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## Nuclear Magnetic Resonance Spectroscopy. Spin-Lattice Relaxation of the Acetic Acid Carboxyl Carbon<sup>1</sup>

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**Abstract:** The carbon-13 spin-lattice relaxation times of the carboxyl carbons of acetic acid and its deuterated analogues ( $\text{CH}_3\text{COOD}$ ,  $\text{CD}_3\text{COOH}$ ,  $\text{CD}_3\text{COOD}$ ) have been measured by nuclear magnetic resonance spectroscopy. These measurements reveal that the methyl protons and the hydroxyl proton contribute roughly equally to the total dipolar relaxation of neat acetic acid. Spin-rotation relaxation is also observed in these molecules. The  $T_1$  and NOE values for the carboxyl carbon of acetic acid in aqueous solution each demonstrate a small concentration dependence in the region of high acid concentration. Although samples of 1 M acetic acid contaminated with paramagnetic metal ions show a marked pH dependence of  $T_1$  and NOE, the  $T_1$  and NOE values for purified samples are invariant with respect to pH. Some chemical implications of the above behavior are discussed.

Traditionally, nuclear magnetic resonance spectroscopists have usually been concerned with the measurement of chemical shifts, resonance intensities, and coupling constants. In recent years, yet another parameter, the spin-lattice relaxation time ( $T_1$ ), has become of interest in carbon-13 NMR spectroscopy.<sup>2</sup> Although  $T_1$  values may yield important information concerning molecular dynamics, their measurement is often fraught with difficulties. The presence of oxygen in solution, contamination by other paramagnetic impurities, vortex formation in the sample, temperature variation, and instrumental problems often lead to variable results for  $T_1$  studies of the same molecule performed under different conditions. The value of  $T_1$  for the carboxyl carbon of neat acetic acid, for example, has been determined by several groups<sup>3</sup> under various conditions, and the reported values range from 18.5 to 41.1 s.

A more detailed study of the spin-lattice relaxation behavior of acetic acid is reported here. Such a study is particularly appropriate in view of the recently discovered extreme sensitivity of carboxyl carbon  $T_1$  values to contamination by trace paramagnetic metal ion impurities.<sup>3e,4,5</sup> By means of selective deuteration experiments, the relative contributions of various mechanisms to the relaxation of the acetic acid carboxyl carbon atom have been studied, and in addition the effects of dilution and pH changes upon the values of  $T_1$  for this nucleus have been investigated.

### Results and Discussion

**Acetic Acid and Its Deuterated Analogues.** In order to investigate the relative contributions to dipole-dipole relaxation ( $T_1^{\text{DD}}$ ) from the methyl and hydroxyl protons of neat acetic acid,  $T_1$  values ( $T_1^{\text{obsd}}$ ) and nuclear Overhauser enhancements ( $\text{NOE} (1 + \eta)$ ) for the carboxyl carbon atom of the molecules  $\text{CH}_3\text{COOH}$ ,  $\text{CH}_3\text{COOD}$ ,  $\text{CD}_3\text{COOH}$ , and  $\text{CD}_3\text{COOD}$  were measured. In each case, the dipole-dipole relaxation arising from the protons and the spin-lat-

tice relaxation due to other mechanisms ( $T_1^{\text{other}}$ ) were calculated by use of eq 1 and 2.

$$T_1^{\text{DD}} = T_1^{\text{obsd}} \frac{1.988}{\eta} \quad (1)$$

$$\frac{1}{T_1^{\text{obsd}}} = \frac{1}{T_1^{\text{DD}}} + \frac{1}{T_1^{\text{other}}} \quad (2)$$

The  $T_1$  values were measured at 15.09 MHz on our "Brukerian" spectrometer by the progressive saturation technique, and are deemed accurate to  $\pm 10\%$ <sup>6</sup> (see Experimental Section). The accuracy of the NOE measurements is approximately  $\pm 5\%$ . Because carboxyl carbon  $T_1$  values are known to be extremely sensitive to paramagnetic metal ion impurities, the samples (obtained from commercial sources) were distilled three or more times, and all glassware, vortex plugs, etc., were decontaminated as described elsewhere,<sup>4</sup> and in the Experimental Section. Deoxygenation was achieved by purging with nitrogen, or by the freeze-thaw technique.

