METAL-ION INDUCED REACTIONS OF β-ALANINE DERIVATIVES

by

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The work presented in this thesis has been undertaken in the Research School of Chemistry and is the author's own unless otherwise stated.

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I appreciate the moral support of my family and of my colleagues throughout the period of this research and especially while this thesis was being written. Finally, I appreciate the effort that John Baker has expended in the organization of this thesis and especially value his many words of encouragement.
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Kinetic data for the hydrolysis of [Co(en)$_2$(β-alaoCH(CH$_3$_2))(ClO$_4$)$_3$] have been obtained in acidic and basic solutions ($\mu$ = 1.0, NaClO$_4$; 25.0°) and fit the rate law, $k_{obsd} = k_0 + (k_1[OH^-] + k_2[OH^-]^2)/(1 + K'[OH^-])$, with $k_0 = 4.7 \times 10^{-5}$ sec$^{-1}$ (the water path), $k_1 = 5.0 \times 10^3$ M$^{-1}$ sec$^{-1}$, $k_2 = 7.0 \times 10^8$ M$^{-2}$ sec$^{-1}$ and $K' \approx 2.3 \times 10^4$ M$^{-1}$. There is a large enhancement in base hydrolysis rate ($\approx 10^5$), relative to that of the free ester. Above pH $\approx 9.2$, a limiting rate is observed and the rate law reduces to $k_{obsd} = k_3 + k_4[OH^-]$, with $k_3 \approx 0.4$ sec$^{-1}$ and $k_4 \approx 3 \times 10^4$ M$^{-1}$ sec$^{-1}$. The change in rate law is strong evidence for a stepwise mechanism involving an intermediate, formed by addition of OH$^-$ at the carbonyl carbon of the ester substrate. Tracer experiments ($^{18}$O) and stereochemical studies show that base-catalyzed hydrolysis of cis-[Co(en)$_2$H$_2$O(β-alaoCH(CH$_3$_2))(NO$_3$)$_3$] occurs by an intramolecular process where bound hydroxide is the nucleophile at the ester substrate. The reaction is proposed to proceed through the same tetrahedral intermediate as that in base hydrolysis of the chelated ester. Kinetic data fit the rate law, $k_{obsd} = k_0 + k_{OH}[OH^-]$, with $k_0 = 1.8 \times 10^{-6}$ sec$^{-1}$ and $k_{OH} \approx 0.18$ M$^{-1}$ sec$^{-1}$ ($\mu$ = 1.0, NaClO$_4$; 25.0°). The rate of intramolecular hydrolysis is governed by the rate of formation of the six-membered chelate ring. The rate constant for the intramolecular reaction is not very different from that for the hydrolysis of the monodentate
ester \( k_{OH} \sim 0.11 M^{-1} \text{sec}^{-1}; \mu = 1.0, \text{NaClO}_4; 25.0^\circ \) so that competition between coordinated and free hydroxide in base hydrolysis leads to \( \sim 60\% \) \([\text{Co(en)}_2(\beta\text{-alaO})]^{2+}\) and \( \sim 40\% \) \([\text{Co(en)}_2\text{OH}(\beta\text{-alaO})]^{+}\). The results for the \( \beta \)-alanine derivatives have been compared with those of the analogous glycine derivatives, to observe the effect of ring size on the rates of the different processes. The significance of the results is discussed in relation to the "bound carbonyl" and "bound hydroxide" mechanisms, entertained for hydrolysis of peptides and esters in hydrolytic enzymes, such as carboxypeptidase A.

In strongly basic solutions (0.1 - 1.0M NaOH), \([\text{Co(en)}_2(\beta\text{-alaO})](\text{ClO}_4)_2 \cdot \text{H}_2\text{O}\) is hydrolyzed to produce both \( \text{cis-}\) and \( \text{trans-}\) \([\text{Co(en)}_2\text{OH}(\beta\text{-alaO})]^{+}\) species, which are subsequently hydrolyzed to \( \text{cis-}\) and \( \text{trans-}\) \([\text{Co(en)}_2\text{OH}_{2}]^{+}\) species. The ring opening is proposed to proceed largely by a dissociative process of deprotonated starting material (S_{N,1CB mechanism). Results of the base hydrolysis of complexes, \([\text{Co(en)}_2 X(\beta\text{-alaOR})] X_2\), \( (X = \text{Cl, Br}; R = \text{H, CH}_{2}\text{CH}_{3}) \) show that the same process is involved and that a common intermediate is formed. Further evidence for the existence of the five-coordinate intermediate, beside competition properties for nucleophiles in the system, comes from stereochemical studies. A new feature of the results is that competition occurs between solvent nucleophiles (\( \text{H}_2\text{O}, \text{N}_3^- \)) and the carbonyl oxygen nucleophile of the N-bound \( \beta \)-alanine ester. Results of stereochemical and competition studies in \( \text{Hg}^{2+}\)-induced halide removal from complexes, \( \text{cis-}[\text{Co(en)}_2 X(\beta\text{-alaOR})] X_2\) \( (X = \text{Cl, Br}; R = \text{H, CH}_3, \text{CH}_2\text{CH}_3) \)
CH(CH₂)₂ are also consistent with a dissociative mechanism involving the formation of a reactive five-coordinate intermediate, the geometry of which is probably square pyramidal. Kinetic data for \( \text{Hg}^{2+} \)-induced bromide removal and base hydrolysis of cis-[Co(en)₂Br(\( \delta \)-alaOR)]Br₂ have also been obtained.
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>aa</td>
<td>amino acid</td>
</tr>
<tr>
<td>β-ala</td>
<td>NH₂CH₂CH₂CO species</td>
</tr>
<tr>
<td>β-alaNH₂</td>
<td>NH₂CH₂CH₂CONH₂</td>
</tr>
<tr>
<td>β-alaO</td>
<td>NH₂CH₂CH₂COO⁻ anion</td>
</tr>
<tr>
<td>arg</td>
<td>arginine</td>
</tr>
<tr>
<td>bu</td>
<td>butyl</td>
</tr>
<tr>
<td>cyclam</td>
<td>1,4,8,11-tetraazacyclotetradecane</td>
</tr>
<tr>
<td>D</td>
<td>optical density</td>
</tr>
<tr>
<td>ε</td>
<td>extinction coefficient, (M⁻¹cm⁻¹)</td>
</tr>
<tr>
<td>en</td>
<td>ethylenediamine</td>
</tr>
<tr>
<td>gem</td>
<td>geminal</td>
</tr>
<tr>
<td>glu</td>
<td>glutamic acid</td>
</tr>
<tr>
<td>gly</td>
<td>NH₂CH₂CO species</td>
</tr>
<tr>
<td>glyNH</td>
<td>NH₂CH₂CONH⁻ anion</td>
</tr>
<tr>
<td>glyO</td>
<td>NH₂CH₂COO⁻ anion</td>
</tr>
<tr>
<td>glyOR</td>
<td>NH₂CH₂COOR</td>
</tr>
<tr>
<td>his</td>
<td>histidine</td>
</tr>
<tr>
<td>L</td>
<td>monodentate ligand</td>
</tr>
<tr>
<td>M</td>
<td>metal</td>
</tr>
<tr>
<td>[M]ₜ</td>
<td>molar rotation at wavelength, λ [deg.M⁻¹m⁻¹]</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>N₄</td>
<td>tetraamine</td>
</tr>
<tr>
<td>N₅</td>
<td>pentaamine</td>
</tr>
<tr>
<td>(±)-NaAsOtarT</td>
<td>(±)-sodium arsenyl tartrate</td>
</tr>
<tr>
<td>NaTPS</td>
<td>sodium 3-(trimethylsilyl)-propanesulphonate</td>
</tr>
<tr>
<td>ORD</td>
<td>optical rotatory dispersion</td>
</tr>
<tr>
<td>ox</td>
<td>oxalate</td>
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<tr>
<td>R</td>
<td>alkyl function</td>
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<tr>
<td>soln</td>
<td>solution</td>
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<tr>
<td>T</td>
<td>temperature</td>
</tr>
<tr>
<td>t</td>
<td>time</td>
</tr>
<tr>
<td>t₁/₂</td>
<td>half-life</td>
</tr>
</tbody>
</table>
tetraen ~ tetraethylenepentaamine
TMS ~ tetramethylsilane
trien ~ triethylenetetraamine
Tris ~ amino-tris(hydroxymethyl)-methane
tyro ~ tyrosine

\[ \mu = \text{ionic strength} \left( \sum c_i z_i^2 \right) \text{ with} \]
\[ c_i, \text{concentration of } i\text{th species and} \]
\[ z_i, \text{charge on } i\text{th species} \]

Specific rotation \([\alpha]_\lambda\) is given by

\[ [\alpha]_\lambda = \frac{\lambda M \alpha_\lambda}{1 \times c} \]
where \(\alpha_\lambda\) is observed rotation (deg.)
\(1\) is path length (dm)
\(c\) is concentration (g/100ml)

and \([M]_\lambda = [\alpha]_\lambda \times \frac{\text{MW}}{100}\)
where MW is the molecular weight

Whence \([M]_\lambda = \frac{\alpha_\lambda}{M \times l}\) where \(M\) is the molar concentration
\(l\) is path length (m)

Values of \([\text{OH}^-]\) throughout this work have been calculated
from pH readings (pH-Stat) using \(pK_w = 13.77 \) (\(\mu = 1.0,\)
NaClO_4; 25.0°) [221].
<table>
<thead>
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1. INTRODUCTION

1.1 Enzyme Reactions and Model Systems

Why do enzymic reactions proceed so rapidly? This is a question which has activated the minds of workers in many branches of science and the result is seen in the overwhelming plethora of publications that are related in some way to this topic. There are two principal facets to the problem of enzyme mechanism to be considered: one is the great rate acceleration and the second is the specificity towards substrates. While the first of these is a more general characteristic applicable to most chemical systems, the second is a property belonging to a certain reaction or class of reactions and is more difficult to solve. For most biological reactions, the two are inseparable.

So that the numerous complications arising from working with large molecular enzyme systems may be reduced, model systems, which may possess the crucial properties of the natural system, have been developed. Four general classifications for enzyme mechanisms leading to rate accelerations have been suggested by Jencks [1]: proximity of reactants, covalent catalysis, general acid-base catalysis and induced strain in reactants. Others include micro-
environmental effects, whereby the local environment of groups in the enzyme-substrate complex is such as to facilitate reaction pathways that are otherwise energetically unfavorable in aqueous solution [2] and also, concerted catalysis [3], but these are not directly relevant to the studies undertaken in this work. The choice of model depends on whether one or more than one of these features is desired to be studied. With only one, the more basic chemistry involved may become clearer. With more than one, the importance of each feature and its significance in rate acceleration may be recognized. Thus, both organic and inorganic chemists have carried out investigations to elucidate mechanisms and have applied them to enzymic systems, even though the rates do not duplicate the enzymic rates. Such examples include studies ranging from reactivities of series of functional groups in organic molecules and of simple coordination complexes to steric effects of substituents at varying distances from reactive sites. In fact, in this context, almost the entire field of chemistry may be considered to be relevant to the understanding of enzyme systems. For example, Kirby and Lancaster have explained increased rate enhancements in intramolecular hydrolysis by steric assistance of alkyl groups in substituted N-methyl maleamic acids. They note that substitution of alkyl functions in methyl maleamic acid enhances the reaction rate by a factor of $10^9$ compared to N-methyl acetamide [4].
Another system which illustrates the importance of proximity or orientation effects is that shown below.

Methyl salicylate, (a), is thought to react with n-butylamine as shown in caption (c) while p-hydroxy-methyl-benzoate, (b), is unreactive in ester aminolysis [5].

Numerous examples of intramolecular catalysis in organic molecules have been collected in some review articles [6-8].

A large number of enzymes appear to operate as purely organic systems, without the presence of a metal ion; examples are pepsin and chymotrypsin. Others require cofactors for their activity, which, in some cases, may be a metal ion [9]. There are several ways in which a metal may activate an enzyme; it may act as an acid, promoting hydrolysis, as an electron donor or acceptor, promoting a redox reaction, as a bridge, connecting substrate and enzyme or as a factor to induce a conformational change necessary for activity.
The earliest recognized metalloenzymes were the peroxidases, catalases and cytochromes [10]. The participation of metal ions in the structure and function of other enzymes is also well documented [11-13]. For example, zinc in dehydrogenases is thought to function by maintaining the tertiary and quaternary structure of the protein [14,15] and by being coordinated to a specific part of the coenzyme and substrate [16], utilizing both oxidized and reduced nicotinamide adenine dinucleotide coenzymes for hydrogen transfer; copper (II) is present in a number of oxidases and is believed to function in oxidizing a number of residues such as tyrosine [17,18]; non-heme iron in the ferredoxins is also thought to act as an electron acceptor between photoactivated molecules and reducing enzymes [19].

One of the most extensively studied enzymes, carboxypeptidase A, is particularly relevant to the work described in this thesis. It is a hydrolytic enzyme, with molecular weight 34,600, catalyzing the hydrolysis of the C-terminal peptide bonds of proteins and peptides [20]; the metal ion, Zn$^{2+}$, being essential for its activity [21]. It is the first metalloenzyme for which the amino acid sequence and high resolution (2Å) structure are known [22] and numerous physical and chemical studies have been undertaken for elucidation of its mode of action [23-27]. On the basis of the molecular structure, Lipscomb has proposed a mechanism for the catalyzed hydrolysis [28]. Zn$^{2+}$ seems to be bound tetrahedrally to one glutamic acid and two histidine residues and in the active site to the acyl residue of the substrate. Two requirements of a suitable mechanism are that a proton be supplied to the
nitrogen of the bond to be cleaved and that attack of \( \text{H}_2\text{O} \)
or another general base at the carbonyl group of the scissile bond occur. These features are illustrated in Fig. 1.1.

Fig. 1.1. A postulated mechanism for peptide hydrolysis in carboxypeptidase A, showing direct metal-carbonyl bonding.

It has been proposed that tyrosine is the proton donor and that an adjacent glutamic acid promotes water attack at the carbonyl centre or alternatively itself acts as the nucleophile [28]. It is also proposed that the major function of the \( \text{Zn}^{2+} \) ion is to polarize the coordinated carbonyl moiety, enhancing its susceptibility to nucleophilic attack [29] for the catalytic effect of metal ions as Lewis acids is well known [30]. Acetylation demonstrates the presence of two reactive tyrosine residues, one or both of which are essential for peptidase activity [31,32]. Nitration experiments pinpoint the active residue as tyrosine 248 [33,34], which moves through about 14Å upon binding to a substrate peptide [35]. The role of such ligand-induced conformational changes in enzymic reactions has been discussed in a review by Koshland and Neet [2]. A more detailed discussion of the importance of other amino acid
residues for activity is presented by Vallee and Riordan [36].

The substrate occupies a long groove and a pocket associated with the zinc coordination site, in the absence of substrate, the pocket is occupied by a water molecule or hydroxide ion [37]. The fact that such spaces are available near the metal ion has been used by Vallee and Williams [38] in support of the theory that enzymes are in their entatic state, that is, that enzymes are in a high-energy state, "poised" for catalytic action.

Alternatively, some parts of the enzyme, beside tyrosine 248, have been observed to change their relative positions when substrates are incorporated [39]; such observations have been used as evidence for induced-fit theories whereby specific interactions between enzyme and substrate result in conformational changes as the substrate is incorporated into the active site [40].

An alternative mechanism, based on that for another zinc enzyme, carbonic anhydrase [41,42], invokes a structure with zinc bound to a hydroxide ion or water molecule, either of which may act as a nucleophile at the carbon atom of the susceptible peptide bond. (Fig. 1.2).

Fig. 1.2. An alternative postulated mechanism for peptide hydrolysis in carboxypeptidase A, with the metal ion bound to a hydroxyl group.
Although spatial considerations in the crystal structure make this arrangement less likely than that in Fig. 1.1, it is still possible that such a mechanism is operative for the system in vivo. In Fig. 1.1, zinc may be considered to act as a Lewis acid while, in Fig. 1.2, the zinc-hydroxo moiety may be considered to act as the nucleophile.

Coleman and coworkers have determined the stability constants for a number of divalent metal ions with carboxypeptidase A [43,44], observing ready interchange between these ions. Moreover, activity is found with all metals except Cu(II), although Hg(II) and Cd(II) catalyze only ester hydrolysis while Zn(II), Mn(II), Co(II) and Ni(II) catalyze both ester and peptide hydrolysis [45]. It has been suggested that not only is the stability of the metal-protein complex a factor but also that the stereochemistry about the metal is important [22].

Vallee and coworkers [46] make the further observation that with various acylation, iodination and photooxidation reactions of carboxypeptidase A, an increase in esterase activity is paralleled by a decrease in peptidase activity. On the basis of these findings and also from a study of pH vs rate profiles for peptide hydrolysis [47] compared with ester hydrolysis, they propose a scheme for ester hydrolysis, where catalysis is effected by hydroxide ions rather than by direct attack of another nucleophile, as in peptide hydrolysis (Figs. 1.3a & b).
Although ester hydrolysis has not been discussed at length, this class of reactions is important both from a biological and purely chemical viewpoint. The mechanisms of ester hydrolysis are closely related to those of peptide hydrolysis and have been examined for a number of esterases [48,49]. Rates are consistent with the formation of a tetrahedral acyl-enzyme intermediate, similar to the type proposed above.

From the example of carboxypeptidase A, a number of facts become clear. A crystal structure contains data for the static molecule and this information alone is not sufficient to elucidate the mechanisms of the dynamic system. Moreover, since acid-base reactions are involved in the enzyme process, a more detailed investigation or reappraisal of presently known facts about such reactions in simple organic and inorganic systems is desirable.

It is now evident that there are several proposals for mechanisms by which ester or peptide bond cleavage may occur. These are summarized pictorially in Fig. 1.4. In all of these possibilities, attack of a nucleophile at the carbonyl carbon leads to a tetrahedral intermediate, which

Fig. 1.3. Proposed mechanisms of (a), esterase and (b), peptidase activity [47]. N is a nucleophile other than OH⁻.
subsequently decomposes to products. With presently known facts about ester hydrolysis, either in a purely organic system or in one that is metal-ion promoted, the proposal of such an intermediate is a reasonable one and this is discussed at greater length in the subsequent Section.

In relation to the foregone discussion, it is logical to choose model systems where (i) the active carbonyl centre is directly attached to the metal ion and (ii) a bound hydroxyl ion is the nucleophile attacking the carbonyl carbon of a free ester. In this manner, the relative efficiencies and rates of the two types of mechanisms may be evaluated. Further,
in many enzymic reactions, functional groups appear to be held together by the combined effect of weak binding to a divalent ion and of the bulk of the surrounding enzyme, so a reasonable choice of a coordination model is one where a small peptide or amino acid derivative is bound to a metal ion with a large stability constant in aqueous solution. Since more highly charged metal ions are generally more stable [50], a suitable choice may be Co(III), the paradigm for mechanistic studies. An important difference between the native enzyme and such a model is that the former has a "ready-made" second coordination sphere consisting of the rest of the protein not directly associated with the active site but perhaps helping to hold the reacting species together; the latter, on the other hand, is an isolated ion in an aqueous medium, the main contributors to the catalysis being closely associated with the metal ion. Eaton has recently emphasized the existence of a second coordination sphere in catalysis [51] and it may well be that such factors are of prime importance.

Reports of recent experiments on metal-ion and enzyme-catalyzed reactions may be found in a number of review articles [52-56]. A great number of model systems comprising Co(III) and α-amino acid derivatives have been probed with a view to answering at least some of the questions associated with the mechanism of enzymic hydrolysis. In the sections which follow, a brief account is presented of the main conclusions of studies of organic and inorganic models of peptidase and esterase activity. Emphasis is placed on the findings of Co(III) promoted reactions of glycine derivatives and, as the discussion develops, the mechanistic
problems left unsolved will become more obvious: from there, the aims of the present work will be outlined.

1.2 The Tetrahedral Intermediate

1.2.1 Ester hydrolysis and related reactions

The most readily observed formation of a tetrahedral intermediate from a carbonyl compound is that resulting from the addition of water or alcohol to a ketone or aldehyde to form a gem-diol, acetal or ketal. The properties of these addition compounds containing a tetrahedral carbon are well-documented [57,58], but, once formed, these compounds do not decompose to produce new compounds but rather revert to the parent compound. Oxygen isotope exchange studies with labelled water have shown that the formation of gem-diols is reversible and so this reaction is the simplest example of a reversible addition to the carbonyl group.

\[
R_1\overset{\text{C} = \text{O}}{\text{C}} + H_2^{18}O \rightleftharpoons R_1\overset{\text{C}}{\text{C}}^{18}\text{OH} \rightleftharpoons R_1\overset{\text{C}}{\text{C}}\overset{\text{OH}}{\text{O}} + H_2O \quad (1.1)
\]

Analogous stable adducts formed by nucleophilic addition at an acyl carbon atom, however, are rare and their existence is inferred by other means. Their instability is due to the fact that energy barriers due to resonance stabilization must be overcome for their formation. This is shown below for an ester.

\[
\overset{\text{R} - \text{C} = \text{O}}{\text{R}} \overset{\text{OR}}{\text{OR}} \rightleftharpoons \overset{\text{R} - \text{C} = \text{O}^-}{\text{R}} \overset{\text{OR}}{\text{OR}} \quad (1.2)
\]
A great number of such nucleophilic addition reactions have been studied and the evidence for the existence of the tetrahedral intermediate is presented in a number of reviews[1,7,52,53,59-62]. In brief, this includes (i) \(^{18}\)O exchange into reactant during hydrolysis in labelled water, (ii) breaks in pH-rate profiles, not directly due to ionization of reagents, (iii) breaks in buffer concentration-rate curves and (iv) discontinuities in reactivity-rate relations.

The strongest evidence for the existence of the metastable tetrahedral intermediate springs from \(^{18}\)O-labelled experiments with esters [63].

\[
\begin{align*}
R-C^{\text{\textsuperscript{18}}O} & + H_2^{\text{\textsuperscript{18}}O} \xrightleftharpoons[k_1]{k_2} \left[ \begin{array}{c}
^{\text{\textsuperscript{18}}O}R-C-OR' \\
^{\text{\textsuperscript{18}}OH}
\end{array} \right] \\
\xrightarrow[k_3]{k_2} & R-C^{\text{\textsuperscript{18}}O} + ROH
\end{align*}
\]

The rate constant for hydrolysis, \(k_h\), is formulated in terms of the individual steps of (1.3) by:

\[
k_h = \frac{k_1 k_3}{k_2 + k_3}
\]

The ratio, \(k_3/k_2\), serves as a measure of the competition between \(-\text{OH}\) and \(-\text{OR}'\) as leaving groups. It is found to increase with decreasing basicity of the leaving group. The appearance of tracer in the reactant strongly suggests the existence of the symmetrical tetrahedral intermediate and proves that the reaction does not proceed by a concerted
$S_N^2$ displacement mechanism. When $k_2 \ll k_3$, that is, when the rate of decomposition of the tetrahedral intermediate to products is greater than to starting material, the hydrolysis constant reduces to $k_1$ and the addition step is rate-determining. When $k_2$ becomes greater than $k_3$, the rate constant reduces to $k_3/k_2$ and both formation of the intermediate and loss of leaving group contribute to the rate constant if $k_1$ is comparable with $k_3$ in (1.4).

The nature of the rate-determining step is readily visualized in a reaction coordinate diagram (Fig. 1.5).

![Reaction coordinate diagrams for a reaction involving an intermediate, $I$, when (a) $k_3 \gg k_2$ and (b) $k_2 \gg k_3$.](image)

When formation of the intermediate is rate-determining, the energy barrier for decomposition to products is smaller than that to starting material, shown in part (a) of the diagram. When decomposition to products is important, the reverse is true of the relative heights of the barriers. In this case, the highest point on the overall free energy diagram determines which step will be rate-determining. Whether or not the intermediate is observable depends on the energy of the intermediate with respect to reactants,
provided the transition state energies are relatively constant.

Base hydrolysis of some esters and amides proceeds without apparent $^{18}$O exchange. This is not interpreted as an indication that an $S_N2$ mechanism is operating but rather that $k_3$ is at least 100 times greater than $k_2$ and hence, that the isotopic exchange methods are unsuitable. The mechanism of both alkaline and acid-catalyzed hydrolysis thus appears to involve addition of the nucleophiles, $OH^-$ or $H_2O$, to the neutral or protonated species, followed by elimination of the leaving group. In aqueous solutions, the common expression for the observed velocity of a hydrolytic reaction may be written as:

$$v_S = (k_0 [H_2O] + f[H^+] + f[OH^-]) [S]$$  \hspace{1cm} (1.5)

where $k_0$ is the constant for spontaneous reaction, $f[H^+]$ and $f[OH^-]$ are functions of hydrogen ion and hydroxide ion concentrations, respectively, and [S] is the concentration of the substrate.

Some examples are now given. The hydrolysis of aminoacetylsalicylamide, under mildly basic conditions, is thought to proceed with the formation of a stable imide intermediate, in an amino acid insertion reaction [64]. The acid hydrolysis of benzamide is also thought to proceed via a tetrahedral intermediate. The experimental results indicate that the addition step is rate-determining in acid hydrolysis but that the degradation of the intermediate is rate-determining in base hydrolysis [65]. Other examples are cited in a review by Bender [59].
While the greatest number of hydrolytic reactions are first order in hydroxide ion concentration, few are known to be second order in hydroxide ion concentration. Biechler and Taft observe a second order term in the rate law for aqueous alkaline hydrolysis of trifluoroacetanilide and a series of N-methylanilides, ROCN(CH₃)C₆H₅ [66]. By comparison with hydrolysis of β-diketones [67] and some Cannizzaro reactions [68], they propose the reaction scheme shown below, where the intermediate is in equilibrium with a deprotonated form.

\[
\begin{align*}
R-C\begin{array}{c}
O\
N-C\end{array}C₆H₅ + OH^- & \quad \overset{K_1}{\rightarrow} \quad R-C\begin{array}{c}
O\
N-C\end{array}C₆H₅ - O R \quad \overset{K_2[OH^-]}{\rightarrow} \quad R-C\begin{array}{c}
O\
N-C\end{array}C₆H₅ - O R + H₂O \\
& \quad \overset{(B)}{\rightarrow} \quad \overset{(C)}{\rightarrow} \\
& \quad R-C\begin{array}{c}
O\
N-C\end{array}C₆H₅ - O R + H₂O
\end{align*}
\]

The existence of the tetrahedral intermediate is inferred from oxygen isotope exchange data [65]. It is more difficult to explain why the reaction proceeds via a deprotonated intermediate. The authors suggest that, if c is to compete favorably with b, its rate of decomposition must be favoured, since it is believed to be present in much smaller concentrations. This may be explained by the formation of the deprotonated acid product from c, compared with the protonated form from b, the extra resonance stabilization of the former perhaps acting to lower the activation energy for the decomposition of c relative to b,
shown below.

\[
\begin{align*}
&\text{HO} \\
&\text{RC-N-C}_6\text{H}_5 \\
&\text{O} \\
&\text{R} \\
&\rightarrow
\end{align*}
\]

\[
\begin{align*}
&\text{O}^- \\
&\text{RC-N-C}_6\text{H}_5 \\
&\text{O} \\
&\text{R} \\
&\rightarrow
\end{align*}
\]

The observed rate expression is:

\[
\kappa_{\text{obsd}} = k_1[\text{OH}^-] + k_2[\text{OH}^-]^2
\]  

The authors compile a list of \( k_1 \) and \( k_2 \) for a series of substituents, \( R \). For \( R = \text{CH}_3 \), \( k_1 = 2.45M^{-1}\text{sec}^{-1} \), \( k_2 = 467M^{-2}\text{sec}^{-1} \) and for \( R = \text{C(CH}_3)_2\text{CN} \), \( k_1 = 0.00062M^{-1}\text{sec}^{-1} \), \( k_2 = 0.00033M^{-2}\text{sec}^{-1} \). The values show that the ratio of the second to first order constant is greater with more electron-withdrawing substituents and that the absolute rates for both paths also increase with the more electron-withdrawing substituents. Bender and Thomas conclude, from isotopic oxygen exchange studies, in the hydrolysis of the \( p \)-substituted acetonilides, that substituent effects are cancelled in the overall process and, further, that breakdown of the tetrahedral intermediate, \( c \), of equation (1.6) should involve the formation of a dipolar ion, \( \text{RC(O\text{^-}})^+\text{C}_6\text{H}_4\text{X} \), for otherwise the leaving group would involve a species of very high basicity [69].

The rate equation for several other amides, urea [70], chloroacetamide [71] and \( N,N \)-diacylamines [72] also contains
a term second order in \([\text{OH}^-]\). Of special interest are the
rate expressions for the alkaline hydrolysis of
2,2,2-trifluoroacetanilide [73] and N-methyl-2,2,2-
trifluoroacetanilide [74,75]. At low hydroxide ion
concentrations, first order hydroxide terms are important.
At higher pH, both first and second order terms are important
and, at even higher pH, the observed rate law reduces to one
that is first order in hydroxide ion. Mader derives a rate
law, (1.11), for the hydrolysis of 2,2,2-trifluoroacetanilide
[73], based on the proposed mechanism below; (1.9 and 1.10).

\begin{align*}
\text{RNHCOR} & \overset{k_1}{\longrightarrow} \text{products} \\
\text{RNHCOR}^+ + \text{OH}^- & \overset{k_2}{\longrightarrow} \text{RNCR} + \text{H}_2\text{O} \\
\text{RNHCR} & \overset{k_3}{\longrightarrow} \text{RNCR}^+ \overset{k_5}{\longrightarrow} \text{OH}^- \overset{k_4}{\longrightarrow} \text{products}
\end{align*}

\begin{equation}
\text{obsd} = \frac{1}{1 + K[\text{OH}^-]} \left[ \frac{k_1 + (k_2 + k_3 K)(k_4[\text{OH}^-] + k_5[\text{OH}^-]^2)}{k_2 + k_3 + k_4 + k_5[\text{OH}^-]} \right] \tag{1.11}
\end{equation}

At pH values where terms in \([\text{OH}^-]^2\) are important, the rate-
limiting step is deduced to be decomposition of the
tetrahedral intermediates while, at even higher pH, the
rate-limiting step becomes addition of \text{OH}^- to the carbonyl
carbon. As \([\text{OH}^-]\) in this equation approaches infinity, a
limiting rate law is observed and the equation reduces to:

\[ k_{\text{obsd}} = \frac{k_2 + k_3 K}{K} \]  

(1.12)

with both experimental and calculated values, \( k_{\text{obsd}} \sim 0.0073 \text{sec}^{-1} \).

Schowen and Zuorick [74] infer that the kinetic law for the N-methyl analogue corresponds to a superposition of general base catalysis on specific hydroxide ion catalysis. The demonstration of a change in rate-limiting step in these reactions is good evidence that at least one intermediate exists.

Bender and Thomas have estimated the upper limit of the lifetime of the tetrahedral intermediate for \( p \)-substituted methylbenzoates to be of the order of \( 10^{-9} \) secs, having deduced that the upper limit for the activation energy from intermediate to products is no greater than 11 kcal/mole [76]. The estimate has kinetic implications in that proton transfer may become significant if the lifetime of the tetrahedral intermediate is less than \( 10^{-9} \) secs, even by a factor of 10. As evidenced in many of the reactions under discussion, proton transfer for base hydrolysis or for acid hydrolysis is important in the mechanism, but assumed to be diffusion-controlled. Cases are known, however, where the claim of Bender and Thomas is justified, that the rate of proton transfer is comparable with other rates in the sequence. These are discussed briefly in a review by Johnson [61].

The mechanisms of acid catalysis in hydrolysis, particularly of esters, are a subject of much discussion and many dissertations are available, generally in close liaison.
with those on base hydrolysis [7, 59, 60, 62, 77, 78]. The mechanism for bimolecular acid-catalyzed ester hydrolysis may be written as shown below, by an $A_{AC}^2$ mechanism, via a symmetrical tetrahedral intermediate, the existence of which has also been inferred by oxygen exchange studies. (1.13).

$$\begin{align*}
R-C^0 &\overset{+H^+}{\longrightarrow} R-C^+ + H^+ \\
&\overset{k_1}{\longrightarrow} R-C^+ OR' + H^+ \\
&\overset{k_1}{\longrightarrow} R-OR' + H^+ \\
&\overset{H_2O}{\longrightarrow} R-C-OR' + H^+
\end{align*}$$

The proton addition is generally believed to be diffusion-controlled; that is, a pre-equilibrium ($K_1$) may be established before nucleophilic addition at the carbonyl carbon occurs. Martin has calculated that the observed rates are explicable in this way, despite the very low concentration of preprotonated ester [79]. A transition state diagram, showing the qualitative relative stabilities of each of the five intermediates of (1.13) is shown in Fig.1.6.

![Fig.1.6. Free energy diagram for acid hydrolysis of an ester in aqueous solution.](image)
The existence of a tetrahedral intermediate has not been inferred, by the aforementioned methods, in the hydrolysis of amino acid esters, but sufficiently close correlations of thermodynamic and kinetic data exist between these esters and structurally similar organic molecules that such intermediates may be postulated. Hydrolysis data for a number of amino acid esters have been accumulated [80-87]. The observed rate constant for base hydrolysis of amino acid esters consists of two terms, one due to hydrolysis of the N-protonated ester and the other, to hydrolysis of the deprotonated form. (This is discussed more fully in Chapter 2).

The functional similarity between amino acid derivatives with a proton on the amino nitrogen and amino acid complexes with the metal ion bound to the amino nitrogen has given rise to a number of studies of the relative efficiencies of \( \text{H}^+ \) and \( \text{M}^{n+} \) in promoting hydrolysis. Interest has been focussed on the study of the metal ion-catalyzed reactions of amino acid derivatives, in the hope that something might be learned of the effectiveness of bound nucleophiles compared with solvent nucleophiles in promoting hydrolysis. The following sections deal, in turn, with various types of metal ion-amino acid complex reactions.

1.2.2 Metal-ion promoted reactions of amino acid derivatives

Metal ions which bind to reactive substrate molecules may be considered to function as Lewis acid catalysts. They introduce positive charge (+1 or greater, depending on the metal ion and its oxidation state) into the system, distorting the electronic distribution in the molecule and
are stable over an extensive range of pH during hydrolysis. A proton, on the other hand, carries only a single positive charge, and is present in significant amounts only in acid solutions. Metal ions, moreover, possess the added properties that their concentrations are readily determinable and remain constant, often over a wide range in pH. Also, stronger binding of metal ions, compared with protons, to substrates is possible, both by σ bonds and by π bonds. Metal ions may bind with more than one species simultaneously, thereby serving to approximate reactants and perhaps even inducing strain in them, favorable for reactivity. In this way, they may act as a template for a reaction, by analogy with proposed schemes for enzyme activity.

Although divalent metal ions are mostly found in enzyme systems, they are generally less suitable in model reactions. The complexes that they form are labile in solution. Thus, the exact cause of an observed effect may not be readily attributed to any of the several possible chemical processes involved. Nevertheless, the presence of divalent metal ions has been shown to promote hydrolysis of a large number of amino acid esters in aqueous solution. Kroll observed the formation of chelates of a number of amino acids with Mn(II) [88] and thence proceeded to study the effect of binding of a number of divalent ions on amino acid ester hydrolysis [89]. He proposed that the carbonyl carbon was more susceptible to attack by hydroxide ion when the amino acid ester was chelated to the metal ion and that a tetrahedral intermediate was formed, but provided no real evidence for such a mechanism. Many other workers have
made various suggestions for these enhancements [83-87,90] with divalent ions, but kinetic studies were hampered by precipitation of metal ion hydroxides at high pH. Bender and Turnquest inferred the existence of a tetrahedral intermediate in the Cu(II)-catalyzed hydrolysis of DL-phenylalanine ethyl ester by oxygen exchange techniques [91]. They indicated that this intermediate was stabilized in the presence of a metal ion.

Hay and Morris [92] considered that it was not necessary to invoke carbonyl-metal ion interaction to explain the catalysis in hydrolysis of a Cu(II)-methylhistididine derivative. They maintained that catalysis was due mainly to electrostatic effects, for the hydroxide ion would be attracted more readily to the substrate attached to a positively charged centre. In the system that they studied, kinetic rates were complicated by the presence of two amino acid esters bound to the metal ion. Angelici and co-workers overcame this problem by studying metal ion complexes with nitriloacetic acid and only one molecule of an amino acid ester [84]. They proposed the formation of a tetrahedral intermediate, but did not differentiate between an intramolecular mechanism involving attack of bound hydroxide ion (1.14) and one involving attack of solvent hydroxide after the carbonyl oxygen of the ester had displaced a bound water (1.15).
From many accumulated rate constants for hydrolysis of esters, both in the absence and presence of metal ions, [80-87,92-95], it is seen that coordination to a divalent metal ion enhances base hydrolysis by a factor of $10^2$ to $10^4$, relative to the N-protonated ester. The N-protonated ester is hydrolyzed about ten times more rapidly than the unprotonated ester. Hix and Jones have found that the base hydrolysis constant for ethyl glycinate, in the presence of divalent metal ions, is $\approx 10^9$ times greater than that for spontaneous hydrolysis [93].

In more recent years, a great deal of work has been carried out on octahedral Co(III) complexes in which four of the coordination positions are occupied by substitution-inert ligands, such as ethylenediamine. The other two positions may be reserved either for a chelated amino acid derivative or for a monodentate derivative, with a potential nucleophile in the sixth position. (See, for example, equation (1.15)). A number of reviews on such Co(III) complexes have recently been published [54-56]. The following Section deals with such complexes in greater detail. The discussion has been limited largely to derivatives of glycine, $\text{H}_3^+\text{NCH}_2\text{COO}^-$, the simplest of the $\alpha$-amino acids.
1.3 Reactions of Octahedral Co(III)-Glycine Derivatives

1.3.1 Substitution chemistry in octahedral Co(III) species

Octahedral Co(III) complexes of the type, [CoN₄LX]²⁺, where N is an amine ligand and L is another monodentate ligand, undergo substitution, with the ligand, X, (X = Cl, Br, NO₃, N₃, etc.), replaced by another ligand, either in basic or acidic conditions. The results of numerous studies of the base hydrolysis of these complexes are consistent with a mechanism involving the formation of a five-coordinate intermediate, the geometry of which may be trigonal bipyramidal [96-98]. Similarly, results of investigations of nitrosation, where an azido complex reacts with nitrous acid to produce an aquo complex, [99,100] and of Hg²⁺-catalyzed aquation in acid of such complexes are consistent with the formation of a five-coordinate intermediate, after removal of the ligand, X. Many examples may be found in the literature [101-105]. More detailed discussions of the proposed mechanisms and stereochemistry are given in Chapters 5 and 6.

When the ligand, L, in [CoN₄LX]²⁺, is an amino acid derivative, there are several interesting features of the two classes of reactions mentioned above. Throughout the course of the reactions, all ligands, except X, remain bound to the metal ion. Thus, the reactivity of the amino acid derivative may be observed, without metal-ligand dissociation, both in acidic and basic conditions. Two prospects may be entertained in a pathway involving the formation of a five-coordinate intermediate. The intermediate may capture the carbonyl oxygen to produce a highly reactive
chelated amino acid derivative or it may capture a solvent molecule to produce a species with a potential nucleophile cis to the monodentate amino acid derivative.

This type of substitution in octahedral Co(III) complexes has been used to generate such species and the following section presents the results of such studies.

1.3.2 Inter-and intramolecular reactions of Co(III)-glycine derivatives

In a study of Hg$^{2+}$-induced hydrolysis of cis-[Co(en)$_2$X(glyOR)]X$_2$ complexes, Alexander and Busch have proposed two structures for the intermediate glycine ester complex, which may be formed after halide is removed [106]. One of these is cis-[Co(en)$_2$H$_2$O(glyOR)]$^{3+}$, formed by solvent entry into the coordination sphere. This species would presumably form the chelated acid by intramolecular attack of bound H$_2$O at the carbonyl carbon. The second postulated structure is the chelated ester, [Co(en)$_2$(glyOR)]$^{3+}$, formed by entry of carbonyl oxygen into the coordination sphere after halide removal. On the basis of visible and infrared spectral data, the authors propose the formation of the chelated ester as the only product of the Hg$^{2+}$-catalyzed reaction. This species is then hydrolyzed rapidly to the chelated acid. Wu and Busch have extended the study to the complex of glycine $t$-butyl ester, which substantiates this claim [107]. The ester intermediate undergoes nucleophilic attack by solvent H$_2$O at the bound carbonyl carbon, to form the chelated acid product.

Buckingham, Foster and Sargeson have studied the base hydrolysis of cis-[Co(en)$_2$X(glyOR)]$^{2+}$ (X = Cl, Br and
$R$ is an alkyl group\cite{108}. For cis-$[\text{Co}(\text{en})_2\text{Br}(\text{glyOCH(CH}_3)_2)]^{2+}$, $^{18}$O tracer experiments have revealed that 50% of the $[\text{Co}(\text{en})_2(\text{glyO})]^{2+}$ product arises from incorporation of the ester carbonyl oxygen, after halide removal, into the coordination sphere. The remaining 50% may be produced by internal nucleophilic attack, on the carbonyl carbon, of a solvent molecule, which has been incorporated into the coordination sphere after bromide removal. The proposed mechanism is shown below.

\begin{align*}
\text{Path A} & \\
\text{Path B}
\end{align*}
The authors have proposed several alternative mechanisms consistent with the $^{18}$O results. One involves a scheme whereby 50% of the chelated acid arises from direct $S_N^2$ attack by the carbonyl oxygen, displacing the bound bromide and the other 50% arises as described before. A second scheme involves formation of a tetrahedral carbon centre of the monodentate ester, where the label is scrambled. Subsequent attack of an OH group would then displace the bound hydroxide. These two mechanisms, however, are not preferred and arguments have been presented in favour of the scheme, (1.16). The results of hydrolysis of chelated glycine dipeptide esters also favour a mechanism involving solvent attack on a chelated species [109,110] rather than bound nucleophile attack on a monodentate species [111].

A number of complexes with formula, $[\text{Co(en)}_2\text{glyOR}]\text{(ClO}_4)$ have subsequently been isolated and studied [112-114]. The great hydrolysis rate of these complexes is strong evidence that they may well be active intermediates in the reactions discussed above. A detailed study of the acid and base hydrolysis of chelated glycine isopropyl ester has been carried out [114]. Rate laws have been established and mechanisms have been proposed, which invoke a tetrahedral intermediate of the type already discussed (Section 1.2.2).

Base hydrolysis of glycine amide derivatives, $\text{cis-[Co(en)}_2\text{Br(glyNR}_1\text{R}_2)]^{2+}$, has led to the formation of both $\text{cis-[Co(en)}_2\text{OH(glyNR}_1\text{R}_2)]^{2+}$ and $[\text{Co(en)}_2\text{(glyNR}_1\text{R}_2)]^{3+}$ [115]. The ratio of the products has been found to be dependent on $R_1$ and $R_2$. The results support the inferences made for base hydrolysis of the analogous glycine isopropyl ester complex.
that both solvent and amino acid carbonyl oxygen may compete for the position vacated by halide. The base hydrolysis of a methyl 6-aminohexanoate complex, \( cis-[Co(en)_2Cl(NH_2(CH_2)_5COOCH_3)]^{2+} \), has recently been studied [116]. The sole product is the \( cis \)-hydroxo ester complex. There is no competition, in this system, by carbonyl oxygen, for the carboxyl group is several atoms removed from the coordination sphere. The formation of a nine-membered ring would certainly be highly improbable.

