Electrostatic Stabilization Can Explain the Unexpected Acidity of Carbon Acids in Enzyme-Catalyzed Reactions

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Abstract: Electrophilic catalysis is inadequate to explain the observed enzymic catalysis of reactions of carboxylic acids as carbon acids, contrary to recent claims (Gerlt, J. A.; Kozarich, J. W.; Kenyon, G. L.; Gassman, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 9667. Gerlt, J. A.; Gassman, P. G. *J. Am. Chem. Soc.* **1992**, *114*, 5928–5934.). The problem is essentially thermodynamic. The negative charge on the enolate allows the possibility of electrostatic stabilization, which can be large in a medium of low polarity; this could supply enough energy to allow rapid reaction.

Introduction

The loss of a proton from the α-position of carbonyl compounds is a key step in many enzyme-catalyzed processes, including carbon–carbon bond formation and racemization.[1] The enol or enolate so formed is generally unstable, and it has been noted that such species may be too high in energy to be on the path for an enzyme catalyzed reaction.[1,2]

It has been proposed[3,4] that the problem of carbon acidity in enzymic reactions, as exemplified by mandelate racemase, can be resolved by mechanisms involving concerted general acid–base catalysis. However, based on published data[5] cited to support such a mechanism, it is clear that the problem is a thermodynamic one and the catalytic scheme proposed does not provide a solution. Enzymes that catalyze reactions of carbon acids must overcome both a kinetic and a thermodynamic problem. Proton abstraction from carbon acids is inherently slow,[6–7,8] which poses a kinetic problem, and the enol or enolate so formed is an unstable intermediate,[5] which poses a thermodynamic problem. To achieve rapid reaction an enzyme must overcome both. The thermodynamic problem is severe for reactions that involve loss of a proton from the α-position of carbonyl compounds, and particularly for carboxylic acids or esters. The equilibrium constants for formation of the corresponding enolates are known to be quite unfavorable ($pK_a > 19$ for ketones or carboxylic acid derivatives[9,10]); it is not obvious how rapid enzymic reactions can proceed via such intermediates. We should emphasize that the problem is most severe for carboxylic acids or esters and less severe for aldehydes, ketones, or thiolesters; in the latter cases, the enol is inherently less unstable, and also less acidic, so that concerted general acid–base catalysis is possible and, in the case of simple ketones, can be observed.[8,11,12]

Results and Discussion

We must point out that, at least for mandelate racemase, and probably for other enzymes of this type, concerted general acid–base catalysis cannot be the explanation because it addresses the wrong problem. (We focus on mandelate racemase because, thanks to the work of Kresge et al.,[5] the energetics of the enol and enolate of mandelate are known.) The difficulty with forming an "enolate" of a carboxylic acid derivative is not that it is an "ate" but that it is an "enol". That is to say, the thermodynamic barrier that the enzyme must overcome is that of making a very unstable tautomer. This tautomer is sufficiently acidic (pK_a = 6.62[5]) that there is negligible energy cost for making the enolate anion from the enol in neutral water. Kresge has shown[13] that, at pH 7, the enolate ion of mandelate lies 25.4 kcal/mol higher in energy than mandelate ion. The transition state for the enzymic reaction is only 13 kcal/mol higher in energy than bound mandelate, based on k_{cat} = 1070 s^{-1}.[14] We will assume that the enzyme active site contains acidic and basic groups, both of pK_a 7.0, and will take account of pK_a values, hydrogen bonding, and electrostatics, initially assuming a dielectric constant of 78.5, the value for water. When we do this, we find that the energies of the species considered are as shown in Figure 1; details of the calculations involved are found in the Appendix. This figure clearly shows the problem: the O-protonated acid, the enolate, and the enol all have very similar energies, and thus there is no way to achieve catalysis by any concerted process involving proton donation and abstraction. Both the enol and the enolate are higher in energy than the observed transition state for k_{cat}. The fundamental problem the enzyme must overcome is thermodynamic. Furthermore, converting the enolate to enol may be counterproductive. It is hard to see how significant stabilization of the enol can be achieved, whereas the negative charge on the enolate allows the possibility of electrostatic stabilization, which can be large in a medium of low polarity.