The results of this study are presented in Table I. It must be emphasized at this point that in spite of the rigorous purification procedures employed, all  $T_1$  values reported in Table I and elsewhere in this paper may be only lower limits. If residual contamination by paramagnetic metal ions or by oxygen is great enough,  $T_1$  values in the absence of contamination may be larger than reported. However, in all cases the reported values are reproducible for multiple samples, and other evidence (see below) indicates that even if some individual  $T_1$  or NOE values are somewhat in error, the trends observed cannot be ascribed to contamination by paramagnetic impurities.

The first line of the table reports results for  $\text{CH}_3\text{COOH}$ . These values for  $T_1$  and NOE agree, within experimental error, with those reported by Farrar et al.<sup>3d</sup> Other values for  $T_1$  and NOE reported in the literature are smaller than

**Table I.**  $T_1$  and NOE Values for Neat Acetic Acids (Carboxyl Carbon)

| Compd                | $T_1^{\text{obsd}}$ , s | NOE (1 + $\eta$ ) | $T_1^{\text{DD}}$ , s | $T_1^{\text{other}}$ , s |
|----------------------|-------------------------|-------------------|-----------------------|--------------------------|
| CH <sub>3</sub> COOH | 40                      | 2.43              | 56                    | 142                      |
| CH <sub>3</sub> COOD | 60                      | 1.95              | 126                   | 115                      |
| CD <sub>3</sub> COOH | 61                      | 2.16              | 105                   | 146                      |
| CD <sub>3</sub> COOD | 137                     | 1.11              | >2000                 | 145                      |

those obtained here, although where NOE values have been measured,<sup>3c</sup> the dipolar relaxation times are in good agreement. These shorter values are likely the result of contamination by oxygen or other paramagnetic impurities, or by vapor formation.<sup>3d</sup> Thus, the dominant relaxation mechanism for the carboxyl carbon atom of CH<sub>3</sub>COOH is dipolar.

The remaining results in Table I refer to specifically deuterated molecules. If we make the reasonable assumption that the major effect of deuteration upon the spin-lattice relaxation time of the carboxyl carbon is loss of dipolar relaxation from the replaced protons, then we may use the results of the deuteration experiments to determine the relative contributions of the various protons to  $T_1^{\text{DD}}$ . Replacement of the hydroxyl proton by deuterium results in a  $T_1^{\text{DD}}$  value of 126 s. This residual dipolar relaxation must arise from the methyl protons. Similarly, replacement of the methyl protons by deuterons yields a value for  $T_1^{\text{DD}}$  of 105 s for relaxation due to the hydroxyl proton. The total dipolar relaxation for this system is given by eq 3, where  $T_1^{\text{DD}}(\text{OH})$  and  $T_1^{\text{DD}}(\text{CH}_3)$  are the contributions to

$$\frac{1}{T_1^{\text{DD}}(\text{total})} = \frac{1}{T_1^{\text{DD}}(\text{OH})} + \frac{1}{T_1^{\text{DD}}(\text{CH}_3)} \quad (3)$$

$T_1^{\text{DD}}(\text{total})$  of the hydroxyl and methyl protons, respectively. Substituting from Table I, a value for  $T_1^{\text{DD}}(\text{total})$  of 57 s is obtained. This value is in good agreement with the value of 56 s found for acetic acid.

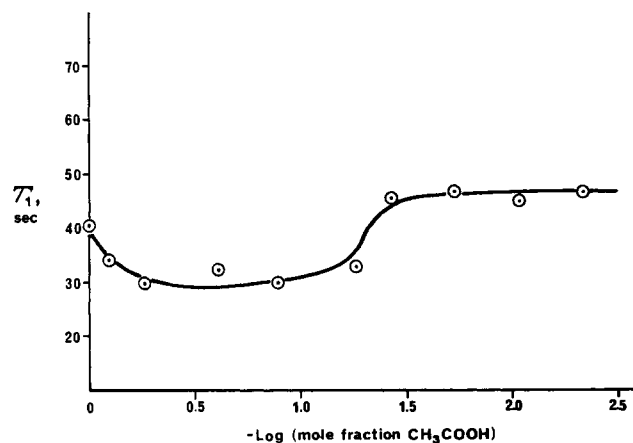
Table I shows that the methyl protons and the hydroxyl protons contribute roughly equally to the relaxation of the carboxyl carbon atom. This is true even though there are three methyl protons and only one hydroxyl proton. Several factors make the hydroxyl proton more efficient than a single methyl proton. The hydroxyl proton is closer to the carboxyl carbon than is the methyl proton, and  $T_1^{\text{DD}}$  is dependent upon the sixth power of the carbon-hydrogen internuclear distance.<sup>2</sup> In addition, rapid internal rotation of the methyl group may lead to a reduction in  $T_1^{\text{DD}}(\text{CH}_3)$ .<sup>7</sup> Finally, in dimeric acetic acid, hydrogen-bonded protons may help to relax the carboxyl carbon.

