

alcohol-*O-d* to the extent that any resultant conclusions regarding anion stabilities based on its assumed absence may be erroneous. Secondly, and as a corollary, unless evidence establishing the absence of internal return is obtained, the derivation of anion stabilities from exchange rate data on any other sulfoxide is unwarranted. Fortunately, based on the recent studies of Cram and co-workers,²¹ one can test for internal return, at least qualitatively, by examining the effects of adding crown ether on the rates of proton exchange. Such a test should be routinely applied to isotopic exchange studies.

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References and Notes

- (1) A. Rauk, E. Buncler, R. Y. Moir, and S. Wolfe, *J. Am. Chem. Soc.*, **87**, 5498 (1965).
- (2) (a) G. Barbarella, A. Garbesi, and A. Fava, *J. Am. Chem. Soc.*, **97**, 5883 (1975); (b) S. Bory and A. Marquet, *Tetrahedron Lett.*, 4155 (1973); (c) R. Lett and A. Marquet, *ibid.*, 1579 (1975); (d) J. F. King and J. R. Du Manoir, *Can. J. Chem.*, **51**, 4082 (1973); (e) K. Nishihata and M. Nishio, *J. Chem. Soc., Perkin Trans. 2*, 1730 (1972); (f) R. R. Fraser, F. J. Schuber, and Y. Y. Wigfield, *J. Am. Chem. Soc.*, **94**, 8795 (1972), and references therein.
- (3) (a) T. Durst, R. R. Fraser, M. R. McClory, R. B. Swingle, R. Viau, and Y. Y. Wigfield, *Can. J. Chem.*, **48**, 2148 (1970); (b) B. J. Hutchinson, K. K. Andersen, and A. R. Katritzky, *J. Am. Chem. Soc.*, **91**, 3839 (1969).
- (4) T. Durst and M. Molin, *Tetrahedron Lett.*, 63 (1975); J. F. Biellmann and J. Vicens, *ibid.*, 2915 (1974).
- (5) S. Wolfe, A. Rauk, and I. G. Czismadia, *Can. J. Chem.*, **47**, 113 (1969).
- (6) "Internal return" as defined by k_{-1} in Scheme I replaces the term "back reaction" originally introduced by Cram⁷ for the same phenomenon. In a recent review⁸ Hunter examines the influence of solvent on the intramolecular component of a large number of base-catalyzed allylic isomerizations and deduces some generalizations regarding the likelihood of internal return occurring in various solvents.
- (7) D. J. Cram, C. A. Kingsbury, and B. Rickborn, *J. Am. Chem. Soc.*, **83**, 3688 (1961).
- (8) D. H. Hunter in "Isotopes in Organic Chemistry", Vol. I, E. Buncler and C. D. Lee, Ed., Elsevier, Amsterdam, 1975, p 135.
- (9) D. J. Cram, "Fundamentals of Carbanion Chemistry", Academic Press, New York, N.Y., 1965, Chapters 3 and 5.
- (10) (a) A. Streitwieser, Jr., P. H. Owens, G. Sonnichsen, W. K. Smith, G. R. Ziller, H. M. Niemeyer, and T. L. Kruger, *J. Am. Chem. Soc.*, **95**, 4254 (1973); (b) A. Streitwieser, W. B. Hollyhead, H. H. Pudjajamaka, P. H. Owens, T. L. Kruger, P. A. Rubenstein, R. H. MacQuarrie, M. L. Browka, W. K. C. Chu, and H. M. Niemeyer, *J. Am. Chem. Soc.*, **93**, 5088 (1971).
- (11) C. G. Swain, E. C. Stivers, J. F. Reuwer, Jr., and L. J. Schaad, *J. Am. Chem. Soc.*, **80**, 5885 (1958).
- (12) J. E. Baldwin, R. E. Hackler, and R. M. Scott, *Chem. Commun.*, 1415 (1969).
- (13) For simplification purposes we will hereafter refer to the *pro R* in *S* proton as the *fast* proton and the *pro R* in *R* proton as the *slow* proton; i.e., the more slowly exchanging tritium is called T^s, etc.
- (14) The tritium content of the water used in the labeling techniques was adjusted in each case to provide sufficient specific activity in the resultant sample to give short (< 10 min) scintillation counting times.