We will now examine ways that an enzyme might employ to overcome this problem. The thermodynamic barrier must be reduced by 12 kcal. We base this estimate on simple Marcus theory.[15–17] using the intrinsic barrier for enolate formation derived for a partially desolvated hydroxide reacting with simple ketones, namely 10.7 kcal/mol,[18] and the free energy of activation derived

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(1) Kluger, R. *Chem. Rev.* **1990**, *90*, 1151–1169.
(2) Thibblin, A.; Jencks, W. P. *J. Am. Chem. Soc.* **1979**, *101*, 4963–4973.
(3) Gerlt, J. A.; Gassman, P. G. *J. Am. Chem. Soc.* **1992**, *114*, 5928–5934.
(4) Gerlt, J. A.; Kozarich, J. W.; Kenyon, G. L.; Gassman, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 9667–9669.
(5) Chiang, Y.; Kresge, A. J.; Pruszynski, P.; Schepp, N. P.; Wirz, J. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 792–794.
(6) Eigen, M. *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 1–72.
(7) Chiang, Y.; Kresge, A. J.; Santaballa, J. A.; Wirz, J. *J. Am. Chem. Soc.* **1988**, *110*, 5506–5510.
(8) Albery, W. J. *Chem. Soc., Faraday Trans.* **1982**, *78*, 1579–1590.
(9) Chiang, Y.; Kresge, A. J.; Pruszynski, P.; Schepp, N. P.; Wirz, J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1366–1368.
(10) Keefe, J. R.; Kresge, A. J. In *The Chemistry of Enols*; Rappoport, Z., Ed.; Wiley: Chichester, U.K., 1990; pp 399–480.
(11) Dawson, H. M.; Spivey, E. *J. Chem. Soc.* **1930**, 2180.
(12) Hegarty, A. F.; Jencks, W. P. *J. Am. Chem. Soc.* **1975**, *97*, 7188–7189.
(13) Kresge, A. J. *Pure Appl. Chem.* **1991**, *63*, 213–221.
(14) Kenyon, G. L.; Hegeman, G. D. *Adv. Enzymol.* **1979**, *50*, 325–360.
(15) Cohen, A. O.; Marcus, R. A. *J. Phys. Chem.* **1968**, *72*, 4249.
(16) Marcus, R. A. *Annu. Rev. Phys. Chem.* **1964**, *15*, 155.
(17) Marcus, R. A. *J. Am. Chem. Soc.* **1969**, *91*, 7224.
(18) Guthrie, J. P. *J. Am. Chem. Soc.* **1991**, *113*, 7249–7255.

0002-7863/93/1515-11569$04.00/0

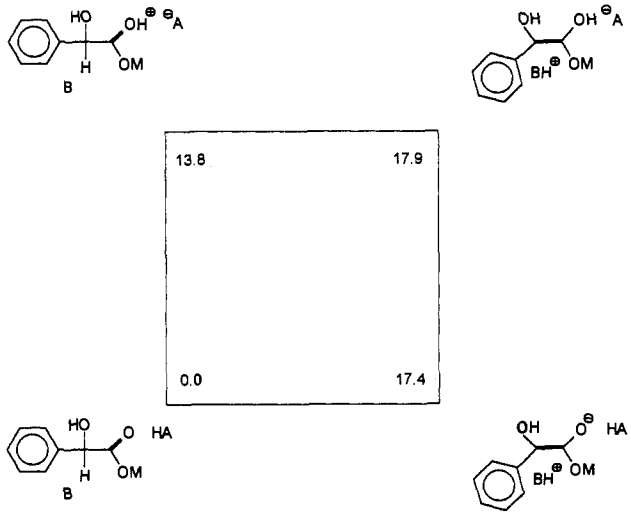
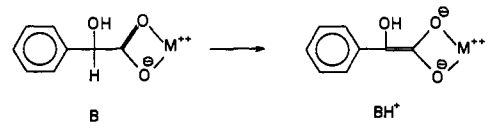


