Strong Hydrogen Bonds in Aqueous and Aqueous−Acetone Solutions of Dicarboxylic Acids: Activation Energies for Exchange and Deuterium Fractionation Factors

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Electrostatic effects and intramolecular hydrogen bonding have been postulated to account for the great differences between pK_1 and pK_2 of dibasic carboxylic acids with closely interacting acidic groups such as maleic acid.[1−4] Calculations indicate that the intramolecular hydrogen bond in hydrogen maleate remains strong in media at the dielectric constant of water.[5] Low-temperature ^{1}H NMR spectra of hydrogen maleate, hydrogen *cis*-cyclohexane 1,2-dicarboxylate, and hydrogen 2,2-dimethylmalonate revealed the presence of slowly exchanging downfield protons ($\delta = 19-20$ ppm) in 10% H_2O/acetone-d_6 at −55 °C.[6] These signals were cited as indicative of strong intramolecular hydrogen bonding. A signal at 19.25 ppm was also observed in a 10% H_2O/acetone-d_6 solution of hydrogen cyclopropane-1,1-dicarboxylate at 25 °C.[7] The downfield signals might represent intramolecular low-barrier hydrogen bonds (LBHBs). In this paper we report low deuterium fractionation factors for the downfield protons in hydrogen *cis*-cyclohexane-1,2-dicarboxylate and hydrogen maleate in aqueous and 10% H_2O/acetone solutions. The low values are compared with the conventional deuterium fractionation factor for *trans*-cyclohexane-1,2-dicarboxylate.[8] We further report high activation energies for the exchange of the downfield protons in hydrogen maleate and hydrogen *cis*-cyclohexane-1,2-dicarboxylate in 10% H_2O/acetone. Ten percent H_2O/acetone is regarded as substantially aqueous on a molar basis, with a mole fraction H_2O of 0.31. The low fractionation factors, high activation energies for exchange, and the downfield ^{1}H NMR chemical shifts support the previously postulated strong hydrogen bonding in aqueous solutions of the intramolecular hydrogen-bonded compounds. The mechanism of proton exchange in these compounds is shown to be acid-catalyzed and not base-catalyzed.

The deuterium fractionation factor of hydrogen *cis*-cyclohexane 1,2-dicarboxylate in 100% aqueous solution was obtained by monitoring the ^{13}C isotope shift on the β carbon of the ring and on the carbonyl carbon at 308 K in H_2O/D_2O mixtures as described by Jarrett and Saunders.[9] The results from several experiments averaged 0.69 ± 0.02. This is well below the value of 1.0 typically observed for conventionally hydrogen-bonded protons and approaches values typical of LBHBs. The fractionation factors for strongly hydrogen-bonded protons tend to decrease in solvents less polar than water.[9,11] The fractionation factor for hydrogen *cis*-cyclohexane-1,2-dicarboxylate, as determined by ^{1}H NMR in 10% H_2O/acetone-d_6 solution at 223 K, was found to be 0.52 ± 0.03, consistent with strong hydrogen bonding. The fractionation factor of hydrogen *cis*-cyclohexane 1, 2-dicarboxylate in 10% H_2O/acetone-d_6 was determined by integration of downfield proton signals in solutions of different H_2O/D_2O composition at 223 K.[11]

Hydrogen *trans*-cyclohexane 1, 2-dicarboxylate does not display a downfield NMR signal under the conditions in which the *cis*-isomer displays a downfield proton.[6] The carbonyl carbon and the α, β, and γ carbons of the ring all show measurable deuterium-induced ^{13}C isotope shifts. The fractionation factors determined at 308 K in 50/50 CD_3CN/H_2O solution averaged 0.90 ± 0.08. A single determination in 100% aqueous solution at 308 K gave a fractionation factor of 1.04. These values represent conventional hydrogen bonding.

Inasmuch as conventional hydrogen bonds display deuterium fractionation factors near unity and LBHBs display low fractionation factors,[12] the data obtained here are consistent with the previous suggestion that an intramolecular LBHB is formed by hydrogen *cis*-cyclohexane 1, 2-dicarboxylate in 10% aqueous/acetone-d_6,[6] and a conventional hydrogen bond is formed by hydrogen *trans*-cyclohexane 1, 2-dicarboxylate in aqueous solutions.

