

"killing" and the time of mixing are negligible if compared with the time of reaction.

The method described here demonstrates that the rate of propagation is first order in monomer at least over a concentration range of 0.02–0.22 *M*. On the other hand, it is found that the rate of propagation is a more complex function of the concentration of "living" ends since the calculated rate constant decreases with increasing concentration of the growing ends (see Table II). Presumably, either the association of the "living" ends decreases their reactivity, or the reactivity is a function of environment, *e.g.*, of ionic strength of the solution. We feel that the latter point of view is probably more nearly correct; however, further work is required to clarify this issue. Variations in the propagation rate constant resulting from changes of counterions and of solvent are under investigation.

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ONE STEP OXIDATION OF THALLIUM(I) TO THALLIUM(III)¹

Sir:

A preliminary report of our investigation of the oxidation of Tl(I) by Ce(IV) in 6.18 *f* HNO₃ at 53.9° is presented here because the results

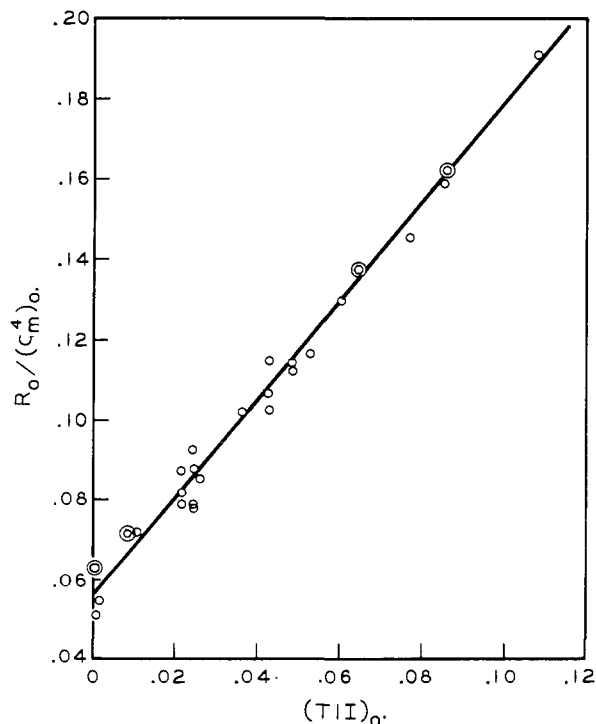


Fig. 1.—Comparison of empirical rate law with experiments: R_0 is initial rate; $(\text{TlI})_0$ is initial Tl(I) concentration; $(C_m^4)_0$ is initial concentration of Ce(IV) monomer.

can be used to prove that a two electron transfer occurs, perhaps by oxygen atom transfer, in some

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oxidations of Tl(I). Whether or not such two electron oxidations occur for metal-ions has been the object of much discussion.²

The procedure used for following the reaction depended upon the initial concentration of reactants. In some experiments Ce(IV) was determined by titration with ferrous sulfate, in others Ce(IV) was determined spectrophotometrically, and in others radioactive Tl(III) was determined after an extraction of Tl(III) into tributyl phosphate. For the last procedure, radioactive Tl(I) and Tl(III) in isotopic equilibrium were used in the reaction mixture and a correction was made for a small extraction of Tl(I). Light was excluded from the reaction mixtures. Chemicals from different lots and from more than one manufacturer were used for each reagent. The thallium and cerium reagents were purified further. Several sources of distilled water were used, with and without additional chemical treatment and distillation. Freshly boiled HNO₃ and HNO₃ direct from reagent bottles were employed. The rate of reaction was insensitive to these variables. The reaction was stoichiometric to within experimental error.

The initial slopes of graphs of (Tl(III)) *vs.* time for over 50 experiments in which (Ce(IV)) varied from 0.00190 to 0.2274 *f*, (Tl(I)) varied from 0.0032 to 0.1074 *f*, (Ce(III)) varied from 0.0 to 0.1560 *f*, and (Tl(III)) varied from 0.0 to 0.1140 *f* are in agreement with an empirical rate law which, in the absence of (Ce(III)), has the form

$$R = \frac{d(\text{Tl(III)})}{dt} = k(\text{Ce(IV)}) + k'(\text{Ce(IV)})(\text{Tl(I)})$$

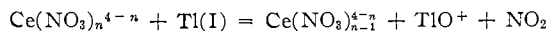
where k and k' are constants and (Ce(IV)) is the concentration of monomeric³ Ce(IV). Figure 1 shows the agreement between the empirical rate law and the experimental rates when a value of 20 is used for the dimerization constant of Ce(IV). The value of k determined from the intercept of the graph is 0.056 h.⁻¹ and the value of k' is 1.23 l. m.⁻¹ h.⁻¹.

