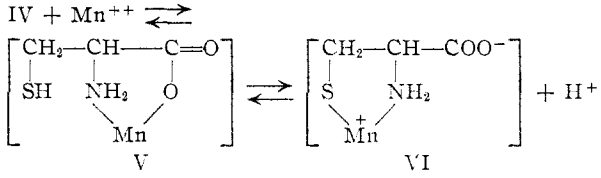
Evidence for this is the low pK value for the α-ammonium group in cysteine, 8.16, as compared to cystine, 9.6.[11] This interaction of sulfhydryl and amino group must be in direct competition with the formation of the manganese complex involving the amino group and the carboxylate ion (IV and V). The conversion of V to VI is favored



by increasing pH, since the higher alkalinity favors the formation of the more stable sulfur–manganese bond. The binding of the manganous ion by the amino group in V increases the acidity of the sulfhydryl group due to the positive inductive effect of the metal ion, thus favoring the conversion of V to VI.

Attempts to measure the ability of glycine amide, L-leucine amide, glycylglycine[8a] and triglycine to form complexes with manganous ions have been unsuccessful. These complexes are formed in very small amounts and are extremely unstable as compared to the parent amino acids. This point is significant in attempting to elucidate the mechanism of manganese-activated proteolytic enzyme systems where the amino acid derivative is used as a substrate, and the amino acid appears as an end product. Unpublished data from this Laboratory indicate that L-leucine and other amino acids will inhibit the hydrolysis of L-leucineamide by a leucine aminopeptidase, and the extent of the inhibition depends on the affinity of the amino acid for the metal ion.

PROVIDENCE, R. I. RECEIVED MAY 21, 1951

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, YALE UNIVERSITY SCHOOL OF MEDICINE]

The Participation of Heavy Metal Ions in the Hydrolysis of Amino Acid Esters[1]

BY HARRY KROLL[2,3]

The rates of hydrolysis of α-amino acid esters in the presence of heavy metal divalent ions were investigated. A mechanism is postulated which involves a second-order reaction between a metal complex of the amino acid ester and hydroxyl ions. The hydrolytic rate is determined by the nature of the side chain in the alpha amino acid ester and the complexing tendency of the metal ion.

In a continuation of our studies on the effects of metal ions in biological systems,[4] it was observed that heavy metal ions accelerated the hydrolysis of amino acid esters in the pH range of 7.5–8.5. This reaction was investigated more closely in order to obtain information which could contribute to an understanding of the nature of metal ion activation of enzyme catalyzed hydrolytic reactions.[5]

Experimental

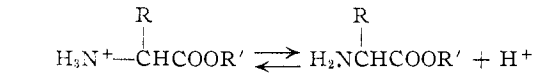
Amino Acid Esters.—The amino acid esters used in this investigation were available in this Laboratory or were prepared by treatment of the appropriate amino acid with methanolic hydrogen chloride. All esters were recrystallized, when possible, from the appropriate solvents. Proline methyl ester hydrochloride and dimethyl aspartate hydrochloride were isolated as viscous oils which could not be crystallized. However, the determination of their nitrogen content showed a satisfactory agreement with the theory.

General Procedure.—All kinetic studies reported were carried out at 25.4°. Four milliliters of a 0.1 M solution of the amino acid ester hydrochloride was pipetted into a 25-ml. volumetric flask, followed by 7.0 ml. of 0.5 M tris-(hydroxymethyl)-aminomethane buffer and by 2.7 ml. of 0.1 N sodium hydroxide. The flask was placed in the water-bath and after five minutes the exact amount of metal ion solution was added plus sufficient water to bring the reaction mixture to volume. After a rapid thorough mixing, a 3-ml. aliquot was withdrawn and titrated immediately to pH 4.0 with 0.05 N hydrochloric acid.

