1968

Metal ion complex catalysis of amino acid ester hydrolysis

Bruce Eugene Leach
Iowa State University

69-4254

LEACH, Bruce Eugene, 1942-

METAL ION COMPLEX CATALYSIS OF AMINO ACID ESTER HYDROLYSIS.

Iowa State University, Ph.D., 1968
Chemistry, inorganic

University Microfilms, Inc., Ann Arbor, Michigan

METAL ION COMPLEX CATALYSIS OF AMINO ACID ESTER HYDROLYSIS

by

Bruce Eugene Leach

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILSOPHY

Major Subject: Inorganic Chemistry

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Ames, Iowa
1968

TABLE OF CONTENTS

INTRODUCTION

Metal ion catalysis of the hydrolysis of α-amino acid esters was discovered in 1952 by Kroll (1). Since that time these reactions have been studied by a number of research groups with hopes of elucidating the nature of the hydrolytic process and the role of metal ions in biological systems. Esters of α-amino acids are an example of a class of important biological compounds which are energy-rich. An energy-rich compound has been defined as one whose reaction with a substance commonly present in the environment is accompanied by a large negative free energy change at physiological pH (2). Amino acid esters which are important intermediates in the biosynthesis of proteins, have a standard free energy of hydrolysis which is comparable to that of adenosine triphosphate, ATP.

At physiological pH in the absence of metal ions the rate of hydrolysis of amino acid esters is very slow. In general, four reactions must be considered. They involve water and hydroxide ion attack upon the protonated and free base esters.

$$EH + H_2O \xrightarrow{k_1} \text{products}$$

$$EH + OH^- \xrightarrow{k_2} \text{products}$$

$$E + H_2O \xrightarrow{k_3} \text{products}$$

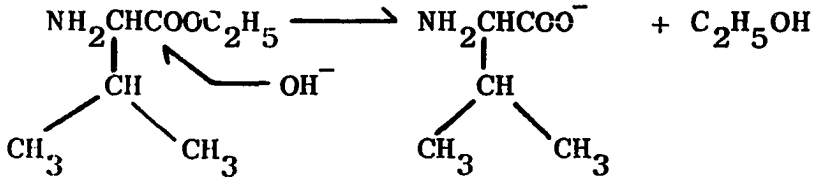
$$E + OH^- \xrightarrow{k_4} \text{products}$$

Conley and Martin (3) have investigated the hydrolysis of glycine ethyl ester and found $k_1 = 5 \pm 3 \times 10^{-9}$ sec.$^{-1}$, $k_2 = 24$ M^{-1} sec.$^{-1}$, k_3 = immeasurably slow, and $k_4 = 0.58$ M^{-1} sec.$^{-1}$. In agreement with the greater nucleophilicity of hydroxide ion, most of the hydrolysis near pH 7 is catalyzed by hydroxide ion.

Hay, Porter and Morris (4) have studied the basic hydrolysis of ethyl glycinate, methyl glycinate, ethyl betaine ethyl ester, ethyl leucinate, methyl leucinate, methyl cysteinate, methyl serinate, ethyl phenylalaninate and dimethyl glutamate at 25.0° and ionic strength 0.1 M and determined the ionization constants of the esters. They found that the ionization constants for amino acid esters were approximately two pK units lower than those for the corresponding amino acids.

Protonation of the amino group of an amino acid ester results in a much greater reactivity of the protonated species in alkaline hydrolysis. The effect of charge on ester hydrolysis was demonstrated (4) by comparison of the rates of hydrolysis for ethyl glycinate, ethyl betaine ethyl ester and cysteine methyl ester. Positively charged betaine ethyl ester, $[(C_2H_5)_3NCH_2COOC_2H_5]^+$, hydrolyzes 36 times faster than ethyl glycinate, $NH_2CH_2COOC_2H_5$, under similar conditions, whereas, the rate of cysteine methyl ester, ($^-SCH_2CH(NH_2)COOCH_3$), hydrolysis was approximately one-eighth as fast as ethyl glycinate.

Angelici and Hopgood (5) have measured the rates of hydrolysis of butyl glycinate, ethyl alaninate, ethyl β-alaninate, and ethyl valinate at 25.0° and ionic strength 0.060 M. They concluded that the steric effect of the R group of ethyl esters of α-amino acids, $NH_2CHRCOOC_2H_5$, is a major factor determining the relative rates. The results indicate that the rates fall into an order predicted by the Newman "rule of six" (6). This rule states that those atoms which are effective in providing steric hindrance are separated from the attacking atom in the transition state by a chain of four atoms, i.e., numbering the oxygen atom of the hydroxyl group attacking the carbonyl atom 1, then the greatest steric hinderance will result from atoms in position 6. For example the rate of hydrolysis of ethyl valinate which has six atoms in position 6 would be expected to be quite slow.



Ester hydrolysis has been observed to be catalyzed by both general base catalysis and nucleophilic catalysis, depending upon the type of ester. Esters activated in the alcohol portion have been found to undergo nucleophilic catalysis (7, 8) whereas esters activated by electron-withdrawing substituents in the acyl portion have been

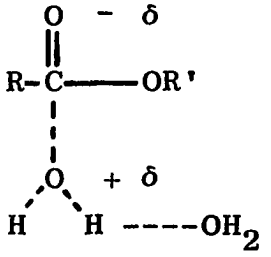
shown to hydrolyze with a mechanism of general base catalysis (9). The nucleophilic catalysis mechanism depicted below in the reactions written for the base hydrolysis of esters is one in which the reagent B directly attacks the sp^2 hybridized carbonyl carbon atom to form an intermediate which is subsequently hydrolyzed by water in a fast step to result in the products of the reaction. In this case, $k_n > k_{OH^-}$, $k_{H_2O} < k'_{OH^-}$, k'_{H_2O} .

$$RCOR' + B: \xrightarrow{k_n} RCB + OR^-$$

$$RCOR' + OH^-, (H_2O) \xrightarrow{k_{OH^-},\ (k_{H_2O})} RCOH + OR^-$$

$$RCB + OH^-, (H_2O) \xrightarrow{k'_{OH^-},\ (k'_{H_2O})} RCOH + B:$$

For general base catalysis the proton transfer from the general base must occur in the rate determining step which is illustrated for the abstraction of a proton by H_2O from the tetrahedral intermediate formed by hydration of the ester carbonyl group.



In general, evidence for the intermediate in the nucleophilic catalysis mechanism is not obtainable since the subsequent hydrolysis is usually faster than its formation. Sometimes the deuterium oxide solvent isotope effect may be used as a criterion to distinguish between general base- and nucleophile-catalyzed reactions (10) but the results may also be ambiguous. One method which has been used is to compare the catalytic effects of two bases of similar pK_a but widely different nucleophilicity (11). Bruice and Lapinski (8) found for example that whereas imidazole and phosphate dianion, $HPO_4^{=}$, have similar pK_a values the coefficients of catalytic activity are 4000 times greater for imidazole toward p-nitrophenyl acetate than for phosphate dianion but similar coefficients are found toward ethyl dichloroacetate. They concluded that p-nitrophenyl acetate was hydrolyzed by nucleophilic catalysis.

Ethyl glycinate was studied (9) and the mechanism was stated to be general base catalysis on the basis of the reaction in the presence of the buffer tris-(hydroxymethyl)-aminomethane. The evidence certainly cannot be considered conclusive in the absence of more data. There is also a problem of definitions as to the function of a general base catalyst (12). If the function of the general base catalyst is not only a rate determining proton transfer but also the

introduction of a base into the substrate to form an unstable intermediate then little difference exists between the two cases.

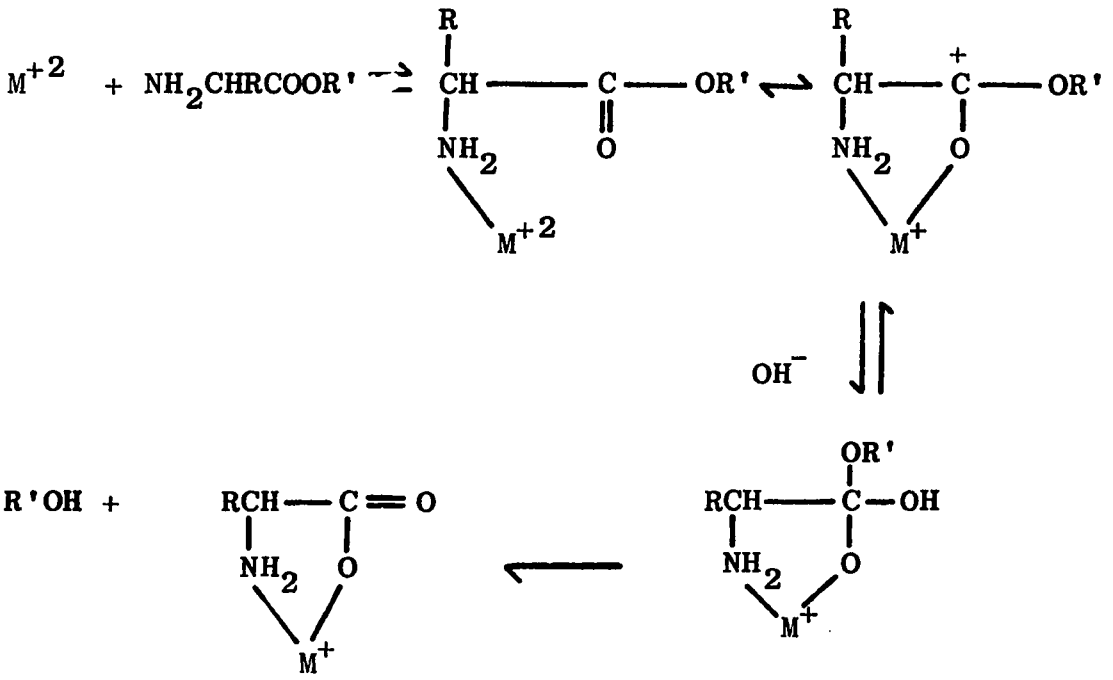
Glycine ethyl ester (13) and ethyl acetate (14) hydrolyses exhibit nearly identical activation energies of 10.9 and 11.0 kcal/mole respectively. The entropy of activation for the basic hydrolysis of ethyl glycinate was -21.7 e.u. (13). The much larger value of 18.1 kcal./mole for the activation energy for ethyl glycinate basic hydrolysis reported by Connor, Jones, and Tuleen (15) is probably due to the contribution of protonated ethyl glycinate to the rate of hydrolysis to give a much larger rate than that reported by a number of other investigators (3,4,13,16,17). Even at pH 9.5 - 11.0 care must be taken that the rate observed does not include contributions from the hydrolysis of small concentrations of the protonated ester. At lower pH values due to the lower concentrations of hydroxide ion the rate observed decreases and at pH 7 the rate observed is too slow to measure by pH stat techniques compared to the metal ion catalyzed hydrolysis (3).

Kroll (1) found that heavy metal ions accelerated the hydrolysis of amino acid esters. It was observed that a maximum rate of hydrolysis occurred when the metal ion : ester ratio approached unity. This was interpreted to mean that the velocity of the hydrolysis at a constant pH maintained by tris-(hydroxymethyl)-aminomethane, tris, buffer

was determined by the equation:

$$v = k_1 (ME) + k_2 (ME_2) + \ldots + k_n (ME_n)$$

where (ME), (ME_2), ... (ME_n) represent concentrations of the several possible chelates, the charges on the species having been omitted. If the ester behaves as a bidentate ligand the maximum value of n is three for six coordinate metal ions. The mechanism proposed for the hydrolytic reaction is:



Later workers tended to ignore possible effects of ME_2 which undoubtedly contributed to the observed rate since copper(II) iminodiacetic acid and nitrilotriacetic acid complexes catalyze ester hydrolysis (5). Kroll (1) also determined that the rates of hydrolysis were second order in hydroxide ion concentration and independent of the buffer concentration. In summary, Kroll claimed that the formation

of a coordination complex, whether or not it was stable, produces a positive center at the carbonyl carbon due to polarization by the electropositive metal ion which is more susceptible to nucleophilic attack.

The use of tris as buffer by Kroll (1) was subsequently criticized by several researchers (18, 19). White, Manning and Li (18) found that formation constants of copper(II) and nickel(II) complexes with tris and with glycine esters are of the same order as values for the corresponding metal ion complexes of ammonia. Bender and Turnquest (19) furthermore showed that the result of using buffers such as tris or glycine decreased the first order hydrolytic rate constants.

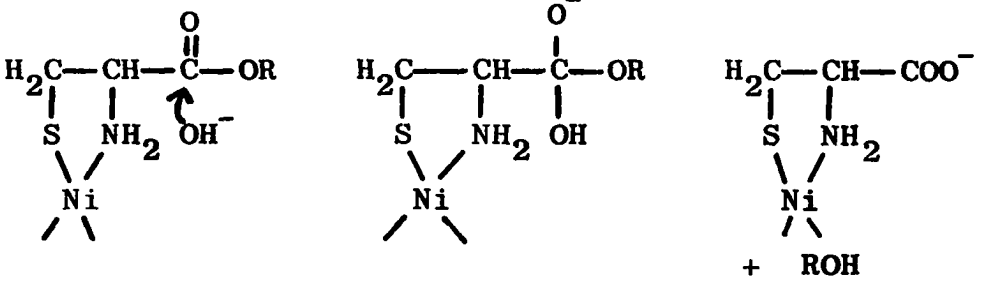
$$\text{ester} + \text{buffer-Cu(II)} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{ester-Cu(II)} \xrightarrow{k_3} \text{products}$$

$$\frac{-d(\text{ester})}{dt} = k_3(\text{ester-Cu(II)}) = k_3 K (\text{ester})(\text{buffer-Cu(II)})$$

where $K = k_1/k_2$. For the observed rate constant, k_{obsd}, to remain constant through the reaction the buffer-copper(II) complex concentration must remain constant. However, since glycine is a better complexing agent for the metal ion than either the ester or buffer, as the copper(II)-glycine complex is formed as the reaction product it decreases the concentration of catalytically active copper complex and therefore a decreasing first-order rate constant is observed as the reaction proceeds. A more serious

criticism to Kroll's interpretation was raised by Bender and Turnquest (19). They found that the back titration to pH 4.0 to determine the amount of unreacted ester did not provid the correct stoichiometry and suggested a different method of back titration than employed by Kroll (1).

These problems exemplify the difficulties in the kinetic measurements before the advent of the pH stat. Using a pH stat, the buffer can be eliminated, the reactions may be followed to completion, and the pH can be controlled more accurately. An investigation of the effect of metal ion interaction with sulfur containing amino acids (18) produced rates of alkaline hydrolysis of glycine and cysteine esters in the presence and absence of metal ions. An attempt was made to correlate formation constants of the metal-ester complexes with the second-order hydrolytic rate constants. From the formation constants with copper(II) and nickel(II) the investigators proposed a mechanism of ester hydrolysis in which the ester group was not coordinated to the metal ion.



The mechanism written for cysteine methyl ester was also postulated for ethyl glycinate. The rate of hydrolysis measured for the basic hydrolysis of cysteine methyl ester in the absence of metal ions determined by White, Manning and Li (18) probably includes zwitterionic species $^{-}SCH_2CH(NH_3^{+})COOCH_3$ as Hay, Porter, and Morris (4) reported a much lower value. The ratio of the catalyzed to uncatalyzed rate of hydrolysis for cysteine methyl ester is 31 which compares favorably with the ratio of the rate of hydrolysis of ethyl betaine ethyl ester to glycine ethyl ester of 36 (4). This gives weight to the argument that cysteine methyl ester does not coordinate via the carbonyl oxygen atom and that the inductive effect of the metal ion acts through the nitrogen atom.

Histidine methyl ester appears to be another example of an amino acid ester in which there is little carbonyl group interaction with metal ions (20) although there is still debate on this point (21). Recent x-ray crystallographic studies (22, 23) of histidine with zinc(II) show that the carboxyl group is weakly coordinated with a Zn-O distance of 2.8-2.9 Angstroms. Chakravorty and Cotton (24) measured the formation constants of nickel(II), copper(II), and zinc(II) ions with a series of ligands related to histidine and came to the conclusion that histidine carboxyl group interactions with copper(II) and nickel(II) are very

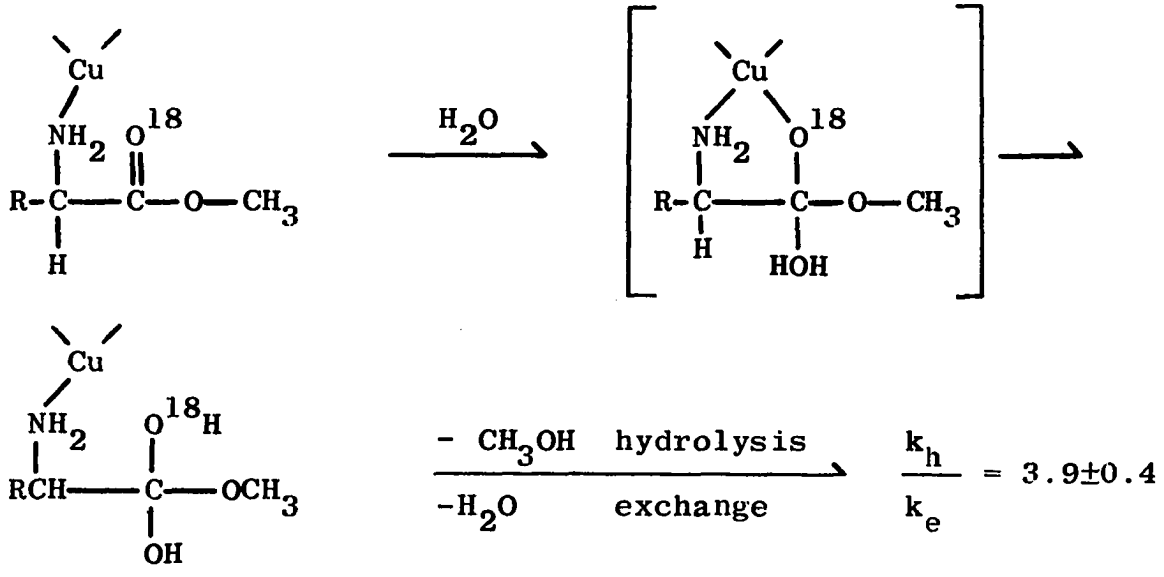
weak if present at all. Hay and Morris (25) did not consider it necessary to invoke ester carbonyl oxygen interaction to explain the degree of catalysis observed in Cu(histidine methyl ester). Angelici and Hopgood (5) also found a low catalysis constant (k_1 catalyzed/k_1) for the [Cu(NTA)(methyl histinate)]$^-$ system.

Evidence has accumulated that the mechanism of ester hydrolysis postulated for cysteine methyl ester cannot be generalized to ethyl glycinate. The ratio of k_1 catalyzed/k_1 for the copper(II) ion catalysis of ethyl glycinate is about 3200 (3). Introduction of a dipositive charge two atoms removed from the carbonyl carbon would not be expected to give catalysis rates of the magnitude observed (19, 26, 27). Such catalysis was demonstrated in the ester hydrolysis of the complex cis-$[Co(en)_2(NH_2CH_2COOC_2H_5)Cl]Cl_2$ where "en" represents ethylenediamine, which was prepared by Alexander and Busch (28). Infrared spectral studies indicated that the amino acid ester functioned as a monodentate ligand bonded through the amino nitrogen. Hydrolysis of this ester was slow (26, 27) compared to that of the complex in which both the amino group and carbonyl oxygen are coordinated cis-$[Co(en)_2(NH_2CH_2COOC_2H_5)]Cl_2$ (26). Whereas spectral data in D_2O solvent showed that cis-$[Co(en)_2(NH_2CH_2COOC_2H_5)]Cl_2$ was coordinated as a bidentate ligand it has been shown that in the solid state bis complexes of methyl and ethyl

glycinate with dihalides of platinum(II), palladium(II), copper(II), zinc(II), cobalt(II), and cadmium(II) and of DL-methyl-α-alaninate with dihalides of copper(II) and palladium (II) form only nitrogen to metal bonds by the ester molecules (29). The complex $[CuCl_2(NH_2CH_2COOC_2H_5)]$ was observed to have a carbonyl oxygen stretching frequency of 1658 $cm.^{-1}$ compared to 1754 $cm.^{-1}$ for ethyl glycinate hydrochloride. The lower carbonyl oxygen stretching frequency has been used as a positive indication of metal-carbonyl oxygen interaction. Hay and Porter (30) have studied a variety of metal ion complexes of α-amino acid esters. They found complexes in which only metal-nitrogen bonding occurs and complexes in which chelate ring formation occurs via secondary donor groups as in methyl L-histidinate, methyl γ-glutamate, and methyl L-cysteinate.

In solution, Klotz, Faller, and Urquhart (31) have concluded from the visible absorption spectrum of an aqueous solution of methyl glycinate and copper(II) that only copper-nitrogen bonds are formed. While the major species appear to be only coordinated through the amino group only a very small amount of carbonyl oxygen coordination has been postulated by most researchers (1,3,19) to explain the metal ion promoted hydrolysis of ethyl glycinate. The amino group of the ester is essential for catalysis of the ester hydrolysis. In the presence of copper(II) ethyl glycinate hydrolyzes readily in contrast to the unreactivity of ethyl acetate (19).

Bender and Turnquest (19) reported a kinetic and isotopic exchange investigation of the copper(II) ion-catalyzed hydrolysis of several α-amino acid esters. It was emphasized that the copper(II) ion catalysis occurred because of interaction of the metal ion with the reaction center, namely the ester group. They did not go as far as Kroll (1) however in postulating bidentate behavior of the ester molecule. The mechanism for ester hydrolysis and oxygen exchange is given for glycine as buffer occupying two coordination positions of copper(II) which for clarity is not shown.

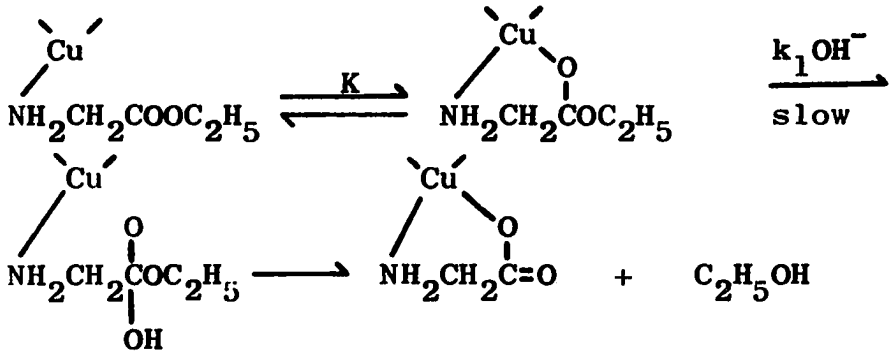


There is a large discrepancy in the values of the rate constants reported by Kroll (1) and Bender and Turnquest (19). This is probably a reflection of experimental difficulties already alluded to.

The most rigorous kinetic study of the copper(II) ion catalyzed hydrolysis of ethyl glycinate (3) made use of pH stat techniques, considered the different possible mechanistic pathways, used equilibrium and formation constants to interpret the kinetic data and corrected for changes in activity coefficients. Conley and Martin (3) concluded that some chelation of the carbonyl oxygen to copper(II) was essential and found the rate law,

$$R_{CuE} = k_{1w} (CuE^{+2}) + k_1 (CuE^{+2}) (OH^-)$$

where the subscript w refers to water as the nucleophile. It was found that the k_1 term dominated and therefore hydroxide ion should be the nucleophile in the mechanisms where water had been previously postulated as the nucleophile. The most probable mechanism is:



A similar study (15) of the copper(II) catalyzed hydrolysis of ethyl glycinate was in good agreement with that of Conley and Martin (3) if the same mechanism of hydroxide ion attack on the copper(II) ester complex is proposed. The values of the second-order hydrolytic rate

constants are as follows: Kroll (1), 10.7×10^4 M^{-1} $sec.^{-1}$, tris buffer, 25.0°, 0.16 M ionic strength; Bender and Turnquest (19), 4.2×10^3 M^{-1} $sec.^{-1}$, tris buffer, 25.0°, 0.16 M ionic strength; Bender and Turnquest (19) 8×10^3 M^{-1} $sec.^{-1}$, glycine buffer, 25.0°, 0.16 M ionic strength; Conley and Martin (3), 7.6×10^4 M^{-1} $sec.^{-1}$, pH stat, 25.0°, 0.16 M ionic strength; and Connor, Jones, and Tuleen (15), 5.9×10^4 M^{-1} $sec.^{-1}$, pH stat, 25.0°, 0.10 M ionic strength.

Connor, Jones, and Tuleen (15) first proposed that the hydroxo complex $[Cu(NH_2CH_2COOC_2H_5)(OH)]^+$ was the reactive species which subsequently was attacked by hydroxide ion to fit the observed rate law. This rate law exhibited a second-order dependence on hydroxide ion concentration. This, however, neglected the first-order hydroxide ion dependence inherent in forming the copper(II) ion-ethyl glycinate complex at pH values where the protonated ester exists in appreciable concentrations. This oversight was corrected later (32) and hydroxo-complex species were not invoked as the reactive species. Hix and Jones (32) extended the study to the metal ions cobalt(II), nickel(II), and zinc(II) and observed the order of hydrolytic rate constants copper(II)>> zinc(II)>cobalt(II)>nickel(II) and the rate law

$$\text{rate} = k_{OH}\,(ME)(OH^-) + k_{H_2O}\,(ME)(H_2O)\,.$$

The order copper(II)>>zinc(II)>cobalt(II)>nickel(II) is not predicted by the stability of the complexes but is

probably related to the oxygen coordinating ability of the metal ion which determines the unknown amount of ester which is chelated through both the carbonyl oxygen and amino nitrogen atoms.

Transition metal ions have been shown to promote the hydrolysis of α-amino acid esters. Several mechanisms have been reviewed. Most explain the promoted hydrolysis by the assumption that polarization of the carbonyl group by the metal ion makes the carbonyl carbon more susceptible to nucleophilic attack. However, most of these studies do not indicate whether coordination of the ester in fact occurs through the amino group or through the amino group and the carbonyl oxygen. There is also uncertainty as to the identity of the nucleophile. Evidence for water (19) and hydroxide (1, 3, 32) attack as well as general base catalysis (26) have been presented.

Determination of a mechanism and treatment of the kinetics have been difficult because of the many labile complexes which result when an α-amino acid ester and a metal ion are placed in aqueous solution and the pH is varied. In general, one or two ester ligands coordinate and these may form hydroxo complexes as well so that determinations of the reactive species and their concentrations is difficult. In the presence of several species it is nearly impossible to rule out contributions to the observed

rate from other than the mono-α-amino acid ester-metal ion complex.

One solution to these problems is to use relatively inert metal complexes such as those of cobalt(III) (26). Another is to simplify the aqueous system under consideration by minimizing prior equilibria. Bidentate ligands usually have second formation constants which are so large that appreciable concentrations of di-ligand-metal ion complexes exist in solution. However some tridentate ligands such as iminodiacetic acid (IMDA) and its derivatives have high first formation constants, K_1, and relatively low second formation constants, K_2 (33, 34, 35, 36, 37). Iminodiacetic acid forms

$$M^{+n} + IMDA^{-2} \underset{}{\overset{K_1}{\rightleftharpoons}} [M(IMDA)]^{n-2}$$

$$[M(IMDA)]^{n-2} + IMDA^{-2} \overset{K_2}{\rightleftharpoons} [M(IMDA)_2]^{n-4}$$

complexes with a variety of metal ions (33, 36, 37, 38) which is an advantage in that comparisons of the rate of ester hydrolysis observed are possible between the various metal ion complexes if an ester can be built into an IMDA framework. It has been possible to prepare these types of compounds and we have investigated a number of amino acid ester-N,N-diacetic acid-metal ion complexes and also metal-ion-substituted iminodiacetic acid complexes with free amino acid esters. The study reported in this thesis was

undertaken to elucidate the kinetics and mechanisms of hydrolysis of amino acid esters catalyzed by metal ion complexes. The amino acid ester derivatives and substituted iminodiacetic acids were prepared and characterized. The formation constants of a number of amino acid and amino acid esters with metal iminodiacetate complexes were determined with the use of a pH meter. The chief instrumentation was a Radiometer TTTlc titrator and SBR2c titrigraph. Rates of basic hydrolysis were measured by pH stat techniques (39) at constant temperature and ionic strength and under a nitrogen atmosphere. Reactions were followed to completion giving conclusive proof of the kinetic order of the reaction. Complete details of the experimental procedures used are given in a subsequent section of this thesis.

Ideally one would like to investigate a relatively simple model system which exhibits some of the important characteristics of enzyme-catalyzed reactions. The systems under investigation possess some of the characteristics which are essential but certainly not all of them. Such a simple model system should exhibit substantial catalytic effects which vary in a manner which can be easily correlated with changes in the substrate. The metal ion or metal complex must have available coordination positions at pH values where hydrolysis occurs. It also should not form complex hydroxo species such as iron(III) and aluminum(III)

do at low pH values. Ideally the product amino acid should form a less stable complex with the metal ion or metal-ligand complex than the reactant so that the system will be truly catalytic. In practice amino acid complexes are more stable than amino acid ester complexes so that a stoichiometric amount of metal ion is consumed. The catalysis should be the result of interaction between the ester group and the metal ion. The catalytic effects should be the result of prior complexation between the metal ion and the substrate ester. It should be possible to determine the stability of this complex in solution and to calculate the concentrations of all species. The reactivity of the free ester toward hydrolysis should be known. The stereochemical aspects should be known of both the metal or metal complex and the metal-ester complex. In practice with labile complexes the exact stereochemistry of a tridentate ligand such as substituted iminodiacetate is seldom known in detail in solution. Stereochemical aspects of both the binding and the subsequent catalysis should be readily explainable on the basis of the geometry of the catalyst and the ester substrate. One would also prefer that the mechanism be similar to those known for the analogous enzyme-catalyzed reactions.

