Soft Stereoelectronic Effects at Carboxyl Oxygen

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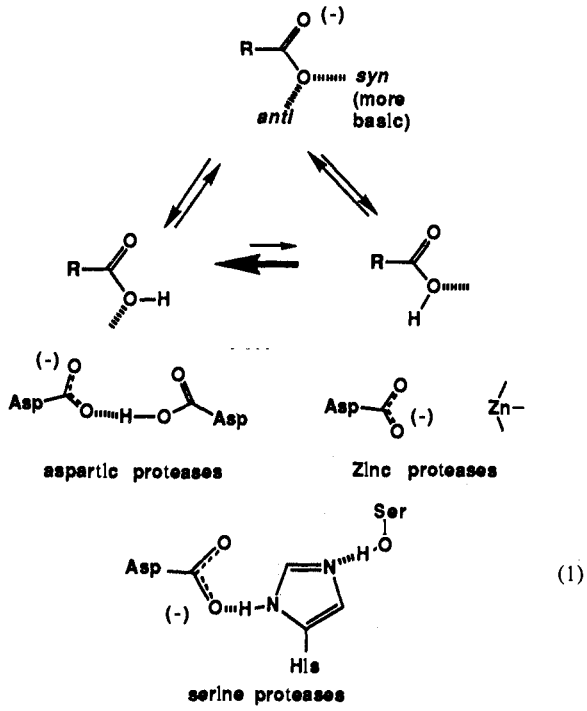
Abstract: The effects of lone pair basicity at carboxyl oxygen were examined in the context of intramolecular general-base catalysis (igbc) of enolization of ketones. Molecules synthesized from Kemp triacid units and xanthene-1,8-dicarboxylic acid were constructed in such a way that the more basic syn lone pair of a carboxylate is directed toward the α-hydrogen of an enolizable ketone. For the Kemp triacid molecules, in which an $11^1/_2$-membered transition structure is involved, the effective molarities (EM's) were 7-17 M, while the xanthene derivative showed 0.5 M for a $12^1/_2$-membered transition structure. The geometries of these processes were evaluated, and it was concluded that stereoelectronic effects in such systems may be softer than previously believed.

Introduction

Nearly a decade has passed since the stereoelectronic features of carboxyl oxygens were brought to light by Gandour,[1] and yet the quantitative evaluation of this effect has been quite elusive. The difference in energy between the two forms of formic acid (eq 1) lies at the heart of the problem. This is reported[2] as being



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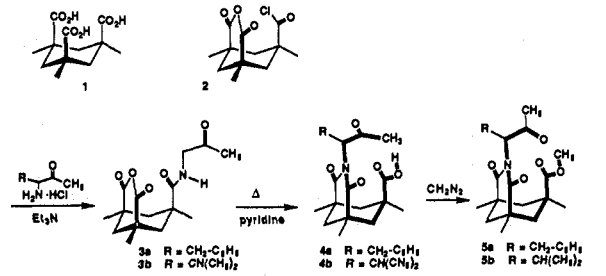
3.9 kcal/mol, whereas recent calculations by Wiberg[3] and Houk[4] suggest that, in the gas phase, 4.6 kcal separate the two forms. As Gandour[1] points out, the difference in basicity of the syn vs anti lone pairs of a carboxylate is suggested by enzyme interiors. For example, the zinc proteases,[5] triosphosphate isomerase,[6] aspartic proteases,[7] lysozyme,[8] and even the serine proteases[9] all present the more basic syn lone pair toward the substrate or the catalytic apparatus. We recently introduced carboxylic acids of the appropriate shape to study these effects as they pertained to hydrogen bonding,[10] metal ion chelation,[11] and stabilizing salt bridges[12] as found in the serine proteases. Here, we discuss the relevance of syn lone pairs in catalytic settings, specifically those involving intramolecular general-base catalysis (igbc).

Previous studies of carboxylate in this context have been summarized by Kirby.[13] Of the many examples cited for igbc, carboxyl groups involved in hydrolysis or enolization of ketones invariably involved the less basic anti lone pairs. As a result, it may be that the low effective molarities (EM's) reported for such processes are an artifact of the stereoelectronic effects, and we have sought to address this issue with our model systems.

Our initial efforts[14] involved the Kemp triacid[15] derivatives. The carboxylate function is rigidly fixed in the ground state of the structure because the plane of its atoms is parallel to those of the imide function. The syn lone pairs are directed toward the α protons of the methyl group in the ketone. More recently, we have examined a system based on the larger xanthene skeleton and find with this some results that suggest that stereoelectronic effects are somewhat softer than previously realized.

Synthesis

The keto acids were prepared from the commercially available Kemp's triacid **1**. Its acid chloride anhydride **2** was condensed with the α-amino ketones under conditions of high dilution (eq 2). The resulting amide anhydrides **3** were rearranged to the corresponding imide acids **4** by heating in pyridine. The methyl ester derivatives **5** were obtained by esterification of the corresponding keto acids with diazomethane. These were intended



(2)

to be suitable controls for reaction of the ketone with external bases such as pivalate. The 1,8-disubstituted xanthene derivative was prepared from the corresponding dicarboxylic acid[16] **6** (eq

(1) Gandour, R. *Bioorg. Chem.* **1981**, *10*, 169.
(2) Meyer, R.; Ha, J. K.; Gunthard, H. S. *Chem. Phys.* **1975**, *9*, 393. Hocking, W. H. *Z. Naturforsch.* **1976**, *31A*, 1113. Bjarnov, E.; Hocking, W. H. *Ibid.* **1978**, *33A*, 610.
(3) Wiberg, K. B.; Laidig, K. E. *J. Am. Chem. Soc.* **1987**, *109*, 5935.
(4) Li, Y.; Houk, K. N. *J. Am. Chem. Soc.* **1989**, *111*, 4505.
(5) Christianson, D. W.; Lipscomb, W. N. *Acc. Chem. Res.* **1989**, *22*, 62-69.
(6) Alber, T.; Banner, D. W.; Bloomer, A. C.; Petsko, G. A.; Philips, D.; Rivers, P. S.; Wilson, I.A. *Philos Trans. R. Soc. London, B* **1981**, *293*, 159-171.
(7) Mathew, E. W.; Braxton, H.; Davies, D. R. *J. Mol. Biol.* **1979**, *44*, 129. James, M. N. G.; Sielecki, A. R. *Ibid.* **1983**, *163*, 199.
(8) Blake, C. C. F.; Johnson, G. A.; North, A. C. T.; Phillips, D. C.; Sarma, V. R. *Proc. R. Soc. London, B* **1967**, 378.
(9) Blow, D. M.; Birktoft, J. J.; Hartley, B. S. *Nature* **1970**, *221*, 337.
(10) Rebek, J., Jr.; Duff, R. J.; Gordon, W. E.; Parris, K. *J. Am. Chem. Soc.* **1986**, *108*, 6068.
(11) Marshall, L.; Parris, K. D.; Rebek, J., Jr.; Luis, S. V.; Burguete, M. I. *J. Am. Chem. Soc.* **1988**, *110*, 5192-5193.
(12) Huff, J. B. Askew, B., Duff, R. J.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1988**, *110*, 5906. Zimmerman, S. C.; Cramer, K. D. *Ibid.* **1988**, *110*, 5904.
(13) Kirby, A. J. *Adv. Phys. Org. Chem.* **1980**, *17*, 183.
(14) Tadayoni, B. M.; Parris, K.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1988**, *111*, 4503.
(15) Kemp, D. S.; Petrakis, K. S. *J. Org. Chem.* **1981**, *46*, 5140.
(16) Nowick, J.; Ballester, P.; Ebmeyer, F.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 8902-8906.

