supports the proposed model in this concentration range. The large deviations from the theory at high drug concentration are consistent with the idea that a more rapid process takes over that does not appear to be aqueous diffusion controlled. More work is needed to explain the latter behavior.

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Keyphrases

Drug release
Matrices–wax
Sulfanilamide–wax system
Tortuosity values–apparent
UV spectrophotometry–analysis

Alpha-Chymotrypsin-Catalyzed Hydrolysis of Some Carbonate Esters

By ATUL A. SHAH* and KENNETH A. CONNORS

The kinetics of the α-chymotrypsin-catalyzed hydrolysis of several carbonate diesters, ROCOOR′, can be described by the Michaelis-Menten equation. *p*-Acetamidophenyltrichloroethyl carbonate shows a sigmoid pH-rate curve with a kinetic dependence on an enzyme function of pKa 7.2. The limiting values of the catalytic rate constant at high pH are, for *p*-acetamidophenyltrichloroethyl carbonate, 8.0×10^{-2} sec.$^{-1}$; for *p*-nitrophenyltrichloroethyl carbonate, 8.2×10^{-2} sec.$^{-1}$; for *p*-nitrophenylethyl carbonate, 0.2×10^{-2} sec.$^{-1}$. The evidence is consistent with the intermediate formation of an alkyl enzyme carbonate, the hydrolysis of which is rate controlling.

CARBONATE DIESTERS are derivatives of carbonic acid, the two hydrogens of the acid being formally replaced by alkyl or aryl groups to give the general structure ROCOOR′. The suggestion has recently been made (1) that such esters may be useful "prodrugs" because they may possess desirable pharmaceutical properties and can release the parent pharmacologically active compounds (ROH and R′OH) upon hydrolysis in the body. The mechanism of enzymatic hydrolysis of carbonate esters is therefore of pharmaceutical interest.

The enzymatic hydrolysis of carbonate esters is also of considerable fundamental importance. α-Chymotrypsin has been selected as the enzyme in the present study because it is commercially available in crystalline form, a good assay method is known, and especially because more is known about the catalytic behavior of chymotrypsin than of any other enzyme. Chymotrypsin is a member of a group of related enzymes known as the serine hydrolases; these enzymes catalyze the hydrolysis of proteins, peptides, amides, and esters. It is now well established that chymotrypsin (and probably other closely related enzymes) functions catalytically by a double displacement type of reaction. With an ester substrate, for example, the reaction may be written as shown in Scheme I.

$$\text{En—OH} + \text{RCOOR}' \rightleftarrows \text{En—OH}\cdot\text{RCOOR}'$$

$$\text{En—OH}\cdot\text{RCOOR}' \xrightarrow{\text{acylation}} \text{EnOCOR} + \text{R}'\text{OH}$$

$$\text{EnOCOR} + \text{H}_2\text{O} \xrightarrow{\text{deacylation}} \text{En—OH} + \text{RCOOH}$$

Scheme I

Here En—OH represents the enzyme, with a serine hydroxyl group specifically indicated. En—OH · RCOOR′ is the enzyme–substrate complex. In the acylation step, the enzyme is acylated with the release of the alcohol portion of the substrate. Deacylation involves hydrolysis of the acyl–enzyme intermediate EnOCOR to give the carboxylic acid portion of the substrate and to regenerate the enzyme. The evidence supporting this interpretation of chymotrypsin-catalyzed reactions has recently been reviewed by Bruice and Benkovic (2). It is now of interest to consider the possible mode of action of chymotrypsin when the substrate is a carbonate ester, ROCOOR′. It is obvious that two possible

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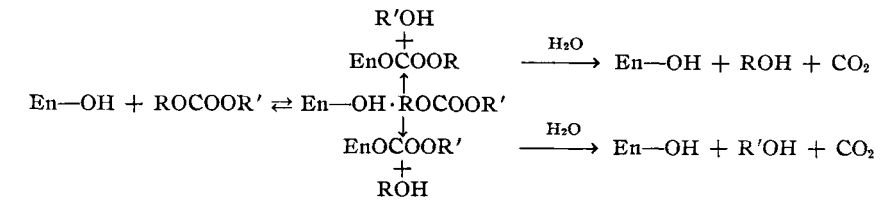


Scheme II

"acyl–enzymes" can be formed from this substrate. The simplest representation of the reaction may therefore be written as shown in Scheme II.