- (15) In the preparation of sample I the selectivity in tritiation to only 3.3% of the tritium pool is assumed to be the same as in deuteration (i.e., the sample should contain 7% of T^s). Then detritiation kinetics were measured to only two half-lives of T^f assuming no change in [T^s]. The rate of deuteration, measured as $-dD_0/dt$, includes formation of both diastereomeric monodeuteriosulfoxides and requires the correction factor $k_H f = 0.94 k_{obsd}$ be included. This correction factor (14.4/15.4) was measured to greater accuracy by Swingle¹⁸ using mass spectral analyses to follow the exchange in D₂O at 24 °C. In the preparation of sample II three consecutive labeling experiments were required. Formation of the anion of 1 in THF at -95° using lithium diisopropyl amide (0.92 of an equivalent), allowing 100 min for equilibration of all possible anionic species then addition of D₂O gave 92% monodeuteriosulfoxide, whose D^s/D^f was shown in separate experiments to be >94/6 by NMR integration. Treatment of this sample with NaOD in D₂O gave mainly dideuteriosulfoxide with no deuterium in the methyl group (MS) and, by NMR, 90% D^s and 95% D^f. Subsequent tritiation in H₂O to 3% of the tritium pool gave selectively labeled sample II, containing 90% D^s and 86% D^f (by NMR). The selectivity in this labeling experiment is assumed to be identical with that measured (MS) separately on a dideuterated sample and found to be (14:1), the same as for deuteration in D₂O. Detritiation kinetics to two half-lives were corrected for the presence of 7% T^s. Loss of deuterium was measured as $-dD_2/dt$ with a correction for loss of D^s included. Sample III, prepared from quenching the anion with tritiated water in THF at -95° was also assumed to contain 6% T^f. Detritiation kinetics were measured after 18% loss of original tritium content (i.e., four half-lives of T^f). Deuteration over the same time period was measured as $-dD_1/dt$ (at beginning of which no undeuterated sample remained). Sample IV from quenching with D₂O + TOD was also assumed to contain 94% D^s + T^s. Loss of tritium and deuterium was measured for kinetics after 25% of total tritium had been lost. The assumption that the selectivity in tritiation is the same as in deuteration is verified by the observed kinetics of detritiation, in particular, by the fact that the largest standard deviation is less than 0.7% of the rate constant. The assumption is, of course, consistent with absence of internal return in the exchange process.
- (16) R. B. Swingle, Ph.D. Thesis, University of Ottawa, 1969.
- (17) Aliquots for tritium and deuterium assay were purified by TLC followed by sublimation. Deuterium analyses were made using an AEI MS-902 mass spectrometer. Scintillation counts were made using a Beckmann LS-150 counter. NMR measurements on selectivity were made using a Varian HA-100 spectrometer equipped with an SD-100 deuterium spin-decoupler.
- (18) Using the theoretical expressions relating $(k_H/k_T)_{obsd}$ and a_1 to k_H/k_T for step I, Scheme I, one can calculate k_H/k_T for step I which turns out to be 2.2 for abstraction of either diastereotopic proton. This very low primary isotope effect is consistent with a highly endothermic proton abstraction process.
- (19) Proton assignments for H₁ and H₂ in 2 have been made earlier.²¹ That selective tritiation is feasible at these two positions was shown by exchange studies in two solvents. Since H₁ exchanges 300 times slower than any other proton in *tert*-butyl alcohol-*O-d*, complete tritiation at all four benzylic positions, followed by detritiation at the three more labile positions gave the sample labeled with tritium in place of H₁. To obtain the tritium label at H₂, the above sample was inverted at sulfur.²⁰
- (20) C. R. Johnson and D. McCants, Jr., *J. Am. Chem. Soc.*, **87**, 5406 (1965).
- (21) J. N. Roitman and D. J. Cram, *J. Am. Chem. Soc.*, **93**, 2231 (1971); S. M. Wong, H. P. Fischer, and D. J. Cram, *ibid.*, **93**, 2235 (1971); J. Almy, D. C. Garwood, and D. J. Cram, *ibid.*, **92**, 4321 (1970); D. H. Hunter, Y.-t. Lin, A. L. McIntyre, D. J. Shearing, and M. Zvagulis, *ibid.*, **95**, 8327 (1973).

