 70 °C.

on the solvent and the sample. The primary isotope shifts for **4** and **9** are negative, but those for **3** are zero within experimental error. For **4** the isotope shifts agree with the −0.11 ppm previously observed.[24] The temperature dependences of the ^1H chemical shifts and of the primary isotope shifts of the monoanions of **2** and **4** are presented in Table 7. Two samples of **2** were examined, on two successive days. There is no apparent relation between chemical shifts and drying procedure. In general, the primary isotope shift becomes more positive as the chemical shift moves further downfield, either from sample to sample or as the temperature decreases. Such a trend parallels a decrease in the O−O distance and an increase in the strength of the hydrogen bond.[1,34]

Discussion

Symmetry of Hydrogen Bonds. In water the uniformly positive $\Delta_o - \Delta_{HA^-}$ at both carboxyl and ipso or α carbons (Table 1) is definitive evidence for an equilibrium between two tautomers with asymmetric hydrogen bonds. This agrees with previous studies.[21,23,26] The asymmetry in **15**−**19** is perhaps expected for these acids, but it must be recognized that they are not so asymmetric that one tautomer is predominant.

In the organic solvents DMSO and THF the carboxyl $\Delta_o - \Delta_{HA^-}$ is always small. The fact that it is uniformly (with one exception) nonnegative might be the result of perturbation of an equilibrium. This is not convincing, since it might simply be a reflection of intrinsic shifts, which happen to be slightly larger in the monoanion, with its intramolecular hydrogen bond, than in the diacid or dianion where Δ_o is calibrated. More information is available from the ipso or α reporter nucleus present in **2**, **3**−**5**, and **9**. In every solvent, not only aqueous but also organic, this $\Delta_o - \Delta_{HA^-}$ is significantly greater than zero (Tables 1−4). These results are consistent with asymmetric hydrogen bonds for all such monoanions. For **15**, **16**, and **19** the results are inconclusive, since $\Delta_o - \Delta_{HA^-}$ at carboxyl and α or ipso carbons is small, but it is unlikely that these have symmetric hydrogen bonds when symmetric dianions have asymmetric ones.

The ipso $\Delta_o - \Delta_{HA^-}$ values are so large that they cannot be attributed to intrinsic isotope shifts that happen to be considerably larger in the monoanion. By independent measure the intrinsic shift in **2** is only 1 ppb per ^{18}O. Besides, although the increase in the isotope shifts from +25 to −55 °C in Table 4 is small, it is in the direction consistent with equilibrium isotope shifts. We conclude that in solution none of the monoanions of the carboxylic acids examined have symmetric hydrogen bonds.

Sensitivity: Carboxyl or Ipso? For phthalate (**2**) in THF-d_8 the isotope shift at carboxyl carbon is hardly greater than the intrinsic shift. Nevertheless, the ipso isotope shift is still 14 ppb, significantly larger than the intrinsic shift. This result is confirmed in all of the organic solvents examined and at different temperatures, as summarized in Table 4. This shows that phthalate does not exist as a single symmetric ion in organic solvents, which is opposite to the original conclusion.[23]

Moreover, the comparison suggests that carboxyl isotope shifts are inadequate to determine the symmetry of the hydrogen bond. Thus, it was misleading to use those to conclude that succinate monoanion has a symmetric hydrogen bond in THF, or even an intramolecular one.[23,26] Nor do we have evidence for a conformational change from that seen in water.[25]

The ipso signals are more sensitive to the state of protonation of the carboxyl group and thus more diagnostic. The difference between the maximum isotope shift and the intrinsic isotope shift of the ipso reporter nucleus in water is 50 ppb, twice the value for the carboxyl nucleus. More importantly, there is no resolvable intrinsic shift of the ipso carbon. Therefore a doubling of the ipso signal is a sure sign of a tautomeric equilibrium, perturbed by ^{18}O.

If the hydrogen bond in the monoanions of dicarboxylic acids is asymmetric in both water and organic solvents, why does the magnitude of the isotope shift differ? One possibility is that in organic solvents there is a range of solvation states. The weaker solvation forces in organic solvents allow locally symmetric environments to coexist with asymmetric ones. The observed isotope shift then is the average over those molecules that have a symmetric hydrogen bond and those that have an asymmetric one.