The deacylation must be more complicated than is implied by Scheme II. The relative rates of formation of the two possible intermediates and their rates of hydrolysis will depend upon the structures R and R'. Structural variation in the substrate should therefore provide useful information on the mechanism of this reaction.

EXPERIMENTAL

Materials—*N-trans*-Cinnamoylimidazole was prepared according to Schonbaum, Zerner, and Bender (3). It was recrystallized twice from cyclohexane, m.p. 133°. [Lit. (3) m.p. 133–135°.] *p*-Nitrophenyl acetate (*p*-NPA) was prepared by the method of Chattaway (4). The crude product was recrystallized twice from Skellysolve B, m.p. 80°. [Lit. (5) m.p. 80°.] *p*-Acetamidophenyl-2,2,2-trichloroethyl carbonate (1) (*p*-APTC), m.p. 153°; *p*-acetaminophenol, m.p. 169–170°; these products were generously supplied by Smith Kline & French Laboratories. *p*-Nitrophenylethyl carbonate (*p*-NPEC): 2.8 Gm. (0.02 mole) of *p*-nitrophenol was dissolved in 20 ml. of pyridine. The solution was cooled in an ice bath and with continuous stirring 2.5 ml. (0.025 mole) of ethyl chloroformate (Aldrich Chemical Co.) was added dropwise. The mixture was allowed to stand 15 min. The pyridine was removed by evaporation under reduced pressure. The oily brown residue was recrystallized twice from methanol–water, m.p. 66°. [Lit. (5) m.p. 67–68°.]

p-Nitrophenyl-2,2,2-trichloroethyl carbonate (*p*-NPTC): to a solution of 120 ml. of toluene and 8.2 ml. of pyridine, cooled to −10°, was added 43 ml. (3.5 mole) of phosgene solution in toluene during about 90 min. Cooling was effected in dry ice–isopropyl alcohol. The reaction mixture was allowed to reach 0°, at which time the addition of 15 ml. (0.1 mole) of trichloroethanol was initiated. This addition required 40 min., and the final temperature was 10°. The mixture was stirred at room temperature for 2 hr. and then 30 ml. of cold water was cautiously added. Considerable CO_2 gas was evolved. The solution was washed with four more 30-ml. portions of water and dried over anhydrous magnesium sulfate. The dry solution was filtered.

Seventy-five milliliters of filtrate was added to a mixture of 4.5 ml. of pyridine, 30 ml. of toluene, and 7.5 Gm. of *p*-nitrophenol; the addition required 1 hr. The mixture was stirred for 90 min. and 0.9 ml. of pyridine was added, followed by 0.5 ml. of pyridine 30 min. later. The mixture was then cooled to 5°, 75 ml. of water was added, and it was stirred for 15 min. The toluene layer was separated and dried over magnesium sulfate. The solvent was removed by reduced pressure and the yellow residue was recrystallized three times from Skellysolve B. Pale yellow needles were obtained, m.p. 58°.

Anal.—Calcd. for $C_9H_6Cl_3NO_5$: C, 34.34; H, 1.91, Cl, 34.97; N, 4.45. Found: C, 34.31; H, 1.85; Cl, 34.85; N, 4.44.

The infrared spectrum showed peaks at 5.65 μ (OCOO), 6.2 and 6.4 μ (aromatic), 8.2 μ (COOR), 9.9, 10.5, and 12.3 μ (*p*-disubstituted phenyl ring), 13 μ (C—Cl), 6.6 and 7.4 μ (aromatic nitro). The nuclear magnetic resonance spectrum yielded τ 5.05 (methyl protons) and 2.5 and 1.67 (aromatic protons). The integrated spectrum gave 1:1:1 ratio for the three types of protons.