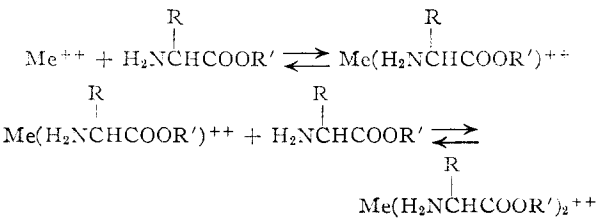
The hydrolytic process was followed by titration of samples at ten-minute intervals. The difference in the amount of 0.05 N hydrochloric acid required to titrate the solution at any given time interval as compared to the value obtained for zero time was used as a measure of the hydrolysis of the amino acid ester. A Cambridge Research Model pH Meter equipped with external glass electrodes was used in the titrations, and all solutions were prepared from oxygen-free distilled water.

Discussion and Results

Amino acid esters in aqueous solutions participate in the equilibrium



Since the process of metal complex formation involves the step-wise addition of the free base of the amino acid ester to the metal ion,[6] it is apparent that, at a pH sufficiently high to allow an appreciable concentration of the free base, a solution of a divalent heavy metal ion and an amino acid ester will contain a series of metal complexes of the type shown below.



At any given pH value, the various species of metal-ester complexes will be determined by the coördination characteristic (affinity) of the metal

(1) Presented before the Division of Organic Chemistry at the Boston Meeting of the American Chemical Society, April, 1951.

(2) Research Fellow, American Cancer Society, 1949–1951.

(3) Alrose Chemical Co., Providence, R. I.

(4) H. Kroll, THIS JOURNAL, **74**, 2034 (1952).

(5) A. L. Lehninger, *Physiol. Rev.*, **30**, 393 (1950).

(6) J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Sons, Copenhagen, 1941.

ion, the concentrations of the reactants, and the stabilities of the complexes formed.

Since, in the absence of a suitable metal ion, the hydrolysis of amino acid esters near *p*H 8 is extremely slow, the rapid reaction observed upon addition of such metal ions must be attributed to the hydrolytic cleavage of a metallic complex. In order to determine the controlling mechanism, it was important to establish the nature of the metal ion–ester complex involved. Since the attainment of equilibrium between metal ion and amino acid ester may be considered to be very rapid, the rate determining step in the hydrolysis must involve the complex. The amount of the intermediate complex at any fixed metal ion concentration is proportional to the concentration of the amino acid ester, and the last-named value could be used therefore in determining the reaction order. At constant *p*H, the rate of hydrolysis was found to follow a first order course with respect to the ester.

In order to determine the ratio of metal ion to ester in the complex undergoing hydrolysis, a study was made of the rate of hydrolysis, at *p*H 7.8–7.9, of glycine ethyl ester in the presence of varying concentrations of cobaltous ion. The data obtained, Fig. 1, indicated that the observed first-order rate approached a maximum as the metal ion:ester ratio approached unity. This

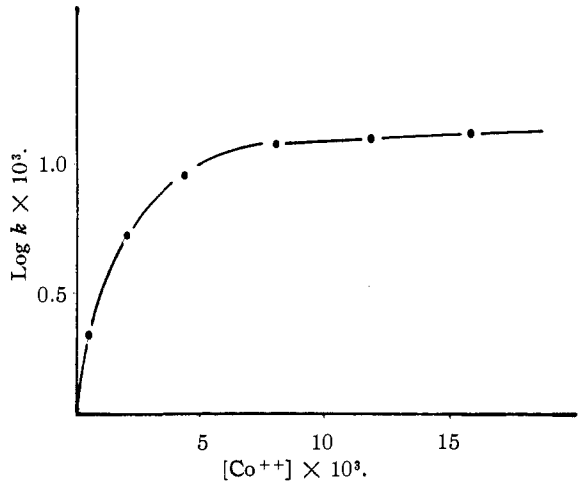


Fig. 1.—Effect of cobaltous ion concentration on the rate of hydrolysis of 0.016 molar glycine ethyl ester at *p*H 7.8 and 25°.

