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Low-Barrier Hydrogen Bonding in Aqueous and Aprotic Solutions of Dicarboxylic Acids: Spectroscopic Characterization

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We present additional spectroscopic evidence for the formation of low-barrier hydrogen bonds (LBHBs) within vicinal and geminal dicarboxylic acid monoanions. Hydrogen *cis*-cyclohexane 1,2-dicarboxylate, displays low-field ^{1}H NMR signals in aprotic solvents at 19.3 to 19.7 ppm, depending on the solvent. The LBHB in hydrogen 2,2-dimethylmalonate is further characterized by the observation of a positive value for the deuterium isotope effect on the chemical shift, $\Delta[\delta_H - \delta_D]$, of 0.8 ± 0.3 ppm. Low-field ^{1}H NMR signals are not observed for hydrogen *trans*-cyclohexane 1,2-dicarboxylate, hydrogen succinate, or hydrogen malonate under the same conditions. These compounds lack internal structural constraints forcing close contact between the caboxylic acids, although close contacts are possible, confirming that compression of carboxylic acid groups facilitates LBHB formation. Internally strained dicarboxylic acid monoanions also display low field ^{1}H NMR signals in aqueous solutions (90/10, acetone-d_6/H_2O) at low temperatures, at which proton exchange is slowed. The low field signals at −50°C are centered at 20.2 ppm for hydrogen maleate, 19.0 ppm for hydrogen 2,2-dimethylmalonate, and at 19.2 ppm for hydrogen *cis*-cyclohexane 1,2-dicarboxylate.

Low-barrier hydrogen bonds (LBHBs) have been postulated to stabilize transition states and intermediates in enzymatic catalysis (*1–4*). LBHBs have been observed in enzymes (*4–9*), and the conditions required for their formation, as well as their functional significance when formed, are currently controversial issues (*10–13*). It has been postulated in the case of chymotrypsin that the LBHB arises from compression between $N^{\delta 1}$ of His 57 and $O^{\delta 1}$ of Asp 102 in the active site brought about by a substrate-induced conformational change (*7*).

Physical chemists have classified hydrogen bonds as three types: weak, strong, and very strong (*14*). Very short hydrogen bonds exist under special conditions, and in the gas phase they may be stronger than weak covalent bonds (*14*, *15*); however, it is questionable whether they can be very strong in solution (*16*). Weak hydrogen bonds are the widely observed, conventional variety. LBHBs are of the intermediate, strong type, in which the barrier in the double minimum potential is low and near the vibrational frequency for hydrogen (*14*). LBHBs display very low field ^{1}H NMR chemical shifts, positive deuterium isotope effects ($\Delta[\delta_H - \delta_D]$) on the chemical shifts, deuterium isotope effects on proton stretching frequencies, and low fractionation factors in D_2O/H_2O mixtures (*14*). We here report a positive deuterium isotope effect on the low field proton NMR signal in hydrogen 2,2-

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dimethylmalonate and very low field ^{1}H NMR signals in hydrogen *cis*-cyclohexane 1,2-dicarboxylate dissolved in aprotic solvents. We further report the observation of downfield ^{1}H NMR signals characteristic of low barrier hydrogen bonding in aqueous acetone solutions of highly strained vicinal and geminal dicarboxylic acids at −50°C.

EXPERIMENTAL PROCEDURES

Materials. *cis*-Cyclohexane 1,2-dicarboxylic anhydride, 2,2-dimethylmalonic acid, maleic acid, and *trans*-cyclohexane 1,2-dicarboxylic acid were purchased from Aldrich and succinic acid from Fisher. All were recrystallized before use, and their purity was verified by ^{1}H NMR. Solutions of tetrabutylammonium hydroxide (1.0 M) in methanol (Aldrich or Sigma) in sealed vials were opened under nitrogen. Anhydrous $CHCl_3$ and $CDCl_3$ (Aldrich) in sealed vials were opened in a dry box and protected from moisture throughout the experiments. Acetonitrile-d_3 (CD_3CN) dimethylsulfoxide-d_6 (DMSO-d_6), and tetrahydrofuran-d_8 (THF-d_8) were purchased from Aldrich in boxes of sealed ampoules.

Preparation of tetrabutylammonium salts of dicarboxylic acids and analysis by ^{1}H NMR. *cis*-Cyclohexane 1,2-dicarboxylic acid was prepared by reaction of the anhydride with 1.0 M acetic acid in an ice bath overnight. The solvent was removed *in vacuo*, and the compound was recrystallized from water. ^{1}H NMR (500 MHz) of *cis*-cyclohexane 1,2-dicarboxylic acid in DMSO-d_6, ambient temperature, recycle time = 1 s: δ 12.03 (2H, s); 2.67 (2H, m); 1.86 (2H, m); 1.66 (2H, m); 1.37 (4H, m).