α-Chymotrypsin, three times recrystallized, was a Worthington product. The purity of the Worthington chymotrypsin was consistently found to be 84–86% by titration against *N-trans*-cinnamoylimidazole, following the procedure of Schonbaum *et al.* (3).

Acetonitrile (Eastman Kodak technical grade) was refluxed over phosphorus pentoxide and distilled twice from P_2O_5, b.p. 81°. Tris(hydroxymethyl)aminomethane (Tris) (Sigma Chemical Co.) was recrystallized from 95% ethanol. Pentaerythritol (Aldrich Chemical Co.) and *n*-butyl alcohol (Mallinckrodt A.R.) were used directly. Buffer chemicals were reagent grade. All water was redistilled from alkaline permanganate in an all-glass still. Buffers were prepared according to published formulas (6, 7). Ionic strength was maintained constant by addition of potassium chloride.

Apparatus—pH was measured with a Radiometer model 25 SE pH meter or with a Sargent model DR pH meter. The meters were standardized against the standard buffers recommended by Bates (8).

Spectral measurements were made with a Cary model 14 recording spectrophotometer equipped with a thermostated cell compartment that maintained temperature to ±0.1°. The absorbance scale was checked against the alkaline chromate solution described by Haupt (9).

Procedures—Reactions were followed by continuous spectrophotometric monitoring at a constant wavelength, which was 240 mμ for *p*-APTC and 400 mμ for *p*-NPA, *p*-NPEC, and *p*-NPTC. The temperature was 25 ± 0.1° in all experiments. Ionic strength was 0.2 *M*, and pH was held constant with buffers. The substrate concentration was much larger than the enzyme concentration. The analysis of the operational normality of the chymotrypsin was made during the same time period as the kinetic measurements.

A typical experiment was conducted as follows. One-centimeter cells, each containing 3.0 ml. of

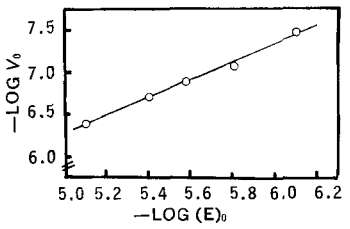


Fig. 1—Plot of $-\log v_0$ against $-\log [E]_0$ for the chymotrypsin-catalyzed hydrolysis of p-*APTC; pH = 7.54, $[S] = 1.13 \times 10^{-4}$ M, $[E]_0 = 7.8 \times 10^{-7}$ to 7.8×10^{-6} M.*

buffer solution, were placed in the reference and sample compartments of the spectrophotometer and were allowed to reach temperature equilibrium. The spectrophotometer signal was balanced and then 50 μl. of substrate stock solution (in acetonitrile) was introduced to the sample cell by means of a glass stirring rod. This was followed by 50 μl. of aqueous enzyme solution. Recording was initiated within 5–10 sec. When rates of nonenzymatic hydrolysis were significant, the enzyme solution was added prior to the substrate. Net enzymatic initial velocities were obtained by subtracting nonenzymatic initial velocities from total initial velocities.

Initial velocities were obtained from the initial zero-order portion of the absorbance-time tracing. The conversion of this quantity, $v_0' = dA/dt$ (initial velocity in absorbance units per second) to $v_0 = dc/dt$ (initial velocity in moles per liter per second) is readily accomplished through application of Beer's law to the substrate and product; the equation obtained is:

$$v_0 = v_0'/b(\epsilon_S - \epsilon_P)$$

where b is the path length, ϵ_S is the molar absorptivity of the substrate, and ϵ_P is the molar absorptivity of the product.

Beer's law is followed, at constant pH, by all substrates and products studied. The molar absorptivity of *p*-APTC at 240 mμ was 1.46×10^4 in the pH range 6.5 to 10.0. For *p*-nitrophenol the molar absorptivity at 400 mμ was 9.52×10^3, 1.75×10^4, and 1.90×10^4 at pH 7.02, 7.84, and 8.91, respectively.