EXPERIMENTAL

Instrumentation

Titrator and assembly

The principle instrumentation consisted of a Radiometer pH-stat with the following components: TTTlc titrator, SBR2c titrigraph, and SBUla syringe burette, all of which were manufactured by Radiometer, Copenhagen, Denmark. The combined equipment has three principal applications.

Titration curve recording The equipment will automatically record potentiometric titrations of any kind. Electrode potential as a function of the volume of titrant added is plotted. The titrant is automatically added slowly at the equivalence points where the buffering capacity is small and more rapidly where the buffering capacity is higher. Therefore titration curves may be recorded in a minimum of time without sacrificing accuracy.

pH-stat recording The titrator will maintain a preselected pH value or electrode potential by continuous control of the feed of corrective reagent. It records on the titrigraph a plot of the supply of correcting reagent added to keep the sample at constant pH vs. time. A number of different machine settings allow one to regulate the rate of addition of reagent within wide limits. The operating

technique and the use of the various controls are fully explained in the instruction manual (40).

pH meter The measuring accuracy, which of course depends on the conditions of the electrodes and the buffer solutions used in the standardization of the titrator, is 0.02 pH under optimum conditions. More accurate reading of the pH scale which is calibrated in units of 0.1 pH is possible with the incorporation of the PHA 630T scale expander into the instrumentation. A meter reading accuracy of 0.003 pH is provided by the scale expander, however, the measuring accuracy of the whole instrumentation is essentially determined by the condition of the electrodes and the accuracy of the buffers used in the pH standardization. The glass electrode may be tested by the use of several different buffer solutions spanning a range of pH values. A poor glass electrode generally will not give consistent pH readings over a range of several pH units. An open liquid junction between electrode liquid and sample provided by the type K101 calomel electrode eliminated the problem of clogging of the porous plug by metal ion complexes and lanthanide ion complexes in particular which occurs with calomel electrodes containing a porous plug. The general condition of the electronic components of the titrator and the probable cause of loss of sensitivity may be readily determined using the tests given in the instruction manual (40).

For pH-stat recording the pH is reproducible to approximately 0.02 pH unit which means that the rate constants determined are reproducible to approximately 10 percent.

Nuclear magnetic resonance

Nmr spectra were obtained on a Varian Associates Model A-60 spectrometer in D_2O using sodium 2,2-dimethyl-2-silapentane-5-sulfonate as internal standard (chemical shift, δ, ppm $\equiv$ 0.0). All chemical shifts are given in ppm downfield from the standard.

Infrared spectra

Infrared spectra obtained in 99.5 % D_2O were made in 0.10 mm cells with Irtran-2 windows using a Beckman IR-12 grating spectrophotometer. Infrared spectra of solids obtained in a KBr pellet were obtained using a Perkin-Elmer Model 21 double beam infrared spectrophotometer.

Visible spectra

Visible spectra were obtained using 1 cm. quartz cells on a Beckman DB-G grating spectrophotometer.

Materials

Water

Water doubly distilled in a glass still was used in the preparation of all aqueous solutions. The conductance of this water was 5×10^4 mho.

Buffer solutions

The titrator was standardized with the following buffer solutions supplied by Matheson, Coleman and Bell: pH 4.00 ± 0.01, pH 7.00 ± 0.01 and pH 10.00 ± 0.01 at 25.0° C. 0.01 M borax (pH 9.177 at 25.0° C) was also used to standardize the titrator.

Metal ion solutions

Analytical grade $Cu(NO_3)_2 \cdot 3H_2O$, $CoCl_2 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, $Pb(NO_3)_2$, $ZnCl_2$, $NiCl_2 \cdot 6H_2O$, $Fe(NO_3)_2$, $CdCl_2 \cdot 2\ 1/2H_2O$, $Al(NO_3)_3 \cdot 9H_2O$, $UO_2(NO_3)_2 \cdot 6H_2O$, $VOCl_2$, $AgNO_3$, $Hg(NO_3)_2$, $Fe(NO_3)_3 \cdot 9H_2O$, $MgCl_2 \cdot 6H_2O$, $Sc(NO_3)_3$, $La(NO_3)_3 \cdot 6H_2O$, and $SnCl_2 \cdot 2H_2O$ were used to prepare metal ion solutions. Solutions of the nitrates of neodymium(III), samarium(III), gadolinium(III), dysprosium(III), erbium(III), ytterbium(III), and lutetium(III) were gifts of Professor Jack Powell, Ames Laboratory, Iowa State University. HfI_4 was a gift of Professor John Corbett, Ames Laboratory, Iowa State University.

The concentration of copper(II) in standard $Cu(NO_3)_2$ solutions was determined by thiosulfate titration of the iodine liberated from the reaction of KI with copper(II). Lanthanide nitrate solutions were standardized by titration with disodium ethylenediaminetetraacetic acid, Na_2H_2EDTA, using xylenol orange indicator (41, 42). The pH of the metal ion solutions is controlled near pH 5 using an acetic acid

buffer solution. The metal ion solution was added to the buffer solution and several drops of indicator and 10 drops of pyridine were added. The solution was pink or purple color and as Na_2H_2EDTA was added the color changed to yellow at the end point. Ten more drops of pyridine were added and if the color changed, the solution was again titrated to the endpoint. The total amount of Na_2H_2EDTA consumed was used to calculate the concentration of the lanthanide solutions.

Nickel(II) metal ion solutions were standardized by ethylenediaminetetraacetic acid, EDTA, titration using naphthyl azoxime S (NAS) as indicator (43). Other salts were weighed without further standardization.

Iminodiacetic acid derivatives

Iminodiacetic acid (IMDA), methyliminodiacetic acid (MeIMDA), thiodiglycolic acid and nitrilotriacetic acid (NTA) were supplied by the Aldrich Chemical Company. [(Hexahydro-2,4,6-trioxo-5-pyrimidinyl)-imino] diacetic acid was supplied by Eastman Organic Chemicals.

Amino acids and esters

DL-valine, L-valine, D-valine, DL-alanine, D-leucine, L-leucine, sarcosine hydrochloride, N-benzoylglycine methyl ester, DL-alanine methyl ester hydrochloride, L-leucine ethyl ester hydrochloride, glycine methyl ester hydrochloride, glycine, and DL-alanine ethyl ester hydrochloride were

obtained from Mann Research Laboratories. Glycine butyl ester hydrochloride, glycine ethyl ester hydrochloride, and betaine hydrochloride were supplied by Eastman Organic Chemicals, β-alanine, 4-aminobutyric acid, and D-(-)-α-phenylglycine by Aldrich Chemical Company.

Preparation and Characterization of Compounds

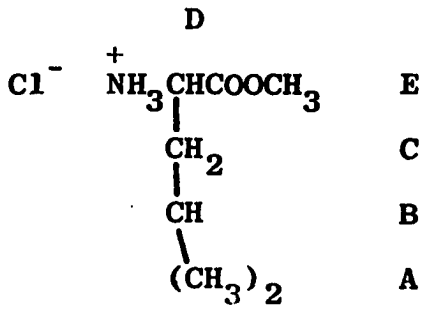
Amino acid esters

Amino acid esters were prepared from the parent amino acid by bubbling HCl gas through an alcohol-amino acid solution according to published techniques (44). Nmr spectra of the esters were obtained in 99.5 % D_2O solutions at a pD of 5-6. A more valid measure of the acidity of the sample solutions was obtained by making use of an equation given by Mikkelson and Nielson (45):

pD = meter reading + 0.44.

Chemical shifts (δ, ppm) are given for the respective alphabetical assignments; the relative intensities as determined by integration are also listed.

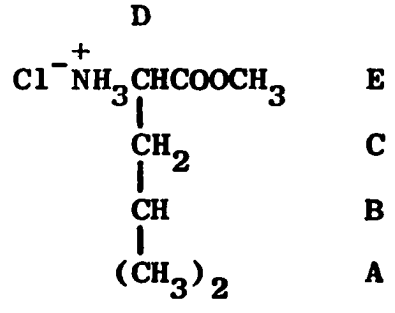
L-leucine methyl ester hydrochloride Dry hydrogen chloride was passed for two hours with continuous stirring into a mixture containing 15 g. of L-leucine suspended in 200 ml. of absolute methanol. The solution was evaporated under reduced pressure and the resulting crystals filtered, recrystallized from 1:5 methanol:diisopropyl ether, and dried under vacuum. The nmr spectrum of



is given below:

δ (ppm)	Relative intensity	Multiplicity	Assignment
0.95	6	doublet	A
1.7-1.9		multiplet	B
~3.85	4	multiplet	C
~3.85		triplet	D
1.8	3	singlet	E

D-leucine methyl ester hydrochloride Dry hydrogen chloride was bubbled for two hours into a suspension of 10 g. D-leucine and 200 ml. of absolute methanol. The solution was evaporated under reduced pressure and the crystals filtered, recrystallized by cooling a hot 1:5 methanol: diisopropyl ether solution of the initial product, and dried under vacuum. The nmr spectrum of



is given below:

δ(ppm)	Relative intensity	Multiplicity	Assignment
0.95	6	doublet	A
1.7-1.9		multiplet	B
~3.8	4	multiplet	C
~3.8		triplet	D
1.8	3	singlet	E

β-alanine ethyl ester hydrochloride Dry hydrogen chloride was bubbled for two hours into a suspension of 15 g. of β-alanine and 200 ml. absolute ethanol. Water produced in the esterification was removed as the benzene-ethanol-water azeotrope at 64.6° C and atmospheric pressure. The alcohol-ester solution volume was reduced to approximately 75 ml. under partial vacuum and ether was added until a precipitate of the amino acid ester hydrochloride formed. Recrystallization was from an ethanol-ether mixture. The procedures for the preparation of the remaining esters are the same except as indicated. The nmr spectrum of

$$Cl^- \overset{+}{N}H_3CH_2CH_2COOCH_2CH_3$$

A B C D

is given below:

δ(ppm)	Relative intensity	Multiplicity	Assignment
2.83	2	triplet	A
3.30	2	triplet	B

4.21	2	quartet	C
1.25	3	triplet	D

Sarcosine ethyl ester hydrochloride The nmr spectrum of

$$Cl^- \overset{+}{N}H_2(CH_3)CH_2COOCH_2CH_3$$

A B C D

was

δ(ppm)	Relative intensity	Multiplicity	Assignment
2.82	3	singlet	A
3.98	2	singlet	B
4.30	2	quartet	C
1.30	3	triplet	D

Ethyl 4-aminobutyrate hydrochloride This ester was used as a reactant in the preparation of ethyl 4-aminobutyrate-N,N-diacetic acid. The ester was very hygroscopic and attempts to crystallize the oily product were unsuccessful.

Betaine ethyl ester hydrochloride The nmr spectrum of

$$Cl^- \ (CH_3)_3\overset{+}{N}CH_2COOCH_2CH_3$$

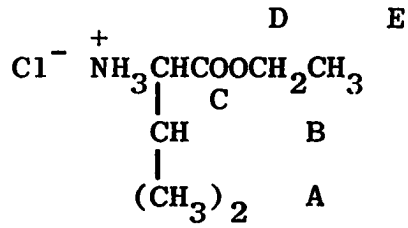
A B C D

was

δ(ppm)	Relative intensity	Multiplicity	Assignment
3.35	9	singlet	A
4.33	2	singlet	B
4.32	2	quartet	C
1.30	3	triplet	D

DL-ethyl valinate and L-ethyl valinate hydrochloride

Dry hydrogen chloride was bubbled into a suspension of the appropriate valine and absolute ethanol for three hours due to the slower rate of esterification of valine. The L isomer crystallized slowly in a refrigerator over a period of two weeks after repeated washing of the oil with ether. The nmr spectra were identical and the spectra of the L isomer is given.



δ(ppm)	Relative intensity	Multiplicity	Assignment
1.05	6	doublet	A
2.32	1	multiplet	B
4.01	1	doublet	C
4.33	2	quartet	D
1.31	3	triplet	E

Amino acid ester-N,N-diacetic acids

Amino acid ester-N,N-diacetic acids were prepared from the parent amino acid ester and reagent grade haloacetic acid in basic solution. Spectral data were used to establish the identity of the compounds. Nmr spectra were sometimes difficult to obtain because of the low solubility of these compounds in water and their insolubility in organic solvents such as dimethylsulfoxide, dimethylformamide, and

acetonitrile. Chemical shifts [δ(ppm)] are given for the respective alphabetical assignments.

Ethyl glycinate-N,N-diacetic acid A 46.5 g. portion (0.25 mole) of iodoacetic acid was dissolved in a minimum amount (~15 ml.) of H_2O and chilled in an ice bath. A solution of 7 N NaOH was added slowly with stirring. The temperature of the solution was kept below 30 ° C to prevent the formation of glycolic acid, $HOCH_2COOH$. When the neutralization was complete, a solution of 17.6 g. of glycine ethyl ester hydrochloride (0.125 mole) was added slowly. The pH was maintained at approximately 9 during the addition of the ester by introduction of NaOH. After stirring the solution for 30 minutes at room temperature, the solution was heated to near boiling and 31.8 g. of $BaCl_2 \cdot 2H_2O$ (0.26 mole) was added to precipitate the product. The precipitate was filtered, washed with hot water, and dried under vacuum.

For identification by nmr it was necessary to convert the barium salt which was only slightly soluble in water into the sodium salt. This was accomplished by adding Na_2SO_4, filtering the $BaSO_4$, and evaporating the resulting solution to dryness at room temperature under vacuum. The nmr spectrum taken in D_2O of

$$\underset{\text{A}}{CH_3}\underset{\text{B}}{CH_2}\underset{\text{C}}{OOCCH_2N(CH_2COO)_2}^{-2}\ \underset{\text{D}}{[Na^+]_2}$$

was:

δ(ppm)	Relative intensity	Multiplicity	Assignment
1.28	3	triplet	A
4.30	2	quartet	B
3.86	2	singlet	C
3.78	4	singlet	D
3.47			NTA

The nmr spectrum clearly identifies ethyl glycinate-N, N-diacetic acid (EGDA) but a weak singlet at δ3.47 indicates a small amount of NTA. Integration showed that 9 % of the sample was NTA; the remaining 89 % of the sample undergoes ester hydrolysis. The presence of some NTA in the sample is not unexpected since at the pH 9 of preparation, EGDA was observed to undergo slow hydrolysis to NTA.

On the basis of the nmr results, the elemental composition was calculated for a mixture of 90 mole % ($C_2H_5OOCCH_2N(CH_2COO)_2$ $Ba \cdot H_2O$) and 10 mole % ($HN(CH_2COO)_3$ $Ba \cdot H_2O$). Analysis: Calculated: C, 25.40; H, 3.42; N, 3.80; Ba, 37.13. Found: C, 25.45; H, 3.93; N, 3.99; Ba, 36.70. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, New York.

The infrared spectrum of the mixture in a KBr pellet showed characteristic absorptions at 3440 (H_2O), 2960 (C-H), 1740 (ester carbonyl), 1570 and 1408 (salt of carboxylic acid), and 1210 and 1142 $cm.^{-1}$ (ester).

Butyl glycinate-N,N-diacetic acid The preparation was analogous to that of EGDA with the substitution of butyl glycinate hydrochloride for ethyl glycinate hydrochloride. The barium salt of butyl glycinate-N,N-diacetic acid (BGDA) was isolated. Lanthanum nitrate was added to a D_2O solution of BGDA to form the more soluble lanthanum complex. An nmr spectrum of

$$[CH_3CH_2CH_2CH_2OOCCH_2N(CH_2COO)_2La]^+$$
$$\quad A \quad B \quad C \qquad D \quad E$$

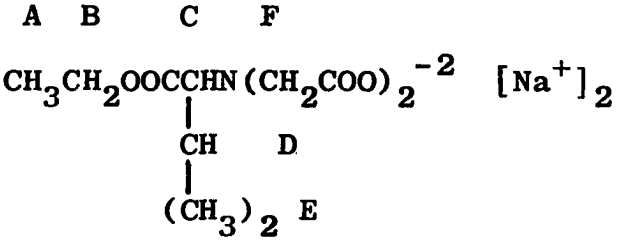
gave the following absorptions:

δ(ppm)	Relative intensity	Multiplicity	Assignment
0.90	3	triplet	A
1.3-1.7	4	multiplet	B
4.30	2	triplet	C
3.82	2	singlet	D
3.55	4	singlet	E
3.47		singlet	NTA

Ethyl valinate-N,N-diacetic acid Ethyl valinate-N,N-diacetic acid (EVDA) was prepared by the reaction of ethyl valinate with iodoacetic acid. The procedure was the same as for ethyl glycinate-N,N-diacetic acid except that the reaction was carried out at pH 10 and at 40-50° C. The unreactivity of the valine ester was very useful in that the barium salt was isolated free of any triacetate contaminant. The elemental composition was calculated on the

basis of $C_2H_5OOCCH(CH(CH_3)_2)N(CH_2COO)_2Ba \cdot H_2O$. Analysis: Calculated: C, 31.90; H, 4.59; N, 3.38. Found: C, 32.00; H, 4.33; N, 3.69.

The barium salt of EVDA was converted to the more soluble sodium salt by stirring with Na_2SO_4 and filtering off the insoluble $BaSO_4$. The resulting solution was evaporated to dryness and an nmr spectrum of

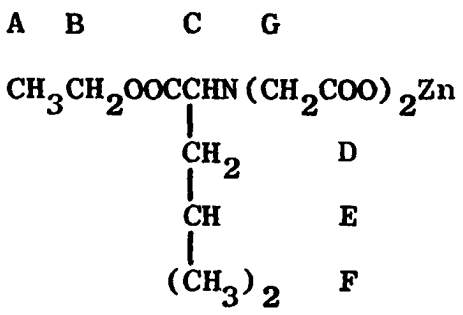


in D_2O was obtained.

δ(ppm)	Relative intensity	Multiplicity	Assignment
1.27	3	triplet	A
4.19	2	quartet	B
3.2	1	multiplet	C
2.0	1	multiplet	D
0.94	6	doublet	E
3.2	4	singlet	F

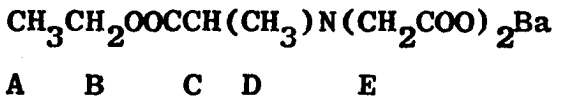
The infrared spectrum of the barium salt of EVDA obtained in a KBr pellet showed characteristic absorptions at 3390 (H_2O), 2900 (C-H), 1725 (ester carbonyl), 1560 and 1405 (salt of carboxylic acid), 1325 (C-H), 1200 and 1142 cm^{-1} (ester).

Ethyl leucinate-N,N-diacetic acid Ethyl leucinate-N,N-diacetic acid (ELDA) was prepared from ethyl leucinate in a manner entirely analogous to that reported for EVDA. To improve the solubility of the barium salt of ELDA it was dissolved in D_2O containing zinc(II) ions. The spectrum of



δ(ppm)	Relative intensity	Multiplicity	Assignment
1.23	3	triplet	A
4.28	2	quartet	B
3.5	1	multiplet	C
3.3	2	multiplet	D
1.6	1	multiplet	E
0.88	6	doublet	F
3.64	4	singlet	G

Ethyl alaninate-N,N-diacetic acid Ethyl alaninate-N,N-diacetic acid (EADA) was prepared analogously to EGDA using ethyl α-alaninate in place of ethyl glycinate. The barium salt was isolated and was soluble enough to obtain weak nmr signals in D_2O.



δ(ppm)	Relative intensity	Multiplicity	Assignment
1.23	3	triplet	A
4.24	2	quartet	B
3.56	1	quartet	C
1.40	3	doublet	D
3.38	4	singlet	E

Ethyl β-alaninate-N,N-diacetic acid Ethyl β-alaninate-N,N-diacetic acid (EβADA) was prepared using ethyl β-alaninate as the parent ester. The procedure for preparation was analogous to that for EGDA. The barium salt was isolated and an nmr spectrum was taken in a D_2O solution containing zinc(II).

$$CH_3CH_2OOCCH_2CH_2N(CH_2COO)_2Zn$$

A B C D E

δ(ppm)	Relative intensity	Multiplicity	Assignment
1.25	3	triplet	A
4.24	2	quartet	B
3.08	2	triplet	C
2.79	2	triplet	D
3.34	4	singlet	E

The infrared spectrum of the barium salt of EβADA obtained in a KBr pellet showed characteristic absorptions at 3450 (H_2O), 2970 (C-H), 1780 (ester carbonyl), 1565 and 1405 (salt of carboxylic acid) and 1185 and 1121 cm^{-1} (ester).

Ethyl 4-aminobutyrate-N,N-diacetic acid Ethyl 4-aminobutyrate was obtained as a crude oily mixture which resisted attempts at crystallization. The crude ester was dissolved in H_2O and reacted with iodoacetic acid in the same manner as described for ethyl glycinate. The barium salt was filtered and dried under vacuum. The barium salt was then converted to the sodium salt by addition of Na_2SO_4 and precipitation of the $BaSO_4$. The nmr of the sodium salt

$$\underset{A\ \ B}{CH_3CH_2}OOC\underset{C\ \ D}{CH_2CH_2}\underset{E}{CH_2}N(\underset{F}{CH_2}COO)_2^{-2}\ [Na^+]_2$$

was determined.

δ(ppm)	Relative intensity	Multiplicity	Assignment
1.25	3	triplet	A
4.17	2	quartet	B
3.22	2	triplet	C
2.0	2	multiplet	D
2.50	2	triplet	E
3.74	4	singlet	F

The infrared spectrum of the barium salt of ethyl 4-aminobutyrate was obtained in a KBr pellet and showed characteristic absorptions at 3400 (H_2O), 2930 (C-H), 1735 (ester carbonyl), 1562 and 1402 (salt of carboxylic acid), 1181 and 1117 $cm.^{-1}$(ester).

Substituted iminodiacetic acids

A number of substituted iminodiacetic acids were prepared from reagent grade primary amines by reaction with reagent grade bromoacetic acid or from amino acids by reaction with bromoacetic acid in basic solution. These compounds were characterized by their nmr spectra and elemental analysis.

Tertiary-butyliminodiacetic acid 38.9 g. (0.28 mole) of bromoacetic acid was dissolved in 15 ml. of H_2O and chilled in an ice bath. A solution of 7 N KOH was added slowly with stirring to neutralize the acid. The temperature was kept below 30° C to minimize the side reaction leading to glycolic acid, $HOCH_2COOH$. After neutralization was complete the remainder (80 ml. 7 N KOH total) of the base was added and an aqueous solution containing about 20 ml. of H_2O and 14.7 ml. of tertiary-butyl amine (0.14 mole) was added over a period of at least 30 minutes. The mixture was allowed to stir two hours at room temperature to complete the reaction and then the iminodiacetate was precipitated as the barium salt with $BaCl_2 \cdot 2\ H_2O$. It was filtered, washed with ethanol and hot water, and dried in a vacuum dessicator. A solution of the barium salt in D_2O containing zinc(II) ion was used to obtain the nmr spectrum of

$$\underset{A}{(CH_3)_3}\underset{B}{CN(CH_2COO)_2Zn}$$

δ(ppm)	Relative intensity	Multiplicity	Assignment
1.48	9	singlet	A
4.25	4	singlet	B

The elemental composition was calculated for $(CH_3)_3CN-(CH_2COO)_2Ba$. Analysis: Calculated: C, 29.60; H, 4.02; N, 4.32. Found: C, 29.52; H, 3.89; N, 4.34.

Cyclohexyliminodiacetic acid 17.0 ml. of cyclohexylamine (0.14 mole) was used to prepare cyclohexyliminodiacetic acid. The procedure was the same as described for tertiarybutyliminodiacetic acid. Zinc(II) ion was added to a D_2O solution of the barium salt to obtain the nmr spectrum of

$$\underset{A}{C_6H_{11}}\underset{B}{N(CH_2COO)_2Zn}$$

δ(ppm)	Relative intensity	Multiplicity	Assignment
1.0-2.1	11	multiplet	A
4.25	4	singlet	B

No attempt was made to resolve the peaks in the spectrum due to the cyclohexyl ring hydrogens due to the low intensity of the nmr signals of these slightly soluble compounds.

The elemental composition based on $C_6H_{11}N(CH_2COO)_2Ba$ was calculated. Analysis: Calculated: C, 34.15; H, 4.28; N, 4.00. Found: C, 34.22; H, 4.33; N, 3.73.

Phenyliminodiacetic acid Phenyliminodiacetic acid was prepared analogously to cyclohexyl and tert-butyliminodiacetic acids with the amine in this case being aniline (12.8 ml., 0.14 mole). Aniline is nearly insoluble in H_2O but as the reaction proceeded the aniline layer was observed to disappear. The nmr spectrum of the zinc(II) complex

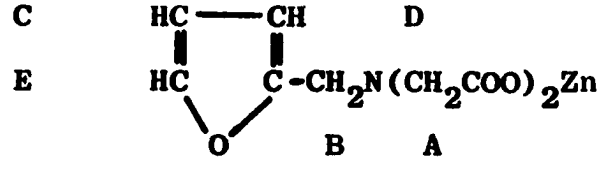
$$\underset{A}{C_6H_5N}\underset{B}{(CH_2COO)_2Zn}$$

formed when zinc(II) was added to a D_2O solution of the barium salt was

δ(ppm)	Relative intensity	Multiplicity	Assignment
6.8-7.6	5	multiplet	A
4.37	4	singlet	B

The elemental composition was calculated on the basis of $C_6H_5N(CH_2COO)_2Ba$. Analysis: Calculated: C, 34.88; H, 2.62; N, 4.07. Found: C, 35.29; H, 2.73; N, 4.32.

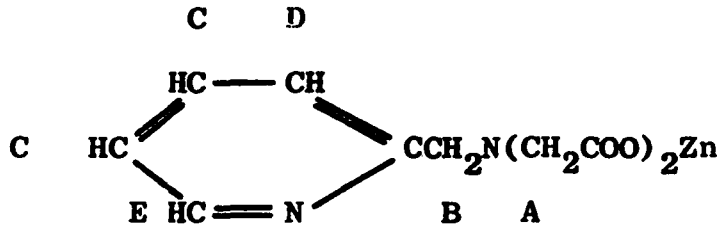
Furfuryliminodiacetic acid Furfuryliminodiacetic acid was prepared analogously to the previous three diacetic acids using 13.6 ml. of furfurylamine (0.14 mole) as the amine. The barium salt was isolated and dissolved in D_2O containing zinc(II) to form the zinc(II) complex



δ(ppm)	Relative intensity	Multiplicity	Assignment
4.28	4	singlet	A
4.72	2	singlet	B
6.60	1	multiplet	C
6.85	1	doublet	D
7.70	1	doublet	E

The elemental composition was calculated on the basis of $C_5H_5ON(CH_2COO)_2Ba \cdot 1/2H_2O$. Analysis: Calculated: C, 30.25; H, 3.00; N, 3.92. Found: C, 30.13; H, 3.15; N, 4.23.

2-picolyliminodiacetic acid 15.1 g. of 2-picolylamine (0.14 mole) was used as the amine in the preparation of 2-picolyliminodiacetic acid. The nmr spectrum of the zinc(II) complex



is given below:

δ(ppm)	Relative intensity	Multiplicity	Assignment
3.74	4	singlet	A
4.43	2	singlet	B
6.2-6.3	2	multiplet	C
6.7-6.8	1	multiplet	D
7.1	1	multiplet	E

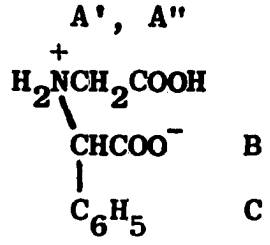
The solubility of these compounds again limits the resolution of absorption peaks with low relative intensity.

The elemental composition was calculated on the basis of $C_5H_4NCH_2N(CH_2COO)_2Ba \cdot 2H_2O$. Analysis: Calculated: C, 30.40; H, 3.54; N, 6.84. Found: C, 29.78; H, 3.48; N, 7.05.

D-(-)-α-phenylglycine-N-monoacetic acid Phenylglycine-N-monoacetic acid was prepared by the reaction in basic solution of bromoacetic acid and phenylglycine. 13.9 g. of bromoacetic acid (0.10 mole) was dissolved in a small amount of H_2O and cooled in an ice bath. A solution of KOH containing 0.30 mole of KOH in approximately 50 ml. of H_2O was slowly added to neutralize the bromoacetic acid. The temperature was kept below 30° C until the neutralization was complete. The phenylglycine (15.1 g., 0.10 mole) was dissolved in about 30 ml. of H_2O and neutralized with the KOH solution. The neutralized bromoacetic acid was then added dropwise to the neutralized phenylglycine solution with addition of KOH to keep the pH at a value near 11. The temperature of the solution was kept near 50° C and the reaction was complete in approximately two hours. The volume of the resulting solution was reduced under vacuum until a precipitate formed. The solution was acidified with HCl, the precipitate filtered, and the solution volume reduced further until another precipitate formed. It was found that the initial precipitates contained a compound or compounds which gave no nmr signal and therefore the initial precipitate was assumed to be KBr. Bromine was liberated from the initial precipitate

when several drops of concentrated sulfuric acid were added which is consistent with the compound being a bromide salt. The final precipitate yielded the desired compound which was subsequently washed with water to remove traces of salt.

