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Conformational Changes of Butanedioic Acid as a Function of pH as Determined from Changes in Vicinal Proton–Proton NMR Couplings[1]

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Abstract: Vicinal proton–proton NMR couplings have been used to estimate the changes in conformational equilibria for butanedioic acid in the progression of its diprotic to monoprotic to di-ionized forms as a function of pH, concentration, and nature of the counterion. The results support earlier conclusions that the K_1/K_2 ratio for butanedioic acid is little, if at all, affected by internal hydrogen bonding in the monoanion. Perhaps surprisingly, the conformational preferences are weak, despite the possibility of steric hindrance and strong stabilizations by hydrogen-bonding, induction, and/or electrostatic effects.

Over the years, very substantial effort has been expended on the determination of conformational equilibria in open-chain organic systems from vicinal proton–proton coupling constants and the Karplus equation, or some variant of it, that relates the couplings to rotational angles.[2] Butanedioic acid would appear to offer an almost ideal opportunity to test and exploit this procedure, because of its simple conformational system and because the changes in charge associated with its ionization, as the pH is increased, might be expected to allow for straightforward prediction of conformational changes that should be reflected in changes of coupling constants. Nonetheless, a more detailed analysis suggests a number of possible complications. How important is intramolecular hydrogen bonding or the nature of the counterion in the various carboxylate species? Will the molecules and ions actually assume conformations with essentially perfectly staggered relationships between the vicinal protons? How important is ionic solvation in increasing the steric bulk of the ionized carboxyls? How much intrinsic change in vicinal couplings should be expected from electronic influences associated with ionization, even if there were no changes in conformational equilibrium? These are by no means simple questions, but they are clearly germane to the general problem of determining conformational preferences by use of vicinal proton–proton couplings.

It can be expected that knowledge of the conformational equilibria for butanedioic acid as a function of its degree of ionization could also shed light on the classic problem posed by the ratio of the first to the second dissociation constant ($K_1/K_2 = 25$) for butanedioic acid. On a statistical basis, diprotic organic acids should have $K_1/K_2 = 4$. Empirically, for short-chain diacids, K_1/K_2 is always greater than 4 and only approaches the statistical ratio for long-chain dicarboxylic acids when the acid groups are far apart. Arguing from this fact, Bjerrum[3] suggested in 1923 that the ratios K_1/K_2 are determined by a combination of the statistical factor and the electrostatic effect of the negative charge of the monoanion inhibiting the ionization of the second carboxyl group. However, his calculations of the effect of the electrostatic charge on K_1/K_2, assuming that the acidic species are in a homogeneous medium of dielectric constant 80, gave much too small values. Kirkwood and Westheimer[4] showed how the discrepancy could be greatly diminished by treating the acid ionizations as occurring in a cavity of low dielectric constant in high dielectric water. Their treatment was bolstered by the finding that substitution with alkyl groups of the chain between the carboxylic groups greatly increased K_1/K_2 as expected for expanding the size of the low dielectric constant cavity.[5] Nonetheless, the treatment has been criticized because the relatively low value of the second ionization in short-chain dicarboxylic acids might at least partly be accounted for by important hydrogen bonding in the monoanion, which would be expected to increase K_1 and decrease K_2.[6] Furthermore, the effect of alkyl substitution was suggested to be accounted for by the well-known influence of alkyl groups in favoring ring formation.[6] However, these latter arguments were countered by further experiments by Westheimer and Benfey.[7]

In 1963, on the basis of thermodynamic studies of a large number of dicarboxylic acids, including butanedioic acid, Eberson and co-workers[8] argued that, while hydrogen bonding might be important in acids with $K_1/K_2 > 10^4$, its effect is likely to be small when K_1/K_2 is much less than 10^4. Clearly, any further question of the importance of hydrogen bonding might be resolved if the conformational equilibria of butanedioic acid and its ionized forms were known.

