A THESIS

entitled

COBALT(III) PROMOTED SYNTHESIS OF PEPTIDES

submitted by

JOHN DEKKERS

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The work described in this Thesis

is the candidate's own work.

JOHN DEKKERS

John Dekkers

Research School of Chemistry,
Australian National University,
Canberra, A.C.T.

DEDICATION

This Thesis is dedicated to my daughter

Kim.

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SUMMARY

A general non-aqueous method for synthesis of dipeptides via cobalt(III) chelated amino acid esters has been developed. It appears that the reaction occurs without racemisation of the chelated and C-terminal amino acid and the chelated amino acid esters should be useful reagents in general peptide synthesis.

The kinetics and mechanism of this reaction has been investigated and the formation and decomposition of an amino-carbinol tetrahedral intermediate has been observed. This intermediate has not been seen in the aminolysis of organic esters and it was deduced that its appearance in this instance arises because of stabilisation with respect to loss of alcohol of the chelated amino-carbinol species.

Since there are chiral centres at both cobalt and the α-carbon atom of the amino acid, the possibility of stereoselective coordination exists. The preferential addition for diastereoisomer pairs was examined in four instances. A rate difference of 3 to 6 was observed and the magnitude of the effect was influenced by the number of chiral centres present. Always the ΛS(S) complexes were formed more rapidly.

The hydrolysis of the peptide bond in complex dipeptides has been examined. Mutarotation and proton exchange of the chelated amino acid were found to be synchronous. These processes are about 8 times faster than hydrolysis of the peptide bond and no stereoselectivity was observed for cleavage. Racemisation of the amino acid during hydrolysis is not important for sequence analysis since the amino acid only has to be identified and its optical purity is irrelevant. Compared to the uncoordinated substrate, a rate enhancement of about 10^6 for hydrolysis and 10^2 for racemisation in the chelated peptide complexes was observed.

Some other observations have been made concerning the formation of chelated amino acids and no stereoselectivity was found in their preparation.

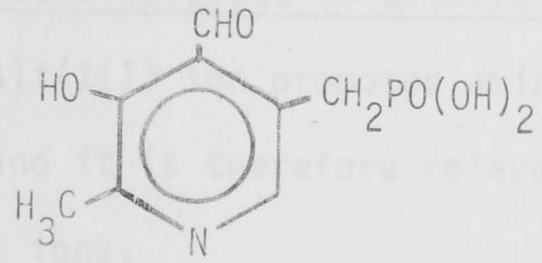
CHAPTER 1

INTRODUCTION

Many organic reactions are catalysed by metal ions and it has been suggested frequently that metal complexes or chelated compounds are the active species (1,2). In recent years, much investigation has taken place into metal ion promoted hydrolysis of amino acid esters (3), amides (3), peptides (3), phosphate esters (4), and carboxylation (5) and decarboxylation (6,7) reactions. An understanding of the mechanisms of these metal ion promoted model reactions may be of value in interpreting the function of metal ions associated with biological systems.

Metal ions are, in many cases, necessary for fulfilling a biological role (8-13) and the metal is believed to reside at, or close to the active site (14). Several recent three dimensional X-ray structural analyses have strongly suggested direct involvement of the metal ion, or alternatively a proton, in the reaction (15).

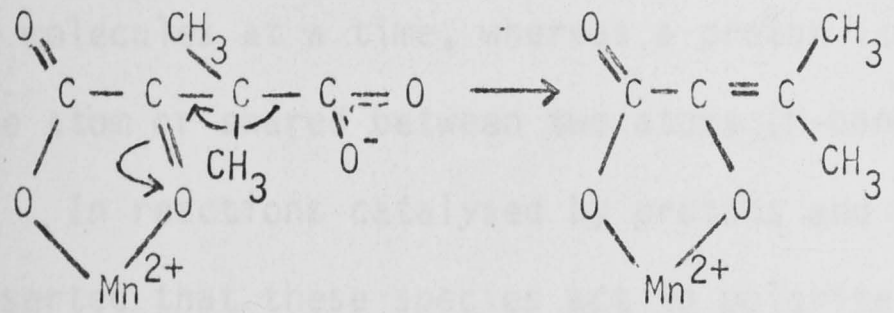
The participation of metal ions in biological systems may be illustrated by their effect on the rate or course of model systems utilising simple organic molecules. For example, several enzymes involved in amino acid metabolism (including amino transferases and decarboxylases) require pyridoxal phosphate (PLP,I) as a co-factor (16).



(I)

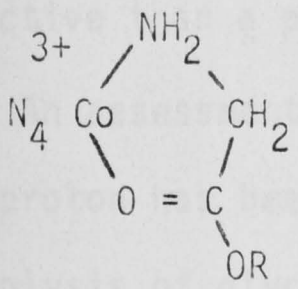
It has been postulated that all such reactions involve the formation of a Schiff-base intermediate between the amino acid and (I) as the first step. Simple systems containing metal ion, amino acid, and an analogue of (I) duplicate the course of many of these enzyme reactions

and the first step in these model reactions is the formation of a Schiff-base complex (17). Also, the Mn^{2+} catalysed decarboxylation of dimethyloxaloacetic acid (1.1), and some of its derivatives (18) may serve as models for oxaloacetate decarboxylase which is known to require a metal ion for activity (19).



$+ CO_2$ (1.1)

Cobalt(III) ion activation in the hydrolysis of chelated glycine isopropyl ester (II) and glycine peptides (III) induces ca. 10^6 fold rate enhancement compared to hydrolysis of the uncoordinated species (20,21). It has been suggested that Co(III) in structures(II) and (III) may serve as a model for Zn (11) in carboxypeptidase A (22) which cleaves the peptide linkage at the carboxyl end of a polypeptide, and for Mn(11), Co(II) or Zn(II) in amino peptidases which cleave N-terminal residues (23).



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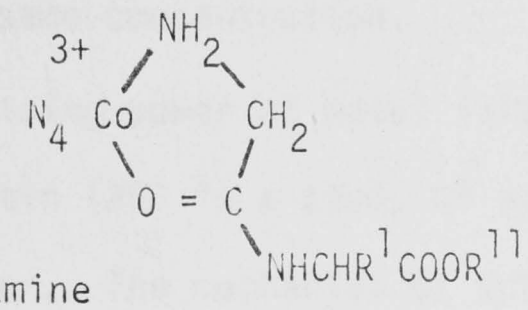


(III)

N_4 = tetramine

Properties of the Metal Ions Relevant to Catalysis

In this Thesis cobalt(III) ion promoted aminolysis and hydrolysis reactions are examined and it is therefore relevant to discuss briefly some properties of metal ions.

The function of a metal ion can be compared to a proton since both are Lewis acids and electrophiles; that is, they accept a share in an electron pair to form a σ bond. However, a metal ion is more versatile than a proton. Firstly, it may exist in constant concentration over a wide range in pH whereas the proton obviously cannot. Secondly, it can

present to the active site a centre of positive charge greater than one, whereas a proton cannot. Also, due to the presence of filled p, d or f orbitals in the valence shell it can donate negative charge to empty orbitals on the ligand to form a π bond whereas a proton does not have this ability (24,25). Finally, a metal ion coordinates to several atoms or molecules at a time, whereas a proton is specifically bound to only one atom or shared between two atoms (H-bond).

In reactions catalysed by protons and metal ions it has been asserted that these species act to polarise the reaction centre. For example, in the decarboxylation of dimethyloxaloacetic acid (1.1), an electron pair initially associated with the carboxyl group is shared with the metal atom (18), and this is considered the driving force for the reaction by weakening the bond adjacent to the carboxyl group and allowing a unimolecular dissociation of CO_2. The effect of different metal ions on the rate of decarboxylation of dimethyloxaloacetic acid is shown in Table 1.1 and it can be seen that Al^{3+} and Fe^{3+} are in general more effective than divalent metal ions, and both are more effective than a proton at the same concentration.

An assessment of the polarising power of metal ions relative to the proton has been made by Martin (26) in a study of acid catalysed hydrolysis of glycine ethyl ester. The mechanism of acid catalysed hydrolysis of esters is generally accepted as occuring via attack of water on the protonated carbonyl oxygen atom of the ester (27), Reaction 1.2. The activation provided by protonation of a small fraction of ester molecules, $pK_a \simeq -6.5$ (28), is sufficient to account for the observed hydrolysis in dilute acid solution.

$$RCOC_2H_5 + H_3^+O \overset{1/K_a}{\rightleftharpoons} \overset{H^+}{\underset{O}{\overset{\|}{RCOC_2H_5}}} + H_2O \longrightarrow \underset{O}{\overset{\|}{RCOH}} + C_2H_5OH + H^+ \qquad (1.2)$$

TABLE 1.1

Effect of Metal Ions on the Rate of Decarboxylation of Dimethyloxaloacetic Acid

Metal Ion	Conc.	pH	k,min^{-1}
Absent	-	4.6	0.0024
Cu^{2+}	0.001	4.6	.143
Al^{3+}	0.001	4.6	.128
Ni^{2+}	.01	4.6	.0216
Mn^{2+}	.01	4.6	.0058
Absent	-	2.4[a]	.0032
Fe^{2+} [b]	.002	2.4[a]	.0102
Fe^{3+}	.002	2.3[a]	.301
Absent	-	0	.00032
Pd^{2+}	.01	0	.00061

[a] Solution unbuffered. [b] Experiment conducted under nitrogen.

TABLE 1.2

Specific Rate Constants for Water Attack on Ethyl Acetate and Glycine Ethyl Ester Complexes at 25°

Species	k_{H_2O} sec^{-1}	Reference
$CH_3C(^{+}OH)OC_2H_5$	$10^{2.5}$[a]	28
$[Cu(NH_2CH_2COOC_2H_5)]^{2+}$ [b]	$10^{-4.4}$	29
$[Co(en)_2NH_2CH_2COOC_2H_5]^{3+}$ [c]	$10^{-2.1}$	30
$NH_3^{+}CH_2COOC_2H_5$ [b]	$10^{-8.3}$	29

[a] The first order rate constant is obtained by using a second order rate constant at 25° of $10^{-4}M^{-1}sec^{-1}$ for acid hydrolysis of ethyl acetate (31) and multiplying by an acid ionisation constant of $10^{6.5}M$ (28). [b] μ = 0.16 (KNO_3). [c] μ = 0.66 ($NaClO_4$), $[H^+]$ = 0.1M.

In Table 1.2, the reactive substrates protonated or coordinated are compared molecule for molecule. The larger rate for water attack on the protonated ester compared with the acid independent path for the hydrolysis of esters when chelated to Co^{3+} and Cu^{2+} (Table 1.2), was attributed to the larger polarising power of the proton relative to a coordinated metal ion (26).

A similar conclusion can be arrived at by comparing the relative acidities of the hydrated proton, Cu^{2+}_{aq}, Co^{3+}_{aq} and $(NH_3)_5CoOH_2^{3+}$, Equilibria (1.3) and (1.4). For H^+, Cu^{2+} and Co^{3+} the respective

$$M(H_2O)_n^{z+} \rightleftharpoons M(H_2O)_{n-1}(OH^-)^{(z-1)+} + H^+ \quad (M = H^+, Cu^{2+} \text{ and } Co^{3+}) \qquad (1.3)$$

$$(NH_3)_5\,Co^{3+}\!-\!OH_2 \rightleftharpoons (NH_3)_5\,Co^{2+}\!-\!OH + H^+ \qquad (1.4)$$

pK_a values (32) are -1.7, 7.3 and 1.7 indicating that the polarising power of the proton is about $10^{9.0}$ and $10^{3.4}$ times greater than for the aquo metal ions. For $(NH_3)_5\,Co^{3+}\!-\!OH_2$, a $pK_a = 6.2$ has been obtained (32). It appears also that the polarising power of the Co(III) ion is greatly reduced in the presence of five coordinated ammonias compared with $Co^{3+}aq$.

Recent kinetic and mechanistic studies (20,21) of the hydrolysis of N-O chelated amide and peptide complexes of the type $[Co(en)_2NH_2CH_2CONHR_1R_2]^{3+}$((III), N_4= 2 en, en=ethylenediamine) and lysis by nitrogen and oxygen bases of Co(III) chelated glycine isopropyl ester ((II), R = C_3H_7, N_4 = 2 en) have been reported. These studies appear to demonstrate the effectiveness of the $Co(en)_2^{3+}$ moiety in polarising the carboxyl group of the peptide or ester. The effect of the metal ion on the rate of hydrolysis of $NH_2CH_2COOC_2H_5$ is large, Table 1.3. Comparison of N-O chelated $[Co(en)_2NH_2CH_2COOC_3H_7]^{3+}$ and $NH_2CH_2COOC_2H_5$ shows a rate enhancement of $\sim 10^6$ in the Co(III) chelate. A comparison of the Co(III) chelate with the N-protonated ester shows that the cobalt is far more

effective in promoting hydrolysis than is the proton. However, it appears that when H^+ and $(NH_3)_5Co^{3+}$ are bound to nitrogen similar rates are observed. The latter observation appears to suggest that the million fold rate enhancement observed in the N-O chelated ester can be attributed to direct metal ion activation of the carbonyl centre. An estimate of $\sim 5\ M^{-1}\ sec^{-1}$ for k_{H_2O} for hydrolysis of $^+NH_3CH_2C(-OR)\!=\!OH^+$ (26,35) is larger than k_{H_2O} for $[Co(en)_2NH_2CH_2COOC_3H_7]^{3+}$ ($k_{H_2O} = 1.9\times10^{-5}\ M^{-1}\ sec^{-1}$ (20)) by a factor of $\sim 10^5$. This again implies that the metal ion is a poor substitute for a proton in hydrolysis reactions.

TABLE 1.3

Comparison of Second Order Rate Constants for Hydroxide Attack on Coordinated and Uncoordinated Glycine Ethyl Ester

At 25° Species	$k_{OH}\ M^{-1}\ sec^{-1}$	Reference
$[Co(en)_2NH_2CH_2COOC_3H_7]^{3+}$ [a]	1.5×10^6	20
$NH_2CH_2COOC_2H_5$ [b]	0.63	33
$^+NH_3CH_2COOC_2H_5$ [c]	24	29
$[(NH_3)_5CoNH_2CH_2COOC_2H_5]^{3+}$ [d]	30	34

[a] $\mu = 1$ ($NaClO_4$); [b] $\mu = 0.1$; [c] $\mu = 0.16$ (KNO_3); [d] $\mu = 0.1$ (KNO_3).

Ester hydrolysis of $(NH_3)_5{}^{3+}Co\text{-}NH_2CH_2COOC_2H_5$ and $NH_3{}^+CH_2COOC_2H_5$ is catalysed in strong acid so that a term first order in acid is likely. This coincides with protonation at the ester carboxyl group. However, when the ester is chelated through the NH_2- and carbonyl oxygen the acid dependent path vanishes since the metal ion substitutes for a proton in the active species. If the equilibrium constant for protonation of ester is accepted (26) then molecule for molecule the protonated species is more effective than the chelated species. The big advantage of the

chelated form is a wide-range of stability at varying pH. Under these conditions the reactive species may be present in high concentration over a wide range of H^+ concentration.

The metal ions which form labile complexes have been most frequently used in metal ion promoted aminolysis and hydrolysis reactions (3). In these systems problems of ligand lability made interpretation of kinetic data difficult and assignment of thermodynamic parameters impractical. Solutions to some of these problems are presented in this Thesis which examines metal ion promoted reactions using cobalt(III) complexes. These complexes are generally relatively inert to substitution of the ligands (36) and kinetic studies of ligand reactivity may be conducted without the complication of pre-equilibria due to ligand dissociation. Furthermore, the properties of these complexes frequently enable reaction intermediates to be isolated and examined independently. In addition, the kinetic patterns for cobalt(III) complexes are well documented and the crystal structures of many cobalt(III) complexes have been determined. Often the stereochemical course of a reaction may be evaluated.

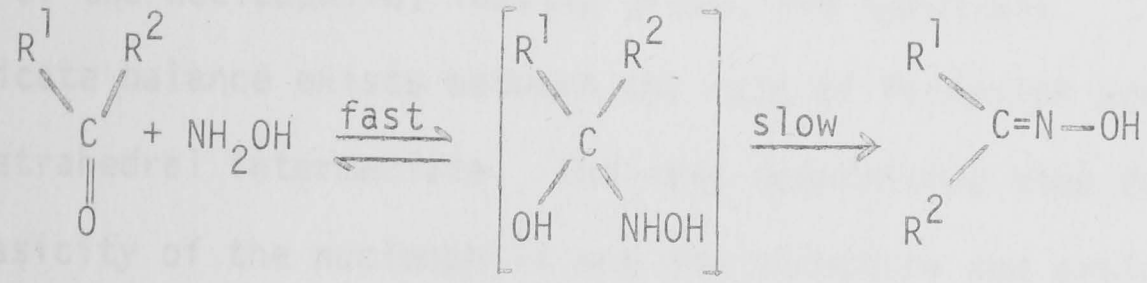
Addition of Nitrogen Nucleophiles to the Carbonyl Centre of Esters

Since much of the work presented in this Thesis is concerned with addition and substitution at carbonyl centres it is pertinent to review briefly their organic chemistry

Ester aminolysis is an example of nucleophilic substitution at unsaturated carbon (37-41). There is general agreement that the substitution proceeds via an addition-elimination mechanism (1.5).

$$R^1\text{-}\overset{\overset{O}{\|}}{C}\text{-}OR + \underset{\text{(nucleophile)}}{\ddot{N}} \rightleftharpoons R^1\text{-}\overset{\overset{O^-}{|}}{\underset{\underset{N}{|}}{C}}\text{-}OR \longrightarrow R^1\text{-}\overset{\overset{O}{\|}}{C}\text{-}N + OR^- \qquad (1.5)$$

with the formation of a chemically distinct tetrahedral intermediate species prior to loss of the ester group. In several instances, such as in the aminolysis of aldehydes and ketones, direct evidence for the formation of a tetrahedral intermediate is available (42,43), Reaction 1.6.



(1.6)

Tetrahedral addition intermediates are much less readily observed in the aminolysis of esters because of resonance stabilisation of the carboxyl group by the -OR group (27) (1.7).

(1.7)

Formation of a well defined addition compound is observed when the acyl group is activated by strongly electron withdrawing substituents or ring structures (44,45). However, the reaction ceases at that stage. Moreover, a variety of indirect evidence exists which implies that aminolysis of esters does proceed via a discrete tetrahedral species (41).

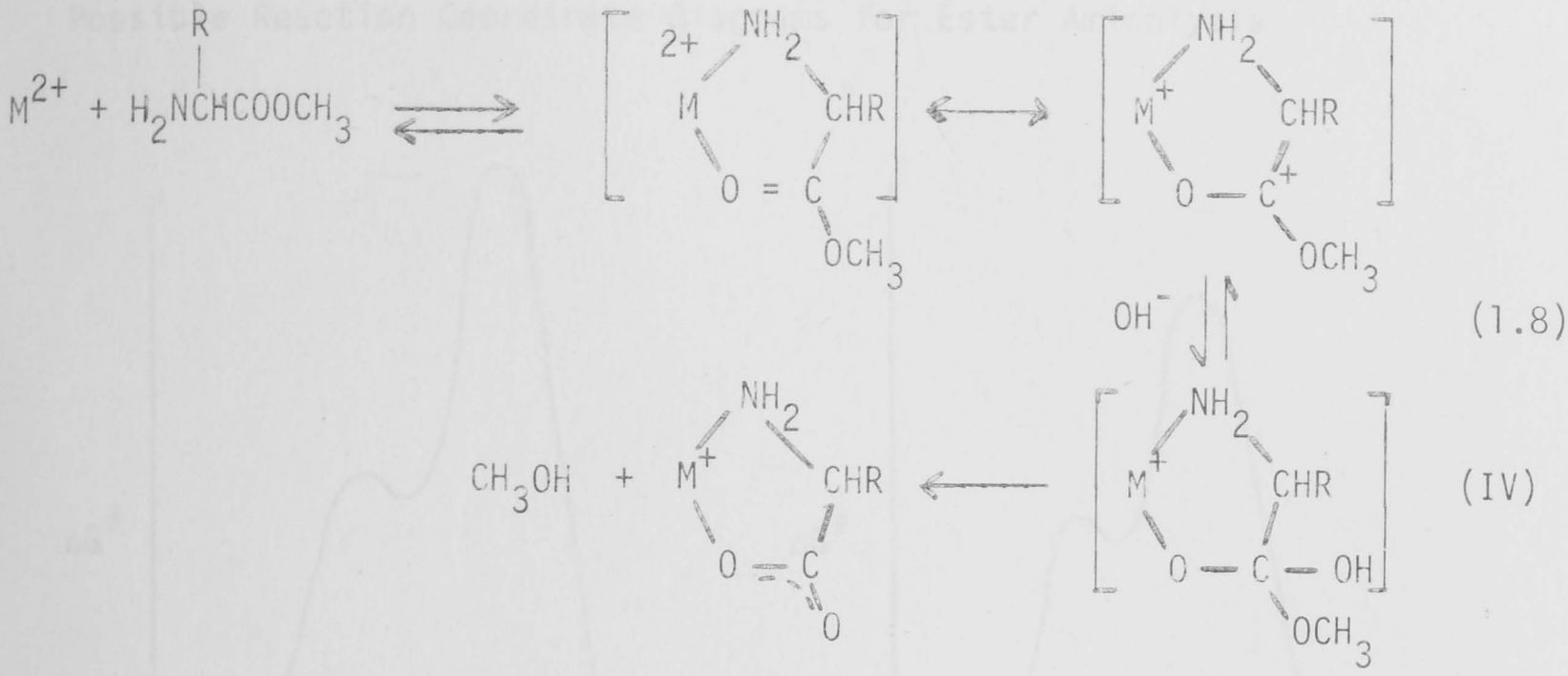
In several studies, breaks in pH-rate profiles not attributable to ionisation of reagents, and breaks in catalyst concentration - rate curves not caused by complexing of reactants have been observed (39,41). Also, breaks in structure - reactivity correlations and partitioning of an intermediate after the rate - determining step have been reported (41). Such characteristic kinetic patterns require that the reaction involves at least two sequential steps and thus provides compelling evidence for the existence of at least one intermediate on the reaction path.

Jencks (46) has made a comprehensive analysis of a number of aminolysis reactions (47) in which the intermediate on the reaction path does not accumulate in detectable concentrations. The author discusses the formation, breakdown and "inherent instability" of this intermediate in terms of the nucleophile, leaving group, and substrate. It appears that a delicate balance exists between the rate of formation and breakdown of the tetrahedral intermediate. The rate determining step depends on the basicity of the nucleophile and the structure and basicity of the leaving group, and on the presence of general bases in solution.

The mechanism of a particular aminolysis reaction may be dictated by changes in the rate determining step under various conditions. For example, in the aminolysis of imidates (47a) at low pH the expulsion of alcohol through a transition state of zero charge is slow and the acid catalysed loss of amine is fast so that the former step is rate determining, Fig. 1.1(a). However at high pH, the attack of amine on the protonated imidate is the rate determing step and the expulsion of alcohol is fast so that the intermediate decomposes to products more often than it reverts to starting materials, Fig. 1.1d. Despite this understanding, the rates of formation and decomposition of the tetrahedral species do not seem to have been observed for any one single substrate (48). For at least one instance it would therefore seem desirable to observe both paths independently so that the effect of changes in conditions for each may be determined.

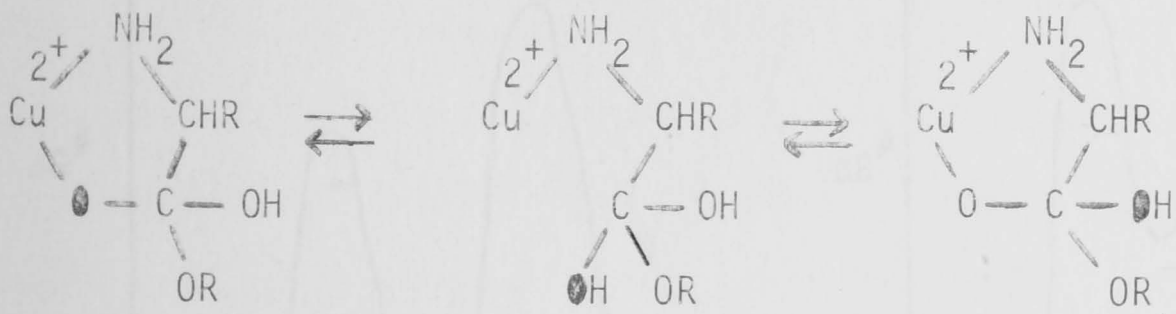
The application of this background to the hydrolysis of chelated acyl species is an obvious step and this has been done in a variety of studies (29,49-56). The metal ion assumes the role of the proton in polarising the C=O centre and a considerable rate enhancement relative to the uncoordinated species is observed. From a study on the hydrolysis of methyl esters in the presence of a divalent metal ion (Cu^{2+}, Co^{2+}, Mn^{2+} and Mg^{2+}) Kroll (49) proposed that the tetratedral intermediate took

the form of the chelated alcohol ester (IV), Reaction 1.8. In a subsequent



(1.8)

study (54) on ethyl phenylalanate hydrolysis in the presence of Cu^{2+} ions, ^{18}O-tracer results demonstrated the requirement for a symmetrical tetrahedral intermediate and it was proposed that addition of water occurred in the chelated ester with rapid equilibration between the coordinated and uncoordinated alcohol functions, Reaction 1.9.



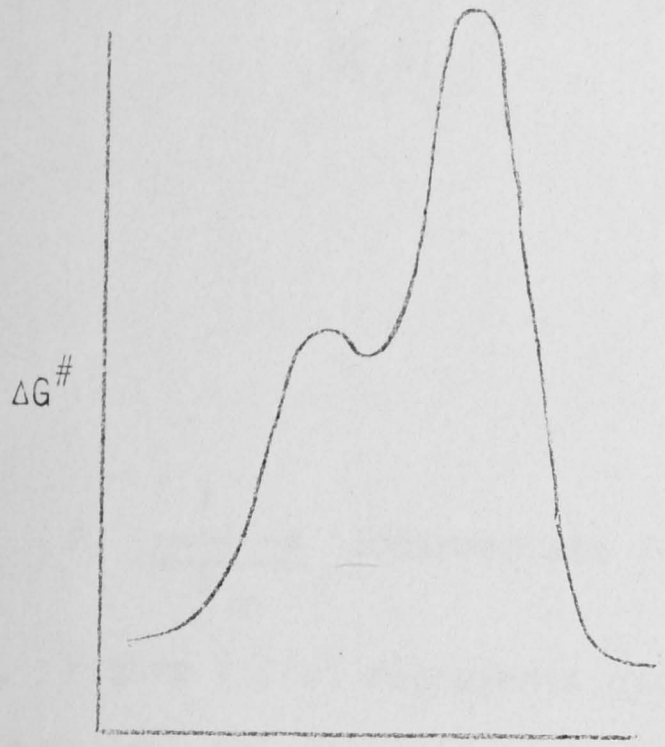
(1.9)

Similar tetrahedral intermediates have been proposed or implied also in cobalt(III) promoted reactions of chelated amino acid esters (20-21, 30, 57-58). Here the addition of amine to the carboxyl centre was considered to produce a tetrahedral amino-carbinol intermediate as the rate determining step and loss of alcohol was fast, Reaction 1.10. However, the results did not absolutely require this to be the case.

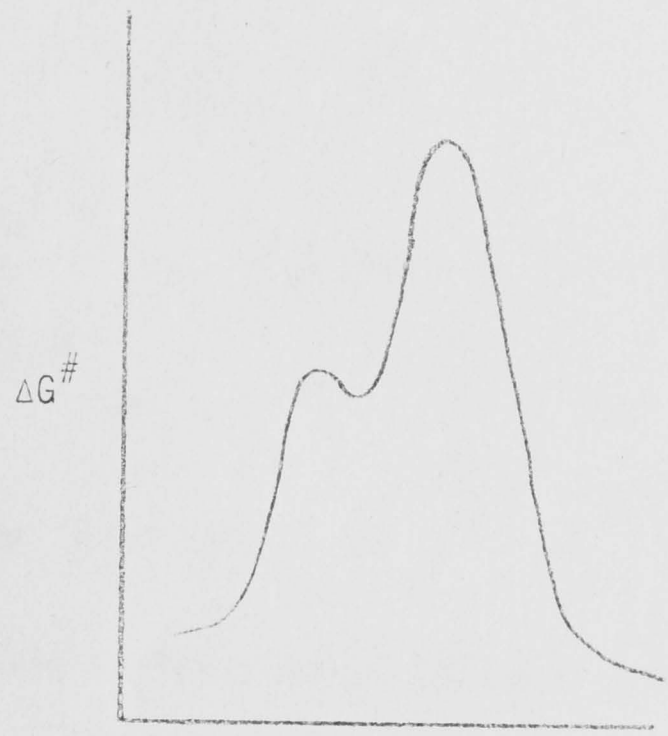
Possible effects of a metal ion on the stability of the tetrahedral intermediate are depicted crudely by Fig. 1.1 (b-d) for Reaction 1.10a.

FIGURE 1.1

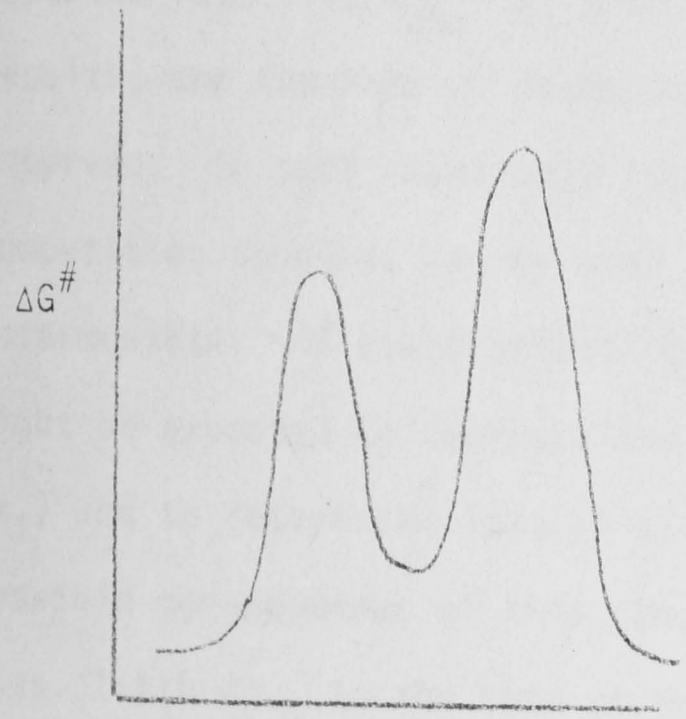
Possible Reaction Coordinate Diagrams for Ester Aminolysis



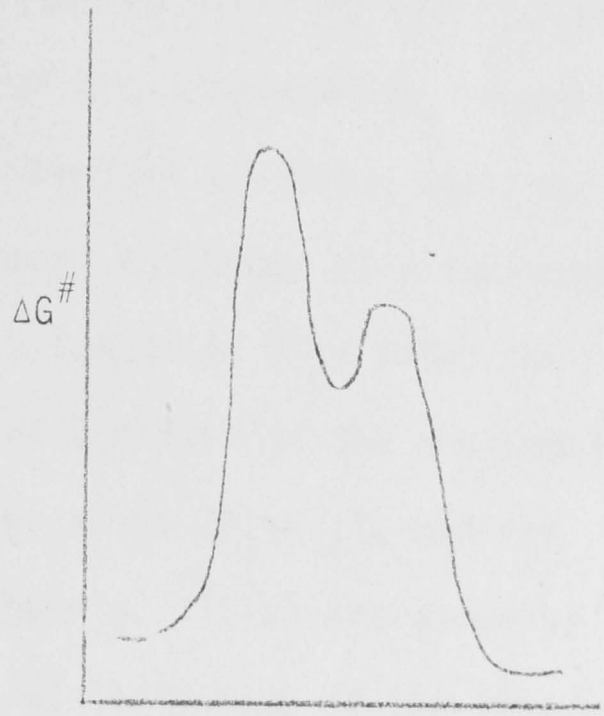
(a) Reaction-coordinate



(b) Reaction-coordinate



(c) Reaction-coordinate



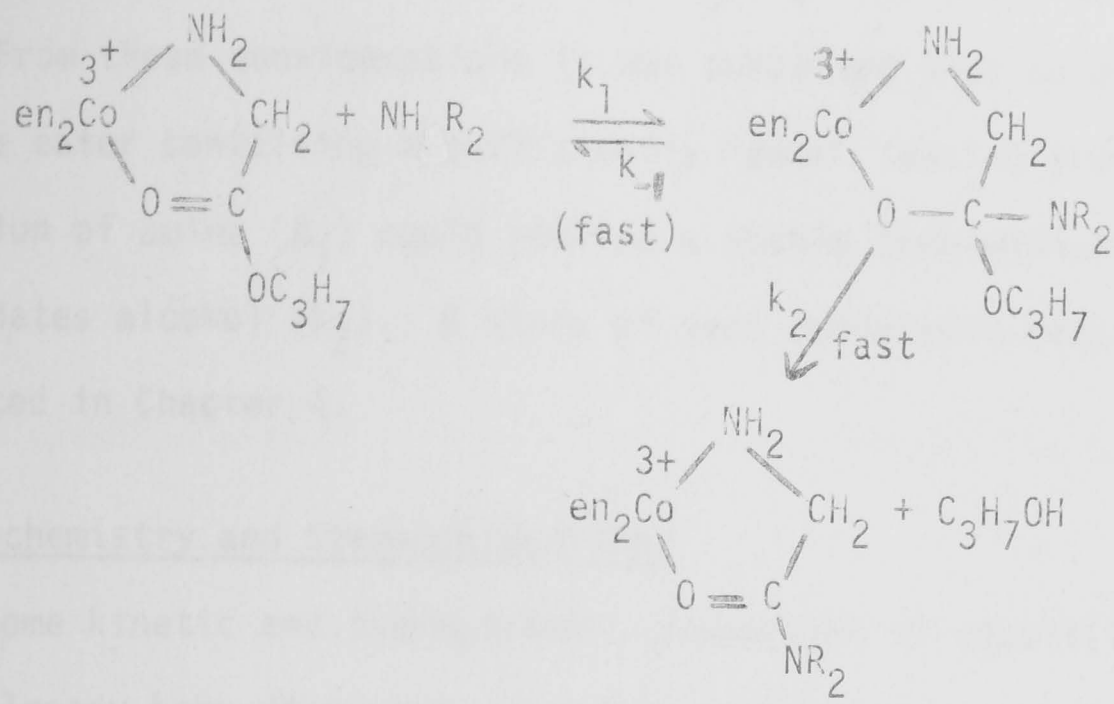
(d) Reaction-coordinate

(a) Aminolysis in absence of a metal ion.

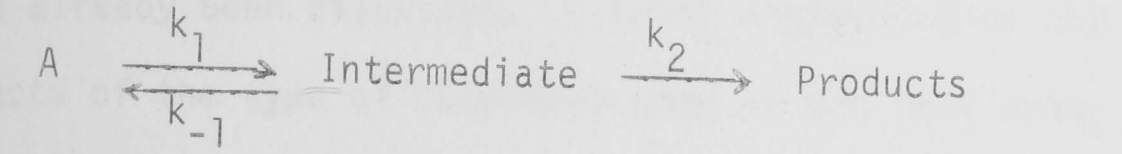
(b) Aminolysis in (a) in the presence of a metal ion with no stabilisation of the tetrahedral intermediate.

(c) Aminolysis in (a) in the presence of a metal ion with stabilisation of the tetrahedral intermediate.

(d) Aminolysis in (a) in the presence of a metal ion with the intermediate after the rate controlling step.



(1.10)



(1.10a)

Figure 1.1(a) represents hydrolysis in the absence of metal ions and using a steady state treatment $k_{obs} = k_1 k_2 / k_{-1} + k_2$. Thus both the addition and elimination rates are important in determining the observed rate. If $k_{obs} = k_1$ then the situation given by Fig. 1.1(d) results, and the rate of decomposition of the intermediate is not observed. In such cases only indirect chemical evidence, such as competition studies, can be used to support existence of a tetrahedral intermediate. On electrostatic grounds, chelation of a metal ion might be expected to increase the rate of addition of the nucleophile (k_1) and to retard the loss of alcohol or amine (k_2,k_{-1}), and the possible consequences of this compared to Fig. 1.1(a) are given by Figs. 1.1(b-d). In the case of no net stabilisation of the intermediate, Fig. 1.1(b), $k_{obs} = k_1 k_{2/k_{-1}+k_2}$ and catalysis is apparent only in k_1. If the intermediate is also stabilised with respect to the transition states by the metal ion then both k_{-1} and k_2 are affected and a complex kinetic situation could arise. In the event of $k_{-1} >> k_2$ then equilibration between the intermediate and reactants arises, and $k_{obs} = Kk_2$ where $K = {}^{k_1}/_{k_{-1}}$, Fig. 1.1(c). Provided k_1 is not very rapid, then the approach to the equilibrium condition may be followed kinetically and therefore the physical and chemical properties of the intermediate

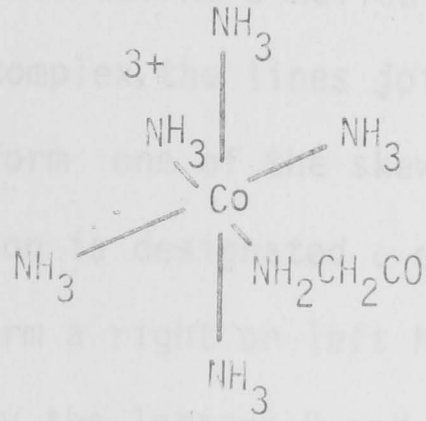
can be investigated. In such a case k_2 may also be measured directly.

From these considerations it was conceived that using a Co(III) active ester containing a sufficiently "poor" leaving group the rapid addition of amine (k_1) could realise a stable intermediate which slowly eliminates alcohol (k_2). A study of such aminolysis reactions (1.10) is reported in Chapter 4.

Stereochemistry and Stereoselectivity

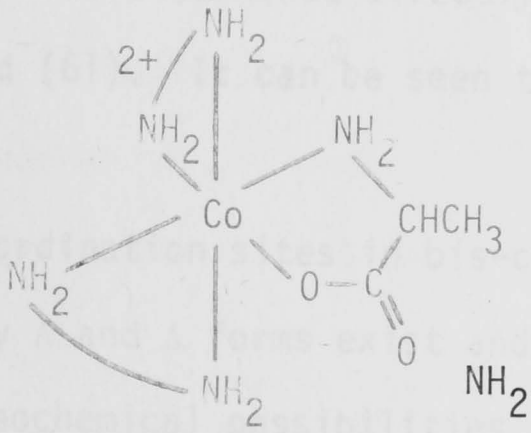
Some kinetic and thermodynamic properties of cobalt(III) complexes have already been discussed. A brief discussion of the stereochemical aspects of the type of complexes used is now pertinent.

Cobalt(III) amine complexes appear to be universally octahedral and $[Co(NH_3)_5NH_2CH_2COOH]^{3+}$ (V) is a typical example. By joining the



(V)

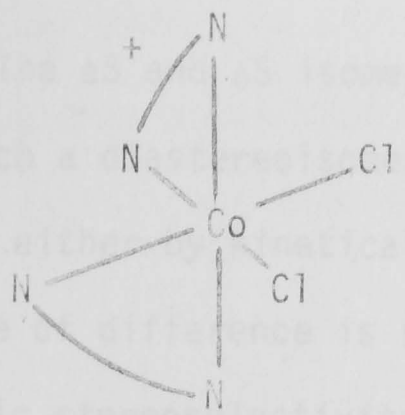
ammonia groups with an alkane chain, chelates are derived, for example $[Co(en)_2ala]^{2+}$ (VI) is a molecule in this class. In the bis- chelate



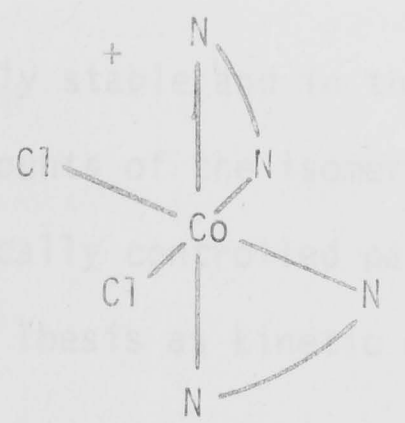
(VI)

$NH_2\text{-}NH_2 = NH_2CH_2CH_2NH_2$

complexes the chelate groups can be arranged either <u>cis</u> or <u>trans</u> to each other about the metal ion and this is demonstrated by the <u>cis</u> and <u>trans</u> isomers of $[Co(en)_2Cl_2]^+$, (VII-IX). It can be seen that <u>cis</u>-$[Co(en)_2Cl_2]^+$ existsin two enantiomeric forms which are mirror images, whereas the <u>trans</u> complex has a plane of symmetry and is not optically active. The <u>cis</u> isomers are designated by the recommended IUPAC nomenclature by the Greek letters Λ and Δ (59,60).

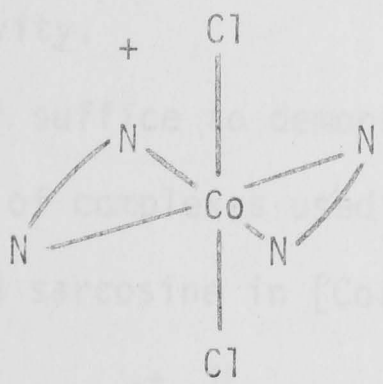


(VII)



(VIII)

Λ-cis$[Co(en)_2Cl_2]^+$ Δ-cis$[Co(en)_2Cl_2]^+$



(IX)

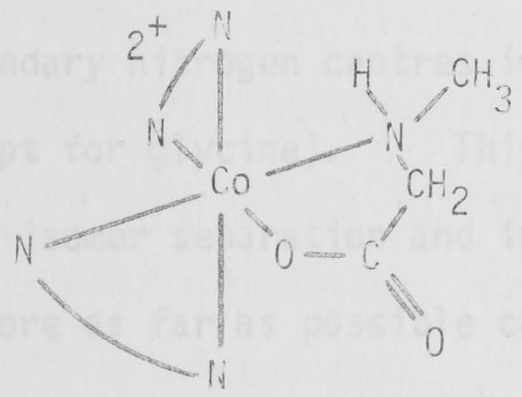
trans-$[Co(en)_2Cl_2]^+$

This designation of isomers is independent of any standard structure, or the nature of ligands. The proposal is based on the idea that two skew and non-orthogonal lines define a helical system. In an octahedral metal chelate complex, the lines joining adjacent ligating atoms of the same ligand form one of the skew lines in bis and tris chelates. The configuration is designated Δ or Λ depending on whether the pairs of skew lines form a right or left handed helical system respectively. Historically, the letters D and L were chosen (61) to assign absolute configuration of structures based on the structure of the D-$[Co(en)_3]^{3+}$ ion which was determined directly using the X-ray anomalous dispersion method (61). It can be seen therefore that Λ ≡ D and Δ ≡ L.

When the remaining coordination sites in bis-chelates are replaced by a bidentate chelate only Λ and Δ forms exist and the trans form vanishes. Additional stereochemical possibilities arise when one of the chelates is asymmetric. For example, in $[Co(en)_2ala]^{2+}$ (VI) there are two chiral centres in the ion and four diastereoisomers may exist, ΛS, ΔR, ΛR and ΔS. These are related in enantiomeric pairs, ΛS ΔR, and ΔS ΛR. The R and S nomenclature is used to define absolute configuration at the asymmetric carbon centres according to the IUPAC nomenclature and sequence rules (62).

The ΔS and ΛS isomers may not be equally stable and in the formation of such a diastereoisomeric pair unequal amounts of the isomers may arise either by kinetically or thermodynamically controlled paths. The degree of difference is referred to in this Thesis as kinetic or thermodynamic stereoselectivity.

Two examples will suffice to demonstrate the extremes which are observed in the types of complexes used in this Thesis. It has been shown that coordinated sarcosine in $[Co(en)_2sar]^{2+}$ ion (XI) is



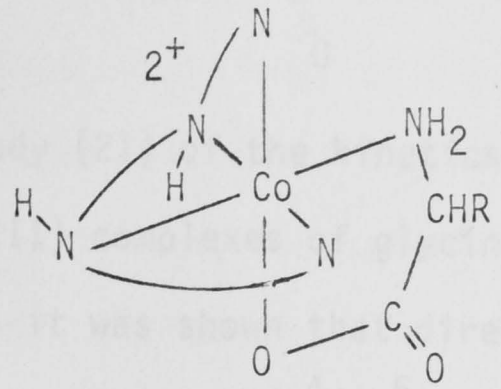
(XI)

stereoselective (63). This was demonstrated by the fact that inversion of the secondary N-methyl centre was not observed in either the Λ- or Δ-$[Co(en)_2sar]^{2+}$ ion even under conditions where N-H was exchanging rapidly (i.e. pH 8.8) (63). A conformational analysis of non-bonded interactions demonstrated that the Δ-$[Co(en)_2(S)\text{-}sar]^{2+}$ ion was considerably more stable than the Λ-$[Co(en)_2(S)\text{-}sar]^{2+}$ ion (63). A crystal structure analysis confirmed the assignment of the most stable isomer as ΔS (64). In contrast, the $[Co(en)_2ala]^{2+}$ and $[Co(en)_2val]^{2+}$ ions have been shown to undergo base-catalysed α-proton exchange and mutarotation with identical rate laws (65). Mutarotation

$$v = k_M\,[complex]\,[OH^-] = k_D\,[complex]\,[OD^-]$$

at the chelated amino acid α-carbon centre provides a method of establishing equilibrium between diastereoisomeric forms and this enables the measurement of their relative stabilities. Under equilibrium conditions the ratios Λ-$[Co(en)_2(R)\text{-}val]^{2+}$/Λ-$[Co(en)_2(S)\text{-}val]^{2+}$ = 1.7 and Λ-$[Co(en)_2(R)\text{-}ala]^{2+}$/Λ-$[Co(en)_2(S)\text{-}ala]^{2+}$ = 1.0 (65).

Some reference will be made to the cobalt(III) trien amino acid complexes which have the configuration (X). This system is akin to the bis(en) complex chemistry but is considerably more complicated



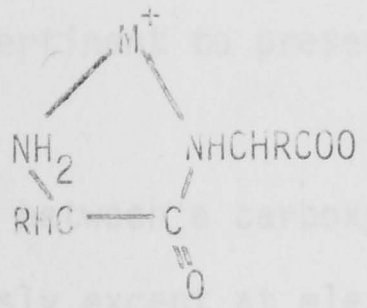
(X)

stereochemically. Chiral centres exist at the cobalt(III) centre, at the coordinated secondary nitrogen centres in the ligand and in the amino acid moiety (except for glycine). This leads to numerous difficulties concerning isomer separation and interpretation of experiments, and therefore as far as possible complexes of this type were avoided.

The above aspects of stereoselectivity are relevant to this Thesis since mutarotation at the chiral carbon centres of the amino acid moieties is examined in Chapters 5 and 6. Also, the addition of amino acid moieties to the chelated amino acid esters is examined, and the possibility of stereoselectivity arises for this addition.

Hydrolysis and Mutarotation of Chelated Peptides.

The effect of metal ions on the hydrolysis of amino acid esters and amides is well known (3,13,66) and a possible significance of these studies is that they may serve as models for the appreciably more complex biological processes whereby peptides are degraded to the parent amino acids. However, there is very little data available on the divalent metal ion catalysed hydrolysis of peptides. Under the conditions required for hydrolysis, it has been demonstrated that the divalent metal ion is coordinated to the amide nitrogen rather than to the carbonyl oxygen as shown in (XI) (29,67,68). This form of coordination stabilises rather than promotes the hydrolysis of the peptide bond (29,34,68-70).



(XI)

In a recent study (21) of the kinetics and mechanism of hydrolysis of N-O chelated Co(III) complexes of glycine amides, glycylglycine and glycylglycine esters it was shown that direct activation of the carboxyl group by the metal results in a 10^4-10^6 fold enhancement in rate compared to the uncoordinated species. In the same study it was also demonstrated that deuteration of the methylene protons of the chelated glycine of the peptide complexes was ca. 8 times faster than peptide bond cleavage. Although this suggests that racemisation of the asymmetric centres in the chelate will precede peptide hydrolysis it is unimportant for N-terminal peptide sequencing procedures. Chapter 6 reports an investigation of the hydrolysis of several N-O chelated peptides of α-amino acids and also investigates the extent of racemisation at the α-carbon centre of the chelated and C-terminal amino acid during hydrolysis.

Peptide Synthesis

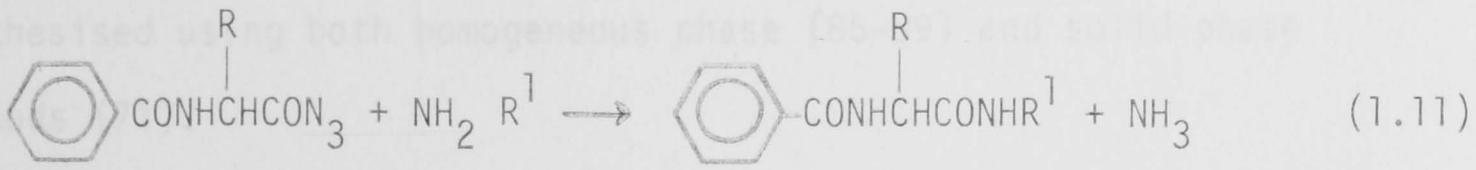
During the last decade synthesis of peptides has developed dramatically. Advances such as solid-phase peptide synthesis (71) now permit the preparation of biologically active peptides for practical purposes. In addition to the already accomplished synthesis of peptide hormones and peptide antibiotics, synthetic work on enzymes is well advanced. New procedures such as coupling methods for forming the peptide bond, protecting groups for preventing unwanted side reactions and selective methods for removing the protecting groups have appeared. Furthermore, new methods have been developed for detecting and maintaining optical purity. These advances and other related aspects are described in numerous reviews (71-76). Since part of this Thesis is concerned with

peptide formation it is pertinent to present a brief summary of some of the methods.

Amide bond formation between a carboxyl group and an amino group does not occur spontaneously except at elevated temperatures and therefore one of the two reactants is converted into a form which is more reactive. Derivatives of the carboxyl component are by far the most commonly employed. Synthesis of a biologically active peptide also requires a method whereby retention of configuration at each asymmetric centre is maintained. Each step in the synthesis therefore requires careful investigation of the possibility of racemisation (72).

The classical method of activating an amino acid is to transform it into its N-protected acid chloride. This technique, first used by Fischer (77), has been adapted in many syntheses using tosylamino acid chlorides. However, they undergo a fragmentation reaction when exposed to alkalis (78),and benzyloxycarbonylamino acid chlorides form the corresponding Leuchs anhydrides on heating, or even long storage at room temperature (72). A further disadvantage of the acid chloride synthesis is the contamination of the acid chlorides with phosphorus oxychloride which results in phosphorus containing peptide products.

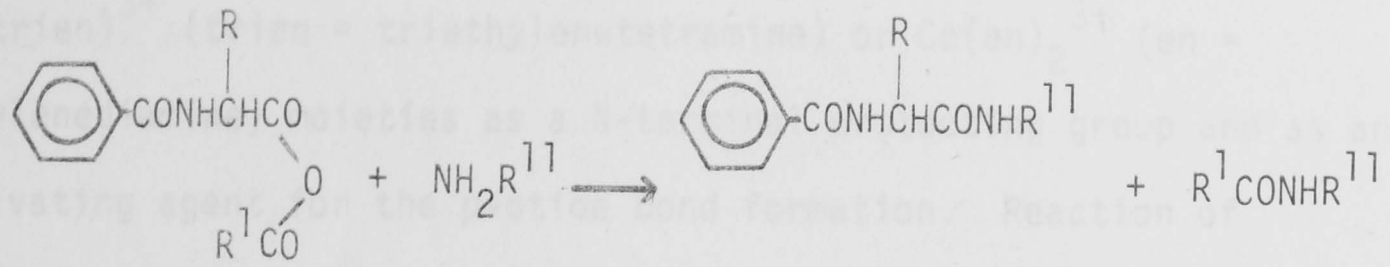
The use of reactive azides to couple amino acids and peptides (1.11) in non-aqueous media was pioneered by Curtius (79). This method has been used extensively for the preparation of optically active peptides since it avoids the racemisation problem (80). However, it can lead to



(1.11)

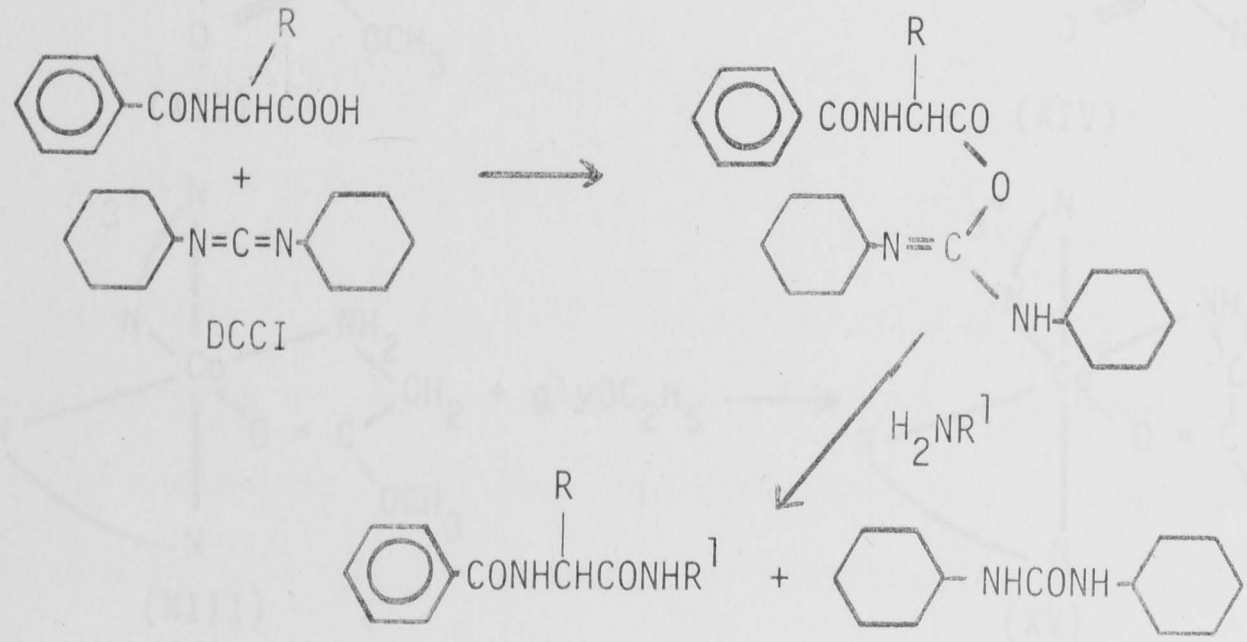
unwanted by-products such as urea derivatives and amides (81) and also the acid azide is difficult to synthesise in many instances (81).

The mixed anhydride method (82) has proved of great practical value in synthesis of small peptides. It leads however to two acylation products as shown in Reaction (1.12).



(1.12)

The use of dicyclohexylcarbodiimide (DCCI) as a coupling agent (83) is currently the most popular method of peptide synthesis. The coupling reaction (1.13) is very rapid and the major side product, dicyclohexyl-urea, can be readily removed from the reaction mixture.

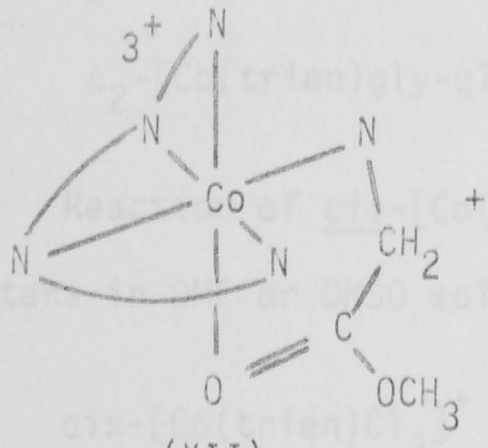


(1.13)

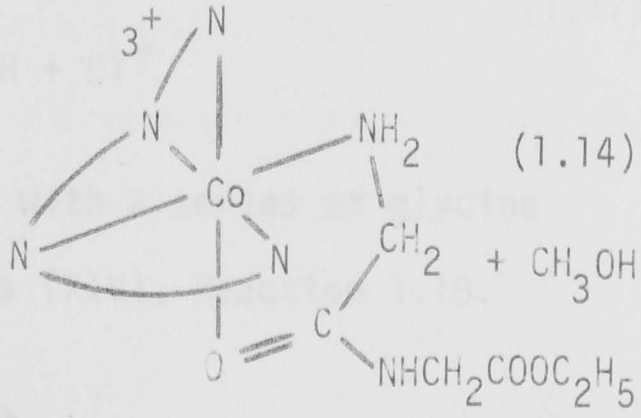
DCCI is also extensively used in the preparation of the active nitro phenyl ester of N-protected amino acids and peptides (74,84). The active nitro phenyl ester of amino acids can be coupled to protected peptide chains giving optically pure products in high yields. The usefulness of this procedure has been demonstrated in the stepwise synthesis of oxytocin and vasopressin and several of their analogues, eledoisin peptides, and insulin derivatives. Many of these polypeptides have been synthesised using both homogeneous phase (85-89) and solid-phase methods (71).