Figure 1. Energies of the enol and the intermediates in the limiting stepwise enolization paths, all relative to enzyme-bound mandelate. The mandelate ion is assumed to be bound to a metal ion which neutralizes the negative charge. (The metal ion is shown coordinated to the carboxylate only; in some enzyme inhibitor complexes, it is also coordinated to the hydroxyl.) The general acid and general base are both assumed to have pKa (or pK_{BH+}) = 7.0. Effects of hydrogen bonding, ion pairing, and desolvation are estimated as described in the text.

from k_{cat}. $\Delta G^{\ddagger} = \tilde{G}(1 + \Delta G^{\circ}/4\tilde{G})^2$: if $\Delta G^{\ddagger} = 13$ and $\tilde{G} = 10.7$, then $\Delta G^{\circ} = 4.4$ kcal/mol, which is 12 kcal/mol less than ΔG° for the proton abstraction estimated above.

First we will consider electrostatic stabilization of an enolate. An enzyme active site could be a relatively nonpolar environment,[19-22] and if both oxygens of the carboxylate/enolate dianion were in contact with a divalent metal, large electrostatic effects are in principle available.[13] Electrostatic stabilization of the enolate could be achieved in various ways, and there are several cationic side chains in the active site. We estimate the electrostatic stabilization involving a divalent metal ion as follows. The ion pair formation constant for Ca^{2+}/AcO^- in water is log $K = 0.78$,[23] which in terms of the Fuoss–Eigen equation[24,25] corresponds to $r = 3.8$ Å. For this contact distance, the extra stabilization for a 2:2 ion pair as opposed to a 2:1 ion pair is 2.2 kcal/mol with a dielectric constant of 78.5. This rises to 11.7 kcal/mol with a dielectric constant of 15, which is lower than sometimes suggested for active sites,[26,27] but not impossibly so.[19-21] If, instead of the contact distance calculated to fit the observed association constant, we use the sum of the ionic radii, 2.43 Å,[28] we find that the extra stabilization becomes 18.24 kcal/mol for a dielectric constant of 15. Thus electrostatic stabilization could provide enough energy to overcome the thermodynamic problem. We should note that one cannot get something for nothing: The same electrostatic effects that stabilize an ion pair make it harder to form the ion pair by altering the acidity of the acid that forms the anion of the ion pair. In order to gain this stabilization of the ion pair, it must be paid for at the beginning by desolvation of the cation; this could be done by trading binding energy for orientation.



Gerlt and Gassman[3] suggested binding of carboxylate ions to a cation (metal ion or amino acid functional group) to cancel the effects of the carboxylate negative charge. We agree that this is very likely to be an important feature of the active site of any enzyme which must remove the α-proton of a carboxylate. Since they argue that the enol, and not the enolate, is the key intermediate, they cannot invoke electrostatic stabilization of the enolate. Instead they suggest (their Scheme III) that a cationic group might stabilize the E–A$^-$·H–enol complex. This means in effect that the cationic group would enhance the acidity of E–AH, but not so much that it was fully ionized before the arrival of the substrate. It is hard to see how a substantial net stabilization can be achieved by this mechanism.

Hydrogen bonding has also been suggested by Gerlt and Gassman[29] as a way to stabilize the enol, but this seems unlikely to provide enough stabilization for enol or enolate. Normal hydrogen bonds are relatively weak and depend on the acidity of the H-donor and the basicity of the H-acceptor.[30] Although there are examples of very strong hydrogen bonds in nonaqueous media or the gas phase, the same hydrogen bonds are much weaker in aqueous solution. A dramatic example if this is FHF^-, for which the hydrogen bond energy in the gas phase is 60 kcal/mol,[31a] while in aqueous solution log $K_f = 0.598$[31b] ($\Delta G = -0.8$). We conclude that, on the basis of present knowledge, hydrogen bonding is unlikely to be able to provide all of the stabilization needed for either enol or enolate, though it doubtless is used to provide some stabilization.[32]