It is not a new idea to determine the conformations of butanedioic acid and its ionized forms by measuring the vicinal proton–proton NMR couplings as a function of pH. Zetta and Gatti[9] made the first measurements for the acid (H_2A) and the dianion (A^{2-}) with the aid of the ^{13}C satellite spectra in dioxane for H_2A and in 1:1 dioxane–water for $K^+{}_2\,A^{2-}$. They concluded from the couplings that the *trans* conformations dominated the

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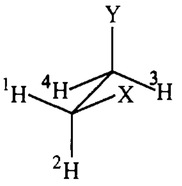
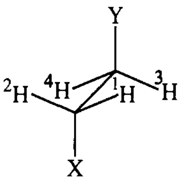
conformational equilibria, despite Raman spectral evidence[10] in favor of the *gauche* forms.

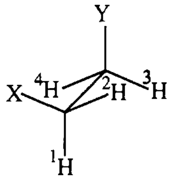
Gil *et al.*[11] have also reported measurements of the proportions of the rotamers of butanedioic acid as a function of pH, based on the measurement of vicinal H–H coupling constants made with ^{13}C satellite spectra. Gil did not, however, take into account expected differences in coupling constants for the diprotic, monoprotic, and di-ionized species but rather used the coupling constants of ethane given by Abraham and Gatti[12] together with orientational-dependent COOH effects as approximate values for the coupling constants of the three rotamers **1**–**3**. Furthermore, Gil and co-workers did not attempt to separate out the couplings for the monoprotic anion, and the measured couplings had substantial error bars in the crucial pH region where the monoprotic species dominates. Nonetheless, the approach appears to offer the possibility of determining whether hydrogen bonding does, in fact, play an important role in making the K_1/K_2 ratio of butanedioic acid be about 25, or whether this ratio, being considerably less than Eberson's cutoff of 10^4, might arise primarily from electrostatic interactions.[13]



1(a-c)



2(a-c)



3(a-c)

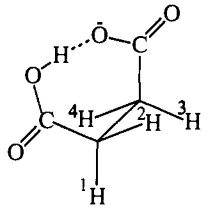
a; X = Y = CO_2H

b; X = CO_2^-, Y = CO_2H

c; X = CO_2^-, Y = CO_2^-

We initially believed that it would be easier to accurately measure the coupling constants of butanedioic acid as a function of pH through a study of the ^{13}C satellites on the proton spectra of *meso*- and *d,l*-2,3-dideuteriobutanedioic acid. However, the broadening of the proton lines by the deuterium attached to the same carbons made this procedure unsuitable. Nevertheless, the results obtained were, within experimental error, consistent with what we obtained later. We subsequently proceeded much along the same lines as Gil with the intent of determining the proportions of the rotamers of butanedioic acid as a function of pH. Where we have differed has been in our approach to the data analysis and the fact that we have been able to use butanedioic-2-^{13}C acid and butanedioic-2,3-$^{13}C_2$ acid for the NMR studies, which greatly simplifies the sensitivity problem associated with measuring the spacing of the ^{13}C satellites on the proton spectra.

If the classical electrostatic effect is indeed the dominant influence in increasing the second ionization constant of butanedioic acid, then one might expect that there would be no great difference in the conformational populations between the diacid and the monoanion. If, however, the monoanion is significantly stabilized by intramolecular hydrogen bonding (**4**), then the *gauche* rotamers should become more favorable. Inspection of models suggests that the most favorable geometry for a hydrogen-bonded monoanion should have the rotational angle θ about the C2–C3 bond rather larger than the perfect staggered value of 60°. Because of the symmetry of butanedioic acid and the ease of rotation about the C2–C3 bond, a *gauche* conformation with θ = 60° will be in rapid equilibrium with an equivalent conformation having θ = –60°. Obviously, any deviations from the perfect staggered rotational angles are hardly expected to be the same for the diprotic, monoprotic, and di-ionized species, which we will henceforth abbreviate as as H_2A (**1a**–**3a**), HA^- (**1b**–**3b**), and A^{2-} (**1c**–**3c**).