The exchange of conventional hydrogen-bonding protons with water is very fast because of the low activation energy (1−3 kcal mol^{-1}). However, the downfield protons of hydrogen maleate and hydrogen *cis*-cyclohexane-1,2-dicarboxylate are observed by ^{1}H NMR at low temperatures in 10% H_2O/acetone-d_6 at 220 K.[6] We have observed a broadened signal at 20.2 ppm for hydrogen maleate at room temperature in the same solvent, and a well-defined signal at 19.25 ppm has been reported for hydrogen cyclopropane-1,1-dicarboxylate under the same conditions.[7] Therefore, the downfield protons in these molecules exchange sufficiently slowly under these conditions to allow the activation energies for exchange to be measured by ^{1}H NMR line-width analysis. The exchange rate was determined for hydrogen maleate by measuring the line-width of the downfield signal as a function of temperature between 233 and 283 K in 10% H_2O/acetone-d_6. The activation energy was calculated from an Arrhenius plot of exchange rate versus temperature.[11] Figure 1 shows the temperature dependence of the exchange rate in one experiment, in which the activation energy was found to be 7.8 kcal mol^{-1}. The activation energies observed in three experiments with hydrogen maleate were 8.1, 7.8, and 7.1 kcal mol^{-1}, and values of 7.4 and 7.1 kcal mol^{-1} were obtained for hydrogen *cis*-cyclohexane 1, 2-dicarboxylate in two runs (see Table 1). The simplest rationale for these high activation energies is that the internal hydrogen bonds in hydrogen maleate and hydrogen *cis*-cyclohexane 1, 2-dicarboxylate are strong in 10% H_2O/acetone-d_6, and this is also consistent with the ^{1}H NMR chemical shifts and fractionation factors. It has also been reported that exchange of the internal hydrogen-bonding proton in sodium 4,5-dihydroxynaphthalene

(1) Westheimer, F. H.; Benfey, O. T. *J. Am. Chem. Soc.* **1956**, *78*, 5309−5311.
(2) Brown, H. C.; McDaniel, D. H.; Häfliger, O. *Determination of Organic Structures by Physical Methods*; Braude, E. A., Nachod, F. C., Eds.; Academic Press: NY, 1955; Vol. 1, Chapter 14, pp 567−662.
(3) McCoy, L. L. *J. Am. Chem. Soc.* **1967**, *89*, 1673−1677.
(4) Frey, P. A.; Cleland, W. W. *Bioorg. Chem.* **1998**, *26*, 175−192.
(5) McAllister, M. A. *Can. J. Chem.* **1997**, *75*, 1195−1202.
(6) Cassidy, J. L.; Lin, J.; Tobin, J. B.; Frey, P. A. *Bioorg. Chem.* **1998**, *26*, 213−219.
(7) Hess, R. A.; Reinhardt, L. A. *J. Am. Chem. Soc.* **1999**, *121*, 9867−9870.
(8) The separation between the carboxylate oxygens in the axial/equatorial isomer of hydrogen *cis*-cyclohexane-1,2-dicarboxylate is slightly less than in the diequatorial isomer of hydrogen *trans*-cyclohexanedicarboxylate.[5] Based on values of ΔpK_a for the ionizations of each compound in water, the $\Delta\Delta G°$ for the *trans*-isomer (pK_a's 3.65 and 5.13) is 2.1 kcal mol^{-1} at 25 °C, and for the *cis*-isomer (pK_a's 3.33 and 6.47) it is 4.4 kcal mol^{-1}. Thus, a substantial contribution to $\Delta\Delta G°$ of ionization is due to hydrogen bonding in the *cis*-isomer. The $\Delta\Delta G°$ for the *trans*-isomer is typical of the contribution due to electrostatic effects in dibasic organic acids.[5]
(9) Jarret, R. M.; Saunders: M. *J. Am. Chem. Soc*. **1985**, *107*, 2648−2654. In this method, introduction of deuterium in a molecule perturbs the ^{13}C NMR peaks of nuclei near the substituted atom. The intrinsic isotopic shifts in the ^{13}C resonance were measured, and the fractionation factor was calculated from the isotopic shifts. The fractionation factor of hydrogen *cis*-cyclohexane 1,2-dicarboxylate in aqueous solution, and the fractionation factor of hydrogen *trans*-cyclohexane 1,2-dicarboxylate in aqueous and in 50/50 CD_3CN/H_2O were obtained by this method at 308 K.
(10) Kreevoy, M. M.; Liang, T. M. *J. Am. Chem. Soc*. **1980**, *102*, 3315−3322.
(11) Markley, J. L.; Westler, W. M. *Biochemistry* **1996**, *35*, 11092−11097.
(12) Hibbert, F. and Emsley, J. *Adv. Phys. Org. Chem.* **1990**, *26*, 255−379.