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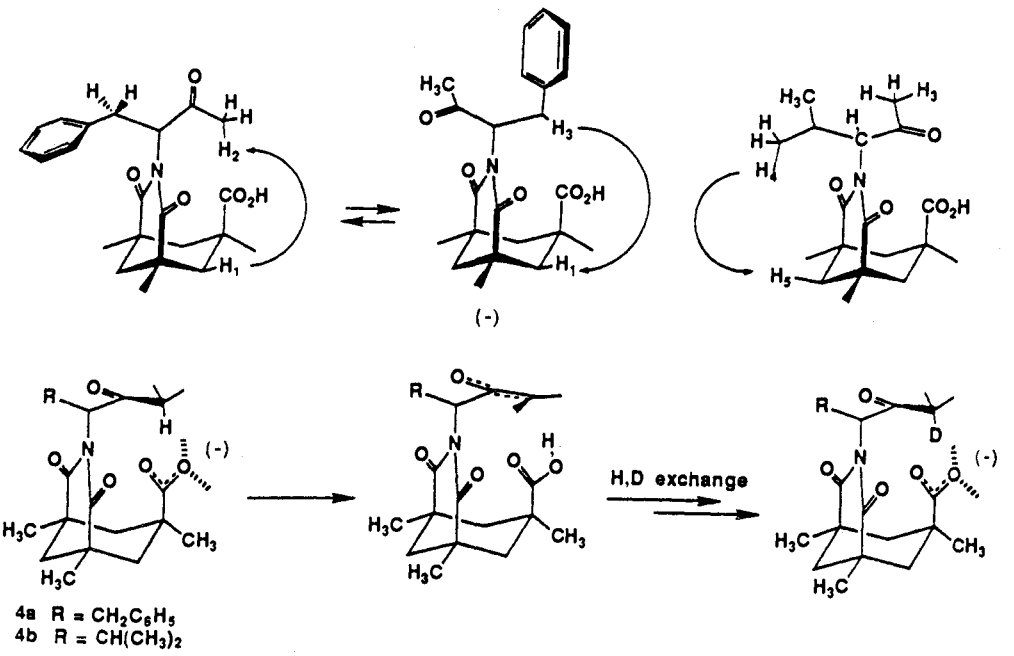
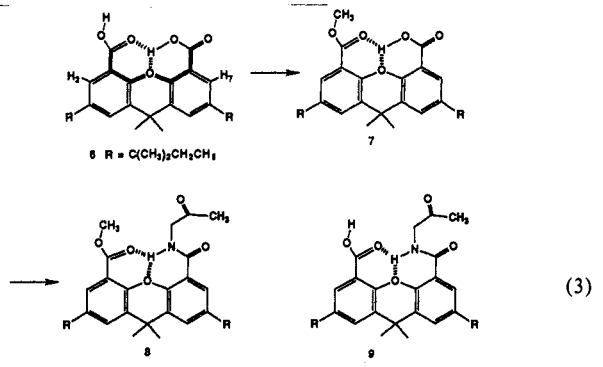
Scheme I



3). Treating the diacid with DCC, DMAP, and methanol gave the acid monoester **7**, the acid chloride of which was used to acylate amino-2-propanone to give **8**. Saponification led to the target keto acid **9**.



(3)

Structure

The solid-phase structure of the keto acid **4a** derived from phenylalanine is shown and features the methyl ketone on the same side (cis) of the imide plane as is the catalytic carboxyl (Figure 1). The conformation of this keto acid was explored in solution by use of nuclear Overhauser effect (NOE) experiments. Irradiation of the most downfield equatorial proton H_1 resulted in signal enhancements of the methyl ketone H_2 and aromatic protons, whereas irradiation of the benzylic proton H_3 gave enhancement of H_1 (Figure 1). Molecular mechanics calculations using MACROMODEL 2.5[17] were performed by rotating around the appropriate single bonds. The trans isomer was suggested to be some kilocalories more stable than the cis isomer (as the carboxylate) from these minimizations. The approach of the carboxylate can be nearly normal to the plane of the ketone carbonyl in such renderings, a situation that resembles that prescribed by Corey[18] for optimal reaction rates (Scheme I). A minimized structure indicated a distance of ~3.1 Å exists between carboxylate oxygen and the methyl carbon. In the valine series, the bulky isopropyl group was expected to increase the population of the cis conformation required for reaction. Indeed, only this conformation was observed through the appropriate NOE experiments.

Scheme II

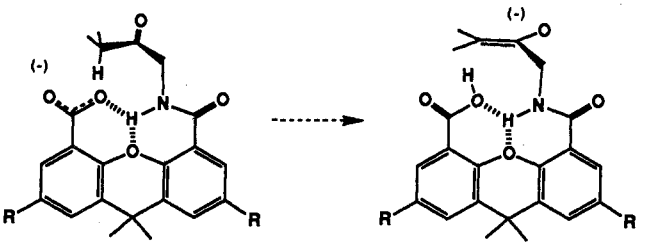


Table I. Equilibrium Acidities of Xanthene and Kemp Triacid Derivatives at 25 °C in Methanol/Water

acid	pK_{a1}	pK_{a2}
6	5.54 ± 0.02	7.62 ± 0.02
7	5.80 ± 0.06	
9	4.75 ± 0.02	
4a	6.23 ± 0.02	
4b	6.23 ± 0.02	

In the xanthene series, there is evidence for extensive intramolecular hydrogen bonding between the amide NH and the xanthene nuclear oxygen.[16] This was indicated by downfield shifts of both ortho hydrogens, H_2 and H_7, and is supported by the pK_a measurements of various derivatives (see the following text). These hydrogen bonds hold many of the functional groups of the system in the aromatic plane. Molecular modeling showed that the methyl ketone can assume a conformation in which a nearly ideal, linear intramolecular proton transfer can be achieved (Scheme II). A planar carboxylic acid can be the initial product of such a transfer, while the methyl ketone can adjust its orientation so that the breaking C–H bond is nearly perpendicular to the plane of the carbonyl, thus enabling maximum resonance overlap in the newly formed enolate. In addition, the less basic anti lone pair of the carboxylate is internally solvated by the amide N–H. Equilibrium pK_a measurements were performed in methanol/H_2O mixtures for these materials.[19] The results are shown in Table I. The Kemp triacid derivatives are unremarkable, where the xanthene

(17) MACROMODEL 2.0: 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.

(18) Corey, E. J.; Sneen, F. A. *J. Am. Chem. Soc.* **1956**, *78*, 6269.

(19) The pK_a's were measured by using the method suggested by W. E. Gordon. For more information, see: Gordon, W. E. *J. Phys. Chem.* **1979**, *83*, 1365. Gordon, W. E. *J. Anal. Chem.* **1982**, *54*, 1595.