1.4 Objectives of the Present Investigation

In the foregoing sections, an account has been given of some important aspects of hydrolytic enzymes, of their activity and of the questions that have arisen about the mechanism of catalysis in such systems. One of the leading problems was shown to be that of establishing whether a nucleophile bound to the metal ion, acting on a free substrate, or whether a free nucleophile acting on a bound substrate was the more effective in promoting hydrolysis. Associated with this problem were those of finding possible mechanisms of hydrolysis. For example, it remained to establish whether a tetrahedral carbon intermediate was formed in the presence of a metal ion. While some of the more basic problems pertinent to these systems may have been solved by studies on model systems, many have been left unsolved. For instance, in the Co(III) glycine ester system, kinetic data were not obtained for an intramolecular process where bound hydroxide was the nucleophile and so a comparison could not be made of the intramolecular rate with that of
hydrolysis of the chelated glycine ester [114]. The rapid
hydrolysis of the latter, however, led the authors to infer
that the enzymic process occurred through carbonyl-bound
substrate. Base hydrolysis of chelated glycine isopropyl ester [114] could be followed only to pH ~ 7, for, at higher
pH, rates were too great to be measured with available
instrumentation. At best, the existence of a tetrahedral
intermediate could only be inferred, on the basis of the
similarity of the Co(III)-amino acid ester moiety with
purely organic molecules. It could not be established,
moreover, whether a mechanism involving nucleophilic addition
or general base catalysis was involved.

An investigation of exactly analogous Co(III)
complexes with β-alanine derivatives, $\text{H}_2\text{NCH}_2\text{CH}_2\text{COOR}$, has
been undertaken in the present work, with the broad aim of
amplifying those details of mechanism, not found for the
glycine ester system. The amino acid, β-alanine, has been
chosen to determine whether enlarged ring size affects the
rate enhancement in hydrolysis. β-alanine is a homologue
of glycine, with similar physical and chemical properties,
although it is not an amino acid commonly found in proteins.
Its reactions are expected to be at least ten times slower
than those of glycine. Thus, it might be anticipated that
the reactions of Co(III) derivatives might be retarded
sufficiently for more detailed kinetic studies to be
undertaken and, also, for otherwise reactive intermediates
to be isolated and investigated.

A study of complexes, which may serve as models for
enzymic hydrolytic processes, has been undertaken, primarily
with the aim of finding a possible mechanism to explain the
great rates of enzymic hydrolysis. Several other facets of
general interest in coordination chemistry have also been
examined. A Co(III) complex containing a potential
nucleophile cis to a bound β-alanine ester has been isolated
(Chapter 4). Another complex, with β-alanine ester, chelated,
has also been prepared (Chapter 2). Kinetic and mechanistic
studies have been carried out with these complexes so that
the effectiveness of hydrolysis in a system containing a
bound nucleophile, relative to one containing carbonyl-
bound ester might be evaluated. Base hydrolysis (Chapter 5)
and induced aquation (Chapter 6) in the complexes,
cis-[Co(en)$_2$X(β-alaOR)]$_2$ have also been studied, to establish
whether these amino acid complexes follow the same
stereochemical course in octahedral substitution as do
simple amine complexes. The results of all of these studies
have been compared with those of the glycine derivatives,
to observe the differences in reactivity between a ligand
leading to a six-membered chelate ring and that of one
leading to a five-membered chelate ring, respectively. Base
hydrolysis, at high pH, of a β-alanine chelate has been
studied (Chapter 3) to ascertain the course of ring opening
and to establish the products formed.

It is well established that the rate of ester
hydrolysis is greatly enhanced when chelation isolates the
reactive site. Acid catalysed hydrolysis is generally
believed to occur by a mechanism involving water attack on
the ester, preprotonated at the carbonyl oxygen (for
esters...
2. HYDROLYSIS OF \([\text{Co(en)}_2(\beta\text{-AlaOR})]^{3+}\)

2.1 Introduction

Although the rates of base hydrolysis of esters of \(\alpha\)-amino acids have been determined at various temperatures, ionic strengths and pH's \([80-83, 89]\), only a few constants for the \(\beta\)-amino acids or higher analogues are available. More specifically, only those for the ethyl \([84]\) and methyl \([85]\) esters of \(\beta\)-alanine, \(\text{H}_3\text{NCH}_2\text{CH}_2\text{COO}^+\), have been obtained. These rates are approximately ten times slower than those for the corresponding esters of glycine \([82]\); using this comparison for isopropyl \(\beta\)-alaninate, it is possible to obtain an approximate rate for its hydrolysis. This method, however, is indirect and in this work is included the determination of the proton ionization constant and rates of alkaline hydrolysis of uncoordinated \(\beta\)-alanine esters under the same conditions as those used in the study of the hydrolysis of the chelated esters.

It is well established that the rate of ester hydrolysis is greatly enhanced when chelation includes the reactive site. Acid catalyzed hydrolysis is generally believed to occur by a mechanism involving water attack on the ester, preprotonated at the carbonyl oxygen \([117]\), whereas
base catalyzed hydrolysis may occur by nucleophilic or general base catalyzed paths. For the \([\text{Co(en)}_2\text{glyOCH(CH}_3\text{)}_2]\)^{3+} ion, it has been demonstrated that the direct nucleophilic path is predominant. This study has been undertaken to observe whether similar mechanisms exist for the analogous \(\beta\)-alanine complex and whether the magnitude of the rate enhancement is affected by the size of the chelate ring. A preliminary study has also been made on the chelated \(\beta\)-alanine amide. Further, the stability of the six-membered chelate ring during and subsequent to hydrolysis in the presence of a number of buffers has been investigated.

2.2 Experimental

2.2.1 Instrumental and general

Visible spectra were recorded on a Cary 14 spectrophotometer, infrared spectra, as nujol mulls on a Perkin-Elmer Model 459 spectrometer and pmr spectra on a Jeol JNM-100MHz Minimar instrument using external TMS or internal NaTPS as references. The Minimar was modified so that spectra were traced on an AEI drop recorder at timed intervals chosen by inserting pegs at suitable points on a large screw; these pegs triggered a microswitch whereby the spectra were obtained. Cobalt estimations were made using a Techtron AA4 atomic absorption spectrophotometer. Spectrophotometric rates were obtained either by hand-mixing the two reactant solutions, each having been filtered, then pouring the mixed solution into a silica cell of suitable path length; otherwise, they were obtained with a stopped-flow apparatus (1cm cell) on a Cary 14 or Cary 16K spectrophotometer or in a Durrum-Gibson stopped-flow reactor (2cm path length). The
Radiometer equipment used in pH-Stat titrations and for buffer pH measurements consisted of a TTA2 electrode assembly, ABU 1 autoburet, TTTI titrator, SBR2 titrigraph, with a pHA scale expander. The solution was continuously stirred in a thermostated container under a nitrogen atmosphere. Periodic checks on the stability of the instrument were made with standard buffer solutions. The Cary 16K spectrophotometer was also fitted with a teflon container (capacity, 50ml) with silica windows of path length 3.2cm; the solution was continuously stirred with a bar magnet, the speed of which was controlled by a Variac resistor unit. The lid of the cell was designed so that the radiometer electrodes, burette and nitrogen supply tube could be inserted.

Products of reactions were sorbed on and eluted from Bio-Rad Analytical Dowex 50Wx2 (200-400 mesh, H+ or Na+-form) cation exchange resin.

2.2.2 Preparation of complexes

Analar reagents were used in all preparations without further purification.

\[ \beta \text{-alanine isopropyl ester} \]

\[ \beta \text{-alanine isopropyl ester} \] was prepared by adding \( \beta \)-alanine (22.3g) to a cold solution (0°) of isopropanol (260ml) to which had been slowly added thionyl chloride (20ml). After the mixture had been refluxed for three hours on a steam bath, it was evaporated to a volume of 50ml and cooled in an ice bath before excess ether was added. The white opalescent crystals were filtered, washed with ether and recrystallized from isopropanol, with ether added to induce crystallization;
the product was collected and dried in an evacuated desiccator.

**Anal.** Calcd for $\beta$-alaOCH(CH$_3$)$_2$.HCl: C, 42.99; H, 8.42; N, 8.35; Cl, 21.15. Found: C, 43.29; H, 8.32; N, 8.36; Cl, 21.13.

**$\beta$-alanine methyl ester**

$\beta$-alanine methyl ester was prepared in a similar manner as the hydrochloride salt. $\beta$-alanine (53.5g) was gradually added to a cold solution (0°) of thionyl chloride (50ml) in methanol (350ml). After being refluxed for 3hrs on a steam bath, the solution was evaporated to a volume of 30ml, cooled in an ice bath, then ether was added. The white opalescent product was recrystallized from methanol, with ether added to induce crystallization, then collected, washed with ether and dried in an evacuated desiccator.

**Anal.** Calcd for $\beta$-alaOCH$_3$.HCl: C, 34.40; H, 7.22; N, 10.04. Found: C, 33.92; H, 7.00; N, 9.52.

**$\beta$-alanine amide**

$\beta$-alanine amide was prepared as the hydrobromide in the following manner. Dimedone $\beta$-alanine ethyl ester hydrochloride was first prepared by adding $\beta$-alanine ethyl ester hydrochloride (0.2 moles) to a solution of dimedone (0.2 moles) in chloroform (600ml), the suspension then being neutralized by the addition of anhydrous triethylamine (0.2 moles) and allowed to stand overnight before being filtered. The filtrate was then evaporated to dryness on a steam bath, the residue dissolved in benzene and triethylamine hydrochloride filtered off.

The dimedone ethyl ester hydrochloride separated out when the solution was cooled in a refrigerator.
overnight. It was collected and dissolved in concentrated ammonia solution to produce dimerdone β-alanine amine. The amine was collected as a solid after the solution was evaporated off and finally, upon dissolution in water (400ml; 70°), was treated with pure Br₂, dropwise, until the yellow colour remained. Crystals of the amide were collected after the solution was cooled to 0°, washed with acetone and dried in an evacuated desiccator.

**Anal.** Calcd for β-alaNH₂··HBr: C, 21.31; H, 5.36; N, 16.57.

Found: C, 20.40; H, 5.05; N, 15.76.

\[
[\text{Co}(\text{en})_2(\beta-\text{alaOR})](\text{ClO}_4)_3 \quad \text{R = CH}_3, \text{CH}(\text{CH}_3)_2
\]

To cis-[Co(en)_2Br(β-alaOCH(CH_3)_2)]Br_2 (22g) (see Section 5.2.2 for preparation) suspended in acetone (25ml), previously dried for 2 days over BDH molecular sieves (type 4A), was added finely ground AgClO_4 (32g), previously dried in an evacuated desiccator, over P_2O_5, for 2 days. The mixture was shaken vigorously in the dark for 1 hour, filtered through "hy-flow" to remove AgBr and the filtrate evaporated to dryness on a rotary evaporator. The crude product was three times redissolved in dry acetone, filtered through Whatman's 542 filter paper and precipitated with anhydrous ether. The final product, which gave a negative result for Ag⁺ with Cl⁻, was then dried in an evacuated desiccator.


The following absorption maxima and extinction coefficients were obtained in H₂O, 0.1M HCl and 1M NaClO₄ at 25.0°:

\[
492 \pm 2\text{nm (ε 98 ± 2)}; \quad 347 \pm 2\text{nm (ε 68 ± 2)}.
\]
[Co(en)$_2$(β-alaOCH$_3$)](ClO$_4$)$_3$ was prepared by an identical procedure using $cis$-[Co(en)$_2$Br(β-alaOCH$_3$)]Br$_2$ (25.6g) (Section 5.2.2, preparation).

Anal. Calcd for [Co(en)$_2$(β-alaOCH$_3$)](ClO$_4$)$_3$: C, 16.55; H, 4.34; N, 12.06. Found: C, 17.05; H, 4.38; N, 11.80.

The absorption spectrum (600-300nm), (0.1M HClO$_4$), gave the following maxima and extinction coefficients: 493 ± 2nm ($\varepsilon$ ± 2); 352 ± 2nm ($\varepsilon$ 72 ± 5).

[Co(en)$_2$(β-alaNH$_2$)](ClO$_4$)$_3$

cis-[Co(en)$_2$Br(β-alaNH$_2$)]Br$_2$ (1.0g) (Section 5.2.2, preparation) was suspended in anhydrous acetone (50ml) and fresh AgClO$_4$ (1.23g) added. It was then allowed to stand in the dark for 3 days. The AgBr was filtered off and, to the filtrate, ether was added, whereupon the crude product separated out. It was collected, redissolved in hot ethanol containing 5% methanol, filtered and allowed to cool. With scratching of the container and addition of ether, the complex began to crystallize. It was cooled in an ice bath, collected, washed with ethanol and ether and air-dried.


The following absorption maxima and extinction coefficients were obtained in 1M NaClO$_4$ at 25.0°: 492 ± 2nm ($\varepsilon$ 117 ± 2); 349 ± 2nm ($\varepsilon$ 92 ± 2).
2.2.3 **Kinetic measurements**

The base hydrolysis of the free ester hydrochloride salts was followed by pH-Stat titration (1.0M NaOH) of an accurately weighed amount of the salt (~0.3g) in 1.0M NaClO₄ (~8ml) at 25.0°C.

The hydrolysis of \([\text{Co(en)}₂(\beta\text{-alaOCH(CH}_₃)_₂)](\text{ClO}_₄)_3\) was followed by pH-Stat titration in the pH range 2 to 7.9. The complex (0.1 to 0.2g) in 1.0M NaClO₄ (~8ml) was titrated at a set pH with 0.1M NaOH at 25.0°C.

Acid and base hydrolysis of the complexes were also followed spectrophotometrically at 346 or 495nm or by recording spectra at timed intervals. For reactions above pH 7, a solution of the complex (10⁻³ to 10⁻² M) was mixed with an equal volume of Tris (0.1 to 0.5M) or glycine (0.1 to 0.25M) buffer at \(\mu=1.0\) (NaClO₄) and 25.0°C, either by hand or in a stopped-flow reactor; the ratio of buffer to complex concentration was at least 30:1. For reactions in acid, accurately weighed quantities of the complexes (4x10⁻⁵ to 4x10⁻³ M) were dissolved in HClO₄ (0.1 to 1.0 M; \(\mu=1.0\), NaClO₄).

For pH measurements of reactant solutions, the error in pH was ±0.01 unit in the range ~3 to ~10; outside of these limits, or for kinetic runs by pH Stat titration, it was ±0.05 unit.

An estimate of the rate of acid hydrolysis of the bidentate isopropyl ester complex (~10⁻⁴ mole, 25.0°C) in 1M DCl (0.6ml) was obtained by recording pmr spectra at timed intervals. The rate of decomposition of the same complex in dilute DCl or D₂O solutions containing NaHCO₃
was obtained by the same technique.

2.2.4 Analysis of the products of hydrolysis of $[\text{Co(en)}_2(\beta\text{-alaOR})(\text{ClO}_4)]_3$

When hydrolysis of the complexes was complete, a spectrum of the solutions (600 to 300nm) was recorded and the product solutions sorbed on H$^+$-form resin and eluted with 2M NaClO$_4$. In all cases, only one band was obtained, the visible spectrum of which was identical with that before ion-exchange chromatography. Analysis for cobalt by atomic absorption spectrophotometry indicated a recovery of >98% from the column. Comparison of the visible spectrum and elution rate of the product with authentic $[\text{Co(en)}_2(\beta\text{-alaO})](\text{ClO}_4)_2$ (see Section 3.2.2 for preparation) confirmed the identity of the product.

The product solution of the hydrolysis of $[\text{Co(en)}_2(\beta\text{-alaOCH(CH}_3)_2)](\text{ClO}_4)_3$ in the presence of bicarbonate was sorbed on Na$^+$-form resin and eluted first with 0.5M NaClO$_4$ at pH~8, then with 2M NaClO$_4$ to remove the more highly charged complexes. Products were identified by visible spectra and elution rates from the ion-exchange column and also by paper chromatography (using butanol-H$_2$O-HCl, 6:3:1, then spraying with ninhydrin) and pmr spectra, compared with authentic materials.

2.2.5 $pK_a$ determinations

$\beta\text{-alaOCH(CH}_3)_2\cdot\text{HCl}$ (0.31g), dissolved in 1.0M NaClO$_4$ (8ml), thermostated at 25.0°C, was titrated with 1.0M NaOH and the pH recorded for each increment of base added.
[Co(en)$_2$(β-alanH$_2$)](ClO$_4$)$_3$ (0.0376g) was dissolved in 1.0M NaClO$_4$ (30ml) in the 3.2cm cell fitted in the Cary 16K spectrophotometer. Absorbance measurements at 330nm were noted as small titres of 1.0M NaOH were added. Acid dissociation constants, in both cases, were calculated by standard methods [118], corrections being made for the increase in volume throughout the titration.

2.3 Results

2.3.1 Kinetic data

Table 2.1 presents data for hydrolysis of the free β-alanine esters at 25.0°, µ=1.0 (NaClO$_4$). The proton ionization equilibrium is given in equation 2.1 and the pK$_a$ has been determined as 9.43±0.05.

\[ \text{H}_3\text{NCH}_2\text{CH}_2\text{CO}_2\text{R} + \text{H}_2\text{NCH}_2\text{CH}_2\text{COOR} + \text{H}^+ \]

\[ \text{(EH}^+) \quad \text{(E)} \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad
TABLE 2.1

Rates of Hydrolysis of β-Alanine Esters; 25.0°C;
\( \mu=1.0, \ NaClO_4^- \).

<table>
<thead>
<tr>
<th>Ester</th>
<th>pH</th>
<th>( 10^4 k_{\text{obsd}} ) (sec(^{-1}))</th>
<th>( k_{\text{obsd}}/[\text{OH}^-] ) (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{H}_2\text{NCH}_2\text{CH}_2\text{COOCH}_3 )</td>
<td>10.53</td>
<td>1.26</td>
<td>0.22</td>
</tr>
<tr>
<td>( \text{H}_2\text{NCH}_2\text{CH}_2\text{COOCH(\text{CH}_3)}_2^a )</td>
<td>11.61</td>
<td>1.56</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>11.60</td>
<td>1.57</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>12.84</td>
<td>21</td>
<td>0.018</td>
</tr>
</tbody>
</table>

\(^a\) \( pK_a = 9.43\pm0.05 \), obtained by the method described in Section 2.2.5.

\(^b\) [\text{OH}^-] \) calculated from pH using a value for \( pK_w = 13.77 \) [221].

is insignificant at high pH.

Table 2.2 lists observed rate constants, \( k_{\text{obsd}} \), obtained spectrophotometrically for the hydrolysis of the chelated esters in acid solutions, while Tables 2.3a,b,c and 2.4 contain observed rate constants obtained either spectrophotometrically with varying buffers, buffer strength, wavelength and cobalt concentration or by \( pH \) titration with NaOH, for hydrolysis under alkaline conditions. Spectra at timed intervals throughout the acid hydrolysis of the isopropyl ester complex are shown in Fig. 2.1. Isosbestic points occur at 382±3nm and 448±3nm, in both acidic and basic solutions confirming that only a single process is involved and that it is the same for both conditions.
## TABLE 2.2

**Spectrophotometric Rate Data for the Hydrolysis, in Acid,** of $[\text{Co(en)}_2(\text{R-alaOR})][\text{ClO}_4]^-$

<table>
<thead>
<tr>
<th>Ester Group, R</th>
<th>[HClO$_4$] M</th>
<th>$10^5 k_{\text{obsd}}$ (sec$^{-1}$)</th>
<th>$10^6 k_{\text{H}_2\text{O}}$ (M$^{-1}$ sec$^{-1}$)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$</td>
<td>0.01</td>
<td>53</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>55</td>
<td>9.9</td>
</tr>
<tr>
<td>CH(CH$_3$)$_2$</td>
<td>0.01</td>
<td>4.6$^c$</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>4.7$^c$</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>1.00$^d$</td>
<td>4.6</td>
<td>0.83</td>
</tr>
</tbody>
</table>

---

**a** $[\text{Complex}]=4 \times 10^{-4}$ to $2 \times 10^{-2}$ M; $\mu=1.0$ (NaClO$_4$); 25.0 ± 0.1$^o$; $\lambda=495$ nm.

**b** $k_{\text{H}_2\text{O}} = k_{\text{obsd}} / 55.5$.

**c** Average of three determinations.

**d** In 1M DCl (D$_2$O), $k_{\text{obsd}}$ $0.96 \times 10^{-5}$ sec$^{-1}$, determined by pmr spectrometry.
TABLE 2.3a

Spectrophotometric Rate Data for the Hydrolysis of

\[ [\text{Co}(en)_2(\beta\text{-alaOCH(CH}_3)_2)](\text{ClO}_4)_3 \] \(^a\) in 0.10M Tris Buffer \(^b\)

<table>
<thead>
<tr>
<th>pH</th>
<th>(10^2k_{\text{obsd}}) ((\text{sec}^{-1}))</th>
<th>(10^2k_{\text{calc.}}) ((\text{sec}^{-1})^c)</th>
<th>(10^2k_1[\text{OH}^-]) ((\text{sec}^{-1}))</th>
<th>(10^2k_2[\text{OH}^-]^2) ((\text{sec}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.19</td>
<td>0.14</td>
<td>0.14</td>
<td>0.13</td>
<td>0.005</td>
</tr>
<tr>
<td>7.24</td>
<td>0.15</td>
<td>0.16</td>
<td>0.14</td>
<td>0.006</td>
</tr>
<tr>
<td>7.70</td>
<td>0.54</td>
<td>0.48</td>
<td>0.42</td>
<td>0.05</td>
</tr>
<tr>
<td>7.82</td>
<td>0.65</td>
<td>0.66</td>
<td>0.57</td>
<td>0.09</td>
</tr>
<tr>
<td>8.20</td>
<td>2.0</td>
<td>1.9</td>
<td>1.3</td>
<td>0.5</td>
</tr>
<tr>
<td>8.55</td>
<td>6.0</td>
<td>5.5</td>
<td>3.0</td>
<td>2.5</td>
</tr>
<tr>
<td>8.68</td>
<td>9.3</td>
<td>8.7</td>
<td>4.1</td>
<td>4.6</td>
</tr>
<tr>
<td>8.75(^d)</td>
<td>11</td>
<td>11</td>
<td>4.8</td>
<td>6.4</td>
</tr>
<tr>
<td>8.94</td>
<td>22</td>
<td>22</td>
<td>7.3</td>
<td>15</td>
</tr>
</tbody>
</table>

\(a\). [Complex]-8\times10^{-4} \text{ to } 2\times10^{-3}\text{M; } \mu=1.0(\text{NaClO}_4); 25.0 \pm 0.1^\circ; \lambda=495\text{nm.}

\(b\). Tris buffer prepared from a stock solution adjusted to the desired pH with HClO\(_4\) and \(\mu\) adjusted to 1.0 with NaClO\(_4\).

\(c\). \(k_{\text{calc.}} = k_0 + (k_1[\text{OH}^-] + k_2[\text{OH}^-]^2)/(1 + K' [\text{OH}^-])\)

\(k_0 = 4.6\times10^{-5}\text{sec}^{-1}\) from Table 2.2;
\(k_1 = 5.0\times10^3\text{M}^{-1}\text{sec}^{-1}\);
\(k_2 = 7.0\times10^8\text{M}^{-2}\text{sec}^{-1}\);
\(K' = 2.3\times10^4\text{M}^{-1}\).

\(d\). Glycine-NaOH buffer.
TABLE 2.3b

Spectrophotometric Rate Data for the Hydrolysis of

$[\text{Co(en)}_2(\beta\text{-alaOCH(CH}_3)_2)](\text{ClO}_4)_3$ in 0.125M Tris Buffer.$^b$

<table>
<thead>
<tr>
<th>pH</th>
<th>$10^2k_{\text{obsd}}$ (sec$^{-1}$)</th>
<th>$10^2k_{\text{calc.}}$ (sec$^{-1}$)$^c$</th>
<th>$10^2k_1[\text{OH}^-]$ (sec$^{-1}$)</th>
<th>$10^2k_2[\text{OH}^-]^2$ (sec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.06</td>
<td>0.10</td>
<td>0.11</td>
<td>0.098</td>
<td>0.002</td>
</tr>
<tr>
<td>7.19</td>
<td>0.14$^e$</td>
<td>0.14</td>
<td>0.13</td>
<td>0.005</td>
</tr>
<tr>
<td>7.61</td>
<td>0.37</td>
<td>0.38</td>
<td>0.34</td>
<td>0.003</td>
</tr>
<tr>
<td>7.68</td>
<td>0.50$^e$</td>
<td>0.46</td>
<td>0.41</td>
<td>0.005</td>
</tr>
<tr>
<td>7.74</td>
<td>0.52</td>
<td>0.53</td>
<td>0.47</td>
<td>0.006</td>
</tr>
<tr>
<td>8.07</td>
<td>1.4</td>
<td>1.3</td>
<td>1.0</td>
<td>0.28</td>
</tr>
<tr>
<td>8.13</td>
<td>1.5</td>
<td>1.5</td>
<td>1.1</td>
<td>0.37</td>
</tr>
<tr>
<td>8.15</td>
<td>1.5</td>
<td>1.6</td>
<td>1.2</td>
<td>0.40</td>
</tr>
<tr>
<td>8.19</td>
<td>2.0$^e$</td>
<td>1.8</td>
<td>1.3</td>
<td>0.50</td>
</tr>
<tr>
<td>8.39</td>
<td>3.2</td>
<td>3.3</td>
<td>2.1</td>
<td>1.2</td>
</tr>
<tr>
<td>8.41</td>
<td>3.7</td>
<td>3.5</td>
<td>2.2</td>
<td>1.3</td>
</tr>
<tr>
<td>8.43</td>
<td>4.4$^e$</td>
<td>3.8</td>
<td>2.3</td>
<td>1.5</td>
</tr>
<tr>
<td>8.47</td>
<td>5.1</td>
<td>5.9</td>
<td>3.1</td>
<td>2.8</td>
</tr>
<tr>
<td>8.59</td>
<td>6.0$^e$</td>
<td>6.4</td>
<td>3.3</td>
<td>3.1</td>
</tr>
<tr>
<td>8.74</td>
<td>12</td>
<td>11</td>
<td>4.7</td>
<td>6.1</td>
</tr>
<tr>
<td>8.75$^d$</td>
<td>15</td>
<td>11</td>
<td>4.7</td>
<td>6.1</td>
</tr>
<tr>
<td>8.76</td>
<td>12</td>
<td>12</td>
<td>5.0</td>
<td>7.0</td>
</tr>
<tr>
<td>8.80</td>
<td>17</td>
<td>13</td>
<td>5.4</td>
<td>8.0</td>
</tr>
<tr>
<td>9.44$^d$</td>
<td>160</td>
<td>170</td>
<td>23</td>
<td>330</td>
</tr>
<tr>
<td>9.80$^d$</td>
<td>390</td>
<td>300</td>
<td>48</td>
<td>640</td>
</tr>
<tr>
<td>10.35$^d$</td>
<td>1000</td>
<td>1040</td>
<td>190</td>
<td>10,100</td>
</tr>
</tbody>
</table>

$^a$. [Complex]$\cdot 5 \times 10^{-3}$ to $10^{-2}$M; $\mu=1.0(\text{NaClO}_4)$; 25.0 ± 0.1°C.

$^b,c,d$. As in Table 2.3a.

$^e$. $\lambda=346$nm; for all other entries, $\lambda=495$nm.
TABLE 2.3c

Spectrophotometric Rate Data for the Hydrolysis of
[Co(en)$_2$(β-alalOCH(CH$_3$)$_2$)](ClO$_4$)$_3$ in 0.25 and 0.5M Tris Buffer.

<table>
<thead>
<tr>
<th>pH</th>
<th>$10^2k_{obsd}$ (sec$^{-1}$)</th>
<th>$10^2k_{calc.}$ (sec$^{-1}$)</th>
<th>$10^2k_1[OH^-]$ (sec$^{-1}$)</th>
<th>$10^2k_2[OH^-]^2$ (sec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.06$^b*$</td>
<td>0.12</td>
<td>0.11</td>
<td>0.098</td>
<td>0.002</td>
</tr>
<tr>
<td>7.23$^b$</td>
<td>0.15</td>
<td>0.16</td>
<td>0.14</td>
<td>0.006</td>
</tr>
<tr>
<td>7.44$^b*$</td>
<td>0.25</td>
<td>0.25</td>
<td>0.23</td>
<td>0.015</td>
</tr>
<tr>
<td>8.15$^b$</td>
<td>2.0</td>
<td>1.6</td>
<td>1.2</td>
<td>0.40</td>
</tr>
<tr>
<td>8.84$^b*$</td>
<td>15</td>
<td>16</td>
<td>5.9</td>
<td>9.7</td>
</tr>
<tr>
<td>8.93$^b*$</td>
<td>22</td>
<td>22</td>
<td>7.2</td>
<td>14.6</td>
</tr>
<tr>
<td>9.12$^b*$</td>
<td>38</td>
<td>33</td>
<td>11</td>
<td>35</td>
</tr>
<tr>
<td>9.13$^b$</td>
<td>42</td>
<td>33</td>
<td>11</td>
<td>36</td>
</tr>
<tr>
<td>9.23$^d$</td>
<td>60</td>
<td>54</td>
<td>14</td>
<td>57</td>
</tr>
</tbody>
</table>

---

a. [Complex]·0.003M; $\mu$=1.0(NaClO$_4$); 25.0 ± 0.1°; $\lambda$=495nm.
b. 0.25M Tris buffer; $b^*$ 0.50M Tris buffer.
c. As in Table 2.3a.
d. 0.25M glycine-NaOH buffer.
TABLE 2.4

Radiometric Rate Data for the Hydrolysis of 

\[ \text{[Co(en)}_2(\beta\text{-alaOCH(CH}_3)_2\text{)](ClO}_4\text{)} \]

<table>
<thead>
<tr>
<th>pH</th>
<th>(10^4 k_{\text{obsd}} \text{(sec}^{-1}))</th>
<th>(10^4 k_{\text{calc}} \text{(sec}^{-1}))</th>
<th>Equivalents of base consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>0.50</td>
<td>0.46</td>
<td>0.72</td>
</tr>
<tr>
<td>3.5</td>
<td>0.72</td>
<td>0.47</td>
<td>0.72</td>
</tr>
<tr>
<td>4.5</td>
<td>0.60</td>
<td>0.49</td>
<td>0.75</td>
</tr>
<tr>
<td>5.1</td>
<td>2.7</td>
<td>0.66</td>
<td>0.80</td>
</tr>
<tr>
<td>6.0</td>
<td>3.1</td>
<td>1.3</td>
<td>0.88</td>
</tr>
<tr>
<td>7.30</td>
<td>21</td>
<td>19</td>
<td>0.96</td>
</tr>
<tr>
<td>7.75</td>
<td>53</td>
<td>53</td>
<td>1.0</td>
</tr>
<tr>
<td>7.92</td>
<td>92</td>
<td>85</td>
<td>1.0</td>
</tr>
</tbody>
</table>

a. Approx. 0.1 g of complex, dissolved in M NaClO\textsubscript{4} (10 ml), titrated with 0.10 M NaOH; \(\mu=1.0\) (NaClO\textsubscript{4}); 25.0 ± 0.1°.

b. \(k_{\text{calc}}\) obtained as explained in footnotes of Table 2.3a.
Fig. 2.1. Visible spectra during hydrolysis, in 0.1M HClO₄, of \( \text{Co(en)}_2(\beta\text{-alaOCH}(\text{CH}_3)_2)(\text{ClO}_4)^- \).
Observed rate constants were obtained from plots of \( \log(D_w - D_t) \) vs \( t \). They were linear for \( 2 \times t \) to \( 3 \times t \). Typical plots are shown in Fig. 2.2.

The \( k_{\text{obsd}} \) values in Tables 2.2 and 2.3 obey the rate law

\[
k_{\text{obsd}} = k_0 + \frac{k_1[\text{OH}^-] + k_2[\text{OH}^-]^2}{1 + k'[\text{OH}^-]} \quad (2.3)
\]

over the pH range 0 to 10.35. The water term, \( k_0 \), is the rate constant for hydrolysis of the chelated isopropyl ester in HClO\(_4\) (4.7 x 10\(^{-5}\) sec\(^{-1}\), Table 2.2), and it is clear that, in the pH range 0 to 4, it is the only important term. Over the pH range, 4 to \( \approx 9.2 \), the denominator is equal to unity and the observed rate law takes the form

\[
k_{\text{obsd}} = k_0 + k_1[\text{OH}^-] + k_2[\text{OH}^-]^2 \quad (2.4)
\]

The constants, \( k_1 \) (5.0 x 10\(^{-3}\) M\(^{-1}\) sec\(^{-1}\)) and \( k_2 \) (7.0 x 10\(^{8}\) M\(^{-2}\) sec\(^{-1}\)) have been evaluated by solving, algebraically, sets of two simultaneous equations of the form (2.4), \( k_{\text{obsd}} \) and [OH\(^-\)] being the variables. The \( k_{\text{calc}} \) values listed in Tables 2.3a, b, c and 2.4 have been obtained by substituting mean values of \( k_1 \) and \( k_2 \), so derived, back into equation (2.4) and the agreement between \( k_{\text{calc}} \) and \( k_{\text{obsd}} \) attests to the accuracy of the rate expression. Above pH \( \approx 9.2 \), the rate law reduces to one that is first order in [OH\(^-\)], of the form

\[
k_{\text{obsd}} = k_3 + k_4[\text{OH}^-] \quad (2.5)
\]

and, from a plot of \( k_{\text{obsd}} \) vs [OH\(^-\)] of the limited data, values
Fig. 2.2. Plots of $\log(D_\infty - D_t)$ vs $t$, from spectrophotometric data, for base hydrolysis (0.1M Tris buffer) and acid-catalyzed hydrolysis of $\text{[Co(en)}_2\beta\text{-alaOCH(CH}_3)_2\text{]}(\text{ClO}_4)_3$. 

- base hydrolysis
- acid hydrolysis
of $k_3 \sim 0.4 \text{sec}^{-1}$ and $k_4 \sim 3 \times 10^4 \text{M}^{-1} \text{sec}^{-1}$ have been obtained. $K'$ in (2.3) is evaluated by neglecting the constant, 1, in the denominator of (2.3) and equating the coefficients,

$$\frac{k_2}{K'} = k_4$$

Thus, $K' \sim 2.3 \times 10^4 \text{M}^{-1}$. The accuracy of these constants is further limited by the error in $[\text{OH}^-]$. At high pH values, this becomes significant, e.g. an error of ±0.05 units at pH~9.8 produces a 20% error in the term squared in $[\text{OH}^-]$ above. In Fig. 2.3 is a curve showing the logarithm of $k_{\text{calc}}$ as a function of pH, with the experimental values also shown.

The rate constants obtained from rate of base uptake on the radiometer agree well with values calculated from spectrophotometric data at pH values above 7 but not below. This may partly be due to the fact that, at lower pH values where the reactions are slow ($t_{1/2} = 40$ to 190 mins), instrumental drift may have occurred with the result that rate data could not be accurately obtained. Furthermore, it is noteworthy that the base consumption per mole of complex decreases with decreasing pH (Table 2.4). Since analysis by ion exchange chromatography, combined with visible spectra of the product solution both before and after elution establish the sole complex product (>98% recovery) to be the acid chelate, it would be expected that one equivalent of base would be consumed according to the equation

$$[\text{Co(en)}_2(\beta-\text{alaOR})]^3^+ + \text{OH}^- \rightarrow [\text{Co(en)}_2(\beta-\text{alaO})]^2^+ + \text{ROH}$$

(2.6)
Fig. 2.3. Profile of \( \log k \) vs pH for base hydrolysis of \([\text{Co}(en)_2(\beta\text{-alaOCH(CH}_3)_2)](\text{ClO}_4)_3\); 25.0\(^\circ\); \( \mu = 1.0 \), NaClO\(_4\). (\( \bullet \) is the calculated curve, with a discontinuity at \( \bullet \); \( \bullet \) are experimental points).
The only other product in the solution is isopropanol. Bell and Coller [119] have pointed out that the amount of alkali consumed in ester hydrolysis is considerably less than the stoichiometric amount of ester, since the acid product becomes protonated, thereby releasing hydroxide. An analogous mechanism for the chelated acid is unlikely since the acid ionization constant lies well below the experimental range of pH. Aliphatic alcohols exhibit basic properties [120] and also are known to be protonated in acid solutions [121-123]. More specifically, Wells has found that isopropanol is protonated in dilute aqueous acidic solutions and is more basic than water, the equilibrium constant in an acidic solution in sodium perchlorate ($\mu = 1.0, 25.0^\circ$) at 2% v/v isopropanol being 0.448 [121]. This value is only small and therefore such a rationale is not expected to provide the only explanation for the observed results. With the presently known facts, the most likely explanation still appears to be that due to the experimental technique.

2.3.2 Infrared spectra

Shown in Fig. 2.4 are the infrared spectra of free $\beta$-alanine, the isopropyl ester as the hydrochloride salt, the coordinated isopropyl ester in $[\text{Co(en)}_2(\beta\text{-alaOCH(CH}_3)_2)](\text{ClO}_4)_3$ and the hydrolysis product, $[\text{Co(en)}_2(\beta\text{-alaO})](\text{ClO}_4)_2\cdot\text{H}_2\text{O}$ in the region 4000 - 250 cm$^{-1}$. For the most part, the spectra are complex and only some of the more characteristic absorptions are discussed.

Free $\beta$-alanine has a broad absorption band from 3500 to 2400 cm$^{-1}$, due to a combination of stretching vibrations
Fig. 2.4. Infrared spectra of
(a) $\beta$-alaOH
(b) $\beta$-alaOCH(CH$_3$)$_2$.HCl
(c) [Co(en)$_2$(\(\beta\)-alaOCH(CH$_3$)$_2$)](ClO$_4$)$_3$
and
d) [Co(en)$_2$(\(\beta\)-alaO)](ClO$_4$)$_2$.H$_2$O.
(Nujol mulls: (a)-(c), CsI plates; (d), KBr plates).

of the NH$_2$ group since $\beta$-alanine exists in the \textit{d}-form, and the distance between the amine and carboxyl groups compared to the \textit{l}-form amino acids [125, 128] gives rise to absorption bands, and antisymmetric stretching modes. There is an additional absorption band from 1520 to 1640 cm$^{-1}$ which is thought to arise from the stretching vibration of the amide group and antisymmetric stretching modes for the \(\beta\)-alanine. An additional strong band appears between 1350 and 1280 cm$^{-1}$ and is assigned to the amide stretching modes of the free amino acid. It may be due to the amide III band and correspond to the amide II band, whereas the NH$_2$ antisymmetric mode of the \textit{d}-form amino acids, [125, 128] may be attributed to the amide I band at 1650 cm$^{-1}$ possibly being a COO$^-$ antisymmetric mode. Other peaks are at 1590 cm$^{-1}$ (NH
of the NH$_3^+$ group (since $\beta$-alanine exists in the zwitterionic form, despite the greater distance between the carboxyl and amino groups compared to the $\alpha$-amino acids [124, 125]) and symmetric and antisymmetric stretching modes of the CH$_2$ moieties. There is an additional sharp band at 2100cm$^{-1}$, the origin of which is not understood. The hydrochloride salt of $\beta$-alanine isopropyl ester similarly has a broad absorption band from 3500 to 2400cm$^{-1}$ with shoulders appearing at 2550 and 2480cm$^{-1}$ (NH$_3^+$). There is also a weak absorption at 1900cm$^{-1}$.

The spectra of the complexes are more difficult to interpret because of the presence of the ethylenediamine ligands. Both complexes have a broad absorption at 3550cm$^{-1}$, which is thought to arise from NH$_2$ vibrations of the coordinated amino acid derivative; lattice water symmetric and antisymmetric stretching modes [126] for the chelated $\beta$-alanine may be hidden under this band. An additional broad band appears between 3300 and 3200cm$^{-1}$ for both complexes and is assigned to NH$_2$ and CH$_2$ vibrations of coordinated ethylenediamine, by comparison with similar complexes [127, 128]. Additional weak bands which may correspond to the unassigned bands of the free amino acid and ester appear at 2380 and 2000cm$^{-1}$ for both complexes.

In the region below 1800cm$^{-1}$, free $\beta$-alanine has two strong absorptions at 1632 and 1572cm$^{-1}$ and a number of other strong absorptions at lower frequencies. Based on assignments made for other amino acids, [125, 129] they may be attributed to carboxyl absorptions, that at 1572cm$^{-1}$ possibly being a COO$^-$ antisymmetric mode. Other peaks are at 1509cm$^{-1}$ (NH
deformation), 1460cm$^{-1}$ (CH$_2$ deformation), 1415cm$^{-1}$ (CH$_2$ scissor), 1402cm$^{-1}$ (symmetric COO$^-$) and 1335, 1295, 1262 cm$^{-1}$ (CH$_2$ wag), 1160cm$^{-1}$ (CH$_2$ twist), 850cm$^{-1}$ (CH$_2$ rock) [130].

In the coordinated β-alaninate complex (Fig. 2.4d), a broad absorption centred around 1600cm$^{-1}$ is evident; the NH deformation band has disappeared, the COO$^-$ symmetric mode vibration appears at 1412cm$^{-1}$ and the various CH$_2$ modes of the amino acid are now complicated by those of coordinated ethylenediamine.

The free β-alanine isopropyl ester exhibits a strong ester carbonyl absorption at 1722cm$^{-1}$ and another characteristic peak at 1200cm$^{-1}$, assigned to the skeletal C=O stretching vibration, by comparison with other esters [131]. In the corresponding ester complex (Fig. 2.4c), these absorptions appear at 1600cm$^{-1}$ and 1210cm$^{-1}$ respectively, the large shift of the former band being indicative of coordination to cobalt of the carbonyl oxygen. Superimposed on the ester carbonyl absorption may also be absorptions due to a COO$^-$ antisymmetric vibration or to an NH bending vibration. In both complexes, the broad double band at 1120cm$^{-1}$ and 1080cm$^{-1}$ and the sharp band at 625cm$^{-1}$ are due to vibrations of the perchlorate anion [132].

The profile of the infrared spectra of the complexes is similar to those of other bis-ethylenediamine complexes [128, 133]. It corresponds to the type A of Powell and Sheppard [128] where the ethylenediamine rings assume a gauche configuration. The strong bands of the complexes at 1455 and 1375cm$^{-1}$, superimposed on the nujol peaks may be attributed
to CH$_2$ (scissor and wag, respectively) of ethylenediamine and also of the amino acid (Figs 2.4c,d). The CH$_3$ scissor modes of the isopropyl group are also expected in this region [130] but cannot be differentiated in the spectra.

The medium bands around 900 cm$^{-1}$ are assigned to CH$_2$ rocking modes and appear at frequencies higher than those of the uncoordinated molecules, since such motions are restricted by bonding to the metal.

