noncooperative nature of the binding also indicates a random, nonspecific interaction between the peptide and the phospholipid surface, without the necessity to invoke the presence of discrete binding sites. The free energy of complex formation is 0.6 kcal/amino acid, a reasonable value if the binding energy is the result of the transfer of a potential amphiphilic helix from water into an amphiphilic surface.[12]

Circular dichroism spectra show that peptides A and D are in a random, rather than in a helical conformation, even in 50% trifluoroethanol. Peptides B and C contain an estimated 10–15% helical structure[13] in aqueous buffers over a wide pH range, the amount of helicity increasing to 40% for peptide B and 33% for C in 50% trifluoroethanol. Thus, the differences in the abilities of the synthetic peptides to bind to lipid vesicles seem to correlate with helix-forming potential. The pH dependency of the binding shows the importance of the state of ionization of the peptides: the isoelectric point is 7.1 for peptide B and 4.6 for peptide C, whereas the vesicles have a negative ζ potential, as indicated by their electrophoretic mobility. Thus, the electrostatic repulsion of negatively charged peptides must reduce their affinity for the vesicles, although the major binding force has to be of a lyophilic nature.

The enzymatic digestion shows that the presence of bound peptide increases the rate of hydrolysis of the phospholipids by a factor of 6, thereby yielding a hydrolytic rate comparable with that observed for human plasma LDL.[14] Thus, the egg lecithin vesicle–peptide complex is a suitable model for the lipoprotein surface by the criterion of this sensitive enzymatic probe. Previous phospholipid–protein binding studies using apoproteins or their fragments and dimyristoyl lecithin vesicles failed to yield a quantitatively analyzable system, mostly because of the major structural reorganization of the vesicles that occurred upon binding.[5] We feel that the present study demonstrates that the synthetic peptide–egg lecithin single bilayer vesicle system provides an excellent model for the study of lipoprotein structure. We are currently pursuing this line of investigations.

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Effect of Phenyl Substituents on the Hydrolyses of Trifluoroacetanilides Catalyzed by Imidazolyl Cation and Hydroxide Ion

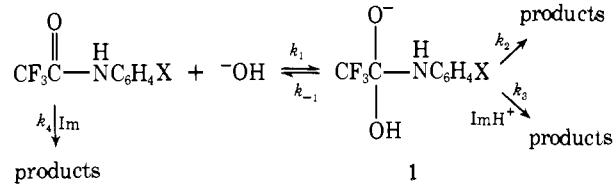
Sir:

We report a result of a simple model study which strongly supports acid catalysis by the imidazolyl cation in serine protease catalyzed hydrolyses of amide compounds, especially anilides. Acid catalysis by the imidazolyl cation of His-57 has been proposed in the α-chymotrypsin-catalyzed hydrolyses of anilides, on the basis of the finding that electron-donating substituents on the phenyl rings of anilides largely facilitated reaction;[1] for example, in the chymotrypsin-catalyzed hydrolyses of *N*-acetyl-L-tyrosine anilides $\rho = -1.8$ at 35 °C.[1e] The imidazolyl cation allegedly catalyzes the breakdown of the tetrahedral intermediate between Ser-195 and the anilide. However, nonenzymatic hydrolyses of trifluoroacetanilides, in which the breakdown of the tetrahedral intermediate between hydroxide ion and the anilide is catalyzed by water (as acid catalyst), showed a *positive* ρ value (+0.69) with respect to phenyl substituents.[2] Thus, there has been a big discrepancy between enzymatic hydrolyses of anilides and nonenzymatic ones previously studied. Here, the alkaline hydrolyses of five trifluoroacetanilides (H, *m*-Cl, *p*-Cl, *m*-NO_2, and *p*-NO_2) catalyzed by imidazolyl cation are reported, showing a large *negative* ρ value consistent with enzymatic reactions.

The alkaline hydrolyses of trifluoroacetanilides in the presence of imidazolyl cation (ImH^+) and imidazole (Im) proceeds as shown in Scheme I.[3,4] The tetrahedral intermediate (**1**) collapses to products via acid catalysis by the imidazolyl cation (k_3) and by water (k_2).[5] Furthermore, neutral imidazole directly catalyzes the hydrolyses (k_4).

The parameters, k_3/k_{-1}, k_1, and k_4, were evaluated from the plot of the observed rate constant of hydrolyses, determined spectrophotometrically, vs. the concentration of imidazole at pH 7.2, 70 °C. As described by Eriksson et al.,[4] this plot gives $k_3/(k_{-1} - k_2)$, k_1, and k_4. Since k_{-1} is much larger than

Scheme I



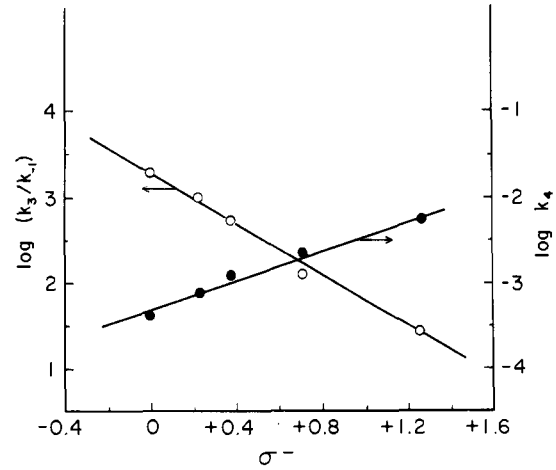


Figure 1. Plots of log (k_3/k_{-1}) and log (k_4) vs. σ^- in the hydrolyses of trifluoroacetanilides at 70 °C; see Scheme I concerning the notation of rate constants.

