BIOORGANIC CHEMISTRY 10, 169–176 (1981)

On the Importance of Orientation in General Base Catalysis by Carboxylate[1]

RICHARD DAVID GANDOUR

Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803

Received April 7, 1980

The carboxylate group serves as a general-base catalyst in numerous chemical and enzymatic reactions. The importance of the direction in which the proton is transferred to the carboxylate is discussed. *syn* (on the same side of the C—O bond as the forming C=O) protonation is estimated to be 10^4-fold more favorable than *anti* protonation. To date, in the models studied where intramolecular reactions involve carboxylate, only *anti* protonation can occur. In these intramolecular models the catalytic efficiency of a carboxylate group may be underestimated due to this inability to achieve an optimal orientation for protonation; whereas for enzymatic reactions involving carboxylate side chains, structural studies support mechanisms involving *syn* protonation.

The past two decades have seen an enormous interest in developing chemical models of enzymatic catalysis (*1*). The high efficiency of enzymatic catalysis has been generally attributed to typical chemical mechanisms, operating under the especially favorable conditions present in the enzyme–substrate complex. Three mechanisms—general acid, general base, and nucleophilic catalysis—appear to be particularly relevant to enzymes. Models of each have been studied with the goal of approximating the catalytic efficiency observed in enzymes. Certain intramolecular models (*2*), where the reacting groups are juxtaposed (and thus serve as a valid analogy for a reaction in an enzyme–substrate complex) have been distinctly successful at achieving this goal. However, in the case of general base catalysis, no intramolecular models have been able to achieve more than a moderate increase in catalytic efficiency (ca. 100 M) when compared to their intermolecular counterparts (*2*e).

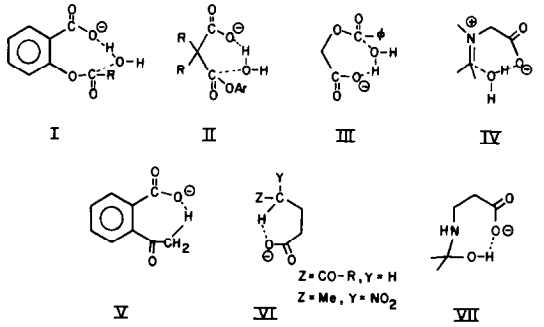
Kirby and Lloyd (*3*) have provided a perspicuous analysis of structure and efficiency in intramolecular general-base catalysis in the discussion of their kinetic studies of the hydrolysis of monoaryl malonates and aryl hydrogen cyclopropane-1,1-dicarboxylates. Intramolecular general base catalysis by carboxylate is less efficient than intramolecular nucleophilic catalysis, possibly because general base catalysis is generally not as efficient as nucleophilic catalysis or because the models studied thus far do not allow for very efficient general base catalysis. Kirby and Lloyd have presented persuasive arguments in support of the former reason. However, the purpose of this paper is to present evidence in support of

[1] Dedicated in loving memory to Ruth Wells Gandour, a superb scientist and devoted supporter and companion.

0045-2068/81/020169-08$02.00/0

the latter argument, especially in reactions where carboxylate functions as an intramolecular general-base catalyst.

Representative chemical models studied (*4*) to date in which intramolecular general base catalysis by carboxylate has been verified are shown below (I–VII).

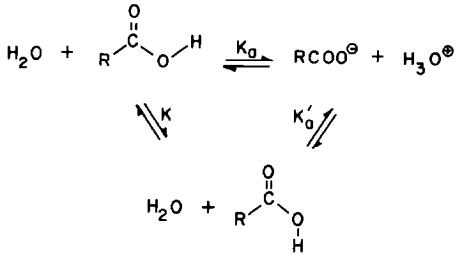


I II III IV V VI VII

Examination of these structures indicates a common geometric feature: the catalytically bridging proton is positioned on the opposite side of the C—O bond from the forming C=O bond. If the proton is fully transferred, a less stable conformation of the carboxyl group is formed.

The most stable conformation for a carboxyl is *syn* (O—H and C=O on the same side of C—O) (*5*). Another energy minima exists on the potential energy curve describing rotation about the C—O bond at a dihedral angle of 180° (*anti*). The top of the barrier to rotation occurs at ca. 90°. At the moment of complete proton transfer to carboxylate in **I–VII**, a carboxyl group in an *anti* (or near *anti*) conformation is produced. Thus, in these intramolecular model reactions, the optimal orientation for proton transfer to carboxylate cannot be achieved. The resultant loss in catalytic power can be assessed from the effective basicity of the carboxylate group and the Brønsted catalysis law.