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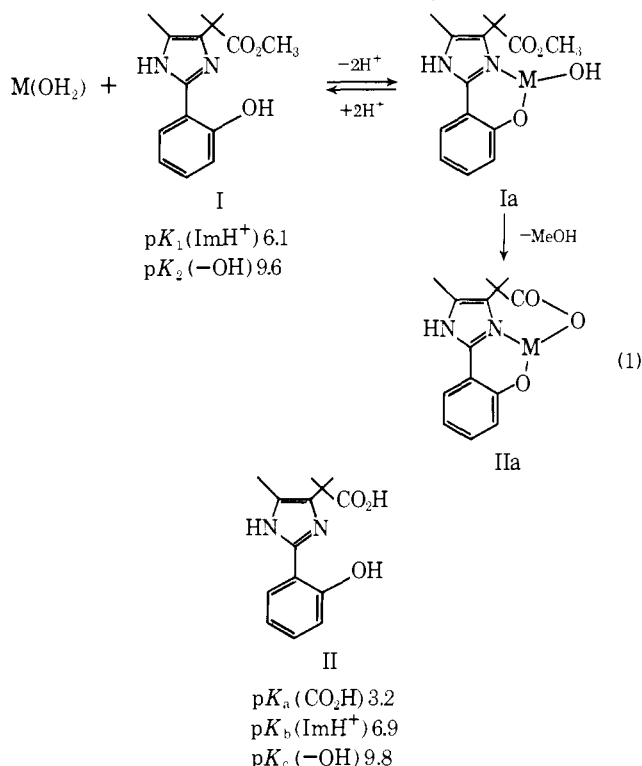
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Intramolecular Hydrolysis of a Methyl Ester by Substrate Bound Metal Hydroxide

Sir:

The role of the metal ion in the mechanism of hydrolytic metalloenzymes continues to receive much attention. Interest in the role of metal bound hydroxides as nucleophiles has been stimulated by recent communications concerning the ability of these species to add to carbonyl compounds¹ and to effect the hydrolysis of carboxylic acid anhydrides.² An ideal model for a metallohydrate centered acyl transferase would be one that directed the attack of the metal bound hydroxyl group in either an intramolecular or an intracomplex reaction, involved the hydrolysis of an acyl function with a poor leaving group (aliphatic carboxylic acid ester or amide), and would allow the determination of all kinetic and thermodynamic constants. In



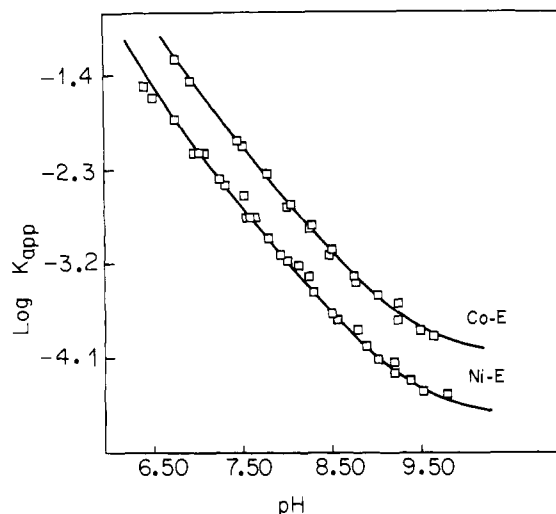
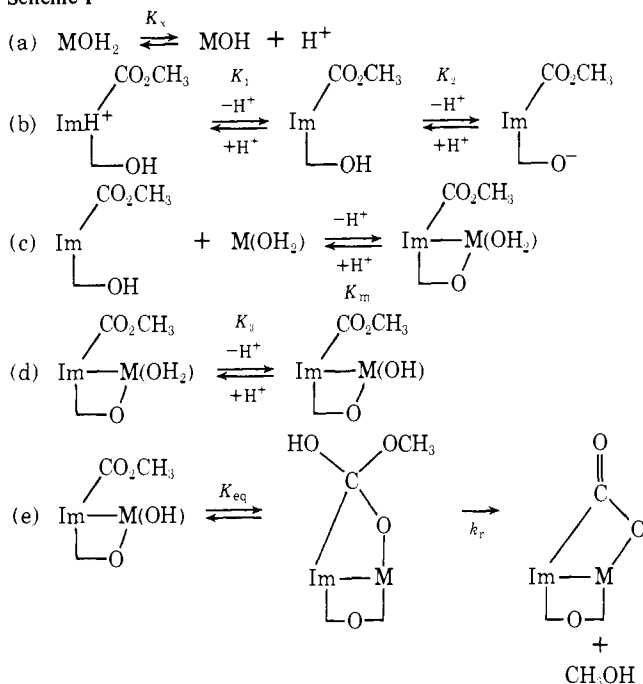