In CH<sub>3</sub>COOD and CD<sub>3</sub>COOH,  $T_1^{\text{DD}}$  and  $T_1^{\text{other}}$  are of roughly equal importance, whereas in CD<sub>3</sub>COOD, where dipolar relaxation is essentially eliminated,  $T_1^{\text{other}}$  accounts for all the relaxation. Chemical-shift anisotropy relaxation has been found to be unimportant for acetic acid.<sup>3c,d</sup> Scalar coupling relaxation has been suggested as a possible contributing mechanism for neat acetic acid.<sup>3d</sup> However, Table I reveals that within experimental error, replacement of the hydroxyl proton by a deuteron has no effect on  $T_1^{\text{other}}$ . Thus, scalar coupling may be ruled out as a significant relaxation mechanism for neat acetic acid, and  $T_1^{\text{other}}$  may be ascribed to spin-rotation relaxation plus any contribution from residual paramagnetic impurities.

The relatively large amount of relaxation in acetic acid arising from the hydroxyl proton means that  $T_1$  values for carboxyl carbon nuclei obtained in aqueous solution cannot be directly compared with values obtained from solutions containing D<sub>2</sub>O. This fact is of special significance for stud-

**Table II.** Concentration Dependence of  $T_1$  and NOE

| Mole fraction acid                       | -Log (mole fraction acid) | $T_1$ , s | NOE (1 + $\eta$ ) |
|--|---------------------------|-----------|-------------------|
| CH <sub>3</sub> COOH in H <sub>2</sub> O |                           |           |                   |
| 1.00                                     | 0.00                      | 40        | 2.43              |
| 0.80                                     | 0.0969                    | 34        | 2.70              |
| 0.561                                    | 0.251                     | 30        | 2.63              |
| 0.245                                    | 0.611                     | 32        | 2.46              |
| 0.129                                    | 0.889                     | 30        | 2.36              |
| 0.054 1                                  | 1.267                     | 33        | 2.39              |
| 0.037 4                                  | 1.427                     | 45        | 2.25              |
| 0.018 7                                  | 1.728                     | 47        | 2.21              |
| 0.009 37                                 | 2.028                     | 45        | 2.11              |
| 0.004 68                                 | 2.330                     | 47        | 2.08              |
| CH <sub>3</sub> COOD in D <sub>2</sub> O |                           |           |                   |
| 1.00                                     | 0.00                      | 60        | 1.95              |
| 0.556                                    | 0.255                     | 60        | 2.11              |

**Figure 1.** Concentration dependence of  $T_1$  of the carboxyl carbon of CH<sub>3</sub>COOH in H<sub>2</sub>O.

ies of compounds of biological interest, because such molecules are often studied in water or D<sub>2</sub>O.

**Concentration Dependence of Acetic Acid  $T_1$  Values.** The results of a study of the concentration dependence of  $T_1$  and NOE for aqueous acetic acid appear in Table II. The usual precautions to avoid contamination by paramagnetic metal ions were observed. In addition, the water used to dilute the samples was doubly distilled, and then extracted with dithizone in carbon tetrachloride to remove metal ions (see Experimental Section).

The changes in  $T_1$  and NOE observed upon dilution of acetic acid with H<sub>2</sub>O are small but outside experimental error. Although the individual  $T_1$  values may be in error by as much as  $\pm 10\%$ , the results reveal a definite trend (Table II and Figure 1). Addition of small amounts of H<sub>2</sub>O (less than about 1 equiv) to neat acetic acid results in a decrease in  $T_1$  accompanied by a small increase in NOE. Subsequent dilution with H<sub>2</sub>O causes an increase in  $T_1$  and decrease in NOE. At concentrations of 0.037 mol fraction of acid or less, dilution has no measurable effect on either  $T_1$  or NOE. Similar nonlinear behavior is manifest in other physical methods for studying acetic acid-water mixtures. For example, dilution curves for acetic acid which have maxima or minima in the region of 0.5 mol fraction acid have been observed in low-frequency Raman spectra,<sup>8</sup> carbon-13 chemical shift measurements,<sup>9</sup> proton chemical shift measurements,<sup>10</sup> hydrogen relaxation time measurements,<sup>11</sup> and viscosity measurements.<sup>12</sup>

The cause is in all probability changes in aggregation of

the molecules in solution as has been postulated by several workers.<sup>13</sup> Thus, the cyclic dimers present in neat acetic acid may, on initial dilution with water, form new species which could be linear dimers, hydrated dimers, acid water polymers, and so on, or mixtures of these. Formation of such aggregates can explain the initial changes on dilution and subsequent further breaking up of aggregates on continued dilution could well account for the maxima or minima in the curves.