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k_2,[2-4] $k_3/(k_{-1} - k_2)$ can be reduced to k_3/k_{-1} to a good approximation.[6]

Figure 1 shows a plot of log (k_3/k_{-1}) vs. σ^-.[7] The slope of the straight line gave the ρ for the (k_3/k_{-1}) of -1.48 ± 0.04. Since the ρ corresponding to k_{-1} was determined to be -0.08, the ρ for k_3 is -1.56 $(= -1.48 + (-0.08))$.[8] This quite large negative value shows that proton donation from the imidazolyl cation to the nitrogen atom of **1** is essentially complete in the transition state, whereas the bond between the carbonyl carbon atom and the nitrogen atom is almost intact. Thus, a large positive charge is located on the nitrogen atom in the transition state. k_3/k_{-1} in the hydrolysis of *m*-nitrotrifluoroacetanilide showed no D_2O solvent isotope effect (1.0 ± 0.2), which also indicates an almost complete proton transfer from the imidazolyl cation to the nitrogen atom of **1.**

The ρ for k_1 was determined to be $+0.57 \pm 0.06$ from the plot of log k_1 vs. σ^-. This value is slightly larger than the ρ obtained at 30 °C in ref 2 (+0.42). The k_1's in D_2O are 1.4 ± 0.2 times larger than those in H_2O, which support nucleophilic attack by hydroxide ion in this reaction. The nucleophilicity of deuteroxide ion is 20–40% higher than that of hydroxide ion.[9]

The rate constant of the reaction, in which the formation of **1** is followed by its rate-determining breakdown catalyzed by imidazolyl cation, is expressed by k_1k_3/k_{-1}. Thus, the ρ for this process is calculated to be -0.91 $(= -1.48 + 0.57)$, using the ρ for k_1 (+0.57). This large *negative* ρ is consistent with the large *negative* ρ observed in the α-chymotrypsin-catalyzed hydrolyses of *N*-acetyl-L-tyrosine anilides (-1.8 at 35 °C).[1e] Furthermore, the magnitude of ρ for the present model reactions, observed at 70 °C, is quantitatively comparable with that in the enzymatic reactions, considering the known decrease of ρ with increasing temperature (-2.2 at 15 °C, -2.0 at 25 °C, and -1.8 at 35 °C).[1e]

On the other hand, the catalysis by neutral imidazole (k_4), which shows general base catalysis followed by water-catalyzed breakdown of the tetrahedral intermediate,[10] exhibited a *positive* ρ value of $+0.84 \pm 0.04$ (Figure 1). This value is close to the value for the reaction in which water catalyzes the breakdown of **1** (+0.69)[2,11] and is far different from the large *negative* ρ in the enzymatic reactions. Thus, the catalysis by water of the breakdown of the tetrahedral intermediate between Ser-195 and the anilide in the enzymatic hydrolyses of anilides is unlikely.

In conclusion, a large *negative* ρ was observed in the imidazolyl cation catalyzed breakdown of **1,** which is consistent with the finding in the enzymatic hydrolyses of anilides. The present result provides strong support for acid catalysis by the imidazolyl cation of His-57 rather than by water in the enzymatic hydrolyses of amides.

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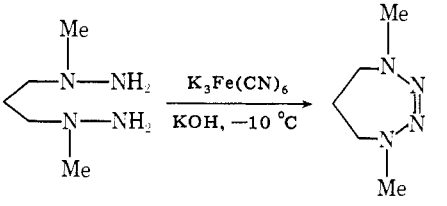
Preparation and Decomposition of a Cis Cyclic Tetrazene, 1,4-Dimethyl-1,2,3,4-tetraaza-2-cycloheptene

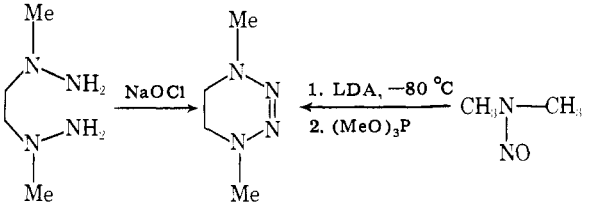
Sir:

We report the preparation and the kinetics of the thermal decomposition of the title compound, **2.** This material was prepared in 18–25% yield by the oxidation of the dihydrazine **1,** using a modification of the procedure of Kreher and Wissman.[1]



1 2

Acyclic tetraalkyl-2-tetrazenes have been shown to prefer the trans configuration.[2] No examples of cis acyclic tetraalkyltetrazenes are known, although Roberts and Ingold[3] suggested that photochemical decomposition of *trans*-tetrazenes involves initial trans → cis isomerization, followed by the dark (thermal) decomposition of the cis isomer. Nelsen and Fibiger,[4]



3

Table I. Thermolysis[a] of 1,4-Dimethyl-1,2,3,4-tetraaza-2-cycloheptene

k, $s^{-1} \times 10^{-4}$ [b]	T, °C, ± 0.1°
1.76 ± 0.08	65
2.79 ± 0.1	70
4.66 ± 0.3	75
6.31 ± 0.5	80
13.05 ± 1.5	90
$\Delta H^{\ddagger} = 18.8 \pm 1$ kcal/mol, $\Delta S^{\ddagger} = -20.2 \pm 1$ eu	

[a] The reaction was carried out on 0.04 M **2** in *n*-hexadecane under nitrogen. The rate constants did not change when the concentration of **2** was doubled to 0.08 M. This indicates the absence of induced decomposition at this concentration range. [b] The rate constants are an average of three independent runs.

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