2.3.3 Pmr spectra

Table 2.5 lists chemical shift data (100 MHz) for the chelated isopropyl ester complex and for the $\beta$-alanine chelate (see Section 3.2.2 for preparation). In dilute DCl or D$_2$SO$_4$ solutions, [Co(en)$_2$(\(\beta\)-alaOCH(CH$_3$)$_2$)]$^{3+}$ shows a broad absorption at 3.4 ppm which integrates for 10 protons; there is a less intense triplet centred around 3.0 ppm, integrating for 2 protons. The former is attributed to the methylene protons of two ethylenediamine ligands and the 2 methylene protons of $\beta$-alanine adjacent to the coordinated nitrogen atom; the latter is attributed to the methylene protons adjacent to the carboxyl function. These assignments are based on the observation that, in the uncoordinated ester, the down-field methylene triplet (3.78 ppm) collapses to a singlet in NaOD solution when the NH$_2$ groups have been deuterated [134], while the up-field triplet remains unchanged. The up-field triplet in the spectrum of the chelated ester also remains unchanged in basic conditions and the absence of broad absorptions at 4-6 ppm demonstrates that the NH$_2$ groups have been deuterated.
In NaN0 solution, integration of the spectrum gives a total of 12 methylene protons; that is, none of the methylene protons are exchanged with deuterium, in particular a methylene proton a to the carbonyl of the amide residue is not exchanged.

The shifts in the spectra relative to the free base. 

### TABLE 2.5

**Pmr Absorptions of β-Alanine Derivatives**

<table>
<thead>
<tr>
<th>R = CH(CH₃)₂</th>
<th>[Co(en)₂]³⁺</th>
<th>[Co(en)₂]²⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₃CH₂CH₂COOR</td>
<td>(β-alaOR)</td>
<td>(β-alaO)</td>
</tr>
<tr>
<td>1.76 (J=6Hz)</td>
<td>1.76 (J=6Hz)</td>
<td>-</td>
</tr>
<tr>
<td>3.28b</td>
<td>3.02b</td>
<td>3.08c</td>
</tr>
<tr>
<td>3.78b</td>
<td>-h</td>
<td>-h</td>
</tr>
<tr>
<td>3.40q</td>
<td>3.34q</td>
<td>-CH₂- adjacent to NH₂</td>
</tr>
<tr>
<td>-f</td>
<td>4.80f</td>
<td>4.60f</td>
</tr>
<tr>
<td>5.52e</td>
<td>5.66g</td>
<td>-CH</td>
</tr>
<tr>
<td>-f</td>
<td>6.00d,f</td>
<td>5.60f</td>
</tr>
</tbody>
</table>

a. Ppm down-field from TMS (100MHz)
b. triplet;
c. quintuplet
d. unsymmetrical; e. septuplet
f. H's exchange with D; signal disappears in NaOD soln.
g. multiplet; it is difficult to determine whether this is a quintuplet or septuplet since the signal appears in the region where there is strong HOD absorption and broad NH₂ absorption.
h. hidden under absorptions due to en.

- Assignment:
  - 1.76 (J=6Hz) = gem-CH₃
  - 3.02b = -CH₂- adjacent to COO
  - 3.08c = -CH₂- adjacent to NH₂
  - 3.34q = -CH₂- of en + -CH₂- of β-ala
  - 4.80f = NH₂ of β-ala
  - 5.66g = CH
  - 5.60f = NH₂ of en

- Notes:
  - Coordination of the water molecule is due to the poor basicity of Co, compared to Cu. Thus, the chemical shift of the methylene protons of the amide residue is in the same solvent mixture, since the exchange of the amide groups is due to the higher basicity of the amide of the Co complex than in the Cu complex.

- Additional information:
  - Assignment of proton absorptions to the amide protons of the Co(en)₂⁺ complex.
  - The spectrum shows a broad NH₂ absorption at 1.34 ppm, in contrast to 1.3 immune shift in the Cu complex. Other methylene groups of the amide acid residue lack in electronic environments which are least affected by the amide acid than in the water.
In NaOD solution, integration of the spectrum gives a total of 12 methylene protons; that is, none of the methylene protons are exchanged with deuterium; in particular a methylene proton α to the carbonyl function of the β-alanine residue is not exchanged.

The shifts in the spectra relative to the free protonated ester provide some information about the nature of the chelate ring formed. The geminal methyl groups are unchanged on coordination; this may be expected since they are fairly distant from the coordination site. The methine proton, however, becomes more acidic, consistent with a withdrawal of electron density from the alcohol function towards the cobalt centre. There is an up-field shift for both methylene absorptions, when the ester is chelated. This may be due to the formation of the chelate ring, but it is more likely to be due to the poorer Lewis acidity of Co$^{3+}$ compared to H$^+$. Thus, the methylene group adjacent to the amino group shifts 0.4 ppm and that adjacent to the carbonyl function, 0.26 ppm.

The chemical shift of the methylene protons of ethylenediamine may not be expected to differ in the acid and ester complexes. Thus, since the band near 3.4 ppm is the sum of methylene protons of ethylenediamine and the methylene of the amino acid adjacent to the amino group, it is reasonable to deduce that the shift up-field from 3.40 in the ester complex to 3.34 ppm in the acid complex may be attributed to an up-field shift in the latter. Furthermore, there is a concomitant shift down-field from 3.02 to 3.08 ppm for the other methylene group of the amino acid. These observations suggest that the two methylene groups of the amino acid residue exist in electronic environments which are less different in the chelated acid than in the ester.
Fig. 2.5. Pmr spectra during hydrolysis of [Co(en)₂β-alaOCH(CH₃)₂](ClO₄)₃ in 1M DCl. (Internal reference, NaTPS; 100MHz).
Only one doublet appears at 1.76 ppm for the methyl protons of the isopropyl group both in the free and chelated \( \beta \)-alanine isopropyl ester. Since the two methyl groups are not expected to be equivalent, it can only be concluded that their shifts are too close for the doublets to be discerned. The spectrum of the analogous glycine complex in acetone-\( d_6 \), on the other hand [114], does show two sets of doublets in this region.

Fig. 2.5 shows pmr spectra at given intervals during acid hydrolysis of the chelated isopropyl ester in 1M DCl. The features mentioned above are evident in this figure. Moreover, as the reaction proceeds, isopropanol is generated, a new \( CH_3 \) doublet appears at 1.61 ppm (\( J = 6 \) Hz) and a methine quintuplet appears at 4.52 ppm. Integration of the isopropyl signals gives a half-life of approximately 20 hours for acid hydrolysis.

2.3.4 Hydrolysis of \([Co(en)_2(\beta\text{-alaOCH(CH}_3)_2)KClO_4]_3\) in the presence of bicarbonate ions

Preliminary pmr investigations have shown that, on addition of sodium bicarbonate to the chelated \( \beta \)-alanine isopropyl ester, at least one additional product, beside the chelated acid, is formed and that it is long-lived, compared to the parent ester. Moreover, this compound is stable in acid, indicating that it is not \([Co(en)_2(HCO_3)(\beta\text{-alaOCH(CH}_3)_2)]^{2+}\) or a similar addition product containing carbonate.

For a more detailed study, spectra have been recorded at intervals in (i) deuterated acetate buffer, pH\( \approx 4 \), (ii) imidazole buffer, pH\( \approx 7 \), (iii) collidine buffer, pH\( \approx 8 \) and
(iv) NaOD/D₂O solution, pD ~ 11, in the absence of added bicarbonate. In each case, there is a decrease in intensity of the isopropyl doublet of the chelated ester with concomitant growth of a new doublet at higher field, consistent with the formation of free isopropanol. The latter assignment is confirmed by adding authentic isopropanol to the reaction mixture. Examples for the imidazole and collidine buffer solutions are shown in Figs 2.6a and 2.7a.

In each case, however, when solid sodium bicarbonate is added, a new doublet appears at a field strength intermediate between those of the chelated ester and isopropanol (1.71ppm, J = 6Hz), (Figs 2.6b, 2.7b,c,d). The doublet increases in intensity together with that of the free alcohol and reaches a maximum when the starting material has disappeared. It then slowly disappears as isopropanol continues to be formed. The rate of growth of this new doublet is dependent on pH, as seen by comparing Figs 2.6b (pH ~ 7) and 2.7b (pH ~ 8) and is also dependent on the amount of bicarbonate added (Figs 2.7b,c and d). Both uncoordinated β-alanine isopropyl ester and cis-[Co(en)₂H₂O(β-alaoCH(CH₃)₂)](NO₃)₃ have isopropyl signals at the same positions as that observed in the above reaction and the results presented below confirm that both compounds are formed.

A sample of chelated ester complex was allowed to react with an aqueous solution of sodium bicarbonate at pH ~ 7.5 for 10 minutes, when all starting material had been consumed, then sorbed on a Na⁺-form column and eluted under basic conditions as outlined in Section 2.2.4. The products were identified as the cis-dihydroxo complex (~10%), the bidentate
Fig. 2.6. Pmr spectra in D$_2$O at intervals during hydrolysis of [Co(en)$_2$(β-alSOCH(CH$_3$)$_2$)](ClO$_4$)$_3$ in (a), imidazole buffer (pD ~ 7) and (b), imidazole buffer in the presence of NaHCO$_3$ (pD ~ 7.5). (Internal reference, NaTPS; 100MHz).
### Table

<table>
<thead>
<tr>
<th>(a)</th>
<th>1 min</th>
<th>4 mins</th>
<th>10 mins</th>
<th>30 mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>(b)</td>
<td>2 mins</td>
<td>42 mins</td>
<td>96 mins</td>
<td>26 hrs</td>
</tr>
<tr>
<td>(c)</td>
<td>2 mins</td>
<td>6 mins</td>
<td>45 mins</td>
<td>26 hrs</td>
</tr>
<tr>
<td>(d)</td>
<td>4 mins</td>
<td>10 mins</td>
<td>34 mins</td>
<td>60 mins</td>
</tr>
</tbody>
</table>

### Figures

**Fig. 2.7.** Pmr spectra in D$_2$O at intervals during hydrolysis of [Co(en)$_2$8-alaOCH(CH$_3$)$_2$](ClO$_4$)$_3$ in
(a), collidine buffer (pD $\sim$ 8),
(b), collidine buffer with a trace of added NaHCO$_3$,
(c), collidine buffer with approximately equimolar NaHCO$_3$,
(d), collidine buffer with excess NaHCO$_3$. (Internal reference, NaTPS; 100MHz).
acid complex (~50%) and the cis-hydroxo ester complex (~40%) by comparison with spectra and elution rates of the authentic materials on ion-exchange columns or by thin layer chromatography. The first product implies the formation of free β-alanine or its ester and this was detected in a thin layer chromatography experiment by the blue coloration that appeared with ninhydrin.

2.3.5 Preliminary study of the base hydrolysis of

\[ [\text{Co(en)}_2(\beta-\text{alaNH}_2)](\text{ClO}_4)_3 \]

The pK_a of the first proton of the amide function in the chelated β-alaNH_2 complex has been determined spectrophotometrically to be 11.05 ± 0.05 by titration against NaOH. During the time required to make this measurement, little hydrolysis (<5%) occurs. Rates of base hydrolysis have been obtained by measurement of base uptake with time. Because only small amounts of complex were used and because there was difficulty in maintaining the electrodes at high pH over extended periods, the data given in Table 2.6 are only approximate. However, it is apparent that a limiting rate is reached at the highest pH.

Ion-exchange chromatography of the product solution could detect only the chelated acid complex as the product; (very little of the starting material was available).
### TABLE 2.6

**Radiometric Rate Data for the Hydrolysis of**

\[ [\text{Co} (\text{en})_2(\beta-\text{alanNH}_2)](\text{ClO}_4)_3 \]

<table>
<thead>
<tr>
<th>pH</th>
<th>(10^3 k_{\text{obsd}} \text{ (sec}^{-1}))</th>
<th>(k_{\text{OH}} \text{ (M}^{-1}\text{sec}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0</td>
<td>0.45</td>
<td>2.7</td>
</tr>
<tr>
<td>10.8</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>11.8</td>
<td>2</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**Notes:**

- \(a\). \(pK_a = 11.05 \pm 0.05\), determined spectrophotometrically (Section 2.2.5). \((\mu = 1.0, \text{NaClO}_4; 25.0^\circ)\)
- \(b\). \(k_{\text{OH}} = \frac{k_{\text{obsd}}}{[\text{OH}^-]}\); (error \(< 20\%\))
2.4 Discussion

2.4.1 The uncoordinated \( \beta \)-alanine esters

The \( pK_a \) of \( \beta \)-alanine isopropyl ester is 9.43 ± 0.05 at 25.0°, \( \mu = 1.0 \) (NaClO4) and is greater than that for the corresponding ethyl ester, \( pK_a = 9.06 \) in aqueous solution at 25.0° [135]; the greater value may be due partly to the greater positive inductive effect of the isopropyl compared with the ethyl group, although thermodynamic factors may be more important. Furthermore, \( \beta \)-alanine (\( pK_a = 10.24 \) [136] and its esters are stronger bases than the corresponding glycine derivatives (\( pK_a = 9.6 \), acid; 7.68 ethyl ester) [89].

Table 2.1 shows that the hydrolysis of \( \beta \)-alanine isopropyl ester at \( \mu = 1.0 \), 25.0° (\( k_{OH} = 0.022M^{-1}sec^{-1} \)) is ten times slower than that of the methyl ester (0.22M\(^{-1}\)sec\(^{-1}\)). The second value is about half that obtained by Hay and Morris [85] where \( \mu = 0.16 \). Further, the rate of \( \beta \)-alanine methyl ester is about one fifteenth that of the glycine analogue (\( k_{OH} = 0.63M^{-1}sec^{-1} \), \( \mu = 0.01 \)) [82,83] if the same allowance is made for the ionic strengths in this comparison.

2.4.2 Acid hydrolysis of chelated \( \beta \)-alanine esters

Tables 2.2 and 2.4 demonstrate that hydrolysis of the complexes, \([\text{Co(en)}_2(\beta\text{-alaOR})](\text{ClO}_4)_3\) where \( R = \text{CH}_3 \), CH(CH\(_3\))\(_2\) is independent of acid concentration from pH 0 to 4. This is consistent with attack of water at the carbonyl centre, with \( k_{H_2O} = 9.7 \times 10^{-6}M^{-1}sec^{-1} \) when \( R = \text{CH}_3 \) and \( 8.3 \times 10^{-7}M^{-1}sec^{-1} \) when \( R = \text{CH(CH}_3)_2 \). A similar proposal, where water is the nucleophile, has been made for uncoordinated esters [59,131], with the difference that hydrolysis is governed by a term first order
in \([H^+]\) in the rate law. This has been interpreted as water attack on the ester, preprotonated at the carbonyl oxygen \([79,117]\). In the complex, the metal ion fulfils the role of the proton, account thus being made for the lack of a term in \([H^+]\). Martin \([79]\) maintains that the \(N_5Co^{3+}\) moiety is less effective in polarizing the carbonyl group than the proton by a factor of \(10^{7.9}\) and this is substantiated in the present work; \(k_{H_2O}\) for the chelated ester is \(8.4 \times 10^{-5}\) M\(^{-1}\) sec\(^{-1}\), while an estimated value, based on Martin's calculations for the carbonyl protonated isopropyl ester is \(\approx 1\) M\(^{-1}\) sec\(^{-1}\).

The stoichiometry for acid hydrolysis of the chelated ester is:

\[
[Co(en)_2(\beta\text{-alaOR})]^{3+} + H_2O \rightarrow [Co(en)_2(\beta\text{-alaO})]^{2+} + ROH + H^+ \quad (2.7)
\]

A possible mechanism, based on proposals for organic esters, is shown below.
In this scheme, (2.8), hydrolysis is shown to proceed without opening of the chelate ring. This is reasonable on the basis of $^{18}O$ tracer experiments on the analogous chelated glycine esters [113], where the chelate ring has been shown to remain intact during acid hydrolysis. Although a six-membered chelate ring is somewhat less stable than a five-membered ring [137], a similar mechanism is likely.

The coordinated $\beta$-alanine methyl ester is hydrolyzed twelve times more rapidly than the isopropyl ester (Table 2.2). Since a similar factor is obtained in the free esters, it is likely that the rate-controlling step is the same in both systems. The large increase in rate from coordinated to free ester may result either from stabilization of the transition state in the latter to destabilization of the former. Buckingham, Foster and Sargeson have shown for chelated glycine esters, that when $R=CH_3$, hydrolysis proceeds via acyl oxygen fission [113], but when $R=C(CH_3)_3$, it proceeds via alkyl oxygen fission [112]. The steric properties and inductive effects of the isopropyl group are expected to lie between those of the abovementioned groups, so that either acyl or alkyl fission may occur [138]. By comparison of the relative rates of methyl and isopropyl esters with those in a homologous series of alkyl acetates [139], however, acyl oxygen cleavage is most likely. (Experiments exhibiting the stability of the six-membered chelate ring during hydrolysis and to show the position of cleavage of the ester function were not carried out, since it was not the direct aim of this work and since reasonable evidence was available from the glycine systems).

The rate of hydrolysis of chelated $\beta$-alanine isopropyl ester in 1M DCl is five times slower than in 1M HCl. This
decrease reflects the importance of the role of H$_2$O in the mechanism, for such a trend would be expected from the poorer basicity of D$_2$O compared to H$_2$O.

It is seen from the overall rate law for hydrolysis of [Co(en)$_2$(β-alaOCH(CH$_3$)$_2$)]$^{3+}$ (equation 2.3) that the contribution from a term first order in [OH$^-$] is already apparent at pH 4 (5%) and is equal to that of the water term at pH 5.7. In the light of the results already presented, the base hydrolysis of the chelated isopropyl ester is discussed in the following section.

2.4.3 Base hydrolysis of [Co(en)$_2$(β-alaOCH(CH$_3$)$_2$)]$^{3+}$

Base hydrolysis of the chelated β-alanine ester proceeds as follows:

$$[\text{Co(en)}_2(\beta-\text{alaOCH(CH$_3$)$_2$})]^{3+} + \text{OH}^- \rightarrow [\text{Co(en)}_2(\beta-\text{alaO})]^{2+} + \text{HOCH(CH$_3$)$_2$}$$  \hspace{1cm} (2.9)

One equivalent of base is consumed and the products are solely the chelated acid and isopropanol.

The rate equation for base hydrolysis contains terms first and second order in [OH$^-$] and is rewritten here. (See Section 2.3.1).

$$k_{\text{obsd}} = k_0 + k_1 [\text{OH}^-] + k_2 [\text{OH}^-]^2 \frac{1}{1 + k' [\text{OH}^-]}$$

The two terms in the numerator contribute equally to $k_{\text{obsd}}$ at pH = 8.63 ($k_1[\text{OH}^-] = k_2[\text{OH}^-]^2 = 0.36$) (See Tables 2.3a to c). Above pH = 9.2, $k_{\text{obsd}}$ deviates from the rate law second order.
in hydroxide (see last three entries in Table 2.3b) and obeys a law, first order in [OH\textsuperscript{-}]), the term with [OH\textsuperscript{-}] in the denominator becoming important.

The problem lies in finding a mechanism and then, in accounting for the term second order in [OH\textsuperscript{-}] and the reversion to a law first order in [OH\textsuperscript{-}] at higher pH.

The requirement of a second hydroxide ion for deprotonation of one of the NH\textsubscript{2} groups of coordinated ethylenediamine or \(\beta\)-alanine ester is unlikely, the first hydroxide ion acting as a nucleophile at the carbonyl carbon. Even though proton exchange is known to occur under the conditions used, there is no obvious reason or evidence that this proton removal would increase the reactivity of the active site. Moreover, this proposal would not accommodate the limiting rate law at high pH (>9.2), for the pK\textsubscript{a} of such coordinated amine groups exceed 14 [140] and the deprotonated reactant would be present only in small concentrations. A similar argument has been used for base hydrolysis of [Co(NH\textsubscript{3})\textsubscript{5}O\textsubscript{2}CCF\textsubscript{3}\textsuperscript{2+}], [141,142], which also obeys a rate law second order in [OH\textsuperscript{-}]; this is also discounted, firstly, because such a deprotonation is unlikely to enhance the reactivity of the active site and secondly, the activation energy required for conjugate base formation (14.5kcal/mole) is higher than the observed value (6.8kcal/mole). The authors suggest a more likely mechanism, involving attack of an OH\textsuperscript{-} at the carbonyl carbon, followed by a second OH\textsuperscript{-} removing an H\textsuperscript{+} from the first, in a concerted process. A concerted mechanism is excluded in the present investigation, for it, too, would not accommodate the rate-limiting situation at higher pH.
A deprotonation more likely to activate hydrolysis is one at the carbon atom α to the carbonyl group for it is known that α-amino acids, such as chelated glycine, have an acidic methylene proton and that the resulting carbanion is reactive \([143,144]\). However, no exchange of the α-methylene protons occurs during base hydrolysis of the chelated β-alanine ester. A similar observation has been made for a Cu(II) complex with β-alanine \([145]\). The proposal of such a deprotonation must, therefore, also be eliminated.

It appears that there is no reasonable mechanism involving deprotonation of the ester complex to account for a term second order in \([OH^-]\), with the subsequent first order limit at higher \(pH\). The observed rate law may be accounted for by invoking a mechanism where an intermediate is deprotonated at the active site.

A possible mechanism is one where the tetrahedral intermediate is formed by addition of \(OH^-\) to the carbonyl centre of the chelated ester, \(CE\). It is proposed that this species is deprotonated at the hydroxyl group and that both the protonated and deprotonated intermediates separately lead to the chelated acid, \(CA\), shown in the sequence, \((2.10)\).

\[
\begin{align*}
(CE) & \\
\xrightarrow{3 + N-CH_2} \xrightarrow{2 + N-CH_2} & \xrightarrow{2 + N-CH_2} \\
\text{OH}^- & \kappa_a & \kappa_b & \kappa_c & \kappa_d \\
\text{OH}^- & \kappa_e & \kappa_f \\
\text{OR} & \\
\text{R} = \text{CH(CH}_3)_2 \\
\end{align*}
\]
There are two derived rate laws which fit the experimental data, and the assumptions and implications of the two differ in a number of ways. The derivations are set out in the Appendix and a discussion of the two laws is set out below.

The first derivation, which assumes a steady state concentration for the intermediates in base hydrolysis, leads to a rate law of the form:

\[
k_{\text{obsd}} = \frac{k_a k_c [\text{OH}^-] + k_a k_d k_e [\text{OH}^-]^2}{k_f + k_d}
\]

In this scheme, the route through the tetrahedral intermediate, IH, gives rise to the term first order in [OH\(^-\)], while the route through the conjugate base, I, gives rise to the term second order in [OH\(^-\)]. It is reasonable to propose the formation of a deprotonated intermediate of the type, I, in the pH range studied, even though its concentration may be small. The hydroxyl group is attached to a carbon atom bonded to the electron withdrawing CoN\(_5\)^{3+} moiety and may well have a proton dissociation constant of about 10\(^{-14}\). This argument is supported by comparison of the structure, IH, with organic molecules containing similar electronegative groups attached to a tetrahedral carbon atom, as illustrated below, in alcohols, hemi-ketals and gem-diols.

\[
\begin{align*}
\text{alcohol} & \quad \text{hemi-ketal} & \quad \text{gem-diol} \\
\begin{array}{c}
R_1-C-R_3 \\
\text{O}_\text{H}
\end{array} & \quad \begin{array}{c}
R_1-C-OR_3 \\
\text{O}_\text{H}
\end{array} & \quad \begin{array}{c}
\text{en}_2\text{Co} \quad \text{CH}_2 \\
\text{O}_\text{H} \quad \text{OR}
\end{array}
\end{align*}
\]
Ballinger and Long have determined the acid ionization constants, $K_a$, of a number of alcohols containing electron-withdrawing functions; they have found trichloroethanol [146] to have a value $5.8 \times 10^{-13}$ (p$K_a = 12.24$) and trifluoroethanol [147], $4.3 \times 10^{-13}$ (p$K_a = 12.37$). Hemi-ketals are known to be unstable and acidity data for them are scarce. However, they are expected to exhibit properties similar to those of gem-diols. Gem-diols are considerably stronger acids than the monohydric alcohols, but few such substances have been investigated [57]. p$K_a$ values for formaldehyde and acetaldehyde hydrates in aqueous solution are 13.27 and 13.57 respectively [148,149] with the value for chloral hydrate at 10.04 [149]. Stewart and Van der Linden have investigated diols containing benzene derivatives and trifluoromethyl groups, obtaining a value as low as 9.18 for $m$-$\text{NO}_2 \cdot \text{C}_6\text{H}_4\text{C(OH)}_2\cdot\text{CF}_3$ [150].

In comparison with the illustrated organic analogues, the intermediate, IH, may be expected to exhibit similar acidic properties, for the tetrahedral carbon atom effectively has three electronegative functional groups. Acidities of the organic molecules can be estimated from Taft's polar substituent constants, $\sigma^*$, [151], but values are not available for groups containing a metal ion, so that only qualitative comparisons can be made amongst them. It can be seen, however, that, while a number of factors may influence the acidity of the proton in question, the effect of a metal ion appears to be less than that of a proton. In fact, an empirical rule for the p$K_a$ of a complex, MAH, in terms of the p$K_a$'s of the free diprotic acid, HAH, has been formulated as
pK (MAH) ≅ \frac{1}{4}[pK_a(\text{HAH})+pK_\text{a}(\text{AH}^-)] [152]. This is apparent in comparing the pK_a's of HOCO_2H, carbonic acid, and [(NH}_3)_5\text{CoO}_2\text{H}^{2+}. In aqueous solution, the pK_a of the first proton of the free acid is 6.35 and that for the second, 10.33 [153] so that pK_a(MAH) is expected to be less than 8.34; in fact, for [(NH}_3)_5\text{CoO}_2\text{H}^{2+}, the value is 6.4 (\mu=0.5, 25.0^\circ) [154]. Discounting the small effect that the different media would have on the quoted values, it appears, in this case, that the effect of the N_5\text{Co}^{3+} moiety is similar to that of the proton. On the basis of the arguments presented, it thus appears reasonable to assume that the pK_a of the tetrahedral intermediate may be about 14.

Turning, now, to the rate equation, (2.11), the experimental data may be discussed. At pH=7.0, 92.6% of the reaction proceeds via the pathway first order in [OH^-] and only 1.3% via that second order in [OH^-]; (the remaining 5% proceeds via the water pathway and becomes negligible at pH>8). At pH=7.0, (k_b + k_c), in the denominator, is far greater than the remaining term, so that the last can be neglected. The second term in the numerator can also be neglected, so that a simple first order law is observed and, equating the coefficients with the experimental data, the following relation is obtained:

$$\frac{k_a}{k_b+k_c} = 5 \times 10^3 \text{M}^{-1}\text{sec}^{-1}$$ (2.12)

At pH values greater than 9.2, the term with hydroxide in the denominator becomes important and the constants appear
to be unimportant, so that equation (2.11) reduces to one that is first order in [OH\(^-\)].

\[ k_{\text{obsd}} = \frac{k_0 k_c}{k_d k_e} (k_f + k_d) + k_a [\text{OH}^-] \]  

(2.13)

It is reasonable to assume that reprotonation of the intermediate, I, occurs at a rate that is far greater than that for loss of the leaving group, that is, \( k_f \gg k_d \), for the former merely involves proton transfer while the latter involves loss of an alkoxyl group. That proton addition is rapid has been shown in a number of systems, specific rates for reprotonation being of the order of \( 10^9 - 10^{10} \text{sec}^{-1} \) [155,156]. Once \( k_d \) is neglected in the brackets of equation (2.13), the ratio \( \frac{k_e}{k_f} \) may be replaced by the constant, \( K \), for it has already been rationalized that the acid dissociation constant may have a value round \( 10^{-14} \) (i.e. \( K \approx 1 \text{ M}^{-1} \)).

On substitution of the experimental and deduced values in equation (2.13), the following ratio is obtained.

\[ \frac{k_c}{k_d} \approx \frac{0.4}{3 \times 10^4} \]

i.e. \( \frac{k_c}{k_d} \approx 10^{-5} \)

The same ratio is also obtained when the ratios of experimental constants, \( k_1 \) and \( k_2 \), are substituted in equation (2.11).
That is,

\[
\frac{k_a k_c}{k_a k_d} \cdot \frac{(k_c+k_d)}{k_a k_d} \approx \frac{k_c}{k_d} = \frac{5 \times 10^3}{7 \times 10^8} \approx 10^{-5} \tag{2.14}
\]

Substituting the value for \( k_a \), found experimentally, into (2.12), a further relation is obtained:

\[
\frac{k_b}{k_c} \approx 4 \tag{2.15}
\]

The ratio in (2.15) implies that \( \text{OH}^- \) is a better leaving group than \( \text{OR}^- \) and this is not unreasonable if a comparison is made of the acid strengths of the resulting species, \( \text{HOH} \) and \( \text{ROH} \); the former is the stronger acid and this correlates with the greater ease of bond breaking in the intermediate \([157]\). The small ratio of \( k_c/k_d \) in (2.14) implies that the leaving group is removed with greater difficulty from the protonated intermediate, \( \text{IH} \), than from the deprotonated species. The former may involve an activated complex with a four- or six-membered ring, as shown below.

In the first illustration, the proton of the hydroxyl group is transferred directly to the leaving group and, in the second, a solvent molecule is the proton donor. An alternative mechanism is one involving an equilibrium, but if this were
the case, the equilibrium would lie far to the left, the electronic configuration without a charge separation being the more stable one.

\[
\text{\begin{align*}
\text{\begin{array}{c}
\text{C} & \text{O} & \text{R} \\
\text{O} & \text{H}
\end{array}}
\quad \rightleftharpoons 
\text{\begin{array}{c}
\text{C} & \text{O} & \text{R} \\
\text{O} & \text{H}
\end{array}}
\end{align*}}
\]

The deprotonated tetrahedral intermediate, on the other hand, may decompose via solvent assisted loss of the alcohol group, with an electron pair shift from the deprotonated oxygen.

\[
\text{\begin{align*}
\text{\begin{array}{c}
\text{C} & \text{O} & \text{R} \\
\text{O} & \text{H}
\end{array}}
\quad \rightarrow 
\text{\begin{array}{c}
\text{C} & \text{OH} \\
\end{array}}
+ 
\text{HOR}
\end{align*}}
\]

An additional factor to account for the small ratio of \( k_c/k_d \) is the charge on the species involved. It may be expected that the leaving group will be lost with greater difficulty from the dipositive species, \( \text{IH} \), compared with the unipositive \( \text{I} \), other things being equal.

The alternative derived rate law, which also fits the experimental data, assumes that the chelated ester complex, \( \text{CE} \), in (2.10), is in equilibrium with the protonated intermediate, \( \text{IH} \), which, in turn, is in equilibrium with the deprotonated species, \( \text{I} \). (See Appendix for derivation). The rate law has the form,

\[
k_{\text{obsd}} = \frac{k'_c k \left[ \text{OH}^- \right] + k'_d K \left[ \text{OH}^- \right]^2}{1 + K' \left[ \text{OH}^- \right]} \quad (2.16)
\]
where $K' = k_a/k_b$. Because a term first order in $[OH^-]$ is the only important one at pH < 7, it follows that $K'[OH^-] << 1$ in the denominator and that the second term in the numerator is also unimportant. The coefficient of the first order term may then be written as:

$$K'k = 5 \times 10^3 \text{ M}^{-1} \text{sec}^{-1}$$  \hspace{1cm} (2.17)

Using the ratios of the experimental values, $k_1$ and $k_2$, and the deduced value, $K_v 1 \text{M}^{-1}$, the following relation for the coefficients in (2.16) is obtained.

$$\frac{k_c}{k_d K} = \frac{5 \times 10^3}{7 \times 10^8}$$

so

$$\frac{k_c}{k_d} \sim 10^{-5}$$  \hspace{1cm} (2.18)

When pH > 9.2, the rate law reduces to a first order so that the constant can be neglected in the denominator and the following relations are obtained.

$$k_{obsd} = k_c + k_d K[OH^-]$$  \hspace{1cm} (2.19)

Whereupon, from the experimental data, the following are obtained, again assuming that $K_v 1$.

$$k_c \sim 0.4 \text{ sec}^{-1}$$  \hspace{1cm} (2.20)

$$k_d \sim 3 \times 10^4 \text{ sec}^{-1}$$  \hspace{1cm} (2.21)

The ratio of the values in (2.20) and (2.21) agrees with that obtained in (2.18).
When $k_C$ is substituted in (2.17), a value for $K'$ is obtained.

$$K' \approx 10^4 \text{ M}^{-1}$$

(2.22)

A comparison can now be drawn between the two derived rate laws, (2.11) and (2.16). The only assumptions made in the derivation of equation (2.11) are that both tetrahedral intermediates are in steady state concentrations, while it is assumed, in the derivation of (2.16), that equilibrium exists between starting material and protonated intermediate and between the intermediates.

The implications of the two derived rate laws are enumerated below.

**Steady State Approximation**

1. $k_C/k_d \approx 10^{-5}$; i.e. the formation of products from the protonated intermediate is far slower than that from the deprotonated form.

2. $k_b/k_c \approx 4$; i.e. reversion of the protonated intermediate to starting material is more rapid than conversion to products. As argued above, this situation is very likely in a complex of this nature.

3. Nothing is learned of the relative rates $k_a/k_b$ from this approximation and hence no estimate is obtained of the stability of the tetrahedral intermediate relative to the starting material.

4. In the rate-limiting case, the coefficient of $[\text{OH}^-]$ has the full form $k_a k_d K/k_d K$, which reduces to $k_a$. The limiting rate may then be either $k_a$ or $k_d$, whichever...
is the smaller, but values for these are not known. Whatever the case, $k_a$ can be rate-determining either at high pH or at low pH but not both, for the relation $k_c/k_d$ eliminates this prospect.

(5) The assumption of an equilibrium between the tetrahedral intermediates, IH and I, eliminates the possibility of $k_e$ or $k_f$ being rate-determining paths.

**Equilibrium Approximation**

(1) $k_c/k_d \approx 10^{-5}$; this result is common to both approximations and appears to be a chemically feasible one. Moreover, values of $k_c$ and $k_d$ are estimated as $\approx 0.4$ sec$^{-1}$ and $\approx 3 \times 10^4$ sec$^{-1}$, respectively.

(2) Because of the nature of the assumption that two equilibria are established, no estimates can be made of the rates associated with chelated ester and intermediates compared with those for decomposition of the intermediates.

(3) The equilibrium constant for formation of the protonated intermediate has a value $10^4$ M$^{-1}$, so that, over the pH range studied, the substrate exists largely as the chelated ester. Moreover, the fact that the metal ion is believed to stabilize the intermediate [158] is used as the basis that this equilibrium exists.

(4) In the rate-limiting case, $k_d$ is the coefficient of the term with [OH$^-$] and becomes rate-determining; that is, loss of the alkoxy group from the deprotonated intermediate is the rate-determining step. At lower pH's (~7), loss of the alkoxy group from the protonated
intermediate is the rate-determining step in this formulation.

While these studies indicate the existence of a tetrahedral intermediate, they do not show whether the addition step or elimination of leaving group is rate-determining. Few examples exist in the literature on similar systems, where the rate-determining step is recognized. From studies of the aminolysis of chelated isopropyl ester, it has been shown that the addition step is rate-determining and it has also been deduced that, in aqueous solution, the elimination step is extremely fast [159].

Some analogous features also occur in organic chemistry. As noted in the Introduction, similar rate laws are observed in the alkaline hydrolysis of a number of amides. The rate law for alkaline hydrolysis of trifluoracetanilides has, in addition, a term, first order in hydroxide, in the denominator [73], the derived expression having the form:

\[
\frac{k_{\text{obsd}}}{1 + k_{1} + (k_{2} + k_{3}k_{4} + k_{5}[\text{OH}^{\text{-}}])[\text{OH}^{\text{-}}]} = \frac{k_{-2} + k_{-3} + k_{4} + k_{5}[\text{OH}^{\text{-}}]}{k_{-2} + k_{-3} + k_{4} + k_{5}[\text{OH}^{\text{-}}]}
\]

This last term in the denominator becomes important at high hydroxide ion concentrations [73-75]. Mader has accounted for the rate law as being due to a limitation on the rate of formation of the tetrahedral intermediate, but other workers present results consistent with elimination being rate determining [74,75].
The features which these compounds and the Co(III) complex of this study have in common are that the substituents on the tetrahedral carbon atom are electron-withdrawing and that one of these substituents has an acidic proton, but they do not help to lead one to some conclusion about the rate-determining step.

This study gives the first reported example of base hydrolysis of a chelated ester which obeys a rate law with a term second order in [OH−] and which shows a change in the rate-determining step with increasing pH. Which of the two derived rate laws gives a better interpretation of the reaction scheme is a matter for contention and more evidence needs to be accumulated before a valid choice can be made.

2.4.4 Hydrolysis of \([\text{Co(en)}_2(\beta\text{-alaOCH(CH}_3)_2)](\text{ClO}_4)_3\) in the presence of bicarbonate ions and hydrolysis of chelated \(\beta\text{-alanine amide}\)

Experiments performed in the absence or presence of several buffers establish that base hydrolysis of the chelated ester proceeds without opening of the chelate ring and that the sole product is the chelated acid complex. In the presence of bicarbonate (or carbonate, at higher pH's), however, opening of the six-membered ring occurs and, to a small extent, also, complete loss of the amino acid ester. Since hydroxide ion alone does not effect ring opening, it follows that bicarbonate or carbonate ions are involved in the rate law; that is, specific base catalysis occurs. This may occur in a number of ways: (a) direct attack at the cobalt atom, with simultaneous cleavage of the
Co-O(amino acid) bond, leading to the formation of a carbonate intermediate, which subsequently decomposes to the hydroxo ester; (b), direct attack at the carbonyl carbon, with cleavage of the C-O bond in the chelate ring and, (c), bicarbonate assisted hydroxide attack, also leading to ring opening.
The third scheme, (c), is least likely, for there is no reason that ring opening should occur in the intermediate with bicarbonate- or carbonate-assisted hydroxide attack compared with hydroxide attack in the absence of bicarbonate.

The first scheme, (a), is also unlikely, since Co(III) amine chemistry appears to be primarily dissociative and little or no evidence exists for direct nucleophilic attack at the cobalt centre in similar complexes. Thus, it seems that specific base catalysis is the most likely mechanism of the three, depicted above.

The dihydroxo species, which appears in small amounts, may be formed either from the hydroxo ester complex by further attack of carbonate or directly from the chelated ester with complete expulsion of the ester from the coordination sphere. Work may help to elucidate this mechanism.

A cursory study of the chelated β-alanine amide complex shows that the rate of amide hydrolysis ($k_{OH} = 2.7 \text{ M}^{-1}\text{sec}^{-1}$ at pH=10) is about $10^4$ times slower than that of the isopropyl ester. This result is similar to that found in the chelated glycine amide system where it is about $10^5$ times slower [156] than that of chelated glycine isopropyl ester. In the β-alanine system, as with the glycine system, a limiting rate is reached at the highest pH studied, in accord with the proposal that the deprotonated amide chelate is unreactive.
2.5 Summary and Conclusions

Shown in Table 2.7 are constants obtained for hydrolysis of β-alanine derivatives compared with those for the glycine analogues.

**TABLE 2.7**

*Summary of Acid and Base Hydrolysis Constants of Amino Acid Derivatives*

<table>
<thead>
<tr>
<th>Compound</th>
<th>( x = 1 )</th>
<th>( x = 2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{H}_2\text{N}(\text{CH}_2)_x\text{COOR} )</td>
<td>( \gamma 0.2^a )</td>
<td>0.02</td>
</tr>
<tr>
<td>( \text{H}_3\text{N}(\text{CH}_2)_x\text{COOR} )</td>
<td>( \gamma 5^a )</td>
<td>( \gamma 1^b )</td>
</tr>
<tr>
<td>( [\text{Co(en)}_2\text{H}_2\text{N}(\text{CH}_2)_x\text{COOR}]^3^+ )</td>
<td>( 1.5 \times 10^6 )</td>
<td>( 5 \times 10^3 )</td>
</tr>
<tr>
<td>( \text{H}_3\text{N}(\text{CH}_2)_x\text{COHOR} )</td>
<td>( \gamma 6^c )</td>
<td>( \gamma 1^c )</td>
</tr>
<tr>
<td>( [\text{Co(en)}_2\text{H}_2\text{N}(\text{CH}_2)_x\text{COOR}]^3^+ )</td>
<td>( 1.9 \times 10^{-5} )</td>
<td>( 8.3 \times 10^{-7} )</td>
</tr>
</tbody>
</table>

- **a.** Calculated on the basis that the isopropyl ester reacts 5 times more slowly than the ethyl ester.
- **b.** Assumes a 50-fold enhancement on protonation of the amino function of the ester.
- **c.** Calculated from the reference.
The effect of the metal ion on the rate of hydrolysis of the chelated amino acid esters is substantial. As seen in Table 2.7, there is a rate enhancement of $\approx 10^6$ for base hydrolysis of the chelated esters compared with the un-coordinated neutral esters and of about $10^4$ to $10^5$ compared with the N-protonated esters. On the other hand, $k_{H_2O}$ for the chelated esters is $10^4$ to $10^5$ times smaller than the calculated values for the free carbonyl-protonated esters. Thus, in the first case, the enhancement is attributed to direct metal ion activation of the reactive carbonyl centre, while, in the second case, the retardation is attributed to the lower polarizing power of the $N_5Co^{3+}$ moiety, compared with $H^+$. 

It is interesting to compare the rates and rate laws, here, with those of the glycine ester chelate. $k_{H_2O}$ (Table 2) for chelated $\beta$-alanine isopropyl ester is some 25 times smaller than that for chelated glycine isopropyl ester.

The constant, $k_1$, for base hydrolysis of chelated $\beta$-alanine ester has been chosen for comparison, since it is the constant of the rate law comparable with that of the rate law observed in the glycine ester chelate. The constant for free $\beta$-alanine ester is 10 times smaller than that for the glycine ester but $k_{OH}$ for the chelated ester is 300 times smaller than that for chelated glycine ester. The difference may reside in enthalpy or entropy factors associated with the chelate rings. Nucleophilic attack, leading to hydrolysis may be less hindered in the five-membered chelate ring, than in the more puckered
six-membered chelate ring, as models show.

Another significant result is that base hydrolysis of \([\text{Co(en)}_2(\beta\text{-alaOCH(CH}_3)_2)](\text{ClO}_4)_3\) proceeds via an intermediate in a stepwise sequence rather than by a single step (S_N2) mechanism. The same observations could not be made for the chelated glycine ester; base hydrolysis could be followed accurately only to pH~7, since, at higher values the rates were too great to be measured [114]. Under the conditions, kinetics only first order in hydroxide were observed. The study of the \(\beta\)-alanine ester chelate suggests that the more complex rate law may well have been seen if measurements could have been made at high pH.

