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Journal

Journal of the American Chemical Society, 141(43)

ISSN

0002-7863

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Publication Date

2019-10-30

DOI

10.1021/jacs.9b08492

Supplemental Material

<https://escholarship.org/uc/item/9br9g3nh#supplemental>

Peer reviewed

Isotopic-Perturbation NMR Study of Hydrogen-Bond Symmetry in Solution: Temperature Dependence and Comparison of OHO and ODO Hydrogen Bonds

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Abstract

Is a hydrogen bond symmetric, with the hydrogen centered between two donor atoms, or is it asymmetric, with the hydrogen closer to one but jumping to the other? The NMR method of isotopic perturbation has been used to distinguish these. Previous evidence from isotope shifts implies that a wide variety of dicarboxylate monoanions are asymmetric, present as a rapidly equilibrating mixture of tautomers. However, calculations of hydrogen trajectories across an anharmonic potential-energy surface could reproduce the observed isotope shifts in phthalate monoanion. Therefore it was concluded that those isotope shifts are instead consistent with isotope-induced desymmetrization on a symmetric potential-energy surface. To distinguish between these two interpretations, the ¹⁸O-induced isotope effects on the ¹³C NMR chemical shifts of cyclohexene-1,2-dicarboxylate monoanion in chloroform-*d* and on the ¹⁹F NMR chemical shifts of difluoromaleate monoanion in D₂O have been investigated. In both cases the

isotope effects are larger at lower temperature and also with deuterium in the hydrogen bond. It is concluded that these behaviors are consistent with the perturbation of an equilibrium between asymmetric tautomers and inconsistent with isotope-induced desymmetrization on a symmetric potential-energy surface.

Introduction

Hydrogen bonds (H-bonds) are the result of an attractive force between a proton and two lone-pair donors.¹ They have a vital role in the structure and reactivity of chemical species.² They contribute to the shape and function of many substances,³ and they have attracted great interest for their possible role in stabilizing intermediates or transition states,⁴ although reasonable alternatives have been proposed.⁵ A fundamental question regarding H-bonds is whether the H is centered between the two donor atoms or is instantaneously closer to one.⁶ This question simplifies if the two donor atoms are of very different basicities. In such cases the potential-energy surface describing the H motion is a double well with unequal well depths, so that the H is always closer to the more basic donor than to the other. This is the situation for some of the most common H-bonds, as in water, proteins, and nucleic acids. They are termed asymmetric H-bonds. In contrast, if the two donor atoms are of equal basicities, the double-well potential becomes symmetric, with equal well depths. If the energy barrier between the wells is substantial, the H may be instantaneously localized in one well and jumping between them, in an asymmetric H-bond. Alternatively, as the distance between the donor atoms decreases, the barrier height drops, and the H may be delocalized across both wells and in a symmetric H-bond. Such H-bonds are often called “short” or “low-barrier” H-bonds, with enhanced strength.⁷

Symmetric species have often been distinguished from asymmetric ones by the NMR method of isotopic perturbation.⁸ This method succeeds even if rapid equilibration coalesces the signals. Our approach has been to apply this method to H-bonds,⁹ in order to distinguish a symmetric species in a single-well potential from a rapidly equilibrating mixture of tautomers in a double-well potential.¹⁰ This requires measuring the isotope shift ${}^n\Delta_X$, which is the change of the NMR chemical shift δ of a reporter nucleus X due to isotopic substitution n bonds away (eq. 1, with n sometimes omitted). In general the isotope shift due to a heavier isotope is negative (greater shielding), although conventions differ.¹¹ There are two contributions to the observed isotope shift, an intrinsic isotope shift Δ_0 and a perturbation shift Δ_{eq} induced by perturbation of an equilibrium (eq. 2). The mere presence of an isotope is responsible for Δ_0 ,¹² while Δ_{eq} is due to an equilibrium isotope effect (EIE) arising from differences in the mass-dependent vibrational frequencies and zero-point energies of the two species in equilibrium.¹³

$${}^n\Delta_X = \delta_{\text{heavy}} - \delta_{\text{light}} \quad (1)$$

$$\Delta_{\text{obs}} = \Delta_0 - \Delta_{\text{eq}} \quad (2)$$

For more than two decades isotopic perturbation has been used to explore the symmetry of H-bonds in the monoanions of dicarboxylic acids and similar species. Although some of them are symmetric in crystals,¹⁴ others, including maleate and phthalate monoanions, are nevertheless asymmetric in aqueous and organic solutions.¹⁵ The asymmetry in solution was attributed to the disordered instantaneous solvation of the local environment,¹⁵⁻¹⁶ leading to solvatomers (isomers that differ in solvation).¹⁷ The disorder of solvation renders the two donor atoms instantaneously inequivalent, whereupon the hydrogen attaches to the less well solvated donor.^{17b,18} Indeed, this interpretation was supported by computer

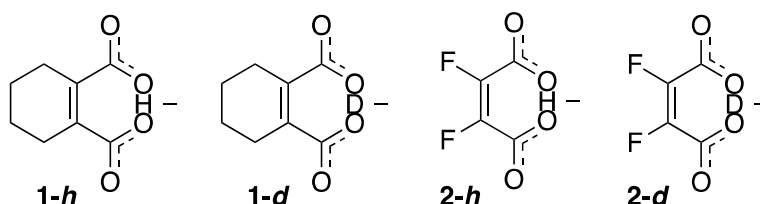
simulations,¹⁹ including the case of otherwise symmetric FHF⁻.²⁰ Further examples show that asymmetry is also seen in NHN and NHO H-bonds.²¹ In summary, we and others have been unable to find evidence for symmetric H-bonds in solution (except at very low temperature, where the solvent becomes well ordered),²² and with only one recent exception that demonstrates the subtlety of solvation.²³ Therefore we concluded that there is no stabilization or enhanced strength associated with symmetric, short, or low-barrier H-bonds.⁹

However, Bogle and Singleton have published an alternative interpretation of those NMR data that were presented as evidence for asymmetric tautomers.²⁴ They calculated the quasiclassical trajectories of hydrogen across the highly anharmonic potential-energy surface in isotopically labeled phthalate monoanion and averaged the ¹³C NMR shifts over those trajectories. They concluded that an ¹⁸O produces a significant intrinsic isotope shift that can account for the results obtained by Perrin and co-workers. If so, phthalate monoanion is symmetric and there is no need to propose equilibrating tautomers.

