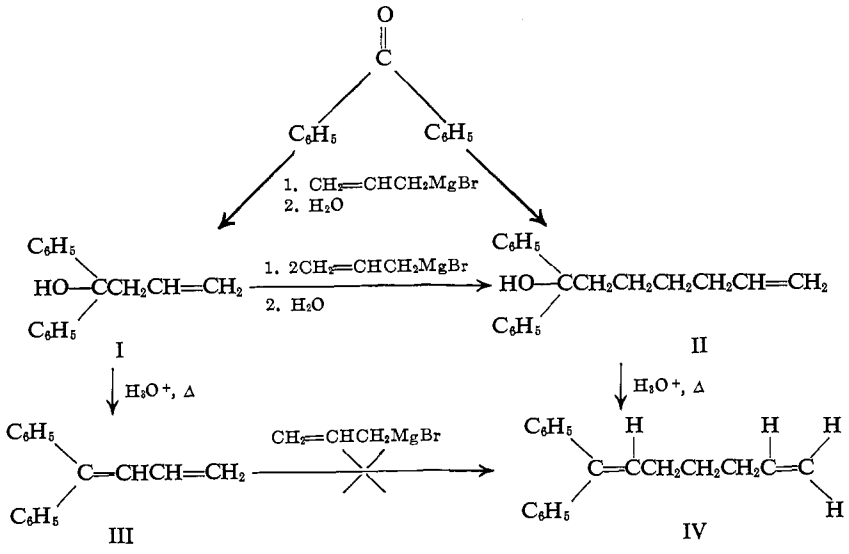
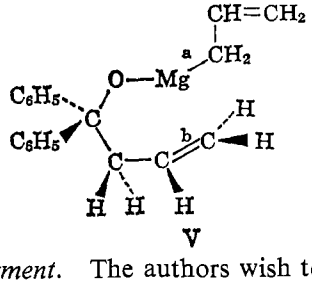
Chart I



participate directly in the formation of II. Clearly the presence of benzophenone is not essential, for the carbinol II could be obtained in good yield (>70%) merely by treating allyldiphenylcarbinol in ether with 2 equiv. of allylmagnesium bromide (25° for 36 hr.). (Incidentally, III was shown not to add allylmagnesium bromide.) There is no reasonable doubt, therefore, that allylmagnesium bromide can add to the unconjugated, unactivated ethylenic linkage of the magnesium carbinolate salt of I.

As a working hypothesis for further synthetic and mechanistic studies of this reaction, we suggest that this addition is facilitated by the intramolecular proximity of the reacting bonds. When allyldiphenylcarbinol is treated with the allyl Grignard reagent, it is reasonable to assume that the presence of diallylmagnesium in the latter[6] would lead to the production of intermediate V. Inspection of a Stuart–Briegleb model of V reveals that the allyl–magnesium bond (a) can easily become contiguous to the vinyl bond (b) of the carbinolate. Further research will probe the generality of this reaction by use of other unsaturated carbinols and amines, as well as a variety of organometallic reagents.



V

Acknowledgment. The authors wish to express their appreciation to the donors of the Petroleum Research Fund for their support of this research by PRF Grant No. 723-A, both at the University of Michigan and The Catholic University of America.

(6) G. O. Johnson and H. Adkins, *J. Am. Chem. Soc.*, 54, 1943 (1932).

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Mixed Metal Complexes as Enzyme Models. I. Intracomplex Nucleophilic Catalysis by an Oxime Anion

Sir:

Studies on esterolytic enzymes and peptidases have proceeded to the point that a general mechanism of their action may be described: acyl transfer occurs to a nucleophilic group of the enzyme within an enzyme–substrate complex, and the acyl group is then hydrolytically removed in a second step. The hope of utilizing such principles to achieve rapid and selective organic reactions has stimulated the construction of various enzyme model systems. We have considered that catalysis by attack of one ligand on another within a mixed metal complex could include the strong catalyst–substrate binding which is a key feature of enzymatic catalysis; furthermore, such enzymes as (zinc-containing) carboxypeptidase use metal bridging between protein and substrate for at least part of the ES binding energy. The problem of providing a strong free nucleophile in a metal complex has been solved by utilizing zinc pyridinecarboxaldoxime anion (I).[1] We wish to report that I is indeed a particularly effective nucleophilic catalyst in the hydrolysis of 8-acetoxyquinoline 5-sulfonate (II), a weakly complexing substrate,[2] by a two-step mechanism involving acyl transfer within a catalyst–substrate complex. Models show