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Experimental Part

A 0.2 M solution of 90%-enriched butanedioic-2-^{13}C acid (ICN Biomedicals, Inc.) was made by dissolving the acid in 99.8% D_2O (Aldrich Chemical Company). A few milligrams of EDTA was added to reduce some line broadening that seemed associated with paramagnetic heavy-metal ion contamination of the original sample of labeled butanedioic acid or D_2O. To maintain a nearly constant concentration of butanedioic-2-^{13}C acid while the pH was changed, part of the labeled butanedioic acid was dissolved in strong sodium hydroxide, and this solution was added in small increments to an equivalent concentration of labeled butanedioic acid in D_2O. This was done 22 times to give 23 samples, and after each addition an NMR spectrum was taken with a Bruker Magnetics AM-500 spectrometer operating at 500.138 MHz. Several typical spectra are shown in Figure 1. The pH (actually pD because the solvent was D_2O) of the solution was determined after each addition with a standard pH meter. Coupling constants were obtained from the observed line positions with the aid of the LAOCN3 program by Bothner-By,[14] modified for use with desktop computers.

In further experiments, 0.02 M solutions were used of 99%-enriched butanedioic-2,3-$^{13}C_2$ acid (Isotec) in D_2O, and the pH was adjusted with NaOH in a similar manner as above. Also, 0.2 M solutions of 99%-enriched butanedioic-2,3-$^{13}C_2$ acid with 0.2 M LiOH (EM Science) and with tetramethylammonium hydroxide (Aldrich Chemical Company) were made in D_2O, and the pH was adjusted by the addition of small amounts of HCl solution. For the solutions with 99%-enriched butanedioic-2,3-$^{13}C_2$ acid, spectra were taken only at high, low, and midrange (about 4.7) pH.

One uncertainty in using NMR for the analysis of the conformational equilibria of butanedioic acid as a function of pH is associated with possible changes in the $^3J_{HH}$ coupling constants with pH as the result of ionization alone. This problem is addressed here with the aid of extensive studies made of the relationship between rotational angles, vicinal proton–proton couplings, and the Huggins[15] electronegativity scale by Haasnoot and co-workers.[16] To use this treatment for butanedioic acid ionization, one needs a reasonable estimate of how the electronegativity of a carboxyl group changes with ionization. To do this, we compared the vicinal proton–proton couplings of propanoic acid at pH 2 and 14, which were 7.47 and 7.77 Hz, respectively. If we now assume the perfect staggered rotational angle of 60° between the protons for both propanoic acid and propanoate anion, then the Haasnoot procedure[16] suggests that there is 0.45 difference between the Huggins[15] electronegativity of COOH and COO^-.

Results and Discussion

The experimental variations of the proton–proton coupling constants obtained for butanedioic acid as a function of pH are shown in Figure 2 and will henceforth be referred to as the *observed* couplings. In analyzing the relationship between the couplings and conformational equilibria, we made what we regard to be a reasonable assumption, namely, that the conformational equilibrium for each rotamer and the respective rotational angles θ will be constant for any given acid species, H_2A, HA^-, and A^{2-},

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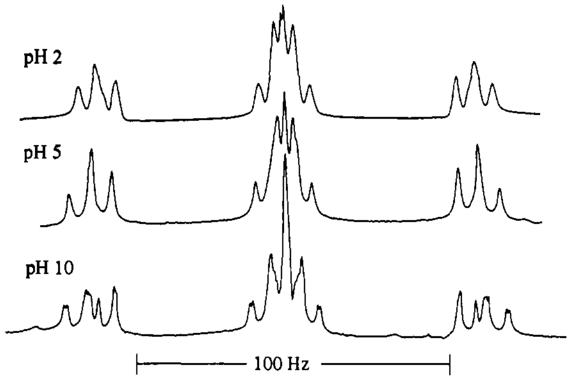

Figure 1. Proton NMR spectra of 0.2 M butanedioic-2-^{13}C acid at different pH values.