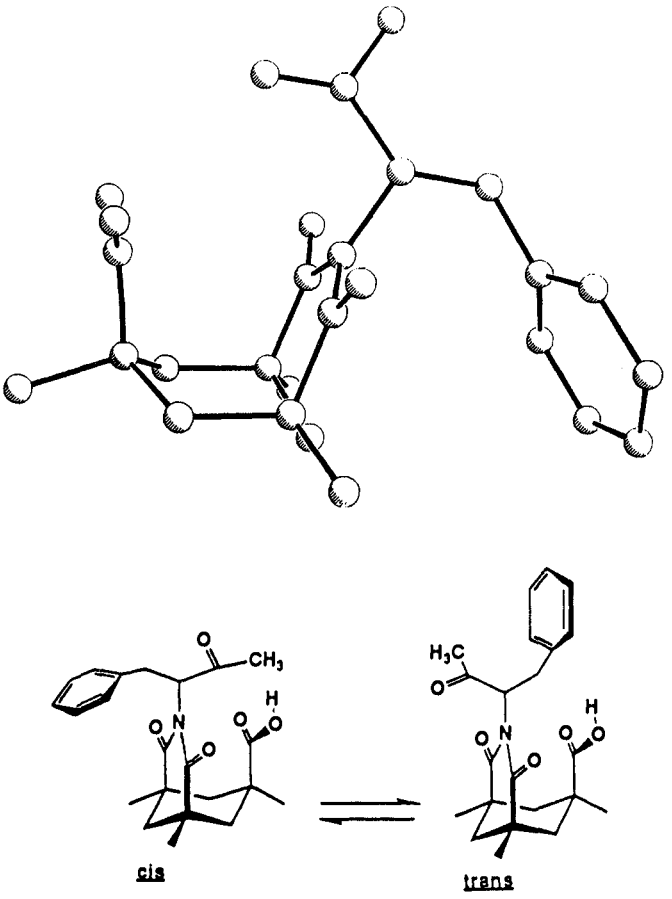


Figure 1.

acids reflect the effects of intramolecular hydrogen bonding. Thus, the large ΔpK_a between the two ionizations of **6** ($\sim$2 units) is due to the difficulty in breaking the intramolecular hydrogen bond in the monoanion and electrostatic repulsion in the dianion. The easy ionization of the monoacid amide **9** reflects the solvation of the carboxylate by the internal NH, while the difficult ionization of the monoester **7** reflects breaking of the intramolecular hydrogen bond.

Kinetics of Exchange

The kinetics of deuteration of the active methyl groups of the keto acids were determined in CD_3OD/D_2O (50:50 v/v) at 60 °C with use of suitable buffers. Four kinetic terms contribute to the enolization process under these conditions (dilute solutions): (1) the reaction of the substrate with the hydronium ion; (2) the reaction of the substrate with hydroxide ion; (3) the reaction of the substrate with the undissociated carboxylic acid; and (4) the reaction of the substrate with carboxylate anion.

The deuteration reaction (Scheme I) was treated as a series of parallel first-order reactions.[20] The first-order rate constants were plotted against pD (Figure 2).[21]

The precision of the first-order rate constants is limited by systematic errors of almost 5% involved in data collection by NMR.

The carboxylic acid **4a** has a pK_a of 6.23 ± 0.02 in $MeOH/H_2O$ and may be expected to catalyze the enolization reaction necessary for deuteration either in its protonated or in its ionized form. The sigmoid curve indicates that the ionized form of the acid is more efficient as an internal catalyst than is the carboxylic acid. The curve has an inflection point at pD 6.0 near the pK_a of the keto acid. The plateau on the curve shows the absence of catalysis by external bases (e.g., buffer).

The pD dependence of the deuteration rate constant may be written as

$$k_{obs} = (K_a/(K_a + [D^+]))k_{COO^-} + ([D^+]/(K_a + [D^+]))k_{COOD}$$

where k_{COO^-} is the rate constant for the anionic form of the keto acid, k_{COOD} is the rate constant of the protonated form of the keto acid, and K_a is the ionization constant of the keto acid. This expression is simply derived from the Henderson–Hasselbalch equation, modified to describe the dependence of k_{obs} on k_{COOD}, k_{COO^-}, and the pD.[22] For keto acid **4a**, the mean of the pD-independent region between 7.02 and 8.70 gave the catalytic constant due to intramolecular general-base catalysis, $k_{COO^-} = (6.6 \pm 0.03) \times 10^{-6}\ s^{-1}$. The rates observed in the plateau region were shown to be independent of the concentration, as would be expected for an intramolecular process. Parallel results were observed for **4b** ($pK_a = 6.23$ in $MeOH/H_2O$); $k_{COO^-} = 2.14 \times 10^{-5}\ s^{-1}$. Although the anions of the two substrates **4a** and **4b** have nearly the same base strength, deuteration of **4b** is faster. Presumably, the isopropyl group of the latter preorganizes the reacting groups with respect to one another.

The general-acid catalysis due to internal carboxylic acid may be determined from the equation

$$k_{obs} = ((k_{COO^-} - k_{obs})/[D^+])K_a + k_{COOD}$$

For the keto acid **4a**, a plot of k_{obs} against $(k_{COO^-} - k_{obs})/[D^+]$ from pD 3.5 to 6.23 was linear (Figure 3). Accordingly, the slope of the line is the kinetically determined ionization constant of 6.00 ± 0.03 for keto acid **4a**,[23] and the intercept gives $k_{COOD} = (7.22 \pm 0.03) \times 10^{-7}\ s^{-1}$. The rate due to specific-base catalysis can be determined by a plot of two rate constants obtained at pD 9.5 and 9.7 vs hydroxide ion concentrations, $k_{OD^-} = (5.44 \pm 0.04) \times 10^{-2}\ M^{-1}\ s^{-1}$. Finally, specific-acid catalysis is obtained by a single experiment at pD 0.7 measured in 0.71 M perchloric acid; $k_{D^+} = (2.67 \pm 0.02) \times 10^{-5}\ M^{-1}\ s^{-1}$. These rate constants are, of course, of limited accuracy. The overall pseudo-first-order rate constant, including the highly acidic and highly basic regions, is given by the following:

$$k_{obs} = k_{D^+}[D^+] + ([D^+]/(K_a + [D^+]))k_{COOD} + (K_a/(K_a + [D^+]))k_{COO^-} + k_{OD^-}[OD^-]$$

Upon insertion of the determined rate constants, this gives the expression for the phenylalanine derivative **4a**

$$k_{obs} = 2.67 \times 10^{-5}[D^+] + 7.22 \times 10^{-7}[D^+]/(K_a + [D^+]) + 6.6 \times 10^{-6}K_a/(K_a + [D^+]) + 5.44 \times 10^{-2}[D^+]$$

and for the valine derivative **4b**

$$k_{obs} = 2.46 \times 10^{-5} + 1.25 \times 10^{-6}[D^+]/(K_a + [D^+]) + 2.14 \times 10^{-5}K_a/(K_a + [D^+]) + 4.19 \times 10^{-2}[OD^-]$$

A comparison of k_{OD^-} for substrates **4a** and **4b** with the base-catalyzed deuteration of 2-butanone[20] at position 1 ($4.50 \times 10^{-2}\ M^{-1}\ s^{-1}$ at 30 °C) shows that the Kemp triacid derivatives react slower than the simple butanone. This difference can be attributed to electrostatic repulsion of the deuteroxide ion by the carboxylate anion of **4a** and **4b**.

