

THE USE OF METAL CHELATES IN THE RESOLUTION OF AMINO

ACIDS AND IN THE MODIFICATION OF THEIR REACTIVITIES

THESIS *signed K.R. Turnbull*

SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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P R E F A C E

The striking changes in properties and reactivities of ligands after co-ordination to metal ions are now receiving increased attention, and this thesis is concerned with one class of ligand, namely amino acids and their derivatives. One problem confronting the investigation was the difficulty of obtaining both optical forms of amino acids, and a general method of resolving these compounds

Apart from the work described in Chapter One, Section IV, which was performed in collaboration with Dr. J.L. Garnett and Mr. S.W. Law of the University of New South Wales, the work is the candidate's own.

Signed *K.R. Lumbull*

November, 1965

The co-ordination of sarcosine (N-methyl glycine) to a metal ion produces an asymmetric nitrogen atom and gives rise to the possibility of resolving the "quaternary ammonium salt" into two optical forms. The resolution of the complex ion  $[Co(NH_3)_4sarc]^{2+}$  has been achieved and the only asymmetric centre in this molecule is the sarcosine nitrogen. This result is interesting because of the consistent failure of organic chemists to resolve quaternary

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The striking changes in properties and reactivities of ligands after co-ordination to metal ions are now receiving increased attention, and this thesis is concerned with one class of ligand, namely amino acids and their derivatives. One problem confronting the investigation was the difficulty of obtaining both optical forms of amino acids, and a general method of resolving these compounds or their derivatives using a metal complex as resolving agent is described in Chapter One. This procedure is a significant practical achievement as both optical forms are now equally accessible and the methods cover a wide range of amino acids. The optical purity of the resolved isomers was extremely high, and because of this property, the resolution method was used to investigate the mechanism of tritiation of amino acid derivatives. The last part of this work was carried out in collaboration with Dr. J.L. Garnett.

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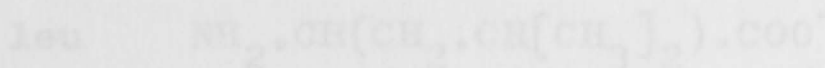
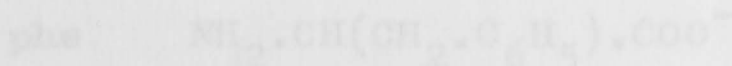
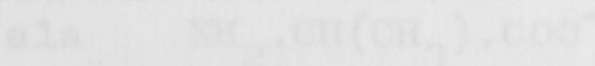
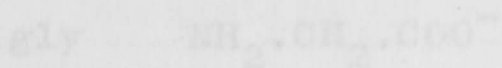
ammonium salts of the form  $[R_1 R_2 R_3 NH]^+$ ; the success in this instance is due to the ability of the metal ion substituent to reduce the rate of dissociation of the proton on the sarcosine nitrogen. The other interesting result in this investigation was the enormous difference between the rate of racemization and the rate of deuteration of the sarcosine  $-NH$ , ( $k_D \sim 4000 k_R$ ). It would appear that the lone pair on the nitrogen helps to preserve the configuration, which does not invert rapidly as is the case in tertiary amines. These results have some relevance to previous efforts to resolve the complex ion  $[Co en_2 sarc]^{2+}$  and are discussed in Chapter Two.

The mechanism of the reaction of co-ordinated glycine with aldehydes and the reactions of co-ordinated Schiff bases of amino acid esters with amines are described in Chapter Three, along with attempts at peptide synthesis. In some of the complexes the amino acid is optically stable and in others racemization occurs; the mechanism for the racemization and the lability of the protons on the  $\alpha$ -carbon atom are discussed, and some observations are made on the stereochemistry of certain Schiff base - amino acid complexes.

This work was supervised by Dr. A.M. Sargeson, Dr. N.S. Gill and Dr. B. Halpern and I am pleased to record my thanks to them for their advice, interest and encouragement.

I thank Mr. S. Brown, Mr. L.B. James and Mr. H. Satrapa for technical assistance with some spectral measurements, and the microanalytical section of the Department of Medical Chemistry for analyses. I am indebted to Miss Pat Campbell for the typing of this thesis and to my wife, Rosemary, for valuable assistance with proof reading.

The financial support provided by a Research Scholarship of the Australian National University is gratefully acknowledged.

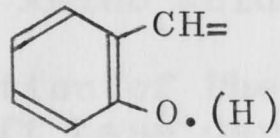


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ABBREVIATIONS

Some abbreviations used in this thesis are:

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IV	en	$\text{NH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{NH}_2$	18
<u>EXPERIMENTAL</u>			
I	ox	$^-\text{OOC} \cdot \text{COO}^-$	25
II	sal		33
IV	gly	$\text{NH}_2 \cdot \text{CH}_2 \cdot \text{COO}^-$	38

			Page
<u>CHAPTER TWO</u>		<u>Resolution of Racemic Amino Acids</u>	
	ala	$\text{NH}_2 \cdot \text{CH}(\text{CH}_3) \cdot \text{COO}^-$	47
	phe	$\text{NH}_2 \cdot \text{CH}(\text{CH}_2 \cdot \text{C}_6\text{H}_5) \cdot \text{COO}^-$	45
	leu	$\text{NH}_2 \cdot \text{CH}(\text{CH}_2 \cdot \text{CH}[\text{CH}_3]_2) \cdot \text{COO}^-$	53
	sarc	$\text{CH}_3 \cdot \text{NH} \cdot \text{CH}_2 \cdot \text{COO}^-$	52
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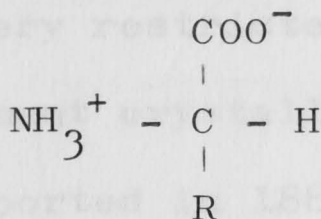
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## CHAPTER ONE

### THE RESOLUTION OF RACEMIC $\alpha$ -AMINO ACIDS

#### INTRODUCTION:

Almost all  $\alpha$ -amino acids may exist in at least two different optically isomeric forms, due to the asymmetry of the molecule



The amino acids found in nature, in the great majority of cases, occur in one form only, usually the -L-configuration; whereas amino acids synthesised chemically from symmetrical compounds are always racemic.

Considerable use is made of optically pure D- and L-amino acids, not only for chemical reactions and physical measurements, but in biochemical and nutrition studies.

Thus considerable effort has been directed to the finding of relatively simple procedures for the separation of racemic amino acids into their optical isomers.

The methods developed are based on principles used by Pasteur for the separation of optical antipodes, and may be broadly classified under the following headings:

- (1) Mechanical separation of crystals which are asymmetric.
- (2) Crystallization methods.
- (3) The action of living organisms or of enzymes which utilize preferentially one isomer or its derivatives.
- (4) Chromatographic adsorption.
- (5) Formation of diastereoisomeric derivatives or salts.

Method (1):

This is of very restricted application and may sometimes be used to supplement crystallization procedures.

Piutti<sup>(1)</sup> reported in 1886 the isolation of D-asparagine from the mother liquor of the crystallization of crude L-asparagine. Hand picking of the D- crystals helped him to obtain optically pure D-isomer.

Method (2):

More recent examples of selective crystallization involve addition of a relatively small amount of crystals of one optical isomer to a supersaturated solution of the racemate. On carefully controlled cooling a quantity of the seeded isomer may be obtained. The filtrate will now be supersaturated with the opposite form, and seeding with crystals of that form may promote its precipitation. This cycle can be repeated after addition of more racemate. A resolution of glutamic acid in this way has been reported<sup>(2)</sup>. In another laboratory resolution of aspartic acid by seeding was unsuccessful, but a good separation was obtained when aqueous solutions of aspartic acid co-ordinated with copper(II) yielded  $\text{Cu}[\text{Cu}(\text{D- or L-aspartate})_2]$  after inocula-

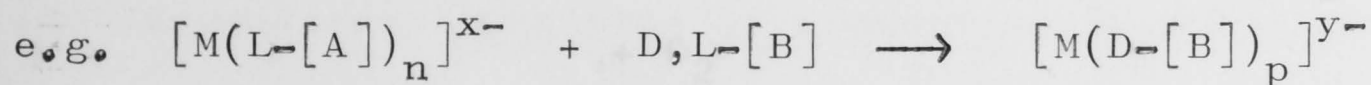
tion with the appropriate crystalline complex<sup>(3)</sup>. Optically active aspartic acid was isolated from each compound.

Although 74% yields of each isomer of the free amino acid were finally obtained, a large number of crystallizations was necessary to achieve this.

One of the authors, Harada, has since reported the successful resolution, by inoculation of free D,L-aspartic acid (as well as D,L-asparagine, D,L-glutamic acid and D,L-glutamine)<sup>(4)</sup> from aqueous solutions containing 20-45% ammonium formate.

Such methods of resolution are dependent upon the relative solubilities of optically active and racemic forms and are not generally applicable.

A rather different technique described as stereoselective ligand exchange has been recently reported. The essence of this method is the reaction of a metal complex of an optical isomer of amino acid [A] with racemic amino acid [B] to yield the metal complex of one isomer of [B].



Shibata and co-workers<sup>(5)</sup> reacted racemic alanine with the ion  $[Co(L\text{-aspartate})_2]^-$  in aqueous alkaline solution.

The compound  $[Co(\text{alanine})_3] \cdot H_2O$  precipitated out, and from it was obtained partly resolved D-alanine of up to 35% optical purity.

Harada<sup>(6)</sup> has similarly reacted racemic aspartic acid with the copper complexes of D- and L- alanine, D- and L-

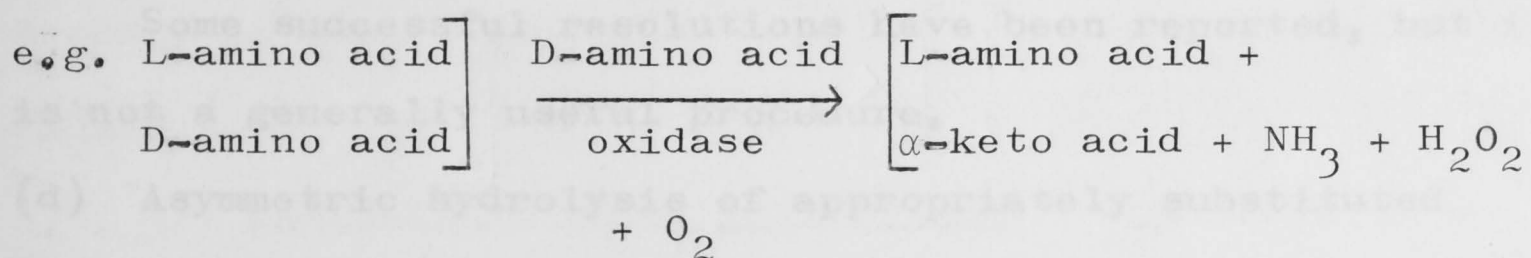
glutamic acid and L-proline to obtain D- and L-aspartic acids having optical purities of 95-100% in yields of up to 50% of the theoretical quantity.

Method (3):

Biological methods may be grouped into four main classes.

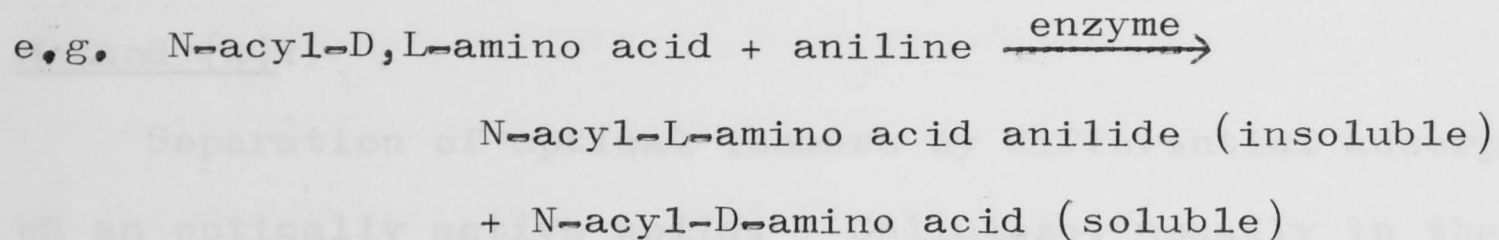
(a) Use of the whole animal, i.e. feeding or injection of a racemic amino acid followed by isolation from the urine of one of the optical antipodes, e.g. after subcutaneous injection of D,L-histidine to rabbits, relatively pure D-histidine was isolated<sup>(7)</sup>. The method is of limited use only, as a resolution procedure.

(b) Asymmetric oxidation or decarboxylation through the action of micro-organisms or tissue fractions, whereby one enantiomorph is unaffected and the other is metabolized to either the corresponding  $\alpha$ -keto acid or amine.



Some micro-organisms may show optical specificity towards some amino acids but not towards others<sup>(8)</sup>. Best results have been obtained with D-amino acid oxidase of mammalian kidney and the L-amino acid oxidase of snake venom, both of which show no measurable attack on the opposite form<sup>(9)</sup>. Disadvantages of this method are that some amino acids react only slowly and some not at all, and that one isomer is always destroyed.

(c) Asymmetric synthesis through the catalytic action of a protease on N-acylated racemic amino acids; one antipode reacts with a base to give an insoluble derivative, while the other antipode remains unreacted in solution.



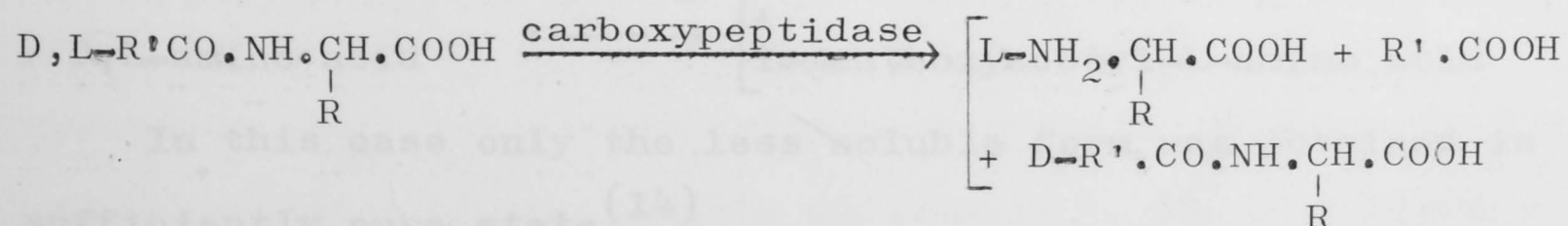
The anilide formed must be sufficiently insoluble for the reaction to go to completion otherwise the soluble D-component will not be pure.

Other factors affect the reaction rate and the separation, e.g. pH and concentration of buffer. Variation of the acyl group may impair the optical specificity of the reaction<sup>(10,11)</sup>. Severe conditions often necessary for hydrolysis to the free amino acid can cause appreciable racemisation.

Some successful resolutions have been reported, but it is not a generally useful procedure.

(d) Asymmetric hydrolysis of appropriately substituted racemic amino acids, in which only the L-antipode is hydrolysed to the free amino acid.

Usually acylases or amidases are used for the removal of N-acyl or amide groups respectively, e.g.



The products may be separated either by differential solu-

bility or by the use of ion exchange resins. Both isomers are obtainable. With a few exceptions this method has been used for the resolution of most of the amino acids, and it may be termed the most useful of the biological procedures.

Method (4):

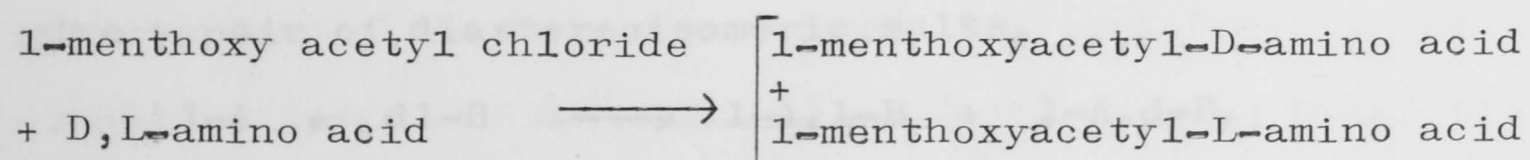
Separation of optical isomers by differential adsorption on an optically active solid. Cellulose, usually in the form of chromatographic paper has been the usual adsorbent. Practically only within the last decade have any successful separations been reported, and to date these involve only a relatively few amino acids or their derivatives<sup>(12)</sup>.

Roberts and Haigh<sup>(13)</sup> have reported the preparation of a synthetic optically active column (actually an ion exchange resin) and its ability to partially resolve D,L-methionine.

In principle the method is an excellent one, since an ideal separation would allow quantitative recovery of both isomers in a pure state.

Method (5):

(a) Diastereoisomeric derivatives: Treatment of a racemic amino acid with an optically active reagent to give diastereoisomeric derivatives which can then be separated by fractional crystallisation, e.g.



In this case only the less soluble form was obtained in sufficiently pure state<sup>(14)</sup>.

Only a few resolutions by this procedure are known. In

a recent application of this method, however, gas chromatography has been used to separate the diastereoisomeric derivatives. Weygand et al.<sup>(15)</sup> reported a method for the investigation of racemization occurring during peptide synthesis. They demonstrated that separation of many diastereoisomeric Trifluoroacetyl-dipeptide methyl esters was possible by gas chromatography. Halpern and Westley<sup>(16)</sup> have applied this technique to the separation of amino acid mixtures. Treatment of the amino acid methyl esters with the resolving agent, N-trifluoroacetyl-L-prolyl chloride produced the diastereoisomeric peptides, N-trifluoroacetyl-L-prolyl-L-amino acid methyl ester and N-trifluoroacetyl-L-prolyl-D-amino acid methyl ester, which were separated on a gas chromatographic column. More recently, they have reported that  $\alpha$ -halogeno-acyl chlorides, in particular L- $\alpha$ -chloroisovaleryl chloride, yield more suitable diastereoisomers<sup>(17)</sup>.

(b) Diastereoisomeric salts: Two salts are formed on treatment of a racemic acid with an optically active base. These are diastereoisomers and may be separated by differential solubility,



Similarly an optically active acid and a racemic base give a pair of diastereoisomeric salts,



Treatment of the separated salts with a suitable reagent will allow isolation of the individual isomers. In 1899 Fischer achieved the first successful resolution of amino acids (as



their N-benzoyl derivatives) using the alkaloid bases brucine and strychnine as resolving agents. The majority of resolutions reported have used N-acylated amino acids, which behave essentially as acids, and so are able to form salts with optically active bases. Some have used amino acid esters or amides able to form salts with optically active acids. Several of the amino acids which are bases (lysine) or acids (aspartic acid) in the free state have been resolved directly<sup>(18,19)</sup>.

In most cases the separation of a pair of diastereoisomeric salts is not ideal. The less soluble salt can usually be freed from the more soluble salt by recrystallisation; however, the more soluble derivative remaining in the mother liquor is contaminated with a small amount of the less soluble material and therefore is difficult to obtain pure, since recrystallisation is not very effective in removing small amounts of an insoluble material from a soluble material.

In 1896, Marckwald<sup>(20)</sup> pointed out that the salts dA.1B and 1A.dB as well as 1A.1B and dA.dB are enantiomorphous and possess the same solubility. Hence if an acid (d1A) is treated with optically active base (1B), the less soluble diastereoisomer (say dA.1B) can be obtained pure. The partially resolved but impure acid, obtained from the more soluble diastereoisomer (1A.1B) can then be recombined with the optical antipode of the original active base (dB). The salt (1A.dB) is now less soluble than its diastereoisomer (dA.dB) and therefore can be obtained pure. In order to

accomplish resolution by Marckwald's method it is necessary to have both d- and l- forms of the resolving agent in the optically pure state.

Most resolutions of amino acids have used as resolving agents the naturally occurring bases, such as brucine, strychnine and quinine, which occur in one form only. More recently several synthetic resolving agents, having both l- and d- isomers, have been used<sup>(21,22)</sup>.

There have also been reports of the resolution of one amino acid derivative by a different derivative of another amino acid, e.g. the resolution of N-acetyl-D,L-tryptophan by L-leucine<sup>(23)</sup>, and the resolution of N-benzoyl aspartic acid by L-leucine ethyl ester or L-leucinamide<sup>(24)</sup>.

The work reported here concerns the successful resolution of a number of amino acid derivatives by optical isomers of metal complex cations.

#### SECTION 1:

##### Neutral Amino Acids:

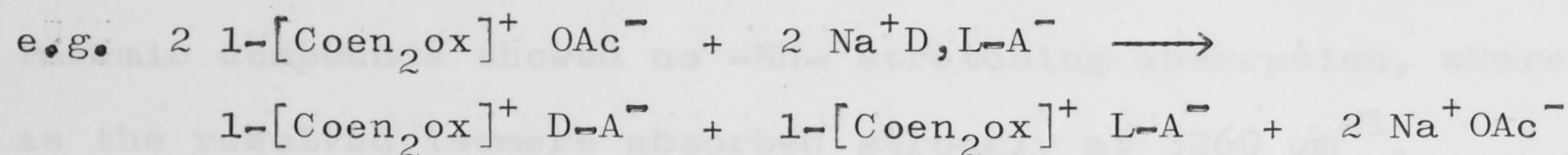
The resolving agents used were the cis-dinitrobis(ethylenediamine) cobalt(III) ion, d- and l-  $[\text{Coen}_2(\text{NO}_2)_2]^+$ , and the oxalatobis(ethylenediamine) cobalt(III) ion, d- and l-  $[\text{Coen}_2\text{ox}]^+$ , both of which are easily prepared and readily resolved to give both enantiomorphs.<sup>(25,26)</sup> Both cations showed satisfactory optical and chemical stabilities under the conditions used here.

Resolution of the free amino acids could not be performed since the diastereoisomers were too soluble.

The benzoyl, tosyl or phthaloyl derivatives were found to be suitable, in that they formed diastereoisomers of which the less soluble forms precipitated from solutions of moderate concentration.

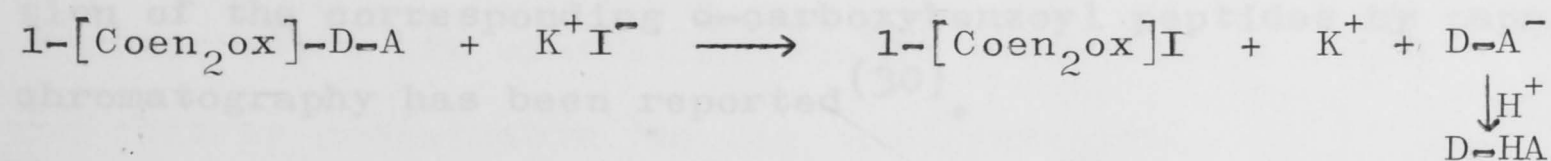
These derivatives were readily prepared by standard methods for such compounds. (27,28,29)

The resolutions were carried out by mixing aqueous solutions of the metal complex acetates with sodium salt solutions of the amino acid derivatives. (The lithium salt of tosyl tryptophan was used, as the sodium salt was insufficiently soluble.) One diastereoisomer precipitated rapidly from solution according to the following equation:



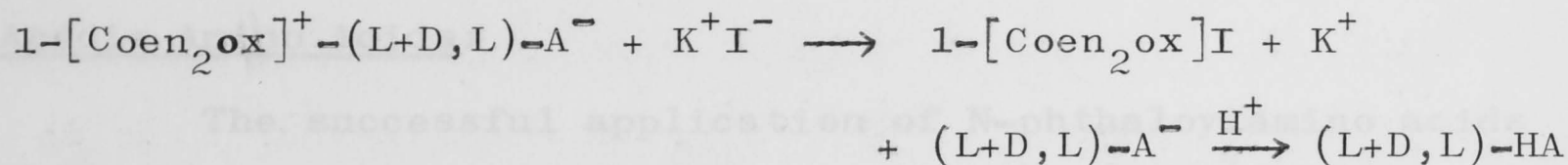
The diastereoisomeric pairs showed widely different solubilities and a sharp separation of isomers was obtained. One recrystallisation was usually sufficient to purify the precipitated salt.

The resolved amino acid derivative was recovered by slurring the diastereoisomer in water with KI, when the insoluble complex iodide was precipitated. After acidification of the filtrate the amino acid derivative usually crystallised.



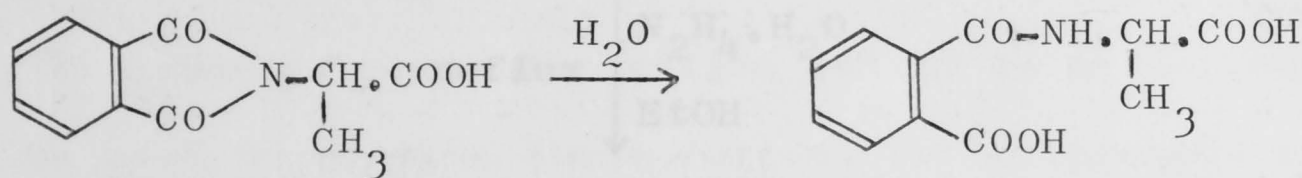
(Several of the resolved tosyl derivatives appeared as oils which required further purification before they solidified.)

From the solution containing the more soluble diastereoisomer the partly resolved amino acid derivative was isolated by acidification, after removal of the resolving agent as its iodide, e.g.



This crude isomer could be obtained optically pure by repeating the resolution procedure with the antipode of the original resolving agent, (*d*-[Coen<sub>2</sub>ox]OAc).

The phthaloyl derivatives of alanine and phenylalanine were resolved but the products were identified as the *o*-carboxybenzoyl derivatives. Infra-red spectra of the racemic compounds showed no  $\text{-NH-}$  stretching absorption, whereas the resolved isomers absorbed strongly at  $3260 \text{ cm}^{-1}$ .



This ring opening is almost certainly due to the slightly alkaline conditions during the resolution procedure (although  $\text{NaHCO}_3$  was used instead of  $\text{NaOH}$  for the preparation of the sodium salt solutions of the racemic phthaloyl derivatives in order to minimize alkalinity during dissolution). The alkali sensitivity of phthaloyl peptides and the identification of the corresponding *o*-carboxybenzoyl peptides by paper chromatography has been reported<sup>(30)</sup>.

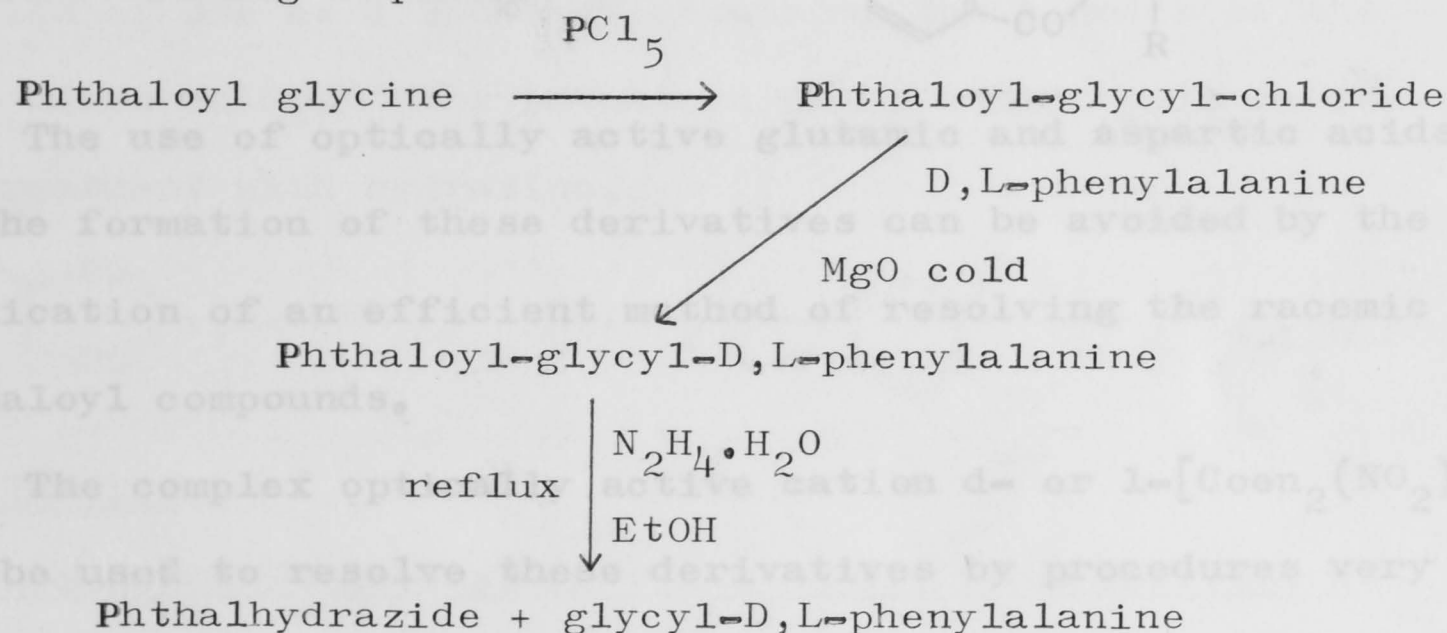
One of the resolved amino acid derivatives, benzoyl-L-alanine was hydrolysed by refluxing in 6N HCl. The isolated

L-alanine was assayed biochemically with D-amino oxidase<sup>(31)</sup> by Professor A.H. Ennor and Dr. H. Rosenberg, and was found to contain less than one part in 500 of the D-antipode.