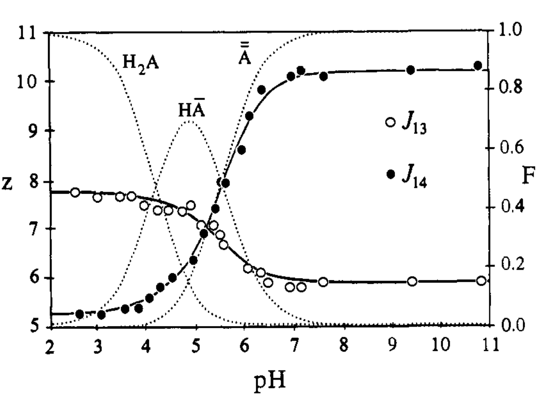

Figure 2. Plots showing the least-squares fits of J_{13} and J_{14} vs pH that were used to determine the coupling constants of the monoprotic species. The dashed lines represent the respective mole fractions F of the H_2A, HA^-, and A^{2-} species calculated from the known ionization constants as a function of pH.

over the pH range at nearly constant concentration and that it is only the *relative* proportions of these species that change with pH.

The fractional concentrations of the various butanedioic acid species are given by

$$[H_2A] = [H^+]^2/(K_1K_2 + K_1[H^+] + [H^+]^2) \quad (1)$$

$$[HA^-] = [H_2A]K_1/[H^+] \quad (2)$$

$$[A^{2-}] = K_2[HA^-]/[H^+] \quad (3)$$

$$[H_2A] + [HA^-] + [A^{2-}] = 1 \quad (4)$$

where, in our case, $[H_2A]$, $[HA^-]$, and $[H^+]$ are actually $[D_2A]$, $[DA^-]$, and $[D^+]$, because the measurements were made in D_2O. The coupling constants of the diprotic and di-ionized species were taken to be those observed at extreme pH values, where the monoprotic species is at extremely small concentration.

Thus, J_{13} (averaged over the rotamer populations) for the diprotic and di-ionized species was found to be 7.8 and 5.9 Hz, respectively, and 5.3 and 10.2 Hz (similarly averaged) were obtained for J_{14}.[17] The coupling constants for the monoprotic species were then calculated by a least-squares fit of all the coupling constants as well as the first and second ionization constants as a function of pH.[18]

The literature pK_1 and pK_2 values of 4.1 and 5.5, respectively, matched well to the calculated values of 4.15 and 5.48 for the best fit of the curve of observed J_{14} with pH. From this treatment, J_{14} of the monoanion came out to be 6.1 Hz, with a correlation coefficient of 0.9933. Also, if the J_{14} for each of the 10 points in which the concentration of the monoanion is greater than 30% (the maximum value is 70%) is calculated individually using the literature pK_1 and pK_2 values, then the average J_{14} also comes out to be 6.1 Hz, with a standard deviation of 0.2 Hz. The variation of J_{13} with pH is smaller than that of J_{14}, so the errors are greater, but using the ionization constants derived from the J_{14} fit, J_{13} of the monoanion could be calculated to be 7.6 Hz, with a correlation coefficient of 0.9703. Point-by-point calculation of J_{13} over the >30% monoanion range gives the average J_{13} of 7.6 Hz, with a standard deviation of 0.2 Hz. The best-fit lines to the experimental data are shown in Figure 2.

Table I. Couplings Calculated for Butanedioic Acid According to Procedures of Haasnoot and Co-Workers[16]

	calculated couplings (Hz)			
	J_{13}		J_{14}	
species	*trans* **2**	*gauche* **1** ↔ **3**	*trans* **2**	*gauche* **1** ↔ **3**
diprotic	3.05	9.05	14.99	3.08
monoprotic	2.89	9.58[a] 10.70[b]	15.80	3.13[a] 1.03[b]
di-ionized	2.73	10.11	16.62	3.17

[a] *Gauche* carboxyl angle = 60°. [b] *Gauche* carboxyl angle = 71°. This value is an average of the different *gauche* values for this rotational angle.