There are several ways in which water-acetic acid aggregates could affect  $T_1$  and NOE values. One way is through slower tumbling rates which in turn would result in more efficient dipolar relaxation. This would cause an increase in NOE values and a decrease in  $T_1$ , as is observed. Subsequent dilution could result in changes in these aggregates and a reversal of  $T_1$  and NOE behavior. Nonlinear changes in macroscopic viscosity as a function of acetic acid concentration would have similar effects of  $T_1$  and NOE. Intimate association of water molecules with the aggregates could also result in an increase in intermolecular dipolar relaxation upon initial dilution. This possibility is supported by observations of intermolecular relaxation by water of the carboxyl carbon of glycine<sup>4</sup> and the carbonyl carbon of perdeuterioacetone.<sup>14</sup> That dilution of  $\text{CH}_3\text{COOD}$  with  $\text{D}_2\text{O}$  produces no change in  $T_1$  or NOE within experimental error (Table II) would appear to support this consideration. However, one must remember that the mechanisms of relaxation are not identical for the deuterated and undeuterated species.

Other changes which would occur as a result of changes in the aggregates with water concentration would be in the exchange rate of the acid hydroxyl protons and the spin-rotation relaxation time. These factors could also affect the measured  $T_1$  values, if they were of the correct magnitude. While residual paramagnetic impurities may contribute to the absolute values of the measured  $T_1$  and NOE values, they cannot explain the observed trends because the initial decrease in  $T_1$  is accompanied by an increase in NOE.

In summary, dilution of acetic acid with  $\text{H}_2\text{O}$  results in an initial small decrease in  $T_1$  values and an increase in NOE for the carboxyl carbon. These changes are presumably due to solvent-solute interactions of some type. Further dilution results in an increase in  $T_1$  until water is present in large excess at which point the  $T_1$  becomes constant. Clearly, if further changes in monomer-dimer-aggregate equilibria occur in the high-water region, the  $T_1$  and NOE values are not sensitive to them, at least to the accuracy of our measurements.

**pH Dependence of  $T_1$  and NOE.** In our initial studies with unpurified solutions of acetic acid-sodium acetate, a strong pH dependence was found for  $T_1$  and NOE of the carboxyl carbon (Table III). Whereas the  $T_1$  values in strongly acid and strongly basic solution were reasonably close to those of 1 M acetic acid (0.0187 mol fraction above), there was a pronounced minimum in  $T_1$  and NOE near pH 4.6, which is approximately the  $pK$  value of acetic acid. An interesting facet of these data is the observation that the dipolar relaxation time remains constant across the whole range of pH and is about 75 s. The same type of behavior occurs for a 1.0 M solution of acetic acid-sodium acetate in  $\text{D}_2\text{O}$  although the observed  $T_1$  values are consistently longer than in  $\text{H}_2\text{O}$  and the NOE values are consistently smaller (Table IV). The dipolar relaxation time is again invariant across the whole pH range, although at 247 s it is significantly longer than in the  $\text{H}_2\text{O}$  solution.

We have observed a similar pH dependence of carboxyl carbon  $T_1$  values in glycine<sup>15</sup> and we have attributed this to contamination by paramagnetic metal ions.<sup>4</sup> Indeed, addition of about  $10^{-4}$  M  $\text{CuCl}_2$  to 1 M acetic acid resulted in a

**Table III.** pH Dependence of  $T_1$  and NOE for Unpurified 1.0 M Acetic Acid-Sodium Acetate in  $\text{H}_2\text{O}$

| pH  | $T_1$ , s | NOE (1 + $\eta$ ) | $T_1^{\text{DD}}$ , s | $T_1^{\text{other}}$ , s |
|-----|-----------|-------------------|-----------------------|--------------------------|
| 0.2 | 38        | 2.10              | 69                    | 84                       |
| 1.4 | 40        | 2.03              | 80                    | 80                       |
| 4.6 | 22        | 1.59              | 72                    | 31                       |
| 5.6 | 31        | 1.76              | 74                    | 50                       |
| 6.2 | 36        | 1.86              | 81                    | 66                       |
| 6.6 | 39        | 2.00              | 77                    | 77                       |
| 9.5 | 47        | 2.25              | 72                    | 151                      |