We do not deny that the intrinsic isotope shift due to an ¹⁸O can be substantial when there is coupling between a desymmetrizing mode and anharmonic isotope-dependent modes. The question remains whether this calculated isotope shift accounts fully for the isotope shifts that were measured.

To address the claim that the measured isotope shifts can be attributed to the isotope effect (IE) on a symmetric but anharmonic potential-energy surface,²⁴ we first studied cyclohexene-1,2-dicarboxylic acid monoanion (**1-h**) in CDCl₃.²⁵ We found that the ¹⁸O-induced ¹³C NMR isotope shifts at the ipso carbons are larger at lower temperature, consistent with

perturbation of an equilibrium between asymmetric H-bond tautomers. We further inferred that this result is inconsistent with the desymmetrizing effect of isotopic substitution on a symmetric potential-energy surface, because the trajectories ought to become less anharmonic at lower temperature. We now extend this study to the temperature dependences of the ^{18}O -induced ^{13}C NMR isotope shifts of **1-d** and of the ^{18}O -induced ^{19}F NMR isotope shifts in difluoromaleate monoanion (**2-h** and **2-d**).



Our second approach is to compare the isotope shifts of diacid monoanions **1-d** and **2-d** with an ODO H-bond to **1-h** and **2-h** with an OHO H-bond. If the isotope shifts are due to the desymmetrizing effect of isotopic substitution on a symmetric but anharmonic potential-energy surface, that effect ought to be smaller with heavier deuterium, whose motion is less anharmonic. Alternatively, if the isotope shifts are due to the perturbation of an equilibrium between asymmetric H-bond tautomers, that equilibrium might become more unbalanced with OD, because the isotope shift is expected to increase with D,^{16a} owing to a larger ^{18}O IE on the acidity of an OD acid.^{26,31} We now report isotope shifts of ^{18}O -labeled protium and deuterium cyclohexene-1,2-dicarboxylate monoanions (**1**) and difluoromaleate monoanions (**2**), in order to compare ODO H-bonds with OHO H-bonds.

Experimental

Synthesis and ^{18}O incorporation

Synthesis of Bu_4N^+ 3,4,5,6-tetrahydrophthalate- d - $^{18}\text{O}_{0-4}$ (**1-d**- $^{18}\text{O}_n$)

A mixture of ^{18}O isotopologues of diacid **1** was synthesized by hydrolyzing the anhydride in H_2^{18}O containing THF (to increase solubility), then adding D_2O and $\text{Bu}_4\text{N}^+ \text{OH}^-$. The solid Bu_4N^+ salt of the monoacid monoanion **1-d- $^{18}\text{O}_n$** was then obtained by removing the solvent in vacuo. Synthesis of difluoromaleic acid, difluoromaleic- $^{18}\text{O}_{0-2}$ acid, Bu_4N^+ difluoromaleate- ^{18}O (**2- $^{18}\text{O}_n$** , $n = 0,1,2$), and of Bu_4N^+ deuterium difluoromaleate- ^{18}O (**2-d- $^{18}\text{O}_n$** , $n = 0,1,2$)

Even though difluoromaleic acid is a "simple" material, it is not readily available, so that it was necessary to give careful consideration to its preparation. Although it has been prepared in one step by oxidation of polyfluorinated aromatics with $\geq 40\%$ peracetic acid,²⁷ this peracid is hazardous and not always available. An alternative small-scale oxidation procedure was reported, but it requires a commercially less accessible phthalocyanine catalyst.²⁸ Instead of these oxidative pathways a multistep procedure is more commonly used.²⁹ However, neither of the requisite precursors, 1,1,2-trichloro-2,3,3-trifluorocyclobutane nor trifluorosuccinic acid, is now available commercially at a reasonable price. Therefore we developed a mild procedure for synthesis and isolation of difluoromaleic acid by oxidation of pentafluorophenol with $\sim 35\%$ peracetic acid.

Difluoromaleic anhydride was then readily prepared by dehydration of difluoromaleic acid with P_2O_5 .³⁰

The procedure for incorporation of ^{18}O was adapted from an earlier one.^{29b} Hydrolysis of difluoromaleic anhydride with H_2^{18}O containing >2 equiv. of Na^{18}OH rapidly produced the mono- ^{18}O -labeled difluoromaleate dianion and avoided equilibration or incorporation of more than one ^{18}O . Excess hydroxide is necessary because with only one equivalent the resulting monoacid monoanion would neutralize a second equivalent of

hydroxide. The use of Na^{18}OH succeeded in furnishing 100% dianion containing $\sim 95\%$ ^{18}O . Acidification with dilute HCl then led to isolation of difluoromaleic acid containing $\sim 80\%$ monolabeled material, along with $\sim 15\%$ of unlabeled and $\sim 5\%$ doubly labeled ^{18}O -diacid. This quality was suitable to clearly observe and interpret the isotope shifts in the ^{19}F NMR spectrum of the monoanion.

Bu_4N^+ protium difluoromaleate- ^{18}O (**2-h- $^{18}\text{O}_n$** ; $n = 0,1,2$) was then readily prepared by the addition of 1 equivalent of $\text{Bu}_4\text{N}^+ \text{CN}^-$ to the solution of difluoromaleic- $^{18}\text{O}_n$ acid in H_2O . However, applying that procedure to the same difluoromaleic- $^{18}\text{O}_n$ acid, but in D_2O , was not successful in producing Bu_4N^+ difluoromaleate- d - $^{18}\text{O}_n$ (**2-d- $^{18}\text{O}_n$** ; $n = 0,1,2$). We surmise that this remarkable feature is because difluoromaleic acid is so strong an acid that it is present in aqueous solution exclusively as its internally hydrogen-bonded monoanion (**2-h**), which is so weak an acid and also so weak a base that neither base- nor acid-catalyzed hydrogen exchange is fast enough to equilibrate the protonated and deuterated isotopologues. However, the equilibrium could be established and shifted toward the desired deuterated isotopologue by heating in D_2O . Evidence for the retention of H within the H-bond and the conditions to remove that H are presented in Supporting Information.