An estimate of the acid dissociation constant, K_a', of a carboxyl group in an anti conformation can be made from the equilibria shown in Scheme 1. The equilibrium constant, K, for the *syn* and *anti* conformational equilibrium can be approximated for formic acid at ca. 10^{-3}, from reported (*5*) energy differences between the two conformations. Calculations of this energy difference for acetic (*6*) and propionic (*6*b) acids suggest an even less favorable equilibrium, $K \approx 10^{-4}$. From Eq. [1] then, K_a' is 10^4-fold larger than K_a.



SCHEME 1

$$K_a' = K_a/K. \qquad [1]$$

Therefore, protonation of a carboxylate in the *anti* direction is less favorable than in the *syn*. In effect, carboxylate is a weaker base when structurally constrained to accept a proton in the *anti* direction.

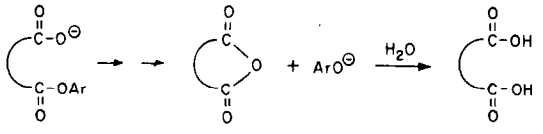
How much this reduction of basicity affects catalytic power can be easily estimated from the Brønsted catalysis law:

$$k = CK_a^{-\beta}, \qquad [2]$$

where k is the rate constant for the reaction, C is a constant of proportionality, and β is the Brønsted coefficient. Assuming β has values in the range of 0.25 to 0.75 and replacing K_a with K_a', leads to the conclusion that the loss of catalytic power can be from 10- to 1000-fold. Thus, one of the reasons for the observed low efficiency of carboxylate in intramolecular general base catalysis is the poor stereochemical arrangement of the carboxylate and the catalytically bridging proton.

The critical value in the above discussion is K_a', which is in fact a reflection of K, the conformational equilibrium constant. In certain intramolecular models, K may be larger than the estimated 10^{-4} due to internal hydrogen bonding which is present in the *anti* conformation but absent in the *syn*. Just how significant an effect this internal hydrogen bonding has on the conformational equilibrium must be evaluated for each model. Intramolecular hydrogen bonding is subject to certain stereochemical requirements as well.

Page (*7*) has emphasized the importance of the stereochemical requirements for intramolecular reactions in evaluating the catalytic efficiency (effective molarity) of a neighboring group. For example, intramolecular nucleophilic catalysis (*2*) by carboxylate of ester hydrolysis frequently involves the formation of a cyclic anhydride (Eq. [3]).



[3]

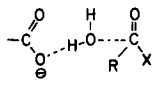
In this example, the *anti* stereochemistry for bonding to the carboxylate is required for formation of the cyclic anhydride. Since the carboxylate is restricted in the model to bonding exclusively in the *anti* direction, the model is predisposed toward nucleophilic catalysis. Page (*7*) has estimated that the entropy loss from this restriction provides $10^{2.6}$ *M* of the expected 10^8 *M* catalytic efficiency (*8*). He has accounted for an additional $10^{3.2}$ *M* from ring strain and loss of internal rotations, while $10^{2.2}$ *M* remains unassigned to stereochemical and structural features inherent to the model. His point that these features generate a significant portion of the expected catalytic efficiency is well taken.

Kirby and Lloyd (*3*) have proposed that the disparity in catalytic efficiencies (100 *M* vs 10^8 *M*) between intramolecular general base and nucleophilic catalysis

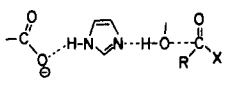
could result from the looser transition state in the former. A loose transition state for intramolecular general base catalysis might be expected, since no fewer than three covalent bonds are being made or broken simultaneously, e.g., **I**. Looseness gives rise to increased residual entropy, hence causes a reduction in the catalytic efficiency of an intramolecular reaction. Kirby and Lloyd have cautioned that their proposal is most valid for fully concerted protolytic reactions. In examples (*9*) of intramolecular general-acid catalysis by carboxyl, where heavy atom motion is believed to be uncoupled from proton transfer, the catalytic efficiencies are 10^3–10^4 *M*. In these examples, where the proton is proposed to be tightly bonded to one atom in the transition state, the internal residual entropy is smaller than in cases with more symmetrical transition states. Therefore for reactions involving tighter transition states increased catalytic efficiency can be expected.