The observed coupling constants were then assumed to be given by

$$J_{\text{obsd}} = J_{\text{d,t}}T_{\text{d}}[H_2A] + J_{\text{m,t}}T_{\text{m}}[HA^-] + J_{\text{i,t}}T_{\text{i}}[A^{2-}] + J_{\text{d,g}}G_{\text{d}}[H_2A] + J_{\text{m,g}}G_{\text{m}}[HA^-] + J_{\text{i,g}}G_{\text{i}}[A^{2-}] \quad (5)$$

where J_{obsd} = the observed J_{13} or J_{14}; $J_{\text{d,t}}$ the calculated J_{13} or J_{14} for *trans* H_2A; $J_{\text{m,t}}$ the calculated J_{13} and J_{14} for *trans* HA^-; $J_{\text{i,t}}$ = the calculated J_{13} or J_{14} for *trans* A^{2-}, and similarly for the *gauche* (g) rotamers. T_{d} is the fraction of the *trans* rotamer for H_2A, and G_{d} is the fraction of the two *gauche* rotamers for H_2A, where, obviously, $G_{\text{d}} + T_{\text{d}} = 1$. T_{m}, G_{m}, T_{i}, and G_{i} are similarly defined for the monoprotic and di-ionized species. $J_{\text{d,t}}$, $J_{\text{m,t}}$, $J_{\text{i,t}}$, $J_{\text{d,g}}$, $J_{\text{m,g}}$, and $J_{\text{i,g}}$ were all calculated using the method outlined by Haasnoot (for the values, see Table I).[16] The Huggins electronegativity of COOH was calculated to be −0.146 using the electronegativities of the C, O, and H as given by Huggins.[15] The previously calculated value of 0.45 for the difference in Huggins electronegativity between protonated and ionized carboxyl groups using propanoic acid gives an electronegativity of −0.596 for COO^-. Both 60° and 71° were used for θ of the monoprotic species. The latter angle was derived from a computer modeling program,[19a] in which a hydrogen bond between the carboxyls was specified with a distance of 2.62 Å between the two oxygens and an angle of 180° at the hydrogen.[19b]

Knowing the coupling constants for each species, eq 5 can be simplified to calculate the percentage of *gauche* and *trans* rotamers for each species. For the example of J_{13} for H_2A, eq 5 becomes

$$J_{13}\,(H_2A) = J_{\text{d,t}}T_{\text{d}} + J_{\text{d,g}}G_{\text{d}} \quad (6)$$

Similar equations give the proportions of *gauche* rotamers shown in Table II. Perhaps surprisingly, there actually appears to be slightly less preference for the *gauche* form of HA^- species than for the *gauche* form of H_2A. Because the determination of the couplings and conformational preference of an "invisible" species such as HA^- might be regarded as tenuous from the data in Figure 2, we show in Figure 3 how the calculated curves for the

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(17) The value for H_2A, but not A^{2-}, is in good agreement with that reported earlier: Abraham, R. J.; Hudson, B. D. *J. Chem. Soc., Perkin Trans. II* **1986**, 1635–1640. Professor Abraham was unable to resolve the discrepancy for A^{2-} but agreed that our value for A^{2-}, which is close to that of Gil and coworkers,[11] is correct.

(18) Roberts, J. D.; Yu, C.; Flanagan, C.; Birdseye, T. R. *J. Am. Chem. Soc.* **1982**, *104*, 3945–3949.

(19) (a) *BioGraf, MacroModel Batchmin Version 2.6*; Columbia University, New York, 1989. (b) Pimentel, G. C.; McClellan, A. L. *The Hydrogen Bond*; W. H. Freeman and Company: New York, 1960; p 268.