Phenylglycine-N-monoacetic acid

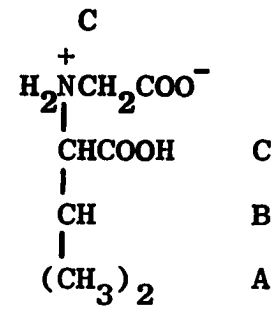


gave the spectrum:

δ (ppm)	Relative intensity	Multiplicity	Assignment
3.08	1	singlet	A' or A"
3.16	1	singlet	A" or A'
4.48	1	singlet	B
7.39	5	singlet	C

The elemental composition was calculated on the basis of $HOOCCH(C_6H_5)N(H)(CH_2COOH)\cdot H_2O$. Analysis: Calculated: C, 53.00; H, 5.75; N, 6.16. Found: C, 54.06; H, 5.25; N, 5.64.

L-valine-N-monoacetic acid L-valine-N-monoacetic acid was prepared analogously to phenylglycine-N-monoacetic acid using 11.7 g. of L-valine (0.10 mole) as the starting amino acid. The nmr spectrum of



was

δ(ppm)	Relative intensity	Multiplicity	Assignment
1.10	6	doublet	A
~2.3	1	multiplet	B
~4.0	3	multiplet	C

The elemental composition was calculated on the basis of $HOOCCH_2N(H)CH(CH(CH_3)_2)COOH$. Analysis: Calculated: C, 48.00; H, 7.43; N, 8.01. Found: C, 48.14, H, 7.60; N, 8.23.

Equilibrium Measurements

Potentiometric measurements were made by using the following Radiometer equipment: TTTlc titrator, P·HA 630T scale expander, and SBUla syringe burette. The electrode system consisted of a G2222C glass electrode and a calomel electrode K101/3.

PK_E values for the esters ethyl glycinate-N,N-diacetic acid, sarcosine ethyl ester, D-leucine methyl ester, L-leucine

$$HE^+ \overset{K_E}{\rightleftharpoons} H^+ + E$$

methyl ester, and alanine methyl ester were determined by manual titration of a 10 ml. aqueous solution containing 0.0067 M ester and 0.050 M KNO_3 with 0.206 M NaOH solution. The pH of the solution was measured, using the scale expander, after each addition of an aliquot of NaOH solution. The formation constants of hydroxo-species of the [M(IMDA)] complexes (0.0067 M) were also determined in this way for

$$M(IMDA) + OH^- \rightleftharpoons^{K_{fOH}} [M(IMDA)(OH)]^-$$

a number of substituted iminodiacetic acids.

The formation constants of ethyl valinate and valine with a series of copper(II) substituted iminodiacetic acid complexes were determined by manual titration. The rates of hydrolysis of ethyl valinate were generally small in the pH range of most of the titrations. Other amino acid esters hydrolyzed too rapidly in the presence of metal-iminodiacetic acid complexes for the results to be meaningful. The concentration of materials in the 10 ml. aqueous solution were 3.0 ml. of a solution of 0.0167 M MCl_2 or $M(NO_3)_2$ and 0.0167 M IMDA, 3.0 ml. of 0.0167 M amino acid or ester, 2.0 ml. of 0.20 M KNO_3 and 2.0 ml. of H_2O. These solutions were titrated with 0.206 M NaOH. Corrections were made for volume increases during the titrations, all of which were carried out under an atmosphere of nitrogen at 25.0° C.

The activity coefficients, $\gamma\pm$, of hydrogen and hydroxide ions were estimated from the Davies equation (46).

$$-\log \gamma_{\pm} = 0.50\ Z_1 Z_2 I^{1/2}/(1 + I^{1/2}) - 0.1\ I$$

This equation is in excellent agreement with the experimental data up to an ionic strength, I, of 0.1 M regardless of the nature of the electrolyte. Hence hydrogen and hydroxide ion concentrations were calculated by using the following relationships:

$$\log [H^+] = - pH - \log \gamma_{\pm}$$

$$\log [OH^-] = pH - pK_w - \log \gamma_{\pm}$$

where K_w is the autoionization constant of water.

Formation constants for ethyl valinate-N,N-diacetate ($EVDA^{-2}$) with metal ions were determined by measuring the pH of partially-neutralized ester-metal ion solutions

$$M^{+2} + EVDA^{-2} \overset{K_1}{\rightleftharpoons} M(EVDA)$$

$$M(EVDA) + EVDA^{-2} \overset{K_2}{\rightleftharpoons} [M(EVDA)_2]^{-2}$$

ranging in concentration from metal:ester of 1:1 to 1:8. The concentration of metal ion used was 0.002 M. Total volume of the sample solutions was 10.0 ml. and the ionic strength was maintained at 0.05 M by the addition of KNO_3 solution. Estimates of the first and second formation constants were determined by conventional computer techniques (47) using a program developed by Stagg and Powell (48). Estimates of the first formation constant, K_1, have an uncertainty of 10 % whereas the second formation constants have an uncertainty of about 25 % due to the approximations made to the rigorous experimental procedures employed by Powell and Rowlands (49).

Kinetic Measurements

Rates of reaction were determined with a Radiometer TTTlc titrator and SBR2c titrigraph. The titrigraph plots per cent volume of an SBUla syringe burette which was determined to deliver 0.497 ml. of solution with 50 turns of the syringe burette micrometer. This volume was determined by weighing the water delivered by the burette when the micrometer screw was manually advanced 50 turns. The titrator was set for pH-stat recording and the pH was maintained at the desired value by the addition of 0.0187 N NaOH to correct for the acid liberated in the ester hydrolysis reaction. The standard 10 ml. Radiometer thermostated reaction vessel was

$$H_3\overset{+}{N}CHRCOOR' + H_2O \longrightarrow H_3\overset{+}{N}CHRCOO^- + R'OH + H^+$$

maintained at 25.0 ± 0.05° by circulating water through the outer jacket, and nitrogen which was saturated with water by bubbling through a water trap was bubbled into the reaction vessel to exclude air. The temperature of the constant temperature water bath was varied to obtain data on the enthalpy and entropy changes associated with certain equilibrium constants and rates of ester hydrolysis. Temperatures were maintained at ± 0.05 degrees of the temperature listed by the use of pre-set thermoregulators. Correction was made for the change in K_w with temperature in the calculation of hydroxide ion concentrations from the relationship

$$\log [OH^-] = pH - pK_w - \log \gamma_{\pm}$$

using the following values of K_w (38): 14.730, 5° C; 14.533, 10°; 14.345, 15°; 14.167, 20°; 13.996, 25°; 13.832, 30°; 13.682, 35°; 13.536, 40°; 13.396, 45°C.

For the amino acid ester-N,N-diacetic acids the composition of the reaction vessel for each kinetic run was 1.0 ml. of 0.0067 M MCl_2 or $M(NO_3)_2$, 1.0 ml. of 0.0067 amino acid ester-N,N-diacetic acid, 2.5 ml. of 0.20 M KNO_3 and 5.5 ml. of H_2O or dilute acid or base to adjust the pH to near the desired value. For the metal ion iminodiacetic acid complexes with amino acid esters the composition of the reaction vessel for each kinetic run was 2.0 ml. of 0.0167 M MCl_2 or $M(NO_3)_2$, 2.0 ml. of 0.0175 M IMDA, 2.5 ml. of 0.2 M KNO_3, 1.0 ml. of 0.0033 M amino acid ester, and 2.5 ml. of H_2O or dilute acid or base to adjust the pH to near the desired value. A slight excess of the iminodiacetic acid was added to insure that no free metal ion would be available to catalyze the ester hydrolysis.

The contents of the reaction vessel (with the exception of the ester solution) were thermostated for 15 minutes and the ester solution was then added and the pH raised to the desired value and the reaction followed automatically by the addition of NaOH solution to maintain the pre-selected pH. One thus obtains a plot of the per cent of the total syringe capacity of NaOH solution delivered vs. time on the titrigraph. The plot obtained for the hydrolysis of ethyl

glycinate-N,N-diacetic acid in the presence of samarium(III) ion at pH 6.3 is depicted in Figure 1.

Since the per cent at the end of the reaction (% end) minus the per cent at any time t (% t) is proportional to the concentration of unreacted ester, the slope of first-order plots of ln (% end - % t) *vs.* time, which are linear to at least 85 % completion of reaction, yielded pseudo-first-order rate constants, k_{obsd}. A plot of ln (% end - % t) *vs.* time for the reaction described by Figure 1 is depicted in Figure 2. Rate constants were almost always reproducible within ± 10 %.

A general nonlinear least-squares computer program (50) was used to calculate the second-order rate constant, k, from the k_{obsd} and pH (i.e., - log $[a_{H+}]$) data. The computer program also calculated rate constants for the individual pathways of a reaction mechanism given the overall rate constant and the experimental rate law.

The slope of plots of ln (% end - % t) *vs.* time yields k_{obsd} values from which the second-order rate constant, k, is obtained in activity units. All rate constants have been converted to molarity units by dividing the k_{obsd} values by the hydroxide ion concentration which was calculated from the expression:

$$\log [OH^-] = \log K_w + pH - \log \gamma_{\pm}$$

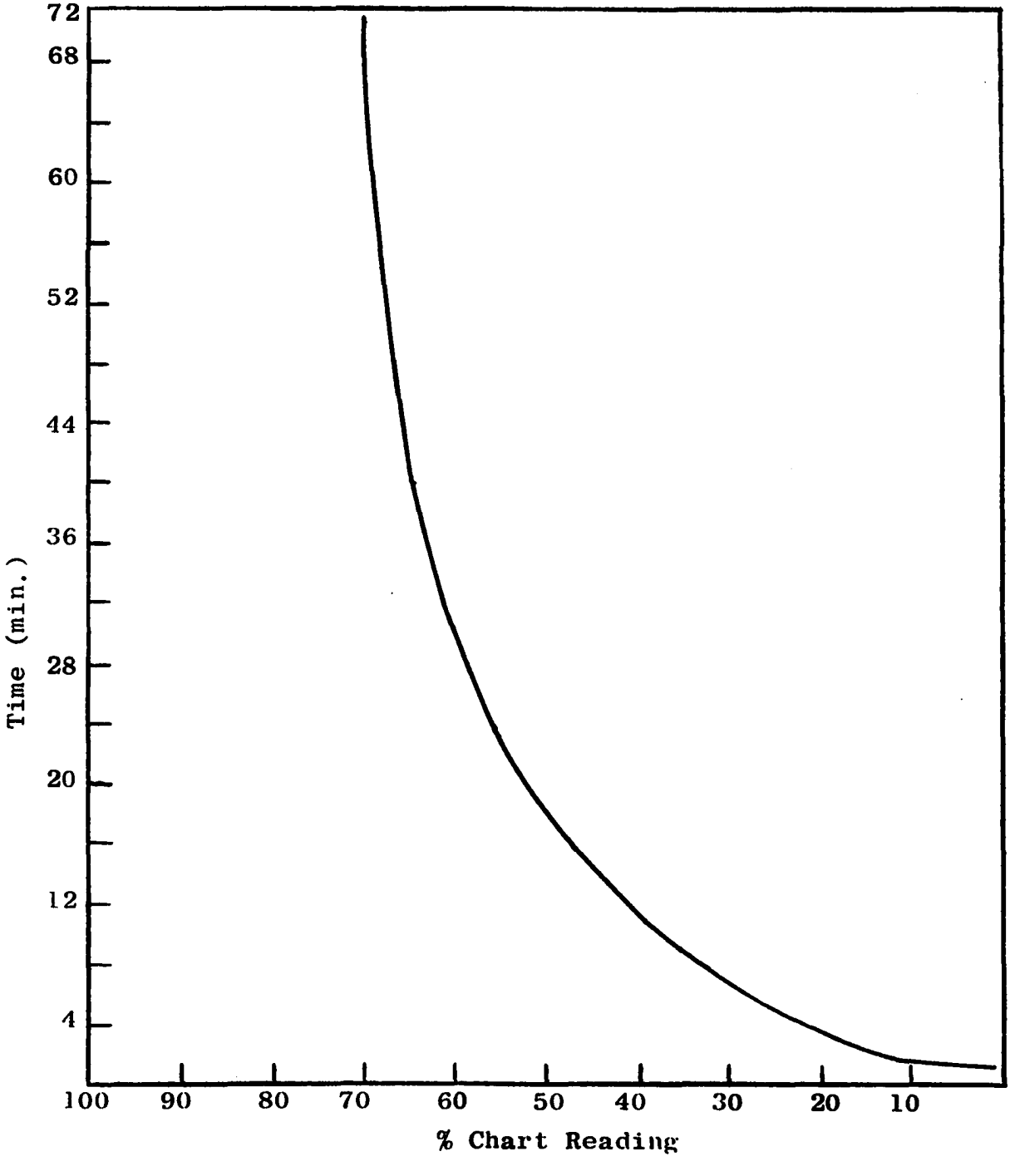


Figure 1. Titrigraph plot for the hydrolysis of $[Sm(EGDA)]^+$, pH 6.3, 25.0° C

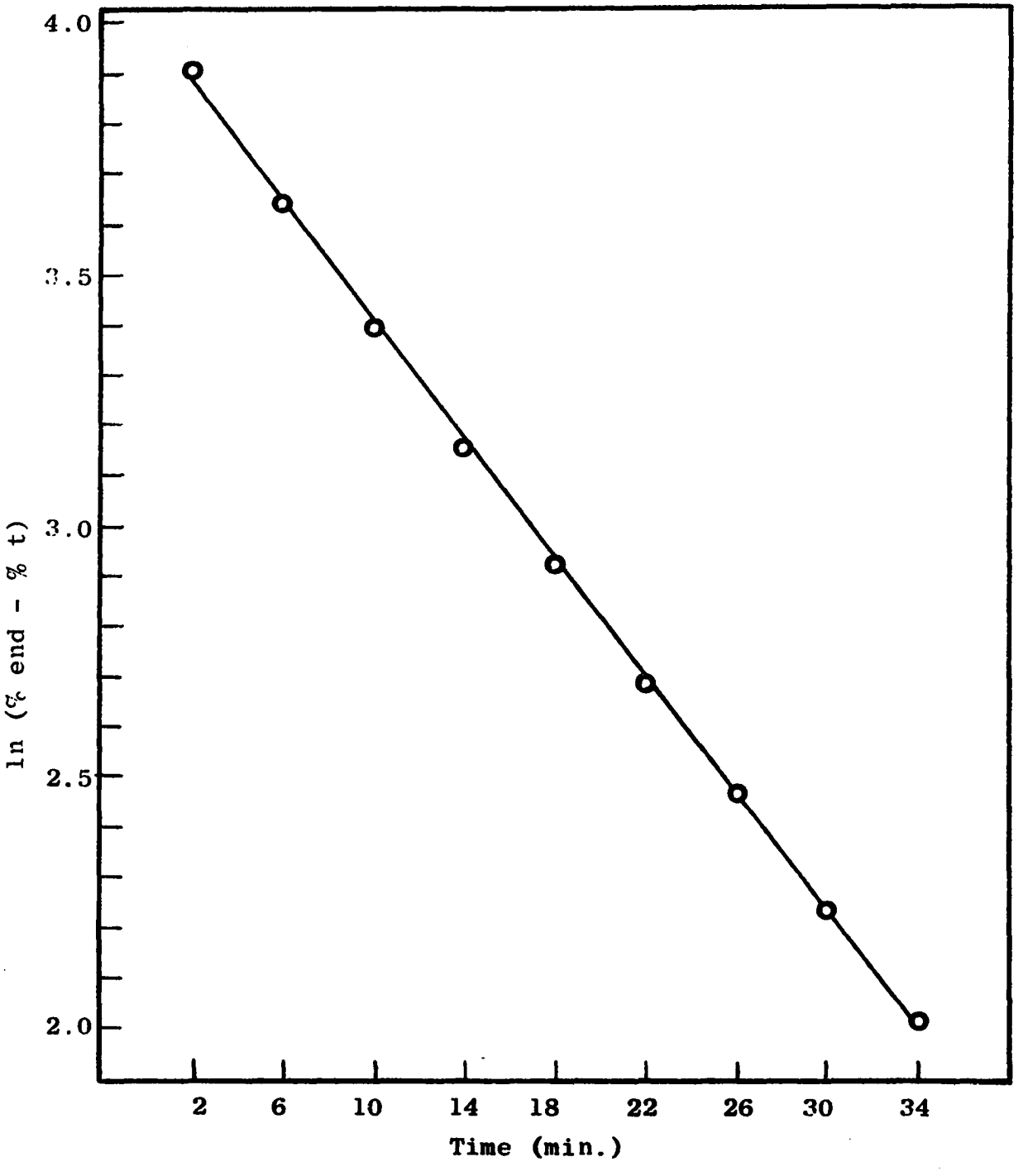


Figure 2. Time vs. ln (% end - % t) for the hydrolysis of $[Sm(\overline{EGDA})]^+$, pH 6.3, 25.0° C

where log $\gamma_{\pm}$ is given by the Guggenheim (51) equation:

$$\log \gamma_{\pm} = - [A \mid Z_1 Z_2 \mid I^{1/2} / (1 + I^{1/2}) + BI]$$

where A and B are constants for water and KNO_3 respectively and have the values of 0.507 and 0.10 at 25.0° C.

The rates of ester hydrolysis obtained at different temperatures were utilized to calculate the enthalpy of activation, $\Delta H^{\ddagger}$, and the entropy of activation, $\Delta S^{\ddagger}$. A general non-linear least squares computer program (50) was used to calculate $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ simultaneously by fitting the data to the following function:

$$k = (k_B T/h) \exp(\Delta S^{\ddagger}/R) \exp(-\Delta H^{\ddagger}/RT)$$

where k is the second-order rate constant, k_B is Boltzmann's constant, h is Planck's constant, T is the absolute temperature, and R is the gas constant in kcal./mole deg.

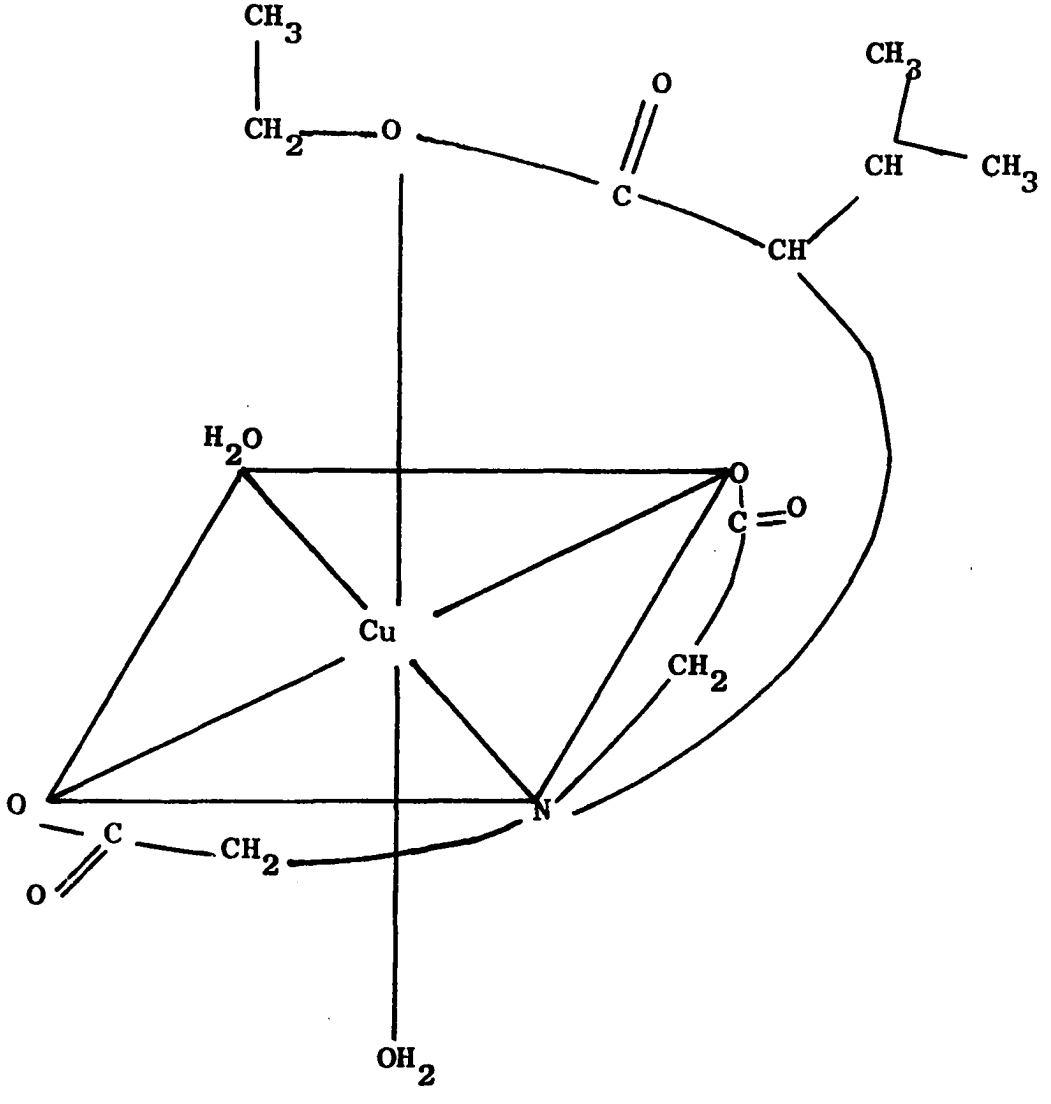
RESULTS

Amino Acid Ester-N,N-diacetic Acids

The amino acid ester-N,N-diacetic acids, ethyl glycinate-N,N-diacetic acid (EGDA), butyl glycinate-N,N-diacetic acid (BGDA), ethyl valinate-N,N-diacetic acid (EVDA), ethyl leucinate-N,N-diacetic acid (ELDA) ethyl alaninate-N,N-diacetic acid (EADA), ethyl β-alaninate-N,N-diacetic acid (EβADA) and ethyl 4-aminobutyrate-N,N-diacetic acid (EABDA), were prepared as the barium salts and found to undergo immeasurably slow ester hydrolysis in the 4.5-7.5 pH range even in the presence of added barium(II) ion. For ethyl glycinate-N,N-diacetic acid which is one of the most susceptible to ester hydrolysis of the substituted iminodiacetic acids, ester hydrolysis becomes significant near pH 8. The pseudo-first-order rate constant, k_{obsd}, for hydrolysis at pH 9.5 is 2.0×10^{-4} $sec.^{-1}$. Undoubtedly this includes contributions from several mechanisms of ester hydrolysis, probably including internal attack by $-COO^-$ (52). In the presence of a number of metal ions ester hydrolysis is measurable in the 5.0-7.0 pH range.

From the known chelating tendencies of substituted iminodiacetic acids (33, 34, 35, 37, 38) it is certain that these amino acid ester N,N-diacetic acids are strongly

coordinated to the metal ions investigated through the iminodiacetate group. The configuration of the iminodiacetic acids about the metal ions in solution is not known. The structure shown below has the three donor atoms occupying meridional positions. While this has been shown not to be



the preferred structure for cobalt(III) iminodiacetic acid complexes (53) it is a probable structure for a metal ion such as copper(II) which has a tendency to form square-planar complexes. This structure has also been confirmed by an x-ray crystallography study of copper(II) ethyl valinate-N,N-diacetic acid by R. A. Jacobson and J. Rodgers[1].

The slow rate of hydrolysis of the sterically hindered ester ethyl valinate-N,N-diacetic acid has allowed the determination of formation constants without appreciable interference from the ester hydrolysis reaction. The calculated values of the stepwise formation constants, (Equation 1), K_1 and K_2 of EVDA with copper(II), lead(II), nickel(II), cobalt(II) and samarium(III) are given in

$$M^{+n} + EVDA^{-2} \underset{}{\overset{K_1}{\rightleftharpoons}} M(EVDA)^{n-2}$$
$$M(EVDA)^{n-2} + EVDA^{-2} \overset{K_2}{\rightleftharpoons} M(EVDA)_2^{n-4} \qquad (1)$$

Table 1. The very high values of K_1 and significantly lower values of K_2 insure that solutions of 1:1 metal:EVDA contain only the complexes, M(EVDA) and $[M(EVDA)(OH)]^-$ depending on the pH range under investigation. Of particular importance for the catalysis of the hydrolysis of these esters (Equation 2) is whether the ester carbonyl group is coordinated to the metal or not. A number of 1:1 metal ion:

---

[1]R. A. Jacobson and J. Rodgers, Iowa State University, Ames, Iowa. Data from the crystal structure of copper(II) ethyl valinate-N,N-diacetic acid. Private Communication. 1968.

disodium ethyl glycinate-N,N-diacetate solutions were prepared using D_2O as solvent and the infrared spectrum was obtained for each and is tabulated in Table 2. If coordination of the ester carbonyl oxygen occurs, the

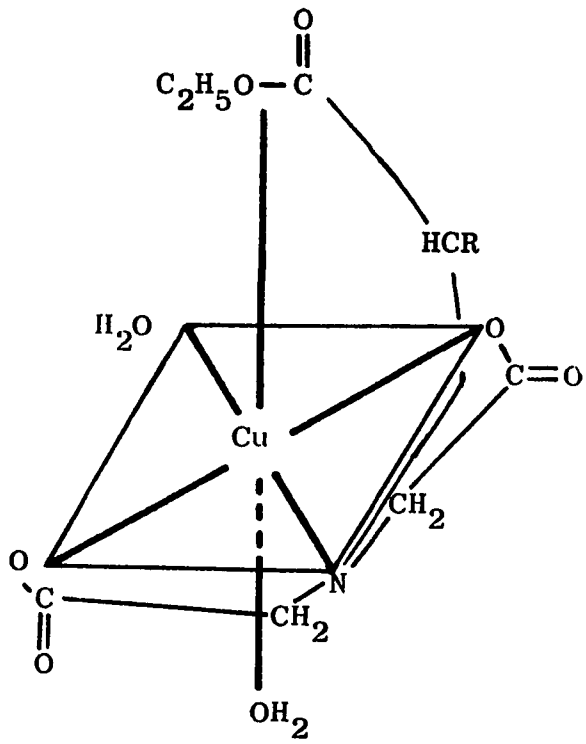
Table 1. Formation constants, K_1 and K_2 for the reaction of EVDA with metal ions

Metal ion	$10^{-6}K_1$	$10^{-3}K_2$	K_1/K_2
Cu(II)[a]	10.9 ± 0.8	1.23 ± 0.36	8,800
Pb(II)	2.2 ± 0.18	35.5 ± 10.0	62
Ni(II)	0.45 ± 0.05	6.2 ± 0.4	72
Sm(III)	0.14 ± 0.01	7.2 ± 0.6	18
Co(II)	0.0825 ± 0.0025	3.5 ± 0.12	24

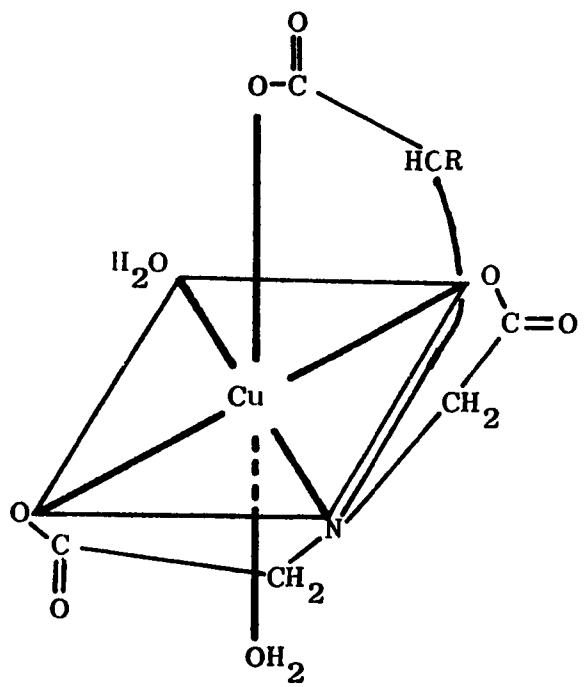
[a]At 25.0° C and 0.05 M ionic strength.

Table 2. Infrared stretching frequencies of metal ion EGDA complexes in D_2O

Metal ion	Carbonyl ester ($cm.^{-1}$)	Carboxylate group ($cm.^{-1}$)
Ba(II)	1722	1595
Cd(II)	1713	1615
Mn(II)	1710	1608
Ni(II)	1738	1625
Co(II)	1696	1607
Zn(II)	1686	1600
Cu(II)	1722	1636
Pb(II)	1703	1612
Gd(III)	1710	1623



+ H_2O ⟶ (2)



+ C_2H_5OH + H^+

Equation 2.

stretching frequency of the carbonyl group should be lowered 50-100 $cm.^{-1}$ from the free ester value (26, 29, 30). No evidence of ester carbonyl oxygen coordination is observed although the infrared peaks are quite broad and are inconclusive.

In an attempt to clarify the situation, nmr studies were undertaken with EGDA. Attention was focused on the methylene protons in the $-NCH_2COOC_2H_5$ grouping. The chemical shifts of the uncoordinated ligand were studied as a function of pD to determine the pD range for the species $(C_2H_5OOCCH_2-N(H)(CH_2COO)_2]^-$ which has the same charge as $[CH_3N(H)(CH_2COO)_2]^-$, methyliminodiacetate (MeIMDA), the model compound which was selected for comparison with EGDA. The methylene of EGDA ($-NCH_2COOC_2H_5$) would be expected to show a downfield shift (provided the ester carbonyl oxygen is coordinated) upon coordination to a metal relative to the change in chemical shift of the methyl group in MeIMDA upon the coordination of MeIMDA to the same metal. Chemical shift parameters in cps were obtained for EGDA and MeIMDA both as free ligand and complexes with lanthanum(III) and are given in Table 3. Lanthanum(III) was used because it is the diamagnetic ion whose complexes were most soluble of the metal ions which catalyze the hydrolysis of EGDA.

