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

$$Ce(NO_3)_n{}^{4-n} + Tl(I) = Ce(NO_3)_{n-1}^{4-n} + TlO^+ + NO_2$$
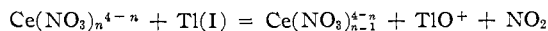
Several mechanisms in agreement with results of experiments in the presence of Ce(III) are possible within the above framework. Experiments are continuing to determine which mechanism is correct. Our results for low concentrations of reactants are in agreement with a mechanism proposed by Halpern[2a] on the basis of unpublished data.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) reaction[4] 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 enyzme; (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).
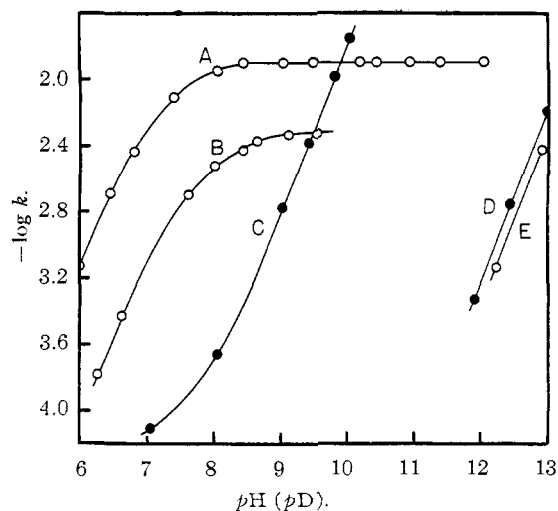
(4) M. L. Bender, Chem. Revs., 60, 53 (1960).

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-chymotrypsin[3] 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.
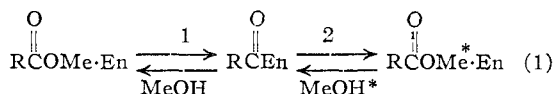
The hydrolysis of N-cinnamoylimidazole (Curve C) exhibits the usual base catalysis. The log k–pH profiles for the alkaline hydrolyses of O-cinnamoyl-N-acetylserinamide and cinnamoyl-α-chymotrypsin in 8 M urea are Curves D and E, respectively. The acylenzyme loses its special hydrolytic properties in 8 M urea because of denaturation, as expected, and exhibits hydrolytic behavior (4.0 × 10^{-2} 1./mole sec.) similar to that of O-cinnamoyl-N-acetylserinamide (5.4 × 10^{-2} 1./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).

Deuterium oxide causes two effects in the deacylation of cinnamoyl-α-chymotrypsin (Curve B): the apparent pK_a is 7.7 in D_2O; and the rate constant at any $pH(pD)$ is 2.5 fold more in H_2O than in D_2O. The shift in the pK_a of the catalytically important group in D_2O is expected.[7] A substantial decrease in rate in D_2O also is found in the acylation of chymotrypsin using p-nitrophenyl trimethylacetate and in the hydrolysis of the specific substrate, N-acetyl-L-tryptophan methyl ester $(k^H/k^D = 2.83)$.[8] These isotope effects cannot be attributable to a pathway involving nucleophilic catalysis alone, which would be expected to exhibit essentially no D_2O isotope effect.[7,9,10] The deuterium oxide and pH results are consistent with a mechanism involving general basic catalysis by a group of pK_a 7.1, or a mechanism involving nucleophilic catalysis together with general acid catalysis by a species of pK_a above 12.5. In either case k^H/k^D would reflect a slow proton transfer and be approximately 2–3.[6,11]

Acylation and deacylation are mechanistically similar: they exhibit similar pH dependencies,[3] deuterium isotope effects and effects of structure on reactivity.[12] Let us assume that acylation and deacylation correctly describe the catalytic process, and that the α-chymotrypsin-catalyzed isotopic exchange reaction[2] proceeds through the same steps as the hydrolysis reaction (Eq. 1). Then it follows that the "acylation" and "deacylation" steps (1 and 2) of the isotopic exchange must be identical. (Step 1 = step −2, but since RCOEn

$$\underset{\text{MeOH}}{\overset{O}{\underset{\rightleftarrows}{\text{RCOMe·En}}}} \overset{1}{\underset{\text{MeOH}}{\rightleftarrows}} \overset{O}{\text{RCEn}} \overset{2}{\underset{\rightleftarrows}{\overset{O}{\text{RCOMe*·En}}}} \quad (1)$$

is also an alkyl ester, step 1 = step 2). A mechanism meeting these requirements must employ at least two simultaneous catalytic functions such as general acidic and general basic catalysis (Eq. 2).[13] In acylation B and HA will be operative while in deacylation the kinetically equivalent combination of BH^+ and A^- will be operative. It is suggested that this form of catalysis be called "conjugate" catalysis for a base and its conjugate acid are operative in the two catalytic steps, as are an acid and its conjugate base.[14]