Table II. Calculated Conformational Preferences

	percent *gauche*		
labeled acid, concn, counterion	H_2A	HA^- (calcd from J_{13})	$A^=$ (calcd from J_{14})
butanedioic-2-C^{13} acid, 0.2 M, Na^+	79.2/81.3	70.4/76.5[a] 60.3/65.7[b]	42.9/47
butanedioic-2,3-$^{13}C_2$ acid, 0.02 M, Na^+	80.3/82.0	71.3/78.4[a] 61.1/66.2[b]	44.2/48.0
butanedioic-2,3-$^{13}C_2$ acid, 0.2 M, Li^+	79.7/81.8	62.9/71.2[a] 53.9/61.1[b]	42.3/45.4
butanedioic-2,3-$^{13}C_2$ acid, 0.2 M, tetramethylammonium ion	79.8/82.1	71.5/77.2[a] 61.2/66.3[b]	44.3/46.7

[a] *Gauche* carboxyl angle = 60°. [b] *Gauche* carboxyl angle = 71°.

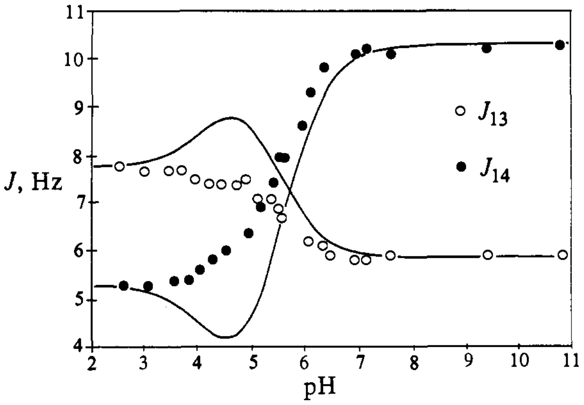

Figure 3. Comparison of the observed couplings with the calculated changes in the coupling constants with pH, if J_{13} and J_{14} are taken to have the values predicted for the *gauche* rotamers of HA^-.

couplings would change with pH if the monoanion had the couplings predicted for the *gauche* conformations.

Our findings support the hypothesis that the K_1/K_2 ratio of butanedioic acid arises primarily from electrostatic effects and not from strong intramolecular hydrogen bonding. It seems likely that one important reason for this is that the hydrogen-bonded form of HA^- would have to have not only the less stereoelectronically favorable *anti* bond configuration for the OH of the un-ionized carboxyl group but also the *anti* orientation at the carboxylate oxygen forming the hydrogen bond.[20]

Again perhaps surprisingly, there is only a relatively small change in conformational preference for butanedioic acid as the result of ionization of H_2A to A^{2-}, but this is in the direction expected for electrostatic repulsions between the carboxylate groups of A^{2-}, which should favor the *trans* conformation. The preferences observed here for H_2A and A^{2-} could be taken to reflect the conclusions of Radom and co-workers[21] that bis-1,2-substituted ethanes with either strongly electron-attracting or electron-donating groups will have unfavorable back-lobe orbital interactions, tending to make the *trans* rotamers less favorable.

However, the situation is not likely to be that simple, because we have found that the ^{13}C satellites on the proton spectrum of butanedinitrile (**5**) in trichloromethane solution indicate the J_{13} and J_{14} vicinal couplings to be nearly equal (6.61 and 7.44 Hz, compared to the respective predicted J_{13} and J_{14} values of 8.5 and 3.1 Hz for the *gauche* average and 3.1 and 13.9 Hz for the *trans* rotamers from the Haasnoot–Altona[16] procedure). The results indicate at most only a few percent deviation from statistical equilibrium in favor of *gauche* or *trans*, the direction of the preference being uncertain because it is not known whether the larger of the observed couplings is associated with which rotamer of **5**. This lack of substantial preference is quite consistent with both dipole moment and infrared measurements on **5**. Thus, the dipole moments of **5** (3.93 D)[22] and propionitrile (3.63 D)[23–26] in benzene correspond to two thirds of the dinitrile existing as the *gauche* rotamer (the expected moment for the pure *trans* rotamer is 0, while that of the pure *gauche* rotamer from the data for propionitrile and assuming tetrahedral angles is 2[3.63(cos(109.5 − 90)(cos(30)))] = 5.93 D). The infrared spectra[27] suggest that the difference in enthalpy between the two kinds of rotamers in the liquid state is only about 360 cal/mol, with the *gauche* being the more stable.

