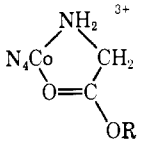
The Role of Oxygen and Nitrogen Bases in the Lysis of Acyl-Activated Esters. Cobalt(III) Chelated Glycine Isopropyl Ester

D. A. Buckingham, D. M. Foster, and A. M. Sargeson

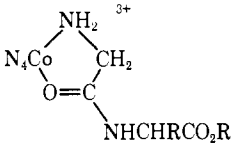
Contribution from the Research School of Chemistry, Australian National University, Canberra, 2600 Australia.
Received December 22, 1969

Abstract: The synthesis of $[Co(en)_2(glyOCH(CH_3)_2)](ClO_4)_3$ is described. Its reactivity toward lysis by nitrogen and oxygen bases has been studied in aqueous solution, and rate enhancements of $>10^6$ are observed, relative to the uncoordinated ester. The activation parameters indicate, for OH_2 and ^-OH, that this enhancement is due to $\Delta S^{\ddagger}$. Nitrogen bases act as nucleophiles producing chelated glycine amides, where $k_{obsd} = k_{N_1}[NHR_2] + k_{N_2}[NHR_2]^2$; oxygen bases produce chelated glycinate only, and appear to act as general bases.

It is now well established that metal ions promote the nonenzymic hydrolysis of amino acid esters and peptides. A detailed analysis of the divalent metal ion (Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}) catalyzed reactions is complicated by ligand lability in the metal complexes,[1,2] but for the related inert Co(III) systems the mechanism is known with some degree of certainty. Thus in the pH range 0–4 water acts as the nucleophile in the hydrolysis of chelated esters[3,4] (I, N_4 = tetramine) and the metal ion



I



II

induces an $\sim 10^7$-fold rate enhancement compared to the same path for the uncoordinated N-protonated ester.[2] The large rate increase results from direct activation of the carbonyl oxygen by the metal ion,[5] and in this regard the metal may be considered a poor substitute for a proton.[6]

In view of the semblance between the possible function of the Zn^{2+} ion in carboxypeptidase A[7] and Co(III) activation in structures I and II, it is of some significance to investigate the mechanisms of lysis in these systems. In particular it was relevant to determine (1) whether bases other than H_2O can promote the lysis of cobalt(III) activated amino acid derivatives in aqueous solution, (2) whether metal ion activation of the acyl function facilitates the direct nucleophilic or general base catalyzed reactions, and (3) whether a charged acyl function discriminates between charged and neutral bases. In this paper we discuss the role of a variety of oxygen and nitrogen bases in the lysis of cobalt(III)-chelated glycine isopropyl ester.

Experimental Section

Analar reagents were used throughout without further purification. Aminoacetonitrile hydrogen sulfate was prepared from methylene aminoacetonitrile[8] by the method of Anslow and King.[9] Pmr spectra were recorded on a Varian-HA100 spectrometer using tetramethylsilane (TMS) as an external (D_2O) and internal (acetone-d_6) reference. Absorption spectra and kinetic data were recorded on a Cary-14 spectrophotometer. Some cobalt estimations were made using a Techtron AA4 atomic absorption spectrophotometer and others were made spectrophotometrically using known extinction coefficients. pH measurements were made using a Radiometer TTA3 electrode assembly, TTT1 titrator, and PHA 630T scale expander. Bio-Rad AG 50W-X2 (200–400 mesh) cation exchange resin was used in chromatographic separations.

Preparation of $[Co(en)_2(NH_2CH_2CO_2CH(CH_3)_2)](ClO_4)_3$. *cis*-$[Co(en)_2Br(glyOCH(CH_3)_2)](ClO_4)_2$[3,4,10] (5 g) was dissolved in Merck acetone (30 ml, predried over BDH molecular sieves type 4A), and passed through a column of molecular sieves. $AgClO_4$ (8 g) dissolved in dry acetone (7 ml) was added, and the resulting solution left to stand for 3 hr. It was then filtered to remove AgBr, and the crude product was precipitated from solution by addition of anhydrous ether (*ca.* 200 ml). The product, initially an oil, solidified on standing under ether overnight. The solid was collected, washed with ether, and twice redissolved in dry acetone and reprecipitated with anhydrous ether. The final product was dried in an evacuated dessicator; yield 2.5 g. Owing to its hygroscopic nature and the presence of traces of Ag, no satisfactory analyses for C, H, and N could be obtained. The complex was instead characterized by infrared spectroscopy in anhydrous acetone[4] (ester carbonyl absorption, 1625 cm^{-1}), by pmr spectroscopy in acetone-d_6, Figure 1, and from the >95% recovery of the dipeptide ester complex ion $[Co(en)_2(glyglyOEt)]^{3+}$ observed upon condensation of the chelated ester complex with glycine ethyl ester in anhydrous acetone (estimated by chromatography of the reaction mixture).

Kinetic Measurements. Rate constants were calculated from spectrophotometric data obtained at 487 nm and in some cases from product analysis data. For buffers of pH <5 a weighed quantity of complex was dissolved in buffer (μ = 1.0 ($NaClO_4$)), and quickly filtered into a thermostated cell. For buffers of pH > 5 a weighed quantity of complex was dissolved in distilled water (pH $\sim$ 5) at 25°, and quickly filtered into the thermostated chamber of a stopped-flow reactor. In the other premixing chamber was buffer of μ = 2.0 ($NaClO_4$) at 25°. After mixing, the change in absorbance was followed. Reactions with $t_{1/2} > 1$ sec could be followed using this technique. Parent buffer solutions were pre-