The published X-ray structure of mandelate racemase with a bound substrate (*p*-iodomandelate) has one oxygen of the carboxylate and the hydroxyl in contact with the metal.[33] This structure is however based on the location of the iodine and a molecular mechanics minimization with the protein structure held fixed. In the absence of the iodine, which is an unnatural perturbation, the phenyl might move farther from the metal, allowing both carboxylate oxygens to make contact with the metal. A more recent X-ray investigation of the complex of one enantiomer of atrolactic acid with the enzyme shows a similar coordination to the metal.[34] The other enantiomer shows disorder in the active site, with atrolactate binding in at least two different conformations, one of which may not have the hydroxyl coordinated to the metal.[34] Furthermore, examination of the structure of the active site using the X-ray coordinates[34] shows that in the active site motion of the substrate and nearby protein side chains is quite possible so that the position of the substrate relative to the metal is not rigidly defined. Thus the coordination seen for poor substrates or for inhibitors may not reflect the coordination at the transition state. In the present paper, our intent is only to show that electrostatic interactions could be large enough to produce the observed rate for mandelate racemase and that these interactions impose no impossible constraints on the geometry of the active site.

(19) Warshel, A. *Biochemistry* **1981**, *20*, 3167–3177.
(20) Warshel, A.; Levitt, M. *J. Mol. Biol.* **1976**, *103*, 227–240.
(21) King, G.; Lee, F. S.; Warshel, A. *J. Chem. Phys.* **1991**, *95*, 4366–4377.
(22) Warshel, A.; Aqvist, J. *Annu. Rev. Biophys. Biophys. Chem.* **1991**, *20*, 267–298.
(23) Davies, C. W. *Ion Association*; Butterworths: Washington, DC, 1962.
(24) Eigen, M. *Z. Elektrochem.* **1960**, *64*, 115–123.
(25) Fuoss, R. M. *J. Am. Chem. Soc.* **1958**, *80*, 5059–5061.
(26) Warshel, A. *Computer Modelling of Chemical Reactions in Enzymes and Solutions*; Wiley: New York, 1991; pp 123.
(27) Russell, S. T.; Warshel, A. *J. Mol. Biol.* **1985**, *185*, 389–404.
(28) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*; Wiley: New York, 1980; pp 14.
(29) Gerlt, J. A.; Gassman, P. G. Manuscript submitted for publication.
(30) Stahl, N.; Jencks, W. P. *J. Am. Chem. Soc.* **1986**, *108*, 4196–4205.
(31) (a) Dixon, H. P.; Jenkins, H. D. B.; Waddington, T. C. *J. Chem. Phys.* **1972**, *57*, 4388–4397. (b) Kresge, A. J.; Chiang, Y. *J. Phys. Chem.* **1973**, *77*, 822.
(32) (a) Fersht et al.[32b] have suggested, on the basis of directed mutagenisis studies of binding to tyrosyl transfer-RNA synthase, that hydrogen bonds involving a charged species are 3 kcal/mol stronger than those involving neutral species. In the present case, two such hydrogen bonds could provide one-half the stabilization needed for the enolate. (b) Fersht, A. R.; Shi, J.-P.; Knill-Jones, J.; Lowe, D. M.; Wilkinson, A. J.; Blow, D.M.; Brick, P.; Carter, P.; Waye, M. M. Y.; Winter, G. *Nature* **1985**, *314*, 235–238.
(33) Neidhart, D. J.; Howell, P. L.; Petsko, G. A.; Powers, V. M.; Li, R.; Kenyon, G. L.; Gerlt, J. A. *Biochemistry* **1991**, *30*, 9264–9273.
(34) Petsko, G. A. Personal communication.

