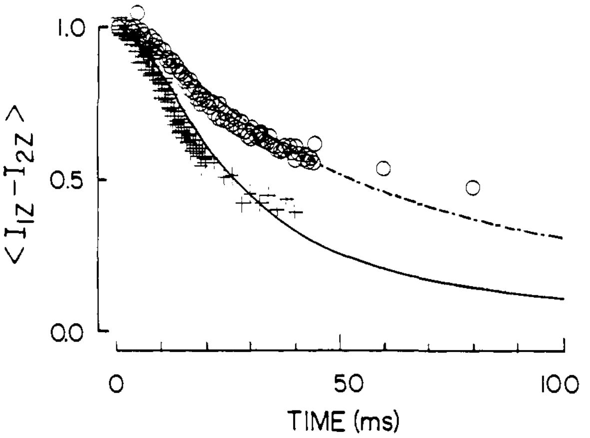

Figure 3. Calculated and experimental evolution of the difference polarization for (a) the $n = 2$ rotational resonance (+, —) and (b) the $n = 3$ rotational resonance (O, — - —) in TEE. Both results were obtained at a field of 7.5 T. Calculations assumed an internuclear separation of 0.505 nm. Other parameters are given in the caption to Figure 2.

TEE crystallizes in a "scorpion"-like conformation where the chain is bent back over the plane of the aromatic ring (Figure 1) and the separation between labeled carbons is 0.505 nm.[15] The magnetization transfer is followed using a simple technique:[13,16] a nonequilibrium initial condition is established by selectively inverting the Zeeman magnetization of one site. After evolution for a variable mixing time τ_m, a nonselective $\pi/2$ pulse is applied to measure the new distribution of magnetization between the two sites. The exchange process is conveniently described as a decay of difference magnetization. Figure 2 displays experimental and calculated curves of the normalized difference magnetization for the $n = 1$ rotational resonance in TEE, and the line for 0.505 nm clearly provides the best fit. In Figure 3, experimental and calculated curves are shown for the $n = 2$ and $n = 3$ resonances. Again, there is good agreement, especially at short times, for a 0.505-nm internuclear separation.

The numerical calculations used to derive the curves (Figures 2 and 3) will be described elsewhere.[17] They differ from previous calculations[13] in that the simultaneous influence of coherent fields and relaxation is incorporated by an integration of the Liouville superoperator over one rotational period. The parameters required, in addition to the internuclear distance, are the isotropic shifts, the principal values of the shift tensors, which are known for many functional groups,[18] and the mutual orientation of the two tensors.[19]

The decay time constant of the zero-quantum coherence of the two spins, T_2^{ZQ}, must also be estimated. Zero-quantum decay influences the magnetization transfer, since the difference polarization is continuously exchanged with zero-quantum coherence under the influence of the dipolar coupling. The zero-quantum relaxation rate is difficult to measure experimentally, but, under the assumption of dephasing by uncorrelated random fields, it may be estimated as the sum of the single-quantum transverse relaxation rates of the two spins.[10] These may be obtained from the spectral line widths, after correcting for magnet inhomogeneity. For the present simulations, the value $T_2^{ZQ} = 6.3$ ms was used.

It is necessary to comment on the sensitivity of the results to errors in the tensor orientations and T_2^{ZQ}. Our calculations suggest that when the sideband intensities are small, as is the case for the TEE $n = 1$ resonance, the results are insensitive to the tensor orientations within 15°. However, the higher order resonances ($n = 2, 3 ...$) are dependent on the relative tensor orientations and thus can potentially provide information on the relative orientation of molecular groups as well as further constraints on the bond distance. As mentioned above, T_2^{ZQ} was estimated as the sum of the single-quantum T_2's, correcting for the small contribution from the inhomogeneous applied magnetic field. The *conservative* error margin of ± 0.05 nm on the measured distance of 0.5 nm reflects an uncertainty of ± 1.4 ms in the value of T_2^{ZQ}. The simultaneous fitting of the data for the $n = 1, 2$, and 3 resonances with the same parameters raises confidence in the values used.