One must first explain the change in chemical shifts with pD. If there are more than one species in solution,

Table 3. Summary of chemical shifts (cps) for MeIMDA and EGDA

pD	A	B	C	D	pD	A	B	C	D
MeIMDA, $CH_3N(CH_2COOH)_2$					La(III) + $CH_3N(CH_2COOH)_2$				
	A	B				A	B		
2.40	181	240			3.00	179	233		
3.05	179	232			4.50	177	228		
4.05	177	227			5.58	170	222		
7.00	177	227			6.70	152	205		
8.35	176	225							
9.12	173	222							
12	134	183							
EGDA, $CH_3CH_2OOCCH_2N(CH_2COOH)_2$					$[La(CH_3CH_2OOCCH_2N(CH_2COO)_2]^+$				
	A	B	C	D	A	B	C	D	
4.00	77	259	234	228	5	77	259	224	211
5.90	77	259	232	227	5[a]	77	260	225	210
7.40	76	255	227	217					
9.50	74	259	209	195					

[a]Using sodium acetate buffer solution at pD 5.

the nmr spectrum gives the time average of the species. To insure that only one species is present, the pD must not be near the value of a pK_a. This is seen in Table 3 for MeIMDA. PD values of 2.40 and 3.05 are too near the pK_a of the acid protons (pK_a = 2.12) and the observed chemical shift contains contributions from $[CH_3N(H)(CH_2COOH)(CH_2COO)]$ as well as $[CH_3N(H)(CH_2COO)_2]^-$. The chemical shift values were relatively constant for MeIMDA in the pD 4-7 range, and this was taken as an indication that only the species

$[CH_3N(H)(CH_2COO)_2]^-$ was present in this pD region and the values of the chemical shifts in this region were used in the comparison. At higher pD values (8-12) the hydrogen on the nitrogen atom is removed (pK_a = 9.65) and an upfield shift with increasing pD was observed. Similarly for EGDA the chemical shift values are relatively constant between pD 4 and 6, and this indicates a single species in solution. The pK_a of the amino group in amino acid esters is about 2 pK_a units lower than that for amino acids (4), and the effects of proton removal from the nitrogen atom are observed at a lower pD (7.40) for EGDA.

La(III) does not complex with MeIMDA at pD 3-4.5, and the chemical shifts were those expected due to free MeIMDA. The addition of excess La(III) at pD 6.70 had no effect on the chemical shifts which implied that complexation was complete. Hence these values of chemical shifts due to $[La(MeIMDA)]^+$ were used in the comparison. For $[CH_3N(H)CH_2COO)_2]^-$ chemical shifts of 177 and 227 cps for the methyl and methylene groups, respectively, were utilized in the comparison between the difference in chemical shift values of free and coordinated MeIMDA and EGDA. Upon coordination to form $[La(CH_3N(CH_2COO)_2)]^+$, the chemical shifts were 152 and 205 cps from the methyl and methylene groups respectively. The upfield shift with coordination is due to the fact that La(III) is apparently not as strong an acid as H^+ in this

complex. EGDA values of chemical shifts are 77, 259, 232 and 227 from A, B, C, D respectively (Table 3) for $[CH_3CH_2COOCH_2N(H)(CH_2COO)_2]^-$ and for the coordinated species $[La(EGDA)]^+$ with corresponding alphabetical assignments the values of the chemical shifts were 77, 259, 224 and 211.

The chemical shifts of the methyl and methylene hydrogens of the ester function do not change on coordination, however, the $-N(CH_2COO^-)_2$ methylene protons of MeIMDA shift 21 cps upfield upon coordination, while the analoguous protons in EGDA undergo a smaller upfield shift of 16 cps. Coordination with La(III) also produces upfield shifts in the CH_3 protons of MeIMDA (25 cps) and the $-NCH_2COOC_2H_5$ methylene protons of EGDA (8 cps). Since the upfield shift is less for the methylene protons of EGDA this suggests that the ester group is losing electron density to La(III) and implies that some La(III) to ester bonding occurs although this interpretation of the chemical shifts may be over simplified and therefore not unambiguous.

Another approach to determine whether the ester is coordinated or not is suggested by the work of Schwarzenbach, Anderegg, Schneider, and Senn (33) who have determined formation constants of metal ions with a number of substituted iminodiacetic acids $RN(CH_2COOH)_2$, containing various functional groups in the R moiety. By comparing the first and second stepwise formation constants he was able to

determine if R was coordinated to the metal or not. The K_1/K_2 ratio was much higher for tetradentate ligands than for tridentates. Using R groups which could not possibly coordinate and R groups which almost certainly did, it was found that R was coordinated to the metal ion when the K_1/K_2 ratio was a) at least 10^6 for copper(II), b) at least 10^5 for lead(II), and c) at least 1000 for cobalt(II) and nickel(II). That the ratios for EVDA (Table 1) are much lower indicates that the ester group is not or only slightly coordinated to the metal ions copper(II), lead(II), nickel(II), and cobalt(II). For samarium(III) one can compare the K_1/K_2 ratios of 290 for $N(CH_2COOH)_3$ (54) and 25 for $HN(CH_2COOH)_2$ (36). The low ratio of 18 for EVDA suggests that this ligand is only tridentate and that the ester group is not coordinated to samarium(III).

Another method (33) used in determining whether the R group in $RN(CH_2COOH)_2$ was coordinated to a metal ion or not has been to observe the linear correlation between log K_1 and the pK_a for the $\overset{+}{N}H$ proton of $[RN(H)(CH_2COO)_2]^-$ when R is a non-coordinating group. The more basic the nitrogen atom in the ligand the more stable is its metal complex. Ligands containing coordinating R groups were considerably more stable than predicted from the correlation. Using the pK_a of 6.75 for EVDA and the formation constants in Table 1 it is clear that EVDA follows the relationships

for tridentate ligands (33) for copper(II), lead(II), cobalt(II) and nickel(II).

The metal ion complexes of EVDA and other amino acid ester-N,N-diacetates increasingly form the hydroxo-complexes $[M(EVDA)(OH)]^-$, as the pH is raised.

$$[M(EVDA)] + OH^- \underset{}{\overset{K_{fOH}}{\rightleftharpoons}} [M(EVDA)(OH)]^- \qquad (3)$$

Values of the equilibrium constants, K_{fOH}, for several metal complexes are given in Table 4. These values show that at pH 6.5 at 25.0° less than 10% of the Cu(EVDA) complex is present as the hydroxo-complex, $[M(EVDA)(OH)]^-$. At pH 7.5, approximately half of the complex, Cu(EVDA), is in the hydroxo-form. The kinetic studies for the amino acid ester-N,N-diacetic acids with the exception of ethyl valinate-N,N-diacetic acids (EVDA) and ethyl leucinate-N,N-diacetic acid (ELDA) were conducted at pH values where the hydroxo-complexes accounted for less than 10% of the total metal ion (amino acid ester-N,N-diacetate) concentration. A higher pH was required for studying the slower rate of hydrolysis of EVDA and ELDA in their metal complexes. At these higher pH values appreciable amounts of both M(EVDA) and $[M(EVDA)(OH)]^-$ were present in solution. The observed rate of hydrolysis is experimentally determined to be, Rate $= k_{obsd} [M(EVDA)]_t$ where $[M(EVDA)]_t$ is the total concentration of both M(EVDA) and $[M(EVDA)(OH)]^-$ and k_{obsd} depends upon the pH of the solution. At low pH,

Table 4. Hydroxo-complex, $[M(EVDA)(OH)]^-$, formation constants, K_{fOH}

Complex	Temperature	K_{fOH}
Ni(EVDA)[a]	25.0° C	3.0×10^2
Co(EVDA)	25.0	6.0×10^2
Pb(EVDA)	25.0	6.0×10^2
Cu(EVDA)[b]	25.0	1.31×10^6
	35.0	7.86×10^5
	45.0	4.80×10^5
$[Sm(EVDA)]^{+c}$	25.0	2.31×10^6
	35.0	2.16×10^6
	45.0	2.04×10^6

[a]Ionic strength 0.05 M for all metal complexes.

[b]ΔH=9.6 kcal./mole, ΔS=4.2 e.u.

[c]ΔH=-1.2 kcal./mole, ΔS=23.6 e.u.

$k_{obsd}=k[OH^-]$, but at high pH it becomes independent of pH (Table 5). Dividing k_{obsd} by OH^- gives the second order rate constant, k, which is constant at low pH but decreases at higher pH.

Two mechanisms can be postulated to account for this rate behavior. Mechanism A assumes a nucleophilic attack

Mechanism A

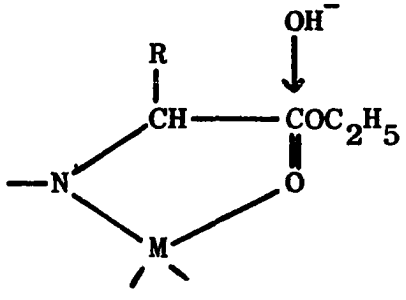
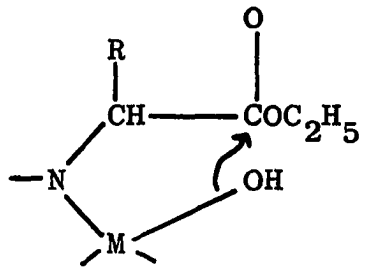


Table 5. Rates of hydrolysis of Cu(EVDA), $[Sm(EVDA)]^+$, Cu(ELDA), and $[Sm(ELDA)]^+$

pH	f_{OH}	$10^4 k_{obsd}$ $sec.^{-1}$	$10^4 k_i$ $M^{-1} sec.^{-1}$	k $M^{-1} sec.^{-1}$	k_e $M^{-1} sec.^{-1}$
Cu(EVDA)[a]					
7.1	0.14	0.280	2.00	182	212
7.5	0.29	0.551	1.90	143	202
7.8	0.45	0.854	1.90	111	200
8.1	0.62	1.18	1.90	77.2	203
8.5	0.80	1.75	2.18	45.2	226
9.0	0.91	1.70	1.87	13.9	155
$[Sm(EVDA)]^+$[a]					
7.5	0.42	0.753	1.79	195	465
Cu(ELDA)[a]					
7.0	0.115	2.52	21.9	2060	2320
7.3	0.206	4.33	21.0	1770	2230
7.5	0.29	5.37	18.6	1390	1960
7.9	0.51	11.5	22.6	1180	2320
8.2	0.67	15.5	23.2	800	2400
$[Sm(ELDA)]^+$[a]					
7.1	0.225	2.84	12.7	1850	2400
7.3	0.315	4.61	14.6	1890	2760

[a]At 25.0° C and 0.05 M KNO_3

Mechanism B



of OH^- on the carbonyl carbon atom of the ester group and that the hydroxo-complex does not react:

$$M(EVDA) + OH^- \xrightarrow{k_e} M(\overset{OH}{|}EVDA) \xrightarrow{\text{fast}} M(VDA) + C_2H_5OH$$

The end products are ethanol and the metal ion complex of valine-N,N-diacetic acid (VDA). This mechanism yields a rate law, Rate=k_e $[M(EVDA)][OH^-]$ in which $[M(EVDA)]$ is the actual concentration of the non-hydroxo-complex. From the formation constant for the hydroxo-complex, K_{fOH}, the fraction, f_{OH}, of the total complex concentration, $[M(EVDA)]_t$, which is in the hydroxo-form, $[M(EVDA)(OH)]^-$, can be calculated. Then $(1-f_{OH})$ is the fraction of $[M(EVDA)]_t$ which is present as M(EVDA). The rate law in terms of these known parameters is

$$\text{Rate} = k_e(1-f_{OH})[M(EVDA)]_t\ [OH^-]$$

Division of k by $(1-f_{OH})$ gives the k_e values shown in Table 5. The constancy of these values indicates that Mechanism A is consistent with the rate data.

A second mechanism which is consistent with the observed rate data is Mechanism B in which internal attack of OH^- which is bound to the metal ion is rate determining.

$$M(EVDA) + OH^- \underset{}{\overset{K_{fOH}}{\rightleftharpoons}} [M(EVDA)(OH)]^-$$

$$[M(EVDA)(OH)]^- \xrightarrow{k_i} \overset{OH}{\underset{|}{M(EVDA)}} \xrightarrow{fast} M(VDA) + C_2H_5OH$$

The rate law for this mechanism is

$$\text{Rate} = k_i[[M(EVDA)(OH)]^-]$$

or in terms of f_{OH} and $[M(EVDA)]_t$,

$$\text{Rate} = k_i \, f_{OH} \, [M(EVDA)]_t$$

In this case $k_{obsd} = f_{OH} \, k_i$. Dividing k_{obsd} by the f_{OH} values calculated from K_{fOH} gives k_i values listed in Table 5. The constancy of these values suggests that Mechanism B as well as Mechanism A is in agreement with the equilibrium and kinetic results.

The values of K_{fOH} for Cu(ELDA) and $[Sm(ELDA)]^+$ were not measured but were assumed to be the same as for Cu(EVDA) and $[Sm(EVDA)]^+$. The constancy of k_e and k_i in Table 5 indicate that this assumption is not unwarranted.

To determine whether Mechanism A or B was correct, the rates of hydrolysis were examined for general nucleophilic catalysis. These studies were conducted at pH values near 7.8 where nearly equal amounts of Cu(EVDA) and $[Cu(EVDA)(OH)]^-$ were present in solution. The observed but small catalytic effects of the nucleophiles listed in Table 6 clearly

Table 6. Rates of hydrolysis of Cu(EVDA), p-nitrophenyl acetate and ethyl chloroformate as catalyzed by several nucleophiles

nucleophile	pK_a	Cu(EVDA)[a]	p-nitrophenyl acetate	ethyl chloro formate
	k, $M^{-1}sec.^{-1}$			
H_2O		-	6×10^{-7}	-
acetate	4.8	0.0002	0.0005	-
HPO_4^{-2}	6.9	0.02	0.007	-
pyridine	5.4	0.01	0.1	-
4-picoline	6.2	0.1	0.5	-
nitrate	3.4	0.38	0.001	32.2
hydroxide	14	209	890	169

[a]At 25.0° C and ionic strength of 0.05 M or more.

eliminate a third possible mechanism of H_2O attack on $[Cu(EVDA)(OH)]^-$. If this were the correct mechanism then the rate constants should have been far greater than those observed in the absence of the added nucleophile. This is because the nucleophiles used are all much more reactive in simple organic systems than H_2O.

Because of the high nucleophilicity of OH^-, relatively high concentrations of other nucleophiles were required to obtain a measurable catalysis. Below concentrations of 1 M acetate ion, no rate enhancement was observed but above this a slight increase was noted. The rate due to OH^- was subtracted from the overall rate to give the rate constant, k_e, for external attack by acetate ion. The margin of

error for acetate ion is large. Because of the buffering action of HPO_4^{-2}, these rates were studied at pH values of 7.0-8.6 and at concentrations of about 0.005 to 0.1 M HPO_4^{-2} which were actual ionic concentrations in solution. For pyridine and 4-methylpyridine, concentrations of the nucleophile at pH 7.5 were less than 0.005 M and 0.001 M respectively. Above these concentrations, k_e decreases probably due to complex formation as noted visibly by a darkening of the solution color. The most clear-cut example of general nucleophilic catalysis is NO_2^-. At pH 7.5, k_e was constant over a nitrite concentration range of 0.0025 to 0.020 M. Values of k_e for these nucleophiles are given in Table 6 along with those for p-nitrophenyl acetate (55, 56) and ethyl chloroformate (57).

The relative nucleophilicities as measured by k_e of these nucleophiles, including OH^-, is roughly the same for Cu(EVDA) and the organic esters. This suggests that ester hydrolysis of Cu(EVDA) proceeds by the same mechanism as for the organic esters, namely general nucleophilic catalysis (Mechanism A), and not by internal hydroxide attack (Mechanism B). That general base catalysis is probably not involved is suggested by the absence of a correlation between the pK_a of the nucleophile and the rate of hydrolysis (58, 59).

The N,N-diacetic acids derived from the amino acid esters butyl glycinate, ethyl alaninate, ethyl-β-alaninate, and ethyl 4-aminobutyrate were complexed with copper(II) and samarium(III) and the rates of ester hydrolysis determined for the hydrolysis reaction at 25.0° and 0.05 M KNO_3. The pseudo-first-order rate constants, k_{obsd}, are given in Table 7. The rates of hydrolysis of ethyl glycinate-N,N-diacetic acid (EGDA) complexed with the metal ions cadmium(II), nickel (II), manganese(II), cobalt(II), iron(II), zinc(II), lanthanum(III), copper(II), lead(II), neodymium(III), gadolinium(III), smarium(III), dysprosium(III), erbium(III), ytterbium(III), and lutetium(III) were studied and found to increase in the order listed. All of these metal-ester complexes hydrolyzed with an observed rate law:

$$\text{Rate} = k\,[M(EGDA)][OH^-].$$

The pseudo-first-order rate constants, k_{obsd}, for the hydrolysis reaction at 25.0° and 0.050 M KNO_3 are given in Table 8. If k_{obsd} depends on the hydroxide ion concentration in the following manner: $k_{obsd} = k\,[OH^-]$, a plot of log k_{obsd} *vs.* pH will give a straight line of slope 1.00. Slopes obtained for all metal ions and esters studied were 1.00 ± 0.05. Figure 3 gives the plot of log k_{obsd} *vs.* pH for the ester hydrolysis of the Cu(EGDA) complex. Table 9 gives the non-linear least squares evaluation of the second-order rate constant, $k(M^{-1}\ sec.^{-1})$, and the standard deviation for

Table 7. Rates of hydrolysis of amino acid ester-N,N-diacetic acids with copper(II) and samarium(III)

pH	$10^4 k_{obsd}$[a] ($sec.^{-1}$)	pH	$10^4 k_{obsd}$ ($sec.^{-1}$)
	Cu(butyl glycinate-N,N-diacetate)		
6.30	2.42	6.70	6.07
6.50	3.54		
	[Sm(butyl glycinate-N,N-diacetate)]		
5.90	3.26	6.60	18.6
6.30	7.50		
	Cu(ethyl alaninate-N,N-diacetate)		
6.00	1.99	6.80	11.9
6.30	4.08	7.00	15.7
6.50	6.38	7.10	19.8
6.70	8.11		
	[Sm(ethyl alaninate-N,N-diacetate)]		
6.40	5.59	6.60	8.63
	Cu(ethyl β-alaninate-N,N-diacetate)		
7.00	0.583	8.00	5.85
7.50	1.88		

[a] At 25.0 C, $[M^{+n}]$=[amino acid ester-N,N-diacetate]= 0.00067 M; $[KNO_3]$ = 0.05 M.

Table 7. (Continued)

pH	$10^4 k_{obsd}$ ($sec.^{-1}$)	pH	$10^4 k_{obsd}$ ($sec.^{-1}$)
	[Sm(ethyl β-alaninate-N,N-diacetate)]$^+$		
6.00	4.76	6.90	37.6
6.50	14.9		
	[Lu(ethyl β-alaninate-N,N-diacetate)]$^+$		
5.40	1.38		
	Pb(ethyl β-alaninate-N,N-diacetate		
6.40	1.52		
	Cu(II), Sm(III), Lu(III) (ethyl 4-aminobutyrate-N,N-diacetate)		
4-8	no reaction		

Table 8. Rates of metal ion-catalyzed hydrolysis of ethyl glycinate-N,N-diacetate acid

Metal ion[a]	pH	$10^4 k_{obsd}$ ($sec.^{-1}$)	pH	$10^4 k_{obsd}$ ($sec.^{-1}$)
Cd(II)	7.40	0.655	8.00	2.54
	7.70	1.45		
Ni(II)	6.80	0.382	7.60	1.64
	7.20	0.780	7.70	2.39
	7.40	1.28	7.80	3.10

[a] At 25.0 and $[M^{+n}]$ = [EGDA] = 0.00067 M; $[KNO_3]$ = 0.05 M.

Table 8. (Continued)

Metal ion[a]	pH	$10^4 k_{obsd}$ ($sec.^{-1}$)	pH	$10^4 k_{obsd}$ ($sec.^{-1}$)
Mn(II)	7.00	0.575	7.50	1.62
	7.30	1.04	7.90	4.04
Co(II)	6.80	0.718	7.50	3.74
	7.10	1.52	7.70	6.45
	7.30	2.44	7.80	7.57
Fe(II)	6.10	0.573	6.40	1.21
	6.20	0.758		
Zn(II)	5.80	0.567	6.60	3.51
	6.00	0.971	6.80	5.72
	6.20	1.33	7.00	8.62
	6.40	2.31	7.20	12.1
	6.50	2.61		
Cu(II)	5.50	0.835	6.50	7.51
	5.80	1.48	6.60	10.7
	6.10	3.03	6.80	15.0
	6.30	4.77	7.00	28.5
	6.40	6.00		
Pb(II)	6.00	3.15	6.40	7.82
	6.10	3.56	6.50	9.45
	6.20	4.75	6.60	14.0
	6.30	6.34	6.80	23.2
La(III)	6.30	2.33	6.60	5.43
	6.60	5.52	6.90	11.3
Nd(III)	5.50	1.47	6.50	12.8
	5.90	3.54	6.80	27.0
	6.20	6.70		

Table 8. (Continued)

Metal ion[a]	pH	10^4 k_{obsd} ($sec.^{-1}$)	pH	10^4 k_{obsd} ($sec.^{-1}$)
Sm(III)	5.50	2.24	6.30	12.1
	5.80	4.01	6.50	16.5
	6.00	5.74	7.10	67.0
Gd(III)	5.30	0.889	6.10	6.10
	5.50	1.41	6.30	11.3
	5.70	2.21	6.50	13.1
	5.90	2.96	6.70	22.9
Dy(III)	5.60	4.76	6.30	21.3
	6.00	10.7		
Er(III)	4.80	1.88	5.70	11.6
	5.10	3.29	6.00	23.3
	5.40	6.15		
Yb(III)	5.00	4.21	5.50	13.3
	5.20	6.70	5.70	21.1
Lu(III)	4.40	0.901	5.30	7.14
	4.60	1.50	5.50	11.3
	4.80	2.20	5.70	17.9
	5.00	4.17	6.00	44.7

Table 9. Rate constants for the metal ion-catalyzed hydrolysis of ethyl glycinate-N,N-diacetic acid

Metal ion[a]	10^{-2} k, $M^{-1}sec.^{-1}$	Metal ion[a]	10^{-2} k, $M^{-1}sec.^{-1}$
Cd(II)	2.14 ± 0.09	La(III)	115 ± 3
Ni(II)	3.89 ± 0.13	Nd(III)	347 ± 5
Mn(II)	4.18 ± 0.05	Sm(III)	447 ± 21
Co(II)	10.1 ± 0.20	Gd(III)	376 ± 16
Fe(II)	38.6 ± 1.0	Dy(III)	877 ± 2
Zn(II)	66.2 ± 2.0	Er(III)	1920 ± 30
Cu(II)	218 ± 7	Yb(III)	3460 ±100
Pb(II)	283 ± 11	Lu(III)	3450 ±230

[a]At 25.0° $[M^{+n}]$ = [EGDA] = 0.00067 M; $[KNO_3]$ = 0.05 M.

each of the metal-ester complexes. Table 10 gives the second-order rate constants, k, for the hydrolysis of various amino acid ester-N,N-diacetate complexes of copper(II) and samarium (III). The following metal ions were observed not to catalyze the ester hydrolysis: aluminum(III), iron(III), magnesium(II), scandium(III), barium(II), hafnium(IV) oxide (HfO^{+2}), vanadyl ion (VO^{+2}), and uranyl ion (UO_2^{+2}) and the following gave precipitates of the diacetic acids: tin(II), silver(I), and mercury(II). The metal ions which did not catalyze the ester hydrolysis form only very weak complexes or undergo extensive hydroxo-complex formation at low pH values.

Table 10. Rates of hydrolysis of amino acid ester-N,N-diacetic acids with copper(II) and samarium(III)

Amino acid ester (-N,N-diacetate)	copper(II) 10^{-2} k $(M^{-1}sec.^{-1})^a$	samarium(III) 10^{-2} $(M^{-1}sec.^{-1})^a$
ethyl glycinate	218	447
butyl glycinate	97.5	326
ethyl alaninate	134	180
ethyl leucinate	22.5	26
ethyl valinate	2.1	4.7
ethyl B-alaninate[b]	4.83	381
ethyl 4-aminobutyrate	no reaction	no reaction

[a]At 25.0° $[M^{+n}]$ = [ester] = 0.00067 M; $[KNO_3]$ = 0.05 M.
[b]Lu(III), 4.50×10^5; Pb(II), 4.93×10^3 M^{-1} $sec.^{-1}$.

Temperature dependence data are given in Table 11 for the hydrolysis of ethyl glycinate-N,N-diacetic acid (EGDA) complexed with the metal ions nickel (II), copper(II), lead(II), samarium(III) and lutetium(III). The data for methyl betaine ethyl ester, $[(CH_3)_3\overset{+}{N}CH_2COOC_2H_5]Cl$, and the parameters determined by Gustafson (13) for ethyl glycinate are included for comparison. Correction has been made for the change in K_w with temperature (38) in the calculation of hydroxide ion concentrations. The enthalpies and entropies of activation for the ester hydrolysis reactions is given in Table 12.

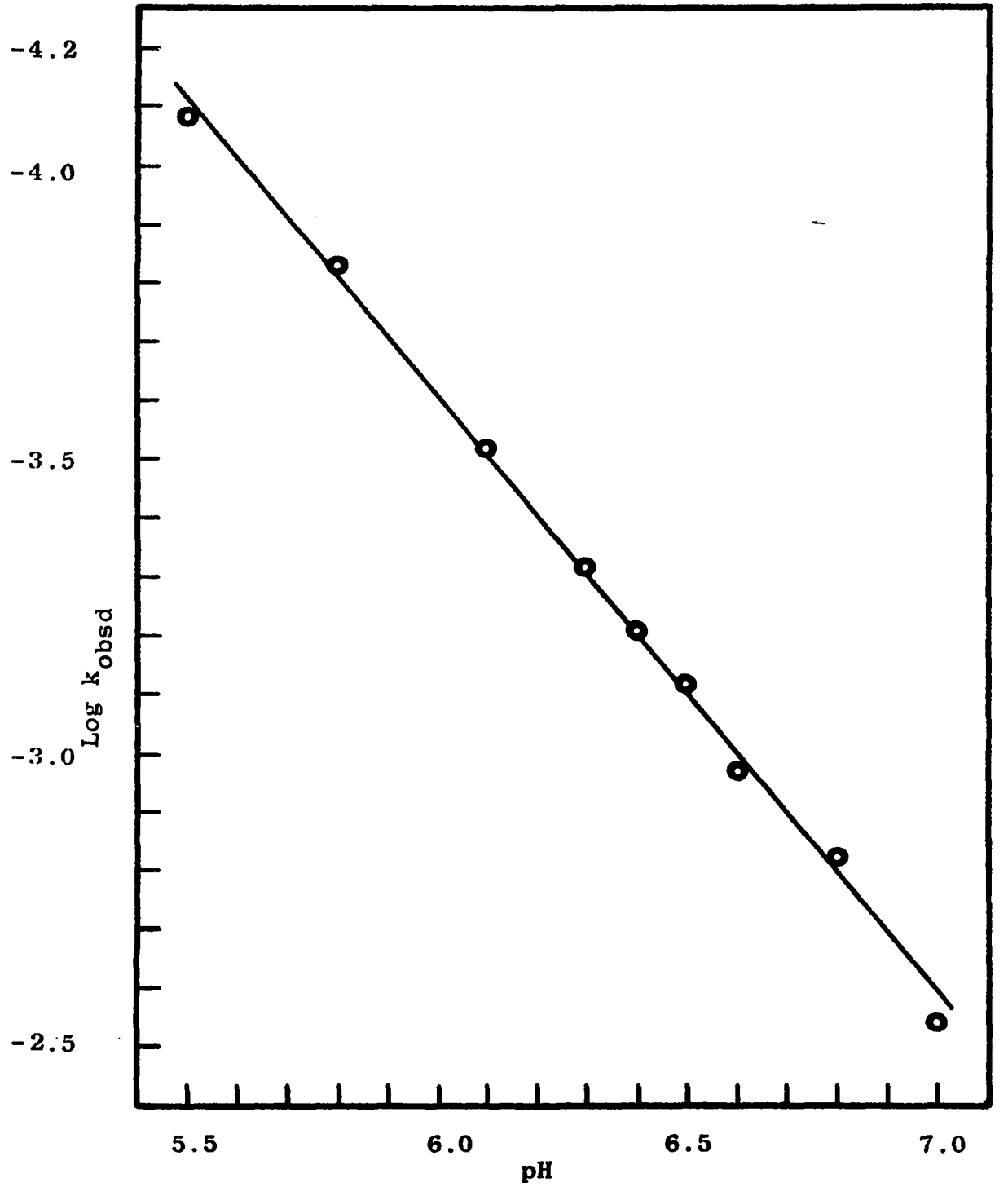


Figure 3. Plot of log k_{obsd} vs. pH for the ester hydrolysis of Cu(EGDA)

Table 11. Temperature dependence of the rate of metal-catalyzed hydrolysis of EGDA

Temperature	$k^a(M^{-1}sec.^{-1})$	Temperature	$k^a(M^{-1}sec.^{-1})$
Betaine ethyl ester (no metal ion present)		Cu(EGDA)	
40.0	99.7, 93.3	40.0	366
30.0	51.7, 52.9	30.0	252
25.0	39.4, 41.0	25.0	218
20.0	32.8, 33.0	20.0	194
13.5	21.2	14.5	178
Ni(EGDA)		$[Sm(EGDA)]^+$	
40.0	6.87, 7.15	40.0	976, 937
35.0	5.82, 5.75	30.0	512, 512
30.0	4.97, 4.77	25.0	447
25.0	3.89	20.0	283, 284
		14.5	221, 197
Pb(EGDA)		8.5	147
40.0	427		
30.0	348	Lu(EGDA)	
25.0	278	30.0	4530
17.0	245, 237	25.0	3700, 3520
		20.0	2410, 2490
		12.5	1530, 1560

[a] $[KNO_3]$ = 0.050 M.