Table I. Equilibria of PCA with Zn^{2+} [a]

	K, *M*
$HA \rightleftarrows H^+ + A^-$	$(9.1 \pm 0.9) \times 10^{-11}$
$HA + Zn^{2+} \rightleftarrows ZnAH^{2+}$	150 ± 15
$A^- + Zn^{2+} \rightleftarrows ZnA^+$	$(5.0 \pm 1.2) \times 10^{5}$
$ZnAH^{2+} \rightleftarrows ZnA^+ + H^+$	$(3.0 \pm 0.7) \times 10^{-7}$
$HA + Zn^{2+} \rightleftarrows ZnA^+ + H^+$	$(4.5 \pm 1.0) \times 10^{-5}$
$ZnA(H_2O)^+ \rightleftarrows ZnA(OH) + H^+$	$(1.8 \pm 0.4) \times 10^{-8}$

[a] At 25.0°. HA = neutral PCA molecule.

(1) *Cf.* S. Balton and A. Beckett, *J. Pharm. Soc.*, 53, 55 (1964).

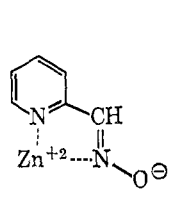
(2) E. J. Corey and R. L. Dawson, *J. Am. Chem. Soc.*, 84, 4899 (1962), report metal-catalyzed hydrolyses of 8-carbamoylquinolines. Compound II was prepared by acetylation and fully characterized. Spectrophotometric studies show that K_s for Zn^{2+} and II is $<5\ M^{-1}$.

Table II. Second-Order Rate Constants[a] for Nucleophilic Attack

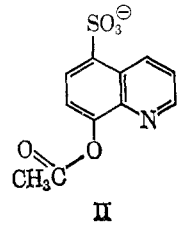
Nucleophile	pK_a	k_2, M^{-1} sec.$^{-1}$ for II	k_2, M^{-1} sec.$^{-1}$ for III	Rate ratio III/II
OH^-	15.7[b]	2.01 ± 0.03	14.8[b]	7.4 ± 0.1
PCA anion	10.04	6.98 ± 0.85	77.2 ± 0.9	11 ± 1
H_2O	−1.7[b]	(7.3 ± 0.9) × 10^{-7}	1.0 × 10^{-8}[b]	0.014 ± 0.002
I	6.5	10 ± 2[c]	10 ± 2[c]	1.0 ± 0.1[d]

[a] At 25°. The pseudo-first-order rate constants for anion liberation from II and III were determined spectrophotometrically in solutions 0.0010 M in $ZnSO_4$, 0.0015 M in PCA, and 0.010 M in 2,6-lutidine buffer, at pH values from 5.89 to 7.53. At any pH, all measurements were made on aliquots of the same reaction mixture, and the substrate concentration was 1.4 × 10^{-4} M or less. [b] W. P. Jencks and J. Carriulo, *J. Am. Chem. Soc.*, **82**, 1778 (1960). [c] Determined over a range of 5.89 to 7.0. Above this pH, nucleophilic attack by ZnPCA(OH) becomes appreciable. [d] The ratio is more accurate than the rate constants, since the latter contain uncertainties in equilibrium constants.

that in a mixed complex of I and II the oxygen atom of I is in a position to attack the acetyl of II.