Results

Carboxyl ^{13}C NMR Chemical-Shift Assignments for ^{18}O Isotopologues of **1-d**

Figure 1 shows the ^{13}C NMR signals for the carboxyl (**A**) carbons of the ^{18}O isotopologues of **1-d- $^{18}\text{O}_n$** at 20°C , -10°C , -20°C , -30°C , and -50°C . The prominent peaks represent unlabeled (**A₀**) and mono- ^{18}O -labeled (**A₁**) carboxyl carbons. These designations are shown in Fig. 2, which is adapted

from Fig. 3 of a previous study.²⁵ The two major signals are separated by an intrinsic isotope shift Δ_0 of -28.5 ppb at 20 °C. Although signals due to a small amount of di- ^{18}O -labeled (\mathbf{A}_2) carboxyl may be present at 171.53 ppm, those signals were ignored. These assignments are based on results obtained from $\mathbf{1-h}$, where addition of unlabeled $\mathbf{1}$ increased the \mathbf{A}_0 intensity.²⁵

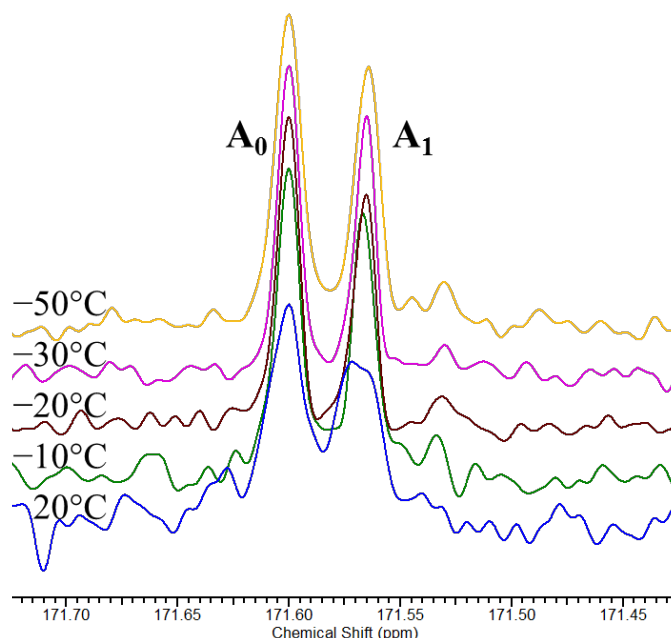


Figure 1. ^{13}C NMR spectrum of the carboxyl region of a mixture of ^{18}O -labeled isotopologues of $\mathbf{1-d-}^{18}\text{O}_n$ in CDCl_3 at 20 °C (blue), -10 °C (green), -20 °C (brown), -30 °C (pink), and -50 °C (yellow).

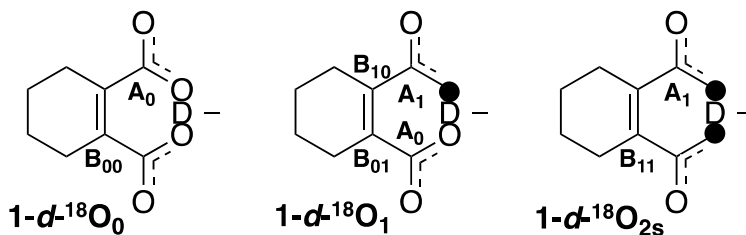


Figure 2. Dominant un-, mono-, and di- ^{18}O -labeled isotopologues of $\mathbf{1-d}$ and designation of distinguishable carbons.

Ipsso ^{13}C NMR Chemical-Shift Assignments for ^{18}O Isotopologues of $\mathbf{1-}$

d

Figure 3 shows the ^{13}C NMR signals for the ipso carbons of the ^{18}O isotopologues of $\mathbf{1-d-}^{18}\text{O}_n$ at 20°C , -10°C , -20°C , -30°C , and -50°C . The ipso designations are also labeled as **B** on the structures in Figure 2. The first subscript represents the number of ^{18}O labels on the carboxyl group adjacent to that ipso carbon, and the second subscript represents the number of ^{18}O labels on the opposite carboxyl. Thus **B₀₀** and **B₁₁** correspond to species where the two carboxyls have the same number of ^{18}O labels. Because these two signals are not resolvable, the intrinsic isotope shift Δ_0 must be too small to measure, in contrast to the intrinsic isotope shift for the carboxyls, and as expected from phthalate monoanion.¹⁵ These signals are therefore designated as **B_{00/11}**. Two other peaks, **B₀₁** and **B₁₀**, correspond to species with one additional ^{18}O label in one of the carboxyls, while we ignore signals **B₁₂** and **B₂₁**. Two further peaks, **B₀₂** and **B₂₀**, correspond to species with two additional ^{18}O labels in one of the carboxyls, but they are too weak to be resolved well. The labeling of the peaks in Fig. 3 is consistent with a negative isotope shift due to a heavy isotope, even though it is not possible to assign which signal is due to the ^{13}C that is closer to the ^{18}O . Finally, it should be noted that isotopologues $\mathbf{1-}^{18}\text{O}_1$ and $\mathbf{1-}^{18}\text{O}_{2s}$, with carboxyls bearing one ^{16}O and one ^{18}O , exist as a mixture of rapidly equilibrating conformational isotopomers related by rotation about the $\text{C}_{\text{ipso}}\text{-C}$ bond, but for brevity only the one with ^{18}O involved in the H-bond is shown. The justification for this simplification is presented in the Supporting Information.