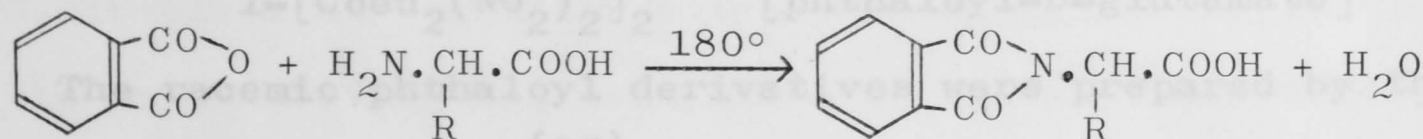
## SECTION II:

### Acidic Amino Acids:

The successful application of N-phthaloylamino acids to synthetic peptide procedures was reported by Kidd and King<sup>(32)</sup> in 1948 and independently by Sheehan and Frank<sup>(33)</sup> in 1949, e.g. the latter prepared glycyl-D,L-phenylalanine by the following sequence:



In a number of cases the phthaloylamino acid can be prepared in good yield by direct fusion of phthalic anhydride with the free amino acid<sup>(29)</sup>.

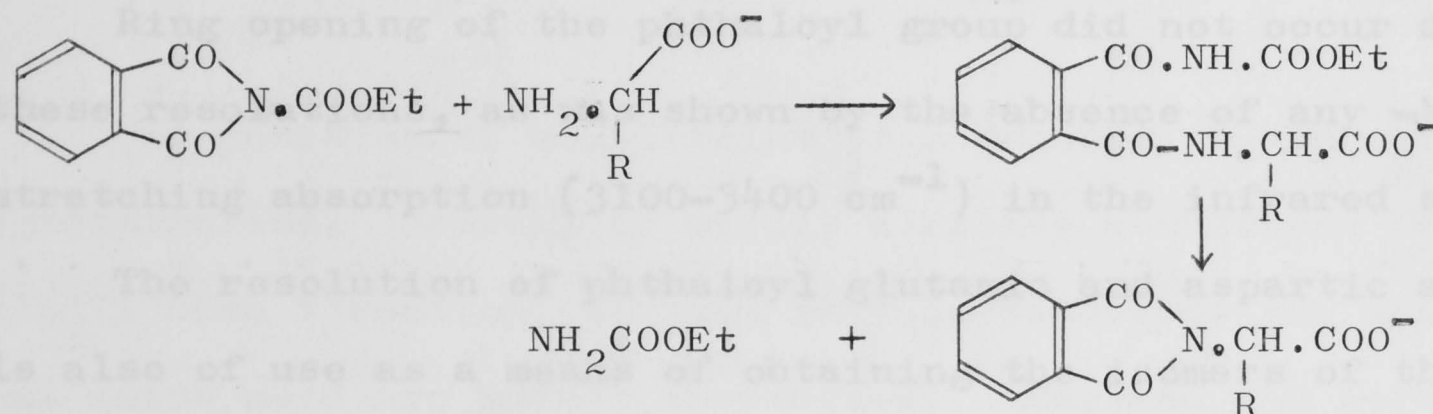


Unfortunately when the optically active glutamic and aspartic acids are used considerable racemization occurs<sup>(34)</sup>, and this is undesirable for peptide synthesis.

Kidd and King<sup>(35)</sup> and Balenovic<sup>(36)</sup> devised alternative routes for these optically active phthaloyl derivatives

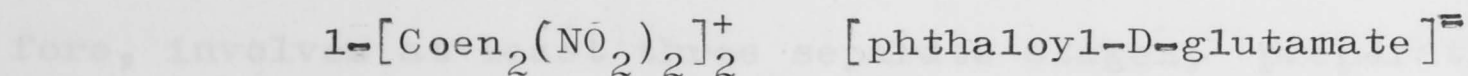
but their methods were long.

Finally Nefkens<sup>(37)</sup> showed that N-carboethoxyphthalimide has excellent properties for the phthaloylation of amino acids under mild conditions; but yields of phthaloyl-L-glutamic acid were only 65%.



The use of optically active glutamic and aspartic acids in the formation of these derivatives can be avoided by the application of an efficient method of resolving the racemic phthaloyl compounds.

The complex optically active cation  $d-$  or  $l-$   $[\text{Coen}_2(\text{NO}_2)_2]^+$  can be used to resolve these derivatives by procedures very similar to those used for the neutral amino acid derivatives. Because these phthaloyl derivatives are dibasic the diastereoisomeric salts have formulae of the type,



The racemic phthaloyl derivatives were prepared by the method of King and Kidd<sup>(35)</sup>.

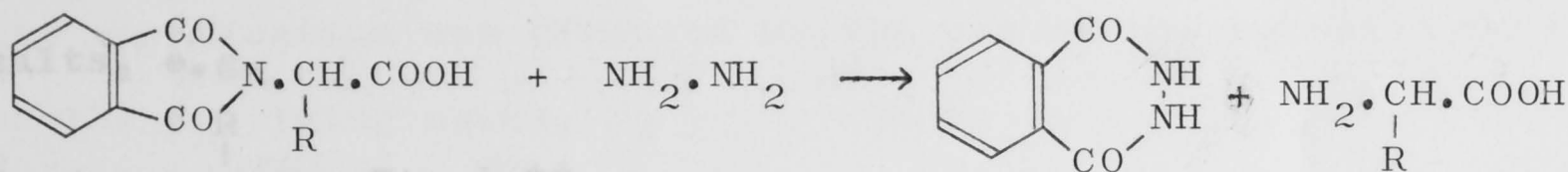
The racemic amino acid was refluxed with phthalic anhydride in dry pyridine. After evaporation in vacuo, boiling with acetic anhydride gave the phthaloyl amino acid anhydride. When this compound was dissolved in boiling water and the

solution was cooled the phthaloyl amino acid crystallised.

The isolated phthaloyl glutamic acid isomers did not precipitate after acidification of their potassium salt solutions. Extraction into ethyl acetate followed by evaporation of this solvent yielded crystalline products.

Ring opening of the phthaloyl group did not occur during these resolutions, as was shown by the absence of any  $\text{-NH-}$  stretching absorption ( $3100\text{-}3400\text{ cm}^{-1}$ ) in the infrared spectra.

The resolution of phthaloyl glutamic and aspartic acids is also of use as a means of obtaining the isomers of the free amino acids. The phthaloyl group was readily removed by treatment with hydrazine,



### SECTION III:

#### Resolution of Phenylalanine via a Schiff base derivative:

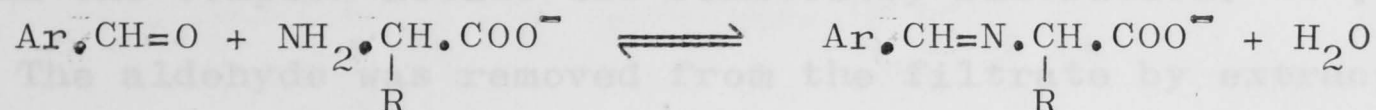
As already mentioned, most chemical procedures for the resolution of neutral amino acids have employed amino acids acylated on the amino groups. The resolution process, therefore, involves at least three separate stages; preparation of the acyl derivatives, resolution of the acyl derivative, and finally hydrolysis of the resolved derivative.

Apart from the length of such an operation, another disadvantage is the possibility of racemization caused by the vigorous conditions often necessary for the removal of the acyl group.

The ideal derivative is one that is easily prepared, and

readily combines with a resolving agent to form the diastereoisomeric salt from which, by simple means, the amino acid can be recovered optically pure.

Aromatic aldehydes combine with amino acids to form Schiff bases according to the equation,



The position of equilibrium is dependent on hydrogen ion concentration; neutral or mildly alkaline conditions favour Schiff base formation, whereas in acid solutions the aldehyde and amino acid are mainly uncombined. Bergman and Zervas<sup>(38)</sup> combined a number of amino acids with several aromatic aldehydes and isolated the products as the calcium and barium salts, e.g.

$(\text{Ar}\cdot\text{CH}=\text{N}\cdot\underset{\text{R}}{\text{CH}}\cdot\text{COO}^-)_2 \text{Ba}^{++}$ , or as salts of the alkaloids brucine, quinine and cinchonidine,  $\text{Ar}\cdot\text{CH}=\text{N}\cdot\underset{\text{R}}{\text{CH}}\cdot\text{COO}^- \text{Brucinium}^+$ .

They realised the possibility that the diastereoisomeric salts formed with the optically active alkaloids might be separable, thus leading to the separation of the amino acid enantiomers. A partial resolution of D,L-serine through the salt salicylidene-(D+D,L)-serinato<sup>-</sup>-Brucinium<sup>+</sup> was achieved, but in other cases only the racemic amino acid was recovered.

Bergman and Zervas concluded their paper thus: "We have therefore described these experiments in the hope that other workers will have more success using this method."

The use of the cation d- or l-[Coen<sub>2</sub>(NO<sub>2</sub>)<sub>2</sub>]<sup>+</sup> has allowed resolution of D,L-phenylalanine via its salicylidene derivative.



Salicylaldehyde was added to an aqueous solution of the sodium salt of D,L-phenylalanine, followed by the resolving agent. The diastereoisomer readily precipitated and was filtered off. After recrystallization, it was treated with KI and the complex iodide was removed by filtration.

The aldehyde was removed from the filtrate by extraction with  $\text{CHCl}_3$  solution containing a stronger primary amine than the amino acid, e.g. cyclohexylamine. The optically active phenylalanine was most easily isolated from the aqueous layer by separation on an ion exchange column.

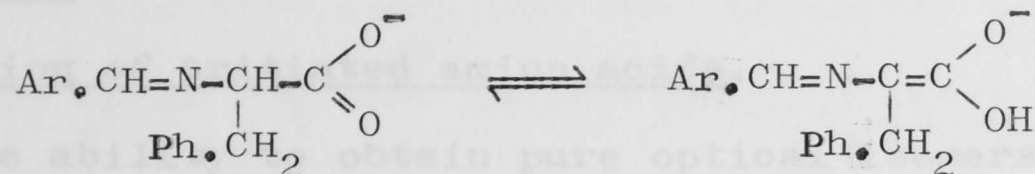
From the mother liquor of the precipitated diastereoisomer the impure enantiomer of the amino acid was isolated; its purification was effected by the use of the opposite form of the resolving agent.

Some difficulties encountered in this procedure should be mentioned.

(1) Recrystallisation of the diastereoisomer from water did not improve its optical purity. Measurement of the specific rotation of aqueous solutions of this salt was made uncertain as rotations of the solutions decreased with time. In one typical case, for a 0.25% solution  $[\alpha]_D^{20}$  decreased from  $-100^\circ$  to  $-50^\circ$  after one hour and then remained constant. The diastereoisomer was successfully recrystallized from hot formamide, by the addition of ethanol. A solution in formamide showed no change in rotation after two hours.

(2) Recovery of the resolved phenylalanine was attempted by acidifying the solution of the Schiff base to separate the

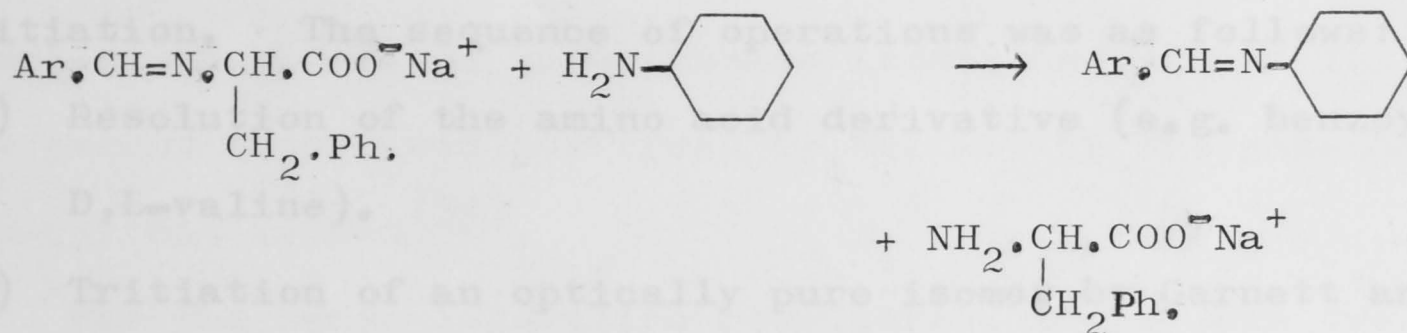
aldehyde and the amino acid. It was found, however, that after acidification with HCl to pH 2-3, the recovered phenylalanine had no optical activity. Racemization possibly involved the tautomeric equilibrium,



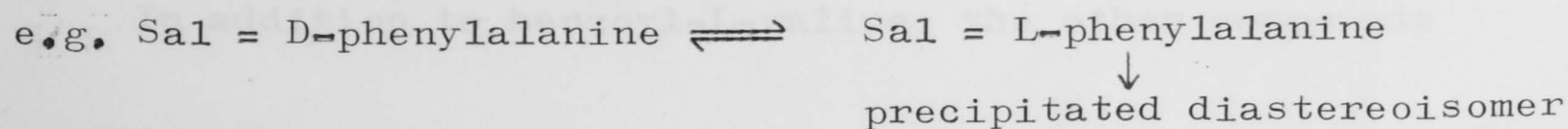
by which the  $\alpha$ -carbon of phenylalanine loses its asymmetry.

After very slow addition of 1N HCl to the Schiff base solution to pH 3.9, the salicylaldehyde was extracted into  $\text{CHCl}_3$  and the L-phenylalanine then isolated was found to be optically pure.

An alternative procedure which dispensed with the need for very carefully controlled acidification was substituted. The Schiff base was broken by treatment with cyclohexylamine which is a stronger base than phenylalanine.



The occurrence of racemization at low pH could, in theory allow a second order asymmetric synthesis to take place. That is, if the diastereoisomer were formed and precipitated at a pH where racemization of the Schiff base occurred, then as one form of the Schiff base was removed from solution in the diastereoisomer, the equilibrium between the enantiomers would convert the excess of the opposite form into racemate.



One attempt to do this was not successful because the diastereoisomer was difficult to precipitate in the acid solution, and the procedure was not pursued.

#### SECTION IV:

##### Resolution of tritiated amino acids.

The ability to obtain pure optical isomers of amino acid derivatives by resolution with the metal complex ions was employed in some studies designed to yield information on the mechanism of tritiation of solid organic compounds by the Wilzbach method.

This work was carried out in collaboration with Dr. J.L. Garnett and Mr. S.W. Law of the Department of Physical Chemistry, University of New South Wales. In essence, the investigation was to determine whether there was any change in the configuration of an optically active compound during tritiation. The sequence of operations was as follows:

- (1) Resolution of the amino acid derivative (e.g. benzoyl-D,L-valine).
- (2) Tritiation of an optically pure isomer by Garnett and Law (benzoyl-L-valine +  $T_2$ ).
- (3) The tritiated compound (benzoyl-L<sup>\*</sup>-valine) was diluted with a large quantity of racemic untritiated compound (benzoyl-D,L-valine) and the resolution was repeated. Both isomers, i.e. benzoyl-(L+L<sup>\*</sup>)-valine, and benzoyl-D-valine, were obtained in an optically pure state.
- (4) Radioactive counting of these resolved fractions.

In addition to benzoyl-L-valine, the other compounds

tritiated were phthaloyl-L-glutamic acid and phthaloyl-D-glutamic acid.

The term "parent" isomer is used to denote the isomeric form which was tritiated, and the "opposite" isomer denotes the other form. d- and l-[Coen<sub>2</sub>(NO<sub>2</sub>)<sub>2</sub>]<sup>+</sup> were the resolving agents used in each case.

For the glutamic acid samples (e.g. L<sup>\*</sup>-), the diastereoisomer precipitated first contained the opposite isomer (D<sub>I</sub>Co). This diastereoisomer was recrystallized three times (D<sub>II</sub>Co, D<sub>III</sub>Co, D<sub>IV</sub>Co) and samples of the opposite isomer were isolated from each of the four fractions (D<sub>I</sub>, D<sub>II</sub>, D<sub>III</sub>, D<sub>IV</sub>). Using the other form of the resolving agent, the precipitated diastereoisomer (L<sub>I</sub>Co) was recrystallized twice (L<sub>II</sub>Co, L<sub>III</sub>Co) and samples of the parent isomer (L<sub>II</sub>, L<sub>III</sub>) were obtained from these last two fractions. Radioactive counting of samples III and IV of the opposite isomer and II and III of the parent isomer was performed by Mr. Law by the gas-phase ion-current method<sup>(39)</sup>.

For the benzoyl-L-valine resolution, there were two main points of difference to the phthaloyl glutamic acid procedures. These were:

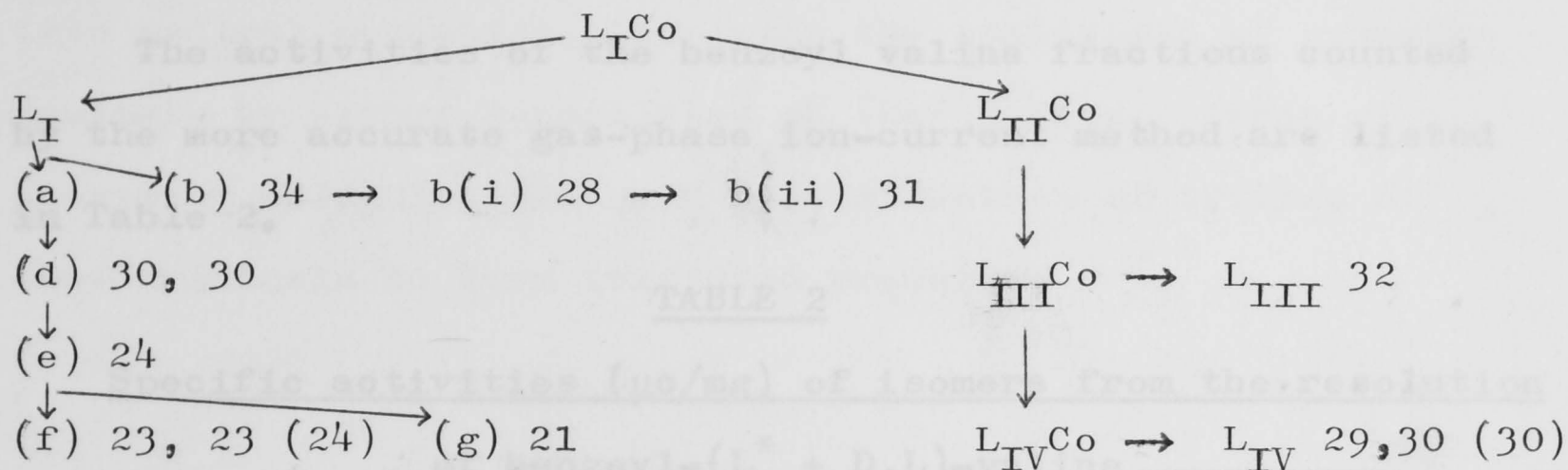
- (1) The initial solution of benzoyl-(L<sup>\*</sup>+D,L)-valine was divided into two equal parts; the L-isomer was isolated from one and the D-isomer was isolated from the other. This ensured that each fraction, when isolated, had been subjected to the same number of solution and



FIG. 1

Specific Activities of L-isomers from the resolution of

benzoyl-(L\* + D,L)-valine



Duplicate measurements were made on several samples.

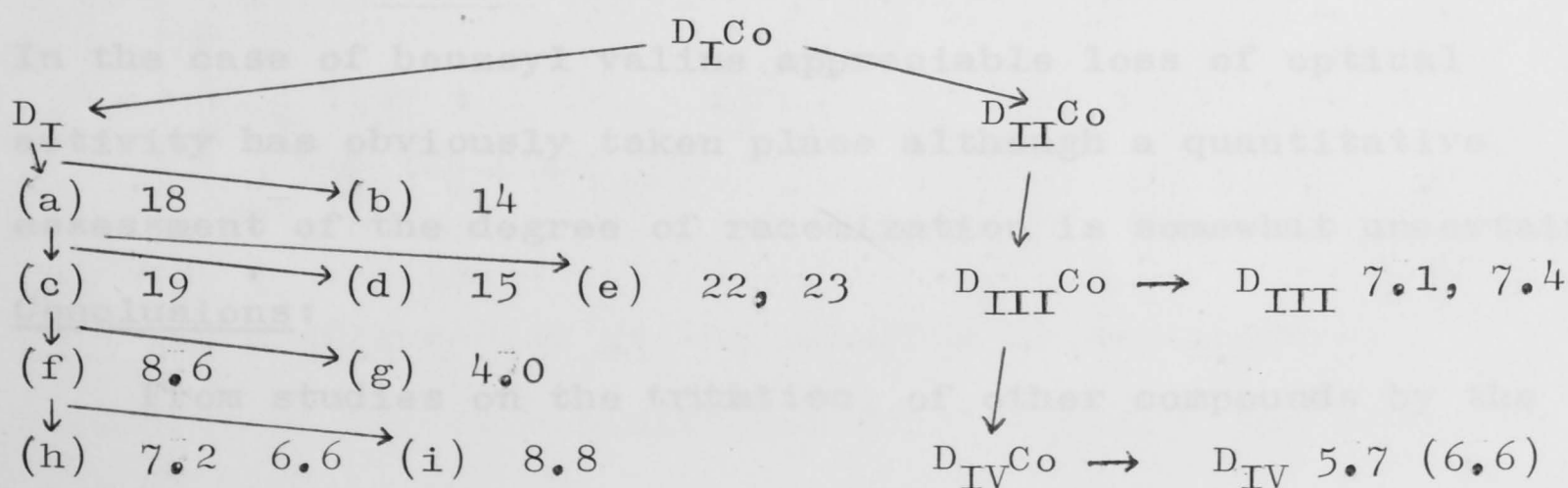
Figures in ( ) were obtained for solutions of concentration 0.5 mg per 10 ml, otherwise concentration was 1.0 mg/10 ml.

The fractions  $L_I$  b(i) and b(ii) were isolated after fraction  $L_I$ (b) had been heated in ethanol-water solution for periods many times greater than that required for a single recrystallization. The small change in the activity from (b) to b(i) and b(ii) indicates that the decrease in activity of fractions  $L_I$  with successive recrystallizations cannot be attributed to exchange of tritium with the solvent.

FIG. 2

Specific Activities of D-isomers from the

resolution of benzoyl (L\* + D,L)-valine



Duplicate measurements were made on several samples. The figure in the ( ) was obtained for a solution of concentration 0.5 mg per 10 ml.

The activities of the benzoyl valine fractions counted by the more accurate gas-phase ion-current method are listed in Table 2.

TABLE 2

Specific activities ( $\mu\text{c}/\text{mg}$ ) of isomers from the resolution of benzoyl-(L<sup>\*</sup> + D,L)-valine

<u>L-isomers</u>	<u>D-isomers</u>
I (d) 0.053	I (a) 0.034
(e) 0.041	(c) 0.019
(f) 0.038	(f) 0.016
	(h) 0.014

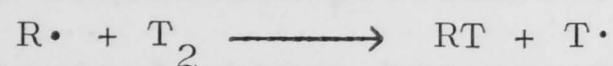
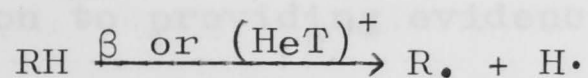
A comparison of these figures with those for the corresponding samples in Figs. 1 and 2 shows that the relative activities obtained by scintillation counting probably have an error of less than 10%.

The results show that very little racemization occurred during the tritiation of the phthaloyl glutamic acids. The activities of the L-isomers from the resolution of phthaloyl-D<sup>\*</sup>-glutamic acid were only twice that of the background count. In the case of benzoyl valine appreciable loss of optical activity has obviously taken place although a quantitative assessment of the degree of racemization is somewhat uncertain.

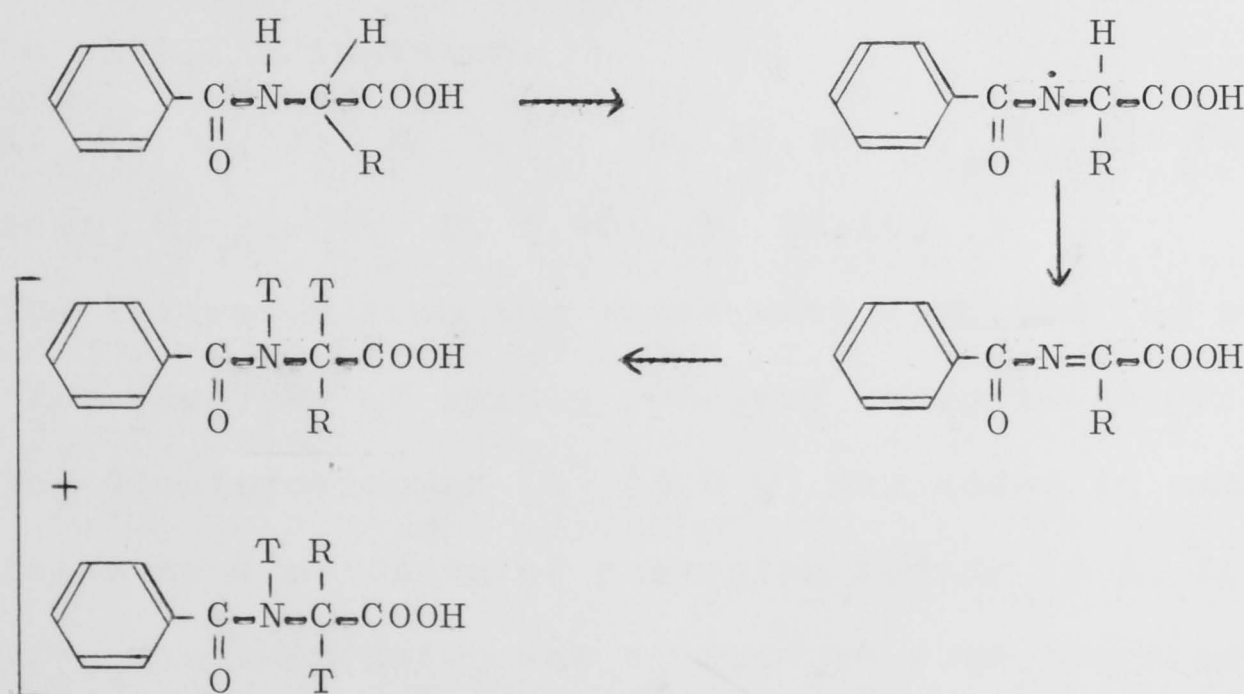
Conclusions:

From studies on the tritiation of other compounds by the

Wilzbach method, notably anthranilic acid, isopropanol and allyl phenyl ether, Garnett, Law and Till<sup>(40)</sup> postulated that incorporation of tritium proceeded predominantly by a free radical mechanism, involving the initiation of organic radicals by either  $\beta$ -particles or  $(\text{HeT})^+$  from decay of a tritium molecule, followed by abstraction of tritium by these radicals to form tritiated products:



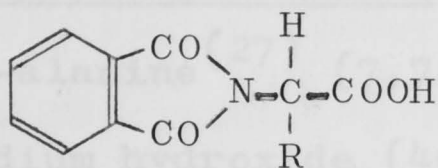
The retention of configuration observed in tritiation of the isomers of phthaloyl glutamic acid is consistent with the observed retentions found when  $(-)$ inositol and octyl phthalates were tritiated in the solid phase<sup>(41)</sup>. Because of the partial racemization found with benzoyl-L-valine, it was considered that some tritiation had occurred through a radical reaction of the type:



The configuration at the  $\alpha$ -carbon is destroyed by formation of the carbon nitrogen double bond. The absence



of racemization for the phthaloyl glutamic acids was consistent with this explanation since the lack of an amino hydrogen would prevent formation of a corresponding symmetrical intermediate,



In addition to providing evidence for mechanisms of tritiation this resolution procedure can determine whether or not stereochemical change has accompanied the labelling of an optically active amino acid derivative.

$[\alpha]_D^{20} = +17^\circ$ , 1% in water.

(Found: C, 36.13; H, 5.64; N, 20.98;  $C_{14}H_{16}O_3N_2$  requires: C, 36.29; H, 5.66; N, 21.16)

The filtrates from the above were combined [B] and set aside for recovery of partly resolved benzoyl-L-alanine.