The possibility of the use of the chelated amino acid esters as activating agents for the synthesis of peptides is examined in this Thesis. It is known that the reaction of the chelated glycine esters with amino acids results in the rapid and quantitative formation of a number of glycine peptides (90,91). These have been synthesised using either the

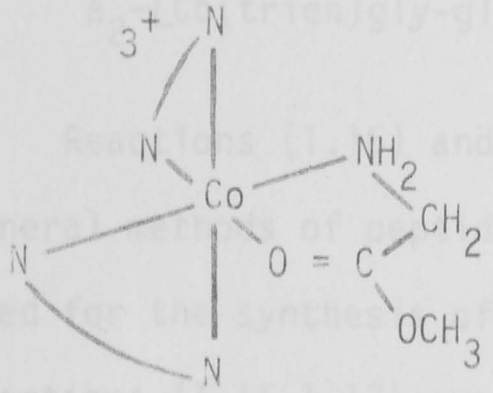
$Co(trien)^{3+}$ (trien = triethylenetetramine) or $Co(en)_2^{3+}$ (en = ethylenediamine) moieties as a N-terminal protecting group and as an activating agent for the peptide bond formation. Reaction of β_2-$[Co(trien)glyOC_2H_5](ClO_4)_3$ (XII) or $[Co(en)_2glyOCH_3](ClO_4)_3$ (XIII)



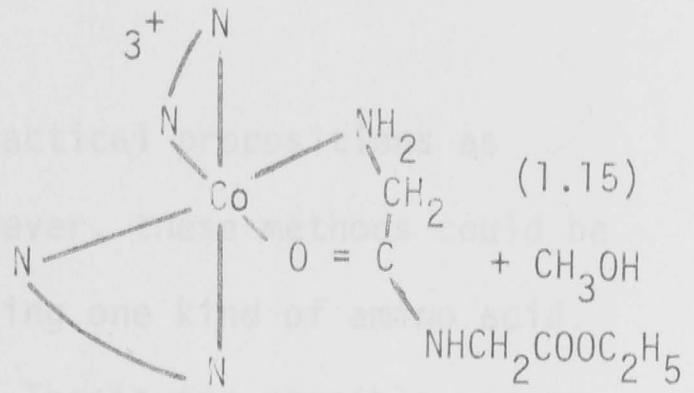
(XII)



(XIV) (1.14)



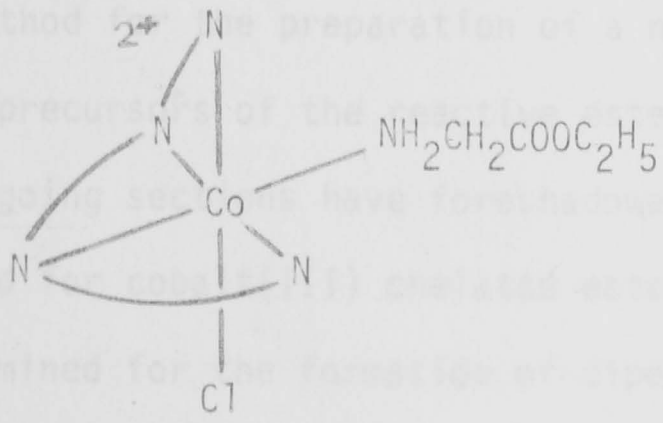
(XIII)



(XV) (1.15)

with glycine ester in anhydrous sulfolane, DMSO or acetone solutions resulted in the rapid and quantitative formation of the peptide complexes (XIV) and (XV) respectively (90). Reactions (1.14) and (1.15) are both complete within 1 min at 20°.

Treatment of α or β-$[Co(trien)(TBP)_2]^{3+}$ (TBP = tri(n-butyl)phosphate) or β_2-$[Co(trien)Cl(glyOC_2H_5)]^{2+}$ (XVI) with glycine ethyl ester in dry



(XVI)

TBP and sulfolane or dimethylformamide (DMF) solutions, Reaction 1.16 and 1.17 respectively, results in the rapid formation of (XIV) (90).

$$\alpha\text{- or } \beta\text{-}[Co(trien)(TBP)_2]^{3+} + 2\ glyOC_2H_5 \xrightarrow{TBP} \beta_2\text{-}[Co(trien)gly\text{-}glyOC_2H_5]^{3+} + C_2H_5OH + 2\ TBP \quad (1.16)$$

$$\beta_2\text{-}[Co(trien)Cl(glyOC_2H_5)]^{2+} + glyOC_2H_5 \longrightarrow \beta_2\text{-}[Co(trien)gly\text{-}glyOC_2H_5]^{3+} + C_2H_5OH + Cl^- \quad (1.17)$$

Reaction of cis-$[Co(trien)Cl_2]Cl$ (92) with a series of glycine esters in DMF or DMSO solutions also yields (XIV), Reaction 1.18.

$$cis\text{-}[Co(trien)Cl_2]^+ + 2\ glyOC_2H_5 \longrightarrow \beta_2\text{-}[Co(trien)gly\text{-}glyOC_2H_5]^{3+} + 2Cl^- \quad (1.18)$$

Reactions (1.16) and 1.18) are not practical propositions as general methods of peptide synthesis. However, these methods could be used for the synthesis of peptides containing one kind of amino acid. Reactions (1.15-1.17) are examined in this Thesis for possible use as methods for synthesis of peptides.

Scope of the Thesis

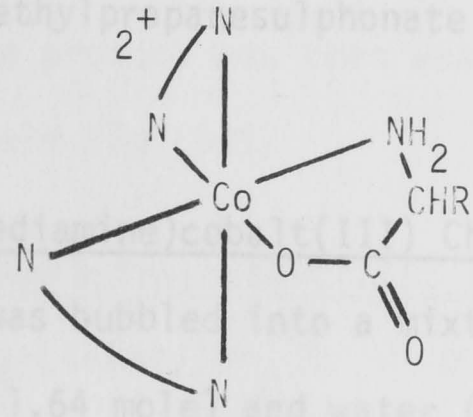
The cobalt(III) complexes discussed to date are generally inert to substitution and exchange of their ligands and this makes these complexes suitable for studies of ligand reactivity without the complication of pre-equilibria due to ligand dissociation. Chapter 2 records a general method for the preparation of a number of amino acid complexes which are precursors of the reactive esters described in Chapter 3. The foregoing sections have foreshadowed the reactivity enhancements expected for cobalt(III) chelated esters, and in Chapter 3 this property is examined for the formation of dipeptide complexes. Chapter 4 then reveals the kinetics and mechanism of the formation of peptide bonds using the cobalt(III) esters, and in Chapter 6 the possibility of cleavage of dipeptides using the Co(III) species is examined.

Apart from the possible practical application, the chelated amino acid moieties have an intrinsic interest, and the mutarotation and proton exchange at the α-carbon centre is one such property which has been examined (Chapter 6). On the whole, isomer stability for complexes of this type has previously been neglected and the possibility of stereoselectivity exists. Similarly, in condensation reactions for the chiral species involved stereoselective addition is possible. These aspects are examined in Chapter 5.

CHAPTER 2

PREPARATION AND CHARACTERISATION OF THE COMPLEX IONS $[Co(en)_2NH_2CHR^1COO]^{2+}$

The $[Co(en)_2NH_2CHRCOO]^{2+}$ salts (I) are used as precursors in one of the methods for synthesis of cobalt(III) chelated amino acid esters required in the peptide synthesis.



(I)

Meisenheimer(93) first prepared cobalt(III) complexes containing chelated amino acid anions by reacting <u>trans</u>-$[Co(en)_2Cl_2]Cl$ with either glycine or sarcosine in aqueous solution. Subsequently a variety of aminoacidobis(ethylenediamine)cobalt(III) ions, $[Co(en)_2aa]^{2+}$, have been prepared using various modifications of this method (94-98). The $[Co(en)_2aa]^{2+}$ ion may be also prepared by reacting an amino acid and <u>cis</u>-$[Co(en)_2OH(H_2O)]^{2+}$ at pH 8 (100).

This Chapter describes the preparation of eighteen different chelated amino acid complexes using a modification of the Meisenheimer method. The resolution of the (S)-proline, (S)-alanine and glycine complexes are also reported.

Experimental

Analar reagents were used throughout without further purification.

Optical rotations were recorded on a Perkin-Elmer P22 polarimeter using 1dcm cells. Rotations of the amino acid complexes were measured for 0.1% solutions.

Visible spectra (300-650 nm) and infrared spectra were recorded on a Cary Model 14 Spectrophotometer and a Perkin-Elmer 457 Infracord respectively.

Reaction mixture products were separated on Bio-Rad analytical Dowex 50W x2 (200-400 mesh, Na^+ or H^+ form) resin.

Proton magnetic spectra were recorded on Varian HA 100-MHz or Jeol C-60HL 60-MHz instruments using external tetramethylsilane (TMS) and internal sodium trimethylpropanesulphonate (NaTPS) respectively as reference standards.

Carbonatobis(ethylenediamine)cobalt(III) Chloride (101)

Carbon dioxide was bubbled into a mixture of ethylenediamine monohydrate (133 ml, 1.64 mole) and water (133 ml) for 2 hr in an ice bath. The cold solution was then treated with cobalt(II) chloride hexahydrate (195g, 0.82 mole) in water (175 ml). A vigorous evolution of carbon dioxide resulted and the solution turned a reddish-violet. The carbon dioxide addition was continued during the preparation. Hydrogen peroxide (30%, 20 ml, 3.9 moles) was then added with stirring over 45 min such that the temperature did not exceed 35° during the addition. The mixture was then heated at 75° for 15 min before cooling to 20°.

The addition of carbon dioxide was then increased and lithium hydroxide monohydrate ($LiOH.H_2O$) (34.4g, 0.82 mole) was added whereupon the temperature rose to ca. 35°. The mixture was left at 20° for 0.5 hr and the $[Co(en)_2CO_3]Cl$ which crystallised was collected. Additional product was obtained by adding methanol (500 ml) to the filtrate and cooling in an ice bath for 2 hr in the presence of carbon dioxide.

The total product was washed with 50% ethanol-water (200 ml) and air dried at 80°, yield 180g. The complex was recrystallised from 50% methanol-water, washed with methanol and air-dried.

Anal. Calcd. for $Co(C_5H_{16}N_4O_3)Cl$: C, 21.87; H, 5.87; N, 20.41; Cl, 12.91. Found: C, 21.8; H, 5.9; N, 20.6; Cl, 13.0.

trans-Dibromobis(ethylenediamine)cobalt(III) Bromide

47% HBr (200 ml) at 70° was treated with $[Co(en)_2CO_3]Cl$ (50g) over 2 hr. Heating at 70° was continued for 4 hr and after a further 2 hr at 20° all the trans-$[Co(en)_2Br_2]Br.HBr$ had crystallised. The product was collected and was washed with ethanol to remove traces of the soluble cis-$[Co(en)_2Br]Br$, HBr of crystallisation and adhering HBr. Washing with ethanol was continued until a solution of the complex in water was pH~7.0. The product was then washed with 80% ethanol-water and air-dried at 30°. Yield 77g (94%).

Aminoacidobis(ethylenediamine)cobalt(III) Iodide

A slurry of trans-$[Co(en)_2Br_2]Br$ (4.2g, 10^{-2} mole) and amino acid (10^{-2} mole) in 50% methanol-water (50 ml) at 50° was treated with $LiOH.H_2O$ (0.41g, 10^{-2} mole) in methanol (25 ml) over 15 min with stirring. The mixture was slowly evaporated to near dryness. Water (25 ml) was added, and after heating at 70° for 15 min the mixture was filtered and the filtrate was treated with excess sodium iodide. On cooling, $[Co(en)_3]I_3$ crystallised; this was removed and more sodium iodide was added to the filtrate and $[Co(en)_2aa]I_2$ crystallised. The complex was recrystallised from warm water by addition of sodium iodide. The final product was washed with ice-cold water and air dried. The absence of $[Co(en)_3]^{3+}$ ion and other possible trace impurities was monitored by ion exchange chromatography of a sample of the amino acid complex sorbed on a H^+ form resin eluted with 1M HCl. The absence of a yellow band, $[Co(en)_3]^{3+}$ and other bands besides that of $[Co(en)_2aa]^{2+}$ was taken as

one criteria of purity. When $[Co(en)_3]^{3+}$ was detected chromatographically the amino acid complex was further recrystallised.

Glycinatobis(ethylenediamine)cobalt(III) Iodide

Molar absorptivities were ε_{487}97 and ε_{347}107 in H_2O at 25°, yield 80%.

Anal. Calcd. for $Co(C_6H_{20}N_5O_2)I_2.H_2O$: C, 13.71; H, 4.21; N, 13.34; Co, 11.22; I, 48.18. Found: C, 13.8; H, 4.3; N, 13.4; Co, 11.2; I, 48.0.

(S)-Alanatobis(ethylenediamine)cobalt(III) Iodide

Molar absorptivities were ε_{487}109 and ε_{348}117 in H_2O at 25°, yield 75%.

Anal. Calcd. for $Co(C_7H_{22}N_5O_2)I_2.H_2O$: C, 16.12; H, 4.24; N, 11.51; Co, 13.10. Found: C, 16.2; H, 4.3; N, 11.3; Co, 13.1.

(S)-Valinatobis(ethylenediamine)cobalt(III) Iodide

Molar absorptivities were ε_{487}101 and ε_{348}110 in H_2O at 25°, yield 80%.

Anal. Calcd. for $Co(C_9H_{26}N_5O_2)I_2$: C, 19.69; H, 4.75; N, 10.92; Co, 10.73. Found: C, 19.6; H, 4.8; N, 10.7; Co, 10.7.

(S)-Leucinatobis(ethylenediamine)cobalt(III) Iodide

Molar absorptivities were ε_{486}109 and ε_{347}131 in H_2O at 25°, yield 75%.

Anal. Calcd. for $Co(C_{10}H_{28}N_5O_2)I_2$: C, 21.33; H, 5.01; N, 12.44; Co, 10.48. Found: C, 21.4; H, 5.3; N, 12.3; Co, 10.5.

(S)-Isoleucinatobis(ethylenediamine)cobalt(III) Iodide

Molar absorptivities were ε_{486}110 and ε_{347}130 in H_2O at 25°, yield 78%.

Anal. Found for $Co(C_{10}H_{28}N_5O_2)I_2$: C, 21.4; H, 5.0; N, 12.2.

(S)-Threoninatobis(ethylenediamine)cobalt(III) Iodide

Molar absorptivities were ε_{486} 102 and ε_{348} 110 in H_2O at 25°, yield 70%.

Anal. Calcd. for $Co(C_8H_{24}N_5O_3)I_2.H_2O$: C, 16.89; H, 4.43; N, 12.31; Co, 10.69; I, 44.60. Found: C, 16.9; H, 4.4; N, 12.1; Co, 10.5; I, 44.3.

A better procedure for isolating the complex was ion exchange chromatography. The complexes in the reaction mixture were sorbed on H^+ form resin and eluted with 1M HCl. The eluate containing $[Co(en)_2(S)\text{-thr}]^{2+}$ was evaporated to dryness, neutralised with 0.1N LiOH to pH 6.0 and was then treated with excess NaI whereupon the complex crystallised. $[Co(en)_2thr]I_2$ was recrystallised from a small volume of water by addition of sodium iodide, washed with ice cold water and methanol, and air dried.

(S)-Serinatobis(ethylenediamine)cobalt(III) Iodide

Molar absorptivities were ε_{486} 118 and ε_{345} 135 in H_2O at 25°, yield 68%.

Anal. Calcd. for $Co(C_7H_{22}N_5O_3)I_2$: C, 15.66; H, 4.13; N, 13.04; Co, 10.97; I, 47.26. Found: C, 15.7; H, 4.0; N, 13.0; Co, 10.9; 46.9.

(S)-Lysinatobis(ethylenediamine)cobalt(III) Iodide

Molar absorptivities were ε_{487} 101 and ε_{346} 120 in H_2O at 25°, yield 76%.

Anal. Calcd. for $Co(C_{10}H_{29}N_6O_2)I_2$: C, 20.78; H, 5.06; N, 14.54; Co, 10.19. Found: C, 20.5; H, 5.1; N, 14.6; Co, 10.2.

(S)-Asparaginatobis(ethylenediamine)cobalt(III) Iodide

Molar absorptivities were ε_{486} 103 and ε_{347} 113 in H_2O at 25°, yield 51%.

Anal. Calcd. for $Co(C_8H_{22}N_6O_3)I_2$: C, 17.04; H, 4.11; N, 14.90; Co, 10.46; I, 45.0; Found: C, 17.0; H, 4.1; N, 14.8; Co, 10.2; I, 45.8.

(S)-Methionatobis(ethylenediamine)cobalt(III) Iodide

Molar absorptivities were ε_{486} 102 and ε_{346} 134 in H_2O at 25°, yield 72%.

Anal. Calcd. for $Co(C_9H_{26}N_5O_2S)I_2 \cdot \frac{1}{2}H_2O$: C, 18.32; H, 4.51; N, 11.87; Co, 9.99; I, 43.02. Found: C, 18.2; H, 4.6; N, 12.0; Co, 10.0; I, 43.2.

(S)-Phenylalaninatobis(ethylenediamine)cobalt(III) Iodide

Molar absorptivities were ε_{487} 103 and ε_{346} 128 in H_2O at 25°, yield 81%.

Anal. Calcd. for $Co(C_{13}H_{26}N_5O_2)I_2$: C, 26.23; H, 4.38; N, 11.72; Co, 9.86. Found: C, 26.6; H, 4.3; N, 11.8; Co, 10.0.

(S)-Tyrosinatobis(ethylenediamine)cobalt(III) Iodide

Molar absorptivities were ε_{490} 108 and ε_{346} 187 in H_2O at 25°, yield 72%.

Anal. Calcd. for $Co(C_{13}H_{26}N_5O_3)I_2$: C, 25.47; H, 4.27; N, 11.42; Co, 9.61; I, 41.39. Found: C, 25.6; H, 4.3; N, 11.2; Co, 9.6; I, 41.0.

(S)-Tryptophanatobis(ethylenediamine)cobalt(III) Iodide

Molar absorptivities were ε_{486} 103 and ε_{340} 298 in H_2O at 25°, yield 65%.

Anal. Calcd. for $Co(C_{15}H_{27}N_6O_2)I_2$: C, 28.32; H, 4.28; N, 13.21; Co, 9.26. Found: C, 28.6; H, 4.4; N, 12.8; Co, 9.2.

(S)-Prolinatobis(ethylenediamine)cobalt(III) Iodide

Molar absorptivities were ε_{488} 103 and ε_{346} 125 in H_2O at 25°, yield 85%.

Anal. Calcd. for $Co(C_9H_{24}N_5O_2)I_2.H_2O$: C, 19.00; H, 4.60; N, 12.30; Co, 10.36; I, 44.91. Found: C, 19.1; H, 4.6; N, 12.4; Co, 10.6; I, 45.3.

(S)-Hydroxyprolinatobis(ethylenediamine)cobalt(III) Iodide

Yield 80%.

Anal. Calcd. for $Co(C_9H_{24}N_5O_3)I_2$: C, 19.17; H, 4.30; N, 12.43. Found: C, 19.3; H, 4.3; N, 12.5.

(S)-Glutaminatobis(ethylenediamine)cobalt(III) Iodide

Molar absorptivities were $\varepsilon_{487}94$ and $\varepsilon_{347}133$ in H_2O at 25°, yield 65%.

Anal. Calcd. for $Co(C_9H_{15}N_6O_3)I_2$: C, 18.70; H, 4.36; N, 14.54; Co, 10.20; I, 43.91. Found: C, 18.8; H, 4.5; N, 14.3; Co, 10.1; I, 43.0.

(S)-Argininatobis(ethylenediamine)cobalt(III) Iodide

It was not possible to isolate the complex as the iodide salt. Instead the reaction mixture was sorbed on a H^+ form resin and eluted with 1.5N HCl. The eluate containing the complex was evaporated to dryness and was then dissolved in water and re-evaporated to dryness (X2) to remove HCl. The product was dried in vacuo over P_2O_5 and was a glassy powder, yield 60%.

Anal. Calcd. for $Co(C_{10}H_{29}O_2N_8)Cl_2.3HCl$: Co, 11.00; C, 22.39; H, 6.19; N, 20.87. Found: Co, 11.3, C, 22.8; H, 6.2; N, 21.5.

(S)-Histinatobis(ethylenediamine)cobalt(III) Iodide

The complex was not obtained analytically pure. However, it was possible to demonstrate that the complex had formed from an analysis of the pmr spectrum and by conversion to the chelated ester used for the formation of peptide complexes.

(S)-Glutamatobis(ethylenediamine)cobalt(III) Perchlorate

The complex was prepared as described by Gillard (99), yield 55%. Anal. Calcd. for $Co(C_9H_{24}N_5O_4)ClO_4.H_2O$: C, 24.41; H, 5.92; N, 15.82; Co, 13.31; Co, 13.31. Found: C, 24.8; H, 5.7; N, 15.7.

It was more convenient to isolate the complex by ion-exchange chromatography. The complexes in the reaction mixture were sorbed on H^+ form resin and eluted with 1M HCl. Five bands separated and Band 2 was identified as $[Co(en)_2gluH]^{2+}$. The eluate of the complex was collected and evaporated to dryness. The complex was then dissolved in water, neutralised with 0.1N LiOH and was converted to a perchlorate salt using Bio-Rad Dowex AG1 x8 (200-400 mesh) resin (ClO_4^- form). The evaporated eluate was dissolved in H_2O (20 ml), treated with $LiClO_4$ when the complex crystallised on standing. It was collected, recrystallised from water, then washed with ethanol and air dried. Yield 1.0g. Anal. Found: C, 24.3; H, 5.8; N, 15.6.

(S)-Aspartatobis(ethylenediamine)cobalt(III) Perchlorate

The complex was prepared as described for $[Co(en)_2glu]ClO_4$, yield 60%.

Anal. Calcd. for $Co(C_8H_{22}N_5O_4)ClO_4$: C, 23.39; H, 5.40; N, 17.05; Co, 14.35. Found: C, 23.7; H, 5.4; N, 16.7; Co, 14.2.

Preparation and Resolution of the $[Co(en)_2gly]^{2+}$ Ion

The complex was prepared and resolved by a modification of a method previously described (96).

cis-$[Co(en)_2Br(glyOCH_3)](ClO_4)_2$ (20), (120g) was dissolved in a solution containing $Hg(NO_3)_2$ (100g) in 0.1M $HClO_4$ (800 ml). The solution was filtered through Hi-Flo and the volume reduced to ca. 150 ml by evaporation at 25°. Addition of NaI precipitated $[Co(en)_2gly](HgI_4)$ which was collected, washed with NaI solution, water, methanol then ether and air dried (yield 198g). The product was shaken

with AgCl (100g) in dilute HCl (600 ml, pH ~3) for 0.5 hr and then filtered. The $[Co(en)_2gly]Cl_2$ concentration in the filtrate was measured spectrophotometrically ε_{487}98.

The salt in H_2O (10 l) was treated with silver antimonyl-(R)-tartrate (82.4g; 2 equivalents) and the suspension was stirred at 50° for 1 hr and mixture was then filtered. The precipitate was warmed with water until most of the adsorbed material had been dissolved. The combined filtrate and washings were let stand overnight, whereupon a fraction of the ΔR diastereoisomer salt crystallised. This was collected, washed with water and acetone and air dried (2.6g $[\alpha]_{546}$ -254°, $[\alpha]_D$ -82°). On reduction of the volume by evaporation under reduced pressure a series of ΔR and ΛR diastereoisomer fractions crystallised in no set order. All fractions of the same sign were combined and recrystallised to constant rotation. For Δ-$[Co(en)_2gly](SbO\text{-}(R)\text{-}tart)_2$, $[\alpha]_D$ -82°, $[\alpha]_{546}$ -253° in H_2O at 25°; $[M]_D$ -691°, $[M]_{546}$ -2133°. Yield 33g.

For Λ-$[Co(en)_2gly](SbO\text{-}(R)\text{-}tart)_2$ $[\alpha]_D$ +291°, $[\alpha]_{546}$ +508°; $[M]_D$ +2453°, $[M]_{546}$ +4283°. Yield 28g.

Anal. Calcd. for $Co(C_6H_{22}N_5O_2)C_8H_8O_{14}Sb_2.H_2O$: C, 19.93, H, 3.59; N, 8.31. Found for the ΔR isomer: C, 19.8; H, 3.8; N, 8.1. Found for the ΛR isomer: C, 20.0; H, 3.4; N, 8.2.

The diastereoisomers were separately dissolved in water and converted to perchlorate salts by passage through a perchlorate form Bio-Rad AG1 x8 (200-400 mesh) anion exchange resin. In each case the eluate volume was evaporated to ca. 50 ml. On addition of NaI and scratching the sides of the vessels the iodide salts crystallised. These were recrystallised from water and then washed with ice cold water and dried *in vacuo* over P_2O_5. Yields, Δ isomer 10.8g; Λ isomer 9.3g.

For the Δ isomer $[\alpha]_D$ -297° $[\alpha]_{546}$ -635°; in H_2O at 25°, $[M]_D$ -1559°, $[M]_{546}$ -3334°.

Anal. Calcd. for $Co(C_6H_{22}N_5O_2)I_2$: C, 13.79; H, 4.03; N, 13.21.

Found for the Δ isomer: C, 13.6; H, 4.1; N, 13.2.

For the Λ isomer $[\alpha]_D$ +293° $[\alpha]_{546}$ +620° in H_2O at 25° $[M]_D$ +1538°, $[M]_{546}$ +3255°.

Anal. Calcd. for $Co(C_6H_{22}N_5O_2)I_2.H_2O$: C, 13.73; H, 4.22; N, 13.34.

Found for the Λ isomer: C, 13.6; H, 4.3; N, 13.1.

The presence of water of crystallisation was confirmed by the presence of an OH stretching absorption band at 3500 cm^{-1}.

Previously reported $[M]_{546}$ values for iodide salts are, ±3450° (96); -3100° (94); -2750° (95).

Preparation and Resolution of ΛΔ-$[Co(en)_2(S)\text{-ala}]I_2$

A hot solution of trans-$[Co(en)_2Br_2]Br$ (83.8g) in 50% methanol-water (800 ml) was treated with (S)-alanine (17.8g) followed by $LiOH.H_2O$ (8.4g) in 50% methanol-H_2O (500 ml). The mixture was evaporated slowly on a steam bath to near dryness and treated with H_2O (100 ml). The evaporation was continued until the volume was ca. 100 ml and sodium iodide was then added. On cooling, $[Co(en)_3]I_3$ crystallised and this was removed and the filtrate was treated with more NaI and $[Co(en)_2ala]I_2$ crystallised. The complex was collected, recrystallised from water by addition of NaI, washed with ice cold water and air dried. Yield 32g. All filtrates were combined and the volume reduced to ca. 60 ml. On cooling, further $[Co(en)_2ala]I_2$ crystallised and this was recrystallised (X2) from water by addition of sodium iodide. Yield 38g. Both fractions were combined and recrystallised from water. No $[Co(en)_3]I_3$ was detected chromatographically when a sample of the final $[Co(en)_2ala]I_2$ was eluted with 1M HCl from a H^+ form resin.

ΛΔ-$[Co(en)_2(S)\text{-ala}]I_2$ (52.1g, 0.1 mole) and silver antimonyl-(R)-tartrate (79.8g, 0.02 mole) in distilled water (1700 ml) was stirred for 0.5 hr at 60°, filtered, and the combined filtrate and washings reduced to ca. 1000 ml by evaporation under reduced pressure. On standing, the Δ-$[Co(en)_2(S)\text{-ala}][SbO\text{-R-tart}]_2$ crystallised and was collected, washed

with cold water, methanol and air dried. The filtrate and washings were combined and the volume reduced as above to ca. 500 ml. A further seven fractions of the same diastereoisomer were collected in this manner and the volume of the filtrate was reduced to ca. 200 ml. All fractions of the same sign were combined and recrystallised to constant rotation. Yield 49g. $[\alpha]_D$ -106°, $[\alpha]_{546}$ -310° in H_2O at 25°; $[M]_D$ -889°, $[M]_{546}$ -2610°. Previously reported value is $[M]_{546}$ -2660° (96).

Anal. Calcd. for $Co(C_7H_{22}N_5O_2)C_8H_8O_{14}Sb_2$: C, 21.48; H, 3.60; N, 8.36. Found: C, 21.7; H, 3.5; N, 8.4.

The remaining filtrate at 45° was treated with methanol until the solution was cloudy. On standing at 20° Λ-$[Co(en)_2(S)\text{-ala}](SbO\text{-}(R)\text{-tart})_2$ crystallised, the product was collected, washed with methanol and then air dried. Five more fractions of the same diastereoisomer were obtained from the filtrate and washings by reducing the volume and adding methanol. All fractions were combined and recrystallised to constant rotation. Yield 35g.

$[\alpha]_D$ +268°, $[\alpha]_{546}$ +506 in water at 25°; $[M]_D$ +2248°; $[M]_{546}$ +4244°. There is no previously reported value for $[M]_{546}$.

Anal. Found for $Co(C_7H_{22}N_5O_2)C_8H_8O_{14}Sb_2$: C, 21.8; H, 3.5; N, 8.7.

The diastereoisomer was converted to the chloride salt using Bio-Rad Dowex AG1 x8 (200-400 mesh, chloride form) anion exchange resin. The eluate was reduced to a small volume and was treated with excess sodium iodide when on cooling Λ-$[Co(en)_2(S)\text{-ala}]I_2$ crystallised. The product was recrystallised to constant rotation from water by adding sodium iodide.

$[\alpha]_D$ 258°, $[\alpha]_{546}$ +607° in water at 25°; $[M]_D$ +1390°; $[M]_{546}$ +3272. Previously reported values are, $[M]_{546}$ +3370° as the iodide salt (96); +3000° as the bromide salt (95); and +2400° as the nitrate salt (94).

Anal. Calcd. for $Co(C_7H_{22}N_5O_2)I_2.H_2O$: Co, 10.93; C, 15.60; H, 4.49; N, 12.99. Found: Co, 10.8; C, 15.4; H, 4.3; N, 12.8. The presence of water of crystallisation was confirmed by an OH stretching absorption at 3500^{-1} cm.

The Δ-$[Co(en)_2(S)\text{-ala}][SbO\text{-}(R)\text{-tart}]_2$ was converted to the iodide salt as described for ΛS isomer. $[\alpha]_D$ -359°, $[\alpha]_{546}$ -710° in water at 25°; $[M]_{546}$ -3700°. Value previously reported is $[M]_{546}$ -3700° as iodide salt (96).

Preparation and Resolution of ΛΔ-$[Co(en)_2(S)\text{-pro}]I_2$

A slurry of trans-$[Co(en)_2Br_2]Br$ (20.9g) and (S)-proline (5.75g) in methanol (800 ml) at 60° was treated with $LiOH.H_2O$ (2.09g) in methanol (200 ml). The mixture was then heated under reflux for 0.7 hr and was then diluted to 3 litre with water. The complexes in solution were sorbed on H^+ form resin and eluted with 1N HCl. The $[Co(en)_2(S)\text{-pro}]^{2+}$ eluate was reduced to dryness, dissolved in water and neutralised with 0.5M LiOH. The solution was then treated with NaI and Δ-$[Co(en)_2(S)\text{-pro}]I_2$ crystallised. The complex was recrystallised from water by addition of NaI, washed with ice cold water and air dried. On addition of more sodium iodide two more fractions of similar rotation were collected. All fractions were combined and recrystallised to constant rotation (3 recrystallisations). $[\alpha]_D$ -428°, $[\alpha]_{546}$ -802° in water at 25°; $[M]_D$ -2343°; $[M]_{546}$ -4410°.

Anal. Calcd. for $Co(C_9H_{24}N_5O_2)I_2.H_2O$: C, 19.00; H, 4.60; N, 12.30. Found: C, 18.8; H, 4.5; N, 12.1.

The filtrate and washings (100 ml) were combined and treated with sodium arsenyl-(R)-tartrate (NaAsO-(R)-tart) (12g) whereupon Δ-$[Co(en)_2(S)\text{-pro}]$ $(NaAsO\text{-}(R)\text{-tart})_2$ crystallised. This was collected and was washed with water and air dried; $[\alpha]_{546}$ -350°. On standing at 20°, three more fractions of the same diastereoisomer were collected. The filtrate was then treated with more NaAsO-(R)-tart (5g) (ratio of no. of equivalents of resolving agent to complex was ca. 2:1) and more diastereoisomer crystallised. On reduction of the filtrate volume a series of ΔSR and ΛSR fractions crystallised in no set order. All fractions of the same sign were combined and recrystallised to constant

rotation. For Λ-$[Co(en)_2(S)\text{-pro}][AsO\text{-}(R)\text{-tart}]_2$, $[\alpha]_D$ +233° and $[\alpha]_{546}$ +526° in water at 25°.

Anal. Calcd. for $Co(C_9H_{24}N_5O_2)C_8H_8O_{14}As_2.H_2O$: C, 25.87; H, 4.34; N, 8.87. Found: C, 25.9; H, 4.4; N, 8.7.

For Δ-$[Co(en)_2(S)\text{-pro}](AsO\text{-}(R)\text{-tart})_2$, $[\alpha]_D$ -204° and $[\alpha]_{546}$ -340° in water at 25°.

Anal. Found: C, 25.6; H, 4.4; N, 8.9.

The diastereoisomers were converted to the iodide salts as described for the resolution of ΛΔ-$[Co(en)_2(S)\text{-ala}]I_2$. For Λ-$[Co(en)_2(S)\text{-pro}]I_2$, $[\alpha]_D$ +312° and $[\alpha]_{546}$ +720° in water at 25°; $[M]_D$ +1716°; $[M]_{546}$ +3960°.

Anal. Calcd. for $Co(C_9H_{24}N_5O_2)I_2.H_2O$: C, 19.00; H, 4.60; N, 12.30. Found: C, 19.0; H, 4.4; N, 12.2.

For Δ-$[Co(en)_2(S)\text{-pro}]I_2$, $[\alpha]_D$ -425° and $[\alpha]_{546}$ -800° in water at 25°, $[M]_D$ -2338°; $[M]_{546}$ -4400°.

Anal. Calcd. for $Co(C_9H_{24}N_5O_2)I_2.H_2O$: C, 18.41; H, 4.78; N, 11.92. Found: C, 18.4; H, 4.8; N, 11.8.

Results and Discussion

Although three different methods (93, 99, 100) have been reported for the preparation of several aminoacidobis(ethylenediamine)cobalt(III) complexes none of the methods are applicable to the preparation of a large number of different amino acid complexes. A general method of preparing $[Co(en)_2aa]^{2+}$ ions by the reaction of trans-$[Co(en)_2Br_2]Br$ with the amino acid in 50% aqueous methanol has been developed which yields eighteen amino acid complexes, (Table 2.1, Reaction 2.1).

$$\text{trans-}[Co(en)_2Br_2]Br + \text{amino acid} \xrightarrow[MeOH]{LiOH} [Co(en)_2aa]Br_2 + LiBr \quad (2.1)$$

All eighteen complexes were isolated as their iodide salts. Their visible spectra are very similar, Table 2.1, and reflect the analogous ligand fields about the central metal atom due to one carboxyl oxygen and five primary amino nitrogen atoms (I).

TABLE 2.1

Absorption Spectra of $[Co(en)_2aa]^{2+}$ ions in H_2O at 25°

Complex[b]	λ_{max} (nm)	ε_{max}	λ_{max}	ε_{max}	% Yield	Ref.[b]
$[Co(en)_2gly]I_2$	487	97	347	107	80	93-96
$[Co(en)_2ala]I_2$	487	109	348	117	75	94-96
$[Co(en)_2val]I_2$	487	101	348	110	80	100
$[Co(en)_2leu]I_2$	486	109	347	130	75	95-107
$[Co(en)_2ile]I_2$	486	110	347	132	78	94-96
$[Co(en)_2thr]I_2$	486	102	347	110	70	100
$[Co(en)_2ser]I_2$	486	118	345	135	68	95,100
$[Co(en)_2lys]I_2$	487	101	348	120	76	97
$[Co(en)_2gln]I_2$	486	94	347	133	65	97
$[Co(en)_2asn]I_2$	486	103	347	113	50	
$[Co(en)_2met]I_2$	486	102	346	134	70	97
$[Co(en)_2phe]I_2$	487	103	346	128	82	95,96,100
$[Co(en)_2tyr]I_2$	490	108	340	187	72	
$[Co(en)_2try]I_2$	486	103	340	298	65	
$[Co(en)_2pro]I_2$	488	103	346	125	85	97,99

[a] Abbreviation used for amino acids are listed in Appendix I.

[b] Reference for previous preparation of complex by a different method.

Their infrared spectra have intense broad absorptions at $1630 \pm 10\ cm^{-1}$ which are characteristic of the C=O stretching mode of a coordinated carboxyl group (30,102).

Complexes of aspartic and glutamic acid could only be prepared by reaction of the respective amino acid with $[Co(en)_2CO_3]ClO_4$ in a neutral solution. All attempts to prepare the cystine and cysteine complexes were unsuccessful. However, recently Kothari and Busch (103) have reported the isolation of these complexes by treating trans-$[Co(en)_2Cl_2]Cl$ with an amino acid in basic solution.

Apart from glycine, (S)-amino acids were used in the preparations and it follows that only two diastereoisomers were produced. These generally have different solubilities and this was most pronounced for the (S)-tyrosine, (S)-tryptophan and (S)-asparagine complexes. Previous investigators (97) have isolated the optically pure Λ isomer containing (S)-serine, (2S, 3R)-threonine and (S)-proline by repeated recrystallisation of the racemic mixtures, or, excepting the (S)-proline complex, by using silver antimonyl-(R)-tartrate as a resolving agent. In the present study the $[Co(en)_2pro]^{2+}$ diastereoisomers were obtained using sodium arsenyl-(R)-tartrate as resolving agent and by exhaustive recrystallisation of the iodide salt. The diastereoisomers were also isolated by ion exchange chromatography on SE Sephadex C-25 Na^+ form resin eluted with 0.05M $NaClO_4$. No stereoselectivity was found in the preparative mixture, since the isomers isolated from the column were present in equal amounts within experimental error, and this confirms previous observations that dissymmetric synthesis does not occur (96,97).

Repeated recrystallisation of ΛΔ-$[Co(en)_2(S)\text{-ala}]I_2$ from water gave Δ-$[Co(en)_2(S)\text{-ala}]I_2$ as the least soluble fraction, while isolation of both diastereoisomers was achieved using silver antimonyl-(R)-tartrate.

The specific and molecular rotations of the resolved (S)-proline, (S)-alanine and glycine complexes are listed in Table 2.2.

TABLE 2.2

Specific and Molecular Rotations of Amino Acid Complexes in Water at 25°

Complexes	589nm		546nm	
	$[\alpha]°$	$[M]°$	$[\alpha]°$	$[M]°$
Δ-$[Co(en)_2gly]I_2$	-297	-1559	-635	-3334
Λ-$[Co(en)_2gly]I_2.H_2O$	+291	+1453	+625	+3301
Δ-$[Co(en)_2(S)\text{-}ala]I_2$	-359	-1870	-710	-3700
Λ-$[Co(en)_2(S)\text{-}ala]I_2.H_2O$	+258	+1390	+607	+3272
Δ-$[Co(en)_2(S)\text{-}pro]I_2.2H_2O$	-425	-2338	-800	-4400
Λ-$[Co(en)_2(S)\text{-}pro]I_2.H_2O$	+312	+1716	+720	+3960

Preparation of the cobalt(III) amino acid complexes in aqueous solution led to disproportionation and in all instances at least four products were obtained. This phenomena has been attributed to the presence of traces of a labile Co(II) intermediate and electron transfer between it and the cobalt(III) complexes allows equilibration of the ligands about the Co(III) ion (104). Some of the possible disproportionation products that can form are indicated in Reaction (2.2) and are in agreement with the +3, +2, +1 and zero charged ions observed when

$$\underline{\text{trans}}\text{-}[Co(en)_2Br_2]Br + aa \xrightarrow[LiOH]{50\%\ MeOH} \begin{cases} [Co(en)_3]^{3+} + [Co(en)_2]^{2+} \\ [Co(en)(aa)_2]^{+} + [Co(aa)_3]^{o} \end{cases} \quad (2.2)$$

complexes in the reaction mixture are eluted from a cation resin, Table 2.3. The major impurity obtained in the present preparation was $[Co(en)_3]^{3+}$ and its amount increased on longer heating times. For example, a 50% methanol-water mixture of trans-$[Co(en)_2Br_2]Br$, proline and LiOH at 70° for 10 hr gave >80% $[Co(en)_3]^{3+}$ while only 30% was formed after 3 hr, Table 2.3. Bands 3 and 5 were identified by spectrophotometric comparison with the authentic complexes, while assignments of bands 2 and 4 were made on the basis of their elution speed relative to $[Co(en)_2pro]^{2+}$, and by their pmr spectra.

TABLE 2.3

Ion Exchange Separation of Products from Preparative Mixture of trans-$[Co(en)_2Br_2]Br$ and Prolinate Anion in Aqueous Solution after 3 hr at 70°.[a]

Band	Colour	Charge	Abundance	Assignment
1	red-violet	0	Trace	$[Co(pro)_3]^{\circ}$
2	purple	+1	10-15%	$[Coen(pro)_2]^{+}$
3	orange	+2	50%	$[Co(en)_2pro]^{2+}$
4	purple	+2	5-10%	$[Co(en)_2(pro)Br]^{2+}$?
5	yellow	+3	30%	$[Co(en)_3]^{3+}$

[a] Chromatography on Bio-Rad Dowex 50W x2 (200-400 mesh) sodium form cation resin and separation obtained with 1M $NaClO_4$ eluant.

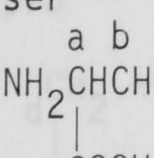
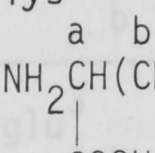
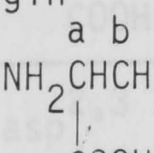
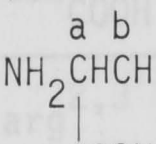
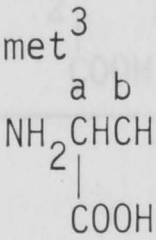
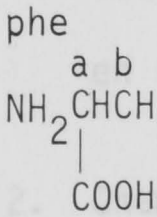
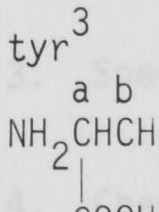
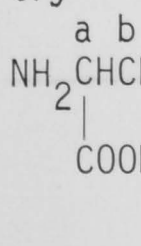
For the more methanol soluble amino acids, a higher yield of amino acid complex was obtained when it was prepared in 80% aqueous methanol. For example, the reaction mixture of trans-$[Co(en)_2Br_2]Br$, proline and LiOH in 80% methanol the yield of $[Co(en)_2pro]Br_2$ was 85%.

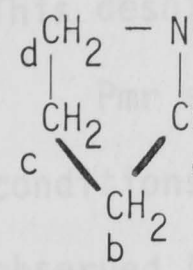
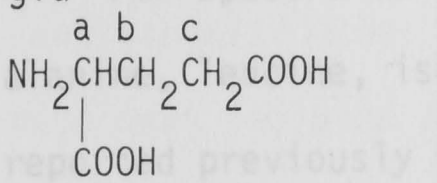
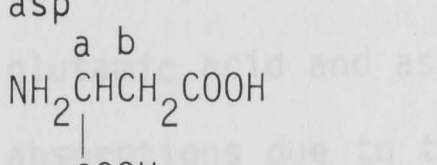
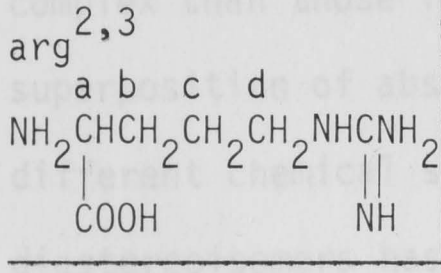
Pmr spectra have been recorded for all the amino acid complexes and are compared with the spectra for the uncoordinated amino acid in Table 2.4. These spectra are useful for the identification of the

TABLE 2.4

Chemical Shifts for Coordinated and Uncoordinated Amino Acids in D_2O at 32°.[1]

Amino Acid	Chemical Shifts[4] for $[Co(en)_2aa]^{2+}$					Chemical Shifts[4] for uncoordinated amino acid				
	a	b	c	d	e	a	b	c	d	e
gly a NH_2CH_2COOH	3.72					3.56				
ala[2] a b NH_2CHCH_3 COOH	4.0	1.93				4.46	1.85			
val c CH_3 a | $NH_2CHCHCH_3$ | b c COOH	3.6	2.20	1.05			3.50	2.15	0.98		
leu a b c d $NH_2CHCHCHCH_3$ | | $COOHCH_3$ e	3.7	1.8	1.8	1.02	1.02	3.60	1.70	1.70	0.96	0.96
ile a b CH_3 NH_2CHCH e | COOH CH_2CH_3 c d	3.6	3.0	1.80	1.12	0.98	3.55	2.30	1.64	1.10	0.97
thr[2] OH a | $NH_2CHCHCH_3$ | b c COOH	4.9	4.25	1.98			4.8	4.20	1.86		

Amino Acid	Chemical Shifts[4] for $[Co(en)_2aa]^{2+}$	Chemical Shifts[4] for uncoordinated amino acid
	a b c d e	a b c d e
ser[2,3] 	4.0 3.92	3.8 3.78
lys[2,3] 	3.7 1.75 3.0	3.62 1.65 2.95
gln[3] 	4.15 3.1 2.60	4.28 2.82 2.63
asn[2,3] 	3.80 3.0	3.91 2.80
met[3] 	3.8 2.8 2.8 2.15	3.7 2.50 2.50 2.08
phe 	4.8 3.51 7.80	4.60 3.44 7.40
tyr[3] 	4.3 3.50 7.30 7.61	4.31 3.24 7.02 7.41
try[2,3] 	4.2 2.30 3.38 7.30	4.12 2.10 3.35 7.15

Amino Acid	Chemical Shifts[4] for $[Co(en)_2aa]^{2+}$ a b c d e	Chemical Shifts[4] for uncoordinated amino acid a b c d e
pro[2,3] 	4.28 2.10 2.10 3.5	4.13 2.1 2.1 3.38
glu[3] 	4.12 2.96 2.56	4.28 2.84 2.65
asp[2,3] 	3.9 3.0	3.92 2.76
arg[2,3] 	3.75 1.90 1.90 3.25	3.65 1.75 1.75 3.18

1. Chemical shifts relative to NaTPS (60 MHz). For $[Co(en)_2aa]^{2+}$ en is a broad resonance 2.6 to 3.2 ppm downfield from NaTPS.

2. Chemical shifts relative to TMS (100 MHz).

3. Spectrum shown in Appendix II.

4. Chemical shifts quoted to one decimal are for broad and/or partially obscurred resonances.

peptide complexes described in Chapter 3.

Table 2.4 indicates that chelation of amino acids has a marked effect on the carbon proton chemical shifts. The result is interpreted as due to the metal ion withdrawing electron density from the ligand protons. This deshielding leads to absorptions at lower fields.

Pmr spectra were recorded in neutral D_2O solutions. Under these conditions NH proton exchange occurs and thereby ND-CH coupling is not observed (105).

Pmr spectra in D_2O for $[Co(en)_2aa]^{2+}$ ions where aa = glycine, alanine, leucine, isoleucine, valine and phenylalanine have been reported previously (105). Pmr spectra of complexes of threonine, serine, lysine, glutamine, asparagine, methionine, tyrosine, tryptophan, proline, glutamic acid and aspartic acid are given in Appendix II. The absorptions due to the chelated amino acid in the racemic ions are more complex than those for the uncoordinated species. This arises from superposition of absorptions for the diastereoisomeric pairs which have different chemical shift values. Spectral non-equivalence of diastereoisomers has been used as a qualitative criterion for optical purity and will be discussed in detail in Chapter 6.

CHAPTER 3

SYNTHESIS OF PEPTIDES VIA COBALT(III) CHELATED AMINO ACID ESTERS

In Chapter 2 a general method for preparing cobalt(III) amino acid complexes was described, and in Chapter 1 the possibility of using these complexes to prepare peptides was foreshadowed. In this Chapter a general procedure for preparing chelated amino acid esters and their subsequent reaction with other amino acids is described. The peptide complexes have been isolated and characterised and in the case of ala-gly experiments designed to investigate the possibility of racemisation during the synthesis have been carried out.

Experimental

Analar reagents were used throughout without further purification.

Cobalt estimations were made using either a Techtron AA4 atomic absorption spectrophotometer or a Cary 14 spectrophotometer using known extinction coefficients for the amino acid and peptide complexes. All optical rotations were measured in 1dcm tube using a Perkin-Elmer P22 Polarimeter and 0.1% solutions.

Proton magnetic resonance spectra were recorded on a Varian HA 100-MHz instrument using external tetramethylsilane (TMS) as a reference standard or on a Jeol C-60HL 60-MHz instrument using sodium trimethylpropanesulphonate (NaTPS) as an internal reference.

Chromatographic separations of the reaction products were made on Bio-Rad analytical Dowex 50W x2 (200-400 mesh, Na^+ or H^+ form) resin unless otherwise stated.

Aminoacidobis(ethylenediamine)cobalt(III) Acetate

$[Co(en)_2aa]I_2$ (10^{-2} mole) in water (20 ml) was treated with silver acetate ($2x10^{-2}$ mole) and the mixture shaken for 15 min. The solution

was then filtered and the silver iodide washed twice with H_2O (5 ml) and then with methanol. The combined filtrate and washings were evaporated to dryness under reduced pressure and the product dried *in vacuo* over P_2O_5.

Aminoacido methyl ester bis(ethylenediamine)cobalt(III) Chloride $[Co(en)_2aaOCH_3]Cl_3$ ($aaOCH_3$ = Amino Acid Methyl Ester)

$[Co(en)_2aa](OAc)_2$ (2×10^{-3} mole) in anhydrous methanol (100 ml) was treated dropwise with PCl_3 (2 ml) at 20°. The mixture was stoppered and kept at 20° for 18 hr to complete the reaction.

Co(III) dipeptide ester Perchlorate $[Co(en)_2aa^{I}aa^{II}OR](ClO_4)_3$ From $[Co(en)_2aa^{I}OCH_3]Cl_3$ in situ

The $[Co(en)_2aaOCH_3]Cl_3$ solution prepared as above was treated with amino acid ester hydrogen chloride (10^{-2} mole) followed by rapid addition of a calculated volume of 1M Et_3N in anhydrous methanol such that the mixture was 0.1M in the amino acid ester. The mixture was neutralised after 30 sec with glacial acetic acid and diluted with water to 500 ml. The reaction products were sorbed on SE Sephadex C-25 Na^+ form resin and eluted with 0.1M $KClO_4$. The eluate of the peptide complex was evaporated to dryness under reduced pressure at ~25°. During evaporation the solution was filtered several times to remove $KClO_4$. The complex was then three times dissolved in methanol and precipitated with ether before drying *in vacuo* over P_2O_5. No satisfactory elemental analyses were obtained for any of the peptide ester complexes because they were all very hygroscopic. However, their integrated pmr spectra showed the absence of other Co(III) compounds.

Co(III) dipeptide acid Chloride $[Co(en)_2aa^{I}aa^{II}O]Cl_2$ From $[Co(en)_2aa^{I}aa^{II}OR]Cl_3$

The reaction mixture of the peptide ester complex after dilution to 500 ml with water was sorbed on H^+ form resin and was eluted with 1.5M HCl. The eluate of the peptide complex was made 3M in HCl and

after 5 hr reduced to dryness. The residue was dissolved in 80% methanol-H_2O, neutralised to pH 6.5 with 0.1M LiOH, precipitated with ether and collected. The precipitation of the complex with ether was repeated, and the product was then dried in vacuo over P_2O_5.

The pmr spectrum integrated correctly and demonstrated the absence of the ester function. Most of the peptide complexes were isolated in this manner since the absence of the ester function simplified their pmr spectra.

$[Co(en)_2aa^{I}aa^{II}O]Cl_2$ From Isolated $[Co(en)_2aaOCH_3]Cl_3$

The following procedures were carried out under dry nitrogen. The $[Co(en)_2aa^{I}OCH_3]Cl_3$ solution was treated with excess ether (sodium dried) to precipitate the complex. The product was collected, washed with methanol-ether (1:10) and then reprecipitated with dry ether from anhydrous methanol before drying under vacuum.

$[Co(en)_2aa^{I}OCH_3]Cl_3$ (10^{-3} mole) in methanol (50 ml) was treated with either the amino acid ester (10^{-2} mole) in methanol or the amino acid ester hydrochloride (10^{-2} mole) neutralised with 0.5M Et_3N in methanol. After 30 seconds the reaction mixture was neutralised with a slight excess of glacial acetic acid and diluted to 500 ml with H_2O. The peptide complex was then isolated as described above.

Condensation of $[Co(en)_2aa^{I}OCH_3]Cl_3$ with an amino acid ester was also carried out in dimethylsulphoxide, acetonitrile, or acetone as a solvent in a similar way to that described above.

Esterification Methods for $[Co(en)_2gly](OAc)_2$ and Testing for Formation of $[Co(en)_2glyOCH_3]^{3+}$

1. $[Co(en)_2gly](OAc)_2$ (0.2g) in anhydrous methanol (100 ml) was treated dropwise with PBr_3 and was kept at 20° for 18 hr. The solution was then treated with glycine ethyl ester hydrogen chloride ($glyOC_2H_5.HCl$) (0.2g) followed by a calculated volume of 0.5M Et_3N in anhydrous methanol such that the reaction mixture was 0.1M in $glyOC_2H_5$. After 30 sec the

solution was quenched to pH ~4 with HOAc and then diluted to 500 ml with water. The complexes in solution were then sorbed on a Na^+ form resin and eluted with 1.5M $NaClO_4$. The resulting orange bands were identified as $[Co(en)_2gly]^{2+}$ and $[Co(en)_2gly\text{-}glyOC_2H_5]^{3+}$ by comparisons of their visible and pmr spectra, and chromatographic behaviour with authentic samples. The relative amounts of the two products were determined by atomic absorption and visible absorption spectroscopy.

2. $[Co(en)_2gly](OAc)_2$ (0.2g) in anhydrous methanol (80 ml) was treated with a methanolic hydrogen chloride solution (15 ml, ~1N in HCl) and kept at 20° for 18 hr. The solution was then treated as in (1).

3. $[Co(en)_2gly](OAc)_2$ (0.2g) in anhydrous methanol (100 ml) was treated with acetyl chloride (CH_3COCl) (1 ml) and the solution kept at 20° for 18 hr. It was then treated as in (1).

4. $[Co(en)_2gly](OAc)_2$ (0.2g) in anhydrous methanol (80 ml) was treated with freshly distilled thionyl chloride ($SOCl_2$) (2 ml) and kept at 20° for 18 hr. The solution was then treated as in (1).

5. $[Co(en)_2gly](OAc)_2$ (0.2g) in anhydrous methanol (100 ml) was treated with 2,2'-dimethoxypropane (1 ml) and kept at 20° for 18 hr. A similar experiment was left for 3 days. The solutions were then treated as in (1).

6. $[Co(en)_2gly](OAc)_2$ (0.2g) in anhydrous methylene chloride-dimethylsulfoxide (30 ml, 10:1) was treated with triethyloxonium fluoroborate (1g) and kept at 20° for 18 hr and in another experiment for 2 days. The solutions were then treated as in (1).

7. $[Co(en)_2gly](OAc)_2$ (0.2g) in anhydrous CH_2Cl_2:DMSO (30 ml, 10:1) was treated with 10% BF_3 in methanol (1 ml) and kept at 20° for 18 hr. The solution was then treated as in (1).

8. $[Co(en)_2gly](OAc)_2$ (0.2g) in anhydrous CH_2Cl_2:DMSO (30 ml, 10:1) was treated with triethylorthoformate (1 ml) and kept at 20° for 18 hr. The solution was then treated as in (1).

All the above alkylation experiments were repeated using $[Co(en)_2ala](OAc)_2$. Cobalt estimations were made to determine the relative amounts of $[Co(en)_2ala]^{2+}$ and $[Co(en)_2ala\text{-}glyOC_2H_5]^{3+}$.

Peptide Formation via a Monodentate Amino Acid Ester

cis-$[Co(en)_2Cl(alaOCH_3)]Cl_2$

$[Co(en)_2ala](OAc)_2$ (0.5g) in methanol (250 ml) was treated dropwise with PCl_3 (5 ml) and kept at 20° for 5 days. The initially orange solution was now deep red and this was treated with ether to precipitate the complex which was collected. After washing with ether-methanol (10:1) the product was dissolved in methanol-isopropanol (1:1). On cooling at 0° the monodentate ester complex crystallised. The complex was recrystallised from methanol-isopropanol (1:1), washed with ether, and dried in vacuo over P_2O_5. Due to its hygroscopic nature elemental analyses were unsatisfactory. Instead the complex was characterised by infrared spectroscopy (ester carbonyl absorption at 1735 cm^{-1}; Nujol mull) and by its pmr spectrum.

cis-$[Co(en)_2Cl(alaOCH_3)]Cl_3$ was also prepared by a modification of a method previously described (106). $[Co(en)_2ala](OAc)_2$ (1g) in methanolic hydrogen chloride (150 ml, 1.5M in HCl) was refluxed for 4 hr and the complex chloride then precipitated by adding ether. The product was collected and washed four times with ether-methanol (30 ml), and then dissolved in anhydrous methanol. On standing $[Co(en)_2ala]Cl_2$ deposited and was removed. The filtrate was treated with isopropanol until cloudy when on cooling at 0° $[Co(en)_2Cl(alaOCH_3)]Cl$ crystallised. The complex was recrystallised (X2) from isopropanol (1:1). The final product was washed with ether-methanol (1:1) and dried in vacuo over P_2O_5. The complex was characterised by IR and pmr spectroscopy and by its visible spectrum; ε_{523} ∿80 and ε_{365} ∿85.

$[Co(en)_2ala\text{-}glyOC_2H_5]Cl_3$

cis-$[Co(en)_2Cl(alaOCH_3)]Cl_2$ (0.1g) in anhydrous methanol (30 ml)

was treated with anhydrous $AgClO_4$ (0.5g) and kept at 20° for 24 hr. The solution was filtered to remove AgCl in a dry nitrogen atmosphere and the filtrate was treated with 5 ml 0.1M glycine ethyl ester in methanol. After 30 seconds the solution was quenched with HOAc and diluted to 100 ml with water. The complexes in solution were sorbed on Na^+ form resin, eluted with 1.5M $NaClO_4$ and two orange bands separated. The $[Co(en)_2ala\text{-}glyOC_2H_5]^{3+}$ and $[Co(en)_2ala]^{2+}$ fractions were collected and cobalt estimations indicated ca. 95% of the product was the dipeptide complex.

$[Co(en)_2gly\text{-}aaO]Cl_2$ and $[Co(en)_2gly\text{-}aaOR]Cl_3$

N-terminal glycine peptides were prepared by a modification of a method described previously (90).

$[Co(en)_2glyOCH_3](ClO_4)_3$ (20) (0.56g, 1 m mole) in anhydrous methanol (50 ml) was treated with amino acid ester hydrochloride (1 m mole), followed by rapid addition of a calculated volume of 0.5M Et_3N in methanol such that the reaction mixture was 0.1M in amino acid ester. After 30 sec the solution was quenched to pH ~4 with HOAc and then diluted with water. The dipeptide acid and ester complexes were isolated as described above for the preparations of $[Co(en)_2aa^{I}aa^{II}O]Cl_2$ and $[Co(en)_2aa^{I}aa^{II}OR]Cl_3$ respectively.

