Table I. Deuterium Incorporation into *trans*-**1** and Calculated Isotope Effects

% D in TFA[a,e]	% D in *trans*-**1**[b]	k_H/k_D[c]
99.3 ± 0.3	71.5 ± 0.3	40 ± 13
92.7 ± 0.3	30.4 ± 0.3	27 ± 1
84.5 ± 0.3[f]	17.0 ± 0.3	27 ± 1
75.7 ± 0.3	10.4 ± 0.3	27 ± 1
59 ± 1[d]	6.1 ± 0.4	22 ± 2

[a] The deuterium content of the TFA was analyzed by addition of a known amount of chloroform and integration of the resonances due to acid and chloroform protons. [b] Obtained from measurement of at least four slow scans of the M − 3CO (*m/e* 276–277) peak; the molecular ion was very weak even at low voltages. The ratios of peak intensities in this cluster in undeuterated *trans*-**1** were in accord with those calculated from known isotope abundances, demonstrating that hydrogen loss is not a source of error in these measurements. [c] See text. [d] This sample of acid contained approximately 20 mol % of water. [e] The mole ratio of acid to *cis*-**1** was about 100. [f] Chloroform solution of complex added to acid (inverse addition).

isomerization under our standard conditions in labeled TFA (specific activity = (6.62 ± 0.02) × 10^{10} cpm/mol). The specific activity of carefully purified *trans*-**1** was (6.36 ± 0.34) × 10^8 cpm/mol (average of two trials), whence $k_H/k_T = 104 \pm 6$.

This value corresponds to a value of $r = 1.41 \pm 0.04$ in eq 2, well within the expected range for a kinetic isotope effect.

The large magnitude of the isotope effect can be at least partially rationalized in terms of the proposed mechanism. The change in vibrational frequencies involved in the proton transfer between oxygen (ν_{OH} ~3600 cm^{-1}) and metal (ν_{MH} 1800–2000 cm^{-1}) is much larger than between oxygen and, say, carbon (ν_{CH} ~3000 cm^{-1}), and thus the ability to discriminate between hydrogen and deuterium in a transfer from oxygen to metal would be expected to be correspondingly greater. Therefore, the observation of a large isotope effect is consistent with the arguments above, and with the essential correctness of the mechanism in Scheme I.

Acknowledgment. This work was supported by grants from the National Science Foundation (GP 16358) and the Chevron Research Corporation.

Supplementary Material Available. Appendix I, the derivation of eq 1, will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-75-907.

References and Notes

(1) The synthesis of this compound will be published later.

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(5) Direct evidence for the relative rates of intra- and intermolecular exchange is obtained from examination of the NMR spectrum of cyclohexadiene iron tricarbonyl in TFA. Two sets of resonances are observed: one (~90%) due to unprotonated complex; the other (~10%) due to the *averaged* spectrum of the metal-protonated species. Similar results have been obtained by B. F. G. Johnson, J. Lewis, and D. Yarrow (*J. Chem. Soc., Chem. Commun.*, 235 (1972)) for (cyclohexadiene)(cyclopentadienyl)rhodium.

(6) If *trans*-**1** isomerized back to *cis*-**1** appreciably rapidly, deuterium would be incorporated into *trans*-**1** by the reverse of Scheme I.

(7) Since the equilibrium concentration of *cis*-**1** is very low,[7] the steric congestion in *cis*-**1** must be large. This congestion is largely produced in the reverse of reaction c.

(8) This expression was derived by integrating the differential equations corresponding to

$$CF_3CO_2H + cis\text{-}\mathbf{1} \xrightarrow{k_H} trans\text{-}\mathbf{1}\ (H)$$

$$CF_3CO_2D + cis\text{-}\mathbf{1} \xrightarrow{k_D} trans\text{-}\mathbf{1}\ (D)$$

The second term results from the fact that in highly deuterated TFA the hydrogen concentration changes appreciably during the reaction even with a large excess of TFA. The correction introduced is negligible for <90% D_1 TFA, and eq 1 reduces to

$$\frac{k_H}{k_D} = \frac{T_H}{T_D}\frac{D_0}{H_0}$$

A detailed derivation will appear as an appendix in the microfilm edition of this journal.

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(10) We would like to thank Professor Howard Whitlock and Ms. Mary Dampier of this department for their assistance in performing this experiment.

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pH Dependence of the ^{13}C Spin–Lattice Relaxation Rate of the Carboxyl Carbon of Acetic Acid

Sir:

In a recent study of the pH dependence of the spin-lattice relaxation time (T_1) of the C2 proton of imidazole, it was concluded that paramagnetic metal ion impurities down to 10^{-7} *M* dominate the relaxation time in the neutral pH range.[1] This was concluded to be the origin of the minimum observed in the pH dependence of the T_1 value, since additions of Cu^{2+} ion at 10^{-6} to 10^{-4} *M* caused a more pronounced minimum, while the chelating agent EDTA reduced the effect.

In view of the relevance of such studies to biological molecules in aqueous solutions, and the extensive use of pulse techniques to measure ^{13}C relaxation times in peptides and proteins,[2-7] it was felt worthwhile to extend these studies to the observation of the ^{13}C resonance of a titrating functional group. The carboxyl group was chosen in view of the availability of highly enriched (90%) $CH_3{}^{13}COOH$ (Merck & Co., Rahway, N.J.). This reduced the time taken to measure the T_1 values by the spin inversion-recovery method,[1] so that only one sequence of pulses (d, π, τ, $\pi/2$) was generally required.