Internal entropy is undoubtedly an important factor in determining catalytic efficiency. The question remains as to whether or not it is the dominant factor. First, the models studied to date have clearly favored nucleophilic catalysis. Furthermore, in many of these models part of the catalytic efficiency is due to relief of strain. Second, the proposal of fully concerted (proton transfer and heavy atom motions being highly coupled) general-base catalysis in acyl transfer reactions remains controversial (*10–12*). Mechanisms where these motions are uncoupled have been suggested (*13, 14*) for other protolytic reactions. An intramolecular model would be ideally suited for a preassociation mechanism (*13*), hydrogen-bonding catalysis (*9*a, *13*), or spectator catalysis (*14*); and as suggested (*3*) these mechanisms would have transition states with smaller internal entropies than transition states in fully concerted mechanisms. Thus, it would appear premature to ascribe the relative catalytic inefficiency of carboxylate as an intramolecular general base totally to the loose-transition-state internal entropy proposal.

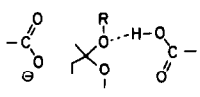
Strong support for *syn* orientation of carboxylate for catalysis comes from examination of structures of the most efficient catalysts—enzymes. General base catalysis by a side chain carboxylate has been proposed for a number of enzymes. Representations **VIII–XIII** summarize the proposed mechanistic roles of carboxylate in these enzymes.



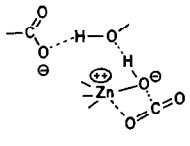
VIII



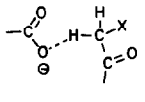
IX



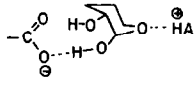
X



XI



XII



XIII

The clearest example for protolytic catalysis of hydrolysis of acyl derivatives, **VIII**, is the recent proposal for penicillopepsin (*15*). The carboxylate from Asp-32 has been proposed to catalyze the attack of H_2O at the carbonyl carbon of a peptide, **VIII** (X = NHR). Transfer of the proton on the *syn* side of the carboxylate is favored. A similar mechanism has been suggested for pepsin (*16*). Support for protolytic catalysis by carboxylate of deacylation of substituted benzoyl papains has recently appeared (*17*). The suspected residue, Asp-158, is positioned in the active site (*18*) in such a way that representation **VIII** (X = SR) is the most likely description of the mechanism. The suggestion (*19*), albeit controversial (*20*), has been made that peptidase activity of carboxypeptidase involves protolytic catalysis by carboxylate. Structural studies (*21*) indicate that the carboxylate, Glu-270, is situated for a *syn* attachment, **VIII** (X = NHR).

The best evidence for the *syn* orientation in hydrogen bonding by carboxylate comes from crystallographic studies (*22*) of the charge relay system, **IX**, in serine proteases. A recent comparison (*22g*) of charge relay systems in a number of serine proteases questions the existence of a Ser–His hydrogen bond. In contrast, the His–Asp hydrogen bond is in all cases a strong bond having the geometry expected for a normal unbifurcated hydrogen bond to carboxylate, **IX**. The catalytic function of the His–Asp couple is the subject of considerable speculation (*23*). An ^{15}N NMR study (*24*) of α-lytic protease supports a mechanism where the imidazole functions as a general base without the transfer of the proton bridging His and Asp. Whether or not this mechanism applies to all serine proteases remains to be seen; however, the structural details concerning the geometry of the His–Asp couple appear to be general features of this class of enzymes.

The study of hen egg-white lysozyme is a unique example in enzymology where the elucidation of the crystal structure predated and stimulated most of the solution studies. The originally proposed mechanism, **X**, based on the structure of the active site (*25*), remains the most likely. Two carboxy groups are involved: Asp-52, as a carboxylate, provides electrostatic stabilization of the putative carboxonium ion, while Glu-35 serves as a general-acid catalyst. Support for the latter comes from a study of the reverse reaction, general-base-catalyzed attack of an alcohol on the carboxonium ion (*26*), and application of the principle of microscopic reversibility. Examination of structures of the active site suggest that both carboxy groups are positioned in the active site with *syn* orientation towards the substrate. This is most clearly seen in gadolinium complexes of the enzyme (*27*).