Removal of ala-gly from Δ-$[Co(en)_2(S)\text{-}ala\text{-}glyO]Cl_2$

1. Δ-$[Co(en)_2(S)\text{-}ala\text{-}glyO]Cl_2$ (0.10g, 250 μmoles) in water (5 ml) was treated with 0.05M VSO_4 in 0.05M $HClO_4$ (4 ml). After ca. 4 min the initially orange solution had turned pale green due to reduction of Co(III) to Co(II). The solution was diluted to 50 ml and was passed through a Na^+ form resin. The V^{2+}, V^{3+} and Co^{2+} ions were sorbed on the resin and the ethylenediamine and peptide eluates were collected using a fraction collector. The combined fractions were evaporated to ca. 1 ml under reduced pressure at 30°.

The same procedure was used with the other peptide complexes.

2. $[Co(en)_2ala\text{-}glyO]Cl_2$ (0.10g, ~250 μmoles) in H_2O (5 ml) at pH ~4 (acetic acid) was treated with sodium borohydride (0.1g) over 5 min. Acetic acid was added to the solution during $NaBH_4$ addition to keep the solution acid. The black products formed during the $NaBH_4$ addition dissolved at 40°. The solution was then treated as in (1).

3. Alanylglycine could also be removed from its Co(III) complex with the following reducing agents: lithium aluminium hydride, lithium tri-t-butoxy aluminium hydride, sodium thiosulphate, sodium cyanide, ammonium polysulphide, H_2S soln and Zn/HCl.

$[Co(en)_2ala\text{-}glyO]Cl_2$ in water was treated with excess of the reagent and the solution kept at 20° until the orange colour of the complex had disappeared. The solution was then treated as in (1).

Isolation of Ala-gly Obtained From Δ-$[Co(en)_2(S)\text{-}ala\text{-}glyO]Cl_2$

The solution of ala-gly and en (ca. 1 ml) obtained from $[Co(en)_2ala\text{-}glyO]Cl_2$ was spread on a thin layer chromatography plate (KGF_{254}, 0.5mm) in a continuous streak. The chromatogram was eluted in an ascending manner with n-butanol:acetic acid:water (5:2:4) in a tank lined with 1/8" filter paper soaked with eluant to aid saturation. A separate chromatogram was spotted with (S)-ala-gly (Fluka), en(Fluka), and the preparative mixture of ala-gly and en were eluted as above. This chromatogram was then treated with ninhydrin spray; en and ala-gly gave purple and pink spots respectively. Their R_F values were 0.38 and 0.46 respectively. After elution, the preparative chromatogram was dried at 60° and the ala-gly and en bands were detected under ultraviolet light. The ala-gly band was removed and treated with water (20 ml). The mixture was filtered and the adsorbent was washed with water (4x5 ml). The combined filtrates were evaporated to dryness and the ala-gly was dried in vacuo over P_2O_5.

Detection of Possible Racemisation in a Preparative Sample of (S)-alanyl-glycine

Samples (~ 200 μmoles) of (S)-ala-gly (Fluka, $[\alpha]_D$ +49±1.0° for a 10% solution in H_2O) and the isolated ala-gly from above, each in 5M HCl (5 ml) in sealed vials, were kept at 110° for 4 hr. After cooling, the samples were taken to dryness under reduced pressure at 30° and dissolved in 0.2 ml of 0.05M sodium pyrophosphate buffer at pH 8.21. They were then tested for their (R)-alanine content in the following manner using sheep kidney (R)-amino acid oxidase. The activity of the (R)-amino acid oxidase was determined using a standard solution of (R)-alanine. The procedure measured the uptake of oxygen at an oxygen electrode for a sample containing (R)-alanine treated with (R)-amino acid oxidase. The instrument was calibrated such that a full scale deflection represented 1.3 μmoles (R)-alanine and the detection limit was ±0.02 μmoles (R)-alanine. All test samples were equilibrated at 33°. The oxygen uptake was then recorded for the hydrolysed Fluka ala-gly and the synthetic ala-gly (20 μmole of each) on treatment with the enzyme. At the completion of each run the test sample was treated with 2 μmole of (R)-alanine to determine whether or not (R)-amino acid oxidase activity was still present. From the amounts of (R)-alanine present in the commercial and synthetic dipeptide samples the amount of racemisation in the synthetic ala-gly was calculated.

Testing for Possible Racemisation During Formation of Δ-$[Co(en)_2(S)\text{-}alaOCH_3]Cl_3$

Δ-$[Co(en)_2(S)\text{-}alaOCH_3]Cl_3$ (0.4g) was prepared from Δ-$[Co(en)_2(S)\text{-}ala](OAc)_2$ and PCl_3 in methanol as described above. The solution of the complex was treated with water (50 ml) and the mixture was then sorbed on a H^+ form resin and eluted with 1.5N HCl. Only one band appeared on the column. The eluate of $[Co(en)_2ala]^{2+}$ was collected and was evaporated to dryness under reduced pressure (X3) to remove

excess HCl. The product was dissolved in water (10 ml), neutralised with 0.5M LiOH to pH 6.5, evaporated to dryness and dried in vacuo over P_2O_5.

Absorption maxima were at 487 nm (ε 98) and 346 nm (ε 108); $[\alpha]_{546}$ -709° and $[\alpha]_D$ -360° in H_2O at 25°.

Test for Racemisation During Formation of Λ-$[Co(en)_2(S)$-ala-gly$OC_2H_5]Cl_3$ from Λ-$[Co(en)_2(S)$-ala$OCH_3]Cl_3$

A solution of Λ-$[Co(en)_2(S)$-ala$OCH_3]Cl_3$ in methanol was treated with glyOC_2H_5.HCl (0.1g), followed by rapid addition of a calculated volume of 0.05M Et_3N in methanol such that the mixture was 0.05M in glyOC_2H_5. After ca. 3 sec the solution was quenched to pH ∿4 and was diluted to 500 ml with water. The complexes in the solution were sorbed on H^+ form resin and eluted with 1.5N HCl. The eluate containing Λ-$[Co(en)_2(S)$-ala$]^{2+}$ was collected and evaporated to dryness (X3) to remove the acid. It was then dissolved in H_2O (10 ml), neutralised with 0.5N LiOH, evaporated to dryness and dried in vacuo. A 0.1% solution in H_2O at 25° gave α_{546} + 0.606° and α_D +0.259°; $[\alpha]_{546}$ +606° and $[\alpha]_D$ +259°.

Kinetics of Mutarotation of Λ-$[Co(en)_2(S)$-ala-gly$OC_2H_5]Cl_3$ Under the Conditions for its Formation

1. A solution of Λ-$[Co(en)_2(S)$-ala$OCH_3]Cl_3$ (0.5g) in methanol (250 ml) was treated with glycine ethyl ester hydrochloride (2.5g) and then by 0.5M Et_3N in methanol such that the solution was 0.1M in glyOC_2H_5. Aliquots were removed after 100 sec, 17 min 20 sec, 90 min and 25 hr, quenched with 10.6M HCl to pH ∿4 and diluted to 500 ml. The samples were then sorbed on H^+ form resins, eluted with 1.5N HCl and the eluates containing the peptide complexes were collected and evaporated to dryness. The products were dissolved in D_2O and their 100 MHz spectra recorded in the vicinity of the methyl protons of alanine using a 500 Hz sweep width.

2. The rate of mutarotation was also followed polarimetrically at 450 nm in a 1 dcm tube. The same experimental conditions were used.

Results and Discussion

The general reaction scheme developed for the synthesis and isolation of dipeptides is presented in Fig. 3.2 using the synthesis of glycylglycine as an example. Table 3.1 gives peptides prepared by this scheme.

In principle, the synthesis involves five steps:

(a) Chelation of the amino acid to Co(III);

(b) esterification of the chelated amino acid;

(c) formation of the dipeptide;

(d) removal of the peptide from Co(III);

(e) isolation of the peptide.

Although the steps are not elaborate, a considerable amount of exploratory and developmental effort was necessary to evolve suitable working methods.

TABLE 3.1

Peptides Synthesised *via* Their Cobalt(III) Chelates

1. Dipeptide Complexes $[Co(en)_2NH_2CHR^{I}CONHCHR^{II}COOR^{III}]^{3+}$		
gly-glyOC_2H_5	leu-glyOC_3H_7	arg-glyOCH_3
gly-alaOC_2H_5	leu-valOC_2H_5	arg-lysOC_2H_5
gly-sarOC_2H_5	val-serOC_2H_5	met-glyOC_2H_5
gly-pheOC_2H_5	val-glyOC_2H_5	hist-glyOC_2H_5
gly-histOC_2H_5	val-alaOC_2H_5	phe-glyOC_3H_7
gly-proOCH_3	thr-glyOC_2H_5	phe-valOC_2H_5
gly-valOC_2H_5	ser-glyOC_2H_5	pro-lysOC_2H_5
ala-glyOC_3H_7	lys-valOC_2H_5	pro-serOC_2H_5
ala-alaOC_2H_5	lys-glyOC_2H_5	pro-valOC_2H_5
ala-valOC_2H_5	gln-glyOC_2H_5	tyr-glyOC_2H_5
ala-proOCH_3	asp-glyOCH_3	tyr-serOC_2H_5
leu-alaOCH_3	asn-glyOC_2H_5	try-glyOCH_3

cont.

TABLE 3.1 (cont.)

2. Tripeptide and Tetrapeptide Complexes

gly-gly-glyOC_2H_5	ala-gly-glyOCH_3
gly-gly-gly-glyOC_2H_5	lys-gly-pheOC_2H_5
ala-gly-pheOC_2H_5	

Choice of Cobalt(III) Moiety

Several Co(III) complexes were examined as possible N-terminal protecting and activating groups for peptide bond formation. The $Co(trien)^{3+}$ (trien=triethylenetetramine) was investigated since with the exception of cysteine, cystine and histidine all the amino acid complexes have been prepared (107). However, esterification of the complexes was difficult and other problems arose due to the presence of stereo- and diastereoisomers some of which appeared to resist esterification. Several $[Co(tren)aa]^{2+}$ complexes (tren = 2,2',2"-triaminotriethylamine) can be prepared (108) and these complexes could be suitable for preparing peptide complexes. However, the synthesis of the tren ligand (108) requires several steps and it is therefore much less accessible than the $Co(trien)^{3+}$ and $Co(en)^{3+}$ moieties. Several tetramine amino acid complexes $[Co(NH_3)_4aa]^{2+}$ have been prepared (109). However, the $Co(NH_3)_4^{3+}$ moiety was not used because the amino acid complexes are difficult to prepare in good yield and on the whole their solubility in organic solvents is much less than en and trien analogues (109). The $[Co(en)_2aa]^{2+}$ complexes did not possess the difficulties mentioned above and were therefore the most convenient starting materials for peptide synthesis.

Esterification of Chelated Amino Acids

Development of a convenient method for the preparation of chelated amino acid esters $[Co(en)_2aaOR]^{3+}$ was in essence the most important aspect of the synthesis since a reactive carbonyl function is necessary for formation of the peptide bond, Figure 3.1, Step III to IV. The

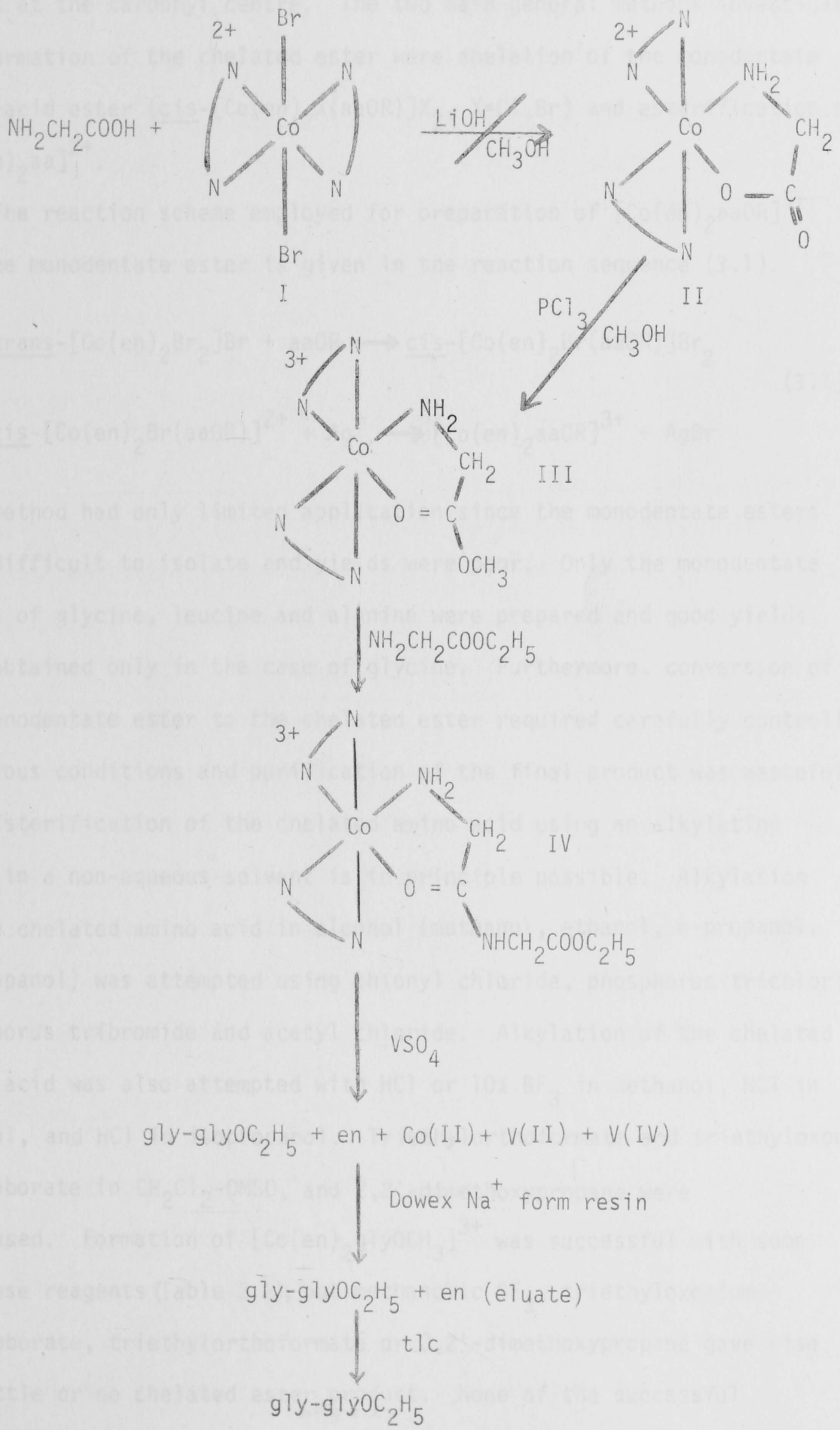


Fig. 3.1 Synthesis of glycyl glycine ethyl ester.

chelated amino acids are themselves unreactive towards substitution by amines at the carbonyl centre. The two main general methods investigated for formation of the chelated ester were chelation of the monodentate amino acid ester (cis-$[Co(en)_2X(aaOR)]X_2$, X=Cl,Br) and esterification of $[Co(en)_2aa]^{2+}$.

The reaction scheme employed for preparation of $[Co(en)_2aaOR]^{3+}$ via the monodentate ester is given in the reaction sequence (3.1).

$$\text{trans-}[Co(en)_2Br_2]Br + aaOR \longrightarrow \text{cis-}[Co(en)_2Br(aaOR)]Br_2$$
$$\text{cis-}[Co(en)_2Br(aaOR)]^{2+} + Ag^+ \longrightarrow [Co(en)_2aaOR]^{3+} + AgBr \qquad (3.1)$$

This method had only limited application since the monodentate esters were difficult to isolate and yields were poor. Only the monodentate esters of glycine, leucine and alanine were prepared and good yields were obtained only in the case of glycine. Furthermore, conversion of the monodentate ester to the chelated ester required carefully controlled anhydrous conditions and purification of the final product was wasteful.

Esterification of the chelated amino acid using an alkylating agent in a non-aqueous solvent is in principle possible. Alkylation of the chelated amino acid in alcohol (methanol, ethanol, n-propanol, isopropanol) was attempted using thionyl chloride, phosphorus trichloride, phosphorus tribromide and acetyl chloride. Alkylation of the chelated amino acid was also attempted with HCl or 10% BF_3 in methanol, HCl in ethanol, and HCl in isopropanol. Triethylorthoformate and triethyloxonium fluoroborate in CH_2Cl_2-DMSO, and 2,2'-dimethoxypropane were also used. Formation of $[Co(en)_2glyOCH_3]^{3+}$ was successful with some of these reagents (Table 3.2), but methanolic BF_3, triethyloxonium fluoroborate, triethylorthoformate or 2,2'-dimethoxypropane gave rise to little or no chelated ester product. None of the successful alkylation methods for the bis(en) amino acid complexes were efficient for $\beta_1[Co(trien)gly]^{2+}$ or effective for $\beta_2[Co(trien)gly]^{2+}$.

TABLE 3.2

Formation of $[Co(en)_2glyOCH_3]^{3+}$ from $[Co(en)_2gly]^{2+}$ in Methanol at 20°. [a]

Alkylating Agent	% Yield $[Co(en)_2glyOCH_3]^{3+}$ [b]
PCl_3	95%
PBr_3	30%
CH_3COCl	50%
HCl in Methanol (1.5M in HCl)	85%
$SOCl_2$	40%

[a] Reaction mixture of $[Co(en)_2gly](OAc)_2$ in methanol treated with 40 fold excess of alkylating agent and kept at 20° for 18 hr.

[b] Yield based on % formation of $[Co(en)_2gly\text{-}glyOC_2H_5]^{3+}$ when the chelated ester was treated with $glyOC_2H_5$.

Esterification was possible in both methanol and ethanol using the reagents listed in Table 3.2. However, the low solubility of the amino acid complexes in ethanol was a disadvantage.

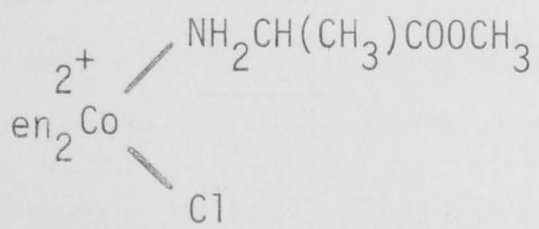
Esterification in methanol required strictly anhydrous conditions since $[Co(en)_2aaOCH_3]^{3+}$ hydrolyses rapidly even in the presence of traces of water, Reaction (3.2). The success of a particular alkylating

$$[Co(en)_2aaOCH_3]^{3+} + H_2O \longrightarrow [Co(en)_2aa]^{2+} + CH_3OH + H^+ \qquad (3.2)$$

procedure was determined by treating the esterified reaction mixture with excess $glyOC_2H_5$. Formation of the dipeptide ester from chelated amino acid ester is known to be quantitative (Chapter 4). The reaction mixture was then neutralised and the complexes were sorbed on a cation exchange resin. In all cases on elution with either $NaClO_4$ or HCl two bands separated. These were identified as $[Co(en)_2gly]^{2+}$ and $[Co(en)_2gly\text{-}glyOC_2H_5]^{3+}$ by comparison of their spectral properties and

chromatographic behaviour with those for authentic compounds. Their relative amounts were calculated from known extinction coefficients. The former species represented starting material, while the latter represented $[Co(en)_2glyOCH_3]^{3+}$ produced by esterification of $[Co(en)_2gly]^{2+}$. Similar qualitative results were obtained for the esterification of $[Co(en)_2ala]^{2+}$.

The most suitable combination of the amino acid complex and alcohol for esterifications using the alkylating agents listed in Table 3.2 is the acetate salt of the complex in methanol. This combination ensured that the amino acid complex remained dissolved during addition of the alkylating agent and during the formation of the chelated ester complex, thereby maximising the yields of chelated ester. Treatment of more than a 0.2M methanol solution of $[Co(en)_2gly](OAc)_2$ with a 40 fold excess of PCl_3, $SOCl_2$ or methanolic HCl (ca. 0.3M HCl) deposited $[Co(en)_2gly]Cl_2$. When the complex precipitates it appears that it is no longer able to form the chelated ester. From Table 3.2 it can be seen that best yields of chelated amino acid ester are obtained using PCl_3. More than 95% $[Co(en)_2glyOCH_3]^{3+}$ and $[Co(en)_2alaOCH_3]^{3+}$ were obtained after ca. 8 hrs on treatment of a 0.01M solution of the respective amino acid complex with a 40 fold excess of PCl_3 in methanol. Warming a solution of the amino acid complex in methanol after addition of either PCl_3 or methanolic HCl results in the more rapid formation of the chelated ester complex but also results in the formation of the monodentate ester (V).



(V)

The complex was identified by pmr (Fig. 3.2), by its IR spectrum (carboxyl absorption at 1735 cm^{-1}) and by conversion to $[Co(en)_2ala]Cl_2$ with Hg^{2+} (57). Furthermore, treatment of the cis-$[Co(en)_2Cl(alaOCH_3)]Cl_2$

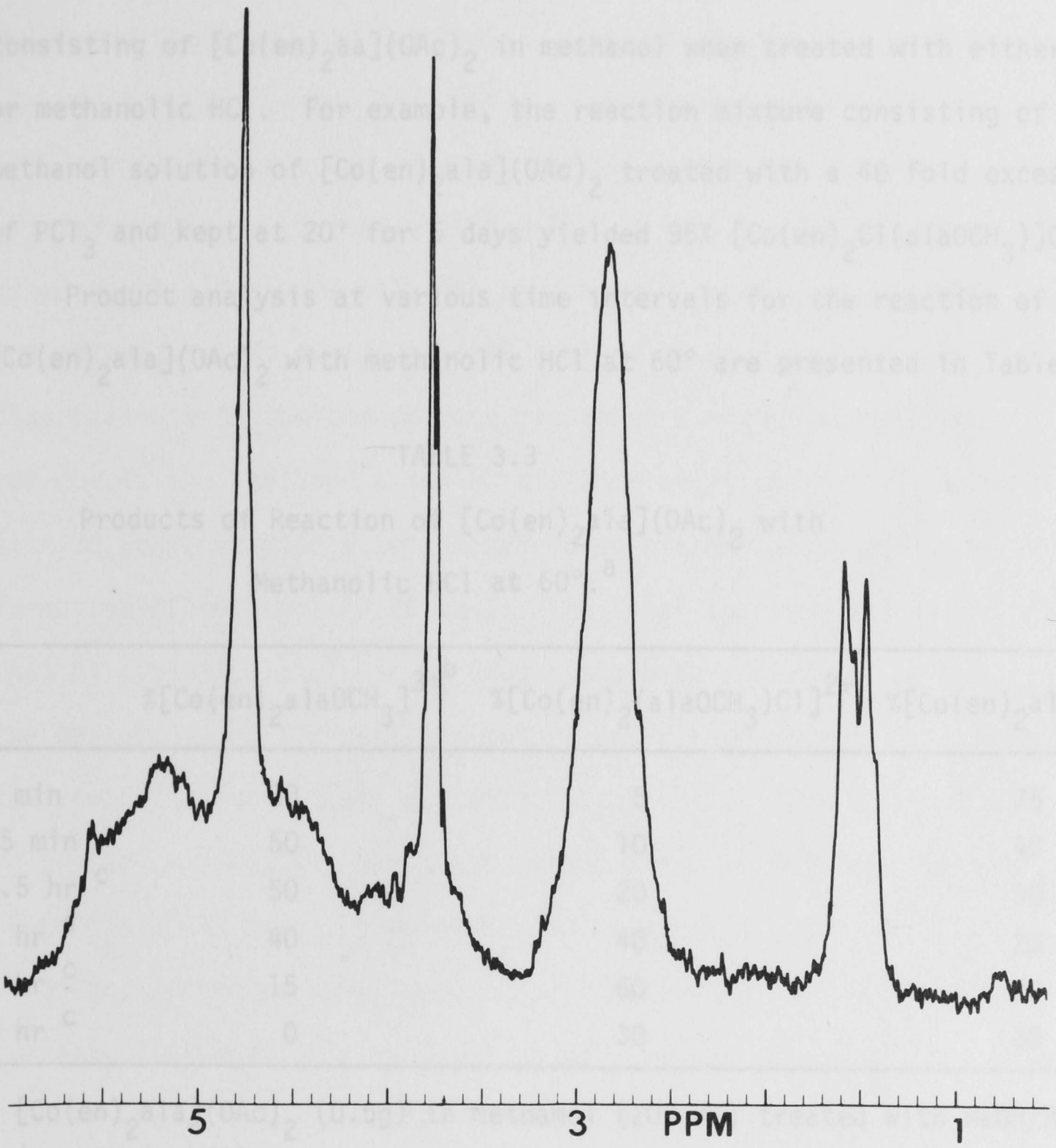


FIGURE 3.2 PMR SPECTRA (60 MHz) OF $[Co(en)_2Cl(alaOCH_3)]Cl_2$ IN D_2O; $-CH_3$ (ALANINE) AT 1.52 ppm; $\rangle\!=\!\langle$ c-H (ALANINE) AT ~4.5 ppm; $-OCH_3$ AT 3.75 ppm (REFERENCE NaTPS).

product with $AgClO_4$ in anhydrous methanol gave an orange solution which when treated with excess $glyOC_2H_5$ gave $[Co(en)_2gly\text{-}glyOC_2H_5]Cl_3$ (ca. 95%). The monodentate complex was the major product of the reaction mixture consisting of $[Co(en)_2aa](OAc)_2$ in methanol when treated with either PCl_3 or methanolic HCl. For example, the reaction mixture consisting of a 0.1M methanol solution of $[Co(en)_2ala](OAc)_2$ treated with a 40 fold excess of PCl_3 and kept at 20° for 5 days yielded 95% $[Co(en)_2Cl(alaOCH_3)]Cl_2$.

Product analysis at various time intervals for the reaction of $[Co(en)_2ala](OAc)_2$ with methanolic HCl at 60° are presented in Table 3.3.

TABLE 3.3

Products of Reaction of $[Co(en)_2ala](OAc)_2$ with Methanolic HCl at 60°.[a]

	$\%[Co(en)_2alaOCH_3]^{3+}$ [b]	$\%[Co(en)_2(alaOCH_3)Cl]^{2+}$	$\%[Co(en)_2ala]^{2+}$
5 min	20	5	75
15 min	50	10	40
0.5 hr [c]	50	20	30
1 hr [c]	40	40	20
3 hr [c]	15	60	20
8 hr [c]	0	30	30

[a] $[Co(en)_2ala](OAc)_2$ (0.5g) in methanol (200 ml) treated with MeOH/HCl (ca. 3M in HCl).

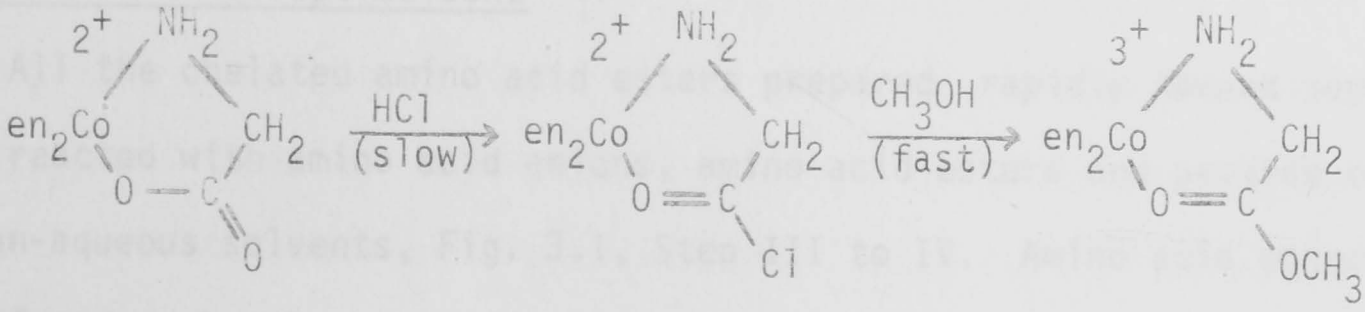
[b] $\%[Co(en)_2ala\text{-}glyOC_2H_5]^{3+}$ formed when the reaction mixture was treated with excess $glyOC_2H_5$.

[c] After 8 hr 40% the product is $[Co(en)_2Cl_2]Cl$.

The chelated ester is initially the major product but after one hour the amount of chelated ester has decreased and the mixture contains about equal amounts of chelated ester and monodentate ester. This requires that the monodentate ester complex is formed by anation of the chelated ester.

The chelated ester complexes were not isolated analytically pure. Instead, it was shown that these complexes had formed as the major product by their conversion to the dipeptide complexes and by pmr and infra-red studies. Pmr spectra (60 MHz) of the product isolated from the reaction of $[Co(en)_2ala](OAc)_2$ in methanol treated with PCl_3 in d_6-DMSO containing a trace of water resembled the spectra obtained during the hydrolysis of $[Co(en)_2glyOCH_3](ClO_4)_3$ (57). The HOD signal slowly moved downfield as the solution became more acidic and the signal for the methyl protons of the alanine doublet centred at 2.05 ppm disappeared with the concomitant growth of a methyl absorption at 1.75 ppm due to the chelated amino acid. Similarly, the methyl absorption at 4.30 ppm (chelated ester) disappeared synchronously with the formation of methanol (3.26 ppm). The final spectrum was identical to that of $[Co(en)_2ala]Cl_2$ in d_6 DMSO containing a trace of methanol. The assignment of the chelated methyl ester resonance is in accord with that reported for $[Co(en)_2glyOCH_3](ClO_4)_3$ (57). The infrared spectrum of $[Co(en)_2alaOCH_3]Cl_3$ has a sharp absorption at 1630 cm^{-1} whereas $[Co(en)_2ala]Cl_2$ has a broad absorption centred at 1635 cm^{-1}. The low frequency of the carbonyl absorption relative to that for $[Co(en)_2Cl(alaOCH_3)]Cl_2$ at 1730 ± 5 cm^{-1} is consistent with coordination of the carboxyl function of the ester (106,110).

The reaction sequence for the esterification with either PCl_3 or MeOH/HCl was not established with certainty but the formation of a reactive acid chloride intermediate <u>en route</u> to the formation of the chelated ester is possible, Reaction (3.3). It is possible that the



(3.3)

HCl slowly attacks the carboxyl group of the amino acid complex to form an acid chloride which then rapidly reacts with methanol to form the chelated methyl ester. Formation of an acid chloride intermediate would greatly enhance the electrophilic character of the carboxyl carbon atom because of the negative inductive effect of the chlorine atom. Once formed the acid chloride is expected to be very susceptible to nucleophilic attack by methanol. Reaction of primary alcohols with PCl_3 at room temperature gives rise to alkyl phosphites and HCl (111) and for methanol this reaction is very rapid at room temperature, Reaction (3.4).

$$3CH_3OH + PCl_3 \longrightarrow 3HCl + (CH_3O)_3P \qquad (3.4)$$

Therefore it is likely that when PCl_3 is added to the amino acid complex in methanol that HCl is the active reagent, (3.3). Since PCl_3 is also a very efficient dehydrating agent, Reaction (3.5), the higher yields

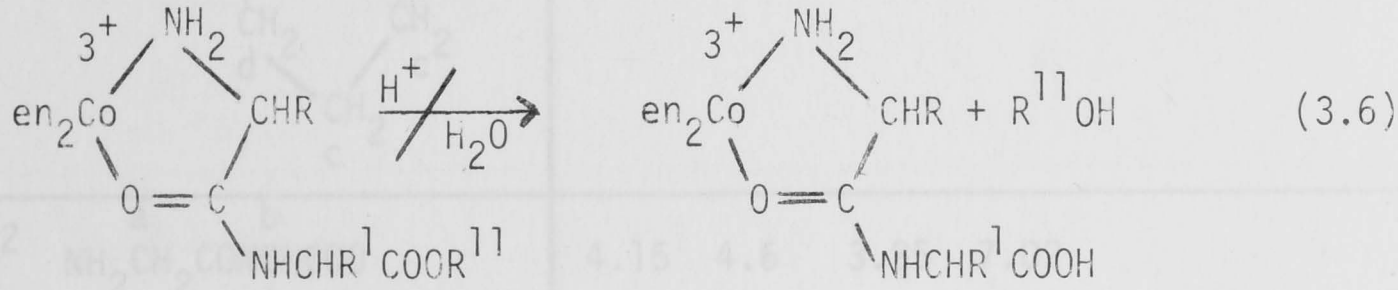
$$3H_2O + PCl_3 \longrightarrow 3HCl + P(OH)_3 \qquad (3.5)$$

of chelated methyl ester obtained with this reagent (Table 3.2) are probably due to the removal of traces of water. All reagents in Table 3.2 are capable of forming acid chlorides and this further suggests that esterification of the amino acid proceeds via this route. Triethylorthoformate, triethyloxonium fluoroborate, BF_3 in methanol, and 2,2'-dimethoxypropane failed to esterify the amino acid complexes. These reagents are most suitable for esterifications that proceed by direct methylation and their failure with cationic amino acid complexes implies that this is an unfavourable process.

Formation of the Peptide Bond

All the chelated amino acid esters prepared rapidly formed peptides when reacted with amino acid anions, amino acid esters and peptide esters in non-aqueous solvents, Fig. 3.1, Step III to IV. Amino acid esters were found to be the most suitable since they are very soluble in the

non-aqueous solvents used. In all preparations the chelated amino acid ester was treated with ca. 10 fold excess of amino acid ester. The peptide complexes prepared are listed in Table 3.1 and their yields were usually greater than 70%. The products were difficult to crystallise and satisfactory elemental analyses were not obtained. Also, quantitative analysis of the peptide ethyl ester complexes by pmr spectroscopy was not very satisfactory because their spectra were complicated by the ester absorptions. To overcome this problem, the ester was removed by acid hydrolysis and the complex was recovered as the peptide acid, Reaction (3.6). Usually the acid hydrolysis was completed



(3.6)

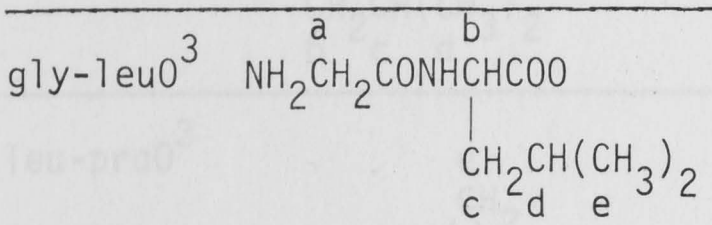
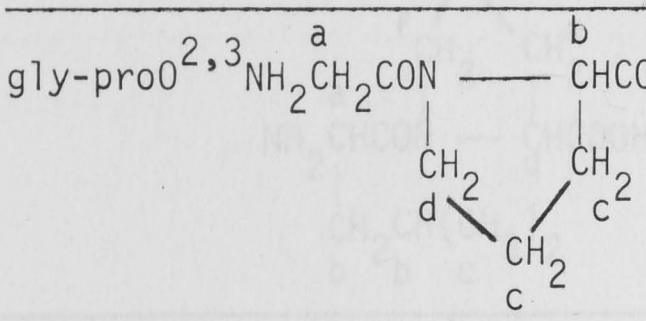
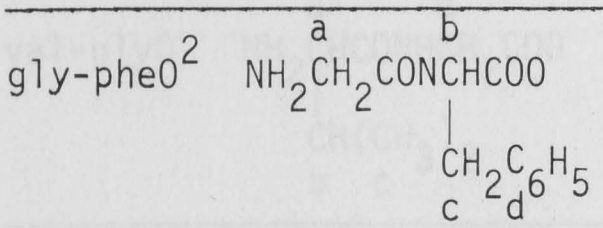
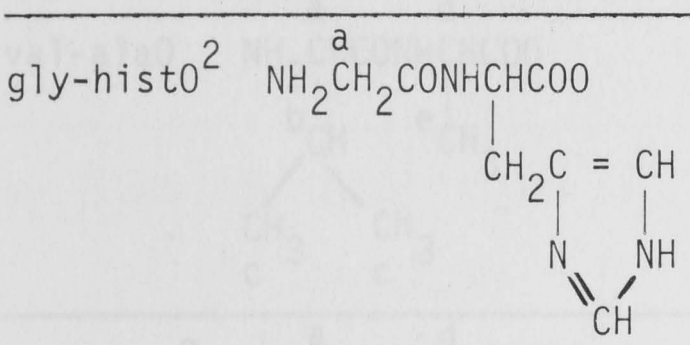
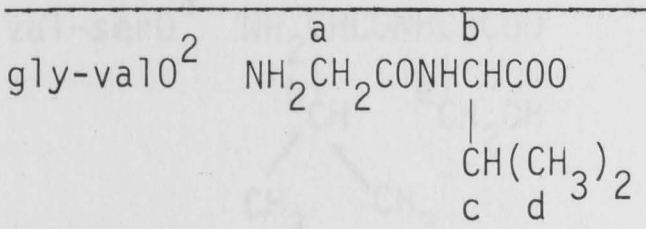
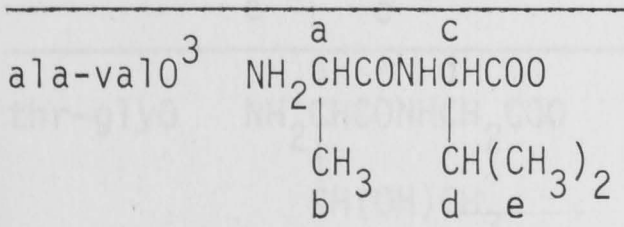
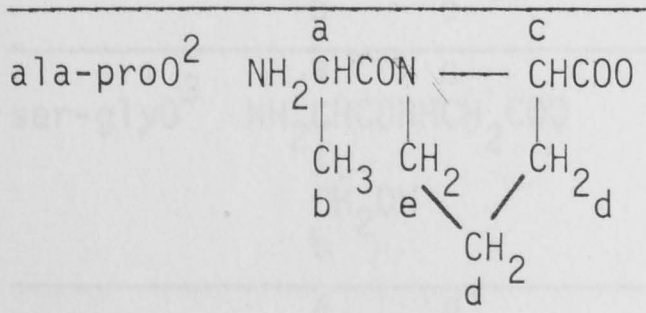
during the chromatographic separation procedure, but occasionally it was done subsequently. It was possible to crystallise and obtain elemental analytical data on some of the peptide acid complexes (Chapter 6), but a more convenient method of identification was by pmr spectroscopy. The chemical shifts for the peptide protons are listed in Table 3.4 and the spectra for a number of the complexes are given in Appendix II.

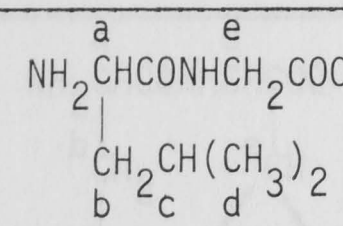
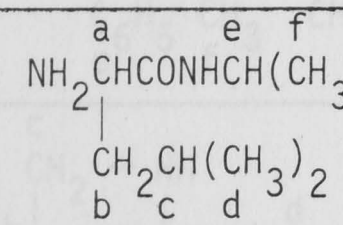
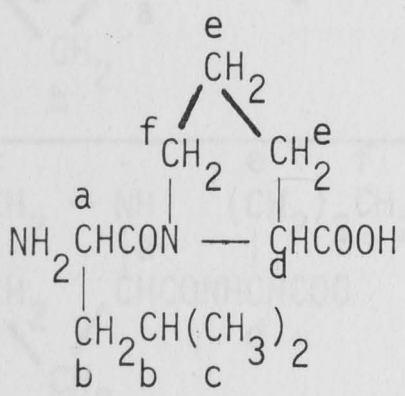
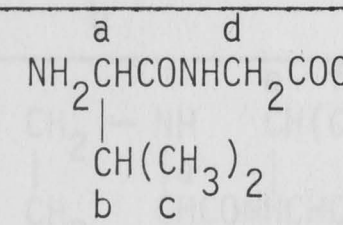
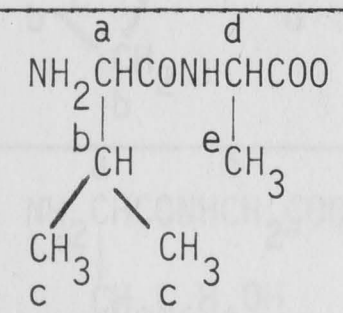
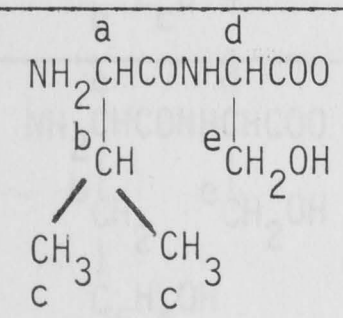
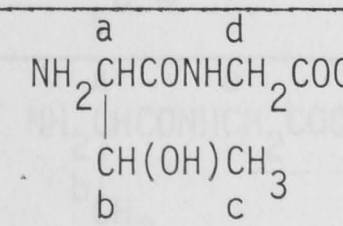
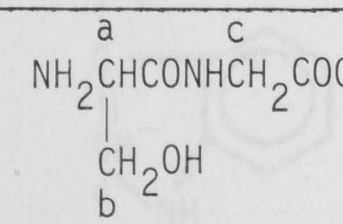
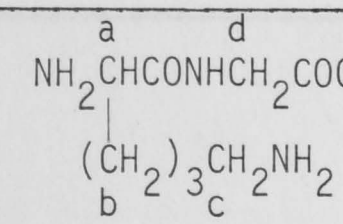
All the spectra integrated correctly for the proton absorptions of the peptide. The chemical shift assignments were made on the basis of a comparison with chemical shifts for the chelated and uncoordinated amino acids listed in Table 2.4. In nearly all instances the chemical shifts of the chelated amino acid of the peptide are very similar to those of the chelated amino acid in $[Co(en)_2aa]Cl_2$ and are at substantially lower fields than those of the uncoordinated amino acid. However, the C-terminal amino acid of the chelated peptide absorbs at a similar field to the uncoordinated species. These results demonstrate that Co(III) deshields only those groups directly coordinated to it.

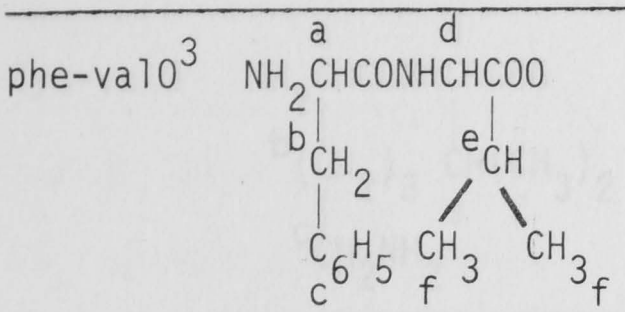
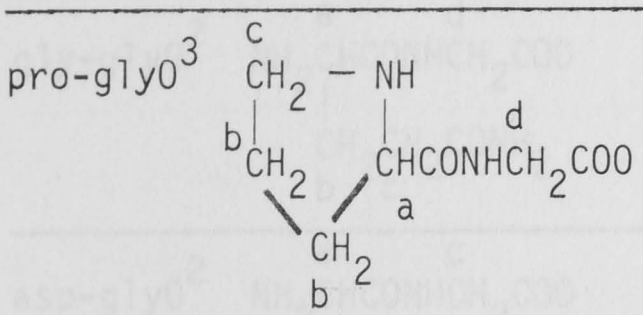
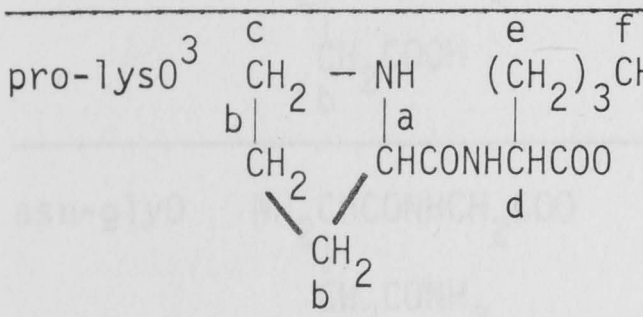
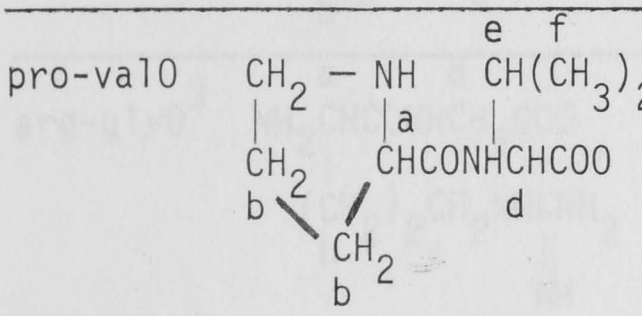
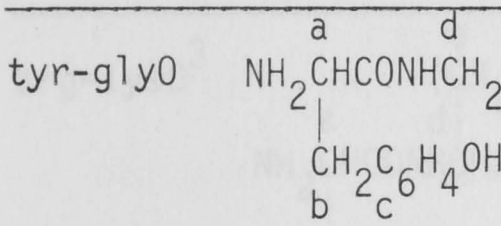
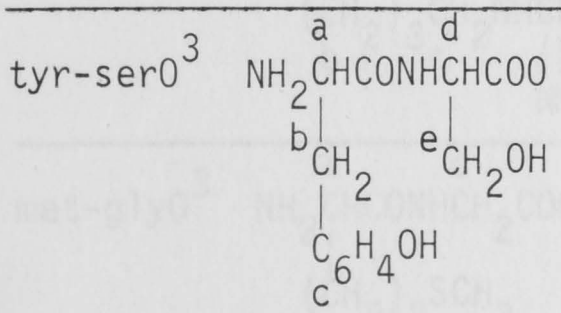
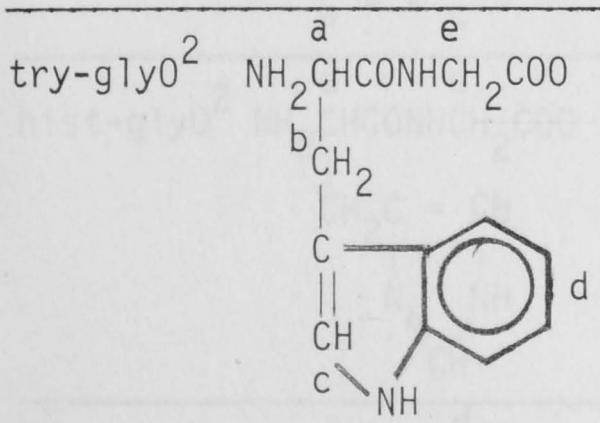
TABLE 3.4

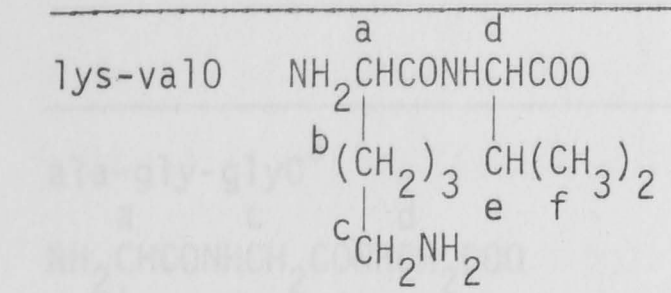
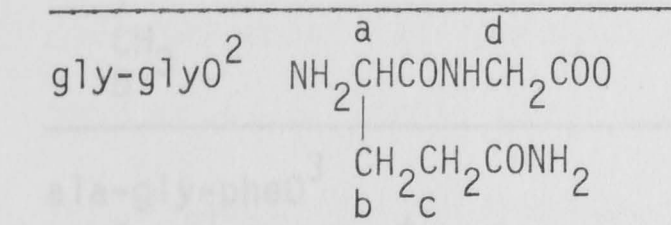
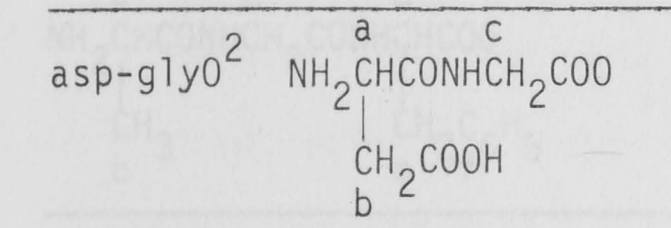
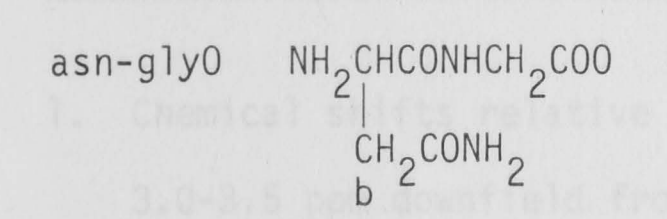
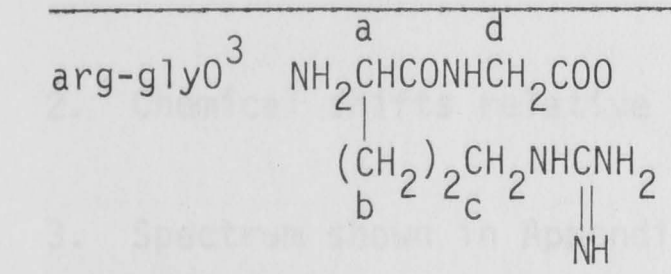
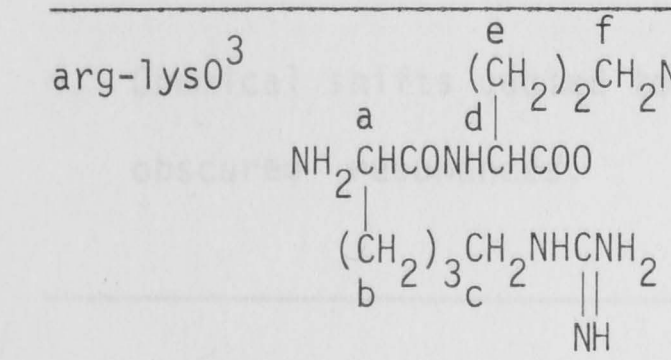
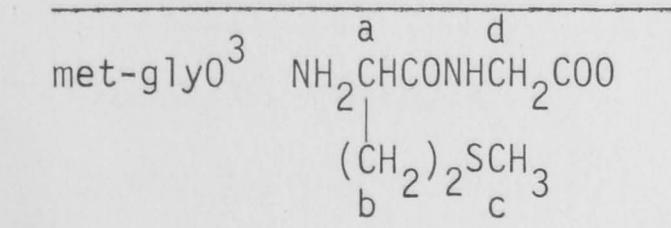
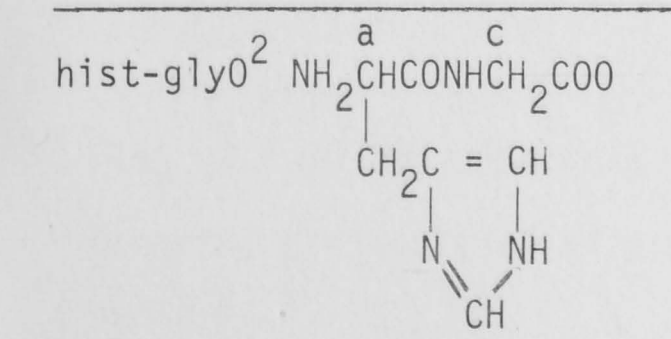
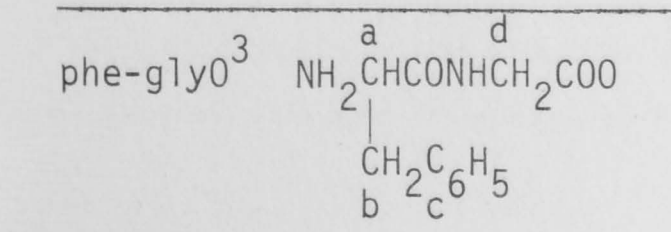
Chemical Shifts for the Peptide Protons in $[Co(en)_2NH_2CHR^{1}CONHCHR^{11}COO]Cl_2$ in D_2O at 32°.[1]

DIPEPTIDES

Peptide anion	Chemical Shifts for Carbon Protons in ppm.[4] a	b	c	d	e	f
gly-leuO[3] 	4.65	5.0	2.2	2.2	1.40	
gly-proO[2,3] 	4.2	4.4	2.15	2.15	3.71	
gly-pheO[2] 	4.15	4.6	3.25	7.02		
gly-histO[2] 	4.17	4.5	3.91			
gly-valO[2] 	4.20	3.71	2.2	1.01		
ala-valO[3] 	4.9	2.15	4.9	2.70	1.38	
ala-proO[2] 	4.4	1.80	4.4	2.15	2.15	3.60

Peptide anion	Chemical Shifts for Carbon Protons in ppm.[4] a	b	c	d	e	f
leu-gly0[3] 	4.9	2.4	2.4	1.50	4.64	
leu-ala0 	4.8	2.4	2.4	1.48	4.8	1.91
leu-pro0[3] 	4.7	2.3	1.42	4.7	3.6	4.1
val-gly0 	4.9	3.0	1.65	4.63		
val-ala0 	4.9	3.0	1.62	4.9	1.93	
val-ser0[3] 	4.3	3.0	1.50	4.3	4.3	
thr-gly0 	4.9	4.28	1.98	4.61		
ser-gly0[3] 	5.0	4.62	4.50			
lys-gly0 	4.6	1.80	2.8	4.15		

Peptide anion	Chemical Shifts for Carbon Protons in ppm.[4] a	b	c	d	e	f
phe-val0[3] 	4.7	3.95	7.90	4.8	2.62	1.34
pro-gly0[3] 	4.9	2.60	4.0	4.38		
pro-lys0[3] 	4.7	2.5	3.8	4.7	2.0	2.5
pro-val0 	4.7	2.62	3.9	4.7	2.70	1.36
tyr-gly0 	4.9	3.80	7.82	4.21		
tyr-ser0[3] 	4.9	3.80	7.51	4.9	4.66	
try-gly0[2] 	4.4	2.3	3.41	7.32	4.16	

Peptide anion	Chemical Shifts for Carbon Protons in ppm.[4] a	b	c	d	e	f
lys-val0 	4.5	1.80	2.8	4.5	2.3	1.95
gly-gly0[2] 	4.6	3.2	2.62	4.16		
asp-gly0[2] 	4.4	3.0	4.13			
asn-gly0 	4.5	3.0	4.15			
arg-gly0[3] 	4.7	2.39	3.72	4.60		
arg-lys0[3] 	4.7	2.2	3.0	4.7	2.2	3.0
met-gly0[3] 	4.95	2.2	2.66	4.68		
hist-gly0[2] 	4.20	4.5	3.91			
phe-gly0[3] 	4.8	3.90	7.92	4.72		

TRIPEPTIDES

Peptide anion	Chemical Shifts for Carbon Protons in ppm.[4] a	b	c	d	e	f
ala-gly-glyO^{3} a c d $NH_2CHCONHCH_2CONHCH_2COO$ CH_3 b	4.9	2.20	4.51	4.68		
ala-gly-pheO^{3} a c d $NH_2CHCONHCH_2CONHCHCOO$ CH_3 $CH_2C_6H_5$ b e f	4.8	2.15	4.52	4.8	3.60	7.81

1. Chemical shifts relative to TMS (100 MHz), en is a broad resonance 3.0-3.5 ppm downfield from TMS.

2. Chemical shifts relative to NaTPS (60 MHz).

3. Spectrum shown in Appendix II.

4. Chemical shifts quoted to one decimal are for broad and/or partially obscured resonances.

This particular aspect will also be discussed in Chapter 6.

The most convenient method for peptide formation was the in situ reaction of $[Co(en)_2aaOCH_3]^{3+}$ with amino acid esters. This procedure avoided isolating $[Co(en)_2aaOCH_3]Cl_3$ and thus reduced the possibility of hydrolytic side reactions. However, in those cases where the chelated ester was isolated, condensation was possible in CH_2Cl_2-DMSO mixtures, DMSO, DMF, acetone, N-dimethylacetamide, acetonitrile and hexamethylphosphoramide.

Reaction times for complete formation of the peptide were determined in acetone, methanol, acetonitrile and DMSO and the results are listed in Table 3.5. The data indicates that the rate is solvent dependent and is most rapid in methanol. The rates of condensation of $glyOC_2H_5$ with

TABLE 3.5

Rate of Peptide Complex Formation in Non-Aqueous Solvents at 25°.[a]

Peptide Complex Synthesised	Solvent	Reaction Time[b]
$[Co(en)_2gly\text{-}glyOC_2H_5]^{3+}$	Methanol	<1 sec[c]
"	Acetone	10 sec
"	DMSO	30 sec
"	Acetonitrile	25 sec
$[Co(en)_2(S)\text{-}ala\text{-}glyOC_2H_5]^{3+}$	DMSO	2000 sec
$[Co(en)_2gly\text{-}(S)\text{-}alaOC_2H_5]^{3+}$	"	300 sec
$[Co(en)_2gly\text{-}(S)\text{-}valOC_2H_5]^{3+}$	"	340 sec

[a] [Co] $\sim 2.5 \times 10^{-3}$M; [amino acid ester] = 2.0×10^{-2}M, μ = 0.15.

[b] 10$t_{½}$ for the addition of amino acid ester to chelated ester (Chapter 4).

[c] Product immediately formed when reactants were rapidly mixed.

$[Co(en)_2glyOCH_3]^{3+}$ and $[Co(en)_2alaOCH_3]^{3+}$ differ by a factor ca. 700, whereas the rates of condensation of $[Co(en)_2glyOCH_3]^{3+}$ with $glyOC_2H_5$, $alaOC_2H_5$ and $valOC_2H_5$ differ only by a factor ca. 10. Evidently the introduction of a substituent in the chelated ester moiety hinders attack more than when the substituent is attached to the nucleophilic amino acid ester. This fact possibly explains the difficulty that was experienced in the preparation of leu-val and leu-ala. These dipeptides were obtained in lower yields (ca. 50%) than were gly-ala or gly-val (> 75%). The slower rate of peptide formation in DMSO, acetone, or acetonitrile than in methanol was a further reason for preparing most of the peptide complexes in methanol.

Improved yields of the peptide complex can be obtained by treating the amino acid ester with excess chelated amino acid ester. Preliminary results for the reaction of $glyOC_2H_5$ treated with either ca. 10 fold excess $[Co(en)_2alaOCH_3]Cl_3$ or $[Co(en)_2glyOCH_3](ClO_4)_3$ in methanol indicate that all the $glyOC_2H_5$ had reacted.

