bonded Cl^{36} was stable to 1 N sulfuric acid and labile to 1 N sodium hydroxide at room temperature. These features strongly suggested that the unknown acid was a decarboxylation product of β-ketoadipic acid and that the chlorine was situated on a carbon alpha to the ketone. Assuming a beta decarboxylation, these considerations limited the possibilities to either δ-chlorolevulinic acid or β-chlorolevulinic acid. Consequently dimethyl α-chloro-β-ketoadipate was synthesized by the chlorination of dimethyl β-ketoadipate with 1 equivalent of sulfuryl chloride according to the method of Allihn for the chlorination of ethyl acetoacetate.[2] This intermediate was hydrolyzed and decarboxylated in 7 N sulfuric acid to yield δ-chlorolevulinic acid (m.p. 73–73.5°). *Anal.* Calcd. for $C_5H_7O_3Cl$: C, 39.88; H, 4.69; Cl, 23.55. Found: C, 39.92; H, 4.60; Cl, 23.43. The position of the chlorine in the synthetic product was established by displacement of the chlorine with hydroxide ion and then periodate oxidation to yield formaldehyde which was isolated as the dimedone derivative (m.p. 191°).

The enzymatically synthesized chlorine containing acid and the synthetic product were identical as shown by constant specific radioactivity on repeated crystallization (synthetic δ-chlorolevulinic acid (24.74 mg.) was added to a purified preparation of the unknown radioactive acid (24,000 + 1,000 c.p.m.) the specific activity (c.p.m./mg.) after successive crystallizations from hexane-ether was 770, 1016, 1081 and 1021). In addition, paper chromatography of the natural and synthetic compounds revealed identical behavior in several solvent systems. Chromatography of the natural and synthetic compounds on silicic acid yielded fractions having a constant ratio of weight to radioactivity.

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(2) F. Allihn, *Ber.*, **11**, 567 (1878).

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ACTIVATION OF AN ENZYME CATALYZED REACTION BY EXCESS SUBSTRATES

Sir:

In the course of an investigation of the kinetics of the α-chymotrypsin catalyzed hydrolysis of methyl aceturate, in aqueous solutions at 25.0° and pH 7.90 and 0.50 M in sodium chloride, we have encountered what appears to be the first example of activation of an enzyme catalyzed reaction, of the type $E + S \rightleftarrows ES \rightarrow E + P$, by excess substrate.

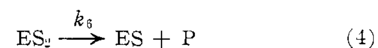
Methyl aceturate is sufficiently soluble in water to permit the examination of its hydrolysis in the presence of α-chymotrypsin over a wide range of substrate concentrations. For the case at hand such reactions were followed with a pH-stat[1] under conditions where $[E] = 4.54 \times 10^{-5}\ M$[2] and $[S]_0$, was varied from $5.0 \times 10^{-3}\ M$ to $6.0 \times 10^{-1}\ M$. When the results of these experiments were presented in a v_0 *vs.* $v_0/[S]_0$ plot,[3,4] it became apparent that the plot consisted of two essentially linear segments, of differing slope and intercept, and a non-linear connecting segment. The slope and intercept of the linear segment associated with values of $[S]_0 = 5.0 \times 10^{-3}\ M$ to $3.0 \times 10^{-2}\ M$ led to values of $K_S = 1.0 \times 10^{-2}\ M$ and $k_3 = 1.2 \times 10^{-4}\ M$/min./mg. protein nitrogen per ml. The slope and intercept of the other linear segment, associated with values of $[S]_0 = 5.0 \times 10^{-2}\ M$ to $6.0 \times 10^{-1}\ M$, gave values of $K_S = 4.1 \times 10^{-1}\ M$ and $k_3 = 1.0 \times 10^{-3}\ M$/min./mg. protein nitrogen per ml.

For a system represented by equations 1 to 4, inclusive, it can be shown that the rate equation for such a representation is given by equation 5

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \quad (1)$$

$$ES \xrightarrow{k_3} E + P \quad (2)$$

$$ES + S \underset{k_5}{\overset{k_4}{\rightleftarrows}} ES_2 \quad (3)$$



$$ES_2 \xrightarrow{k_6} ES + P \quad (4)$$

$$v_0 = \{(K_S''k_3 + k_6[S]_0)[E][S]_0\}/\{K_S''[S]_0 + [S]_0^2 + K_S'K_S''\} \quad (5)$$

where $K_S' = (k_2 + k_3)/k_1$ and $K_S'' = (k_5 + k_6)/k_4$.

With $K_S' = 1.0 \times 10^{-2}\ M$, $K_S'' = 4.1 \times 10^{-1}\ M$, $k_3 = 1.2 \times 10^{-4}\ M$/min./mg. protein nitrogen per ml. and $k_6 = 1.0 \times 10^{-3}\ M$/min./mg. protein nitrogen per ml. it was found that equation 5 provided a satisfactory quantitative description of the dependence of v_0 upon $[S]_0$ over the 120-fold range of substrate concentration that had been studied. Therefore, we conclude that the α-chymotrypsin catalyzed hydrolysis of methyl aceturate may be represented by equations 1 to 4, inclusive, and that evidence has been obtained with respect to the formation of an ES_2 complex that is capable of yielding reaction products at a greater rate than the corresponding ES complex. That is, activation by excess substrate has been demonstrated.

The extension of these studies to systems involving two different substrates, in order to explore the possibility of demonstrating synergism in an isolated enzyme system, and to systems involving a substrate and a substrate analog that is incapable of yielding reaction products, in order to investigate the possibility of observing inhibition at low concentrations of the analog and activation at high concentrations, is in progress.

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(1) T. H. Applewhite, R. B. Martin and C. Niemann, THIS JOURNAL, **80**, 1457 (1958).

(2) Based upon an assumed molecular weight of 22,000 and a nitrogen content of 16.0% for monomeric α-chymotrypsin.

(3) G. S. Eadie, *J. Biol. Chem.*, **146**, 85 (1942).

(4) All values of v_0 were corrected for the non-enzyme catalyzed hydrolysis of the substrate.