was interpreted to indicate that the velocity of the hydrolysis at constant *p*H was determined by the equation

$$v = k_1[\mathrm{MeB}^{++}] + k_2[\mathrm{MeB_2}^{++}] \ldots + k_n[\mathrm{MeB_n}^{++}]$$

where MeB^{++}, $\mathrm{MeB_2}^{++} \ldots \mathrm{MeB_n}^{++}$ represent the several possible metal complexes. As the metal ion concentration was increased, the formation of the 1:1 ester–metal complex was favored at the expense of those complexes containing a higher ratio of amino acid ester to metal. The attainment of a maximal hydrolytic rate, unaffected by further addition of metal ion, was further evidence to support the existence of the postulated intermediate.

The reaction was found to be sensitive to changes in *p*H. An increase of one *p*H unit produced a fourfold increase in the rate of hydrolysis (Fig. 2); this can be attributed to a second-order reaction between metal ion complex and hydroxyl ion, superimposed on the very slow breakdown of the amino acid ester in the absence of added metal ion.

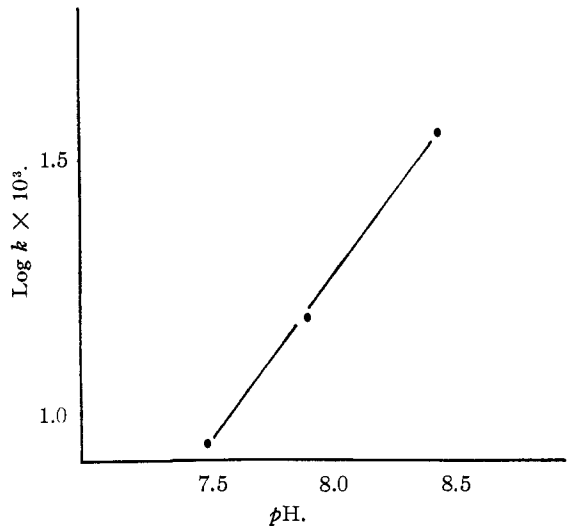


Fig. 2.—Effect of *p*H on the rate of hydrolysis of 0.016 molar glycine ethyl ester in the presence of 0.016 molar cobaltous ion at 25°.

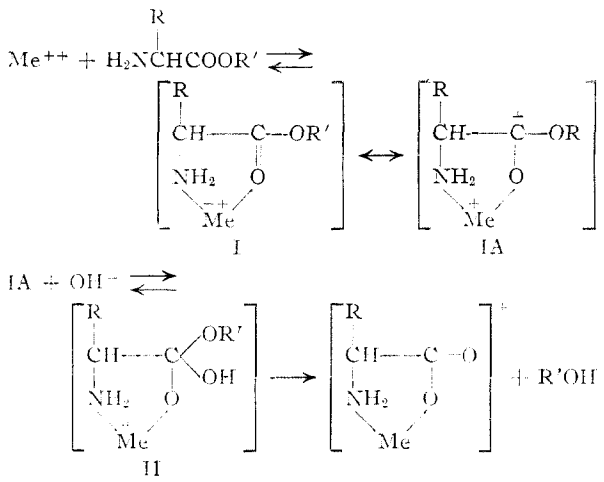
This interpretation is supported by several considerations. The participation of the solvent in the actual hydrolytic process can be ruled out; otherwise the observed first-order constant would be independent of the *p*H. The reaction is not a general base catalyzed process, since doubling the buffer concentration had no effect on the rate.

In the formation of either stable or unstable coördination complexes, the driving force is the strong electrophilic character of the metal, and this process is favored by the formation of five- or six-membered chelate rings. The formation of stable five-membered chelate complexes of alpha amino acids[7] is well known, and it is possible to postulate a mechanism which involves the conversion of a five-membered metal chelate complex of an alpha amino acid ester by hydroxyl ions into the corresponding complex of the amino acid.