Removal of the Peptide from the Cobalt(III) Complex

The major method used to remove the peptide from its cobalt(III) complexes was by reduction to a labile cobalt(II) species. All reagents used together with comments regarding their use are listed in Table 3.6. The reducing agents VSO_4 and $NaBH_4$ were most efficient with less than five minutes (20°) being required to complete the reduction of a 0.05M Co(III) solution using a 50% excess of either VSO_4 (Reaction 3.7) or $NaBH_4$. When $NaBH_4$ was used in the presence of HOAc

$$V(H_2O)_6^{2+} + [Co(en)_2\ peptide]^{3+} \longrightarrow V(H_2O)_6^{3+} + Co(H_2O)_6^{2+} + peptide + en \quad (3.7)$$

an insoluble black precipitate formed but this dissolved when the solution was warmed. Although H_2S has been found useful for recovering peptides from their Cu(II) complexes (112) it was found not as efficient as V^{2+} or $NaBH_4$ in the present experiments.

TABLE 3.6

Reducing Agents Used For Removal of Peptide From Co(III) Complex.[a]

Reagent	Comment
Chromous Chloride	Oxygen free conditions required. Rapid reaction.
Lithium Aluminium Hydride	Reduction complete in 5 min.
Sodium Boro Hydride	"
Lithium tri-(t-butoxy)-Aluminium Hydride	"
Sodium Thiosulphate	Very slow reduction ca. 20 hrs.
Sodium Cyanide	Rapid reduction. Handling problems.
Ammonium Sulphide	Reduction complete after 30 min at 40°.
Hydrogen Sulphide	"
Vanadous Sulphate	Reduction complete in 5 min.
Zn/HCl	Reduction complete after 18 hr.

[a] [Complex] ~0.05M, reducing reagent 50% excess.

Isolation of Dipeptides

The metal ions in the mixture after the reaction of the Co(III) peptide complex with either VSO_4 or $NaBH_4$ were removed by ion exchange chromatography. When the solution (pH ~5) was passed through a Na^+ form cation exchange resin Co(II) and V(IV) ions were sorbed on the resin and the peptide and ethylenediamine were eluted with the solvent front. The peptide and ethylenediamine were then separated by thin layer chromatography using a butanol-water-acetic acid eluant. The isolated peptides were identified by comparisons with authentic samples using thin layer chromatography and by amino acid analysis after acid hydrolysis. The dipeptides ser-gly, ala-gly, gly-ala and leu-gly were analysed in this manner.

Test for Possible Racemisation During Peptide Synthesis

Possible racemisation during formation of Δ-$[Co(en)_2(S)\text{-}alaOCH_3]^{3+}$ from Δ-$[Co(en)_2(S)\text{-}ala]^{2+}$ was tested by hydrolysing the former complex in dilute acid to give starting material. Results given in Chapter 6

and a previous study (65) suggest that racemisation of (S)-ala in Δ-$[Co(en)_2(S)\text{-}alaOCH_3]^{3+}$ is not expected to occur in mild acid conditions and the specific rotation of the product isolated as Δ-$[Co(en)_2ala]Cl_2$ was the same as that for an authentic sample of Δ-$[Co(en)_2(S)\text{-}ala]Cl_2$. Possible racemisation during peptide bond formation was tested by quenching a reaction mixture consisting of Λ-$[Co(en)_2(S)\text{-}alaOCH_3]^{3+}$ and $glyOC_2H_5$ in methanol after ca. one half life for the condensation. The products were isolated as Λ-$[Co(en)_2ala]Cl_2$ and Λ-$[Co(en)_2ala\text{-}glyO]Cl_2$ and their measured rotations were the same as those for authentic samples of optically pure Λ-$[Co(en)_2(S)\text{-}ala]Cl_2$ and Λ-$[Co(en)_2(S)\text{-}ala\text{-}glyO]Cl_2$ However, it is unlikely that this method could detect less than 2% racemisation since either the optically active amino acid or peptide contribute only ca. 8% of the total rotation at 546 nm in their respective complexes. Measured rotations were usually of the order of 0.500° for 0.1% solutions in water (546 nm), and were reproducible to ±0.002°.

Possible racemisation in the peptide complex was also tested by pmr spectroscopy. Any racemisation of (S)-ala in Δ-$[Co(en)_2(S)\text{-}ala\text{-}glyOC_2H_5]^{3+}$ results in the formation of its diastereoisomer Δ-$[Co(en)_2(R)\text{-}ala\text{-}glyOC_2H_5]^{3+}$. The methyl absorptions of the diastereoisomers differ by ca. 4 cps (Chapter 6). Optically pure ΔS and ΔR species yield methyl absorptions (doublets) at 2.17 and 2.21 ppm downfield from TMS and amounts of ΔR peptide greater than 5% could be detected in the ΔS peptide. The detection of less than 5% racemisation was not possible because the methyl absorptions of the minor product could not be observed against the background absorptions of the major component. A very sensitive method for detection of racemisation in (S)-amino acids is enzymically using (R)-amino acid oxidase. However, use of this method required the acid hydrolysis of the isolated ala-gly and this limited the accuracy of the method. The amount of racemisation detected was 0.7%. This value was obtained by comparing the amount of racemisation that had occurred on

acid hydrolysis of an authentic sample of (S)-ala-gly (Fluka) (8.2%) with that obtained using the synthetic product (8.9%).

The mutarotation of Λ-$[Co(en)_2(S)\text{-ala-gly}OC_2H_5]^{3+}$ prepared *in situ* from Λ-$[Co(en)_2(S)\text{-ala}OCH_3]^{3+}$ and 0.1M $glyOC_2H_5$ in methanol was followed by pmr spectroscopy. The pmr spectra (100 MHz) of the methyl protons of alanine in $[Co(en)_2ala\text{-}glyOC_2H_5]^{3+}$ for samples removed from the reaction mixture at various time intervals, are shown in Fig. 3.3. Initially one methyl doublet with J=7 cps is present, but another doublet appears as (S)-alanine mutarotates as shown in subsequent spectra. After 25 hr the spectrum consists of 2 sets of doublets of equal intensity and no further change is then observed. This implies that the complex has equilibrated and requires that the mixture contains approximately equal amounts of the ΔS and ΔR diastereoisomers. This particular aspect will be discussed in detail in Chapter 6. However, the significance of the result, in regard to the synthesis of peptides, is that under the conditions used for the condensation in methanol, racemisation of the product does not occur. Racemisation is *ca*. 4000 times slower than the formation of the peptide bond. The k_{obs} for mutarotation of Λ-$[Co(en)_2(S)\text{-ala-gly}OC_2H_5]^{3+}$ in 0.1M $glyOC_2H_5$ in methanol is $1.5\pm0.2\times10^{-4}$ sec^{-1} (polarimetric rate data) whereas the formation of the peptide complex in 0.1M $glyOC_2H_5$ is *ca*. 0.6 sec^{-1}.

Obviously little racemisation of the (S)-alanine in the peptide complex occurs under experimental conditions and the 0.7% racemisation detected by the (R)-amino acid oxidase is an upper limit. This particular result is from a single experiment and its confirmation is necessary using a more refined procedure. If it is established that the isolated ala-gly is not optically pure, further obvious experiments to be performed are the detection of possible racemisation in $[Co(en)_2ala\text{-}OCH_3]^{3+}$ and its parent amino acid complex using (R)-amino acid oxidase. These particular aspects are currently being investigated.

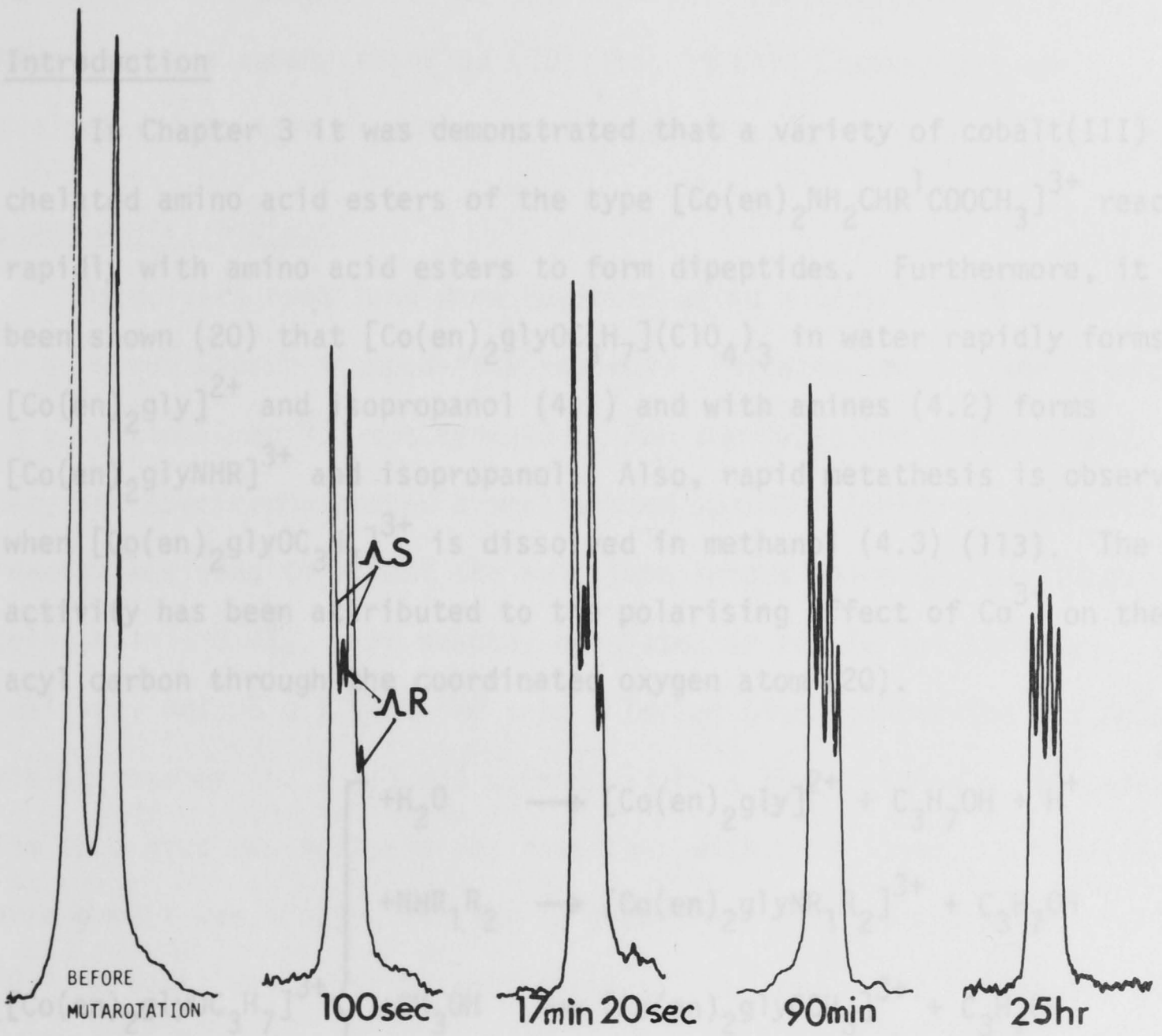


FIGURE 3.3 PMR SPECTRA OF THE METHYL ABSORPTIONS OF ALANINE IN Λ-$[Co(en)_2ala\text{-}glyOC_2H_5]Cl_3$ REMOVED FROM THE REACTION MIXTURE Λ-$[Co(en)_2(S)\text{-}alaOCH_3]Cl_3$ AND 0.1M $glyOC_2H_5$ IN METHANOL AT DIFFERENT TIMES.

CHAPTER 4

AMINOLYSIS OF $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ KINETICS AND MECHANISM

Introduction

In Chapter 3 it was demonstrated that a variety of cobalt(III) chelated amino acid esters of the type $[Co(en)_2NH_2CHR^1COOCH_3]^{3+}$ react rapidly with amino acid esters to form dipeptides. Furthermore, it has been shown (20) that $[Co(en)_2glyOC_3H_7](ClO_4)_3$ in water rapidly forms $[Co(en)_2gly]^{2+}$ and isopropanol (4.1) and with amines (4.2) forms $[Co(en)_2glyNHR]^{3+}$ and isopropanol. Also, rapid metathesis is observed when $[Co(en)_2glyOC_3H_7]^{3+}$ is dissolved in methanol (4.3) (113). The activity has been attributed to the polarising effect of Co^{3+} on the acyl carbon through the coordinated oxygen atom (20).

$[Co(en)_2glyOC_3H_7]^{3+}$ (in non-aqueous solvent)

$$+H_2O \longrightarrow [Co(en)_2gly]^{2+} + C_3H_7OH + H^+ \quad (4.1)$$

$$+NHR_1R_2 \longrightarrow [Co(en)_2glyNR_1R_2]^{3+} + C_3H_7OH \quad (4.2)$$

$$+CH_3OH \longrightarrow [Co(en)_2glyOCH_3]^{3+} + C_3H_7OH \quad (4.3)$$

$$+glyOC_2H_5 \longrightarrow [Co(en)_2gly\text{-}glyOC_2H_5]^{3+} + C_3H_7OH \quad (4.4)$$

$$+alaOC_2H_5 \longrightarrow [Co(en)_2gly\text{-}alaOC_2H_5]^{3+} + C_3H_7OH \quad (4.5)$$

This Chapter reports a kinetic study of reactions (4.4) and (4.5). In DMSO and in other non-aqueous solvents aminolysis of $[Co(en)_2glyOC_3H_7]^{3+}$ is not complicated by the competitive hydrolysis path found in water (20). It was anticipated that an understanding of the mechanism would assist in defining the most suitable conditions for synthesis of Co(III) peptide complexes. Furthermore, such a study serves not only to improve our understanding of the process of peptide bond formation in the coordination sphere of a metal ion, but it can also be of value in

elucidating the aminolysis of esters in organic systems where formation of stable tetrahedral intermediates are rare (Chapter 1). Activation parameters for reactions (4.1), (4.4) and (4.5) have been determined. The activation parameters for hydrolysis of $[Co(en)_2glyOC_3H_7](ClO_4)_3$ have been previously reported (20), but in this Chapter are re-evaluated from more precise experimental data.

Experimental Section

Aminolysis reactions were followed using a Cary 16K spectrophotometer or a Durrum-Gibson stopped-flow reactor. Infrared spectra were recorded on a Perkin-Elmer 457 spectrometer. For the reactions followed with the Cary 16K spectrophotometer a small, hand operated Teflon stopped-flow reactor was used to inject the solutions into a Helma QS flow-through cell (vol. < 2 ml). The reactor consisted of two Teflon syringes (delivery vol. 5 ml) inserted into a Teflon block containing a simple mixing chamber (~0.2 ml) and surrounded by a thermostated metal case. The apparatus was suitable for reactions with $t_{\frac{1}{2}} > 1$ sec. A similar arrangement was adopted for the IR experiment except that a NaCl cell (0.1 mm path) was substituted for the silica cell. The hydrolysis of $[Co(en)_2glyOC_3H_7](ClO_4)_3$ was followed by pH-Stat titration with the following Radiometer apparatus: a TTA_3 electrode assembly, a ABU 1 autoburet, a TTT1 titrator, and SBR_2 titrigraph. A constant pH was maintained on a pHA 630T scale expander linked to a TTT1 titrator.

Cobalt estimations were made with a Techtron AA4 atomic absorption spectrometer. Products from kinetic experiments were separated on Bio-Rad analytical Dowex 50W x2, (200-400 mesh, Na^+ or H^+ form) resin or on SE-Sephadex C-25 Na^+ form resin. Absorption spectra of eluate fractions were recorded on a Cary 14 spectrometer.

Glassware used in the preparations of the chelated ester complex, kinetics, and product analysis experiments was rinsed with water, methanol and dried in an oven at 200° for at least one day. It was

transferred to a dry box (N_2 atmosphere, desiccant P_2O_5) shortly before use.

The Teflon stopped-flow reactor was dissembled after each kinetic run, rinsed with dry AR methanol and dried in a vacuum dessicator. The apparatus was reassembled in the dry box.

Dimethyl sulphoxide

DMSO (Mallinchrodt AR,21) was dried over Linde molecular sieves (type 4A) for 2 days. It was then shaken intermittently with calcium hydride (10g) for 12 hr and fractionally distilled from fresh calcium hydride (10g) under reduced pressure. The first fraction (100 ml) was discarded. The fraction used for kinetic experiments gave bp 48° at 3mm and n_D^{25} 1.4785 (114), and was stored over Linde sieves (4A) previously preheated at 300° for 2 days.

Tetraethylammonium perchlorate

Tetraethylammonium chloride (31g) in 50% ethanol (100 ml) was treated with $LiClO_4$ (22g) in ethanol (20 ml). The perchlorate salt which immediately deposited was collected and thoroughly washed with cold water and twice recrystallised from 80% ethanol. The final product was washed with ether and dried under vacuum at 60° for 2 days over P_2O_5.

Glycine ethyl ester hydrogen chloride

$GlyOC_2H_5.HCl$ was prepared by a modification of a previous method (115).

A slurry of glycine (75.1g) in AR ethanol (437 ml) was treated with LR thionyl chloride (131g) at 20° over 20 min. The mixture was refluxed for 30 min and then evaporated to dryness under reduced pressure. The product was dissolved in hot ethanol (100 ml), treated with ether until cloudy and cooled slowly, whence the ester hydrochloride crystallised. The product was thoroughly washed with ether-

ethanol (10:1) and air dried. The combined filtrate and washings were treated with more ether when a further crop of ester hydrochloride was obtained. Both fractions of $glyOC_2H_5$.HCl were combined and twice recrystallised from ether-ethanol (5:1), air dried overnight, and further dried in a vacuum oven at 80° over P_2O_5 for 5 hr. Yield 128g (95%).

Anal. Calcd. for $C_4H_9NO_2$.HCl: C,33.38; H, 6.50; N, 10.03. Found: C, 33.3; H, 6.5; N, 10.0.

Alanine ethyl ester hydrogen chloride

$AlaOC_2H_5$.HCl was prepared by using alanine in place of glycine. Elemental analyses were not obtained. However, the pmr spectrum in D_2O intergrated correctly for $[ND_3^+CH(CH_3)COOC_2H_5]C\bar{l}$.

Ethyl glycinate

Glycine ethyl ester hydrogen chloride (30g) was added with stirring to a saturated sodium hydroxide solution (50 ml). The ester which separated was extracted with ether (100 ml). This, and two further ether extractions (100 ml) were dried over anhydrous Na_2CO_3 for 2 hr. The ether extract was then filtered and the ether evaporated under reduced pressure. Glycine ethyl ester was fractionally distilled under reduced pressure and three fractions collected, bp 40-42° at 7mm, yield 18g. Only the middle fraction was used for kinetics, bp. 41.0° at 7mm, n_D^{25} 1.4236 . The dry ester was stored at -20°.

Anal. Calcd. for $NH_2CH_2COOC_2H_5$: C, 46.59; H, 8.80; N, 13.58. Found: C, 46.3; H, 8.8; N, 13.7: Solutions of the ester were freshly made up in dry DMSO.

Ethyl alaninate

The procedure used for ethyl glycinate was employed. Ethyl alaninate bp 37.1-37.2° at 5.5mm.

$\underline{[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3}$

The complex was prepared in a dry box (N_2 atmosphere) by a modification of a previous method (20).

$\underline{cis}$-$[Co(en)_2Br(glyOCH(CH_3)_2)](ClO_4)_2$ (57) (18g) in methanol (50 ml, Grignard-dried) was treated with $AgClO_4$ (17g, dried over P_2O_5 for 4 days). The mixture was shaken intermittently for 30 min and filtered. The solid product was collected, washed with two portions of methanol-ether (100 ml, 1:5). This removed traces of any unreacted $\underline{cis}$-$[Co(en)_2Br(glyOCH(CH_3)_2)](ClO_4)_2$. $[Co(en)_2glyOCH(CH_3)_3](ClO_4)_3$ was dissolved in anhydrous acetone (30 ml) and anhydrous ether ($\underline{ca}$. 300 ml) was added to yield an oily product. The ether layer was removed and the oily product dissolved in acetone-methanol (150 ml, 1:4) and treated with ether. Crystallisation was induced by scratching the beaker with a glass rod. The product was recrystallised from acetone-methanol (1:4) and adhering solvent removed under vacuum. The complex was stored in an evacuated desiccator over P_2O_5. Yield 12g.

$\underline{Anal}$. Calcd. for $Co(C_{10}H_{25}N_5O_2)(ClO_4)_3$: C, 18.17; H, 4.56; N, 11.79; Co, 9.91. Found: C, 18.1; H, 4.8; N, 12.1; Co, 9.9.

Kinetic measurements

The aminolysis of $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ with $glyOC_2H_5$ and $alaOC_2H_5$ was followed spectrophotometrically at 450 nm. Solutions of the complex ($5.0x10^{-3}$M) and $glyOC_2H_5$ in DMSO were prepared and loaded into the reactor in the dry box. The reactor was transferred to the spectrophotometer and equilibrated at the reaction temperature (15 min). After each reaction, the reactor was washed with dry methanol and redried for 6 hr in an evacuated desiccator over P_2O_5. For reactions with $t_{1/2}$ < 1 sec the Durrum-Gibson stopped-flow reactor was used and a similar procedure was adopted for drying the syringe assembly and reservoirs.

Rate data were not obtainable for $t_{\frac{1}{2}} < 0.1$ sec because of the high viscosity of the reactant solutions (mixing time ca. 0.1 sec). The aminolysis of $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ was also followed quantitatively by infra-red spectroscopy. The reactor was charged with a solution of the complex (0.4M) and ester (0.6M) in the dry box and the solutions injected into the NaCl cell. Data were recorded at 1630 cm^{-1}.

The hydrolysis of $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ was followed by pH-Stat titration of 0.01M NaOH using the Radiometer apparatus. The complex (0.06g) was dissolved in 1M KNO_3 (20 ml) in the thermostated reaction vessel and the solution continuously stirred as the titrant was added under a nitrogen atmosphere. The hydrolysis of the complex was also followed spectrophotometrically. A weighed quantity of the complex dissolved in 0.1M $HClO_4$ (μ=1, $NaClO_4$ or KNO_3) at a desired temperature was quickly transferred to a 1 cm thermostated cell. An absorbance change of ca. 0.08 OD unit was then followed at 487 nm.

Product analysis

Product analysis during the aminolysis reactions were determined by quenching at time intervals, the reaction mixture of the chelated ester (25 ml, $5x10^{-3}$M) and $glyOC_2H_5$ (25 ml, $4.0x10^{-2}$M) with 10.6M HCl HCl to pH ~4 or pH 1, or with water (Table 4.4). The solutions were diluted with H_2O and the complexes sorbed on either H^+ or Na^+ form resin. Elution from Sephadex SE, C-25 resin (Na^+ form) with 0.05M $KClO_4$ allowed recovery of the complex peptide ester and $[Co(en)_2gly](ClO_4)_2$ free of most of the $KClO_4$. Complexes isolated from Dowex resin (H^+ form) following elution with 2N HCl, were $[Co(en)_2gly]Cl_2$ and $[Co(en)_2gly\text{-}glyOH]Cl_3$. The total amounts of 2^+ and 3^+ ions recovered exceeded 98% and the cobalt estimations for the bands eluted from the columns were made by atomic absorption and visible spectroscopy.

Detection of water in DMSO

$[Co(en)_2glyOC_3H_7](ClO_4)_3$ (0.14g) was dissolved in DMSO (50 ml) and kept for 4 hr in the dry box. Fresh $glyOC_2H_5$ (0.1 ml) was then added and after 10 sec the mixture was quenched with 10.6M HCl to pH ~4 and diluted with water. The complexes in solution were sorbed on H^+ form resin and eluted with 1.5N HCl. When water was present both the glycinato and dipeptide products were observed; when water was absent only the dipeptide complex was formed. The glycinato complex arises by traces of water in the DMSO, and the solvent was periodically checked in this manner.

Results

Choice of reagents and experimental conditions

The condensation of $[Co(en)_2glyOCH(CH_3)_2]^{3+}$ with other amino acid esters (4.6) and amines (4.2) was followed in a number of non-aqueous

$$[Co(en)_2glyOR^{I}]^{3+} + NH_2CH_2COOR^{II} \rightarrow [Co(en)_2glyglyOR^{II}]^{3+} + R^{I}OH \quad (4.6)$$

solvents and shown to form quantitatively the dipeptide ester or amino acid amide respectively. However, the relatively basic amines, methylamine, n-butylamine, diethylamine and NH_3 also decomposed the product and this complicated the kinetics analysis. The amino acid ester chosen to follow the kinetics was ethyl glycinate primarily because of its ease of preparation and purification, and because it was relatively stable towards 1,4-diketopiperazine formation when stored below 0°. It was found that in DMSO, $glyOC_2H_5$ produced the dipeptide slightly more rapidly with $[Co(en)_2glyOCH_3](ClO_4)_3$ than with $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$. (Table 4.2). The major part of the study was carried out with the isopropyl ester.

The solvents examined were dimethyl formamide (DMF), acetonitrile, N-dimethylacetamide, acetone, methanol, and dimethylsulphoxide (DMSO).

TABLE 4.1

Kinetics of the Reaction Between $[Co(en)_2glyOCH(CH_3)_2]^{3+}$ and $GlyOC_2H_5$ in DMSO at 25°.[a]

	Rate I		Rate II	
$GlyOC_2H_5$ [M]	k_{obs} sec^{-1}	$k_1 = k_{calc}$[b] M^{-1} sec^{-1}	k_{obs} sec^{-1}	k_{calc}[c] sec^{-1}
0.01	0.13±0.01(4)	13	$1.87\pm0.10\times10^{-3}$(3)	1.82×10^{-3}
0.02	0.28±0.01(5)	14	$3.65\pm0.10\times10^{-3}$(3)	3.23 "
0.04	0.58±0.01(2)	14	5.78±0.10 " (3)	5.27 "
0.08	1.1 ±0.05(3)	14	7.20±0.05 " (3)	7.70 "
0.1	1.5 ±0.05(5)	15	8.50±0.05 " (4)	8.50 "
0.2	3.0 ±0.03(4)	15	$1.05\pm0.05\times10^{-2}$(4)	1.07×10^{-2}
0.25	3.3 ±0.03(4)	13	1.10±0.05 " (2)	1.13 "
0.37			1.20±0.05 " (3)	1.21 "
0.5			1.25±0.05 " (2)	1.26 "
1.0			1.40±0.05 " (3)	1.35

[a] $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3 = 2.5\times10^{-3}$M. Measured at 450 nm.

[b] $k_{calc} = \frac{k_{obs}}{[glyOC_2H_5]}$ M^{-1} sec^{-1}.

[c] $k_{calc} = \frac{k_2K[base]}{1+K[base]}$ sec^{-1}, k_{calc} was calculated using K = 14.5 $mole^{-1}$, $k_2 = 1.45\times10^{-1}$ sec^{-1} and program

Condensation occurred in all cases but some solvents showed more desirable characteristics than others. The complex was not sufficiently soluble in DMF or acetone, acetonitrile and N-dimethylacetamide all reacted slowly with the base forming intense brown solutions or slowly decomposed the product dipeptide complex, and this prevented collection of useful kinetic data. Acetone also condensed with the methylene group of the chelated ester by a Knoevenagel type reaction. The addition of amine ($5x10^{-2}M$) to the orange chelated ester complex ($5x10^{-3}M$) in methanol gave immediately a relatively intense red colour which faded quickly ($\sim$30 sec) to the orange solution of the dipeptide complex (quantitative). It is possible that the red colour arises by deprotonation of the ethylenediamine of chelated amino acid ester amino groups or by the formation of the tetrahedral intermediate. This reaction has not been further examined.

Dimethylsulphoxide was chosen as the solvent for the kinetic analysis since it did not appear to generate any dubious or unwanted characteristics apart from a very slow decomposition of the peptide product at high base concentrations (> 1M). However, this did not interfere with the rate measurements.

Kinetics

The aminolysis of $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ with $glyOC_2H_5$ was followed spectrophotometrically at 450 nm; two rate processes differing considerably in velocity were observed (Fig. 4.1). The optical density change for each process was ca. 0.05 O.D. units. The reactions were measured under pseudo first order conditions of amine and the rate constants for varying amine concentrations (separated graphically) are given in Table 4.1. For the first process, plots of log $(D_\infty - D_t)$ against time were linear for at least $4xt_{\frac{1}{2}}$. The kinetics under each condition were at least duplicated and the same results were obtained with both types of stopped-flow reactor under the same conditions.

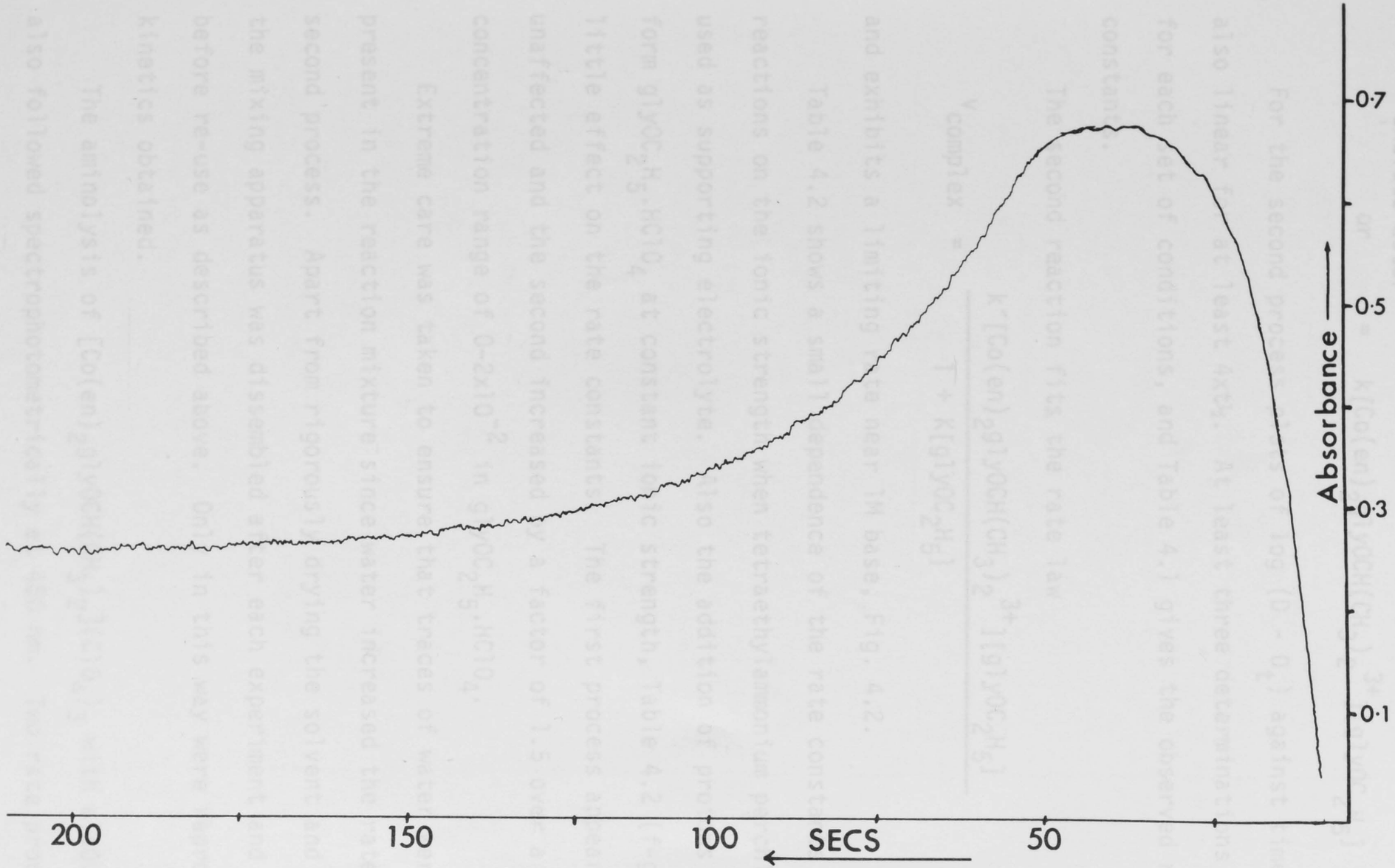


FIGURE 4.1 CHANGE IN OPTICAL DENSITY AGAINST TIME FOR THE CONDENSATION OF $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ (2.0×10^{-3}M) WITH $glyOC_2H_5$ (1.0×10^{-2}M) IN DMSO AT 450 mm at 25°.

The first process obeyed the rate law

$$v_{\text{chelate ester}} = k[\text{chelated ester}][\text{amine}]$$

$$\text{or} \quad = k[Co(en)_2glyOCH(CH_3)_2^{3+}][glyOC_2H_5]$$

For the second process plots of log $(D - D_t)$ against time were also linear for at least $4xt_{\frac{1}{2}}$. At least three determinations were made for each set of conditions, and Table 4.1 gives the observed rate constants.

The second reaction fits the rate law

$$v_{\text{complex}} = \frac{k'[Co(en)_2glyOCH(CH_3)_2^{3+}][glyOC_2H_5]}{1 + K[glyOC_2H_5]}$$

and exhibits a limiting rate near 1M base, Fig. 4.2.

Table 4.2 shows a small dependence of the rate constants for both reactions on the ionic strength when tetraethylammonium perchlorate was used as supporting electrolyte. Also the addition of protons in the form $glyOC_2H_5.HClO_4$ at constant ionic strength, Table 4.2 (f-g), had little effect on the rate constants. The first process appeared to be unaffected and the second increased by a factor of 1.5 over a concentration range of $0\text{-}2x10^{-2}$ in $glyOC_2H_5.HClO_4$.

Extreme care was taken to ensure that traces of water were not present in the reaction mixture since water increased the rate of the second process. Apart from rigorously drying the solvent and reagents, the mixing apparatus was dissembled after each experiment and dried before re-use as described above. Only in this way were reproducible kinetics obtained.

The aminolysis of $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ with $alaOC_2H_5$ was also followed spectrophotometrically at 450 nm. Two rate processes were also observed and the O.D. changes for each was ca. 0.08 O.D. units. As for the aminolysis of $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ with $glyOC_2H_5$, each reaction was measured under pseudo first order conditions of amine.

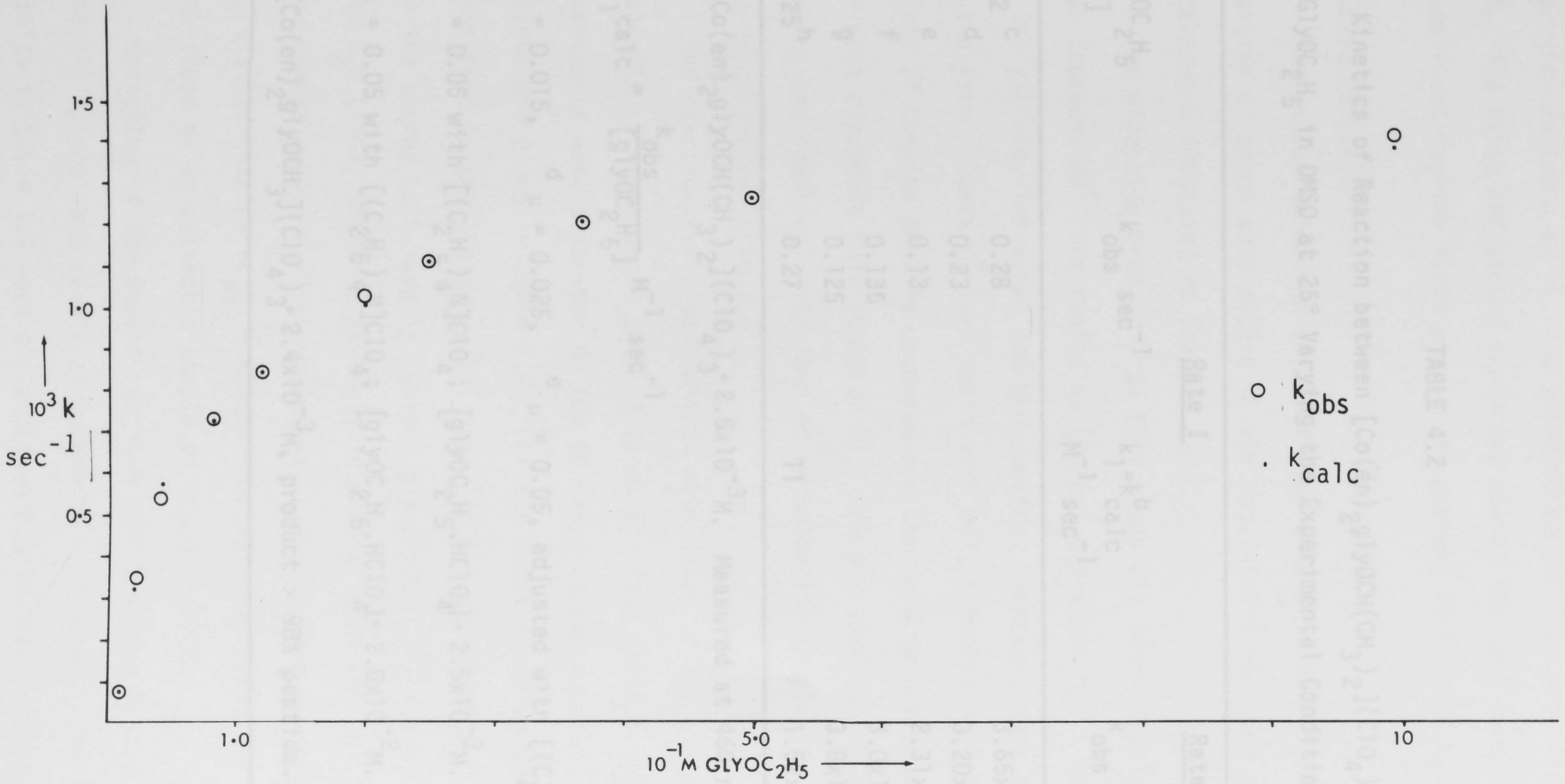


FIGURE 4.2 PLOT OF k_{obs} AND k_{calc} AGAINST [$glyOC_2H_5$] FOR THE DECOMPOSITION OF THE TETRAHEDRAL AMINO-CARBINOL INTERMEDIATE IN DMSO TO FORM $[Co(en)_2gly\text{-}glyOC_2H_5](ClO_4)_3$.

TABLE 4.2

Kinetics of Reaction between $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ and $GlyOC_2H_5$ in DMSO at 25° Varying the Experimental Conditions.[a]

$GlyOC_2H_5$ [M]	Rate I k_{obs} sec^{-1}	Rate I $k_1=k_{calc}^{b}$ M^{-1} sec^{-1}	Rate II k_{obs} sec^{-1}
0.02 [c]	0.28		3.65×10^{-3}
" [d]	0.23		3.20×10^{-3}
" [e]	0.13		2.31×10^{-3}
" [f]	0.135		3.0×10^{-3}
" [g]	0.125		3.8×10^{-3}
0.025 [h]	0.27	"	1.52×10^{-3}

[a] $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3 = 2.5\times10^{-3}$M. Measured at 450 nm.

[b] $k_{1calc} = \frac{k_{obs}}{[glyOC_2H_5]}$ M^{-1} sec^{-1}

[c] μ = 0.015, [d] μ = 0.025, [e] μ = 0.05, adjusted with $[(C_2H_5)_4N]ClO_4$.

[f] μ = 0.05 with $[(C_2H_5)_4N]ClO_4$; $[glyOC_2H_5.HClO_4] = 2.5\times10^{-3}$M.

[g] μ = 0.05 with $[(C_2H_5)_4N]ClO_4$; $[glyOC_2H_5.HClO_4] = 2.0\times10^{-2}$M.

[h] $[Co(en)_2glyOCH_3](ClO_4)_3 = 2.4\times10^{-3}$M, product > 98% peptide.

The rate constants at varying amine concentrations are given in Table 4.3. The first and second processes obeyed the same rate laws as those found for the first and second process with $glyOC_2H_5$.

Product Analysis

The isolation of the chelated dipeptide complexes formed by the addition of amino acid esters to the cobalt(III) chelated amino acid esters were discussed in Chapter 3.

The dipeptide product from this study was identified after isolation from Sephadex Na^+ form resin, as $[Co(en)_2gly\text{-}glyOC_2H_5]^{3+}$ by analysis of its visible, infra-red and pmr spectra. However, when the complex was eluted from a Dowex H^+ form resin with HCl, hydrolysis of the ester moiety of complex peptide occurred and the complex was then isolated as chelated dipeptide acid (Chapter 3). The pmr spectrum of the isolated complex was identical with that of an authentic sample of $[Co(en)_2gly\text{-}glyOH](ClO_4)_3$.

The reaction was also quenched with water or HCl at selected time intervals. The products (98% of total Co) from these quenching experiments were separated by ion exchange chromatography, and their amounts are given in Table 4.4. For the first reaction they were identified as the $[Co(en)_2gly]^{2+}$ and $[Co(en)_2gly\text{-}glyOC_2H_5]^{3+}$ ions. For the second path largely $[Co(en)_2gly\text{-}glyOC_2H_5]^{3+}$ arose on quenching with H_2O (>90%) while in strongly acid conditions $[Co(en)_2gly]^{2+}$ was also formed (~30%). This product was characterised by comparison of its visible spectra (ε_{487} = 98), chromatographic behaviour and pmr spectra with those of an authentic sample of $[Co(en)_2gly]I_2$.

Quenching of the second reaction with a slight excess of acid gave essentially the same result as quenching with water. The two sets of results in Table 4.4 were collected with different batches of chelated ester and the slight discrepancy reflects the presence of a 3-4% $[Co(en)_2gly]^{2+}$ impurity in the second reactant.

TABLE 4.3

Spectrophotometric Rate Data for the Condensation of ΛΔ-$[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ with (RS)-$AlaOC_2H_5$ in DMSO at 25°.[a]

Base	Rate I		Rate II	
	$10^2 k_{obs}$ sec^{-1}	$k_{obs}/[alaOC_2H_5]$ M^{-1} sec^{-1}	$10^4 k_{obs}$ sec^{-1}	$k^b_{calc} = \frac{k_2 K[alaOC_2H_5]}{1+K[alaOC_2H_5]}$ sec^{-1}
$2.0x10^{-3}$	0.27±0.05[c]	1.3		
$5.0x10^{-3}$	0.69±0.05[c]	1.4	1.3±0.1	1.2
$1.0x10^{-2}$	1.4 ±0.05	1.4	1.6±0.1	1.5
$2.0x10^{-2}$	2.7 ±0.1	1.3	1.8±0.1	1.8
$5.0x10^{-2}$	5.3 ±0.1	1.1	2.0±0.1	2.0
$1.0x10^{-1}$	8.7 ±0.1	0.9	2.2±0.1	2.2

[a] Rates measured at 450 nm in a 1 cm flow-through cell, [complex] = $2.5x10^{-3}$M, μ = 0.015.

[b] Calculated using K = 244 $mole^{-1}$, k_2 = $5.2x10^{-2}M^{-1}$ sec^{-1}.

[c] Rates measured in a 5 cm cell, [complex] = $2.5x10^{-4}$M.

TABLE 4.4

Products of the Reaction of $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ with $GlyOC_2H_5$ in DMSO at 25°.[a]

t sec	$[Co(en)_2gly\text{-}glyOC_2H_5]^{3+}$		$[Co(en)_2gly]^{2+}$		Quenching Method
A	50		48		d
5[b]	53	(47)[h]	47	(53)	d
	52		45		d
10	70	(68)	26	(32)	d
30	95	(97)	5	(3)	d
32	95	(97)	4	(3)	d
120	98	(100)	-		d
500[c]	99	(100)	-		d
1090[c]	98	(100)	-		d
B 5[b]	46		54		e
40[c]	89		11		e
	71		29		f
	73		27		g
300[c]	93		7		e
1860[c]	97		3		e
1860	96		4		f

[a] $[complex] = 2.5 \times 10^{-3}M$, $[glyOC_2H_5] = 1.0 \times 10^{-2}M$.

[b] $t_{\frac{1}{2}}$ 5.5 sec rate I.

[c] $t_{\frac{1}{2}}$ 370 sec rate II.

[d] Solution added to calculated amount of 11N HCl to neutralise base to pH~4.

[e] Water 50 ml added to reaction solution.

[f] Solution added to 11N HCl (1.5 ml).

[g] HCl (50 ml 0.2M) added to solution.

[h] Values in brackets are calculated product ratios using the measured rate constants given in Table 4.1.

Product analysis for the formation of $[Co(en)_2gly\text{-}alaOC_2H_5]^{3+}$ following quenching with water or HCl gave the same result as for the formation of $[Co(en)_2gly\text{-}glyOC_2H_5]^{3+}$. The $[Co(en)_2gly\text{-}alaOC_2H_5]^{3+}$ ion was characterised by elemental analysis, visible and pmr spectra as the chelated dipeptide acid (Chapter 6).

Infrared Experiments

IR spectra of $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ in anhydrous DMSO, and $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ treated with $glyOC_2H_5$ in anhydrous DMSO are given in Figs. 4.3 and 4.4 respectively. An absorption band at 1630 cm^{-1} is present in the spectrum of both the chelated ester and resulting peptide complex and they are of approximately the same intensity. This frequency for the ester and peptide complexes has been observed before, (90) and is consistent with C=O coordination. The sharp absorption band at 1740 cm^{-1} in the peptide spectrum (Fig. 4.4) attributed to both the C=O stretch of the uncoordinated moiety of the peptide and of unreacted $glyOC_2H_5$.

On mixing the ester complex with glycine ester a rapid and substantial decrease in the absorption band at 1630 cm^{-1} was observed, Fig. 4.5. This was followed by a slower increase back to almost the same intensity, Fig. 4.5. This experiment was reproduced several times. It did not give good kinetic data since the cell could not be thermostated and the IR beam caused substantial changes in the temperature of the solution during the course of the reaction. However the rate of change of the C=O absorption was roughly consistent with the accurate rate data obtained in the visible region.

Activation Parameters

Rate data for the condensation of $[Co(en)_2glyOC_3H_7]^{3+}$ with $alaOC_2H_5$ and $glyOC_2H_5$ in DMSO at different temperatures are listed in Table 4.5A and 4.5B. Accurate rate data at 30° were not obtained for the initial

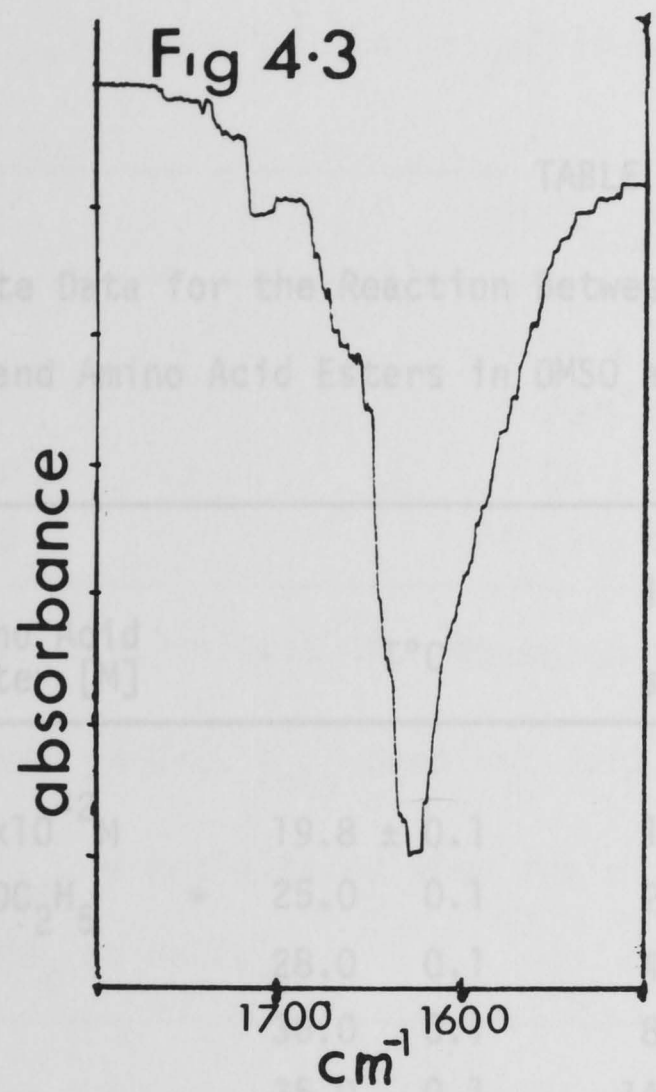


FIGURE 4.3 INFRA-RED SPECTRUM OF $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ (0.2M) IN DMSO (NaCl CELL, 0.1 mm PATH).

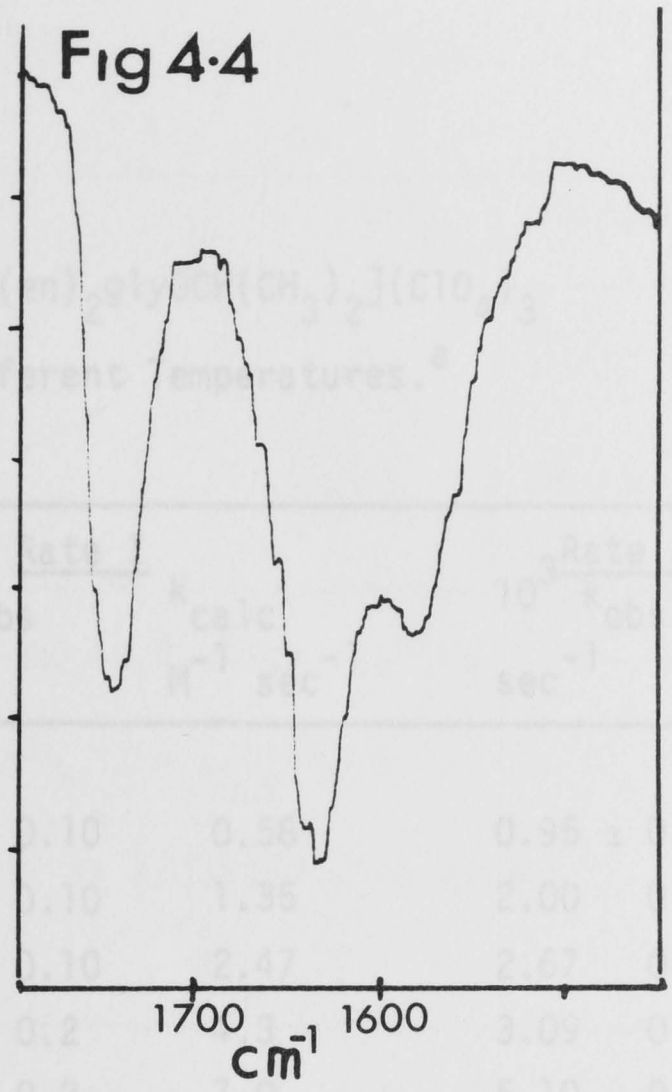


FIGURE 4.4 INFRA-RED SPECTRUM OF PRODUCTS OF THE REACTION BETWEEN $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ (0.2M) AND $glyOC_2H_5$ (0.3M) IN DMSO (NaCl CELL, 0.1 mm PATH).

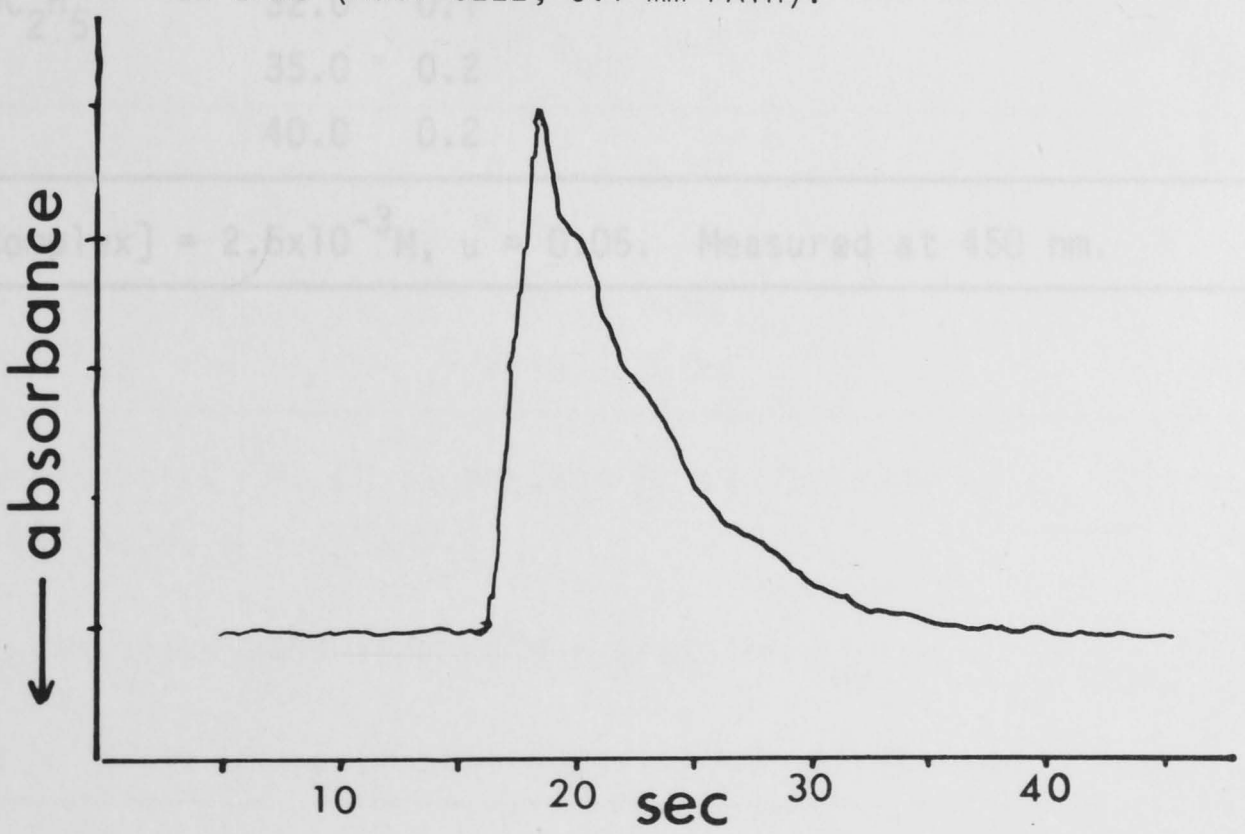


FIGURE 4.5 CHANGE IN ABSORBANCE AGAINST TIME FOR THE CONDENSATION OF $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ (0.2M) WITH $glyOC_2H_5$(0.3M) IN DMSO AT 1630 cm^{-1} AT ca. 25° (NaCl CELL, 0.1 mm PATH).

TABLE 4.5

Rate Data for the Reaction Between $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ and Amino Acid Esters in DMSO at Different Temperatures.[a]

Amino Acid Ester [M]	T°C		Rate I $10^2 k_{obs}$ sec^{-1}		k_{calc} M^{-1} sec^{-1}	Rate II $10^3 k_{obs}$ sec^{-1}	
A							
$2.0x10^{-2}M$	19.8	± 0.1	1.16	± 0.10	0.58	0.95	± 0.05
$alaOC_2H_5$	25.0	0.1	2.70	0.10	1.35	2.00	0.05
	28.0	0.1	4.94	0.10	2.47	2.67	0.05
	30.0	0.1	8.6	0.2	4.3	3.09	0.05
	35.0	0.1	14.0	0.2	7.0	5.10	0.05
B							
	19.8	0.1	1.1	0.1	1.1	0.96	0.05
	25.0	0.1	13.0	1.0	13	1.87	0.05
$10^{-2}M$	30.0	0.1	35	10	35	3.09	0.10
$glyOC_2H_5$	32.0	0.1				4.81	0.5
	35.0	0.2				5.33	0.5
	40.0	0.2				13.30	0.5

[a] [Complex] = $2.5x10^{-3}M$, μ = 0.05. Measured at 450 nm.

TABLE 4.6A

Lysis of $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ in H_2O.[a]

T	$10^3 k_{obs}$ sec^{-1}	$10^5 k_{H_2O}$ M^{-1} sec^{-1}
16	0.56 ± 0.05	1.00
25	1.20 ± 0.05 [c]	2.18
32.2	2.04 ± 0.05	3.68
41.8	5.33 ± 0.05	9.62

[a] [Complex] = 5×10^{-3}M. Measured at 487 nm in 1 cm cell.

[b] Over pH 0-2, k_{obs} does not vary, hence $k_{H_2O} = k_{obs}/55.5$.

[c] k_{obs} is not altered when ionic strength is adjusted with either KNO_3 or $NaClO_4$.

TABLE 4.6B

Lysis of $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ in H_2O at pH 5.[a]

T[c]	$10^3 k_{obs}$ sec^{-1}	$10^3 k_{OH^-}$[b] M^{-1} sec^{-1}
10.5 ± 0.1	0.44 ± 0.05	1.2 ± 0.1
20.0 ± 0.1	1.52 ± 0.05	7.1 ± 0.1
25.0 ± 0.1	3.01 ± 0.05	16.9 ± 0.2
30.0 ± 0.2	4.95 ± 0.05	30.2 ± 0.2

[a] [Complex] 5×10^{-3}M, pH 5, pH-Stat titration rate data. Titration with 0.01M NaOH, μ = 1 (KNO_3).

[b] k_{OH} is calculated from rate expression $k_{obs} = k_{H_2O} + k_{OH}[OH^-]$.

[c] At pH 5 the OH^- path constitutes > 85% of the total reaction.

reaction of $[Co(en)_2glyOCH(CH_3)_2]^{3+}$ with $glyOC_2H_5$ because the reaction was too fast to measure accurately (<2 sec) on the Cary 16 spectrophotometer. The enthalpies of activation for the formation and decomposition of the amino-carbinol intermediate were calculated from the slopes of Arrhenius plots. The entropies of activation, $\Delta S^{\dagger}_{298}$, were then obtained from the Eyring equation,

$$k = (KT/h)exp/-\Delta H^{\dagger}/RT)exp(\Delta S^{\dagger}/R)$$

where T = 298°K. The $\Delta H^{\dagger}$ and $\Delta S^{\dagger}$ values for the reactions of $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ with $alaOC_2H_5$ and $glyOC_2H_5$ are listed in Table 4.7. Similarly, $\Delta H^{\dagger}$ and $\Delta S^{\dagger}$ values for the k_{OH} and k_{H_2O} paths were obtained from radiometric data (Table 4.6A and 4.6B) for the hydrolysis of $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ at different temperatures, Table 4.7. Previously reported values (20) obtained in acetate buffers for the k_{H_2O} path are $\Delta H^{\dagger}$ = 14.6±0.1 kcal $mole^{-1}$ and $\Delta S^{\dagger}$ = -25±1 cal $mole^{-1}$ deg^{-1} and the k_{OH} path are $\Delta H^{\dagger}$ = 14.3±1 kcal $mole^{-1}$ and $\Delta S^{\dagger}$ = 15±4 cal $mole^{-1}$ deg^{-1}.