The diastereoisomer [A] (5.0 g) was added in small quantities to a solution of potassium iodide (5 g) in water (20 ml.), the suspension was stirred at room temperature for 15 minutes and the d-cis-dinitroethylenediamine cobalt(III) iodide was filtered. The filtrate was acidified with

## EXPERIMENTAL

All measurements of optical rotation were made with a 1 dm. tube.

### SECTION I - NEUTRAL AMINO ACIDS.

#### Resolution of Benzoyl-D,L-Alanine:

Benzoyl-D,L-alanine<sup>(27)</sup> (7.72 g) was dissolved in sufficient 1N sodium hydroxide (40 ml.) to give an exactly neutral solution, which was added to a solution of d-cis-dinitrobis(ethylenediamine) cobalt(III) acetate [prepared by shaking the d-iodide (9.0 g) and silver acetate (3.73 g) in warm water (25 ml.) for 10 minutes and then filtering off the precipitated silver iodide]. The solution was evaporated to 30 ml. under reduced pressure at 30°. After cooling to 5° the yellow diastereoisomeric salt d-cis-dinitrobis(ethylenediamine) cobalt (III) - benzoyl-D-alaninate [A] was filtered, dried and recrystallised by dissolving in warm water (30 ml.) evaporating to 20 ml. and cooling to 5°. (5.0 g, 54%)

$[\alpha]_D^{20} = +17^\circ$ , 1% in water.

(Found: C, 36.13; H, 5.64; N, 20.98;  $C_{14}H_{26}O_7N_7Co$ . requires: C, 36.29; H, 5.66; N, 21.16)

The filtrates from the above were combined [B] and set aside for recovery of partly resolved benzoyl-L-alanine.

The diastereoisomer [A] (5.0 g) was added in small quantities to a solution of potassium iodide (5 g) in water (20 ml.), the suspension was stirred at room temperature for 15 minutes and the d-cis-dinitrobis(ethylenediamine) cobalt(III) iodide was filtered. The filtrate was acidified with

6N-hydrochloric acid, cooled at 5° for 15 minutes and the colourless crystals were filtered. Recrystallisation from water gave benzoyl-D-alanine (1.9 g)  $[\alpha]_D^{20} = -34^\circ$ , 1% in 1N sodium hydroxide, (Dunn and co-workers<sup>(27)</sup> reported  $[\alpha]_D^{25} = -32.5^\circ$ , 1% in 1.05N sodium hydroxide).

The combined filtrates [B] were evaporated to 20 ml. under reduced pressure at 30°, and potassium iodide (5 g) was added. After removal of the precipitated d-cis-dinitrobis(ethylenediamine) cobalt (III) iodide, the filtrate was acidified with 6N-hydrochloric acid, cooled at 5° for 15 minutes and the partially resolved benzoyl-L-alanine was collected (4.5 g). This product was reresolved with l-cis-dinitrobis(ethylenediamine) cobalt(III) acetate using the same procedure as described above. Recrystallisation from water gave benzoyl-L-alanine. (2.0 g)  $[\alpha]_D^{20} = +33^\circ$ , 1% in 1N sodium hydroxide. (Dunn and co-workers<sup>(27)</sup> reported  $[\alpha]_D^{22.5} = +33.4^\circ$ , 0.48% in 1N sodium hydroxide.)

#### Resolution of Benzoyl-D,L-valine:

Benzoyl-D,L-valine (Nutritional Biochemical Corporation) (2.21 g) was dissolved in 1N sodium hydroxide (10 ml.) to give an exactly neutral solution, which was added to a solution of d-cis-dinitrobis(ethylenediamine) cobalt(III) acetate [ex d-iodide (4.0 g) and silver acetate (1.67 g) in water (20 ml.).]

After scratching and standing for two hours the yellow diastereoisomeric salt d-cis-dinitrobis(ethylenediamine) cobalt(III) benzoyl-L-valine was filtered, dried and recryst-

stallised from water (1.0 g)  $[\alpha]_D^{20} = +42^\circ$ , 0.45% in water.  
 (Found: C, 39.27; H, 6.11; N, 19.83;  $C_{16}H_{30}O_7N_7Co$   
 requires: C, 39.10; H, 6.15; N, 19.96).

A suspension of the diastereoisomer (0.9 g) in water (10 ml.) was treated with potassium iodide (0.9 g) stirred at room temperature for 15 minutes and the d-cis-dinitrobis(ethylenediamine) cobalt(III) iodide was collected. The filtrate was acidified with 6N hydrochloric acid, cooled at  $5^\circ$  for 15 minutes and the colourless crystals were filtered. Recrystallisation from aqueous ethanol gave benzoyl-L-valine (0.3 g)  $[\alpha]_D^{20} = +19.6^\circ$ , 1.1% in ethanol.  $[\alpha]_D^{20} = +53^\circ$ , 1.0% in chloroform.

Fox<sup>(42)</sup> reported  $[\alpha]_D^{25} = +21.8^\circ$ , 4.9% in 95% ethanol and Hinman<sup>(43)</sup> reported for benzoyl-D-valine,  $[\alpha]_D^{31} = -51.0^\circ$ , 1.04% in chloroform.

#### Resolution of Tosyl-D,L-Methionine:

Tosyl-D,L-methionine (1.54 g)<sup>(28)</sup> was dissolved in 1N sodium hydroxide (5 ml.) to give an exactly neutral solution, which was added to a solution of d-cis-dinitrobis(ethylenediamine) cobalt(III) acetate [ex d-iodide (2.00 g) and silver acetate (0.83 g) in water (15 ml.)].

After standing for one hour the yellow diastereoisomeric salt d-cis-dinitrobis(ethylenediamine) cobalt(III)-tosyl-D-methioninate was filtered, dried and recrystallised from water (0.5 g)  $[\alpha]_D^{20} = +22^\circ$ , 0.5% in water.

(Found: C, 33.50; H, 5.76; N, 16.20;  $C_{16}N_{32}O_8N_7S_2Co$   
 requires: C, 33.51; H, 5.63; N, 17.10).

A suspension of the diastereoisomer (0.5 g) in water (10 ml.) was treated with a solution of potassium iodide (0.5 g) in water (10 ml.), stirred at room temperature for 15 minutes and the d-cis-dinitrobis(ethylenediamine) cobalt(III) iodide was filtered. Acidification of the filtrate with 6N hydrochloric acid produced an oil which was extracted with chloroform. The chloroform solution was washed with water and re-extracted with aqueous sodium bicarbonate solution. The aqueous alkaline solution was acidified with 6N hydrochloric acid and the resulting oil solidified after several days at 2°. The product was recrystallised by dissolution in chloroform, extraction with sodium bicarbonate solution, acidification with hydrochloric acid followed by cooling at 2° until solidification occurred. The colourless crystals of tosyl-D-methionine were filtered, washed with cold water and dried at room temperature.

(0.2 g)  $[\alpha]_D^{20} = -12.4^\circ$ , 1.5% in ethanol.

Tosylation of L-methionine (Fluka, puriss) by the same method, followed by recrystallisation as above gave crystals having  $[\alpha]_D^{20} = +12.6^\circ$ , 1.0% in ethanol.

Resolution of Tosyl-D,L-Proline:

Tosyl-D,L-proline<sup>(28)</sup> (2.14 g) was dissolved in a solution of sodium bicarbonate (0.67 g) in water (10 ml) to give an exactly neutral solution which was added to a solution of l-cis-dinitrobis(ethylenediamine) cobalt(III) acetate [ex l-iodide (4.00 g) and silver acetate (1.67 g) in water (20 ml)]. Water was added to the solution to give a total volume of

100 ml. After standing for one hour the flocculant yellow diastereoisomeric salt 1-cis-dinitrobis(ethylenediamine) cobalt(III)-tosyl-L-prolinate was filtered, dried and recrystallised from water (1.1 g)  $[\alpha]_D^{20} = -81^\circ$ , 0.26% in water. (Found: C, 35.59; H, 5.24; N, 18.17;  $C_{16}H_{30}O_8N_7S$  Co requires: C, 35.62; H, 5.61; N, 18.18).

A suspension of the diastereoisomer (1.0 g) in water (50 ml) was treated with a solution of potassium iodide (1.0 g) in water (20 ml.), stirred at room temperature for 30 minutes and the 1-cis-dinitrobis(ethylenediamine) cobalt(III) iodide was filtered. The filtrate was evaporated to 10 ml. under reduced pressure at  $30^\circ$ . Acidification with 6N hydrochloric acid produced an oil which was extracted with chloroform. The chloroform solution was washed with water and re-extracted with aqueous sodium bicarbonate solution. The aqueous alkaline solution was acidified with 6N hydrochloric acid and the resulting oil solidified after several days at  $2^\circ$ . The product was recrystallised by dissolution in chloroform, extraction with sodium bicarbonate solution, acidification with hydrochloric acid followed by cooling at  $2^\circ$ .

The colourless crystals of tosyl-L-proline mono hydrate were filtered, washed with cold water and dried at room temperature and atmospheric pressure. (0.1 g)  $[\alpha]_D^{20} = -92.8^\circ$ , 0.8% in ethanol. Izumiya<sup>(44)</sup> reported  $[\alpha]_D^{10} = -92.5^\circ$ , 1.78% in ethanol).

Resolution of Phthaloyl-D,L-Phenylalanine:

Phthaloyl-D,L-phenylalanine<sup>(29)</sup> (5.90 g) was suspended in water (120 ml.) and sodium bicarbonate (1.68 g) was added to give an exactly neutral solution. After filtering to remove a small amount of undissolved material, the solution was added to a solution of 1-cis-dinitrobis(ethylenediamine) cobalt(III) acetate [ex 1-iodide (5.00 g) and silver acetate (2.07 g) in water (25 ml.)].

After standing for 30 minutes the yellow diastereoisomeric salt 1-cis-dinitrobis(ethylenediamine) cobalt(III)-o-carboxy-benzoyl-D-phenylalaninate was filtered, dried and recrystallised from water (3.2 g)  $[\alpha]_D^{20} = +72^\circ$ , 0.3% in water.

(Found: C, 43.27; H, 5.11; N, 16.46;  $C_{21}H_{30}O_9N_7Co$  requires: C, 43.22; H, 5.18; N, 16.81).

A suspension of the diastereoisomer (2.9 g) in water (50 ml.) was treated with a solution of potassium iodide (2.9 g) in water (30 ml.), stirred at room temperature for 20 minutes and the 1-cis-dinitrobis(ethylenediamine) cobalt(III) iodide was filtered. The filtrate was evaporated to 10 ml. under reduced pressure at 30°, acidified with 6N hydrochloric acid, cooled at 5° for 15 minutes and the colourless crystals were filtered. Recrystallisation from aqueous ethanol gave o-carboxy-benzoyl-D-phenylalanine (1.29 g)  $[\alpha]_D^{20} = +213^\circ$ , 1% in ethanol. The infrared spectrum showed absorption at  $3260\text{ cm}^{-1}$  (-NH- stretching).

Resolution of Phthaloyl-D,L-Alanine:

Phthaloyl-D,L-alanine<sup>(29)</sup> (2.20 g) was suspended in water

(12 ml) and sodium bicarbonate (0.84 g) was added to give an exactly neutral solution, which was added to a solution of d-cis-dinitrobis(ethylenediamine) cobalt(III) acetate [ex d-iodide (4.00 g) and silver acetate (1.67 g) in water (20 ml.)].

After standing for one hour the flocculant yellow diastereoisomeric salt d-cis-dinitrobis(ethylenediamine) cobalt(III)-o-carboxy-benzoyl-D-alaninate was filtered, dried and recrystallised from water (1.4 g)  $[\alpha]_D^{20} = +38^\circ$ , 0.48% in water.

(Found: N, 19.55, Calc. for  $C_{15}H_{26}O_9N_7Co$ ; N, 19.33).

A suspension of the diastereoisomer (1.3 g) in water (10 ml) was treated with potassium iodide (1.3 g), stirred at room temperature for 15 minutes and the d-cis-dinitrobis(ethylenediamine) cobalt(III) iodide was collected. The filtrate was acidified with 6N hydrochloric acid, cooled at  $5^\circ$  for 15 minutes and the colourless crystals were filtered. Recrystallisation from aqueous ethanol gave o-carboxy-benzoyl-D-alanine (0.26 g)  $[\alpha]_D^{20} = +24^\circ$ , 2.6% in ethanol. The infrared spectrum showed absorption at  $3260\text{ cm}^{-1}$  (-NH-stretching).

#### Resolution of Tosyl-D,L-Tryptophan:

Tosyl-D,L-tryptophan<sup>(28)</sup> (2.75 g) was dissolved in a solution of lithium hydroxide (0.32 g) in water (80 ml) to give an exactly neutral solution, which was added to a solution of d-oxalatobis(ethylenediamine) cobalt(III) acetate [ex d-bromide (2.80 g) and silver acetate (1.27 g) in water (25 ml).]



After standing for 30 minutes the pink diastereoisomeric salt d-oxalatobis(ethylenediamine) cobalt(III)-tosyl-L-tryptophanate was filtered, dried and recrystallised from water (1.5 g)  $[\alpha]_D^{20} = +437^\circ$ , 0.25% in water.

A suspension of the diastereoisomer (1.4 g) in water (20 ml.) was treated with a solution of potassium iodide (1.4 g) in water (20 ml.), stirred at room temperature for 30 minutes and the d-oxalatobis(ethylenediamine) cobalt(III) iodide was filtered. Acidification of the filtrate with 6N hydrochloric acid produced an oil which was extracted with chloroform. The chloroform solution was washed with water and re-extracted with aqueous sodium bicarbonate solution. The aqueous alkaline solution was acidified with 6N hydrochloric acid and the resulting oil solidified after several days at 2°. The product was recrystallised by dissolution in chloroform, extraction with sodium bicarbonate solution, acidification with hydrochloric acid followed by cooling at 2° until solidification occurred. The colourless crystals of tosyl-L-tryptophan were filtered, washed with cold water and dried at room temperature. (0.47 g)  $[\alpha]_D^{20} = -42^\circ$ , 1.1% in ethanol.

Tosylation of L-tryptophan ( $[\alpha]_D^{20} = -33^\circ$ , 0.5% in water) by the same method followed by recrystallisation as above gave crystals having  $[\alpha]_D^{20} = -42^\circ$ , 1.1% in ethanol.

Resolution of Tosyl-D,L-Serine:

Tosyl-D,L-serine<sup>(28)</sup> (2.59 g) was dissolved in 1N sodium hydroxide (10 ml.) to give an exactly neutral solution which was added to a solution of l-cis-dinitrobis(ethylenediamine)

cobalt(III) acetate [ex l-iodide (2.27 g) and silver acetate (0.95 g) in water (20 ml.)].

After standing for 2 hours the yellow diastereoisomeric salt l-cis-dinitrobis(ethylenediamine) cobalt(III)-tosyl-L-serinate was filtered, dried and recrystallised from water (1.36 g)  $[\alpha]_D^{20} = +4^\circ$ , 0.4% in water.

(Found: C, 31.44; H, 5.42; N, 18.27;  $C_{14}H_{28}O_9N_7S$  Co requires: C, 31.76; H, 5.33; N, 18.52).

A suspension of the diastereoisomer (1.2 g) in water (10 ml.) was treated with potassium iodide (1.2 g) stirred at room temperature for 15 minutes and the l-cis-dinitrobis(ethylenediamine) cobalt(III) iodide was collected.

The filtrate was acidified with 6N hydrochloric acid cooled at  $5^\circ$  for 15 minutes and the colourless crystals were filtered. Recrystallisation from methanol gave tosyl-L-serine (0.42 g)  $[\alpha]_D^{20} = -33^\circ$ , 1% in pyridine.

Hofman<sup>(45)</sup> reported  $[\alpha]_D^{25} = -32.3^\circ$ , 2% in pyridine.

## SECTION II - ACIDIC AMINO ACIDS.

### Resolution of Phthaloyl-D,L-glutamic Acid:

A suspension of phthaloyl-D,L-glutamic acid<sup>(35)</sup> (5.54 g) in water (15 ml.) was exactly neutralised with sodium bicarbonate (3.36 g) and the resulting solution was added to a solution of d-cis-dinitrobis(ethylenediamine) cobalt(III) acetate, [prepared from d-iodide (9.0 g) and silver acetate (3.73 g) in water (25 ml.)]. After filtration from a small insoluble residue the filtrate was precipitated with ethanol (130 ml.) and the yellow diastereoisomeric salt d-cis-dinitrobis

(ethylenediamine) cobalt(III) phthaloyl-L-glutamate [A] was collected, dried and recrystallised by dissolving in warm water (40 ml.) and reprecipitating with ethanol (130 ml.) (6.5 g, 80%)  $[\alpha]_D^{20} = +26^\circ$ , 1% in water. (Found: C, 30.08; H, 5.48; N, 21.52;  $C_{21}H_{41}O_{14}N_{13}Co_2 \cdot H_2O$  required: C, 30.18; H, 5.19; N, 21.79).

The filtrates from the above were combined [B] and set aside for recovery of partly resolved phthaloyl-D-glutamic acid. The diastereoisomer [A] (6.5 g) was added in small quantities to a solution of potassium iodide (5 g) in water (20 ml.), the suspension was stirred at room temperature for 15 minutes and the d-cis-dinitrobis(ethylenediamine) cobalt(III) iodide (4 g) was collected. The filtrate was acidified with 6N hydrochloric acid and the phthaloyl-L-glutamic acid was extracted several times with ethylacetate. The combined extracts were dried ( $Na_2SO_4$ ). Evaporation of the ethylacetate extracts followed by recrystallisation from water yielded phthaloyl-L-glutamic acid m.p. 158-159°,  $[\alpha]_D^{20} = -45.4^\circ$  1% in ethanol. Tipson<sup>(34)</sup> recorded m.p. 158-159° and  $[\alpha]_D = -42.6^\circ$ , 1% in 95% ethanol. The combined filtrates [B] were evaporated to dryness at 30° under reduced pressure, redissolved in water (30 ml.) and potassium iodide (5 g) was added. After removal of the precipitated d-cis-dinitrobis(ethylenediamine) cobalt(III) iodide the filtrate was acidified with 6N hydrochloric acid and the partially resolved phthaloyl-D-glutamic acid was extracted with ethylacetate. Evaporation of the extract yielded 2.7 g. The product was

reresolved with 1-cis-dinitrobis(ethylenediamine) cobalt(III) acetate using the same procedure as described above. Recrystallisation from water gave optically pure phthaloyl-D-glutamic acid (2 g) m.p. 158-159°  $[\alpha]_D^{20} = +45.4^\circ$ , 1% in ethanol.

Recovery of D-glutamic acid from Phthaloyl-D-glutamic acid:

The phthaloyl derivative (1 g) was suspended in water (5 ml.) and the pH of the mixture adjusted to pH 6.5 by the addition of  $\text{Na}_2\text{CO}_3$  (0.4 g). Hydrazine hydrate (1 g, 80%) was then added and the solution was left at room temperature for 2 days. The reaction mixture was then acidified with HI to a pH of 3.5, the precipitated phthalhydrazide was filtered off, the filtrate was concentrated in vacuo and the D-glutamic acid was precipitated by the addition of alcohol. After recrystallisation from aqueous ethanol optically pure D-glutamic acid was obtained; (0.4 g),  $[\alpha]_D^{20} = -31^\circ$ , 1.4% in 5N HCl. Greenstein<sup>(46)</sup> reports  $[\alpha]_D^{25} = +31.8^\circ$ , 0.5-2.0% in 5N HCl for L-glutamic acid.

Resolution of Phthaloyl-D,L-Aspartic Acid:

Phthaloyl-D,L-aspartic acid<sup>(35)</sup> (6.09 g) was suspended in water (40 ml.), and  $\text{NaHCO}_3$  (3.98 g) was added to give a neutral solution which was added to a solution of 1-[Coen<sub>2</sub>(NO<sub>2</sub>)<sub>2</sub>]OAc [ex 1-iodide (9.60 g) and silver acetate (3.99 g) in water (30 ml.)]. The solution was concentrated to 40 ml. in vacuo and the diastereoisomer 1-[Coen<sub>2</sub>(NO<sub>2</sub>)<sub>2</sub>]<sub>2</sub>-phthaloyl-D-aspartate was filtered and recrystallised from water (2.58 g).  $[\alpha]_D^{20} = -18^\circ$ , 1.0% in water.

(Found: C, 28.8; H, 5.1; N, 22.0;  $C_{20}H_{39}O_{14}N_{13}Co_2 \cdot H_2O$  requires: C, 29.2; H, 5.0; N, 22.1).

To the diastereoisomer (2.5 g) was added KI (1.5 g) in water (12 ml.), the suspension was stirred for 15 minutes and the  $1-[Coen_2(NO_2)_2]I$  was filtered. The filtrate was acidified with 6N HCl, cooled at 5° for 15 minutes and colourless crystals were filtered off. ( $D_I$ ) (0.26 g). Recrystallisation from aqueous ethanol gave optically impure phthaloyl-D-aspartic acid (0.22 g)  $[\alpha]_D^{20} = +48^\circ$ , 1.0% in methanol.

The filtrate from  $D_I$  was extracted three times with ethyl acetate, the combined extracts were dried over  $Na_2SO_4$  and evaporated in vacuo. Addition of one drop of water caused crystallisation of optically pure phthaloyl-D-aspartic acid which was collected by filtration. (0.51 g)  $[\alpha]_D^{20} = +66^\circ$ , 1.0% in methanol. Balenovic et al. reported  $[\alpha]_D^{17} = -58^\circ$ , 0.4% in methanol for phthaloyl-L-aspartic acid. (36)

### SECTION III.

#### Resolution of Phenylalanine via a Schiff base derivative:

D,L-phenylalanine (3.30 g) was dissolved in 1.0N NaOH (20.0 ml.) and salicylaldehyde (Fluka, puriss.) (2.44 g) was added. The solution was stirred until the aldehyde had completely dissolved. To this yellow solution was added a solution of  $d-[Coen_2(NO_2)_2]OAc$  [ex d-iodide (4.00 g) and  $AgOAc$  (1.67 g) in water (30 ml.)]. After 15 minutes the yellow diastereoisomeric salt  $d-[Coen_2(NO_2)_2]$ -salicylidene-L-phenylalaninate [A] was filtered and washed with acetone

(4.0 g). Recrystallisation was effected by solution in hot formamide (35 ml.) and addition of ethanol (220 ml.). After several hours the product was filtered, washed with ethanol and acetone and dried under vacuum. (2.0 g)  $[\alpha]_D^{20} = -73^\circ$ , 1.0% in formamide.

(Found: C, 44.6; H, 5.5; N, 18.3;  $C_{20}H_{30}N_7O_7Co$  requires: C, 44.5; H, 5.6; N, 18.2%)

The diastereoisomer (1.80 g) was stirred in warm water (30 ml. at  $35^\circ$ ), NaI (0.9 g) was added and stirring was continued for a further 15 minutes before cooling and filtering off the  $d-[Coen_2(NO_2)_2]I$ .

The filtrate, which contained salicylidene-L-phenylalanine as its sodium salt, was extracted four times with cyclohexylamine (2 ml.) in  $CHCl_3$  (25 ml.) and then three times with  $CHCl_3$  (25 ml.).

The aqueous solution was then passed through a column of DOWEX 50W (acid form) and washed with water until the eluate was no longer acidic, i.e. until HI had been removed. The adsorbed L-phenylalanine was eluted with 2.5% pyridine in water.

After evaporation to dryness and recrystallisation from aqueous ethanol optically pure L-phenylalanine was obtained (0.45 g)  $[\alpha]_D^{20} = -33^\circ$ , 1.5% in water.

To the filtrate of the crude diastereoisomer [A] was added KI, and remaining resolving agent was removed as its Iodide. The filtrate was extracted as above with cyclohexylamine in  $CHCl_3$ , and very crude D-phenylalanine (1.0 g) was isolated from the aqueous phase.

D,L-phenylalanine (2.30 g) was combined with this crude D-phenylalanine and treated with NaOH, salicylaldehyde and 1-[Coen<sub>2</sub>(NO<sub>2</sub>)<sub>2</sub>]OAc exactly as before. In this case, however, the diastereoisomer yielded optically pure D-phenylalanine (0.45 g)  $[\alpha]_D^{20} = +33^\circ$ , 1.5% in water.

Alternative Hydrolysis of the Schiff base without Racemisation:

After treatment of the diastereoisomer with KI to remove the resolving agent, the solution of the sodium salt of the Schiff base was acidified to pH 3.9 by slow addition with rapid stirring of 1N HCl and was then extracted four times with CHCl<sub>3</sub>. The aqueous phase was treated exactly as before to yield optically pure L-phenylalanine.

SECTION IV.

Resolution of Tritiated Amino Acids:

(1) Resolution of untritiated amino acid derivatives:

Benzoyl-L-valine and phthaloyl-D- and L-glutamic acids were obtained by resolution procedures exactly as described in Sections I and II respectively.

(2) Tritiation:

Each of the optically active amino acid derivatives was exposed to tritium gas (one C. of 98% purity) for two weeks at room temperature.

(3) Resolution of the tritiated compound:

(a) Resolution of Phthaloyl-D,L-Glutamic Acid in the presence of Phthaloyl-L<sup>\*</sup>-Glutamic Acid:

A suspension of phthaloyl-D,L-glutamic acid (5.34 g) and phthaloyl-L<sup>\*</sup>-glutamic acid (0.20 g) in water (15 ml.) was

exactly neutralised with sodium bicarbonate (3.36 g) and the resulting solution was added to a solution of 1-cis-dinitrobis(ethylenediamine) cobalt(III) acetate [ex 1-iodide (9 g) and silver acetate (3.73 g) in water (25 ml.)].

After filtration, the filtrate was precipitated with ethanol (130 ml.) and the yellow diastereoisomeric salt 1-cis-dinitrobis(ethylenediamine) cobalt(III)-phthaloyl-D-glutamate ( $D_I\text{Co}$ ) was collected and dried, (6.9 g, 85%). The filtrate [B] was set aside for recovery of partly resolved phthaloyl-L<sup>\*</sup>-glutamic acid. Portion of the diastereoisomer ( $D_I\text{Co}$ ) (1.2 g) was set aside to give fraction  $D_I$  of phthaloyl-D-glutamic acid.

The remainder of  $D_I\text{Co}$  (4.7 g) was recrystallised by dissolving in warm water (30 ml.) and reprecipitating with ethanol (100 ml.), yielding 4.3 g ( $D_{II}\text{Co}$ ), 1.2 g of which were set aside.

The remaining 3.1 g of  $D_{II}\text{Co}$  were recrystallised twice to give fractions  $D_{III}\text{Co}$  (2.5 g) and  $D_{IV}\text{Co}$  (1.2 g).

The four diastereoisomer fractions  $D_I\text{Co}$ ,  $D_{II}\text{Co}$ ,  $D_{III}\text{Co}$ ,  $D_{IV}\text{Co}$  (each 1.2 g) were in turn added to solutions of potassium iodide (1.5 g) in water (5 ml.) and the suspensions were stirred for 15 minutes. The 1-cis-dinitrobis(ethylenediamine) cobalt(III) iodide was collected and the filtrate was acidified with 6N hydrochloric acid. The phthaloyl-D-glutamic acid was extracted with ethyl acetate. Evaporation of the dried extract followed by recrystallisation yielded phthaloyl-D-glutamic acid, fractions  $D_I$ ,  $D_{II}$ ,  $D_{III}$ ,  $D_{IV}$ .



The filtrate [B] was evaporated to dryness at 30° under reduced pressure, redissolved in water (30 ml.) and potassium iodide (5 g) was added. After removal of the precipitated *l*-*cis*-dinitrobis(ethylenediamine) cobalt(III) iodide the filtrate was acidified with 6N hydrochloric acid and the partially resolved phthaloyl-(L\* + D,L)-glutamic acid was extracted with ethyl acetate. Evaporation of the extract yielded 2.7 gm. [Fraction V]

This product was re-resolved with *d*-*cis*-dinitrobis(ethylenediamine) cobalt(III) acetate using the same procedure as described above. Two recrystallisations only of the diastereoisomer (L<sub>I</sub>Co) were carried out giving Fractions L<sub>II</sub>Co and L<sub>III</sub>Co from which fractions L<sub>II</sub> and L<sub>III</sub> of phthaloyl-L\* -glutamic acid were obtained.

(b) Resolution of Phthaloyl-D,L-Glutamic Acid in the presence of Phthaloyl-D\* -Glutamic Acid:

A suspension of phthaloyl-D,L-glutamic acid (5.35 g) and phthaloyl-D\* -glutamic acid (0.20 g) in water (15 ml.) was exactly neutralised with sodium bicarbonate (3.36 g) and the resulting solution was added to a solution of *d*-*cis*-dinitrobis(ethylenediamine) cobalt(III) acetate [ex *d*-iodide (9 g) and silver acetate (3.73 g) in water (25 ml.)]. After filtration the filtrate was precipitated with ethanol (130 ml.) and the diastereoisomeric salt *d*-*cis*-dinitrobis(ethylenediamine) cobalt(III)-phthaloyl-L-glutamate (L<sub>I</sub>Co) was collected and dried (6.8 g, 84%).