The variations of the isotope shifts can also be explained by comparing the variability of chemical shifts in water and organic solvents. According to eq 2, the perturbation shift is proportional to *D*, the difference between the chemical shifts of protonated and unprotonated carboxyl groups in the monoanion, which can be approximated by the chemical shifts of diacid and dianion (eq 4). The latter could not be measured in organic solvents, but the difference between chemical shifts of diacid and monoanion is another measure of the sensitivity of chemical shift to the state of protonation. In water the magnitude of this difference, for all the entries in Table 1, averages 2.3 ppm, but in DMSO it averages only 0.2 ppm, from Table 2, and the average is <0 for carboxyl carbons. If *D* is correspondingly reduced in organic solvents, then so is the isotope shift. Hence even though there might be an equilibrium between two tautomers, it does not manifest itself by an isotope shift, especially at carboxyl carbon. It was therefore misleading to rely on carboxyl isotope shifts in organic solvents to conclude that hydrogen bonds were symmetric.[23,26]

(34) Hibbert, F.; Emsley, J. *Adv. Phys. Org. Chem.* **1990**, *26*, 255.

For ipso and α carbons D (eq 3) represents the difference between chemical shifts adjacent to carboxyl and adjacent to carboxylate in the monoanion. For ipso carbons, which show a large downfield shift on deprotonation, D seems to be large enough to lead to appreciable isotope shifts. Thus in contrast to the carboxyl isotope shifts, the ipso isotope shifts, when observable, can provide unequivocal evidence for asymmetric hydrogen bonds. In further contrast, the α carbons of **15** and **16** have almost the same chemical shift in dianion as in diacid. Consequently they show no resolvable isotope shift in the monoanion, unlike the otherwise similar ipso carbons of phthalate (**2**), but also unlike **9**, whose chemical shifts give no indication of the large isotope shift of the α carbon.

Our ability to estimate D may be even more compromised. If the approximation in eq 4 is valid, then the chemical shift of the monoanion must also be close to the average of those of the diacid and dianion (eq 9). From the variations of chemical

$$\delta_{\text{monoanion}} = {}^1/_2(\delta_{\text{COOH}} + \delta_{\text{CO}_2^-}) \approx {}^1/_2(\delta_{\text{diacid}} + \delta_{\text{dianion}}) \quad (9)$$

shifts of phthalate with water content in THF-d_8 (Figure 1) and DMSO-d_6, this latter approximation holds better in water than in organic solvents and better for ipso carbons than for carboxyl. Thus we conclude that we cannot use measured carboxyl chemical shifts to estimate D in organic solvents.

Primary Isotope Shifts. For the monoanions of 3,4-furandicarboxylic (**4**) and 3,4,5,6-tetrahydrophthalic (**9**) acids the primary isotope shifts at room temperature (Table 6) are negative in all solvents. This is in agreement with the asymmetric hydrogen bonds found by the method of isotopic perturbation of equilibrium. For hydrogen 1,2-cyclopentenedicarboxylate (**3**) the primary isotope shift is near zero, which might suggest that it has a weak hydrogen bond despite the downfield chemical shift.

Hydrogen phthalate (**2**) shows large variations (Table 5) with solvent and even within the same solvent. In DMSO-d_6 the primary isotope shift is negative and of the magnitude observed for β-diketones, which have asymmetric hydrogen bonds. In $CDCl_3$ the shift is sometimes zero, which might suggest a weak hydrogen bond. In both CD_2Cl_2 and THF-d_8 the shifts range from positive to negative, the latter corresponding to a symmetric hydrogen bond. A change of sign with solvent has also been seen for salicylaldehyde and for ethyl acetoacetate.[24,33,35] Yet by the method of isotopic perturbation of equilibrium (Table 4) hydrogen phthalate is asymmetric in all solvents studied. This contradiction cannot be attributed to the possibility that the samples examined by the method of isotopic perturbation were wetter, with weakened intramolecular hydrogen bonds, since the ^{1}H chemical shifts, which are thought to be a measure of hydrogen bond strength, are identical to those in the CD_2Cl_2 and THF-d_8 samples that exhibit positive primary isotope shifts.