(1) H. Kroll, *J. Amer. Chem. Soc.*, **74**, 2036 (1952).
(2) D. A. Buckingham, to be submitted for publication.
(3) M. D. Alexander and D. H. Busch, *J. Amer. Chem. Soc.*, **88**, 1130 (1966).
(4) D. A. Buckingham, D. M. Foster, and A. M. Sargeson, *ibid.*, **90**, 6032 (1968).
(5) For R = CH_3, the ester carbonyl absorption occurs at 1630 cm^{-1} (ref 4), as compared with 1735 cm^{-1} in the monodentate ester complex (ref 3), suggesting partial carbonium ion character at the carbonyl carbon atom in the chelate complex.
(6) R. B. Martin, *J. Amer. Chem. Soc.*, **89**, 2501 (1967).
(7) W. N. Lipscomb, M. L. Ludwig, J. A. Hartsuck, T. A. Steitz, H. Muirhead, J. E. Coppola, G. N. Reeke, and F. A. Quiocho, *Federation Proc.*, **26**, 385 (1967); T. A. Steitz, M. L. Ludwig, F. A. Quiocho, and W. N. Lipscomb, *J. Biol. Chem.*, **242**, 4662 (1967).
(8) P. Adams and W. D. Langley, "Organic Syntheses," Coll. Vol. I, N. Rapjohn, Ed., John Wiley & Sons, Inc., New York, N. Y., 1941, p 355.
(9) W. K. Anslow and H. King, in ref 8, p 298.
(10) Nomenclature and abbreviations used in this paper are described in D. A. Buckingham, D. M. Foster, and A. M. Sargeson, *J. Amer. Chem. Soc.*, **91**, 4102 (1969).

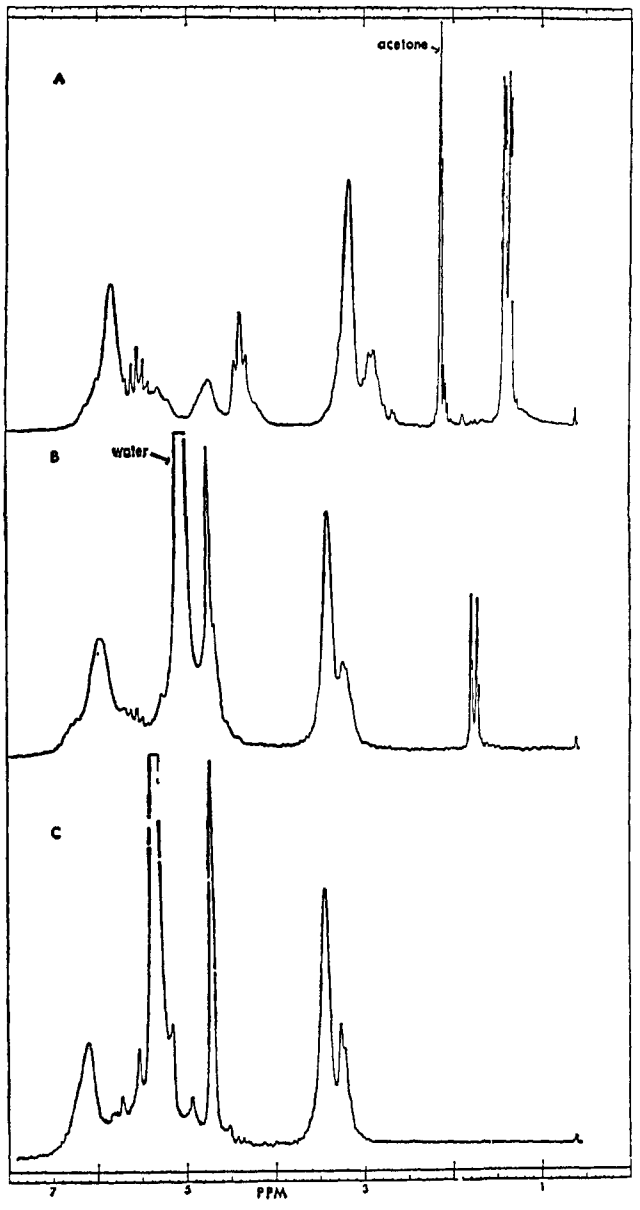


Figure 1. Pmr spectra (a) in acetone-d_6 of $[Co(en)_2(glyOCH(CH_3)_2)](ClO_4)_3$; (b) in D_2O of the 3+ adduct isolated following reaction of $[Co(en)_2(glyOCH(CH_3)_2)](ClO_4)_3$ with glycine isopropyl esters in aqueous solution; (c) in D_2O of the 3+ adduct isolated following reaction of $[Co(en)_2(glyOCH(CH_3)_2)](ClO_4)_3$ with aminoacetonitrile in aqueous solution (reference, TMS).

pared by stoichiometric addition of standardized sodium hydroxide or perchloric acid to the protonated or free bases, respectively. The pH of the resultant solution was then measured. Other buffer solutions were made by dilution.

Product Analysis. Treatment of $[Co(en)_2(glyOCH(CH_3)_2)](ClO_4)_3$ with ammonia, dimethylamine, glycine ethyl ester, glycine isopropyl ester, aminoacetonitrile, and aniline buffers in aqueous solution gave two products. The complex (0.3–0.4 g) was dissolved in the buffer (prepared and described above) and quenched to pH 2–3 with 70% $HClO_4$ after 15 sec (ammonia, dimethylamine), 10 min (glycine esters, imidazole), 15 min (aminoacetonitrile, pyridine), and 30 min (aniline). The solution was diluted with water and sorbed onto an H^+-form resin. Elution in 1.5 *N* HCl gave two orange bands, corresponding to 2+ and 3+ species. The 2+ species were identified in all cases as $[Co(en)_2gly]^{2+}$ by comparison of spectral properties (ϵ_{487} 98) and chromatographic behavior with that of an authentic sample. The amounts of 2+ and 3+ ions recovered represented >90% of the total Co, and small varying amounts of brown immobile material were observed on the top of the column as a reaction by-product.[11] The relative percentages of 2+ and 3+ ions formed were calculated assuming 100% recovery. For glycine isopropyl and ethyl esters and aminoacetonitrile, the eluate containing the 3+ ion was taken several times to dryness from water by vacuum evaporation, and the pmr spectrum of the product measured in D_2O.

For the oxygen bases the sole product of hydrolysis was $[Co(en)_2gly]^{2+}$. This was established variously by pmr spectroscopy, visible spectra, and chromatographic behavior. Recovery was >90%.

Reaction with Aniline in Acetone. $[Co(en)_2(glyOCH(CH_3)_2)](ClO_4)_3$ (0.5 g) was dissolved in dry acetone (*ca.* 20 ml) and aniline (1.8 g) was added. After 5 min at *ca.* 20° glacial acetic acid was added in excess to convert the excess aniline to acetanilide, and the solution taken to dryness, dissolved in water, and sorbed on the H^+-form resin. On elution with 1.5 *M* HCl, 2+ and 3+ products separated. The major 3+ band was difficult to elute compared to 3+ products formed from other amines, but was eluted with 3 *M* HCl. It was immobile with 1 *M* $NaClO_4$ as eluate, pH 4–5. The eluted product was taken several times to dryness, and its pmr spectrum run in D_2O.