The deuterium isotope effect was measured for substrate **4b**; $k_{C-H}/k_{C-D} = 5.2$, where k_{C-D} is proton exchange of the deuterated keto acid at 60 °C in CH_3OH/H_2O (50:50, v/v) at pH 7.4. The magnitude of the isotope effect clearly shows that the rate-determining step for general-base-catalyzed enolization of **4b** involves breaking of the C–H bond. Presumably, internal return is slower than the exchange of the carboxylic acid proton with solvent.

(20) Rappe, C. *Acta Chem. Scand.* **1966**, *20*, 2236.

(21) pD = pH + 0.4. This relationship has been found in water; see: Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188. It has, however, not been tested in CD_3OD/D_2O mixture and is therefore an assumption.

(22) Jencks, W. P. *Catalysis in Chemistry and Enzymology*, 2nd ed.; Dover Publication: New York, 1987; p 583.

(23) The pK_a that is obtained kinetically in CD_3OD/D_2O is different from that obtained titrimetrically in H_2O/CH_3OH. The ionization constants of acids in H_2O and D_2O are related by the equation $\Delta pK_a = 0.41 + 0.02pK_{AH}$, where $\Delta pK_a = pK_{AD} - pK_{AH}$. These equations were suggested by: Bell, R. P. *The Proton in Chemistry*; Cornell University: Ithaca, NY, 1959; p 188. In a number of cases, large discrepancies have been observed; for example, see: McDougel, A. O.; Long, F. A. *J. Phys. Org. Chem.* **1962**, *66*, 429.

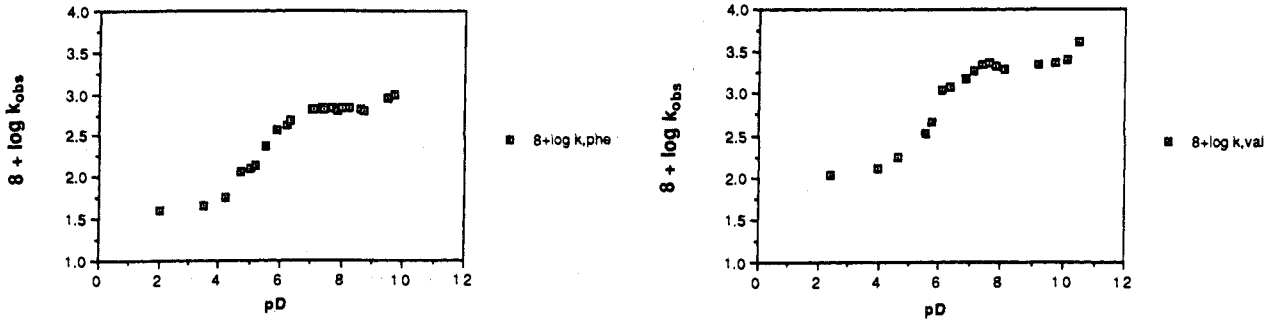


Figure 2. pD–rate profiles for the deuteration of keto acids **4a** (left) and **4b** (right).

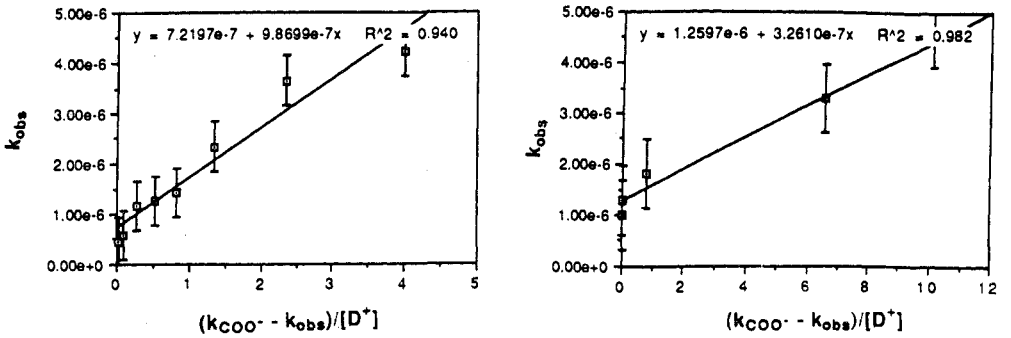
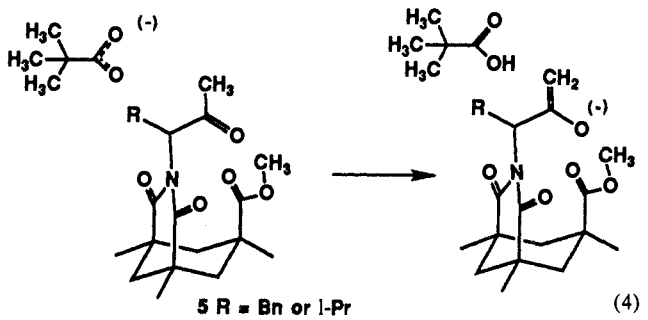


Figure 3. Kinetically determined pK_a (slope) and general-acid catalysis (intercept) for the deuteration of keto acids **4a** (left) and **4b** (right) at 60 °C in CD_3OD/D_2O.

Table II. Effective Molarities

keto ester	pD	[buffer], M	k_2, M^{-1} s^{-1}	EM, M
6a	7.53	0.088	0.90×10^{-6}	7.0
6b	7.90	0.097	1.25×10^{-6}	17

To assess the rate acceleration due to the carboxylate function in the intramolecular case, the effective molarity of carboxylate may be calculated. Effective molarity is formally defined as the concentration of the catalytic group required to make an intermolecular reaction proceed at the observed rate of a similar intramolecular process.[13] The significance of the intramolecular general-base catalysis of keto acids **4a** and **4b** was investigated by comparing the rate of deuteration of these substrates with the rate of deuteration of their methyl esters **5a** and **5b**. These undergo intermolecular exchange in presence of the sodium salt of trimethylacetic acid (eq 4). Since the basicity of pivalate (pK_a = 6.40 ± 0.02) differs from the carboxylate groups in the substrates **4a** and **4b** under identical conditions, bimolecular rate constants in Table II were corrected for the basicity by use of the Bronsted equation[24] and a β value of 0.80. The percentage of free base in the buffer was 88%. These bimolecular reactions were treated as pseudo-first-order reactions. The rate constants and the EM's are given in Table II.