Discussion

An obvious interpretation of the two rate processes is the formation and decomposition of the tetrahedral addition complex, Fig. 4.1. This is partly supported by the types of rate laws observed for similar systems with purely organic substrates (41). In those processes where addition is the rate determining step, a term, first order in base is usually observed. Occasionally, an additional term second order in base also appears (46). In those processes where the leaving group determines the rate a more complicated rate law is frequently seen, and this is usually associated with some method of protonating the leaving group (47a).

The interpretation of the kinetics is supported by the product analysis derived from the quenching experiments. If the analysis is correct, quenching with water on the way to maximum optical density should

produce $[Co(en)_2gly]^{2+}$ and $[Co(en)_2gly\text{-}glyOC_2H_5]^{3+}$. The former arises from unreacted chelated ester which hydrolyses rapidly in the presence of water, Reaction 4.1. The latter arises rapidly from the tetrahedral intermediate in the presence of a plentiful supply of a proton donor. Clearly the experimental data (Table 4.4) supports this view. After all the chelated ester has been transformed to the tetrahedral intermediate, quenching with water produces largely the chelated dipeptide ester (>90%).

Finally, a consequence of the formation of the tetrahedral species is that the C=O stretching frequency for chelated ester at 1630 cm^{-1} should vanish. The experiment where this frequency was monitored showed a sharp initial decrease in this absorption (Fig. 4.5), followed by a slower growth back to approximately the initial intensity. This result is consistent with the kinetic data since at the amine concentration used the IR spectrometer would not be able to follow the rate of formation of the tetrahedral intermediate ($t_{\frac{1}{2}}$ ~0.1 sec), but should observe a reasonable concentration of this species, and its relatively slower decay to chelated dipeptide ester (C=O stretch 1630 cm^{-1}) ($t_{\frac{1}{2}}$ ~7 sec).

The three sets of experiments give unqualified support for the existence of the chelated tetrahedral intermediate, and it remains to discuss these results in the light of previous studies on related organic substrates (41). Over the range of amine concentration used the attack of amine is first order, and it is asserted that the process is bimolecular. The rate for the decomposition of the intermediate, however, implies some pre-equilibrium process and this is in agreement with previous proposals for reactions of this type (20,41). A proton transfer to the leaving group is indicated such that isopropanol is lost as $(CH_3)_2CHOH$. The argument that this is a better leaving group than the isopropoxide ion is especially strong in this instance where the acyl fragment has an overall +3 charge.

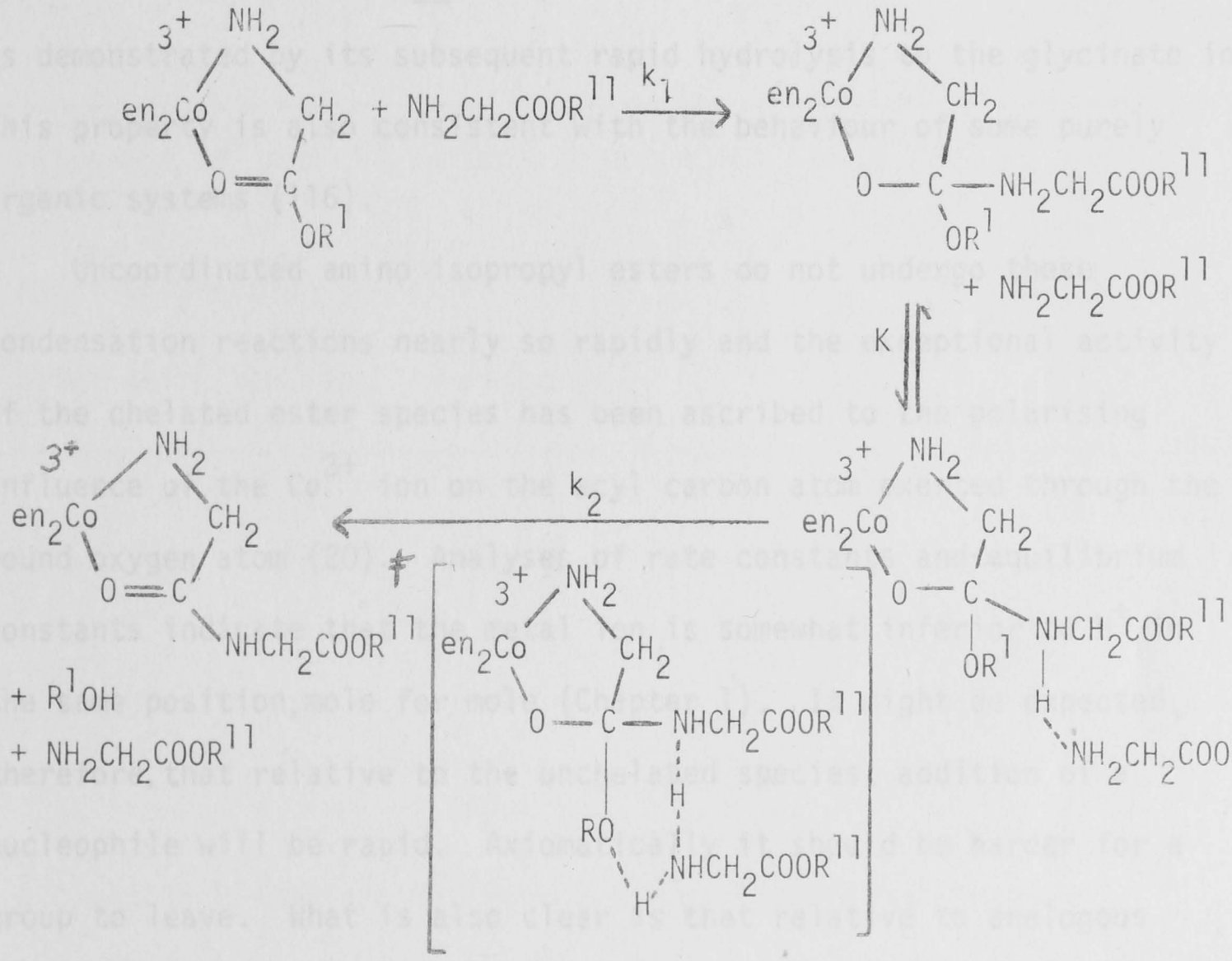
The mechanism in Fig. 4.6 yields the rate laws as shown and these are

$$v_{(\text{chelate ester})} = k_1[\text{ester chelate}][NH_2CH_2COOC_2H_5]$$

$$v_{(\text{amino carbinol})} = \frac{Kk_2[\text{amino-carbinol}][NH_2CH_2COOC_2H_5}{1 + K[NH_2CH_2COOC_2H_5]}$$

FIGURE 4.6

The Proposed mechanism for the aminolysis of $[Co(en)_2glyOR^{I}]^{3+}$ with $NH_2CH_2COOR^{II}$ in DMSO



in agreement with all observations. Since base is involved in the second reaction a mechanism requiring two equilibria involving transfer of H^+ from the amide N atom to the $-OR^1$ group followed by rate determining loss of R^1OH cannot accommodate the observed rate law. A simple mechanism involving amine bonded in the transition state with a concerted proton transfer and loss of ROH as the rate determining step will satisfy the observations. This is preferred to a pre-equilibrium for removal of a

proton from the aminol-carbinol complex followed by attack of protonated amine at OR^1 and simultaneous loss of R^1OH, since this will not accommodate the small effect observed by adding protonated base, Table 4.2. The concerted proton transfer and loss of R^1OH *via* the bonded amine of the previous mechanism will not be affected by protonated base. It appears from the quenching experiments at pH >4 in aqueous solution that alcohol is a much better leaving group than amine (a factor of at least 30). However, in strongly acid conditions the protonated amine competes more effectively (a factor of 2.5 in favour of alcohol) and appreciable quantities (*ca*. 30%) of the chelated ester are regenerated, as demonstrated by its subsequent rapid hydrolysis to the glycinato ion. This property is also consistent with the behaviour of some purely organic systems (116).

Uncoordinated amino isopropyl esters do not undergo these condensation reactions nearly so rapidly and the exceptional activity of the chelated ester species has been ascribed to the polarising influence of the Co^{3+} ion on the acyl carbon atom exerted through the bound oxygen atom (20). Analyses of rate constants and equilibrium constants indicate that the metal ion is somewhat inferior to H^+ in the same position, mole for mole (Chapter 1). It might be expected, therefore, that relative to the unchelated species, addition of a nucleophile will be rapid. Axiomatically it should be harder for a group to leave. What is also clear is that relative to analogous organic reactions, a pronounced stabilisation of the tetrahedral intermediate has occurred. This effect is illustrated by the reaction-coordinate diagram for the ester aminolysis (Fig. 4.7) which allows both rates to be observed and permits the formation of large amounts (>80%) of the intermediate. In Fig. 4.7 addition of amine to the chelated ester to give the amino-carbinol intermediate is governed by k_1. This species is then involved in a pre-equilibrium step prior to

TABLE 4.7

Activation Parameters for Lysis of $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ with Different Nucleophiles in H_2O and DMSO at 25°

Nucleophile	Medium	$\Delta H^{\dagger}$ kcal $deg^{-1}mol^{-1}$	$\Delta S^{\dagger}$ $cal.mol^{-1}deg^{-1}$
H_2O	H_2O	15.4	-29.0
OH^-	H_2O	15.5 [a]	+57.0 [a]
$alaOC_2H_5$	DMSO	31.3 [b]	+48
		18 [c]	
$glyOC_2H_5$	DMSO	19±3 [b]	+30±5
		20 [c]	

[a] Value for $\Delta H^{\dagger}$ and $\Delta S^{\dagger}$ quoted are corrected for $\Delta H^{\dagger}$ and $\Delta S^{\dagger}$ for dissociation of water.

[b] Addition reaction (reaction 1).

[c] Elimination reaction (reaction 2).

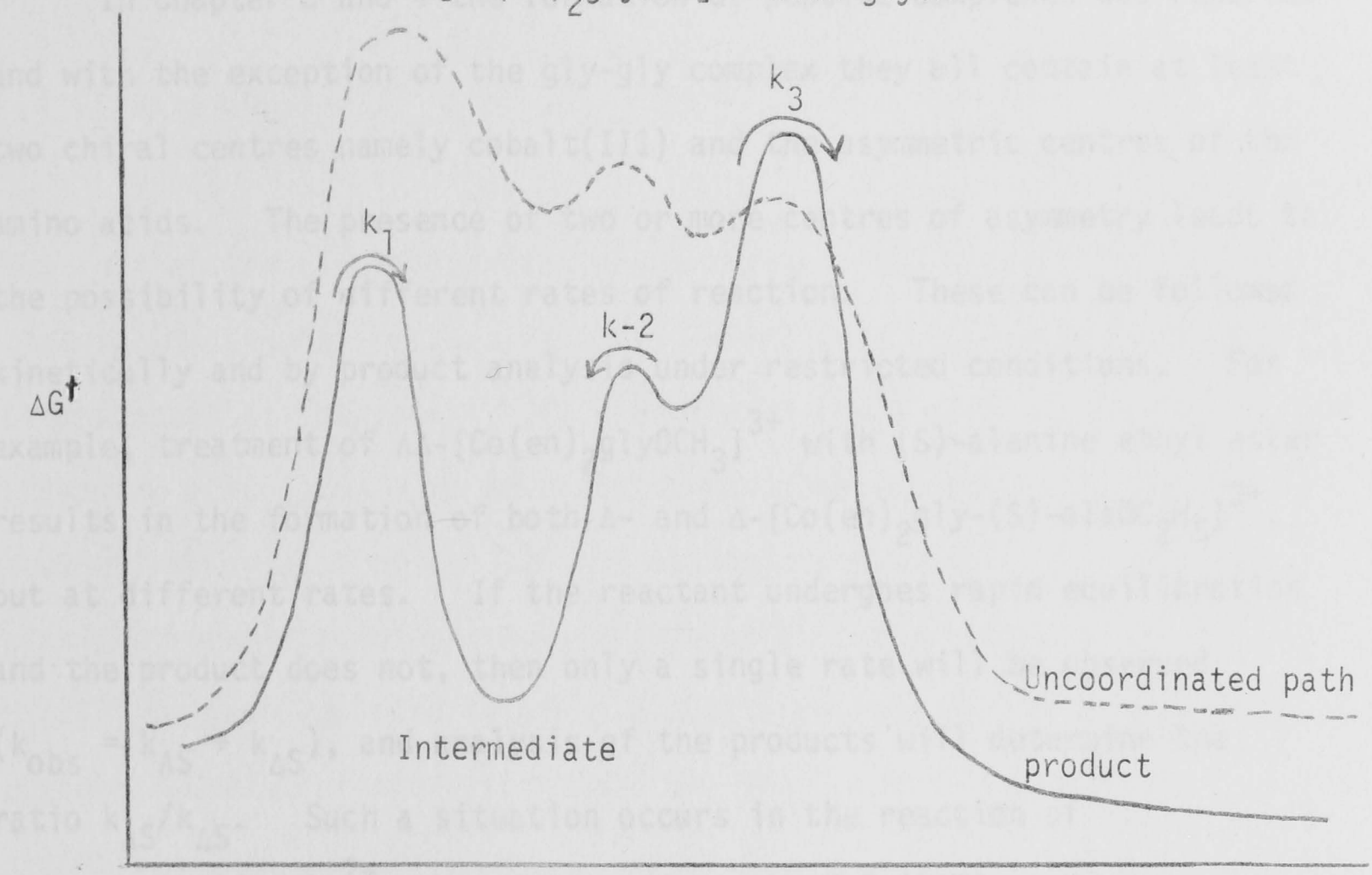
TABLE 4.8

Activation Parameters for Hydrolysis of Organic Substrates

Substrate	Medium	$\Delta H^{\dagger}$	$\Delta S^{\dagger}$
$C_2H_5OCOCH_2NH_2$ (33)	H_2O	9.0	-32
$C_2H_5OCOCH_2NH_3^+Cl^-$ (117)	H_2O	10	-12
$C_2H_5OCOCH_2N^+Et_3Cl^-$ (118)	H_2O	12.2	- 3
$C_2H_5OCOCH_2CH_3$ (119)	H_2O	11.2	-29

FIGURE 4.7

Proposed Reaction-Coordinate Diagram for the Reaction of $[Co(en)_2glyOR^{I}]^{3+}$ with $glyOR^{II}$



rate determining loss of alcohol, k_3. Stabilisation of the intermediate relative to transition states and relative to the uncoordinated reaction may act to lower $\Delta G^{\ddagger}$ for both processes relative to the uncoordinate species. The activation parameters obtained for the aminolysis and hydrolysis of the chelated esters (Table 4.7) show that acceleration for addition of a nucleophile (NH_2R,H_2O,OH^-) arises exclusively via an increase in $\Delta S^{\ddagger}$ relative to the organic reactions in the same solvent (Table 4.8). It can be seen that hydrolysis of organic substrates are all associated with negative $\Delta S^{\ddagger}$ values. These observations are inconsistent with the polarisation argument above which might be expected to appear in the enthalpy term. The entropy effect may be associated with a substantial increase in the stability of the chelate in the transition from the planar glycinato chelate ring to a pronounced gauche conformation in the tetrahedral intermediate.

CHAPTER 5

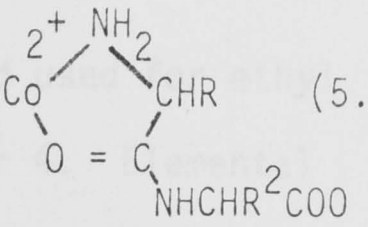
STEREOSELECTIVITY

In Chapter 3 and 4 the formation of peptide complexes was reported and with the exception of the gly-gly complex they all contain at least two chiral centres namely cobalt(III) and the asymmetric centres of the amino acids. The presence of two or more centres of asymmetry leads to the possibility of different rates of reaction. These can be followed kinetically and by product analysis under restricted conditions. For example, treatment of ΛΔ-$[Co(en)_2glyOCH_3]^{3+}$ with (S)-alanine ethyl ester results in the formation of both Λ- and Δ-$[Co(en)_2gly\text{-}(S)\text{-}alaOC_2H_5]^{3+}$, but at different rates. If the reactant undergoes rapid equilibration and the product does not, then only a single rate will be observed ($k_{obs} = k_{\Lambda S} + k_{\Delta S}$), and analysis of the products will determine the ratio $k_{\Lambda S}/k_{\Delta S}$. Such a situation occurs in the reaction of ΛΔ-$[Co(en)_2OH(H_2O)]^{2+}$ with amino acids at pH 6-7 (100). If however, the products mutarotate rapidly under experimental conditions, then product analysis will determine only their relative energy differences and will not provide information on possible kinetic selectivity. An example of this is found in the reaction of ΛΔ-$[Co(en)_2OH(H_2O)]^{2+}$ with (S)-sarcosine at pH 9 (63). It follows that in this type of chemistry these possibilities have to be examined before kinetic selectivity can be ascertained.

In the purely organic systems the selective formation of R-S (or S-R) dipeptides from S and R or racemic amino acid derivatives has been shown by several authors (120-123). It has been demonstrated recently (124) that the presence of an isotactic unit (S-S or R-R peptide) at the reaction centre increases selectivity when compared to a syndiotactic unit (R-S or S-R peptide). The selectivity was ascribed to differences in the dipeptide conformations (125).

There appear to be no reports in the literature on stereoselective formation of peptides promoted by metal ions. However, studies (126) on the formation of cobalt(III) dipeptide complexes, Reaction 5.1,

$$[Co(en)_2OH(H_2O)]^{2+} + NH_2CHR^1CONHCHR^2COO^- \longrightarrow \quad (5.1)$$



report that the Δ-configuration predominates at the cobalt(III) centre when the C-terminal residue is an (S)-amino acid. Likewise Λ-chirality predominates at the cobalt(III) centre when the C-terminal residue is an (R)-amino acid (126). No selectivity was observed when only the N-terminal amino acid of the peptide was asymmetric (127). In contrast, alkaline hydrolysis of pentaglycylglycine in the presence of Cu(II) and Ni(II) has been reported to give selective release of the C-terminal residue. Chelation protected the other four peptide bonds from hydrolysis (128).

This Chapter presents kinetic and product analysis studies of the formation of peptide complexes aimed at determining whether or not the reaction is stereoselective. Also, the reported stereoselective formation of Λ-$[Co(en)_2glu]^+$ from ΛΔ-$[Co(en)_2CO_3]^+$ at pH 6 (99) has been investigated.

Experimental

Spectrophotometric rates were followed on a Cary 16K spectrophotometer using a hand operated Teflon rapid-reaction mixer (see Chapter 4) to inject the mixed solution into a 1 cm Helma QS flow-through cell. Spectropolarimetric rate data and the rotations of the complexes were measured in a 1dcm cell using a Perkin-Elmer P22 polarimeter. For spectropolarimetric rate data the Teflon rapid reaction mixer was also used to inject the mixed solutions into the polarimeter cell.

Chromatographic separations of reaction products were made on Bio-Rad analytical Dowex 50W x2 (200-400 mesh, H^+ form) resin.

Pmr spectra were recorded on a Varian HA 100-MHz instrument using external TMS as a standard reference.

Ethyl (S)-Valinate

The ester was prepared and purified by the method used for ethyl glycinate and ethyl (S)-alaninate described in Chapter 4. Elemental analysis were not obtained. However, the pmr spectrum in D_2O integrated correctly for ethyl valinate.

Phosphorus Trichloride

Unilab reagent (min 98% PCl_3) was distilled over an atmosphere of dry nitrogen at 760mm. The fraction bp 74-74.5° was collected and stored in a sealed container.

Methanol

AR methanol (2 l) was stored over Linde molecular sieves (type 4A) for 2 days. It was then transferred to a distillation flask and magnesium turnings (10g, dried in an oven for 1 hr) and iodine (1g) were added. The mixture was gently warmed until reaction started. When all the magnesium and iodine had reacted the mixture was refluxed for 3 hr. The first 100 ml of the distillate was discarded. The methanol used for kinetics had bp 64.3-64.5 at 760mm and was stored over Linde molecular sieves (4A).

Dimethylsulphoxide

DMSO (Mallinchrodt, AR) was purified as described in Chapter 4.

$GlyOC_2H_5$ Solutions in Methanol

A freshly prepared stock solution of 0.5M Et_3N (AR) in methanol (Grignard dried) was titrated against aliquots of $[Co(en)_2(S)\text{-}alaOCH_3]Cl_3$ prepared *in situ* as described in Chapter 3 and diluted with water. Bromo-phenol blue was used as an indicator to detect the end point. The same Et_3N was also used to prepare $glyOC_2H_5$ solutions in methanol from

a stock solution of 0.1M $glyOC_2H_5$.HCl in methanol. The $glyOC_2H_5$ solution used for condensation with $[Co(en)_2(S)\text{-}alaOCH_3]Cl_3$ contained sufficient 0.5M Et_3N to neutralise the reaction mixture of the complex and to produce a specified concentration of $glyOC_2H_5$.

Resolution of cis-$[Co(en)_2Br(glyOC_3H_7)](ClO_4)_2$

Racemic cis-$[Co(en)_2Br(glyOC_3H_7)](ClO_4)_2$ (1.15g) in water (10 ml, pH ~4 (HOAc)) at 50° was treated with sodium arsenyl(R)-tartrate (NaAsO-(R)-tart) (0.10g). On standing at room temperature Δ-$[Co(en)_2Br(glyOC_3H_7)]$ $(AsO\text{-}R\text{-}tart)_2$ crystallised and this was collected, washed with ice cold water and air dried. On reduction of the solution volume and addition of more resolving agent (0.1g) another two crops of the same diastereoisomer crystallised. This procedure was repeated three more times. All fractions were combined and recrystallised to constant rotation (0.5g). A 0.1% solution at 25° in 0.01M $HClO_4$ gave α_{589} -0.081°, whence $[\alpha]_{589}$ -81°.

Anal. Calc. for $Co(C_9H_{27}N_5O_2Br)C_8H_{18}O_{14}As_2$: C, 24.77; H, 4.28; N, 8.49; Co, 7.15. Found: C, 24.3; H, 4.2; N, 8.4; Co, 7.2.

The diastereoisomer was converted to the bromide salt by trituration with excess NaBr in water (1 ml) and was recrystallised from hot dilute hydrobromic acid by addition of NaBr and cooling. The product was washed with methanol and air dried. A 0.1% solution at 25° in 0.01M $HClO_4$ gave α_{589} -0.110°, whence $[\alpha]_{589}$ -110°.

Anal. Calc. for $Co(C_9H_{27}N_5O_2Br)Br_2$: C, 20.15; H, 5.08; N, 13.07; Co, 10.99. Found: C, 20.2; H, 5.1; N, 13.1; Co, 11.1.

The filtrate remaining after removal of the diastereoisomer was reduced to 5 ml and on addition of excess NaBr three fractions of Λ-$[Co(en)_2Br(glyOC_3H_7)]Br_2$ crystallised. These were combined and recrystallised to constant rotation from hot 0.01M HBr by addition of NaBr and cooling. The product was washed with methanol and air dried. A 0.1% solution at 25° in 0.01M $HClO_4$ gave α_{589} +0.109° whence $[\alpha]_{589}$ +109°.

Anal. Calc. for $Co(C_9H_{27}N_5O_2Br)Br_2.H_2O$: C, 19.51; H, 5.27; N, 12.64; Co, 10.63. Found: C, 19.4; H, 5.2; N, 12.4; Co, 11.0.

ΛΔ-$[Co(en)_2glyOC_3H_7](ClO_4)_3$

The complex was prepared as described in Chapter 4. The complex was characterised by pmr spectroscopy and from the >95% recovery of the dipeptide ester complex $[Co(en)_2gly\text{-}glyOC_2H_5](ClO_4)_3$ from the reaction of $[Co(en)_2glyOC_3H_7](ClO_4)_3$ with $glyOC_2H_5$ in methanol.

ΛΔ-$[Co(en)_2$(RS)-ala$](OAc)_2$

ΛΔ-$[Co(en)_2$(RS)-ala$]I_2$ was prepared as described in Chapter 2 and was converted to the acetate salt as described in Chapter 3. No optical rotation was observed for a 0.2% solution at 546 nm and 450 nm. The pmr spectrum also confirmed that the complex was racemic at both asymmetric centres (2 sets of methyl absorptions of equal intensity at ∿1.75 ppm).

ΛΔ-$[Co(en)_2$(RS)-ala$OCH_3]Cl_3$, Λ$[Co(en)_2$(S)-ala$OCH_3]Cl_3$ and Δ-$[Co(en)_2$(S)-ala$OCH_3]Cl_3$

Racemic or optically pure $[Co(en)_2$(S)-ala$](OAc)_2$ (0.5g) in dry methanol (300 ml) was treated dropwise with PCl_3 (2 ml) and kept at 20° for 18 hr. The complexes were all reacted in situ with amino acid esters as described below.

Kinetic Measurements

(a) Condensation of Λ-and Δ-$[Co(en)_2$(S)-ala$OCH_3]Cl_3$ with $glyOC_2H_5$ in Methanol

Solutions of the chelated ester complex and $glyOC_2H_5$ in methanol were equilibrated at 25° in separate premixing chambers of the rapid-reaction mixer. After mixing, the change in rotation was followed at 480 nm using a 0.1° full scale chart expansion.

(b) Condensation of ΛΔ-$[Co(en)_2glyOC_3H_7](ClO_4)_3$ with (S)-alaOC_2H_5 and (S)-valOC_2H_5 in DMSO

The condensation of the chelated ester complex with either (S)-alaOC_2H_5

or (S)-valOC_2H_5 was followed spectrophotometrically at 450 nm. Solutions of the complex ($5x10^{-3}$M) and ester ($4x10^{-2}$M) were prepared in a dry box (N_2 atmosphere) and syringes of the rapid reaction mixer were also charged and loaded in the dry box. The reactor was then transferred to the spectrophotometer and reactants equilibrated at 25° before injection into the cell.

Product Analysis

All the peptide complexes were isolated as described in Chapter 3. The amount of peptide complex formed was determined by Co analyses and they were identified by pmr spectroscopy (Chapter 3).

Product Analysis at Various Times During the Condensation of $\Lambda\Delta$-$[Co(en)_2(RS)\text{-ala}OCH_3]Cl_3$ with $glyOC_2H_5$, (RS)-$alaOC_2H_5$ and (S)-$alaOC_2H_5$

The same procedure was used for each condensation reaction except that the reacting solutions were quenched at different times. Aliquots (5 ml) of $\Lambda\Delta$-$[Co(en)_2(RS)\text{-ala}OCH_3]Cl_3$ in methanol prepared as described above were diluted to 20 ml with distilled water and titrated against a stock solution of 0.5M Et_3N in methanol using bromo-phenol blue as indicator. The volume of Et_3N required to neutralise a known volume of the chelated ester solution was calculated.

1. Addition of $glyOC_2H_5$

A 50 ml aliquot of the chelated ester was rapidly treated with 50 ml of 0.02M $glyOC_2H_5$, neutralised after ca. 5 sec with HOAc and diluted to 500 ml with water. The complexes were sorbed on a H^+ form resin and eluted with 1.5M HCl. The eluate of the peptide complex was collected, made 3N in HCl, left for 6 hr and then reduced to dryness. The sample was dissolved in D_2O and a pmr spectrum of the methyl protons of alanine recorded using a 500 Hz sweep width expansion.

The same procedure was repeated for samples quenched after 10 sec, 22 sec and 3 min.

2. Addition of (RS)-alaOC$_2$H$_5$ and (S)-alaOC$_2$H$_5$-

The same procedure as in (1) was used except that aliquots of the chelated ester were quenched after 20 sec, 60 sec and 3 min. The pmr spectrum was recorded for both sets of methyl absorptions.

Mutarotation of [Co(en)$_2$ala-alaOH]Cl$_3$ Synthesised from ΛΔ-[Co(en)$_2$(RS)-alaOCH$_3$]Cl$_3$ and (S)-alaOC$_2$H$_5$-

The complex (0.1g) in glycine buffer (20 ml, pH 10.4, μ = 1 ($NaClO_4$)) was kept at 20° for 50 min and then neutralised with HOAc and diluted to 100 ml with H_2O. The complexes were sorbed on a H^+ form resin and eluted with 1.5M HCl. The eluates of the peptide complex and $[Co(en)_2ala]^{2+}$ were collected and their relative amounts estimated by Co analyses; $[Co(en)_2ala\text{-}alaOH]^{3+}$ 55%, $[Co(en)_2ala]^{2+}$ 45%. The pmr absorptions due to the alanine signals were recorded on a 500Hz sweep width expansion.

Product Analysis During the Condensation of ΛΔ-[Co(en)$_2$glyOC$_3$H$_7$](ClO$_4$)$_3$- with (S)-alaOEt in DMSO

A solution of $[Co(en)_2glyOC_3H_7](ClO_4)_3$ (0.1g in 50 ml DMSO) was treated rapidly with 50 ml 0.04M (S)-alaOC$_2$H$_5$ in DMSO and quenched after 20 sec with water (50 ml). The solution was acidified with HOAc to pH ~4 and the complexes sorbed on a H^+ form resin and eluted with 2N HCl. The eluates of the peptide complex and $[Co(en)_2gly]^{2+}$ were collected. The eluate of the peptide complex was reduced to dryness after 10 hr and was then dissolved in water (50 ml). Visible spectra and the optical rotations at 546 nm and 589 nm for both complexes were recorded. The same procedure was repeated for samples quenched with water after 40 sec and 5 min.

Product Analysis at Various Times for the Reaction Between ΛΔ-[Co(en)$_2$CO$_3$]ClO$_4$ and (S)-Glutamic Acid

1. The procedure described by Gillard (99) was used. ΛΔ-$[Co(en)_2CO_3]ClO_4$ (6.7g) in water (400 ml) was treated with (S)-glutamic

acid (2.9g) and the mixture was then refluxed. After 15 min a 50 ml aliquot was withdrawn from the reaction mixture, diluted to 200 ml and the complexes were sorbed on a H^+ form resin and eluted with 1N HCl. The eluate of the band identified as $[Co(en)_2(S)\text{-gluH}]^{2+}$ (band 4) was collected and reduced to dryness several times to remove HCl. The pmr and visible spectra of the product was identified as $[Co(en)_2(S)\text{-gluH}]Cl_2$ by comparison of its visible and pmr spectra and chromatographic behaviour with that of an authentic sample (Chapter 2). The optical rotations for a 0.1% solution were recorded at 546 and 589 nm.

The above procedure was repeated for samples removed from the reaction mixture after 30 min, 65 min, 2 hr, 4 hr, 8 hr and 16 hr.

2. ΛΔ-$[Co(en)_2CO_3]ClO_4$ (6.7g) in water (100 ml) was treated with (S)-glutamic acid (2.9g) and the solution taken to dryness. The product at this stage was a red gum and this was taken up in water (50 ml) and a 2 ml aliquot removed and diluted to 100 ml. The complexes were sorbed on H^+ form resin and eluted with 1N HCl. $[Co(en)_2gluH]^{2+}$ was identified as above and the optical rotations of a 0.1% solution were recorded at 546 and 589 nm. The evaporation of the reaction mixture was continued and three more aliquots were removed and treated as in (1). After four evaporations an orange gum remained and this was taken up in water (100 ml). The solution was then evaporated at 25° and Λ-$[Co(en)_2(S)\text{-glu }]ClO_4$ crystallised and was removed. Two more fractions of the same diastereoisomer crystallised and were combined and recrystallised to constant rotation. $[\alpha]_D$ = +461° and $[\alpha]_{450}$ = +903°. Elemental analyses are given in Chapter 2. It was not possible to obtain the pure ΔS isomer from the reaction mixture.

Results

Polarimetric rate data for the formation of $[Co(en)_2ala\text{-}glyOC_2H_5]^{3+}$ in methanol were collected at 480 nm and for a 10^{-3}M solution of the complex a rotation change of the order 0.060° - 0.100° was observed.

The rate constants (Table 5.1) for the condensation reaction were obtained from plots of log $(\alpha^\circ_\infty - \alpha_t{}^\circ)$ against time which were linear for at least 3 half-lives. At least 4 runs were made for reactions with $t_{\frac{1}{2}}<5$ sec and reproducibility was better than ±10%. For condensation reactions with $t_{\frac{1}{2}}>5$ sec at least 3 runs were made and rates were reproducible to better than ±5%. For the formation of Λ-$[Co(en)_2(S)\text{-ala-gly}OC_2H_5]^{3+}$, the observed rates fit the rate law

$$v = k[\text{complex}][\text{gly}OC_2H_5]$$

and the second order rate constants are given in Table 5.1. The deviation from this rate law for the formation of the ΔS complex is difficult to explain, Table 5.1. However, at low glycine ethyl ester concentrations (<0.05M) the reaction rate could possibly be influenced by the large quantities of Et_3N present relative to $\text{gly}OC_2H_5$. At the lowest concentration of $\text{gly}OC_2H_5$, ca. 95% of the total amount of Et_3N added was required to neutralise the reaction mixture of the chelated ester, and only 5% to produce $\text{gly}OC_2H_5$.

Pmr spectra of the methyl proton absorptions in the peptide complexes isolated from the reaction of $\Lambda\Delta$-$[Co(en)_2(S)\text{-ala}OCH_3]^{3+}$ and $\text{gly}OC_2H_5$ in methanol quenched at different times are shown in Fig. 5.1. Chemical shifts (100 MHz) for the $[Co(en)_2(S)\text{-ala-gly}O]^{2+}$ diastereoisomers are summarised in Table 6.2, and show a separation of ca. 4 cycles. Figure 5.1 shows that when $\Lambda\Delta$-$[Co(en)_2(RS)\text{-ala}OCH_3]^{3+}$ is reacted with $\text{gly}OC_2H_5$ the methyl doublet at 2.21 ppm is more predominant than the doublet centred at 2.17 ppm in all spectra except that recorded for the complete reaction. In the latter case the methyl absorptions have the same intensity. The doublet centred at 2.21 ppm corresponds to that for Λ-$[Co(en)_2(S)\text{-ala-gly}O]^{2+}$ and Δ-$[Co(en)_2(R)\text{-ala-gly}O]^{2+}$ based on the assignment of the methyl doublet in an authentic sample of Λ-$[Co(en)_2(S)\text{-ala-gly}O]^{2+}$, Table 6.3. Similarly, the doublet at 2.17 ppm

TABLE 5.1

Spectropolarimetric Rate Data for the Condensation of Λ-and Δ-$[Co(en)_2(S)\text{-}alaOCH_3]Cl_3$ with $glyOC_2H_5$ in Methanol (25°, 480 nm, [Complex] $\sim 10^{-3}$M)

$10^2[glyOC_2H_5]$	$10\ k_{obs}\ sec^{-1}$	$\frac{k_{obs}}{[glyOC_2H_5]}\ M^{-1}sec^{-1}$
Λ-$[Co(en)_2(S)\text{-}alaOCH_3]^{3+}$		
4.5	4.6	10.1 ± 1.0
3.4	3.5	10.1 ± 1.0
3.0	2.8	9.8 ± 1.0
2.6	2.9	11.0 ± 1.0
2.2	2.4	10.7 ± 0.8
1.7	2.0	11.9 ± 0.5
1.2	1.4	11.3 ± 0.5
Δ-$[Co(en)_2(S)\text{-}alaOCH_3]^{3+}$		
7.5	3.8	5.0 ± 0.5
6.0	2.8	4.7 ± 0.5
5.0	2.4	4.7 ± 0.5
5.2	2.4	4.6 ± 0.5
4.5	2.2	4.8 ± 0.5
4.0	1.9	4.8 ± 0.5
3.0	1.2	4.0 ± 0.3
2.7	1.0	3.7 ± 0.1
2.5	0.82	3.3 ± 0.1
2.0	0.61	3.5 ± 0.1
1.4	0.31	2.3 ± 0.1
1.0	0.25	2.5 ± 0.1

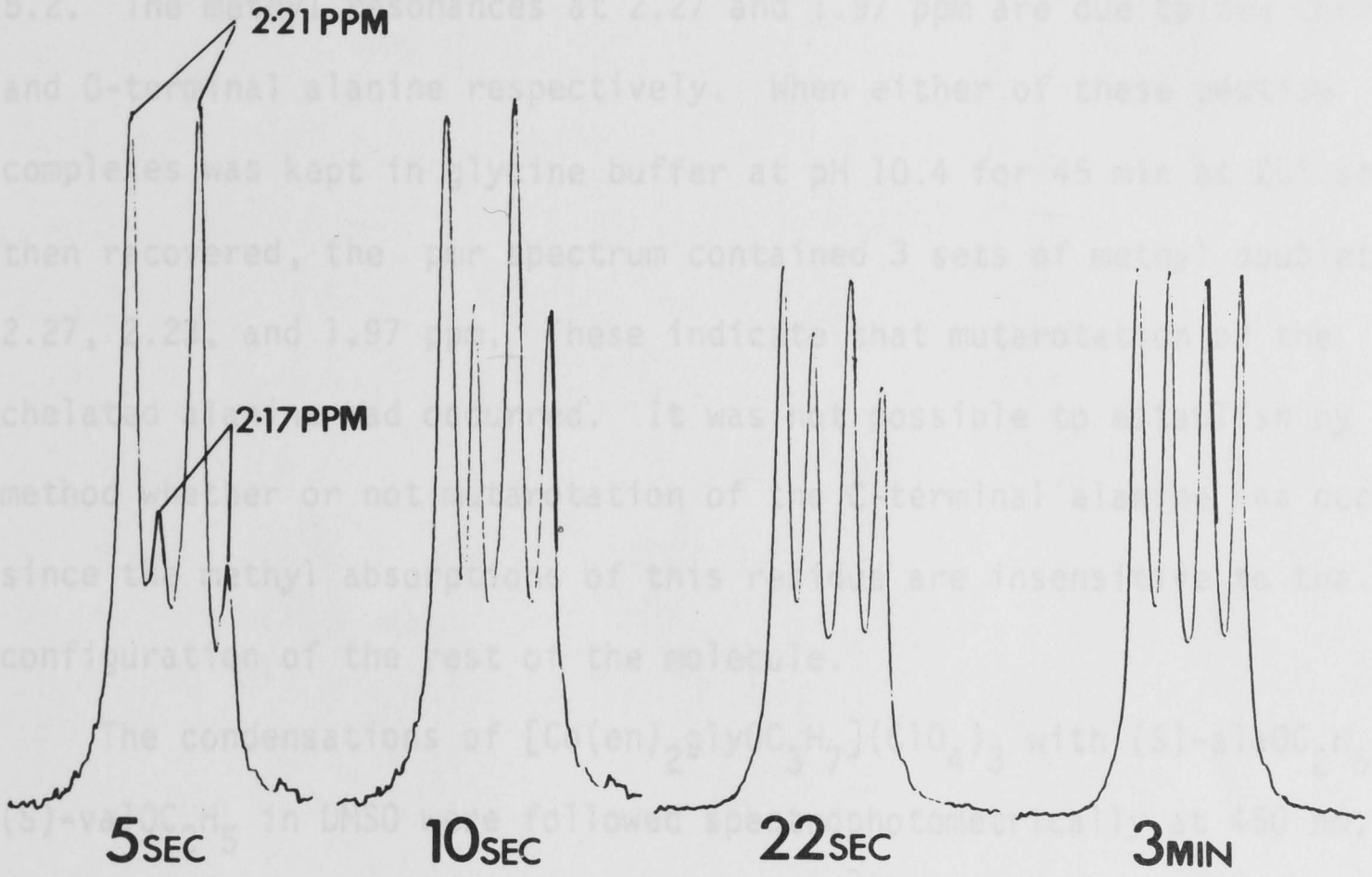


FIGURE 5.1 PMR SPECTRA (100 MHz, 500 Hz SWEEP WIDTH) IN D_2O OF THE METHYL PROTON ABSORPTIONS IN THE PEPTIDE COMPLEXES ISOLATED FROM THE REACTION OF $\Lambda\Delta$-$[Co(en)_2(RS)\text{-}alaOCH_3]Cl_3$ AND $GLYOC_2H_5$(0.02M) IN METHANOL AT DIFFERENT TIMES. DOUBLET AT 2.21 ppm (7 cps) CORRESPONDS TO THE ΛS + ΔR PEPTIDES AND DOUBLET AT 2.17 ppm (7 cps) CORRESPONDS TO THE ΛS + ΛR PEPTIDES (EXTERNAL REFERENCE TMS).

is due to the formation of Δ-$[Co(en)_2(S)\text{-}ala\text{-}glyO]^{2+}$ and Λ-$[Co(en)_2(R)\text{-}ala\text{-}glyO]^{2+}$.

The pmr spectra for the $[Co(en)_2ala\text{-}alaO]^{2+}$ ion isolated following the reaction of $\Lambda\Delta$-$[Co(en)_2(RS)\text{-}alaOCH_3]^{3+}$ with either (RS)- or (S)-$alaOC_2H_5$ for various lengths of time gave identical results, Figure 5.2. The methyl resonances at 2.27 and 1.97 ppm are due to the chelated and O-terminal alanine respectively. When either of these peptide complexes was kept in glycine buffer at pH 10.4 for 45 min at 25° and then recovered, the pmr spectrum contained 3 sets of methyl doublets at 2.27, 2.23, and 1.97 ppm. These indicate that mutarotation of the chelated alanine had occurred. It was not possible to establish by this method whether or not mutarotation of the C-terminal alanine had occurred since the methyl absorptions of this residue are insensitive to the configuration of the rest of the molecule.

The condensations of $[Co(en)_2glyOC_3H_7](ClO_4)_3$ with (S)-$alaOC_2H_5$ and (S)-$valOC_2H_5$ in DMSO were followed spectrophotometrically at 450 nm. For the formation of $[Co(en)_2gly\text{-}valOC_2H_5]^{3+}$ two rate processes similar to those described in Chapter 3 for the formation of $[Co(en)_2gly\text{-}glyOC_2H_5]^{3+}$ and $[Co(en)_2gly\text{-}(S)\text{-}alaOC_2H_5]^{3+}$ were observed. For all reactions, plots of log $(D_\alpha - D_t)$ against time for both processes were linear for at least four half lives, and at least two runs were carried out under each set of conditions with similar results. The observed first order rate constants are listed in Table 5.2.

Optical rotations of the products isolated from the reaction of $\Lambda\Delta$-$[Co(en)_2glyOC_3H_7]^{3+}$ with (S)-$alaOC_2H_5$ in DMSO quenched at different times are listed in Table 5.3. These results are consistent with the kinetic data and demonstrate that Λ-$[Co(en)_2gly\text{-}(S)\text{-}alaOC_2H_5]^{3+}$ forms more rapidly than Δ-$[Co(en)_2gly\text{-}(S)\text{-}alaOC_2H_5]^{3+}$.

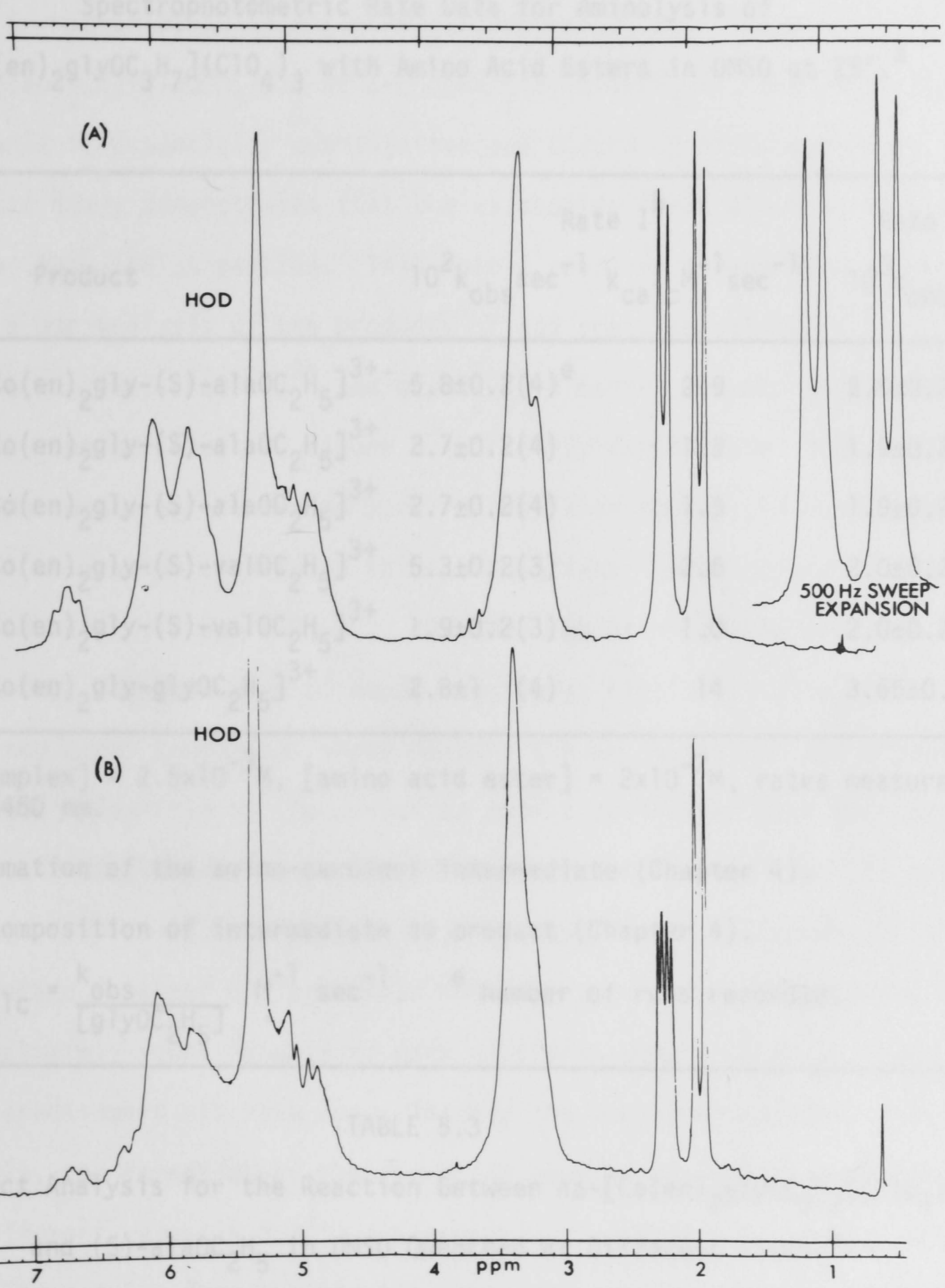


FIGURE 5.2 PMR SPECTRA (100 MHz) IN D_2O OF $[Co(en)_2ala\text{-}(S)\text{-}alaO]Cl_2$:
(A) SPECTRUM OF THE PRODUCT ISOLATED FROM THE REACTION MIXTURE OF $\Lambda\Delta\text{-}[Co(en)_2(RS)\text{-}alaOCH_3]Cl_3$ AND $(S)\text{-}alaOC_2H_5$ IN METHANOL:
(B) PEPTIDE PRODUCT (A) ISOLATED AFTER BEING KEPT IN GLYCINE BUFFER pH 10.5 FOR 45 MIN.

TABLE 5.2

Spectrophotometric Rate Data for Aminolysis of $[Co(en)_2glyOC_3H_7](ClO_4)_3$ with Amino Acid Esters in DMSO at 25°.[a]

Product	Rate I[b] $10^2 k_{obs}$ sec^{-1}	k_{calc} $M^{-1}sec^{-1}$	Rate II[c] $10^3 k_{obs}$ sec^{-1}
Λ-$[Co(en)_2gly$-(S)-$alaOC_2H_5]^{3+}$	5.8±0.2(4)[e]	2.9	2.0±0.2(4)[e]
Δ-$[Co(en)_2gly$-(S)-$alaOC_2H_5]^{3+}$	2.7±0.2(4)	1.3	1.9±0.2(2)
$\Lambda\Delta$-$[Co(en)_2gly$-(S)-$alaOC_2H_5]^{3+}$	2.7±0.2(4)	1.3	1.9±0.2(6)
Λ-$[Co(en)_2gly$-(S)-$valOC_2H_5]^{3+}$	5.3±0.2(3)	2.6	2.0±0.2(3)
$\Lambda\Delta$-$[Co(en)_2gly$-(S)-$valOC_2H_5]^{3+}$	1.9±0.2(3)	1.0	2.0±0.2(2)
$\Lambda\Delta$-$[Co(en)_2gly$-$glyOC_2H_5]^{3+}$	2.8±1 (4)	14	3.65±0.10(4)

[a] [Complex] = 2.5×10^{-3}M, [amino acid ester] = 2×10^{-2}M, rates measured at 450 nm.

[b] Formation of the amino-carbinol intermediate (Chapter 4).

[c] Decomposition of intermediate to product (Chapter 4).

[d] $k_{calc} = \frac{k_{obs}}{[glyOC_2H_5]}$ M^{-1} sec^{-1}. [e] Number of runs recorded.

TABLE 5.3

Product Analysis for the Reaction Between $\Lambda\Delta$-$[Co(en)_2glyOC_3H_7](ClO_4)_3$ and (S)-$alaOC_2H_5$ in DMSO Quenched at Different Times.[a]

t sec	$[Co(en)_2gly$-(S)-alaO$]^{2+}$ α^{b}_{546}	$[Co(en)_2gly]^{2+}$ α^{b}_{546}
10	+0.089	-0.043
40	+0.050	-0.023
300	+0.015	-0.010

[a] [Complex] ~5×10^{-3}M, [$alaOC_2H_5$] = 2×10^{-2}M, $t_{1/2}$ for fastest reaction is ca. 12 sec.

[b] Rotation measured in 1 dcm cell, values are normalised for solutions in H_2O at 25° with an O.D. = 0.5 at 487 nm in a 1 cm cell.

Discussion

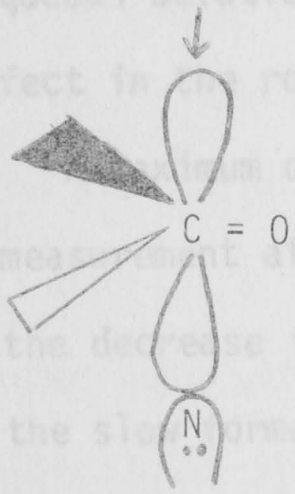
Formation of Λ- or Δ-$[Co(en)_2(S)\text{-}ala\text{-}glyOC_2H_5]^{3+}$ from Λ-$[Co(en)_2(S)\text{-}alaOCH_3]Cl_3$ or Δ-$[Co(en)_2(S)\text{-}alaOCH_3]Cl_3$ and $glyOC_2H_5$ in methanol is essentially quantitative and occurs at different rates. The kinetic study demonstrates that the ΛS peptide forms about three times faster than the ΔS peptide. This result receives independent support from a pmr analysis of the products of the reaction between ΛΔ-$[Co(en)_2(RS)\text{-}alaOCH_3]^{3+}$ and $glyOC_2H_5$ in methanol quenched at different times. The pmr spectra, Figure 5.1, clearly demonstrate that the ΛS, ΔR peptide complexes are formed more rapidly than the ΔS, ΛR complexes. The methyl absorption of alanine in each enantiomeric set occurs at 2.21 ppm (ΛS, ΔR) and 2.17 ppm (ΛR, ΔS). At the completion of the reaction the two methyl doublets were of equal magnitude, Fig. 5.1. This result was obtained under conditions where neither the reactants nor the dipeptide products mutarotate at the carbon or cobalt centres, so that the observed product distribution at the end of the reaction is required. In glycine buffers (pH 8.6-10.5) Λ-$[Co(en)_2(S)\text{-}ala\text{-}glyO]^{2+}$ and Δ-$[Co(en)_2(S)\text{-}ala\text{-}glyO]^{2+}$ mutarotate (Chapter 6), and their equilibrium mixtures also consist of approximately equal amounts of each diastereoisomer. That is, the diastereoisomers all have approximately the same thermodynamic stability. Clearly, the formation of the dipeptide complex $[Co(en)_2ala\text{-}glyOC_2H_5]^{3+}$ at different rates is due to a stereoselective effect of kinetic origin

The results from the kinetic study and product analysis (Table 5.2 and 5.3) for the condensation in DMSO of Λ- and Δ-$[Co(en)_2glyOC_3H_7]^{3+}$ with $(S)\text{-}alaOC_2H_5$ and $(S)\text{-}valOC_2H_5$ respectively reveal two pertinent discoveries concerning properties of the Co(III) moiety in regard to its role in peptide formation. Firstly, the different chiralities of the Co(III) moieties give rise to the selective formation of peptides. For the formation of the gly-(S)-ala and gly-(S)-val complexes, the ΛS complexes were formed about three times more rapidly in both instances.

Secondly, Table 5.2 demonstrates that differences in rate only occur during the addition of the (S)-amino acid to the chelated ester moiety and not in the decay of the tetrahedral intermediate to produce the peptide. That is, the stereoselectivity arises when the (S)-amino acid ester attacks the carbonyl centre of the chelated amino acid ester to form the tetrahedral intermediate.

Results in Table 5.1 and 5.2 both indicate that the ΛS peptide complexes are formed more rapidly. These results suggest that a larger stereoselective effect may be observed if both amino acid centres of the peptide are optically active and this was demonstrated for the formation of $[Co(en)_2ala\text{-}alaOC_2H_5]^{3+}$. Product analysis by pmr of the reaction mixture containing ΛΔ-$[Co(en)_2(RS)\text{-}alaOCH_3]Cl_3$ and (S)-$alaOC_2H_5$ demonstrated that Λ-$[Co(en)_2(S)\text{-}alaO\text{-}(S)\text{-}alaOC_2H_5]^{3+}$ is formed about six times faster than Δ-$[Co(en)_2(S)\text{-}ala\text{-}(S)\text{-}alaOC_2H_5]^{3+}$.

It remains to discuss the factors which may cause this stereoselectivity. Nucleophilic attack at the carbonyl carbon is most facile in the direction perpendicular to the trigonal plane, i.e. at the carbon p-orbital. When this occurs maximum overlap of the filled sp^3 orbital of the nucleophile with the carbon p-orbital is possible (5.3). Attack



(5.3)

at the carbonyl centre of $[Co(en)_2glyOR]^{3+}$ by either $alaOC_2H_5$ or $valOC_2H_5$ results in the stereoselective formation of the diastereoisomers presumably due to non-bonded interactions between the hydrogen atoms of the adjacent ethylenediamine chelate and the incoming amino acid. Such interactions were not apparent from an examination of Drieding models of Λ- and Δ-$[Co(en)_2gly\text{-}(S)\text{-}alaOC_2H_5]^{3+}$ and Λ- and

Δ-$[Co(en)_2gly$-(S)-$valOC_2H_5]^{3+}$. The inability to observe such interaction in these models was not altogether unexpected because the differences in rates of condensation do not represent very large energy differences. For the formation of $[Co(en)_2ala$-$glyOC_2H_5]^{3+}$ attack at the carbonyl centre of the chelate by $glyOC_2H_5$ may be hindered by interactions between the ethylenediamine chelate and the methyl group of the chelated ester. The more pronounced rate differences for the formation of diastereoisomers of $[Co(en)_2ala$-$alaOC_2H_5]^{3+}$ suggest that interaction between the chelated alanine and ethylenediamine and the incoming amino acid residue act cooperatively.

Kinetic stereoselective formation of Λ-$[Co(en)_2$(S)-glu$]^+$ and Δ-$[Co(en)_2$(R)-tartrate$]^+$ from racemic carbonato-bis(ethylenediamine) cobalt(III) ion and (S)-glutamic and (R)-tartaric acid has been reported (99). The authors report that the reaction between $\Lambda\Delta$-$[Co(en)_2CO_3]^+$ and (S)-glutamic acid in H_2O produces as the first product Λ-$[Co(en)_2$(S)-glu$]^+$ which shows a strong Cotton effect for its d-d band at ca. 490 nm. Product analysis, for this reaction carried out in boiling aqueous solution, was done by measuring the amplitude of the Cotton effect in the rotary dispersion spectra at 491 nm at different times. A maximum optical activity was reached after 2 hr and one further measurement after 12 hr indicated no activity. It was stated that the decrease in optical activity after a maximum at 2 hr was due to the slow formation of the Δ-$[Co(en)_2$(S)-glu$]^+$. A crystal structure of Λ-$[Co(en)_2$(S)-glu$]ClO_4$ indicated the presence of hydrogen bonded interactions between the γ-carboxyl group of the chelated glutamate and hydrogen atoms of the adjacent ethylenediamine

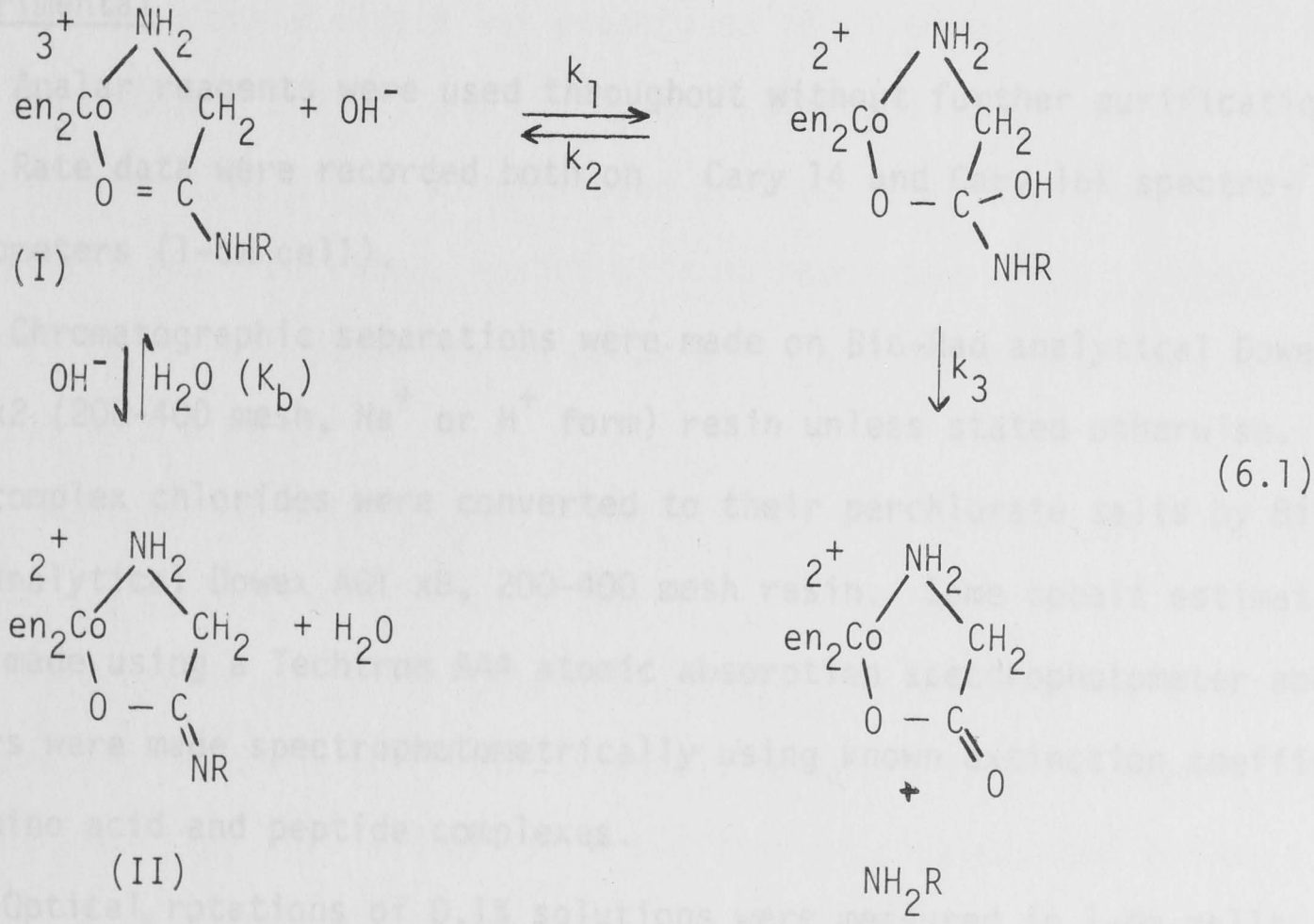
ring (129) and it was suggested that the kinetic stereoselectivity arose from this favourable interaction. Gillard's preparation(s) (99) of $[Co(en)_2(S)\text{-glu}]^+$ has now been repeated several times. Preferential crystallisation of Λ-$[Co(en)_2(S)\text{-glu}]ClO_4$ occurred and has been previously reported (99). The products of the reaction mixture were also sorbed on H^+ form resin and eluted with 1M HCl. Almost complete separation of the ΛS and ΔS glutamato complexes was obtained and the ΛS glutamato complex of the same optical purity as previously reported (99) was eluted preferentially and crystallised. Complete separation of the diastereoisomers was not possible but it was estimated by cobalt analysis that the ratio of ΛS:ΔS was ca. 1:1. Yields from all preparations gave less than 60% $[Co(en)_2(S)\text{-glu}]^+$ and at least four species were present in the reaction mixture. The experiments described by Gillard (99) to establish the kinetic stereoselectivity were repeated but in this instance the $[Co(en)_2(S)\text{-gluH}]^{2+}$ was separated from the other reaction mixture products by ion exchange chromatography. $[Co(en)_2(S)\text{-glu}]Cl_2$ isolated from the reaction mixture after 15 min, 30 min, 65 min, 2 hr, 4 hr and 16 hr did not have any appreciable activity for 0.1% solution at 546 nm and 589 nm. Considerable disproportionation occurred for the above reaction similar to that described for $[Co(en)_2(S)\text{-pro}]^{2+}$ ion (Chapter 2).