Results

Rate Data. Observed rate constants (k_{obsd}, Table I) for the loss of $[Co(en)_2(glyOCH(CH_3)_2)]^{3+}$, were obtained from plots of log $(D_\infty - D_t)$ against time, which were linear for at least $2 \times t_{1/2}$ and in most cases $3 \times t_{1/2}$. Three runs were made under each set of conditions and reproducibility varied from ±5% at low pH to ±15% at high pH. The k_{obsd} values obey the rate expression

$$k_{obsd} = k_{hyd} + k_N'[N]_T \quad (1)$$

where k_{hyd} and k_N' are, respectively, rate constants for the lyate and buffer species, and $[N]_T$ is the total buffer concentration. At constant pH, extrapolation to $[N]_T = 0$ gave k_{hyd} and the slope gave k_N'. The rapidity of the hydrolysis reaction ($t_{1/2}$ (pH 1) ~ 12 min; $t_{1/2}$ (pH 7) ~ 40 sec) and the small optical density changes observed (0.02–0.06) limited the reproducibility of the data and restricted the practicable range of free base concentrations used. The second-order rate constants given in Table I were calculated using the equations

$$k_{hyd} = k_{H_2O}[H_2O] + \frac{10^{-14}k_{OH}}{[H^+]} \quad (2)$$

$$k_N = k_N'\frac{K_a + [H^+]}{K_a} \quad (3)$$

where $[H^+]$ is the hydrogen ion concentration as measured by the glass electrode and K_a is the acid dissociation constant for the conjugate acid of the buffer. In $HClO_4$ solutions, in the absence of buffer, k_{obsd} was constant over the pH range 0–2 and k_{H_2O} was obtained by dividing k_{obsd} by 55.5. At other pH's in the presence of buffer, k_{OH} was obtained from k_{hyd} using eq 2. The uncertainty in k_{OH} ($1.5 \pm 0.5 \times 10^6$ M^{-1} sec^{-1}) arises from the small differences in the extrapolated value of k_{hyd} for the different buffers, and from the reproducibility in the measurement of pH (±0.05). For the other bases, errors in k_N represent maximum probable deviations, and arise from the uncertainty in [N], the uncertainty in pH, and the uncertainty in the slope k_N'.

Included in Table I are rates for chloroacetate-catalyzed hydrolysis in D_2O. From the intercept at $[chloroacetate]_T = 0$ a value for $k_{hyd} = 5.4 \times 10^{-4}$ sec^{-1} was obtained, giving $k_{H_2O}/k_{D_2O} \sim 2$. The pK_a for chloroacetate in H_2O and D_2O was measured with a glass electrode at $\mu = 1.0$ ($NaClO_4$) by titration against 1 *M* NaOH, giving pK_a (H_2O) = 2.60 ± 0.02, pK_a

(11) See Buckingham, *et al.*, ref 10.

Table I. Rate Data for the Hydrolysis of $[Co(en)_2(glyOCH(CH_3)_2)](ClO_4)_3$[a]

Buffer	pK_a	[N][c]	pH	k_{obsd}, sec^{-1} ($\times 10^4$)	k_N,[d] M^{-1} sec^{-1}
Water[e]	−1.7	55.4	0	10.5	
		55.4	2.0	10.5	
		55.4[i]	1.0	10.5	$1.9 \pm 0.1 \times 10^{-5}$
		55.4[f]	1.0	4.8	$8.7 \pm 0.1 \times 10^{-6}$
		55.4[g]	1.0	19.3	$3.5 \pm 0.1 \times 10^{-5}$
Trichloroacetate	0.7	0.22	0.8	14.7	
		0.11	0.8	12.0	
		0.05	0.8	11.1	
		0	0.8	10.4	$1.6 \pm 0.3 \times 10^{-3}$
Dichloroacetate	1.4	0.2	1.4	11.9	
		0.1	1.4	11.5	
		0.05	1.4	10.8	
		0	1.4	10.4	$0.9 \pm 0.2 \times 10^{-4}$
Chloroacetate	2.6	0.25	2.6	44.4	
		0.2	2.6	36.8	
		0.075[h]	2.27	21.7	
		0.05[h]	2.25	19.2	
		0.025[h]	2.25	15.8	
		0.0125[h]	2.02	13.2	
		0	2	10.5	$1.4 \pm 0.1 \times 10^{-2}$
Chloroacetate[b]	2.7	0.56	2.8	19.7	
		0.28	2.8	12.6	
		0.14	2.8	8.75	
		0	2.8	5.4	$2.6 \pm 0.1 \times 10^{-3}$
Azide	4.0	0.2	4.0	17.7	
		0.1	4.0	14.4	
		0.05	4.0	13.0	
		0	4.0	11.7	$3.2 \pm 0.1 \times 10^{-3}$
Aniline	4.6	0.2	4.6	33.4	
		0.1	4.6	25.7	
		0.05	4.6	22.1	
		0	4.6	18.3	$0.7 \pm 0.1 \times 10^{-3}$
Acetate	4.8	0.06	4.1	28.8	
		0.03	4.1	18.5	
		0.015	4.0	17.7	
		0	4	12.2	$2.9 \pm 0.6 \times 10^{-2}$
		0.25	5.0	93.6	
		0.24[f]	5.0	49.5	
		0.24[g]	5.0	182	
		0.125	5.1	55.3	
		0.12[f]	5.0	34.1	
		0.12[g]	5.0	121	
		0.06	5.0	42.8	
		0.06[f]	5.0	18.7	
		0.06[g]	5.0	77	
		0	5	31.6	$2.1 \pm 0.4 \times 10^{-2}$
		0[f]	5	12.0	$1.5 \pm 0.3 \times 10^{-2}$
		0[g]	5	47	$5.6 \pm 0.4 \times 10^{-2}$
Pyridine	5.2	0.2	5.2	84.5	
		0.1	5.2	66.0	
		0.05	5.2	49.5	
		0	5.2	40.0	$2.3 \pm 0.3 \times 10^{-2}$
Aminoacetonitrile	5.3	0.17	6.0	86.6	
		0.08	5.9	65.4	
		0.04	5.9	57.8	
		0	5.9	48.5	$2.2 \pm 0.1 \times 10^{-2}$
Imidazole	7.0	0.08	6.3	460	
		0.04	6.3	400	
		0.02	6.3	190	
		0	6.3	180	0.4 ± 0.2
Hydroxide[i]	15.7		0–7		$1.5 \pm 0.5 \times 10^6$
			5[f]	7.2	$7.2 \pm 0.5 \times 10^5$
			5[g]	27.7	$2.8 \pm 0.5 \times 10^6$