5 R = Bn or i-Pr (4)

The exchange reactions of **9** were studied by use of the same experimental conditions; again, the reaction was followed by NMR

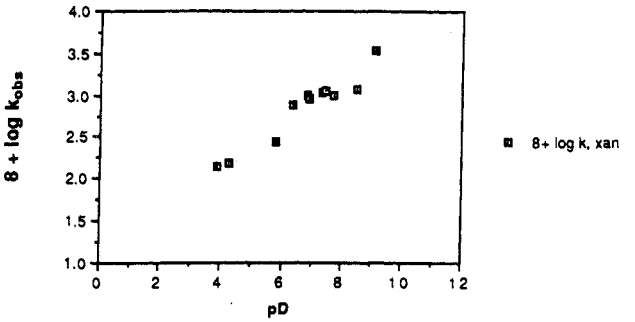
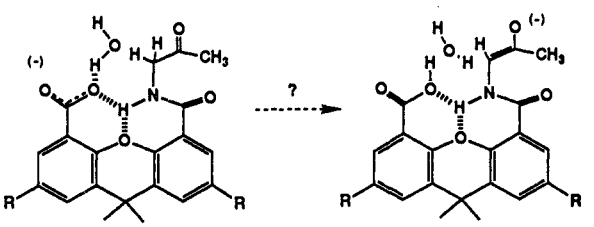


Figure 4. pD–rate profile for deuteration of keto acid **9** in CD_3OD/D_2O at 60 °C, μ = 0.10 M.

through the disappearance of the CH signals of the methyl ketone. The intramolecular rate constant was $k_{COO^-} = 10.36 \times 10^{-6}$ s^{-1}. The pD–rate profile is shown in Figure 4. Slow reaction times and solubility problems precluded examining the reaction at lower pH's, but the pH-independent region that represents intramolecular catalysis by the carboxylate is quite clear.

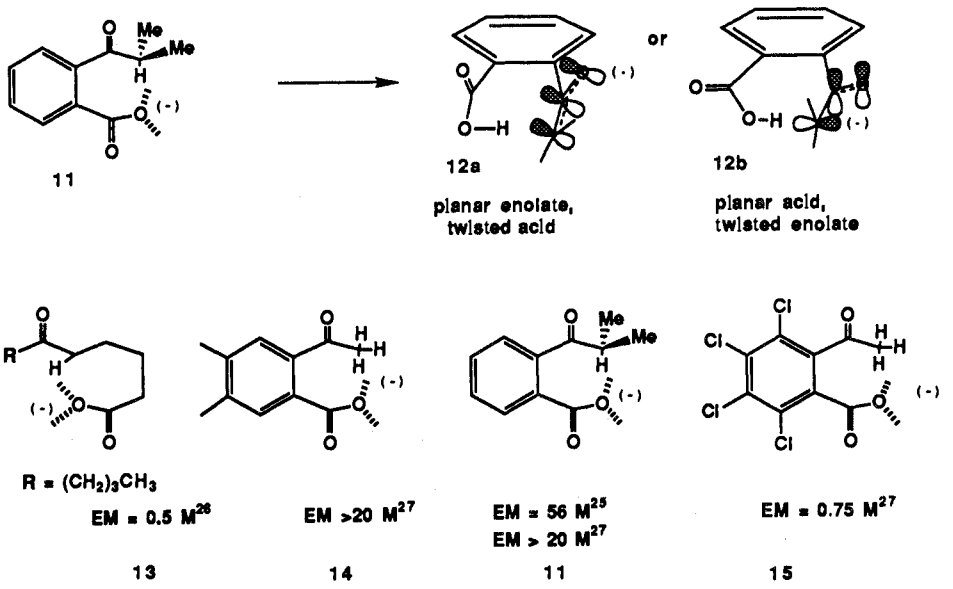
We were surprised to find that not only the methyl group exchanged under these conditions. The *methylene* protons also showed exchange, but at a somewhat slower rate, $k_{COO^-} = 3.55 \times 10^{-6}$ s^{-1}. That the methylene exchanges at all is understandable by its enhanced acidity through the inductive effect of the α-arylamido group. However, *no amount of modeling produced a transition structure for proton transfer that was geometrically reasonable*, with respect to either a stable carboxylic acid product or the trajectory for the proton transfer. One possibility is an "outer sphere" reaction mediated by a water molecule as shown in eq 5 (we thank Prof. R. Breslow for this suggestion).



(5)

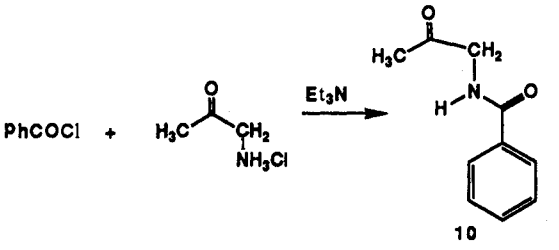
(24) Bronsted, J. N.; Guggenheim, E. A. *J. Am. Chem. Soc.* **1927**, *49*, 2554. Bell, J. N.; Lidwell, O. M. *Proc. R. Soc. London* **1940**, *A176*, 88.

Scheme III



The exchange of the methyl ketone **9** occurred at a rate quite comparable to our previous system **4a**, even though the carboxylate is a weaker base by nearly 2 orders of magnitude (pK_a 4.7 vs 6.2). Also, more degrees of rotational freedom exist in the xanthene ketone than in the original Kemp triacid derivative. Therefore, the exchange rate must also be a result of the enhanced acidity of the new methyl ketone.

Low solubility prevented the use of the methyl ester methyl ketone **8** as the substrate for the bimolecular process. Instead, a model substance **10**, the benzoyl derivative of the amino-2-propanone was prepared (eq 6). The base-catalyzed exchange of this substance was studied with use of pivalate buffers in the pD-independent region. Good kinetic behavior for exchange was observed under pseudo-first-order conditions.



(6)

It was shown that the methyl hydrogens of the benzoyl compound exchanged some 19 times *slower* than the methylene hydrogens (Figure 5), again reflecting the enhanced acidity of the latter. This number is corrected for the number of protons involved; the rates are 8.5×10^{-3} M^{-1} s^{-1} for the methylene vs 4.5×10^{-4} M^{-1} s^{-1} for the methyl hydrogens. The calculated effective molarity for the deuteration of methyl protons of **9** is therefore only 0.5 M. This is still a reasonably large number considering the $12^1/_2$-membered transition state involved. The effective molarity involved in the methylene protons can be calculated to be 1/40th that of the methyl group, since the decrease in rate per hydrogen was a factor of 2 despite the nearly 20-fold enhanced acidity. This 40-fold drop, which corresponds to $\Delta\Delta G^{\ddagger}$ of $\sim$2.2 kcal/mol is a measure of the energetic price paid for poor reaction stereoelectronics.

Discussion and Perspective

In 1965, Bender,[25] in his study of ketone enolization in *o*-isobutyroylbenzoic acid (**11**), established an effective molarity of 56 M for intramolecular catalysis. In this system, the reactants are hardly in a favorable arrangement for enolization: either the nonplanar carboxyl **12a** (Scheme III) or a poorly stabilized enolate **12b** must be the initial product. Moreover, the latter process must involve the less basic lone pair. This study is therefore all the more remarkable because it reports the highest EM for any intramolecular general-base-catalyzed process. Structurally related systems, **13**–**15** are less effective.[26,27] For example, the flexible **13** shows a low EM even though its exocyclic transition structure permits stable geometries for both carboxyl and enolate functions. The relatively low EM's observed in the present systems–despite their apparent stereoelectronic propriety–deserve some comment.