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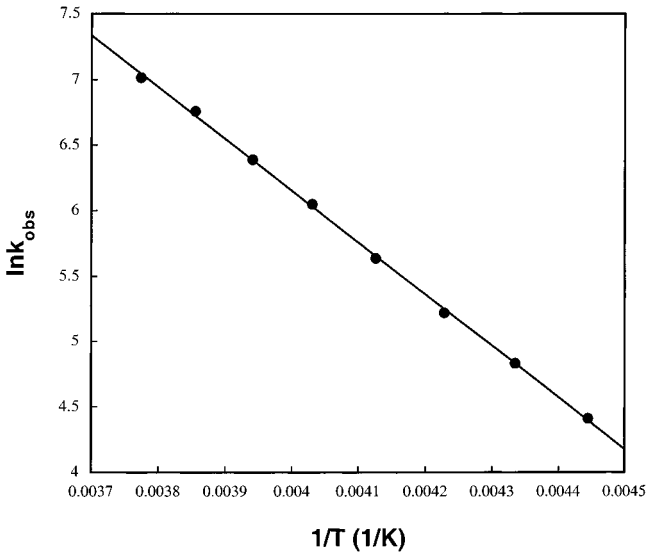


Figure 1. Activation energy for exchange of the downfield proton of hydrogen maleate with H_2O. The exchange rate of the downfield-field proton in hydrogen maleate in 10% H_2O/acetone-d_6 (0.31 mole-fraction H_2O) was measured by line-width analysis as a function of temperature between 222 and 270 K. Shown is the Arrhenius plot of the exchange rate as a function of temperature. The activation energy determined from this plot is 7.8 kcal mol^{-1}.

Table 1. Physicochemical Properties of Intramolecular Hydrogen Bonds in Aqueous and Aqueous−Acetone Solutions of Dicarboxylic Acids

	deuterium fractionation factor	activation energy (kcal mol^{-1})
hydrogen *cis*-cyclohexane 1,2 dicarboxylate	0.69 ± 0.02 [a] 0.52 ± 0.03 [b]	7.4, 7.1
hydrogen *trans*-cyclohexane 1, 2 dicarboxylate	0.90 ± 0.08 [c] 1.04 [1, d]	--
hydrogen maleate	0.77 ± 0.05 [e]	8.1, 7.8, 7.1

[a] In H_2O at 308 K. [b] In 90% acetone-d_6/10% H_2O at 223 K. [c] In 1/1 CD_3CN/H_2O at 308 K. [d] Single determination at 308 K. [e] Jarret and Saunders, 1985.

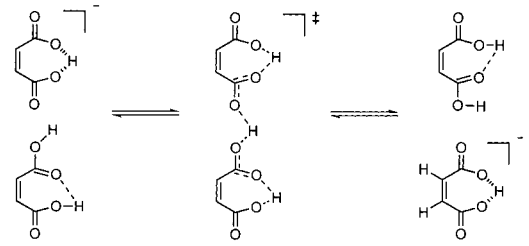
2,7-disulfonate proceeds with an activation energy of 9.9 kcal mol^{-1} in 9% DMSO-d_6 aqueous solution.[13]

Fractionation factors and activation energies for exchange measured in 10% H_2O/acetone-d_6 do not represent the behavior of molecules deprived of water in organic solvents. On a molar basis, 10% H_2O/acetone-d_6 is 0.31 mole-fraction water. Therefore, molecules such as hydrogen maleate and hydrogen *cis*-cyclohexane-1,2-dicarboxylate should be well solvated with water in this solvent. Despite this fact, the intramolecular hydrogen bond displays spectroscopic and deuterium fractionation properties characteristic of strong hydrogen bonding, as well as high activation energies for proton exchange.