RESULTS[1]

Order with Respect to Enzyme—Because carbonate esters are unusual substrates for chymotrypsin, it was considered important to investigate the validity of the usual rate relationships. When the logarithm of the initial velocity of the chymotrypsin-catalyzed hydrolysis of *p*-APTC was plotted against the logarithm of the enzyme concentration at constant substrate concentration, straight lines of unit slope were obtained. Figure 1 and Table I show these results. It can be concluded that under these conditions the chymotrypsin-catalyzed hydrolysis of *p*-APTC is first order with respect to the enzyme.

Stoichiometry—Hartley and Kilby (10, 11) studied the chymotrypsin-catalyzed hydrolysis of *p*-NPA and *p*-NPEC. They observed, with both of these substrates, an initial rapid release of *p*-nitrophenol (the "burst effect") followed by a slower, zero-order production of *p*-nitrophenol. These experiments have been repeated, with identical results. Extrapolation of the linear portions of the plot of *p*-nitrophenol concentration *versus* time to zero time gave finite intercepts. When these intercept values (expressed as molar concentration of product) are plotted against the molar concentration of enzyme, straight lines of unit slope are obtained (12). The stoichiometry of the reactions between chymotrypsin and *p*-NPA or *p*-NPEC is therefore 1:1.

The manifestation of the burst effect by *p*-NPEC is pertinent in the present study because it suggests similar mechanisms for the chymotrypsin-catalyzed hydrolyses of *p*-NPEC and *p*-NPA.

Kinetic Behavior—The adherence of carbonate ester systems to the Michaelis-Menten kinetic scheme was investigated. Plots of initial velocity against substrate concentration show the characteristic "saturation" effect (Fig. 2). Linear Lineweaver-Burk plots of $1/v_0$ *versus* $1/[S]$ were obtained, and it was from these plots that the kinetic parameters were determined. The kinetic scheme is:

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} ES \xrightarrow{k_{cat.}} \text{products} \qquad \text{(Eq. 1)}$$

where ES is the enzyme–substrate complex. From the Lineweaver-Burk plots can be obtained the quantities $V_{max.}$ and $K_{m(app.)}$, where:

$$V_{max.} = k_{cat.}[E]_0 \qquad \text{(Eq. 2)}$$

$$K_{m(app.)} = \frac{k_{-1} + k_{cat.}}{k_{+1}} \qquad \text{(Eq. 3)}$$

Since the enzyme concentration is known, $k_{cat.}$ can be calculated.

Table II gives values of $k_{cat.}$ and $K_{m(app.)}$ for the chymotrypsin-catalyzed hydrolysis of *p*-APTC

TABLE I—ORDER WITH RESPECT TO ENZYME IN THE HYDROLYSIS OF *p*-APTC[a]

pH	Order (Slope)[b]	Order (Slope)[c]
7.06	1.01	0.93
7.54	1.06	1.03
8.15	0.99	1.01

[a] 25.0°; ionic strength = 0.20; 3.2% acetonitrile. [b] $[S]_0 = 1.13 \times 10^{-4}$ *M*; $[E]_0 = 7.8 \times 10^{-7}$ to 7.8×10^{-6} *M*. [c] $[S]_0 = 1.17 \times 10^{-4}$ *M*; $[E]_0 = 8.3 \times 10^{-7}$ to 8.3×10^{-6} *M*.

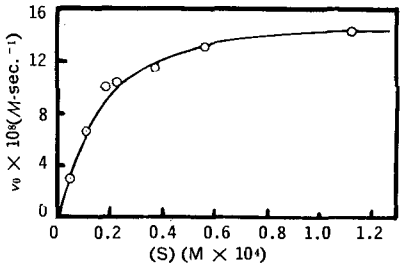


Fig. 2—Initial velocity against substrate concentration for the chymotrypsin-catalyzed hydrolysis of p-*APTC; pH = 7.05, $[E]_0 = 4.02 \times 10^{-6}$ M.*

[1] These abbreviations are used: *p*-acetamidophenyltrichloroethyl carbonate, *p*-APTC; *p*-nitrophenyl acetate, *p*-NPA; *p*-nitrophenylethyl carbonate, *p*-NPEC; *p*-nitrophenyltrichloroethyl carbonate, *p*-NPTC.