The filtrate [B] was set aside for recovery of partly resolved phthaloyl-D\* -glutamic acid.

Diastereoisomer  $L_I Co$  was recrystallised three times to give Fractions  $L_{II} Co$ ,  $L_{III} Co$ ,  $L_{IV} Co$ . Each of these (plus Fraction  $L_I Co$ ) was worked up to give fractions  $L_I$ ,  $L_{II}$ ,  $L_{III}$ ,  $L_{IV}$  of phthaloyl-L-glutamic acid.

The partly resolved phthaloyl-D<sup>\*</sup>-glutamic acid obtained from [B] gave Fraction V (2.6 g). This was re-resolved with 1-cis-dinitrobis(ethylenediamine) cobalt(III) acetate to give phthaloyl-D<sup>\*</sup>-glutamic acid, fractions  $D_{II}$  and  $D_{III}$ .

Resolution of Benzoyl-D,L-Valine in the presence of Benzoyl-L<sup>\*</sup>-valine:

Benzoyl-D,L-valine (30.59 g) and benzoyl-L<sup>\*</sup>-valine (0.3837 g) were suspended in water (340 ml) and a neutral solution was obtained after slow addition of  $NaHCO_3$  (11.76 g).

Isolation of Benzoyl-L-valine:

One half of this solution (170 ml.) was treated with an aqueous solution of  $d-[Coen_2(NO_2)_2]OAc$  in water (130 ml.) [ex d-iodide (27.86 g) and  $AgOAc$  (11.63 g)]. After one hour the diastereoisomeric salt  $d-[Coen_2(NO_2)_2]$ -benzoyl-L-valinate was filtered, washed well with ethanol and acetone and dried. ( $L_I Co$ ) (12.50 g)  $[\alpha]_D^{20} = +41^\circ$ , 0.5% in water. This salt was divided into two portions, (A) and (B), for subsequent treatment.

(A): 6.0 g diastereoisomer was stirred in water (50 ml.) with  $NaI$  (3.6 g) for 15 minutes, cooled to  $5^\circ$  and the  $d-[Coen_2(NO_2)_2]I$  was filtered. The filtrate was acidified to pH 4 by the slow addition of 2N  $HCl$ , cooled in ice for 10 minutes; benzoyl-L-valine was filtered, washed twice with ice cold

water (5 ml.) and dried. ( $L_I$ ) (2.21 g).

This compound was recrystallised from ethanol-water four times and a number of fractions were obtained. Their identities are shown in Fig.1. Fraction (a) was the first precipitate from the recrystallisation of  $L_I$ , and (b) was a second fraction. Similarly (f) and (g) are the first and second fractions respectively from the recrystallisation of (e).

(B): 6.5 g diastereoisomer ( $L_I$ Co) was recrystallised from hot water to give  $L_{II}$ Co (5.11 g) which was recrystallised from hot water twice more to give  $L_{III}$ Co and  $L_{IV}$ Co.

0.60 g portions of the diastereoisomers,  $L_{III}$ Co and  $L_{IV}$ Co, were treated with NaI (0.36 g) in warm water (5 ml.) stirred for 5 minutes, cooled to 5° and the  $d$ -[Coen<sub>2</sub>(NO<sub>2</sub>)<sub>2</sub>]<sub>2</sub>I was filtered. The filtrate was acidified to pH 4 with 2N HCl, filtered within 10 minutes, and the benzoyl-L-valine was washed with cold water. Fractions  $L_{III}$  and  $L_{IV}$  were obtained.

Rotations measured on these fractions of benzoyl-L-valine showed that all were optically pure.

$[\alpha]_D^{20} = +53^\circ$ , 1.0% in chloroform.

#### Isolation of Benzoyl-D-Valine:

The other half of the solution (170 ml.) of benzoyl-D,L-valine and benzoyl-L<sup>\*</sup>-valine was treated with a solution of 1-[Coen<sub>2</sub>(NO<sub>2</sub>)<sub>2</sub>]OAc in water (110 ml.) [ex 1-iodide (27.86 g) and AgOAc (11.63 g)]. After one hour the diastereoisomeric salt 1-[Coen<sub>2</sub>(NO<sub>2</sub>)<sub>2</sub>]-benzoyl-D-valinate was filtered,

washed well with ethanol and acetone and dried. ( $D_I Co$ )

(13.46 g)  $[\alpha]_D^{20} = -43^\circ$ , 0.5% in water.

This diastereoisomer was divided into two portions which were treated in the same manner as the diastereoisomer  $L_I Co$ , i.e. one portion was treated to give benzoyl-D-valine ( $D_I$ ) which was recrystallised from ethanol-water to give a number of fractions  $D_{I(a)}$ ,  $D_{I(b)}$ , etc, while the other portion was recrystallised three times to give  $D_{II} Co$ ,  $D_{III} Co$  and  $D_{IV} Co$ ;  $D_{III} Co$  and  $D_{IV} Co$  were then treated to give benzoyl-D-valine samples,  $D_{III}$  and  $D_{IV}$ .

Fraction  $D_{I(b)}$  (0.12 g) was dissolved in ethanol-water and heated in an open beaker on a steam bath for 12 hours. During this time water and ethanol were added to replace solvent lost by evaporation. After cooling the solution, fraction  $D_{I b(i)}$  (0.02 g) was filtered and washed with ethanol. The filtrate was reheated as before for another 12 hours, the solution was then evaporated to a small volume and fraction  $D_{I b(ii)}$  (0.10 g) was precipitated by the addition of water. Fig. 2 shows the relationship of these various D- fractions.

#### (4) Radioactive Counting:

The phthaloyl-glutamic acid fractions and seven fractions of benzoyl valine were counted by the gas-phase ion-current method<sup>(39)</sup> and activities were recorded as micro-curies per milligram.

For the scintillation counting, 1.0 mg (or, in several cases, 0.5 mg) benzoyl-D- or L-valine was dissolved in 10.0ml.

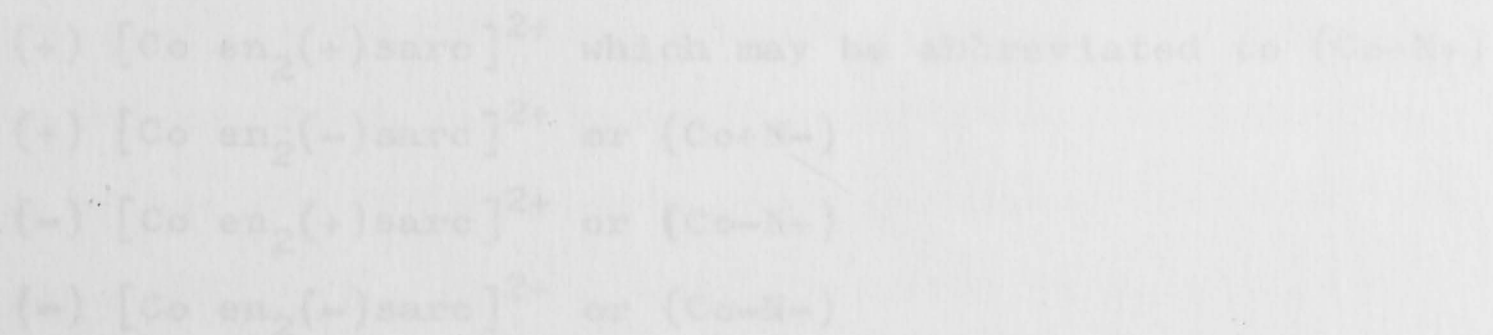
solvent. The solvent was prepared by dissolving PPO (2.00 g) and POPOP (0.200 g) in toluene (500 ml).

Activities were measured by a Packard Tri Carb Liquid Scintillation Spectrometer Model 314 and were given as counts per minute.

carbon atom in that the tetrahedral disposition of four different substituents about the central atom should allow for the existence of two optical isomers. Such a situation could occur after co-ordination of the nitrogen of a suitable secondary or tertiary amine to a metal ion:



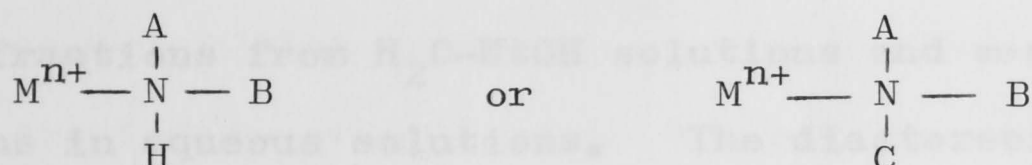
This possibility was appreciated by Mislow, who had worked extensively on the stereochemistry of the saturated trivalent nitrogen in organic compounds (47,48) and these studies led him to co-ordinate sarcosine to cobalt in the complex ion  $(\text{Co en}_2 \text{sarc})^{2+}$  (49). This ion is of interest as it has two centres of asymmetry, one due to the disposition of the groups around the nitrogen atom of the sarcosine and the other due to the arrangement of ligands about the Co(III) atom. Disregarding the conformations of the ligands, there are four active forms (two diastereoisomeric pairs) possible:



## CHAPTER TWO

STEREOCHEMISTRY AND MECHANISM OF RACEMISATIONOF CO-ORDINATED SARCOSE COMPLEXESINTRODUCTION:

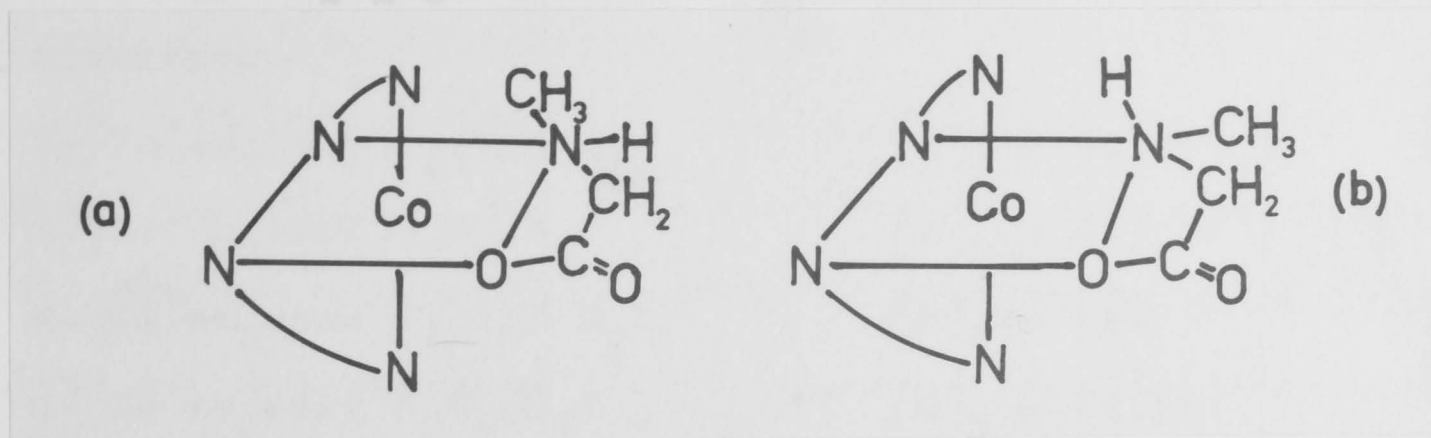
A quaternary nitrogen atom is similar to the saturated carbon atom in that the tetrahedral disposition of four different substituents about the central atom should allow for the existence of two optical isomers. Such a situation could occur after co-ordination of the nitrogen of a suitable secondary or tertiary amine to a metal ion:



This possibility was appreciated by Meisenheimer, who had worked extensively on the stereochemistry of the saturated tervalent nitrogen in organic compounds<sup>(47,48)</sup> and these studies led him to co-ordinate sarcosine to cobalt in the complex ion  $(\text{Co en}_2 \text{sarc})^{2+}$ <sup>(49)</sup>. This ion is of interest as it has two centres of asymmetry, one due to the disposition of the groups around the nitrogen atom of the sarcosine and the other due to the arrangement of ligands about the Co(III) atom. Disregarding the conformations of the ligands, there are four active forms (two diastereoisomeric pairs) possible:

- (+)  $[\text{Co en}_2(+)\text{sarc}]^{2+}$  which may be abbreviated to (Co+N+)
- (+)  $[\text{Co en}_2(-)\text{sarc}]^{2+}$  or (Co+N-)
- (-)  $[\text{Co en}_2(+)\text{sarc}]^{2+}$  or (Co-N+)
- (-)  $[\text{Co en}_2(-)\text{sarc}]^{2+}$  or (Co-N-)

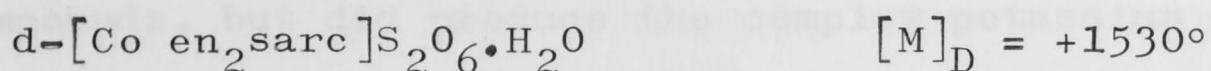
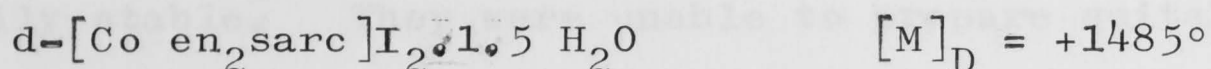
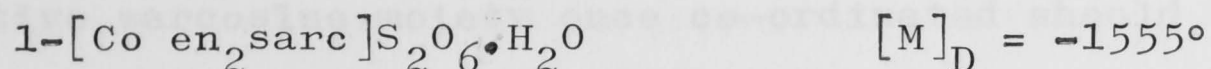
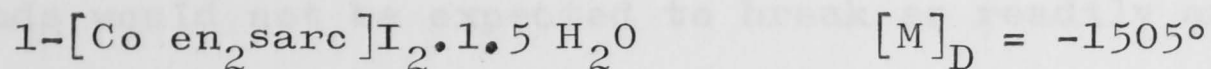
For racemic sarcosine the compound would be designated  $(Co+N^{\pm})$  or  $(Co-N^{\pm})$ . Each of the two  $(Co+)$  isomers shown below has a nonsuperposable mirror image.



Meisenheimer resolved the complex with the d-bromo-camphor sulphonate anion; he recrystallized the diastereoisomer fractions from  $H_2O-EtOH$  solutions and measured their rotations in aqueous solutions. The diastereoisomers obtained, designated as  $(Co-N^{\pm})$  and  $(Co+N^{\pm})$ , gave  $[M]_D$  values of  $-923^{\circ}$  and  $+2020^{\circ}$  respectively. In one instance, recrystallization of the diastereoisomer  $(Co+N^{\pm})$  gave a fraction having  $\alpha_D = +4.14^{\circ}$ ,  $[M]_D = +2290^{\circ}$  which decreased to  $+3.85^{\circ}$ ,  $[M]_D = +2130^{\circ}$  after 2 hours, and then to  $+3.66^{\circ}$ ,  $[M]_D = +2020^{\circ}$  after standing overnight. Meisenheimer considered that this fraction contained the  $(Co+N^+)$  isomer and that the activity of the nitrogen was lost on standing in aqueous solution. Another fraction of diastereoisomer, claimed to be  $(Co+N^-)$ , had  $\alpha_D = +2.84^{\circ}$ ,  $[M]_D = +1775^{\circ}$  which rose to  $+2.92^{\circ}$ ,  $[M]_D = +1825^{\circ}$  after 2 hours standing, but did not increase any further. In another instance, recrystallization of the  $(Co-N^{\pm})$  diastereoisomer yielded a fraction having  $\alpha_D = -1.41^{\circ}$ ,  $[M]_D = -860^{\circ}$  which reverted to  $\alpha_D = -1.53^{\circ}$ ,  $[M]_D = -930^{\circ}$  after 2 hours standing. This fraction was

considered to contain impure (Co-N+) isomer.

The diastereoisomers (Co-N $\pm$ ) and (Co+N $\pm$ ) were treated with NaI or Na<sub>2</sub>S<sub>2</sub>O<sub>6</sub> to give the optically active iodides or dithionates.



Meisenheimer assigned the configurations (Co-N $\pm$ ) and (Co+N $\pm$ ) to these compounds also.

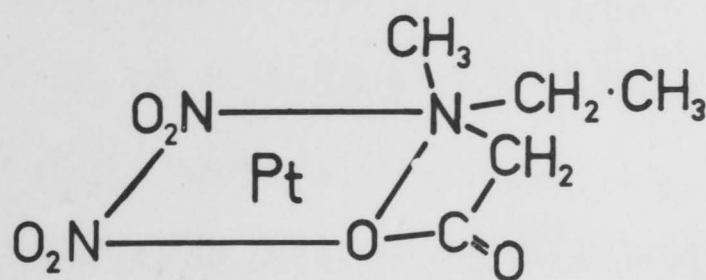
He reported that treatment of the (Co+N+) diastereoisomer to give the iodide or dithionate did not give consistent results. In one instance a d-dithionate isomer showed, on standing, a decrease in rotation of the expected order. On the other hand some l-dithionate fractions [obtained from the (Co-N $\pm$ ) diastereoisomer] gave initial  $[M]_D$  values from  $-1565^\circ$  to  $-1100^\circ$  which changed to  $-1300^\circ$  in 24 hours; however, in most cases the rotation of the l-dithionate was constant at  $[M]_D \sim -1500^\circ$ .

Although it was claimed, on the basis of the mutarotations, that the asymmetry of the Sarcosine nitrogen had been demonstrated, only two isomers of the complex were isolated and the mutarotations were not always reproducible. A later attempt to repeat Meisenheimer's work was unsuccessful. (50)

This may be explained by the possibility that the hydrogen attached to the nitrogen of co-ordinated sarcosine is labile and exchanges rapidly with the aqueous phase. This



could lead to rapid racemisation or mutarotation of the active nitrogen centre, and to circumvent this problem Kuebler and Bailar attempted to co-ordinate N-methyl N-ethyl glycine (N-ethyl sarcosine).<sup>(51)</sup> Here the C-N bonds would not be expected to break so readily and the active sarcosine moiety once co-ordinated should be optically stable. They were unable to prepare suitable Co(III) compounds, but did produce the complex potassium dinitro (N-methyl N-ethyl glycinato) platinum-(II)-ate, where the N of the chelate is the only source of optical activity.



This complex was resolved with quinine bisulphate and several fractions of diastereoisomer finally yielded solutions of the potassium salt of the complex ion which had slight levo rotations ( $0.012-0.021^\circ$ ) - the same sign as the resolving agent. However these rotations fell to zero in five or six hours. In another experiment, aqueous solutions of the racemic complex, after shaking with d- or l- quartz, showed slight rotations in the directions opposite to that of the quartz used ( $0.014-0.034^\circ$ ). These rotations also disappeared after five to six hours. It seems unlikely that in this instance the C-N bond would break; the racemisation then would be attributed to the rupture of the Pt-N bond. Although

the rotations observed were low, it would seem that the co-ordinated tertiary N atom can be resolved.

Returning to the co-ordinated sarcosine complexes, the possibility of stabilizing the proton on the sarcosine nitrogen arose from a consideration of studies made by Anderson, Briscoe and Spoor on the kinetics of interchange of hydrogen isotopes with complex ions<sup>(52)</sup>. For the ions  $[\text{Co}(\text{NH}_3)_6]^{3+}$  and  $[\text{Co en}_3]^{3+}$  the rate of exchange of hydrogen bonded to nitrogen was inversely proportional to the hydrogen ion concentration

i.e. Rate =  $k [\text{OH}^-]$

For  $[\text{Co}(\text{NH}_3)_6]^{3+}$  at pH = 4.55 and T = 20°, the half life of the reaction was 79 minutes.

More recent investigations by different methods have confirmed this rate dependence on  $[\text{OH}^-]$  for these two complexes, and for a number of other ammine complexes<sup>(53,54,55,56)</sup>.

These results<sup>(52)</sup> indicated that in solutions of moderate acidity (pH = 3),  $t_{1/2}$  for the exchange of hydrogen with  $[\text{Co}(\text{ND}_3)_6]^{3+}$  would be of the order of 40 hours at room temperature and the inverse reaction would not be expected to differ by more than an order of magnitude.

If Meisenheimer's inability to obtain the four isomers of  $[\text{Co en}_2 \text{sarc}]^{2+}$  was due to lability of the hydrogen attached to the sarcosine nitrogen, and if this hydrogen exchanges at a rate similar to those in  $[\text{Co}(\text{NH}_3)_6]^{3+}$ , then at pH = 3 the exchange rate should be sufficiently slow to allow isolation of optical isomers due to an asymmetric nitrogen.

With this possibility in mind the Meisenheimer resolution of  $[\text{Co en}_2\text{sarc}]^{2+}$  was repeated.

Resolution of  $[\text{Co en}_2\text{sarc}]^{2+}$ :

Silver d-bromo camphor sulphonate was used as the resolving agent and the diastereoisomer fractions were recrystallised from  $\text{H}_2\text{O}$ -EtOH solutions to constant activity,  $[\text{M}]_D = -970^\circ$  and  $+2050^\circ$  (see Experimental). Further recrystallization of these fractions from  $0.001\text{M HClO}_4$ -EtOH solutions did not change their rotations, measured either in  $\text{H}_2\text{O}$  or  $0.001\text{M HClO}_4$  solution. Also, no change in rotation was observed after the solutions had stood for 12 hours.

The optically active ions d- and l- $[\text{Co en}_2\text{sarc}]^{2+}$  were isolated as the iodides from  $0.001\text{M HClO}_4$  solutions.

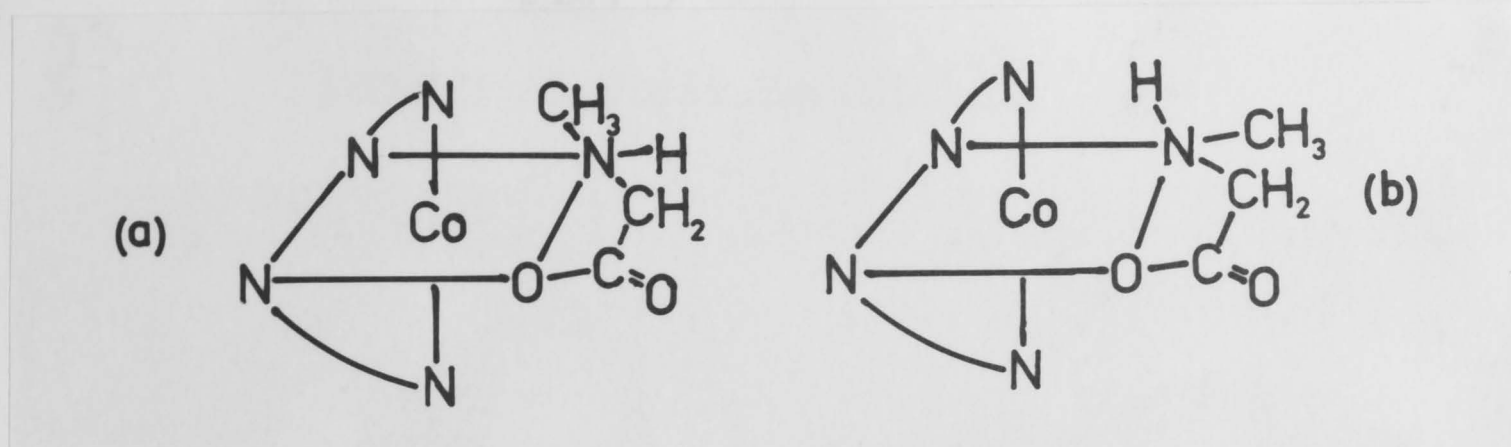
l- $[\text{Co en}_2\text{sarc}]\text{I}_2 \cdot 1.5 \text{H}_2\text{O}$ ,  $[\text{M}]_D = -1540^\circ$ , and

d- $[\text{Co en}_2\text{sarc}]\text{I}_2 \cdot 1.5 \text{H}_2\text{O}$ ,  $[\text{M}]_D = +1510^\circ$ . Identical rotations were recorded in  $0.001\text{M HClO}_4$ ,  $\text{H}_2\text{O}$  and  $0.1\text{M NaHCO}_3$  solutions, and they were unchanged after recrystallisation from  $0.001\text{M HClO}_4$  solution. These  $[\text{M}]_D$  values are substantially the same as those reported by Meisenheimer (p.47).

If the sarcosine in these isomers was resolved and was capable of being racemized it was expected that this racemization would occur in  $0.1\text{M NaHCO}_3$  solution (pH = 8.8).

The failure to observe any activity definitely attributable to the nitrogen can be accounted for by examination of the Leybold space filled molecular models of the four possible isomers. For each configuration of the cobalt atom one configuration of the sarcosine nitrogen will be more favoured

than the other.



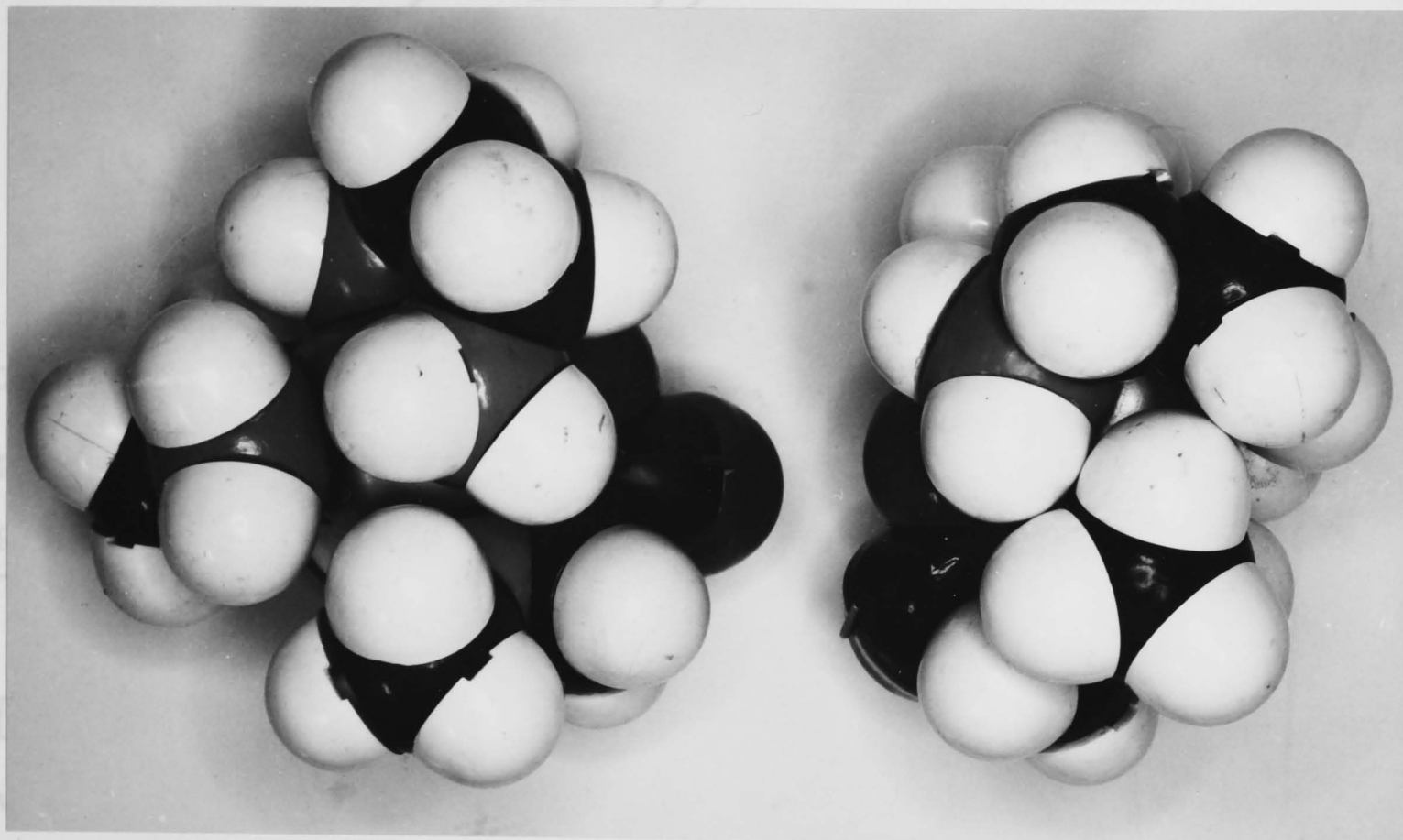
In structure (b) the CH<sub>3</sub> group of the sarcosine is crowded by one of the ethylenediamine ligands, whereas structure (a) is relatively strainless as the CH<sub>3</sub> group lies between the two ethylenediamine rings. (See Fig.3)

It is considered likely therefore, that the sarcosine ligand is co-ordinated stereospecifically, so giving rise to only two isomers [Co + N +] and [Co - N -] or [Co + N -] and [Co - N +]; thus it is one of these isomer pairs which have been obtained from this resolution and from Meisenheimer's.