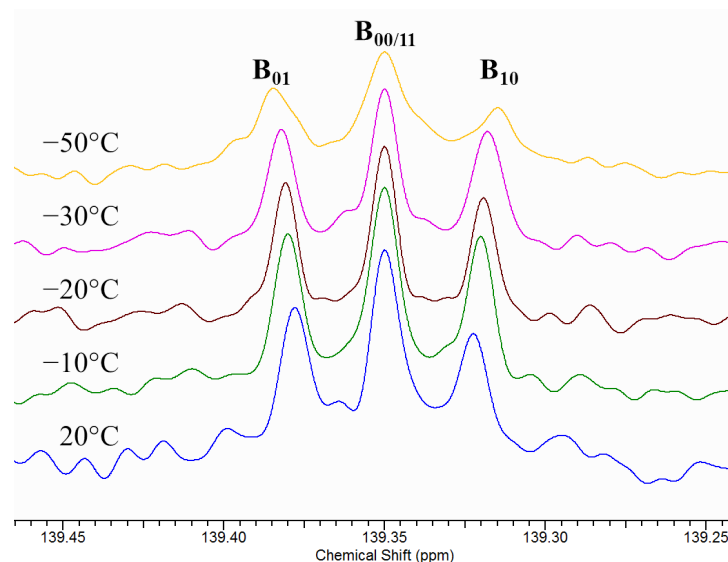


Figure 3. ^{13}C NMR spectrum of the ipso region of a mixture of ^{18}O -labeled isotopologues of $\mathbf{1-d-}^{18}\text{O}_n$ in CDCl_3 at 20°C (blue), -10°C (green), -20°C (brown), -30°C (pink), and -50°C (yellow).

Temperature Dependence of Chemical-Shift Differences in $\mathbf{1-d}$ and $\mathbf{1-h}$

Tables S5 and S6 list the chemical shifts of the carboxyl (**A**) and ipso (**B**) carbons in ^{18}O isotopologues of $\mathbf{1-h}$ and $\mathbf{1-d}$, respectively, at various temperatures. The carboxyl (**A**) chemical-shift differences are dismissed as less reliable, and we focus on the **B** signals of ipso carbons. The ipso chemical-shift differences, $\mathbf{B}_{01}-\mathbf{B}_{10}$, for both $\mathbf{1-h}$ and $\mathbf{1-d}$ are listed in Table 1. Because the signal assigned as \mathbf{B}_{01} is more deshielded than that assigned as \mathbf{B}_{10} , these differences represent negative isotope shifts.

Table 1. Chemical-shift differences (ppb) for ipso (**B**) carbons of $\mathbf{1-h}$ and $\mathbf{1-d}$ at various temperatures.

Anio		20°	-10°	-20°	-30°	-50°
n	Δ	C	C	C	C	C
	$\mathbf{B}_{01}-\mathbf{B}_{10}$	46.				
$\mathbf{1-h}^{25}$	$_{10}$	0	51.2	52.2	53.5	55.3

B₀₁–B		55.				
1-d	₁₀	6	60.0	61.5	64.1	69.9

All the chemical-shift differences for **1-h** and **1-d** in Table 1 increase with decreasing temperature. This confirms what had been observed for **1-h**,²⁵ while the result for **1-d** is new. Figure 4 shows a plot vs $1000/T$ of the observed chemical-shift differences Δ for the **B₀₁ – B₁₀** ipso separations of both **1-h** and **1-d**. The plots are adequately linear, with slopes of 8.6 ± 1.3 and 13.3 ± 0.8 ppm-K, respectively. The intercepts of 17 ± 5 and 9.5 ± 3 ppb represent intrinsic shifts that are higher than the expected 3 ppb, but the values are quite uncertain because they depend on extrapolating to infinite temperature from data over a small range. Besides, correcting for that discrepancy hardly changes the slope. The key result is that in both cases the increase of Δ with decreasing temperature can be attributed to the perturbation of an equilibrium between tautomers.

Figure 4. Linear fit of chemical-shift difference Δ vs $1000/T$ for the ipso carbons ($\mathbf{B}_{01} - \mathbf{B}_{10}$) of $\mathbf{1-h-}^{18}\text{O}$ (x) and $\mathbf{1-d-}^{18}\text{O}$ (+) in CDCl_3 .

^{19}F Chemical Shifts and ^{18}O -Induced ^{19}F Isotope Shifts of $\mathbf{2-h}$ and $\mathbf{2-d}$

The ^{19}F NMR spectra of difluoromaleate monoanion-*h*, monoanion-*d*, and dianion (without ^{18}O incorporation) in D_2O are singlets with chemical shifts -126.45, -126.33, and -140.79 ppm, respectively. These are in good agreement with previously reported values.^{29b} Although difluoromaleic acid is such a strong acid that it does not persist in aqueous solution so that its chemical shift cannot be measured, that chemical shift can be estimated as -112 ppm, assuming an additive contribution from each proton. From the ^{19}F NMR chemical shifts of monoanion and dianion, a perturbation shift of 0.07 ppm between the two fluorines of difluoromaleate- ^{18}O monoanion can be estimated from eq. 3, if the equilibrium constant between the two tautomers, one with H on ^{18}O and the other with H on ^{16}O , is taken as 1.01, based on the ^{18}O EIE on the acidity of simple carboxylic acids.²⁶

$$\Delta_{\text{eq}} = D (K - 1)/(K + 1) \quad (3)$$

The ^{19}F NMR spectrum of the disodium salt of a 15:80:5 mixture of un-, mono-, and di- ^{18}O -labeled difluoromaleate dianion in D_2O shows a singlet at δ -140.785 ppm. In contrast, the ^{19}F NMR spectra of the Bu_4N^+ salts of 15:80:5 mixtures of un-, mono-, and di- ^{18}O -labeled protium- and deuterium-difluoromaleate monoanion ($\mathbf{2-h-}^{18}\text{O}_n$ and $\mathbf{2-d-}^{18}\text{O}_n$, $n = 0,1,2$) in D_2O at 20°C are shown in Fig. 5. The chemical shifts of $\mathbf{2-h-}^{18}\text{O}_n$ and $\mathbf{2-d-}^{18}\text{O}_n$ taken from those spectra are included in Tables S7 and S8. The individual signals of $\mathbf{2-h-}^{18}\text{O}_n$ are approximately twice as broad as those of $\mathbf{2-d-}^{18}\text{O}_n$, as rationalized below, but the overall spans are almost the same (17.6 Hz) for both multiplets. It should also be noted that even in D_2O the H does not exchange

out of the H-bond of $\mathbf{2}$ - h - $^{18}\text{O}_n$, because this monoanion is too weak an acid to lose its H. Nor is it sufficiently basic to be converted to the diacid, from which that H can be removed.