The proposed mechanism is in agreement with a general principle which states that the metal complexes are stronger Lewis type acids[8] than the uncombined ligand molecule, and the increase in acid strength is related to the complexing tendency of the metal ion. Thus, in the hydrolysis of amino acid esters in the presence of divalent heavy metal ions, the formation of a metal–ester complex gives an acid (I and IA) which has a greater affinity for hydroxyl ions than the free amino acid ester. The product (II) of this acid–base neutralization is unstable and undergoes a rapid decomposition to the end products of the reaction.

(7) D. M. Greenberg, "Amino Acids and Proteins," Charles C Thomas, Springfield, Illinois, 1951, p. 472.

(8) G. Schwarzenbach, *Chimia*, 3, 1 (1949).



Effect of Metal Ion.—A series of five divalent metal ions were studied. It will be noted from Table I that the ester cleavage increased with the

TABLE I

EFFECT OF SEVERAL METAL IONS ON THE HYDROLYSIS OF GLYCINE METHYL ESTER

Temp., 25.4°; metal ion concn., 0.016 mole/l.; ester concn., 0.016 mole/l.

Metal ion	*p*H	$k_{obsd.}$, sec.$^{-1}$
Cu^{+2}	7.3	0.0425
Co^{+2}	7.9	.0156
Mn^{+2}	7.9	.00351
Ca^{+2}	7.9	.0007
Mg^{+2}	7.9	.0007

increasing tendency of the metal ion to enter into complex formation.[9] The effectiveness of cupric ions to induce the hydrolysis of glycine ethyl ester is in agreement with its tendency to form stable complexes, whereas calcium and magnesium ions, which form unstable complexes, were without effect.

Effect of Ester Radical—In Table II are given

TABLE II

COBALT-INDUCED HYDROLYSIS OF SEVERAL GLYCINE ESTERS AT *p*H 7.9, 25°

Co^{+2}, 0.016 mole/l.; ester concn., 0.016 mole/l.

Ester	$k_{obsd.}$, sec.$^{-1}$
Ethyl	0.0156
Methyl	.0262
Benzyl	.0254

data on the hydrolysis of three glycine esters, which were studied in order to determine the effect of the alcohol radical on the hydrolytic rate. The data approximate the results obtained by the alkaline hydrolysis of similarly substituted acetic acid esters.[10]

Effect of Amino Acid Side Chain.—The influence of the amino acid side chain in the hydrolysis of the corresponding ester is of interest because the information so obtained can contribute to an understanding of the chemical function of these groups in more complicated systems. In column 3 of Table III the relative rates of hydrolysis of the methyl esters of a series of amino acids by

(9) J. Bjerrum, *Chem. Revs.*, **46**, 381 (1950).

(10) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, pp. 211–212.

TABLE III

COBALT INDUCED HYDROLYSIS OF METHYL ESTERS OF AMINO ACIDS AT *p*H 7.9, 25°

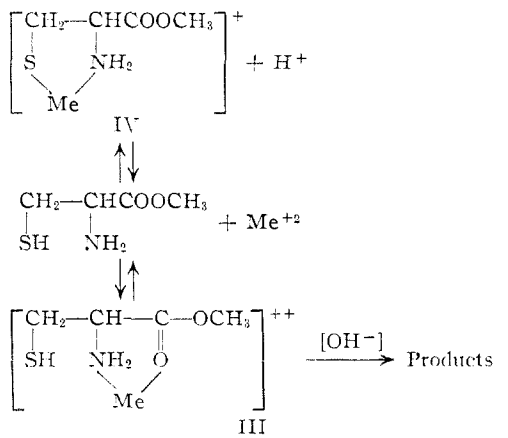
Co^{++}, 0.016 mole/l.; ester concn. 0.016 mole/l.