It is apparent that the potential asymmetry of co-ordinated nitrogen could be demonstrated unequivocally by the resolution of a complex in which the co-ordinated nitrogen is the sole source of asymmetry. Such a compound is the Pt(II) complex for which Kuebler and Bailar reported a partial resolution. (p.48) These workers also reacted N, N-diethyl glycine with  $\text{cis-}[\text{Co}(\text{NH}_3)_4\text{Cl}_2]^+$ ,  $[\text{Co}(\text{NH}_3)_4(\text{SO}_3)_2]^-$  and  $[\text{Co}(\text{NO}_2)_4(\text{NH}_3)_2]^-$  but did not obtain the desired complexes  $[\text{Co}(\text{NH}_3)_4(\text{Et}_2\text{-gly})]^{2+}$  and  $[\text{Co}(\text{NO}_2)_4(\text{Et}_2\text{-gly})]^{2-}$ . They therefore did not attempt the preparation of the analogous complexes with N-methyl N-ethyl glycine which would have possessed asymmetry only at the co-ordinated nitrogen, e.g.

FIG. 3

Isomers of  $d\text{-}[\text{Co en}_2 \text{sarc}]^{2+}$



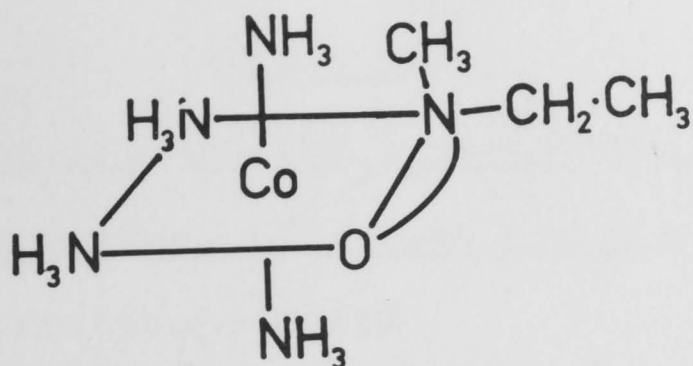
(a)

(b)

The N-methyl groups appear at the lower right of each model. In configuration (a) this methyl group lies between the ethylenediamine rings, but in (b) the methyl group is crowded by one of the ethylenediamine rings.

Rates of Racemization of  $d\text{-}$  or  $l\text{-}[\text{Co}(\text{NH}_2)_4 \text{sarc}]^{2+}$

In neutral solutions these isomers racemized readily and completely. The final solutions showed no rotation at any wavelength (e.g. Table 3). The rates of racemization were measured in buffered solutions over the pH range 4.44 to 8.02



Following the resolution of  $[\text{Co en}_2\text{sarc}]^{2+}$  it appeared likely that if the complex ion  $[\text{Co}(\text{NH}_3)_4\text{sarc}]^{2+}$  could be prepared, it might be resolved from acid solution.

Resolution of  $[\text{Co}(\text{NH}_3)_4\text{sarc}]^{2+}$ :

The complex ion  $[\text{Co}(\text{NH}_3)_4\text{sarc}]^{2+}$  was prepared from  $[\text{Co}(\text{NH}_3)_4\text{Cl OH}_2]\text{SO}_4$  and isolated as its nitrate. After conversion to the more soluble chloride it was resolved with  $d\text{-Na} [\text{Co en ox}_2]\text{H}_2\text{O}$ . The diastereoisomer  $1\text{-}[\text{Co}(\text{NH}_3)_4\text{sarc}]^{2+}$   $d\text{-}[\text{Co en ox}_2]^- \text{Cl}^- \text{H}_2\text{O}$  was treated with  $\text{NH}_4\text{NO}_3$ , and the most soluble fraction of  $[\text{Co}(\text{NH}_3)_4\text{sarc}](\text{NO}_3)_2$  obtained by fractional crystallization was the pure levo isomer,  $[\alpha]_D = -51^\circ$  and  $[\alpha]_{436} = -220^\circ$ , 0.1% solution in 0.001M  $\text{HClO}_4$ .

The most soluble portion of nitrate obtained from the filtrate of the diastereoisomer,  $d\text{-}[\text{Co}(\text{NH}_3)_4\text{sarc}](\text{NO}_3)_2$ , gave  $[\alpha]_D = +48^\circ$  and  $[\alpha]_{436} = +206^\circ$ , 0.1% in 0.001M  $\text{HClO}_4$ .

Rates of Racemization of  $d\text{-}$  or  $1\text{-}[\text{Co}(\text{NH}_3)_4\text{sarc}]^{2+}$ :

In neutral solutions these isomers racemized readily and completely. The final solutions showed no rotation at any wavelength (e.g. Table 3). The rates of racemization were measured in buffered solutions over the pH range 4.44 to 8.02

and at temperatures between 20° and 40°.

TABLE 3

Racemization of  $d\text{-[Co(NH}_3\text{)}_4\text{sarc] (NO}_3\text{)}_2$  in 0.1M

Phosphate buffer, pH = 8.02 and T = 20.2°

Complex concentration = 0.11%

Time (min.)	$\alpha_{436}$ (deg.)
1.5	0.236
3	0.222
6	0.193
10	0.159
16.5	0.118
22	0.091
345	0.000 = $\alpha_{313}$ , $\alpha_{364}$ , $\alpha_{546}$ and $\alpha_{578}$ .

If the racemization rate were proportional to  $[\text{OH}^-]$ , as was found for the hydrogen exchange of Co(III) amines<sup>(52-56)</sup> then

$$\frac{d\alpha}{dt} = -k \alpha [\text{OH}^-],$$

since  $\alpha$  is proportional to the concentration of optically active isomer. Rearrangement and integration gives

$$\ln \alpha = -k [\text{OH}^-] t + \text{const.}$$

For each kinetic run,  $[\text{OH}^-]$  is constant throughout the reaction, so that for each experiment  $k[\text{OH}^-]$  will be constant ( $k'$ ).

$$\text{Thus } \ln \alpha = -k' t + \text{const.}$$

$$\text{or } \log \alpha = -\frac{k'}{2.303} t + \text{const.}$$

In every case a plot of  $\log \alpha_{436}$  against time gave a straight line (Fig. 4) in agreement with the above rate law. From these plots,  $k'$  ( $\text{min}^{-1}$ ) the pseudo-first order rate constant was calculated, thence the overall rate constant  $k$  ( $\text{M}^{-1} \text{min}^{-1}$ ).

Rates of racemization measured at  $30.3^\circ$  in 0.1M acetate buffers over a pH range of 1.8 units gave  $k$  values which were nearly constant, thus showing a first order dependence on  $[\text{OH}^-]$ . Dilution of the buffer concentration by a factor of 10 did not affect the rate, which therefore was independent of acetate ion concentration. These results are given in Table 4.

TABLE 4

Rate Constants for the Racemization of d- or l- $[\text{Co}(\text{NH}_3)_4\text{sarc}]$   
 $(\text{NO}_3)_2$  at  $30.3^\circ$  in 0.1M acetate buffer

pH	$t_{1/2}$ (min)	$k' \times 10^2$ ( $\text{min}^{-1}$ )	$[\text{OH}^-] \times 10^8$	$\frac{k'}{[\text{OH}^-]} = k$ ( $\text{M}^{-1} \text{min}^{-1}$ )
4.80	864	.0802	.0631	$12.7 \times 10^5$
5.31	284	.244	.204	$12.0 \times 10^5$
5.83	90	.770	.676	$11.4 \times 10^5$
6.62	14.9	4.65	4.17	$11.2 \times 10^5$
<u>0.01M Acetate</u>				
*5.80	98.5	.704	.631	$11.2 \times 10^5$

Complex concentrations were 0.0032 - 0.0035M.

\*NaCl was added to the 0.01M acetate solution to maintain the Ionic Strength equal to that of the 0.1M acetate at pH = 5.83,  $\mu = 0.10$ .



Fig. 4a

Racemization of  $d\text{-}[\text{Co}(\text{NH}_3)_4 \text{sarc}]^{2+}$

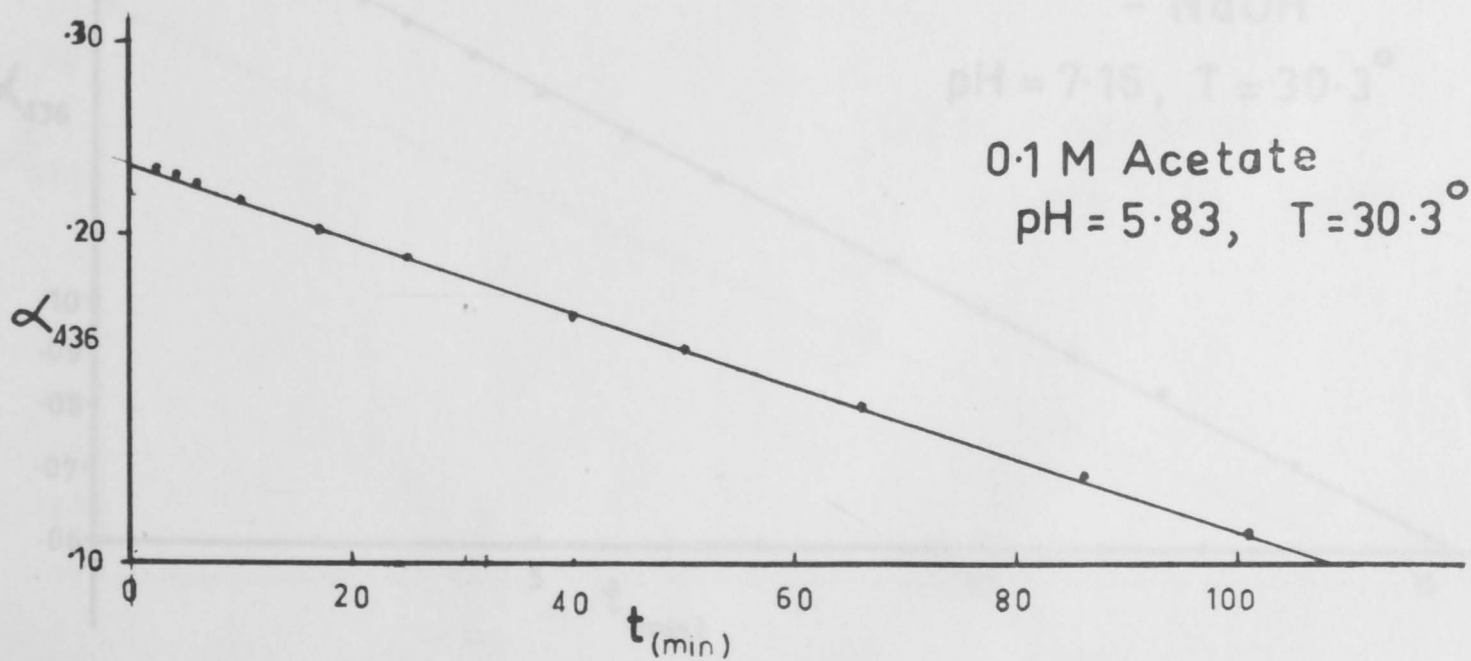
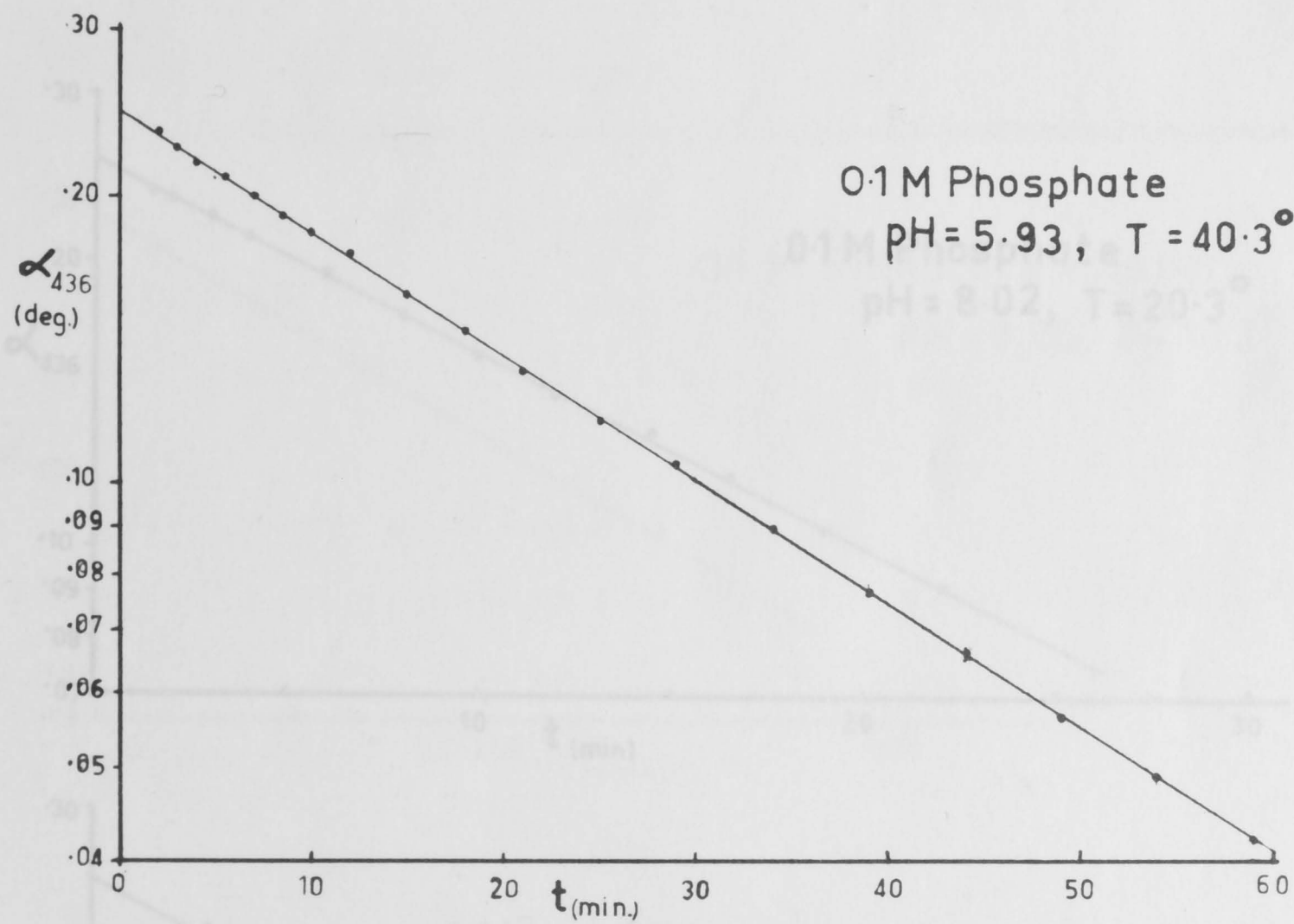


Fig. 4b

Racemization of  $d\text{-}[\text{Co}(\text{NH}_3)_4 \text{sarc}]^{2+}$

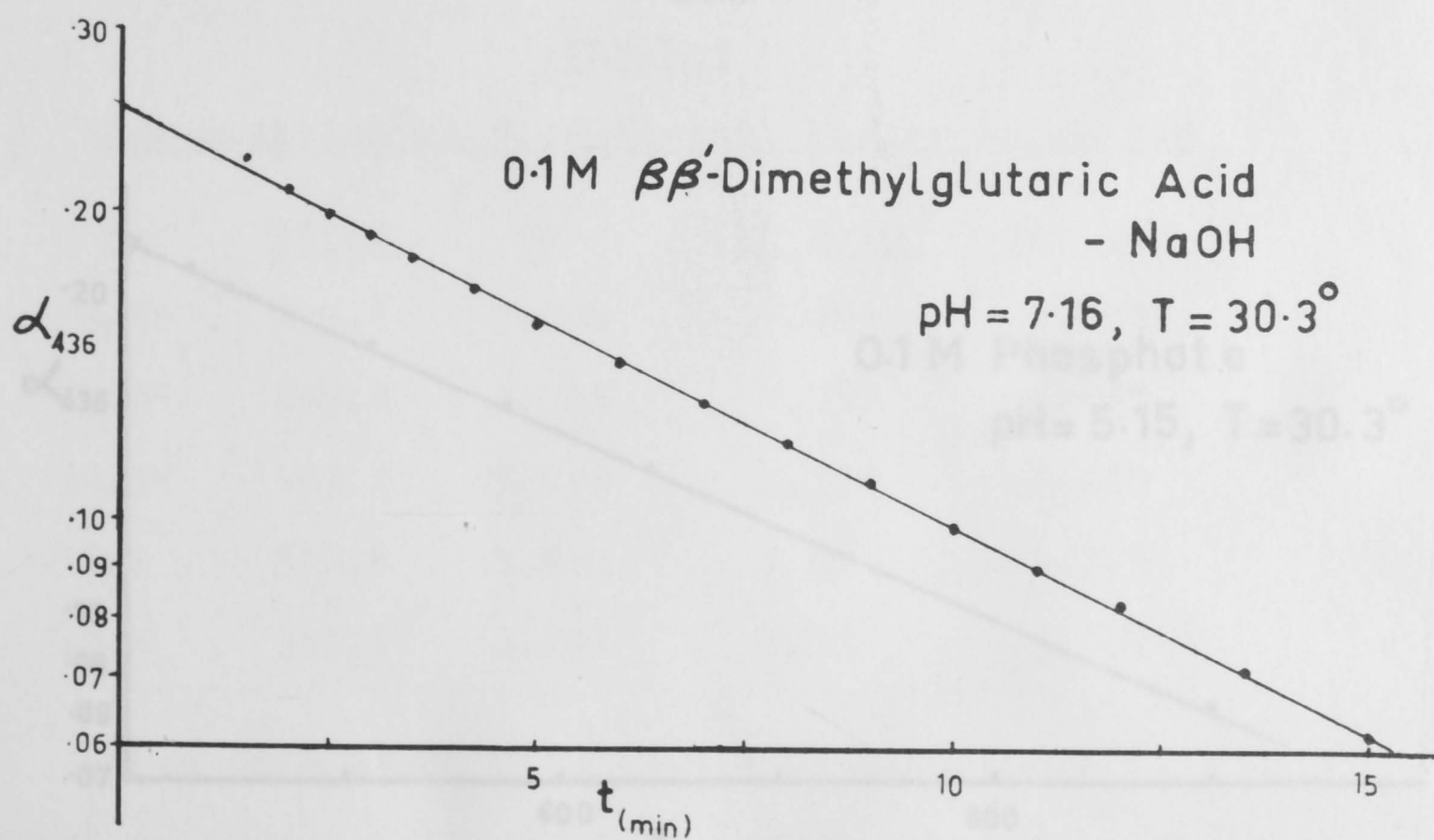
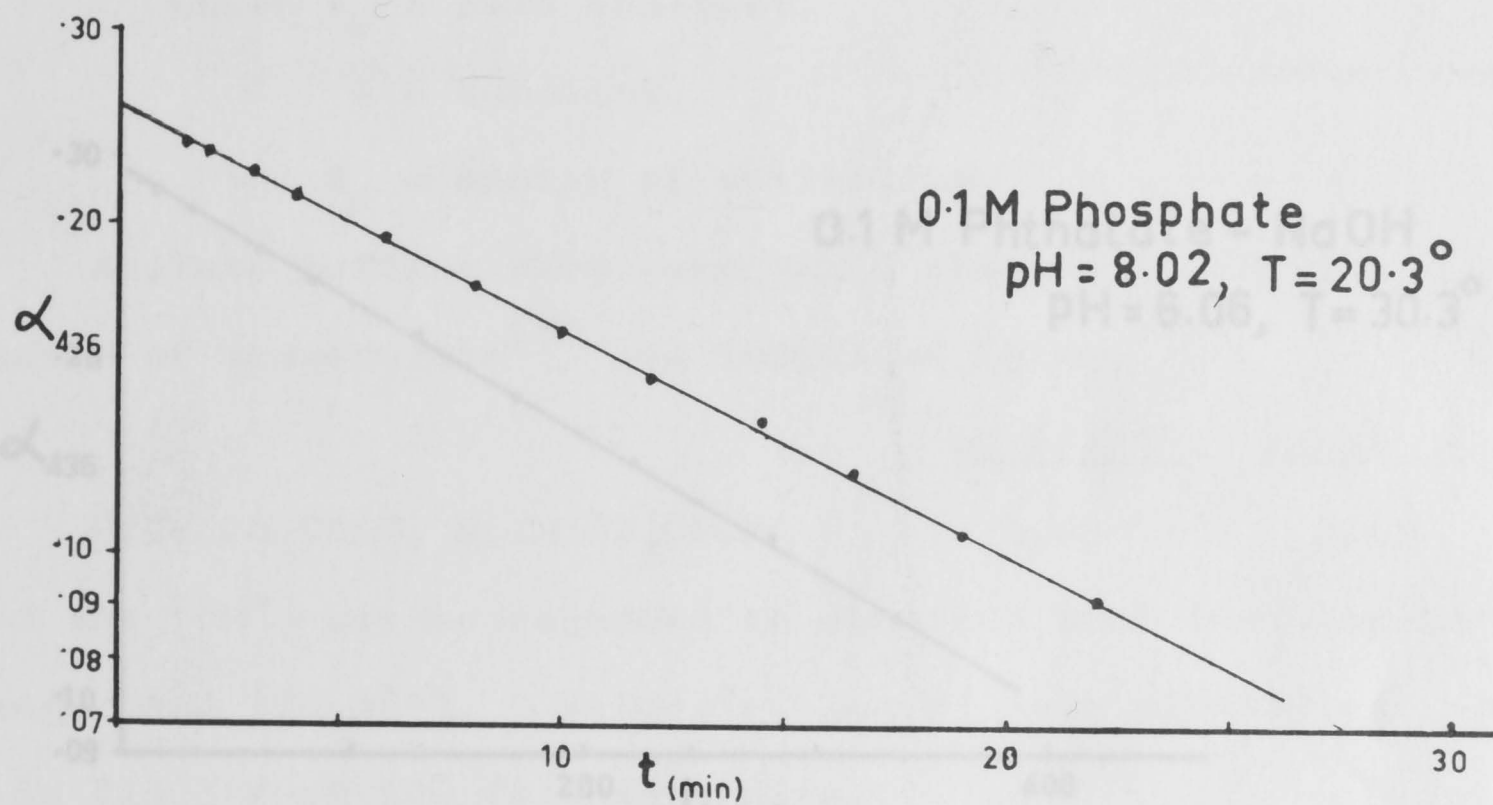


Fig. 4c

Racemization of  $d-[Co(NH_3)_4 \text{ sarc}]^{2+}$

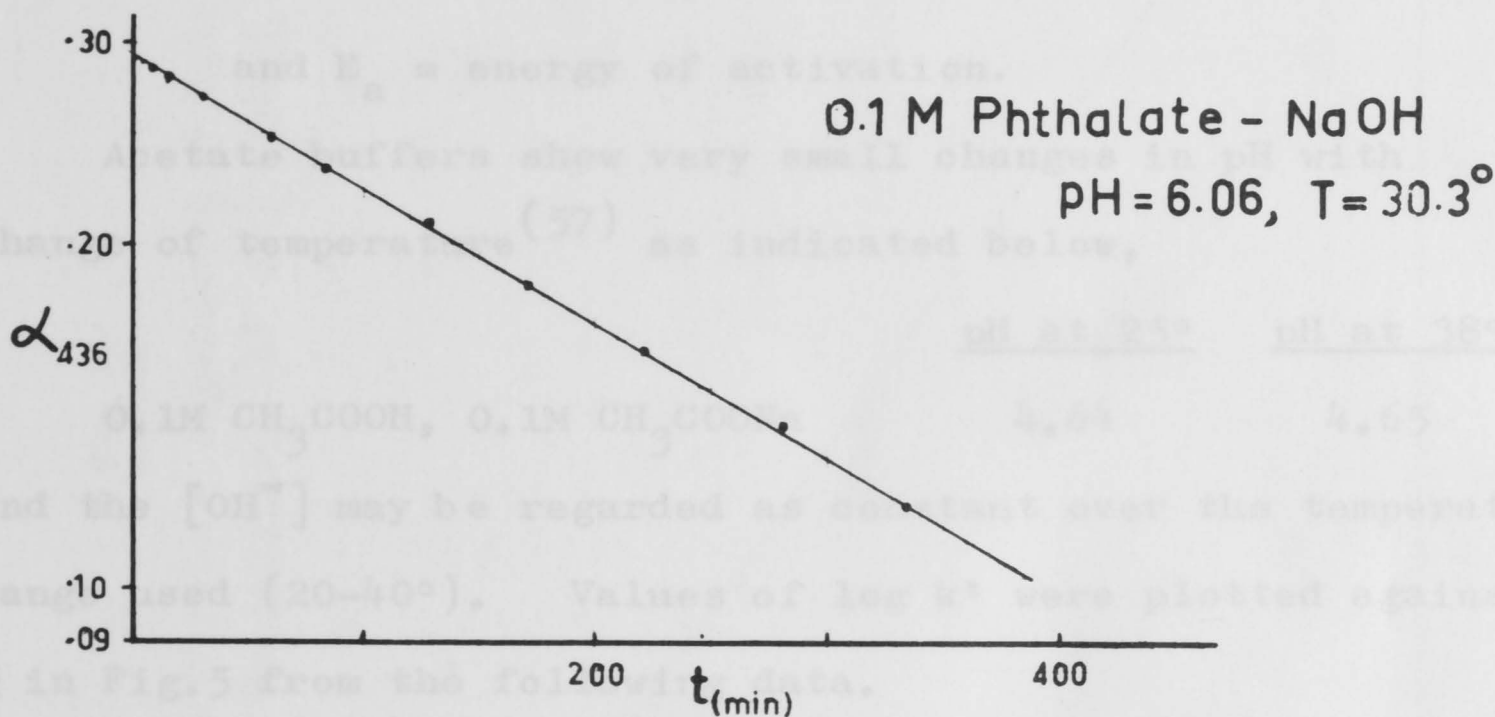
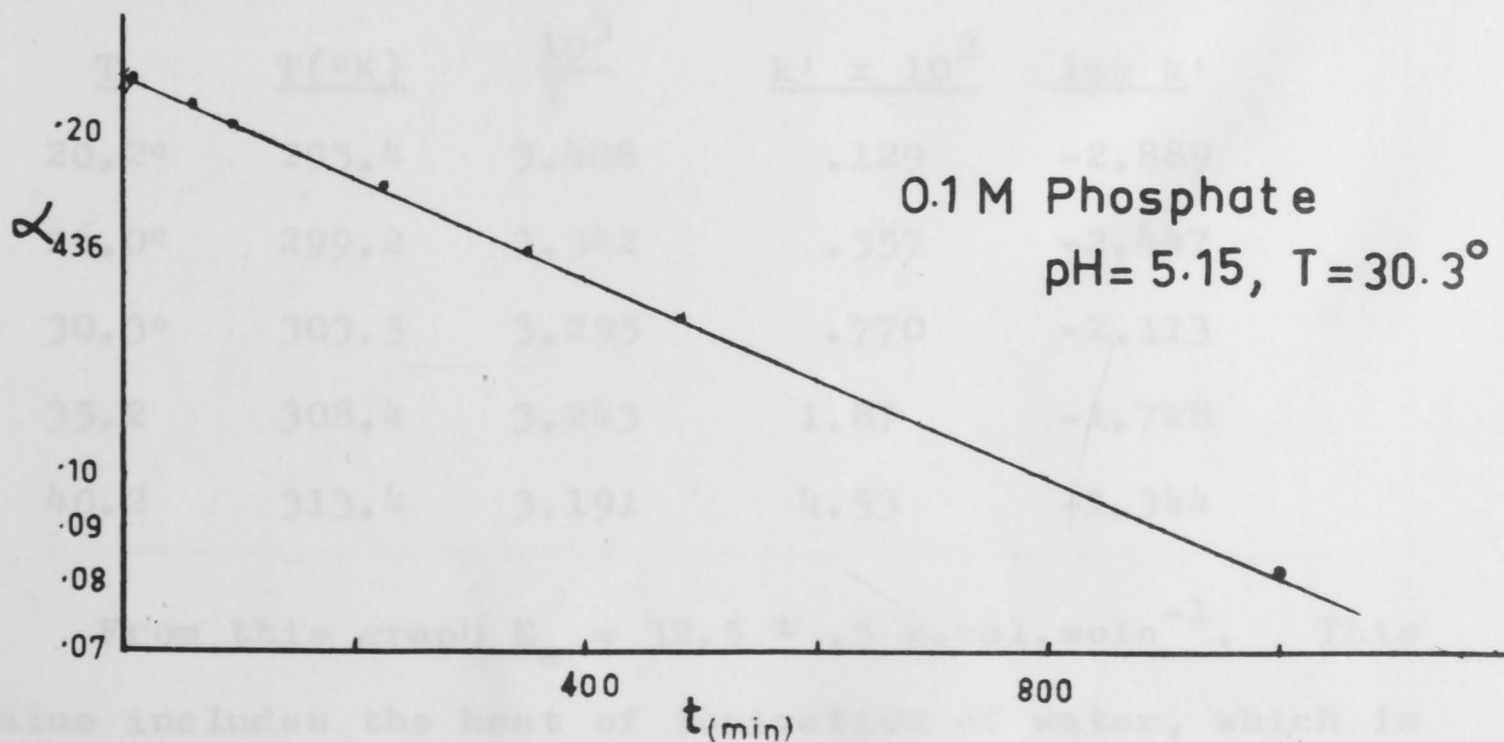


TABLE 5

Racemization in 0.1M Acetate buffer at pH 5.83



Energy and Entropy of Activation:

From the measurement of rates at various temperatures in acetate (pH = 5.83) the activation energy for the racemization was calculated, using the Arrhenius equation,

$$\log k_r = \log A - \frac{E_a}{2.303R} \frac{1}{T}$$

where  $k_r$  = rate constant,

A = constant,

and  $E_a$  = energy of activation.