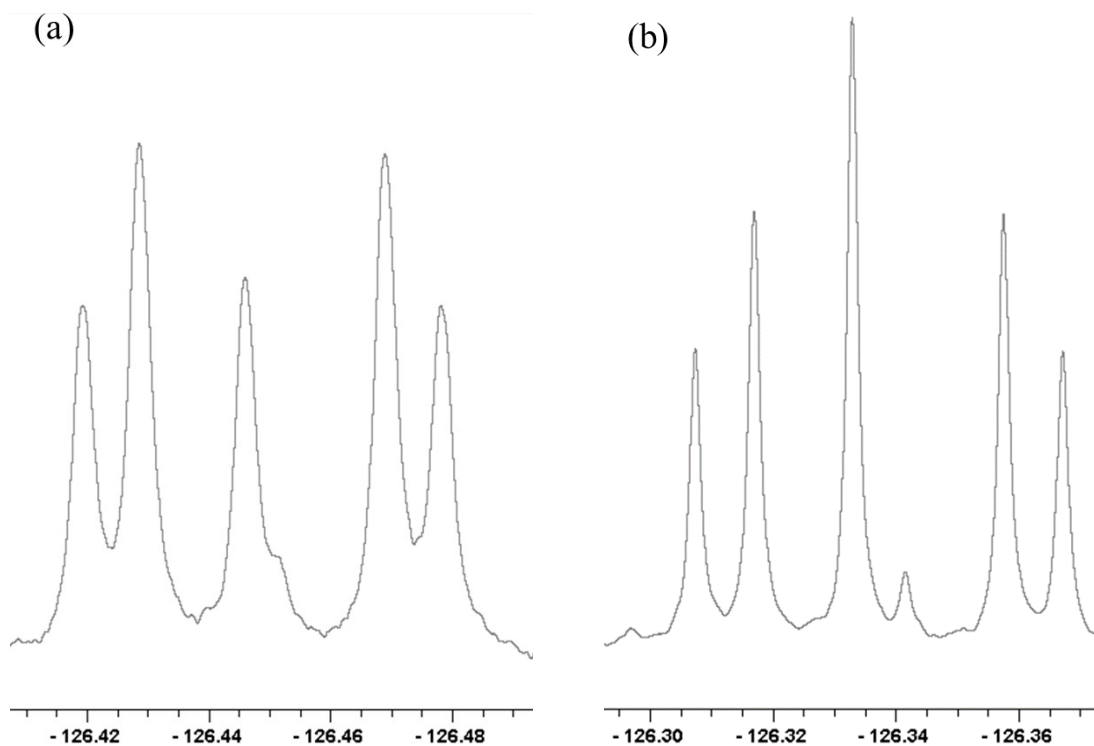


Figure 5. ^{19}F NMR spectra of (a) $\mathbf{2}$ - h - $^{18}\text{O}_n$ and (b) $\mathbf{2}$ - d - $^{18}\text{O}_n$ in D_2O at 20°C .

Of the two central signals in Fig. 5a or Fig. 5b the more intense can be assigned to unlabeled ($\mathbf{2}$ - $^{18}\text{O}_0$) and the less intense to di- ^{18}O -labeled ($\mathbf{2}$ - $^{18}\text{O}_2$), based on the mass-spectral data in Table S4 and as in a previous study.^{29b} The outer four signals, which can be assigned to monolabeled $\mathbf{2}$ - ^{18}O , can be recognized as an AB spin system. The spectra in Fig. 5 are very similar to each other, except that the peaks of $\mathbf{2}$ - h - ^{18}O are slightly broader. The broadening can be attributed to unresolved HF coupling, with $J_{\text{HF}} \ll 1$ Hz. Actually that coupling is an average of a four-bond $^4J_{\text{HF}}$ and a five-bond $^5J_{\text{HF}}$ coupling, each of which is generally ~ 1 Hz, but it is quite possible that they

are of opposite signs, as in trifluoromethylbenzene,³¹ so that they nearly cancel.

The ^1H and ^{19}F NMR spectra of the Bu_4N^+ salts of **2-*h*- $^{18}\text{O}_n$** and **2-*d*- $^{18}\text{O}_n$** in CD_3CN are shown in Figs. S2 and S3. The incorporation of deuterium in the latter is confirmed by the absence of the ^1H signal at 20.33 ppm that was present in the former. Again the individual ^{19}F signals of **2-*h*- $^{18}\text{O}_n$** are broader than those of **2-*d*- $^{18}\text{O}_n$** , as can be attributed to unresolved J_{HF} .

Second-order analysis was used to analyze the AB patterns in Fig. 5 of monolabeled **2- ^{18}O** and **2-*d*- ^{18}O** in D_2O , as described in Supporting Information. Values of the coupling constants J_{AB} , chemical-shift differences $\Delta\nu_{\text{AB}}$, and intrinsic isotope shifts Δ_0 are in Table 2. The fact that $\Delta\nu_{\text{AB}}$ is so much larger than Δ_0 is strong evidence that $\Delta\nu_{\text{AB}}$ is not an intrinsic shift but must be due to the perturbation of an equilibrium. Presumably this perturbation shift has a negative value, but its sign cannot be determined because it is not possible to assign which ^{19}F is closer to the ^{18}O and subject to the more negative isotope shift.