The primary isotope shifts in CD_2Cl_2 are temperature-dependent (Table 7). For 3,4-furandicarboxylate (**4**) the values are always negative, corresponding to a strong, asymmetric hydrogen bond, as found from the ^{18}O-induced isotope shifts. For hydrogen phthalate (**2**) there are variations that may correspond to vagaries of sample preparation and dryness. Sample 1 has an isotope shift of +0.18 ppm and a ^{1}H chemical shift of 20.95 ppm at −55 °C, which confirms the shifts of +0.15 ppm and 21.0 ppm previously reported[24] and long accepted as evidence of a symmetric hydrogen bond. Nevertheless, the ^{18}O-induced isotope shifts (Table 4) indicate an asymmetric hydrogen bond.

We consider the ^{18}O-induced isotope shifts more direct and more reliable than the primary isotope shift for assessing the symmetry of hydrogen bonds. Although eq 8 represents a model whereby a centered hydrogen leads to positive primary isotope shift, such a shift depends on the sign of $d^2\sigma/dr^2$. Besides, it could have other origins, such as the Ubbelohde effect, wherein the ODO distance is greater than the OHO.[36] An ^{18}O-induced isotope shift depends on the magnitude of D in eq 2 but is otherwise model-independent, in that the observation of such a shift, beyond the intrinsic shift, is strong and direct evidence for an equilibrium between two tautomers, and therefore an asymmetric hydrogen bond.

Solution vs Solid. It is often hoped that the crystal structure will describe a molecule in solution. Indeed the prior conclusion was that there are nonpolar solvents with an environment similar to the crystal.[21] Now none of our results in solution conform to the symmetric hydrogen bonds seen in some crystals.[5] It is reasonable that hydrogen bonding by water decreases the strength of the intramolecular bond. Alternatively, the hydrogen bonding to the two carboxyls may not be identical, creating a preference for protonation of the less solvated one. Even in other solvents weak solvation interactions may act similarly. The difference might thus be attributed to the polarity of the solvents, inasmuch as ab initio calculations show that a polarizable medium or a neighboring ion stabilizes the asymmetric structure.[37] However, a crystal is also a polarizable medium with neighboring ions.

A clue to this difference is that crystals are found with asymmetric hydrogen bonds, owing to asymmetrically placed counterions.[6] We have assumed that the bulky Bu_4N counterion used in the organic solvents does not associate closely with the carboxyl groups. In support, the isotope shift in DMSO-d_6 is the same with K as with Bu_4N. Nevertheless our assumption may not be valid. We have been restricted to anions that are paired with counterions in nonpolar solvents. If that counterion is placed asymmetrically relative to the two carboxyls, then one tautomer will be favored. This has the same consequence as unequal hydrogen bonding to the two carboxyls in disordered protic solvents.

What are the implications for the hypothesis of SSHBs in enzyme reactions? It is conceivable that an enzyme can create a sufficiently symmetric local environment to permit a symmetric SSHB. However, our inability to detect any in solution makes this unlikely. Alternatively, symmetry, covalent character, and resonance may not be necessary to the strength of these hydrogen bonds.

Conclusions

By the method of isotopic perturbation of equilibrium the symmetry of hydrogen bonds in the monoanions of diacids can be determined. In aqueous solution all monoanions examined have asymmetric hydrogen bonds. This is attributed to the competing hydrogen bonding by water, which favors an asymmetric bond. The salts of diacids whose carboxylic groups differ in acidity have asymmetric hydrogen bonds in organic solvents. In addition, the monoanions of diacids such as phthalic acid also have asymmetric hydrogen bonds in all organic solvents. This is opposite the previous conclusion, but it is firmly based on isotope shifts at the ipso carbons that can be attributed only to perturbation of an equilibrium between two

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tautomers. The asymmetry is attributed to disordered hydrogen bonding in aqueous solution and to a counterion effect in nonpolar solvents.

Since this conclusion contradicts a previous one based on primary isotope shifts, we have reinvestigated these. Three monoanions have negative or zero primary isotope shifts in organic solvents, in agreement with the conclusion from isotopic perturbation. Hydrogen phthalate (**2**) is confirmed as having a positive primary isotope shift, but it also shows an ^{18}O-induced isotope shift, which is direct evidence for an asymmetric hydrogen bond. We therefore conclude that primary isotope shifts are not reliable in judging hydrogen bonds as symmetric.

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