The tetrabutylammonium (NBu_4) salts of *cis*- and *trans*-cyclohexane 1,2-dicarboxylic acid, succinic acid, 2,2-dimethylmalonic acid, and succinic acid were prepared by dissolving the purified acids in methanol, adding one equivalent of tetrabutylammonium hydroxide from a 1.0 M stock solution, and stirring for 20 min. The solvent was removed by rotary evaporation *in vacuo*, and the salt was dried under vacuum. Preliminary ^{1}H NMR samples were prepared at 15 mM in anhydrous $CDCl_3$ or DMSO-d_6 and diluted to 1.5 or 0.5 mM. Samples of 15 mM hydrogen *cis*-cyclohexane 1,2-dicarboxylate were also prepared in acetonitrile-d_3 and THF-d_8. ^{1}H NMR (500 MHz) of 15 mM hydrogen *cis*-cyclohexane 1,2-dicarboxylate in $CDCl_3$, ambient temp., recycle time = 1s: δ 19.75 (1H, s); 3.29 (8H, t, NBu_4 CH_2's); 2.74 (2H, m); 2.00 (4H, m); 1.66 (8H, q, NBu_4 CH_2's); 1.44 (8H, m, NBu_4 CH_2's); 1.44 (2H, m); 1.00 (12H, t, NBu_4 CH_3's). ^{1}H NMR of 15 mM hydrogen 2,2-dimethylmalonate in $CDCl_3$: δ 19.6 (1H, s); 3.26 (8H, t, NBu_4 CH_2's); 1.16 (8H, m, NBu_4 CH_2's), 1.44 (8H, m, NBu_4 CH_2's and 6H (acid 2-CH_3's)); 1.04 (12H, t, NBu_4 CH_3's).

Preparation of O-deuterated 2,2-dimethylmalonate salt. The O-deuterated tetrabutylammonium hydrogen 2,2-dimethylmalonate was prepared by dissolving hydrogen 2,2-dimethylmalonic acid in D_2O, freezing the sample, and drying under vacuum. Exchange of deuterium into the sample was verified by the disappearance of the ^{1}H NMR signal for –COOH in the ^{1}H NMR spectrum. The ^{2}H NMR spectrum was obtained on the 500 MHz DMX Bruker NMR spectrometer through the BroadBand channel with the ^{2}H lock channel detuned. A 1% solution of $CDCl_3$ in $CHCl_3$ was used as an external reference. The low-field deuterium signal was not observable

TABLE 1
Values of Chemical Shift for the Low-Field Proton of Hydrogen *cis*-Cyclohexane 1,2-Dicarboxylate in Various Organic Solvents

Solvent	Concentration (mM)	δ_H (ppm)[a]
cis-Cyclohexane 1,2-dicarboxylate		
$CDCl_3$	15	19.75
$CDCl_3$	1.5	19.71
DMSO-d_6	15	19.77
DMSO-d_6	1.5	19.76
THF-d_8	15	19.35
CD_3CN	15	19.34

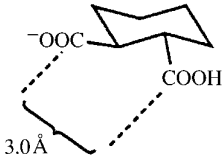
[a] Spectra were accumulated at ambient temperature with a delay time of 1 s.

at 15 mM, but at 0.1 M a broad resonance was observed at 18.83 ppm ($\nu_{1/2}$ = 1366 Hz) at 25°C. The line width of this signal was decreased to 784 Hz at 5°C and the chemical shift of the low-field deuteron was 19.2 ppm. The difference in the values of chemical shift at 25 and 5°C was used to estimate the error on δ_D.

RESULTS AND DISCUSSION

The deuterium isotope effect on the chemical shift of the low field proton in hydrogen 2,2-dimethylmalonate. Hydrogen 2,2-dimethylmalonate was previously studied in $CDCl_3$, DMSO-d_6, CD_3CN, and THF-d_8, and it displayed a low field resonance at δ_H 19.4 to 19.6 ppm in these solvents (*4*). We have confirmed the value of δ_H 19.6 ppm for this compound at higher concentrations (100 and 15 mM). We have now observed a broad signal for the low field deuteron in the deuterium NMR spectrum of hydrogen O-deutero-2,2-dimethylmalonate (100 mM). The relaxation rate T_1 is a function of the electric quadrupole moment and the molecular symmetry. Deuterium is a quadrupolar nucleus with a spin of one, and the short relaxation time may result in significant line broadening relative to the corresponding proton signal. The width of the signal may be accentuated by chemical exchange of the deuteron with adventitious D_2O in the samples. The width of the low-field ^2H NMR signal at 25°C (δ_D 18.8 ppm; $\nu_{1/2}$ = 1366 Hz) made it difficult to obtain an accurate value for $\Delta[\delta_H - \delta_D]$. The line width at 5°C was narrower (δ_D = 19.2, $\nu_{1/2}$ = 784 Hz), allowing a calculation of $\Delta[\delta_H - \delta_D]$ with a reasonable estimate on the error for the δ_D measurement. The deuterium isotope effect, $\Delta[\delta_H - \delta_D]$, was estimated to be $+0.8 \pm 0.3$ ppm. The positive isotope effect is in the range for LBHBs (*14*) and supports the assignment of the bridging proton in hydrogen 2,2-dimethylmalonate as an LBHB.