[a] [Complex] $\sim 5 \times 10^{-3}$ to 1×10^{-2} M, μ = 1.0 ($NaClO_4$), T = 25.0 ± 0.1°. [b] Rate in 99% D_2O. [c] Concentration of buffer conjugate base. [d] Rate extrapolated to [N] = 0; $k_N = (k_{obsd} - k_{N=0})/[N]$. [e] Perchloric acid solutions. [f] 16.2 ± 0.1°. [g] 32.8 ± 0.1°. [h] Data taken from M. D. Alexander and D. H. Busch, *J. Amer. Chem. Soc.*, **88**, 1130 (1966), μ = 0.66. [i] Calculated from intercepts of plots of k_{obsd} *vs.* [N] by subtraction of k_{OH_2}. [j] μ = 0.1 and 1.0.

(95% D_2O) = 2.67 ± 0.03.[11a] Using these constants to calculate chloroacetate base concentrations a value of 5 for $k_{OClAc}(H_2O)/k_{OClAc}(D_2O)$ was obtained. The agreement between k_{OAc} values obtained at pH 4 and 5 indicates that for this base at least this path is largely acid independent. Data in perchloric acid at μ = 1.0 and

(11a) Note Added in Proof. The latter value was evaluated using the expression pK_a = pH + 0.4: P. K. Glascoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).

Table II. Product Analysis of Nitrogen Base Catalyzed Hydrolysis of $[Co(en)_2(glyOCH(CH_3)_2](ClO_4)_2$[a]

Base	[N][b]	pH[c]	% 3+ product	% $[Co(en)_2gly]^{2+}$	k_{N_1}, M^{-1} sec^{-1}	k_{N_2}, M^{-2} sec^{-1}
Dimethylamine	2.0	11.0	15	85	87	29
	1.0	11.0	7	93		
Ammonia	2.0	9.6	77	23	36	31
	1.0	9.6	53	47		
	0.25	9.6	17	83		
	0.25[d]	8.5	66	34		
			66	34		
Glycine ethyl ester	1.0	7.6	54	46	0.25	0.39
	0.5	7.6	29	71		
Glycine isopropyl ester	1.0	7.5	56	44	~0.25	~0.4
Imidazole	1.0	7.1	0	~100		
Amino acetonitrile	0.5	6.3	33	67	1.4×10^{-2}	5.6×10^{-3}
	0.25	6.3	19	81		
Pyridine	2.0	5.0	0	~100		
Aniline	0.1	4.6	Trace	~100		

[a] [Complex] $\sim 3 \times 10^{-2}$ M, 25°. [b] [N] = conjugate base concentration; $[N]_{total} = 2[N]$. [c] pH = pK_a. [d] $[N]_{total} = 2.75$ M.

0.1 (Table I) indicate that k_{OH_2} is independent of ionic strength within this range.

Activation Parameters. From data in acetate buffers at 25, 16.2, and 32.8°, energies of activation for k_{OH} and k_{OH_2} were calculated from the slope of an Arrhenius plot. Enthalpies of activation were obtained from the equation $\Delta H^{\pm} = E_a - RT$, and entropies of activation, $\Delta S^{\pm}_{298}$ from the Eyring equation, $k_2 = (KT/h)\exp(-\Delta H^{\pm}/RT)\exp(\Delta S^{\pm}/R)$, where $T = 298.2°$ K. For k_{OH_2}, $\Delta H^{\pm} = 14.6 \pm 0.1$ kcal mol^{-1}, $\Delta S^{\pm} = -25 \pm 1$ cal mol^{-1} deg^{-1}; for k_{OH}, $\Delta H^{\pm} = 14.3 \pm 1$ kcal mol^{-1}, $\Delta S^{\pm} = 15 \pm 4$ cal mol^{-1} deg^{-1}. Acetate buffers show negligible pH variation over this temperature range.[12]

Product Analysis. The formation of chelated dipeptides by addition of amino acid derivatives to the carbonyl atom of cobalt(III) chelated glycine esters in nonaqueous solvents has already been reported,[13] and the results presented in Table II show that such reactions also occur in water. A similar reaction was presumed to account for the formation of chelated peptide ester complexes from reaction in aqueous solution of glycine esters with β-$[Co(trien)Cl_2]Cl$.[14]

The pmr spectrum of the 3+ ion formed from reaction with glycine isopropyl ester is given in Figure 1. This spectrum is consistent with the 3+ species being $[Co(en)_2(glyglyOCH(CH_3)_2)]^{3+}$ (isopropyl CH_3 doublet ~1.7 ppm, CH (quartet ~5.5 ppm). In the case of glycine ethyl ester, the product dipeptide ester complex undergoes acid-catalyzed ester hydrolysis on the H^+-form resin, and the isolated product was identical, in its visible spectrum, chromatographic behavior, and pmr spectrum with that formed from passage of authentic $[Co(en)_2(glyglyO)](ClO_4)_2$[15] through an H^+-form resin. Under the conditions of isolation, the α- and β-CH_2 absorptions coincide in the pmr spectrum at 4.7 ppm. The 3+ product formed in the reaction with aminoacetonitrile, after elution from the H^+-form resin, was also identical with $[Co(en)_2(glyglyOH)]^{3+}$ (Figure 1) which must be ascribed to acid hydrolysis of the coordinated glycylaminonitrile during isolation.

(12) R. G. Bates, "Determination of pH," John Wiley & Sons, Inc., New York, N. Y., 1964, p 117.
(13) D. A. Buckingham, L. G. Marzilli, and A. M. Sargeson, *J. Amer. Chem. Soc.*, **89**, 2772 (1967).
(14) J. P. Collman and E. Kimura, *ibid.*, **89**, 6096 (1967).
(15) D. A. Buckingham, C. E. Davis, D. M. Foster, and A. M. Sargeson, *J. Amer. Chem. Soc.*, in press.