Table 12. Enthalpies and entropies of activation for ester hydrolysis

Ester[a]	$\Delta H^{\ddagger}$ (kcal/mole)	$\Delta S^{\ddagger}$ (e.u.)
Ethyl glycinate	10.6 ± 0.5	-21.7 ± 1.0
Betaine ethyl ester	9.7 ± 0.3	-18.5 ± 1.1
Cu(EGDA)	5.0 ± 0.5	-21.9 ± 1.5
Ni(EGDA)	6.7 ± 0.4	-24.2 ± 1.2
Pb(EGDA)	4.4 ± 0.6	-23.5 ± 2.1
$[Sm(EGDA)]^+$	10.0 ± 0.4	- 4.0 ± 1.2
$[Lu(EGDA)]^+$	10.2 ± 0.6	0.8 ± 2.0

[a] $[KNO_3]$ = 0.050 M.

The dependence of the rate of hydrolysis on the ionic strength of the medium was determined at pH 6.30 for the hydrolysis reaction involving copper(II) and EGDA. Concentrations of KNO_3 of 0.025, 0.050, and 0.100 M were used to give values of ionic strength, I [KNO_3 + Ba(II) from BaEGDA], of 0.028, 0.053 and 0.103 M. Values of 10^4 k_{obsd} ($sec.^{-1}$) at these respective ionic strengths were 3.86, 3.90 and 3.78, which are the same within experimental error. Replacement of KNO_3 with KCl had no effect. The corresponding study with samarium(III) and EGDA with values of ionic strength of 0.003, 0.028, 0.053, 0.103 and 0.203 gave values of 10^4 k_{obsd} ($sec.^{-1}$) of 10.9, 8.90, 9.81, 7.20, and 5.84 respectively. A plot of

log k_{obsd} *vs.* $I^{1/2}/(1+I^{1/2})$ gave a straight line of slope 1.04. A medium of high dielectric constant stabilizes charged species, and the effects can be predicted by considering the following reaction:

$$[M^{+n}(EGDA)]^{n-2} + OH^- \rightleftarrows [M^{+n}(EGDA)(OH)]^{n-3} \qquad (4)$$

which corresponds to formation of the transition state. For copper(II) and EGDA, the Cu(EGDA) complex is neutral and since both the right and left sides of equation 4 each bear a single charge, neither side of the equation is favored by changing the ionic strength, and no effect is observed. For samarium(III) there are two unit charged species on the left and a neutral species on the right side of Equation 4, and therefore increasing ionic strength should stabilize the charged species and the observed rate constant k_{obsd} should decrease as was observed. Quantitatively (59) $\log k_1/k = -A\Delta(Z)^2 I^{1/2}/(1+I^{1/2})$ where $A = 0.507$ for aqueous solutions and $\Delta(Z)^2$ is defined as usual as the charges on products minus reactants (i.e., $(n-3)^2 -(1)^2-(n-2)^2$ for Equation 4.

Excess copper(II) ion had no effect on the rate of hydrolysis. With a concentration of copper(II) ion from 0.00067 to 0.0011 to 0.0015 M, the pseudo-first-order rate constants (3.90, 3.68, and 3.65×10^{-4} $sec.^{-1}$, respectively) remained, within experimental error, unchanged.

The addition of excess samarium(III) even for [Sm(III)] = 0.00134 M and [EGDA] = 0.00067 M had no effect within experimental error, on the rate of the Sm(III) catalyzed hydrolysis of EGDA. Using a deficiency of metal the rate of hydrolysis was that expected for the particular concentration of metal-ester complex. However, after the complexed ester had hydrolyzed, the reaction continued at a much slower rate until all the ester was hydrolyzed. The slow terminal hydrolysis was presumably a result of some complexation with excess EGDA instead of NTA by the metal ion. Addition of other chelating groups such as glycine to a solution of Cu(EGDA) slowed the rate of hydrolysis presumably by complexation.

Ethyl glycinate-N,N-diacetic acid was also hydrolyzed in the presence of zinc(II) ion and the proton nmr spectrum obtained. The final product when the solution was evaporated under reduced pressure and dissolved in D_2O gave a singlet at δ 3.65 ppm which was in good agreement with the nmr spectrum obtained from a D_2O solution prepared from zinc(II) and disodium nitrilotriacetate directly. The hydrolysis reaction stoichiometry and the nmr spectrum of the hydrolysis product indicate that the product of the hydrolytic reaction is the metal complex of NTA.

Amino Acid Esters

The rates of hydrolysis of various amino acid esters catalyzed by copper(II) iminodiacetate complexes were followed by pH stat techniques. The equilibria involved in these systems were extensively studied. The ionization constants of the amino acid esters, $EH^+ \rightleftarrows H^+ + E$, were calculated using the equation:

$$pK_E = pH + \log \gamma_{\pm} + \log \frac{E_{Tot} - ([Na^+] + [H^+] - [OH^-])}{[Na^+] + [H^+] - [OH^-]} \qquad (5)$$

This equation was derived from the following expressions for electroneutrality, total ester concentration and the ionization constant.

$$EH^+ + Na^+ + H^+ = [OH^-] + [Cl^-]$$

$$E_{Tot} = [EH^+] + [E] = [Cl^-] = [ester]$$

$$K_E = [E]\ [H^+] \ / \ [EH^+]$$

The values of pK_E given in Table 13 are an average of 8-10 determinations calculated over the range of 20-80% of titration of the ester hydrochloride with sodium hydroxide.

Hydroxo-complex formation constants, K_{fOH}, $CuIMDA + OH^- \rightleftarrows Cu(IMDA)(OH^-)$, were determined by pH titration for the hydroxo-complexes, $[Cu(IMDA)(OH)]^-$. These values were used as a correction factor where small amounts of the

Table 13. Ionization constants of amino acid esters at 25.0° and 0.05 M KNO_3

Ester	pK_E	Ester	pK_E
methyl glycinate	7.62[a]	ethyl leucinate	7.64[a]
ethyl glycinate	7.68[a]	ethyl valinate	7.75[b]
n-butyl glycinate	7.78[b]	ethyl B-alaninate	9.13[b]
methyl alaninate	7.85[b]	ethyl valinate-N,N-diacetate	6.75
ethyl alaninate	7.91[b]	ethyl glycinate-N,N-diacetate	6.60
ethyl sarcosinate	8.10		
L-methyl leucinate	7.63		
D-methyl leucinate	7.63		

[a](4).

[b](5).

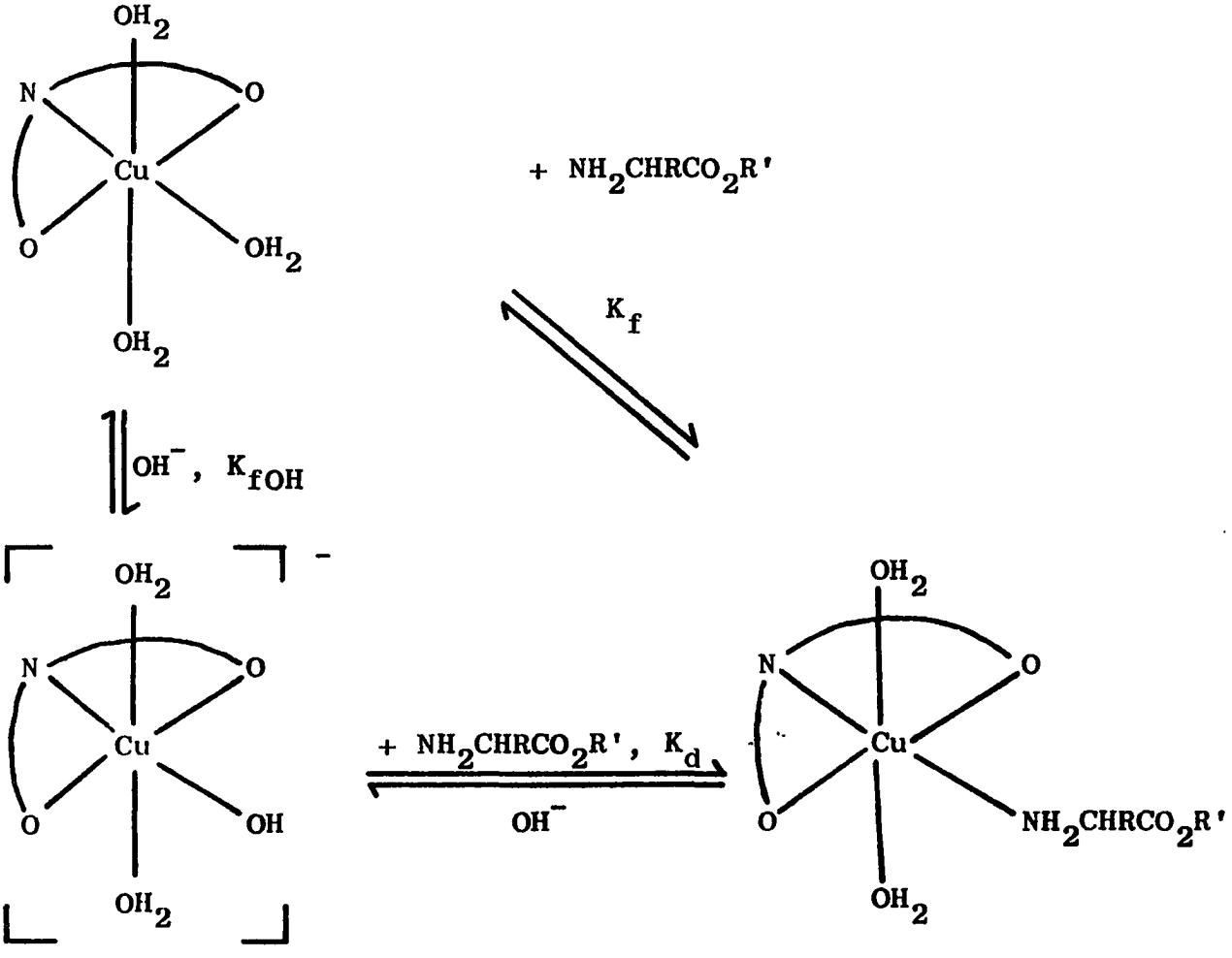
hydroxocomplex were formed and are valid only over approximately the first 25% of the titration of the copper(II) iminodiacetate complex with sodium hydroxide. Table 14 gives the values of K_{fOH} which were determined.

Table 14. Hydroxo-complex formation in copper(II) complexes of substituted iminodiacetates at 25.0°[a]

Cu(iminodiacetate)	K_{fOH}
iminodiacetate	1.8×10^6
methyliminodiacetate	6.4×10^5
cyclohexyliminodiacetate	6.0×10^5
tert-butyliminodiacetate	1.9×10^5
furfuryliminodiacetate	1.1×10^5
phenyliminodiacetate	2.5×10^6
D-phenylglycine-N-monoacetate	5.0×10^4
L-valine-N-monoacetate	6.2×10^4
[(hexahydro-2,4,6-trioxo-5-pyrimidinyl)-imino]diacetate(uramildiacetic acid)	3.8×10^4

[a][KNO_3] = 0.05 M.

Potentiometric titrations of 1:1 mole ratios of [Cu(IMDA)] with ethyl valinate show that two equivalents of NaOH are added in the pH range up to about pH 10. The addition of a second equivalent is postulated as due to formation of an hydroxo species. The data from these titrations show that the following equilibria are involved:



$$K_d = [M(IMDA)(OH)]^-[E] \;/\; [M(IMDA)(E)][OH^-] = K_{fOH}/K_f$$

where K_f is the formation constant of the ester with Cu(IMDA). The value of K_d can be calculated from the expression:

$$\log K_d = \log ([E]_{Tot}/2) + pK_w - pH_{3/2\ equiv} + \log \gamma\pm$$

where $pH_{3/2\ equiv}$ is the pH of the solution when 3/2 mole of NaOH has been added per mole of Cu(IMDA). The values obtained for log K_d were 2.7 and 3.1 using the values of K_{fOH} and K_f and the equation given above respectively. Considering the error, particularly in K_{fOH}, these values are in fair agreement.

Formation constants were calculated by a computer for 20-25 experimental points in the 20-80% completion range of the potentiometric titration with NaOH of solutions of Cu(IMDA) and the amino acid or ester. The average formation constant and its standard deviation were computed for the range 20 to 80% titration. The average K_f value was used to calculate theoretical titration data which in all cases reproduced the experimental data quite well as shown in Figure 4.

The computable relationship for amino acid esters, HE^+, complexing with CuZ in a 1:1 mole ratio is

$$K_f = \frac{(\beta-\delta)[(\beta-\delta)\ M_{Tot} - \alpha\gamma(1+\delta)]}{\alpha\gamma^2(1+\delta)} \qquad (6)$$

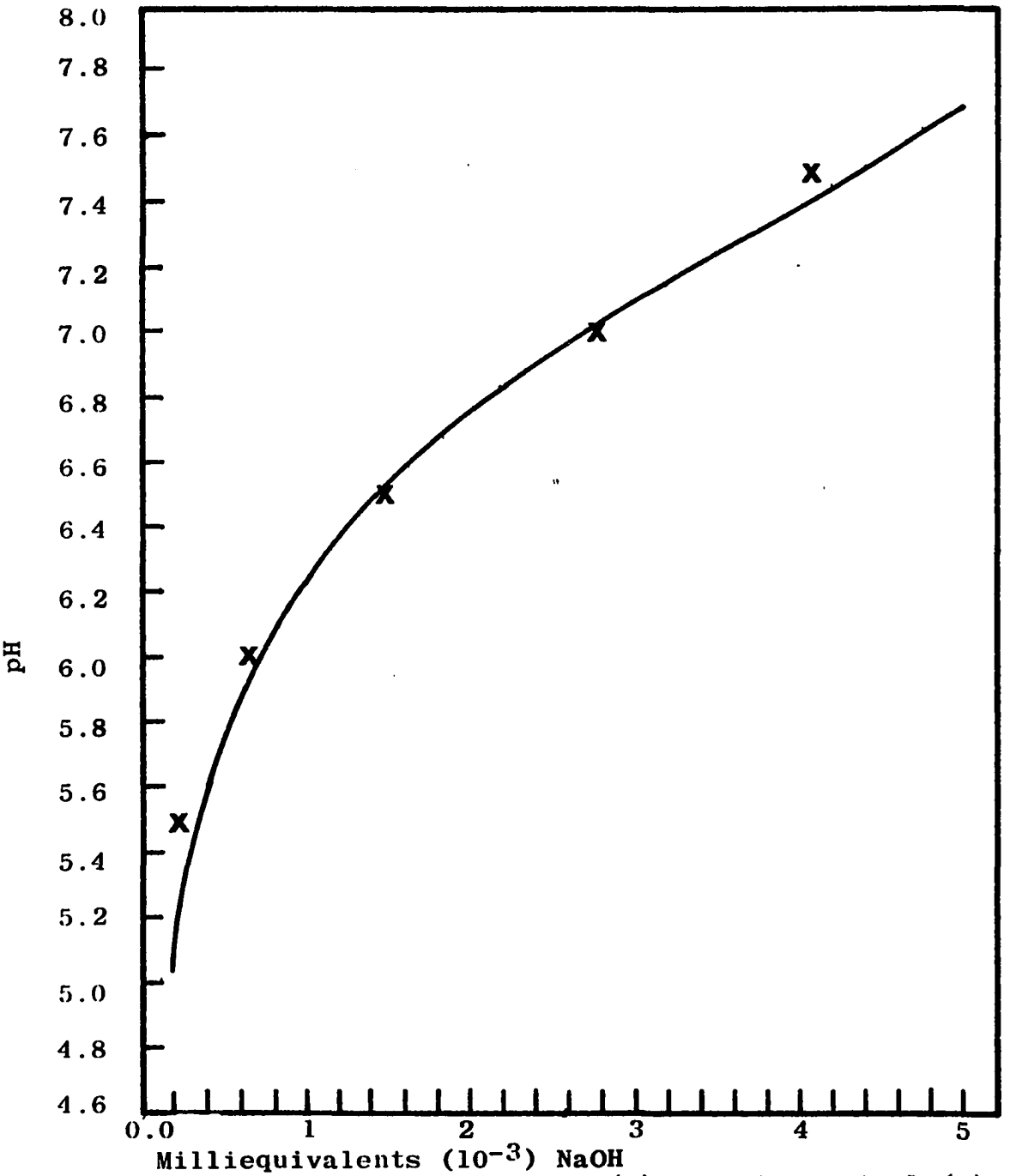


Figure 4. Plots of experimental (-) and theoretical (x) titration curve for 0.005 meq. Cu(L-valine-N-monoacetate) and L-leucine ethyl ester + NaOH

where $\alpha = 1 + [H^+]/K_E$

$\beta = [H^+]/K_E$

$\gamma = M_{Tot} + [OH^-] - [Na^+] - [H^+]$

and $\delta = K_{fOH}[OH^-]$

was obtained from the definitions of the ionization constant of the amino acid ester, K_E; the hydroxo-formation constant, K_O ($CuZ + OH^- \rightleftarrows CuZOH^-$); and the formation constant, K_f, for coordination of the amino acid ester to CuZ, together with the equations for total amino acid ester concentration, total CuZ concentration and electroneutrality.

$$K_f = \frac{[CuZE]}{[CuZ][E]} ; \quad K_{OH} = \frac{[CuZ(OH)^-]}{[CuZ][OH^-]} ;$$

$$K_E = \frac{[E][H^+]}{[HE^+]}$$

Total ester:

$$E_{Tot} = [E] + [EH^+] + [CuZE] = [Cl^-]$$

Total CuZ:

$$CuZ_{Tot} = [CuZ] + [CuZE] + [CuZOH^-]$$

Electroneutrality:

$$[Na^+] + [EH^+] + [H^+] = [CuZOH^-] + [OH^-] + [Cl^-].$$

The corresponding computable relationship for K_f using 1:1 mole ratios of CuZ and an amino acid ester is

$$K_f = \frac{[\beta + (\beta-\delta)\delta][\beta + (\beta - \alpha)\delta]\ CuZ_{Tot} - \alpha\gamma(1 + \delta)]}{\alpha\gamma^2(1 + \delta)}$$

where

$$\alpha = 1 + [H^+]/K_a2 + [H^+]^2/K_a1\ K_a2$$

$$\beta = [H^+]/K_a2 + 2\ [H^+]^2/K_a1\ K_a2$$

$$\gamma = 2\ M_{Tot} + [OH^-] - [Na^+] - [H^+]$$

$$\delta = K_{OH}\ [OH^-]$$

and the following definitions of equilibrium constants were utilized together with equations for total amino acid concentration, total CuZ concentration and electroneutrality.

$$K_f = \frac{[CuZA^-]}{[CuZ][A^-]}\ ; \quad K_{OH} = \frac{[CuZ\ OH^-]}{[CuZ][OH^-]}\ ;$$

$$K_{a1} = \frac{[AH][H^+]}{[AH_2^+]}\ ; \quad K_{a2} = \frac{[A^-][H^+]}{[HA]}$$

Total acid:

$$A_{Tot} = [A^-] + [AH] + [AH_2^+] + [CuZA^-]$$

Total CuZ:

$$CuZ_{Tot} = [CuZ] + [CuZA^-] + [CuZOH^-]$$

Electroneutrality:

$$[Na^+] + [H^+] + [AH_2^+] = [A^-] + [CuZA^-] + [CuZOH^-]$$

No deviations were observed that would indicate formation of a bis ester or acid complex under the conditions used.

Calculated values of K_f for several amino acids and esters with Cu(IMDA) and IMDA derivatives are given in Table 15. The formation constants for the complexation of the ester to the metal iminodiacetate complex are needed if the rates of hydrolysis are dependent on the concentration of the metal iminodiacetate complex, MZ, to calculate the concentration of $MZ(NH_2CHRCOOR')$. Hydrolysis of complexes $MZ(NH_2CHRCOOR')$

$$MZ + \overset{+}{N}H_3CHRCOOR' \rightleftarrows MZ(NH_2CHRCOOR') + H^+ \qquad (7)$$

where M was copper(II) and Z was a substituted iminodiacetate, $RN(CHR'COOH)(CH_2COOH)$, gave pseudo-first-order rate constants at various pH values at 25.0° and 0.05 M ionic strength which are listed in Table 16. Under the experimental conditions no hydrolysis due to uncoordinated ester was observed or expected at the relatively low pH values of this study (3,4,5). The pH dependence observed and the effect on the pseudo-first-order rate constant of doubling the concentration of the copper(II) substituted iminodiacetate complex is given in Table 17. The non-linear least squares evaluation of k and k_{H_2O} which are presumably the rate of attack by hydroxide ion and water respectively (see Discussion and Conclusion) on the ester complex is given in Table 18. The ester may or may not be completely coordinated to the copper(II) iminodiacetate complex (Equation 7). If it is completely coordinated, the dependence of k_{obsd} on hydroxide ion concentration is k_{obsd} =

Table 15. Formation constants of CuZ with amino acids and esters at 25.0° and 0.05 M KNO_3

Z	ligand	log K_f	Stand. dev.
IMDA	glycine	6.42	0.06
IMDA	alanine	6.27	0.06
IMDA	leucine	6.53	0.04
IMDA	valine	6.17	0.06
IMDA	ethyl valinate	3.57	0.06
IMDA	butyl glycinate	3.69	0.06
methylIMDA	valine	6.19	0.02
methylIMDA	butyl glycinate	4.33	0.07
methylIMDA	ethyl valinate	3.19	0.02
cyclohexylIMDA	valine	6.58	0.03
cyclohexylIMDA	ethyl leucinate	3.21	0.05
cyclohexylIMDA	butyl glycinate	4.05	0.04
tert-butylIMDA	valine	5.54	0.07
tert-butylIMDA	ethyl leucinate	2.61	0.02
tert-butylIMDA	butyl glycinate	3.50	0.02
phenylIMDA	valine	6.77	0.02
phenylIMDA	ethyl leucinate	2.60	0.01
phenylIMDA	butyl glycinate	3.52	0.01
furfurylIMDA	valine	6.64	0.03
furfurylIMDA	ethyl leucinate	2.42	0.01
furfurylIMDA	butyl glycinate	3.88	0.06
uramilidiacetic acid	valine	4.57	0.04
uramilidiacetic acid	ethyl leucinate	3.05	0.02
uramilidiacetic acid	butyl glycinate	3.38	0.02
L-valine-N-monoacetate	L-valine	5.41	0.01
L-valine-N-monoacetate	D-leucine	4.93	0.10
L-valine-N-monoacetate	L-leucine	5.74	0.05

Table 15. (Continued)

Z	ligand	log K_f	Stand. dev.
L-valine-N-monoacetate	ethyl L-leucinate	3.73	0.06
L-valine-N-monoacetate	ethyl D-leucinate	3.20	0.06
L-valine-N-monoacetate	L-alanine	5.29	0.02
L-valine-N-monoacetate	D-alanine	4.74	0.10
D-phenylglycine-N-monoacetate	L-valine	4.35	0.04
D-phenylglycine-N-monoacetate	L-ethyl leucinate	3.02	0.03

Table 16. Rates of ester hydrolysis as catalyzed by copper(II) complexes of substituted iminodiacetates at 25.0°

pH	10^4 k_{obsd}(sec.$^{-1}$)[a]	pH	10^4 k_{obsd}(sec.$^{-1}$)[2]
Cu(iminodiacetate)(methyl glycinate)			
4.80	0.0650	6.10	2.84
5.00	0.0973	6.20	4.10
5.20	0.172	6.30	6.00
5.30	0.201	6.40	9.34
5.36	0.226	6.50	13.6
5.50	0.350	6.60	19.8
5.70	0.622	6.70	33.8
5.80	1.07	6.80	42.5
6.00	1.68	6.90	71.0
6.10	2.86		
Cu(iminodiacetate)(ethyl glycinate)			
5.70	0.200	6.30	2.00
5.90	0.342	6.40	3.27
6.00	0.512	6.50	4.92
6.10	0.769	6.60	7.92
6.20	1.30	6.70	11.3
Cu(iminodiacetate)(butyl glycinate)			
6.10	0.708	6.40	2.80
6.20	0.927	6.50	4.05
6.30	1.74	6.60	5.72
Cu(iminodiacetate)(ethyl L-leucinate)			
6.10	0.428	6.50	1.45
6.20	0.615	6.60	2.05
6.30	0.755	6.70	3.22
6.40	1.02	6.80	5.74
Cu(iminodiacetate)(ethyl sarcosinate)			
6.10	0.680	6.40	1.76
6.20	0.859	6.50	2.65
6.30	1.15	6.60	4.27

[a][Cu(IMDA)] = 0.0033 M; [ester] = 0.00033 M; [KNO_3] = 0.05 M.

Table 16. (Continued)

pH	10^4 k_{obsd}(sec.$^{-1}$)[a]	pH	10^4 k_{obsd}(sec.$^{-1}$)[a]
Cu(iminodiacetate)(methyl alaninate)			
5.50	0.406	6.40	4.87
5.70	0.581	6.50	8.54
5.90	0.912	6.60	11.1
6.10	1.58	6.70	43.8
6.30	3.43		
Cu(methyliminodiacetate)(methyl glycinate)			
5.60	0.125	6.40	0.905
5.70	0.153	6.50	1.08
5.80	0.204	6.60	1.42
5.90	0.266	6.70	1.55
6.00	0.300	6.80	2.12
6.10	0.426	6.90	2.37
6.20	0.550	7.00	3.43
6.30	0.656		
Cu(tert-butyliminodiacetate)(methyl glycinate)			
6.10	1.05	6.50	2.62
6.20	1.40	6.60	3.31
6.30	1.74	6.70	4.17
6.40	2.10	6.80	5.30
Cu(cyclohexyliminodiacetate)(methyl glycinate)			
5.70	0.830	6.30	4.17
5.80	1.06	6.40	5.18
5.90	1.45	6.50	6.36
6.00	1.75	6.60	8.10
6.10	2.32	6.70	9.79
6.20	3.16	6.80	12.1
Cu(furfuryliminodiacetate)(methyl glycinate)			
6.40	0.570	7.30	4.32
6.70	1.11	7.60	8.23
7.00	2.01		

Table 16. (Continued)

pH	10^4 k_{obsd}(sec.$^{-1}$)[a]	pH	10^4 k_{obsd}(sec.$^{-1}$)[a]
Cu(phenyliminodiacetate)(methyl glycinate)			
6.30	2.55	6.90	11.3
6.60	5.50		
Cu(uramildiacetate)(methyl glycinate)			
8.30	12.2	8.80	35.7
8.50	16.8		
Cu(D-phenylglycine-N-monoacetate)(methyl glycinate)			
6.10	1.49	6.40	6.65
6.30	3.64	6.40	6.35
6.30	3.98		
Cu(L-valine-N-monoacetate)(methyl glycinate)			
6.80	1.52	7.30	5.68
7.00	2.13	7.40	7.64
7.10	2.88	7.50	10.1
7.20	3.98		

$k[OH^-]$ and the overall rate of hydrolysis is given by the expression:

$$\text{Rate} = k\,[Cu(IMDA)(NH_2CHRCOOR')]\,[OH^-]$$

If the ester is not completely coordinated, according to Equation 7, the concentration of $Cu(IMDA)(NH_2CHRCOOR')$ depends on the hydroxide ion concentration. Thus k_{obsd} depends on the hydroxide ion concentration as $k_{obsd} = k\,[OH^-]^2$ and the overall rate of hydrolysis is given by the expression:

Table 17. Hydroxide and copper(II) substituted iminodiacetate concentration dependence of the rates of hydrolysis of methyl glycinate

Z	pH	10:1 Cu:ester 10^4 k_{obsd} $(sec.^{-1})^a$	20:1 Cu:ester 10^4 k_{obsd} $(sec.^{-1})^b$	Kinetic order in OH^-
IMDA	6.3	6.00	12.00	first,second
methylIMDA	6.6	1.32	1.55	first
phenylIMDA	6.3	2.55	2.68	first
furfurylIMDA	6.4	0.570	0.555	first
cyclohexylIMDA	6.1	2.32	2.42	first
tert-butylIMDA	6.1	1.05	1.10	first
uramildiacetate	8.3	12.2	15.7	first
D-phenylglycine-N-monoacetate	6.3	3.98	7.90	second
L-valine-N-monoacetate	7.4	7.64	8.24	first
	7.2	3.98	4.95	first
	7.0	2.13	3.42	second
	6.8	1.52	2.40	second

[a] [CuZ] = 0.0033 M; [ester] = 0.00033 M; [KNO_3] = 0.050 M.

