Thus the acid-dissociation of this type of compound may be explained on the basis of either "protonated" or "half-bond" intermediates.

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## THE CALCIUM CHLORIDE AND HYDROXYL ION CATALYZED HYDROLYSIS OF SEVERAL ACYLATED $\alpha$-AMINO ACID ESTERS

*Sir:*

The availability of a pH-Stat[1,2] has permitted us to determine the base catalyzed hydrolysis of several acylated $\alpha$-amino acid esters, to the acylated $\alpha$-amino acid and alcohol, in the presence of calcium chloride and in the absence of conventional buffers. Under these conditions it has been found that the initial rates of hydrolysis are first order with respect to ester and hydroxyl ion but decrease from first order with respect to calcium chloride as the mean ion activity of the latter species is increased. From the reactions $S + M \underset{k_2}{\overset{k_1}{\rightleftharpoons}} MS$; $MS + OH^- \xrightarrow{k_3} M + P$; $S + OH^- \xrightarrow{k_4} P$, it follows that $v' = d[P]/dt = k_3[MS][OH^-] + k_4[S][OH^-]$. Experiments conducted under conditions where $[M] >> [MS]$, when [M] is the mean ion activity of calcium chloride,[3] allowed us to establish the relationship $v' = k_3[MS][OH^-] + k_4[\sigma][OH^-] - k_4[MS][OH^-]$, when $[\sigma] = [S] + [MS]$. Assuming a steady state process for MS, it follows that $v' - k_4[\sigma]\cdot[OH^-] = v = \{(k_3 - k_4)[OH^-][M][\sigma]\}/\{[M] + ((k_2 + k_3[OH^-])/k_1)\}$. Since $k_4[\sigma][OH^-]$ was determinable and the reactions were first order with respect to hydroxyl ion, $k_2 >> k_3[OH^-]$ and the second term in the denominator of the preceeding equation may be taken as the equilibrium constant $K = k_2/k_1 = [M][S]/[MS]$. Setting $k_3' = k_3[OH^-]$, $k'_4 = k_4[OH^-]$ and $k_3'' = (k_3' - k_4') = (k_3 - k_4)[OH^-]$ it follows that $[M]/(v/[\sigma]) = ([M]/k_3'') + K/k_3''$ and a plot of the left hand term *vs.* [M] will give a line of slope $1/k_3''$ and intercept $K/k_3''$. Such linear relationships were observed and led to the values of $K$ and $k_3$ given in Table I. The constancy of $K$, for benzoylglycine methyl ester at *p*H 7.90 and 8.40, confirms the conclusion that $k_2 >> k_3[OH^-]$ and $K = k_2/k_1$.

While calcium chloride acting in conjunction with hydroxyl ion is not a very efficient catalyst for the hydrolysis of acylated $\alpha$-amino acid esters, it is just this property that permits the separate evaluation of $K$ and $k_3$. In addition the use of a *p*H-Stat[1,2] has avoided complications arising from specific buffer effects such as those encountered by Bender and Turnquest[4] in their studies on the cupric ion catalyzed hydrolysis of several $\alpha$-amino acid esters, a reaction examined earlier by Kroll.[5]

TABLE I

VALUES OF $K$ AND $k_3$[a,b]

| Ester | *p*H | $K$, $M$ | $k_3$ $M^{-1}$, sec.$^{-1}$ |
|---|---|---|---|
| Benzoylglycine methyl ester | 7.90 | 25 ± 2 | 680 ± 30 |
| | 8.40 | 25 ± 2 | 670 ± 30 |
| Acetyl-L-valine methyl ester | 7.90 | 2.8 ± 0.1 | 7.2 ± 0.2 |

[a] In aqueous solutions at 25.0° and containing calcium chloride. [b] Constants evaluated from a minimum of eight experiments conducted under conditions where the concentration of ester was varied from 5 to 40 × $10^{-3}$ $M$ and that of calcium chloride from 0.6 to 3.0 $M$.

The observation that a more stable MS complex may react with hydroxyl ion to give hydrolysis products at a slower rate than a less stable MS complex, *cf.* Table I, is but one example of the information that can be gained from knowledge of the constants $K$ and $k_3$. The fact that the reaction system described in this communication is an almost complete analog of the simple enzyme-catalyzed reaction creates further interest in a system which in its own right is of importance in developing an understanding of the characteristics of an important class of solvolytic reactions.

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## SYNTHESIS OF AMINO ACIDS CATALYZED BY AMINO ACID OXIDASES[1]

*Sir:*

The oxidation of amino acids by the general L- and D-amino acid oxidases[2] has been considered to be an irreversible process involving an unstable imino acid intermediate which hydrolyzes spontaneously to the corresponding $\alpha$-keto acid and ammonia in accordance with the reactions

$$RCHNH_2COOH + Enzyme\text{-}FAD \longrightarrow RC{=}NHCOOH + Enzyme\text{-}FADH_2$$

$$RC{=}NHCOOH + H_2O \longrightarrow RCOCOOH + NH_3$$

$$Enzyme\text{-}FADH_2 + O_2 \longrightarrow Enzyme\text{-}FAD + H_2O_2$$

In the presence of oxygen the reaction will proceed in the direction of $\alpha$-keto acid formation because of continuous oxidation of enzyme-bound reduced coenzyme.

We have carried out experiments in which a purified amino acid oxidase preparation and an amino acid were incubated with the $\alpha$-keto acid analog of another amino acid substrate. Under anaerobic conditions, L-amino acid oxidase and D-amino acid oxidase catalyzed the formation of L- and D-amino acid isomers, respectively, from the corresponding $\alpha$-keto acids. Thus, D-alanine was formed when D-amino acid oxidase,[3] pyruvate, and D-phenylalanine were incubated in the absence of oxygen; the reverse reaction was observed with D-alanine

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(2) H. A. Krebs, in J. B. Sumner and K. Myrbäck, "The Enzymes," Vol. II, Part 1, Academic Press, Inc., New York, N. Y., 1951, p. 499.

(3) Prepared from sheep kidney by the procedure of E. Negelein and H. Brömel, *Biochem. Z.*, **300**, 225 (1939); the purified preparation was treated with an excess of FAD, and then dialyzed against 0.1 $M$ sodium pyrophosphate buffer (*p*H 8.3) to remove free FAD and free ammonia.