Table II contains product percentages of 2+ ($[Co(en)_2gly]^{2+}$) species and 3+ ($[Co(en)_2glyNR_2]^{3+}$) species analyzed (chromatographically) after reaction of the chelated ester complex with nitrogen bases, NHR_2. The results may be treated by considering ester lysis as arising from competition amongst water, hydroxide ion, and the nitrogen base for the chelated ester, the two former paths producing $[Co(en)_2gly]^{2+}$ and the latter $[Co(en)_2glyNR_2]^{3+}$. In all cases, the observed product percentages for varied [N], and in the case of ammonia for varied [$^-$OH], may be fitted to the following expression

$$\%\,[Co(en)_2gly]^{2+}/\%[Co(en)_2glyNR_2]^{3+} = (k_{OH_2}[OH_2] + k_{OH}[^-OH])/(k_{N_1}[N] + k_{N_2}[N]^2) \quad (4)$$

where k_{N_1} and k_{N_2} are, respectively, the second- and third-order rate constants for $[Co(en)_2glyNR_2]^{3+}$ production, and [N] is the concentration of free amine. In the pH range quoted in Table II, the water path is negligible and only the hydroxide ion path effectively produces the $[Co(en)_2gly]^{2+}$ product. Little or no $[Co(en)_2gly]^{2+}$ results from subsequent hydroxide ion promoted hydrolysis of $[Co(en)_2glyNR_2]^{3+}$, since the reaction solutions were rapidly quenched, and the rate constants for these reactions are relatively small (k_{OH} = 1–25 M^{-1} sec^{-1} at 25°, μ = 1.0).[15]

The values for k_{N_1} and k_{N_2} given in Table II were obtained by solving expression 4 for the observed product distributions and using pK_w = 14.0, $k_{H_2O} = 1.9 \times 10^{-5} M^{-1}$sec^{-1} and $k_{OH} = 1.5 \times 10^6 M^{-1}$sec^{-1}. The observed product percentages obtained chromatographically were reproducible to within ±2%. For ammonia, observed and calculated product percentages are as follows: [N] = 2, pH 9.6; % 3+, calcd 77 (obsd 77); [N] = 1, pH 9.6; % 3+, calcd 53 (obsd 53); [N] = 0.25, pH 9.6; % 3+, calcd 13 (obsd 17); [N] = 0.25, pH 8.5; % 3+, calcd 67 (obsd 66, 66, duplicate runs).

Two other rate expressions may be excluded by the observed product distributions. If it were assumed that $[Co(en)_2gly]^{2+}$ resulted in part from general base catalysis by the nitrogen base (expression 5, where k_B is the second-order rate for general base catalysis by N), or that $[Co(en)_2glyNR_2]^{3+}$ resulted in part from a term first order in hydroxide and first order in N (expression 6, with k_{NOH} the relevant rate constant), then solutions to eq 5 and 6 result in negative values for k_B and k_{NOH}.

$$\%[Co(en)_2gly]^{2+}/\%[Co(en)_2glyNR_2]^{3+} = (k_{OH_2}[OH_2] + k_{OH}[^-OH] + k_B[N])/k_N[N] \quad (5)$$

$$\%[Co(en)_2gly]^{2+}/\%[Co(en)_2glyNR_2]^{3+} = (k_{OH_2}[OH_2] + k_{OH}[^-OH])/(k_N[N] + k_{NOH}[N][^-OH]) \quad (6)$$

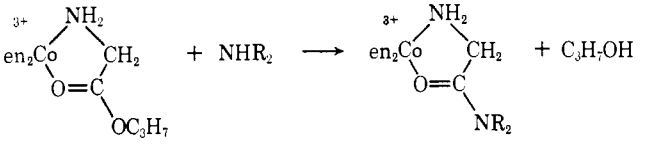
An expression such as eq 7, in which $[Co(en)_2glyNR_2]^{3+}$ is produced exclusively by a term first order in amine, leads in all cases to larger values of k_N for larger values of [N], at the same pH. Thus, it is necessary to invoke terms both first order and second order in amine for $[Co(en)_2glyNR_2]^{3+}$ production. However, small contributions from other terms cannot be excluded.[16] With the small concentrations of amine used in the spectrophotometric rate evaluations, Table I, the term second order in amine becomes negligibly small. Reasonable agreement is seen between k_N for aminoacetonitrile obtained spectrophotometrically (Table I) and k_{N_1} obtained by product distribution (Table II).

$$\%[Co(en)_2gly]^{2+}/\%[Co(en)_2glyNR_2]^{3+} = (k_{OH_2}[OH_2] + k_{OH}[^-OH])/k_N[N] \quad (7)$$

The low solubility of aniline in water and small k_N value (Table I) limited the quantity of 3+ ion to 2–4%. Only a trace was detected on the ion exchange column. However the $[Co(en)_2(glyNHPh)]^{3+}$ ion, prepared by condensation in acetone, was stable to hydrolysis at pH 5, and its pmr spectrum in D_2O showed the glycine CH_2 absorption at 4.7 ppm (2 H) and phenyl ring protons (5 H) at 7.8 ppm from external TMS. Using pyridine and imidazole, only $[Co(en)_2gly]^{2+}$ was observed on the column.

Discussion

Nitrogen Bases. Ester lysis by nitrogen bases corresponds to nucleophilic substitution at the carbonyl carbon to form the chelated glycine amide. This has



been demonstrated for $NH(CH_3)_2$, NH_3, glyOEt, glyOPr^i, NH_2CH_2CN, and aniline, and the approximately linear correlation between the pK_a and log k_{N_1} values for all nitrogen bases, Figure 2, implies that a similar result holds for pyridine and imidazole. For these bases, a slope of $\beta \sim 0.8$ is obtained, similar to that found for other nucleophilic aminolysis reactions.[17] The observed rate law, $k_N' = k_{N_2}[Am] + k_{N_2}[Am]^2$ is that usually found for aminolysis of esters with "poor" leaving groups.[16] However, a recent study on a series of acyl-activated *o*-nitrophenyl acetates proposed that, apart from the methoxyamine reaction (charged esters), aminolysis by imidazole, 2-aminopyridine, glycineOEt, and ethylenediamine was not subject to general base catalysis by a second molecule of amine.[18]

(16) W. P. Jencks and J. Carriuolo, *J. Amer. Chem. Soc.*, **82**, 675 (1960); T. C. Bruice and M. Mayahi, *ibid.*, **82**, 3067 (1960); J. F. Bunnett and G. T. Davis, *ibid.*, **82**, 665 (1960); G. M.Blackburn and W. P. Jencks, *ibid.*, **90**, 2638 (1968); J. F. Kirsch and A. Kline, *ibid.*, **91**, 1841 (1969); T. C. Bruice and S. M. Felton, *ibid.*, **91**, 2799 (1969).