In the presence of Ce(III) the rate of the reaction is inhibited markedly. Thallium(III) has no effect on the rate. The first term in the empirical rate law requires a mechanism involving an intermediate formed by a reaction of Ce(IV). This intermediate cannot contain thallium. In order to account for the inhibition by Ce(III), competition between Ce(III) and Tl(I) for the intermediate must be invoked. Assuming that the precautions taken to eliminate the necessity for considering impurities were successful, we conclude that the only possible reacting substances are H₂O and NO₃⁻. Reasonable intermediates would be OH or NO₃. The reaction between Tl(I) and the intermediate must be either a one-electron transfer to give Tl(II) and the original compound from which the intermediate was formed or a two electron

(2) (a) J. Halpern, *Can. J. Chem.*, **37**, 148 (1959); (b) W. C. E. Higginson and J. W. Marshall, *J. Chem. Soc.*, 447 (1957); (c) F. H. Westheimer in "The Mechanism of Enzyme Action," edited by W. D. McElroy and H. B. Glass, The Johns Hopkins University Press, Baltimore, Md., 1954, pp. 321–352.

(3) B. D. Blaustein and J. W. Gryder, *J. Am. Chem. Soc.*, **79**, 540 (1957).

transfer to give Tl(III) and a new intermediate, perhaps NO_2 . If the first alternative is operative, Tl(II) must react rapidly with Ce(IV) to give Tl(III) and Ce(IV). If the second alternative is operative, Ce(IV) reacts rapidly with the new intermediate to give Ce(III) and the compound from which the first intermediate was formed, perhaps NO_3^- . The second term in the empirical rate law implies a bimolecular reaction between Tl(I) and Ce(IV) which must be either a one electron transfer to give Tl(II) or a two electron reaction, for example



Several mechanisms in agreement with results of experiments in the presence of Ce(III) are possible within the above framework. Experiments are continuing to determine which mechanism is correct. Our results for low concentrations of reactants are in agreement with a mechanism proposed by Halpern^{2a} on the basis of unpublished data.

Now, if Tl(II) is involved in both the Ce(IV) reaction and the exchange reaction between Tl(I) and Tl(III), the Ce(IV) reaction in the presence of Tl(III) could be no slower than the exchange reaction because the exchange could feed Tl(II) to the Ce(IV) reaction. However, the exchange is much more rapid than the Ce(IV) reaction⁴ and hence the two reactions cannot both involve Tl(II). We therefore conclude that either the Tl(I)-Tl(III) exchange reaction or the Ce(IV)-Tl(I) reaction, or both, must involve a two electron transfer of some sort, probably an oxygen atom transfer.

(4) (a) R. J. Prestwood and A. C. Wahl, *J. Am. Chem. Soc.*, **71**, 3137 (1949); (b) G. Harbottle and R. W. Dodson, *ibid.*, **73**, 2442 (1951). Since the conditions used by these workers were different from those employed here, the exchange was measured under our experimental conditions. When Tl(I) = 0.0086 *f* and Tl(III) = 0.0077 *f* the half life of the exchange is 0.25 hour, while the Ce(IV)-Tl(I) reaction under the same conditions with Ce(IV) = 0.050 has a half time of 10 hours.

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THE INFLUENCE OF pH AND DEUTERIUM OXIDE ON THE KINETICS OF α -CHYMOTRYPSIN-CATALYZED REACTIONS^{1,2}

Sir:

The action of α -chymotrypsin on phenyl esters has been shown to proceed in three steps: (1) adsorption of the substrate on the enzyme; (2) acylation of the enzyme releasing the phenol; and (3) deacylation of the acyl-enzyme producing the carboxylic acid and regenerating the enzyme.^{3,4} Most studies of the effect of pH on α -chymotrypsin action may be analyzed in terms of dependency on

(1) This research was supported by Grant H-5726 of the National Institutes of Health, and by grants from the Upjohn Company, and the Lilly Research Laboratories.