Finally, it is apparent that the six-membered chelate ring is stable during hydrolysis by hydroxide and in several buffers, but that it opens in the presence of bicarbonate ion. A similar reaction does not occur with the five-membered glycinate system. This suggests that the six-membered ring is significantly less stable in alkaline solution and the following chapter is devoted to a study of this topic.
3. BASE HYDROLYSIS OF \([\text{Co(en)}_2(\beta\text{-AlaO})]^{2+}\)

3.1 Introduction

Few studies have been carried out on opening of chelate rings and none appear to have been done with amino acid derivatives. On the other hand, many investigations have been aimed at determining formation rates and stability constants of metal chelates. Tables of stability constants for a large number of divalent metal ion chelates of \(\alpha\)-amino acids have been compiled [161-162], since the values may have some relevance to the understanding of the activity of enzyme systems. Data have also been accumulated for \(\beta\)-alanine [163-165]. For complexes of the type \(M(aa)_2\), where aa is an \(\alpha\)- or \(\beta\)-amino acid, the stability diminishes in the following order [161].

\[
\text{glycine} > \alpha\text{-alanine} > \beta\text{-alanine}
\]

From this order, it is inferred that a five-membered chelate ring is generally more stable than a six-membered chelate ring. Boyd and co-workers find that \(\Delta S\) for formation of the \(\beta\)-alanine complex is considerably smaller than that for glycine and suggest that this reflects a greater loss of librational entropy for the former [165]. Kustin et al.
make a similar deduction from the data for complexation rate for \( \alpha - \) and \( \beta - \) alanine [164] and, in addition, maintain that the resulting greater ring strain for \( \beta - \) alanine contributes to the higher barrier for ring closure, compared with glycine. It is also clear, from crystal structures, that \( \beta - \) amino acid chelate rings exist in skew boat conformations [166]. They depart, much more so than \( \alpha - \) amino acid chelate rings from the requirement that the \(-{C}O-{C}O-M\) moiety be planar for maximum electron delocalization [167]. This factor may well be an important one in accounting for the lower stability of the six-membered, compared with the five-membered chelate ring.

Complexes of \( \alpha - \) amino acids with Co(III) are considerably more robust than those with divalent metal ions and remain intact even at high pH (~12). Only recently have preliminary observations of \([\text{Co(en)}_2(\text{glyO})]^2+\) complexes in 1M NaOH shown that a Co(II) species is formed and that the amino acid chelate ring may be opened [168].

Previously, (Section 2.3.4), the \( \beta - \) alanine ester chelate ring was shown to open in the presence of bicarbonate, even at moderate pH (~8) but to remain stable in aqueous solutions to pH ~10 in the absence of bicarbonate. A study of the reactivity of \([\text{Co(en)}_2(\beta - \text{alaO})](\text{ClO}_4)_2\cdot\text{H}_2\text{O} \cdot \text{H}_2\text{O}\) in strongly basic solutions (0.5 - 1.0M NaOH) has thus been undertaken to observe the reactivity of the complex at even higher pH. The primary aim, in this chapter, is to establish the products formed on base hydrolysis of this complex and to obtain estimates of specific rates for the processes.
3.2 Experimental

3.2.1 Instrumental and general

Visible spectra were recorded on a Cary 14 spectrophotometer, infrared spectra on a Perkin-Elmer Model 459 spectrophotometer and pmr spectra on a Jeol JNM-100MHz Minimar or Varian HA-100MHz instrument using TMS as external or NaTPS as internal reference. Cobalt estimations were made by visible spectrophotometry or by atomic absorption spectrophotometry with a Techtron AA4 Model instrument. The radiometer equipment used has already been described (Section 2.2.1).

Bio-Rad Analytical Dowex 50WX2 (200-400 mesh, Na+ form) cation exchange resin was used for the separation of products.

\[ [\text{Co(en)}_2(\text{glyO})]Cl_2 \] was obtained from this laboratory.

The complexes, cis-[Co(en)_2(H_2O)_2](NO_3)_3 and trans-[Co(en)_2(H_2O)_2](ClO_4)_3, were donated by Dr. J. Harrowfield; the desired hydroxo complexes were generated by dissolving these complexes in basic solutions.

3.2.2 Preparation of complexes

\[ [\text{Co(en)}_2(\beta-\text{alaO})](\text{ClO}_4)_2 \cdot \text{H}_2\text{O} \]

(a) \[ [\text{Co(en)}_2(\beta-\text{alaOCH(\text{CH}_3}_2)](\text{ClO}_4)_3 \] was dissolved in a minimum volume of water, the pH of which was adjusted to ~1 with 70% HClO_4. The solution was gently warmed on a steam bath for three hours, and, on addition of NaClO_4, cooled in an ice bath until the product separated out. This was filtered, washed with a little ethanol and ether, then dried in a vacuum desiccator.
(b) \([\text{Co(en)}_2(\beta\text{-alaOCH}(\text{CH}_3)_2)](\text{ClO}_4)_3\) was dissolved in a minimum volume of water and a concentrated solution of sodium hydroxide added until the pH of the solution was about 9. It was left to stand for one hour at room temperature and the procedure as in (a) then followed.

**Anal.** Calcd for \([\text{Co(en)}_2(\beta\text{-alaO})](\text{ClO}_4)_2\cdot\text{H}_2\text{O}:\) Co, 12.17; C, 17.36; H, 5.00; N, 14.47. Found: Co, 11.92; C, 17.24; H, 5.02; N, 14.31.

\([\text{Co(en)}_2(\beta\text{-alaO})]\text{Cl}_2\cdot\text{H}_2\text{O}\)

\([\text{Co(en)}_2(\beta\text{-alaO})](\text{ClO}_4)_2\cdot\text{H}_2\text{O}\) was dissolved in water, sorbed on H\(^+\)-form Dowex 50Wx2 resin, then eluted with 3M HCl before being evaporated to dryness on a rotary evaporator. At this stage, elemental analyses of the hygroscopic crystals were obtained and indicated the presence of HCl in stoichiometric amounts.

**Anal.** Calcd for \([\text{Co(en)}_2(\beta\text{-alaO})]\text{Cl}_2\cdot\text{HCl}\cdot\text{H}_2\text{O}:\) C, 20.48; H, 6.38; N, 17.06; Cl, 25.90. Found: C, 20.85; H, 6.58; N, 16.57; Cl, 26.48.

The crystalline solid was then washed several times with water and again evaporated to dryness to remove all traces of HCl. To a solution of the residue in a minimum volume of H\(_2\)O was added methanol and ethanol to induce crystallization; after cooling, the orange-pink product was collected, washed with ethanol and ether and dried in an evacuated desiccator.

**Anal.** Calcd for \([\text{Co(en)}_2(\beta\text{-alaO})]\text{Cl}_2\cdot\text{H}_2\text{O}:\) C, 23.61; H, 6.79; N, 19.67. Found: C, 23.66; H, 6.70; N, 19.72.
The visible spectrum from 600 to 300 nm gave the following maxima and extinction coefficients, which were independent of the anion and of the solution medium, 0.1 to 3 M HCl, 0.5 to 3 M NaClO₄, H₂O, 0.01 to 1 M HClO₄, Tris buffer: 495 ± 1 nm (ε 28.0 ± 0.5); 351 ± 1 nm (ε 88 ± 1).

3.2.3 Procedure for the base hydrolysis study

[Co(en)₂(β-alan)](ClO₄)₂·H₂O was dissolved in 0.5 M NaOH, µ=1.0, NaClO₄, or in 1.0 M NaOH at 25.0°C such that the concentration was in the range 0.01 to 0.04 M. Aliquots, each containing approximately 0.1 g complex, were quenched at various time intervals to pH~8 with 1 M HClO₄ and diluted ten-fold before being sorbed on a column of Na⁺-form resin (1 x 20 cm). The band was eluted with 0.5 M NaClO₄, adjusted to pH~9 with NaOH, and, when the unipositive species had been separated, with 1 M and/or 2 M NaClO₄. Alternatively, for better separation of the unipositive species, aliquots were quenched to pH~4, first sorbed on a short column of Na⁺-form resin (1 x 7 cm) as a narrow band, then converted to the basic form with 0.1 M NaOH. The resin with the sorbed product was then transferred to a Na⁺-form column and the bands eluted, as above. Under these conditions the species, trans-[Co(en)₂(OH)₂]⁺ or trans-[Co(en)₂OH(β-alan)]⁺, cis-[Co(en)₂(OH)₂]⁺ and cis-[Co(en)₂OH(β-alan)]⁺ could be separated. An even longer column would have been necessary for separation of the two trans species but this would have decreased elution rates considerably. The rate of base hydrolysis was estimated from the proportion of complex that remained unreacted. Absorption spectra (600 to 300 nm)
were recorded simultaneously to enable a more complete interpretation of the reaction sequence to be made. Spectra, at timed intervals, of the complex $(1.3 \times 10^{-3} \text{M})$ in $0.1 \text{M} \ NaOH (\mu=1.0, \text{NaClO}_4, 25.0^\circ)$ were also recorded. In a similar manner, spectra of $[\text{Co(en)}_2(\text{glyO})]Cl_2$ in $1 \text{M NaOH} (6.2 \times 10^{-3} \text{M})$ were obtained.

Selected eluted fractions containing the hydroxo $\beta$-alaninato isomers were adjusted to $\mu=1$ and mixed with an equal volume of $1.0 \text{M NaOH}$. Spectra of the solutions were then recorded at intervals. For the cis isomer, aliquots were quenched and analyzed, as above, by atomic absorption and visible spectrophotometry.

For determination of base consumption for the initial reaction, the $\beta$-alanine chelate (0.1g) was dissolved in $1 \text{M NaClO}_4$ solution (8ml) and titrated with $0.1 \text{M NaOH (}\mu=1.0, \text{NaClO}_4)$ at pH = 12.0 for 106 minutes, then quenched and analyzed as above. The base consumption was obtained after correction for a blank titration.

$3.2.4$ Characterization and analysis of products

As mentioned in Section 3.2.3, aliquots of the reacting solution were quenched at intervals and eluted with $\text{NaClO}_4(0.5 - 2.0 \text{M; pH~9})$, as above. Cobalt concentrations in the eluant fractions were determined by atomic absorption spectrophotometry and also from visible spectra, using known extinction coefficients.

The $[\text{Co(en)}_2(OH)_2]^+$ isomers, formed in the latter stages of the base hydrolysis, were identified by comparison of elution rates and visible spectra with the authentic
materials. The hydroxo \( \beta \)-alanimato isomers were similarly identified by comparison of visible spectra and relative elution rates with the same isomers obtained after base hydrolysis of \([\text{Co(en)}_2\text{Br(}\beta \text{-alaOH)}]\text{Br}_2\) (see Section 5.2.4). The presence of the \( \beta \)-alanine residue was further established from pmr spectra after the eluant fractions containing the isomers were treated in the following manner: the solutions were quenched to pH\,4 with H\(\text{ClO}_4\), sorbed on H\(^+\)-form resin and eluted with 3M HCl, then evaporated to dryness on a rotary evaporator. The temperature of the bath was kept below 30\(^\circ\), during evaporation, to minimize exchange of bound H\(2\text{O}\) with Cl\(^-\). The residues were washed several times and re-evaporated to dryness to remove all traces of HCl.

3.2.5 Resolution of \([\text{Co(en)}_2(\beta \text{-alaO})]\text{Cl}_2\cdot \text{H}_2\text{O}\) and the measurement of optical activity in the hydrolysis products

\([\text{Co(en)}_2(\beta \text{-alaO})]\text{Cl}_2\cdot \text{H}_2\text{O}\) (7.1g) was dissolved in warm water (35ml, \(\sim 40^\circ\)) and \((+)_D\text{-Na}[\text{Co(en)}(\text{ox})_2]\text{Cl}_2\cdot \text{H}_2\text{O}\) \((\alpha)_D = + 490^\circ; 6.35g) added. The diastereoisomer began to crystallize when the solution was cooled to room temperature and the side of the container scratched. After \(\sim 1\) hour, the crystals were collected, washed with aqueous methanol, then methanol and dried with ether (Yield=5.4g). Another fraction was collected after the filtrate was allowed to stand in a refrigerator overnight, then treated as before (Yield=3.75g). The first fraction was re-crystallized from hot water (\(\sim 80\text{ml}\) and three fractions...
collected. The last two of these were combined with the second fraction above and recrystallized in a similar manner. The resolving agent was removed from each of the recrystallized fractions using Dowex anion exchange resin (AG 1x8, 200-400 mesh, Cl⁻-form). The product was eluted with water, reduced to dryness on the rotary evaporator, redissolved in the minimum volume of water and recrystallized as the perchlorate salt, by addition of NaClO₄. When the optical purity of each sample was established by measurement of the rotation (0.1%; H₂O; 10cm cell), samples were once again recrystallized from hot water, the rotation remaining as before, with [α]₅₈₀ = -180° (i.e., α₅₈₀ = -0.180°; 0.1% soln).

Ana1. Calcd for (H₅₈₀ - [Co(en)₂(β-alaO)](ClO₄)₂·H₂O:

C, 17.36; H, 5.00; N, 14.47. Found: C, 17.97;
H, 4.80; N, 14.65.

Molar rotations for this sample in 2M NaClO₄ are given in Table 3.3. (See p. v for definition of molar rotation).

For the base hydrolysis study, the resolved complex was treated as described in Section 3.2.3, using the second method outlined in the previous section for separation of the products; it was thermostated at 25.0° for ~3 hours before being quenched and sorbed on Na⁺-form resin. Eluant fractions were estimated for cobalt by atomic absorption spectrophotometry and rotations measured on the spectropolarimeter.

3.3 Results

Infrared and pmr spectra of the β-alanine chelate have been described in Sections 2.3.2 and 2.3.3 respectively.
In Fig. 3.1 are shown spectra during the decomposition of 
$[\text{Co(en)}_2(\beta\text{-alaO})](\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$ in 1M NaOH. For comparison, 
spectra during decomposition of $[\text{Co(en)}_2(\text{glyO})]\text{Cl}_2$ in 1M NaOH 
are shown in Fig. 3.2. In Table 3.1 are listed product 
analysis results at the given time intervals throughout 
the reaction, in 0.5M and in 1.0M NaOH. The complexes 
are listed, from left to right, in order of elution.

### TABLE 3.1

Product Analysis during Hydrolysis of $[\text{Co(en)}_2(\beta\text{-alaO})]^2+$ 
in Strongly Basic Solutions at 25.0°C, $\mu = 1.0, \text{NaClO}_4$.\(^a\)

<table>
<thead>
<tr>
<th>Time</th>
<th>%trans-$[\text{Co(en)}_2(\text{OH})_2]^{1+}$</th>
<th>%trans-$[\text{Co(en)}_2(\text{OH})(\beta\text{-alaO})]^+$</th>
<th>%cis-$[\text{Co(en)}_2(\text{OH})_2]^{1+}$</th>
<th>%cis-$[\text{Co(en)}_2(\text{OH})(\beta\text{-alaO})]^+$</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>44mins(^b)</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>14</td>
<td>77</td>
</tr>
<tr>
<td>1hr 20mins(^b)</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>30</td>
<td>57</td>
</tr>
<tr>
<td>4hrs40mins(^b)</td>
<td>0</td>
<td>17</td>
<td>3</td>
<td>44</td>
<td>36</td>
</tr>
<tr>
<td>15hrs(^b)</td>
<td>5</td>
<td>15</td>
<td>30</td>
<td>48</td>
<td>7</td>
</tr>
<tr>
<td>28hrs(^b)</td>
<td>23</td>
<td>0</td>
<td>50</td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td>170hrs(^c)</td>
<td>52</td>
<td>0</td>
<td>45</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6hrs20mins(^c)</td>
<td>0</td>
<td>25</td>
<td>0</td>
<td>38</td>
<td>37</td>
</tr>
<tr>
<td>28hrs40mins</td>
<td>17</td>
<td>0</td>
<td>39</td>
<td>39</td>
<td>5</td>
</tr>
</tbody>
</table>

\(^a\) % error = ±5%; duplicate experiments were carried out.

\(^b\) $[\text{OH}^-] = 1.0\text{M}$.

\(^c\) $[\text{OH}^-] = 0.5\text{M}$.

\(^d\) Estimated by collecting 3 fractions of the first band. The first of 
these contained largely the dihydroxo species, as shown by visible 
spectra.
Fig. 3.1. Visible spectra during base hydrolysis, in 1M NaOH of [Co(en)$_2$(β-alao)](ClO$_4$)$_2$.H$_2$O to (a), cis- and trans-[Co(en)$_2$OH(β-alao)]$^+$ and subsequently, (b), to cis- and trans-[Co(en)$_2$(OH)$_2$]$^+$: 25.0°.
**Fig. 3.2. Visible spectra during base hydrolysis, in 1M NaOH, of \([\text{Co(en)}_2\text{glyO}]\text{Cl}_2; 25.0^\circ\).**
### TABLE 3.2
Extinction Coefficients for Complexes Produced during Hydrolysis of \([\text{Co(en)}_2(\beta-\text{AlaO})]^{2+}\)

<table>
<thead>
<tr>
<th>Complex</th>
<th>(\lambda_{\text{max}}) (nm)</th>
<th>(\varepsilon_{\text{max}}) (M(^{-1}) cm(^{-1}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>trans-([\text{Co(en)}_2(\text{OH})_2]^{1+})</td>
<td>518</td>
<td>54</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td>(\approx370)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>520</td>
<td>53</td>
<td>[169]</td>
</tr>
<tr>
<td></td>
<td>380</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>cis-([\text{Co(en)}_2(\text{OH})_2]^{1+})</td>
<td>518</td>
<td>91</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td>(\approx370)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>520</td>
<td>93</td>
<td>[169]</td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>trans-([\text{Co(en)}_2\text{OH}(\beta-\text{AlaO})]^{1+})</td>
<td>500</td>
<td>76</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td>(\approx350)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>trans-([\text{Co(en)}_2\text{H}_2\text{O}(\beta-\text{AlaOH})]^{3+})</td>
<td>490</td>
<td>57</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>cis-([\text{Co(en)}_2\text{OH}(\beta-\text{AlaO})]^{1+})</td>
<td>500</td>
<td>91</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>(\approx350)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>cis-([\text{Co(en)}_2\text{H}_2\text{O}(\beta-\text{AlaOH})]^{3+})</td>
<td>488</td>
<td>70</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>([\text{Co(en)}_2(\beta-\text{AlaO})]^{2+})</td>
<td>495</td>
<td>128</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>90</td>
<td></td>
</tr>
</tbody>
</table>

\(a\) Solutions measured at 25.0° in NaClO\(_4\) (0.5-2M).

\(b\) Error in \(\lambda = \pm2\text{nm}\)

\(c\) Error in \(\varepsilon = \pm2\text{M}^{-1}\text{cm}^{-1}\). Values are averaged from at least 3 different solutions. Positions of maxima in the near ultraviolet region are only approximate, due to the presence of small amounts of organic material from the resin, which have high \(\varepsilon\)'s in basic solutions.

\(d\) Produced by acidifying the solution of the corresponding hydroxo species to \(pH \approx 2\) with HClO\(_4\).
Extinction coefficients, from visible spectra, are listed in Table 3.2. The values obtained for the dihydroxo isomers, eluted in the order, trans, then cis, agree well with literature values [169]. Those for the aquo β-alaninato isomers, also eluted in the above order, are somewhat higher than those of simpler amine complexes; e.g., trans- and cis-[Co(en)$_2$(NH$_3$)$_2$H$_2$O]$^{3+}$, at 480nm, have values, 45 and 66, respectively [140].

In Fig. 3.3 are pmr spectra of the complexes formed during hydrolysis of chelated β-alanine. The spectrum of the starting material has been included for reference. As discussed in Section 2.3.3, the signal near 3.0ppm is characteristic of the presence of β-alanine and identifies the products formed during the initial reaction as isomers still containing the amino acid, rather than as the dihydroxo isomers.

The conclusion that these trans and cis isomers were eluted in the order shown in Table 3.1 is substantiated by the results obtained from hydrolysis of the resolved complex, quenched at a time when they were the only products (2hrs 50 mins). They are shown in Table 3.3, also in order of elution from left to right. (See p.v for definition of [M]$_\lambda$).

**TABLE 3.3**

| Molar Rotations [deg.cm$^{-1}$ m$^{-1}$] of β-Alaninato Complexes |
|-----------------|-----------------|-----------------|
| λ(nm)           | [Co(en)$_2$OH(β-alao)]$^{+a}$ | [Co(en)$_2$(β-alao)]$^{2+b}$ |
|                 | [cis$^-$]        | [eluted$^-$]     |
| 589             | 0                | -109             |
| 546             | 0                | -273             |
| 436             | 0                | +633             |
|                 |                  |                  |

a. In 0.5M NaClO$_4^-$  b. In 2M NaClO$_4^-$
Fig. 3.3. PMR spectra in D₂O of the complexes isolated during base hydrolysis of 
([Co(en)]²⁺(β-alA0))⁴⁺ in 1M NaOH. (Internal reference, NaTPS; 100MHz).
As expected for the eluate containing the trans complex, no optical rotation is observed. It is also seen, from the figures for the eluted chelate (column 3), compared with a solid sample of the chelate, dissolved in 2M NaClO₄ (column 4), that the experimental error is less than 5%. (Further evidence is presented in the following Chapter, Section 4.3.3).

All complexes could be separated cleanly except the trans-dihydroxo and trans-hydroxo β-alaninato species (see Experimental). It was clear, however, from visible spectra, that only the latter was formed during the first 3 hours of hydrolysis and that only the former was present after 28 hours. The existence of the mixture was evidenced by the position of the absorption maxima and extinction coefficients, both of which lay between the values for the pure products. The dihydroxo product was eluted first, for the first eluant fractions of bands containing the two species had visible spectra corresponding closely to the authentic dihydroxo complex. For the purposes of the experiment, however, this separation was not crucial and the reaction sequences could still be established from the other data. This sequence is given in Fig. 3.4.

The facts supporting this sequence are presented below. (For clarity of discussion, the various reactions will be referred to by the relevant rate constants).

(1) The existence of isosbestic points at 543, 421 and 363nm for approximately the first 3 hours of the reaction established that a single process was principally involved in the formation of both cis-
and dihydroxy-8-aminonine species (Fig. 3.1) and that neither of these products was formed to any great extent, in line with the trans isomerism. Some evidence for the presence of isomerization may be shown by the observation that the six-trans ratio (2:1, 2:5, and 2:6) for the first three entries in Table 3.2. This variation, however, falls within the experimental error. Isobestic points also occurred in the transesterification of...en, CH2 COO-

\[ \text{H}_2\text{NCH}_2\text{CH}_2\text{COO}^- + \text{OH}^- \]

\[ \overset{k_3}{\leftrightarrow} \]

\[ \overset{k_{-3}}{\leftrightarrow} \]

\[ \text{H}_2\text{NCH}_2\text{CH}_2\text{COO}^- + \text{OH}^- \]

\[ \overset{k_4}{\rightarrow} \]

\[ \text{H}_2\text{NCH}_2\text{CH}_2\text{COO}^- + \text{NH}_2\text{CH}_2\text{CH}_2\text{COO}^- \]

\[ \overset{k_5}{\rightarrow} \]

\[ \text{H}_2\text{NCH}_2\text{CH}_2\text{COO}^- + \text{NH}_2\text{CH}_2\text{CH}_2\text{COO}^- \]

\[ \overset{k_6}{\rightarrow} \]

\[ \overset{k_{-6}}{\rightarrow} \]

\[ \text{H}_2\text{NCH}_2\text{CH}_2\text{COO}^- + \text{NH}_2\text{CH}_2\text{CH}_2\text{COO}^- \]

Fig. 3.4. Reaction sequence for the base hydrolysis of [Co(en)$_2$(8-alaO)](ClO$_4$)$_2$. 

Two equivalents would have been required if the dihydroxy species were formed directly.

3. As the reaction continued, the isobestic points were lost and the product solution, after some days...
and trans-hydroxo $\beta$-alaninato species (Fig. 3.1) and that neither of these products was formed to any great extent, by subsequent cis-trans isomerization. Some evidence for a small degree of isomerization may be shown by the variation in the cis:trans ratio ($\sim 2:1, \sim 2.5:1, \sim 2.6:1$) for the first three entries in Table 3.1. This variation, however, falls within the experimental error. Isosbestic points also occurred at lower [OH$^-$] (0.1 or 0.5M NaOH; $\mu=1.0$, NaClO$_4$) and appeared to be maintained for longer periods with decreasing [OH$^-$]. This indicates that the first reaction is dependent on [OH$^-$].

No dihydroxo product was formed in any detectable amount in the early stages of the reaction, where the isosbestic points existed. It follows that a process, whereby the amino acid is lost completely from the coordination sphere in a single step, did not occur. Moreover, when a sample of the chelate was titrated at pH9.12 for 100 minutes to produce 15% of the amino acid isomers, only 0.15 equivalent of base was consumed, according to the stoichiometry:

$$[\text{Co(en)}_2(\beta\text{-alaO})]^2+ + \text{OH}^- \rightarrow [\text{Co(en)}_2\text{OH}(\beta\text{-alaO})]^+$$

(3.1)

Two equivalents would have been required if the dihydroxo species were formed directly.

As the reaction continued, the isosbestic points were lost and the product solution, after some 7 days,
contained solely an equilibrium mixture of the *cis-* and *trans-* dihydroxo complexes. The spectra remained constant for at least 7 days after the attainment of equilibrium, with a value, 71.6 ± 0.5 (52% *trans-* complex). This agrees with the average value for the *cis-trans* equilibrium, after 7 days, of 71.7 (55% *trans-* complex) obtained from the tables of Bjerrum and Rasmussen [169].

(4) The *trans*-dihydroxo species was apparently formed largely through isomerization of the *cis* species rather than through the irreversible path from the *trans*-hydroxo *β*-alaninato species, for its concentration was not built up significantly until a reasonable proportion (>20%) of the *cis*-dihydroxo species was formed. This may be due to the fact that the constant, $k_4$, is small compared with $k_{-6}$, but, in any case, with the presently known facts, the path through $k_4$ cannot be eliminated.

(5) The existence of the path through $k_3$ was recognized from the spectra shown in Fig. 3.5. In 0.5M NaOH ($\mu$=1.0, NaCl0.4, 25.0°C) the absorbance of an eluant fraction of *trans*-[$\text{Co(en)}_2\text{OH(β-alaO)}^+$ increased at 500nm for 6 hours, then decreased with a corresponding shift of the maximum to longer wavelengths until the *cis-trans* dihydroxo equilibrium was established after 7 days.

A study of the spectrophotometric data of Table 3.2 suggests that the initial increase was due to isomerization to the *cis*-hydroxo *β*-alaninato complex and, thereafter that the amino acid ligand was completely lost before isomerization of the *cis*-dihydroxo complex.
Fig. 3.5. Visible spectra during reaction of trans-(Co(en)$_2$OH(β-alaO))$^+$ in 0.5M NaOH; 25.0°C; $\nu=1.0$, NaClO$_4$.
occurred. The small initial increase, rather than
decrease, in the absorbance of the reactant solution
implied that the proportion of complex being diverted
through the irreversible route, $k_4$, was small. Product
analysis of a dilute sample supported this; also, no
chelate was detected, indicating that no reverse path
to the starting material existed. Product analysis on
a sufficiently large quantity of $\text{trans-}[\text{Co(en)}_2\text{OH}(\beta\text{-alaO})]^+$
could substantiate these results.

The paths through $k_2$, $k_3$ and $k_5$ were also established
by a separate experiment. A solution $\text{cis-}$
$[\text{Co(en)}_2\text{OH}(\beta\text{-alaO})]^+$ in 0.5M NaOH decomposed to the
extent of $\approx20\%$ after 4 hours, $4\%$ of which comprised
the starting material and the rest, ($\approx13\%$) the $\text{trans}$
isomer; a small amount ($<5\%$) of the dihydroxo isomers
is formed. After 24 hours, $60\%$ of the $\text{cis}$-hydroxo
starting material had decomposed to $\approx3\%$ of the
$\beta$-alanine chelate, $12\%$ of the $\text{trans}$-dihydroxo and $45\%$
of the $\text{cis}$-dihydroxo isomer. It thus appeared that,
in the early stages, the paths via $k_2$ and $k_3$ were
significant but that the paths through $k_4$, $k_5$ and $k_6$
became important as the reaction continued.

Spectra at intervals throughout the reaction are
shown in Fig. 3.6. The initial small increase in
absorbance corresponded to the formation of the amino
acid chelate ($\varepsilon_{\text{max}}^{\text{495}}=128$) from the $\text{cis}$-hydroxo $\beta$-alaninato
complex ($\varepsilon_{\text{max}}^{\text{500}}=91$) through the path, $k_2$, (Fig. 3.4).
Very little change in absorbance, but a subsequent shift
to longer wavelengths corresponded to the $\text{cis}$-dihydroxo
species ($\varepsilon_{\text{max}}^{\text{518}}=91$) being formed in significant amounts.
Finally, the decreasing absorbance was indicative of isomerization of the dimethyl complex, largely through $k_{-2}$. When a solution of cis-[Co(en)$_2$OH(b-alaO)]$^{2+}$ at pH 10 was allowed to stand at room temperature for about two months, the major product (-80%) was found to be the y-alanine chelate. This result suggests that at lower pH, the path through $k_{-2}$ is favoured over the alternative paths, $k_4$ or $k_5$ and also $k_6$ is dependent on $[OH^-]$. The stoichiometry of the reaction for the formation of the dimethyl complex is therefore

$$\text{Co(en)$_2$OH(b-alaO)}^{3+} + 3\text{OH}^- \rightarrow \text{Co(en)$_2$OH(b-alaO)}^{2+} + \text{H}_2\text{O}.$$ (1.2)

With the evidence now presented for the existence of the scheme shown in Fig. 3/4, an estimate can be made of the half-life for the hydrolysis of [Co(en)$_2$OH(b-alaO)]$^{3+}$, is 7.0 min in 0.5M NaOH, to Co(en)$_2$OH(b-alaO) and b-ala.$\text{Co(en)$_2$OH(b-alaO)}$.$^2+ + b$-ala$.$

Simplify calculations, $k_{-2}$ is the rate constant for the rise time more than 5% change, its pseudo-first order rate constant then be used [39] in $A(t) = A(0) + B \exp(-k_{-2}t)$.$^2+ + b$-ala$.$

![Absorbance vs. $\lambda$ (nm) graph](image)

**Fig. 3.6. Visible spectra during reaction of cis- [Co(en)$_2$OH(b-alaO)]$^+$ in 0.5M NaOH; 25.0°; $\mu = 1.0$, NaClO$_4$.**
Finally, the decreasing absorbance was indicative of isomerization of the dihydroxo complex, largely through $k_{-6}$. When a solution of cis-$[\text{Co(en)}_2(\beta\text{-alaO})]^{2+}$ at pH-10 was allowed to stand at room temperature for about two months, the major product (>80%) was found to be the β-alanine chelate. This result suggests that, at lower pH, the path through $k_{-2}$ is favoured over the alternative paths, $k_{-3}$ or $k_{5}$ and also that the path $k_5$ is dependent on [OH$^-\$]. The stoichiometric equation for the formation of the dihydroxo complex is:

$$[\text{Co(en)}_2\text{OH}(\beta\text{-alaO})]^+ + \text{OH}^- \rightarrow [\text{Co(en)}_2\text{(OH)}_2]^+ + \beta\text{-alaO}^-$$

(3.2)

With the evidence now presented for the existence of the scheme shown in Fig. 3.4, an estimate can be made of the half-life for the hydrolysis of $[\text{Co(en)}_2(\beta\text{-alaO})]^{2+}$, in 1.0M NaOH, to give cis- and trans-$[\text{Co(en)}_2\text{OH}(\beta\text{-alaO})]^+$, from equation (3.1). To simplify calculations, $k_{-2}$ can be neglected, for it gives rise to less than 5% chelate; a simple pseudo-first order law may then be used [170].

$$\ln \frac{a}{a-x} = \frac{k_{12}t}{12}$$

(3.3)

where $a$ is the initial concentration, taken here to be unity, $x$, the decrease in concentration of the reactant with time, $t$ and $k_{12}$, the required rate constant. The first two entries of Table 3.1 give values $k_{12} = 5.8 \times 10^{-3} \text{ min}^{-1}$ and $6.2 \times 10^{-3} \text{ min}^{-1}$, respectively; i.e., $t_{1/2} = 120$ and 110 mins, respectively.
A calculation for the third entry gives a value, \( t_{k_2} \approx 180 \text{ mins.} \)

The larger value is probably due to the commencement of the subsequent reactions in this complex scheme so that the first value may be considered to be the true one.

If the two isomers are formed by different paths, the rate constant for the process may be divided into these two paths, according to the ratio of the products \( \frac{k_2}{k_1} = \frac{[\text{cis}]}{[\text{trans}]} \)

and is \( \approx 2.5 \) from No. (1) fact, p.103). The values for \( k_1 \) and \( k_2 \) may then be determined from the relation, \( k_{12} = k_1 + k_2 \), whence \( k_1 = 1.7 \times 10^{-3} \text{ min}^{-1} \) and \( k_2 = 4.3 \times 10^{-3} \text{ min}^{-1} \). Possible mechanisms in the reaction scheme (Fig. 3.4) are now discussed.

3.4 Discussion

The hydrolysis of \([\text{Co(en)}_2(\beta-\text{alaO})]^2+\) in strongly alkaline solution consists of a complex sequence of reactions. Despite this difficulty, the study has led to a number of interesting facts and to an estimate of the relative magnitudes of the various steps.

Consider, first, \( k_1 \) and \( k_2 \) of Fig. 3.4. It is interesting that the opening of the amino acid chelate ring during base hydrolysis results in the formation of both \(\text{trans-}\) and \(\text{cis-hydroxo}\ \beta\)-alaninato species. As already shown, the \(\text{trans}\) species is not formed to any great extent from the \(\text{cis}\) species during the first three hours of the reaction; (\(\approx 13\%\) in 4 hours with \([\text{OH}^-] = 0.5\text{M}\)). If a first order rate law is assumed for the isomerization, as has been done by other workers [169] for related complexes, a rate constant for the isomerization, \( k = k_3 + k_{-3} \approx 10^{-5} \text{ sec}^{-1} \) (\(t_{\frac{k_2}{k_1}} \approx 20 \text{ hrs}\)) is obtained.

If the isomerization is assumed to be independent of \([\text{OH}^-]\) at
high hydroxide ion concentrations, then this rate constant predicts that \( \approx 5\% \) of the \( \text{trans} \) isomer will be formed through the path, \( k_3 \), in 80 minutes. Thus, \( \approx 2\% \) (5\% of 30\%) of the \( \text{trans} \) isomer (Expt. (2), Table 3.1) will be derived from this path.

The value of \( t_{1/2} \approx 20 \) hrs (\( \mu=1.0, 25.0^\circ \) for \( \text{cis-trans} \) isomerization of \([\text{Co(en)}_2\text{OH}(\beta\text{-alaO})]^+ \) is comparable with values for similar complexes, tabulated below.

**TABLE 3.4**

*Half-lives for Isomerization of Co(III) Complexes in Strongly Basic Solutions. ([OH\(^-\]) > 0.01M)*

<table>
<thead>
<tr>
<th>Complex</th>
<th>T</th>
<th>( \mu )</th>
<th>( t_{1/2} ) (hrs)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Co(en)}_2\text{OH}(\beta\text{-alaO})]^+ )</td>
<td>25.0</td>
<td>1.0</td>
<td>( \approx 20 )</td>
<td>This work</td>
</tr>
<tr>
<td>([\text{Co(en)}_2(\text{OH})_2]^+ )</td>
<td>25.0</td>
<td>1.0</td>
<td>29</td>
<td>[171]</td>
</tr>
<tr>
<td>([\text{Co(en)}_2(\text{OH})_2]^+ )</td>
<td>35.0</td>
<td>1.0</td>
<td>4.5</td>
<td>[172]</td>
</tr>
<tr>
<td>([\text{Co(en)}_2(\text{NH}_3)(\text{OH})]^2+ )</td>
<td>30.0</td>
<td>0.1</td>
<td>9</td>
<td>[173]</td>
</tr>
</tbody>
</table>

Tong and Yankwich have observed that the isomerization rate remains constant for \([\text{Co(en)}_2(\text{OH})_2]^+ \) when \([\text{OH}^-]\) > 0.01M [195], so that, in the present system, it was reasonable to make this assumption for calculating the isomerization rate. With differences due to temperature and ionic strength taken into account, it is seen that the complexes in Table 3.4 isomerize at similar rates.

It is of interest to compare the reaction sequence
observed in this system with that of other systems, where ring opening is observed and to discuss the mechanisms that have been proposed for them.

Francis and Jordan have studied the rate of carbonate chelate ring opening and closing in alkaline solution [172]. From an $^{18}$O tracer study, they have shown that the ring opening proceeds with Co-O cleavage. Scheidegger and Schwarzenbach have examined the same system at lower pH (>9) and have obtained estimates of $t_\frac{1}{2}$ and equilibrium constants for the individual steps shown in Fig. 3.7 [173].

Another comparison is the alkaline hydrolysis of carbonate, which may be studied by a macroscopic technique. The reaction proceeds with the stoichiometry:

\[
\begin{align*}
\text{(en)}_2 \text{Co}^2+ \text{O}_2 \text{CO}_3^- + \text{OH}^- & \xrightarrow{t_\frac{1}{2} = 90\text{mins}} \text{cis-(en)}_2 \text{Co}^2 \text{O} \text{C}=\text{O} \xrightarrow{t_\frac{1}{2} = 180\text{mins}} \text{trans-(en)}_2 \text{Co}^2 \text{OCO}_2^- + \text{H}_2\text{O} + \text{HCO}_3^- \n\end{align*}
\]

Fig.3.7. Opening of the four-membered chelate ring of a carbonato complex at pH > 9 [173].

Loss of the bidentate ligand occurs in two steps. Only the cis-hydroxo carbonato isomer is formed in the first step and when $[\text{HCO}_3^-] \approx 10^{-2}\text{M}$, very little of the cis-dihydroxo isomer is formed in a subsequent step. The entire system is reversible and in this respect, differs from the system under present investigation, where only some of the steps have this feature. A relevant feature of Fig.3.7 is that
the reverse step to starting material is twice as slow as the forward step. For the corresponding steps of the present system (Fig. 3.4), the reverse rate from the cis-isomer appears to be slower, by a factor of at least ten, than the forward rate, while a reverse path from the trans-isomer directly to starting material does not exist. That is, ring formation from the trans isomer cannot occur and intramolecular ring closure from the cis-hydroxo β-alaminato species is slower than that for the four-membered carbonato ring, as expected.

Another comparison is the alkaline hydrolysis of Co(III) complexes containing the five-membered oxalate ring system, which has been studied by a number of workers [175-178]. The reaction proceeds with the stoichiometry,

$$[\text{Co(en)}_2\text{C}_2\text{O}_4]^+ + 2\text{OH}^- \rightarrow [\text{Co(en)}_2(\text{OH})_2]^+ + \text{C}_2\text{O}_4^{2-} \quad (3.4)$$

and the observed rate law for the reaction indicates the formation of the cis-hydroxo oxalato complex. Farago and Mason [177] claim to observe this hydroxo intermediate by an increase in absorbance at 330nm of the reactant solution before the dihydroxo product is formed. At low [OH⁻], hydrolysis is slower than isomerization of the dihydroxo product, so that an equilibrium mixture is obtained [178] but, as the temperature is lowered, the isomerization rate is decreased and the cis-isomer is reported as being the substantial product [177]. The results of ¹⁸O tracer experiments indicate that C-O bond fission occurs in the
second, as seen below [176].

\[
(\text{en})_2\text{Co}^+ \left\{ \begin{array}{c}
\text{O} \\
\text{C} \\
\text{O} \\
\text{C}
\end{array} \right\} + \text{OH}^- \rightleftharpoons (\text{en})_2\text{Co}^+ \left\{ \begin{array}{c}
\text{O} \\
\text{C} \\
\text{O} \\
\text{C}
\end{array} \right\} \text{OH}^- + \text{C}_2\text{O}_3\text{O}^{2-} \quad (3.5)
\]

The alkaline hydrolysis of analogous complexes containing the six-membered malonate ring system gives results that are similar to those of the oxalato system in dilute alkali. Up to 0.1M [179], the products are the equilibrium mixture of the isomers of \([\text{Co}(\text{en})_2(\text{OH})_2]^+\).

When substituted malonates of the type shown below are used, ring opening and complete loss of malonato ligand may be studied separately. The cis-dihydroxo isomer, alone, is then formed and it subsequently isomerizes to produce the equilibrium mixture of cis and trans isomers.

\[
(\text{en})_2\text{Co}^+ \left\{ \begin{array}{c}
\text{O} \\
\text{C} \\
\text{C} \\
\text{O}
\end{array} \right\} + \text{OH}^- \rightarrow (\text{en})_2\text{Co}^+ \left\{ \begin{array}{c}
\text{O} \\
\text{C} \\
\text{C} \\
\text{O}
\end{array} \right\} \text{OH}^- \quad (3.6)
\]

Pseudo first order rate constants reach a limiting value when \([\text{OH}^-] \approx 2M\) and the explanation may be that the proton of the malonate residue dissociates to produce an unreactive, resonance-stabilized species [180].
In none of the oxygen chelate ring systems discussed above does there appear to be any evidence for the initial formation of a trans-hydroxo species. In this respect, the amino acid chelate in the present investigation is different. Both trans and cis products are formed in the initial process.

Several mechanisms appear to be consistent with the observations. The most reasonable is an $S_N$ICB mechanism for the base hydrolysis, where an amine function is deprotonated before the leaving group, the bound carboxylate moiety, is expelled to produce a five-coordinate intermediate [181]. Rapid capture of $\text{OH}_2$ by the intermediate could then lead to both cis and trans products. A similar mechanism has been proposed to account for the formation of both cis and trans products from complexes, $[\text{Co(en)}_2(\text{NH}_3)\text{Y}]^{2+}$ [140].

The rate constant in the present system, $k_{12}'$, would then be associated with formation of the intermediate. This mechanism requires Co-O bond cleavage.

A second, but less likely, scheme is one where two separate mechanisms are operative for production of the cis- and trans-hydroxo $\beta$-alaninato complexes. One of the mechanisms may be similar to that proposed for oxygen chelate ring systems (vide supra), where OH$^-$ attacks the bound carbonyl carbon, with subsequent C-O bond cleavage. This mechanism would give rise solely to the cis-isomer. The other isomer may then be formed competitively via the $S_N$ICB mechanism, already described. (It is also possible that this isomer may be formed by a path involving ion-pair formation [182] or by an $S_N2$ process but the evidence for such pathways is scant, if it exists at all). If, then,
two mechanisms co-exist, the constant, $k_{12}$, may be divided into separate constants, $k_1$ and $k_2$, for production of the two isomers, as done in equation (3.3).

These proposals could be tested by $^{18}O$ tracer studies, the label being incorporated in the Co$^{3+}O-C$ position of the starting material. If a single mechanism exists for the production of both isomers, the label will appear in the $\beta$-alanine residue in each of the isomers (see (3.7) below). If two separate mechanisms exist, as described above, only some of the $\beta$-alanine residue, (resulting from the $S_N$1CB mechanism) will be labelled in the cis product, the rest appearing elsewhere (3.8); on the other hand, all of the label will appear in the $\beta$-alanine residue for the trans product.

$$\text{The loss of optical activity (74%, relative to the optically pure, structurally similar cis-hydroxo ester species (Fig.6.3)) of the cis-hydroxo $\beta$-alaninato isomer (Table 3.3) is good evidence for the existence of the dissociative mechanism.}$$
It is interesting to note the spectral changes that occurred in the β-alanine chelate (Fig. 3.1) compared with the glycine chelate (Fig. 3.2) and their implications. At all times throughout the hydrolysis of the β-alanine chelate, the spectra gave absorption maxima, characteristic of octahedral Co(III) complexes. This was not the case for the glycine chelate; the colours of the reactant solution varied from the initial orange-pink through mauve where it appeared that both a Co(III) dihydroxo species ($\lambda_{\text{max}} \approx 520\text{nm}$) and a tetrahedral Co(II) species were present; the latter was present in much smaller concentrations, for the extinction coefficients of such Co(II) species are known to be some 50 times greater than those of the octahedral Co(III) species [183]. As the reaction continued, the absorption bands due to Co(II) species disappeared and the final spectrum had a single band at 520nm.

