being absent in the spectrum of the dihydro derivative. The proton magnetic resonance spectrum (in $CDCl_3$ and relative to external CH_2Cl_2 as reference)[3] indicated the presence of three olefinic hydrogens (−20 to +30 cycles), one methyl attached to carbonyl (+131 cycles; *cf.* acetone in $CDCl_3$ at +133 cycles) and three other methyl groups (+174 and +179 cycles). In accord with physical evidence for the structural feature $R\text{-}CO\text{-}CH_3$, reaction of the unsaturated ketone with sodium hypobromite afforded a nor acid (liquid) which was characterized as the crystalline benzyl isothiouronium salt, m.p. 143–145°; found: C, 67.46; H, 8.80. These facts are uniquely accommodated by formula IV for the cleavage product.

Electrolytic oxidation of the acetate of I (III) affords the onocerane coupling product in good yield, but *none* of the ketone IV, consistent with the view that formation of IV requires hydrogen atom discharge from V.

Electrolytic oxidation of the C_8 epimer of I (II) also produces the cleavage product IV, but in addition considerable amounts of two similar ketonic substances (determined by vapor chromatography), possibly formed from V by migration of hydrogen and methyl and subsequent elimination.

We are currently investigating the scope of this reaction.

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[3] The spectrum was obtained at 40 mc. with a Varian Model V-4300B high-resolution spectrometer fitted with a field-sensing stabilizer. A concentric tube cell was used.

DEPARTMENT OF CHEMISTRY AND
CHEMICAL ENGINEERING E. J. COREY
UNIVERSITY OF ILLINOIS RONALD R. SAUERS
URBANA, ILLINOIS SHERLOCK SWANN, JR.

RECEIVED SEPTEMBER 3, 1957

THE BEHAVIOR OF $[Fe(bipy)_3]^{+2}$ TYPE COMPOUNDS IN STRONG $HClO_4$

Sir:

It has been pointed out[1,2,3] that the mechanisms proposed for the acid hydrolysis[4] of $[Fe(bipy)_3]^{+2}$ do not completely describe the experimental kinetic behavior. As a portion of a study to evaluate the effects of changes in the chelating agent on the rate of acid dissociation of iron(II)-methine type compounds, we have obtained evidence for the existence of protonated species in concentrated acid solutions of $[Fe(PPI)_3]^{+2}$ (I), $[Fe(bipy)_3]^{+2}$ (II), and $[Fe(phen)_3]^{+2}$ (III). The formation of these species is accompanied by changes in spectra and magnetic susceptibility and is reversible upon dilution. The ability to form relatively stable protonated species may clarify the mechanism of the acid dissociation.

The visible spectrum of (I) perchlorate in water shows the maximum at 563 mμ, ϵ = 8,790. It is diamagnetic both in solution ($X_M = 530 \times 10^{-6}$) (slightly positive due to dissociation) and in the solid state ($X_M = -21.5 \times 10^{-6}$). (I) perchlorate dissolved in 8–12 *M* $HClO_4$ slowly changes to (ϵ_{max} at 540 mμ = 40.5), with an effective moment of 5.0 B.M. Dilution with 1 *M* acid results in the rapid regeneration of (I) in yields as high as 95%. Since the ligand hydrolyzes in strong acid, Fe(II) does not react rapidly with the ligand when $H^+ >$ 0.2 *M*, and the original complex can be regenerated, it is improbable that compound B is $[Fe(PPI)_2]^{+2}$ or $[Fe(PPI)]^{+2}$. Neither can a change in oxidation state be considered, for the reaction will take place in the presence of $As(OH)_4^-$ and Sn^{+2}. We interpret these observations on the basis of the formation of a protonated species in which the ligands are bound by $4s4p^34d^2$ hybridization.

The action of concentrated $HClO_4$ on (II) results in a slow reaction. Initial (ϵ_{max} at 522 mμ = 8,700; μ_{eff} = 0), final (ϵ_{max} at 620 mμ = 15; μ_{eff} = 5.2). Although considerable dissociation to Fe(II) takes place, the solution will partially revert to (II) on dilution with 1 *M* acid. In the light of the evidence for the existence of protonated species in the 2,2′,2″-terpyridine-iron(II) system[5] and since rapid disproportionation of $[Fe(bipy)]^{+2}$ to Fe(II) and (II) is highly unlikely in 1 *M* acid, it appears that these complexes associate with strong acids.

(III) chloride on addition to strong acid changes from red (ϵ_{max} at 510 mμ = 11,500) to blue (ϵ_{max} at 605 mμ = 175). A change in μ_{eff} from 0 to 4.9 accompanies the color change. Regeneration by dilution with 1 *M* acid results in 45% of the original (III). Treatment of *d*-$[Fe(phen)_3]^{+2}$ with concd. H_2SO_4 does not result in complete racemization for after regeneration a portion of the optical activity remains.

In the Fe(III)-methine systems, definite absorption shifts are observed but no changes in magnetic susceptibility occur. Lowering the acid concentration regenerates the starting material. The stability toward dissociation of the +2 and +3 complexes is enhanced in concentrated acids.

In both the +2 and +3 states, concentrated solutions of $NaClO_4$ do not cause these changes, whereas association with H^+ or acids also takes place in H_2SO_4 and to a lesser extent in $HClO_4$–HAc and concd. $HCl\text{–}CH_3OH$ mixtures. The spectra of the protonated species in $HClO_4$ and H_2SO_4 are identical in the visible region.

Strong association of the highly charged protonated complex with negative ions is indicated from kinetic measurements. Isolation has not been accomplished. Thus the constitution is not known but the kinetics of formation of the protonated complexes indicate that at least two H^+ are added and the major changes in spectra and magnetic moment take place in the final step.

The knowledge that the complexes have an affinity for concentrated acids supports the theory of the existence of lower protonated species in dilute acid and allows a quantitative explanation of the H^+ dependence of their dissociation without the introduction of "half-bonded" structures.[6]

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[1] P. Krumholz, *J. Phys. Chem.*, **60**, 87 (1956).

[2] F. Basolo, J. C. Hayes and H. M. Neumann, THIS JOURNAL, **70**, 3807 (1954).

[3] J. H. Baxendale and P. George, *Trans. Faraday Soc.*, **46**, 736 (1950).

[4] bipy = 2,2′-bipyridine, phen = 1,10-phenanthroline, PPI = 2-pyridinal-*n*-propylimine.

[5] R. B. Martin and J. A. Lissfelt, THIS JOURNAL, **78**, 938 (1956).

[6] E. A. Healy and R. Kent Murmann, to be published.

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

DEPARTMENT OF CHEMISTRY
THE UNIVERSITY OF CONNECTICUT
STORRS, CONNECTICUT
EDWARD A. HEALY
R. KENT MURMANN

RECEIVED JUNE 27, 1957

THE CALCIUM CHLORIDE AND HYDROXYL ION CATALYZED HYDROLYSIS OF SEVERAL ACYLATED α-AMINO ACID ESTERS

Sir:

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

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

TABLE I
VALUES OF K AND k_3[a,b]

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

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

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

CONTRIBUTION No. 2235 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA
R. BRUCE MARTIN
CARL NIEMANN

RECEIVED AUGUST 12, 1957

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

Sir:

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

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

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

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

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

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

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(1) Supported in part by grants from the National Science Foundation and from the National Heart Institute, National Institutes of Health, Public Health Servce.

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

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