(2) Paper VI in the series "The Mechanism of Action of Proteolytic Enzymes"; previous paper, W. A. Glasson and M. L. Bender, *J. Am. Chem. Soc.*, **82**, 3336 (1960).

(3) G. R. Schonbaum, K. Nakamura and M. L. Bender, *ibid.*, **81**, 4746 (1959).

(4) M. L. Bender, *Chem. Revs.*, **60**, 53 (1960).

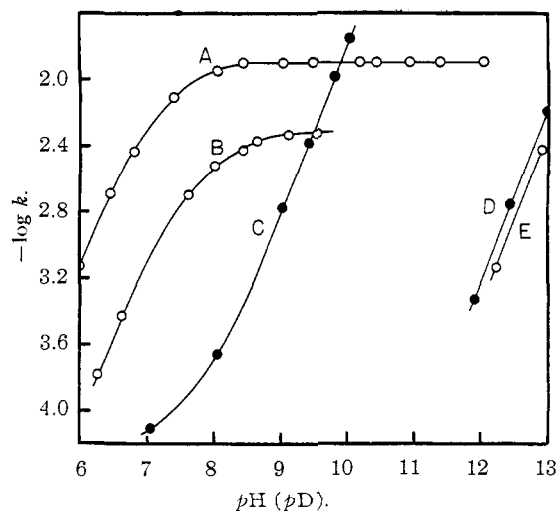


Fig. 1.—The hydrolysis of some cinnamoyl derivatives: (A) cinnamoyl-chymotrypsin in H_2O ; (B) cinnamoyl-chymotrypsin in D_2O ; (C) cinnamoylimidazole; (D) O-cinnamoyl-N-acetylserinamide in 8 *M* urea; (E) cinnamoyl-chymotrypsin in 8 *M* urea.

a base with an apparent pK_a of approximately 7. Since bell-shaped pH-rate profiles are found in most enzymatic processes, we wished to investigate carefully the possibility of a bell-shaped curve in α -chymotrypsin catalysis. We further wished to investigate the function of the base of pK_a 7 by observations of the kinetic effect of D_2O .

The kinetics of the deacylation of cinnamoyl- α -chymotrypsin³ was determined because of the simplicity of the system.⁵ The hydrolysis of cinnamoyl- α -chymotrypsin (Curve A of Fig. 1) shows the usual $\log k$ -pH profile with dependence on a basic group of apparent pK 7.1. Significantly the curve remains flat up to pH 12, in spite of considerable denaturation of the enzyme. This result rules out the participation of the tyrosine hydroxyl groups (pK 10.0) and the ϵ -ammonium ions of lysine (pK 10.2) but leaves the possibility of the participation of a group of pK_a above 12.5 as an (unobserved) general acid catalyst.

The hydrolysis of N-cinnamoylimidazole (Curve C) exhibits the usual base catalysis. The $\log k$ -pH profiles for the alkaline hydrolyses of O-cinnamoyl-N-acetylserinamide and cinnamoyl- α -chymotrypsin in 8 *M* urea are Curves D and E, respectively. The acyl-enzyme loses its special hydrolytic properties in 8 *M* urea because of denaturation, as expected, and exhibits hydrolytic behavior (4.0×10^{-2} l./mole sec.) similar to that of O-cinnamoyl-N-acetylserinamide (5.4×10^{-2} l./mole sec.). These rate data indicate that cinnamoyl- α -chymotrypsin structurally resembles an ester (of serine)⁶ and not a cinnamoylimidazole derivative, confirming earlier degradative evidence.⁴

(5) It can be shown that the catalytic processes of both the acylation and deacylation steps must utilize the same enzymatic components, from a consideration of α -chymotrypsin-catalyzed isotopic exchange reactions and the principle of microscopic reversibility. See M. L. Bender, G. R. Schonbaum and G. A. Hamilton, *J. Polymer Sci.*, in press (1961).

(6) Similar behavior of acetyl-chymotrypsin in 8 *M* urea has been noted: B. M. Anderson, E. H. Cordes and W. P. Jencks, *J. Biol. Chem.*, in press (1961).