Errors in any of a number of the parameters used could affect the final percentage of *gauche* rotamer calculated from the NMR couplings for each species. For example, it is hard to know the degree to which the *gauche* conformers favor, if at all, the perfect staggered rotational angle of 60°. However, if θ for the diprotic species is not 60°, but is 55° or 65°, then the corresponding percentage of *gauche* would still be 87% or 73%, respectively, as calculated from J_{13}. Percentages calculated from J_{14} for the same deviations would be 89% or 75%, respectively. Even with these values, the percent *gauche* for the diprotic species is still higher than that for the monoprotic species with either $\theta = 60°$ or $\theta = 71°$.

Another possible source of error might be in the values of the coupling constants obtained from the curves in Figure 2 for each of the species, especially in the case of J_{13}, as it spans a smaller range than J_{14}. It was found that the percentages obtained from J_{13} will converge with those obtained from J_{14}, with changes in the observed J_{13} of 0.13, −0.22, and 0.36 Hz for the H_2A, HA^-, and A^{2-} species, respectively. Because the observed couplings for the H_2A and A^{2-} are believed to be accurate to about ±0.1 Hz, these changes do not seem to account for all, if any, of the discrepancy between the percentages of *gauche* as calculated using J_{13} and J_{14}.

Also, the electronegativity of COOH could be altered so that the percent *gauche* for H_2A calculated from J_{13} converges with that calculated from J_{14}. The electronegativity of COO^- can be similarly "fudged" so that the *gauche* percentages for A^{2-} also converge. However, when the altered electronegativities are then used to calculate the percent *gauche* for HA^-, the discrepancy between the values derived from J_{13} and J_{14} actually increases almost 6%. This suggests that the original derived electronegativities are more accurate than the "fudged" electronegativities.

It has been suggested that the concentrations of our samples were sufficiently high or the nature of the cation such as to cause the observed conformational preferences to deviate from what would be expected for hydrogen-bonded HA. Because we were unable to obtain additional enriched butanedioic-2-^{13}C acid, we used 99%-enriched butanedioic-2,3-$^{13}C_2$ acid solutions to check these possibilities. The proton spectra derived from 0.2 M 99%-enriched butanedioic-2,3-$^{13}C_2$ acid are much more complex than those from butanedioic-2-^{13}C acid (see Figure 4), but the couplings calculated from the spectrum of the dilabeled acid as H_2A were the same as those from the monolabeled acid to within 0.05 Hz. The spectra of solutions of 0.02 M 99%-enriched butanedioic-2,3-$^{13}C_2$ acid showed no significant differences in the couplings or the conformational preferences for any of the acidic species (see Table II). Also, solutions of 0.2 M 99%-enriched butanedioic-

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[21] Radom, L.; Pople, J. A. *J. Am. Chem. Soc.* **1970**, *92*, 4786–4795. We are indebted to Dr. J. R. Murdoch for calling our attention to this reference. An apparent spectacular example is provided by $CF_3CCl_2CCl_2CF_3$, for which the *gauche* conformation predominates to better than 90%, see: Weigert, F. J.; Roberts, J. D. *J. Am. Chem. Soc.* **1968**, *90*, 3577. However, this example could be complicated by the observed strong F–F interaction, which might stabilize the *gauche* rotamer.