[b] [CuZ] = 0.0067 M; [ester] = 0.00033 M; [KNO_3] = 0.040 M.

$$\text{Rate} = k' [Cu(IMDA)][HE^+][OH^-]^2$$

or

$$\text{Rate} = k_2 \frac{K_f K_E}{K_w} [Cu(IMDA)(NH_2CHRCOOR')][OH^-]$$

where K_w is the autoionization constant of H_2O, K_f is the formation constant of the amino acid ester with the Cu(II) complex of the particular IMDA derivative, and K_E is the ionization constant of the amino acid ester. In the cases

that water attack on the ester complex (see Discussion and Conclusion) is appreciable the observed rate is

$$\text{Rate} = (k_{H_2O} + k\,[OH^-])\,\frac{K_f K_E}{K_w}\,[Cu(IMDA)(NH_2CHRCOOR')]$$

Water attack was measurable only for Cu(IMDA) itself complexed with esters with methyl esters being most susceptible to H_2O attack. Using the values of the autoionization constant of H_2O, K_w, the ionization constant of the amino acid ester, K_E, and the formation constant of the amino acid ester with the Cu(II) complex of the particular IMDA derivative, K_f, the values of the second-order rate constant due to water attack and the second-order rate constant due to hydroxide ion attack on the ester carbonyl carbon atom were calculated from the observed rate constant, k_{obsd}. The nitrogen-substituted iminodiacetates were shown (Table 17) to exhibit the experimental rate law

$$\text{Rate} = k\,[Cu(IMDA)(NH_2CHRCOOR')][OH^-]$$

That the rate constants, k_{obsd}, did not change within experimental error when the CuZ concentration at the given pH was increased from 10:1 MZ: ester to 20:1 was taken to indicate that at the given pH and higher pH values the ester was totally complexed. With few exceptions the formation constant data support the kinetic evidence, however; in the case of tert-butyl iminodiacetate and phenyliminodiacetate the ester would not be completely coordinated at pH 6.1 with a MZ: ester ratio of 10:1 according to the calculated formation

Table 18. Rates of hydrolysis of amino acid esters with copper(II) complexes of substituted iminodiacetates at 25.0°[a]

Amino acid ester[c]	Cu(iminodiacetate)	10^{-4} k[b] ($M^{-1}sec.^{-1}$)	10^{6} k_{H_2O} ($M^{-1}sec.^{-1}$)
MeGly	Cu(IMDA)	3.21	4.05
EtGly	Cu(IMDA)	1.85	2.70
BuGly	Cu(IMDA)	1.39	1.39
EtLeu	Cu(IMDA)	0.435	----
EtSar	Cu(IMDA)	2.10	1.75
MeAla	Cu(IMDA)	2.62	6.42
EtBetaine	Cu(IMDA)	no reaction	
benzoyl-glycine methylester	Cu(IMDA)	no reaction	
MeGly	Cu(MeIMDA)	0.329	<0.1
MeGly	Cu(PhIMDA)	1.41	
MeGly	Cu(t-BuIMDA)	0.838	
MeGly	Cu(cyclohexylIMDA)	1.97	
MeGly	Cu(furfurylIMDA)	0.208	
MeGly	Cu(uramildiacetate)	0.056	
MeGly	Cu(D-phenylglycine-N-monoacetate)	2.3	
MeGly	Cu(L-valine-N-monoacetate)	0.317	

[a][CuZ] = 0.0033 M, [ester] = 0.00033 M, [KNO_3] = 0.050 M.

[b]Rate constants have standard deviations $\leq$10%.

[c]Common abbreviations, Me = methyl, Ph = phenyl, Et = ethyl, Bu = butyl, Gly = glycine, Leu = leucine, Ala = alanine, Sar = sarcosine.

constants. However, the formation constants for the complexation of methyl glycinate may be slightly larger than that for butyl glycinate. The formation constants of the

Cu(II) N-substituted iminodiacetates with amino acid esters vary considerably with the steric hinderance of the ester as shown in Table 15. Iminodiacetate itself exhibits small changes in log K_f for various amino acids and amino acid esters so that the log K_f value of 3.57 obtained for ethyl valinate was used for all the amino acid esters studied. For methyl glycinate the value of log K_f of 3.57 was also used for Cu(D-phenylglycine-N-monoacetate). Comparison of the formation constants of Cu(substituted iminodiacetates) with ethyl leucinate and butyl glycinate indicate that substituents on the nitrogen atom of the iminodiacetate sterically interact with large bulky groups on the amino acid or amino acid ester. In such cases the formation constants for butyl glycinate are substantially higher than those for ethyl leucinate.

Cu(L-valine-N-monoacetate) was found to show stereoselectivity in coordinating to D and L amino acids and esters with the formation constant of the L isomer being greater than that for the D isomer. The difference in formation constant between the D and L isomers is less for D- and L-alanine than for D- and L-leucine indicating that large bulky groups on the ester favor stereoselectivity with Cu(L-valine-N-monoacetate). Kinetic data were obtained for the hydrolysis of [Cu(L-valine-N-monoacetate)(L-(D)-leucine methyl ester)]. These are given in Table 19. A plot showing the pH dependence of the observed rate constants is given in Figure 5. The

Table 19. Rates of copper(L-valine-N-monoacetate) catalyzed hydrolysis of D- and L-leucine methyl ester at 25.0°[a]

D-leucine methyl ester			L-leucine methyl ester		
pH	ratio[b]	10^4 k_{obsd}(sec.$^{-1}$)	pH	ratio[b]	10^4 k_{obsd}(sec.$^{-1}$)
6.80	10:1	0.333	6.70	10:1	0.445
6.90	10:1	0.519	6.80	10:1	0.616
7.00	10:1	0.795	6.90	10:1	0.806
7.10	10:1	0.979	7.00	10:1	1.01
7.20	10:1	1.41	7.10	10:1	1.42
7.20	20:1	2.54	7.20	10:1	1.85
7.20	30:1	4.10	7.30	10:1	2.22
7.20	40:1	5.27	7.40	10:1	2.79
7.20	60:1	5.42	7.50	10:1	3.48
7.40	10:1	2.78	7.60	10:1	4.55
7.50	10:1	4.25	7.90	10:1	9.36
7.60	10:1	5.14			
7.70	10:1	6.14			
7.90	10:1	10.1			
8.20	10:1	20.3			

[a]10:1 = [CuZ] : [ester] = 0.0033 M : 0.00033 M; [KNO_3] = 0.05 M.

[b]20:1 = [CuZ] : [ester] = 0.0067 M: 0.00033 M, etc.

second-order rate constants for the hydrolysis of the complexes with D- and L-leucine methyl ester are different. The second-order rate constant for the D isomer is 1.3×10^3 M^{-1} sec.$^{-1}$ whereas for the L isomer the second-order rate constant is 1.0×10^3 M^{-1} sec.$^{-1}$. At pH 7.4 using a 2:1 ratio of Cu(L-valine-N-monoacetate) to amino acid ester the observed rates of hydrolysis were 1.80×10^{-4} sec.$^{-1}$ and 1.04×10^{-4} sec.$^{-1}$ for the L and D isomers of leucine methyl ester respectively.

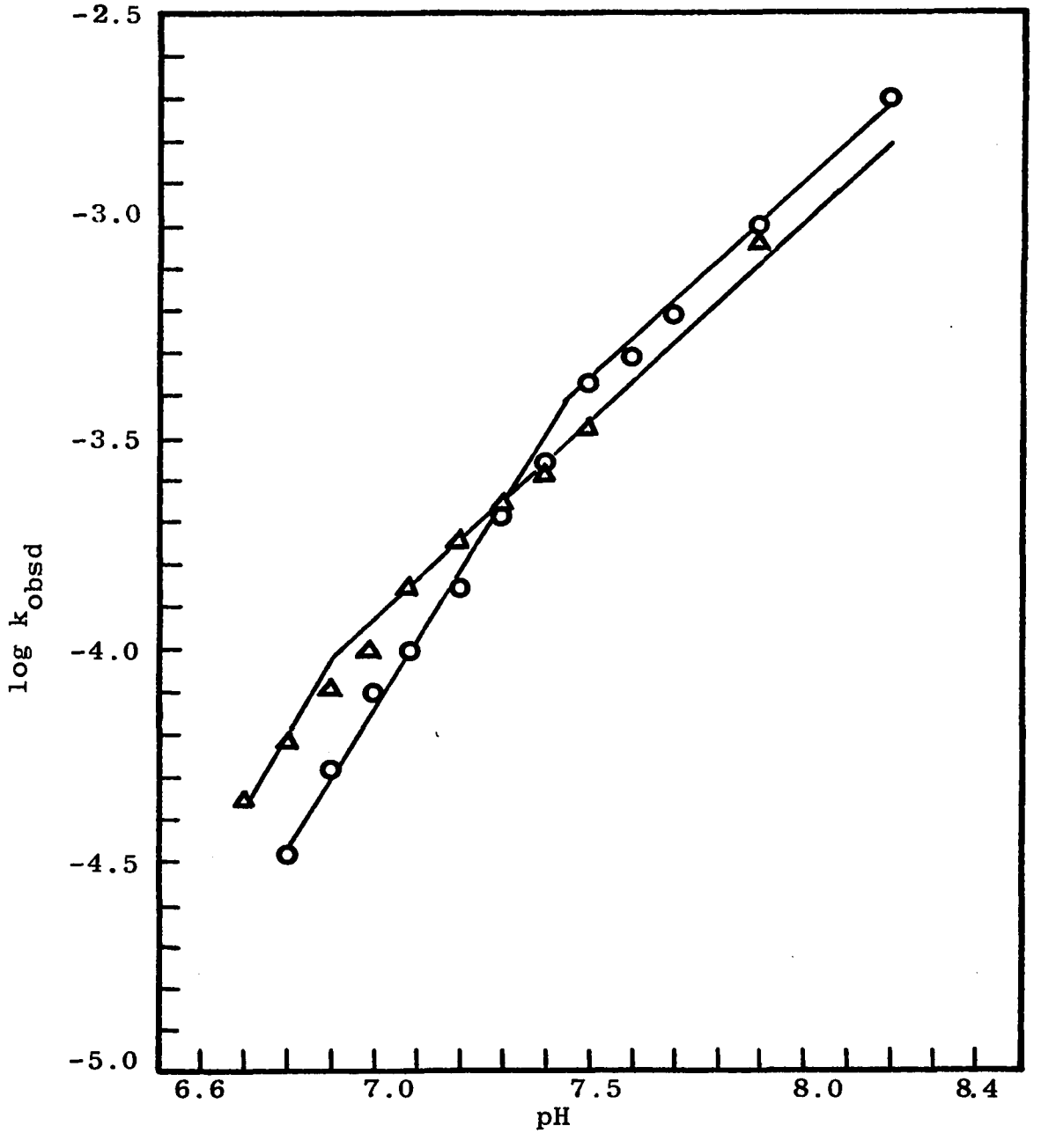


Figure 5. Plot of log k_{obsd} *vs.* pH for the hydrolysis of Cu(L-valine-N-monoacetate) complexes of D(O) and L(Δ) methyl leucinate

Thus by a choice of optimum reaction conditions one may obtain a 1.75 factor between the rates of hydrolysis of D- and L-leucine methyl ester complexed to Cu(L-valine-N-monoacetate).

Measurements obtained on a Jasco Optical Rotatory Dispersion recorder Model ORD/UV-5 indicated that neither D-phenylglycine nor L-valine racemized under the conditions for the preparation of the N-monoacetates, namely, 3 hours at pH 11 and a temperature of 70°C. ORD measurements further indicated that there was no change in optical rotation of a freshly prepared copper(II) complex of D-phenylgycine-N-monoacetate and L-valine-N-monoacetate with time over a period of several days and at a higher pH of 7.5 no change in optical rotation was observed in a five hour period. No stereoselectivity was observed for the hydrolysis of D- and L-leucine methyl ester complexed to Cu(D-phenylglycine-N-monoacetate) as at pH 6.3 the pseudo-first-order rate constants for D- and L-leucine methyl ester hydrolysis were 3.08×10^{-4} sec.$^{-1}$ and 2.86×10^{-4} sec.$^{-1}$ respectively which are the same within experimental error. D- and L-methyl leucinate exhibited pseudo-first-order rates of hydrolysis which were the same within experimental error (1.13×10^{-3} sec.$^{-1}$ and 1.17×10^{-3} sec.$^{-1}$ at pH 6.8 respectively) when these esters were complexed with

Cu(cyclohexyliminodiacetate) which has no optical activity. This suggests that steric interaction between optically active centers on a complex and an ester bearing an optical center may lead to a difference in formation constants for the two optically active isomers of amino acid ester..

DISCUSSION AND CONCLUSION

Amino Acid Ester-N,N-diacetic Acids

The amino acid ester-N,N-diacetic acids investigated (60,61) are strongly coordinated to many metal ions through the iminodiacetate group (see Experimental and Results). The configuration of the amino acid ester-N,N-diacetate about the metal ion in these labile complexes is unknown although for copper(II) the most likely structure is a meridional arrangement of the iminodiacetate group. Cobalt(III), however, has been shown to prefer a facial configuration of the iminodiacetate group (53) indicating that different metal ions may prefer different configurations in aqueous solutions.

In aqueous solution the amino acid ester-N,N-diacetate complexes exist in a form in which the ligand which has four potential coordinating sites is tridentate. The low ratio of the first formation constant, K_1, to the second stepwise formation constant, K_2, indicates (33) that while the ester function is a potential fourth coordinating site (Equation 2) it is not or only slightly coordinated to the metal ions copper(II), lead(II), nickel(II), cobalt(II) and samarium(III). The linear correlation for tridentate substituted imino-diacetates, where R is a non-coordinating group, between log K_1 and the pK_a for the ^+NH proton of $[RN(H)(CH_2COO)_2]^-$ is

followed also by ethyl valinate-N,N-diacetate for copper(II), lead(II), cobalt(II), and nickel(II).

No evidence for ester carbonyl oxygen coordination was observed in the ir spectra of a number of ethyl glycinate-N,N-diacetate complexes in D_2O (Table 2). If coordination of the ester carbonyl oxygen occurs the stretching frequency of the carbonyl group should be lowered 50-100 $cm.^{-1}$ from the free ester value (26,29,30). No lower stretching frequency due to ester carbonyl coordination was observed in D_2O solution and the small variations which occurred among the metal ion complexes did not correlate with the second-order rate constants determined for ester hydrolysis. The infrared peaks were broad and inconclusive as was an nmr study (Table 3) of the effect of coordination to a metal ion on the methylene protons in the $-NCH_2COOC_2H_5$ group in ethyl glycinate-N,N-diacetate which showed small upfield shifts for these protons upon coordination to lanthanum(III) compared to a somewhat larger upfield shift in the corresponding methyl group in methyliminodiacetate.

The ir spectrum of solid $Ba[C_2H_5OOCCH(CH(CH_3)_2)N(CH_2COO^-)_2]$ taken in a KBr pellet compared to the spectrum obtained for $Cu[C_2H_5OOCCH(CH(CH_3)_2)N(CH_2COO^-)_2]$ indicates that no change in the stretching frequency of either the ester carbonyl oxygen or ether oxygen occurs when the ligand is complexed with copper(II) in the solid state. The characteristic

absorptions were 1725 $cm.^{-1}$ (C=O ester) and 1200 and 1142 $cm.^{-1}$ (ester). This is consistent with the long Cu-O distance of approximately 2.85 Å found for the Cu-ether oxygen atom by R. A. Jacobson and J. Rodgers[1] in an x-ray crystallography study of copper(II) ethyl valinate-N,N-diacetic acid. In this structure the carbonyl ester oxygen was further away at over 3.0 Å from the metal ion. At these long distances presumably little Cu-O bonding occurs and in solution water molecules would compete for the coordination position. While the preferred coordination site in the ester group is ether oxygen in this particular case in the solid state, this may not be true in general due to the steric hinderance of ethyl valinate-N,N-diacetate and packing considerations of the DL isomers in this study. More crystallographic evidence would help substantiate the preferred configuration in the solid state. Even then extrapolation to aqueous solution would be questionable due to the weak metal to ester interactions.

In aqueous solution less Cu-O coordination would be expected and no direct evidence for metal ion-carbonyl ester oxygen coordination was found in this study. Carbonyl ester oxygen coordination in the inert complex of cobalt(III) cis-$[Co(en)_2(NH_2CH_2COOC_2H_5)]Cl_2$ was postulated to account for

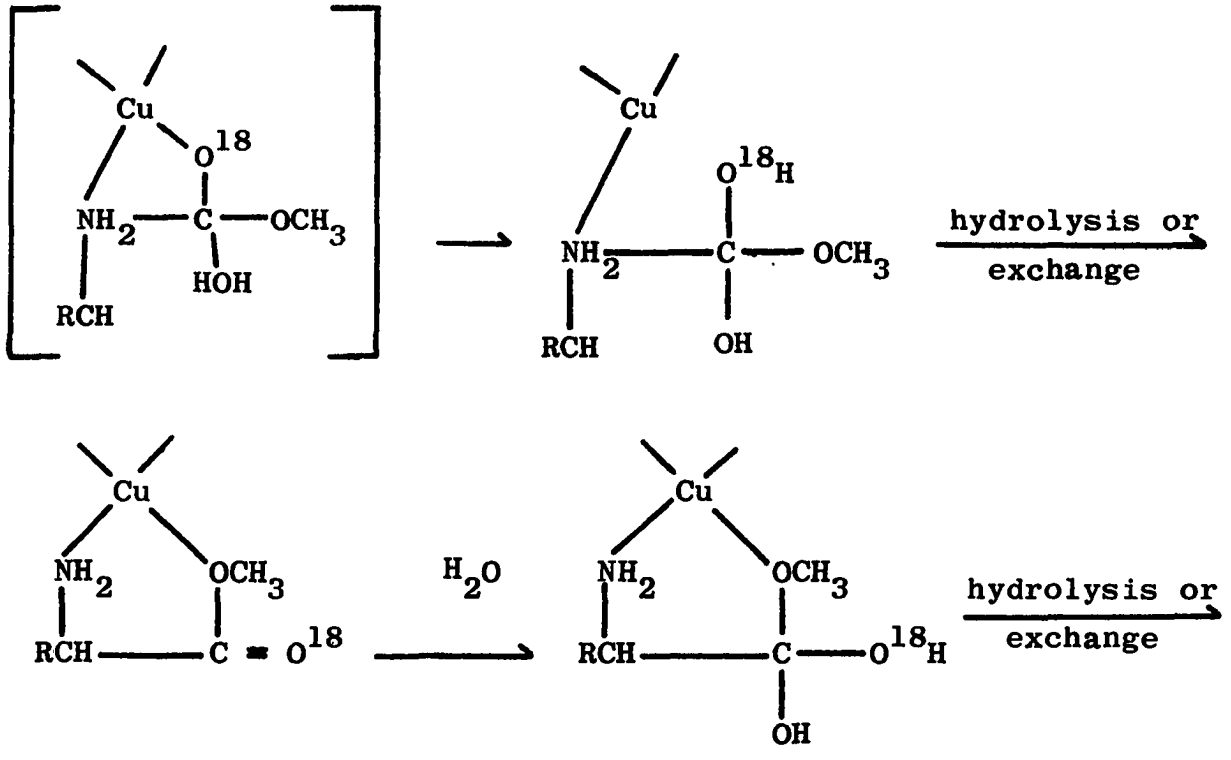
---

[1]R. A. Jacobson, and J. Rodgers, Iowa State University, Ames, Iowa. Data for the crystal structure of copper(II) ethyl valinate-N,N-diacetic acid. Private Communication. 1968.

the lowering of the C=O infrared stretching frequency. This suggests that a rapid equilibrium exists in labile systems between chelated and monodentate amino acid ester and that this undetermined constant, K, is very small in most instances. However, as was pointed out by Bender and Turnquest (19) either oxygen atom could coordinate to the metal atom and withdraw electron density from the carbonyl carbon atom thereby facilitating nucleophilic attack at the carbonyl carbon. The carbon oxygen π system would be expected to be the better nucleophile toward the electrophilic metal ion and for this reason carbonyl ester oxygen coordination has been proposed most frequently with generally no supporting experimental evidence. If analogy is made to the mechanism of acid catalyzed hydrolysis of esters in which protonation occurs at the carbonyl ester oxygen to render the carbonyl carbon more susceptible to nucleophilic attack by H_2O and to give a symmetrical tetrahedral intermediate which has been established beyond all reasonable doubt by O^{18} exchange experiments, then the metal ion acting as an acid would be expected to coordinate to the same position. E. L. Smith and D. H. Spackman (62) have studied the action of leucine aminopeptidase in the presence of Mn(II) or Mg(II) to hydrolyze amino acid amides. They concluded the metal interaction is with the nitrogen of the susceptible bond as is expected due to the greater bascity of the nitrogen compared to the carbonyl oxygen atom. This also explains

the absence of esterase action on amino acid esters by leucine aminopeptidase. In amino acid esters the most basic site in the ester function is the carbonyl oxygen atom. Collman and Kimura (63) have obtained proof of peptide formation through activation of the glycine ester group by coordination with Co(III) followed by nucleophilic attack by the amino group on another amino acid or peptide reagent in cis-chlorotriethylene-tetramine-glycine ester cobalt(III) complexes.

Unfortunately all the proposed arguments in favor of carbonyl ester oxygen coordination lack experimental evidence in the systems under consideration and although ester carbonyl oxygen coordination is the favored mechanism because the transition state would contain a partially negative carbonyl oxygen atom which could be stabilized to a greater extent electrostatically than the ether oxygen which is essentially neutral in the transition state it is not possible to decide between these two possible mechanisms for both should lead to an enhanced reaction rate due to polarization of the carbonyl carbon atom. The transition state involving coordination of the ether oxygen atom would unambiguously permit the formation of a tetrahedral intermediate in which two oxygen atoms are equivalent and therefore capable of exchange as found by Bender and Turnquest (19) and would be in agreement with the x-ray structure of the solid [Cu(ethyl valinate-N,N-diacetate)$(H_2O)_2$].



The amino group has been shown to be essential to the promotion of the ester hydrolysis (19). As will be discussed subsequently, the chelating of the amino acid ester may be strongly entropy controlled and the function of the amino group is to coordinate the ester initially and provide an inductive effect which enhances the rate of hydrolysis. This inductive effect has been found to be on the order of 30-100 fold increase in the rate of hydrolysis. Increasing the charge on the nitrogen atom has been shown (4) to give a catalysis constant (rate catalyzed/rate uncatalyzed) of 36 for betaine ethyl ester compared to ethyl glycinate.

Similarly where no metal-ester oxygen interaction is involved, namely for cysteine methyl ester (4,18), histidine methyl ester (25), Cu(NTA)(methyl histidinate) (5), and cis-chlorobis-(ethylenediamine) ethyl glycinate cobalt(III) chloride (26, 27) low catalysis constants were obtained. In contrast, copper(II) catalyzes the rate of hydrolysis of ethyl glycinate by a factor of 1.2×10^5 (3) and catalysis factors of the magnitude of $10^4 - 10^5$ were found in this investigation. The rate constants for the hydrolysis of [Cu(ethyl glycinate-N,N-diacetate)]^{o} and [Cu(ethyl glycinate)]$^{+2}$ are surprisingly similar in view of the formal charges of zero and +2 on the respective complexes. This indicates that an inductive model is not adequate to explain the experimental data and some metal ion-ester oxygen interaction is necessary to further withdraw electron density from the carbonyl carbon thereby facilitating nucleophilic attack.

The kinetic investigation of the hydrolysis of metal ion amino acid ester-N,N-diacetates to form the corresponding amino acid-N,N-diacetate complexes indicated that the rate of hydrolysis was first-order in metal-ester complex and first-order in hydroxide ion concentrations. Several mechanisms were postulated in the Results section of this thesis which are consistent with the experimental data. The most probable mechanisms are as follows: A) hydroxide ion attack at the carbonyl carbon atom of the ester group of a metal-ester

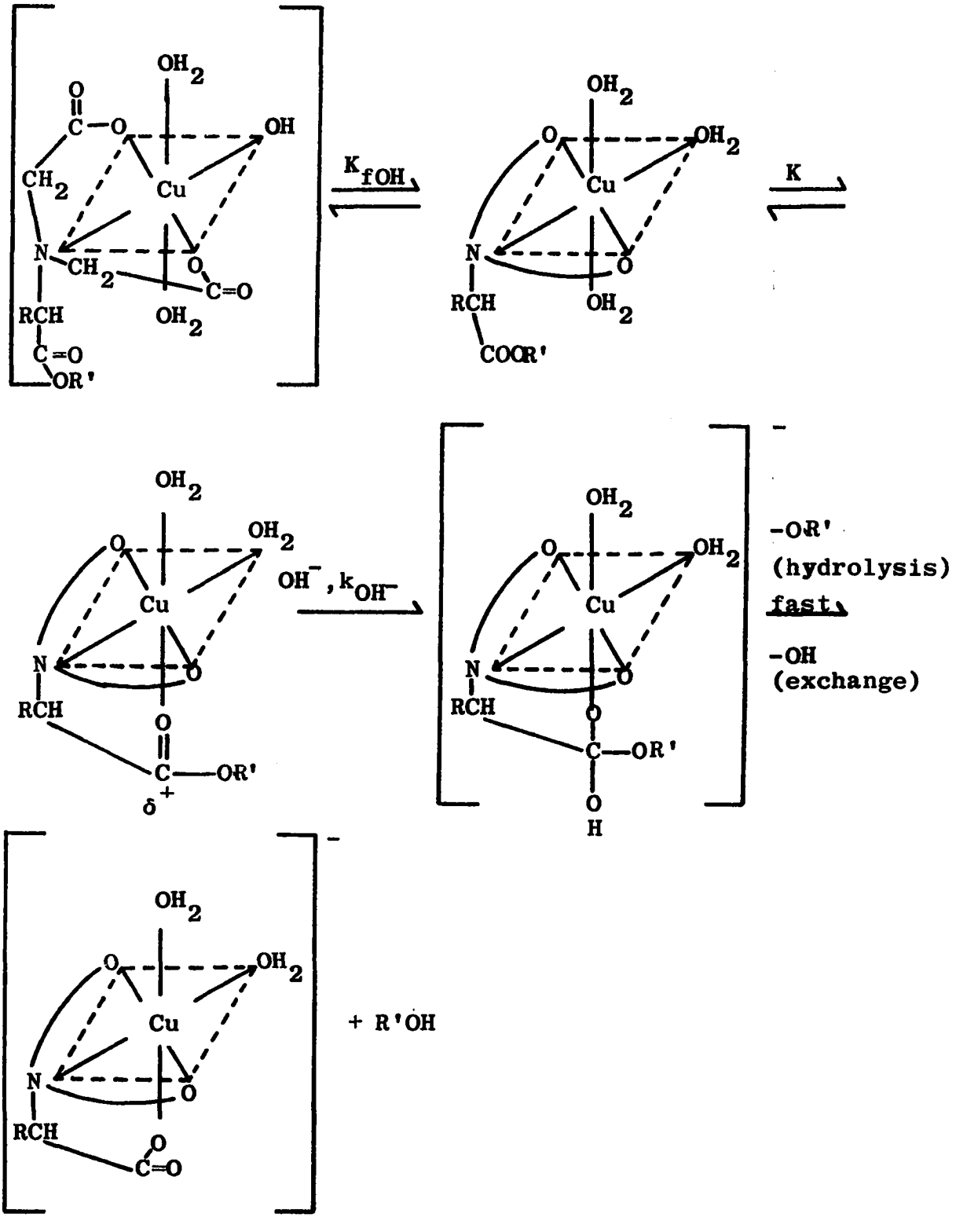
complex in which metal ion-ester oxygen coordination exists in the transition state, B) internal attack of hydroxide ion which is bound to the metal ion, C) hydroxide ion attack at the carbonyl carbon atom of the ester group of a metal-ester complex in which no metal-ester oxygen interaction exists, and D) water attack at the carbonyl carbon of the ester function on a metal-ester hydroxo-complex, [M(amino acid ester-N,N-diacetate)(OH)]$^-$.