The results presented here demonstrate that rotationally enhanced magnetization transfer can be used to measure specific internuclear separations up to at least 0.5 nm for $^{13}C-^{13}C$ spin pairs. The main requirement for quantitative experiments is an isolated two-spin system, which can be achieved with isotopic labeling.[20] The technique is expected to be most useful for distinguishing among a set of possible distances and conformations in biochemical systems, where the menu of labeling schemes is expanding rapidly.[21-23]

Acknowledgment. F.C. is the recipient of an MIT Bantrell Fellowship. We thank the Los Alamos Stable Isotope Laboratory for the [4'-$^{13}C_1$]tyrosine from which TEE was synthesized. This research was supported by the National Institutes of Health (GM-23403, GM-25505, GM-36920, and RR-00995).

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[16] The experiments described in this note were performed on two home-built spectrometers with fields of 9.4 and 7.5 T. Sample spinning was performed in rotors and stators purchased from Doty Scientific (Columbia, SC). In all cases the spinning speed was stabilized within $\pm 0.05\%$ (see: de Groot, H. J. M.; Copié, V.; Smith, S. O.; Winkel, C.; Lugtenburg, J.; Herzfeld, J.; Allen, P. J.; Griffin, R. G. *J. Magn. Reson.* **1988**, *77*, 251). Double ^{13}C-labeled TEE was prepared by esterifying [4'-$^{13}C_1$]Tyr with [1'-$^{13}C_1$]-ethanol. Data were collected on two samples containing 100% doubly labeled TEE, diluted to either 5% or 20% in nonlabeled material. The results were indistinguishable, suggesting that the ^{13}C spin pairs were effectively well isolated from one another in both cases.

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[19] The relative orientation of the shift tensors has been determined from single-crystal experiments. Special thanks are accorded to Prof. G. S. Harbison for communicating these results prior to publication.

[20] Rotational resonance is also possible between naturally occuring spin pairs—$^{31}P-^{31}P$, $^{19}F-^{19}F$—thus circumventing the requirement of labeling. Moreover, since these nuclei have larger γ's, it should be possible to measure distances which are correspondingly larger than 0.5 nm.

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Intramolecular Catalysis of Enolization: A Probe for Stereoelectronic Effects at Carboxyl Oxygen

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Catalysis by neighboring carboxylate has been examined in a number of contexts, and the results have been summarized by Kirby.[1] The behavior of carboxylate in such systems is frequently expressed as "effective molarity" (EM), in which the rate for an intramolecular reaction is compared to that of an appropriate bimolecular case. The usefulness of EM for reactions of carboxylates has been criticized by Gandour[2] since the less basic anti lone pair (eq 1) is invariably involved in the intramolecular cases, whereas the more basic syn lone pair is likely to be involved in the bimolecular cases. Such stereoelectronic effects at carboxylate oxygen may be responsible for the low EM's observed in intramolecular general base catalyzed (igbc) processes, e.g., **1** and **2**.[2] It has not been possible to assess the importance of lone pair

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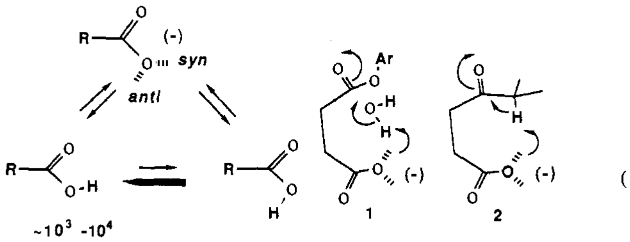
[1] Kirby, A. *J. Adv. Phys. Org. Chem.* **1980**, *17*, 183-278.

[2] Gandour, R. *Bioorg. Chem.* **1981**, *10*, 169-176; for a recent theoretical study see ref 10.

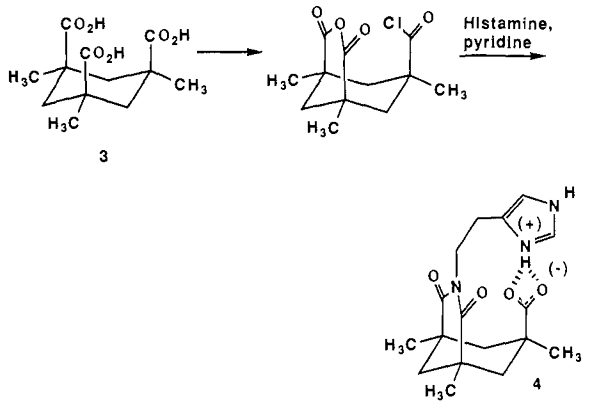
0002-7863/89/1511-4503$01.50/0



(1)