(17) S. L. Johnson, *Advan. Phys. Org. Chem.*, **5**, 237 (1967).

(18) B. Holmquist and T. C. Bruice, *J. Amer. Chem. Soc.*, **91**, 2985 (1969).

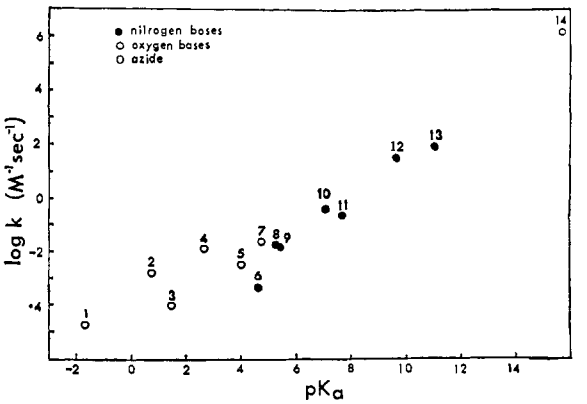
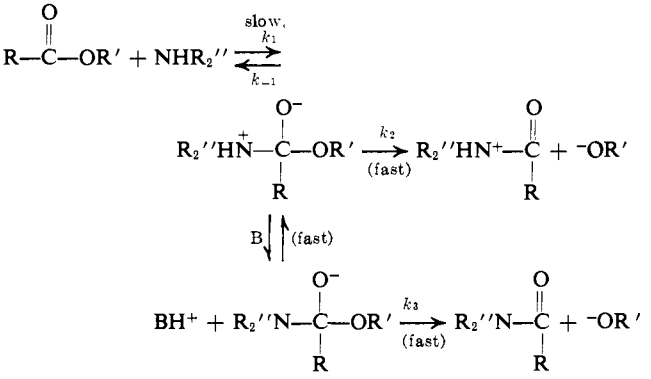


Figure 2. Plot of base pK_a *vs.* log k for the lysis of $[Co(en)_2(glyOCH(CH_3)_2)](ClO_4)_3$ in aqueous solution, where k is the second-order rate constant: 1, water; 2, trichloroacetate; 3, dichloroacetate; 4, chloroacetate; 5, azide; 6, aniline; 7, acetate; 8, pyridine; 9, aminoacetonitrile; 10, imidazole; 11, glycine ethyl ester; 12, ammonia; 13, dimethylamine; 14, hydroxide; 25°, μ = 1–4.

The mechansim of aminolysis at unsaturated carbon remains uncertain. In a small number of instances changes in rate law on changing the pH, or buffer concentration at constant pH, have been interpreted in terms of the existence of a tetrahedral addition intermediate.[19] Other results are usually interpreted assuming the formation of this intermediate following the rate-controlling transition state.[20] This mechanism is represented as follows and accommodates the second-order path. The third-order path is attributed to general base catalyzed addition of amine.



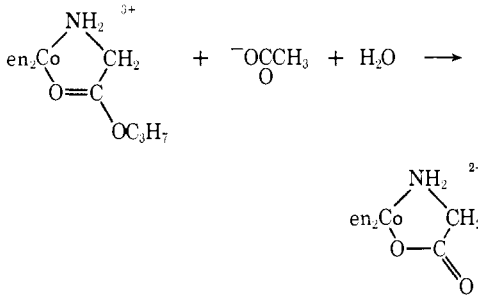
While the present results may be interpreted in this manner, an alternative proposal consisting of rate-determining acid-catalyzed removal of alcohol[21] is also consistent with the results. Indeed, acyl activation might be expected to accelerate addition of the amine (k_1) and retard loss of alcohol as $^-OR'$ (k_2,k_3) or HOR' and amine (k_{-1}). Provided an intermediate is formed, a sufficiently reactive ester containing a "poor" leaving group could realize the situation where rapid addition of amine forms a *stable intermediate*, which slowly eliminates alcohol. These possibilities arise for the Co(III) activated systems, and a detailed discussion of the aminolysis mechanism will be reserved until studies of this nature, now in progress, are reported.

(19) G. M. Blackburn and W. P. Jencks, *ibid.*, **90**, 2638 (1968), and examples quoted therein.

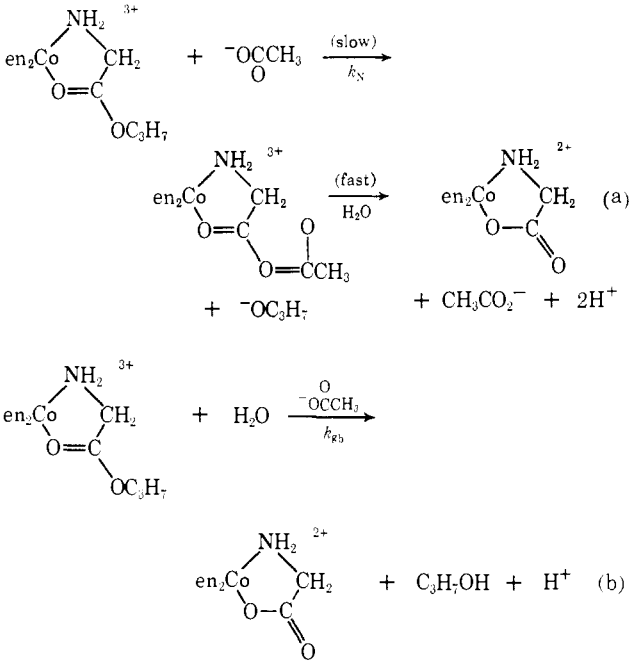
(20) W. P. Jencks and M. Gilchrist, *ibid.*, **90**, 2622 (1968).