Catalysis by a lone general base[6] (e g , in Eq. 2) would fit the kinetic results but would violate the chemical symmetry required by the exchange

(7) The shift of 0.6 ± 0.1 pK unit is within the experimental error of the predicted shift of 0.56 pK unit for a group of pK_a 7: R. P. Bell, "The Proton in Chemistry," Cornell University Press, Ithaca, N. Y., 1959, p. 188.

(8) J. F. Thompson, Arch. Biochem. Biophys., 90, 1 (1960), reports $k^H/k^D = 2.5$ for the fumarase-catalyzed conversion of fumarate to malate.

(9) Unpublished observations of M. L. Bender and M. C. Neveu.

(10) D_2O stabilizes the helical form of ribonuclease, relative to its uncoiled form; J. Hermans and H. A. Scheraga, Biochim. et Biophys. Acta, 36, 534 (1959).

(11) B. Zerner and M. L. Bender, J. Am. Chem. Soc., 83, in press (1961); Y. Pocker, Proc. Chem. Soc., 17 (1960); A. R. Butler and V. Gold, ibid., 15 (1960).

(12) Unpublished observations of G. A. Hamilton and K. Nakamura.

(13) This treatment assumes no tetracovalent intermediates such as $RC(OH)(OR)_2$.

(14) A referee has suggested that in addition to an enzymatic group, HA could be a water molecule.

$$(2)$$
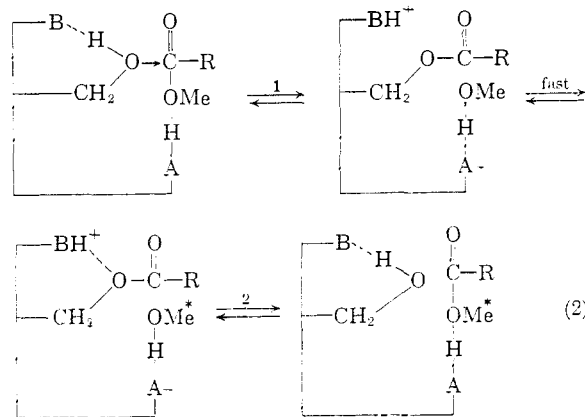
reaction A lone general base would remove a proton from the attacking alcohol in step 1 (but not in step 2) and its conjugate acid (BH^+) would donate a proton to the alkoxyl group of the ester in step 2 (but not in step 1), whereas in this exchange chemical symmetry requires that exactly the same processes take place in both halves of the over-all exchange reaction. Therefore it must be concluded that a single kind of catalysis (either acid, base or nucleophile) is not sufficient and that one must postulate a scheme such as eq. 2.

If extrapolation to the hydrolytic system is allowed (substitution of H_2O for MeOH in the deacylation step) the enzymatic participants in acylation and deacylation must still bear the same relationship to one another, requiring that a general base (or nucleophile) and a general acid again be involved.

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ORGANIC DISULFIDES AND RELATED SUBSTANCES. III. ONE-STEP PREPARATION OF SULFINIC ESTERS FROM LEAD TETRAACETATE AND DISULFIDES OR THIOLS[1]

Sir:

We wish to report a novel means of preparing sulfinic esters by oxidizing disulfides with lead tetraacetate (I) in chloroform–methanol, probably according to the equation

$RSSR + 3Pb(OAc)_4 + 4CH_3OH \longrightarrow$

     I

$2RS(O)OCH_3 + 3Pb(OAc)_2 + 4AcOH + 2AcOCH_3$
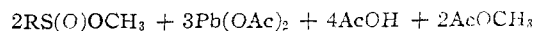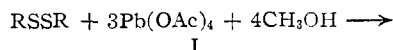
Thiols also can be used, since the agent I oxidizes them to disulfides.[2] In view of the stability and ready availability of disulfides and thiols, the present process should make sulfinic esters much

(1) Research supported by the Office of Ordnance Research, U. S. Army. Presented at the Southeastern Regional Meeting of the American Chemical Society, Birmingham, Ala., Nov. 3–5, 1960. Part II, D. E. Pearson, D. Caine and L. Field, J. Org. Chem., 25, 867 (1960).

(2) L. Field and J. E. Lawson, J. Am. Chem. Soc., 80, 838 (1958).