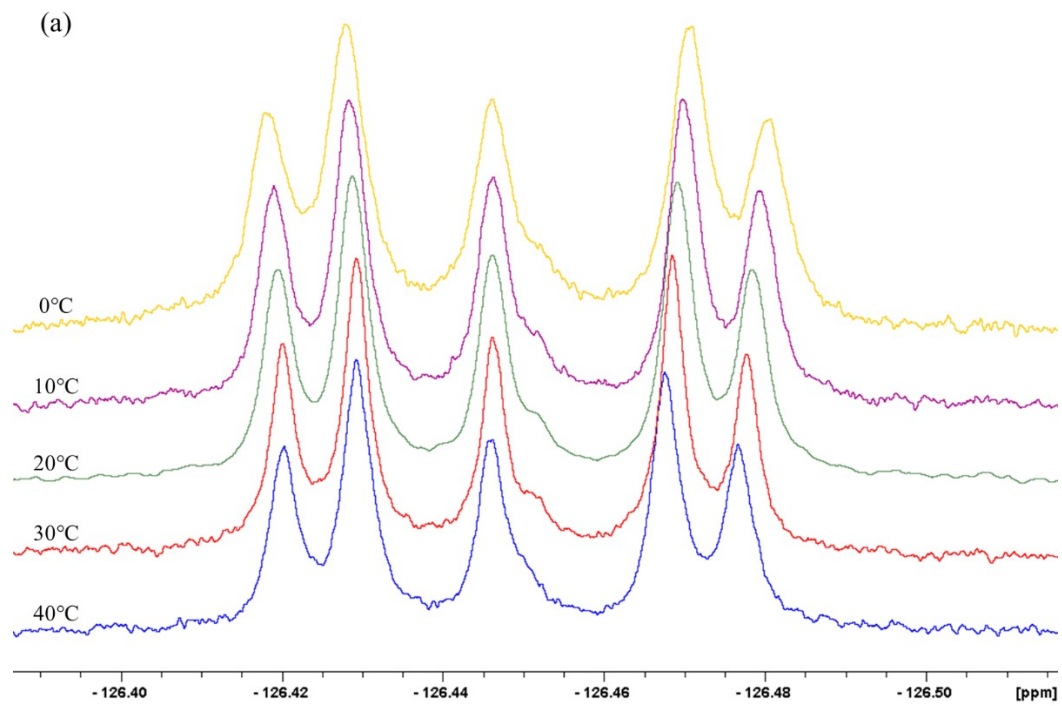
Table 2. Analysis of AB patterns of monolabeled **2- ^{18}O** and **2-*d*- ^{18}O** in Fig. 5.

Parameter	2-<i>h</i>-$^{18}\text{O}_n$	2-<i>d</i>-$^{18}\text{O}_n$
J_{AB} , Hz	2.62	2.71
$\Delta\nu_{\text{AB}}$, ppb	48.72	49.27
$-\Delta_0$, ppb	2.95	4.30

Temperature Dependence of ^{19}F Isotope Shifts of **2-*h*- $^{18}\text{O}_n$** and **2-*d*- $^{18}\text{O}_n$**

Figure 6 shows ^{19}F NMR spectra of **2-*h*- $^{18}\text{O}_n$** and **2-*d*- $^{18}\text{O}_n$** in D_2O from 0 to 40°C. As the temperature decreases, the chemical shifts of the AB

patterns of both samples move apart slightly, with no deterioration in resolution, and with maintenance of the symmetry. As in Fig. 5 the peaks of **2-*h*-¹⁸O** are slightly broader, owing to unresolved HF coupling. Tables S7 and S8 list the chemical shifts for every signal in Fig. 6. Values of the coupling constants J_{AB} , chemical-shift differences Δ_{VAB} , and intrinsic isotope shifts Δ_0 are in Table 3. The slightly lower J_{AB} for **2-*h*-¹⁸O** may be an artifact of the broadening due to unresolved HF coupling.



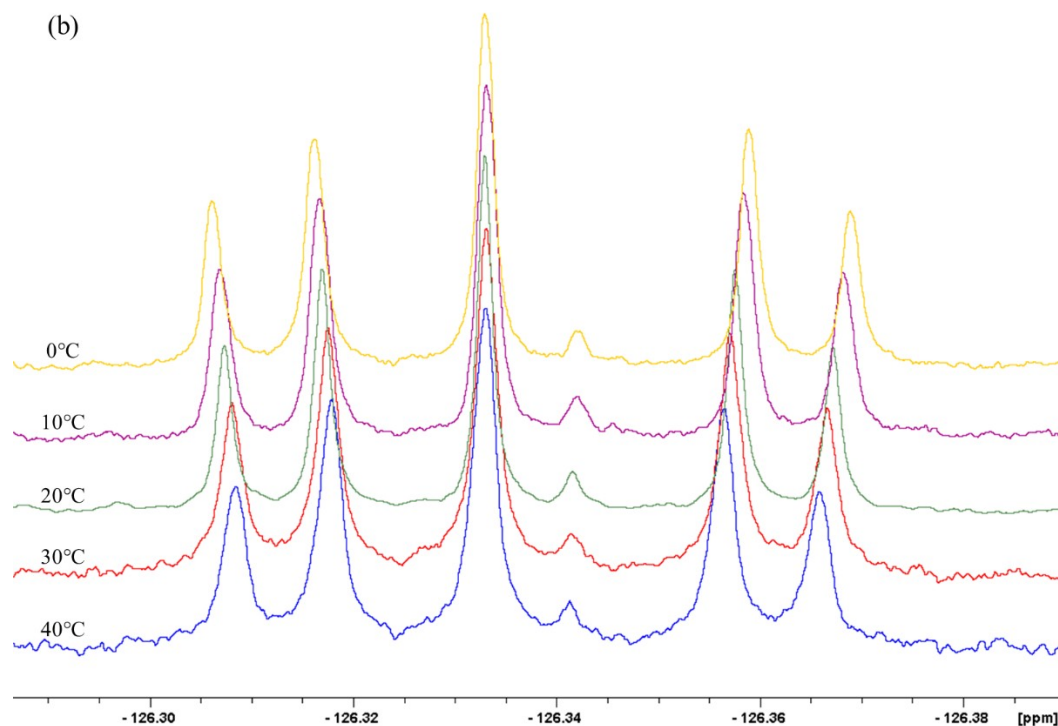


Figure 6. Variable-temperature ^{19}F NMR spectra in D_2O of (a) Bu_4N^+ protium difluoromaleate- $^{18}\text{O}_n$ ($\mathbf{2-h-}^{18}\text{O}_n$) and (b) Bu_4N^+ deuterium difluoromaleate- $^{18}\text{O}_n$ ($\mathbf{2-d-}^{18}\text{O}_n$).