TABLE II—$k_{cat.}$ AND $K_{m(app.)}$ VALUES FOR THE CHYMOTRYPSIN-CATALYZED HYDROLYSIS OF *p*-APTC[a]

pH	No. of Detns.	Buffer	$10^2 k_{cat.}$ (sec.$^{-1}$) Mean[b]	$10^2 k_{cat.}$ (sec.$^{-1}$) Range	$10^6 K_{m(app.)}$ (M) Mean[b]	$10^6 K_{m(app.)}$ (M) Range
6.57	1	Phosphate	1.8	—	12.5	—
6.85	2	Phosphate	3.3	0.2	9.8	1.6
7.05	2	Phosphate	3.5	0.4	11.0	0.5
7.48	2	Phosphate	5.0	0.8	13.1	—
7.79	1	Phosphate	7.8	—	—	—
8.11	5	Phosphate	7.9 (±0.8)	1.0	16.2 (±3.7)	8.5
8.11	1	Borate	7.5	—	18.6	—
8.25	1	Botate	7.0	—	13.9	—
8.36	3	Borate	7.5 (±0.6)	1.1	15.9 (±1.2)	1.5
8.49	3	Borate	6.8 (±0.4)	0.7	15.3 (±5.3)	10.5
8.75	3	Borate	7.7 (±0.4)	0.7	19.8 (±1.7)	3.1
8.95	3	Borate	8.1 (±0.3)	0.5	21.0 (±1.7)	3.2
9.17	3	Carbonate	7.5 (±0.4)	0.6	28.9 (±3.0)	5.7
9.34	1	Carbonate	6.8	—	28.0	—
9.80	1	Carbonate	6.4	—	—	—

[a] $[E]_0 = 4 \times 10^{-6} M$; $[S] = 10^{-5}$ to $10^{-4} M$; 1.6% acetonitrile. [b] Uncertainties listed are standard deviations.

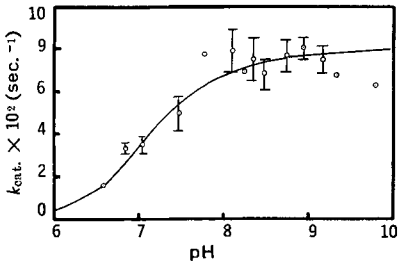


Fig. 3—pH-rate profile for the chymotrypsin-catalyzed hydrolysis of p-*APTC.*

over the pH range 6.5 to 9.8. The rate constant $k_{cat.}$ shows a sigmoid dependence on pH (the values above pH 9 are not considered as reliable as the others). If the enzyme is treated as a monobasic acid with characteristic dissociation constant, K_a, and the catalytic activity of the enzyme is assigned solely to its basic form, it is easily shown that $k_{cat.}$ is related to the pH through the equation:

$$k_{cat.} = \frac{k' K_a}{[H^+] + K_a} \qquad \text{(Eq. 4)}$$

where k' is a rate constant that is independent of pH. Figure 3 shows the data of Table II with a smooth curve calculated with Eq. 4 and the values pKa = 7.2 and $k' = 8.0 \times 10^{-2}$ sec.$^{-1}$.

The hydrolysis of *p*-NPEC and of *p*-NPTC were studied at several pH's. These results are given in Tables III and IV. It is seen that $k_{cat.}$ increases with pH in much the same manner as is observed with *p*-APTC. *p*-NPTC and *p*-APTC constitute an interesting pair of substrates because they contain the common trichloroethyl group. It is noted that the limiting value of $k_{cat.}$ (that is, the value at high pH) is 8.2×10^{-2} sec.$^{-1}$ for *p*-NPTC; this value is essentially the same as the limiting value of 8.0×10^{-2} sec.$^{-1}$ found for *p*-APTC. *p*-NPEC and *p*-NPTC are also a pair of substrates with a common group; the limiting rate constants are quite different for these compounds. The consequences of these comparisons will be discussed later.