Observation of low field protons in hydrogen cis-cyclohexane 1,2-dicarboxylate. A low field proton NMR signal was observed for tetrabutylammonium hydrogen *cis*-cyclohexane 1,2-dicarboxylate in aprotic solvents (Table 1). The signal in $CDCl_3$



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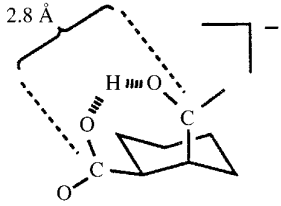

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and DMSO was nearly concentration-independent in the mM range, and the signal was also present in THF-δ_8 and CD_3CN.

Low field proton NMR signals were not observed in $CDCl_3$ solutions of tetrabutylammonium hydrogen *trans*-cyclohexane 1,2-dicarboxylate or of tetrabutylammonium hydrogen succinate. The signals for COOH appeared at 12–13 ppm. Similar, negative results were reported for hydrogen malonate (*4*). The carboxylic acid and carboxylate groups of these dicarboxylic acids can, in principle, interact with each other, although they are not forced into contact by internal structural constraints. Inasmuch as intramolecular LBHBs are not found, the potential strength of an intramolecular LBHB must not be sufficient to draw these molecules into internally hydrogen bonded conformations in competition with intermolecular, hydrogen bonded complexes. In the cases of hydrogen 2,2-dimethylmalonate and hydrogen *cis*-cyclohexane 1,2-dicarboxylate, internal structural factors tend to force the carboxylate and carboxylic acid groups into close contact, and these constraints appear to be necessary to induce the formation of the LBHB. The role of compression in the induction of LBHBs has been discussed elsewhere (*7*, *17*).

The hydrogen cyclohexane 1,2-dicarboxylates offer an interesting case of transition between LBHB formation in the *cis*-isomer and conventional hydrogen bonding in the *trans*-isomer. In the *trans*-isomer **1** the preferred geometry is diequatorial (*18–20*), in which the carboxylate and carboxylic acid groups can potentially interact, with the carboxyl-carbons separated by only 3.0 Å. However, an LBHB is not observed. Molecular models of hydrogen *cis*-cyclohexane 1,2-dicarboxylate **2** show that the carbon atoms of the carboxylate and carboxylic acid groups are closer (2.8 Å) than in the *trans*-isomer, and the NMR spectrum indicates the presence of an LBHB. It appears that the small difference in carboxyl-separation for the *cis*- and *trans*-isomers may be a factor in LBHB formation. Owing to free rotation about the bonds to the cyclohexane backbone, the carboxylic and carboxylate groups in the *trans*-isomer should be able to assume an appropriate contact geometry for strong hydrogen bonding, but a potential LBHB is not strong enough to bring the two groups close enough together for it to exist. In the case of the *cis*-isomer, the cyclohexane backbone appears to force the two groups sufficiently close together for LBHB formation.

The significance of internal compression in the formation of LBHBs is further highlighted by the observation of an LBHB in hydrogen 2,2-dimethylmalonate but not in hydrogen malonate (*4*). Similar enforced interactions of surface carboxylic