An inductive effect through the nitrogen atom has been shown to be insufficient to cause the large catalytic effects found and therefore mechanism C is unlikely. A study of the rates of hydrolysis of Cu(EVDA) catalyzed by several nucleophiles indicated that general nucleophilic catalysis rather than internal hydroxide ion attack was involved as mechanism B postulates. If water were the nucleophile as postulated in mechanism D, then much larger rates would have been expected for the better nucleophiles studied. The relative nucleophilicities, as measured by the second-order rate constants, of these nucleophiles, including OH^-, is roughly the same for Cu(EVDA) and for organic acetates (Table 6). This suggests that ester hydrolysis of Cu(EVDA) proceeds by general nucleophilic catalysis. That general base catalysis is probably not involved is suggested by an absence of a correlation between pK_a of the nucleophile and the rate of hydrolysis (11). The most probable mechanism consistent with the rate

law; Rate = k [M(EGDA)] [OH^-] is mechanism A in which an hydroxide ion attacks the carbonyl carbon of the ester which has coordinated to the metal ion in a rapid prior equilibrium. As has been discussed, either oxygen atom in the ester group could coordinate although carbonyl ester oxygen coordination is shown. The experimental second-order rate constant, k, is Kk_{OH} in terms of this mechanism. Experimentally it has not been possible to determine K but the value of K is probably small as inferred from the formation constant studies of EVDA with copper(II), lead(II), nickel(II), cobalt(II), and samarium(III). In the presence of hydroxo complexes this mechanism yields a rate law; Rate = k_e [M(EGDA)] [OH^-] in which [M(EGDA)] is the actual concentration of the non-hydroxo-complex. From the formation constant for the hydroxo-complex, K_{fOH}, the fraction, f_{OH}, of the total complex concentration, $[M(EGDA)]_t$ which is in the hydroxo-form, $[M(EGDA)(OH)]^-$ can be calculated. Then $(1-f_{OH})$ is the fraction of $[M(EGDA)]_t$ which is present as M(EGDA). The rate law in terms of these known parameters is

$$\text{Rate} = k_e\ (1-f_{OH})\ [M(EGDA)]_t\ [OH^-]$$

The rates of hydrolysis of amino acid ester-N,N-diacetic acids with copper(II) and samarium(III) (Table 10) indicate that changing from an ethyl to a butyl ester (EGDA to BGDA) slows the rate of hydrolysis by a small factor, 1.4-2.2. The


K_{fOH}
K
OH^-, k_{OH^-}
-OR'
(hydrolysis)
fast
-OH
(exchange)
+ R'OH

R group in the ester ligand, $C_2H_5OOCCH(R)N(CH_2COO^-)_2$, affects the rates of hydrolysis depending on its nature. Thus rates are very similar for R=-H or $-CH_3$ (EGDA or EADA) for either copper(II) or samarium(III) complexes, but for R = $-CH_2CH(CH_3)_2$ or $-CH(CH_3)_2$ (ELDA or EVDA) the rates are considerably slower as observed for the non-metal ion-catalyzed reactions (4,5) and discussed in terms of Newman's "Rule of Six" (6) which is an empirical rule which states that in reactions involving addition to an unsaturated function containing a double bond, those atoms which are effective in providing steric hinderance are separated from the attacking atom in the transition state by a chain of four atoms, i.e. numbering the oxygen atom of the hydroxyl group attacking the carbonyl carbon atom 1, then the greatest steric hinderance will result from atoms in position 6. The number of methylene groups separating the nitrogen atom and the ester group is very important in determining the amount of catalysis exerted by the metal ion. The greatest catalysis is observed in complexes of the ligands, $C_2H_5OOC(CH_2)_nN(CH_2COOH)_2$, when n = 1. Increasing n to a value of 2 decreases the rate for the copper(II) complex by a factor of 45 but only a factor of 5.7 for the larger lead(II) ion. For samarium(III) such a change decreases the rate by only a factor of 1.2, while the rate actually increases for lutetium(III) by a factor of 1.3 in going from n =1 to n = 2. Extending the chain to n = 3 yields complexes which undergo

no measurable hydrolysis up to pH 8. Models of these complexes show that it is very difficult for the ester carbonyl group to coordinate to the metal ion. Important factors in the n = 1 or 2 series are the stability of 5 and 6 membered chelate rings with various metal ions and the entropy associated with the chelation.

The rate of ester hydrolysis of EGDA metal complexes (Table 9) increases with the metal ion in the following order: Cd(II) < Ni(II) < Mn(II) < Co(II) < Fe(II) < Zn(II) < La(III) < Cu(II) < Pb(II) < Nd(III) < Gd(III) < Sm(III) < Dy(III) < Er(III) < Yb(III) < Lu(III). In general the +3 lanthanides are somewhat better catalysts. Within the lanthanide group, the relative catalytic properties increase with increasing atomic number except for the decrease at Gd(III). Numerous properties of the lanthanides follow a similar trend. One example is the trend in formation constants, K_f, of these ions with the iminodiacetate ion [L^{+3} + $IMDA^{-2}$ = $[L(IMDA)]^{+}$] which is shown in Figure 6. This implies that the same factors which influence the coordination of the iminodiacetate group or the iminodiacetate portion of the ester ligands may be operative in promotion of the ester hydrolysis. The +3 lanthanide ion catalyzed reactions have virtually the same $\Delta H^{\ddagger}$ (Table 12) as for ethyl glycinate (10.6 kcal/mole). That the activation energy for the reaction remains unchanged

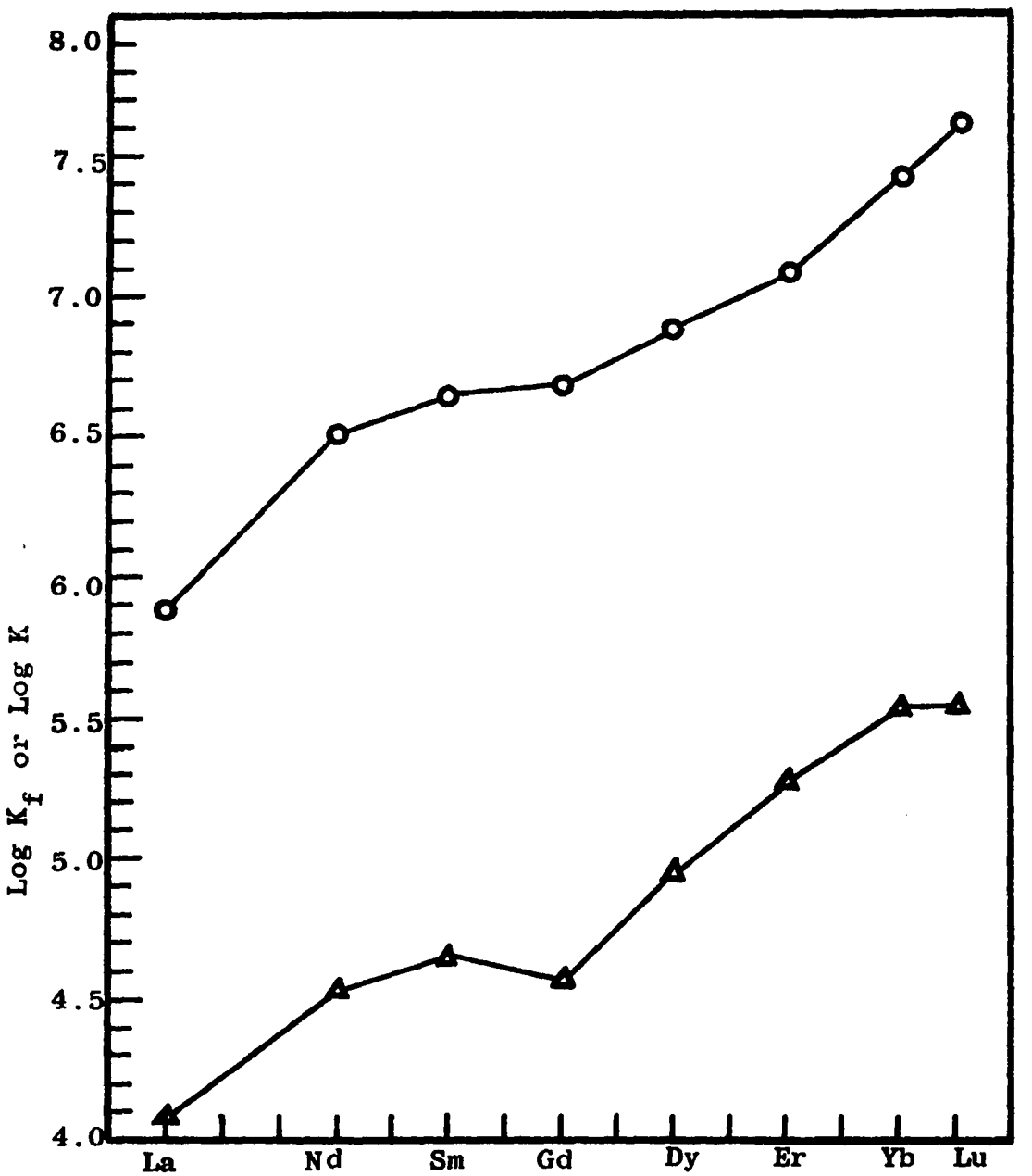


Figure 6. Plot of atomic number of lanthanides vs. log K_f for M(III) + IMDA (O); vs. log K for $[\overline{M(EGDA)}]^{+f}$ ester hydrolysis (Δ)

suggests that the energy required to remove the water of solvation from the charged ions in going to the neutral and presumably less solvated transition state is roughly the same as that gained by coordinating the ester to the lanthanide ion. Whereas $\Delta H^{\ddagger}$ remains virtually constant for the lanthanide ions the entropy of activation, $\Delta S^{\ddagger}$, (Table 12) increases drastically from -21.7 e.u. for ethyl glycinate (13) to -4.0 for $[Sm(EGDA)]^{+}$ and +0.8 for $[Lu(EGDA)]^{+}$. Hence, the catalytic effect of the lanthanides results from an increase in $\Delta S^{\ddagger}$ with $\Delta H^{\ddagger}$ remaining essentially unchanged. The increase in $\Delta S^{\ddagger}$ is not unexpected when one considers that a positive $[Sm(EGDA)]^{+}$ and negative OH^{-} are reacting in the rate determining step to give a neutral and considerably less solvated activated complex.

The order of increasing hydrolytic second-order rate constants for the +2 ions is Cd(II) < Ni(II) < Mn(II) < Co(II) < Fe(II) < Zn(II) < Cu(II) < Pb(II). The catalytic properties of the +2 ions do not parallel the formation constants of M(IMDA) complexes as found for the lanthanide ions. There does, however, appear to be limited correlation with the hydroxo-formation constant for $[M(OH)]^{+}$ as pointed out by Hix and Jones (32). They found that metal ions were increasingly active in the hydroxide-catalyzed hydrolysis of ethyl glycinate in the order: Ni(II) < Co(II) < Zn(II) <<

Cu(II) although this is also the order of increasing hydroxo-complex stability (32,64) it is uncertain what this limited correlation means since the observed general nucleophilic catalysis of the hydrolysis of Cu(EVDA) minimized the importance of hydroxo-complexes. In addition the rates of hydrolysis do not correlate well with the hydroxo-formation constants of M(EVDA) given in Table 4. Currently the limited and approximate correlation between the catalytic ability and the hydroxo-formation constant of Ni(II), Co(II), Zn(II) and Cu(II) appears to be a fortuitous relationship.

The enthalpy of activation of the hydroxide ion-catalyzed hydrolysis of ethyl glycinate is 10.6 kcal/mole (13). For $(CH_3)_3\overset{+}{N}CH_2COOC_2H_5$ the activation energy is slightly lower at 9.7 kcal/mole. The $\Delta H^{\ddagger}$ of the +2 metal ion-catalyzed hydrolysis of EGDA are lower than that of ethyl glycinate (Table 12) and decrease in the order: Ni(EGDA) > Cu(EGDA) > Pb(EGDA). The entropies of activation for ethyl glycinate and the EGDA complexes are all -22.5 ± 1 e.u. Thus the increased rate of ester hydrolysis of the EGDA is a result of substantial lowering of $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ remaining virtually unchanged.

An examination of the thermodynamics of ion association involving +2 ions and methyliminodiacetate ion (65) and iminodiacetate ion (66) for the 1:1 complexes indicates that the thermodynamic functions $-\Delta H$ and $-\Delta G$ do not in general

correlate with the rates of hydrolysis. However the function ΔS and the function $\Delta S + S^{o} (M^{+2})$ which removes the variable cationic aqueous entropy and is defined below where M is a

$$\Delta S + S^{o} (M^{+2}) = [S_{g}(MA) - S_{g}(A^{-2})] + [S_{hyd}(MA) - S_{hyd} (A^{-2})]$$

+2 metal ion and A^{-2} is an iminodiacetate and S_{g} and S_{hyd} refer to the gaseous and hydration entropies respectively do, perhaps fortuitously, correlate fairly well with the observed order of catalytic activity in the ester hydrolysis reactions as shown in Figure 7 and Figure 8. That the rate of hydrolysis of the +2 metal ion promoted hydrolysis of amino acid ester-N, N-diacetates shows a correspondence to an entropy function suggests that chelation of the ester group through either the carbonyl ester oxygen or ether oxygen is strongly dependent upon the entropy change associated with the chelation to the particular metal ion perhaps resulting in a larger value of K, the equilibrium constant for ester oxygen chelation, as well as the enthalpy change associated with going from the reactants to the transition state.

Amino Acid Esters

The substituted iminodiacetates complex strongly to copper(II) ion and 1:1 mole ratios of metal ion: substituted iminodiacetate maximize the amount of 1:1 Cu(IMDA) complex formed in aqueous solution. Increasing the copper(II):

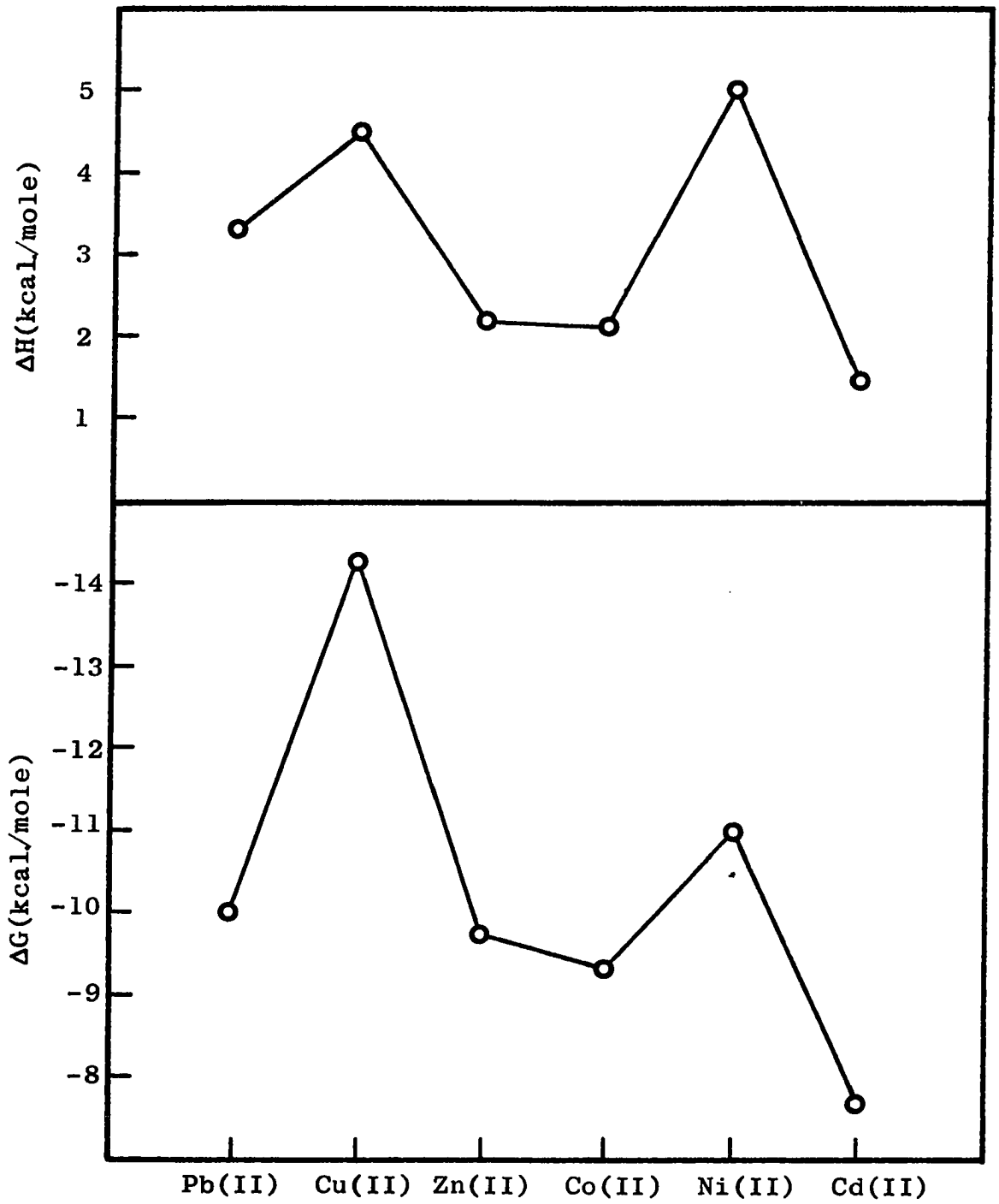


Figure 7. Enthalpy and free energy changes for the formation of complexes M^{+2} + IMDA $\rightleftharpoons$ M(IMDA)

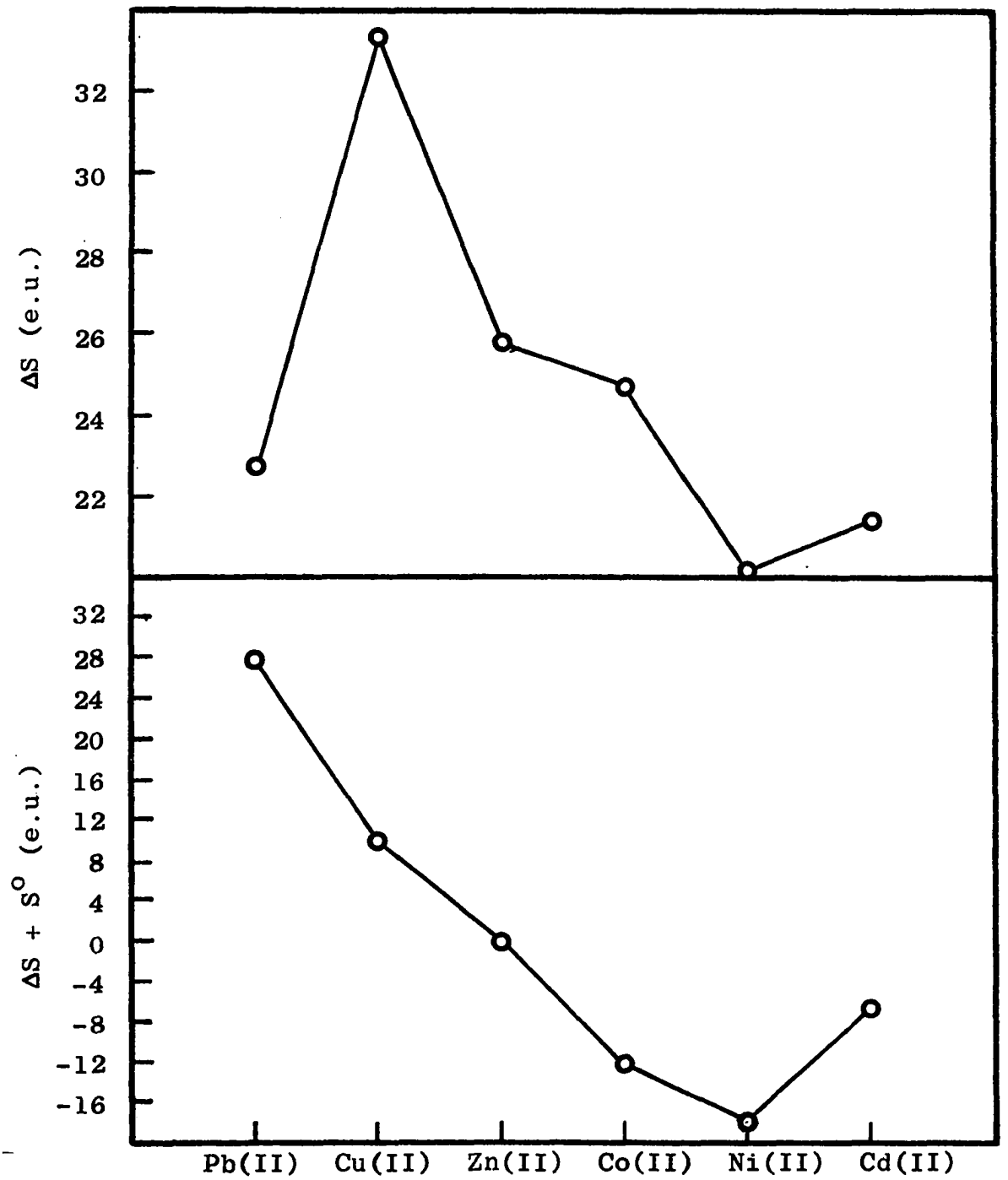
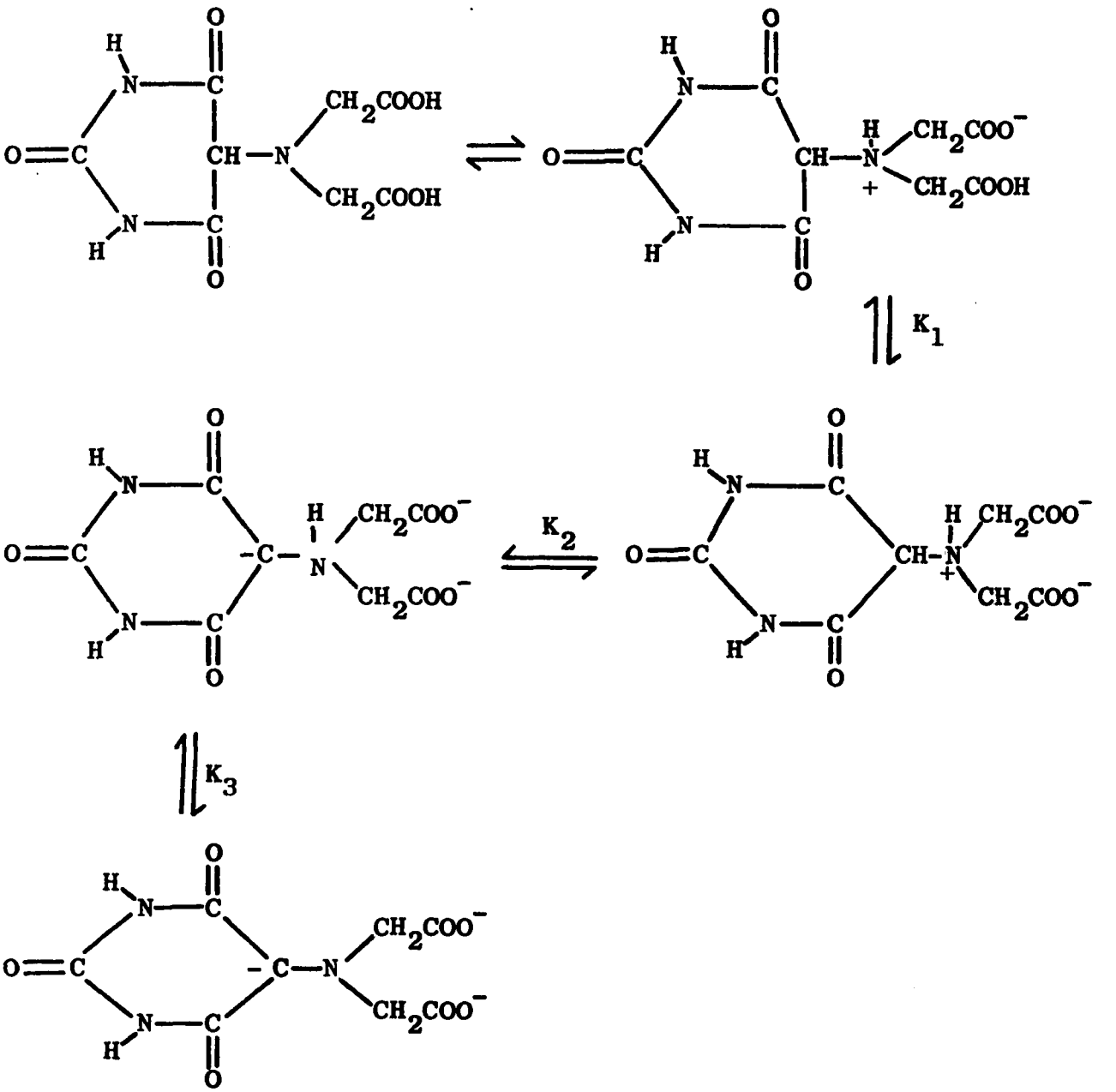


Figure 8. Entropy changes for the formation of complexes M^{+2} + IMDA $\rightleftharpoons$ M(IMDA)

substituted iminodiacetate mole ratio to 1.0:1.1 decreased the observed rate of hydrolysis of methyl glycinate in the presence of the complex by approximately 10% indicating that the assumption of stoichiometric formation of a copper(II) iminodiacetate complex is reasonable. Formation constants of these copper(II) substiuted iminodiacetate complexes with amino acids and amino acid esters (Table 15) were determined in order to allow us to calculate the amount of $[Cu(IMDA)(\text{amino acid})]^-$ or [Cu(IMDA)(amino acid ester)] present under various concentration and pH ranges. The formation constants for the complexation of amino acids with Cu(IMDA) are similar indicating little if any steric hinderance between iminodiacetate ion and amino acid. The formation constants for complexation of ethyl valinate and butyl glycinate to Cu(IMDA) are the same within experimental error also. However, if the iminodiacetate is substituted with a large bulky group particulary on the nitrogen atom, $RN(CH_2COO^-)_2$, then formation constants are much larger by as much as a factor of ten for the complexation of the smaller butyl glycinate than for ethyl leucinate to Cu(methyliminodiacetate), Cu(cyclohexyliminodiacetate, Cu(tert-butyliminodiacetate), and Cu(uramilidiacetate), Cu(U). This indicates that there is steric repulsion between the R groups of the iminodiacetates, $RN(CH_2COO^-)_2$, and amino acid esters, $NH_2CHRCOOR'$. The formation

constant for Cu(uramildiacetate) complexing with the amino acid esters is considerably lower than that found for other iminodiacetate derivatives. This iminodiacetate is best represented as H_3L, however, with three acid dissociation constants (67,68) of $pK_1 = 1.7$, $pK_2 = 2.67$ and $pK_3 = 9.63$.



The formal charge on the copper(II) complex is minus one and a similar formation constant (log K_f=3.33) is obtained for the complexation of butyl glycinate to $[Cu(NTA)]^-$ (69) as for the complexation of butyl glycinate to $[Cu(uramildiacetate)]^-$ (log K_f=3,38).

The presence of the phenyl group in D-phenylglycine-N-monoacetate lowers the formation constant for the complexation of valine and ethyl leucinate with Cu(D-phenylglycine-N-monoacetate) indicating some steric hinderance between the R groups of substituted iminodiacetates, $[HN(CHRCOO^-)(CH_2COO^-)]$, and amino acid esters, $NH_2CHRCOOR'$, is operative in lowering the value found for the formation constant. The similar rates of hydrolysis of methyl glycinate complexed with Cu(IMDA) and Cu(D-phenylglycine-N-monoacetate) suggests that electron withdrawal by the phenyl group enhances the rate of ester hydrolysis. The steric hinderance of the phenyl group may not be as great as expected for although Cu(D-phenylglycine-N-monoacetate) contains an optically active center, no difference in the rate of hydrolysis of D- and L-leucine methyl ester complexed with Cu(D-phenylglycine-N-monoacetate) was observed.

L-valine-N-monoacetate, $HN(CH_2COO^-)(CH(CH(CH_3)_2)COO^-)$, complexes with copper(II) indicate little if any steric hinderance between the R group ($-CH(CH_3)_2$) of the iminodiacetate and the R group on the L isomer of the amino acid or amino

acid ester but considerable steric hinderance with large R groups on D isomers of amino acids and amino acid esters as shown by the lower formation constants for the complexation of D-leucine, D-leucine ethyl ester, and D-alanine compared to the corresponding L isomers with Cu(L-valine-N-monoacetate). This difference in formation constants between D and L isomers results in different pH dependences of the rate of hydrolysis under certain pH conditions (Figure 5).

In the presence of a metal iminodiacetate complex, MZ, amino acid esters complex with the metal ion complex according to Equation 7. The equilibrium constant for the

$$MZ + \overset{+}{N}H_3CHRCOOR' \rightleftarrows MZ(NH_2CHRCOOR') + H^+ \qquad (7)$$

reaction, K_{eq} is given by

$$K_{eq} = [MZ(NH_2CHRCOOR')]\ [H^+]/[MZ]\ [\overset{+}{N}H_3CHRCOOR'] = K_fK_E$$

where K_f is the formation constant of the amino acid ester with the metal iminodiacetate complex and K_E is the ionization constant of the amino acid ester. With few exceptions the formation constant data agrees fairly well with the experimental kinetic evidence concerning the degree of completeness of the complexation of the amino acid ester to the metal iminodiacetate complex (Table 17) at the pH values and concentrations of reactants given.

Kinetic studies indicated that the hydrolytic reaction is first-order in total ester concentration. Since free amino acid esters are not observed to hydrolyze at an appreciable rate under the experimental conditions a Cu-(iminodiacetate)(amino acid ester) complex is postulated to be the reactive species. If the formation of the ester complex is complete as given by Equation 7 and hydrolysis proceeds by OH^- attack, the dependence of k_{obsd} on the hydroxide ion concentration is $k_{obsd} = k\,[OH^-]$ and the overall rate of hydrolysis is given by the expression:

$$\text{Rate} = kK\,[Cu(IMDA)(NH_2CHRCOOR')][OH^-]$$

The most probable mechanism involves hydroxide ion attack on the carbonyl carbon atom of the ester which has been polarized by a rapid prior equilibrium chelation of the carbonyl ester or ether oxygen atom by analogy to the mechanism proposed for hydrolysis of metal ion complexes of amino acid ester-N,N-diacetic acids.