Figure 1. pH dependence of the apparent dissociation constant (K_{app}) of metal complexes of ester I. The solid curves represent the best fit of eq 5 to the experimental data.

Scheme I



this communication we report the successful study of such a model.

Hydrolysis of I³ (eq 1) has been investigated as a function of pH and metal ion concentration. Our experimental results find quantitative expression in the reactions of Scheme I. The values of the determined constants at 30° in H₂O at $\mu = 0.2$ (NaClO₄) are: $pK_x(Co^{2+}) = 9.4$, $pK_x(Ni^{2+}) = 9.4$; $\log K_m(Co^{2+}) = 5.42$, $\log K_m(Ni^{2+}) = 4.85$; $pK_3(Co^{2+}) = 9.6$; $pK_3(Ni^{2+}) = 9.6$; $K_{eq}(Co^{2+}) = 14.0$, $K_{eq}(Ni^{2+}) = 2.0$; $k_r(Co^{2+}) = 1.05 \text{ min}^{-1}$, $k_r(Ni^{2+}) = 0.60 \text{ min}^{-1}$. A description of the means by which the various constants were obtained and justification for the proposal that a stable tetrahedral intermediate occurs along the reaction path follow.

The thermodynamic constants of eq a to d (Scheme I) can be determined titrimetrically⁴ since proton transport and metal ion complexation are rapid compared to the hydrolytic steps of reaction e. The complexation of I with metal ions is evidenced by a lowering of the pK of the phenolic hydroxyl which may be quantitatively monitored at 292 nm (an isobestic point for ionization of the imidazolyl group). The pH dependent

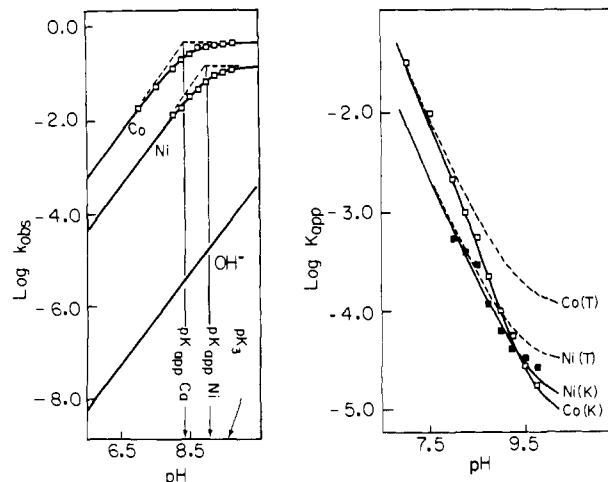


Figure 2. Left Panel: pH-rate profile for the hydrolysis of ester I in the presence of Co^{2+} , Ni^{2+} , and the OH^- catalyzed reaction. The arrows on the pH ordinate show the kinetically determined pK for $Co(H_2O)$ and $Ni(H_2O)$, as well as the thermodynamic pK_3 . Right Panel: pH dependence of the kinetically determined dissociation constant of metal complexes of ester I: (\square) Co^{2+} , (\blacksquare) Ni^{2+} . Solid lines labeled $Co(K)$ and $Ni(K)$ represent the fit of eq 8 to the data. Dotted lines labeled $Co(T)$ and $Ni(T)$ represent the best fit of the data in Figure 1 to eq 5.