Proton exchange between hydrogen maleate and maleic acid can only be observed in the presence of very low concentrations of free maleic acid (<1% of hydrogen maleate), as indicated by broadening of the downfield signal of hydrogen maleate. With the addition of acetic acid, the downfield signal persists but gradually broadens with increasing acetic acid. The broadened signal is observable at 1:1 hydrogen maleate:acetic acid. This slight broadening is due to the acid-catalyzed exchange of the downfield proton.

Disodium maleate has limited solubility in 10% aqueous acetone-d_6, so that little information is available on the effect of maleate dianion on the exchange of hydrogen maleate with water

Scheme 1



in 10% H_2O/acetone-d_6. However, 1% maleate dianion relative to hydrogen maleate does not broaden the downfield signal. In numerous experiments, no indication of base-catalyzed exchange could be obtained with disodium maleate.

The results on hydrogen maleate indicate that exchange of the downfield proton in 10% aqueous acetone-d_6 is catalyzed by the traces of maleic acid present at equilibrium according to the mechanism of Scheme 1, in which hydrogen maleate is depicted with an LBHB in a notation that is intended to denote strong hydrogen bonding but not necessarily symmetrical hydrogen bonding. In the mechanism, exchange of the internally hydrogen-bonded proton does not require cleavage of the LBHB prior to exchange; instead disruption of the LBHB in one molecule is partially compensated by the simultaneous formation of a new LBHB in the other molecule in the transition state. Therefore, the activation energy for exchange (7.7 ± 0.4 kcal mol^{-1}) does not represent the hydrogen-bonding energy of the LBHB and should be regarded as lower than the stabilization energy of the intramolecular hydrogen bond.

Low-barrier hydrogen bonds (LBHBs) are strong hydrogen bonds, in which the barrier in the double minimum potential is low and near the vibrational frequency for hydrogen.[12] The properties of LBHBs include very short contacts between donor and acceptor, very low field ^{1}H NMR chemical shifts, positive deuterium isotope effects on the chemical shifts, deuterium isotope effects on proton-stretching frequencies, low deuterium fractionation factors, and high activation energies for exchange with medium protons.[11,12,14,15] LBHBs have been postulated to play important roles in certain enzymatic reactions by stabilizing metastable intermediates and transition states.[16−19] Opposing arguments have been presented.[20−23] Moreover, the absence of evidence for strong hydrogen bonding in aqueous solutions has been cited in a review.[23] The present findings indicate that strong, intramolecular hydrogen bonding can exist in aqueous−acetone solutions (0.31 mole-fraction water) of hydrogen maleate and hydrogen *cis*-cyclohexane-1,2-dicarboxylate.

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(13) Zhao, Q.; Abeygunawardana, C.; Talalay, P.; Mildvan, A. S. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8220−8224.
(14) Jeffrey, G. A. *An Introduction to Hydrogen Bonding*; Oxford University Press: New York, 1997.
(15) Harris, T. K.; Abeygfunawardana, C,; Mildvan, A. S. *Biochemistry* **1997**, *36*, 14661−14665.
(16) Cleland, W. W. *Biochemistry* **1992**, *31*, 317−319.
(17) Gerlt, J. A.; Gassman, P. G. *Biochemistry* **1993**, *32*, 11943−11952.
(18) Cleland, W. W.; Kreevoy, M. M. *Science* **1994**, *264*, 1887−1890.
(19) Frey, P. A.; Whitt,. S. A.; Tobin, J. B. *Science* **1994**, *264*, 1927−1930.
(20) Guthrie, J. R. *Chem. Biol.* **1996**, *3*, 163−170.
(21) Warshel, A.; Papazyan, A.; Kollman, P. A. *Science* **1995**, *269*, 102−104.
(22) Shan, S.; Herschlag, D. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14474−14479.
(23) Perrin, C. L. *Annu. Rev. Phys. Org. Chem.* **1997**, *48*, 511−544.