(21) J. F. Bunnett and G. T. Davis, *ibid.*, **82**, 665 (1960).

Oxygen Bases. The product of ester lysis by a variety of substituted acetates, H_2O, ^-OH, and N_3^-, is $[Co(en)_2gly]^{2+}$, *e.g.*

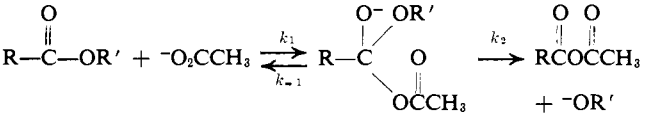


The mechanistic pathway for hydrolysis has not been established, and at least two paths are consistent with the results: (a) the nucleophilic addition of acetate (k_N) followed by rapid hydrolysis of the resulting anhydride; (b) the general acetate catalyzed addition of water (k_{gb}).



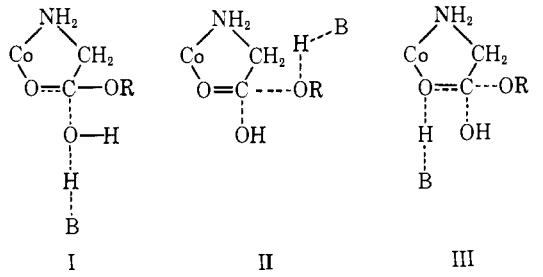
The following evidence, however, is consistent with general base catalyzed addition of water. (1) The β value[17] for the oxygen bases ($\sim$0.4) appears to be smaller than that for the nitrogen bases ($\sim$0.8) (Figure 2). This may suggest a change in mechanism or rate-determining step. (2) Whereas the N_3^- ion is a very powerful nucleophile for carbonyl carbon compared to its affinity for a proton,[17] the present result shows that it is no more effective than the oxyanions. This suggests that azide and oxyanions are not here functioning as nucleophiles. (3) The large deuterium isotope effect for $ClCH_2CO_2^-$ ($k(H_2O)/k(D_2O) \sim 5$) is consistent with this base's primary role in proton abstraction. Large kinetic isotope ratios (>2) are commonly found for general base catalyzed reactions, whereas direct nucleophilic addition usually involves little or no isotopic distinction ($k(H_2O)/k(D_2O) \sim 1$).[17]

General base catalysis by acetate has been demonstrated for the hydrolysis of phenyl acetates with poor leaving groups.[22] This is consistent with a preferred decomposition of the nucleophilic addition product to reactants rather than to products when the nucleophilicity of the base for carbon is less than that of the alcoholate ion ($k_{-1} > k_2$). This situation appears to prevail in the



acetate-catalyzed hydrolysis of the chelated isopropyl ester.

At the present time we do not wish to speculate further on the mechanism. The kinetically indistinguishable paths[23] involving oxyanion-catalyzed addition of water to the carbonyl carbon (I) or reversible addition of hydroxide and general acid catalyzed removal of alcohol (II), both may be accommodated by the present results. However, mechanisms involving general acid



catalyzed protonation of the carbonyl oxygen (III),[23] seem less likely, since the carbonyl oxygen when coordinated to cobalt will be a very much weaker base than the already weakly basic carbonyl oxygen of uncoordinated esters ($pK_a \sim -6.5$).[24]

General Remarks. The effect of the metal ion on the hydrolysis rate is large. Comparison of the present value of k_{OH} with the similar rate constant for hydrolysis of $NH_2CH_2CO_2Et$ ($k_{OH} \sim 0.6\ M^{-1}\ sec^{-1}$)[25] shows a rate enhancement of $\sim 10^6$ in the cobalt(III) chelate, and a similar comparison with the k_{OH} path for $^+NH_3CH_2CO_2Et$ ($k_{OH} \sim 24\ M^{-1}\ sec^{-1}$)[26] shows that the cobalt is far more effective in promoting hydrolysis than a proton on the nitrogen of the ester. However, a comparison of the latter rate with that for ester hydrolysis in the $(NH_3)_5Co(NH_2CH_2CO_2Et)^{3+}$ ion ($k_{OH} = 30\ M^{-1}\ sec^{-1}$)[27] suggests that when H^+ and $(NH_3)_5Co^{3+}$ are bound to nitrogen, their effect on the rate is similar. The additional enhancement of $\sim 10^5$ for cobalt(III) can therefore be attributed to direct metal ion activation of the carbonyl center. In this regard the metal ion appears to be much less efficient than the proton, since an estimate of $\sim 6\ M^{-1}\ sec^{-1}$ for k_{H_2O} for the carbonyl oxygen protonated ester[6] $^+NH_3CH_2C(—OR)=OH^+$ is larger than k_{H_2O} for the chelated ester by a factor of $\sim 10^5$. The significance of the cobalt(III) promoted reaction is that the activated $^{3+}Co—O=C<$ system is present under all pH conditions.

Finally, the $\Delta H^{\ddagger}$ values for both the water and ^-OH paths are similar ($\sim$14.5 kcal mol^{-1}) and not very different from those obtained for the base-catalyzed hydrolysis of ethyl glycinate (8.8 kcal mol^{-1}),[25] N-pro-

(22) D. G. Oakenfull, T. Riley, and V. Gold, *Chem. Commun.*, 385 (1966).

(23) W. P. Jencks, *Progr. Phys. Org. Chem.*, 2, 63 (1964).
(24) E. M. Arnett, *ibid.*, 1, 223 (1963).
(25) R. W. Hay and L. J. Porter, *J. Chem. Soc.*, 1261 (1967).
(26) H. A. Conley and R. B. Martin, *J. Phys. Chem.*, **69**, 2914 (1965).
(27) D. A. Buckingham, D. M. Foster, and A. M. Sargeson, *J. Amer. Chem. Soc.*, **91**, 3451 (1969).

tonated ethyl glycinate (11.8 kcal mol^{-1}),[25] ethyl acetate (11.4 kcal mol^{-1}),[28] and $Et_3N^+CH_2CO_2Et$ (12.7 kcal mol^{-1}).[29] The large increase in rate observed for the chelated ester results entirely from the large positive entropy of activation (~15 cal mol^{-1} deg^{-1}). The above hydrolysis reactions are all associated with negative $\Delta S^{\pm}$ values: glyOEt (−32 cal mol^{-1} deg^{-1}),[25] EtOAc (−27 cal mol^{-1} deg^{-1}),[28] HglyOEt$^+$ (−6 cal mol^{-1} deg^{-1}),[25] $^+NEt_3CH_2CO_2Et$ (−3 cal mol^{-1} deg^{-1}).[29] Also, the ~10^{11} rate increase for ^-OH compared to H_2O for the chelated ester may be attributed largely to a positive entropy charge of ~40 cal mol^{-1} deg^{-1}.