The above experiments demonstrate that the stereoselective formation of Λ-$[Co(en)_2(S)\text{-glu}]^+$ does not occur.

CHAPTER 6

HYDROLYSIS, MUTAROTATION AND PROTON EXCHANGE STUDIES OF ALANINE PEPTIDE COMPLEXES

Rate enhancements of ca. 10^4-10^6 for hydrolysis of N-O chelated glycine amide complexes of the type $[CoN_4glyNR_1R_2]^{3+}$ (I) over uncoordinated substrates have been reported (21). In this study it was shown that attack of hydroxide ion occurred at the carbonyl carbon of the chelated amide and the results required that the conjugate base (II) be unreactive, Reaction 6.1. In Chapter 3, N-O chelated



(S)-ala-glyO, (S)-ala-glyOC_3H_7, gly-(S)-alaO and (S)-ala-(S)-alaO complexes were prepared and it was considered desirable to determine if similar rate enhancements occurred for peptide cleavage in these complexes. In this Chapter the rates of hydrolysis for the above alanine peptide complexes are reported and also the possibility of stereoselective bond cleavage is investigated. The previous Chapter reports their stereoselective formation.

The extent of racemisation at the asymmetric carbon centres of amino acids in chelated dipeptide complexes is not known. However, labilisation of the α-CHR proton in chelated amino acid complexes has been observed (65,130) and studies of the hydrolysis of N-O chelated gly-gly complexes (21) indicated that racemisation of the asymmetric centres in the chelate will compete with peptide hydrolysis. A kinetic study aimed at determining what relationship exists between the α-proton exchange and mutarotation in the $[Co(en)_2(S)\text{-ala-gly}]^{2+}$ ion as well as the extent of racemisation of (S)-alanine in $[Co(en)_2\text{gly-(S)-alaO}]^{2+}$ and $[Co(en)_2(S)\text{-ala-glyO}]^{2+}$ during peptide hydrolysis is reported.

Experimental

Analar reagents were used throughout without further purification.

Rate data were recorded both on Cary 14 and Cary 16K spectrophotometers (1-cm cell).

Chromatographic separations were made on Bio-Rad analytical Dowex 50W x2 (200-400 mesh, Na^+ or H^+ form) resin unless stated otherwise. The complex chlorides were converted to their perchlorate salts by Bio-Rad analytical Dowex AG1 x8, 200-400 mesh resin. Some cobalt estimations were made using a Techtron AA4 atomic absorption spectrophotometer and others were made spectrophotometrically using known extinction coefficients of amino acid and peptide complexes.

Optical rotations of 0.1% solutions were measured in 1-dm cells with a Perkin-Elmer P22 polarimeter.

Proton magnetic resonance spectra were recorded on both a Varian HA 100-MHz instrument using external tetramethylsilane (TMS) as a standard reference and a Jeol C-60HL 60 MHz instrument using sodium trimethylsilylpropanesulphonate (NaTPS) as an internal standard reference.

pH measurements were made using a Radiometer pH meter incorporating a pH A630T scale expander. For the Na_2CO_3:$NaHCO_3$ buffers in D_2O, the

pH was measured with a glass electrode and the pD evaluated from the empirical expression pD = pH + 0.4 (131).

ΛΔ-$[Co(en)_2(S)\text{-ala-glyO}](ClO_4)_2$

$[Co(en)_2(S)\text{-ala}](OAc)_2$ (2g) in anhydrous methanol (400 ml) was treated dropwise with PCl_3 (4 ml) over 15 min and kept at 20° for 18 hr. The mixture was treated with $glyOC_2H_5$ (4g), neutralised after 30 sec with HOAc and diluted to 500 ml with water. The complexes were sorbed on a H^+ form resin and eluted with 2M HCl. The eluate of the peptide complex was collected, neutralised to pH 6.5 with 0.5M LiOH and was converted to the perchlorate salt using an anion form resin. The complex perchlorate eluate was evaporated to dryness, dissolved in 50% aqueous methanol (20 ml) and $LiClO_4$ was added whereupon $[Co(en)_2ala\text{-glyO}](ClO_4)_2$ crystallised slowly. The complex was recrystallised from water, washed with methanol and dried in vacuo over P_2O_5 (1.5g). Absorption maxima occurred at 487 nm (ε 115) and 345 nm (ε 130) in H_2O at 25°.

Anal. Calcd. for $Co(C_{10}H_{25}N_6O_3)(ClO_4)_2$: C, 20.64; H, 4.81; N, 16.06. Found: C, 20.8; H, 4.7; N, 15.8.

Alternatively, $[Co(en)_2ala\text{-glyO}](ClO_4)_2$ was prepared by treating the reaction mixture of $[Co(en)(S)\text{-ala}](OAc)_2$ and PCl_3 in methanol with $glyOC_2H_5HCl$ (2g) followed by rapid addition of a calculated volume of 0.5M Et_3N in methanol such that the reaction mixture was 0.5M in $glyOC_2H_5$. After 30 sec the mixture was neutralised with HOAc and the peptide complex was then isolated as described above.

Isolation of Λ-$[Co(en)_2(S)\text{-ala-glyO}](ClO_4)_2$ from ΛΔ-$[Co(en)_2(S)\text{-ala-glyO}](ClO_4)_2$

The racemate (3g) in 50% aqueous ethanol (50 ml) was treated with $LiClO_4$ (2g) and crystallisation of Λ-$[Co(en)_2(S)\text{-ala-glyO}](ClO_4)_2$ was induced by scratching the vessel's walls with a glass rod. The 4 fractions

collected were further fractionated. Fractions with the same sign were then combined and recrystallised to a constant rotation (0.6g); $[\alpha]_{546}$ +635° and $[\alpha]_D$ +331° in water at 25°.

Anal. Calcd. for $Co(C_9H_{25}N_6O_3)(ClO_4)_2$: C, 20.64; H, 4.81; N, 16.06; Co, 11.26. Found: C, 20.3; H, 4.9; N, 16.0; Co, 11.1.

The very soluble salt Δ-$[Co(en)_2(S)\text{-ala-glyO}](ClO_4)_2$ was not isolated even from solvent mixtures by this method. Partial separation of diastereoisomers was achieved chromatographically on Sephadex G-100 resin. The complex (0.1g) in water (1 ml) was sorbed on the resin and eluted slowly with water (∿1 ml/½ hr). The initial fractions representing ∿10% total cobalt contained the ΛS isomer. The maximum specific rotation was $[\alpha]_{546}$ +629° in water at 25°.

Δ-$[Co(en)_2(S)\text{-ala-glyO}](ClO_4)_2$

The complex was prepared and isolated as described for ΛΔ-$[Co(en)_2(S)\text{-ala-glyO}](ClO_4)_2$. Yield 1.5g. Specific rotations in water at 25° were $[\alpha]_{546}$ -690° and $[\alpha]_D$ -356°.

Anal. Calcd. for $Co(C_{10}H_{25}N_6O_3)(ClO_4)_2$: C, 20.64; H, 4.81; N, 16.06; Co, 11.26. Found: C, 20.5; H, 4.5; N, 17.1; Co, 11.3.

Λ-$[Co(en)_2(S)\text{-ala-glyO}](ClO_4)_2$

The complex was also prepared as above. Specific rotations in water were $[\alpha]_{546}$ +638° and $[\alpha]_D$ +330°.

Anal. Found for $Co(C_9H_{25}N_6O_3)(ClO_4)_2$: C, 21.0; H, 4.8; N, 17.0; Co, 11.0.

ΛΔ-$[Co(en)_2(S)\text{-ala-glyOCH}(CH_3)_2](ClO_4)_3$

$[Co(en)_2alaOCH_3]Cl_3$ was prepared using the PCl_3 method as described above. The chelated alanine ester solution was treated rapidly with 1M $glyOCH(CH_3)_2$ in methanol to make the reaction mixture 0.1M in ester. After 30 sec the mixture was neutralised with HOAc, diluted with 200 ml

distilled water and the complexes were sorbed on SE Sephadex C-25 Na^+ form resin and eluted with 0.1M $KClO_4$. The eluate of the tripositive band was collected and was evaporated to near dryness. The product was diluted with methanol and treated with excess ether to precipitate the complex. This was collected, precipitated twice more with ether as above, collected and then dried in vacuo over P_2O_5. It was not possible to recrystallise the complex and satisfactory elemental analyses were not obtained. However, pmr spectra in D_2O integrated correctly for $[Co(en)_2ala\text{-}glyOCH(CH_3)_2](ClO_4)_3$, Fig. 6.2. Absorption maxima occurred at 487 nm (ε 120) and 345 nm (ε 150) in 1.0M $NaClO_4$ at 25°.

Λ-$[Co(en)_2gly\text{-}(S)\text{-}alaO](ClO_4)_2$

$[Co(en)_2glyOCH_3]Cl_3$ was prepared using the PCl_3 method. A solution of the complex was treated with $alaOC_2H_5$.HCl followed by rapid addition of a calculated volume of 0.5M Et_3N in methanol with stirring such that the reaction mixture was 0.1M in $alaOC_2H_5$. After 30 sec the mixture was neutralised with HOAc, diluted to 500 ml with water, the complexes were sorbed on a H^+ form resin and eluted with 1.5M HCl. The eluate of the peptide complex was collected and after 8 hr was evaporated to dryness. The complex was dissolved in 50% aqueous methanol, neutralised with 0.5M LiOH to pH 6.5 and was converted to the perchlorate salt by passage through an anion form resin. Λ-$[Co(en)_2gly\text{-}(S)\text{-}alaO](ClO_4)_2$ was then isolated as described above for ΛΔ-$[Co(en)_2(S)\text{-}ala\text{-}glyO](ClO_4)_2$. The specific rotations were $[\alpha]_{546}$ +611° and $[\alpha]_D$ +324° in water at 25°. Absorption mixima occurred at 487 nm (ε 116) and 345 nm (ε 135) in H_2O at 25°. These values were the same in 1M $NaClO_4$.

Anal. Calcd. for $Co(C_9H_{25}N_6O_3)(ClO_4)_2$: C, 20.64; H, 4.81; N, 16.06.
Found: C, 21.1; H, 5.0; N, 16.0.

Δ-$[Co(en)_2gly\text{-}(S)\text{-}alaO](ClO_4)_2$

This complex was prepared as described for Λ-$[Co(en)_2gly\text{-}(S)\text{-}alaO](ClO_4)_2$

$[\alpha]_{546}$ -673° and $[\alpha]_D$ -330° in water at 25°.

Anal. Found for $Co(C_9H_{25}N_6O_3)(ClO_4)_2$: C, 21.2; H, 4.9; N, 16.3.

ΛΔ-$[Co(en)_2gly\text{-}(S)\text{-}alaO](ClO_4)_2$

Racemate $[Co(en)_2glyOCH_3](ClO_4)_3$ (1.0g) in Grignard dried methanol (50 ml) was treated with (S)-$alaOC_2H_5$ (0.5g). After 10 sec the mixture was neutralised with 10.6M HCl. The complex was then isolated as described above. Absorption maxima occurred at 487 nm (ε 116) and 345 nm (ε 135) in H_2O at 25°.

Anal. Found for $Co(C_9H_{25}N_6O_3)(ClO_4)_2$: C, 21.1; H, 4.8; N, 16.5.

ΛΔ-$[Co(en)_2gly\text{-}glyO](ClO_4)_2$

$[Co(en)_2glyOCH_3]Cl_3$ was prepared using the PCl_3 method as described above. A solution of the chelated ester was then treated with excess $glyOC_2H_5$, neutralised after 30 sec with HOAc and was diluted to 500 ml with water. The complexes were sorbed on a H^+ form resin and eluted with 1.5M HCl. The eluate of the peptide complex was collected, evaporated to dryness, dissolved in water and neutralised with 0.5M LiOH. On treatment with $LiClO_4$ and cooling, $[Co(en)_2gly\text{-}glyO](ClO_4)_2$ crystallised. The complex was recrystallised from water by addition of $LiClO_4$, washed with methanol and dried in vacuo over P_2O_5. Absorption maxima occurred at 487 nm (ε 95) and at 344 nm (ε 104) in H_2O at 25°.

Anal. Calcd. for $Co(C_8H_{23}N_6O_3)(ClO_4)_2$: C, 18.87; H, 4.55; N, 16.50. Found: C, 18.6; H, 4.6; N, 16.3.

The complex was also prepared from either $[Co(en)_2glyOCH_3](ClO_4)_3$ or $[Co(en)_2glyOCH(CH_3)_2](ClO_4)_3$ in acetone treated with $glyOC_2H_5$ as previously described (90).

Kinetic Measurements

The hydrolysis of all complexes was followed spectrophotometrically at 310 nm. A 5 ml aliquot from a stock solution of the complex

(2.5×10^{-3}M, μ = 1 ($NaClO_4$) and 5 ml 0.05M glycine buffer of known pH (μ = 1 ($NaClO_4$)) at the desired temperature were rapidly mixed and the solution transferred to a thermostated 1 cm cell in the spectrophotometer. Absorbance changes of the order 0.06 to 0.25 optical density units were followed. At the completion of the hydrolysis the pH was measured and [OH^-] calculated using pK_w = 13.37 (32).

The mutarotation of Λ- and Δ-[$Co(en)_2$(S)-ala-glyO]$(ClO_4)_2$ and Λ-[$Co(en)_2$(S)-ala-glyOC_3H_7]$(ClO_4)_3$ were followed polarimetrically at 480 nm. Equal volumes of the complex (0.1% solution, μ = 1 ($NaClO_4$)) and 0.05M glycine buffer of known pH (μ = 1, $NaClO_4$) at the desired temperature were rapidly mixed and the solution transferred to a thermostated 1dcm cell. Rotation changes of the order of 0.1° to 0.15° were observed. The equilibrium rotation for each run was also recorded and was reproducible to ±0.005°. At the completion of the mutarotation the pH was measured and [OH^-] calculated using pK_w = 13.37 (32).

Mutarotation of Λ- and Δ-[$Co(en)_2$(S)-ala]I_2 were followed polarimetrically at 450 nm. The complex (∿0.02g) in water (10 ml) and 0.1M NaOH (10 ml) each at 25° were rapidly mixed and the solution transferred to a thermostated 1dcm cell. Rotation changes of the order of 0.08° to 0.09° were observed. The equilibrium rotation for each run was recorded. The ionic strength was adjusted with KCl to 0.5.

Pmr Equilibrium Studies

1. Λ- and Δ-[$Co(en)_2$(S)-ala-glyO]$(ClO_4)_2$ and Λ-[$Co(en)_2$(S)-ala-glyOC_3H_7]$(ClO_4)_3$. The same procedure was used for Λ- and Δ-[$Co(en)_2$(S)-ala-glyO]$(ClO_4)_2$. The complex (0.1g) in 0.5M glycine buffer (20 ml, pH 9.82, μ = 1 ($NaClO_4$)) was kept at 25° for 50 min and was then neutralised with HOAc to pH ∿4. The solution was diluted to 100 ml and the complexes sorbed on a H^+ form resin and eluted with 1.5M HCl. The eluates of the peptide complex and $[Co(en)_2ala]^{2+}$ were collected and cobalt determinations (atomic absorption) carried out. The peptide

complex was then evaporated to dryness and a pmr spectrum in D_2O recorded of the methyl protons of alanine using a 500 Hz full sweep width. The same procedure was used for Λ-$[Co(en)_2(S)\text{-ala-glyOC}_3H_7](ClO_4)_3$ except that the complex was kept at 25° in 0.5M glycine buffer (pH 9.45) for 1 hr.

2. Λ- and Δ-$[Co(en)_2(S)\text{-ala}]I_2$. The same procedure was used for both complexes. The complex (0.1g) in 0.1M NaOH (30 ml) was kept at 25° for 1 hr, then neutralised with 0.1N HCl to pH ∿3 and diluted to 100 ml with H_2O. The product was sorbed on a H^+ form resin and eluted with 1.5M HCl. The eluate of $[Co(en)_2ala]^{2+}$ was collected and evaporated to dryness. The complex was dissolved in D_2O and the pmr spectrum recorded.

Proton Exchange

1. $[Co(en)_2\text{gly-glyO}](ClO_4)_2$ (0.06g) was dissolved in 0.5 ml 2M Na_2CO_3:$NaHCO_3$ buffer at 32°. The solution was then transferred rapidly to a pmr tube and exchange of the α- and O-terminal $-CH_2-$protons of the peptide followed by scanning the spectrum between 4.0 to 4.8 ppm downfield from TMS at regular time intervals until no further change in the spectrum was observed. The pH was then measured at 32° and $[OD^-]$ calculated using pK_{D_2O} = 14.31 (132). The areas under the $-CH_2-$ absorptions were measured at least 5 times using a Gelman planimeter.

2. Λ-$[Co(en)_2(S)\text{-ala-glyO}](ClO_4)_2$ (0.1g) in 0.5M glycine buffer in D_2O (pD 10.01) was kept at 25° for 5.5 min and then neutralised with HOAc to pH ∿4 and diluted to 100 ml. The complexes were sorbed on a H^+ form resin and eluted with 1.5M HCl. The eluate of the peptide complex was collected and evaporated to dryness. It was then dissolved in D_2O and a pmr spectrum recorded of the methyl protons of alanine using a 500 Hz full sweep width expansion. For another sample Λ-$[Co(en)_2(S)\text{-ala-glyO}](ClO_4)_2$ in the same buffer in D_2O, the pH at 25°

was measured and $[OD^-]$ calculated using pK_{D_2O} = 14.71 (132). The same procedure was repeated for samples neutralised after 30 min and 1 hr.

Product Analysis

1. $[Co(en)_2gly\text{-}glyO](ClO_4)_2$. The complex (0.2g) in 0.5M glycine buffer (20 ml, pH 10.2, μ = 1 ($NaClO_4$)) was kept at 25° for 4 hrs and then neutralised with HOAc to pH ∿4 and diluted to 100 ml. The product was sorbed on H^+ form resin and eluted with 1.5M HCl. Only one band was present and this was collected. The volume was reduced to dryness and the residue dissolved in water (10 ml). The solution was then neutralised with 0.5M LiOH and after treatment with excess NaI and cooling, $[Co(en)_2gly]I_2$ crystallised. The complex was recrystallised from H_2O by addition of NaI, washed with ice cold water and dried in vacuo over P_2O_5. The chromatographic behaviour, visible spectrum (ε_{487}98, and ε_{345}107) and pmr spectrum of the isolated complex were identical with that of an authentic sample of $[Co(en)_2gly]I_2$.

2. $[Co(en)_2gly\text{-}(S)\text{-}alaO](ClO_4)_2$. The same procedure was used except the complex was kept in buffer at 25° for 4 hr. A visible and pmr spectrum of the isolated complex were identical with that of an authentic sample of $[Co(en)_2gly]I_2$.

3. $[Co(en)_2(S)\text{-}ala\text{-}glyO](ClO_4)_2$ and $[Co(en)_2(S)\text{-}ala\text{-}glyOC_3H_7](ClO_4)_3$. The same procedure was used for both complexes. The complex (0.1g) in 0.5M glycine buffer (20 ml, pH 10.30, μ = 1 ($NaClO_4$)) was kept at 25° for 1 hr and was then quenched with HOAc to pH ∿4. Only one cobalt complex was present and was isolated as described in (1). The visible spectrum (ε_{487}109 and ε_{348}117) and pmr spectrum were identical with that of an authentic sample of $[Co(en)_2ala]I_2$.

Detection of Possible Racemisation of (S)-alanine in Λ-$[Co(en)_2gly(S)\text{-}alaO](ClO_4)_2$ During Hydrolysis

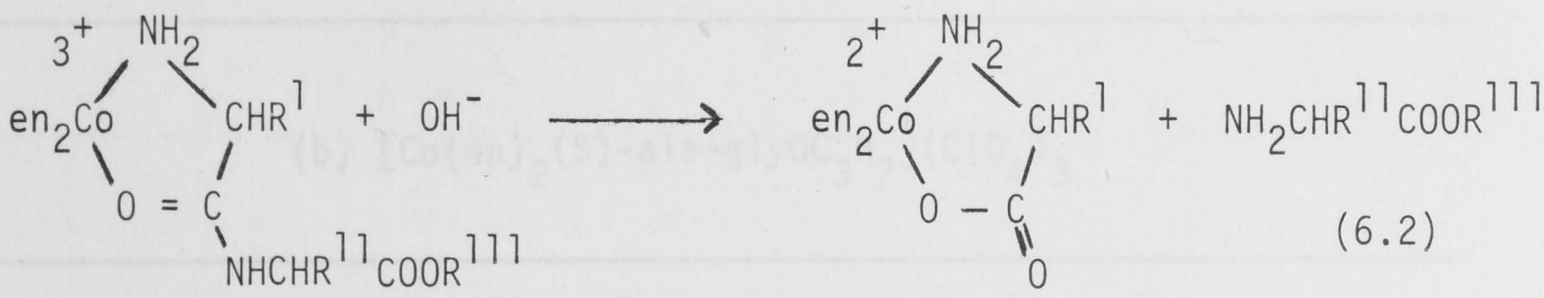
1. Rotations at 450 nm for a 0.1% solution of the complex in 0.5M glycine buffer (pH 10.20) at 25° were recorded over 1 hr.

2. The complex (0.1g) in 0.5M glycine buffer (pH 10.20) was kept at 25° for 1 hr and was then neutralised with HOAc to pH ∿4 and diluted to 100 ml. The complexes were sorbed on H^+ form resin and eluted with 1.5M HCl. The eluate of the peptide complex was collected, evaporated to dryness and the perchlorate salt then isolated as described for $\Lambda\Delta$-$[Co(en)_2(S)\text{-ala-glyO}](ClO_4)_2$. A pmr spectrum was recorded. Absorption maxima occurred at 487 nm (ε 115) and 345 nm (ε 134) in H_2O at 25°. Specific rotations for the complex were $[\alpha]_{546}$ +609° and $[\alpha]_D$ +325° in H_2O at 25°.

Results

Base Hydrolysis

In general, hydrolysis of the chelated dipeptides to give the chelated amino acid complex and amino acid (Reaction 6.2) was followed



(6.2)

spectrophotometrically at 310 nm. However, the hydrolysis of $[Co(en)_2ala\text{-}glyO](ClO_4)_2$ was also followed spectropolarimetrically at 450 nm. No difficulty was encountered in following hydrolysis at pH <9.8 as previously reported for the hydrolysis of $[Co(en)_2gly\text{-}glyO](ClO_4)_2$ (21), although smaller changes in optical density occurred at low pH.

The observed rate constants for peptide cleavage (Table 6.1 and 6.2) were obtained from plots of log $(D_t - D_\alpha)$ against time. These plots were linear for at least four half lives. The rate constants fit a rate law of the form

TABLE 6.1

Spectrophotometric Rate Data for Hydrolysis of Peptide Complexes
[μ = 1.0, 310 nm, [Complex] $\sim 10^{-3}$M, 0.05M Glycine Buffer]

(a) $[Co(en)_2(S)\text{-ala-glyO}](ClO_4)_2$

t	$[OH^-]$ 10^4M	k_{obs} 10^3 sec^{-1}	$\frac{k_{obs}}{[OH^-]}$ $M^{-1}sec^{-1}$
25°	5.9	1.4	2.4
	2.0	8.3×10^{-1}	4.3
	6.3×10^{-1}	2.8×10^{-1}	4.5
	4.8×10^{-1}	2.4×10^{-1}	4.7
	3.9×10^{-1}	1.9×10^{-1}	4.9
	3.5×10^{-1}	1.7×10^{-1}	4.8
	1.8×10^{-1}	8.6×10^{-2}	4.7
	1.0×10^{-1}	4.8×10^{-2}	4.8
16°	6.4×10^{-1}	8.9×10^{-1}	1.4
33°	3.9×10^{-1}	4.7×10^{-1}	12.1
	2.5	3.0×10^{-1}	12.0

(b) $[Co(en)_2(S)\text{-ala-glyOC}_3H_7](ClO_4)_3$

25°	6.2	1.4	2.2
	2.2	1.6	7.2
	1.7	1.2	7.2
	1.3	8.2×10^{-1}	7.5
	8.3×10^{-1}	5.8×10^{-1}	7.0
	5.7×10^{-1}	4.1×10^{-1}	7.3
	3.3×10^{-1}	2.1×10^{-1}	7.2
	2.2×10^{-1}	1.5×10^{-1}	7.0

TABLE 6.1 continued

(c) Λ-$[Co(en)_2gly\text{-}(S)\text{-}alaO](ClO_4)_2$

t	$[OH^-]$ $10^4 M$	k_{obs} $10^4\ sec^{-1}$	$\frac{k_{obs}}{[OH^-]}$ $M^{-1}sec^{-1}$
25°	3.9	2.3	0.60
	1.6	1.4	0.91
	1.2	1.3	1.1
	6.7×10^{-1}	6.8×10^{-1}	1.0
	5.3×10^{-1}	5.3×10^{-1}	1.0
	3.1×10^{-1}	3.4×10^{-1}	1.1
	2.0×10^{-1}	2.8×10^{-1}	1.4

(d) Δ-$[Co(en)_2gly\text{-}(S)\text{-}alaO](ClO_4)_2$

25°	3.9	2.3	0.58
	1.5	1.4	0.95
	6.7×10^{-1}	6.7	1.0
	3.1×10^{-1}	3.0	1.0

(e) $[Co(en)_2gly\text{-}glyO](ClO_4)_2$

25°	6.3×10^{-1}	1.5	2.5
	3.5×10^{-1}	8.9×10^{-1}	2.5
32°	1.6	1.5	0.98
	3.9×10^{-1}	3.9×10^{-1}	1.0

μ = 3 ($NaClO_4$)

TABLE 6.2

Spectropolarimetric Rate Data for the Hydrolysis of Λ-$[Co(en)_2(S)\text{-ala-glyO}](ClO_4)_2$ (μ=1, 450 nm, [Complex] $\sim 10^{-3}$M, 0.05M Glycine Buffer)[a]

t	$[OH^-]$ 10^4M	k_{obs} 10^3 sec^{-1}	$\frac{k_{obs}}{[OH^-]}$ $M^{-1}sec^{-1}$
33°	1.0	1.3	13
	7.6×10^{-1}	9.1	12
	3.8×10^{-1}	4.9×10^{-1}	13
	2.5×10^{-1}	2.8×10^{-1}	11
	1.5×10^{-1}	1.8×10^{-1}	12
	7.1×10^{-2}	9.9×10^{-2}	14

[a] Rotation change observed for a 0.1% [Co] soln was of the order of 0.1 to 0.2° in a 1-cm cell.

$$v = k[complex][OH^-]$$

and for $[Co(en)_2gly\text{-}glyO](ClO_4)_2$ they have a similar value in H_2O and D_2O (Table 6.1(e) and 6.8). Hydrolysis of $[Co(en)_2(S)\text{-}ala\text{-}glyOCH(CH_3)_2](ClO_4)_3$ occurred mainly at the peptide bond and little or no concommitant hydrolysis was observed. This was established by product analysis and pmr studies.

In all instances the chelated amino acid complex was the sole Co(III) product of the hydrolysis. Hydrolysis of $[Co(en)_2ala\text{-}glyO]^{2+}$ and $[Co(en)_2ala\text{-}glyOC_3H_7]^{3+}$ gave $[Co(en)_2ala]^{2+}$, whereas $[Co(en)_2gly\text{-}alaO]^{2+}$ and $[Co(en)_2gly\text{-}glyO]^{2+}$ yielded $[Co(en)_2gly]^{2+}$. The formation of chelated glycine as the only complex remaining after hydrolysis of $[CoN_4gly\text{-}glyOR]^{3+}$ has been reported previously (21).

Pmr Spectra

Chemical shift data (100 MHz) for the dipeptide complexes are listed in Table 6.3. Assignments are based on chemical shift data for the uncoordinated peptide and on related trien and bis(en) glycine complexes previously described (21,92). In alkaline D_2O solutions amine and amide protons deuterate rapidly and the protons attached to the α-carbon atom of the chelated amino acid are also extremely labile compared to both the uncoordinated species and to the α-carbon protons of the non-chelated amino acids. This selective activation of the chelated α-protons therefore permits an unambiguous assignment of these protons in the peptide complexes.

The methylene protons of ethylenediamine absorb in the region 3.0-3.7 ppm downfield from TMS. The carbon protons of the chelated amino acid in the peptide complexes are further downfield than when the same amino acid is O-terminal. The latter protons have approximately the same chemical shift as those for the uncoordinated species. For example, in Λ-$[Co(en)_2(S)\text{-}ala\text{-}glyOH]^{3+}$, (S)-ala-gly,

TABLE 6.3

Chemical Shifts for the Carbon Protons in
Coordinated and Uncoordinated Amino Acids and Peptides.[a]

Species	glycine	alanine[c]		Ester function
	$-CH_2-$	$-CH_3$	-CH	
[b] Λ-$[Co(en)_2gly$-(S)-alaO$]^{2+}$	4.56s	1.84d	4.68	
Δ-$[Co(en)_2gly$-(S)-alaO$]^{2+}$	4.48s	1.78d	4.65q	
$\Lambda\Delta$ -$[Co(en)_2$(RS)-ala-glyO$]^{2+}$	4.66s	2.21d 2.17d	4.84q	
Λ-$[Co(en)_2$(S)-ala-glyO$]^{2+}$	4.66s	2.21d	4.86q	
Δ-$[Co(en)_2$(S)-ala-glyO$]^{2+}$	4.64s	2.17d	4.84q	
$[Co(en)_2$(S)-ala-gly$OC_3H_7]^{2+}$	4.70s	2.19d	5.50qi	1.75 d(gem-CH_3)
[d] $\Lambda\Delta$-$[Co(en)_2gly$-glyO$]^{2+}$	α4.53s β4.33s			
$\Lambda\Delta$-$[Co(en)_2$(RS)-ala$]^{2+}$		1.90d	4.83q	
glycine	4.29			
gly-glyOC_3H_7	α4.40s β4.44s			1.69d (gem-CH_3) 5.45 qi(-CH<)
gly-glyOC_3H_7-HCl	α4.43s β4.55s			1.75d (gem-CH_3) 5.50 qi (-CH<)
ala-glyOH	4.58s	2.03d	4.90q	
gly-alaOH	4.37s	1.87d	4.68q	

[a] Values (ppm) are downfield from TMS (100 MHz) in D_2O solutions; s = singlet, d = doublet, t = triplet, q = quartet, qi = quintet, gem = geminal.

[b] $-CH_2-$ protons of en exhibit a broad resonances in the region 3.0-3.7 ppm downfield from TMS.

[c] The methyl doublets of alanine in the complexes all have J = 7 c.p.s.

[d] Assignment of $-CH_2-$ resonances are based on the lability of α-CH_2-protons in alkaline D_2O.

Λ-$[Co(en)_2gly\text{-}(S)\text{-}alaOH]^{3+}$ and gly-(S)-ala the chemical shift values of the methyl protons are 2.21, 2.03, 1.84 and 1.87 ppm respectively downfield from TMS, Table 6.3.

Different chemical shifts for the methyl protons of alanine and the methylene protons of glycine are observed in the spectra of diastereoisomers of $[Co(en)_2gly\text{-}(S)\text{-}alaOH]^{3+}$ and $[Co(en)_2(S)\text{-}ala\text{-}glyOH]^{3+}$. In both instances the amino acid proton resonances for the ΛS isomer are downfield from the ΔS isomer, Table 6.3. The nonequivalence of resonances in diastereoisomers of this type has not been previously clearly observed (65). In Λ- and Δ-$[Co(en)_2(S)\text{-}ala\text{-}glyOH]^{3+}$ the methyl resonances, which appear as doublets arising from coupling with the α-proton, are sufficiently well separated to enable equilibration and proton exchange studies to be carried out.

Mutarotation

Over the pH range 8.85-10.77 coordinated alanine in $[Co(en)_2(S)\text{-}ala\text{-}glyO]^{2+}$ mutarotates about eight times faster than hydrolysis of the peptide bond (Table 6.1 and 6.4). The equilibria that result are given by Eq. 6.3 and 6.4 where $k_1/k_2 = K$. When either Λ-$[Co(en)_2(S)\text{-}ala\text{-}glyO](ClO_4)_2$ (ΛS) or

$$\Delta\text{-}[Co(en)_2(S)\text{-}ala\text{-}glyO]^{2+} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \Delta\text{-}[Co(en)_2(R)\text{-}ala\text{-}glyO]^{2+} \qquad (6.3)$$

$$\Lambda\text{-}[Co(en)_2(S)\text{-}ala\text{-}glyO]^{2+} \underset{k_1}{\overset{k_2}{\rightleftharpoons}} \Lambda\text{-}[Co(en)_2(R)\text{-}ala\text{-}glyO]^{2+} \qquad (6.4)$$

Δ-$[Co(en)_2(S)\text{-}ala\text{-}glyO](ClO_4)_2$ (ΔS) were permitted to equilibrate, the final rotations at 480 nm (Table 6.4), were equal but of opposite sign. Analysis by pmr of the product demonstrated that the equilibrium mixtures contained 50±5% of each component, K = 1.0.

The same equilibrium mixture was obtained when Λ- or Δ-$[Co(en)_2(S)\text{-}ala]I_2$ were allowed to equilibrate in basic solutions.

TABLE 6.4

Spectropolarimetric Rate Data for Mutarotation of Peptide Complexes
[μ=1.0 ($NaClO_4$), 480 nm, [Complex] ~0.1% soln, 0.05M glycine buffer]

(a) Λ-$[Co(en)_2(S)\text{-ala-glyO}](ClO_4)_2$

t	$[OH^-]$ $10^4 M$	k_{obs} 10^3 sec^{-1}	$\frac{k_{obs}}{[OH^-]}$ $M^{-1}sec^{-1}$
25°	2.5	8.2	33
	1.6	5.0	32
	4.5×10^{-1}	1.5	33
	1.4×10^{-1}	0.48	34
	8.9×10^{-2}	0.35	35
	7.1×10^{-2}	0.25	35
	Using 0.1M glycine buffer		
	2.6×10^{-2}	1.00	38
	Equilibrium rotation = -0.995 ± 0.005° for 0.1% solution		
34°	0.17	1.7	100
	Equilibrium rotation = -0.945 ± 0.005° for 0.1% solution		

(b) Δ-$[Co(en)_2(S)\text{-ala-glyO}](ClO_4)_2$

34°	5.5×10^{-1}	5.6	102
	1.7×10^{-1}	1.7	100
	6.6×10^{-2}	6.8	103
	3.2×10^{-2}	3.2	100
25°	2.6	0.98	37
16°	5.5×10^{-1}	0.66	12
	Equilibrium rotation at 25° = +0.950 ± 0.005°		

TABLE 6.4 continued

(c) Λ-$[Co(en)_2(S)\text{-ala-glyOCH}(CH_3)_3](ClO_4)_3$

$[OH^-]$ $10^4 M$	k_{obs} $10^2\ sec^{-1}$	$\frac{k_{obs}}{[OH^-]}$ $M^{-1}\ sec^{-1}$
4.5	2.6	57.7
1.1	1.5	128
8.5×10^{-1}	1.3	157
6.7×10^{-1}	3.4	200
4.5×10^{-1}	1.1	260
2.7×10^{-1}	9.3×10^{-1}	270
2.6×10^{-1}	9.6×10^{-1}	271
1.6×10^{-1}	4.6×10^{-1}	290
6.3×10^{-2}	1.9×10^{-1}	287
3.0×10^{-2}	8.8×10^{-2}	290

TABLE 6.5

Mutarotation of Λ-$[Co(en)_2(S)\text{-ala-glyO}](ClO_4)_2$ (25°, 450 nm, [Complex] ∿0.05% soln, 0.05M Glycine Buffer pH 9.59)

Dependence on Ionic Strength

μ	$[OH^-]$ M	k_{obs} $10^3\ sec^{-1}$	$\frac{k_{obs}}{[OH^-]}$ $M^{-1} sec^{-1}$
0.02	3.9×10^{-5}	3.12	80
0.12	"	2.14×10^{-1}	54
1.02	"	1.38×10^{-2}	35

Equilibrium rotation -0.990° for 0.1% solution

Table 6.6 gives the rate data for the equilibration of these isomers together with the respective equilibrium rotations in 0.05M NaOH. It can be seen that the final rotations at 450 nm were equal but of opposite sign, and pmr analysis of the equilibrated mixture demonstrated that equivalent amounts of ΛS and ΛR (ΔS, ΔR) isomers were present. This result has been reported previously for a pmr analysis (60 mc) for the equilibrium mixture obtained when Δ-$[Co(en)_2(S)\text{-ala}]I_2$ was allowed to equilibrate in basic solution (65).

TABLE 6.6

Rate Data of Equilibration of Λ- and Δ-$[Co(en)_2(S)\text{-ala}]I_2$ at 25°. [a]

Complex	Λ-$[Co(en)_2(S)\text{-ala}]I_2$	Δ-$[Co(en)_2(S)\text{-ala}]I_2$
$10^4 k_{obs}$ sec^{-1}	9.7	9.6
$10^2 k_{calc}$ [b] M^{-1} sec^{-1}	1.9	1.9 1.78 [c]
$[\alpha]_{450}$ deg at equilibrium rotation	-517	+514
$[\alpha]_{450}$ deg extrapolated t = 0 sec	-562	+472

[a] μ = 0.5 (KCl), 450 mm, [Complex] = 0.1% solution, 0.05M NaOH.

[b] Ref. 65, $k_{calc} = \frac{kobs}{[OH^-]}$ M^{-1} sec^{-1}.

[c] Ref. 65, equilibrium rotations not given.

Reactions 6.3 and 6.4 were carried out on a larger scale and the peptide complexes isolated from the equilibrium mixture. Figure 6.1 shows the pmr spectrum of Λ-$[Co(en)_2(S)\text{-ala-glyO}]^{2+}$ and the methyl absorption of alanine of the peptide product obtained from the equilibrium mixture. When ΛS was equilibrated in glycine buffer the methyl doublet of alanine centred at 2.21 ppm (Fig. 6.1) concomitantly disappeared as a methyl

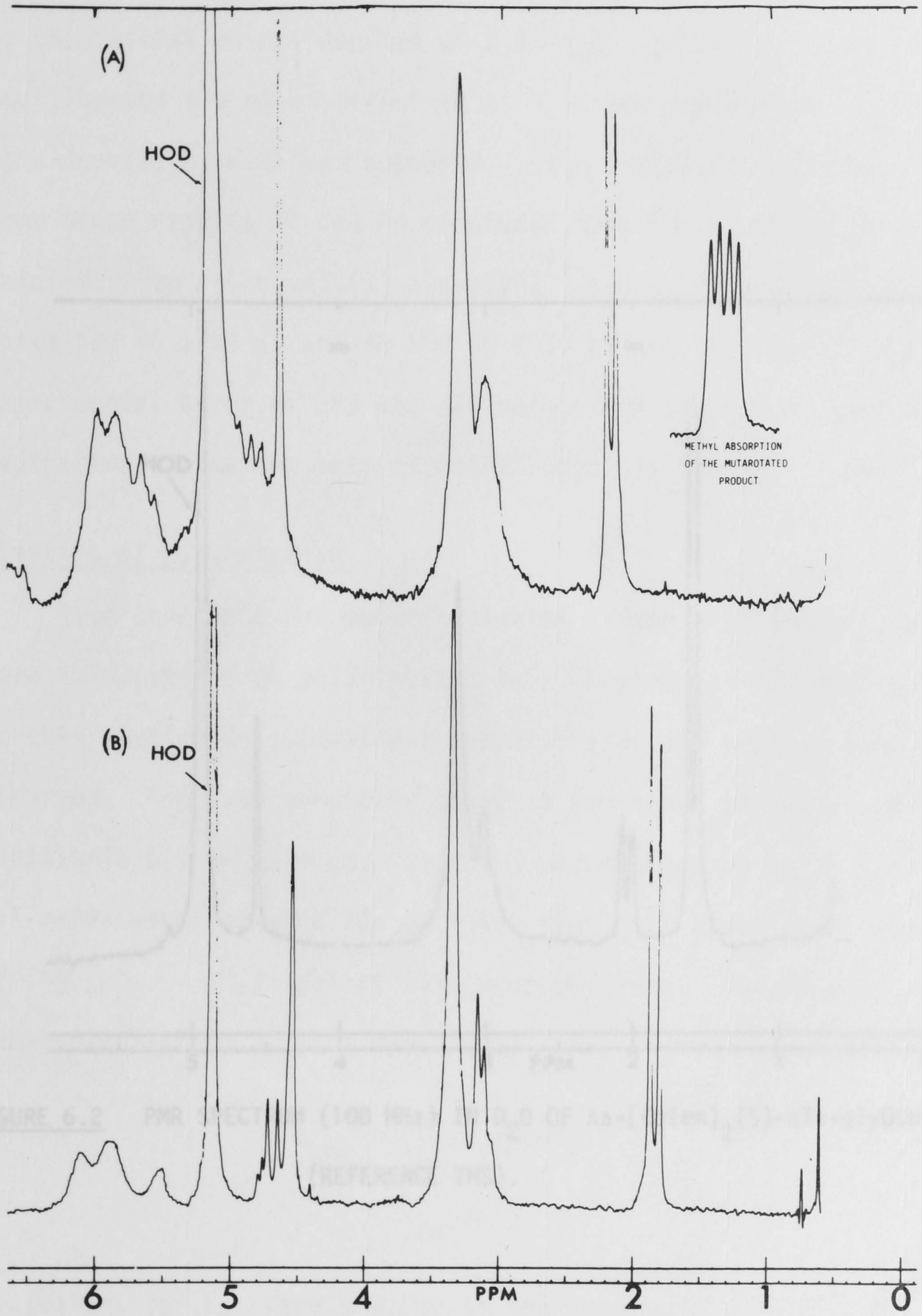


FIGURE 6.1 PMR SPECTRA (100 MHz) IN D_2O OF (A) Λ-$[Co(en)_2(S)\text{-ala-glyO}](ClO_4)_2$ CAPTION SHOWS METHYL ABSORPTION (500 Hz SWEEP WIDTH) AFTER MUTAROTATION IN GLYCINE BUFFER: (B) Δ-$[Co(en)_2\text{gly-(S)-alaO}](ClO_4)_2$ (REFERENCE TMS).

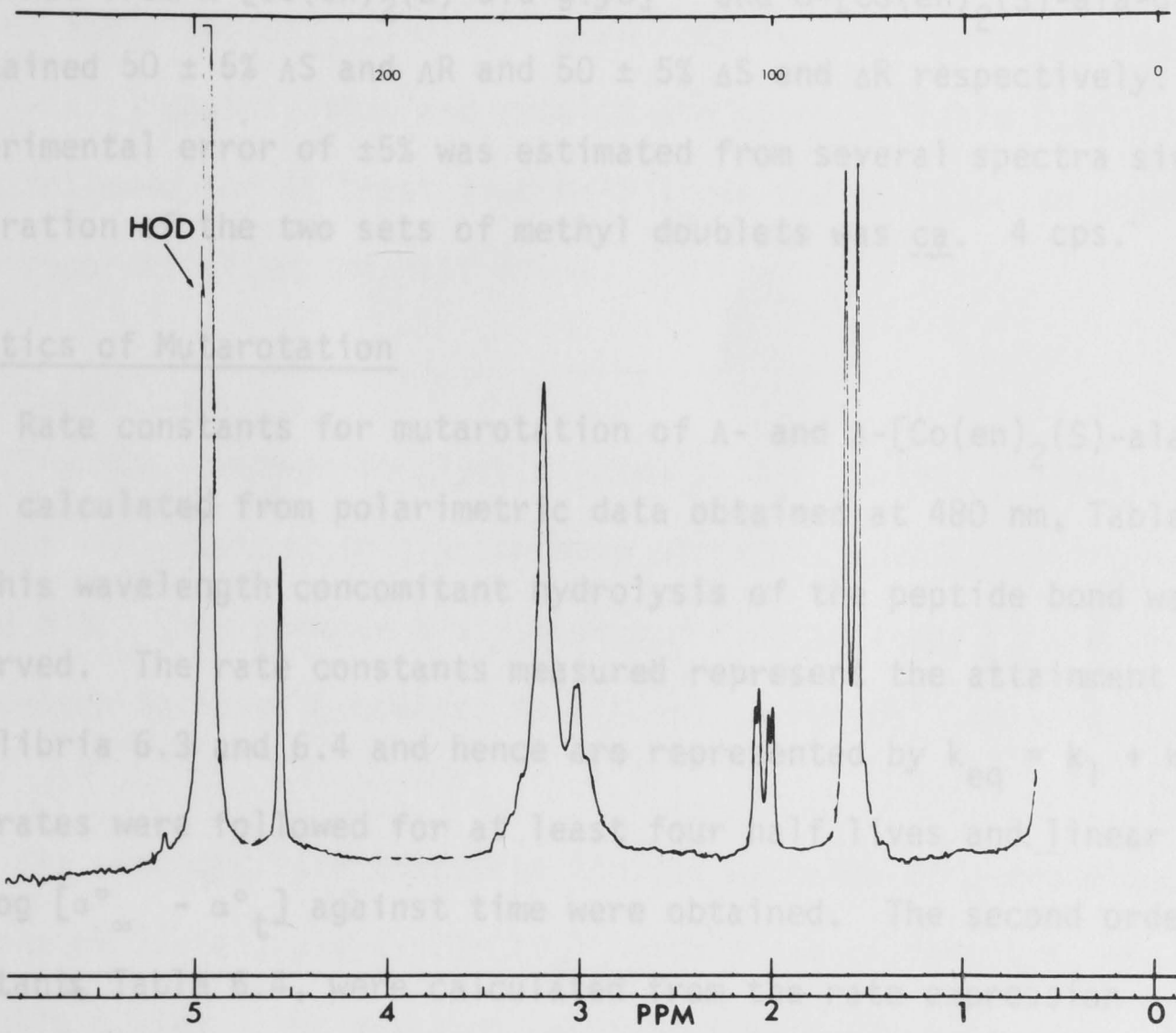


FIGURE 6.2 PMR SPECTRUM (100 MHz) IN D_2O OF $\Lambda\Delta$-$[Co(en)_2(S)\text{-ala-glyOCH}(CH_3)_2]^{3+}$ (REFERENCE TMS).

doublet formed at 2.17 ppm. At equilibrium the height of each set of methyl signals was the same and corresponded to approximately ½ height of the initial methyl doublet at 2.21 ppm. Similarly, when ΔS was equilibrated the doublet centred at 2.17 ppm concomitantly disappeared as a doublet at 2.21 ppm appeared. Each methyl doublet has J = 7 cps. From these results it can be concluded that the equilibrium mixture obtained from Λ-$[Co(en)_2(S)\text{-ala-glyO}]^{2+}$ and Δ-$[Co(en)_2(S)\text{-ala-glyO}]^{2+}$ contained 50 ± 5% ΛS and ΛR and 50 ± 5% ΔS and ΔR respectively. An experimental error of ±5% was estimated from several spectra since separation of the two sets of methyl doublets was ca. 4 cps.

Kinetics of Mutarotation

Rate constants for mutarotation of Λ- and Δ-$[Co(en)_2(S)\text{-ala-glyO}]^{2+}$ were calculated from polarimetric data obtained at 480 nm, Table 6.4. At this wavelength concomitant hydrolysis of the peptide bond was not observed. The rate constants measured represent the attainment of Equilibria 6.3 and 6.4 and hence are represented by $k_{eq} = k_1 + k_2$. All rates were followed for at least four half lives and linear plots of log $[\alpha^\circ_\infty - \alpha^\circ_t]$ against time were obtained. The second order rate constants, Table 6.4, were calculated from the rate expression

$$k_{obs} = [k_1+k_2][OH^-]$$
$$= k_{eq}[OH^-]$$

The values for k_{eq} were similar at the same ionic strength but increased as μ decreased, Table 6.5.

The observed rate constants for the mutarotation of Λ-$[Co(en)_2(S)\text{-ala-glyOC}_3H_7]^{3+}$ (Table 6.4(c)) were obtained from plots of log $[\alpha^\circ_\infty - \alpha^\circ_t]$ against time and these were all linear for at least three half lives over the pH range examined (pH 8.5 - 10.4). The observed rate constants obey the rate expression

$$v = k_{eq}[complex][OH^-]$$

A considerable deviation from the rate law occurred for pH > 9.8 and this was primarily due to hydrolysis of the ester function to yield $[Co(en)_2ala\text{-}glyO]^{2+}$. This product mutarotates at a much slower rate (Table 6.3).

The observed rate constants measured for attainment of equilibria for Λ- and Δ-$[Co(en)_2(S)\text{-}ala]^{2+}$ were obtained from plots of log $[\alpha^\circ_\infty - \alpha^\circ_t]$ against time and results are given in Table 6.6. Rates were followed for at least four half lives and equilibrium rotations were recorded after ten half lives.

Proton Exchange

The $-CH_2-$protons in glycylglycine in the $[Co(en)_2gly\text{-}glyO]^{2+}$ ion and the $-CH_2-$protons in glycine have different chemical shift values, Table 6.3. This permits a simultaneous kinetic study of the hydrolysis and proton exchange processes in $[Co(en)_2gly\text{-}glyO]^{2+}$ in alkaline D_2O by pmr.

The observed rate constants for the exchange of α-methylene protons of $[Co(en)_2gly\text{-}glyO](ClO_4)_2$ with D^+ at different pD are presented in Table 6.7(a). The data are consistent with the rate law

$$v = k[complex][OD^-]$$

The kinetic data for the α-CH_2-proton exchange were obtained by following the collapse of the methylene protons absorption as they deuterated, Fig. 6.3. Plots of log (area of absorption $_t$ - area of absorption $_\infty$) against time were linear for at least two half lives. Proton exchange of the O-terminal $-CH_2-$protons was not apparent. However, this absorption slowly decreased in intensity synchronous with the appearance of free glycine at 4.20 ppm. This change corresponded to peptide hydrolysis and the observed rate constants (Table 6.8) were in accord

TABLE 6.7

Proton Exchange Data for Peptide Complexes in D_2O
(32.4°, μ=3.0 ($NaClO_4$), [Complex] ≈ 0.25M, and 1M
Na_2CO_3:$NaHCO_3$ Buffer)

(a) $[Co(en)_2gly\text{-}glyO](ClO_4)_2$ Exchange of α-CH_2-protons

$[OD^-]$ M	k_{obs} sec^{-1}	$\frac{k_{obs}}{[OD^-]}$ M^{-1} sec^{-1}
2.0×10^{-5}	3.47×10^{-4}	17
8.5×10^{-5}	1.16×10^{-4}	14
1.5×10^{-4}	2.20×10^{-3}	15

(b) Λ-$[Co(en)_2(S)\text{-}ala\text{-}glyO](ClO_4)_2$ Exchange of α-proton

2.0×10^{-5}	2.0×10^{-3}	100 ± 20

TABLE 6.8

Rate Data for Hydrolysis of $[Co(en)_2gly\text{-}glyO](ClO_4)_2$ in D_2O
(32.4°, μ=3.0 ($NaClO_4$), [Complex] ≈ 0.25M, 1M Na_2CO_3:$NaHCO_3$ Buffer)

$[OD^-]$ M	k_{obs} sec^{-1}	$\frac{k_{obs}}{[OD^-]}$ M^{-1} sec^{-1}
2.0×10^{-5}	1.9×10^{-5}	1.0 ± 0.5
8.5×10^{-5}	1.4×10^{-5}	1.5 ± 0.5
1.5×10^{-4}	2.0×10^{-4}	1.2 ± 0.5

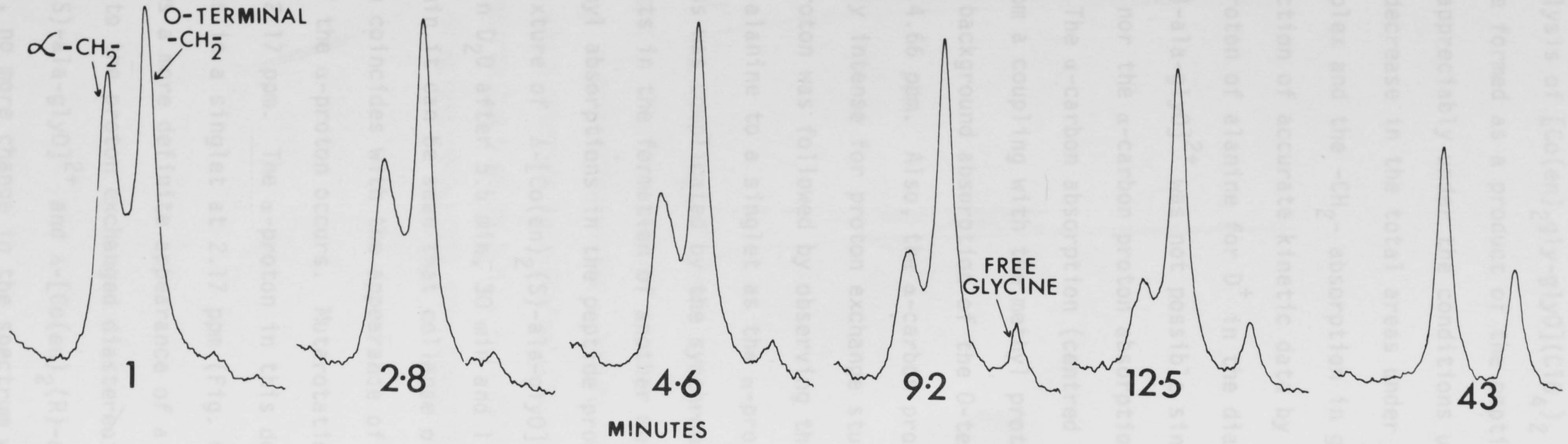


FIGURE 6.3 EXCHANGE OF THE α-PROTON FOR A DEUTERON AND HYDROLYSIS OF $[Co(en)_2gly\text{-}glyO]ClO_4)_2$ IN Na_2CO_3:$NaHCO_3$ IN D_2O (pH = 10.20, μ = 3, 32°). THE PROTON EXCHANGE IS FOLLOWED BY THE COLLAPSE OF THE α-CH_2-ABSORPTION, WHEREAS THE HYDROLYSIS OF THE PEPTIDE FOLLOWED BY THE COLLAPSE OF THE O-TERMINAL-CH_2-ABSORPTION AND THE CONCOMMITANT APPEARANCE OF FREE GLYCINE.

with hydrolysis of $[Co(en)_2gly\text{-}glyO](ClO_4)_2$ in water, Table 6.1(e). The glycine formed as a product of the peptide hydrolysis did not deuterate appreciably under the conditions used since there was less than a 3% decrease in the total areas under the O-terminal absorption in the complex and the $-CH_2-$ absorption in glycine.