The observations of the glycine and β-alanine systems, show that five-membered amino acid chelate ring opening may proceed via a different pathway from six-membered amino-acid chelate ring opening.

The rate constants for the various reactions that occur during base hydrolysis of $[\text{Co(en)}_2(\beta\text{-alaO})]^2^+$ are summarized in Table 3.5. (Refer to Fig. 3.4 for reaction sequence).

In conclusion, it is seen that the hydrolysis of $[\text{Co(en)}_2(\beta\text{-alaO})]^2^+$, in highly alkaline solution, involves a complex sequence of reactions. Nevertheless, approximate rate constants have been obtained for the various steps and, more important, the origin of the products has been established.
TABLE 3.5

Rate Constants for Steps in Base Hydrolysis of
\([\text{Co(en)}_2(\beta\text{-alaO})]^2+\) in 1M NaOH; 25.0°; \(\mu = 1.0, \text{NaClO}_4\).

<table>
<thead>
<tr>
<th>Reaction</th>
<th>(k(\text{min}^{-1}))</th>
<th>(t_\frac{1}{2}) (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opening of (\beta)-alanine ring</td>
<td>(6 \times 10^{-3})</td>
<td>2</td>
</tr>
<tr>
<td>Ring closure of (\text{cis}-[\text{Co(en)}_2\text{OH}(\beta\text{-alaO})]^+)</td>
<td>(&lt; 6 \times 10^{-4})</td>
<td>(&gt; 20)</td>
</tr>
<tr>
<td>(\text{cis-trans isomerization of})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>([\text{Co(en)}_2\text{OH}(\beta\text{-alaO})]^+)</td>
<td>(6 \times 10^{-4})</td>
<td>20</td>
</tr>
<tr>
<td>Loss of (\beta)-alanine from (\text{cis}-[\text{Co(en)}_2\text{OH}(\beta\text{-alaO})]^+)</td>
<td>(&lt; 6 \times 10^{-4})</td>
<td>(&gt; 20^a)</td>
</tr>
<tr>
<td>Loss of (\beta)-alanine from (\text{trans}-[\text{Co(en)}_2\text{OH}(\beta\text{-alaO})]^+)</td>
<td>(6 \times 10^{-4})</td>
<td>(&gt; 20^b)</td>
</tr>
<tr>
<td>(\text{cis-trans isomerization of})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>([\text{Co(en)}_2\text{OH}]^+)</td>
<td>(4 \times 10^{-4})</td>
<td>29</td>
</tr>
</tbody>
</table>

- **a** Deduced from fact (6), Section 3.3 (p. 106)
- **b** From the observation that \(k(\text{trans}) < k(\text{cis})\).
- **c** Reference [171].

It is important to note that the tracer studies show that 50% of \([\text{Co(triaa)}\text{Cl(glyOC}_2\text{H}_3)]^2+\) product arises from coordination of the ester carbonyl oxygen and the rest by intramolecular hydrolysis of bound solvent. Thus, for 16% of the product also arises by intervention of solvent to the reaction mechanism.
4. HYDROLYSIS AND RING CLOSURE IN cis-[Co(en)$_2$OH($\beta$-alaOR)]$^{2+}$

AND cis-[Co(en)$_2$H$_2$O($\beta$-alaOR)]$^{3+}$

4.1 Introduction

In some investigations involving reactions of coordinated ligands, it has been proposed that the active nucleophile is also bound to the metal ion, in a position cis to that ligand. Experimental evidence for this proposition in metal-ion catalysis is rare. One example is seen in the Co(III)-promoted amidolysis of glycine ethyl ester, which proceeds, in 0.1M NaOH, according to the equation:

$$[\text{Co(NH}_3)_5(\text{glyOC}_2\text{H}_5)]^{3+} + \text{OH}^- \rightarrow [\text{Co(NH}_3)_4(\text{glyNH})]^{2+} + \text{H}_2\text{O} + \text{HOC}_2\text{H}_5$$ (4.1)

The chelated glycine imide product must be formed by attack of a cis-deprotonated ammine ligand at the carbonyl carbon of the dangling amino acid [184]. Another example is the base hydrolysis of cis-[Co(en)$_2$Br(glyOCH(CH$_3$)$_2$)]$^{2+}$ [108], where $^{18}$O tracer studies show that 50% of [Co(en)$_2$(glyO)]$^{2+}$ product arises from coordination of the ester carbonyl oxygen and the rest, by intramolecular hydrolysis of bound solvent.

$\beta_2$-[Co(trien)Cl(glyOC$_2$H$_5$)]$^{2+}$, in base hydrolysis, is similar, for 16% of the product also arises by intervention of solvent.
oxygen in the coordination sphere [185]. The Co(III)-
promoted hydrolysis of glycine amides [115] and lysis of
bromide in cis-[Co(en)$_2$Br(NH$_2$CH$_2$CH$_2$Br)]$^{2+}$ are similar.

The intermediate, with a bound oxygen nucleophile,
in metal-ion promoted hydrolysis of $\alpha$-amino acid esters,
is very reactive (vide supra) and thus has not been isolated
and studied separately. This chapter presents the prepara-
tion of such an intermediate containing $\beta$-alanine, cis-
[Co(en)$_2$H$_2$O($\beta$-alaOCH(CH$_3$)$_2$)](NO$_3$)$_3$, and also the details
of its reactivity and stereochemistry during acid and base
hydrolysis.

4.2 Experimental

4.2.1 Instrumental and general

Visible and infrared spectra were recorded on Cary
14 and Perkin-Elmer Model 459 instruments, respectively and
pmr spectra on a Jeol JNM-100MHz Minimar using TMS as
external or NaTPS internal reference. The attachments for
pmr kinetics experiments, described in Section 2.2.1, were
also used. Cobalt estimations were made using a Techtron
AA4 atomic absorption spectrophotometer. Spectrophotometric
rates were obtained by the methods already described in
Section 2.2.1. The $^{18}$O content of CO$_2$, recovered from the
labelled compounds, was determined using an Atlas M-86
mass spectrometer.

Product separations were effected with Bio-Rad
Analytical Dowex 50Wx2 (200-400 mesh, H$^+$- or Na$^+$- form)
cation exchange resin.
4.2.2 Preparation of complexes

cis-\([\text{Co(en)}_2\text{H}_2\text{O}(\beta-\text{alaOCH(CH}_3)_2)](\text{NO}_3)_3\)

To a suspension of \(\text{cis-[Co(en)}_2\text{Br(\beta-alaOCH(CH}_3)_2]Br}_2\)
(38.5g; preparation in Section 5.2.2) in \(\text{H}_2\text{O}\) (50ml) was added a solution of \(\text{HgO}\) (45.5g, 3 equivs) in 70% \(\text{HClO}_4\) (37ml). The mixture was shaken vigorously for 20 minutes until the complex had dissolved and the purple colour had changed to pink-orange, then cooled in an ice bath before being filtered to remove the \(\text{HgBr}_2\). To the filtrate, excess crystalline lithium nitrate was added, followed by a fifty-fold excess of ethanol with a little methanol. Crystallization was induced by scratching the vessel and, after further cooling in an ice bath, the product was collected, washed with methanol, ethanol and ether, then recrystallized from a minimum volume of water with further addition of lithium nitrate. The solid was dried in an evacuated desiccator.

**Anal.** Calcd for \([\text{Co(en)}_2\text{H}_2\text{O}(\beta-\text{alaOCH(CH}_3)_2)](\text{NO}_3)_3\):

\[
\begin{align*}
\text{Co, 11.46; C, 23.35; H, 6.08; N, 21.79.} \\
\text{Found: Co, 12.05; C, 23.36; H, 5.76; N, 22.09.}
\end{align*}
\]

The spectrum from 600 to 300nm gave the following absorption maxima and extinction coefficients in \(\text{H}_2\text{O, 1M NaClO}_4\) and 3M \(\text{HCl}\):

- 489 ± 2nm (\(\varepsilon\) 72 ± 2), 351 ± 2nm (\(\varepsilon\) 68 ± 2).

The \(^{18}\text{O}\)-labelled complex was prepared by two methods.

1. To a suspension of \(\text{cis-[Co(en)}_2\text{Br(\beta-alaOCH(CH}_3)_2]Br}_2\)
(4.1g) in 1.5% \(^{18}\text{O}\) enriched \(\text{H}_2\text{O}\) (15ml) was added a solution of \(\text{HgO}\) (4.9g) in 70% \(\text{HClO}_4\) (3.9ml). The mixture was shaken vigorously for 10 minutes and cooled in an ice bath before the \(\text{HgBr}_2\) was filtered off. To the
filtrate was added excess crystalline lithium nitrate, a little methanol and a fifty-fold excess of ethanol. Crystallization was induced by scratching the vessel; after standing in an ice bath for ~30 minutes the pale pink-orange product was collected, washed with methanol, ethanol and ether and dried in an evacuated desiccator.

(2) To a solution of cis-[Co(en)$_2$Br($\beta$-alaOCH(CH$_3$)$_2$)](ClO$_4$)$_2$ (10.0g; preparation in Section 5.2.2) in 1.5% $^{18}$O enriched H$_2$O (25ml) was added a solution of HgO (3.7g) in 70% HClO$_4$ (3ml). The procedure of (1), above, was then followed.

Spectra of both products gave extinction coefficients of 76 and 75 at 489nm for (1) and (2), respectively. A solution of each complex, brought to pH~9 for 60 seconds, quenched to pH~2 with HClO$_4$, sorbed on and eluted from H$^+$ form resin, showed that 10±2% chelated ester was present in the original sample. This appeared in the product after hydrolysis of the labelled starting materials and thus, corrections were applied, as described in Section 4.3.4.

4.2.3 Kinetic measurements

Acid hydrolysis rate measurements were made by dissolving cis-[Co(en)$_2$H$_2$O($\beta$-alaOCH(CH$_3$)$_2$)](NO$_3$)$_3$ (~0.2g) in HClO$_4$ (0.01 to 1M; $\mu$=1.0, NaClO$_4$; 25.0$^0$) so that the ratio of complex to acid was not greater than 1:10 and recording visible spectra at intervals. Rates were obtained from a plot of log($D_\infty$-$D_t$) vs t. Rates were also obtained by calculating the percentage of the product fraction after an
An aliquot of the reaction mixture had been sorbed on H+-form resin and eluted with 2M NaClO₄ at pH ≈ 4.

Acid and base hydrolysis of the cis-[Co(en)₂H₂O-(β-alaOH)]³⁺ ion were followed spectrophotometrically, at a fixed wavelength. The species was obtained directly from cis-[Co(en)₂Br(β-alaOH)]Br₂, as described in Section 5.2.4, the solution then being mixed with HCl or HClO₄ (final concentration, 0.05 to 1.0M), or with Tris buffer (1.0M) so that the concentration of Co(III) was ≈ 10⁻³M and µ=1.0 (NaClO₄) at 25.0°C. Acid hydrolysis of the isopropyl ester complex in 1M DCIO₄ was also followed by recording NMR spectra at intervals and measuring the rate of growth or disappearance of isopropyl signals.

Base hydrolysis of the complexes was followed, spectrophotometrically, by mixing, manually or in a stopped-flow reactor, equal volumes of the complexes (≈ 5x10⁻³M) with triethylamine buffer (0.5M) or with NaOH solution (0.05-1.0M), all at µ=1.0, NaClO₄ and 25.0°C.

Rate measurements were also obtained, spectrophotometrically, by following the absorbance change at a fixed wavelength of a solution of cis-[Co(en)₂Br(β-alaOR)]²⁺, mixed with buffer at µ=1.0, 25.0°C, after bromide removal was complete. (See Section 5.2.3). Cells of suitable path length were chosen so that absorbance changes were ≈ 0.2 units, wherever possible.

Base hydrolysis was also followed by pH-Stat titration of an accurately weighed sample of complex (0.2 to 0.5g) in 1M NaClO₄ (≈ 10ml) with NaOH (0.1 to 1.0M, µ=1.0, NaClO₄) at 25.0°C.
When the radiometer was coupled with the spectrophotometer, the complex (~0.08g), dissolved in 1M NaClO\textsubscript{4} (30ml) was titrated with 1M NaOH, at 25.0\degree. Solvent blank titrations were carried out and corrections applied.

4.2.4 Product analysis

The products of acid hydrolysis of \textit{cis-[Co(en)}\textsubscript{2}H\textsubscript{2}O-(\beta\text{-alaOR})\textsuperscript{3+} and of base hydrolysis of \textit{cis-[Co(en)}\textsubscript{2}OH-(\beta\text{-alaO})\textsuperscript{+} at lower pH were identified by comparison of the visible spectra with those calculated for mixtures of known possible products, and also by elution from H\textsuperscript{+}-form resin with 2M NaClO\textsubscript{4} (pH~2) and analysis of the eluted solutions by visible and atomic absorption spectrophotometry.

After base hydrolysis of \textit{cis-[Co(en)}\textsubscript{2}OH(\beta\text{-alaOCH-(CH\textsubscript{3})\textsubscript{2})\textsuperscript{2+}}, the reaction mixture was either quenched to pH~9 with 1M HClO\textsubscript{4}, diluted and sorbed on Na\textsuperscript{+}-form resin, or just diluted and sorbed directly on Na\textsuperscript{+}-form resin. The mauve and cerise unipositive species were eluted with 0.5M NaClO\textsubscript{4} at pH~9 and the orange bipositive species with 2M NaClO\textsubscript{4} at pH~8. Cobalt estimations on each fraction were carried out both by visible spectrophotometry and by atomic absorption analysis. Products were identified by comparison of their spectra and elution rates with those of the authentic materials, as also done in Section 3.24.

4.2.5 $pK_a$ determinations

The aquo isopropyl ester complex (0.2g), dissolved in 10ml 1M NaClO\textsubscript{4} at 25.0\degree was titrated with NaOH of known concentration. The $pK_a$ was then calculated by the usual
method, a correction being made for the volume of base added [118]. The pK\textsubscript{a} was also determined by recording spectra (600-300nm) of a solution of the complex ([Co] \textasciitilde 10^{-2}\text{M}) in 1M NaClO\textsubscript{4} and measuring the pH as small particles of solid NaOH were added. A plot of absorbance at 380nm vs pH gave the pK\textsubscript{a} at the inflection point.

The pK\textsubscript{a}'s of cis-[Co(en)\textsubscript{2}H\textsubscript{2}O(\beta-alaOH)]\textsuperscript{3+} were determined by the titrimetric procedure given above. A solution (14ml; \(\mu = 1.0,\text{NaClO}_4\)), containing the doubly deprotonated species, cis-[Co(en)\textsubscript{2}OH(\beta-alaO)]\textsuperscript{+}, was obtained by the method described in Section 5.2.4. ([Co] \textasciitilde 10^{-2}\text{M}). It was then titrated with 1.0M HClO\textsubscript{4} at 25.0\,^\circ, a correction being made for the volume of acid added.

4.2.6 Measurement of optical activity in \(\text{(+)}\,\text{cis-}[\text{Co(en)}\textsubscript{2}\text{H\textsubscript{2}O(\beta-alaOCH(CH\textsubscript{3})\textsubscript{2})}]\textsuperscript{2+}\) formed on hydrolysis of \(\text{(+)}\,\text{cis-}[\text{Co(en)}\textsubscript{2}\text{H\textsubscript{2}O(\beta-alaOCH(CH\textsubscript{3})\textsubscript{2})}]\textsuperscript{3+}\) was prepared \textit{in situ} by the Hg\textsuperscript{2+}-catalyzed removal of bromide from \(\text{(+)}\,\text{cis-}[\text{Co(en)}\textsubscript{2}\text{Br(\beta-alaOCH(CH\textsubscript{3})\textsubscript{2})}]\text{Br}_2\), (see Section 5.2.5 for preparation of the resolved material), in the following manner. The resolved complex (\textasciitilde 0.2g) was dissolved in 0.2M HgClO\textsubscript{4}/0.4M HClO\textsubscript{4} solution (10ml; \(\mu = 1.0,\text{NaClO}_4\)) and immediately sorbed on H\textsuperscript{+}-form resin and eluted with 3M HCl or with 2M NaClO\textsubscript{4}. The band, which moved as a 3+ species, contained both cis-[Co(en)\textsubscript{2}H\textsubscript{2}O(\beta-alaOCH(CH\textsubscript{3})\textsubscript{2})]\textsuperscript{3+} (90\%) and [Co(en)\textsubscript{2}(\beta-alaOCH(CH\textsubscript{3})\textsubscript{2})]\textsuperscript{3+} (10\%) (see Table 6.2) which were not separated for these experiments.
For the acid hydrolysis experiment, two runs were done. In the first, the band was eluted with 2M NaClO₄ (pH ~1) and in the second, it was eluted with 3M HCl. Both solutions were allowed to stand for about 8 weeks. The rotation of these solutions was measured in a 10cm cell on the spectropolarimeter, periodically, until no further change was observed. Analysis of the solutions, at this stage, by ion exchange chromatography and by visible spectra showed that only one product was present (>98%). Cobalt estimations were made by the usual methods, ([Co]~1.5x10⁻³M) and final sets of readings for molar rotations are shown in Table 4.9 (Section 4.3.5).

For the base hydrolysis experiment, the product band from the Hg²⁺/H⁺ experiment, still sorbed on the resin, was transferred to a beaker, washed several times with H₂O, then treated with 1M NaOH at pH ~13 for 5 minutes, reacidified to pH ~2 with 1M HClO₄, then transferred to the top of a column of H⁺-form resin and eluted with 2M NaClO₄ (pH ~2). The [Co(en)₂(β-alaO)]²⁺ product, the first band to be eluted, was collected, estimated for cobalt [1.49x10⁻³M] and the rotation measured in a 10cm cell on the spectropolarimeter. Molar rotations are given in Table 4.9. (Section 4.3.5).

4.2.7 Analytical procedures for ^1⁸O estimations

The ^1⁸O content of the solvent from which the aquo-¹⁸O-cis-[Co (en)₂H₂O(β-alaOCH(CH₃)₂)](NO₂)₃ was prepared, was determined by equilibrating a sample of the reaction
solution with CO$_2$ (~ 0.01mole) overnight at 80° and the $^{18}$O content in the CO$_2$ determined by the mass spectrometer as C$^{18}$O$^{16}$O.

The $^{18}$O enrichment in the starting material was determined in the following manner: $^{18}$O-labelled cis-[Co(en)$_2$H$_2$O($\beta$-alaOCH(CH$_3$)$_2$)](NO$_3$)$_3$ (0.4g) was first dried for 2 hours at room temperature on the vacuum line (10$^{-4}$ mm) in a Urey tube. It was then heated at 80° for 2 hours and the $^{18}$O-labelled coordinated water distilled into a break-seal tube containing an equimolar mixture of HgCl$_2$ and HgCN$_2$. The tube was then heated at 400° for 10 hours and the $^{18}$O content, produced on pyrolysis, determined as above.

Acid Hydrolysis of $^{18}$O-Labelled cis-[Co(en)$_2$H$_2$O($\beta$-alaOCH-$(CH_3)_2$)](NO$_3$)$_3$

Aquo-$^{18}$O-cis-[Co(en)$_2$H$_2$O($\beta$-alaOCH(CH$_3$)$_2$)](NO$_3$)$_3$ (1.4g) was dissolved in 1M HCl (25ml), thermostated at 25.0° for 17 hours, then sorbed on H$^+$-form resin; the first band, the [Co(en)$_2$(\beta-alaO)]$^{2+}$ product was eluted with 2M HCl, rapidly evaporated to dryness on a rotary evaporator, washed twice with water to remove the acid and again evaporated to dryness, before being recrystallized from a minimum volume of water, with methanol and ethanol added. The solid was dried at 110° for 12 hours in an evacuated oven at 1mm, then dried on the vacuum line at 10$^{-3}$ mm for 2 days to ensure the removal of all traces of solvents and of the H$_2$O of crystallization.

A sample of this complex (~ 0.1g) was sealed into a break-seal tube, under vacuum, containing an equimolar
mixture of HgCl₂ and Hg(CN)₂, heated overnight at 400°
and the ¹⁸O content of the CO₂, formed on pyrolysis,
determined as previously.

The ¹⁸O exchange rate of labelled coordinated
water in the complex in 1M HCl at 25.0° was determined
as follows: the unreacted material, sorbed on H⁺-form
resin in the above reaction, was washed several times,
with water, transferred to a beaker, slowly neutralized
with 0.1M NaOH, then made basic to pH=13 and maintained
at that pH for ~5 minutes. The contents of the beaker
were then quenched to pH~4 with 1M HCl and transferred
to the top of a fresh column of H⁺-form resin. The
desired labelled product, [Co(en)₂(β-alaO)]²⁺, was
eluted with 2M HCl and treated as above for determination
of ¹⁸O enrichment.

Base Hydrolysis of ¹⁸O-Labelled [Co(en)₂H₂O(β-alaOCH-(CH₃)₂]
(NO₃)₃

To aquo-¹⁸O-cis-[Co(en)₂H₂O(β-alaOCH(CH₃)₂)](NO₃)₃
(0.4g), dissolved in 1M NaClO₄ was added 1M NaOH and the
pH maintained at 12.3 for 7 minutes. The solution was
quenched to pH~8 with 1M HClO₄, diluted five-fold and
sorbed on Na⁺-form resin. The unipositive mauve and
cerise species were separated first from the desired orange
dipositive species with 2M NaClO₄ at pH=9. The orange
complex, still sorbed on the resin, was manually transferred
to a new column, washed several times with water to remove
perchlorate anion, then eluted with 2M HCl and treated, as
above, for determination of the ¹⁸O enrichment.
4.3 Results

4.3.1 Infrared, pmr and visible spectra

Infrared and pmr spectra of cis-[Co(en)$_2$H$_2$O-$(\beta$-alaOCH(CH$_3$)$_2$](NO$_3$)$_3$ are shown in Figs. 4.1a and b, respectively. In the infrared spectrum, the intense absorption at 1725 cm$^{-1}$ is assigned to the stretching mode of the ester carbonyl function. The free isopropyl ester hydrochloride also has a band at the same wavenumber (Fig. 2.4b); this is expected, for Co(III) bonded to NH$_2$- has an effect similar to a proton on the bond strength of the carbonyl function. The broad absorption near 1350 cm$^{-1}$ is assigned to the $\nu_3$ vibration of the nitrate anion [127]. The pmr spectrum (Fig. 4.1b) has features similar to those of the chelated ester complex (c.f., Fig. 2.5, Table 2.5). The signal for gem-CH$_3$ appears at 1.71 ppm ($J=6$ Hz) down-field from TMS, intermediate between the corresponding signal in the ester chelate (1.76 ppm; $J=6$ Hz) and free isopropanol (1.61 ppm; $J=6$ Hz). The splitting in the methylene proton signals (3.0-3.4 ppm) is apparent. The triplet at 3.02 ppm may be attributed to the methylene protons adjacent to the carboxyl group; that at 3.28 ppm to the methylene protons of the ethylenediamine ligands and that at 3.40 to the methylene protons adjacent to the amino group of the bound $\beta$-alanine ester. Unlike the chelated $\beta$-alanine derivatives (Table 2.5), where the two last-mentioned signals merge together, all of the methylene protons for the monodentate ester are able to be distinguished.
Fig. 4.1. Spectra of cis-\([\text{Co(en)}H_2O(\beta-\text{alaOCH(CH}_3)_2)](\text{NO}_3)_3\) (---) and \([\text{Co(en)}_2(\beta-\text{alaOCH(CH}_3)_2)](\text{ClO}_4)_2\) (-----).

(a), infrared spectra (nujol mulls; CsI plates) and (b), pmr spectra in \(D_2O/DC1\) (internal reference, NaTPS; 100 MHz).
Visible spectra of cis-[Co(en)$_2$H$_2$O(B-alaOCH(CH$_3$)$_2$)](NO$_3$)$_3$ in 1.0M NaClO$_4$ as a function of pH, are shown in Fig.4.2. Maxima for the aqueous species occur at 493 ± 2 nm (pH 2.5) and 351 ± 2 nm (pH 9.0); and, for the hydroxy species, at 489 ± 2 nm (pH 2.5) and 369 ± 2 nm (pH 9.0). The pK$_a$ for B-alaOCH(CH$_3$)$_2$ for cis-[Co(en)$_2$H$_2$O(B-alaOCH(CH$_3$)$_2$)](NO$_3$)$_3$ derivatives are tabulated below.

**Table 4.1:** The pK$_a$ values for cis-[Co(en)$_2$H$_2$O(B-alaOCH(CH$_3$)$_2$)](NO$_3$)$_3$ in 1.0M NaClO$_4$.

<table>
<thead>
<tr>
<th>pH</th>
<th>H$_2$O/B-alaOCH(CH$_3$)$_2$ Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>pH = 2.5</td>
</tr>
<tr>
<td>9.0</td>
<td>pH = 9.0</td>
</tr>
</tbody>
</table>

**Fig. 4.2.** Visible spectra of cis-[Co(en)$_2$H$_2$O(B-alaOCH(CH$_3$)$_2$)](NO$_3$)$_3$ in 1.0M NaClO$_4$ with increasing pH. (pK$_a$ = 6.05).
Visible spectra of cis-$[\text{Co(en)}_2\text{H}_2\text{O}(\beta-\text{alaCH}_3\text{)}_2]\{\text{NO}_3\}_3$

in 1.0M NaClO$_4$, as a function of pH, are shown in Fig 4.2. Maxima for the aquo species occur at 489 ± 2nm ($\varepsilon$ 72 ± 2) and 351 ± 2nm ($\varepsilon$ 68 ± 2) and, for the hydroxo species, at 500 ± 2nm ($\varepsilon$ 93 ± 2) and 360 ± 2nm ($\varepsilon$ 83 ± 2). The pK$_a$'s for the $\beta$-alanine derivatives are tabulated below.

### TABLE 4.1

The pK$_a$'s of cis-$[\text{Co(en)}_2\text{H}_2\text{O}(\beta-\text{alaOR})]^{3+}$ at 25.0° and $\mu = 1.0$, NaClO$_4$.

<table>
<thead>
<tr>
<th>R</th>
<th>pK$_a$ (H$_2$O)</th>
<th>pK$_a$ (β-alaOH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH(CH$_3$)$_2$</td>
<td>6.05 ± 0.05$^a$</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6.08 ± 0.07</td>
<td>-</td>
</tr>
<tr>
<td>H</td>
<td>6.46 ± 0.07</td>
<td>3.9 ± 0.1</td>
</tr>
</tbody>
</table>

$^a$ Determined spectrophotometrically; $\lambda = 380$nm, [Co] = 0.0091M. Other values were obtained by the titrimetric method; [Co] $\sim$ 0.035M. For $R = \text{CH(}\text{CH}_3\text{)}_2$ [OH$^-$] = 0.1M. For $R = \text{H}$, [OH$^-$] = 0.5M and [H$^+$] = 1.0M.

#### 4.3.2 Kinetic data

Table 4.2 presents data for acid-catalyzed hydrolysis of cis-$[\text{Co(en)}_2\text{H}_2\text{O}(\beta-\text{alaOR})]^{3+}$. Plots of log $(D_0 - D)/D$ or of % product vs time were linear for $> 3 \times t_1/2$. The data fit the rate law, $k_{\text{obsd}} = k_{\text{H}_2\text{O}}[\text{H}_2\text{O}]$, with $k_{\text{H}_2\text{O}} = 3.24 \times 10^{-8}$M$^{-1}$sec$^{-1}$ for $R = \text{CH(}\text{CH}_3\text{)}_2$ and $k_{\text{H}_2\text{O}} = 3.24 \times 10^{-7}$M$^{-1}$sec$^{-1}$ for $R = \text{H}$. When $R = \text{CH(}\text{CH}_3\text{)}_2$, spectra of the solutions, after $10 \times t_1/2$, corresponded to that of $[\text{Co(en)}_2(\beta-\text{alaO})]^{2+}$ (±3%). Ion exchange chromatography also indicated a single product. When $R = \text{H}$, spectra of the product solution had maxima at 493 ± 2nm
### TABLE 4.2

Data for Acid Hydrolysis/Ring Closure of cis-[Co(en)$_2$H$_2$O(β-alaOR)]$^{3+}$ at 25.0$^\circ$C; µ = 1.0, NaClO$_4$.

<table>
<thead>
<tr>
<th>R</th>
<th>[HClO$_4$]M</th>
<th>$t_H$ (hrs)</th>
<th>$10^6 k_{obsd}$ (sec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH(CH$_3$)$_2$</td>
<td>0.01</td>
<td>105</td>
<td>1.8</td>
</tr>
<tr>
<td>CH(CH$_3$)$_2$</td>
<td>0.10</td>
<td>110</td>
<td>1.8</td>
</tr>
<tr>
<td>CH(CH$_3$)$_2$</td>
<td>0.50</td>
<td>100</td>
<td>1.8</td>
</tr>
<tr>
<td>CH(CH$_3$)$_2$</td>
<td>1.00</td>
<td>110</td>
<td>1.8</td>
</tr>
<tr>
<td>CH(CH$_3$)$_2$</td>
<td>0.01</td>
<td>100</td>
<td>1.8$^b$</td>
</tr>
<tr>
<td>CH(CH$_3$)$_2$</td>
<td>1.00</td>
<td>95</td>
<td>2.0$^b$</td>
</tr>
<tr>
<td>CH(CH$_3$)$_2$</td>
<td>-</td>
<td>~100</td>
<td>1.8$^c$</td>
</tr>
<tr>
<td>H</td>
<td>0.05</td>
<td>10.0</td>
<td>18</td>
</tr>
<tr>
<td>H</td>
<td>0.50</td>
<td>10.5</td>
<td>18</td>
</tr>
<tr>
<td>H</td>
<td>1.00</td>
<td>10.1</td>
<td>18</td>
</tr>
<tr>
<td>H</td>
<td>0.50</td>
<td>10</td>
<td>18$^d$</td>
</tr>
</tbody>
</table>

---

- **a.** [Co] $\approx$ 4x10$^{-4}$ to 4x10$^{-3}$M; $\lambda$ = 495 nm.
- **b.** Rates determined by ion-exchange chromatography; others obtained spectrophotometrically.
- **c.** A saturated solution of complex in ~1M DC1O$_4$.
- **d.** Fraction obtained, by ion exchange, after base hydrolysis of the corresponding ester, treated in the same manner as those above.
<table>
<thead>
<tr>
<th>λ (nm)</th>
<th>pH</th>
<th>10^3 k_{obsd} (sec^{-1})</th>
<th>k_{OH} (M^{-1}sec^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>495</td>
<td>10.36</td>
<td>0.120</td>
<td>0.27</td>
</tr>
<tr>
<td>355</td>
<td>10.42</td>
<td>0.128</td>
<td>0.29</td>
</tr>
<tr>
<td>495</td>
<td>10.58</td>
<td>0.177</td>
<td>0.28c</td>
</tr>
<tr>
<td>350</td>
<td>10.64</td>
<td>0.196</td>
<td>0.27</td>
</tr>
<tr>
<td>495</td>
<td>11.44</td>
<td>1.32</td>
<td>0.28</td>
</tr>
<tr>
<td>495</td>
<td>11.49</td>
<td>1.60</td>
<td>0.30</td>
</tr>
<tr>
<td>495</td>
<td>11.59</td>
<td>1.91</td>
<td>0.29</td>
</tr>
<tr>
<td>(II)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>495</td>
<td>12.42</td>
<td>12</td>
<td>0.26</td>
</tr>
<tr>
<td>495</td>
<td>12.62</td>
<td>25</td>
<td>0.28</td>
</tr>
<tr>
<td>495</td>
<td>13.15</td>
<td>66</td>
<td>0.27</td>
</tr>
<tr>
<td>495</td>
<td>13.46</td>
<td>140</td>
<td>0.28</td>
</tr>
<tr>
<td>(III)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>495</td>
<td>10.62</td>
<td>0.201</td>
<td>0.28</td>
</tr>
<tr>
<td>495</td>
<td>11.47</td>
<td>1.23</td>
<td>0.25</td>
</tr>
<tr>
<td>495</td>
<td>11.49</td>
<td>1.49</td>
<td>0.29</td>
</tr>
</tbody>
</table>

a. Each value is an average of at least two experiments.
b. pH readings made on the radiometer. Triethylamine stock solution was adjusted to the desired pH with 1M HClO₄ c. Obtained subsequent to bromide removal in cis-[Co(en)₂Br(β-alaOCH(CH₃)₂)]⁺. A similar experiment, beginning with the cis-bromo acid complex (0.5M Tris buffer, pH=9.39 gave k_{obsd}=1.0\times10^{-6} sec^{-1}, with ε_{495}^{max}=129 at ω.
Ion exchange chromatography indicated that \( \approx 15\% \) \( \text{trans-}[\text{Co(en)}_2\text{H}_2\text{O}(\beta-\text{alaOH})]^3+ \) was formed, as well as \( [\text{Co(en)}_2(\beta-\text{alaO})]^2+ \).

Table 4.3 presents rate data for base hydrolysis of \( \text{cis-}[\text{Co(en)}_2\text{OH}(\beta-\text{alaOCH(CH}_3)_2)]^2+ \). The data fit the rate law,

\[
k_{\text{obsd}} = k_{\text{OH}} [\text{OH}^-]
\]

with \( k_{\text{OH}} = 0.28 \pm 0.02 \text{M}^{-1}\text{sec}^{-1} \). An estimate of the rate of ring closure for the hydroxo acid complex is \( k_{\text{obsd}} = 1.0 \times 10^{-6} \text{sec}^{-1} \) at pH 9.39 (Table 4.3; footnote c).

The rate of base consumption by \( \text{cis-}[\text{Co(en)}_2\text{OH-}(\beta-\text{alaOCH(CH}_3)_2)]^2+ \) could not be measured accurately for a number of reasons. Reactions were slow at moderately high pH (e.g., at pH = 10.42, \( t_{1/2} = 90 \text{ mins} \)) and instrumental drift generally occurred; for pH > 10.4, the accuracy of the instrument was lower for pH readings, even though reactions were faster; diffusion of titrant through the nozzle, during slow reactions, incurred greater error in pH readings. Results from rate of base consumption are given in Table 4.4. The rate law is of the form of equation (4.2), with \( k \approx 0.25 \pm 0.06 \text{M}^{-1}\text{sec}^{-1} \).
### Table 4.4

**Radiometer Data for Base Hydrolysis of cis-**

\[
{[\text{Co}(en)_2\text{OH}(\beta-\text{alaOCH(CH}_3)_2)]^2+; \quad \mu=1.0, \text{NaClO}_4; \quad 25.0^\circ.}^a
\]

<table>
<thead>
<tr>
<th>[NaOH]M titrant</th>
<th>pH</th>
<th>(10^3 k_{\text{obsd}}(\text{sec}^{-1}))</th>
<th>(k_{\text{OH}}(\text{M}^{-1}\text{sec}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>10.55</td>
<td>0.14</td>
<td>0.22</td>
</tr>
<tr>
<td>0.25</td>
<td>10.61</td>
<td>0.19</td>
<td>0.28</td>
</tr>
<tr>
<td>0.50</td>
<td>11.27</td>
<td>0.72</td>
<td>0.23</td>
</tr>
<tr>
<td>1.00</td>
<td>11.47</td>
<td>1.2</td>
<td>0.24</td>
</tr>
<tr>
<td>0.10</td>
<td>11.56</td>
<td>1.9</td>
<td>0.29</td>
</tr>
</tbody>
</table>

^a. Values are averages of two or three runs; % error in \(k_{\text{OH}}\) ~25%.

### 4.3.3 Product analysis

Table 4.5 gives product analyses after base hydrolysis of the cis-hydroxo ester complex at varying pH on the radiometer or after hand-mixing a solution of the complex with NaOH solution of known concentration. Recovery of cobalt from ion exchange columns was better than 95%. The uni-positive mauve coloured \([\text{Co}(en)_2\text{OH}(\beta-\text{alaO})]^+\) isomers could not be separated well on short columns (~12x1cm) but were recovered pure, on longer columns (~20x1cm) using 0.5M NaClO\(_4\) (pH ~9).
### TABLE 4.5

**Product Analysis after Radiometric Base Hydrolysis**

of cis-$[\text{Co(en)}_2\text{OH}(\beta-\text{alaOCH(CH}_3)_2)]^{2+}$ at $\mu=1.0$,

$\text{NaClO}_4$; $25.0^\circ a$

<table>
<thead>
<tr>
<th>pH</th>
<th>% trans-</th>
<th>% cis-</th>
<th>% trans-acoH(β-alaO)]$^+$</th>
<th>% cis-[Co(en)$_2$($\beta$-alaO)]$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>10.4</td>
<td>8</td>
<td>31</td>
<td>61</td>
</tr>
<tr>
<td>(2)</td>
<td>10.6</td>
<td>6</td>
<td>34</td>
<td>60</td>
</tr>
<tr>
<td>(3)</td>
<td>10.8</td>
<td>8</td>
<td>31</td>
<td>61</td>
</tr>
<tr>
<td>(4)</td>
<td>11.5</td>
<td>11</td>
<td>32</td>
<td>58</td>
</tr>
<tr>
<td>(5)</td>
<td>11.6</td>
<td>10</td>
<td>32</td>
<td>55</td>
</tr>
<tr>
<td>(6)</td>
<td>11.7</td>
<td>8</td>
<td>34</td>
<td>58</td>
</tr>
<tr>
<td>(7)</td>
<td>11.9</td>
<td>11</td>
<td>38</td>
<td>51</td>
</tr>
<tr>
<td>(8)</td>
<td>12.3</td>
<td>6</td>
<td>33</td>
<td>61$^b$</td>
</tr>
<tr>
<td>(9)</td>
<td>12.5</td>
<td>16</td>
<td>33</td>
<td>50</td>
</tr>
<tr>
<td>(10)</td>
<td>12.8$^c$</td>
<td>18</td>
<td>34</td>
<td>48</td>
</tr>
<tr>
<td>(11)</td>
<td>13.5$^c$</td>
<td>16</td>
<td>32</td>
<td>51</td>
</tr>
</tbody>
</table>

a. Analysis, after hydrolysis, of 0.1g of complex. Values shown are averages of 3 experiments.

b. Determination on the $^{18}$O-enriched sample (0.4g). Reaction quenched before being sorbed on column. See, also, footnotes to Table 4.8.

c. Solution of complex, hand-mixed with a ten-fold excess of NaOH.

The order of elution of products was as shown in Table 4.5: trans isomer, cis isomer, then chelate. Table 4.6 gives absorption maxima and extinction coefficients, where possible, of the products. Spectra of the aquo species were obtained by acidifying solutions of the corresponding hydroxo...
species to pH 2 with HClO₄.

The acidified eluant fractions of both isomers when sorbed on 8⁻-form resin, were immobile with 2M NaClO₄ (pH 2) eluant, i.e., the complexes behaved as though there were no charges, showing that there were no charge assignment made.

### TABLE 4.6
**Visible Absorption Spectral Properties of the Products of Hydrolysis of cis-[Co(en)₂OH(β-alalOCH(CH₃)₂)]²⁺**

<table>
<thead>
<tr>
<th>Complex</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt; (nm)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>ε&lt;sub&gt;max&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>trans-[Co(en)₂OH(β-alalO)]&lt;sup&gt;+&lt;/sup&gt;</td>
<td>500</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>-</td>
</tr>
<tr>
<td>trans-[Co(en)₂H₂O(β-alalOH)]&lt;sup&gt;3+&lt;/sup&gt;</td>
<td>495</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>345</td>
<td>-</td>
</tr>
<tr>
<td>cis-[Co(en)₂OH(β-alalO)]&lt;sup&gt;+&lt;/sup&gt;</td>
<td>500</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>-</td>
</tr>
<tr>
<td>cis-[Co(en)₂H₂O(β-alalOH)]&lt;sup&gt;3+&lt;/sup&gt;</td>
<td>490</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>352</td>
<td>-</td>
</tr>
<tr>
<td>[Co(en)₂(β-alalO)]&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>495</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>-</td>
</tr>
</tbody>
</table>

---

**a.** Solutions measured at 25.0° in NaClO₄ (0.4-2.0M).

**b.** Error in λ = ±2nm. Below 400nm, it is ±3nm, due to absorbance by organic material from the resin; similarly, error in ε=±2; at the lower wavelengths, it is much greater, for the same reason and, thus, not included here.

Charge assignments have been made on the basis of elution speed relative to the well-authenticated β-alanine chelate. The absorption maxima and extinction coefficients of the products agree well with those obtained on base hydrolysis...
of \([\text{Co(en)}_2(\text{S-alaO})]^{2+}\), (Table 3.2). The acidified eluant fractions of both isomers when sorbed on \(H^+\)-form resin, were immobile with 2M NaClO\(_4\) (pH\(\approx\)2) eluant, i.e., the complexes behaved as 3+ ions, showing that there were two deprotonable groups present in the pH range, 2 to 8.

The following observations also support the assignments made.

(1) Spectra of the first eluant fraction, in 0.2M HC10\(_4\) (\(\mu=0.5\), NaClO\(_4\), 25.0°), changed only slowly with time. (See Fig. 4.3). An estimate of \(t_{1/2} \approx 40\) days was obtained (\(k_{\text{obsd}} \approx 2 \times 10^{-7}\) sec\(^{-1}\)). This rate is \(\approx 100\) times slower than that for the characterized cis isomer in acid (see Table 4.2), indicating that the first band was the trans isomer.

(2) The spectra of acidified and basic solutions (Fig. 4.4) of the two bands show features which are consistent with cis and trans configurations. The \(^1T_{1g}\) state of regular octahedral symmetry is split when this symmetry is lowered, but to a greater extent in a trans complex than in a cis complex. Thus, the trans complex is expected to have a less symmetrical absorption band near 500nm than the cis complex. Also, because the cis isomer lacks a centre of symmetry, it is expected to have absorption bands of greater intensity [194]. The acidified solution of the first band shows an unsymmetrical band at 495nm, with an extinction coefficient that is lower than that of the second band and thus is characterized as the trans isomer.
Fig. 4.3. Spectra throughout cis-trans isomerization and subsequent ring closure in trans-[Co(en)$_2$H$_2$O($\beta$-alaOH)]$^{3+}$ in 0.2M HClO$_4$ ($\mu = 1.0$, NaClO$_4$, 25.0°C).
Fig. 4.4. Visible absorption spectra of
(a) trans-\([\text{Co(en)}_2\text{H}_2\text{O}(\text{6-alaOH})]^3+\) & trans-\([\text{Co(en)}_2\text{OH}(6-\text{alaO})]^3+\); (b) cis-\([\text{Co(en)}_2\text{H}_2\text{O}(6-\text{alaOH})]^3+\) & cis-\([\text{Co(en)}_2\text{OH}(6-\text{alaO})]^3+\). (0.5M NaClO\_4; 0.01M).
The corresponding basic solutions show the same features.

(3) Finally, the second eluate, in acid solution, reacts with a rate corresponding to that of the characterized cis isomer, as seen by the last entry of Table 4.2.

Table 4.7 shows base consumption data over a range of pH for hydrolysis of cis-[Co(en)$_2$OH(β-alaOCH(CH$_3$)$_2$)]$^{2+}$, determined from base uptake on the radiometer.