A crystal structure study of human carbonic anhydrase B has led to a proposed mechanism, **XI**, involving a carboxylate, Glu-106 (*28*). The question of whether or not a proton is fully transferred to the carboxylate remains unanswered. It appears that Glu-106 is hydrogen bonded to the hydroxyl of Thr-199 in a *syn* orientation. A further hydrogen bond between Thr-199 and a hydroxyl bound to Zn completes the chain. The suggested function of the carboxylate is to provide electron density to the nucleophilic hydroxyl as it is transferred from Zn to the electropositive carbon of CO_2. A further suggestion (*29*) has been made that the ionizing group of

pK_a of 7 might be Glu-106, since it is located in a hydrophobic environment. If this is correct, then attack of a Zn-bound water activated by general-base catalysis from Glu-106 transmitted by the intervening Thr-199 would be a likely mechanism (*29*). If so, **XI** would need modification by an additional proton attached to the hydroxyl bonded to Zn.

Carboxylate-catalyzed proton transfer from a carbon has been suggested for (1) the isomerization of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate, **XII** (X = OH), catalyzed by triose phosphate isomerase (*30*); and (2) the enolization of 2-benzyl-3-*p*-methoxypropionate, **XII** (X = CH(COOH)Bz), catalyzed by carboxypeptidase A (*31*). In the case of triose phosphate isomerase, a proposed positioning of the substrate in the active site (*30*b) indicates an orientation with respect to Glu-165 that is neither *syn* or *anti*. Since the resolution in the study was 0.6 nm, detailed comments concerning orientation are premature. In a clever study to probe the stereochemistry of the active site of carboxypeptidase A, Sugimoto and Kaiser (*31*) have studied H–D exchange in a ketonic analog of the enzyme's ester and peptide substrates. They have concluded that Glu-270 is the functional group which abstracts the proton from the ketone. Assuming the same placement of this substrate with other substrates from crystallographic studies on peptide–enzyme complexes (*21*), a *syn* orientation for proton transfer to carboxylate would be favored.

The final example of carboxylate catalysis in enzymes involves glucose phosphate isomerase. Shaw and Muirhead have put forth a mechanism, based on their structural studies (*32*), which is partly illustrated by **XIII**. These authors support a *syn* orientation for proton transfer. In fact, their complete mechanism involves the carboxylate group functioning as a polyfunctional tautomeric catalyst (*33*). Thus the carboxylate is believed to be hydrogen bonded to both hydroxy groups in **XIII**. This hydrogen bonding arrangement could only occur with a *syn* orientation to the carboxylate.

In summary, the structural data for enzymes tend to support the consideration that *syn* orientation is favored for protolytic catalysis by carboxylate. This conclusion is not surprising, given the relative energetics of *syn* vs *anti* and the general belief that enzymes have been developed in such a manner as to optimize their catalytic machinery. Theories have appeared which emphasize orientation as a critical factor for catalysis. Koshland's orbital-steering hypothesis (*34*) and Wang's directed-proton-transfer (*35*) proposal are most notable for this discussion. Koshland's ideas have been the subject of considerable controversy, (*36*) largely directed toward his quantitative assessment of orientation factors and the chemical models used as the basis for the hypothesis. Few would deny that orientation is important. The disagreement lies in whether or not orientation is an enormous factor contributing to the catalytic power in enzymes.

In applying this question to protolytic catalysis by carboxylate, we suggest that *syn* vs *anti* orientation is worth $10^{2\pm1}$ in catalytic power. Experimental demonstration of this suggestion remains to be done. Work is in progress in our laboratory on the synthesis of chemical models in which the carboxy group is oriented *syn* to the reacting group. Subsequent kinetic studies on these models should begin to answer this question.

ACKNOWLEDGMENT

Acknowledgment is made of support for this research to Donors of the Petroleum Research Fund, administered by the American Chemical Society.