**Table IV.** pH Dependence of  $T_1$  and NOE for Unpurified 1.0 M Acetic Acid-Sodium Acetate in  $\text{D}_2\text{O}$

| pH   | $T_1$ , s | NOE (1 + $\eta$ ) | $T_1^{\text{DD}}$ , s | $T_1^{\text{other}}$ , s |
|------|-----------|-------------------|-----------------------|--------------------------|
| 1.1  | 64        | 1.69              | 186                   | 98                       |
| 5.62 | 27        | 1.23              | 237                   | 31                       |
| 5.88 | 33        | 1.26              | 255                   | 38                       |
| 6.09 | 47        | 1.41              | 231                   | 60                       |
| 6.19 | 51        | 1.42              | 242                   | 64                       |
| 9.83 | 91        | 1.68              | 269                   | 147                      |

**Table V.** pH Dependence of  $T_1$  and NOE for Purified 1.0 M Acetic Acid-Sodium Acetate in  $\text{H}_2\text{O}$

| pH   | $T_1$ , s | NOE (1 + $\eta$ ) | $T_1^{\text{DD}}$ , s | $T_1^{\text{other}}$ , s |
|------|-----------|-------------------|-----------------------|--------------------------|
| 2.1  | 43        | 2.29              | 67                    | 121                      |
| 2.4  | 45        | 2.25              | 72                    | 120                      |
| 3.7  | 42        | 2.24              | 68                    | 110                      |
| 4.3  | 45        | 2.20              | 75                    | 113                      |
| 5.4  | 40        | 2.23              | 65                    | 104                      |
| 5.6  | 45        | 2.16              | 78                    | 107                      |
| 13.1 | 46        | 2.27              | 72                    | 126                      |
| 13.1 | 44        | 2.35              | 65                    | 135                      |

greatly accentuated pH dependence with  $T_1$  values on the order of 1 s at pH 4.5 and negligible NOE. A similar study was recently reported by Cohen and co-workers<sup>3e</sup> who also found a large pH dependence of  $T_1$  for acetic acid solutions doped with  $\text{Cu(II)}$ . For nondoped solutions these authors obtained much larger  $T_1$  values, although from pH 4 to 7.6 their data varied somewhat randomly from 33.1 to 60.3 s with an average relaxation time of 44 s. Unfortunately these authors did not measure nuclear Overhauser enhancements and so were unable to make estimates of the true dipolar relaxation times.

Even in such heavily doped samples as those just described it is still possible to obtain meaningful dipolar relaxation times if one has reliable NOE data. For example, in our purposely doped sample of acetic acid-sodium acetate at pH 1.7 we find  $T_1$  to be 12.4 s and the NOE to be 1.4. These data yield a dipolar relaxation time of 62 s, which is in good agreement with that of our "nondoped" sample.

We finally examined the pH dependence of  $T_1$  and NOE in acetic acid solutions which had been purified as discussed above and in the Experimental Section. The results appear in Table V. Within experimental error, there is no pH dependence of either  $T_1$  or NOE. Considering the relatively large error in  $T_1^{\text{DD}}$  the agreement over the whole pH range is quite striking. The average  $T_1^{\text{DD}}$  of 70 s is in excellent agreement with the average  $T_1^{\text{DD}}$  of 75 s from the unpurified sample.

Since it was found that in neat acetic acid replacement of the hydroxyl proton by a deuteron resulted in an increase in  $T_1$ , it might be expected that removal of the hydroxyl pro-

ton by the formation of the acetate ion would have a similar effect, and that  $T_1$  values should increase with increasing pH. Since this is not observed, there may be some compensatory effect. This could be an increase in viscosity and such increases have been observed for acetic acid–sodium acetate solutions.<sup>16</sup> However, it must be remembered that whereas neat acetic acid is mostly dimeric, 1 M acetic acid may well be mostly monomeric, and the relative importance of the various relaxation mechanisms need not be the same for the two situations.