<table>
<thead>
<tr>
<th>pH</th>
<th>Fraction [Co(en)$_2$(β-alaO)]$^{2+}$ produced</th>
<th>Equivs base consumed/mole complex.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Obsd</td>
</tr>
<tr>
<td>10.4</td>
<td>0.62</td>
<td>1.34</td>
</tr>
<tr>
<td>11.3</td>
<td>0.58</td>
<td>1.35</td>
</tr>
<tr>
<td>11.7</td>
<td>0.58</td>
<td>1.43</td>
</tr>
</tbody>
</table>

a. Determinations on 0.2-0.5g sample; $\mu$=1.0, NaClO$_4$; 25.0°.

One equivalent of base is required for deprotonation of the complex to produce the reactant hydroxo ester complex. Within experimental error it is seen that the remaining fraction of base is consumed in the formation of the hydroxo complexes, which must therefore have an overall +1 charge. The stoichiometric equations for the formation of the
products are given below.

\[
[\text{Co(en)}_2\text{OH}(\beta-\text{alaOCH(CH}_3)_2)]^{2+} + \text{OH}^- \rightarrow [\text{Co(en)}_2(\beta-\text{alaO})]^{2+} + \text{HOCH(CH}_3)_2\text{OH}^- \quad (4.3)
\]

\[
[\text{Co(en)}_2\text{OH}(\beta-\text{alaOCH(CH}_3)_2)]^{2+} + \text{OH}^- \rightarrow [\text{Co(en)}_2\text{OH}(\beta-\text{alaO})]^{2+} + \text{HOCH(CH}_3)_2\text{OH}^- \quad (4.4)
\]

Formation of the chelate requires no base, as seen in equation (4.3) whereas that of the hydroxo products does.

The observed rate law is first order in [OH\text{\textsuperscript{−}}] for formation of all products. This means that the ratio of products formed is independent of pH and that the formation of each product, individually, also obeys a rate law, first order in [OH\text{\textsuperscript{−}}]. This is the case, on the whole, over the pH range 10.4 to 12.3 but certainly does not hold at higher pH's where reactant solutions were mixed with excess NaOH. (See Table 4). There are two reasons for this. Firstly, in strongly basic solution, the cis isomer and β-alanine chelate themselves then react, as shown in Chapter 3; the former isomerizes to a small degree and the latter undergoes base hydrolysis to form both cis- and trans-hydroxo β-alaninato complexes. These reactions, however, are much slower than that being observed and do not interfere with the spectrophotometric rates to any great degree. Secondly, wherever the product solution was just diluted and sorbed on Na\textsuperscript{+}-form resin, decomposition continued to occur, but to a far lesser extent, with resultant increase in the percentage of trans product but with no apparent variation in the amount of cis product. This was
seen in the experiment with the $^{18}$O-enriched sample which was quenched to pH ~7 after 7 half-lives before being sorbed on Na$^+$-form resin (No(8), Table 4.5). The degree of decomposition of the β-alanine chelate is reduced, compared with experiments at similar pH.

The analyses from pH 10.4 to 11 may be taken to represent the product distribution over the entire pH range studied, when the above factors are taken into account. As may be expected, very little trans hydroxo β-alaninato product is formed in the reaction and the major products are those resulting from chelate ring formation or from hydrolysis of a monodentate ester function.

The reaction is shown below.

\[
\text{Prep. of labelled complex (1) Reaction with OH}^{-} \xrightarrow{k''} (\text{en})_2\text{Co(OH)}((\text{β-alanO}) + \text{ROH}
\]

\[
\text{cis} (33\%) + \text{trans} (\sim 7\%)
\]

Fig. 4.5. Product distribution in base hydrolysis of cis-\{(\text{en})_2\text{Co(OH)(β-alanOCH(CH}_3\text{)}}_2\}^{2+}; \mu = 1.0$, NaClO$_4$; 25.0$^\circ$.

The observed rate constant (0.28 M$^{-1}$ sec$^{-1}$) may be divided according to the product distribution shown in Fig. 4.5, with $k'_{OH}$(chelated acid) $\sim 0.17$ M$^{-1}$ sec$^{-1}$ and $k''_{OH}$(cis- + trans-hydroxo acids) $\sim 0.11$ M$^{-1}$ sec$^{-1}$.
4.3.4 $^{18}$O tracer experiments

Table 4.8 presents data for the water exchange rate and for hydrolysis, in acidic and basic conditions, of $^{18}$O-labelled $\text{cis-}[\text{Co(en)}_2\text{H}_2\text{O}(\beta\text{-alaOCH(CH}_3)_2)](\text{NO}_3)_3$. The $^{18}$O enrichment, in atom %, was calculated using the formula [186],

$$%^{18}O = \frac{100R}{2 + R} - N$$

where $R$ is the 46/44 ratio obtained from the mass spectrometer measurements and $N$ is the atom % $^{18}$O in CO$_2$ of normal isotopic composition (0.1936). Two separate samples, (1) and (2), were used in the experiments.

**TABLE 4.8**

Results of Experiments on Aquo-$^{18}$O-Labelled $\text{cis-}[\text{Co(en)}_2\text{H}_2\text{O}(\beta\text{-alaOCH(CH}_3)_2)](\text{NO}_3)_3$.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Cpd analyzed</th>
<th>$%^{18}$O</th>
<th>$%^{18}$O corr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prep. of labelled complex (1)</td>
<td>Reaction soln.</td>
<td>1.045</td>
<td>1.045</td>
</tr>
<tr>
<td></td>
<td>Complex (1)</td>
<td></td>
<td>0.71$^b$</td>
</tr>
<tr>
<td>Base hydrolysis (1)$^c$</td>
<td>[Co(en)$_2$(β-alaO)]$^{2+}$</td>
<td>0.321</td>
<td>0.380</td>
</tr>
<tr>
<td>Prep. of labelled complex (2)</td>
<td>Reaction soln.</td>
<td>1.240</td>
<td>1.240</td>
</tr>
<tr>
<td></td>
<td>Complex (2)</td>
<td></td>
<td>0.837</td>
</tr>
<tr>
<td>Acid hydrolysis (2)$^d$</td>
<td>[Co(en)$_2$(β-alaO)]$^{2+}$</td>
<td>0.186</td>
<td>0.355</td>
</tr>
<tr>
<td>Unreacted (2) of acid</td>
<td>[Co(en)$_2$(β-alaO)]$^{2+}$</td>
<td>0.289</td>
<td>0.289</td>
</tr>
</tbody>
</table>

Note: $^a$ Two samples used, as described in Section 4.2.7; $u = 1.0, \text{NaClO}_4; 25.0^\circ$.

$^b$ Calculated on the assumption that the $%$ incorporation of label in complex (1) is the same as that in (2); i.e., $1.045 \times (0.837/1.240)$.

$^c$ At pH 12, all of the chelated ester impurity (10%) was hydrolyzed and appeared in the 65$%$ chelate product. Hence, the corrected value is $0.321 \times (65/55)$.

$^d$ Quenched after 17hrs. During this time, all of the chelated ester impurity (10%) had reacted ($t_k = 4$ hrs; Table 2.2) to form the chelated acid. Thus, of the 21$%$ formed, 11$%$ was derived from the aquo ester. The corrected value is $0.186 \times (21/11)$.

$^e$ Determined at $t = 18$ hrs.
As explained in Footnote \( d \) of Table 4.8, under the experimental conditions of acid hydrolysis, the chelated ester impurity was also hydrolyzed and appeared in the recovered \([\text{Co(en)}_2(\beta-\text{alaO})]\text{Cl}_2\). Calculation of the expected amount of chelate produced from the aquo ester complex after 17hrs, using \( t_{1/2} = 100\text{hrs} \) (Table 4.2) gives a value of 11.1\%, in agreement with the corrected amount of chelated acid produced in this experiment. Similarly, for the base hydrolysis, at pH \( \approx 12 \), the 10\% chelated ester impurity was converted to the chelated acid. A blank determination for the starting material, (1), has been computed, since insufficient starting material was available for an experimental determination to be made.

The last experiment of Table (4.8) gives an estimate of the \( \text{H}_2\text{O} \) exchange rate in acid solution. Assuming a first-order rate law, by analogy with related studies [171], the rate constant for exchange \( k_{\text{ex}} \), is given by

\[
\ln \frac{1}{1-x} = k_{\text{ex}} t
\]

where \((1-x)\) is the proportion of labelled complex remaining after time, \( t \), and, from the data, is \((2 \times 0.289)/0.837\); i.e., 67\% of the complex is still labelled after 18hrs. The factor of 2 is inserted because 2 O's are present in the product being analyzed whereas only one is present in the starting material. Hence, \( k_{\text{ex}} = 6.3 \times 10^{-6} \text{sec}^{-1} \) (\( t_{1/2} = 3\text{hrs} \)).

The derivation of the rate equations in this system is set out in the Appendix, with calculations made for expected enrichment, assuming that bound water is the nucleophile. After 17hrs, it is calculated that 29\%
unlabelled aquo ester starting material should be present and that 76% labelled complex should be formed. The experimental values are 33% and 84%, respectively and agree with those calculated within experimental error. Hence, the proposal that acid hydrolysis proceeds by intramolecular attack of bound H₂O at the ester is supported by the results. The results show that base hydrolysis of the aquo ester complex also proceeds with retention of label ((2 × 0.38)/0.71).

The % enrichment in the products of hydrolysis has been based on the figure for the ¹⁸O-labelled starting material, rather than on that for the solvent. Strictly, these two values should be the same, but it is seen that the value for the enriched material is only 67% of that for the reaction solution from which it was isolated. This loss may be due either to water exchange of the solid sample with the atmosphere, or, more likely, of exchange of label with NO₃⁻ anion, while the sample was being dried under vacuum, before the blank determination and hydrolysates were carried out.

By calculation of the % enrichment in the products of both acid and base hydrolysis in the manner described, it is seen that both reactions proceed with retention of label; that is, that hydrolysis occurs by attack of bound water or hydroxide ion at the substrate ester in an intramolecular process.

4.3.5 Experiments on (H)₅₉⁹-Co(ën)₂Br(β-alαOCH(CH₃)₂)Br₂

Fig. 4.6 gives a flow chart for the reactions studied. In Table 4.9 are molar rotations for product
Fig. 4.6. Retention of configuration on Hg$^{2+}$-catalyzed removal of bromide from $(\theta)_{569}$-cis-[$Co(en)_2Br(\beta$-alaOCH(CH$_3$)$_2$)]$^{2+}$ and subsequent hydrolysis of the products.

**TABLE 4.9**

Molar Rotations (deg.$\cdot$m$^{-1}$ · cm$^{-1}$) of Solutions after Hydrolysis of $\beta$-alanine Complexes, at 25.0°C.

<table>
<thead>
<tr>
<th>$\lambda$(nm)</th>
<th>$\theta$(A + C)$^a$</th>
<th>$\theta$(B + C)$^b$</th>
<th>Authentic$^d$ [Co(en)$_2$(B-alaO)]$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>589</td>
<td>+920</td>
<td>+920</td>
<td>+920</td>
</tr>
<tr>
<td>546</td>
<td>+1480</td>
<td>+1260</td>
<td>+1270</td>
</tr>
<tr>
<td>436</td>
<td>-1710</td>
<td>-1300</td>
<td>-1240</td>
</tr>
</tbody>
</table>

$^a$ Acid hydrolysis experiment.

$^b$ Base hydrolysis experiment.

$^c$ Smaller values (96%), relative to authentic material, may be due to the presence of some cis-chloro $\beta$-alanine complex, formed during hydrolysis.

$^d$ The $\Delta\theta$ configuration would give exactly the same values at the given wavelengths, but with opposite sign.
solutions of the reactions shown, together with that of optically pure $\left[\text{Co(en)}_2 (\beta\text{-alaO})\right]^{2+}$, in the same media. The data show that both the aquo ester complex and the chelated ester are formed from the resolved bromo ester with complete retention of configuration and that subsequent hydrolysis in acid and base, of both complexes, proceeds with complete retention of configuration.

4.4 Discussion

4.4.1 Properties of the complexes

The positions of the absorption maxima and magnitudes of extinction coefficients for the aquo and hydroxo β-alanine complexes are characteristic of octahedral low-spin $d^6$ Co(III) complexes. The spectra of the monodentate acid and ester complexes are very similar. This is not unexpected, since the acid and ester groups are several atoms removed from the metal centre. (For both aquo complexes, $\lambda_{\text{max}} = 490\text{nm}$ ($\epsilon = 72$) and for both hydroxo complexes, $\lambda_{\text{max}} = 500\text{nm}$ ($\epsilon = 92$)). When β-alanine is chelated, however, the presence of the ester group, compared to the acid, does have an effect (see Chapter 2), for it is now closer to the coordination sphere. (In the ester chelate, $\lambda_{\text{max}} = 490\text{nm}$ ($\epsilon = 99$) and in the acid chelate, $\lambda_{\text{max}} = 495\text{nm}$ ($\epsilon = 128$)). Presumably, electron delocalization is made possible, once the ester function is lost, so that the energy of the electronic transition becomes smaller and the intensity increases.

The presence of the ester function in the monodentate complexes, $\text{cis-[Co(en)}_2 \text{H}_2\text{O(β-alaOR)\}^{3+}}$, has little effect on the acid strength of coordinated $\text{H}_2\text{O}$ ($pK_a = 6.06$ when
R = CH(CH₃)₂; pKₐ = 6.46 when R = H. The pKₐ values determined in this work are similar to those of similar aquo complexes. For example, Tobe has found that the corresponding aquo amine complex, cis-[Co(en)₂H₂O(NH₃)]³⁺ has pKₐ = 6.1 at 20°C [188].

4.4.2 Acid hydrolysis of cis-[Co(en)₂H₂O(β-alaOR)]³⁺

Oxygen tracer experiments, coupled with work on resolved complexes, provide valuable information about the mechanism of acid-catalyzed hydrolysis of the complex species, cis-[Co(en)₂H₂O(β-alaOR)]³⁺. Most important is the result, from the tracer experiment, that coordinated H₂O is the nucleophile attacking the carbonyl carbon centre, to effect ring closure and ester hydrolysis. The reaction is independent of pH in the range 0–2. A suggested mechanism for the reaction may be one involving rate-determining hydrolysis of the monodentate ester function, followed by more rapid ring closure of the resulting cis-aquo acid. (See Table 4.2). This, however, is not considered very likely, for the following reason. Free β-alanine ester hydrochloride is prepared at high temperatures over a period of 3hrs (100°C; Section 2.2.2), and so it cannot be hydrolyzed at the same time. At 25°C, it would not, then, be expected to be hydrolyzed with a half-life of 100hrs, under much milder conditions. A preferred mechanism is set out in (4.5). On formation of the tetrahedral intermediate, the pKₐ of the first proton will drop well below the initial value of 6.06, while the acidity of the second should also be enhanced. By analogy with Chapter 2, a six-membered ring, incorporating a solvent H₂O, is proposed to assist in the protonation of the -OR.
function, making for a far better leaving group. As shown in the sequence (4.5), the ring is made to include the proton attached to the coordinated oxygen, but it may just as well include the proton of the solvent. The conversion of one to the other would merely involve a proton transfer. If the latter is the case, the intermediate becomes identical with that proposed for acid hydrolysis of the chelated ester (Chapter 2). If a common intermediate exists, $k_2$ cannot be rate-determining in the present case, since, otherwise, the same rate would be observed for acid hydrolysis of the chelated ester and of the aquo ester complex. Hence, the intramolecular nucleophilic attack of $H_2O$ ($k_1$) is rate-
determining in the sequence (4.5).

Studies on the resolved complex show that the intramolecular reaction proceeds with retention of configuration about the cobalt atom, even though oxygen exchange occurs at a rate that is four times greater than that of ring closure. The first result is in agreement with retention of all six bonds to the metal atom. The second result requires water exchange to occur without racemization. The agreement between molar rotations of the product in acid hydrolysis and authentic $\text{H}_{589}-[\text{Co(en)}_2(\beta-\text{alaO})]^2^+\text{ also show that no isomerization to the trans-hydroxo ester has occurred.}$

The rate constant for solvent exchange in $\text{cis-}[\text{Co(en)}_2\text{H}_2\text{O}(\beta-\text{alaOCH(CH}_3)_2\text{})]^3^+\text{ (}k_{\text{ex}}=6.3 \times 10^{-6}\text{ sec}^{-1}\text{) is similar to that for } [\text{Co(NH}_3)_5\text{H}_2\text{O}]^3^+\text{ (}k_{\text{ex}}=5.9 \times 10^{-6}\text{ sec}^{-1}\text{)} [189]$ and for $\text{cis-}[\text{Co(en)}_2(\text{H}_2\text{O})_2]^3^+\text{ (}k_{\text{ex}}=7.5 \times 10^{-6}\text{ sec}^{-1}\text{)} [171]$ and not very different from that for $\text{cis-}[\text{Co(en)}_2(\text{NH}_3)_2\text{H}_2\text{O}]^3^+\text{ (}k_{\text{ex}}=10^{-6}\text{ sec}^{-1}\text{)} [190]. (This last value is not very accurate, due to experimental difficulties). Also, racemization and isomerization in $\text{cis-}[\text{Co(en)}_2(\text{NH}_3)_2\text{H}_2\text{O}]^3^+$ are similar to those of the $\text{cis-aquo }\beta\text{-alanine ester complex.}$ Martin and Tobe have obtained an extrapolated value for racemization rate in $\text{cis-}[\text{Co(en)}_2(\text{NH}_3)_2\text{H}_2\text{O}]^3^+\text{ (}k = 3 \times 10^{-8}\text{ sec}^{-1}; 25.0^\circ\text{), which is about 30 times slower than the exchange rate, and have observed that only 1% isomerization occurs during exchange [190]. In the present system, the observed retention of configuration during solvent exchange requires the racemization rate to be $< 6 \times 10^{-7}\text{ sec}^{-1}\text{ (i.e., at least a factor of 10 slower than exchange).}$

An additional result obtained from studies on the resolved complexes is that acid hydrolysis of the chelated
ester (path C of Fig. 4.6) also proceeds with retention of configuration about the metal. This is in accord with no Co-O bond cleavage during hydrolysis.

Ring closure of the cis-aquo acid complex has a rate that is 10 times faster than that of the corresponding ester (Table 4.2). This may be due to the enhanced electrophilic character of the carbonyl carbon adjacent to a proton compared to an alkyl group [189] or to decreased steric crowding, but, with presently known facts, no convincing rationalization can be made for such a process.

4.4.3 Base hydrolysis of cis-[Co(en)$_2$OH(β-alalOCH(CH$_3$)$_2$)]$^{2+}$

Oxygen tracer work indicates that, in base hydrolysis of cis-[Co(en)$_2$OH(β-alalOCH(CH$_3$)$_2$)]$^{2+}$, the product, the [Co(en)$_2$(β-alalO)]$^{2+}$ ion, results from intramolecular nucleophilic attack of bound hydroxide at the carbonyl centre of the ester. It is then reasonable to propose the formation of a tetrahedral intermediate during hydrolysis and, provided that proton exchange is fast, this implies that the cis-hydroxo β-alanine ester and the chelated ester form a common intermediate. This is depicted in a reaction profile (Fig. 4.7).

![Fig. 4.7. Reaction profile for hydrolysis of monodentate β-alanine ester, ME, and chelated β-alanine ester, CE, via a common intermediate, I, to produce the chelated acid, CA.](image-url)
The lower energy of the chelated ester compared with the monodentate ester is in accord with the observation that chelated complexes are generally more stable than their non-chelated analogues [223,224]. The higher energy barrier for reversion of intermediate to monodentate ester may be attributed to the energy required to open the six-membered chelate ring.

It is seen, in Chapter 2, that base hydrolysis of the chelated ester has a second order rate constant, $3 \times 10^4 \text{M}^{-1} \text{sec}^{-1}$. For the hydroxo ester complex, however, the much smaller rate constant (0.28M$^{-1}$ sec$^{-1}$) requires the rate-determining step to be formation of the intermediate; that is, nucleophilic attack of bound hydroxide is rate-determining. It remains to propose a mechanism for the intramolecular hydrolysis, that requires intervention of OH$^-$ for the formation of the tetrahedral intermediate.

Experimental findings eliminate a mechanism whereby solvent OH$^-$ attacks the carbonyl carbon to effect hydrolysis before ring closure occurs. (As already observed (Chapter 3), the cis-hydroxo β-alaninato species produces ≤5% of the chelated acid, at pH > 12, over several hours, and is rather decomposed to the dihydroxo species. At pH = 9.39 (Table 4.3, Footnote c) the reaction is very slow indeed ($k_{OH} \sim 0.025 \text{M}^{-1} \text{sec}^{-1}$) leading to the chelated acid).

A mechanism which is consistent with the observations is shown in (4.6). The derivation for the rate law is given in the Appendix. It assumes the steady state approximation for the concentration of the deprotonated ester complex, $E$. 
Another considered mechanism for the reaction is one involving a concerted process, which may occur readily possible because of the slight stabilization of the zwitterion by the chelate ring. This would require that (1) the active site undergoes a large change in $pK_a$ in the course of the reaction and (2) bonded $pK_a$ of the catalyst lie between the initial and final $pK_a$ of the active centre [219]. In the present system, the $pK_a$ of the catalyst is 14 and that of the intermediate I would be well below 6. An estimate of 4 has been made for the $pK_a$ of the starting complex, by analogy with similar organic systems [119], so that the acid-base rule appears to be satisfied. However, in this case, the intermediate and transition states may be stabilized by the presence of Co(III)[196] so that a path through the deprotonated intermediate of (4.6) might well be accessible.

Results from the optically active $\eta^3$-cis-[Co(en)$_2$O(5-AlaOCH(CH$_3$)$_2$)]$^{2+}$ species indicate that no racemization occurs during chelate ring formation in alkaline solution. This is in accord with an intramolecular $\text{C}-\text{O}$ bond cleavage.

The rate law is

$$k_{\text{obsd}} = k^\# [\text{OH}^-]$$

(4.7)

where $k^\#$ is a composite first order rate constant.

One aspect of the proposed mechanism, (4.6), is noteworthy. The backward path from the deprotonated intermediate I, to the hydroxo species, E, may be neglected, since no hydroxo species was formed during base hydrolysis of the chelated ester. Indeed, such a process involving ring opening is not expected to occur when there is an alternate path, in which the chelate ring persists.
Another considered mechanism for the reaction is one involving a concerted process, which might avoid possible formation of highly unstable intermediates [218]. The concerted step would require that (1) the active site undergo a large change in pK$_a$ in the course of the reaction and (2), the pK$_a$ of the catalyst lie between the initial and final pK$_a$ of the active centre [219]. In the present system, the pK$_a$ of the catalyst is 14 and that of the intermediate, I, would be well below 6. An estimate of ~20 has been made for the pK$_a$ of the starting complex, by analogy with organic systems [168], so that the *ad libido* rule above appears to be satisfied. However, in this case, the intermediate and transition states may be stabilized by the presence of Co(III) [159] so that a path through the deprotonated species, E, of (4.6) might well be accessible.

Results from the optically active \( \theta_{\text{cis}} \text{cis-}[\text{Co(en)}_2\text{H}_2\text{O}(\beta\text{-alaOCH(CH}_3)_2)]^{2+} \) species indicate that no racemization occurs during chelate ring formation in alkaline solution. This is in accord with an intramolecular process without Co-O bond cleavage.

It is interesting, now, to look at the overall reaction for base hydrolysis of the *cis*-hydroxo ester complex. From product analyses (Table 4.5), it is clear that solvent competes with bound hydroxide for the monodentate ester, but about 67% as effectively; 40% hydroxo β-alaninato complex is formed, while 60% chelate is formed. The *trans*-hydroxo β-alaninato product (~7%) is most likely to have been formed from isomerization of both the *cis*-hydroxo acid and ester complexes during the reaction.
4.4.4 General remarks

Table 4.10 lists hydrolysis constants for reactions leading to the β-alanine chelate.

**TABLE 4.10**

Hydrolysis Rates for Formation of [Co(en)$_2$(β-alaO)]$^{2+}$ from Complexes, A; $\mu = 1.0, NaClO_4$; 25.0°.

<table>
<thead>
<tr>
<th>A (R = CH(CH$_3$)$_2$)</th>
<th>$k_{H_2O}$(M$^{-1}$ sec$^{-1}$)</th>
<th>$k_{OH}$(M$^{-1}$ sec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="ClO$_4$">Co(en)$_2$(β-alaOR)</a>$_3$</td>
<td>$8.3 \times 10^{-7}$</td>
<td>$3 \times 10^4$</td>
</tr>
<tr>
<td>cis-<a href="NO$_3$">Co(en)$_2$H$_2$O(β-alaOR)</a>$_3$</td>
<td>$3.2 \times 10^{-8}$</td>
<td>$0.17$</td>
</tr>
</tbody>
</table>

Although hydroxide seems to be more effective in promoting hydrolysis in both complexes (Table 4.10), the relative magnitudes are vastly different. This may be a consequence of the greater susceptibility of the substrate ester to hydrolysis in basic conditions compared with acidic conditions, when it is activated by the metal ion. Another comparison may also be made. The ratios, $k_{H_2O}$ and $k_{OH}$ of chelate to monodentate ester are, respectively, 26 and $1.8 \times 10^5$. These figures may indicate that solvent H$_2$O is somewhat more effective in promoting hydrolysis at the activated substrate than is bound water at the dangling substrate, but that the basic conditions are far more effective. No direct correlation of nucleophilicity with base strength [192,193] can be made at all in this case, for other factors, such as reactivity of substrate and energy factors associated with ring closure are more important.
The second order rate constants for base hydrolysis of the free N-protonated ester and of the monodentate β-alanine ester are, respectively, ~1 M$^{-1}$ sec$^{-1}$ (Table 2.7) and 0.17 M$^{-1}$ sec$^{-1}$. Thus, there is little difference in the rate of ester hydrolysis when Co(III) is bound to the amino group of β-alanine ester (monodentate) or when a proton is attached to the amino group. This situation is similar for an analogous glycine complex, [Co(NH$_3$)$_5$(glyOC$_2$H$_5$)]$^{3+}$, where base hydrolysis to the monodentate acid complex proceeds with a rate that is not very different from that of the N-protonated ester [114,184]. On the other hand, intramolecular hydrolysis, in base, of the cis-hydroxo glycine ester complex, where bound hydroxide is the nucleophile, proceeds much more rapidly than solvent hydroxide-promoted hydrolysis of the monodentate ester, since no [Co(en)$_2$OH(glyO)]$^+$ is formed [108]. Although five- and six-membered chelate rings have different stabilities, the differences in rate between the glycine and β-alanine systems cannot be due to an unfavorable pre-equilibrium associated with the rate constant for formation of the tetrahedral intermediate. This prospect has already been eliminated by showing that formation of the tetrahedral intermediate is rate-determining. The most likely cause for the difference may arise from the differences in the rate, associated with ring formation. This would comprise two factors. Firstly, a small kinetic chelate effect [196] would be operative; this would contribute to the entropy of activation, $\Delta S^+$, which relates to the probability of collisions between reacting species [212]. Secondly, there may be a contribution to the enthalpy of
activation, $\Delta H^+$, associated with the strain in forming the chelate ring. Kustin and co-workers have argued that ring strain associated with six-membered $\beta$-alanine chelate ring formation is indeed greater than that with five-membered chelate ring formation for $\alpha$-alanine, and have noticed, also, a small difference in the chelate effect for the two systems [164,213].

In the glycine ester system, base hydrolysis seems to be more efficient, in that the major product is the glycine chelate, whereas, in the $\beta$-alanine system, only $60\%$ chelate is formed. For acid hydrolysis, in the glycine ester system, little is known as to whether solvent water is even incorporated into the coordination sphere, but, in the $\beta$-alanine system, the reaction is $100\%$ efficient, leading exclusively to the chelated acid.

Certainly, then, the lower reactivity of $\beta$-alanine derivatives has allowed the isolation of a monodentate ester with a potential nucleophile $cis$ to it and has also made possible the study of both intramolecular and intermolecular paths.
5. **BASE HYDROLYSIS OF cis-[Co(en)₂X₂(S-AlaOR)]²⁺**

### 5.1 Introduction

The mechanism and steric course of base hydrolysis of complexes of the type \([\text{CoN}_5X]^2+\) (N = nitrogen donor ligand; X = I, Br, Cl, NO₃, etc.) have been the subjects of numerous investigations and recent reviews summarize the important features of the reactions [197,198]. The overall process is given by the equation:

\[
[\text{CoN}_5X]^2+ + \text{OH}^- \rightarrow [\text{CoN}_5\text{OH}]^2+ + X^- \quad (5.1)
\]

It is now generally agreed that the mechanism of base hydrolysis involves a dissociative process of a conjugate base. This is called the S_N1CB mechanism, shown below for a pentaamine complex.

\[
[\text{Co(NH}_3)_5X]^2+ + \text{OH}^- \xrightarrow{\text{k}_1} \frac{1}{\text{k}_2} [\text{Co(NH}_3)_4\text{NH}_2X]^+ + \text{H}_2\text{O} \\
\frac{1}{\text{k}_3} \text{k}_3 \\
[\text{Co(NH}_3)_4\text{NH}_2]^2+ + X^- \quad (5.2)
\]

\[
\text{fast} \\
\text{H}_2\text{O} \\
[\text{Co(NH}_3)_5\text{OH}]^2+
\]

The salient features are that:

1. Deprotonation occurs in the initial step of the reaction.
2. Loss of X occurs from the deprotonated reactant to proceed the reaction.
3. The intermediate reacts with a nucleophile, either anionic or neutral, leading to products.

The deprotonation step is usually fast and so metastable species are not observed. There was no significant evidence for base exchange in the reactant, even though X-change was at least tens of times faster than base hydrolysis.
The salient features are that:

1. Deprotonation occurs in the initial step of the reaction;
2. Loss of X occurs from the deprotonated reactant to produce a five-coordinate intermediate, and
3. The intermediate reacts with a nucleophile, either anionic or neutral, leading to products.

The deprotonation step is usually fast and is generally incorporated as a pre-equilibrium [181]. It can, however, be rate-determining, as was asserted for base hydrolysis of [Co(cyclam)Cl₂]⁺ [199]. Considerably more proton exchange occurred in the product than in the reactant. This was interpreted as being a consequence of the base hydrolysis involving a deprotonation step. A more definite experiment is the base hydrolysis of the diastereoisomeric forms (R and S) of α,β-[Co(tetraen)Cl]²⁺. There was no mutarotation in the reactant, even though H-exchange was at least one hundred times faster than base hydrolysis. The product ratio of the hydroxo products, α,β-[Co(tetraen)OH]²⁺, was found to be S/R = 13/6, different from the equilibrium ratio, S/R = 1/9, in favour of the less stable isomer. The rearrangement about the secondary N occurred after Cl⁻ was lost and before H₂O entered the coordination sphere. These results showed that deprotonation was required in the process and were also consistent with the formation of a five-coordinate intermediate [200]. Other evidence for the existence of an intermediate of reduced coordination number comes from stereochemical and competition studies [98, 140, 201]. A common result was obtained with different leaving groups, X [98, 140, 202, 203], and competition between various
nucleophiles occurred. For the system, cis- and trans- [Co(en)$_2$NH$_3$X]$^{2+}$ [140] (X = Cl, Br, NO$_3$, SCN), not only did the stereochemistry of the hydroxo product remain constant, but, in the presence of azide, the competition ratio, azido/hydroxo and the stereochemistry of the azido product remained constant, independent of the leaving group. The results also showed that there is little discrimination between nucleophiles, so it was deduced that the intermediate is highly reactive, with little opportunity for the nucleophile to exert its power. Kinetic studies on the systems referenced above also support this claim, for loss of X$^-$ has been found to be the rate-determining step, with entry of solvent or other competitor occurring at the same rate as ligand loss. If the process is dissociative, it is expected that a sterically crowded complex will be hydrolyzed much more rapidly than one without crowding. The strain will be relieved as the reactant proceeds through the activated complex to the relatively stabilized five-coordinate intermediate. Such a large enhancement in rate ($>10^5$) was observed in base hydrolysis of a series of pentakisalkylamine complexes, relative to [Co(NH$_3$)$_5$Cl]$^{2+}$ [204]. Evidence for ground-state steric effects was seen in a crystal structure of [Co(NH$_2$CH$_3$)$_5$Cl](NO$_3$)$_2$. The Co-N-C angles were in the range 120-124°; that is, distortions were as much as 15° from the expected tetrahedral values [205]. The geometry of the proposed five-coordinate intermediate in base hydrolysis has been discussed by Basolo and Pearson[206]. When the ligand, L, in complex ions, [CoN$_4$LX]$^{2+}$,
contains a potential donor atom, a new possibility arises in base hydrolysis. This donor may compete, in an intramolecular process, with solvent and added anions, for the vacated position in the proposed five-coordinate intermediate to produce a chelate exclusively or as well as the expected hydroxo or anion products. In the base hydrolysis of cis-[Co(en)$_2$X(glyOR)]$^{2+}$ [108], tracer experiments ($^1$H O) indicated that ~50% carbonyl oxygen was incorporated into the coordination sphere and that ~50% solvent was also included.

The aims of this chapter are (i), to establish whether base hydrolysis of cis-[Co(en)$_2$X(β-alaOR)]$_2$ is consistent with the $S_N$$^1$CB mechanism for other amine complexes, (ii), to show that competition between solvent and carbonyl oxygen of the bound β-alanine derivative does occur in base hydrolysis, (iii), to ascertain whether added anions milk both the intermolecular and intramolecular paths in hydrolysis and (iv), to study the stereochemical course of the reaction with the aim of learning something about the possible geometry of the intermediate.

5.2 Experimental

5.2.1 Instrumental and general

Visible spectra were recorded on a Cary 14 spectrophotometer, infrared spectra of nujol mulls on a Perkin-Elmer Model 459 spectrometer and pmr spectra on a Jeol JNM-100MHz Minimar or Varian HA-100MHz, using TMS as external or NaTPS as internal references. Cobalt estimations were made using a Techtron AA4 atomic absorption spectrometer.
Spectrophotometric rates were obtained on a Cary 16K spectrophotometer by mixing the two reactants in a stopped-flow reactor or by titration of a solution, in the spectrophotometer, with NaOH(1.0M), with the equipment described in Section 2.2.1. Rates were also obtained by measurement of rate of base uptake on the pH-Stat apparatus. The equipment used for this purpose and for pH measurements has also been described in Section 2.2.1. $\alpha_\lambda$ values for optically active complexes were measured at 25.0° in a Perkin-Elmer P22 spectropolarimeter in a 10cm cell.

Bio-Rad Analytical Dowex 50Wx2 (200-400mesh, Na⁺-form) or CM Sephadex C25 (Na⁺-form) cation exchange resin was used for separation of the products of the reactions observed. Analar reagents were used throughout, without further purification.

5.2.2 Preparation of complexes

$[\text{Co(en)}_2\text{CO}_3]\text{Cl}$

The method of Schäfer was used [207]. A stream of CO₂ was bubbled into a mixture of ethylenediamine monohydrate (133ml; 1.64mole) in H₂O (133ml), in an ice bath, for two hours. A solution of CoCl₂.6H₂O (195g; 0.82mole) in H₂O (175ml) was then added, whereupon CO₂ was vigorously evolved and the solution became red-violet in colour. To oxidize the Co(II), 30% H₂O₂ (200ml; 3.9 mole) was added, the solution being continually stirred, mechanically, in an ice bath. In the initial stages, the solution became gelatinous, so that manual stirring was necessary. As the temperature increased to 35°, the solution became more
intensely red. When addition of $H_2O_2$ was complete, the mixture was heated to 75° for 15mins, then cooled to room temperature. Some LiOH (34.4g; 0.82mole) was then added and the rate of addition of $CO_2$ increased, whereupon the temperature rose to 35°. The mixture was then left to stand at room temperature for ~1hr, when crystallization began to occur. The yield was increased by quick addition of methanol (500ml), followed by cooling in an ice bath for ~2hrs, $CO_2$ still being bubbled into the solution. The solid was collected, washed with ethanol and air-dried.


$trans-[Co(en)_2X_2]X$. $X = Br, Cl.$

To a solution of conc. HX (200ml), preheated to 70°, was added $[Co(en)_2CO_3]Cl$ (50g), over a period of lhr. The solution was allowed to stand for 30mins, then cooled in an ice bath for 2hrs. The product was filtered, washed with ethanol, then acetone and dried in an oven (100°) for 3hrs. These complexes were used directly in the following preparations.

$cis-[Co(en)_2X(\beta-alaOR)]X_2$. $X = Br, Cl.$ $R = CH_3, CH(CH_3)_2$.

The halo ester complexes were prepared by a modification of conventional methods [208,209]. The complexes, $cis-[Co(en)_2Br(\beta-alaOCH_3)]Br_2$ and $cis-[Co(en)_2X(\beta-alaOCH(CH_3)_2)]X_2$ were prepared by triturating the corresponding $\beta$-alanine ester hydrochloride (1.0equiv.) with $trans-[Co(en)_2X_2]X$ (1.0equiv.) in the minimum volume of
$\text{H}_2\text{O}$ required to make a paste. A trace of $\text{CoX}_2$ was added to catalyze the reaction. Diethylamine (1 equiv.) was added dropwise and the mixture continuously ground until it was uniformly purple, small aliquots of $\text{H}_2\text{O}$ being added to maintain miscibility. After the reaction mixture was cooled in an ice-bath and ethanol added, the crude product was collected, washed with ethanol and acetone and air-dried. It was then dissolved in a minimum volume of boiling $\text{H}_2\text{O}$, acidified with conc. HX and recrystallized by adding excess NaX and cooling the solution to 0°. It was collected again, washed with ethanol and acetone, then dried in an evacuated desiccator. (Yields > 70%).

**Anal.** Calcd for $[\text{Co(en)}_2\text{Br(β-alaOCH}_3\text{)}]\text{Br}_2$: C, 18.41; H, 4.83; N, 13.42. Found: C, 18.14; H, 4.58; N, 13.51. Calcd for $[\text{Co(en)}_2\text{Br(β-alaOCH(CH}_3\text{)}_2\text{)}]\text{Br}_2$: Co, 10.71; C, 21.09; H, 5.13; N, 12.30; Br, 43.58. Found: Co, 10.89; C, 21.14; H, 5.30; N, 12.25; Br, 43.77. Calcd for $[\text{Co(en)}_2\text{Cl(β-alaOCH(CH}_3\text{)}_2\text{)}]\text{Cl}_2\cdot\frac{1}{2}\text{H}_2\text{O}$: C, 28.22; H, 7.09; N, 16.46; Cl, 25.00. Found: C, 28.61; H, 7.11; N, 16.73; Cl, 25.05.

The following absorption maxima and extinction coefficients were obtained, both in 0.1M HCl and in 1M NaClO$_4$ at 25.0°: 

- $\text{cis-[Co(en)}_2\text{Br(β-alaOCH}_3\text{)}]\text{Br}_2$: 540±2nm ($\varepsilon$ 81±2); $\text{cis-[Co(en)}_2\text{Br(β-alaOCH(CH}_3\text{)}_2\text{)}]\text{Br}_2$: 540±2nm ($\varepsilon$ 81±2); $\text{cis-[Co(en)}_2\text{Cl(β-alaOCH(CH}_3\text{)}_2\text{)}]Cl}_2\cdot\frac{1}{2}\text{H}_2\text{O}$, 527±2nm ($\varepsilon$ 78±2), 367±2nm ($\varepsilon$ 86±2).

The perchlorate salt of $\text{cis-[Co(en)}_2\text{Br(β-alaOCH(CH}_3\text{)}_2\text{)}]^{2+}$ was obtained in the following manner. The corresponding bromide salt (22 g) was dissolved in a minimum volume of
A hot solution of AgClO₄ (16.6g) in a minimum volume of hot H₂O was added in small aliquots, the mixture being kept hot throughout (80°). The AgBr was filtered off and to the filtrate was added excess NaClO₄. The solution was cooled in ice and the purple-red product collected, washed with cold H₂O and ethanol, then dried in an evacuated desiccator.

**Anal.** Calcd for [Co(en)$_2$Br(β-alaOCH(CH₃)$_2$)](ClO₄)$_2$: C, 20.39; H, 4.96; N, 11.89; (ClO₄)$_2$, 29.62. Found: C, 20.72; H, 5.14; N, 12.02; (ClO₄)$_2$, 29.02.

The sample was analyzed for perchlorate gravimetrically, by addition of tetraphenylarsonium chloride to an accurately weighed sample of the complex (0.1g), collecting the precipitate (tetraphenylarsonium perchlorate) and drying to constant weight in an evacuated desiccator.

**cis-[Co(en)$_2$Br(β-alaOH)]Br$_2$**

This complex was prepared by warming a solution of **cis-[Co(en)$_2$Br(β-alaOCH₃)]Br$_2$** (7g) in 7.6M HBr (70ml) at 50° for 15mins, then shaking at room temperature for 70hrs. The volume of the solution was then reduced to 30ml on a rotary evaporator. The solution was cooled in an ice bath and ethanol added. The crystals which separated were washed with ethanol, twice recrystallized from H₂O (80°), with HBr and NaBr added, cooled in an ice bath and the product collected, washed with ethanol and ether and dried in an evacuated desiccator.

**Anal.** Calcd for [Co(en)$_2$Br(β-alaOH)]Br$_2$: Co, 11.60; C, 16.55; H, 4.56; N, 13.79; Br, 47.20. Found: Co, 11.90; C, 16.61; H, 4.69; N, 13.64; Br, 47.30.
From a spectrum between 600 and 300 nm, the absorption maximum in 0.1 M HCl at 25.0 °C was found at 540 ± 2 nm (ε 81 ± 2).

\textit{cis-[Co(en)}_2\text{Br(β-alaNH}_2\text{)]Br}_2\text{]Br}_2

Trans-[Co(en)}_2\text{Br}_2\text{]Br}(12.4 g) was ground to a paste with β-alaNH \text{HBr}(5.0 g) in the minimum volume of H\text{H}_2\text{O}.

Triethylamine (3.0 g) in methanol (15 ml) was stirred in over a period of 1 hr and the mixture then ground for a further hour, when it thickened and became deep purple in colour. Small aliquots of H\text{H}_2\text{O} were added to facilitate grinding. The mixture became solid after 3 hrs. Methanol was then added, the crude product collected and washed with methanol, ethanol and acetone.

The product was recrystallized from a minimum volume of H\text{H}_2\text{O} (80 °C), acidified with HBr. Some NaBr was added, whereupon the product crystallized when the solution was allowed to stand in the refrigerator overnight. It was collected, washed with methanol and ether, then dried in an evacuated desiccator.

Anal. Calcd for [Co(en)}_2\text{Br(β-alaNH}_2\text{)]Br}_2\text{H}_2\text{O}: C, 16.01; H, 4.99; N, 16.00. Found: C, 16.07; H, 5.01; N, 16.07.

The complex was subsequently used in the preparation of the β-alanine amide chelate (Section 2.2.2).

5.2.3 Kinetic measurements

For \textit{cis-[Co(en)}_2\text{Br(β-alaOR)]Br}_2\text{, }R=H, CH_3, CH(CH_3)_2,\text{ spectrophotometric rates were obtained by mixing a solution of the complex }(^{\text{4}}\times 10^{-3} \text{ M})\text{ in 1 M NaClO}_4\text{ with an equal volume of glycine }0.2 \text{ M, }µ=1.0, \text{NaClO}_4\text{ or Tris } (1.0 \text{ M})\text{ buffer }25.0 °\text{C and following absorbance changes at a fixed wavelength.}
Rates were also obtained by measuring rate of base uptake on the pH-Stat. The complex (0.2-0.4g) was dissolved in 1.0M NaClO₄ (~10ml) and titrated with NaOH of accurately known concentration (0.2-1.0M), also with \( \mu = 1.0 \) at 25.0°. Base hydrolysis of the resolved isopropyl ester complex was carried out on the pH-Stat in the same manner and also analyzed as for the unresolved material.