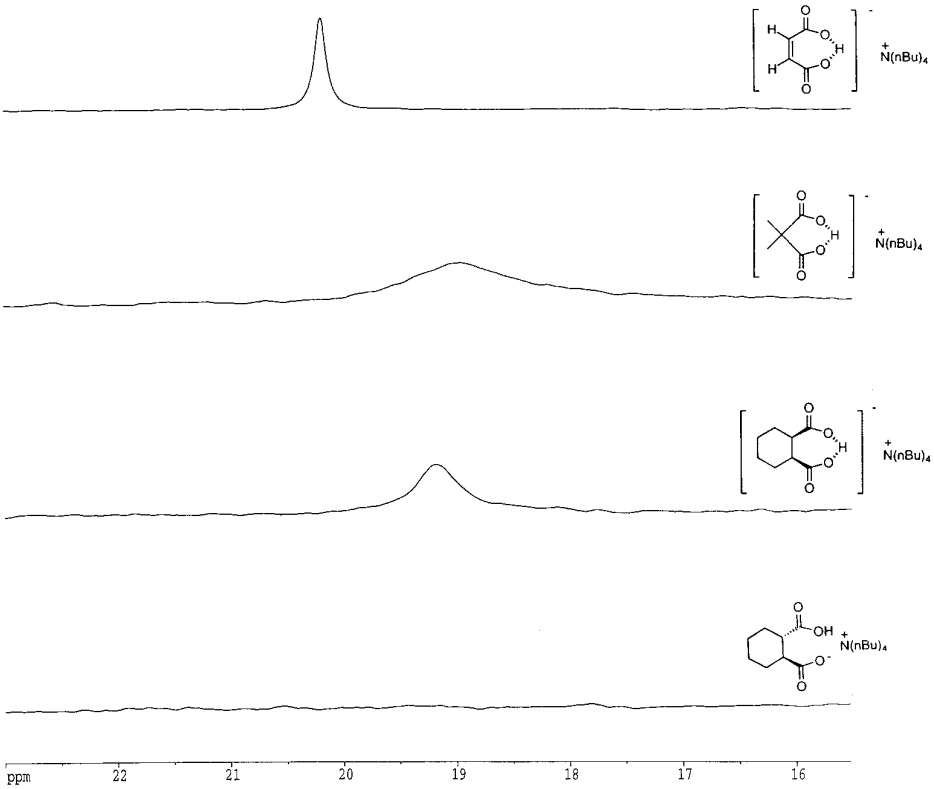

FIG. 1. Low field, low temperature ^{1}H NMR spectra of dicarboxylic acid monoanions in aqueous acetone. The tetrabutylammonium salts of hydrogen maleate, hydrogen 2,2-dimethylmalonate, hydrogen *cis*-cyclohexane 1,2-dicarboxylate, and hydrogen *trans*-cyclohexane 1,2-dicarboxylate were dissolved at 0.1 to 0.2 M concentrations in 90% acetone-d_6/H_2O, with 2,2-dimethyl-2-silapentane-5-sulfonate as the internal standard. The low field region of the ^{1}H NMR spectra showed no signals at room temperature. The low field spectra obtained at −55°C are shown in the figure. The low field signals are centered at 20.2 ppm for hydrogen maleate, 19.0 ppm for hydrogen 2,2-dimethylmalonate, and at 19.2 ppm for hydrogen *cis*-cyclohexane 1,2-dicarboxylate. Hydrogen *trans*-cyclohexane 1,2-dicarboxylate does not show a low field signal.

acid groups in proteins have recently been analyzed (*21*), and substrate-induced compression between His 57 and Asp 102 is thought to be essential in the formation and function of the LBHB in chymotrypsin (*7*).

Low field protons in aqueous solutions of strained dicarboxylic acid monoanions. Low field protons assigned as LBHBs in NMR spectra are commonly observed for the monoanions of strained vicinal and geminal dicarboxylic acids dissolved in aprotic solvents (*4*, *13*, *14*). However, they are not observed in aqueous solutions of the same species. We have considered whether they might exist in aqueous solutions but are not easily observable in NMR spectra owing to fast exchange with water protons. In such cases, low field protons might be observed if their exchange

rates could be lowered enough. This approach has succeeded in the observation of a downfield proton in the case of 4,5-dihydroxynaphthalene-2,7-disulfonate in 10/90 water/acetone-d_6 at low temperature (*22*). To examine this issue, we prepared the tetrabutylammonium salts of dicarboxylic acid monoanions in aqueous acetone (10/90 water/acetone-d_6) and recorded the low field proton NMR spectra at temperatures ranging from ambient to −50°C. The low field regions at −50°C are shown in Fig. 1 for hydrogen maleate, hydrogen 1,2-dimethylmalonate, hydrogen *cis*-cyclohexane 1,2-dicarboxylate, and hydrogen *trans*-cyclohexane 1,2-dicarboxylate. Low field ^{1}H NMR signals were observed in all cases except *trans*-cyclohexane 1,2-dicarboxylate. Therefore, LBHBs appear to form in strained dicarboxylic acids in aqueous solutions. The signals in aqueous acetone are broadened relative to those in aprotic solutions, which may be attributed to chemical exchange with water. The field positions of the low field protons in aqueous acetone are 0.2 to 0.4 ppm upfield from those found in aprotic solvents, which may represent the effects of variant solvation in aqueous and aprotic solvents. However, the signals in Fig. 1 are clearly low field and well within the range observed in aprotic solvents for low barrier hydrogen bonding (*14*).

The possibility of strong intramolecular hydrogen bonding in aqueous solutions has been discussed elsewhere (*17*). Strong intramolecular hydrogen bonding in water was postulated on the basis of an analysis of the large differences between values of pK_1 and pK_2 for internally strained, geminal and vicinal dicarboxylic acids. The downfield ^{1}H NMR signals of hydrogen maleate, hydrogen *cis*-cyclohexane-1,2-dicarboxylate, and hydrogen 2,2-dimethylmalonate in Fig. 1 confirm these expectations.

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