REFERENCES

1. (a) T. C. BRUICE AND S. J. BENKOVIC, "Bioorganic Mechanisms," Vols. 1 and 2, Benjamin, New York, 1966; (b) W. P. JENCKS, "Catalysis in Chemistry and Enzymology," McGraw–Hill, New York, 1969; (c) M. L. BENDER, "Mechanisms of Homogeneous Catalysis from Protons to Proteins," Wiley–Interscience, New York, 1971; (d) "Transition States of Biochemical Processes" (R. D. Gandour and R. L. Schowen, Eds.), Plenum, New York, 1978.
2. For reviews see: (a) A. J. KIRBY AND A. R. FERSHT, *Progr. Bioorg. Chem.* **1**, 1 (1971); (b) B. CAPON, "Proton-Transfer Reactions" (E. F. Caldin and V. Gold, Eds.), Chap. 11, Chapman & Hall, London, 1975; (c) T. H. FIFE, *Advan. Phys. Org. Chem.* **11**, 1 (1975); (d) R. D. GANDOUR, Ref. (*1d*), Chap. 14; (e) A. J. Kirby, *Advan. Phys. Org. Chem.* **17**, 183 (1980).
3. A. J. KIRBY AND G. J. LLOYD, *J. Chem. Soc. Perkin Trans. 2*, 1753 (1976).
4. Pertinent literature references for **I** can be found in Ref. (*2*); for **V, VI** in Ref. (*2b*) and for **II**, Ref. (*3*). For **III** see: A. ARCELLI AND C. CONCILLO, *J. Chem. Soc. Perkin Trans. 2*, 1563 (1977). For **IV** see: R. M. POLLACK, R. H. KAYSER, AND J. R. DAMEWOOD, JR. *J. Amer. Chem. Soc.* **99**, 8232 (1977). For **VII** see: A. J. KIRBY, R. S. MCDONALD, AND C. R. SMITH, *J. Chem. Soc. Perkin Trans. 2*, 1495 (1974).
5. M. R. PETERSEN AND I. G. CSIZMADIA, *J. Amer. Chem. Soc.* **101**, 1076 (1979) and references therein.
6. (a) R. MEYER, T.-K. HA, AND HS. H. GÜNTHARD, *Chem. Phys.* **9**, 393 (1975); (b) N. L. ALLINGER AND S. H. M. CHANG, *Tetrahedron* **33**, 1561 (1977).
7. M. I. PAGE, *Chem. Soc. Rev.* **2**, 295 (1973).
8. W. P. JENCKS, *Advan. Enzymol.* **43**, 219 (1975).
9. (a) R. H. BROMILOW AND A. J. KIRBY, *J. Chem. Soc. Perkin Trans. 2*, 149 (1972); (b) G.-A. CRAZE AND A. J. KIRBY, *J. Chem. Soc. Perkin Trans. 2*, 61 (1974); (c) B. M. DUNN AND T. C. BRUICE, *J. Amer. Chem. Soc.* **92**, 2410 (1970) and **93**, 5725 (1971).
10. L. H. FUNDERBURK, L. ALDWIN, AND W. P. JENCKS, *J. Amer. Chem. Soc.* **100**, 5444 (1978).
11. M.-U. CHOI AND E. R. THORNTON, *J. Amer. Chem. Soc.* **96**, 1428 (1974).
12. R. L. SCHOWEN, *Progr. Phys. Org. Chem.* **9**, 275 (1972).
13. W. P. JENCKS, *Accounts Chem. Res.* **9**, 425 (1976).
14. L. D. KERSHNER AND R. L. SCHOWEN, *J. Amer. Chem. Soc.* **93**, 2014 (1971).
15. M. N. G. JAMES, I.-N. HSU, AND L. T. J. DELBAERE, *Nature (London)* **267**, 808 (1977).
16. V. K. ANTONOV, L. M. GINODMAN, Y. K. KAPITANNIKOV, T. N. BARSHEVSKAYA, A. G. GUROVA, AND L. D. RUMSH, *FEBS Lett.* **88**, 87 (1978).
17. V. I. ZANNIS AND J. F. KIRSCH, *Biochemistry* **17**, 2669 (1978).
18. J. DRENTH, J. N. JANSONIUS, R. KOEKOEK, L. A. AE. SLUYTERMAN, AND B. G. WOLTHERS, *Phil. Trans. Roy. Soc. Ser. B* **257**, 231 (1970).
19. R. BRESLOW AND D. L. WERNICK, *Proc. Nat. Acad. Sci. USA* **74**, 1303 (1977).
20. M. W. MAKINEN, K. YAMAMURA, AND E. T. KAISER, *Proc. Nat. Acad. Sci. USA* **73**, 3882 (1976).
21. J. A. HARTSUCK AND W. N. LIPSCOMB, "The Enzymes" (P. D. Boyer, Ed.), Vol. III, 3rd ed., pp. 1–56. Academic Press, New York, 1971.
22. (a) J. J. BIRKTOFT AND D. M. BLOW, *J. Mol. Biol.* **68**, 187 (1972); (b) G. H. COHEN, E. W. SILVERTON, B. W. MATTHEWS, H. BRAXTON, AND D. R. DAVIES, *J. Mol. Biol.* **44**, 129 (1979); (c) H. FEHLHAMMER AND W. BODE, *J. Mol. Biol.* **98**, 683 (1975); (d) W. BODE AND P. SCHWAGER, *J. Mol. Biol.* **98**, 693 (1975); (e) H. C. WATSON, D. M. SHOTTON, J. M. COX, AND H. MUIRHEAD, *Nature (London)* **225**, 806 (1970); (f) D. M. SHOTTON AND H. C. WATSON, *Nature (London)* **225**, 811 (1970); (g) D. A. MATTHEWS, R. A. ALDEN, J. J. BIRKTOFT, S. T. FREER, AND J. KRAUT, *J. Biol. Chem.* 252, 8875 (1977); (h) R. M. STROUD, L. M. KAY, AND R. E. DICKERSON, *J. Mol. Biol.*