Effects of Additives—During the pH-dependence study of the chymotrypsin–*p*-APTC system an unusual acceleration effect was observed in 0.05 M Tris buffers of pH 8 to 9. Values of $k_{cat.}$ were found to be about threefold greater in the presence of Tris than were values observed in other buffers. Figure 4 shows this effect; the constants observed in Tris buffers are plotted with the normal pH-rate profile. In attempting to account for this acceleration, some structurally related compounds were investigated as additives in the chymotrypsin–*p*-APTC system. The additives used were: (*a*) pH 8.15 ammonia buffer; (*b*) 0.15 M methanol; (*c*) pH 8.15 ammonia buffer and 0.15 M methanol; (*d*) pH 8.03 0.1 M glycine–NaOH buffer; (*e*) pH 8.0 0.1 M imidazole–HCl buffer; (*f*) 0.01 M pentaerythritol; (*g*) 0.15 M *n*-butyl alcohol. Only *n*-butyl alcohol caused an acceleration of the enzymatic reaction, but it was not nearly as effective as was Tris. Studies were carried out to evaluate $k_{cat.}$ and $K_{m(app.)}$ at several values of *n*-butyl alcohol concentration; both quantities increased with increase in the alcohol concentration (12). *p*-NPA was found to be a competitive inhibitor of the chymotrypsin-catalyzed hydrolysis of *p*-APTC. Since *p*-NPA is known to be

TABLE III—$k_{cat.}$ AND $K_{m(app.)}$ VALUES FOR THE CHYMOTRYPSIN-CATALYZED HYDROLYSIS OF *p*-NPEC[a]

pH	Buffer	$10^3 k_{cat.}$ (sec.$^{-1}$)[b]	$10^6 K_{m(app.)}$ (M)
7.02	Phosphate	0.6	6.4
7.81	Phosphate	1.3	4.2
8.35	Borate	1.5	5.0
8.83	Borate	1.9	11.9

[a] $[E]_0 = 3.89 \times 10^{-6} M$; $[S] = 2.9 \times 10^{-6}$ to $1.4 \times 10^{-3} M$; 1.6% acetonitrile. [b] Literature value (10) is 1.31×10^{-3} sec.$^{-1}$ at pH 7.6 (turnover number = 0.078 mole *p*-NPEC/min./mole enzyme).

TABLE IV—$k_{cat.}$ AND $K_{m(app.)}$ VALUES FOR THE CHYMOTRYPSIN-CATALYZED HYDROLYSIS OF *p*-NPTC[a]

pH	Buffer	$10^2 k_{cat.}$ (sec.$^{-1}$)	$10^7 K_{m(app.)}$ (M)
7.02	Phosphate	4.1	5.5
7.84	Borate	6.6	4.7
8.91	Borate	8.2	9.1

[a] $[E]_0 = 4.14 \times 10^{-7} M$; $[S] = 6.5 \times 10^{-7}$ to $4 \times 10^{-5} M$; 1.6% acetonitrile. The 0.0–0.1 absorbance slide wire of the spectrophotometer was used.

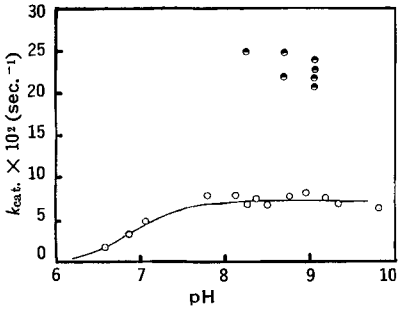


Fig. 4—Acceleratory effect of TRIS on the chymotrypsin-catalyzed hydrolysis of p-*APTC. Key:* ○, *values in phosphate, borate, and carbonate buffers;* ◒, *values in Tris buffers.*

a normal substrate of chymotrypsin, this result implies that the hydrolysis of *p*-APTC is also catalyzed in a normal manner.