The most relevant theoretical treatment is due to Li and Houk,[4] who studied the deprotonation of acetaldehyde with formate ion in the gas phase with computational methods (Figure 6). In the early stage of the reaction, the syn deprotonation was only slightly preferred over the anti deprotonation, but as the reaction proceeded, the anti conformation became favored. This was attributed to electrostatic repulsion between the enolate ion and the distal oxygen. This repulsive interaction was diminished when the acid was anti. This effect was expected to disappear in the enol, and upon the solvation of the enolate, the syn deprotonation was favored by 5 kcal/mol.

According to these calculations, the energy barrier involving the syn lone pair is just 1.0 kcal/mol more favorable than anti lone pair in an intermolecular reaction. The stereoelectronics of the oxygen lone pairs do not appear to be a critical parameter; rather, electrostatics are involved and distance is more important than orientation. In **4**, proton transfer to the carboxylate leads to a somewhat nonplanar carboxyl acid (Figure 7) that could slow the rate of the intramolecular reaction. The magnitude of these effects was estimated to be 2–4 kcal/mol. However, the energetic cost of the dreadful reaction trajectory for the exchange of the CH_2 of the xanthene **9** was less than 2.5 kcal/mol. In retrospect, the derivatives **4a** and **4b**, in which only slight distortions were calculated, seem adequate to assess the lone pair effect. The rate retardation due to nonideal geometries was probably quite minimal, and the effective molarity is a reasonable measure of Gandour's hypothesis.[1]

In summary, the current results, along with the earlier ones of Bell[26,27] and Bender,[25] suggest a new interpretation: *stereoelectronic effects in igbc processes may be much softer than previously appreciated.* This view is further supported by recent observations concerning proton relay[28] and displacement reactions.[29] A similar conclusion was reached earlier by Kirby[30] in discussing the unimportance of the syn lone pair, but perhaps the right experiments have yet to be performed.

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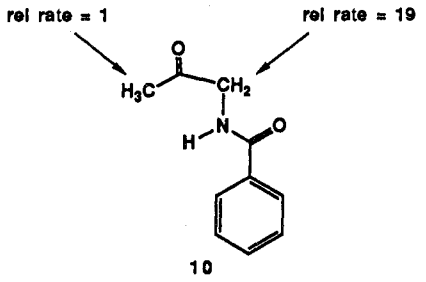

Figure 5.

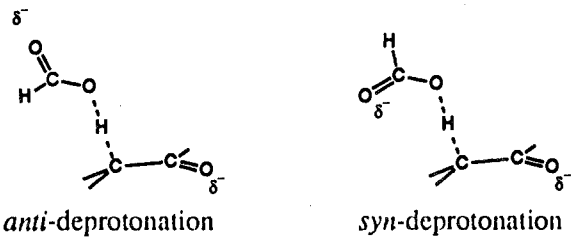

Figure 6. Deprotonation of acetaldehyde by formate anion.

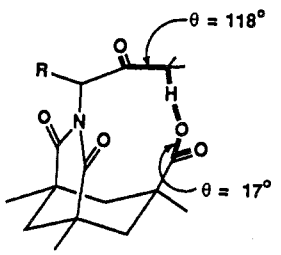

Figure 7. Nonplanar carboxylic acid that forms during the enolization process of keto acid **4a**.

Experimental Section

Buffer solutions were made in D_2O by partial neutralization of 0.1 M potassium hydrogen phthalate or potassium dihydrogen phosphate with 0.1 M sodium hydroxide. Tris(hydroxymethyl)aminomethane (Tris, 0.1 M) was neutralized with 0.1 M HCl. Sodium bicarbonate (0.05 M) was neutralized with 0.1 M sodium hydroxide.

A reaction mixture contained keto acid (1.17×10^{-2} M) and sodium perchlorate (ionic strength 0.1 M) in deuterated methanol/buffer (50:50).

A sample of 0.5 mL of this reaction was added into the NMR tube and frozen. The frozen tube was left on the vacuum line and sealed.

The disappearance of methyl protons of the ketone at 2.19 ppm was followed with use of a Bruker 300-MHz spectrometer. The solvent peak HOD was used as lock signal. Since the equatorial protons did not change under the reaction conditions, the peak corresponding to the most downfield equatorial protons at 2.67 ppm was used as reference. The NMR tube containing the sample was placed in an oil bath that was thermostated at 60.0 ± 0.2 °C. The reactions were ordinarily followed up to 75% completion except for very slow reactions, which were followed up to the first half-life.

Anhydride Amide 3a. A 331.95-mg (1.28-mmol) sample of anhydride acid chloride **2** was suspended in dry methylene chloride in a 250-mL three-neck flask equipped with a drying tube. A 300-mg (1.50-mmol) portion of the amino ketone–HCl from phenylalanine was dissolved in 150 mL of dry methylene chloride. In a separate flask, 363 mg (2.9 mmol) of triethylamine (freshly distilled from calcium hydride) was dissolved in 50 mL of dry methylene chloride. These two solutions were placed in separate dropping funnels and were simultaneously added dropwise to the acid chloride solution. This solution was allowed to stir for 1 h at room temperature. The reaction mixture was then washed with 10% citric acid and dried over sodium sulfate. The solvent was removed by rotary evaporation at room temperature, and the compound was purified by recrystallization from methylene chloride/hexanes: yield 400 mg (81%) of **3a** in the form of white crystals; mp 134–135 °C. The spectral data were the following: ^{1}H NMR (CD_2Cl_2, 300 MHz): δ 1.16 (s, 3 H), 1.27 (d, 1 H, J = 14 Hz), 1.32 (d, 2 H, J = 15 Hz), 1.36 (s, 3 H), 1.38 (s, 3 H), 2.03 (d, 1 H, J = 13.6 Hz), 2.08 (s, 3 H), 2.56 (d, 1 H, J = 14.4 Hz), 2.65 (d, 1 H, J = 14.2 Hz), 3.02 (dd, 1 H, J_1 = 7.95 Hz, J_2 = 5.94 Hz), 3.18 (dd, 1 H, J_1 = 4.47 Hz, J_2 = 9.48 Hz), 4.80 (m, 1 H), 6.25 (d, 1 H, J = 6.18 Hz), 7.14 (d, 2 H, J = 6.5 Hz), 7.27 (m, 3 H); IR (CH_2Cl_2) 3395, 3020, 2850, 1456, 1423, 1327, 1221, 1182, 1797, 1763, 1662, 1080, 1010, 702, 662 cm^{-1}; MS m/e 385 (M^+), 342, 297, 258, 212, 167, 147 amu; HRMS for $C_{22}H_{27}O_5N$ calcd 385.1889, found 385.1889.