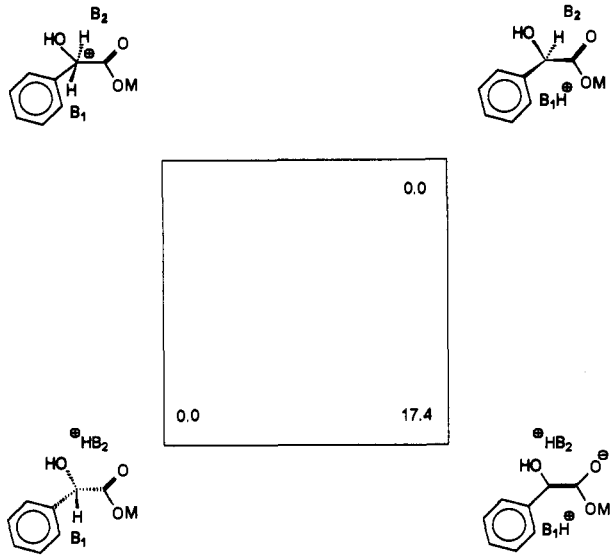


Figure 2. Possible concerted path for racemization of mandelate. The limiting stepwise intermediates are the enolate and the pentacoorinate carbocation. A concerted path would avoid both intermediates, proceeding through the center of the diagram. The energy of the intermediate is calculated as described in the text. The energy of the carbocation is unknown, but as described in the text, it seems very unlikely that the intrisic barrier for the proton-transfer process can be low enough to allow a concerted path with the observed activation energy and a plausible carbocation energy.

Electrostatic stabilization (not necessarily by a metal ion) provides a convenient way to stabilize an enolate. It is difficult to see how a similarly large stabilization could be obtained for the enol. Binding energy of the substrate could be used by the enzyme to set up a situation in the enzyme–substrate complex where various cationic and catalytic groups were desolvated in ways relieved upon conversion to the enolate. Thus binding energy would be converted to catalytic effect.[35]

We should also consider another alternative, which in principle could provide a way to avoid the thermodynamic problem posed by the instability of the enol or enolate of a carboxylic acid. This is the use of a concerted mechanism, involving simultaneous loss of a proton from one side of the stereocenter of mandelate and donation of a carbon to the other side. Thibblin and Jencks have provided compelling evidence that the ring opening of cyclopropanol involves concerted C-protonation and C–C bond cleavage[2] and proposed that enzyme-catalyzed aldol condensations might involve a concerted mechanism.[2] Albery and Knowles suggested that proline racemase might involve a concerted mechanism, although they gave no detailed structural or energetic analysis of what such a mechanism might involve.[36]

We wish to show that, for the case of mandelate racemase, consideration of the energetics and of the implications of Marcus theory suggests that the concerted mechanism appears improbable. Since such mechanisms have been proposed for a number of enzymes[1,2,3,36] and experiments have been designed to test for the existence of such mechanisms,[36] having a simple criterion for the likelihood of a concerted path should prove useful in focusing attention on those cases where a concerted path is most likely or, conversely, in focusing attention on the logical implications in terms of intermediate energies and intrinsic barriers if evidence for a concerted path is found.

A concerted mechanism for racemization of mandelate, as shown in Figure 2, implies two limiting stepwise paths through the enolate carbanion and the pentacoordinate carbocation.[37] For such a mechanism to be faster than the stepwise mechanism by way of the enolate requires two things that look improbable. First, the pentacoordinate carbocation cannot be too high in energy; we will derive relations showing that, except for extremely low intrinsic barriers, the energy of the pentacoordinate carbocation must be improbably low. Second, the intrinsic barrier for the proton-transfer events must be low. With the intrinsic barrier observed in nonenzymic systems, i.e. 10.7 kcal/mol for the reaction of a partly desolvated hydroxide ion with a carbonyl compound,[18] a concerted pathway leading to the observed rate of reaction is impossible. In terms of two-dimensional Marcus theory, a concerted reaction requires a low intrinsic barrier for at least one of the limiting stepwise processes.[38,39] For the substantial intrinsic barrier for proton removal in simple cases to become very small seems quite improbable. The concerted path still requires substantial geometry change and nuclear motion, and this seems to demand a large intrinsic barrier.[40]

From the equation for the energy along the disparity coordinate[41] in the quadratic model for two-dimensional Marcus theory,[38] we can derive an equation relating the energy at the minimum along the disparity coordinate to the intrinsic barrier and the corner energies. For a concerted exchange, the initial and final states are essentially the same and the intrinsic barriers for both edge reactions are the same.