dissociation constant of the metal-substrate complex (K_{app}) is defined as in eq 2

$$K_{app} = \frac{[M_T][E]}{[E-M]} \quad (2)$$

($[M_T]$ = total metal concentration, $[E]$ = concentration of total uncomplexed I, and $[E-M]$ = concentration of complexed I). Since $[M_T] \gg [I]$, one is justified in equating free metal ion with $[M_T]$. ($[I] = 1.25 \times 10^{-5} \text{ M}$, $[M_T] = 1 \times 10^{-4}$ to $1 \times 10^{-3} \text{ M}$). K_{app} was determined in two ways which gave excellent agreement. (1) By monitoring the change in A_{292} upon addition of increments of metal perchlorates at constant pH, $\alpha = [E-M]/[E + E-M]$ was determined. A plot of $1/\alpha$ vs. $1/[M_T]$ (see eq 3) gives K_{app} as the slope.

$$\frac{1}{\alpha} = 1 + \frac{K_{app}}{[M_T]} \quad (3)$$

(2) Alternatively, α was determined as a function of pH at constant metal ion concentration; since $1 - \alpha = ([E])/([E] + [E-M])$, eq 2 becomes eq 4.

$$\log K_{app} = \log [M_T] - \log \frac{\alpha}{1 - \alpha} \quad (4)$$

According to reactions a to d of Scheme I, the dependence of K_{app} on pH is given by eq 5.

$$\log K_{app} = \log K_m - pH + \log \left(1 + \frac{a_H}{K_1} + \frac{K_2}{a_H} \right) + \log \left(1 + \frac{K_x}{a_H} \right) - \log \left(1 + \frac{K_3}{a_H} \right) \quad (5)$$

The fit of experimental data, obtained by iteration in K_m and K_3 to eq 5 is shown in Figure 1.

Conversion of I \rightarrow IIa was followed spectrophotometrically (335 nm formation of IIa or 285.5 nm disappearance of I) at various pH's and metal ion concentrations; ($[I] = 6.25 \times 10^{-6} \text{ M}$, $[M_T] = 5 \times 10^{-5}$ to $1 \times 10^{-3} \text{ M}$) first-order kinetics were observed for at least 2-3 half-lives. The rate constant (k_{app}) increased with increasing metal concentration until saturation of substrate by metal ion. Plots of $1/k_{app}$ vs. $1/[M_T]$ at each pH investigated were linear and gave $1/k_{obsd}$ as the intercept at $1/[M_T] = 0$ while the intercept on the $-1/[M_T]$ axis provided the kinetically determined metal substrate dissociation

constant (K_{app}). Plots of $\log k_{obsd}$ vs. pH in the presence of Co^{2+} or Ni^{2+} and for the OH^- catalyzed reaction in the absence of metal ($k_{OH} = 1.63 \text{ min}^{-1} \text{ M}^{-1}$) are shown in Figure 2. The metal ion data were fit to eq 6 using $k = 0.98 \text{ min}^{-1}$, $pK_3' = 8.4$ for Co^{2+} , and $k = 0.4 \text{ min}^{-1}$, $pK_3' = 9.1$ for Ni^{2+} .

$$k_{obsd} = k \frac{K_3'}{K_3' + a_H} \quad (6)$$

The fact that pK_3' is less than pK_3 allows distinction to be made between attack of the substrate bound metal-hydroxide (Scheme I) and the apparently identical kinetic mechanism which involves attack of external OH^- on the carbonyl group activated by the bound metal.⁶