If the ester is not completely coordinated, the amount coordinated depends on the hydroxide ion concentration and experimentally k_{obsd} is found to exhibit second-order dependence on hydroxide ion concentration and the overall rate of hydrolysis is given by the expression:

$$\text{Rate} = k'K\,[Cu(IMDA)]\,[\overset{+}{N}H_3CHRCOOR']\,[OH^-]^2$$

or

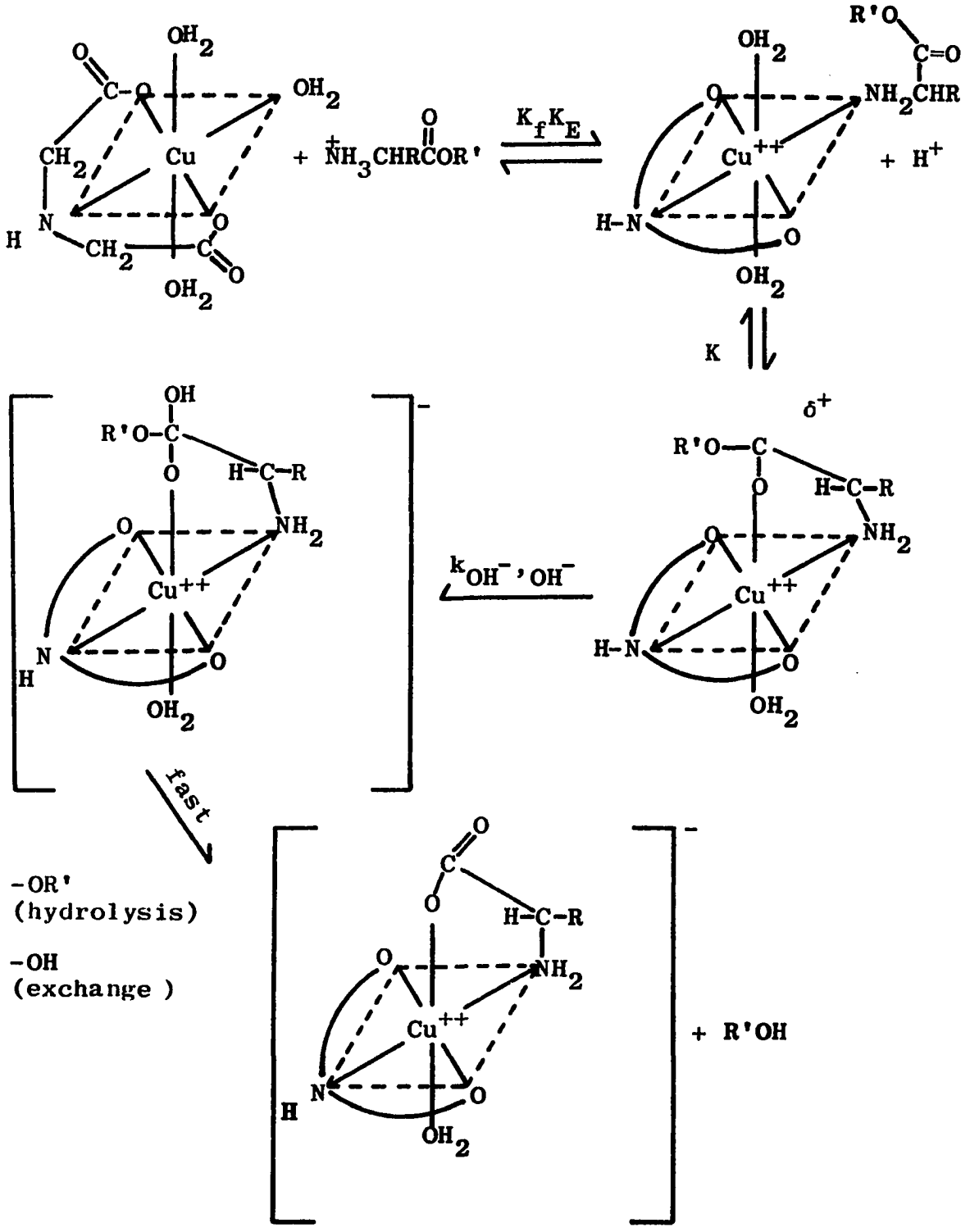
$$\text{Rate} = kK(K_f K_E/K_w)\ [Cu(IMDA)(NH_2CHRCOOR')][OH^-]$$

Cu(IMDA) complexed with methyl esters of amino acids, in particular, exhibited water attack in which experimentally it was found that the rate expression:

$$\text{Rate} = (k_{H_2O} + k\ [OH^-])\ (KK_f K_E/K_w)[Cu(IMDA)(NH_2CHRCOOR')]$$

best fit the data. Appreciable rates of water attack have also been found for $[Cu(NTA)(NH_2CHRCOOR')]^-$ ester hydrolysis (5). The rate of water attack found for $Cu(IMDA)(NH_2CH_2COOCH_3)$ hydrolysis of $4.05 \times 10^{-6}\ M^{-1}\ sec.^{-1}$ (Table 18) is consistent with the relative nucleophilicities of water and hydroxide ion, the catalysis factor being approximately 10^4, the rate of water-catalyzed hydrolysis of $\overset{+}{N}H_3CH_2COOC_2H_5$ (3) in the absence of metal ions being $5 \times 10^{-9} sec.^{-1}$. The rate of ester hydrolysis of $Cu(IMDA)(NH_2CH_2COOCH_3)$ due to nitrite ion attack was found to be $0.38\ M^{-1} sec.^{-1}$, the same as found for the hydrolysis of Cu(EVDA) which suggests that the mechanisms involved are very similar. The mechanism proposed for the hydrolysis of amino acid esters in the presence of copper(II) iminodiacetate complexes is given on the following page.

The first step in the proposed mechanism is coordination of the amino acid ester through the nitrogen atom in agreement with the UV spectra of copper(II) ion solutions containing amino acid esters (31) and the absence of a


$K_f K_E$
K
k_{OH^-}, OH^-
fast
-OR'
(hydrolysis)
-OH
(exchange)
+ R'OH
+ H+

lower carbonyl ester oxygen stretching frequency in D_2O solutions of these complexes. In the second step chelation of the carbonyl ester oxygen atom or ether ester oxygen atom is postulated to occur with an equilibrium constant, K, which probably has a very small but undetermined value. This metal ion-ester oxygen interaction is necessary to explain the relatively large catalysis factors which cannot be ascribed to an inductive effect through the nitrogen atom of the ester alone. In the rate determining step, hydroxide ion attacks the polarized carbonyl ester carbon atom to give the transition state intermediate which can then lose an alkoxide ion to undergo hydrolysis or loss of OH^- to undergo exchange as found in the copper(II) ion-catalyzed hydrolysis of ethyl glycinate studied by Bender and Turnquest (19). Other nucleophiles such as water may attack the carbonyl carbon atom as general nucleophilic catalysis is the proposed mechanism. The final product of the hydrolysis reaction is the copper(II)(iminodiacetate)(amino acid) complex and the appropriate alcohol.

The rates of hydrolysis (Table 18) of various amino acid esters complexed to Cu(IMDA) follow the customary trends of methyl > ethyl > butyl esters and decreasing second-order rate constants with the size of the R group, $NH_2CHRCOOR'$, of the ester. The unreactivity of betaine ethyl ester and

benzoyl-glycine methylester are undoubtedly due to groups attached to the nitrogen atom which prevent coordination of the ester to Cu(IMDA). The second-order rate constants of the hydrolysis of methyl glycinate complexed to various copper(II) substituted iminodiacetate complexes are not readily interpretable. Steric hinderance has a large effect, however electron releasing or withdrawing groups on the iminodiacetate may be involved as well.

In general, the lowest rates of ester hydrolysis were observed for complexes in which the ligand is four coordinate and the formal charge on the metal ion complex is minus one. Examples of this category are $[Cu(NTA)]^-$ and $[Cu(U)]^-$ which are relatively poor catalysts for ester hydrolysis.

Low rates of ester hydrolysis are also observed for copper(II) complexes of four coordinate ligands with zero formal charge. Hydroxyethyliminodiacetate ion (33) which occupies four coordination sites on the metal ion gave a first-order rate constant of methyl glycinate hydrolysis of 1.1×10^{-4} sec.$^{-1}$ at pH 6.8 which is comparable to that for the Cu(furfuryliminodiacetate) complex at the same pH value. The first and second formation constants of furfuryliminodiacetate, K_1 and K_2, with copper(II) ion were determined using the methods described for EVDA in the Experimental section of this thesis. The values of $\sim 8 \times 10^9$

and ~4 x 10^6 for K_1 and K_2 respectively indicate (33) that the ligand is tridentate rather than tetradentate but models indicate that the furfuryl group may be effective in steric blocking of a fourth coordination site on the metal ion. Complexes which cannot be four coordinate but which models indicate substantial steric blocking of a fourth coordinating position (copper(II) complexes of methyliminodiacetate, t-butyliminodiacetate, and L-valine-N-monoacetate) exhibit slower rates of ester hydrolysis than complexes with little or no steric interaction with a fourth coordination position (iminodiacetate, cyclohexyliminodiacetate, and phenyliminodiacetate) which were found to be the best catalysts for ester hydrolysis. Since the second-order rate constant, k, is a product of Kk_{OH} where k_{OH} is the rate of hydroxide ion attack on the ester complex and K is the equilibrium constant for chelation of the amino acid ester oxygen atom it is not known whether the steric effects reduce K or k_{OH} or both.

Crystal structures of some of these compounds together with more formation constant data for similar systems would give a better understanding of the steric requirements of the various substituents and the role they have in the hydrolysis mechanism.

The hydrolysis if (R)-(-)- and (S)-(+)-histidine methyl esters in the presence of catalytically active complexes $[Ni((R)-(-)-histidinate)]^+$ and $[Ni(S)-(+)-histidine)]^+$ has

been studied by Hix and Jones (70). They found the rate of hydrolysis of the (R)-(-) ester greater in the presence of the $[Ni((S)-(+)-histidinate)]^+$ ion and the (S)-(+) ester greater in the presence of the $[Ni((R)-(-)-histidinate)]^+$ ion such that approximately 40% greater observed rate constants were found with opposite ester-histidinate configurations than for identical ester-histidinate configurations. The stereoselectivity was postulated to arise from differences in the degree of interaction between the ester carbonyl group and the coordination center. Titration data indicated no difference in the stabilities of the histidinato(methyl histidinate)nickel(II) complexes such as were found for L-valine-N-monoacetate(methyl leucinate) copper(II) complexes.

The second-order rate constant, k, is a product of Kk_{OH} where k_{OH} is the rate of hydroxide ion attack on the ester complex and K is the equilibrium constant for chelation of the amino acid ester oxygen atom. If the second-order rate constants for the hydrolysis of different optical isomers of amino acid esters are different, it suggests that there is a difference in the steric hinderance of the chelation of the ester or a difference in the rate of hydroxide ion attack. The second-order rate constants for the hydrolysis of Cu(L-valine-N-monoacetate)(L-leucine methyl ester) and

Cu(L-valine-N-monoacetate)(D-leucine methyl ester) are 1.00×10^3 $M^{-1}sec.^{-1}$ and 1.30×10^3 $M^{-1}sec.^{-1}$ respectively which indicates that either the chelation of the D isomer of the ester is favored or the rate of hydroxide ion attack is different for the two complexes. It would seem reasonable that the same effects should be observed in the value of the formation constant, K_f, and the chelation constant, K, and on this basis the lower value of K_f for D-leucine methyl ester but larger second-order rate constant, k, would appear difficult to resolve until more stereochemical information is available on the optically active ligands in aqueous solution.

BIBLIOGRAPHY

1. H. Kroll, J. Am. Chem. Soc., 74, 2036 (1952).

2. H. R. Mahler and E. H. Cordes. Biological Chemistry. Harper and Row, New York, N.Y. 1966.

3. H. L. Conley, Jr. and R. B. Martin, J. Phys. Chem., 69, 2914 (1965).

4. R. W. Hay, L. J. Porter and P. J. Morris, Australian J. Chem., 19, 1197 (1966).

5. R. J. Angelici and D. Hopgood, J. Am. Chem. Soc., 90, 2514 (1968).

6. M. S. Newman. Steric Effects in Organic Chemistry. John Wiley, Inc., New York, N.Y. 1956.

7. M. L. Bender and M. C. Neveu, J. Am. Chem. Soc., 80, 5388 (1958).

8. T. C. Bruice and R. Lapinski, J. Am. Chem. Soc., 80, 2265 (1958).

9. W. P. Jencks and J. Carriuolo, J. Am. Chem. Soc., 83, 1743 (1961).

10. M. L. Bender, E. J. Pollock and M. C. Neveu, J. Am. Chem. Soc., 84, 595 (1962).

11. T. C. Bruice and S. Benkovic. Bioorganic Mechanisms. Vol. 1. W. A. Benjamin, Inc., New York, N.Y. 1966.

12. M. L. Bender and B. W. Turnquest, J. Am. Chem. Soc., 79, 1656 (1957).

13. C. Gustafson. Suomalaisen Tiedeakatemian Toimituksia, Ser. A II, 15, (1945).

14. J. E. Potts, Jr. and E. S. Amis, J. Am. Chem. Soc., 71, 2112 (1949).

15. W. A. Connor, M. M. Jones and D. L. Tuleen, Inorg. Chem., 4, 1129 (1965).

16. J. M. White, R. A. Manning and N. C. Li, J. Am. Chem. Soc., 79, 5859 (1957).

17. R. P. Bell and B. A. W. Coller, Faraday Soc. Trans., 60, 1087 (1964).

18. J. M. White, R. A. Manning and N. C. Li, J. Am. Chem. Soc., 78, 2367 (1956).

19. M. L. Bender and B. W. Turnquest, J. Am. Chem. Soc., 79, 1889 (1957).

20. D. D. Perrin and U. S. Sharma, J. Chem. Soc., (A), 724 (1965).

21. A. C. Andrews and D. M. Zebolsky, J. Am. Chem. Soc., 742 (1965).

22. M. M. Hardin and S. J. Cole, Acta Cryst., 16, 643 (1963).

23. R. Kretzinger, F. A. Cotton, and E. F. Bryan, Acta Cryst., 16, 651 (1963).

24. A. Chakravorty and F. A. Cotton, J. Phys. Chem., 67, 2878 (1963).

25. R. W. Hay and P. J. Morris, Chem. Comm., 23 (1967).

26. M. D. Alexander and D. H. Busch, J. Am. Chem. Soc., 88, 1130 (1966).

27. R. W. Hay, M. L. Jansen and P. L. Cropp, Chem. Comm., 621 (1967).

28. M. D. Alexander and D. H. Busch, Inorg. Chem., 5, 602 (1966).

29. M. P. Springer and C. Curran, Inorg. Chem., 2, 1270 (1963).

30. R. W. Hay and L. J. Porter, Australian J. Chem., 20, 675 (1967).

31. I. M. Klotz, I. L. Faller and J. M. Urquhart, J. Phys. Chem., 54, 18 (1950).

32. J. E. Hix, Jr. and M. M. Jones, Inorg. Chem., 5, 1863 (1966).

33. G. Schwarzenbach, G. Anderegg, W. Schneider and H. Senn, Helv. Chim. Acta, 38, 1147 (1955).

34. H. Irving and J. J. R. F. da Silva, J. Chem. Soc., 3308 (1963).

35. H. Irving and J. J. R. F. da Silva, J. Chem. Soc., 945 (1963).

36. L. C. Thompson, Inorg. Chem., 1, 490 (1962).

37. G. Furlani, Chim. Ital., 97, 1423 (1967).

38. Chemical Society (London), Chem. Soc. (London), Special Publication 17, 1 (1964).

39. R. J. Angelici and B. E. Leach, J. Am. Chem. Soc., 89, 4605 (1967).

40. Instrumentation Manual for SBR2/SBU1/TTT1 Titration Equipment. 2nd Ed. Radiometer. Copenhagen, Denmark. 1961.

41. J. Korbl and R. Pribil, Chemist-Analyst, 45, 102 (1956).

42. J. Kinnunen and B. Wennerstand, Chemist-Analyst, 46, 92 (1957).

43. J. S. Fritz, J. E. Abbink and M. A. Payne, Anal. Chem., 33, 1381 (1961).

44. J. P. Greenstein and M. Winitz. Chemistry of the Amino Acids. Vol. 2. John Wiley, Inc., New York, N.Y. 1961.

45. K. Mikkelson and S. O. Nielson, J. Phys. Chem., 64, 632 (1960).

46. C. W. Davies, J. Chem. Soc., 2093 (1938).

47. J. C. Sullivan, J. Rydberg and W. F. Miller, Acta. Chem. Scand., 13, 2023 (1959).

48. W. R. Stagg and J. E. Powell. U.S. Atomic Energy Commission Report IS-727 [Iowa State University of Science and Technology, Ames. Inst. for Atomic Research]. 1963.

49. J. E. Powell and D. L. G. Rowlands, Inorg. Chem., 5, 819 (1966).

50. R. H. Moore and R. K. Zeigler. U.S. Atomic Energy Commission Report LA-2367, plus addenda [Los Alamos Scientific Lab., N. Mex.]. 1959.

51. E. A. Guggenheim, Phil. Mag., 22, 322 (1936).

52. M. L. Bender, Chem. Rev., 60, 53 (1960).

53. D. W. Cooke, Inorg. Chem., 5, 1141 (1966).

54. T. Moeller and R. Ferrus, Inorg. Chem., 1, 49 (1962).

55. W. P. Jencks and J. Carriuolo, J. Am. Chem. Soc., 82, 1778 (1960).

56. T. C. Bruice and G. L. Schmir, J. Am. Chem. Soc., 79, 1663 (1957).

57. M. Green and R. F. Hudson, Chem. Soc. Proc., 149 (1959).

58. M. L. Bender, F. J. Kezdy and B. Zerner, J. Am. Chem. Soc., 85, 3017 (1963).

59. S. W. Benson. The Foundations of Chemical Kinetics. McGraw-Hill Book Co., Inc., New York, N.Y. 1960.

60. R. J. Angelici and B. E. Leach, J. Am. Chem. Soc., 90, 2499 (1968).

61. B. E. Leach and R. J. Angelici, J. Am. Chem. Soc., 90, 2504 (1968).

62. E. L. Smith and D. H. Spackman, J. Biol. Chem., 212, 271 (1955).

63. J. P. Collman and E. Kimura, J. Am. Chem. Soc., 89, 6096 (1967).

64. S. Chaberek, R. C. Courtney and A. E. Martell, J. Am. Chem. Soc., 74, 5057 (1952).

65. G. H. Nancollas and A. C. Park, Inorg. Chem., 7, 58 (1968).

66. G. Anderegg, Helv. Chim. Acta, 47, 1801 (1964).

67. H. Irving and J. J. R. F. da Silva, J. Chem. Soc., 448 (1963).

68. H. Irving and J. J. R. F. da Silva, J. Chem. Soc., 458 (1963).

69. D. Hopgood and R. J. Angelici, J. Am. Chem. Soc., 90, 2508 (1968).

70. J. E. Hix, Jr. and M. M. Jones, J. Am. Chem. Soc., 90, 1723 (1968).

ACKNOWLEDGMENTS

To my wife, Sharon, for her help, encouragement and understanding during graduate school and for her companionship which has enriched my life.

To my parents for their encouragement and financial help which has made this pursuit of higher education possible.

To my major professor, Dr. R. J. Angelici, for all of his help and guidance throughout my graduate work. It is an honor and enjoyable experience to work for an individual possessing such a dynamic and congenial personality.

I thank the Universities of Nebraska and Colorado for financial assistance in my undergraduate work and Dr. R. Keller for encouraging me to attend graduate schoool at Iowa State and developing my interests in Inorganic Chemistry.

I thank Dr. J. Graham, Dr. L. Bussetto, Mrs. J. Ingemanson, Miss J. Williams, and Mssrs. W. Loewen, E. Siefert, J. Ingemanson, J. Del Gaudio, D. Thomas, A. Kruse, and J. Feinblatt for many helpful suggestions.

To Dr. Hopgood for his most helpful discussions concerning the equilibrium and kinetic studies.

To my typist, Mrs. P. Toms, for a job well done.

I also thank the Department of Health, Education and Welfare for support of this research.

VITA

The author was born in Wichita, Kansas on November 16, 1942 to Mr. and Mrs. Leonard E. Leach. He received his elementary education at the rural District 19 School and his secondary education at Dorchester High School. He attended the University of Nebraska in Lincoln where his interest in chemistry grew. At the end of his sophomore year the family moved to Longmont, Colorado where he attended the University of Colorado in Boulder, graduating with a B.A. degree in chemistry in 1964.

The author entered the Graduate College at Iowa State University in 1964 where he majored in Inorganic Chemistry. On June 24, 1966 he married Sharon K. Westegaard, daughter of Mr. and Mrs. Niels N. Westegaard of Hurley, South Dakota.

His research under the direction of Dr. Robert J. Angelici resulted in six publications, five in the area of metal ion catalysis of amino acid ester hydrolysis and one in the area of nickel carbonyl substitution reactions.

After receiving his Ph.D. degree in August, 1968 the author will begin work as a post-doctoral research associate under the direction of Dr. Daniel Leussing at Ohio State University, Columbus, Ohio.

APPENDIX

Derivation of a Calculable Expression for the Formation Constant of Amino Acid Esters with $[MZ]^o$

The equations which follow represent an exact solution to the formation constant, K_f, of 1:1 MZ: amino acid ester, E, complexes where M is a +2 metal ion and Z is an iminodiacetate. The concentrations of species, equilibrium constants and electroneutrality condition apply to the reaction MZ + $Cl\overset{-}{\overset{+}{H}}_3NCHRCOOR'$ + NaOH → $MZ(NH_2CHRCOOR')$ + Na^+ + Cl^- + H_2O. Equation numbers given to the left of a particular equation indicate those equations from which it is derived.

$$K_f = \frac{[MZE]}{[MZ][E]} \quad (1) \qquad K_{OH} = \frac{[MZOH^-]}{[MZ][OH^-]} \quad (2)$$

$$K_E = \frac{[E][H^+]}{[EH^+]} \quad (3)$$

Total ester:

$$E_{Tot} = [E] + [EH^+] + [MZE] = [Cl^-] \quad (4)$$

Total metal:

$$M_{Tot} = [MZ] + [MZE] + [MZOH^-] \quad (5)$$

Electroneutrality:

$$[Na^+] + [H^+] + [HE^+] = [MZOH^-] + [Cl^-] + [OH^-] \quad (6)$$

(3,4) $E_{Tot} = [E] \left(1 + \frac{[H^+]}{K_E}\right) + [MZE] = [E] (1 + \beta) + [MZE]$

$$E_{Tot} = [E]\alpha + [MZE] \quad (7)$$

where

$$\beta = \frac{[H^+]}{K_E} \quad \text{and} \quad \alpha = (1 + H^+/K_E)$$

(5,7) $M_{Tot} = [MZ] + E_{Tot} - [E]\alpha + [MZOH^-]$ (8)

(2,8) $M_{Tot} = [MZ] + E_{Tot} - [E]\alpha + [MZ]\ K_{OH}\ [OH^-]$ (9)

$$M_{Tot} = E_{Tot} \quad (10)$$

(9,10) $0 = [MZ] - [E]\alpha + [MZ]\ K_{OH}\ [OH^-]$

rearranging:

$$[E] = \frac{[MZ]\ (1 + K_{OH}\ [OH^-])}{\alpha} \quad (11)$$

(2,3,6) $[Na^+] + [E]\beta + [H^+] = [MZ]\ (K_{OH}[OH^-]) + [Cl^-] + [OH^-]$ (12)

rearranging and using $M_{Tot} = E_{Tot} = [Cl^-]$

$$[E]\beta = [MZ]\ (K_{OH}[OH^-]) + [OH^-] + M_{Tot} - [Na^+] - [H^+] \quad (13)$$

Let $\gamma = M_{Tot} + [OH^-] - [Na^+] - [H^+]$ and

$$\delta = K_{OH}[OH^-]$$

$$[E]\beta = [MZ]\delta + \gamma \quad (14)$$

(11, 14) $\beta[MZ](1 + \delta) = \alpha\gamma + \alpha[MZ]\delta$

$$[MZ]\ (\beta + \beta\delta - \alpha\delta) = \alpha\gamma \quad (15)$$

since $\alpha = 1 + \beta$, (15) reduces to

$$[MZ]\ (\beta - \delta) = \alpha\gamma \qquad (16)$$

(16) $$[MZ] = \frac{\alpha\gamma}{(\beta-\delta)} \qquad (17)$$

(2,5) $$M_{Tot} = [MZ]\ (1+\delta) + [MZE] \qquad (18)$$

(17,18) $$[MZE] = M_{Tot} - \frac{\alpha\gamma(1+\delta)}{(\beta-\delta)} \qquad (19)$$

(11,17) $$[E] = \frac{\gamma(1+\delta)}{(\beta-\delta)} \qquad (20)$$

(1,17,19,20)

$$K_f = \frac{M_{Tot} - \frac{\alpha\gamma(1+\delta)}{(\beta-\delta)}}{\frac{\alpha\gamma}{(\beta-\delta)}\ \frac{\gamma(1+\delta)}{(\beta-\delta)}}$$

$$K_f = \frac{(\beta-\delta)\ [(\beta-\delta)\ M_{Tot} - \alpha\gamma(1+\delta)]}{\alpha\gamma^2(1+\delta)} \qquad (21)$$

Derivation of a Calculable Expression for the Formation Constant of Amino Acids with $[MZ]^o$

The equations which follow represent an exact solution to the formation constant, K_f, of 1:1 MZ: amino acid, A, complexes where M is a +2 metal ion and Z is an iminodiacetate.

$$MZ + ClH_3NCHRCOOH + NaOH \rightarrow [MZ(NH_2CHRCOO)]^- + Na^+ + Cl^- + H_2O.$$

$$K_f = \frac{[MZA^-]}{[MZ][A^-]} \quad (1) \qquad K_{OH} = \frac{[MZOH^-]}{[MZ][OH^-]} \quad (2)$$

$$K_{A_1} = \frac{[AH][H^+]}{[AH_2^+]} \quad (3) \qquad K_{A_2} = \frac{[A^-][H^+]}{[HA]} \quad (4)$$

Total Acid:

$$A_{Tot} = [A^-] + [AH] + [AH_2^+] + [MZA^-]$$

Total Metal:

$$M_{Tot} = [MZ] + [MZA^-] + [MZOH^-]$$

Electroneutrality:

$$[Na^+] + [H^+] + [AH_2^+] = [A^-] + [MZA^-] + [MZOH^-] + [OH^-] + [Cl^-] \quad (7)$$

(3,4,5)

$$A_{Tot} = [A^-]\,[1 + [H^+]/K_{A_2} + [H^+]^2/K_{A_1}K_{A_2}] + [MZA^-] \quad (8)$$

let

$$\beta = \frac{[H^+]}{K_{A_2}} + \frac{2[H^+]^2}{K_{A_1}K_{A_2}}$$

$$\alpha = 1 + \frac{[H^+]}{K_{A_2}} + \frac{[H^+]^2}{K_{A_1}K_{A_2}}$$

$$A_{Tot} = [A^-]\alpha + [MZA^-] \quad (9)$$

(6,9) $$M_{Tot} = [MZ] + A_{Tot} - [A]\alpha + [MZOH^-] \quad (10)$$

$$M_{Tot} = A_{Tot} \quad (11)$$

(2,10,11) $$[MZ] - [A]\alpha + K_{OH}[MZ][OH^-] = 0 \quad (12)$$

Let $K_{OH}[OH^-] = \delta$

$$[MZ](1+\delta) - [A^-]\alpha = 0$$

$$[A^-] = \frac{[MZ](1+\delta)}{\alpha} \qquad (13)$$

(2,3,4,7)

$$[Na^+] + [AH_2^+] + [H^+] = [MZ]\delta + [MZA^-] + [OH^-] + [Cl^-] + [A^-] \qquad (14)$$

(2,6) $$M_{Tot} - [MZ](1+\delta) = [MZA^-] \qquad (15)$$

(14,15)

$$[AH_2^+] - [A^-] = M_{Tot} - [MZ] + [OH^-] + [Cl^-] - [Na^+] - [H^+] \qquad (16)$$

Let $\gamma = 2\,M_{Tot} + [OH^-] - [Na^+] - [H^+]$ where $[Cl^-] = M_{Tot}$

$$[AH_2^+] - [A^-] = \gamma - [MZ] \qquad (17)$$

$$[A^-](\beta-\alpha) = [AH] + 2[AH_2^+] - [A] - [AH] - [AH_2^+] \qquad (18)$$

(17,18) $$[A^-](\beta-\alpha) = \gamma - [MZ] \qquad (19)$$

(13,19) $$[MZ](1+\delta)(\beta-\alpha) = \alpha(\gamma - [MZ]) \qquad (20)$$

rearranging: $[MZ](\beta - \alpha\delta + \beta\delta - \alpha) = \alpha\gamma - \alpha[MZ]$

$$[MZ](\beta + \beta\delta - \alpha\delta) = \alpha\gamma$$

$$[MZ] = \frac{\alpha\gamma}{(\beta+\beta\delta - \alpha\delta)} \qquad (21)$$

Substitution of (21) in (13) yields:

$$[A^-] = \frac{\gamma(1+\delta)}{(\beta+\beta\delta - \alpha\delta)} \qquad (22)$$

Substitution of (21) in (15) yields:

$$[MZA^-] = M_{Tot} - \frac{\alpha\gamma(1+\delta)}{(\beta+\beta\delta - \alpha\delta)}$$

$$[MZA^{-}] = \frac{(\beta+\beta\delta - \alpha\delta)\, M_{Tot} - (1+\delta)\alpha\gamma}{(\beta+\beta\delta - \alpha\gamma)} \qquad (23)$$

(1, 21, 22, 23)

$$K_f = \frac{[(\beta + \beta\delta - \beta\delta)\, M_{Tot} - (1+\delta)\alpha\gamma]\ [\beta+\beta\delta - \alpha\delta]}{\alpha\gamma^2(1+\delta)}$$

$$K_f = \frac{[\beta+(\beta-\delta)\delta]\{[\beta+(\beta-\alpha)\delta]M_{Tot} - \alpha\gamma(1+\delta)\}}{\alpha\gamma^2(1+\delta)} \qquad (24)$$