Acetic acid was one of the molecules studied in a consideration of the origins of ^{13}C relaxation phenomena in small molecules, in which it was concluded that for the carboxyl carbon atom the predominant relaxation mechanism was dipolar.[8] In a recent report, Armitage et al.[9] described a minimum in the pH dependence of the ^{13}C T_1 value of the carboxyl carbon atoms of various amino acids, which they attributed to a spin rotation mechanism.

In the present work ^{13}C relaxation times were measured at 55 MHz using a probe constructed at NIH and interfaced to the Varian HR 220 FT system. Pulse widths were typically 14 μsec for a 90° pulse, and delay times of the order of 50–250 sec ($\simeq 5\ T_1$) were used. Long T_1 values are especially sensitive to the absorption of oxygen from the air. To reduce this effect samples were transferred under a stream of nitrogen gas, and NMR tubes were filled with nitrogen before and after each pH measurement and were kept stoppered. Samples were 1 *M* $CH_3{}^{13}COOH$ in 0.1 *M* NaCl, and pH was adjusted with 1 *N* NaOH. Above the p*K* value (4.5) buffering capacity was minimal and it was difficult to obtain pH values in the range 5–11. The standard er-

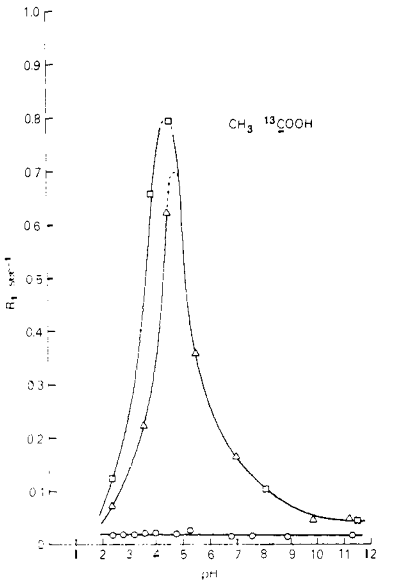

Figure 1. Spin-lattice relaxation rate of the carboxyl carbon atom of 90% enriched $CH_3{}^{13}COOH$ (1 *M* in 0.1 *M* NaCl solution) as a function of pH, alone (○), and in the presence of 3.8×10^{-4} *M* Cu^{2+} (△) and 7.6×10^{-4} *M* Cu^{2+} (□) ions.

Table I

pH	T_1	pH	T_1
3.95	42.2 ± 1.0	5.28	36.4 ± 2.4
4.14	33.1 ± 2.6	6.75	60.3 ± 1.4
4.35	35.8 ± 0.9	7.55	58.8 ± 2.0
4.74	44.6 ± 2.7		

rors of T_1 determinations were 10% or less. Results are reported as relaxation rates, R_1 ($\equiv 1/T_1$).[8]

The R_1 value measured for the carboxyl carbon of neat $CH_3{}^{13}COOH$ was 0.054 sec^{-1}, in good agreement with a previous value for an undegassed sample (0.05 sec^{-1}),[8] although this is naturally higher than the value recorded in degassed samples.[8,10] ^{13}C relaxation rates of 1 *M* aqueous solutions of acetic acid at different pH values are shown in Figure 1. A sample at pH 2.8 diluted to 0.15 *M* showed no significant change giving an R_1 value of 0.028 sec^{-1}.

No significant pH dependence is observed in the R_1 values. A good deal of scatter was observed in the T_1 values using several different samples (Table I). It is not considered warranted to make deductions on the basis of such differences (cf. ref 9) since long T_1 values are known to be very sensitive to the uptake of oxygen from the air and the presence of minute traces of paramagnetic metal ion impurities in solution.

On the addition of Cu^{2+} at 10^{-4} *M* concentration a distinct maximum is observed in the pH dependence of the ^{13}C R_1 value (Figure 1). At intermediate pH values the R_1 value is raised by complexation of Cu^{2+} with the carboxylate anions, whereas at low pH the Cu^{2+} ions are not bound to acetic acid since protons compete effectively, and at high pH hydroxyl ions compete with the acetate ions for the metal ions. The reason for the minimum being close to the p*K* value of the titrating group in this and the previous study[1] is under investigation. It is possible that the decrease in R_1 at high pH reflects a slow rate of exchange of Cu^{2+} between carboxylate anions.

These results do not eliminate spin rotation as a possible source of relaxation for carboxyl carbon atoms in the ostensible absence of paramagnetic metal ions. Nevertheless, it is concluded that low concentrations of paramagnetic metal ions can dominate relaxation in the intermediate pH range leading to a maximum in the spin-lattice relaxation rate.

Acknowledgment. We thank Roderick Wasylishen and Edwin Becker for helpful discussions.

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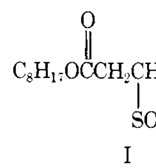
Motional Freedom and Polarity within Water Pools of Different Sizes. Spin Label Studies

Sir:

Di-2-ethylhexyl sodium sulfosuccinate (I) has the ability to solubilize large amounts of water in heptane.[1] This water



$$C_8H_{17}O\overset{O}{\overset{\|}{C}}CH_2\underset{SO_3^-Na^+}{\underset{|}{C}H}\overset{O}{\overset{\|}{C}}OC_8H_{17}$$

I

is confined in spherical "pools" encased by the surfactant. The size of the pools depends on the $[H_2O]/[I]$ ratio. For example, light scattering data[1] have revealed that ratios of 2.2, 8.9, and 36 lead to pools containing roughly 40, 400, and 6400 water molecules, respectively. We report here an ESR study of three spin labels (II) dissolved in the water pools. These nitroxide radicals display simple three-line spectra; the hyperfine coupling constants depend on the local polarity whereas the line widths reflect the rate of mo-