$$G = \Delta G^\circ_y + (\Delta G^\circ_{rxn} - 2\Delta G^\circ_y + 4(\tilde{G}_x + \tilde{G}_y))q + (\Delta G^\circ_x + \Delta G^\circ_y - \Delta G^\circ_{rxn} - 4(\tilde{G}_x + \tilde{G}_y))q^2$$

$$q^* = \frac{(\Delta G^\circ_{rxn} + 2(2(\tilde{G}_x + \tilde{G}_y) - \Delta G^\circ_y)}{2(\Delta G^\circ_{rxn} - \Delta G^\circ_x - \Delta G^\circ_y + 4(\tilde{G}_x + \tilde{G}_y))}$$

$$\Delta G^* = \frac{\Delta G^\circ_{rxn}{}^2 + 8\Delta G^\circ_{rxn}(\tilde{G}_x + \tilde{G}_y) + 4(4(\tilde{G}_x + \tilde{G}_y)^2 - \Delta G^\circ_x \Delta G^\circ_y}{4(\Delta G^\circ_{rxn} - \Delta G^\circ_x - \Delta G^\circ_y + 4(\tilde{G}_x + \tilde{G}_y))}$$

$$\Delta G^\circ_y = \frac{\Delta G^* \Delta G^\circ_x + 8\tilde{G}_y(2\tilde{G}_y - \Delta G^*)}{\Delta G^\circ_x - \Delta G^*}$$

For the energy that we estimated for the "carbanion" corner, concerted reactions are only possible for $\tilde{G} \leq 3$, and to obtain the experimental free energy of activation at $\tilde{G} \approx 3$ requires that the "pentacoordinate carbocation" lie only 13 kcal higher in energy than the starting point, which implies a pK_a for this "pentacoordinate carbocation" of only −2, which seems improbably low. If the intrinsic barrier were lower, the pentacoordinate carbocation could be higher in energy and still satisfy the constraints: 31.3 kcal/mol at $\tilde{G} = 1$ and 51 kcal/mol at $\tilde{G} = 0$. It must be emphasized that such low intrinsic barriers appear improbable for a process involving so much nuclear motion and bond reorganization.

Although the concerted alternative seems improbable, we cannot rule it out at this time. We can however say quite confidently that, IF the enzyme is to use a concerted path of the sort discussed above in order to avoid the carbanion, THEN it must achieve a dramatic reduction in intrinsic barrier. Studies by Landro et al. of a mutant form of the enzyme which catalyzes exchange but not racemization strongly suggest that the reaction is in fact stepwise.[41a]

(35) Jencks, W. P. In *Advances in Enzymology*; Meister, A., Ed.; Wiley: New York, 1975; Vol. 43, pp 219–410.
(36) Belasco, J. G.; Albery, W. J.; Knowles, J. R. *Biochemistry* **1986**, *25*, 2552-2558.

(37) (a)In Olah's [37b] nomenclature, the pentacoordinate species would be called a carbonium ion as opposed to a carbenium ion. (b) Olah, G. *J. Am. Chem. Soc.* **1972**, *94*, 808–820.
(38) Guthrie, J. P. *Can. J. Chem.* **1987**, *65*, 1951–1969.
(39) Guthrie, J. P. *J. Am. Chem. Soc.* **1991**, *113*, 3941–3949.
(40) Mitchell, D. J.; Schlegel, H. B.; Shaik, S.; Wolfe, S. *Can. J. Chem.* **1985**, *63*, 1642–1648.
(41) (a) Grunwald, E. *J. Am. Chem. Soc.* **1985**, *107*, 125. (b) Landro, J. A.; Kallarakai, A. T.; Ransom, S. C.; Gerlt, J. A.; Kozarich, J. W.; Neidhart, D. J.; Kenyon, G. L. *Biochemistry* **1991**, *30*, 9274–9281.