A stable tetrahedral intermediate along the reaction path (Scheme I) could account for the fact that pK_3' determined kinetically is significantly different from the thermodynamic constant pK_3 . The nonrate determining formation of an additional intermediate after ionization of the $M(OH_2)$ moiety would result in the apparent ionization constant (pK_3') of the $M-OH_2$ moiety of the complex being less than pK_3 .^{7,8} On the basis of Scheme I, k_{obsd} is given by eq 7.

$$k_{obsd} = \frac{k_r K_{eq}}{(1 + K_{eq})} \left[\frac{K_3(1 + K_{eq})}{a_H + K_3(1 + K_{eq})} \right] \quad (7)$$

From eq 7 it can be seen that K_3' of the complexed $M(OH_2)$ moiety is provided by $K_3(1 + K_{eq})$ and that $k = k_r K_{eq}/(1 + K_{eq})$. The data in Figure 2 can be fitted to eq 7 using the thermodynamic pK_3 and $k_r = 1.05 \text{ min}^{-1}$, $K_{eq} = 14.5$ for Co^{2+} , and $k_r = 0.6 \text{ min}^{-1}$, $K_{eq} = 2.5$ for Ni^{2+} .

According to Scheme I the value of K_{app} determined kinetically is given by eq 8 which may be compared to eq 5 which provides the value of K_{app} determined from the titrimetric data.

$$\log K_{app} = \log K_m - pH + \log \left(1 + \frac{a_H}{K_1} + \frac{K_2}{a_H} \right) + \log \left(1 + \frac{K_x}{a_H} \right) - \log \left[1 + \frac{K_3(1 + K_{eq})}{a_H} \right] \quad (8)$$

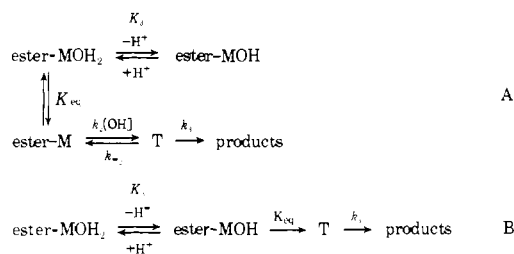
The fit of the experimental data to eq 8 is shown in Figure 2; $K_{eq} = 13.5$ for Co^{2+} and 1.5 for Ni^{2+} . The agreement between K_{eq} determined from eq 7 and 8 is reasonable. Examination of Stuardt and Breigle models of metal complexes of I reveal that once complexation with the imidazole nitrogen and phenolic hydroxyl occurs, it is not possible for the metal to coordinate with the ester carbonyl. This taken in conjunction with the observed pH-rate profile which shows that ionization of the $M-OH_2$ moiety is associated with catalysis eliminates metal ion activation of the carbonyl to intermolecular attack by OH^- as a contributing factor in this model system. Catalysis and complexation both exhibit a degree of specificity for the metal ion. No detectable complexation or catalysis was observed with Mg^{2+} , Ca^{2+} , or Mn^{2+} . Zn^{2+} forms a catalytically inactive dimeric complex $(I)_2Zn$, whereas, the 1:1 Cd^{2+} complex is catalytically inactive, perhaps due to steric requirements.

At pH values below pK_3 , intramolecular $M-OH$ hydrolysis of I exceeds that for OH^- mediated hydrolysis by 2×10^5 (Co^{2+} complex) and 1.2×10^4 (Ni^{2+} complex). The specific rate constants for intramolecular catalysis by the $M-OH$ moiety ($K_{eq}k_r = 14.7$ and 1.2 min^{-1} for Co^{2+} and Ni^{2+} , respectively) are ca. 10^3 less than the reported values of k_{cat} for the hydrolysis of a good ester substrate by carboxypeptidase.⁹ This rate ratio of 10^3 (ca. 4 kcal M^{-1}) is not large when taken with the fact that some distortion of the metal ion geometry is required in the model system¹⁰ in order to effect the formation of the tetrahedral intermediate. Without this requirement the intramolecular catalysis by $M-OH$ may be expected to approach that of carboxypeptidase.