Table 3. Parameters describing the AB patterns in Fig. 6 from $\mathbf{2-h-}^{18}\text{O}$ and $\mathbf{2-d-}^{18}\text{O}$.

Parameter	2	0°C	10°C	20°C	30°C	40°C
J_{AB} , Hz	<i>h</i>	2.75	2.68	2.62	2.57	2.54
	<i>d</i>	2.82	2.77	2.71	2.68	2.65
$\Delta\nu_{\text{AB}}$, ppb	<i>h</i>	51.4	49.9	48.7	47.5	46.3
	<i>d</i>	51.9	50.4	49.2	48.1	47.0
$-\Delta_0$, ppb	<i>h</i>	3.38	2.85	2.75	2.75	2.30
	<i>d</i>	4.55	4.40	4.30	4.20	4.20

All of these parameters decrease with increasing temperature for both **2-h**- ^{18}O and **2-d**- ^{18}O . The scalar coupling constants J_{AB} seem to decrease with increasing temperature, but very slightly, and they ought to be constant. Although the intrinsic isotope shifts Δ_0 appear to increase with decreasing temperature, this increase is due to poor spectral resolution, so that we can take the intrinsic isotope shifts as nearly constant. The decrease of Δ_{VAB} with increasing temperature is considerably larger and more significant. It can be attributed to the perturbation of an equilibrium between tautomers. Figure 7 shows a plot of the chemical-shift differences Δ_{VAB} for **2-h**- ^{18}O and **2-d**- ^{18}O versus $1000/T$, both with correlation coefficient 0.999. The slopes are 10.75 ± 0.19 and 10.3 ± 0.19 ppm-K, respectively, with intercepts 12 ± 0.6 and 14 ± 0.6 ppb. The slope for **2-d** is greater than that for **2-h**, but the difference is barely significant.

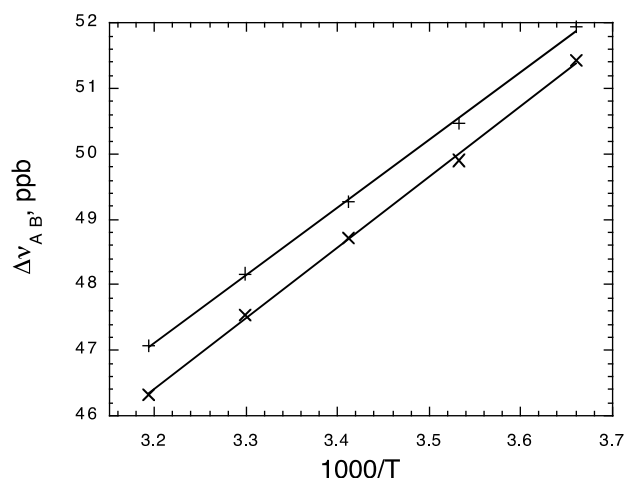


Figure 7. Linear fit of chemical-shift differences Δ_{VAB} for **2-h**- ^{18}O (x) and **2-d**- ^{18}O (+) versus $1000/T$.

To the extent that the temperature dependences in Table 3 are manifestations of an equilibrium, the slopes in Fig. 7 correspond to a $\Delta\Delta G^\ominus$ of

–1.41 cal/mol for **2-h**-¹⁸O and –1.47 cal/mol for **2-d**-¹⁸O, or to K_{16}/K_{18} at 20°C of 1.0024 or 1.0025 per ¹⁸O, respectively. These values are in qualitative agreement with the typical ¹⁸O IE of ~1.01 on acidity.^{26,32} The intercepts, 12 and 14 ppb, which ought to equal the intrinsic isotope shifts Δ_0 , are much higher than the values in Table 2. However, the errors in the intercepts are quite large, owing to an extrapolation over a large range.

It was previously demonstrated that not only in aqueous solution but also in the aprotic organic solvents CD₃CN and CD₂Cl₂ the ¹⁹F NMR spectrum of difluoromaleate-¹⁸O monoanion **2-h**-¹⁸O exhibits an AB spin system.^{29b} The ¹⁸O-induced chemical-shift difference between the two fluorines at 20°C are 0.046, 0.029, and 0.028 ppm in D₂O, CD₃CN and CD₂Cl₂, respectively. It was confirmed that these are not because of an intrinsic isotope shift but because of an equilibrium isotope shift, due to perturbation of an equilibrium by the isotopic label. It was further concluded that the H-bonded monoanion exists as a pair of tautomeric structures which are asymmetric due to the disorder of the instantaneous solvation environment. The results here now show that the ¹⁹F NMR spectrum of difluoromaleate-¹⁸O monoanion **2-d**-¹⁸O also exhibits an AB spin system, which is taken as evidence that this H-bonded monoanion exists as a pair of tautomeric structures that are asymmetric.

In summary, the chemical-shift separations for both **1** and **2** increase with decreasing temperature. This demonstrates that these separations are not intrinsic isotope shifts but must be due to the perturbation of an equilibrium.

Comparison of OHO and ODO H-bonds

According to the data in Table 1 the ipso chemical-shift separation for **1-d** at all temperatures is at least 9 ppb larger than for **1-h**. Moreover,

although the slope for **2-d** in fig. 7 may not be significantly greater than that for **2-h**, the chemical-shift differences $\Delta\sigma_{AB}$ in Table 3 are larger at all temperatures for **2-d**- ^{18}O than for **2-h**- ^{18}O . In both anions a larger difference is seen with the ODO H-bond than with the OHO.