Collection of accurate kinetic data by pmr for exchange of the α-carbon proton of alanine for D^+ in the diastereoisomers of $[Co(en)_2(S)\text{-}ala\text{-}glyO]^{2+}$ was not possible since neither the methyl absorption nor the α-carbon proton absorption was clearly defined during exchange. The α-carbon absorption (centred at 4.84) is a quartet arising from a coupling with the methyl protons and is not well defined due to the background absorption of the O-terminal $-CH_2-$ resonance of glycine at 4.66 ppm. Also, the α-carbon proton absorption was not sufficiently intense for proton exchange studies. However, the exchange of the α-proton was followed by observing the collapse of the methyl doublet of alanine to a singlet as the α-proton exchanged with D^+, but this process was complicated by the synchronous process of mutarotation which results in the formation of another methyl doublet. The spectra of the methyl absorptions in the peptide product isolated from the reaction mixture of $\Lambda\text{-}[Co(en)_2(S)\text{-}ala\text{-}glyO](ClO_4)_2$ in glycine buffer (pH 9.65) in D_2O after 5.5 min, 30 min and 1 hr are shown in Fig. 6.4. After 5.5 min it can be seen that collapse of the methyl doublet centred at 2.21 ppm coincides with the appearance of a singlet at 2.21 ppm as exchange of the α-proton occurs. Mutarotation results in a doublet centred at 2.17 ppm. The α-proton in this doublet also exchanges and this results in a singlet at 2.17 ppm (Fig. 6.4(c) and (d)). Figure 6.4(c) shows a more definite appearance of a set of singlets which correspond to the proton exchanged diastereoisomers of $\Lambda\text{-}[Co(en)_2(S)\text{-}ala\text{-}glyO]^{2+}$ and $\Lambda\text{-}[Co(en)_2(R)\text{-}ala\text{-}glyO]^{2+}$. After 1 hr, Fig. 6.4(d), no more change in the spectrum was observed and two singlets

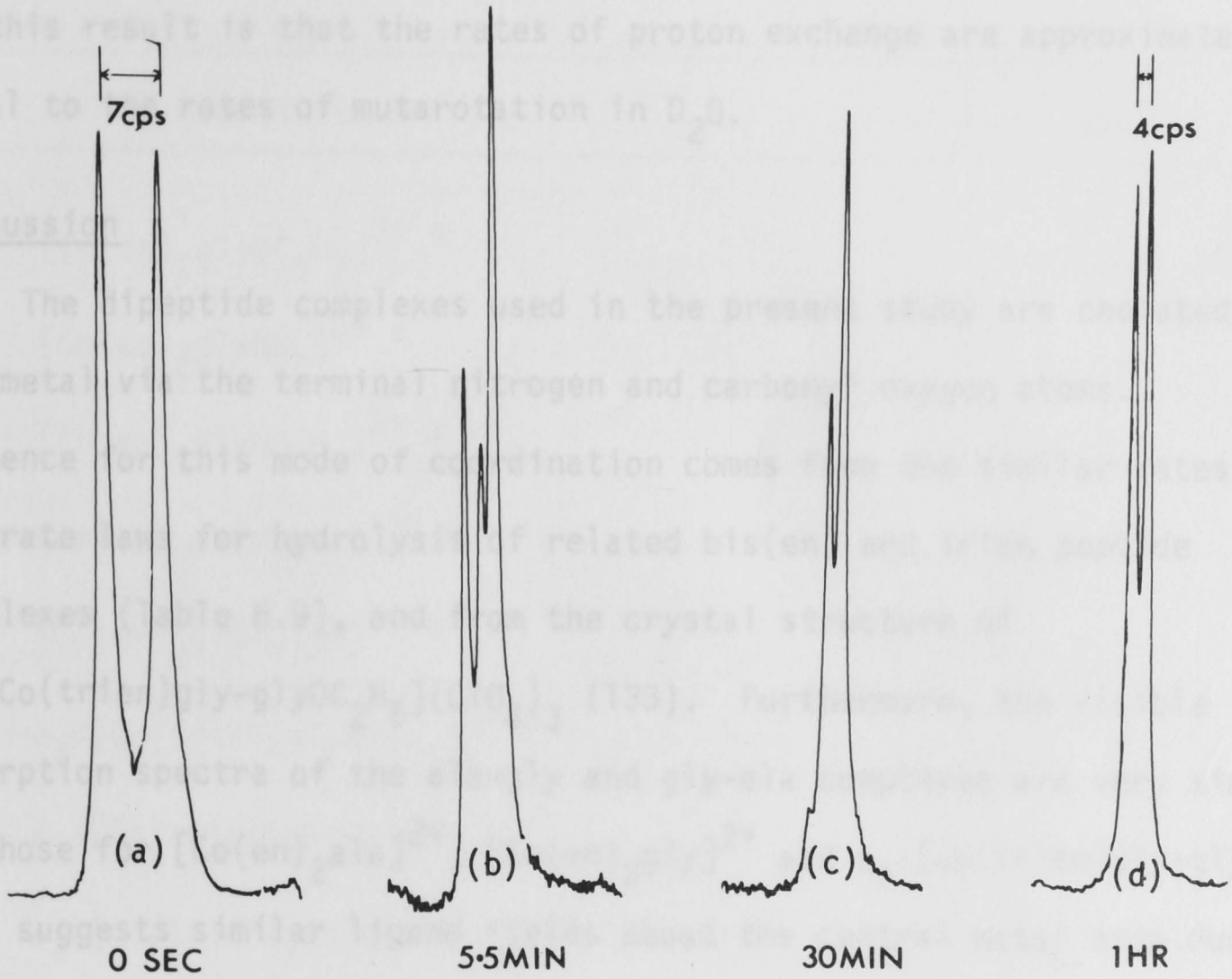


FIGURE 6.4 MUTAROTATION OF THE α-PROTON AND THE CONCURRENT α-PROTON EXCHANGE FOR A DEUTERON IN Λ-$[Co(en)_2(S)\text{-ala-glyO}](ClO_4)_2$ IN GLYCINE BUFFER (pH 9.65 IN D_2O, μ = 1, 25°) FOLLOWED BY THE COLLAPSE OF METHYL DOUBLET(S) INTO SINGLET(S)

(2.21 and 2.17 ppm) were in the spectrum. These products correspond to the Λ-$[Co(en)_2(S)\text{-}ND_2CD(CH_3)CONDCH_2COO]^{2+}$ (2.21 ppm) and Λ-$[Co(en)_2(R)\text{-}ND_2CD(CH_3)CONDCH_2COO]^{2+}$ ions (2.17 ppm). Spectrum Fig. 6.4(b) corresponds to about one half life for the mutarotation of Λ-$[Co(en)_2(S)\text{-ala-glyO}]^{2+}$ in the same buffer at 25°. The significance of this result is that the rates of proton exchange are approximately equal to the rates of mutarotation in D_2O.

Discussion

The dipeptide complexes used in the present study are chelated to the metal via the terminal nitrogen and carbonyl oxygen atoms. Evidence for this mode of coordination comes from the similar rates and rate laws for hydrolysis of related bis(en) and trien peptide complexes (Table 6.9), and from the crystal structure of β_2-$[Co(trien)gly\text{-}glyOC_2H_5](ClO_4)_3$ (133). Furthermore, the visible absorption spectra of the ala-gly and gly-ala complexes are very similar to those for $[Co(en)_2ala]^{2+}$, $[Co(en)_2gly]^{2+}$ and β_2-$[Co(trien)gly\text{-}glyOC_2H_5]^{3+}$. This suggests similar ligand fields about the central metal atom due to one oxygen and five nitrogen atoms. Also, it has been demonstrated that N-O chelated peptides base hydrolyse with greater ease than the N-N chelated peptides (21,67,68). In N-O peptides the chelate ring is not required to break and hydrolysis rates are enhanced by the positively charged metal ion which renders the carbonyl carbon atom more susceptible to attack by the nucleophilic reagent. However, in N-N peptide complexes, for example $[Co(gly\text{-}glyO)_2]^-$(67) and $[Cu(gly\text{-}glyO)_2]^-$(68) and in N-N bonded $[Co(NH_3)_4glyNH]^{2+}$ (34), there is a considerable resistance to hydrolysis and endo chelate ring cleavage is required if this is to occur. Furthermore, ^{18}O results for the hydrolysis of the corresponding $[Co(en)_2glyNH_2]^{3+}$ ion require the amide to be oxygen bonded (134).

The rates of amide hydrolysis in the dipeptide ester and dipeptide acid complexes are all very similar, Table 6.9. The slower rates found

TABLE 6.9

Comparison of Second-Order Rate Constants for Ester, Amide and Peptide Hydrolysis Promoted by Co(III) at 25°, μ = 1

Substrate	k M^{-1} sec^{-1}
$[Co(en)_2glyOC_3H_7]^{3+}$ [a]	1.5 ± 0.5 x 10^6
$[Co(en)_2glyNH_2]^{3+}$ [a]	2.5 ± 1
$[Co(en)_2glyNHCH_3]^{3+}$ [a]	1.6 ± 0.2
$[Co(en)_2glyN(CH_3)_2]^{3+}$ [a]	1.1 ± 0.2
$[Co(en)_2gly\text{-}glyO]^{2+}$ [a]	2.6 ± 0.2
β_2-$[Co(trien)glyNHCH_3]^{3+}$ [a]	2 ± 1
β_2-$[Co(trien)gly\text{-}glyOCH_3]^{3+}$ [a]	5 ± 1
β_2-$[Co(trien)gly\text{-}glyOC_3H_7]^{3+}$ [a]	3 ± 1
β_2-$[Co(trien)gly\text{-}glyO]^{2+}$ [a]	3 ± 1
$[Co(en)_2gly\text{-}(S)\text{-}alaO]^{2+}$	1 ± 0.1
$[Co(en)_2(S)\text{-}ala\text{-}glyO]^{2+}$	4.8 ± 0.1
$[Co(en)_2(S)\text{-}ala\text{-}glyOC_3H_7]^{3+}$	7.2 ± 0.2
$[Co(en)_2(S)\text{-}alaOCH_3]^{3+}$	5 ± 2 x 10^6
$[Co(en)_2(RS)\text{-}ala\text{-}(RS)\text{-}alaO]^{2+}$	0.8 ± 0.2
$[Co(NH_3)_5glyOC_2H_5]^{3+}$ [b]	30 ± 1

[a] Reference 20.

[b] Reference 34, μ = 0.1 (KNO_3).

for chelated gly-ala and ala-ala could possibly be due to hindered attack of OH^- at the carbonyl centre by the methyl group of alanine or simply due to a stronger amide bond. Steric hindrance of attack of an amine was proposed for peptide bond formation in Chapter 3 and this could operate in cleaving the peptide bond. The results for the hydrolysis of the alanine peptide complexes supports the mechanism established for the hydrolysis of $[Co(en)_2gly\text{-}glyO]^{2+}$ (21), Reaction 6.1. In the present study data was collected mainly in the pH range 9.0-10.3 and a rate law which was first order in hydroxide ion concentration was obtained (Table 6.1). However, the rate limiting behaviour observed in the data at higher pH supports the formation of the unreactive deprotonated reactant (II) in Reaction 6.1.

Table 6.9 shows that amide hydrolysis is 10^6 times slower than ester hydrolysis. Similar rate differences appear in comparisons of uncoordinated peptides with uncoordinated amino acid esters (Table 6.10) and

TABLE 6.10

Rate Data for the Hydrolysis of Peptides and Amino Acid Esters at 25°

Substrate	$10^6 k_{obs}$ sec^{-1}
glycyl-glycine	9.0 [a]
glycyl-glycinato anion	4.8 [b]
glycyl-(RS)-alanine	0.99 [a]
(RS)-alanyl-glycine	0.85 [a]
methyl (S)-alanate	1.1×10^6 [c]
methyl glycinate	1.3×10^6 [c]

[a] Reference 135, 0.1M peptide, 0.5M NaOH.

[b] Reference 67, 10^{-2}M glyglyOH, 0.5M NaOH, 10^{-2}M $CuSO_4$.

[c] Reference 33, μ = 0.1, pH-Stat measurement at pH 11.

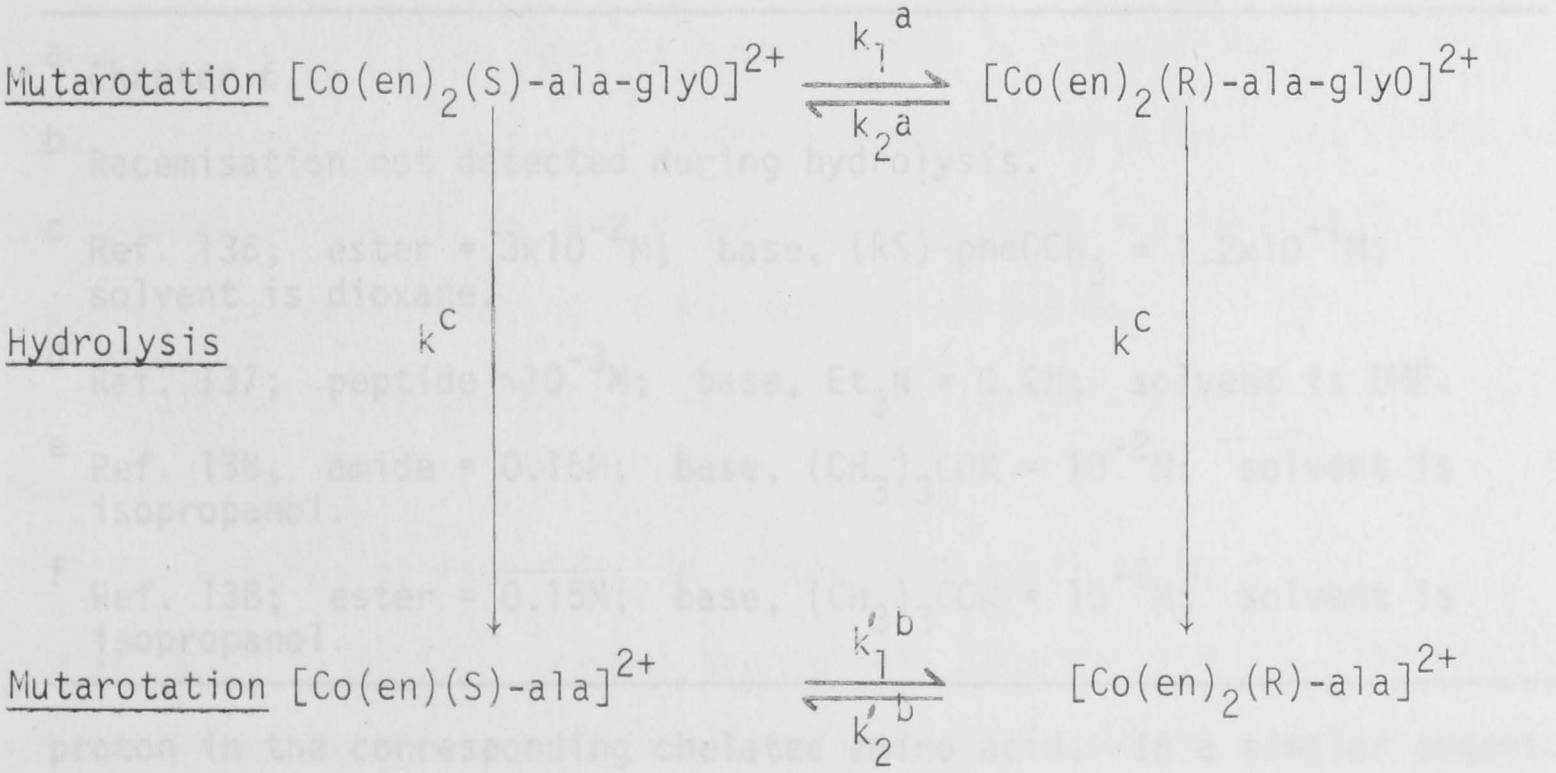
between the uncoordinated molecule, Table 6.9 and 6.10.

No kinetic stereoselectivity for hydrolysis of Λ- and Δ-$[Co(en)_2gly\text{-}(S)\text{-}alaO]^{2+}$ was observed, both rates being the same, Table 6.1(c) and (d). This contrasts with the different rates for formation of these diastereoisomers reported in Chapter 5.

Cleavage of the peptide bond and mutarotation at the α-carbon centre in $[Co(en)_2(S)\text{-}ala\text{-}glyO]^{2+}$ and $[Co(en)_2(S)\text{-}ala\text{-}glyOC_3H_7]^{3+}$ are competitive. The kinetic and product analysis results demonstrate that the mutarotation of the chelated (S)-ala-gly is about eight times faster than its hydrolysis. Thus, product analysis of the reaction mixture of $[Co(en)_2(S)\text{-}ala\text{-}glyO](ClO_4)_2$ in glycine buffer after about ten half lives for mutarotation gave 45% $[Co(en)_2(RS)\text{-}ala\text{-}glyO]^{2+}$ and 55% $[Co(en)_2ala]^{2+}$. The relationship between hydrolysis and mutarotation in Δ-$[Co(en)_2(S)\text{-}ala\text{-}glyO]^{2+}$ is shown in Fig. 6.5.

FIGURE 6.5

Relationship Between Hydrolysis and Mutarotation of $[Co(en)_2(S)\text{-}ala\text{-}glyO](ClO_4)_2$ in Glycine Buffer



[a] $k_{obs/[OH^-]} = k_1+k_2 \simeq 2k_1 = 35 M^{-1}\ sec^{-1}$.

[b] $k_{obs/[OH^-]} = k'_1+k'_2 \simeq 2k'_1 = 1.9\times10^{-2} M^{-1}\ sec^{-1}$.

[c] $k_{[OH^-]} = k_{obs}/[OH^-] = 4.8\ M^{-1}\ sec^{-1}$.

From Table 6.11 it can be seen that the α-carbon proton of the chelated peptide racemises some $2x10^3$ times faster than the α-carbon

TABLE 6.11

Comparison of Second Order Rate Constants for the Hydrolysis of Peptide Complexes and Racemisation in Chelated and Uncoordinated Amino Acids and Peptides at 25°.

	Racemisation M^{-1} sec^{-1}	Hydrolysis M^{-1} sec^{-1}
$[Co(en)_2(S)\text{-ala}]^{2+}$ [a]	1.9×10^{-2}	
$[Co(en)_2(S)\text{-ala-glyO}]^{2+}$	35	4.8
$[Co(en)_2(S)\text{-ala-glyOC}_3H_7]^{3+}$	250	7.2
$[Co(en)_2\text{gly-(S)-alaO}]^{2+}$	- [b]	1.0
α-amino iso-butyl-(S)-pheOCH$_3$ [c]	1.2×10^{-2}	
O-(carbobenzoxy gly-(S)-phe)--(N)-ethylsalicylamide [d]	1.3×10^{-2}	
$C_6H_5.CHCH_3.CON(C_2H_5)_2$ [e]	7.6×10^{-1}	
$C_6H_5.CHCH_3.COOCH(CH_3)_2$ [f]	5×10^{-3}	

[a] Chapter 6.

[b] Racemisation not detected during hydrolysis.

[c] Ref. 136; ester = $3x10^{-2}$M; base, (RS)-pheOCH$_3$ = $1.2x10^{-1}$M; solvent is dioxane.

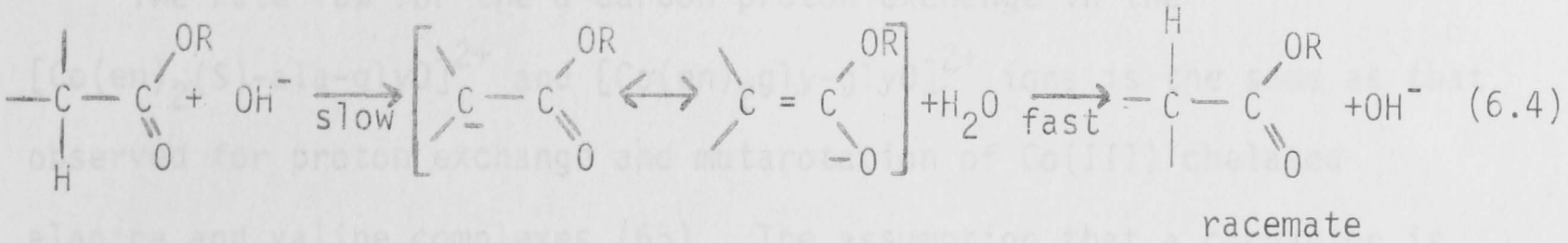
[d] Ref. 137; peptide $\sim 10^{-3}$M; base, Et_3N = 0.2M; solvent is DMF.

[e] Ref. 138; amide = 0.15M; base, $(CH_3)_3COK$ = 10^{-2}M; solvent is isopropanol.

[f] Ref. 138; ester = 0.15M; base, $(CH_3)_3COK$ = 10^{-2}M; solvent is isopropanol.

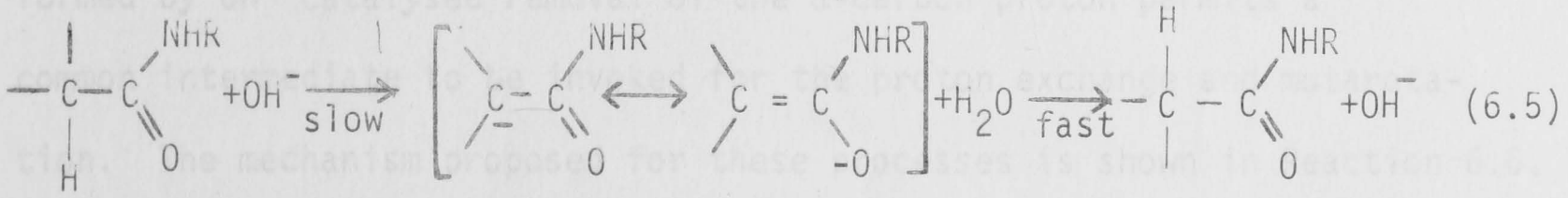
proton in the corresponding chelated amino acid. In a similar organic example, racemisation of diethyl phenylmethylacetamide is about 150 times faster than in the corresponding isopropyl ester (138), Table 6.11. Presumably the α-carbon centre is more acidic in amides than in esters.

In both systems the rate determining step for racemisation is the base catalysed removal of the α-carbon proton to form a carbanion, Eq. 6.4 and 6.5. This carbanion is stabilised in the symmetrical oxyanion and necessarily gives rise to a racemic product (139).



(6.4)

racemate



(6.5)

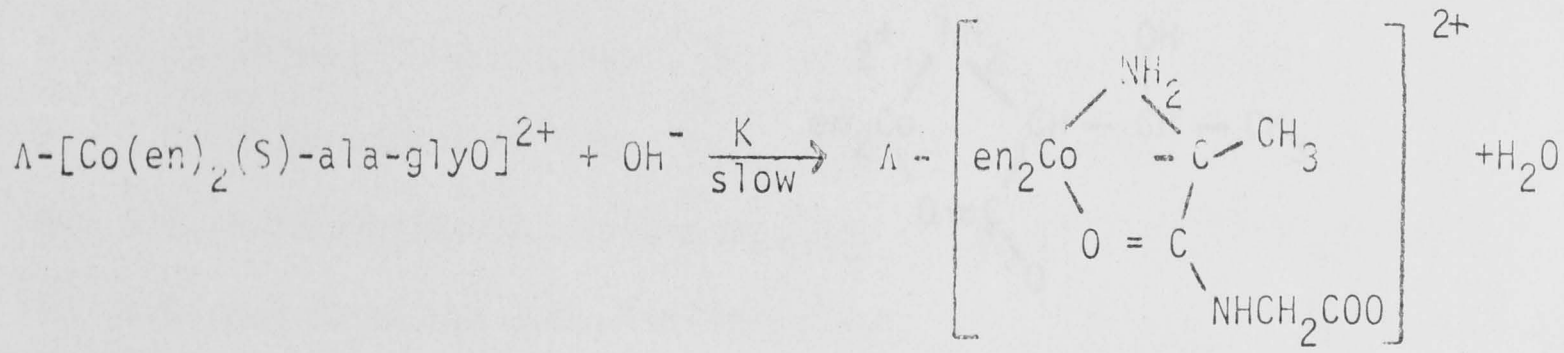
racemate

The more rapid mutarotation of the peptide ester complex compared to the amino acid complex is attributed to the higher formal charge on the former complex. This higher charge might be expected to increase the acidity of the α-carbon proton. When the carboxyl group is three carbon atoms removed from the asymmetric centre, as in $[Co(en)_2(S)\text{-ala-glyO}]^{2+}$, compared to a carboxyl group adjacent to an asymmetric centre, as in $[Co(en)_2(S)\text{-ala}]^{2+}$, the rate of racemisation is enhanced by a factor of 2000. This may be attributed to the higher effective positive charge at the asymmetric centre in the former complex. This argument is further supported by neutralisation of the carboxyl group as in $[Co(en)_2(S)\text{-ala-glyOCH}(CH_3)_2]^{3+}$ where a smaller enhancement in rate is found.

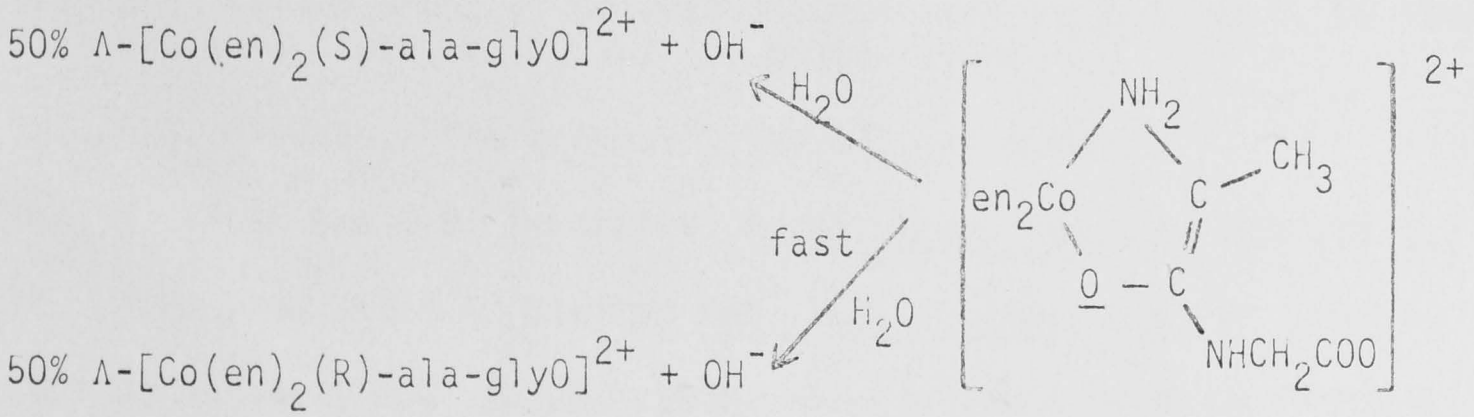
The absence of racemisation of the O-terminal amino acid in $[Co(en)_2gly\text{-}(S)\text{-alaO}]^{2+}$ was not unexpected. The pmr spectra (Table 6.6) of the O-terminal amino acid residue in the chelated and uncoordinated peptide species were very similar which suggests similar chemical environments and thus similar chemical properties. Furthermore, in proton exchange studies of the $[Co(en)_2gly\text{-glyO}]^{2+}$ and $[Co(en)_2(S)\text{-ala-glyO}]^{2+}$

ions, less than 3% exchange of the O-terminal $-CH_2-$ glycine protons was observed in the time required for complete hydrolysis. It appears that the influence of the metal ion in these complexes does not extend appreciably beyond the chelate ring.

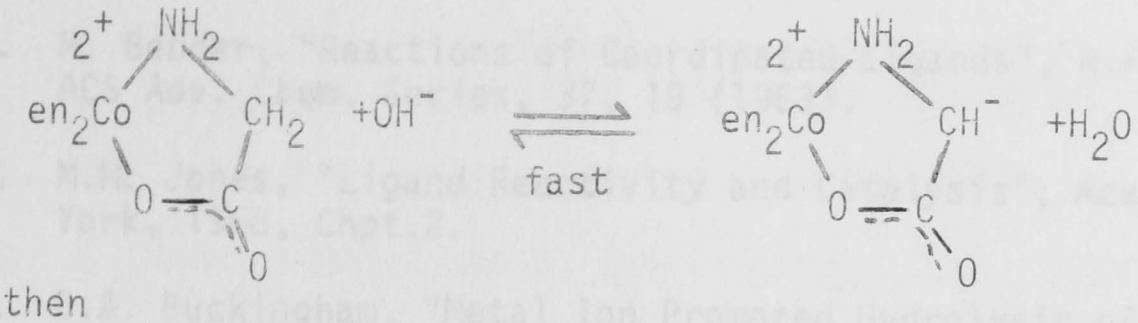
The rate law for the α-carbon proton exchange in the $[Co(en)_2(S)\text{-ala-glyO}]^{2+}$ and $[Co(en)_2\text{gly-glyO}]^{2+}$ ions is the same as that observed for proton exchange and mutarotation of Co(III) chelated alanine and valine complexes (65). The assumption that a carbanion is formed by OH^- catalysed removal of the α-carbon proton permits a common intermediate to be invoked for the proton exchange and mutarotation. The mechanism proposed for these processes is shown in Reaction 6.6.



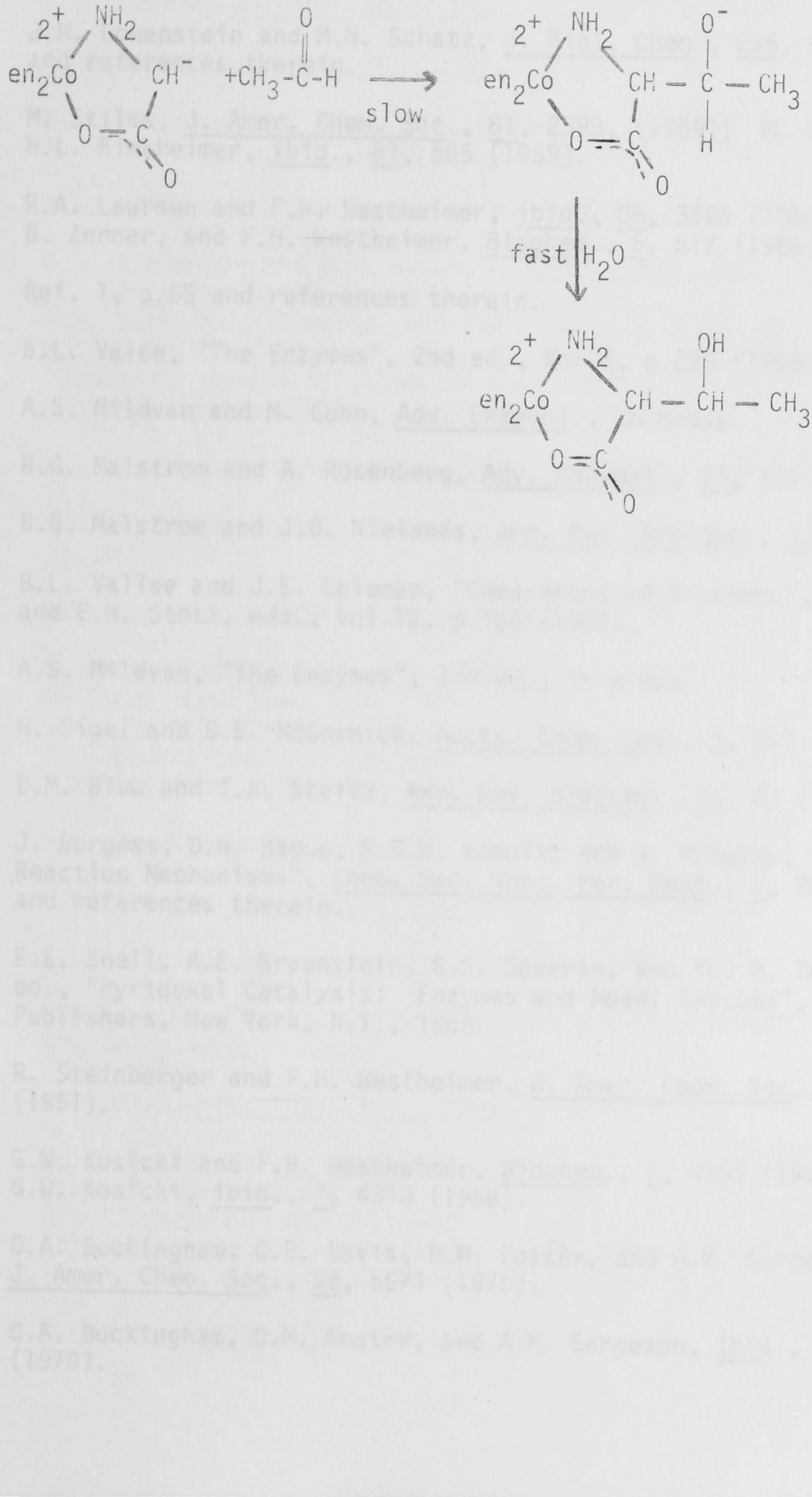
(6.6)



A similar mechanism has been proposed for H-exchange and mutarotation in the chelated α-amino acids (65). Evidence for the formation of the carbanion intermediate in the latter reaction comes from the condensation reaction of $[Co(en)_2\text{gly}]^{2+}$ with acetaldehyde in basic solution to form the threonine and allothreonine complexes (140). A kinetic study on the formation of the threoninato complex ion is consistent with a mechanism involving base catalysed abstraction of an α-carbon proton followed by insertion of a molecule of aldehyde (141), Reaction 6.7.


2+
NH2
en2Co
CH2
+OH-
fast
CH-
+H2O
O
C
then

(6.7)


2+
NH2
en2Co
CH-
+CH3-C-H
O
slow
O-
CH
C
CH3
H
fast
H2O
OH
CH
CH3

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APPENDIX I

ABBREVIATIONS USED FOR AMINO ACIDS ARE THOSE PROPOSED BY IUPAC (142)

Alanine	Ala	Leucine	Leu
Arginine	Arg	Lysine	Lys
Asparagine	Asn	Methionine	Met
Asparatic Acid	Asp	Phenylalanine	Phe
Cysteine	Cys	Proline	Pro
Glutamic Acid	Glu	3-Hydroxyproline	3-Hyp
Glutamine	Gln	Serine	Ser
Glycine	Gly	Threonine	Thr
Histidine	His	Tryptophan	Trp
Isoleucine	Ile	Tyrosine	Tyr
		Valine	Val

ABBREVIATIONS USED FOR AMINO ACIDS AND PEPTIDES IN COMPLEXES

aa	amino acid anion
aaOR	N-monodentate, or N-O chelated amino acid ester
$aa^{I}aa^{II}O$	peptide anion
$aa^{I}aa^{II}OR$	peptide ester

APPENDIX II

PMR SPECTRA (100 MHz) OF AMINO ACID AND PEPTIDE COMPLEXES.[a]

Amino Acid Complexes[c]

1. $[Co(en)_2thr]^{2+}$
2. [b] $[Co(en)_2ser]^{2+}$
3. [b] $[Co(en)_2lys]^{2+}$
4. $[Co(en)_2gln]^{2+}$
5. $[Co(en)_2asn]^{2+}$
6. [b] $[Co(en)_2met]^{2+}$
7. $[Co(en)_2tyr]^{2+}$
8. [b] $[Co(en)_2try]^{2+}$
9. [b] $[Co(en)_2pro]^{2+}$
10. $[Co(en)_2glu]^{+}$
11. [b] $[Co(en)_2asp]^{2+}$
12. [b] $[Co(en)_2arg]^{2+}$

Peptide Complexes[c]

1. $[Co(en)_2gly\text{-}leuO]^{2+}$
2. [b] $[Co(en)_2gly\text{-}proO]^{2+}$
3. $[Co(en)_2ala\text{-}valO]^{2+}$
4. $[Co(en)_2val\text{-}serO]^{2+}$
5. $[Co(en)_2leu\text{-}glyO]^{2+}$
6. $[Co(en)_2leu\text{-}alaO]^{2+}$
7. $[Co(en)_2leu\text{-}proO]^{2+}$
8. $[Co(en)_2ser\text{-}glyO]^{2+}$
9. $[Co(en)_2met\text{-}glyO]^{2+}$
10. $[Co(en)_2pro\text{-}glyO]^{2+}$
11. $[Co(en)_2pro\text{-}lysO]^{2+}$
12. $[Co(en)_2phe\text{-}glyO]^{2+}$
13. $[Co(en)_2phe\text{-}valO]^{2+}$
14. $[Co(en)_2phe\text{-}serO]^{2+}$
15. $[Co(en)_2tyr\text{-}glyO]^{2+}$
16. $[Co(en_2)tyr\text{-}serO]^{2+}$
17. $[Co(en)_2arg\text{-}glyO]^{2+}$
18. $[Co(en)_2arg\text{-}lysO]^{2+}$
19. $[Co(en)_2ala\text{-}gly\text{-}pheO]^{2+}$
20. $[Co(en)_2ala\text{-}gly\text{-}glyO]^{2+}$

---

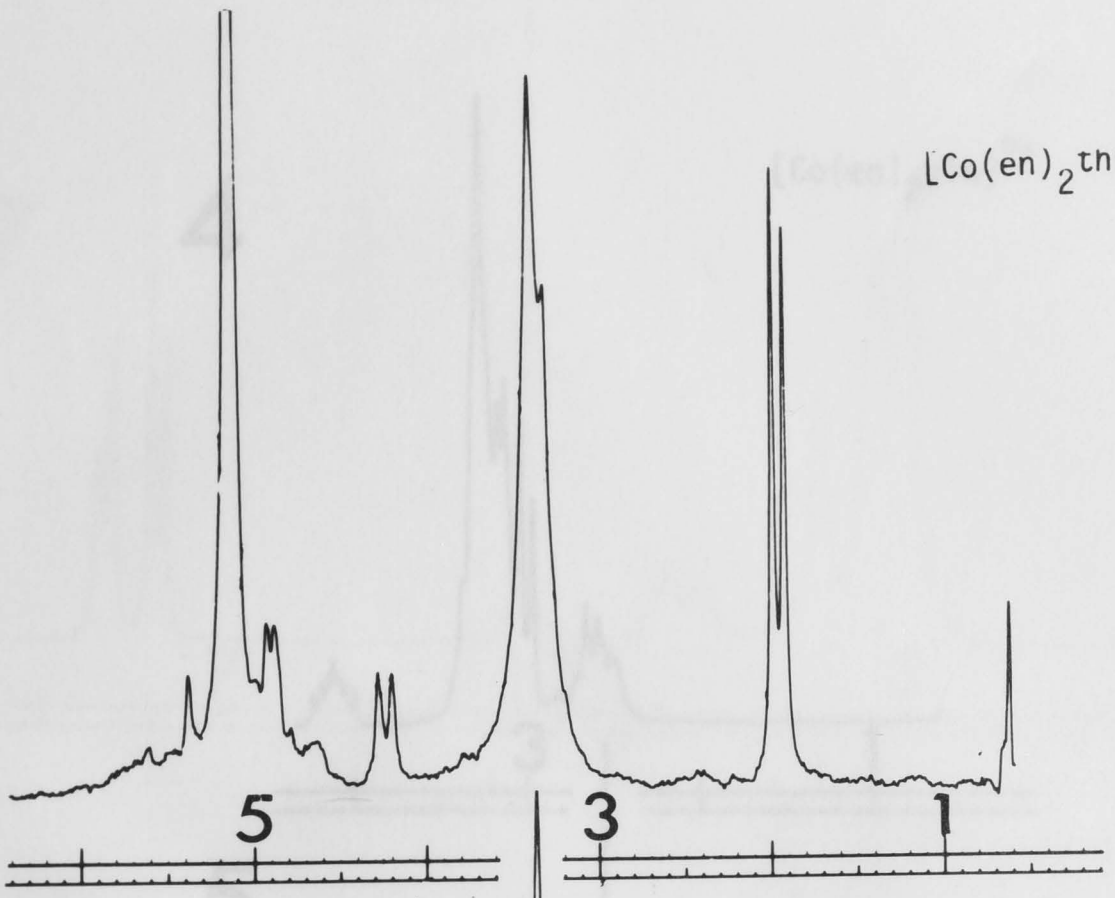
[a] Chemical shifts for the carbon protons in the amino acid complexes and in the peptide complexes are listed in Table 2.4 and 3.4 respectively; external reference is TMS.

[b] 60 MHz spectra (internal reference NaTPS).

[c] (S)-amino acids in all complexes

AMINO ACID COMPLEXES

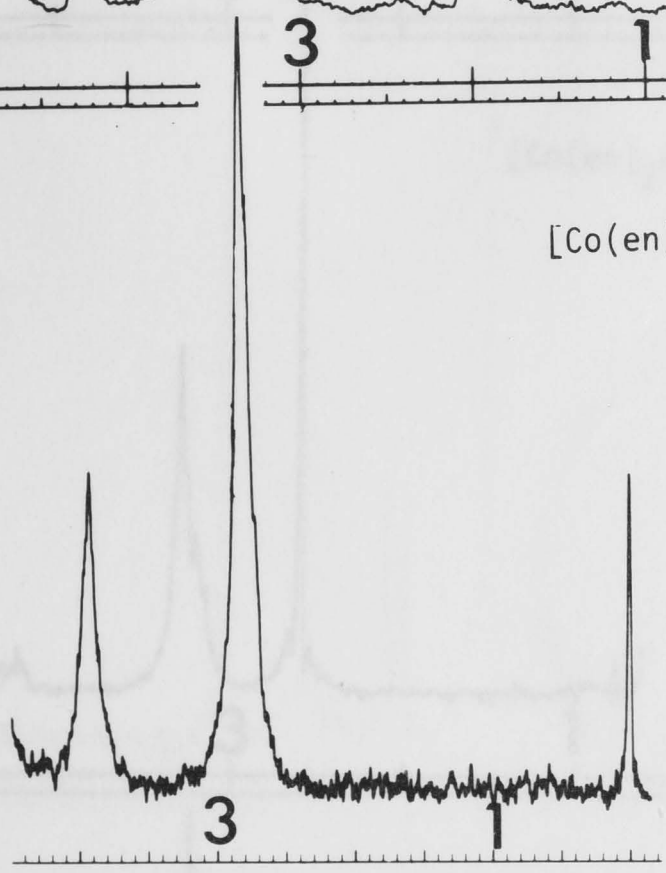
1



$[Co(en)_2thr]^{2+}$

$[Co(en)_2ser]^{2+}$

2



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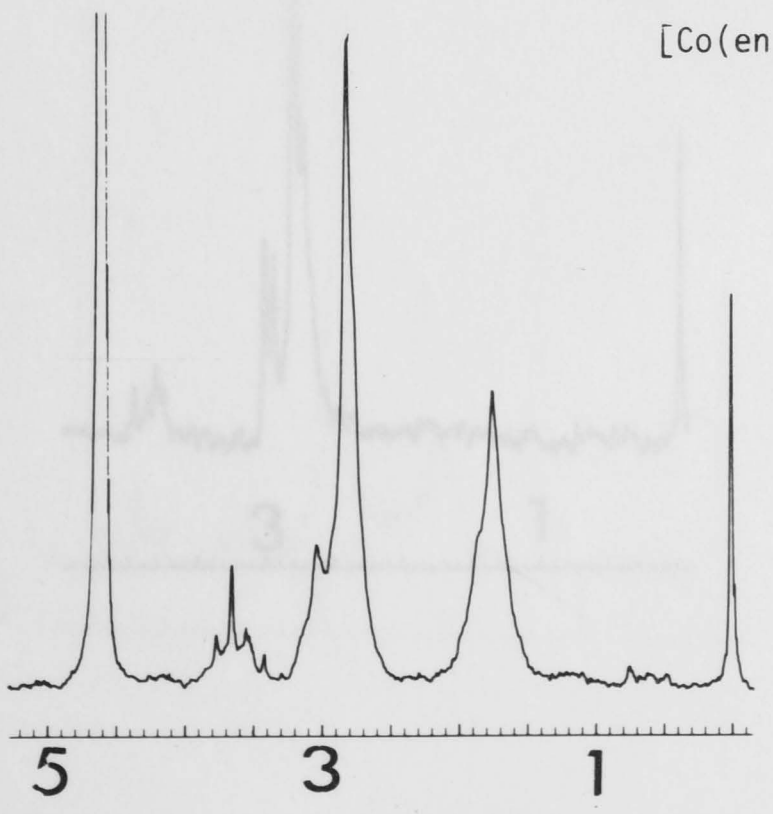

$[Co(en)_2lys]^{2+}$

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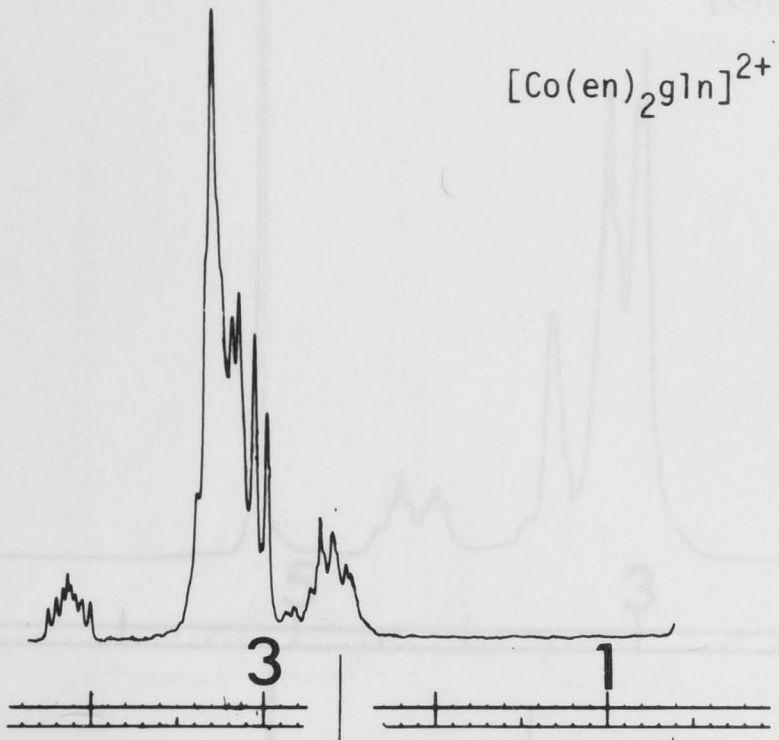

$[Co(en)_2gln]^{2+}$

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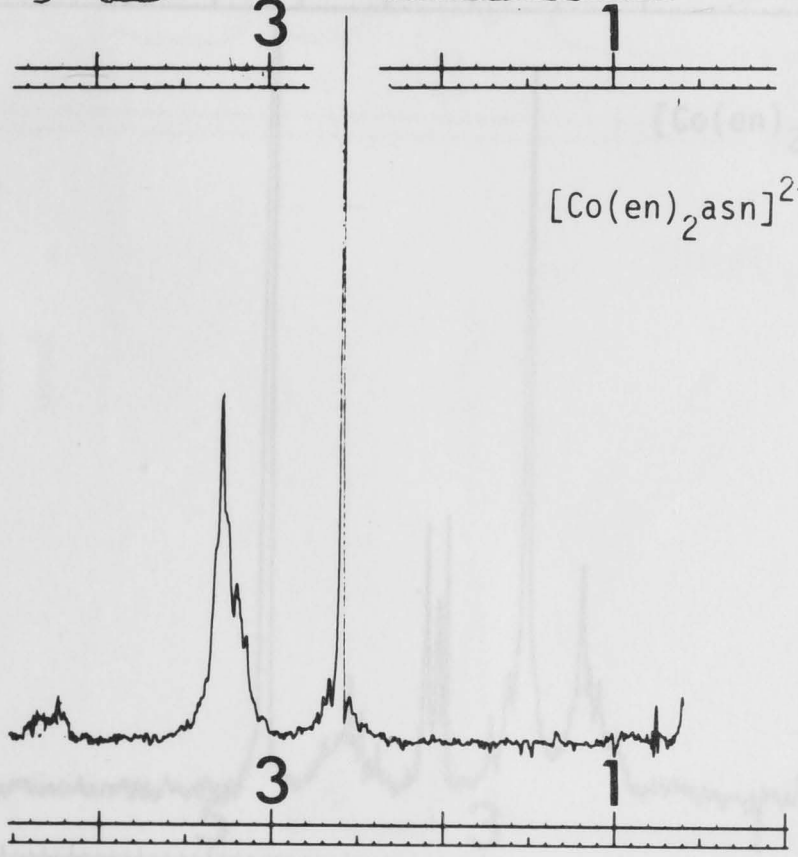

$[Co(en)_2asn]^{2+}$

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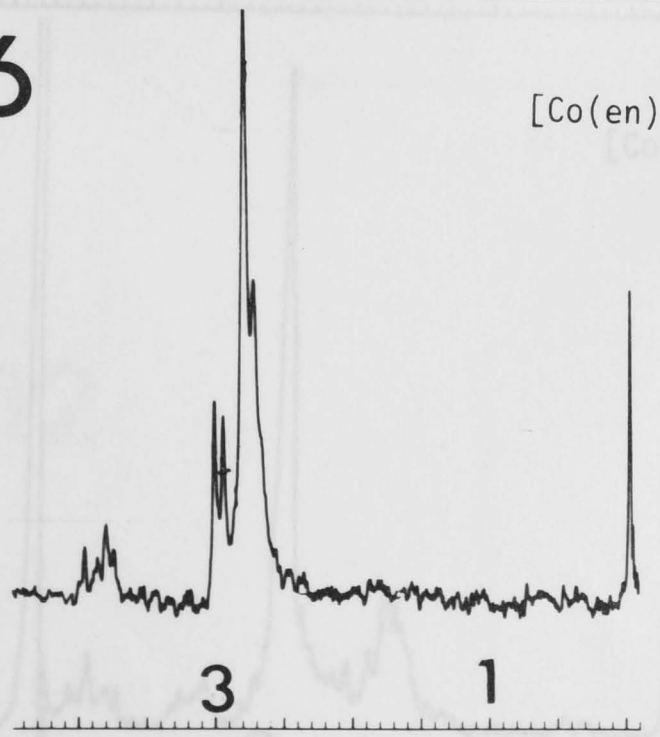

$[Co(en)_2met]^{2+}$

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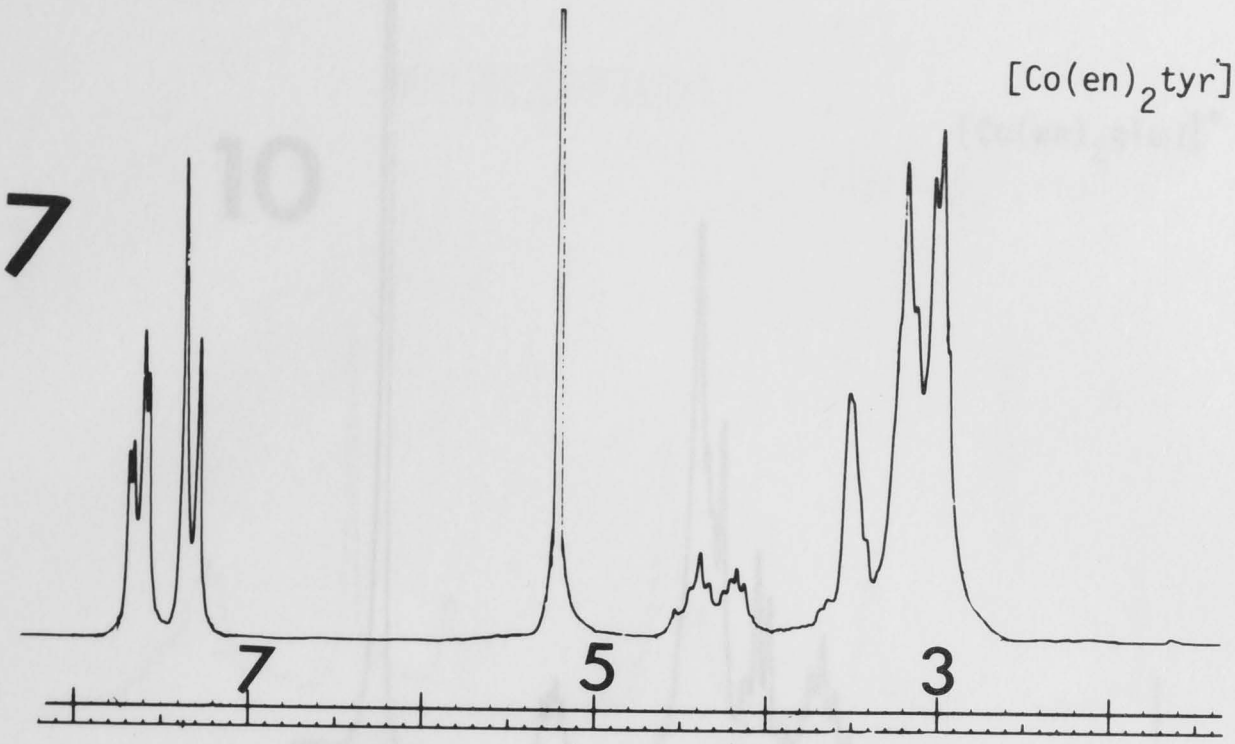

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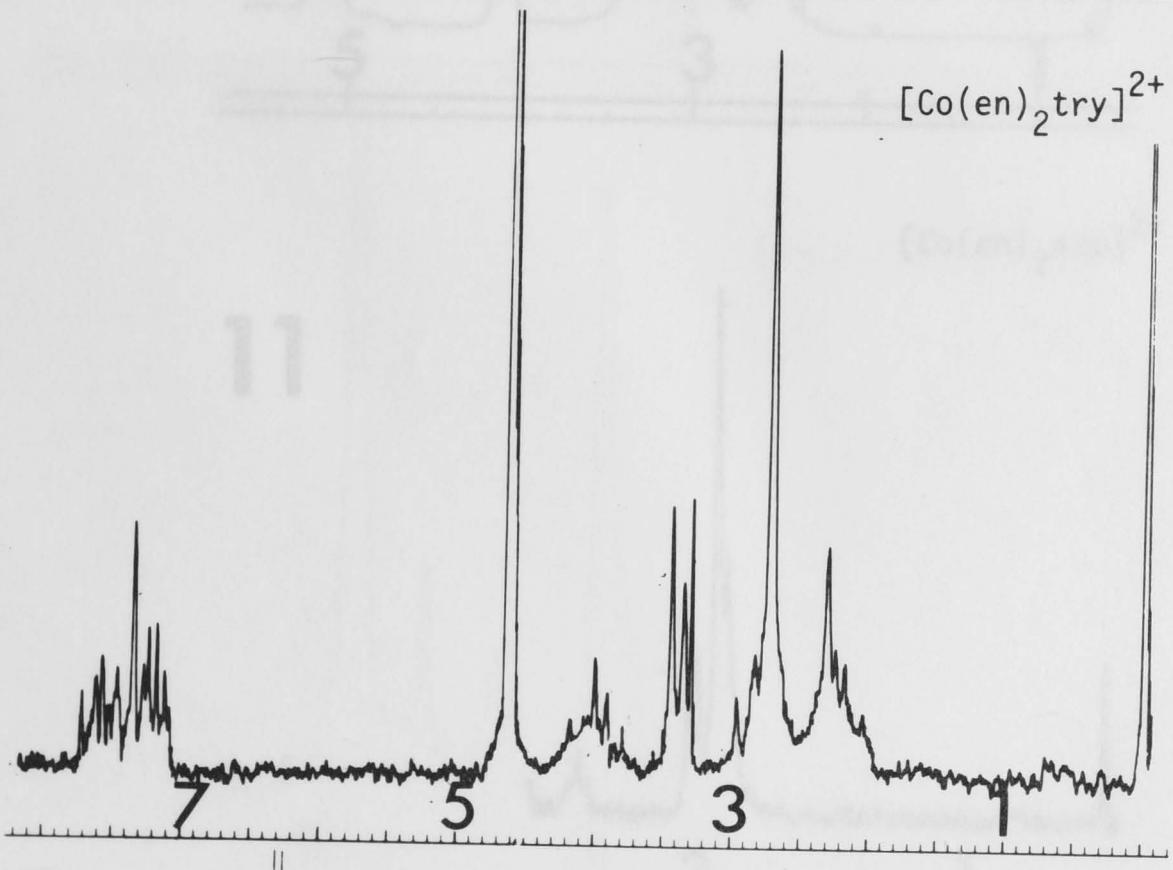

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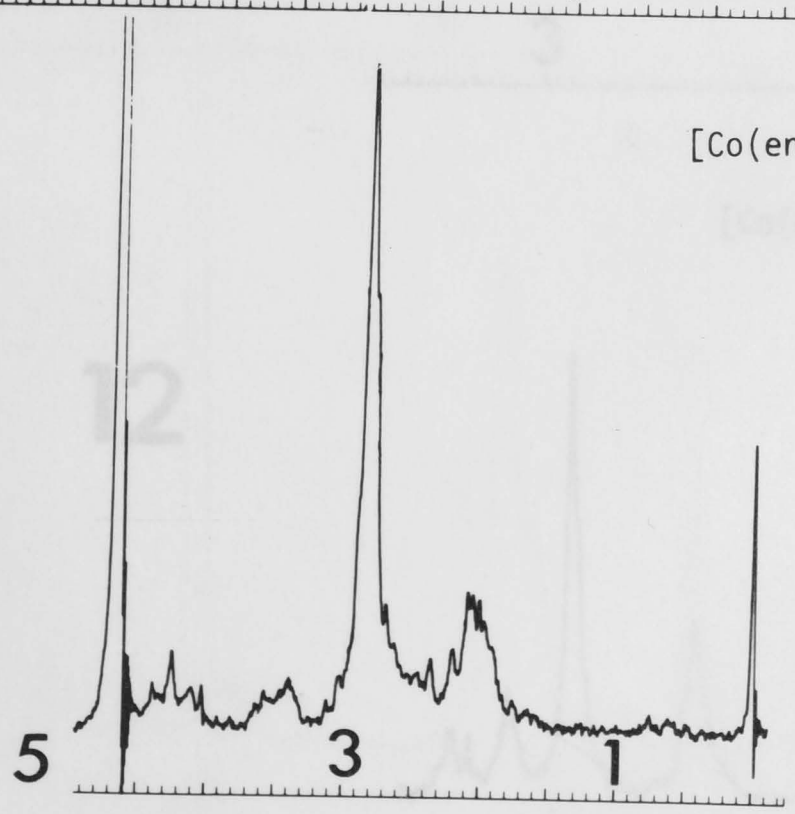
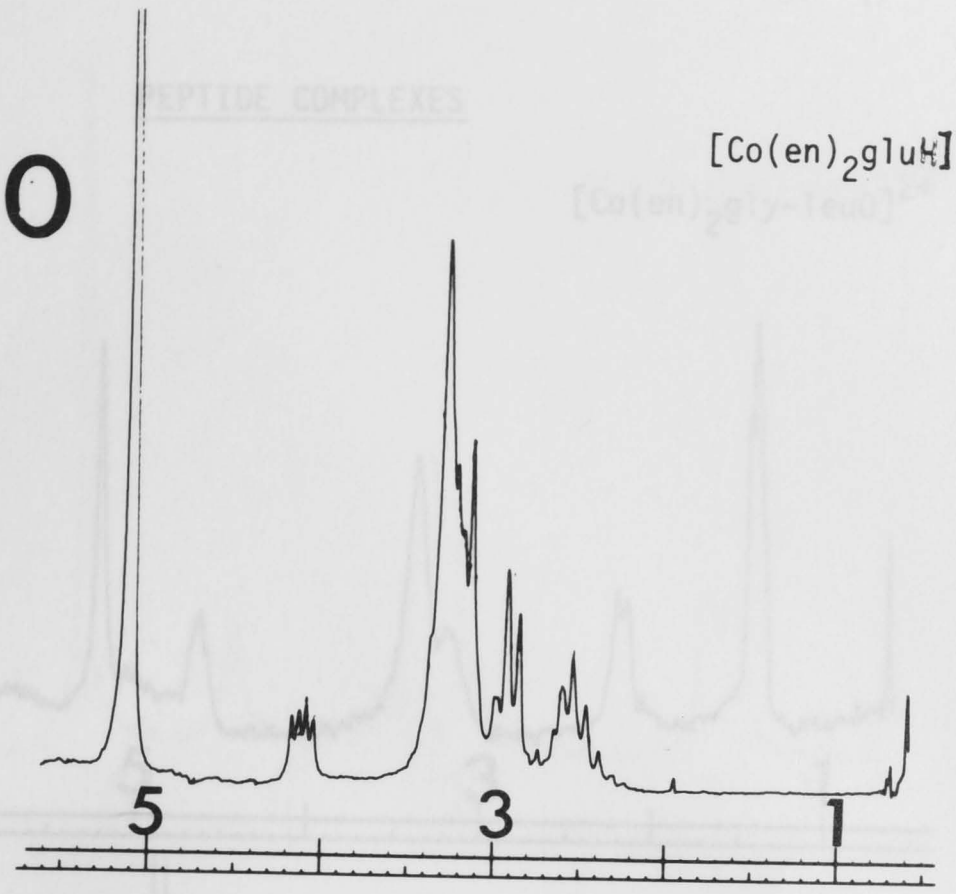