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References and Notes

- (1) E. Chafee, T. P. Dasgupta, and G. M. Harris, *J. Am. Chem. Soc.*, **95**, 4169 (1973); D. A. Palmer and G. M. Harris, *Inorg. Chem.*, **13**, 965 (1974).
- (2) (a) R. Breslow, D. E. McClure, R. S. Brown, and J. Eisenbach, *J. Am. Chem. Soc.*, **97**, 194 (1975); (b) D. A. Buckingham and L. M. Engelhardt, *ibid.*, **97**, 5915 (1975).
- (3) G. A. Rogers and T. C. Bruice, *J. Am. Chem. Soc.*, **96**, 2473 (1974).
- (4) All studies were carried out in the Cary pH-stat cell⁹ under N_2 . A modified buret assembly prevented precipitation of $M(OH)_2$ and the calomel electrode was separated from the reaction by a liquid junction through which 0.2 M NaClO_4 was passed. This apparatus prevents leakage of Cl^- into the reaction.
- (5) T. C. Bruice and J. R. Maley, *Anal. Biochem.*, **34**, 275 (1970).
- (6) Thus, mechanisms A and B (where T = tetrahedral intermediate) both provide the kinetic expression of eq 6. For A, where ester-M is the carbonyl



activated intermediate, two cases can be considered. (1) k_2 is rate determining, thus k of eq 6 = $k_2 K_w K_{eq} / K_3$ and $K_3' = K_3 / (1 + K_{eq})$. (2) k_3 is rate determining and steady state in T is assumed. $k = k_2 k_3 K_w K_{eq} / (k_3 + k_2) K_A$ and $K_3' = K_3 / (1 + K_{eq})$. Assumption of steady state in ester-M shows that $K_3' = K_3$. For B assuming k_3 is rate determining eq 7 is derived. Assumption of steady state in T shows $K_3' = K_3$. Therefore, only mechanism B can account for the observation that pK_3' is less than pK_3 . In addition only mechanism B can account for the observation that the kinetically determined metal substrate dissociation constant is less than the thermodynamically determined constant (see eq 8). More importantly, mechanism A is eliminated by observations that metal ion does not accelerate the reaction of NH_2OH with the ester. For a fuller discussion of the influence of preequilibria on kinetically pK_3' 's see ref 7.

- (7) T. C. Bruice and G. L. Schmir, *J. Am. Chem. Soc.*, **81**, 4552 (1959).
- (8) D. A. Buckingham, J. Dehhers, and A. M. Sargeson, *J. Am. Chem. Soc.*, **95**, 4173 (1973), have presented evidence for a metal stabilized tetrahedral intermediate in the aminolysis of $Co(III)$ complexes of glycine esters.
- (9) D. S. Auld and B. Holmquist, *Biochemistry*, **13**, 4355 (1974).
- (10) Evidence has been presented for the existence of ground state distortion of metal ion geometry in carboxypeptidase. B. L. Vallee and R. J. P. Williams, *Proc. Natl. Acad. Sci. U.S.A.*, **59**, 498 (1968).
- (11) Macy Faculty Scholar 1975-1976.

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The Diphenylvinylene Carbonate-Diene Exciplex. Solvent Dependence of Radiative and Nonradiative Decay Processes

Sir:

There is growing evidence that excited state π complexes or exciplexes are intermediates in numerous photochemical cycloaddition reactions.¹⁻⁴ Previous investigations of the stereospecific $\pi_2s + \pi_2s$ cycloaddition reactions of singlet *trans*-stilbene and diphenylvinylene carbonate (V) with electron rich alkenes⁵ and dienes⁶ provided indirect evidence for exciplex intermediates. The high quantum yields (~ 1.0) for adduct formation in nonpolar solvents^{5a,b,6c,e} seemed to preclude the observation of exciplex fluorescence. We now wish to report the observation of solvent sensitive exciplex fluorescence from singlet V and 2,5-dimethyl-2,4-hexadiene (D). The effect of solvent upon exciplex fluorescence, cycloaddition, and non-radiative decay pathways provides important insights into the behavior of the exciplex intermediate.

Quenching of the fluorescence of V⁷ is accompanied by the appearance of a new structureless emission at longer wave-