In some experiments, the rate subsequent to bromide removal was also measured by monitoring the absorbance change at \( \lambda = 495 \text{nm} \) (See Chapter 4).

5.2.4 Product analysis

On completion of bromide removal by base hydrolysis on the pH-Stat, indicated by negligible uptake of base, the product solutions were quenched to pH ~8 with HClO₄, diluted and sorbed on Na⁺-form resin. For reactions at pH >12, the complexes (~0.3g) were dissolved in H₂O (~2ml) and mixed with a ten-fold excess of base, (whereupon the pH of the solution was calculated) and also quenched with HClO₄ or CH₃COOH to pH ~8, immediately sorbed on Na⁺-form resin and eluted with 0.5M NaClO₄ (pH ~8), then with 2M NaClO₄. For the ester complexes, elution of bands from the Na⁺-form resin with 0.5M NaClO₄ (pH ~8) indicated that less than 3% of a unipositive species was formed. This was the hydrolysis product of the monodentate ester function, \( \text{cis-}[\text{Co(en)}₂\text{OH(β-alaOH})]⁺ \), formed during bromide removal. It was identified by comparison of spectra and elution rates with the authentic material, prepared from \( \text{cis-}[\text{Co(en) Br(β-alaOH})_{2}]² \). When it was established, in this manner, that only bipositive products were formed under
these conditions, the product solutions of the ester complexes were treated in the following manner. They were quenched to pH ≈ 4 with HClO₄ or CH₃COOH, sorbed on H⁺-form resin and eluted with 2M NaClO₄ at pH ≈ 2 or with 3M HCl. This method had the advantage that the terpositive cis-aquo ester product could be separated more effectively from the bipositive β-alanine chelate product, that shorter columns could be used and hence, that elution rates could be increased. On a Na⁺-form column, both species were bipositive charged at pH ≈ 8. The eluted fractions were analyzed for [Co] by atomic absorption spectrophotometry and characterized by comparison of elution rates and visible spectra (600-300nm) with those of the authentic materials. The presence of cis-[Co(en)₂OH(β-alanOCH(CH₃)₂)]²⁺ as a product after bromide removal was also established by the value of the subsequent rate constant for base hydrolysis, obtained spectrophotometrically. It was identical with that for the authentic material (See Chapter 4).

Wherever cis-[Co(en)₂OH(β-alanO)]⁺ was required for further reaction (see Chapters 3 and 4), it was obtained after elution from the Sephadex resin, where eluants of lower ionic strength were used (0.1-0.2M).

5.2.5 \( pK_a \) determinations

An accurately weighed sample of cis-[Co(en)₂Br(β-alanOH)]Br₂ (0.2-0.4g) in 1.0M NaClO₄ (10ml) was titrated with NaOH of known concentration (0.2-1.0M) at 25.0 °C. The pH was then raised to 10 and maintained there until bromide removal was complete. The solution was then back-titrated with HClO₄ (0.5M). The \( pK_a \) values of the coordinated
water and β-alanine were then estimated by standard methods [118], volume corrections being applied.

5.2.6 Resolution of cis-[Co(en)$_2$Br(β-alacOCH(CH$_3$)$_2$)]Br$_2$

The complex (7.75g), dissolved in H$_2$O, was converted to the acetate salt by the addition of CH$_3$COOAg (5.00g, 2equivs). The solid AgBr was removed, θ-NaAsOtartr. (3.9g) added to the filtrate and washings and the solution then cooled in an ice bath. Several fractions of crystallized material were collected and those with similar optical rotations combined and recrystallized to constant rotation from a minimum volume of hot H$_2$O. The product was converted to the bromide salt by being dissolved in a minimum volume of H$_2$O (90°), acidified with HBr. Excess NaBr was then added and the solution was cooled in an ice bath. The solid was collected, washed with ethanol and acetone, then dried in an evacuated desiccator. A 0.1% solution of the product in H$_2$O gave $\alpha$$_{590}$ = +0.064° and $\alpha$$_{546}$ = -0.038° in a 10cm cell.

5.2.7 Competition with azide

The resolved complex (0.2g) was dissolved in H$_2$O (5ml) and 0.5M NaOH (5ml) and 4M NaN$_3$ (5ml) added. The solution was quenched after 5secs with 1M CH$_3$COOH to pH ~ 5.5. The solution was diluted five-fold, sorbed on a column (1 x 70cm) of Na$^+$-form resin and eluted with 1M NaClO$_4$ (pH ~ 5.5). When the dipositive bands were separated from the stationary terpositive band, the stationary band was transferred to a new column and eluted with 2M NaClO$_4$ (pH ~ 5.5). The bands on the long column were then eluted with 1M NaClO$_4$ (pH ~ 5.5).
As before, visible spectra of the eluted bands were recorded and cobalt estimations made by atomic absorption spectrophotometry.

5.3 Results

5.3.1 Infrared, pmr and visible spectra

Fig. 5.1 shows infrared spectra of the cis-bromo β-alanine acid and ester complexes. As for the cis-aquo β-alanine ester complex, the intense sharp band at 1725 cm⁻¹ is assigned to the stretching mode of the ester carbonyl function in both the methyl and isopropyl ester complexes, while that at 1700 cm⁻¹ is attributed to the stretching mode of the acid carbonyl function in the corresponding acid complex. The broad absorptions near 1560 cm⁻¹ are possibly due to COO⁻ antisymmetric modes.

Table 5.1 gives pmr absorptions for the bromo β-alanine complexes.

**TABLE 5.1**

*pmr Absorptions of cis-\([\text{Co(en)}_2\text{Br(β-alaOR)}]\text{Br}_2* in D₂O. \(^a\)

<table>
<thead>
<tr>
<th>R = H</th>
<th>R = CH₃</th>
<th>R = CH(CH₃)₂</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>1.73 (J = 6Hz)</td>
<td><em>gem-CH₃</em></td>
</tr>
<tr>
<td>3.28(^b)</td>
<td>3.28(^b)</td>
<td>3.28(^b)</td>
<td>-CH₂ of en + β-ala.</td>
</tr>
<tr>
<td>-</td>
<td>4.25</td>
<td>-</td>
<td>CH₃.</td>
</tr>
<tr>
<td>4.6(^c)</td>
<td>4.6(^c)</td>
<td>4.6(^c)</td>
<td>NH₂ of β-ala.</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>5.6(^d)</td>
<td>-CH-</td>
</tr>
<tr>
<td>5.8(^c)</td>
<td>5.8(^c)</td>
<td>5.8(^c)</td>
<td>NH₂ of en.</td>
</tr>
</tbody>
</table>

\(^a\) Ppm downfield from TMS (100MHz).
\(^b\) broad, multiplet.
\(^c\) broad.
\(^d\) multiplet.
Infrared spectra of cis-\([\text{Co(en)}_2\text{Br(alaOR)}])\text{Br} \quad (a), \quad \text{R} = \text{H}, \quad (b), \quad \text{R} = \text{CH}_3, \quad (c), \quad \text{R} = \text{CH(CH}_3\text{)}_2^2.

(Nujol mulls; CsI plates).
TABLE 5.2

Rate Constant Data for Base Hydrolysis of

cis-\([\text{Co(en)}_2\text{Br(\beta-alaOH)})\text{Br}_2\); \( \mu = 1.0, \text{NaClO}_4 \); 25.0°.

<table>
<thead>
<tr>
<th>pH</th>
<th>(\lambda) (nm)</th>
<th>(10^2 k_{\text{obsd}}) (sec(^{-1}))</th>
<th>(k_{\text{Br}}) (M(^{-1}) sec(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) Spectrophotometric data.(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.05</td>
<td>355(^b)</td>
<td>0.081</td>
<td>43</td>
</tr>
<tr>
<td>9.17</td>
<td>361(^c)</td>
<td>0.127</td>
<td>50</td>
</tr>
<tr>
<td>9.20</td>
<td>536(^c)</td>
<td>0.119</td>
<td>44</td>
</tr>
<tr>
<td>9.39</td>
<td>355(^b)</td>
<td>0.188</td>
<td>45</td>
</tr>
<tr>
<td>9.49</td>
<td>361(^c)</td>
<td>0.248</td>
<td>47</td>
</tr>
<tr>
<td>9.52</td>
<td>536(^c)</td>
<td>0.247</td>
<td>44</td>
</tr>
<tr>
<td>10.02</td>
<td>361(^c)</td>
<td>0.837</td>
<td>47</td>
</tr>
<tr>
<td>10.05</td>
<td>536(^c)</td>
<td>0.825</td>
<td>44</td>
</tr>
<tr>
<td>10.30</td>
<td>536(^c)</td>
<td>1.63</td>
<td>48</td>
</tr>
<tr>
<td>10.44</td>
<td>361(^c)</td>
<td>2.06</td>
<td>44</td>
</tr>
<tr>
<td>10.62</td>
<td>355(^d)</td>
<td>3.07</td>
<td>43</td>
</tr>
<tr>
<td>11.24</td>
<td>355(^d)</td>
<td>13.7</td>
<td>46</td>
</tr>
<tr>
<td>11.64</td>
<td>355(^d)</td>
<td>33.5</td>
<td>45</td>
</tr>
<tr>
<td>(II) pH-Stat data.(^f)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.40</td>
<td>-</td>
<td>0.020</td>
<td>48</td>
</tr>
<tr>
<td>8.75</td>
<td>-</td>
<td>0.043</td>
<td>45</td>
</tr>
<tr>
<td>9.00</td>
<td>-</td>
<td>0.075</td>
<td>44</td>
</tr>
<tr>
<td>9.12</td>
<td>-</td>
<td>0.109</td>
<td>48</td>
</tr>
<tr>
<td>9.23</td>
<td>-</td>
<td>0.146</td>
<td>50</td>
</tr>
<tr>
<td>9.38</td>
<td>-</td>
<td>0.175</td>
<td>46</td>
</tr>
<tr>
<td>9.61</td>
<td>-</td>
<td>0.330</td>
<td>47</td>
</tr>
<tr>
<td>9.65</td>
<td>-</td>
<td>0.316</td>
<td>42</td>
</tr>
<tr>
<td>10.30</td>
<td>-</td>
<td>1.60</td>
<td>48</td>
</tr>
</tbody>
</table>

\(a\) [Complex] \(\sim 1 \times 10^{-3}\) to \(2 \times 10^{-3}\) M; pH measurements were made at the conclusion of the reaction for buffer solutions.

\(b\) 0.5M Tris buffer. \(c\) 0.1M glycine-NaOH buffer.

\(d\) 0.25M triethylamine buffer.

\(e\) Solution titrated with NaOH to constant pH.

\(f\) 0.2 to 0.4g complex, titrated with NaOH (0.2 to 1.0M).

\(g\) \(k_{\text{Br}} = k_{\text{obsd}}/ [\text{OH}^-]\).
TABLE 5.3

Rate Constant Data for Base Hydrolysis of

cis-[Co(en)$_2$Br($\beta$-alaOCH(CH$_3$)$_2$)]Br$_2$; $\mu$ = 1.0, NaClO$_4$; 25.0$^\circ$.

<table>
<thead>
<tr>
<th>pH</th>
<th>$\lambda$ (nm)</th>
<th>$10^2 k_{obsd}$ (sec$^{-1}$)</th>
<th>$k_{Br}$ (M$^{-1}$sec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I)</td>
<td>Spectrophotometric data.$^a$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.80</td>
<td>536$^b$</td>
<td>0.076</td>
<td>69</td>
</tr>
<tr>
<td>8.86</td>
<td>361$^c$</td>
<td>0.079</td>
<td>65</td>
</tr>
<tr>
<td>9.23</td>
<td>536$^d$</td>
<td>0.199</td>
<td>69</td>
</tr>
<tr>
<td>9.25</td>
<td>361$^d$</td>
<td>0.210</td>
<td>69</td>
</tr>
<tr>
<td>9.37</td>
<td>536$^b$</td>
<td>0.257</td>
<td>72</td>
</tr>
<tr>
<td>9.38</td>
<td>355$^c$</td>
<td>0.263</td>
<td>65</td>
</tr>
<tr>
<td>9.55</td>
<td>361$^d$</td>
<td>0.418</td>
<td>69</td>
</tr>
<tr>
<td>10.06</td>
<td>361$^d$</td>
<td>1.29</td>
<td>66</td>
</tr>
<tr>
<td>10.50</td>
<td>361$^d$</td>
<td>3.52</td>
<td>66</td>
</tr>
<tr>
<td>10.58</td>
<td>355$^e$</td>
<td>4.35</td>
<td>68</td>
</tr>
<tr>
<td>11.30</td>
<td>355$^e$</td>
<td>23.5</td>
<td>70</td>
</tr>
<tr>
<td>(II)</td>
<td>pH-Stat data.$^f$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.98</td>
<td>-</td>
<td>0.104</td>
<td>66$^h$</td>
</tr>
<tr>
<td>9.00</td>
<td>-</td>
<td>0.113</td>
<td>66$^h$</td>
</tr>
<tr>
<td>9.08</td>
<td>-</td>
<td>0.140</td>
<td>72</td>
</tr>
<tr>
<td>9.37</td>
<td>-</td>
<td>0.246</td>
<td>72</td>
</tr>
<tr>
<td>9.91</td>
<td>-</td>
<td>0.880</td>
<td>65</td>
</tr>
</tbody>
</table>

$^a$ [Complex] $\sim$ 1 x 10$^{-3}$ to 2 x 10$^{-3}$M; pH measurements were made at the conclusion of the reaction for buffer solutions.

$^b$ Solution titrated to constant pH with NaOH.

$^c$ 0.5M Tris buffer.

$^d$ 0.1M glycine-NaOH buffer.

$^e$ 0.25M triethylamine buffer.

$^f$ 0.2 to 0.4g complex, titrated with NaOH (0.2 to 1.0M).

$^g$ $k_{Br} = k_{obsd}/[OH^-]$.

$^h$ Reaction carried out on resolved starting material.
TABLE 5.4

Base Consumption and Product Analysis in Base Hydrolysis of cis-[Co(en)$_2$X(β-alaOR)]X$_2$; $\mu = 1.0$, NaClO$_4$; 25.0º.$^a$

<table>
<thead>
<tr>
<th>pH</th>
<th>Equiv base consumed.$^b$</th>
<th>[Co(en)$_2$OH(β-alaOR)]$^{n+}$</th>
<th>% trans</th>
<th>% cis</th>
<th>Total.$^d$</th>
<th>%[Co(en)$_2$(β-alaO)]$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) $X = Br$, $R = H$; ($n = 1$ for pH &gt; 7).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.80</td>
<td>-</td>
<td>14</td>
<td>77</td>
<td>91</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>9.02</td>
<td>1.92</td>
<td>-</td>
<td>-</td>
<td>91</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>9.21</td>
<td>-</td>
<td>15</td>
<td>75</td>
<td>90</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>9.35</td>
<td>1.90</td>
<td>10</td>
<td>80</td>
<td>90</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>9.60</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>91</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>9.82</td>
<td>-</td>
<td>15</td>
<td>75</td>
<td>90</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>9.97</td>
<td>1.85</td>
<td>-</td>
<td>-</td>
<td>90</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>11.02</td>
<td>1.90</td>
<td>-</td>
<td>-</td>
<td>92</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>11.96</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>92</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>13.2 $^c$</td>
<td>-</td>
<td>16</td>
<td>76</td>
<td>92</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>13.3 $^c$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>93</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>13.5 $^c$</td>
<td>-</td>
<td>12</td>
<td>79</td>
<td>91</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>14.0</td>
<td>-</td>
<td>24</td>
<td>72</td>
<td>96</td>
<td>4$^e$</td>
<td></td>
</tr>
<tr>
<td>(II) $R = CH(CH_3)_2$; ($n = 2$).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.50</td>
<td>0.98</td>
<td>X = Br</td>
<td>-</td>
<td>-</td>
<td>65$^e$</td>
<td>35</td>
</tr>
<tr>
<td>9.20</td>
<td>1.0</td>
<td>X = Br</td>
<td>-</td>
<td>-</td>
<td>67</td>
<td>32</td>
</tr>
<tr>
<td>9.91</td>
<td>1.0</td>
<td>X = Br</td>
<td>-</td>
<td>-</td>
<td>68</td>
<td>32</td>
</tr>
<tr>
<td>13.0$^c$</td>
<td>-</td>
<td>X = Br</td>
<td>-</td>
<td>-</td>
<td>68</td>
<td>31</td>
</tr>
<tr>
<td>10.40</td>
<td>-</td>
<td>X = Cl</td>
<td>-</td>
<td>-</td>
<td>67</td>
<td>32</td>
</tr>
<tr>
<td>13.0$^c$</td>
<td>-</td>
<td>X = Cl</td>
<td>-</td>
<td>-</td>
<td>66</td>
<td>32</td>
</tr>
</tbody>
</table>

---

$^a$ When $R = H$, products eluted at pH > 7; otherwise, eluted at pH < 4. %Error in values = ± 2%.

$^b$ Only for some experiments was this obtained. Otherwise, 1M NaOH was added dropwise, without measurement, to the required pH before the titrator was used.

$^c$ Excess NaOH (≥10 times; 0.2-1.0M) mixed with soln of complex (~0.2g).

$^d$ The cis and trans products, in some experiments, were collected together.

$^e$ Calculated, from ε's of pure isomers, to contain ~40% trans isomer. See Text.

$^f$ The complex, (0.205g), left in 1M NaOH (10ml) (3½hrs), then analyzed.
In contrast with the aquo complex (Section 4.3.1) and the β-alanine chelates (Section 2.3.3), the methylene signals in the bromo complexes (3.28ppm) appear as a broad band. Presumably, the methylene protons of the ethylenediamine ligands and of the β-alanine residue are in similar electronic environments in the presence of the coordinated bromide, compared with H₂O or with chelated β-alanine.

The visible spectra of cis-[Co(en)₂Br(β-alaOR)]Br₂ were identical for R = H, CH₃, CH(CH₃)₂, with λmax = 540nm (ε 81). A similar observation has been made for the aquo and hydroxo complexes (Chapter 4), for the function, R, is not expected to affect the energies of the metal orbitals when it is several atoms removed from the coordination sphere.

The pKa of cis-[Co(en)₂Br(β-alaOH)]Br₂ has been determined as 3.85 ± 0.05 at 25.0°C and µ = 1.0, NaClO₄. This value is similar to that for the corresponding aquo complex (pKₐ = 3.9 ± 0.1; Table 4.1).

5.3.2 Kinetic data

Tables 5.2 and 5.3 present rate constants for bromide removal in cis-[Co(en)₂Br(β-alaOR)]²⁺ for R = H and R = CH(CH₃)₂, respectively. For the spectrophotometric data, plots of log (D₀₀-Dₜ) vs time were linear for at least 3×t½. Similarly, for the pH-Stat data, plots of log (V₀₀-Vₜ), where V is volume of base consumed, were linear for at least 3×t½. The agreement between the spectrophotometric and radiometric rates for both complexes indicates that the reaction observed was the same and that it consumed base. The similarity of kBr (kobsd/[OH⁻])
between the two complexes indicates that the same reaction was being observed; viz., bromide removal. The results in the two tables also show that the same reaction was being observed at several wavelengths.

For the acid and ester complexes, there were isosbestic points at 527 ± 3nm, 410 ± 3nm (Tris buffer) and 530 ± 3nm, 410 ± 3nm (Tris buffer) and 541 ± 3nm, 422 ± 3nm (glycine buffer), respectively. Fig. 5.2 shows visible spectra during base hydrolysis, in Tris buffer, of the cis-bromo β-alanine acid complex.

Fig. 5.2. Visible spectra during base hydrolysis of cis-[Co(en)Br(β-alam)Br₂ in 0.5M Tris buffer (pH = 9.39; 25.0°C; µ = 1.0, NaClO₄).
The reaction was not subject to general base catalysis since the rate was unchanged for different buffers and buffer strengths. The presence of buffers in the solution did not enhance or inhibit the reaction, for the rate was the same in the absence of buffers. The consistency of the rate data for the optically pure \((\text{cis})-\text{Co(en)}_2\text{Br}(_2\text{alaOCH(CH}_3)_2\text{)}\text{Br}_2\) with those for the unresolved complex suggests that the reactions of only the \text{cis} compound have been studied. The rate law for the complexes is:

\[
k_{\text{obsd}} = k_{\text{Br}} [\text{OH}^-]
\]

From Tables 5.2 and 5.3, \(k_{\text{Br}} = 46 \pm 2 \text{M}^{-1}\text{sec}^{-1}\) when \(R=H\) and \(k_{\text{Br}} = 68 \pm 2 \text{M}^{-1}\text{sec}^{-1}\) when \(R=\text{CH(CH}_3)_2\).

5.3.3 Product analysis

Table 5.4 gives product analyses by ion exchange chromatography and base consumption data for base hydrolysis of the complexes studied. The products from the reaction of the \text{cis}-bromo acid complex were separated on a column under alkaline conditions, in exactly the same manner as were the products of hydrolysis of the \text{cis}-aquo ester complex (Chapter 4). In fact, elution of the product solutions of these two complexes gave exactly the same bands, all with the same elution rates, but in different proportions. The products from the reaction of the \text{cis}-bromo ester complex were separated on a column under acidic conditions, for the ester products separated as 3+ aquo species, rather than 2+ hydroxo species, from the 2+ chelated acid product. Under the conditions, the \text{cis} and \text{trans} aquo
ester products could not be separated but, rather, were collected as a single band. In acidic conditions, a long column would have been required for the separation, and, with the slower elution rate due to the extra length, the isomers would have been hydrolyzed. Similarly, if a long column had been used and all products eluted as 2+ species under alkaline conditions, hydrolysis would have caused smearing in the bands and inaccurate product analyses.

The first two bands (Table 5.4) in the solution after hydrolysis of the β-alanine acid complex were identified by their visible spectra and relative reactivities in acidic and basic solutions, as already described (Section 4.3.3). The 2+ product (acid- and base-form columns), [Co(en)$_2$(β-alaO)]$^{2+}$, was identified by comparison of its elution rate and visible spectrum with that of the authentic material. The other major product was identified as the cis-hydroxo β-alanine ester complex by following the subsequent, slower reaction, after bromide removal, at $\lambda = 495$nm (Table 4.3, Footnote c). The rate corresponded to that for the authentic cis-aquo ester complex and also showed that the trans complex present did not interfere, for reasons already explained (Section 4.3.3).

Table 5.4 shows that the product distribution after base hydrolysis of cis-[Co(en)$_2$X(β-alaOR)]$^{2+}$ was independent of pH, within experimental error, over a range of [OH$^-$] from $6.3 \times 10^{-6}$M to 0.5M. The extent of formation of the chelate, however, was dependent on R, increasing from 9% when R = H to 33% when R = CH(CH$_3$)$_2$. Base consumption data agreed well with values expected from product analyses.
For the cis-bromo acid complex, 1 equivalent of base was required for deprotonation of the acid proton and 0.90 equivalents for the formation of 90% hydroxo acid product. No base was required for the formation of the chelate. The stoichiometry for the reactions is given in equations (5.4) to (5.6).

\[
[\text{Co(en)}_2\text{Br(β-alaOH)}]^2+ + \text{OH}^- \rightarrow [\text{Co(en)}_2\text{Br(β-alaO)}]^+ + \text{H}_2\text{O} \quad (5.4)
\]

\[
[\text{Co(en)}_2\text{Br(β-alaO)}]^+ + \text{OH}^- \rightarrow [\text{Co(en)}_2\text{OH(β-alaO)}]^+ + \text{Br}^- \quad (5.5)
\]

\[
[\text{Co(en)}_2\text{Br(β-alaO)}]^+ + \text{OH}^- \rightarrow [\text{Co(en)}_2(β-alaO)]^{2+} + \text{Br}^- + \text{OH}^- \quad (5.6)
\]

For the cis-bromo ester complex, a total of 1 equivalent of base was required for production of the hydroxo species and for hydrolysis of the reactive chelated ester intermediate, according to the following equations; \( R = \text{CH(CH}_3\text{)}_2 \).

\[
[\text{Co(en)}_2\text{Br(β-alaOR)}]^2+ + \text{OH}^- \rightarrow [\text{Co(en)}_2\text{OH(β-alaOR)}]^2+ + \text{Br}^- \quad (5.7)
\]

\[
[\text{Co(en)}_2\text{Br(β-alaOR)}]^2+ + \text{OH}^- \rightarrow [\text{Co(en)}_2(β-alaOR)]^{3+} + \text{Br}^- + \text{OH}^- \quad (5.8)
\]

\[
[\text{Co(en)}_2(β-alaO)]^{2+} + \text{ROH} + \text{Br}^- 
\]

Table 5.4(II) also shows that the product distribution after base hydrolysis of \( \text{cis-}[\text{Co(en)}_2\text{X(β-alaOR)}]^{2+} \) was independent of the leaving group, \( X \).

5.3.4 Experiments on the resolved complex

Table 5.5 gives molar rotations (see p.v) of resolved \( \theta_{589} - \text{cis-}[\text{Co(en)}_2\text{Br(β-alaOCH(CH}_3\text{)}_2)]\text{Br}_2 \) and for the bands obtained on elution of the products of base hydrolysis of
### TABLE 5.5

Molar Rotations (deg.M⁻¹m⁻¹) of Products of Hydrolysis of

\( \text{cis-[Co(en)}_2 \text{Br(β-alaOR)}] \text{Br}_2' \) \((R = \text{CH(CH}_3)_2)\).

<table>
<thead>
<tr>
<th>(\lambda (\text{nm}))</th>
<th>cis-[Co(en)]_2Br-(β-alaOR)]²⁺</th>
<th>cis-[Co(en)]_2H₂O-(β-alaOR)]³⁺</th>
<th>Products of base hydrolysis</th>
<th>Resolved⁹,¹¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>589</td>
<td>+350</td>
<td>+350</td>
<td>-900</td>
<td></td>
</tr>
<tr>
<td>546</td>
<td>-207</td>
<td>+60</td>
<td>+400</td>
<td>-1260</td>
</tr>
<tr>
<td>436</td>
<td>-1250</td>
<td>-500</td>
<td>-740</td>
<td>+1280</td>
</tr>
</tbody>
</table>

⁹ In H₂O.

¹⁰ Calculated for 60% cis product. Error = ± 30 deg.M⁻¹m⁻¹.

¹¹ Error for other readings = ±10%.

The (H₅₈₉) isomer would give the same values, but with opposite sign.

### TABLE 5.6

Product Analysis and Molar Rotations of Products after Base Hydrolysis of \( \text{cis-[Co(en)}_2 \text{Br(β-alaOR)}] \text{Br}_2' \) in the Presence of Azide; \( (R = \text{CH(CH}_3)_2)\).

<table>
<thead>
<tr>
<th>[Co(en)]_2⁻</th>
<th>trans-[Co(en)]_2⁻</th>
<th>cis-[Co(en)]_2⁻</th>
<th>cis- + trans-[Co(en)]_2⁻</th>
<th>N₃(β-alaOR)²⁺</th>
<th>N₃(β-alaOR)²⁺</th>
<th>H₂O(β-alaOR)³⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) Product distribution.</td>
<td>10%</td>
<td>14%</td>
<td>22%</td>
<td>50%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(II) Molar Rotations (deg.M⁻¹m⁻¹).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>589nm</td>
<td>+350</td>
<td>0</td>
<td>(\nu+30)</td>
<td>+90⁹</td>
<td></td>
<td></td>
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<tr>
<td>546nm</td>
<td>+390</td>
<td>0</td>
<td>(\nu+50)</td>
<td>+150</td>
<td></td>
<td></td>
</tr>
<tr>
<td>436nm</td>
<td>-500</td>
<td>0</td>
<td>(\nu-140)</td>
<td>-380</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

⁹ Error in readings ≥ 20%. See p.v for definition.

Calculated on the basis that the mixture contains 40% trans isomer.
the complex at pH = 9.02. Table 5.6 gives the product distribution and molar rotations of the products obtained in a competition experiment with azide ion in basic solution. The order of elution was (i), chelated β-alanine complex, (ii), trans-azido β-alaninato complex, (iii), cis-azido β-alaninato complex and (iv), aquo ester complex. The complexes, (i) and (iv), were identified by their visible spectra, colour and elution rates, compared with the authentic materials. The aquo ester complex contained both cis and trans isomers, as recognized by the lower value for ε (63), compared with the observed value (ε = 72) for the authentic cis-isomer and the deduced value (ε = 55) for the trans isomer, assuming that the extinction coefficient for the latter would be identical with that for the corresponding β-alanine acid complex (Table 4.6). From the ε's of freshly eluted solutions, it is calculated that ~40% trans-aquo ester was present. The \([\text{Co(en)}_2N_3(\beta-\text{alaOCH(CH}_3)_2)]^{2+}\) isomers had the following absorptions: 

\[
\lambda_{\text{max}}(\text{trans}) = 518 \pm 3 \text{nm; (ε 220 ± 10)} ; \quad \lambda_{\text{max}}(\text{cis}) = 510 \pm 3 \text{nm (ε 360 ± 10)}.
\]

These isomers were identified by their colour (mauve) and by the similarity of their absorption spectra with those of the isomers, \(\text{trans-[Co(en)}_2N_3(\text{NH}_3)]^{2+}\) (\(\lambda_{\text{max}} = 510 \text{nm; ε 270}\)) and \(\text{cis-[Co(en)}_2N_3(\text{NH}_3)]^{2+}\) (\(\lambda_{\text{max}} = 510 \text{nm; ε 350}\)) [140]. Unlike the corresponding glycine ester system [108], no trans- or cis-\([\text{Co(en)}_2(N_3)_2]^+\) seemed to be formed, for the spectra of the complexes in the present investigation did not correspond to those of the characterized diazido complexes [210]. The reaction for the glycine system was reported to yield only the cis-azido glycine ester complex [108], unlike
the present system, where both trans and cis isomers were formed.

The duration of the elution (~40hrs) and the length of column used caused the greatest errors overall. Bands tended to be smeared and, hence, eluted as dilute solutions ([Co] ~ 5 x 10^{-4} M), incurring greater errors in cobalt estimations by atomic absorption spectrophotometry and also in measurement and calculation of molar rotations.

5.4 Discussion

5.4.1 Mechanism and stereochemistry of the base hydrolysis of cis-[Co(en)_2X(β-alaOR)]X_2

Base hydrolysis of cis-[Co(en)_2Br(β-alaOR)]Br_2 proceeds with rate constants, \( k_{Br} = 46 \pm 2 \text{M}^{-1} \text{sec}^{-1} \) when \( R = H \)
and \( k_{Br} = 68 \pm 2 \text{M}^{-1} \text{sec}^{-1} \) when \( R = \text{CH(CH}_3)_2 \) (\( \mu = 1.0, \text{NaClO}_4; 25.0^\circ \))

These values are not too different from those obtained for cis-[Co(en)_2Br(NH_2CH_2CH_2Br)]Br_2 (\( k_{Br} = 180 \text{M}^{-1} \text{sec}^{-1} \)) [226] and for cis-[Co(en)_2Br(glyOCH(CH}_3)_2]Br_2 (\( k_{Br} = 280 \text{M}^{-1} \text{sec}^{-1} \)) [108].

The results of Table 5.4 show that the product distribution after base hydrolysis of cis-[Co(en)_2X(β-alaOR)]^{2+} is independent of the leaving group (X = Cl, Br) and this suggests that a common intermediate of reduced coordination number is formed. The formation of such an intermediate allows for both solvent H_2O and carbonyl oxygen to compete for the vacated coordination position, since both hydroxo ester and chelate products are formed directly. The results of Chapter 4 preclude any of the chelate product from being formed by subsequent intramolecular hydrolysis by bound hydroxide, since this reaction is 380 times slower (\( k_{OH} = 0.18 \text{M}^{-1} \text{sec}^{-1} \); Chapter 4) than bromide removal.
The proposed pathways for the reaction are shown in Fig. 5.3.

The results of Table 5.4 show that the extracoordinated \( \alpha \)-alanine \( \alpha \)-alanine competes just as effectively as the \( \alpha \)-alanine \( \alpha \)-alanine, despite the negligible solubility of formation of some acid derivatives, since the percentage of product, compared with the \( \alpha \)-alanine \( \alpha \)-alanine, is not a true competitive procedure. On the other hand, the extracoordinated \( \alpha \)-alanine \( \alpha \)-alanine, since only 15% \( \alpha \)-alanine \( \alpha \)-alanine was added to the solution, compared with a total of 15% \( \alpha \)-alanine \( \alpha \)-alanine. This may be due to a more effective competitive procedure for the \( \alpha \)-alanine \( \alpha \)-alanine, but on an equimolar scale, the \( \alpha \)-alanine \( \alpha \)-alanine is highly reactive, as discussed in Section 5.1.

The studies with resolved optically active complexes have produced results for the stereochemical course of the reactions of complexes of octahedral Co(II) ions. The data of octahedral Co(II) ions have been assigned the same configuration, if they have the same sign for optical rotation at wavelengths above Table 5.4.

\[ \text{Fig. 5.3 Reaction scheme for base hydrolysis of}\]
\[ \text{cis-} \{\text{Co(en)}_2\text{Br}(\beta\text{-alaOCH(CH}_3)_2)\}_2\text{Br}^+.\]
The results of Table 5.4 show that the ester carbonyl oxygen competes just as effectively as solvent H₂O for the vacated coordination position cis to the amino acid derivative, since 33% chelate product, compared with ~37% cis-hydroxo ester product (i.e.~60% × 67%) was formed. On the other hand, carbonyl oxygen of the coordinated β-alanine acid is a less effective competitor for the vacated coordination position, since only 10% chelate was formed, compared with a total of 90% cis-hydroxo acid product. This may be due to a more impenetrable solvation sheath around the deprotonated carboxyl group.

On the whole, there is little discrimination for competitors, whether intermolecular anionic, neutral or intramolecular, on an equimolar scale. This supports the claim that the five-coordinate intermediate is highly reactive, as discussed in Section 5.1.

Studies with resolved optically active complexes have produced results for the stereochemical course of the base hydrolysis of the complexes. The rotatory dispersion curves of octahedral Co(III) complexes have been used in the assignment of a configuration (Δ or Λ) about the metal ion [228]. On this basis, the complexes in the present work, of the general type, [Co₅O]ⁿ⁺ have been assigned the same configuration, if they have the same sign for optical rotation at wavelengths above 500nm (i.e. at 589nm and 546nm as shown in the Tables).
The results (Tables 5.5 and 5.6) show that the steric course for formation of the chelate is the same, within experimental error, in the absence and in the presence of azide, with \( \sim 34\% \) retention of activity. The cis-aquo product, too, is formed with only \( \sim 15\% \) retention of activity. (See Fig.6.3 for the rotation curve of the optically pure cis-aquo \( \beta \)-alanine ester complex). In the competition experiment with azide, the cis-aquo product is formed with 33\% retention of activity, if it is calculated that \( \sim 40\% \) trans product is formed. The cis-azido product is formed with \( <10\% \) retention of activity, if it is assumed that the rotation for the fully resolved cis-isomer would be equal to that for the complex, cis-[Co(en)\(_2\)N\(_3\)(NH\(_3\))]\(^+\), with [M] \(_{946}\) \( \sim 500\text{deg.}\text{M}^{-1}\text{m}^{-1}\) [140]. (The cis-aquo \( \beta \)-alanine ester ammine complex has a visible spectrum (600 - 300nm) and ORD curve that is similar to those of the corresponding cis-aquo ammine complex and thus the comparison of the azido complexes is reasonable). There is some conjecture about the geometry of the five-coordinate intermediate or intermediates formed during base hydrolysis [140,203,211]. Pearson and Basolo [206] have proposed that, after loss of the leaving group, a complex, cis-[Co(en)\(_2\)XL\]^\text{2+}\) may give rise to two trigonal bipyramidal structures, one with L in the trigonal plane and the other with L in an axial position.

Two such structures, A (asymmetric) and B (symmetric) are shown in Fig.5.4. For base hydrolysis of the cis-bromo ester complex, A would lead to trans-aquo ester (\( \sim 30\% \)) and cis-aquo ester with retention of configuration (\( \sim 34\% \) of 37\%) while B would lead to racemate (\( \sim 66\% \) of 37\%). Both A and B would lead to chelate (33\%).
Fig. 5.4. Proposed structures for five-coordinate intermediates formed simultaneously in base hydrolysis of [Co(en)$_2$LX]$^{n+}$ [206].

An alternative proposal is one where a single intermediate is invoked, with a geometry that is intermediate between trigonal bipyramidal and square pyramidal, as shown below.

Fig. 5.5. Proposed structure of an asymmetric five-coordinate intermediate to account for observed cis and trans product formation and inversion of configuration in base hydrolysis of cis-[Co(en)$_2$X(β-alamOR)]$X_2$. The formation of such an asymmetric intermediate could accommodate the formation of both trans and cis products and of retention and inversion of configuration, depending on the direction of the incoming nucleophile, from $a$, $b$ or $c$. Inversion would require that the incoming ligand, from $a$ or $b$, change the configuration of an amine.
It is seen that 33% chelate was formed in base hydrolysis of \( \text{cis-}[\text{Co}(\text{en})_2X(\beta-\text{alaOCH(CH}_3)_2)]^{2+} \) (Table 5.4(II)) and that only 10% was formed when azide was present (Table 5.6). Similarly, 67% aquo products were formed in the absence of azide, while only 50% were formed when azide was present. Azide ion thus depletes both intramolecular and intermolecular paths by carbonyl oxygen and \( \text{H}_2\text{O} \) attack, respectively, at the vacated position, but depletes the former to a greater extent. The total amount of \( \text{cis} \) product is, however, not altered greatly in the presence of azide competitor, when the chelate is also considered as \( \text{cis} \) product (22% \( \text{cis} \)-azido \( \beta \)-alaninato + 30% \( \text{cis} \)-aquo \( \beta \)-alaninato + 10% chelate species, in the presence of added azide, compared with 40% \( \text{cis} \)-aquo \( \beta \)-alaninato + 33% chelate species in base hydrolysis in the absence of azide). The limited experimental results also indicate that azide competes with solvent for the \( \text{trans} \) position, relative to the \( \text{cis} \) position, by an equivalent amount, since the \( \text{trans}/\text{cis} \) ratio for both hydroxo and azido isomers is much the same (~0.65).

5.4.2 Comparison with other systems

The behaviour of \( \text{cis-}[\text{Co}(\text{en})_2\text{Br}(\text{glyOCH(CH}_3)_2)]\text{Br}_2 \) [108], compared with that of \( \text{cis-}[\text{Co}(\text{en})_2\text{Br}(\beta-\text{alaOCH(CH}_3)_2)]\text{Br}_2 \) in base hydrolysis is given here.
\[\text{cis-}[\text{Co(en)}_2 \text{Br(glyOR)}] \text{Br}_2\]

\[\text{cis-}[\text{Co(en)}_2 \text{Br(\beta-alaOR)}] \text{Br}_2\]

\[R = \text{CH(CH}_3)_2\]

\((1)\) \(k_{\text{Br}} = 280 \text{M}^{-1} \text{sec}^{-1}\).

\((2)\) \% chelate formed = 69%.

- 34\% is derived from hydrolysis of the chelated ester intermediate;
- 34\% is derived from hydrolysis of the proposed cis-hydroxo ester intermediate.

\(\% \text{ chelate formed} = 33\%.\) This is derived entirely from chelated ester intermediate;

~~37\% cis-hydroxo ester product is formed and is stable to hydrolysis under the conditions.

\((3)\) Results of experiments on the resolved complex require that base hydrolysis occur with 50\% racemization.

Results of experiments on the resolved complex require that base hydrolysis occur with 66\% racemization.

\((4)\) Competition experiments in the presence of azide lead to:

\[\text{trans-}[\text{Co(en)}_2 (\text{N}_3)_2]^{2+} (21\%),\]

\[\text{trans-}[\text{Co(en)}_2 \text{N}_3(\text{glyO})]^+ (14\%),\]

\[\text{cis-}[\text{Co(en)}_2 (\text{glyO})]^{2+} (47\%),\]

\[\text{cis-}[\text{Co(en)}_2 \text{N}_3(\text{glyOR})]^{2+} (9\%).\]

\[\text{trans-} (14\%) + \text{cis-} (22\%)\]

\[\text{trans-} (23\%) + \text{cis-} (37\%)\]

\[\text{cis-}[\text{Co(en)}_2 \text{OH}_2(\text{\beta-alaOR})]^{3+},\]

\[\text{cis-}[\text{Co(en)}_2(\text{\beta-alaO})]^{2+} (10\%).\]

From (2), it is seen that the ester carbonyl oxygen of both the glycine and \(\beta\)-alanine derivatives competes equally with solvent \(\text{H}_2\text{O}\) for the vacated position of the intermediate, for both give essentially the same distribution of products. This result is unexpected, for it might have been thought that the carbonyl oxygen of the glycine derivative would have been a somewhat better competitor than that of the corresponding \(\beta\)-alanine derivative, due to its shorter distance from the coordination sphere. The same effect is seen in (4), above, with azide competitor. The 47\% glycine chelate, derived from both chelated ester
and from the proposed reactive hydroxo glycine ester intermediate, equates well with the total 10% β-alanine chelate + 37% cis-hydroxo β-alanine ester complex.

It was postulated (Chapter 3) that ring opening in base hydrolysis of \([\text{Co(en)}_2(\beta\text{-alaO})]^{2+}\) could proceed by an $S_N^1$CB mechanism, involving the formation of a highly reactive five-coordinate intermediate. If this is so, the product distribution should be independent of the way in which the intermediate was generated, whether from \([\text{Co(en)}_2(\beta\text{-alaO})]^{2+}\) or from cis-\([\text{Co(en)}_2\text{Br(β-alaO})]^-\). Base hydrolysis of the former gave a ratio for the hydroxo β-alaninato isomers, cis/trans of $\approx 2.5/1$ (fact (1), p.103). When the latter was left to stand for the same period of time under the same experimental conditions, the ratio, cis/trans was $\approx 3/1$ (Footnote f, Table 5.4), not very different from that in the former. The reduction in the ratio for the latter, from 5/1, immediately after bromide removal, to $\approx 3/1$ after prolonged standing in the strongly basic solution, was due to depletion of the cis product, largely by subsequent isomerization to trans product and by some reversion to starting material. Certainly, the similarity of these ratios is evidence for the existence of the $S_N^1$CB mechanism involving a common intermediate in base hydrolysis of these two complexes.