Acetate buffers show very small changes in pH with change of temperature<sup>(57)</sup> as indicated below,

	<u>pH at 25°</u>	<u>pH at 38°</u>
0.1M CH <sub>3</sub> COOH, 0.1M CH <sub>3</sub> COONa	4.64	4.65

and the [OH<sup>-</sup>] may be regarded as constant over the temperature range used (20-40°). Values of log k' were plotted against  $\frac{1}{T}$  in Fig.5 from the following data.

TABLE 5

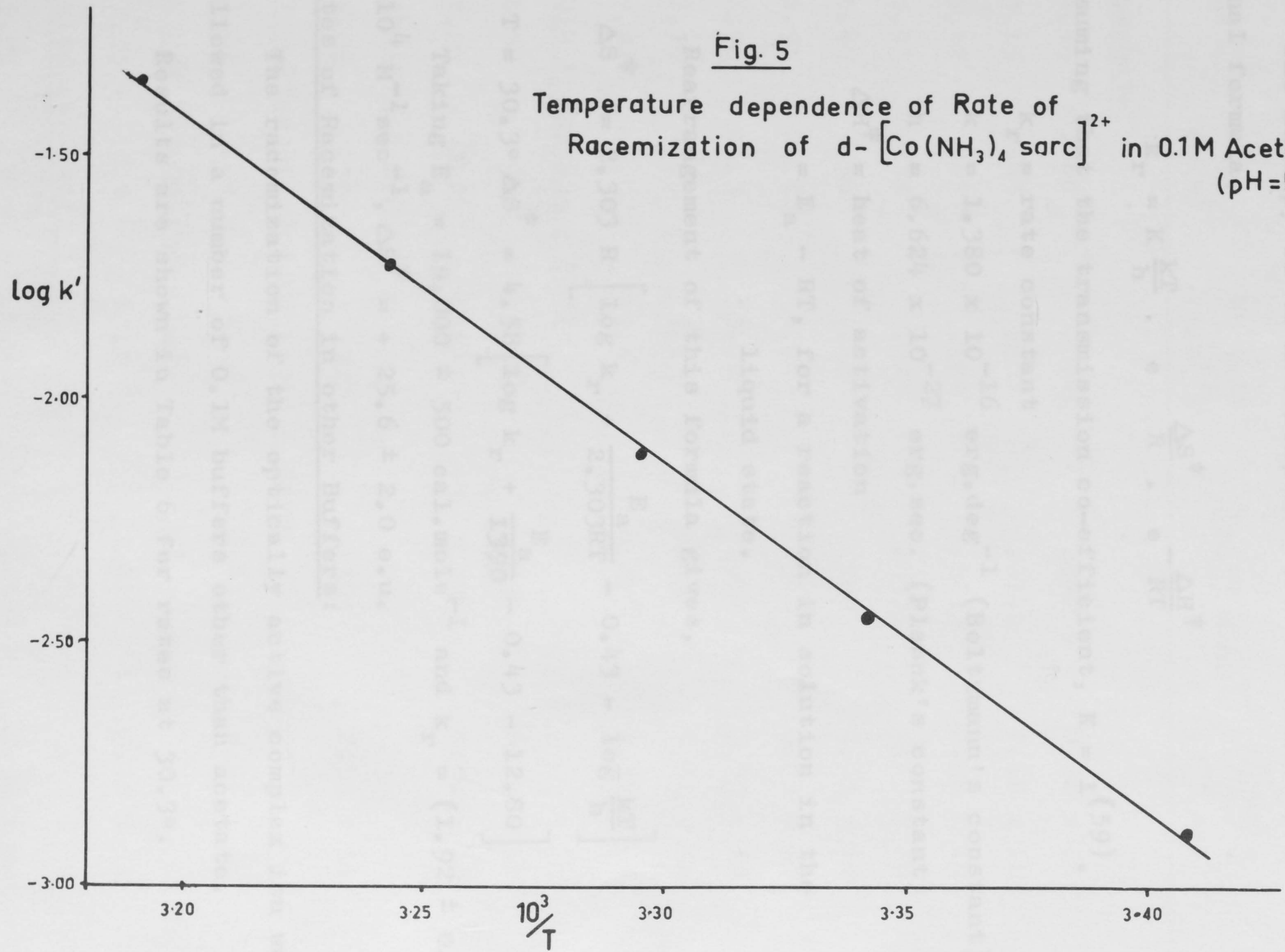
Racemization in 0.1M Acetate buffer at pH 5.83

<u>T</u>	<u>T(°K)</u>	$\frac{10^3}{T}$	<u>k' x 10<sup>2</sup></u>	<u>log k'</u>
20.2°	293.4	3.408	.129	-2.889
26.0°	299.2	3.342	.357	-2.447
30.3°	303.5	3.295	.770	-2.113
35.2	308.4	3.243	1.87	-1.728
40.2	313.4	3.191	4.53	-1.344

From this graph  $E_a = 32.5 \pm .5 \text{ k.cal.mole}^{-1}$ . This value includes the heat of ionization of water, which is

Fig. 5

Temperature dependence of Rate of  
Racemization of  $d\text{-}[\text{Co}(\text{NH}_3)_4 \text{sarc}]^{2+}$  in 0.1M Acetate  
(pH=5.83)



$13.5 \text{ k.cal.mole}^{-1}$  (58), thus the corrected value of  $E_a$  is  $19.0 \pm .5 \text{ k.cal.mole}^{-1}$ .

The Entropy of Activation,  $\Delta S^\ddagger$  was calculated from the usual formula,

$$k_r = K \frac{kT}{h} \cdot e^{\frac{\Delta S^\ddagger}{R}} \cdot e^{-\frac{\Delta H^\ddagger}{RT}}$$

assuming that the transmission co-efficient,  $K = 1$  (59).

$k_r$  = rate constant

$k = 1.380 \times 10^{-16} \text{ erg.deg}^{-1}$  (Boltzmann's constant)

$h = 6.624 \times 10^{-27} \text{ erg.sec.}$  (Planck's constant)

$\Delta H^\ddagger$  = heat of activation

=  $E_a - RT$ , for a reaction in solution in the

liquid state.

Rearrangement of this formula gives,

$$\Delta S^\ddagger = 2.303 R \left[ \log k_r + \frac{E_a}{2.303RT} - 0.43 - \log \frac{kT}{h} \right]$$

$$\text{At } T = 30.3^\circ \Delta S^\ddagger = 4.58 \left[ \log k_r + \frac{E_a}{1390} - 0.43 - 12.80 \right]$$

Taking  $E_a = 19,000 \pm 500 \text{ cal.mole}^{-1}$  and  $k_r = (1.92 \pm 0.04) \times 10^4 \text{ M}^{-1} \text{sec}^{-1}$ ,  $\Delta S^\ddagger = + 25.6 \pm 2.0 \text{ e.u.}$

#### Rates of Racemization in other Buffers:

The racemization of the optically active complex ion was followed in a number of 0.1M buffers other than acetate.

Results are shown in Table 6 for rates at  $30.3^\circ$ .

TABLE 6

Rate Constants for the Racemization of  $[\text{Co}(\text{NH}_3)_4\text{sarc}]^{2+}$   
 in 0.1M buffers at 30.3°

pH	$t_{1/2}$ (min)	$k' \times 10^2$ ( $\text{min}^{-1}$ )	$[\text{OH}^-] \times 10^7$	$\frac{k'}{[\text{OH}^-]} = k$ ( $\text{M}^{-1} \text{min}^{-1}$ )
<u>Phosphate:</u>				
4.44	3060	.0226	.00275	$8.22 \times 10^5$
5.15	685	.101	.0141	$7.18 \times 10^5$
5.42	367	.189	.0263	$7.18 \times 10^5$
5.93	135	.523	.0851	$6.15 \times 10^5$
6.43	56	1.24	.269	$4.61 \times 10^5$
6.96	21.5	3.22	.912	$3.53 \times 10^5$
* 6.96	21.5	3.22	.912	$3.53 \times 10^5$
7.16	13.5	5.13	1.45	$3.54 \times 10^5$
7.61	6.0	11.6	4.07	$2.84 \times 10^5$
# <u>0.01M Phosphate + NaCl:</u>				
6.38	34	2.04	.234	$8.70 \times 10^5$
7.03	8.2	8.45	1.07	$7.90 \times 10^5$
<u>Phthalate - NaOH:</u>				
6.06	256	.271	.115	$2.35 \times 10^5$
<u><math>\beta\beta'</math> - Dimethylglutaric Acid - NaOH:</u>				
7.16	7.4	9.36	1.45	$6.46 \times 10^5$
<u>N-Ethyl Morpholine - HCl:</u>				
7.15	5.2	13.2	1.41	$9.36 \times 10^5$
<u>Collidine - HCl:</u>				
5.97	80	.866	.0933	$9.28 \times 10^5$
7.14	5.6	12.3	1.38	$8.91 \times 10^5$

TABLE 6 (Cont'd)

Imidazole - HCl:

6.23	52.5	1.32	.170	$7.76 \times 10^5$
6.23	52.5	1.32	.170	$7.76 \times 10^5$
6.71	16.6	4.17	.513	$8.13 \times 10^5$
7.15	5.6	12.4	1.41	$8.77 \times 10^5$
7.15	5.4	12.8	1.41	$9.06 \times 10^5$

\* Complex concentration was 0.0015M. All other complex concentrations were 0.0032 - 0.0035M.

# Sufficient NaCl was added to maintain the Ionic Strength equal to that of the 0.1M phosphate buffer,  $\mu = 0.11$  and 0.18 for pH = 6.43 and 7.16, respectively.

It can be seen that all the values of k for these buffers were lower than those for the acetate buffers. While k was reasonably constant with changing pH for the Collidine and Imidazole buffers, in phosphate buffer k decreased with increasing pH (Fig.6). The racemization in phosphate buffer did not therefore fit the rate law,

$$\frac{d\alpha}{dt} = -k \cdot \alpha \cdot [\text{OH}^-]$$

If the reaction were catalysed by the phosphate ions as well as hydroxyl ion, the rate law might be expected to have the form,

$$-\frac{d\alpha}{dt} = \left( k [\text{OH}^-] + k_1 [\text{H}_2\text{PO}_4^-] + k_2 [\text{HPO}_4^{2-}] \right) \alpha$$

A decrease in the buffer concentration, i.e.  $[\text{H}_2\text{PO}_4^-]$  and  $[\text{HPO}_4^{2-}]$ , would then result in a slower rate of reaction. In Table 6 it is shown that in 0.01M buffers the rates of

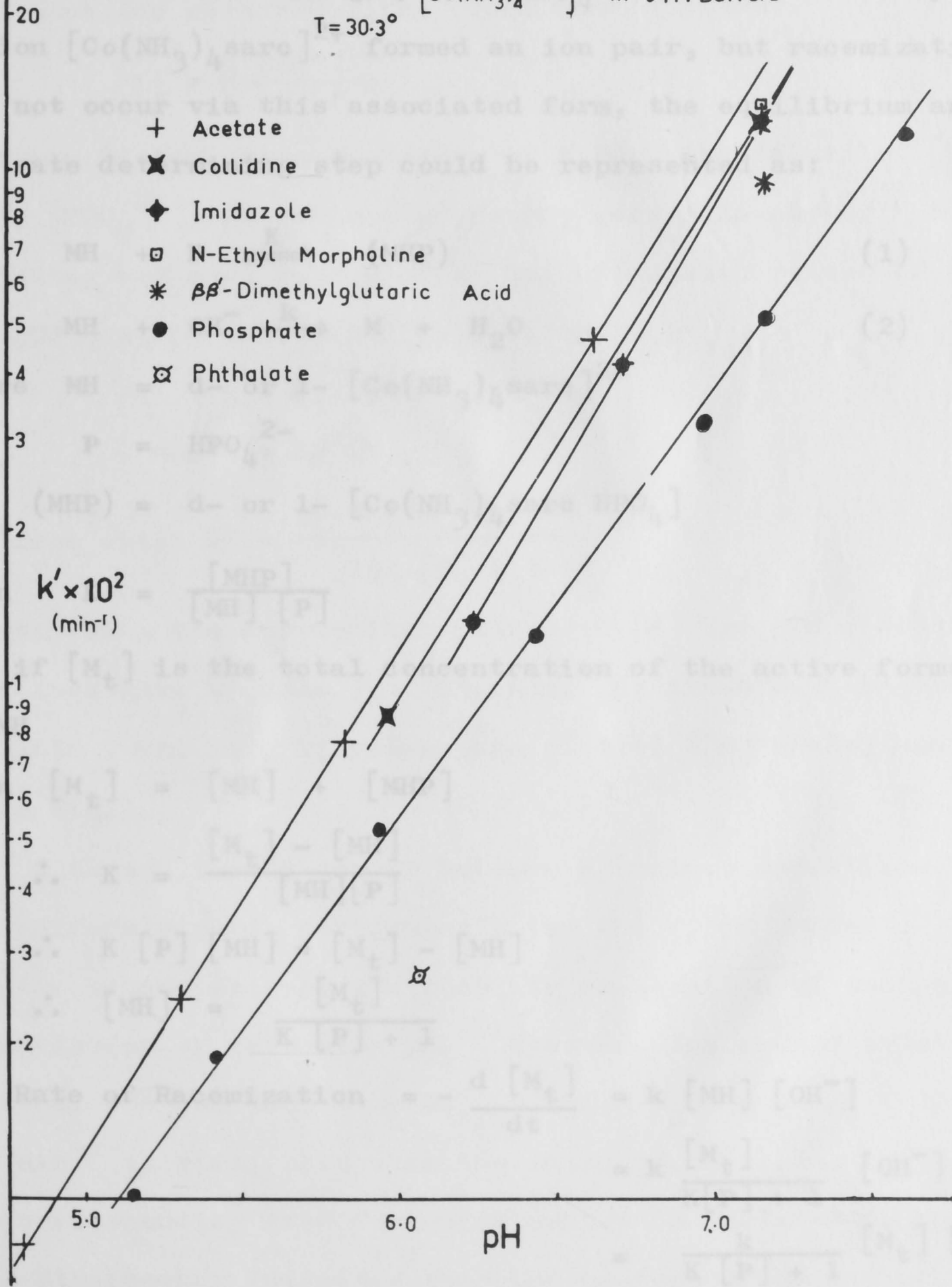


reaction were greater than those in  $\text{O}_2\text{P}^-$  buffers, which indicates that there is no catalysis by phosphate ions. Ion Pair Formation with  $\text{HPO}_4^{2-}$ :

**Fig. 6**

Racemization of  $d\text{-}[\text{Co}(\text{NH}_3)_4\text{sarc}]^{2+}$  in 0.1M Buffers

$T = 30.3^\circ$

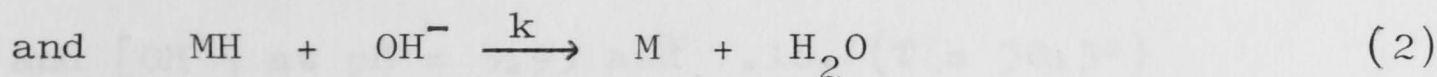


The rate constant  $k'$  is defined as  $k' = \frac{d[\text{MIP}]}{dt} \cdot \frac{[\text{M}][\text{P}]}{[\text{MIP}]}$ . Now  $[\text{M}_t]$  is the total concentration of the active forms of the metal ion,  $[\text{M}_t] = [\text{M}] + [\text{MIP}]$ .  $K = \frac{[\text{MIP}]}{[\text{M}][\text{P}]}$ .  $\therefore [\text{MIP}] = \frac{[\text{M}_t][\text{P}]}{K[\text{P}] + 1}$ . The rate of racemization  $= -\frac{d[\text{M}_t]}{dt} = k[\text{M}][\text{OH}^-] = k \frac{[\text{M}_t][\text{P}]}{K[\text{P}] + 1} [\text{OH}^-]$ .  $\therefore \ln[\text{M}_t] = -\frac{k[\text{OH}^-]}{K[\text{P}] + 1} t + \text{const.}$

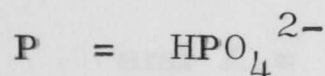
reaction were greater than those in 0.1M buffers, which indicates that there is no catalysis by phosphate ions.

Ion Pair Formation with  $\text{HPO}_4^{2-}$ :

If the double charged anion  $\text{HPO}_4^{2-}$  and the free complex cation  $[\text{Co}(\text{NH}_3)_4\text{sarc}]^{2+}$  formed an ion pair, but racemization did not occur via this associated form, the equilibrium and the rate determining step could be represented as:



where  $\text{MH} = \text{d- or l- } [\text{Co}(\text{NH}_3)_4\text{sarc}]^{2+}$



and  $(\text{MHP}) = \text{d- or l- } [\text{Co}(\text{NH}_3)_4\text{sarc HPO}_4]$

$$\text{Then } K = \frac{[\text{MHP}]}{[\text{MH}][\text{P}]}$$

Now if  $[\text{M}_t]$  is the total concentration of the active forms of MH

$$\text{then } [\text{M}_t] = [\text{MH}] + [\text{MHP}]$$

$$\therefore K = \frac{[\text{M}_t] - [\text{MH}]}{[\text{MH}][\text{P}]}$$

$$\therefore K [\text{P}] [\text{MH}] = [\text{M}_t] - [\text{MH}]$$

$$\therefore [\text{MH}] = \frac{[\text{M}_t]}{K [\text{P}] + 1}$$

$$\begin{aligned} \text{The Rate of Racemization} &= - \frac{d[\text{M}_t]}{dt} = k [\text{MH}] [\text{OH}^-] \\ &= k \frac{[\text{M}_t]}{K [\text{P}] + 1} [\text{OH}^-] \\ &= \frac{k}{K [\text{P}] + 1} [\text{M}_t] [\text{OH}^-] \end{aligned}$$

$$\therefore \ln [\text{M}_t] = - \frac{k [\text{OH}^-]}{K [\text{P}] + 1} t + \text{const.}$$

From this equation it can be seen that a plot of  $\ln [M_t]$  (i.e.  $\ln \alpha$ ) against  $t$  would give a straight line of gradient  $-\frac{k [\text{OH}^-]}{K [\text{P}] + 1}$ .

Thus the observed first order rate constant,

$$k' = \frac{k [\text{OH}^-]}{K [\text{P}] + 1} \quad (3)$$

From the Dissociation Constants of  $\text{H}_3\text{PO}_4$ , the values of  $[\text{P}]$  (i.e.  $[\text{HPO}_4^{2-}]$ ) at various pH values were calculated. Substituting observed values of  $k'$  and calculated values of  $[\text{P}]$  and  $[\text{OH}^-]$  at pH = 5.93 and 7.16, ( $T = 30.3^\circ$ )

$$k = 6.7 \times 10^5 \text{ (M}^{-1} \text{ min}^{-1}\text{)}$$

$$\text{and } K = 19 \text{ (M)}$$

$$\text{from which } k' = \frac{(6.7 \times 10^5) [\text{OH}^-]}{19 [\text{P}] + 1}$$

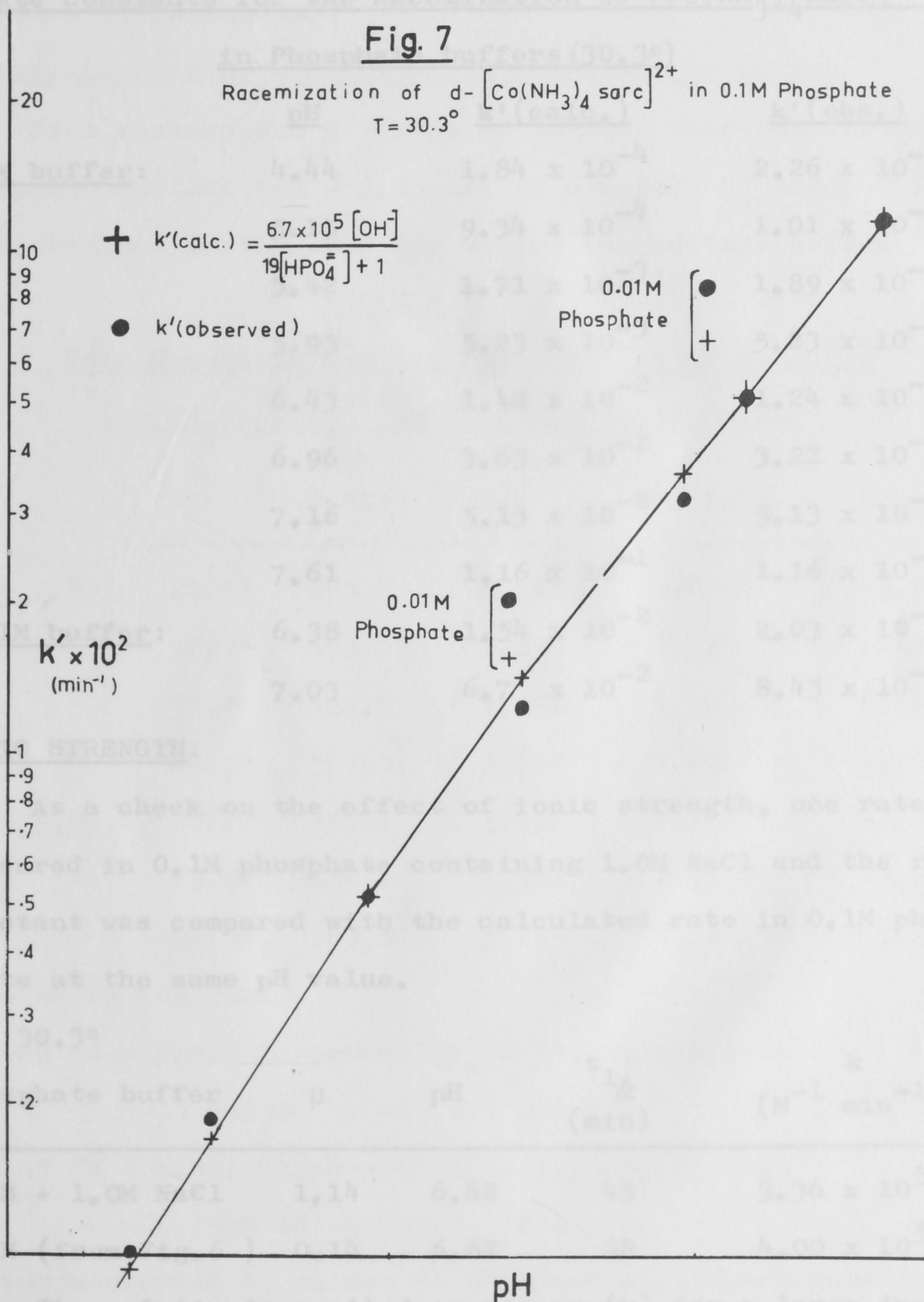
Substituting the appropriate values of  $[\text{P}]$  and  $[\text{OH}^-]$  at other pH values gave  $k'$ (calc.).  $k'$ (calc.) is shown with  $k'$ (obs) in Table 7 and both  $k'$  values are plotted (log scale) against pH in Fig.7.

Although the agreement between  $k'$ (calc.) and  $k'$ (obs) is not uniformly good, particularly for the 0.01M buffers, this kinetic scheme does give a possible explanation of the observed variation of rate with pH. However, the second order rate constant for the step  $\text{MH} + \text{OH}^- \longrightarrow \text{M} + \text{H}_2\text{O}$ ,  $k = 6.7 \times 10^5 \text{ M}^{-1} \text{ min}^{-1}$  is still less than the value of  $k$  ( $11.4 \times 10^5$ ) for the corresponding step for racemization in the acetate buffers. This discrepancy indicates that the concept of an ion pair equilibrium with  $\text{HPO}_4^{2-}$  in conjunction with reaction of the

free complex ion with OH<sup>-</sup> does not adequately explain the racemization process in phosphate buffer.

TABLE 7

Rate Constants for the Racemization of  $[\text{Co}(\text{NH}_3)_4 \text{sarc}]^{2+}$



The relatively small decrease in  $k'$  for a large increase

free complex ion with  $\text{OH}^-$  does not adequately explain the racemization process in phosphate buffer.

TABLE 7

Rate Constants for the Racemization of  $[\text{Co}(\text{NH}_3)_4\text{sarc}]^{2+}$   
in Phosphate buffers (30.3°)

	<u>pH</u>	<u>k' (calc.)</u>	<u>k' (obs.)</u>
<u>0.1M buffer:</u>	4.44	$1.84 \times 10^{-4}$	$2.26 \times 10^{-4}$
	5.15	$9.34 \times 10^{-4}$	$1.01 \times 10^{-3}$
	5.42	$1.71 \times 10^{-3}$	$1.89 \times 10^{-3}$
	5.93	$5.23 \times 10^{-3}$	$5.23 \times 10^{-3}$
	6.43	$1.42 \times 10^{-2}$	$1.24 \times 10^{-2}$
	6.96	$3.63 \times 10^{-2}$	$3.22 \times 10^{-2}$
	7.16	$5.13 \times 10^{-2}$	$5.13 \times 10^{-2}$
	7.61	$1.16 \times 10^{-1}$	$1.16 \times 10^{-1}$
<u>0.01M buffer:</u>	6.38	$1.54 \times 10^{-2}$	$2.03 \times 10^{-2}$
	7.03	$6.7 \times 10^{-2}$	$8.45 \times 10^{-2}$

IONIC STRENGTH:

As a check on the effect of ionic strength, one rate was measured in 0.1M phosphate containing 1.0M NaCl and the rate constant was compared with the calculated rate in 0.1M phosphate at the same pH value.

T = 30.3°

Phosphate buffer	$\mu$	pH	$t_{1/2}$ (min)	k ( $\text{M}^{-1} \text{min}^{-1}$ )
0.1M + 1.0M NaCl	1.14	6.62	45	$3.36 \times 10^5$
0.1M (from Fig.6)	0.14	6.62	38	$4.00 \times 10^5$

The relatively small decrease in (k) for a large increase

in ( $\mu$ ) indicates that differences in ( $\mu$ ) would not account for either the decrease in ( $k$ ) with increasing  $[\text{OH}^-]$  in phosphate buffers or the larger values of ( $k$ ) in acetate relative to phosphate.

Energy and Entropy of Activation in Phosphate Buffer:

From measurement of rates at various temperatures in phosphate buffers (pH = 5.93 and pH = 6.96),  $E_a$  and  $\Delta S^\ddagger$  were obtained in the same way as for the acetate buffers.

TABLE 8

Rate Constants for the Racemization of d- or l-

$[\text{Co}(\text{NH}_3)_4\text{sarc}]^{2+}$  in 0.1M Phosphate buffer

	T	T(°K)	$\frac{10^3}{T}$	$k' \times 10^2$	log k'
<u>pH = 5.93</u>	20.2°	293.4	3.408	0.0783	-3.106
	30.3°	303.5	3.295	0.523	-2.282
	35.7°	308.9	3.237	1.33	-1.876
	40.3°	313.5	3.190	3.01	-1.521
<u>pH = 6.96</u>	20.2°	293.4	3.408	0.521	-2.283
	25.5°	298.7	3.348	1.41	-1.851
	30.3°	303.5	3.295	3.22	-1.492

From these figures:

$$\text{at pH} = 5.93, \text{ "apparent" } E_a = 33.0 \text{ k.cal.mole}^{-1}$$

$$\text{and } \Delta S^\ddagger = +24.3 \pm 2.0 \text{ e.u.}$$

$$\text{and at pH} = 6.96, \text{ "apparent" } E_a = 32.0 \text{ k.cal.mole}^{-1}$$

$$\text{and } \Delta S^\ddagger = +23.2 \pm 2.0 \text{ e.u.}$$

giving corrected  $E_a$  values of 19.5 and 18.5 k.cal.mole<sup>-1</sup>.