Amino acid	$k_{obsd.}$, sec.$^{-1}$	$k_{ester}/k_{glycine\ ester}$
Glycine	0.0262	1.0
L-Alanine	.0195	0.74
L-Leucine	.0099	.38
DL-Phenylalanine	.0236	.90
L-Tyrosine	.0100	.38
L-Cysteine	.0121	.46
L-Arginine	.0147	.56
L-Histidine	.0117	.45
L-Aspartic acid	.0172	.65
DL-Serine	.0253	.96
L-Proline	.0186	.71

cobaltous ion are listed on the basis of an arbitrary value of 1.0 for glycine methyl ester.

The mechanism proposed for the metal ion induced hydrolysis of amino acid esters postulates that the observed first-order rate constant depends on the reaction of the metal complex with hydroxyl ions. Accordingly, any substituent on the alpha carbon of the amino acid ester which decreases the general acid properties of the complex will decrease the rate of hydrolysis. If one excludes those amino acid esters (histidine methyl ester, cysteine methyl ester, etc.) which contain side chain residues capable of combining with the metal ion, the compounds containing electron-attracting substituents in the side chain (*e.g.*, serine methyl ester and phenylalanine methyl ester) are hydrolyzed at approximately the same rate as is glycine methyl ester. However, the side chains of L-proline, L-alanine and L-leucine are electron-repelling. The esters of these compounds are hydrolyzed at a slower rate than that of glycine methyl ester, and the accumulation of alkyl groups in the side chain potentiates this effect. These observations are in complete agreement with the effect of substituents on the alkaline hydrolysis of the ethyl esters of substituted carboxylic acids.[8] In terms of the mechanism proposed above, the acid strength of the metal complex is increased by electron-attracting substituents and decreased by electron-repelling groups.

When the amino acid ester molecule contains, in addition to the α-amino group, another group



which can compete for the metal ion, there results a competitive reaction which can reduce the concentration of the actual complex required for the hydrolysis.[3] Cysteine methyl ester is an example of this type of inhibition of the ester hydrolysis. The strong affinity of the sulfhydryl group for the metal ion results in a complex (IV) which is much more stable than the complex (III) required for the hydrolysis.

Other amino acid esters which are capable of reacting with the metal ion in a manner other than that indicated for glycine are histidine, tyrosine and aspartic acid.

Glutamic acid dimethyl ester was found to undergo a relatively rapid conversion to the methyl ester of pyrrolidone carboxylic acid. The addition of cobaltous ion accelerated the rate of breakdown of the dimethyl ester so that the over-all effect resulted in an observed first order constant 1.1 times greater than found with glycine methyl ester.

NEW HAVEN, CONN. RECEIVED MAY 25, 1951

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

An Acidity Function in Aqueous Hydrazine[1]

BY N. C. DENO

An acidity function (H_-) has been determined from 5–60% (by weight) hydrazine by the use of indicators. This function is identical with pH (measured by the glass electrode) from pH 11 to 12, and reaches the value 15.93 in 60% hydrazine. This H_- function is used to establish a scale of pK values for weak acids.

Hammett[2–4] showed that an acidity function defined by equation (1) could be determined in media whose acidities were outside the pH range in water. It was necessary to the success of this acidity function that the term $\log f_B/f_{HB}$ be independent of the indicator used. Hammett found this to be true in sulfuric acid–water mixtures providing indicators of a single charge type were employed.

$$H = -\log a_{H^+} - \log f_B/f_{HB} \quad (1)$$

It was the purpose of the present work to extend Hammett's concept of an acidity function into strongly basic media. Such a function would be of use in determining the relative strengths of weak acids, and with certain base-catalyzed reactions, it should bear a linear relationship to the log of the rate constant. This would be analogous to the linear relationship between H_0 and $\log k$ which Hammett[4] and others[5] have found for certain acid-catalyzed reactions.