The system under present investigation has the novel feature that one of the competitors for the vacated site in the proposed five-coordinate intermediate is already on the molecule and leads to chelate ring formation. The slight discrimination between nucleophiles in the system also shows that the intramolecular path, leading to the six-membered chelate ring is less favoured than the intermolecular path.
6. **INDUCED AQUATION OF cis-\(\text{Co(en)}_2 X(\beta-\text{AlaOR})\)^{2+}**

### 6.1 Introduction

The induced aquation of complexes \([\text{CoN}_4 XL]^{n+}\) by \(\text{Hg}^{2+}\) ions in acid solutions [101], with \(L\), a monodentate ligand and \(X = \text{Cl, Br, I}\), proceeds by the equation

\[
[\text{CoN}_4 XL]^{n+} + \text{Hg}^{2+} + \text{H}_2\text{O} \rightarrow [\text{CoN}_4 (\text{H}_2\text{O})L]^{(n+1)+} + \text{HgX}^+. \quad (6.1)
\]

It is proposed that \(\text{Hg}^{2+}\) binds with the halide, generating an especially good leaving group. The results of competition experiments in \(\text{Hg}^{2+}\)-induced aquation and in nitrosation of azido complexes, with added anions and other potential ligands in solution [214-216] are consistent with the proposal that a five-coordinate intermediate is formed during the reaction. They show that there is little discrimination between anions for the intermediate and that anions are much better competitors than neutral molecules. The constant ratios, \(^{18}\text{O}/^{16}\text{O}\), for the aquo products in the \(\text{Hg}^{2+}\)-induced reactions [101] also indicate a common intermediate of reduced coordination number. Studies of the \(\text{Hg}^{2+}\)-induced aquation of the \(\text{trans}\) deuterated complex, \([\text{Co(NH}_3)_4 X(\text{ND}_3)]^{2+}\), have shown that there is little or no stereochemical rearrangement and so it is inferred that the intermediate adopts a square pyramidal geometry, with the \(\text{ND}_3\) ligand at the apex [100].
When the ligand, L, cis to the leaving group, is a potential chelating ligand, such reactions, involving a five-coordinate intermediate are of special interest, for the second donor atom may compete with potential donors in the solvent for the vacated coordination position. Alexander and Busch have postulated that the carbonyl oxygen of bound glycine esters in \( \text{Hg}^{2+}\)-induced reactions of complexes, \( \text{cis-[Co(en)}_2\text{X(glyOR)}\text{]}\times_2 \) exclusively takes up the vacated sixth coordination position to form a highly reactive chelated ester. This species is subsequently rapidly hydrolyzed to yield the chelated glycine product [106]. Such chelated esters have since been isolated and their reactivity shown to be high [109,110,114,217]. For the \( \text{Hg}^{2+}\)-induced hydrolysis of \( \text{cis-[Co(en)}_2\text{Br(glyOCH(CH}_3)_2\text{)]Br_2} \), it was inferred, from \(^{18}\text{O}\) experiments, that, if solvent \( \text{H}_2\text{O} \) was ever incorporated into the sixth coordination position, it was rapidly expelled by carbonyl oxygen of the bound ester [114].

The present study presents the results of kinetic and stereochemical studies in the \( \text{Hg}^{2+}\)-catalyzed aquation of \( \beta\)-alanine derivatives, \( \text{cis-[Co(en)}_2\text{X(\beta-alaOR)}\text{]}\times_2 \). It aims to establish whether the same stereochemical course is followed, as has been observed for similar amine complexes and to determine to what extent intramolecular competition by carbonyl oxygen vs intermolecular competition by solvent occurs, after halide is removed.
6.2 Experimental

6.2.1 Instrumental and general

Visible spectra were recorded on a Cary 14 spectrophotometer and cobalt estimations were made using a Techtron AA4 atomic absorption spectrophotometer. Spectrophotometric rates were obtained with a stopped-flow apparatus (1cm cell) on a Cary 16K spectrophotometer.

Products of the reactions were eluted from Bio-Rad Analytical Dowex 50Wx2 (200-400 mesh, H⁺-form) cation exchange resin.

Analar reagents were used throughout, without further purification. A stock solution of Hg²⁺ in HClO₄ (µ = 1.0, NaClO₄) was prepared by dissolving HgO in HClO₄ of known concentration, then diluting the concentrated solution so that [Hg²⁺] = 0.2M and [H⁺] = 0.3M and adjusting the ionic strength with NaClO₄. For the reaction at pH ~ 4 (Table 6.2), Hg(CH₃COO)₂ was dissolved in H₂O, such that [Hg²⁺] = 0.025M and ionic strength adjusted to µ = 1.0 with NaClO₄.

6.2.2 Preparation of complexes

Details of the preparation of complexes used for the Hg²⁺-catalyzed reactions have already been presented in Section 5.2.2.

6.2.3 Kinetic measurements

A solution of cis-[Co(en)₂Br(β-alaOR)]Br₂, R = H, CH₃, CH(CH₃)₂ in 1.0M NaClO₄ (5 × 10⁻⁴ to 8 × 10⁻⁴ M) was mixed with an equal volume of Hg²⁺/H⁺ solution of known concentration µ = 1.0, NaClO₄, at 25.0°C. Rates were followed spectrophotometrically at several wavelengths in the range, 600 to 300nm.
6.2.4 Product analysis

The complexes, cis-[Co(en)$_2$X(β-alaOR)]X$_2$, (\~0.3g) were dissolved in H$_2$O (\~2ml) and treated with the Hg$^{2+}$/H$^+$ stock solution (15ml; [Hg$^{2+}$] = 0.2M; [H$^+$] = 0.3M; µ = 1.0, NaClO$_4$). In another experiment, the bromo acid complex (0.05g) was dissolved in 0.025M Hg(CH$_3$COO)$_2$ (50ml) and the pH adjusted to \~4.5 with 4M NaOH. For the bromo acid complex, the product solution was sorbed directly on H$^+$-form resin and eluted with 2M NaClO$_4$ at pH \~2. The first band, the orange [Co(en)$_2$(β-alaO)]$^{2+}$ product, was eluted with 2M NaClO$_4$ (pH \~2). The second band (the aqua acid product) was eluted with either 2M NaClO$_4$ (pH \~2) or with 3M HCl. For the cis-halo ester complexes, the product solutions were immediately titrated to pH = 9 for \~30secs, then quenched to pH \~3 with 1M HClO$_4$ and sorbed on H$^+$-form resin. The product bands were eluted as above.

To determine the dependence of product distribution on [Hg$^{2+}$] and [H$^+$], a second set of experiments was carried out. The complexes, cis-[Co(en)$_2$Br(β-alaOR)]Br$_2$, (\~0.05g), dissolved in 1M NaClO$_4$ (50ml) were reacted with 0.02M Hg$^{2+}$ solution ([H$^+$] = 0.3M; 25.0$^\circ$), then treated as above. Cobalt estimations were made by atomic absorption spectrophotometry and complexes were characterized by comparison of elution rates and visible spectra with those of the authentic materials.

6.2.5 Experiments with (H)$_{565}$-cis-[Co(en)$_2$Br(β-alaOCH(CH$_3$)$_2$)]Br$_2$

The resolved β-alanine isopropyl ester complex was treated in the following manner. A sample (0.1g) was dissolved in 0.2MHg$^{2+}$/0.3M H$^+$ solution (5ml; µ = 1.0, NaClO$_4$;
An aliquot was diluted twenty-fold ([Co] = 0.00242 M) and its rotation measured in a 10cm cell on the spectropolarimeter. The rest of the solution was sorbed on H+ form resin and eluted with 2M NaClO₄ (pH ~3). Its rotation was measured in the same way and [Co] determined by atomic absorption spectrophotometry ([Co] = 0.000880M).

The rotation of this eluted solution was subsequently measured at intervals, for several weeks, until no further change was observed. Curves are shown in Fig.6.2.

A second sample (0.1g) was dissolved in 0.2M Hg²⁺/0.3M H⁺ solution (10ml; µ = 1.0, NaClO₄; 25.0°). It was then titrated to pH = 9.0 for ~30secs, to hydrolyze the chelated ester product to the chelated acid, quenched to pH~ 2 with 1M HClO₄ and sorbed on H⁺-form resin. The first band, the [Co(en)₂(β-alaO)]²⁺ ion, was eluted with 2M NaClO₄ (pH ~3). The pH of the eluant was then increased to 9 with 0.5M NaOH to remove the second band more quickly as a 2+ species, cis-[Co(en)₂OH(β-alaOCH(CH₃)₂)]²⁺. The rotation of the solution was immediately measured in a 10cm cell on the spectropolarimeter and the cobalt concentration determined from visible spectra. The solution was then acidified with 1M HClO₄, the spectrum recorded and the rotation measured. ([Co] in basic soln = 0.00216M; [Co] in acidic soln = 0.00162M).

Molar rotations of these solutions are shown in Fig. 6.3.
6.3 Results

6.3.1 Kinetic data and product analysis

Table 6.1 presents rate data for the Hg^{2+}-induced bromide removal in cis-[Co(en)₂Br(β-alaOR)]Br₂. Plots of \((D∞-Dt)\) vs \(t\) were linear for at least \(3 \times t\). The observed rate law is first order in \([\text{Hg}^{2+}]\).

\[
\text{k}_{\text{obsd}} = k_{\text{Hg}}[\text{Hg}^{2+}] \tag{6.2}
\]

The constants are, \(k_{\text{Hg}} = 2.9 \pm 0.1\text{M}^{-1}\text{sec}^{-1}\) for \(R = \text{H}\),
\(k_{\text{Hg}} = 2.39 \pm 0.03\text{M}^{-1}\text{sec}^{-1}\) for \(R = \text{CH₃}\), and \(k_{\text{Hg}} = 2.44 \pm 0.03\text{M}^{-1}\text{sec}^{-1}\) for \(R = \text{CH(}\text{CH₃})₂\). There was no noted dependence on the acidity of the solutions for \(\text{pH} < 2\). Above this \(\text{pH}\), the reaction appeared to be complex and no simple rate law could be obtained. The same rate was observed at different wavelengths, indicating that no other reaction was occurring to any significant extent during bromide removal.

Fig.6.1 shows pmr spectra of the cis-bromo β-alanine isopropyl ester complex and of the product solution after bromide removal. The spectrum of the starting material has already been discussed (Section 5.3.1). The features characteristic of the cis-aquo ester (Section 4.3.1) and of the chelated ester (Section 2.3.3) are evident in the spectrum of the product solution. The spectra of the product solutions of the corresponding methyl and acid complexes show the same trends, compared with the parent bromide.

Table 6.2 gives product distributions after halide removal in \(\text{cis-[Co(en)}₂\text{X(β-alaOR)}]\text{X}₂\). As mentioned in the footnotes, the method of analyzing for the chelated ester was to hydrolyze it to the chelated acid (pH ~ 9).
### TABLE 6.1

Spectrophotometric Rate Constant Data for Hg$^{2+}$-catalyzed Bromide Removal from cis-[Co(en)$_2$Br(β-alaOR)]Br$_2$. $^a$

<table>
<thead>
<tr>
<th>R</th>
<th>[Hg$^{2+}$]$^b$</th>
<th>[H$^+$]$^b$</th>
<th>$10^{-1}$k$_{\text{obsd}}$ $^c$ (sec$^{-1}$)</th>
<th>k$_{\text{Hg}}$$^d$ (M$^{-1}$sec$^{-1}$)</th>
</tr>
</thead>
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<tr>
<td>H</td>
<td>0.010</td>
<td>0.015</td>
<td>0.310</td>
<td>3.10</td>
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<tr>
<td>&quot;</td>
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<td>0.148</td>
<td>0.294</td>
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<tr>
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<td>2.93</td>
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<td>CH$_3$</td>
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<td>&quot; (b)</td>
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<td>2.39</td>
</tr>
<tr>
<td>&quot;</td>
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<td>0.148</td>
<td>1.20</td>
<td>2.40</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.101</td>
<td>0.148</td>
<td>2.38</td>
<td>2.38</td>
</tr>
<tr>
<td>CH(CH$_3$)$_2$</td>
<td>0.010</td>
<td>0.015</td>
<td>0.252</td>
<td>2.52</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.010</td>
<td>0.148</td>
<td>0.241</td>
<td>2.41</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.025</td>
<td>0.148</td>
<td>0.616</td>
<td>2.47</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.045</td>
<td>0.134</td>
<td>1.07</td>
<td>2.37</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.050</td>
<td>0.148</td>
<td>1.17</td>
<td>2.35</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.101</td>
<td>0.148</td>
<td>2.48$^e$</td>
<td>2.48</td>
</tr>
</tbody>
</table>

$^a$ [Complex] $\sim 5 \times 10^{-4}$ to $8 \times 10^{-4}$ M; $\lambda = 322$nm; ΔOD $\geq 0.4$units; μ = 1.0,NaClO$_4$; 25.0°.

$^b$ Perchlorate anion.

$^c$ Values are averages of 3 runs.

$^d$ k$_{\text{Hg}}$ = k$_{\text{obsd}}$/[Hg$^{2+}$]

$^e$ Average of 5 runs, with differing $\lambda$ (322,350,495nm).
Fig. 6.1. Pmr spectra of (a), cis-[Co(en)$_2$Br(β-alactOCH(CH$_3$)$_2$)]Br$_2$ in D$_2$O and (b), the product solution, after reaction with Hg$^{2+}$/D$_2$O containing cis-[Co(en)$_2$H$_2$O(β-alactOCH(CH$_3$)$_2$)]$^{3+}$ (90%) and [Co(en)$_2$(β-alactOCH(CH$_3$)$_2$)]$^{3+}$ (10%). (Internal reference, NaTPS; 100MHz).
TABLE 6.2

Product Analysis after Hg\(^{2+}\)-catalyzed Halide Removal, in Acid, of cis-[Co(en)\(_2\)X(\(\beta\)-alaOR)]\(X_2\).\(^a\)

<table>
<thead>
<tr>
<th>X</th>
<th>R</th>
<th>%[Co(en)(_2)((\beta)-alaOR)](^{3+})(^b)</th>
<th>%[Co(en)(_2)H(_2)O((\beta)-alaOR)](^{3+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Br</td>
<td>H</td>
<td>18(^c)</td>
<td>80</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>20(^d)</td>
<td>80</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>19(^e)</td>
<td>80</td>
</tr>
<tr>
<td>&quot;</td>
<td>CH(_3)</td>
<td>11(^c)</td>
<td>87</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>9(^d)</td>
<td>90</td>
</tr>
<tr>
<td>&quot;</td>
<td>CH(CH(_3))(_2)</td>
<td>10(^c)</td>
<td>88</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>9(^d)</td>
<td>88</td>
</tr>
<tr>
<td>Cl</td>
<td>&quot;</td>
<td>10(^c)</td>
<td>90</td>
</tr>
</tbody>
</table>

\(^a\) \(\mu = 1.0, \text{NaClO}_4; 25.0^\circ\). Values are averages of 2 experiments.

\(^b\) Estimated as the \(\beta\)-alanine chelate, after hydrolysis of the product solution at pH = 9.

\(^c\) \([\text{Hg}^{2+}] = 0.1\text{M}; [\text{H}^+] = 0.15\text{M}; \approx 0.3\text{g complex used.}\)

\(^d\) \([\text{Hg}^{2+}] = 0.01\text{M}; [\text{H}^+] = 0.15\text{M}; \approx 0.06\text{g complex used.}\)

\(^e\) \([\text{Hg}^{2+}] = 0.025\text{M (acetate salt); pH } \approx 4.5.\)

This was necessary for two reasons. Firstly, separation of the chelated ester and aquo ester or acid complex products (both 3+ species) would not have been possible on the lengths of columns used and secondly, hydrolysis of the chelated esters occurred, at least in part, during the time that product solutions were sorbed on and eluted from the resin, thus causing a smearing in the columns. For all bromo complexes, a trace of orange material (<5%) remained on the column under the conditions of elution. This may have been some highly charged polymer, but insufficient material could be collected for characterization.
Table 6.2 shows that the extent of formation of the ester chelate was independent of the leaving group, X, and independent of the ester, R, (10% formation), but that it varied when R = H (20% formation). It also shows that the product distribution is independent of [Hg\(^{2+}\)] and of [H\(^{+}\)]. The reaction stoichiometry is given by equations (6.3) to (6.5).

For R = H, CH\(_3\), CH(CH\(_3\))\(_2\),
\[
\text{[Co(en)]}_2 X (\beta\text{-alaOR})^{2+} + \text{Hg}^{2+} + \text{H}_2\text{O} \rightarrow [\text{Co(en)}_2\text{H}_2\text{O}(\beta\text{-alaOR})]^{3+} + \text{HgX}^+
\]

For R = CH\(_3\), CH(CH\(_3\))\(_2\),
\[
[\text{Co(en)}_2 X (\beta\text{-alaOR})]^{2+} + \text{Hg}^{2+} \rightarrow [\text{Co(en)}_2 (\beta\text{-alaOR})]^{3+} + \text{HgX}^+
\]

For R = H,
\[
[\text{Co(en)}_2 X (\beta\text{-alaOH})]^{2+} + \text{Hg}^{2+} \rightarrow [\text{Co(en)}_2 (\beta\text{-alaOH})]^{2+} + \text{HgX}^+ + \text{H}^+
\]

6.3.2 *Experiments on (\text{cis})_599-[Co(en)]_2 Br(\beta\text{-alaOCH}(CH\(_3\))\(_2\))Br\(_2\)*

Fig. 6.2 shows ORD curves of the complex, (\text{cis})_599-[Co(en)]_2 Br(\beta\text{-alaOCH}(CH\(_3\))\(_2\)) Br\(_2\), of the product solution after Hg\(^{2+}\)-catalyzed bromide removal and after subsequent hydrolysis in acidic conditions and of authentic, resolved (\text{cis})_599-[Co(en)]_2 (\beta\text{-alaOH})Cl\(_2\). Fig. 6.3 shows ORD curves of the cis-aquo \(\beta\)alanine ester product of Hg\(^{2+}\)-catalyzed aquation, in acidic and in basic solutions. Some results of studies on resolved complexes have already been presented (Fig. 4.6; Table 4.9). The flow diagram for Hg\(^{2+}\)-catalyzed aquation and subsequent hydrolysis is shown in Fig 6.4.
Fig. 6.2. Rotatory dispersion curves of
(+)-H$_2$O-cis-[(Co(en)$_2$Br($\beta$-alaOCH(CH$_3$)$_2$)]Br, (--), in 0.3M HClO$_4$,
(---), in Hg$^2+$/H$^+$, immediately after bromide removal,
(----) in Hg$^2+$/H$^+$, after subsequent hydrolysis of the products
of bromide removal. (-----) is authentic [Co(en)$_2$(\beta-alaO)](ClO$_4$)$_2$
(resolved) in 2M NaClO$_4$ at pH ~ 3.
Fig. 6.3. Rotatory dispersion curves of

(---) cis-[Co(en)$_2$H$_2$(β-alaOCH(CH$_3$)$_2$)]$^{3+}$, in 0.5M NaClO$_4$ at pH = 2.,

(-----) cis-[Co(en)$_2$OH(β-alaOCH(CH$_3$)$_2$)]$^{2+}$, in 0.5M NaClO$_4$ at pH = 8.
Discussion

Mechanism and stereochemistry of the Hg$^{2+}$-induced halide removal from cis-[Co(en)$_2$X(β-alaOR)]$_2^+$ interacts with a rate constant $k_{Hg} = 2.4M^{-1}\text{sec}^{-1}$ when R is an ester function and $k_{Hg} = 2.9M^{-1}\text{sec}^{-1}$ when R = H. These values are consistent with those extrapolated by Alexander and Busch [106] for the corresponding glycine ester system, where an estimate of $k_{Hg} \approx 1M^{-1}\text{sec}^{-1}$ was obtained, due to interference by the subsequent hydrolysis of the chelated ester formed.

The results of Table 6.2 lend support to the existence of a five-coordinate intermediate, for the product distribution is independent of the leaving group, X, (X = Br, Cl). This is consistent with the results for other amine complexes (see Section 6.1). The observed competition between carbonyl oxygen and solvent is also
good evidence that the reaction proceeds via a five-coordinate intermediate. The stereochemical study allows a postulate to be made about the nature of the five-coordinate intermediate. As shown in the Results, there was no evidence that any trans aquo ester product was formed. In addition, the reaction proceeded with retention of configuration about the cobalt atom, for the end-product, [Co(en)₂(β-alaO)]²⁺, after bromide removal and hydrolysis, was optically pure (Chapter 4). These two facts preclude the formation of trigonal bipyramidal intermediates of the type shown in Fig.5.4. The structure of Fig.5.5 (asymmetric structure, intermediate between square pyramidal and trigonal bipyramidal) would account for the results, provided that the incoming group enters only in the cis position. However, there is no reason to believe that such a discrimination should exist for entering groups. A more likely possibility is a square pyramidal structure, shown in Fig.6.5.

![Proposed mechanism for Hg²⁺-induced halide removal from cis-[Co(en)₂X(β-alaOR)]X₂, invoking a five-coordinate square pyramidal intermediate.](image-url)
For the halo ester and acid complexes, solvent $H_2O$ competes far more effectively for the vacated position than does carbonyl oxygen. The former give rise to 90% aquo species, while the latter give rise to 80% aquo species.

6.4.2 Comparison of $Hg^{2+}$-assisted halide removal with base-catalyzed halide removal

Table 6.3 lists product analyses for halide removal in $\beta$-alanine complexes.

**TABLE 6.3**

Comparison of Data for Halide Removal in $\beta$-Alanine Derivatives in Acidic and Basic Conditions.

<table>
<thead>
<tr>
<th>Complex $[Co(en)_2X(\beta$-alaOR)]$</th>
<th>% monodentate product</th>
<th>% chelate product</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X$</td>
<td>$R$</td>
<td>$80$</td>
</tr>
<tr>
<td>$Br$</td>
<td>$H$</td>
<td>$90$</td>
</tr>
<tr>
<td>$Br$</td>
<td>$CH_3$</td>
<td>$67$</td>
</tr>
<tr>
<td>$Br$</td>
<td>$CH(CH_3)_2$</td>
<td>$90$</td>
</tr>
<tr>
<td>$Cl$</td>
<td>$CH(CH_3)_2$</td>
<td>$90$</td>
</tr>
</tbody>
</table>

The following features are evident from a comparison of the values in Table 6.3.

(1) When $R = H$, compared with $R = alkyl$, twice as much chelate $(20\% \text{ vs } 10\%)$ is formed in $Hg^{2+}$-catalyzed halide removal, but only one-third as much $(10\% \text{ vs } 33\%)$ is formed in base hydrolysis.
hydrolysis. This reversal from acidic to basic conditions may be due to the fact that, in basic conditions, the monodentate \( \beta \)-alanine acid, which is deprotonated, is surrounded by a hydrated sheath which hinders intramolecular nucleophilic attack by carboxylate oxygen at the vacated coordination site in the five-coordinate intermediate. (2) The evidence for the existence of five-coordinate intermediates is strong, both in Hg\(^{2+} \)-induced halide removal and in base hydrolysis. Different leaving groups give the same result and competition occurs between nucleophiles in solution and a neutral competitor already on the molecule. As discussed in the relevant sections, the results of these investigations indicate that the five-coordinate intermediate assumes a different geometry in acidic and in basic conditions and this factor may partly account for the different product distributions in acidic and basic conditions.

6.4.3 Comparison with the glycine system

From the results accumulated for Hg\(^{2+} \)-induced hydrolysis and similar reactions of cis-\([\text{Co(en)}_2 \text{Br(glyOR)}] \text{Br}_2\), where R is an ester group \([106,107,113]\), it was inferred that the intermediate was solely chelated glycine ester and that solvent \( \text{H}_2\text{O} \) did not appear to be incorporated into the coordination sphere. On the other hand, bromide removal in base hydrolysis of the same complex led to 50% incorporation of solvent \([108]\). In the \( \beta \)-alanine system, a reverse in this trend occurred. Solvent was incorporated in the coordination sphere to a greater extent in acidic conditions than in basic conditions (Table 6.3). It may be that the difference between the glycine ester and \( \beta \)-alanine ester
systems, in passing from acidic to basic conditions, could be explained by differences in free energy terms due to five-membered compared with six-membered chelate ring formation.

In conclusion, the study of the Hg$^{2+}$-assisted halide removal in β-alanine derivatives, \(\text{cis-}[\text{Co(en)}_2 X(\beta\text{-alaOR})]X_2\), has presented a new aspect in the study of mechanisms involving the formation of a five-coordinate intermediate. It has shown that competition for the intermediate may occur by both intermolecular and intramolecular pathways.

Reactive five-coordinate intermediates appear to be formed during Hg$^{2+}$-induced addition of X in \(\text{cis-}[\text{Co(en)}_2 X(\beta\text{-alaOR})]X_2\) \((X = \text{Br, Cl}; X = \text{I, CI(CH}_3)_2\) and during hydrolysis of the same complexes by dissociation of X, induced by OH$^{-}$, from the conjugate base (S$_{\text{N}}$ICP mechanism). Evidence for the existence of these five-coordinate intermediates comes from stereochemical studies and their competition properties for the nucleophiles in the system. Different leaving groups, X, give a common result and, on an equimolar basis, little discrimination between these nucleophiles is observed (<ten-fold difference). A new feature of the results is that competition occurs between solvent nucleophiles and a nucleophile attached to a bound group, leading to chelate formation. The S$_{\text{N}}$ICP mechanism is also supported by the product analysis and stereochemical studies in base hydrolysis of the species, \([\text{Co(en)}_2(\beta\text{-alaO})]^+\). The existence of dissociative mechanisms for Hg$^{2+}$-induced halide removal and base hydrolysis of the β-alanine derivatives could be tested further by studying the competition on
7. COMMENTS AND CONCLUSIONS

7.1 General

A summary of some reactions of Co(III) complexes of β-alanine is shown in a flow-chart (Fig 7.1), and the salient features and implications of these reactions follow.

Reactive five-coordinate intermediates appear to be formed during Hg²⁺-induced aquation of X in

\[ \text{cis-}[\text{Co(en)}_2X(\beta\text{-alaOR})]_2 \]

\((X = \text{Br, Cl}; \ R = \text{H, CH(CH}_3)_2 \)) and during hydrolysis of the same complexes by dissociation of X, induced by OH⁻, from the conjugate base (SN₁CB mechanism). Evidence for the existence of these five-coordinate intermediates comes from stereochemical studies and their competition properties for the nucleophiles in the system. Different leaving groups, X, give a common result and, on an equimolar basis, little discrimination between these nucleophiles is observed (<ten-fold difference). A new feature of the results is that competition occurs between solvent nucleophiles and a nucleophile attached to a bound group, leading to chelate formation. The SN₁CB mechanism is also supported by the product analysis and stereochemical studies in base hydrolysis of the species, \([\text{Co(en)}_2(\beta\text{-alaO})]^2+\).

The existence of dissociative mechanisms for Hg²⁺-induced halide removal and base hydrolysis of the β-alanine derivatives could be tested further by studying the competition on
Fig. 7.1. Flow chart for induced removal of $X$ from $\text{cis-}[\text{Co(en)}_2X(\beta\text{-alaOR})]^{2+}$ and Co(III)-promoted hydrolyses of $\beta$-alanine derivatives.
nitrosation and hydrolysis of \( \text{cis-}[\text{Co}(\text{en})_2\text{N}_3(\beta-\text{alaOR})]^{2+} \).

The induced bromide removal in the \( \text{cis-} \)bromo \( \beta \)-alanine complexes has been used to produce new reactive species under especially mild conditions, necessary because of the sensitivity of the ester function to hydrolysis. These new reactive complexes are the chelated ester complex and the monodentate \( \beta \)-alanine ester complex, with a potential oxygen nucleophile adjacent to it (Fig. 7.1). The ester function of the N-bound \( \beta \)-alanine derivative is hydrolyzed by solvent nucleophile at a rate that is not very different from that of the free protonated ester. It is hydrolyzed by bound nucleophile at much the same rate and it has been shown that the rate of ring closure, leading to the six-membered chelate ring, determines the rate of hydrolysis. However, when the carbonyl function is activated by being bound to the metal ion, the ester function is hydrolyzed with a rate that is very much greater (\( \sim 10^5 \) times).

On an absolute scale, free \( \beta \)-alanine ester is hydrolyzed more slowly (\( \sim 10 \) times) than the analogous glycine ester. When both are chelated, the former reacts more slowly (\( \sim 380 \) times). However, intramolecular hydrolysis of the monodentate \( \beta \)-alanine ester complex is very much slower (\( > 10^4 \) times) than that of the analogous glycine ester complex. This has been attributed to the greater difficulty in forming a puckered six-membered ring compared with the essentially planar five-membered ring of the glycine derivative. The great accelerations in base hydrolysis of carbonyl bound esters may be attributed to the stabilization by the metal ion, of the tetrahedral carbon centre in the intermediate and also of the transition state \([158,159]\).
Evidence, in the present work, for the existence of the tetrahedral intermediate comes from the complexity in the rate law for base hydrolysis of chelated β-alanine ester, especially from the reversion from a rate law second order in [OH\(^-\)] to one first order in [OH\(^-\)] at higher pH (~9.2). It is proposed that the two reactions – base hydrolysis of chelated ester and cyclization of hydroxo monodentate ester – are related by a common intermediate, containing a tetrahedral carbon centre, on the way to the chelated acid. The interconversion of the two species, via the intermediate, was not achieved, but it was seen that chelation was rate-determining. However, the rate-determining step for hydrolysis of the chelated ester was not established, but, clearly, interconversion was slower than product formation.

7.2 Possible Applications to Hydrolytic Enzyme Mechanisms

With the results of this investigation now presented, it is possible to speculate on the relative chemical efficacies of the mechanisms, proposed in the Introduction (Fig.1.4, p.9), for enzyme catalysis in hydrolysis of peptides or esters, namely (a), attack at bound carbonyl by OH\(^-\), (b), attack at free adjacent carbonyl by bound OH\(^-\), (c), attack at bound carbonyl in a general base mechanism and (d), attack at bound carbonyl by carboxylate nucleophile, resulting in anhydride formation. Of the possibilities, (a) and (b), the results of the present investigation favour (a) for the following reasons. (1) An M–OH moiety might not be expected to be as effective a
nucleophile as solvent \( \text{OH}^- \), other things being equal.

(2) The rate of intramolecular base hydrolysis is sensitive to conformational effects, since intramolecular ring closure in glycine ester complexes appears to be more rapid than intermolecular hydrolysis of carbonyl-bound ester, while, for the \( \beta \)-alanine derivative, the reverse is true. Although chelate rings are probably not formed in the enzyme system, an analogy may still be drawn, for the intramolecular processes in model systems simulate proximity effects in the enzyme. Thus, the evidence suggests that a process so sensitive to conformational effects might not generally operate in hydrolytic enzymes.

(3) The rate enhancement of hydrolysis of chelated esters, relative to free esters, is substantial \((\sim 10^5 - 10^6)\) and appears to be largely independent of conformational effects. Presumably, only bound carbonyl is required to account for the rate enhancement. This is shown for base hydrolysis of \([\text{Co(NH}_3]_5\text{OCHN(CH}_3)_2]\)\(^{3+}\) to the products, \([\text{Co(NH}_3]_5\text{OOCH}]^{2+}\) and \(\text{HN(CH}_3)_2\) \([225]\), where the same rate enhancement \((\sim 10^4)\) is observed, relative to free \(\text{HCON(CH}_3)_2\), as that of chelated glycine dimethyl amide, compared with free glycine dimethyl amide \([115]\). This proposition could be tested further, in the present system, by preparing and studying the reactivity of ester complexes, bound to a metal ion only through the carbonyl oxygen. Thus, the evidence, as it stands, does not favour possibility (b).

It remains to deduce which of the suggested species in (a), (c) or (d) might be the most effective nucleophile at the active site in a hydrolytic metalloenzyme such as
carboxypeptidase A. Investigations of base hydrolysis of chelated glycine ester, in the presence of acetate, have shown that nucleophilic addition, leading to anhydride formation, occurs to the exclusion of a general base mechanism and this seems true of all carboxylate-containing species [159]. An analysis of rate constants [159] indicates that carboxylate ion (1M) would be as effective as OH$^-\$ ions at physiological pH ($\sim$7.5; [OH$^-\$] $< 10^{-6}$ M) in promoting hydrolysis and much more effective than H$_2$O.

The models thus indicate that a reasonable mechanism to account for the rapid rates of peptide or ester hydrolysis is one where the carbonyl-bound substrate is hydrolyzed by nucleophilic attack of the proximal glutamic-270 carboxylate ion. This assumes, of course, that the carbonyl oxygen to metal bond is already formed at the active site. The activated carbonyl mechanism can account for a rate enhancement, relative to free substrate, of the order of $10^6$. The enhancement in hydrolytic enzyme systems is $>10^9$ [227]. This large value may be due to additional factors which have not been simulated in the model systems chosen. For instance, the surrounding protein may organize the nucleophile so that its effective concentration at the active site is far higher than that which can be attained in a model system ($\sim$50M).

It is known that the metal ions found in enzyme systems are labile towards substitution in simple complexes. This property of lability is necessary for catalytic activity. Once a reaction of a bound nucleophile has occurred, the remaining part must be released from the coordination sphere,
allowing new substrate to undergo the same reaction. The Co(III) complexes have been organized to mimic the Zn(II) chemistry with respect to the possible dissociative processes, for example, where bound H\textsubscript{2}O is replaced by carbonyl oxygen, but there, the similarity ends. Co(III) complexes, in hydrolysis, react irreversibly to form kinetically inert chelates of amino acid residues and so cannot act catalytically. It is clear, however, that the kinetic robustness of Co(III) complexes has allowed mechanistic detail to be followed and, in this respect, the Co(III) chemistry has proved to be truly invaluable. Conversely, it is the lability of the metal ion in the hydrolytic enzyme, coupled with the effect of the residues surrounding the substrate, that makes it difficult, if not impossible, to unravel the detail in mechanisms associated with enzyme processes, at the present time.
APPENDIX

Derivation of Rate Laws

Al (Section 2.4.3)

Rate law for base hydrolysis of the [Co(en)$_2$(β-alaOCH(CH$_3$)$_2$)]$^{3+}$ ion.

The reaction scheme, (2.10), is set out below. CE is the chelated ester; IH is the protonated tetrahedral intermediate and I is the deprotonated form; CA is the chelated acid product.

\[
\begin{align*}
CE + OH^- & \xrightarrow{k_a} IH + OH^- \xrightarrow{k_c} CA \\
& \xrightarrow{k_b} CA \quad (2.10)
\end{align*}
\]

Steady State Approximation

The rate of formation of each of the species in (2.10) is given by:

\[
\begin{align*}
\frac{d[CE]}{dt} &= -k_a[OH^-][CE] + k_b[IH] \quad (A1.1) \\
\frac{d[IH]}{dt} &= k_a[OH^-][CE] + k_f[I] - (k_e[OH^-] + k_c + k_d)[IH] \quad (A1.2) \\
\frac{d[I]}{dt} &= k_e[OH^-][IH] - (k_d + k_f)[I] \quad (A1.3) \\
\frac{d[CA]}{dt} &= k_c[IH] + k_d[I] \quad (A1.4)
\end{align*}
\]

and

\[
\frac{d[CA]}{dt} = -\left(\frac{d[CE]}{dt} + \frac{d[IH]}{dt} + \frac{d[I]}{dt}\right) \quad (A1.5)
\]

Applying the steady state approximation to the two intermediates, IH and I, the following are obtained.
\[
\frac{d[IH]}{dt} = 0 \quad \text{(Al. 6)}
\]

\[
\frac{d[I]}{dt} = 0 \quad \text{(Al. 7)}
\]

Thus, substituting (Al. 6) and (Al. 7) into (Al. 5),

\[
\frac{-d[CA]}{dt} = \frac{d[CE]}{dt} \quad \text{(Al. 8)}
\]

Substituting (Al. 6) into (Al. 2),

\[
k_a[OH^-][CE] = (k_e[OH^-] + k_c + k_d)[IH] - k_f[I] \quad \text{(Al. 9)}
\]

Substituting (Al. 7) into (Al. 3),

\[
k_e[OH^-][IH] = \left( \frac{k_f + k_d}{k_f + k_d} \right)[I]
\]

\[
\therefore [I] = \frac{k_e[OH^-][IH]}{k_f + k_d} \quad \text{(Al. 10)}
\]

Substitute (Al. 10) into (Al. 9),

\[
k_a[OH^-][CE] = \left( k_e[OH^-] + k_c + k_d - \frac{k_e k_f[OH^-]}{k_f + k_d} \right)[IH]
\]

\[
= \left( k_b + k_c + \frac{k_d k_e[OH^-]}{k_f + k_d} \right)[IH]
\]

\[
\therefore [IH] = \frac{k_a[OH^-]}{k_b + k_c + \frac{k_d k_e[OH^-]}{k_f + k_d}}[CE] \quad \text{(Al. 11)}
\]

Substitute (Al. 11) into (Al. 1) and use (Al. 8).
\[-\frac{d\left[\text{CA}\right]}{dt} = \frac{d\left[\text{CE}\right]}{dt} = k_a [\text{OH}^-]\left(1 - \frac{k_b}{k_b + k_c + \frac{k_d k_e [\text{OH}^-]}{(k_f + k_d)}\right)\]

\[\frac{d\left[\text{CA}\right]}{dt} = -k_a [\text{OH}^-]\]

Assume an equilibrium to exist between starting material and tetrahedral intermediate. The equilibrium constant, \(K'\), is given by [168]:

\[K' = \frac{k_a}{k_b} = \frac{[\text{IH}]}{[\text{CE}] [\text{OH}^-]}\]
Also, assume an equilibrium to exist between the tetrahedral intermediates; the equilibrium constant, $K$, is given by:

$$K = \frac{[I]}{[IH][OH^-]}$$

$$\therefore [I] = K[IH][OH^-] \quad (Al.13)$$

The rate of formation of product, $CA$, is given by the relation:

$$\frac{d[CA]}{dt} = k_c [IH] + k_d [I] \quad (Al.14)$$

And, from (Al.13),

$$\frac{d[CA]}{dt} = \left( k_c + k_d K[OH^-] \right) [IH] \quad (Al.15)$$

The total concentration of Co is given by:

$$[Co] = [CE] + [IH] + [I] \quad (Al.16)$$

Substituting (Al.12) and (Al.13) into (Al.16),

$$[Co] = \frac{1}{K[OH^-]} + 1 + K[OH^-] [IH]$$

$$= \left( \frac{1 + K[OH^-] + KK'[OH^-]^2}{K'[OH^-]} \right) [IH]$$

$$\therefore [IH] = \left( \frac{K'[OH^-]}{1 + K[OH^-] + KK'[OH^-]^2} \right) [Co] \quad (Al.17)$$

Substitute (Al.17) into (Al.15),

$$\frac{d[CA]}{dt} = \left( \frac{k_c K'[OH^-] + k_d KK'[OH^-]^2}{1 + K[OH^-] + KK'[OH^-]^2} \right) [Co]$$
If \([I]\) is small compared to \([IH]\), (i.e., \([Co] = [CE] + [IH]\)), the expression reduces to:

\[
\frac{d[CA]}{dt} = \frac{K'k_c[OH^-] + K'dK[OH^-]^2}{1 + K'[OH^-]} [Co]
\]

Thus,

\[
k_{\text{obsd}} = \frac{K'k_c[OH^-] + K'dK[OH^-]^2}{1 + K'[OH^-]}
\]

The reaction sequence is shown below.

The constant, \(k_1\), may be neglected, for the resulting concentration of label in solution, due to the forward reaction, will be very small indeed.
The differential equations for the system are as follows:

\[
\frac{dL}{dt} = -(k_1 + k_2)L 
\]  
(A2.1)

\[
\frac{dU}{dt} = k_1 L - k_2 U
\]  
(A2.2)

\[
\frac{dL_p}{dt} = k_2 U
\]  
(A2.3)

\[
\frac{dL}{dt} = k_2 L
\]  
(A2.4)

The equation, (A2.1) integrates to:

\[
L = L_0 e^{-(k_1 + k_2) t}
\]  
(A2.5)

where \( L_0 \) is the initial concentration.

Substitute (A2.5) into (A2.2).

\[
\frac{dU}{dt} = k_1 L_0 e^{-(k_1 + k_2) t} - k_2 U
\]  
(A2.6)

The general solution [187] for an equation of this form is:

\[
U = K_1 e^{-(k_1 + k_2) t} + K_2 e^{-k_2 t}
\]  
(A2.7)

Differentiate (A2.7) and equate the coefficients with those of (A2.6).

\[
U = L_0 \left( e^{-k_2 t} - e^{-(k_1 + k_2) t} \right)
\]  
(A2.8)

Substitute (A2.5) into (A2.4) and integrate; boundary conditions are: when \( t = 0 \), \( L = L_0 \).

\[
L_p = \frac{k_2 L_0}{k_1 + k_2} \left( 1 - e^{-(k_1 + k_2) t} \right)
\]  
(A2.9)

The value for \( U_p \) may readily be found from the relation:

\[
U_p = L_0 - \left( L + L_p + U \right)
\]  
(A2.10)
Substitution of $k_1 \ (6.3 \times 10^{-6} \text{sec}^{-1})$ and $k_2 \ (1.8 \times 10^{-6} \text{sec}^{-1})$, with $t = 17 \text{hrs} \ (6.12 \times 10^4 \text{secs})$ into (A2.5), (A2.8), (A2.9) and (A2.10) yield, respectively:

\[ L = 0.609L_0 \quad \text{(A2.11)} \]
\[ U = 0.287L_0 \quad \text{(A2.12)} \]
\[ L_p = 0.078L_0 \quad \text{(A2.13)} \]
\[ U_p = 0.026L_0 \quad \text{(A2.14)} \]

The total product formed is:

\[ (L_p + U_p) = 0.104L_0 \]

Thus, the percentage of labelled product is:

\[ \%L_p = \frac{0.078}{0.104} \times 100 = 76\% \quad \text{(A2.13)} \]

The percentage of unlabelled starting material, after 17hrs, is calculated to be 29\%, as seen above (A2.12).
Rate law for base-catalyzed intramolecular hydrolysis of the cis-[Co(en)$_2$OH(β-alacOCH(CH$_3$)$_2$)]$^{2+}$ ion.

The reaction scheme, (4.6), is set out below.

HE is the hydroxo ester starting material; E is the deprotonated hydroxo ester species; I is the deprotonated intermediate and CA is the chelated acid product.

\[
\begin{align*}
\text{HE} + \text{OH}^- & \xrightleftharpoons[k_-]{k_1} \text{E} + \text{H}_2\text{O} \xrightarrow[k']{} \text{I} + \text{H}_2\text{O} \\
& \xrightarrow[k_d]{k} \text{CA}
\end{align*}
\]  

(4.6)

The rate of formation of CA and E are given by:

\[
\frac{d[CA]}{dt} = k'[E] 
\]  

(A3.1)

\[
\frac{d[E]}{dt} = k_1[\text{HE}][\text{OH}^-] - k_-[E] - k'[E] 
\]  

(A3.2)

The steady state approximation is applied to the deprotonated starting material, E:

\[
\frac{d[E]}{dt} = 0 
\]  

(A3.3)

Substitute (A3.3) into (A3.2)

\[
[E] = \frac{k_1[\text{OH}^-][\text{HE}]}{k' + k_-} 
\]  

(A3.4)

Substitute (A3.4) into (A3.1)

\[
\frac{d[CA]}{dt} = \left(\frac{k'k_1[\text{OH}^-]}{k' + k_-}\right)[\text{HE}] 
\]
Thus,

\[
k_{\text{obsd}} = \frac{k'k_1 [\text{OH}^-]}{k' + k_{-1}}
\]

where \( k' \) is the composite rate constant, \( \frac{k'k_1}{k' + k_{-1}} \).
REFERENCES


34. M. Sokolovsky & B.L. Vallee, ibid., 6, 700 (1967).


89. H. Kroll, *ibid.*, 74, 2036 (1952).


168. D.A. Buckingham, private communication.


207. C.E. Schäffer, private communication.


220. W. Marty, private communication.


227. W. O'Sullivan, private communication.