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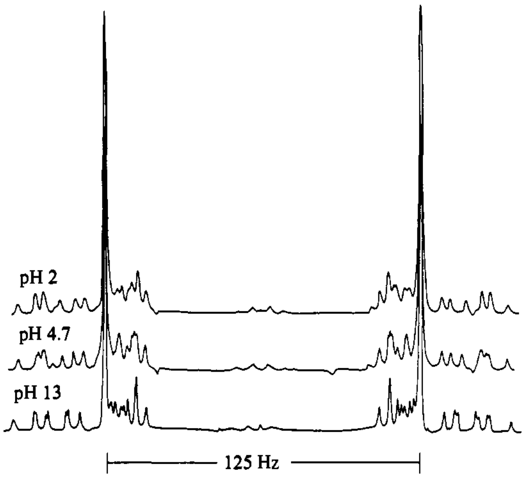

Figure 4. Proton NMR spectra of 0.2 M butanedioic-2,3-$^{13}C_2$ acid at different pH values.

2,3-$^{13}C_2$ acid with either lithium or tetramethylammonium ion as the counterion showed little change in conformational preference (see Table II). The single exception was with lithium and HA^-, where it appears that there is perhaps 5% less *gauche* compared to HA^- with tetramethylammonium as the counterion. This is perhaps surprising, because one could envision an increase in preference for the *gauche* rotamer if Li^+ were to intramolecularly bridge the oxygens of the 1,4-carboxyl groups, even though this, like intramolecular hydrogen bonding, would not involve the most stereoelectronically favorable $Li^+ \cdots O^-$ interaction. The slightly increased amount of *trans* rotamer, however, may reflect instead a more favorable Li^+ intermolecular bridging similar to that which occurs in the crystal structures of alkali hydrogen butanedioates in which the conformational preference is exclusively *trans*.[28]

One rather obvious other culprit for the failure to observe both strong hydrogen-bonding and/or electrostatic effects is our choice of solvent, water. The dielectric constant is high, although the Kirkwood–Westheimer treatment[4] would suggest that the acid species being in a cavity of low dielectric constant would certainly not experience an effective dielectric constant of 80 as might be expected for solutions in pure water. Furthermore, water will be a good competitor in hydrogen-bond formation so that external hydrogen bonds to water could well be favored over intramolecular hydrogen bonding. If this is so, then there needs to be caution in postulating hydrogen bonding as an influence in determining conformations in water solutions. With respect to electrostatics, Gil[11] has reported that there seemed to be little if any change in the conformational mix when the butanedioate dianion is dissolved in dioxane. The solubility is not high, but we, too, observed very little change in the ^{13}C satellites on the proton spectra for the dianion in dioxane.

Perhaps the most important contributions to the low conformational preferences observed here and elsewhere[17,29,30] come from unfavorable entropy changes associated with the loss of rotational freedom that would be associated with strong conformational preferences. The recent work of Williams and coworkers[31] on complexation of peptides with vancomycin antibiotics indicates that free energy contributions equivalent to 0.8–1.2 kcal/mol at 300 K are associated with the loss of rotational freedom about each C-C single bond in binding where rotations are resticted. Presumably, comparable influences would be expected for equilibria between specific conformations that involved strong hydrogen-bonding or electrostatic interactions. Even taking the low value of 0.8 kcal/mol at 300 K, formation of an intramolecular $O^- \cdots H{-}O$ bond in the butanedioate monoanion would be inhibited by 2.4 kcal/mol on this score, in addition to the likelihood that this is not the most stereoelectronically favorable configuration for hydrogen-bond formation.

In summary, it seems reasonable to conclude that the generally close agreement in the percentages of *gauche* rotamer calculated from either J_{13} or J_{14} is a positive indicator for the use of proton coupling constant data to determine conformational equilibrium constants. Perhaps more importantly, it is clear that, despite the possibility of strong stabilizations amounting to 2–10 kcal/mol by hydrogen bonding, as well as of strong induction and/or electrostatic effects, the conformational preferences in the systems studied here are weak. However, there is growing evidence that hydrogen-bonding and electrostatic effects are not as important as might be expected on intuitive grounds, and other factors such as steric hindrance and rotational entropy changes may play the really significant roles in determining the conformational preferences in diamines,[17,29] amino alcohols,[30] and amino acids.[17,29]

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