83, 185 (1974); (i) P. W. CODDING, L. T. J. DELBAERE, K. HAYAKAWA, W. L. B. HUTCHEON, M. N. G. JAMES, AND L. JURÁSEK, *Canad. J. Biochem.* **52**, 208 (1974); (j) C. S. WRIGHT, R. A. ALDEN, AND J. KRAUT, *Nature (London)* **221**, 235 (1969); (k) R. A. ALDEN, C. S. WRIGHT, AND J. KRAUT, *Phil. Trans. Roy. Soc. London Ser. B* **B257**, 119 (1970); (l) S. T. FREER, J. KRAUT, J. D. ROBERTUS, H. T. WRIGHT, AND NG. H. XUONG, *Biochemistry* **9**, 1977 (1970); (m) G. D. BRAYER, T. J. DELBACRE, M. N. G. JAMES, *J. Mol. Biol.* **131**, 743 (1979).

23. J. KRAUT, *Annu. Rev. Biochem.* **46**, 331 (1977).
24. W. W. BACHOVCHIN AND J. D. ROBERTS, *J. Amer. Chem. Soc.* **100**, 8041 (1978).
25. C. C. F. BLAKE, L. N. JOHNSON, G. A. MAIR, A. C. T. NORTH, D. C. PHILLIPS, AND V. R. SARMA, *Proc. R. Soc. London Ser. B* **167**, 378 (1967); C. A. VERNON, *Proc. R. Soc. London Ser. B* **167**, 389 (1967).
26. J. A. RUPLEY, V. GATES, AND R. BILBREY, *J. Amer. Chem. Soc.* **90**, 5633 (1968).
27. S. J. PERKINS, L. N. JOHNSON, P. A. MACHIN, AND D. C. PHILLIPS, *Biochem. J.* **181**, 21 (1979).
28. K. K. KANNAN, M. PETEF, K. FRIDBORG, H. CID-DRESNER, AND S. LÖVGREN, *FEBS Lett.* **73**, 115 (1977).
29. A. FERSHT, "Enzyme Structure and Mechanism," p. 336. Freeman, Reading, United Kingdom 1977.
30. (a) J. R. KNOWLES AND W. J. ALBERY, *Accounts Chem. Res.* **10**, 105 (1977); (b) D. C. PHILLIPS, P. S. RIVERS, M. J. E. STERNBERG, J. M. THORNTON, AND I. A. WILSON, *Biochem. Soc. Trans.* **5**, 642 (1977).
31. T. SUGIMOTO AND E. T. KAISER, *J. Amer. Chem. Soc.* **101**, 3946 (1979).
32. P. J. SHAW AND H. MUIRHEAD, *FEBS Lett.* **65**, 50 (1976).
33. P. R. RONY AND R. O. NEFF, *J. Amer. Chem. Soc.* **95**, 2896 (1973).
34. D. E. KOSHLAND, JR., *Proc. Robert A. Welch Found.* **15**, 53 (1972).
35. J. H. WANG, *Proc. Nat. Acad. Sci. USA* **66**, 874 (1970).
36. For a review see Ref. (*2d*).