Imide Acid 4a. A sample of 192 mg (0.5 mmol) of anhydride amide **3a** was dissolved in 5 mL of dry pyridine and the solution heated under reflux for 2 h. The solvent was removed under reduced pressure, and the yellow oil that remained was dissolved in methylene chloride. The solution was washed with 10% aqueous citric acid and then dried over sodium sulfate. The solvent was removed by evaporation. A white solid remained that was purified by recrystallization from methanol/water: yield 85%; mp 209–210 °C. The spectral data were the following: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.92 (s, 3 H), 0.98 (d, 2 H, J = 13.4 Hz), 1.03 (d, 1 H, J = 14 Hz), 1.09 (d, 1 H, J = 14.2 Hz), 1.11 (s, 3 H), 1.18 (s, 3 H), 2.19 (s, 3 H), 2.62 (d, 1 H, J = 10.3 Hz), 2.67 (d, 1 H, J = 10.3 Hz), 3.13 (dd, 1 H, J_1 = 9.6 Hz, J_2 = 4.3 Hz), 3.40 (dd, 1 H, J_1 = 6.8 Hz, J_2 = 7.1 Hz), 5.38 (dd, 1 H, J_1 = 6.9 Hz, J_2 = 2.7 Hz), 7.11 (d, 2 H, J = 6.6 Hz), 7.23 (m, 3 H); IR ($CHCl_3$) 2970, 2932, 1728, 1705, 1678, 1462, 1429, 1381, 1367, 1319, 1221, 1022, 945, 852 cm^{-1}; MS m/e 385 (M^+), 342, 297, 268, 212, 167, 147, 121 amu; HRMS for $C_{22}H_{27}O_5N$ calcd 385.1889, found 385.1889.

Anhydride Amide 3b. The Dakin–West reaction of valine with acetic anhydride and pyridine did not proceed as smoothly as did the reaction of phenylalanine. A sample of 3.0 g of valine was heated under reflux in acetic anhydride (10.0 mL) and pyridine (10.0 mL) overnight. Then, it was neutralized with saturated sodium bicarbonate solution and extracted (5 × 50 mL) with methylene chloride. The pooled organic solvent was removed and dried over sodium sulfate. The crude reaction mixture was purified by recrystallization from methylene chloride/hexanes and yielded 2.0 g (49.0%) of white crystals, mp 63 °C. The spectral data were the following: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.80 (d, 3 H, J = 6.9 Hz), 1.01 (d, 3 H, J = 9.3 Hz), 2.04 (s, 3 H), 2.2 (s, 3 H), 2.24 (m, 1 H), 4.69 (dd, 1 H), 6.05 (broad, 1 H); IR ($CHCl_3$) 3296, 2965, 2934, 2876, 1718, 1653, 1466, 1431, 1371, 1307, 1184 cm^{-1}; MS m/e 157 (M^+), 149, 133, 124, 114, 72, 43 amu; HRMS for $C_8H_{15}O_2N$ calcd 157.1103, found 157. 1103.

A sample of 800 mg of this solid was dissolved in 2 N HCl and the resultant mixture heated under reflux for 48 h. The solvent was removed under reduced pressure. The hydrochloride was obtained in 95% yield, mp 150–153 °C. The spectral data were the following: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.07 (d, 3 H, J = 7.00 Hz), 1.24 (d, 3 H, J = 7.07 Hz), 2.32 (s, 3 H), 2.47 (m, 1 H), 4.24 (m, 1 H), 8.53 (broad s, 3 H); IR ($CHCl_3$) 2913–2967, 1727, 1521, 1405 cm^{-1}; MSFAB m/e for $C_6H_{12}ONCl$ calcd 151.0526, found 151.0526.

The preparation of **3b** was carried out under the same conditions as those used for anhydride amide **3a**. It was purified by recrystallization from toluene: yield 75%; mp 154–157 °C. The spectral data were the following: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.79 (d, 3 H, J = 6.96 Hz), 1.0 (d, 3 H, J = 6.75 Hz), 1.25 (s, 3 H), 1.28 (d, 1 H, J = 11.0 Hz), 1.34 (s, 3 H), 1.35 (s, 3 H), 1.36 (d, 2 H, J = 13.2 Hz), 2.02 (d, 1 H, J = 13.5 Hz), 2.17 (m, 1 H), 2.22 (s, 3 H), 2.62 (d, 1 H, J = 14.1 Hz), 2.67 (d, 1 H, J = 13.2 Hz), 4.57 (dd, 1 H), 6.22 (d, 1 H); IR (CH_2Cl_2) 3399, 2970, 2936, 1799, 1767, 1716, 1466, 1423, 1387, 1306, 1215 cm^{-1}; MS m/e 337 (M^+), 322, 309, 294, 248, 220, 167 amu; HRMS for $C_{18}H_{27}O_5N$ calcd 337.1889, found 337.1889.

Imide Acid 4b. A sample of 250.0 mg (0.74 mmol) of anhydride amide **3b** was dissolved in 3 mL of pyridine and the solution heated under reflux overnight. The pyridine was removed by rotary evaporation, and the crude reaction mixture was dissolved in methylene chloride, washed with 10% citric acid solution, and then dried over sodium sulfate. Purification was accomplished by recrystallization from chloroform/hexanes and yielded 185.0 mg (74%) of white crystals, mp 193–195 °C. The spectral data were the following: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.69 (d, 3 H, J = 6.8 Hz), 0.96 (d, 3 H, J = 6.6 Hz), 1.15 (d, 1 H, J = 7.0 Hz), 1.20 (d, 1 H, J = 7.0 Hz), 1.24 (s, 6 H), 1.29 (s, 3 H), 1.36 (d, 1 H, J = 13.8 Hz), 1.94 (d, 1 H, J = 13.2 Hz), 2.15 (s, 3 H), 2.50 (m, 1 H), 2.73 (d, 1 H, J = 14.3 Hz), 2.81 (d, 1 H, J = 14.2 Hz), 4.66 (d, 1 H, J = 8.10 Hz); IR ($CHCl_3$) 3500 (broad), 2860–2967, 1727, 1705, 1683, 1464, 1429, 1365, 1213, 1196 cm^{-1}. MS m/e 337 (M^+), 322, 294, 278, 220, 194, 167 amu; HRMS for $C_{18}H_{27}O_5N$ calcd 337.1889, found 337.1888.

Imide Methyl Ester 5a. A 200.0-mg sample of imide acid **4a** was dissolved in ether/methylene chloride (50:50), and a solution of diazo-

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methane was added slowly until a yellow solution persisted. The excess diazomethane was quenched with acetic acid, and the solvent was removed at reduced pressure. The pale yellow oil obtained was crystallized from methanol/water to give 180 mg (90%) of white crystals, mp 88-90 °C. The spectral data were the following: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.06 (s, 3 H), 1.12 (d, 1 H, J = 14.4 Hz), 1.17 (d, 2 H, J = 12.5 Hz), 1.20 (s, 3 H), 1.26 (s, 3 H), 1.53 (d, 1 H, J = 13.1 Hz), 2.03 (s, 3 H), 2.75 (d, 1 H, J = 12.9 Hz), 2.80 (d, 1 H, J = 13.2 Hz), 2.95 (dd, 1 H, J_1 = 5.9 Hz, J_2 = 8.6 Hz), 3.44 (dd, 1 H, J_1 = 8.10 Hz, J_2 = 6.4 Hz), 3.60 (s, 3 H), 5.25 (dd, 1 H, J_1 = 5.9 Hz, J_2 = 2.10 Hz), 7.22 (m, 5 H); IR (CH_2Cl_2) 2966, 2932, 1728, 1680, 1496, 1462, 1429, 1383, 1319, 1207, 1169, 1093, 1020, 736, 702 cm^{-1}; MS m/e 399 (M^+), 356, 340, 296, 268, 212, 194, 181 amu; HRMS for $C_{23}H_{29}NO_5$ calcd 399.2046, found 399. 2046.