DISCUSSION

The conventional Michaelis-Menten kinetic scheme is represented by Eq. 1. It has been shown above that the chymotrypsin-catalyzed hydrolysis of several carbonate diesters can be accounted for by this overall scheme, and the enzymatic behavior of these substrates is therefore normal. The relationship between initial velocity and substrate concentration is the well-known Michaelis-Menten equation, Eq. 5:

$$v_0 = \frac{k_{cat.}[S][E]_0}{K_{m(app.)} + [S]} \qquad \text{(Eq. 5)}$$

where $K_{m(app.)}$ is defined in Eq. 3.

If the reaction proceeds through an acyl–enzyme intermediate, as with ester and amide substrates of chymotrypsin, $k_{cat.}$ may be a complex constant. The reaction then becomes Scheme III.

It can be shown (2, 12, 13) that the $k_{cat.}$ of Eq. 1 is related to the constants of Scheme III by $k_{cat.} = k_{+2}k_{+3}/(k_{+2} + k_{+3})$, and the implications of this relationship for chymotrypsin reactions have been discussed in detail (14).

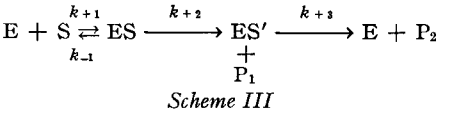
The situation is more complicated with carbonate diesters. Now the kinetic scheme is shown in Scheme IV.

Application of the steady-state approximation to ES, ES′, and ES″ (where ES′ and ES″ are the two possible "acyl–enzyme" intermediates) leads to an equation of the same form as Eq. 5, and comparison shows that the experimental $k_{cat.}$ can be expressed by Eq. 6:

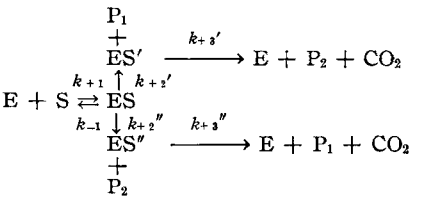
$$k_{cat.} = \frac{k'_{+3}k_{+3}''(k_{+2}' + k_{+2}'')}{k_{+2}'k_{+3}'' + k_{+2}''k_{+3}' + k_{+3}'k_{+3}''} \qquad \text{(Eq. 6)}$$

It is therefore clear that $k_{cat.}$ cannot, in general, be given a simple interpretation. If, however, one of the possible pathways in Scheme IV is greatly favored (*e.g.*, let $k_{+2}' \rangle\rangle\rangle k_{+2}''$), then Eq. 6 simplifies to $k_{cat.} = k_{+2}'k_{+3}'/(k_{+2}' + k_{+3}')$, which is identical with the corresponding equation for Scheme III. If this condition holds and, in addition, the deacylation of ES′ is rate determining (*i.e.*, $k_{+2}' \rangle\rangle\rangle k_{+3}'$) then $k_{cat.} = k_{+3}'$.

The similar behavior of *p*-NPA and *p*-NPEC suggests very strongly that the carbonate ester is



Scheme III



Scheme IV

TABLE V—LIMITING $k_{cat.}$ VALUES

Substrate	$10^2k_{cat.}$(sec.$^{-1}$)
p-APTC	8.0
p-NPTC	8.2
p-NPEC	0.2

hydrolyzed by the same overall reaction path as the carboxylic ester. Each substrate exhibits a rapid "burst" release of a stoichiometric amount of *p*-nitrophenol, which is followed by a steady-state zero-order production of *p*-nitrophenol. This behavior is consistent with Scheme III, where P_1 = *p*-nitrophenol and ES′ = $EnOCOCH_3$ (for *p*-NPA) or $EnOCOOCH_2CH_3$ (for *p*-NPEC).

The limiting $k_{cat.}$ values for *p*-APTC, *p*-NPTC, and *p*-NPEC provide the interesting comparison shown in Table V.