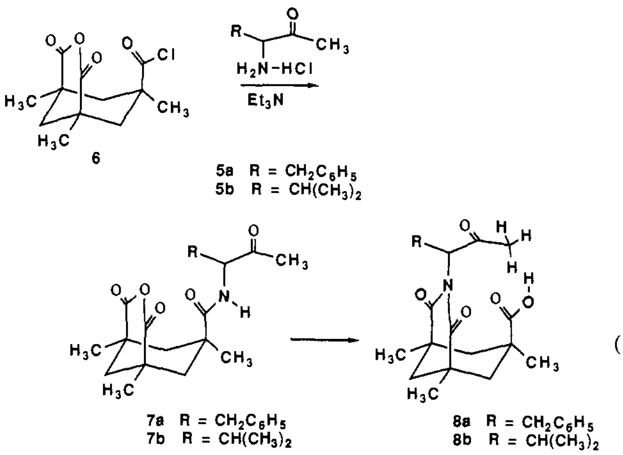
orientation because appropriate structures were unavailable.

We recently introduced a series of compounds in which carboxyl groups could be oriented with respect to other structural elements in the same molecule.[3] Specifically, condensation of Kemp's[4] triacid **3** and its derivatives with a number of amines yields structures in which carboxyl O-H groups are directed toward well-defined chemical microenvironments, e.g., **4** (eq 2). We now have prepared related systems in which the appropriate (syn) lone pair of a carboxylate is directed toward the α-hydrogen of an enolizable ketone. Here we report our preliminary results.



(2)

The new compounds are prepared by condensation of α-amino ketones **5** with the anhydride acid chloride[4] **6** (eq 3) under conditions of high dilution. The resulting amide anhydrides **7** are rearranged to the corresponding imide acids **8** by mere heating in pyridine.[5] The appropriate amino ketones are obtained as their hydrochlorides from Dakin–West reactions[6] of phenylalanine or valine, followed by hydrolysis in aqueous HCl.



5a R = $CH_2C_6H_5$
5b R = $CH(CH_3)_2$

7a R = $CH_2C_6H_5$
7b R = $CH(CH_3)_2$

8a R = $CH_2C_6H_5$
8b R = $CH(CH_3)_2$

(3)

An X-ray crystallographic study[7] gave the structure of the keto acid **8a** as shown in Figure 1. In this structure the carbonyl of the ketone is directed toward the OH group of the acid. Hydrogen bonding occurs in an intermolecular sense between the acid and the ketone of a second molecule. Molecular mechanics calculations using the AMBER[8] force field gave an energy minimized structure for the carboxylate **9a** (eq 4) in which a distance of ~3.1 Å exists between carboxylate oxygen and methyl carbon. The approach of the carboxylate in this structure is nearly normal to the plane of the ketone carbonyl. This situation resembles that prescribed