Within experimental error these values are the same as

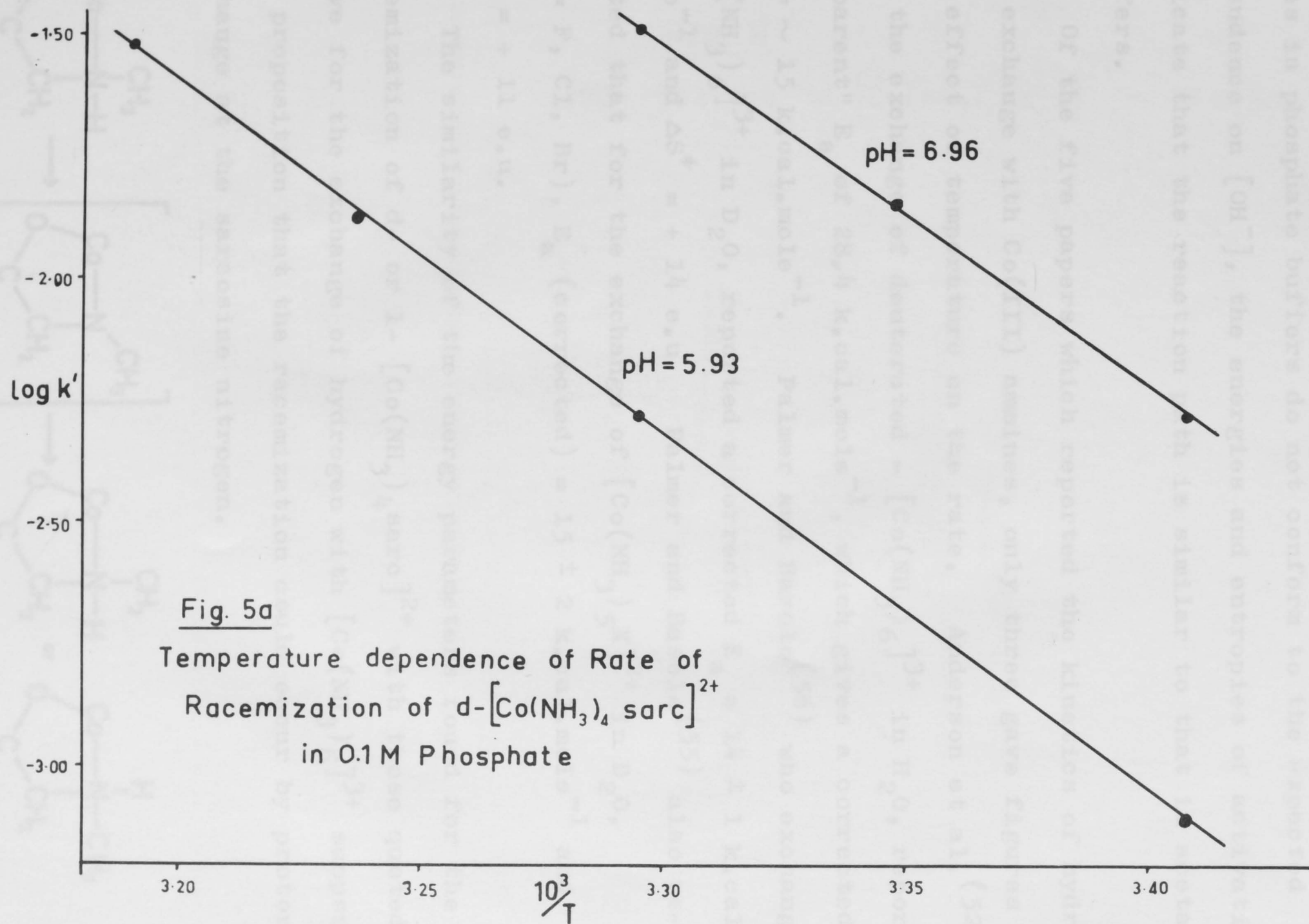


Fig. 5a

Temperature dependence of Rate of  
 Racemization of  $d\text{-}[\text{Co}(\text{NH}_3)_4 \text{sarc}]^{2+}$   
 in 0.1M Phosphate

those obtained in acetate buffer at pH = 5.83 ( $E_a = 19.0 \pm 0.5$  k.cal.mole<sup>-1</sup> and  $\Delta S^\ddagger = +25.6 \pm 2.0$  e.u.). Although the rates in phosphate buffers do not conform to the expected dependence on  $[\text{OH}^-]$ , the energies and entropies of activation indicate that the reaction path is similar to that in acetate buffers.

Of the five papers which reported the kinetics of hydrogen exchange with Co(III) amines, only three gave figures on the effect of temperature on the rate. Anderson et al.<sup>(52)</sup> for the exchange of deuterated -  $[\text{Co}(\text{NH}_3)_6]^{3+}$  in  $\text{H}_2\text{O}$ , reported "apparent"  $E_a$  of 28.4 k.cal.mole<sup>-1</sup>, which gives a corrected  $E_a = \sim 15$  k.cal.mole<sup>-1</sup>. Palmer and Basolo<sup>(56)</sup> who exchanged  $[\text{Co}(\text{NH}_3)_6]^{3+}$  in  $\text{D}_2\text{O}$ , reported a corrected  $E_a = 14 \pm 1$  k.cal.mole<sup>-1</sup> and  $\Delta S^\ddagger = +14$  e.u. Palmer and Basolo<sup>(55)</sup> also reported that for the exchange of  $[\text{Co}(\text{NH}_3)_5\text{X}]^{2+}$  in  $\text{D}_2\text{O}$ , (X = F, Cl, Br),  $E_a$  (corrected) =  $15 \pm 2$  k.cal.mole<sup>-1</sup> and  $\Delta S^\ddagger = +11$  e.u.

The similarity of the energy parameters found for the racemization of d- or l-  $[\text{Co}(\text{NH}_3)_4\text{sarc}]^{2+}$  with those quoted above for the exchange of hydrogen with  $[\text{Co}(\text{NH}_3)_6]^{3+}$  supports the proposition that the racemization could occur by proton exchange at the sarcosine nitrogen.

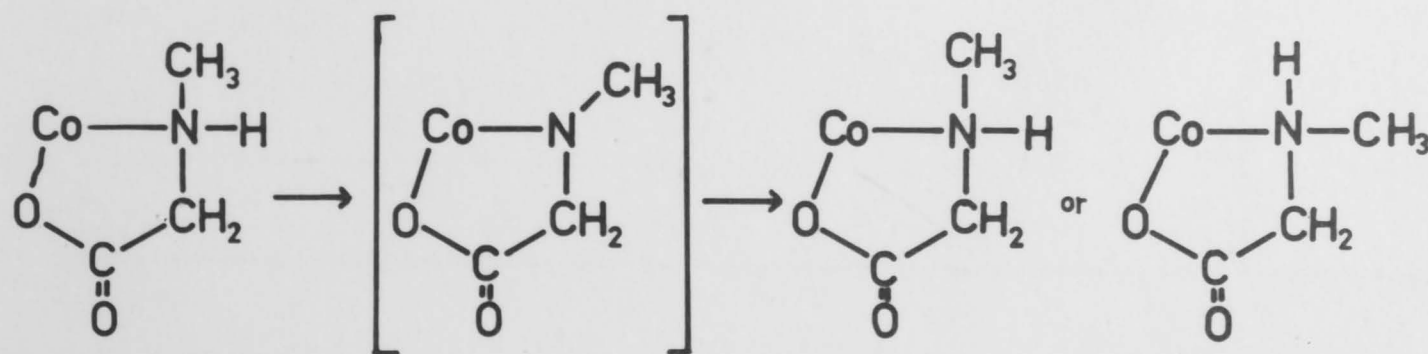
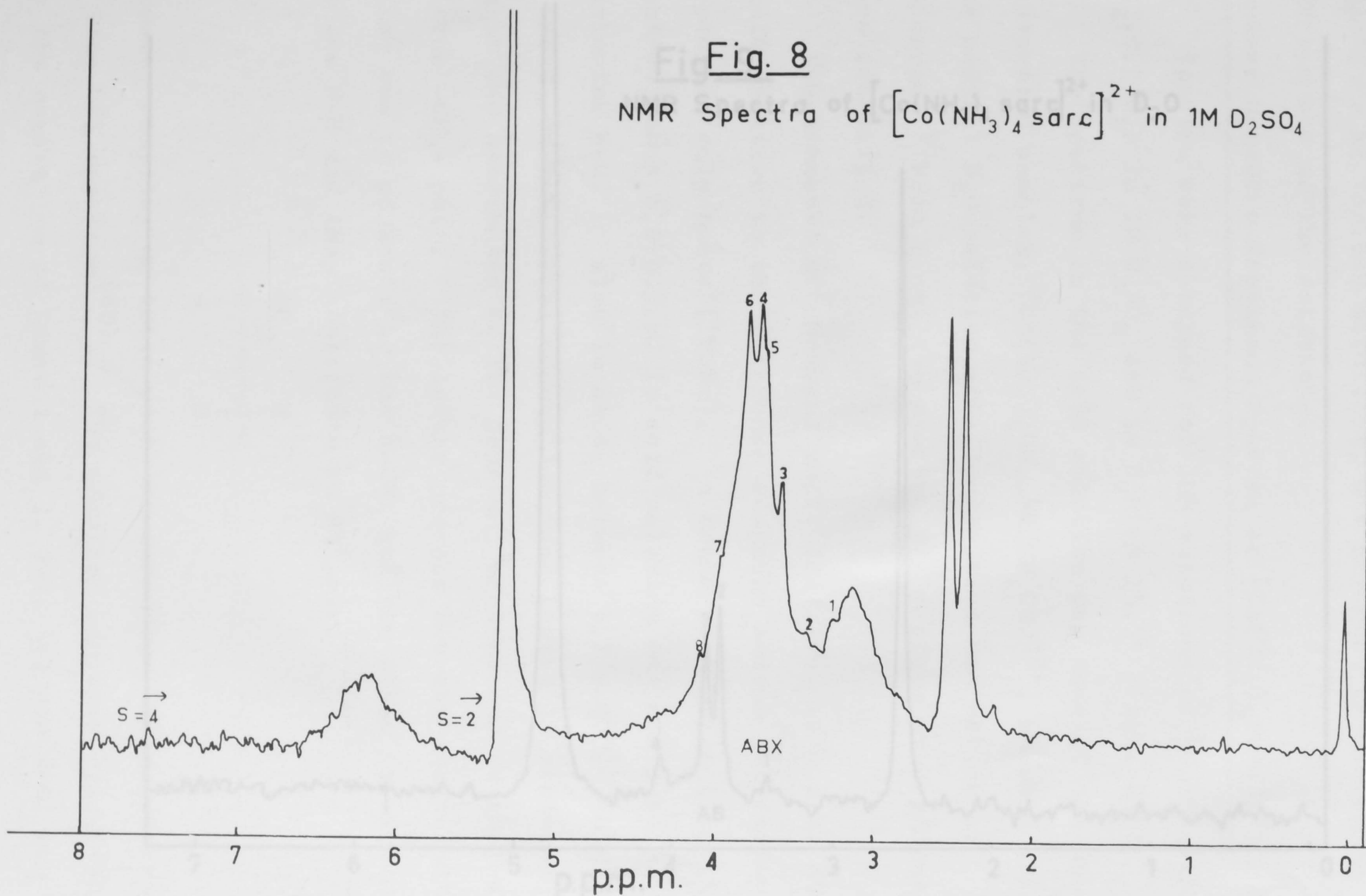


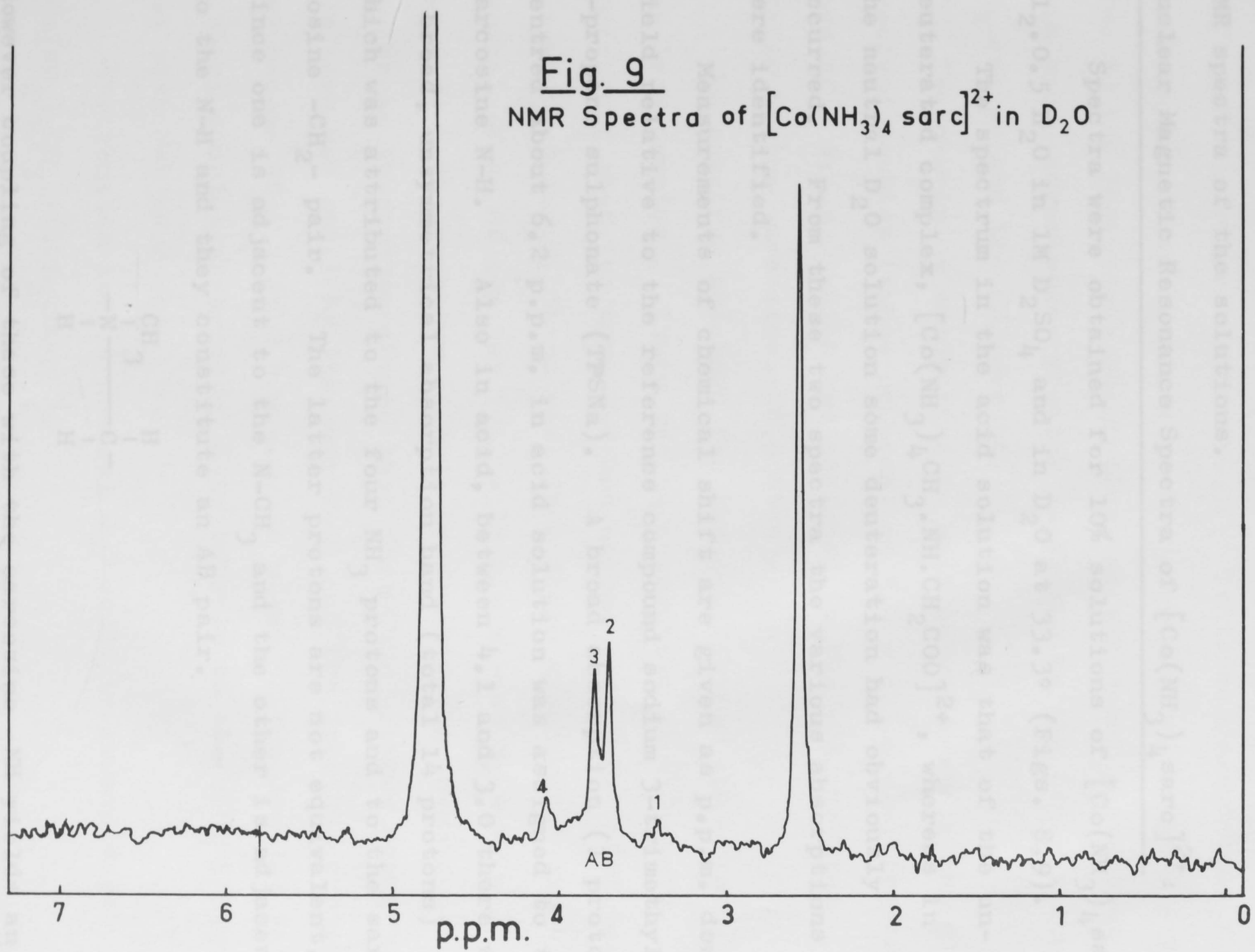


Fig. 8

NMR Spectra of  $[\text{Co}(\text{NH}_3)_4 \text{sarc}]^{2+}$  in 1M  $\text{D}_2\text{SO}_4$



**Fig. 9**  
 NMR Spectra of  $[\text{Co}(\text{NH}_3)_4 \text{sarc}]^{2+}$  in  $\text{D}_2\text{O}$



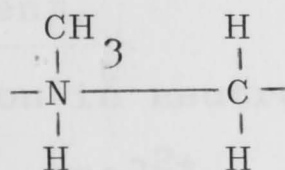
The exchange of this proton with deuterium in  $D_2O$  solutions at various acidities, was followed by recording the NMR spectra of the solutions.

Nuclear Magnetic Resonance Spectra of  $[Co(NH_3)_4sarc]^{2+}$ :

Spectra were obtained for 10% solutions of  $[Co(NH_3)_4sarc]Cl_{12} \cdot 0.5 H_2O$  in 1M  $D_2SO_4$  and in  $D_2O$  at  $33.3^\circ$  (Figs. 8,9).

The spectrum in the acid solution was that of the undeuterated complex,  $[Co(NH_3)_4CH_3 \cdot NH \cdot CH_2COO]^{2+}$ , whereas in the neutral  $D_2O$  solution some deuteration had obviously occurred. From these two spectra the various absorptions were identified.

Measurements of chemical shift are given as p.p.m. downfield relative to the reference compound sodium 3-trimethyl-1-propane sulphonate (TPSNa). A broad absorption (1 proton) centred about 6.2 p.p.m. in acid solution was assigned to the sarcosine N-H. Also in acid, between 4.1 and 3.0 there was a broad, unsymmetrical absorption band (total 14 protons) which was attributed to the four  $NH_3$  protons and to the sarcosine  $-CH_2-$  pair. The latter protons are not equivalent, since one is adjacent to the  $N-CH_3$  and the other is adjacent to the N-H and they constitute an AB pair.



However coupling of these with the sarcosine  $-NH$  yields an 8 line ABX spectrum<sup>(60)</sup>. The coupling constant  $J_{AB}$  given by the separation of lines 1 and 3, 2-4, 5-7 and 6-8, was 18 cps. (Fig.8). The broad  $NH_3$  band may be roughly resolved

into one peak due to 9 protons and another due to 3 protons, which indicates that three  $\text{-NH}_3$  groups have the same chemical shift but the other is different. It is suggested that the ammine group which is trans to the co-ordinated oxygen, gives rise to the individual absorption, as the model shows that this group experiences greater shielding by the other three  $\text{NH}_3$  groups and the  $\text{CH}_3$  groups.

The remaining absorption in acid solution was a sharply resolved doublet (three protons) at 2.4 - 2.5 p.p.m. (separation of 6 c.p.s.) which was assigned to the  $\text{-CH}_3$  protons (split by the sarcosine  $\text{-NH}$  proton).

The spectrum obtained for the equilibrium state in  $\text{D}_2\text{O}$  was less complex. There was no absorption at 6.2 p.p.m. indicating that the sarc N-H had become sarc N-D. Between 4.1 and 3.0 p.p.m. there were four sharp symmetrical lines, typical of an AB quartet ( $J_{\text{AB}} = 18$  c.p.s. and  $\delta_{\text{A}} - \delta_{\text{B}} = 16$  c.p.s.). The presence of this system and the absence of any broad absorption showed that the  $\text{-CH}_2\text{-}$  protons remained, but were not split by the sarc N-D, and the four  $\text{NH}_3$  groups had become  $\text{ND}_3$ . At 2.4 p.p.m. in place of the doublet was a sharp singlet absorption, attributable to the  $\text{-CH}_3$  protons, but with N-D adjacent.

The complex ion in neutral  $\text{D}_2\text{O}$  could be regarded as  $[\text{Co}(\text{ND}_3)_4\text{CH}_3\cdot\text{ND}\cdot\text{CH}_2\cdot\text{COO}]^{2+}$ .

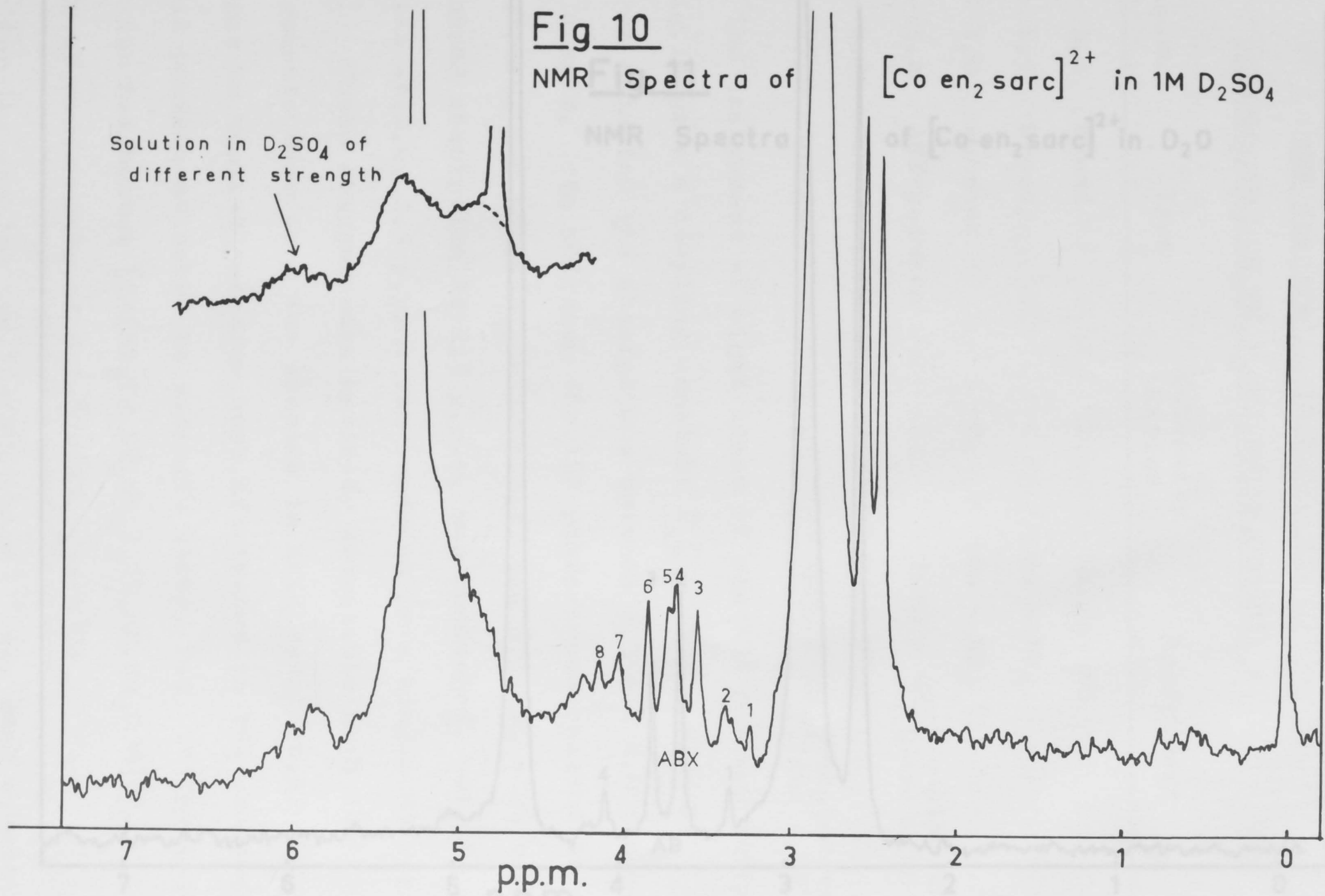
The above interpretation of the spectra of  $[\text{Co}(\text{NH}_3)_4\text{sarc}]^{2+}$  was supported by the spectra of  $[\text{Co en}_2\text{sarc}]\text{Cl}_2$  in 1M  $\text{D}_2\text{SO}_4$  and in  $\text{D}_2\text{O}$  (Figs. 10,11). Integration of the absorptions

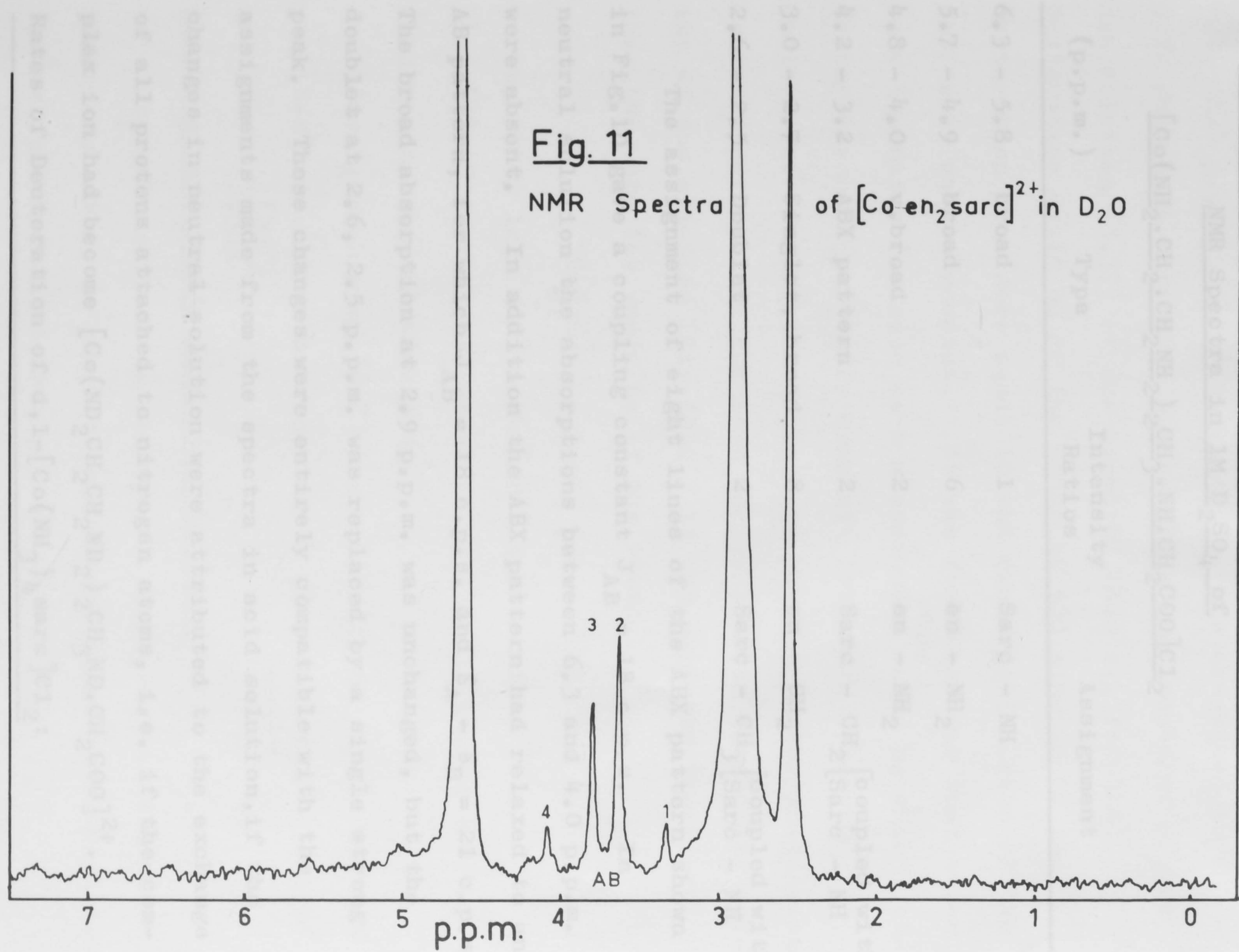
Fig 10

NMR Spectra of

$[\text{Co en}_2 \text{sarc}]^{2+}$  in 1M  $\text{D}_2\text{SO}_4$

Solution in  $\text{D}_2\text{SO}_4$  of  
different strength

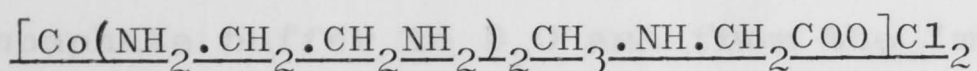




in acid solution allowed the analysis shown in Table 9.

TABLE 9

NMR Spectra in 1M  $D_2SO_4$  of



(p.p.m.)	Type	Intensity Ratios	Assignment
6.3 - 5.8	broad	1	Sarc - NH
5.7 - 4.9	broad	6	en - $NH_2$
4.8 - 4.0	v. broad	2	en - $NH_2$
4.2 - 3.2	ABX pattern	2	Sarc - $CH_2$ [coupled with Sarc - NH
3.0 - 2.7	Singlet, broad	8	en - $CH_2$
2.6 - 2.5	Doublet	2	Sarc - $CH_3$ [coupled with Sarc - NH

The assignment of eight lines of the ABX pattern shown in Fig. 10 gave a coupling constant  $J_{AB} = 18$  c.p.s. In neutral solution the absorptions between 6.3 and 4.0 p.p.m. were absent. In addition the ABX pattern had relaxed to an AB pattern, for which  $J_{AB} = 18$  c.p.s. and  $\delta_A - \delta_B = 21$  c.p.s. The broad absorption at 2.9 p.p.m. was unchanged, but the doublet at 2.6, 2.5 p.p.m. was replaced by a single strong peak. These changes were entirely compatible with the assignments made from the spectra in acid solution, if the changes in neutral solution were attributed to the exchange of all protons attached to nitrogen atoms, i.e. if the complex ion had become  $[Co(ND_2CH_2CH_2ND_2)_2 \cdot CH_3ND \cdot CH_2COO]^{2+}$ .