These relative rates can be accounted for with the assumptions that Scheme IV is the pertinent reaction scheme, that formation of the alkyl enzyme–carbonate is greatly favored over the aryl enzyme–carbonate, and that deacylation is rate controlling. Then, as shown above, $k_{cat.} = k_{+3}'$. The essential identity of $k_{cat.}$ for *p*-APTC and *p*-NPTC leads to these assumptions, for this identity is readily explicable on the basis that $k_{cat.}$ is a measure of the reaction rate of an intermediate common to the two substrates; this intermediate would be $EnOCOOCH_2CCl_3$. This interpretation is consistent with the fortyfold greater value of $k_{cat.}$ for *p*-NPTC relative to *p*-NPEC.

Because of the greater complexity of Scheme IV compared with Scheme III, the argument presented in the preceding paragraph, implicating a common intermediate, is not so compelling as it is for the carboxylic acid derivatives. However, the greatly different chemical natures of the R and R′ groups in these ROCOOR′ substrates probably favor a considerable disparity in the rate constants k_{+2}' and k_{+2}'' in Scheme IV. If R and R′ were similar (both aryl groups, for example) it is possible that very complex kinetics might be observed, and that $k_{cat.}$ could only be interpreted in terms of Eq. 6.

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Keyphrases

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α-Chymotrypsin catalyzed hydrolysis
UV spectrophotmetry, continuous-analysis
Stoichiometry
pH-dependence of hydrolysis

Effect of Certain Drugs on Perfused Human Placenta VIII

Angiotensin-II Antagonists

By CHARLES O. WARD and RONALD F. GAUTIERI

The majority of the drugs tested as angiotensin antagonists in this investigation were from two pharmacologic classes: negative musculotropic agents (papaverine, sodium cobaltinitrite, isosorbide dinitrate, nitroglycerin, sodium nitrite, and dipyridamole) and agents which selectively block α-adrenergic receptors (dibenamine, phentolamine, tolazoline, and hydralazine). Of the direct smooth muscle depressants, sodium nitrite and dipyridamole were the most effective; dibenamine and phentolamine were the most potent adrenergic blocking agents employed. Atropine, cocaine, and cyproheptadine were also tested as antagonists of the pressor effect of angiotensin and exhibited only moderate effectiveness. Lidoflazine, a specific angiotensin antagonist, was the most potent compound tested in this study; the degree of antagonism exhibited by it, however, was only slightly greater than either dibenamine or phentolamine. The results indicate that the pressor effect of angiotensin in the perfused human placenta is primarily the result of a direct stimulation of vascular smooth muscle and secondarily to a stimulation of α-adrenergic receptors.

WHILE RESEARCH with angiotensin in the last two decades has dealt principally with its effects on innervated preparations, there still exists in the literature a paucity of information on the actions of this polypeptide in the more common nerve-free preparations, the perfused human placenta and umbilical cord. Eliasson and Astrom (1) could detect only moderate increases in perfusion pressure using angiotensin in the former preparation; others have reported little or no effect with angiotensin on either the perfused umbilical cord (2) or helical strips of both the human umbilical artery and vein (3). In contrast to these investigations, Gautieri and Mancini (4) recently reported an increase in perfusion pressure of the human placenta that ranged from 11 to 51 mm. Hg; this response to angiotensin was reduced but not completely blocked by either 3-or 6-mg. doses of phentolamine.

In addition to the effects of angiotensin on the above isolated preparations, many recent investigations have linked abnormally high blood levels of angiotensin and renin with the pathogenesis of the toxemias of pregnancy (5–8). The symptomology of clinical toxemia has been mimicked by the injection of renin-containing renal extracts into rats made hypertensive by the administration of desoxycorticosterone (DCA); renal damage similar to that of toxemia as well as convulsions, anasarca, oliguria or anuria, sodium retention, and increased blood urea nitrogen were reported (5). Also, an increased pressor response to angiotensin administration was found by Chesley (6) in women with pre-eclampsia. However, a decreased sensitivity to this autocoid was observed in normal pregnancy when this group was compared with

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