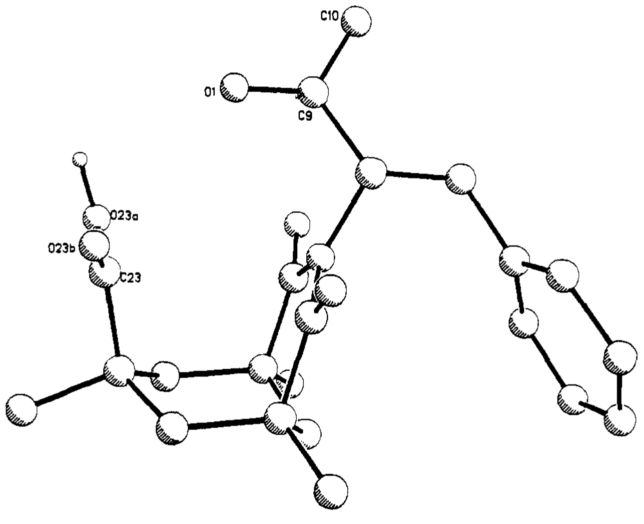
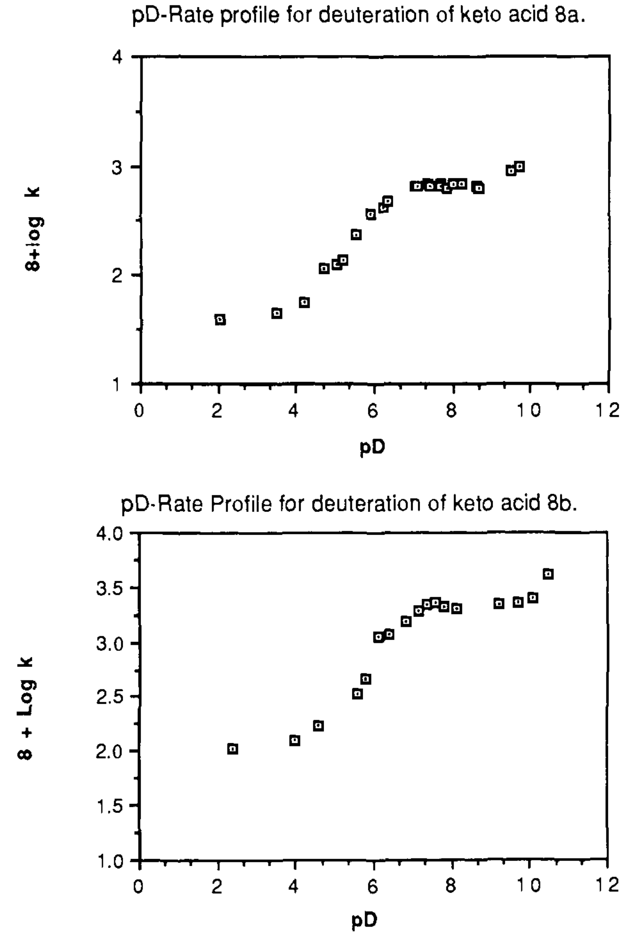


Figure 1. Crystal structure of **8a**. Only the carboxylic acid proton is shown.



pD-Rate profile for deuteration of keto acid 8a.

pD-Rate Profile for deuteration of keto acid 8b.

Figure 2. pD rate profile for deuteration of keto acid **8a** (top) and **8b** (bottom).

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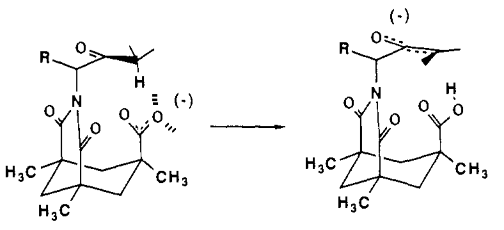
(4) Kemp, D. S.; Petrakis, K. S. *J. Org. Chem.* **1981**, *46*, 5140–5143.

(5) All new compounds were characterized by a full set of high-resolution spectra. For **8a**, mp 209–210 °C; **8b**, 193–195 °C; **11a**, 88–90 °C; **11b**, 98–100 °C.

(6) Dakin, H. D.; West, R. *J. Biol. Chem.* **1928**, *78*, 91.

(7) Details of the crystallographic study will be published elsewhere.

(8) MACROMODEL, 1.5; Still, W. C. Columbia University. Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Chio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765–784.



(4)

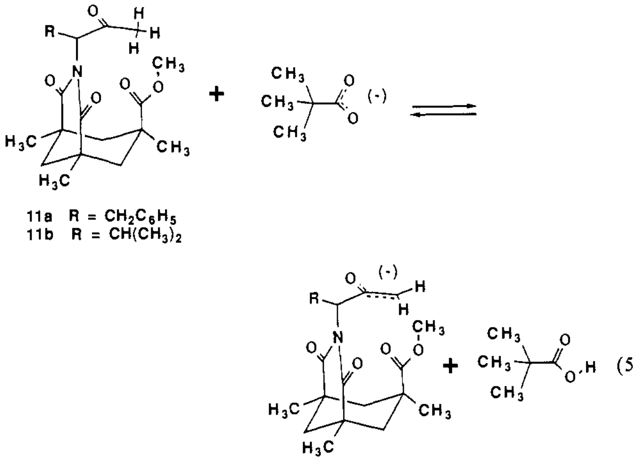
9a R = $CH_2C_6H_5$
9b R = $CH(CH_3)_2$

10a R = $CH_2C_6H_5$
10b R = $CH(CH_3)_2$

by Corey[9] for an optimal reaction rate.