Conclusions

We have shown that it is in principle possible for mandelate racemase to achieve its observed catalytic effect using a mechanism already known to physical organic chemistry, namely electrostatic stabilization of the enolate. It remains to be discovered what the enzyme actually does. Other enzymes catalyzing reactions of carbon acids have similar problems to overcome. Any mechanistic proposal must address the thermodynamic as well as the kinetic aspects of these problems. Energetic considerations combined with Marcus theory provide a powerful tool for examining the likelihood of proposed mechanisms for enzymic catalysis.

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Appendix

Detailed Calculation of the Energies of the Corner Intermediates for the Enolization of Mandelate. We use the following equilibrium constants, given as $pK = -\log K$: Ph—CHOH—COOH = Ph—C(OH)=C(OH)$_2$, $pK_E = 15.4$;[13] Ph—CHOH—COOH = Ph—CHOH—COO^- + H^+, $pK_a = 3.41$;[42] Ph—CHOH—COOH = Ph—C(OH)=C(O^-)(OH) + H^+, $pK_a = 22.0$;[13] Ph—CH(OH)=C(OH)$_2$ = PhC(OH)=C(O^-)(OH) + H^+, $pK_a = 6.62$;[13] Ph—CHOH—C^+(OH)$_2$ = Ph—CHOH—COOH + H^+, $pK_a = -8$, estimated from the pK_a of protonated acetic acid, −6.56[38,43] Taft σ^* values for phenyl and hydroxyl,[44] and a generalized Taft ρ^* value;[44] for the acid and base in the enzyme active site which catalyze enolization at pH 7, we assume pK_a values of 7.

For each corner structure, we allow for the stabilizing effects of hydrogen bonding involving the general acid or its conjugate base; energies of hydrogen bonds are estimated using the equation of Stahl and Jencks.[30] Since the Stahl and Jencks equation is for intermolecular complexation and we are here concerned with hydrogen bond formation within the enzyme substrate complex, we correct for the statistical cost of bringing two species together; following Hine[45] we take this as +2.42 kcal/mol. For the general base (assumed to be an amine) or its conjugate acid, which is in contact with a hydrocarbon-like portion of the molecule, we assume a partial desolvation. The energy cost of this desolvation for an amine is taken as the difference between ΔG_t, the free energy of transfer from gas to water, of an amine and that of the analogous hydrocarbon. We used *N*,*N*-dimethylaniline as a suitable moderately basic amine and calculated ΔG_t from the solubility[46] and the vapor pressure[47] with isopropylbenzene as the analogous hydrocarbon;[48] the hydrogen bond energy so calculated was about 3 kcal/mol. For lack of a better value, the same is used for the ammonium ion. For species with charge separation, we include an ion pairing contribution, estimated using a value for the contact ion pair taken as equal to that for *tert*-butylcation–chloride[49] and corrected for longer distances by assuming that all heavy atom separations are equal. These ion pair energies must also be corrected for statistical costs.

(42) Jencks, W. P.; Regenstein, J. In *Handbook of Biochemistry and Molecular Biology*, 3rd ed.; Fassman, G., Ed.; Chemical Rubber Company: Cleveland, OH, 1976; Vol. 1, pp 305–451.

(43) Deno, N. C.; Pittman, C. U.; Wisotsky, M. J. *J. Am. Chem. Soc.* **1964**, *86*, 4370.

(44) Perrin, D. D.; Dempsey, B.; Serjeant, E. P. *pKa Prediction for Organic Acids and Bases*; Chapman and Hall: London, 1981.

(45) Hine, J. *J. Am. Chem. Soc.* **1971**, *93*, 3701–3708.

(46) Chebib, H.; Jambon, C.; Merlin, J.-C. *J. Chim. Phys. Phys.-Chim. Biol.* **1982**, *79*, 157–160.

(47) Weast, R. C., Ed. *Handbook of Chemistry and Physics*, 48 ed.; Chemical Rubber Co.: Cleveland, OH, 1967.

(48) Cabani, S.; Gianni, P.; Mollica, V.; Lepori, L. *J. Solution Chem.* **1981**, *10*, 563.

(49) Abraham, M. H. *J. Chem. Soc., Perkin Trans. 2* **1973**, 1893.