For the unpurified sample in D<sub>2</sub>O (Table IV) the constancy of  $T_1^{DD}$  over the whole pH range is again apparent; the average value of 247 s, however, is considerably greater than in the H<sub>2</sub>O case. The shorter  $T_1$  values for H<sub>2</sub>O solutions at low pH are due, at least in part, to the presence of a proton rather than a deuteron on the hydroxyl oxygen. Because acetic acid is present mainly as an anion at pH 9 and above, the shorter  $T_1^{DD}$  for the H<sub>2</sub>O solutions in this region must be due to intermolecular relaxation from the protons of water (assuming that the correlation times of CH<sub>3</sub>COO<sup>−</sup> in water and D<sub>2</sub>O are approximately equal). The magnitude of this intermolecular effect may be calculated from

$$1/T_1^{DD(inter)} = 1/T_1^{DD(H_2O)} - 1/T_1^{DD(D_2O)}$$

and comes out to be about 100 s. We have recently observed intermolecular effects of similar magnitude in aqueous solutions of glycine.<sup>4</sup> If one assumes that at pH 9 and above all paramagnetic metal ions are present as hydroxides<sup>17</sup> and do not affect  $T_1$  and NOE values, then the larger Overhauser enhancement observed for the acetic acid carboxyl carbon in H<sub>2</sub>O as opposed to D<sub>2</sub>O constitutes a direct observation of an intermolecular Overhauser enhancement. This intermolecular carbon-13 NOE finds its analogy in proton NMR in the classic double resonance experiment of Kaiser<sup>18</sup> on a chloroform–cyclohexane solution. Germane to the intermolecular relaxation noted in the acetic acid–sodium acetate system is the conclusion of von Goldammer and co-workers<sup>14</sup> that intermolecular relaxation from water is important for the carbonyl carbon of perdeuterioacetone. von Goldammer's results indicated that the magnitude of the intermolecular relaxation was about 100 s.

It remains to make some comments about the effect of the paramagnetic metal impurities on the carboxyl carbon  $T_1$  values in the unpurified samples. Since the  $T_1$  and NOE values for the purified and unpurified samples at pH 9 and above are in close agreement, the assumption that paramagnetic metal impurities are present as hydroxides in this pH region and hence do not affect relaxation appears to be a good one. It is evident from the tables that paramagnetic metal ions have a maximal effect near pH 4.5, and that some residual contribution to relaxation may still be present at low pH.

The results reported above for acetic acid serve to emphasize the extreme precautions which must be observed if meaningful  $T_1$  values for carboxyl nuclei are desired. However, although absolute values for  $T_1$  may sometimes be suspect, meaningful trends can often still be observed if nuclear Overhauser enhancements are measured.

## Experimental Section

**NMR Measurements.** All carbon-13 spin-lattice relaxation times were measured using the progressive saturation method,<sup>19</sup> with our pulse Fourier transform modified "Brukarian" spectrom-

eter operating at 15.09 MHz. The 90° pulse was 12 μs. The lock signal was obtained from external D<sub>2</sub>O in a capillary except when a deuterium-containing solution was being studied. Teflon vortex plugs were used to ensure that the samples were confined within the transmitter coils. The temperature was 30 ± 1°.

**Studies of Neat Acids.** Acetic acid and its deuterated analogues were all obtained from commercial sources, and were distilled three or more times before use. All tubes, glassware, vortex plugs, etc., were soaked for at least 24 h in alkaline EDTA solution to remove metal ions, and were rinsed thoroughly with doubly distilled water which was stored in a polyethylene bottle. Samples were run either in 10-mm tubes, with deoxygenation using a nitrogen purge, or in sealed bulbs, with degassing using four freeze–thaw cycles. Identical results were obtained from the two methods.

**Dilution Studies.** The same precautions were taken as are described above for neat acids. In addition, the water and D<sub>2</sub>O used for dilution were doubly distilled and then extracted five times with a 0.05% solution of dithizone in carbon tetrachloride. The acetic acid used was either the triply distilled acid mentioned above, or a triply distilled sample about 90% carbon-13 enriched at the carboxyl carbon.

**pH Studies.** The acetic acid used in these studies was about 90% carbon-13 enriched at the carboxyl carbon, and was triply distilled before use. In addition to the precautions described above, the sodium hydroxide solution used to adjust pH was the "ultrapure" grade supplied by Alfa Inorganics. The pH data for unpurified samples were obtained with 90% carbon-13 enriched sodium acetate. The pH values were measured on a Radiometer pH meter 26 using a glass electrode.

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