The pD rate profile for enolization of this ketone is given in Figure 2. The rate of enolization was determined in 50:50 CD_3OD/D_2O at 60 °C using suitable buffers. The reaction was followed by NMR through the disappearance of the signal for the methyl ketone. The plateau region corresponds to the titrimetrically determined[10] pK_a (6.2). The kinetically determined pK_a of **8a** is 6.00 in the reaction medium. The rate is 6.6×10^{-6} s^{-1}. Parallel results were observed for **8b** ($pK_a = 6.47$ in this medium), although the rate constants for exchange of **8b** were ~3 times faster than those for **8a**. The rates observed in the plateau region were shown to be independent of concentration, as would be expected for an intramolecular process.

The calculation of an EM for the process requires an adequate bimolecular control. Accordingly, we have studied the enolization of the corresponding methyl esters **11** using pivalate[10] as the base under the same conditions, pD = 7.5, 60 °C (eq 5). The bimolecular rate measured for **11a** was 5.2×10^{-6} M^{-1} s^{-1}, and the EM involved in eq 4 is thus 7.17 M. For the valine derivative **11b** the corresponding values are $k_1 = 2.14 \times 10^{-5}$ s^{-1}, $k_2 = 3.1 \times 10^{-6}$ M^{-1} s^{-1}, and the EM is therefore 17 M.



11a R = $CH_2C_6H_5$
11b R = $CH(CH_3)_2$

(5)

These are the first cases of igbc in which the more basic lone pair of the carboxylate is involved, and the EM's are about an order of magnitude larger than most values reported[1] for the anti lone pairs. Yet, generalizations must be made with caution. It is possible that the rigidity of these structures does not permit facile intramolecular reaction. For example, proton transfer to the carboxylate would lead to a somewhat nonplanar carboxylic acid.[11] The energetic cost of such distortions has been explored with appropriate computations and is modest (2-4 kcal/mol) in the case at hand.[12] It appears that stereoelectronic effects at carboxylate oxygen do contribute to the low EM's involving igbc with these functional groups, but the full extent has yet to be determined. The current skeleton also appears suitable for the study of intramolecular nucleophile processes; such studies are in progress, and we shall report on them in due course.

Acknowledgment. We are grateful to the National Institutes of Health for support of this research and to R. Duff and Dr. W. Gordon for advice. We thank Professor K. N. Houk and Dr. Y. Li for sharing the results of their computations with us prior to publication.

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Theoretical Assessments of the Basicity and Nucleophilicity of Carboxylate Syn and Anti Lone Pairs

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Carboxyl groups at active sites of enzymes may be involved in general acid-base or nucleophilic catalysis.[1] Intramolecular reactions of carboxyl groups sometimes approach the efficiency of enzymes when the carboxyl groups are involved in intramolecular nucleophilic catalysis, but intramolecular general base catalysis usually occurs with only moderate rate acceleration as compared to the intermolecular cases.[2] Gandour pointed out that the syn lone pair of a carboxylate is $>10^4$ more basic than the anti.[3] He attributed the inefficient general base catalysis observed in most intramolecular models to poor geometric design. An anti lone pair is the base in these chemical models, whereas nature's designer uses syn lone pairs in enzymatic catalysis.[1,3] There have been several recent reports of chemical models to measure the relative hydrogen bonding and basicity of syn and anti lone pairs.[4,5] The surprisingly small differences observed prompt us to report our theoretical assessments of the stereoelectronic effects of syn and anti lone pairs of carboxyl oxygen.

The syn conformations of simple carboxylic acids and esters are 6-8 kcal/mol more stable than the anti.[6] There is less electron repulsion between lone pair electrons on the two oxygens in the syn conformation.[6] The importance of this effect in transition states or in hydrogen bonding was evaluated theoretically using ab initio molecular orbital calculations. Geometry optimizations were performed with the 3-21G or 6-31G* basis sets, and single-point energy calculations were carried out on these geometries with the 6-31G* basis set or 6-31+G* basis set for anionic systems.[7] Such calculations are known to reproduce energetics of hydrogen bonding.[8,9]

Two complexes of formate with a planar ammonia, to mimic imidazole in Zimmerman's model,[4c] are shown in Figure 1. The syn lone pair of carboxylate forms a slightly stronger hydrogen

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(3) Gandour, R. D. *Bioorg. Chem.* **1981**, *10*, 169.

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