Discussion

Temperature Dependence of Isotope Shifts

According to the data in Table 1, the chemical-shift differences for **1-d** increase with decreasing temperature, as had been observed for **1-h**.²⁵ This is further strong evidence for the perturbation of an equilibrium between tautomers that differ in whether the proton or deuteron is bonded to the ^{18}O -labeled carboxyl. Most diagnostic is the separation between **B**₀₁ and **B**₁₀ ipso ^{13}C NMR signals, for which the slopes in Fig. 4 correspond to $\Delta\Delta G^\circ$ of -5.1 and -7.9 cal/mol for **1-h** and **1-d** and corresponding equilibrium IETs K_{16}/K_{18} of 1.009 and 1.015.

For **2** the decrease of the ^{18}O -induced ^{19}F equilibrium isotope shifts $\Delta\nu_{AB}$ with increasing temperature, as shown in Table 3, is also significant. It too is strong evidence for the perturbation of an equilibrium between tautomers. Moreover, as can be seen in Fig. 7, the dependence is adequately linear in $1/T$, with slopes corresponding to EIEs K_{16}/K_{18} at 20°C of 1.0024 per ^{18}O for **2-h** or 1.0025 for **2-d**. The lower EIE, relative to **1**, may be attributed to the high acidity of difluoromaleic acid, which reduces the sensitivity of acidity to ^{18}O substitution.

This is the same tautomeric equilibrium that was described for many dicarboxylate monoanions.^{15-16,17b,18,25,29b} Although the H-bond is intrinsically symmetric, with a single-well potential, asymmetry arises from the disorder of solvation and the presence of solvatomers (isomers, or tautomers, that

differ in solvation).^{17b} Thus we conclude that the H-bonds in protium and deuterium tetrahydrophthalate-¹⁸O monoanions (**1-h**-¹⁸O and **1-d**-¹⁸O) and in protium and deuterium difluoromaleate-¹⁸O monoanions (**2-h**-¹⁸O and **2-d**-¹⁸O) are asymmetric. Moreover, because the Born-Oppenheimer Approximation guarantees that the potential-energy surface is independent of isotopic substitution,³³ this same conclusion holds for unlabeled **1** and **2**, without the asymmetry of one ¹⁶O and one ¹⁸O and regardless of whether H or D is in the H-bond.

Comparison of OHO and ODO H-bonds

The slope of Fig. 4 corresponds to an EIE per ¹⁸O for **1-d** of 1.015, significantly larger than the EIE of 1.009 for **1-h**. The increase can be attributed to the presence of D in the H-bond instead of H, as had been found for the (empirical and computed) ¹⁸O EIEs on the acidity of HCOOD (1.015,1.018) versus HCOOH (1.011,1.015).^{32a,c} Similarly, the EIEs per ¹⁸O for **2-d** is 1.0025, larger than the 1.0024 for **2-h**. This small difference is truly significant, not because it derives from the slopes in Fig. 7 but because it derives from the observation that $\Delta_{V_{AB}}$ for **2-d** in Table 3 is greater than for **2-h** at all temperatures, just as the ipso chemical-shift separations for **1-d** are greater than for **1-h**.

In both cases the larger ¹⁸O EIE for the ODO H-bond than for the OHO H-bond is consistent with perturbations of equilibria between tautomers. The equilibria become more unbalanced with OD, because the acidity of an OD acid shows a larger ¹⁸O IE than does an OH acid.^{26,31}

It should be noted that this result represents an isotope effect on an isotope effect ("IE/IE"). According to the Rule of the Geometric Mean,³⁴ multiple isotopic substitutions act independently, so that there should never be any IE/IE.³⁵ Yet they have often been observed. The most common

examples are of secondary deuterium IEs on the large primary kinetic IE of deuterium, as in hydride transfer reactions,³⁶ and of the decrease of the secondary tritium IE on E2 elimination of deuterium.³⁷ Because IEs other than primary IEs are generally small, an IE/IE is a second-order effect that can be difficult to detect. One remarkable example is the observation of the nonadditivity of secondary deuterium IEs on the basicity of trimethylamine,³⁸ which was made possible by a highly accurate NMR titration method. Here it is the sensitivity of ^{13}C and ^{19}F NMR that permits detection of different ^{18}O EIEs for ODO and OHO H-bonds.

Conclusions

The larger isotope shifts at lower temperature are consistent with the perturbation of an equilibrium that becomes more unbalanced at lower temperature. Likewise, the larger isotope shifts with deuterium within the H-bond are consistent with perturbation of an equilibrium because the ^{18}O IE on the acidity of an OD is greater than its effect on an OH. These are exactly the results to be expected if the observed isotope shifts are due to the perturbation of an equilibrium between tautomers. Moreover, these are results from two independent methods, both of which support the conclusion that each of these two dicarboxylate monoanions is a mixture of tautomers, not a single symmetric species.

It is not entirely clear what the experimental results would be if the observed isotope shifts are due to the anharmonicity of hydrogen motion in a symmetric H-bond, as proposed by Bogle and Singleton.²⁴ Our expectations were that anharmonicity would be more significant at higher temperature, where vibrational modes are excited, and with lower-mass protium in the H-bond. These expectations are exactly opposite to the observed results.

Although those are only our expectations, evidence for a greater anharmonicity with H than with D is presented in the Supporting Information.

Therefore we recommend that further computations be undertaken to assess the effects on isotope shift of both the temperature dependence of anharmonicity and the comparison of ODO and OHO H-bonds. If those computations support our expectations, then we can reject anharmonicity as primarily responsible for the observed isotope shifts. We do not deny that anharmonicity can contribute to the isotope shifts, but only that it is not the dominant contribution. If this is correct, then we must conclude that the observed isotope shifts are due to the perturbation of an equilibrium between tautomers, and that the H-bonds in tetrahydrophthalate monoanion (**1**) and difluoromaleate monoanion (**2**) are instantaneously asymmetric.

Acknowledgments

This research was supported by NSF Grant CHE11-48992 and by grants CHE234B and CHEFFPE from the UCSD Academic Senate and Department of Chemistry & Biochemistry. We sincerely thank Dr. Anthony Mrse and Dr. Yongxuan Su for facilitating the use of NMR and mass spectrometry.

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TOC Graphic