I



II

Spectroscopic and titrimetric methods establish the equilibrium constants listed in Table I for complexes between zinc and pyridinecarboxaldoxime (PCA). Using these constants and the pseudo-first-order rates of phenoxide formation, we have derived the second-order rate constants listed in Table II for attack on II and on *p*-nitrophenyl acetate (III).[3] These are rates for the spectrophotometric appearance of zinc–oxyquinolinesulfonate complex (λ_{max} 365 mμ) and *p*-nitrophenoxide ion. Under our conditions, subsequent hydrolysis of acetylated I is about ten times slower than acetyl transfer from II. It is still fast enough that the over-all titrimetric rate of hydrolysis of II with 0.01 M Zn^{2+} and 0.015 M PCA is increased by a factor of 10 over the uncatalyzed rate (which has k_1 = 4.6 × 10^{-3} min.$^{-1}$) at neutrality, and a factor of 2 over the rate in 0.01 M Zn^{2+} and 0.015 M glycine. It is apparent that I is an extraordinary[4] nucleophile toward both substrates; it is comparable in reactivity to hydroxide ion, although its pK_a is only 6.5.

The data in Table II show that *p*-nitrophenyl acetate (III) is more reactive than is II toward OH^- and PCA anion; this is as expected since the *p*-nitrophenoxide ion (pK_a = 7.0) is a better leaving group than is the oxyquinolinesulfonate ion (pK_a = 8.4). The very high reactivity of II toward water probably reflects intramolecular general base catalysis by the quinoline nitrogen. We suggest that the increased reactivity of II toward I (shown by the III/II reactivity ratio) reflects an improved rate because of I–II complex formation. Control runs have established that the acetyl transfer from II to I has the correct apparent kinetic order in zinc (calculated from the equilibria in Table I) and completely exclude a mechanism with 2 zinc atoms in the transition state. Furthermore, by quenching with EDTA and isolation, PCA acetate[5] has been identified (infrared, n.m.r., thin layer chromatography) as an intermediate in the hydrolyses, found in 84 ± 4% of the theoretical maximum yield.

The suggestion that the relatively high reactivity of II toward I is a reflection of reaction within a mixed complex is supported by kinetic runs in the presence of 1 mole of *o*-phenanthroline/mole of Zn^{2+}. The *o*-phenanthroline complex of I is actually four times more nucleophilic toward *p*-nitrophenyl acetate, apparently because of increased basicity, but the rate ratio for *p*-nitrophenyl acetate/II is again up to 5.6 (pH 7.51) to 6.1 (pH 7.22). *o*-Phenanthroline is a competitive inhibitor which at least partially blocks binding of II.

The effects seen here are relatively modest since II is a poor ligand with high intrinsic reactivity. We are currently extending these studies to more firmly bound substrates, with I and other chelate catalysts, to examine the scope of this approach to enzyme models.

(3) All kinetic runs were performed with the substrate at less than one-fifth the concentration of zinc to avoid inhibition by product.

(4) Although *p*-nitrophenyl acetate is used as an example of an uncomplexing substrate, the high reactivity toward I probably indicates that here too there is stabilizing interaction between zinc and the leaving group during acetyl transfer.

(5) S. Ginsburg and I. B. Wilson, *J. Am. Chem. Soc.*, 79, 481 (1957).

(6) Predoctoral Fellow of the National Science Foundation. Partial support of this work by the National Institutes of Health is also gratefully acknowledged.

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The Use of the Tyrocidines for the Study of Conformation and Aggregation Behavior

Sir:

Protein studies undertaken from many viewpoints have focused attention on the importance of interacting forces other than those involved in covalent linkages. These forces largely determine the specific physical and biological properties of a given protein. The terms hydrogen bonds, hydrophobic bonds, hydrophilic bonds, π bonds, and ionic bonds in recent years have been widely used in the attempt to describe more precisely the nature of the interactions which determine the stability, conformation, solubility, and state of aggregation of such large polymeric molecules. Obviously a number of parameters, some perhaps not yet even defined, are involved in the concerted interactions to be considered.

This communication is written to point out the value of the tyrocidines and the related peptide gramicidin S-A in this connection and the reason they can serve as particularly valuable models. The tyrocidines are a unique class of naturally occurring cyclic decapeptides. Thus far the individual members can be isolated in preparative amounts only by countercurrent distribution probably because of their very strong degree of aggre-