A series of neutral weak acids were chosen as indicators. Using Hammett's notation, this acidity function is an H_- function and would be defined by equation (2). The indicators would react as in equation (3).

$$H_- = pK_{HA} + \log c_{A^-}/c_{HA} = -\log a_{H^+} - \log f_{A^-}/f_{HA} \quad (2)$$

$$A^- + H^+ = HA \quad (3)$$

Experimental

All spectral measurements were made by means of a Beckman model DU spectrophotometer. With each indicator the spectrum above 400 mμ was recorded for anion and neutral acid. The principal absorption maxima of the anions were chosen as the wave lengths to measure c_{A^-}/c_{HA}. This decreased the error due to the change in spectrum with changing media. With all indicators except number VIII, the absorption of the neutral indicator was zero at the wave length employed. The maxima, log e, and slit widths employed are given in Table II.

It was assumed that all indicators ionized according to equation 3 except indicator VIII. Indicator VIII belongs to the charge type A^- since its first acidic hydrogen is known to ionize at a much lower pH. The acidity function defined by VIII would be an H_- function. It is remarkable that this H_- acidity function defined by VIII follows so closely the H_- function given in Table I.

Stock acetone solutions were used to introduce the indicators. One or two drops of the acetone solution (concentration about 0.2%) were added to the hydrazine solution. This provided a rapid method of dispersing the indicators into the solution. Since most of the indicators slowly reacted with hydrazine, this rapid dispersal was necessary. With p-nitrobenzyl cyanide (indicator I), an ethanol solution was used in hydrazine concentrations below 20%. Although acetone reacts with hydrazine, it is believed that the amount added, 0.02 g. per 10 g. of hydrazine solution, had a negligible effect on the basicity of the media.

3,6-Dinitrocarbazole (II).—The carbazole to be nitrated was prepared by the method of Horning[6] to ensure absence of other aromatic compounds. Nitration was effected with 70% nitric acid in acetic acid.[7] The m.p. has been reported as 320°.[7] The initial product does melt around 320°, but after vacuum sublimation and several crystallizations from nitrobenzene, the fine needles melt at least above 340° and retain only a cream color (previously gold to brown).

1- and 3-Nitrocarbazole.—The procedure of Morgan and Mitchell[8] was used. The small amount of 1-nitrocarbazole is easily separated by their procedure. However, the 3-nitrocarbazole, which was implied to be the major product and easily obtainable, was impure. After considerable recrystallization only a small amount was finally obtained in fairly pure form.

Discussion

The system hydrazine–water was chosen for the following reasons.[9] Hydrazine is basic and is unique in possessing a m.p. (2°), b.p. (117°), and dielectric constant (ϵ = 53 at 20°),[10] close to the values of the same properties for water. This similarity in dielectric constant is particularly advantageous since in going from pure water to pure hydrazine there will be relatively small changes in a large dielectric constant. This circumstance should

(1) This work was performed under Contract AF33(038)-20391 between The Pennsylvania State College and the Office of Air Research.

(2) L. P. Hammett, THIS JOURNAL, **50**, 2666 (1928).

(3) L. P. Hammett and A. J. Deyrup, *ibid.*, **54**, 2721 (1932).

(4) L. P. Hammett and M. A. Paul, *ibid.*, **56**, 827 (1934).

(5) N. C. Deno and M. S. Newman, *ibid.*, **72**, 3852 (1950).

(6) E. C. Horning, M. G. Horning and G. W. Walker, *ibid.*, **70**, 3935 (1948).

(7) P. Ziersch, *Ber.*, **42**, 3799 (1909).

(8) G. T. Morgan and J. G. Mitchell, *J. Chem. Soc.*, 3283 (1931).

(9) The author is indebted to Dr. W. C. Fernelius for suggesting the use of hydrazine in these studies.

(10) H. Ulich and W. Nespital, *Z. physik. Chem.*, **16B**, 221 (1932).