(28) E. Tommila, A. Loivisto, J. P. Lyyra, K. Antrell, and S. Heimo, *Ann. Acad. Sci. Fenn., Ser. A*, II, 47 (1952).

(29) R. P. Bell and F. J. Lindars, *J. Chem. Soc.*, 4601 (1954).

Mechanisms of Thiamine-Catalyzed Reactions. A Kinetic Analysis of the Decarboxylation of Pyruvate by 3,4-Dimethylthiazolium Ion in Water and Ethanol[1]

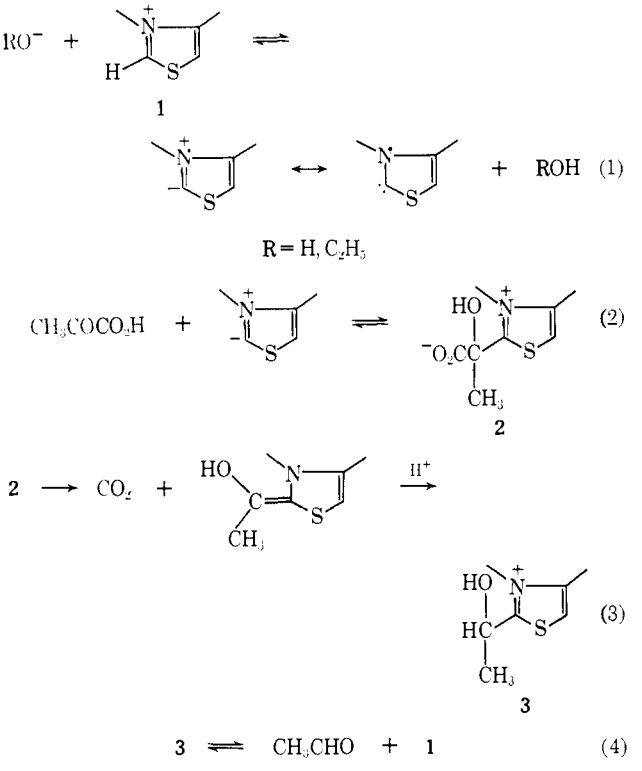
John Crosby and Gustav E. Lienhard

Contribution from the James Bryant Conant Laboratory, Harvard University, Cambridge, Massachusetts 02138. *Received February 13, 1970*

Abstract: This paper reports the kinetics of the hydrogen–deuterium exchange of 3,4-dimethylthiazolium ion (**1**) in ethanol and the kinetics of the breakdown of 2-(1-carbethoxy-1-hydroxyethyl)-3,4-dimethylthiazolium ion (**4**) and of 2-(1-hydroxyethyl)-3,4-dimethylthiazolium ion (**3**) to form **1** and ethyl pyruvate or acetaldehyde, in ethanol and in water. All three reactions are catalyzed by lyate ion. The rate constants for catalysis by ethoxide ion in ethanol are: for the hydrogen–deuterium exchange reaction of **1**-2-*d*, 4.6 × 10^9 M^{-1} min^{-1} at 25°; for the breakdown of **4**, 2.6 × 10^8 M^{-1} min^{-1} at 44.6°; for the breakdown of **3**, 1.1 × 10^5 M^{-1} min^{-1} at 45.6°. The lyate ion catalyzed hydrogen–deuterium exchange of **1**-2-*d* occurs 500 times more rapidly in ethanol than in water. The lyate ion catalyzed elimination reactions of **3** and **4** are 10^4–10^5 times faster in ethanol than in water. Also, the addition of **1** to the keto group of ethyl pyruvate to form **4**, the equilibrium constant for which is 20 M^{-1} in ethanol at 25.9°, probably is about 10^4 times faster in ethanol than in water when the rates are compared at the same concentration of lyate ion. In conjunction with earlier work, these results provide a kinetic analysis of the decarboxylation of pyruvate by **1** and provide further support for the hypothesis that catalysis in thiamine pyrophosphate dependent enzymatic reactions may be due in large part to binding of the thiazolium nucleus in a hydrophobic region of the enzymes.

The decarboxylation of α-keto acids is catalyzed by thiamine and other thiazolium salts. This catalysis is a model for the thiamine pyrophosphate dependent enzymatic decarboxylations of α-keto acids, which, however, are much more rapid. The major covalent changes which occur during catalysis have been clearly established, both for the model and the enzymatic reactions, largely through the efforts of Breslow, Krampitz, and Holzer.[2] These are given in eq 1–4 for the case of the decarboxylation of pyruvate catalyzed by 3,4-dimethylthiazolium ion (**1**).

The kinetics of ionization in aqueous solution of the hydrogen atom at C-2 of thiamine **1** and other thiazolium compounds have been previously determined.[3–6] The reaction is, as shown in eq 1, catalyzed by hydroxide ion. We have recently studied the kinetics



(1) This research was supported by grants (GB4848 and GB12848) from the National Science Foundation and a fellowship to J. C. from the William F. Milton Fund of Harvard University. Address inquiries to G. E. L.

(2) L. O. Krampitz (*Ann. Rev. Biochem.*, **38**, 213 (1969)) thoroughly reviews the enzymatic and model reactions.

(3) R. Breslow and E. McNelis, *J. Amer. Chem. Soc.*, **81**, 3080 (1959).

(4) P. Haake, L. P. Bauscher, and W. B. Miller, *ibid.*, **91**, 1113 (1969).

(5) J. Ullrich and A. Mannschreck, *Biochim. Biophys. Acta*, **115**, 46 (1966).

(6) W. Hafferl, R. Lundin, and L. L. Ingraham, *Biochemistry*, **2**, 1298 (1963).