10

$[Co(en)_2gluH]^{+}$



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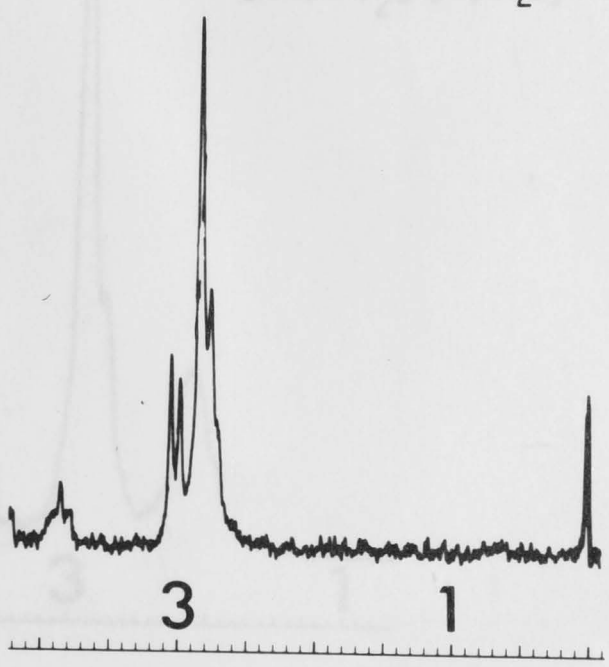
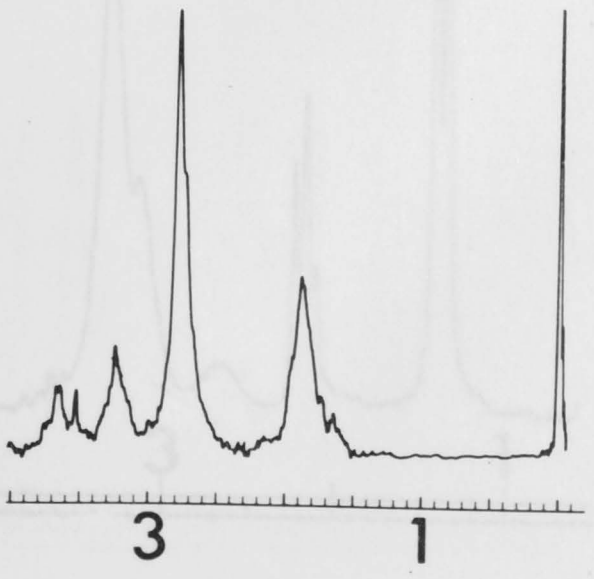
$[Co(en)_2asp]^{2+}$



$[Co(en)_2arg]^{2+}$

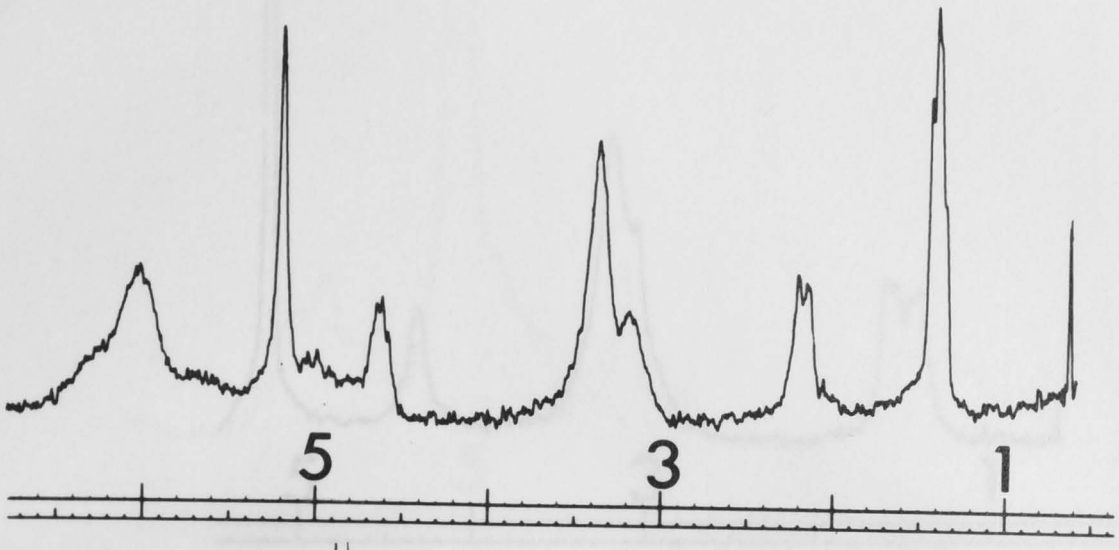
12



PEPTIDE COMPLEXES

1

$[Co(en)_2gly\text{-}leuO]^{2+}$



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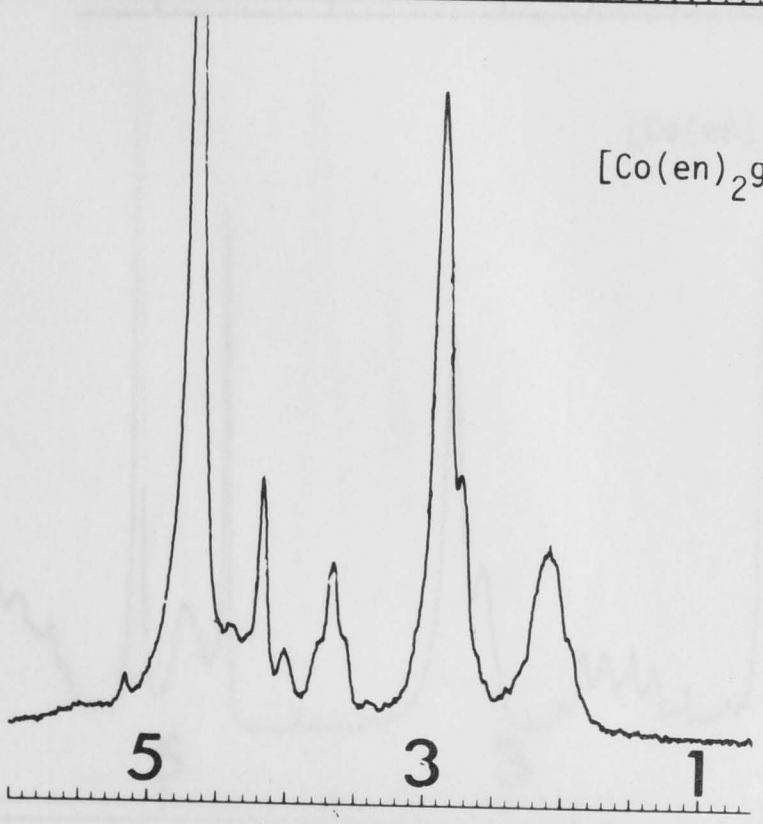
$[Co(en)_2gly\text{-}proO]^{2+}$



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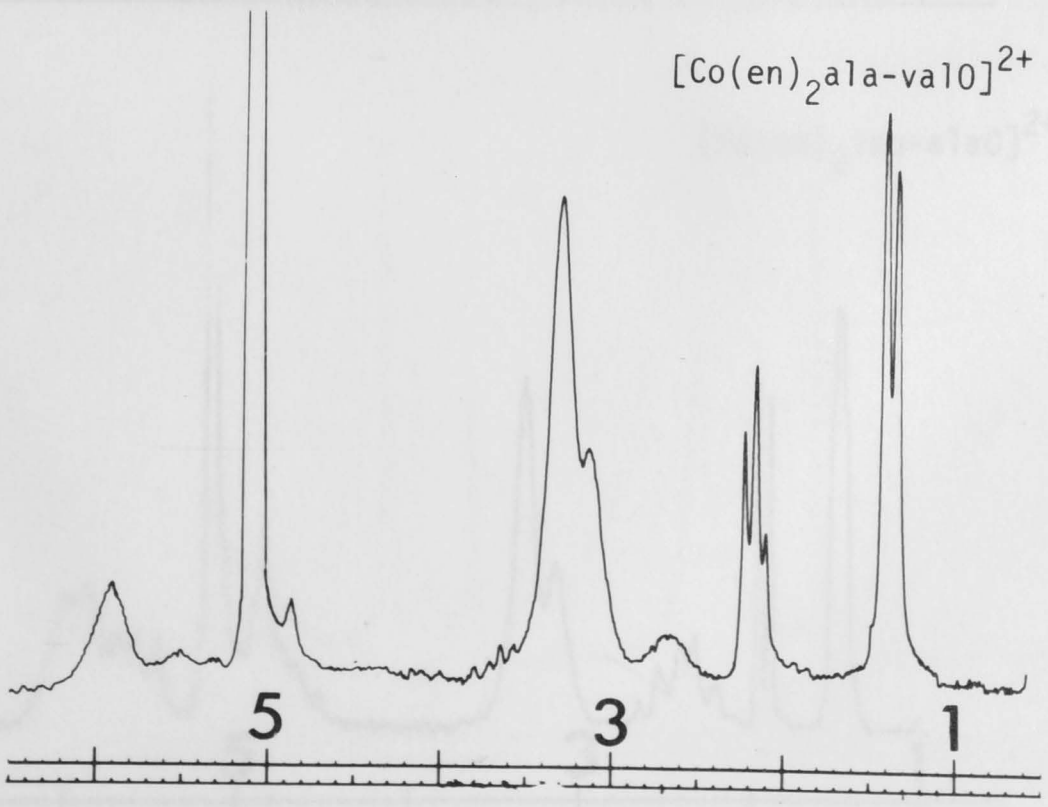
$[Co(en)_2ala\text{-}valO]^{2+}$



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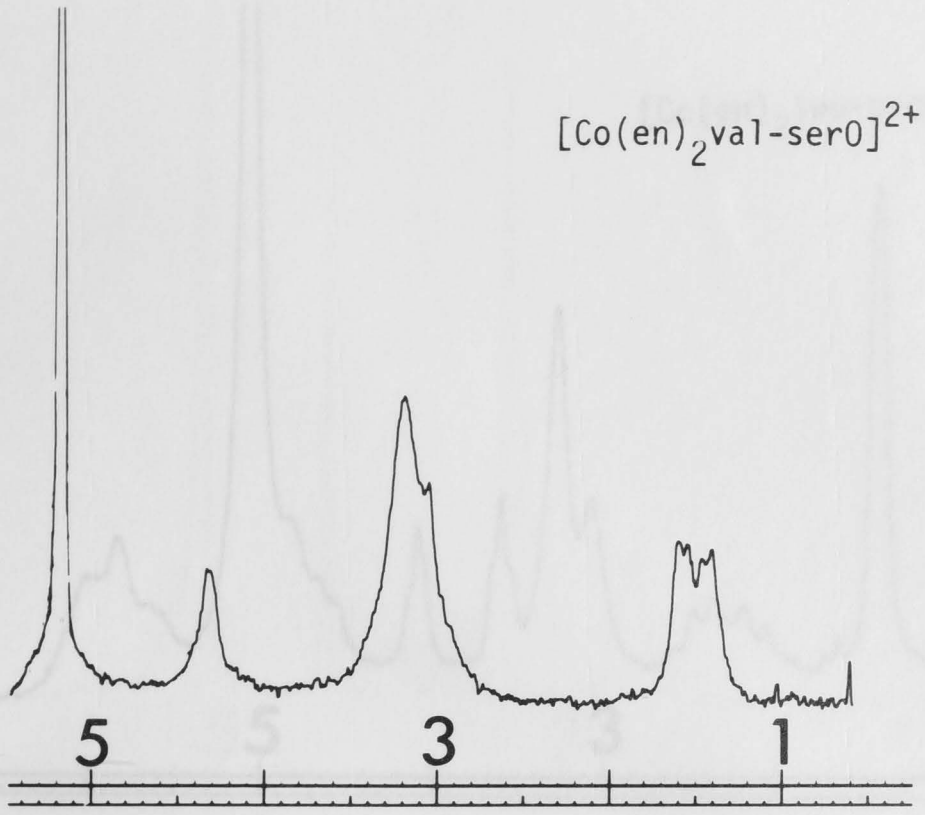

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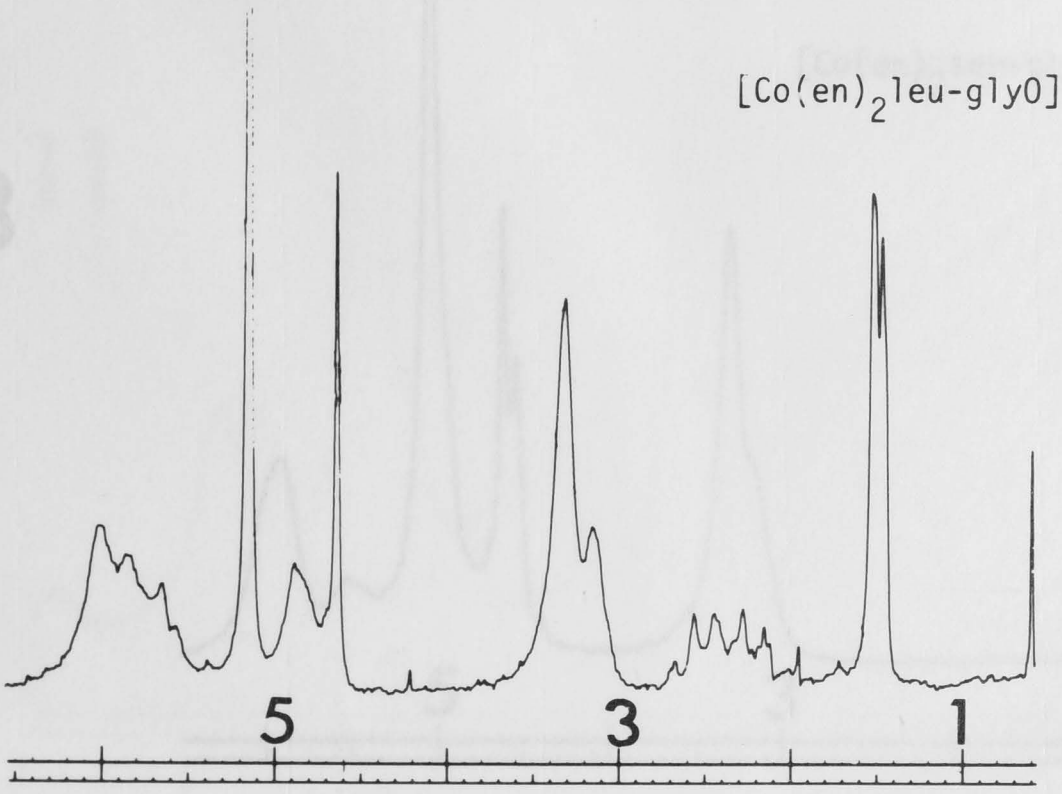

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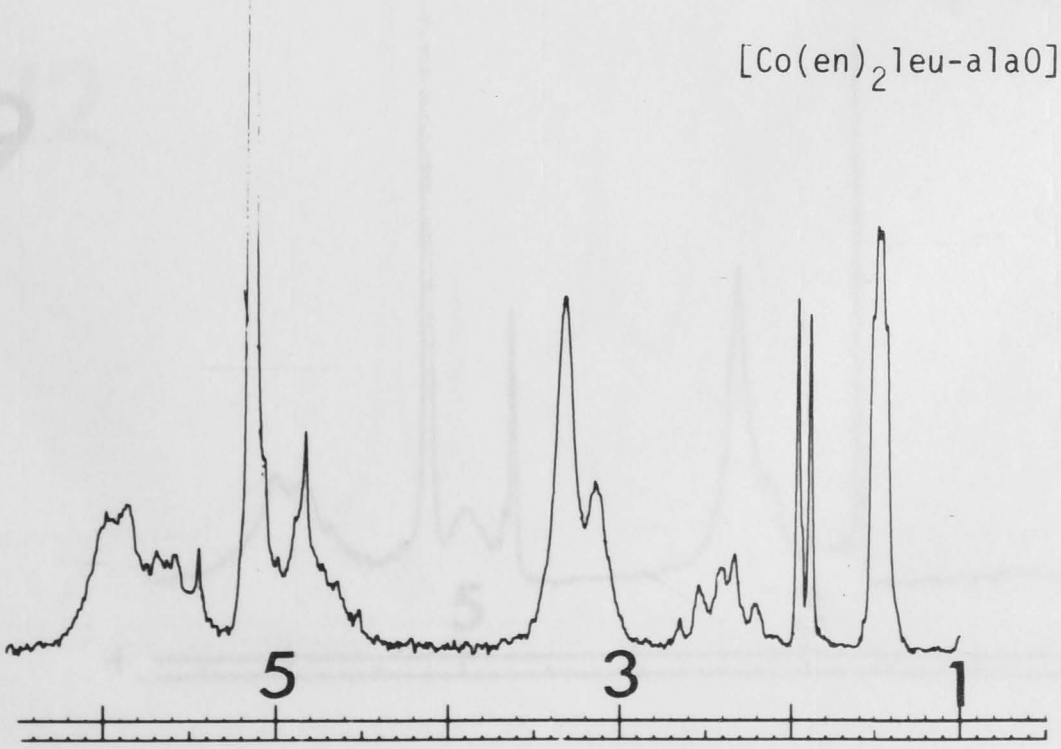

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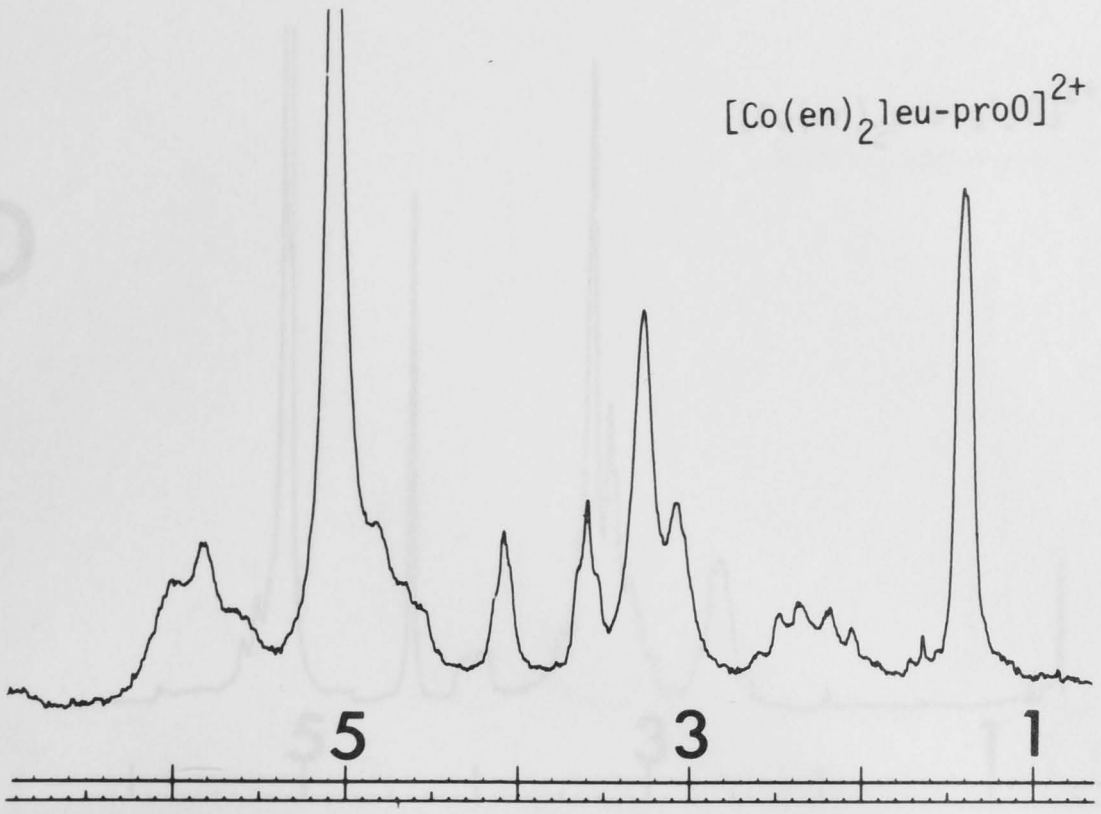

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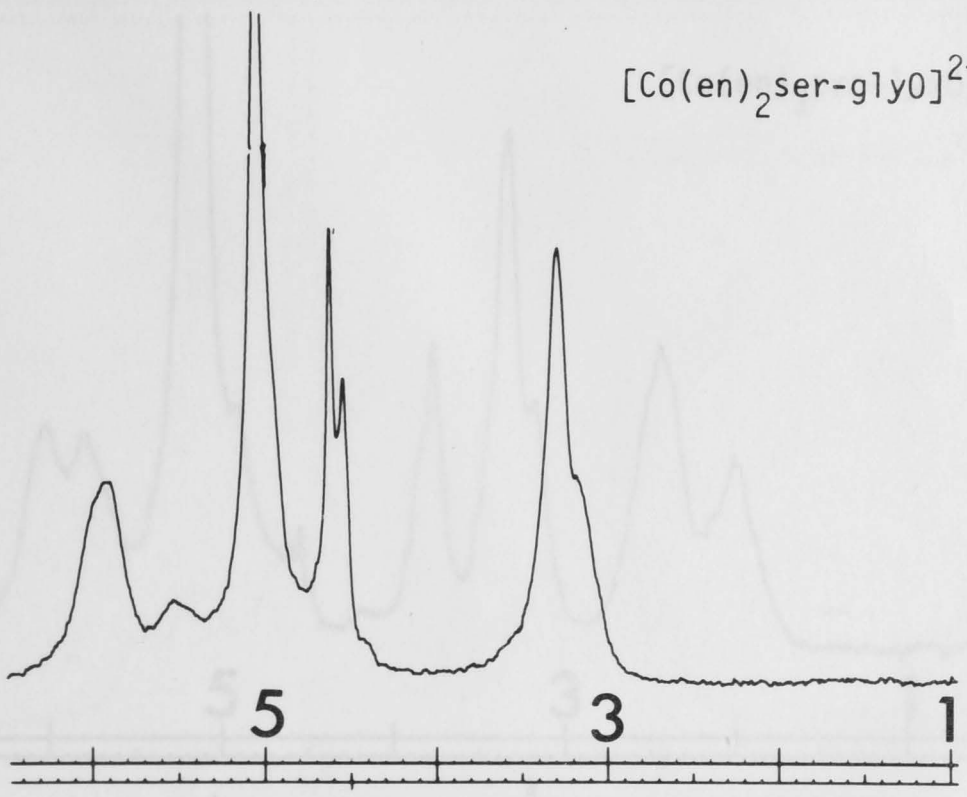

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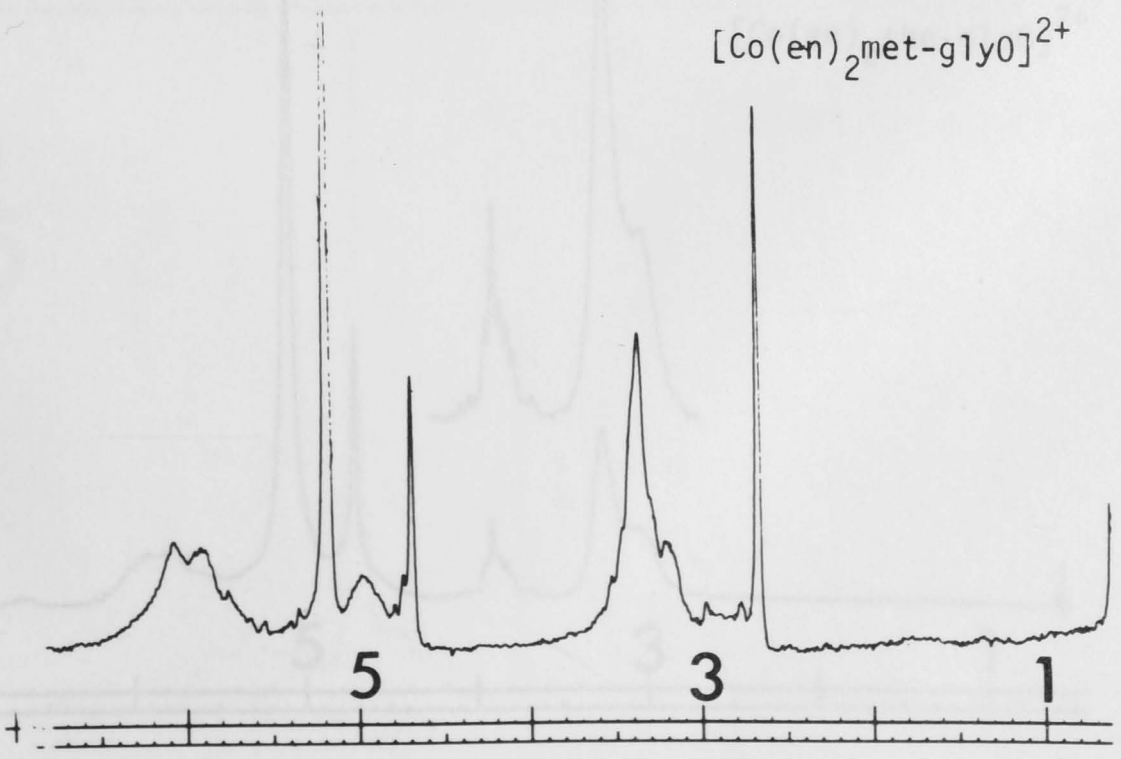

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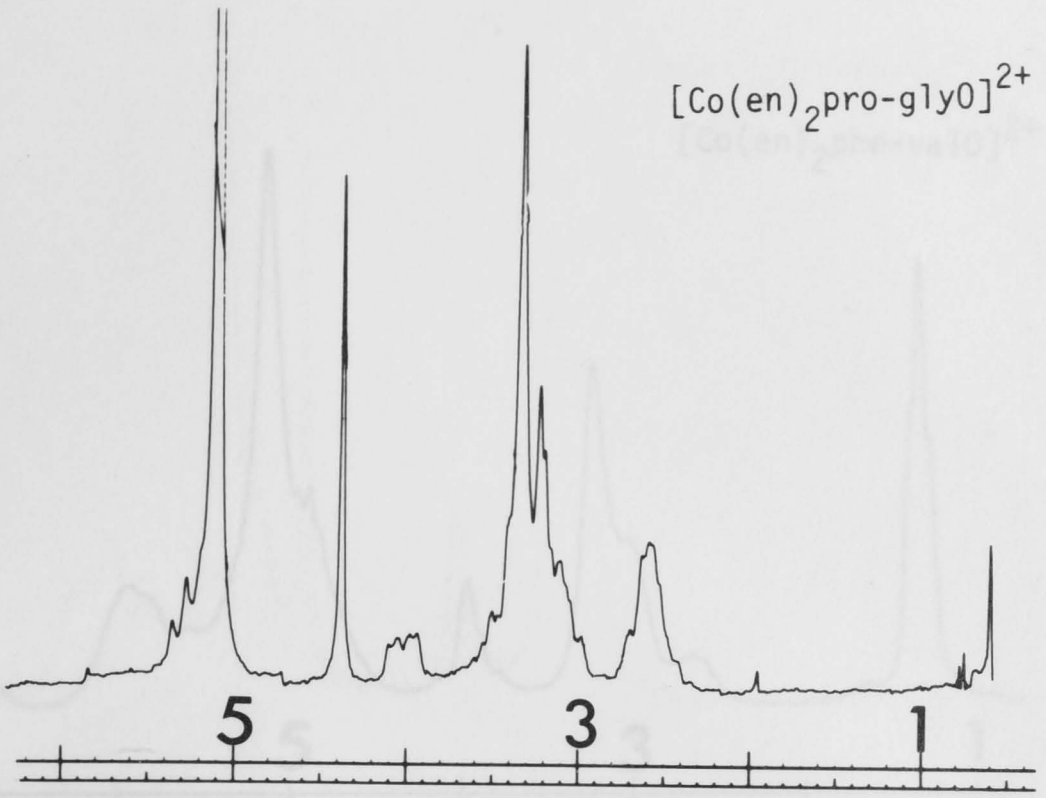

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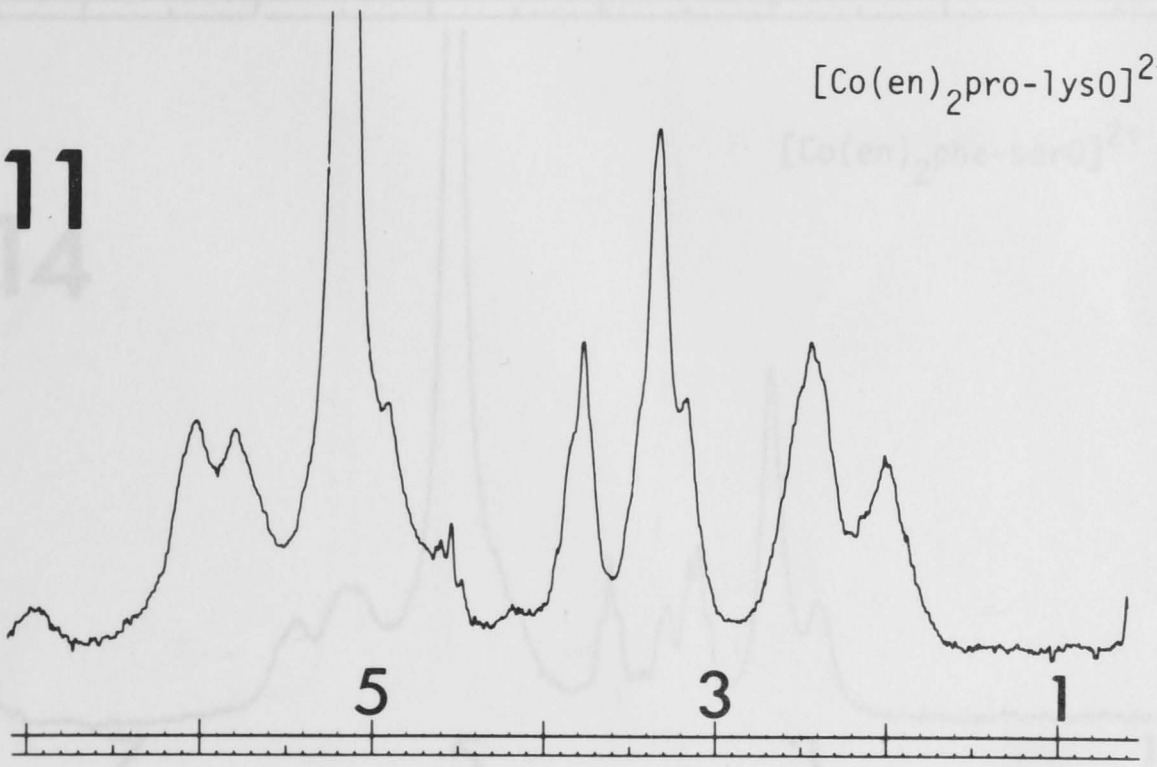

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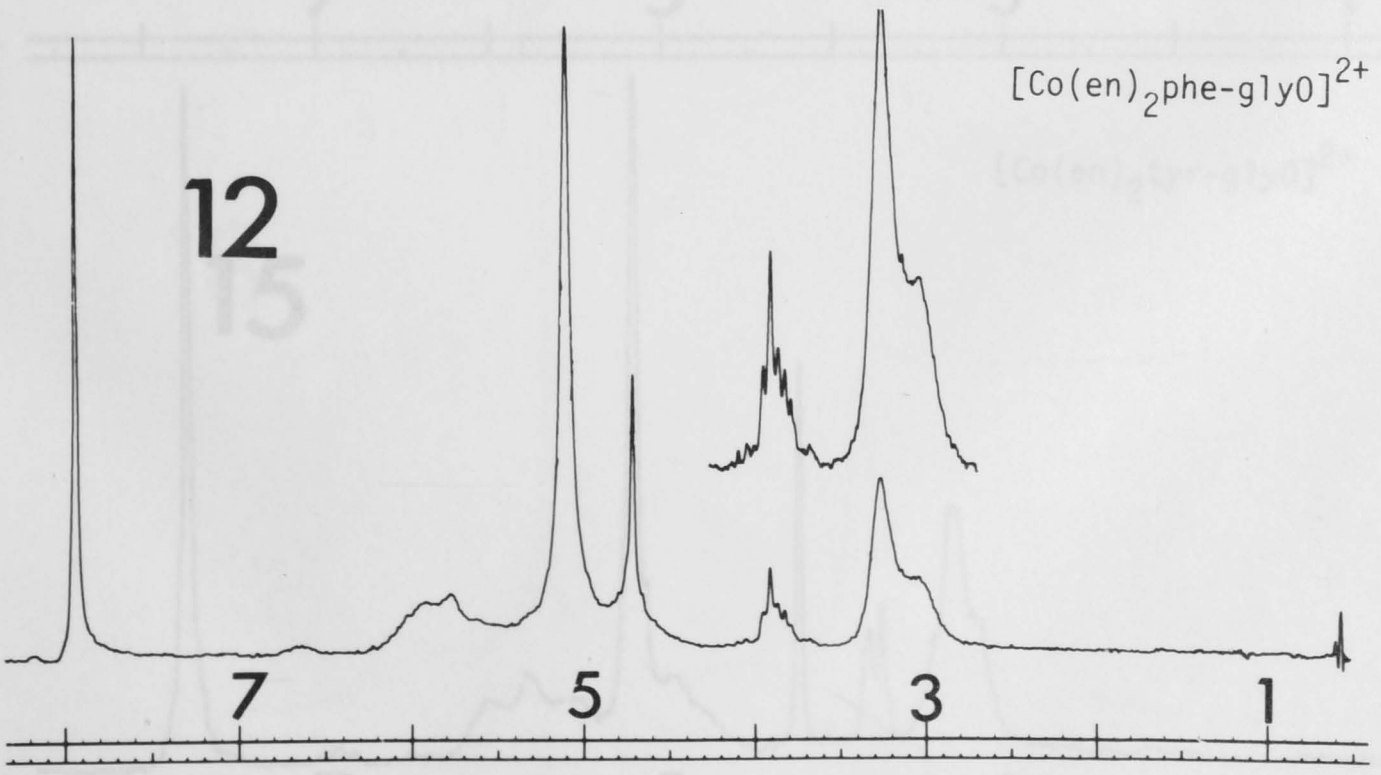
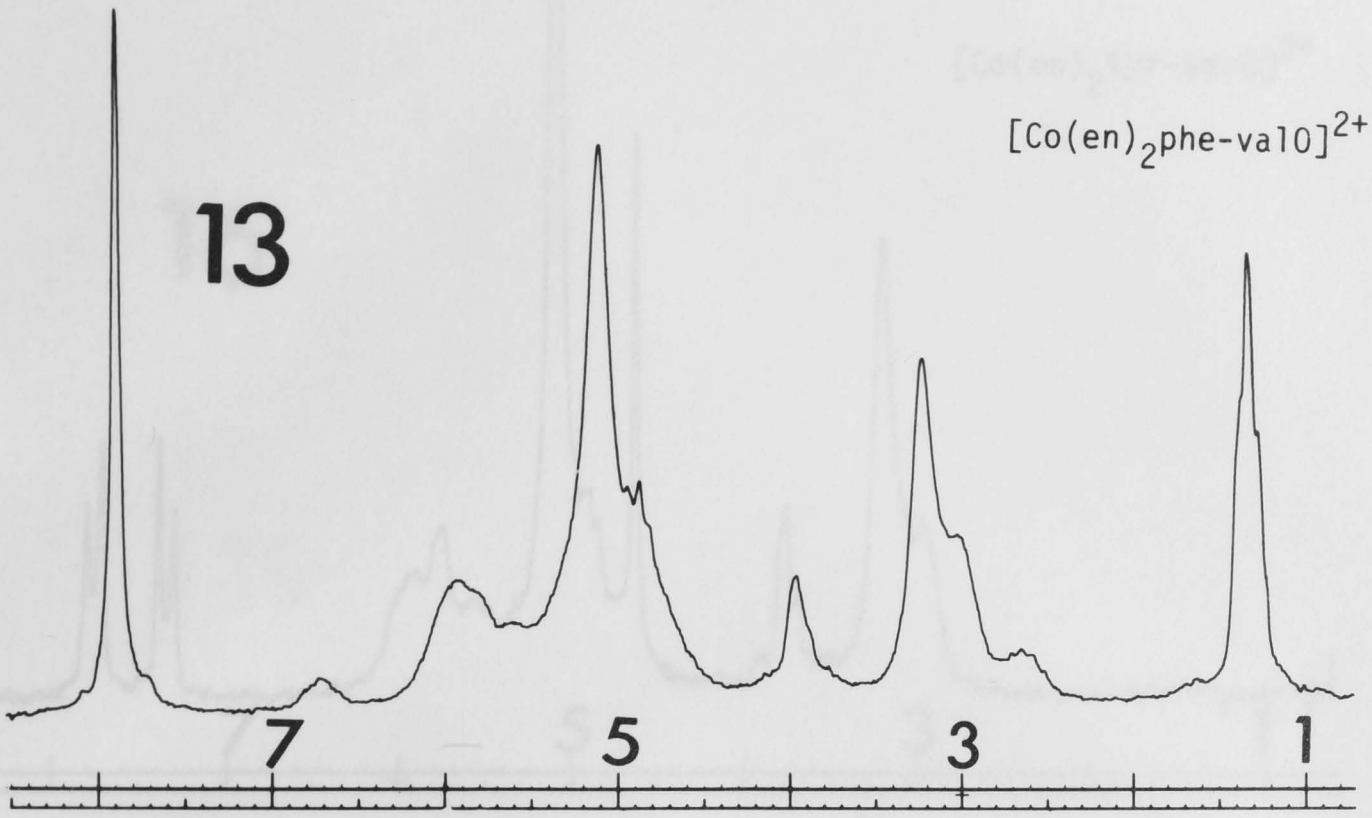


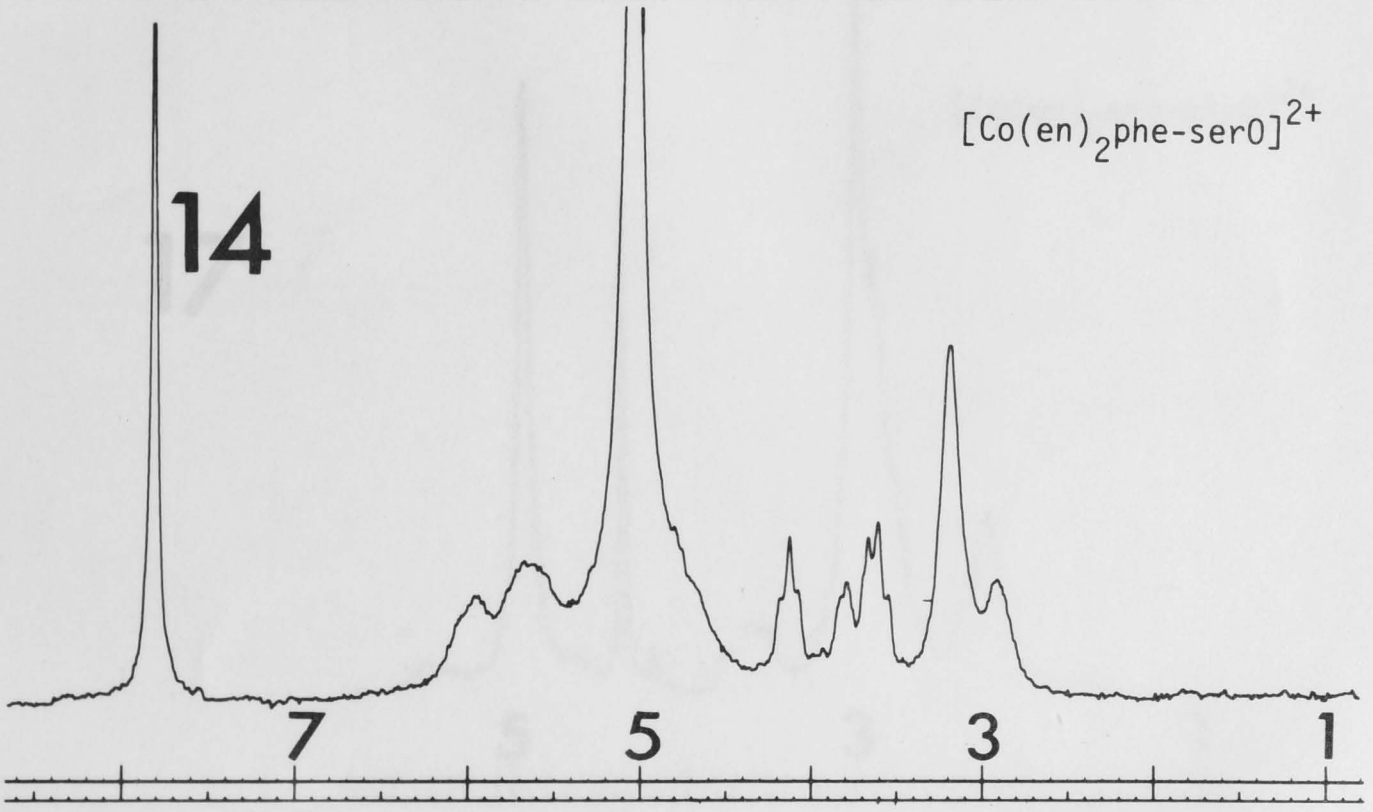
13

$[Co(en)_2phe\text{-}val0]^{2+}$



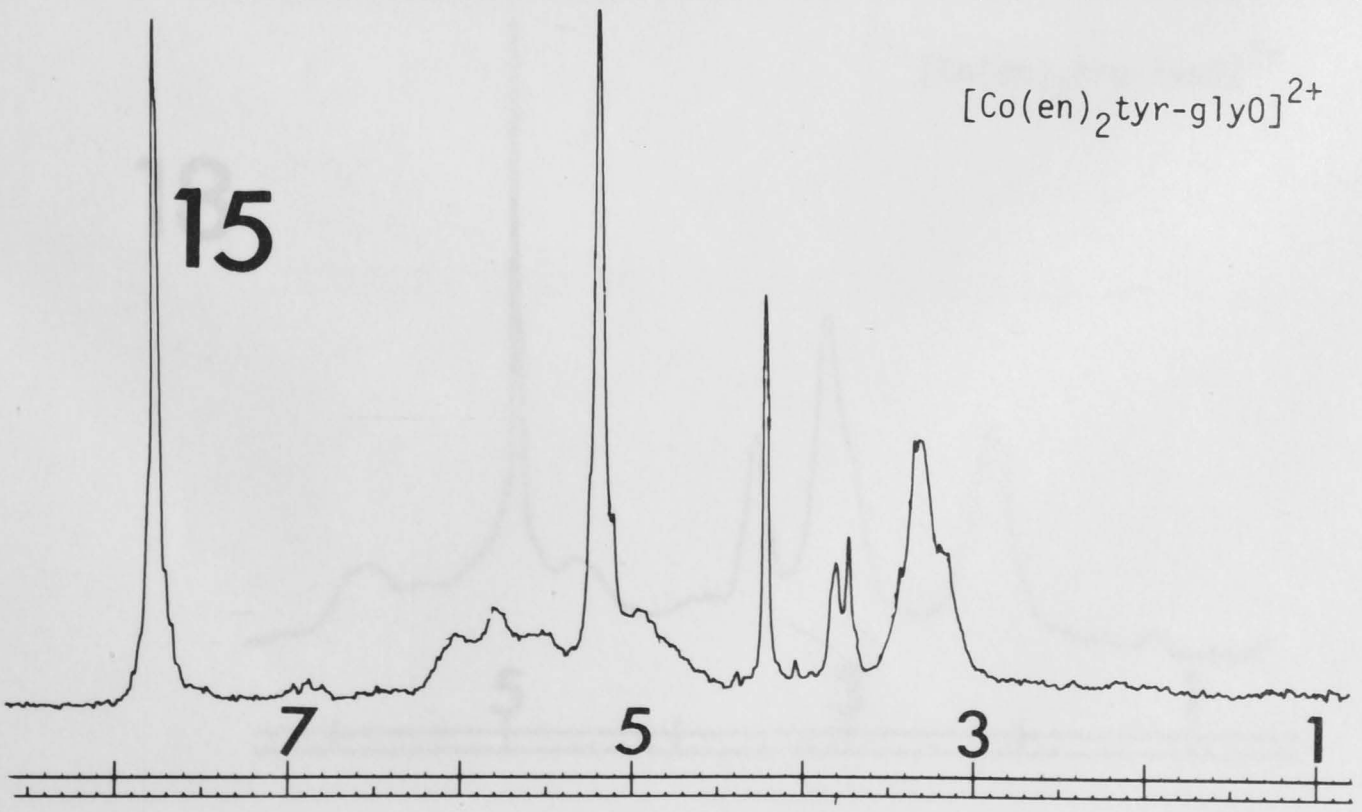
14

$[Co(en)_2phe\text{-}ser0]^{2+}$



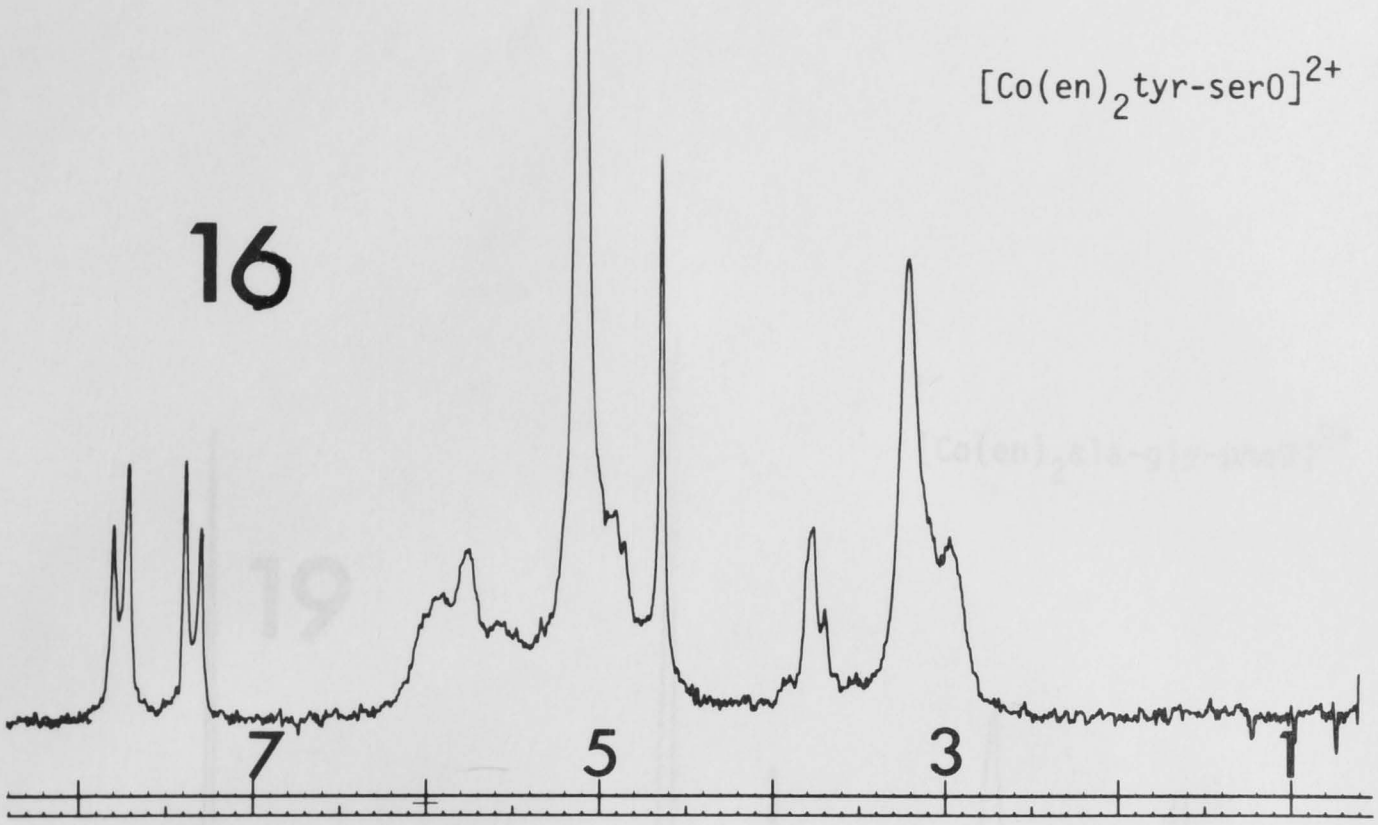
15

$[Co(en)_2tyr\text{-}gly0]^{2+}$



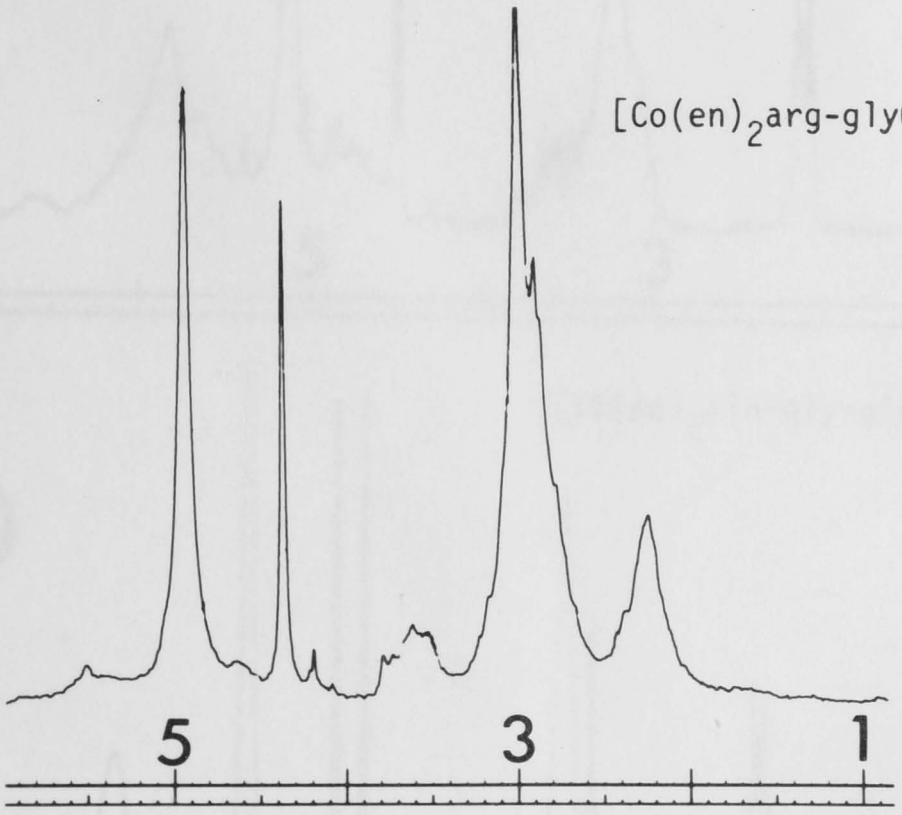
16

$[Co(en)_2 tyr\text{-}serO]^{2+}$



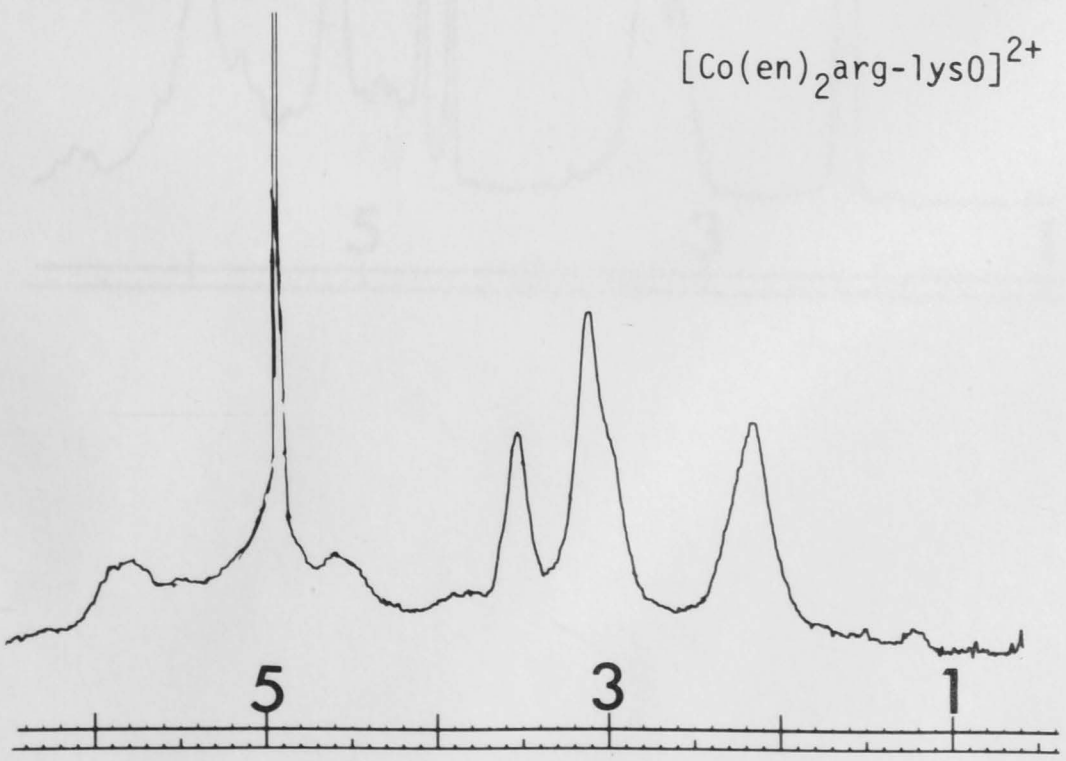
17

$[Co(en)_2 arg\text{-}glyO]^{2+}$

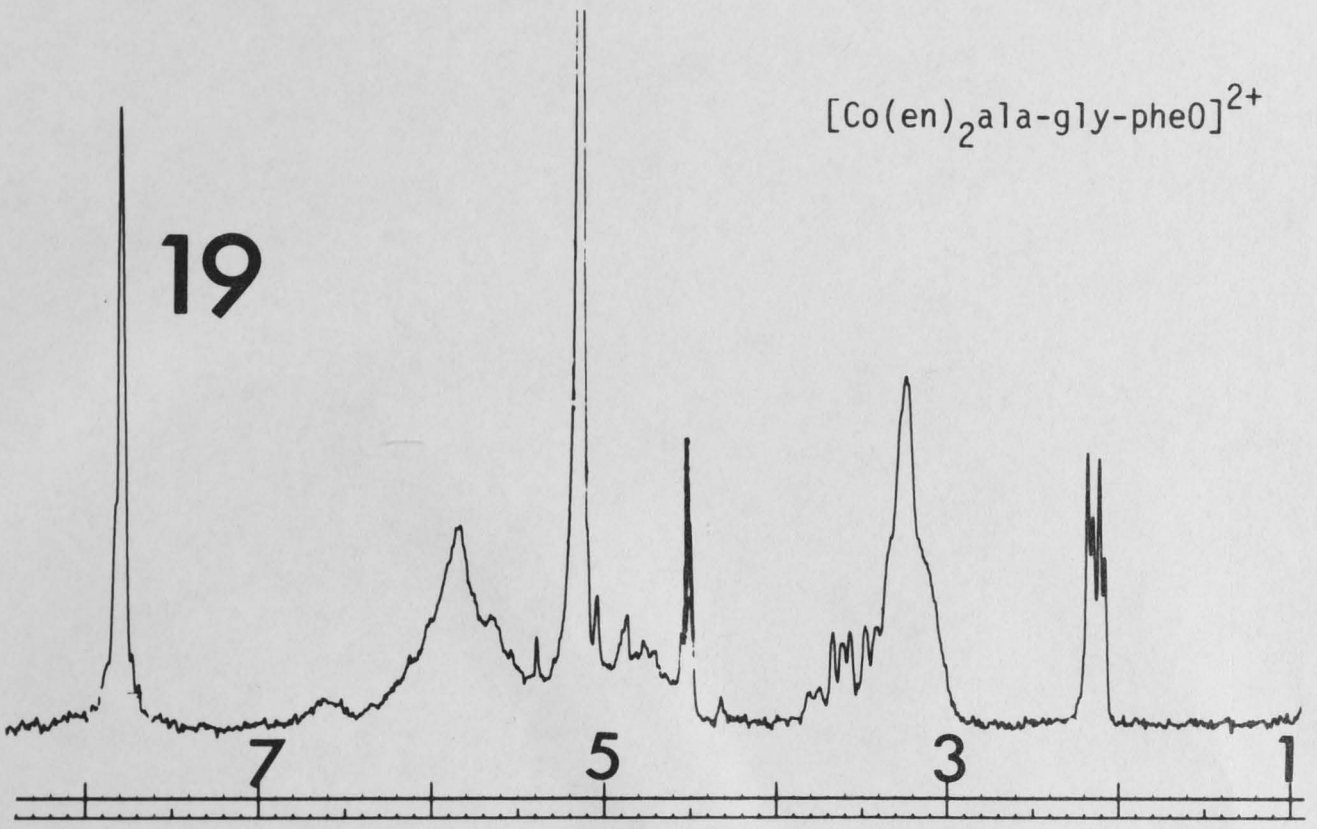


18

$[Co(en)_2 arg\text{-}lysO]^{2+}$



19



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