Imide Methyl Ester 5b. This compound was synthesized and purified by use of the same conditions as those used for **5a**. The yield was 75%; mp 98-100 °C. The spectral data were the following: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.75 (d, 3 H, J = 6.8 Hz), 0.93 (d, 3 H, J = 6.7 Hz), 1.2 (d, 2 H, J = 14.8 Hz), 1.22 (s, 3 H), 1.27 (s, 3 H), 1.31 (s, 3 H), 1.37 (d, 1 H, J = 13.2 Hz), 1.98 (d, 1 H, J = 13.23 Hz), 2.12 (s, 3 H), 2.49 (m, 1 H), 2.79 (d, 1 H, J = 14.5 Hz), 2.86 (d, 1 H, J = 14.31 Hz), 3.62 (s, 3 H); IR (CH_2Cl_2) 3026-2947, 1734, 1718, 1684, 1489, 1458, 1431, 1363, 1317, 1259, 1172 cm^{-1}. MS m/e 351 (M^+), 336, 308, 348, 220, 194, 121 amu; HRMS for $C_{19}H_{29}O_5N$ calcd 351.2046, found 351.2046.

Keto Acid 9. The synthesis of this compound was carried out with the procedure described for **3**, starting with the acid chloride[16] of **7** and the hydrochloride of aminoacetone. The compound **8** was obtained as a crystalline solid and was used without further purification. A sample (49 mg) of **8** was dissolved in 8 mL of methanol. To this was added 2 mL of 1 N NaOH, and the solution was heated at reflux for 3 h. Methanol was removed under reduced pressure, and the crude mixture was acidified with 1 N HCl at 15 °C. The aqueous solution was extracted with methylene chloride (3 × 15 mL), and the combined organic layers were dried over sodium sulfate, filtered, and then concentrated to dryness. The compound **9** was obtained in 70% yield. The spectral data were the following: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.7 (m, 6 H), 1.35 (m, 12 H), 1.7 (m, 10 H), 2.29 (s, 3 H), 4.38 (d, 2 H, J = 7.5 Hz), 7.50 (d, 1 H, J = 3.0 Hz), 7.66 (d, 1 H, J = 3.0 Hz), 7.98 (d, 1 H, J = 3.0 Hz), 8.05 (d, 1 H, J = 3.0 Hz), 9.2 (broad, 1 H); ^{13}C (CD_3OD) δ 9.5, 27.5, 29.0, 31.5, 33.3, 33.35, 38.5, 40.0, 52.0, 129.3, 130.0, 130.3, 130.6, 145.0, 146; IR (CH_2Cl_2) 3314, 2965-2876, 1717, 1706, 1684, 1521, 1447, 1191, 1103 cm^{-1}.

Ketone 10. A sample (120 mg, 0.855 mmol) of benzoyl chloride and 0.34 mL (2.5 mmol) of freshly distilled triethylamine were dissolved in 40.0 mL of CH_2Cl_2. A 168.9-mg (0.94-mmol) portion of α-aminoacetone hydrochloride was added to the reaction mixture followed by stirring overnight. The crude mixture was washed with a saturated solution of sodium carbonate followed by 10% HCl and then dried over sodium sulfate. The solvent was removed by rotary evaporation at room temperature. Purification by flash column chromatography on silica gel with 75:25 hexanes/ethyl acetate as the solvent system afforded **10** in 68% yield. The spectral data were the following: ^{1}H NMR ($CDCl_3$, 270 MHz) δ 2.35 (s, 3 H), 4.35 (d, 2 H, J = 8 Hz), 6.9 (broad, 1 H), 7.45 (m, 3 H), 7.85 (m, 2 H); IR (CH_2Cl_2) 3437, 3332-3300, 1720, 1639, 1540, 1405, 1019 cm^{-1}.

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Registry No. 2, 79410-29-0; **3a**, 120881-32-5; **3b**, 120881-33-6; **4a**, 120881-34-7; **4b**, 120881-35-8; **5a**, 120881-36-9; **5b**, 120904-96-3; **6**, 130525-40-5; **7**, 132103-56-1; **8**, 132103-57-2; **9**, 132103-58-3; **10**, 132103-59-4; D_2, 7782-39-0; α-aminoacetone hydrochloride, 7737-17-9.

Substrate Structure and Solvent Hydrophobicity Control Lipase Catalysis and Enantioselectivity in Organic Media

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Abstract: The lipase from *Candida cylindracea* catalyzes the enantioselective esterification of 2-hydroxy acids in nearly anhydrous organic solvents with primary alcohols as nucleophiles. The nature of the 2-hydroxy acid and organic reaction medium affects the efficiency of catalysis and the enantioselectivity. Straight-chain 2-hydroxy acids are highly reactive and give nearly 100% enantioselectivities in esterification reactions with 1-butanol. Slight branching with a methyl group adjacent to the 2-hydroxy moiety in toluene causes a substantial loss (up to 200-fold) in the lipase's catalytic efficiency with a concomitant loss in enantioselectivity. Losses in catalytic efficiency and enantioselectivity are also observed when the lipase is employed in hydrophilic organic media such as dioxane or tetrahydrofuran as compared to hydrophobic solvents such as toluene. With straight-chain substrates, the lipase is over 100-fold more active in toluene than in tetrahydrofuran or dioxane, while optimal enantioselectivity is observed in toluene. The loss in enantioselectivity in hydrophilic solvents is mainly due to a drop in the catalytic efficiencies of the *S* isomers, as the *R* isomers' catalytic efficiencies remain largely unchanged. In highly apolar solvents, such as cyclohexane, enantioselective relaxation occurs due to an increase in the reactivity of the *R* isomers relative to that of their *S* counterparts. These findings enabled a rational selection of substrates and solvents for a two-step, chemoenzymatic synthesis of optically active 1,2-diols to be carried out, the first step being the aforementioned enantioselective esterification of 2-hydroxy acids followed by reduction with $LiAl(OCH_3)_3H$ to give the optically active 1,2-diol. Diols such as (*S*)-(+)-1,2-propanediol, (*S*)-(-)-1,2-butanediol, (*S*)-(-)-1,2-hexanediol, and (*S*)-(-)-4-methyl-1,2-pentanediol were produced in high optical purities (at least 98% enantiomeric excess (ee)).

Introduction

The substrate specificity and enantioselectivity of enzymatic catalysis in aqueous solutions have been well-studied.[1] Both characteristics owe their existence to the remarkable capability of enzymes to discern structural deviations in related molecules.[2] These factors can be quantitatively described by kinetic analyses through differences in the catalytic efficiency constant or specificity constant (k_{cat}/K_m or V_{max}/K_m) between two or more substrates or between a given pair of stereoisomers. Alterations in substrate specificity or enantioselectivity can be induced by changes in either the enzyme structure or the reaction medium. The former requires protein engineering,[3] is not applicable to all enzymes, is tedious,

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