Rates of Deuteration of d,1- $[Co(NH_3)_4sarc]Cl_2$ :

For the complex ion  $[Co(NH_3)_4sarc]^{2+}$  the most sensitive

measure of the degree of deuteration of the sarcosine nitrogen was obtained from the change, doublet  $\rightarrow$  singlet at 2.5 - 2.4 p.p.m.  $[\text{Co}(\text{NH}_3)_4\text{sarc}]\text{Cl}_2$  was dissolved in a 0.1M phosphate buffer in  $\text{D}_2\text{O}$  and from the "measured pD" of the solution (5.37) it was expected that  $t_{1/2}$  for the deuteration would be of the order of 500 minutes if the deuteration rate were equal to the racemization rate of the optically active complex. It was found however that after only 5 minutes (as soon as a measurement could be taken) the deuteration was virtually complete (Fig.12). Several reactions were followed in solutions of much higher acidity and it was apparent that the rate decreased with increasing acidity of the solution (Fig.13). It was expected therefore that the rate law would be of the form:

$$-\frac{d[\text{N-H}]}{dt} = k [\text{OD}^-] [\text{N-H}]$$

$$\text{or } 2.303 \log [\text{N-H}] = -k [\text{OD}^-] t + \text{const.}$$

where  $[\text{N-H}]$  is the concentration of complex having the sarcosine nitrogen undeuterated.

During each run,  $[\text{OD}^-]$  was constant, so that a plot of  $\log [\text{N-H}]$  against  $t$  was expected to give a straight line of gradient  $-\frac{k[\text{OD}^-]}{2.303}$ .

Choice of a suitable parameter for  $[\text{N-H}]$ :

The intensity of an absorption band, that is the area under the curve, is proportional to the concentration of the species producing the absorption.

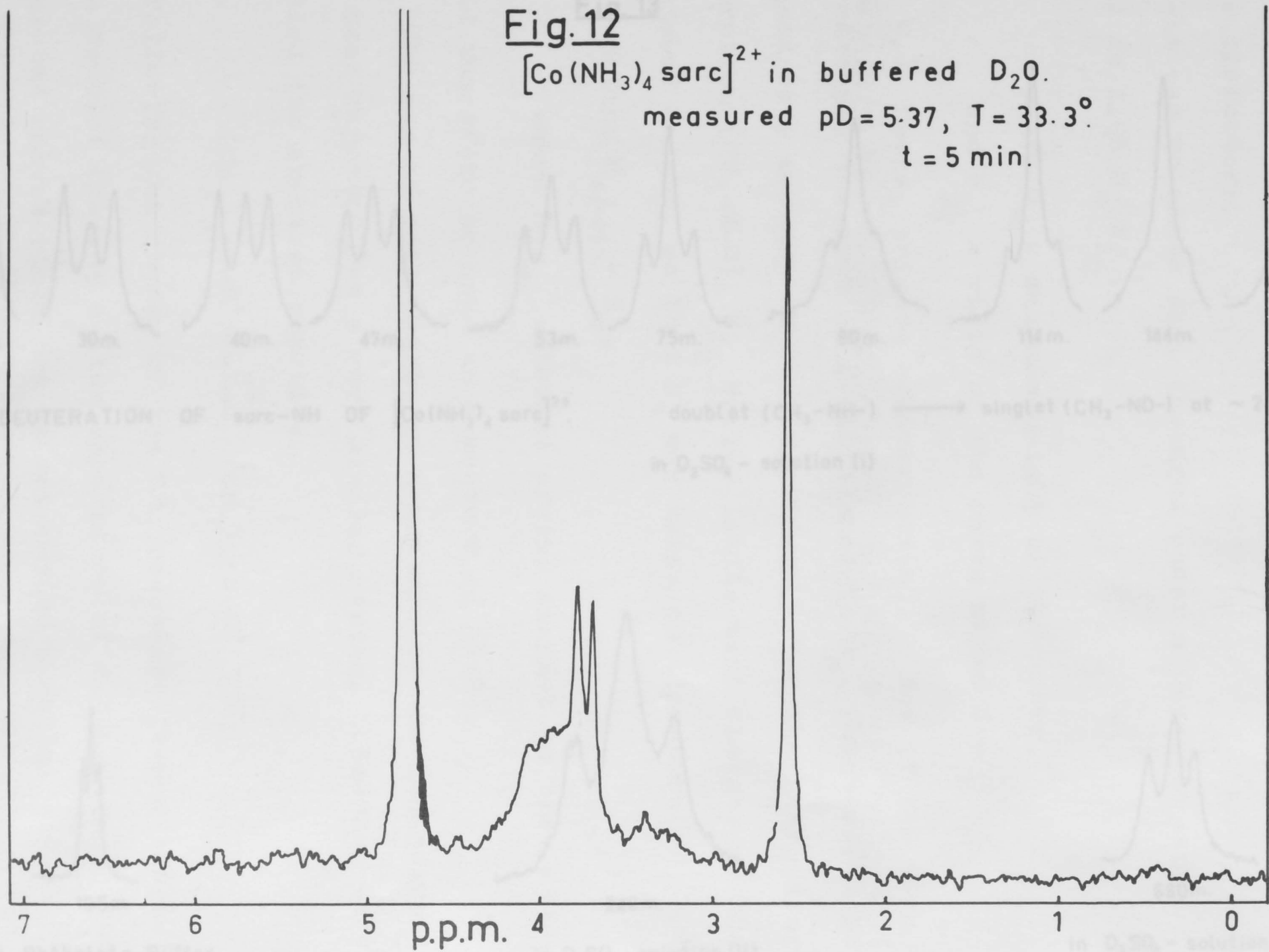
$$\text{The area of the doublet, } A_d = c[\text{N-H}]$$

$$\text{and area of the singlet, } A_s = c[\text{N-D}]$$

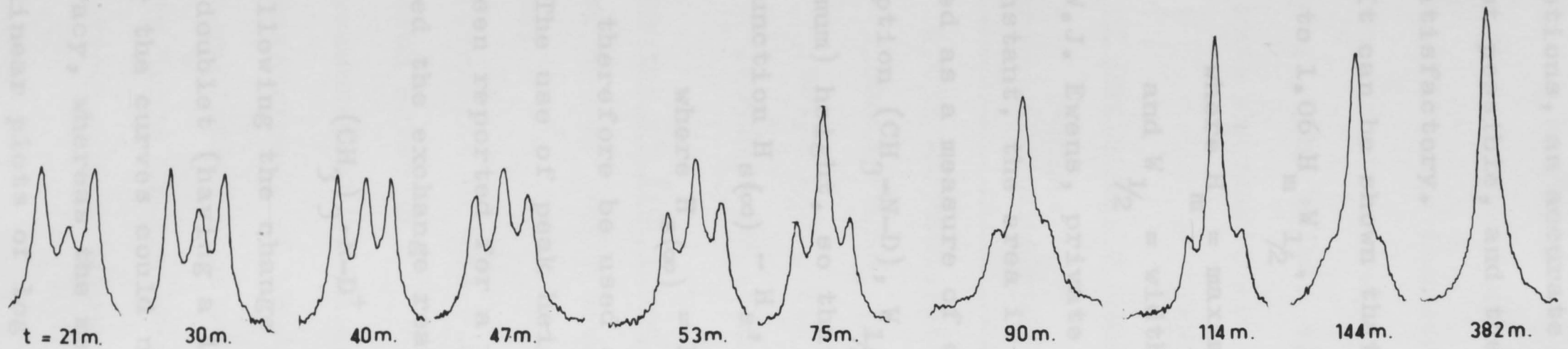


Fig. 12

$[\text{Co}(\text{NH}_3)_4 \text{sarc}]^{2+}$  in buffered  $\text{D}_2\text{O}$ .  
measured  $\text{pD} = 5.37$ ,  $T = 33.3^\circ$   
 $t = 5 \text{ min.}$



**Fig. 13**



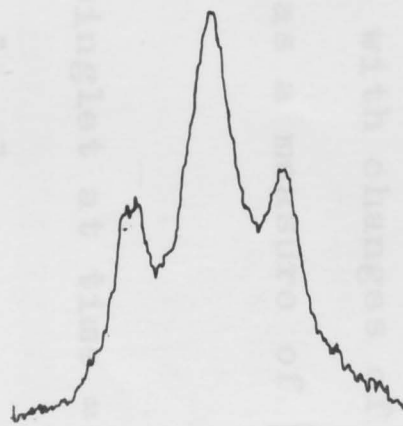
DEUTERATION OF sarc-NH OF  $[\text{Co}(\text{NH}_3)_4 \text{sarc}]^{2+}$

doublet ( $\text{CH}_3\text{-NH-}$ )  $\longrightarrow$  singlet ( $\text{CH}_3\text{-ND-}$ ) at  $\sim 2.5$  ppm.  
in  $\text{D}_2\text{SO}_4$  - solution (i)



10.5m.

in Phthalate Buffer



246m.

in  $\text{D}_2\text{SO}_4$  - solution (ii)



680m.

in  $\text{D}_2\text{SO}_4$  - solution (iii)

However, because of the overlapping of the singlet and doublet absorptions, an accurate measurement of their respective areas was not possible, and the  $\log A$  vs.  $t$  plots for the rates were not satisfactory.

It can be shown that the area under a Gaussian curve is equal to  $1.06 H_m W_{1/2}$ ,

where  $H_m$  = maximum height of the curve,

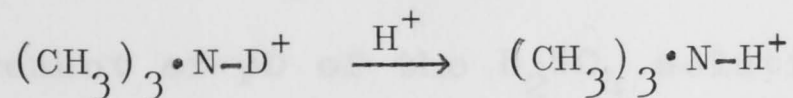
and  $W_{1/2}$  = width of the curve where  $H = \frac{1}{2} H_m$ .

(Dr. W. J. Ewens, private communication). Now if the term  $W_{1/2}$  is constant, the area is proportional to  $H_m$ , which may then be used as a measure of concentration. For the sharp singlet absorption ( $\text{CH}_3\text{-N-D}$ ),  $W_{1/2}$  varied little with changes of the (maximum) height, so that  $H_s$  was taken as a measure of  $[\text{N-D}]$ . The function  $H_s(\infty) = H_s$ ,

where  $H_s(\infty)$  = height of the singlet at time =  $\infty$

could therefore be used as a measure of  $[\text{N-H}]$ .

The use of peak height as a measure of the concentration has been reported for a very similar system. Emerson et al.<sup>(61)</sup> studied the exchange reaction.



by following the change of the  $\text{CH}_3$  resonance from a singlet to a doublet (having a line separation of 6.5 c.p.s.). Areas under the curves could not be determined with sufficient accuracy, whereas the measurement of peak heights was simple, and linear plots of  $\log (H_\infty - H_t)$  against  $t$  were claimed.

For the deuteration reactions studied here, plots of  $\log (H_{\infty} - H_t)$  against  $t$  gave straight lines, indicating pseudo first order kinetics (Fig.14).

One rate was measured in 0.05M potassium hydrogen phthalate solution and three rates were measured in  $D_2SO_4$  solution. The values of  $t_{1/2}$  and  $k'$ , the observed first order rate constant, are given in Table 10.

TABLE 10

Rates of Deuteration (sarc N-H) of  $[Co(NH_3)_4sarc]Cl_2$  at  $33.3^\circ$

Medium (in order of increasing acidity)	$t_{1/2}$ (min)	$k'$ ( $min^{-1}$ )
0.05M KH Phthalate	10	$6.9 \times 10^{-2}$
.00044M $D_2SO_4$ (i)	52	$1.3 \times 10^{-2}$
.0017M $D_2SO_4$ (ii)	200	$3.5 \times 10^{-3}$
.0096M $D_2SO_4$ (iii)	750	$9.2 \times 10^{-4}$

Measurement of  $[OD^-]$  and calculation of the rate constant,  $k$ :

Values of  $[OD^-]$  were obtained from three different sources:

- Titration of the  $D_2SO_4$  solutions against a standard NaOH solution,
- measurement of pD of the  $D_2SO_4$  solutions (pH meter) and
- measurement of pD of the reaction solutions (pH meter).

The Dissociation constant of  $D_2O$  was taken as  $1.54 \times 10^{-15}$  (62).

In Table 11 are shown the different values of  $[OD^-]$  and the corresponding values of  $k$ .

Fig. 14a

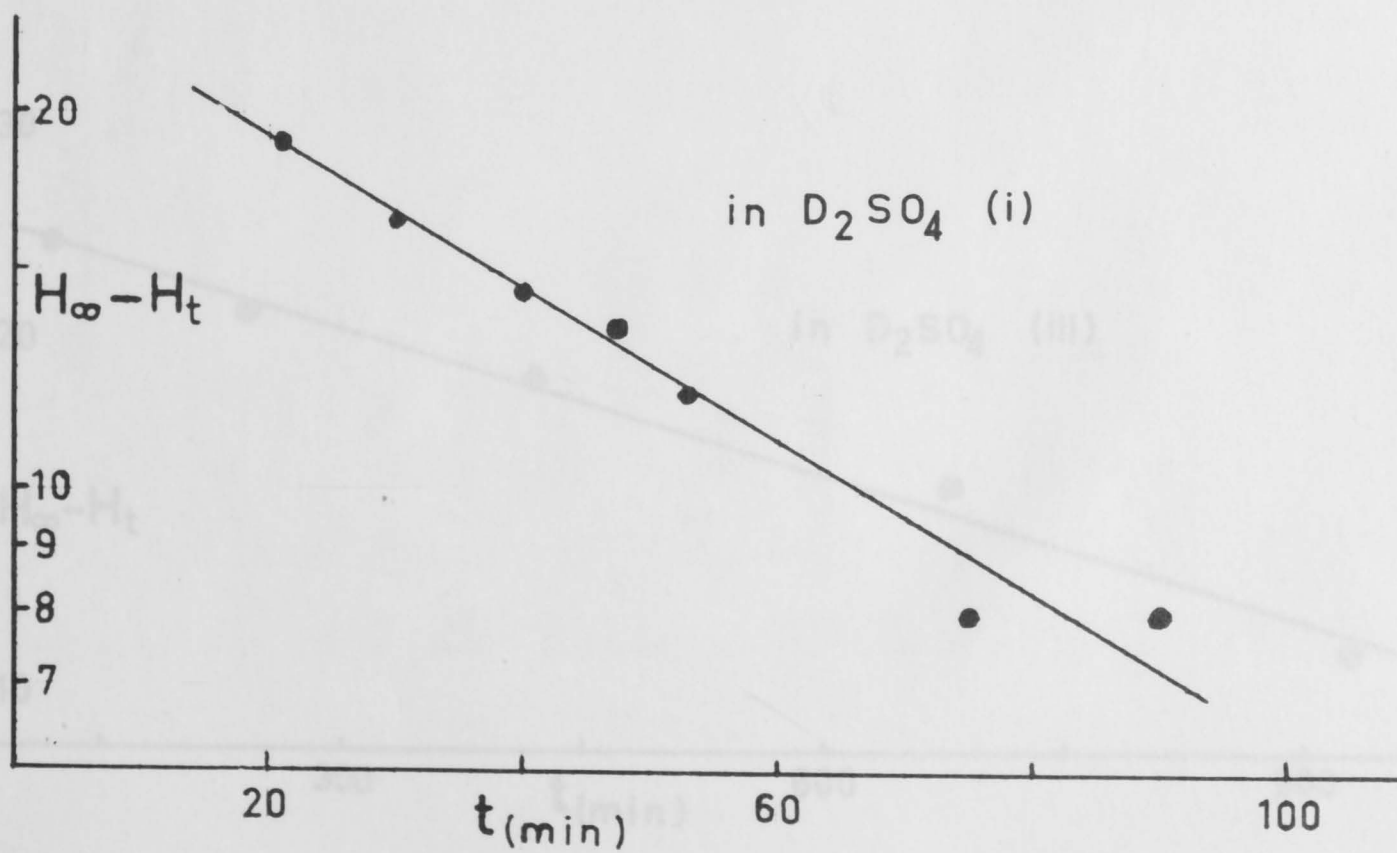
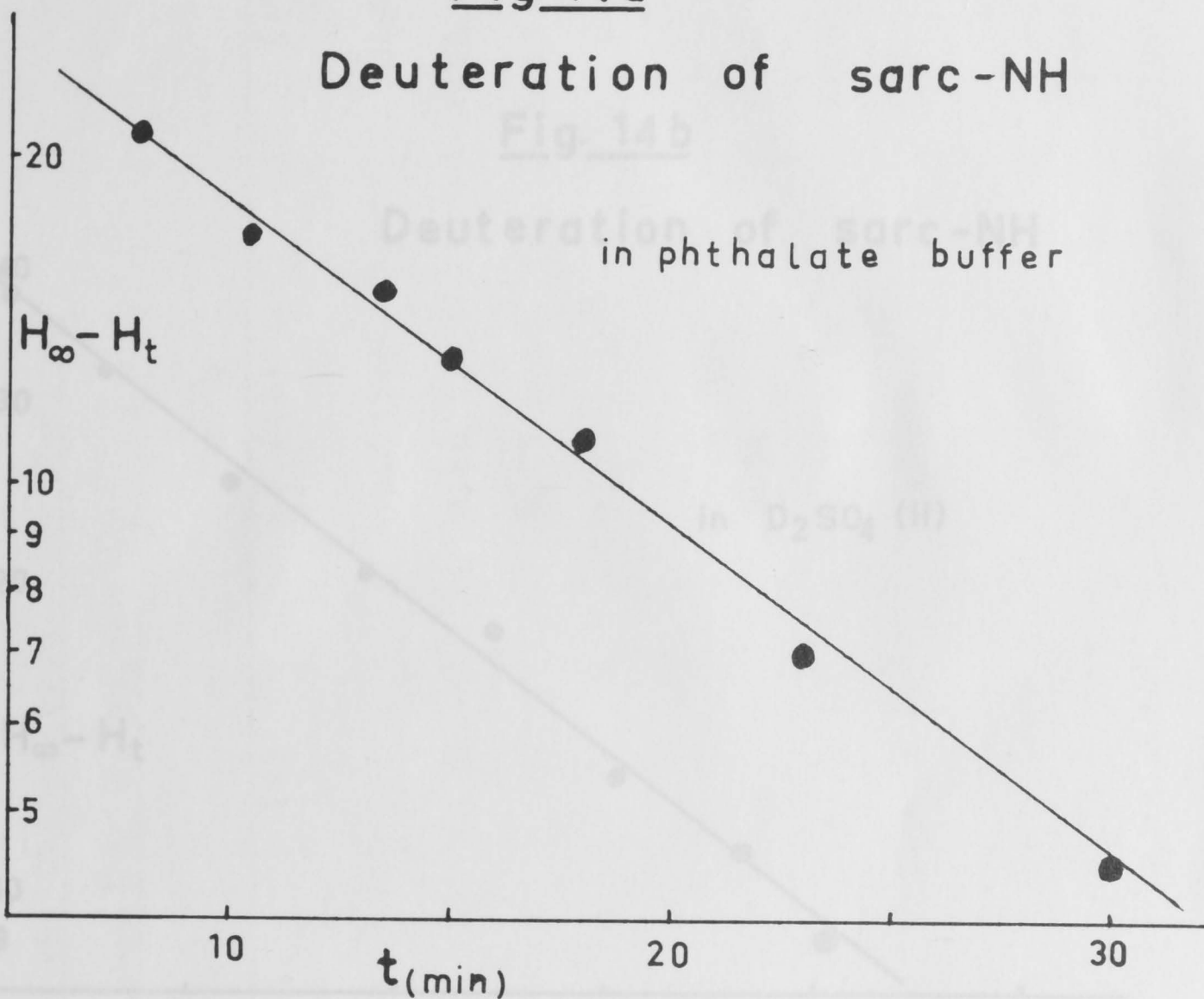


Fig. 14b

Deuteration of sarc-NH

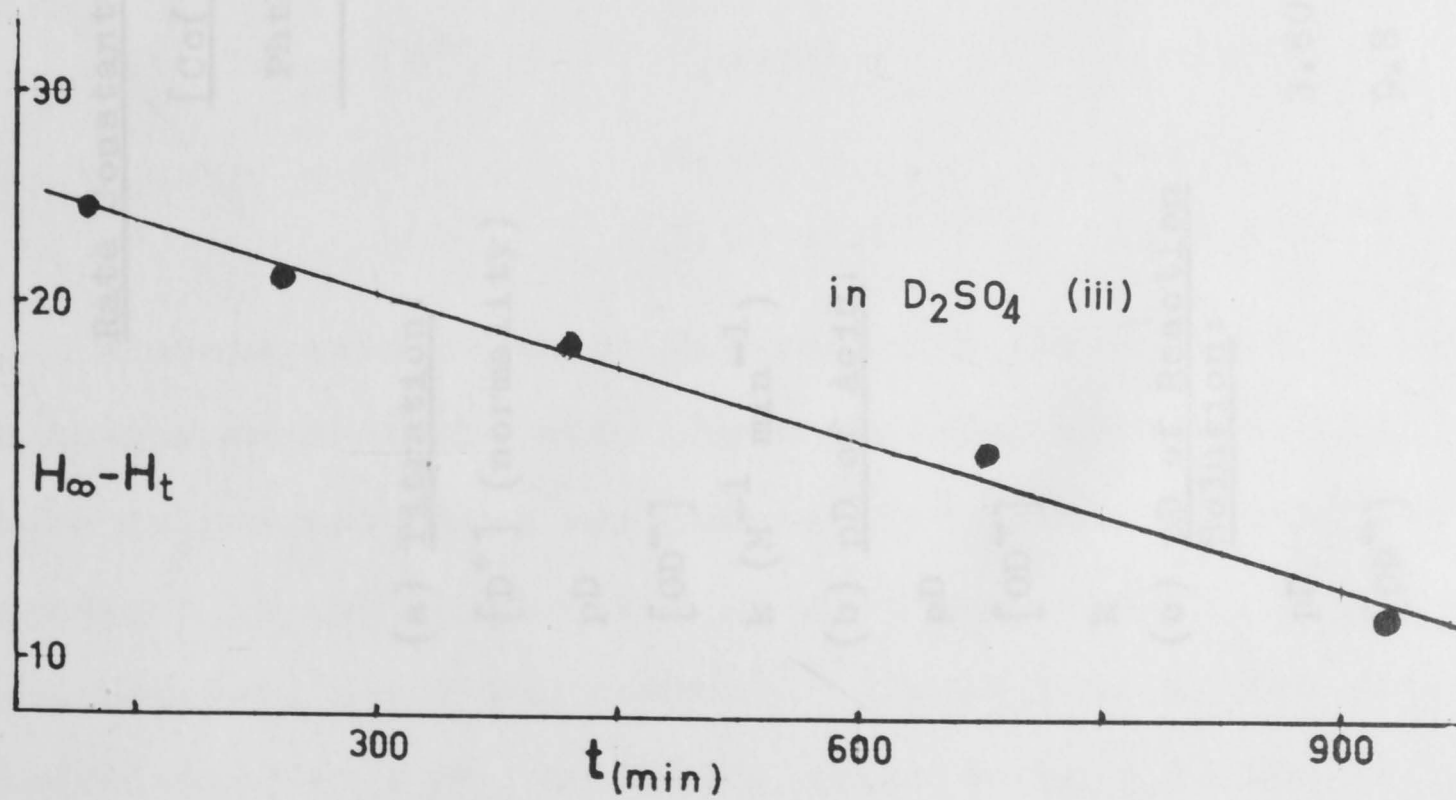
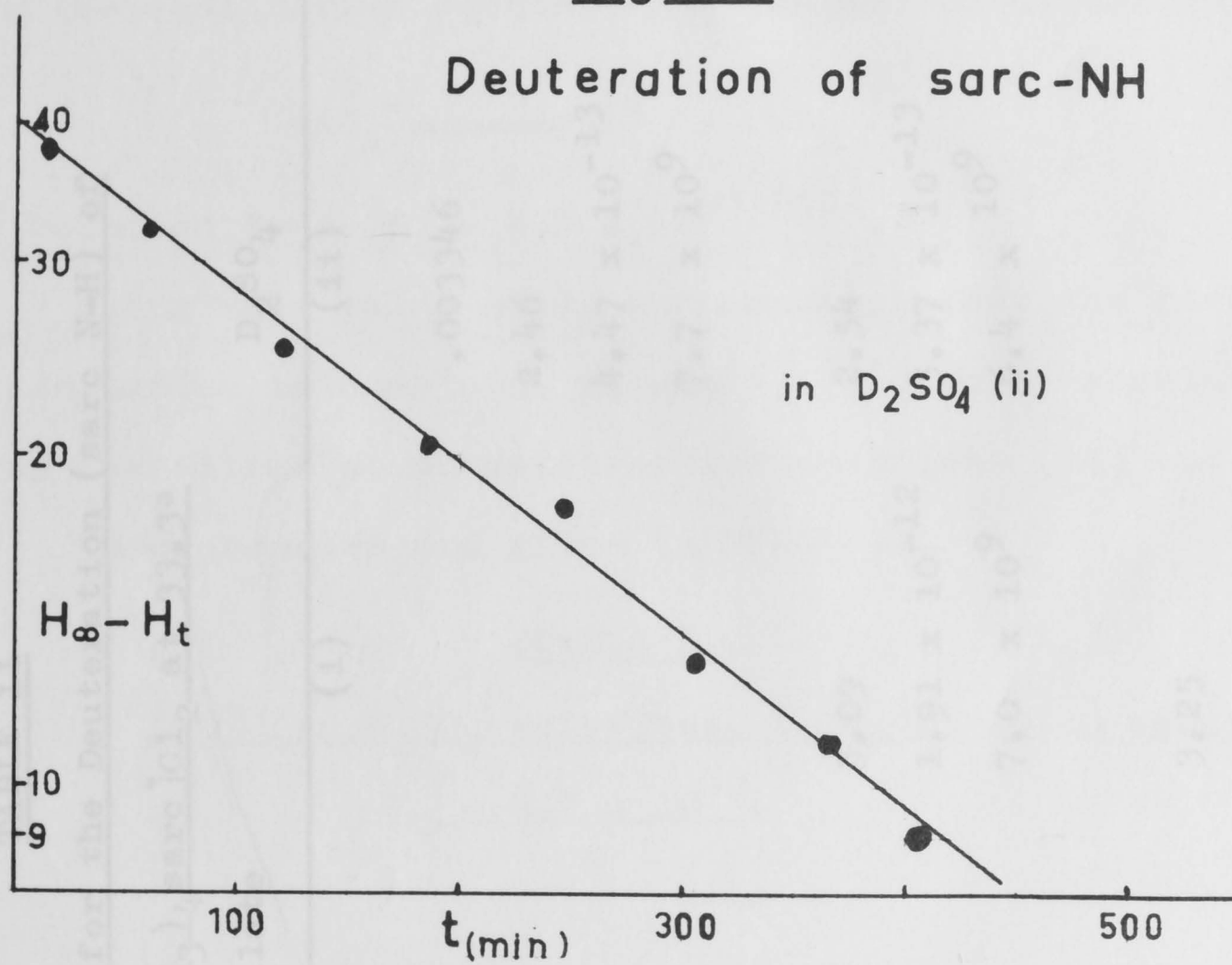


TABLE 11

Rate Constants for the Deuteration (sarc N-H) of

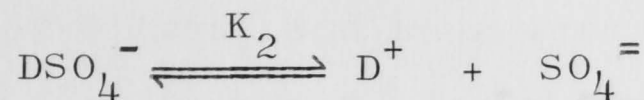
 $[\text{Co}(\text{NH}_3)_4\text{sarc}]\text{Cl}_2$  at  $33.3^\circ$ 

	Phthalate	$\text{D}_2\text{SO}_4$	
	(i)	(ii)	(iii)
(a) <u>Titration:</u>			
$[\text{D}^+]$ (normality)		.003346	.01921
pD		2.46	1.72
$[\text{OD}^-]$		$4.47 \times 10^{-13}$	$8.13 \times 10^{-14}$
$k$ ( $\text{M}^{-1} \text{min}^{-1}$ )		$7.7 \times 10^9$	$11 \times 10^9$
(b) <u>pD of Acid:</u>			
pD	3.09	2.54	2.04
$[\text{OD}^-]$	$1.91 \times 10^{-12}$	$5.37 \times 10^{-13}$	$1.70 \times 10^{-13}$
$k$	$7.0 \times 10^9$	$6.4 \times 10^9$	$5.4 \times 10^9$
(c) <u>pD of Reaction Solution:</u>			
pD	3.80	3.25	2.02
$[\text{OD}^-]$	$9.8 \times 10^{-12}$	$2.75 \times 10^{-12}$	$1.62 \times 10^{-13}$
$k$	$7.0 \times 10^9$	$4.8 \times 10^9$	$5.7 \times 10^9$

Some comment on these numbers is required.

Titration:

Titration gives an accurate measure of the total acid concentration, but this quantity (the Normality) will be equal to  $[D^+]$  only if the acid is completely dissociated. Now the equilibrium constant for the second dissociation:



is reported to be  $K_2 = 4.7 \times 10^{-3}$  (63).

Using this value of  $K_2$  and assuming that the first dissociation is complete, values of  $[D^+]$  were calculated from the titration normalities for solutions (ii) and (iii).

These results are given in Table 12.

TABLE 12

$[OD^-]$  in  $D_2SO_4$  solutions, from titration using

$$\underline{K_2 = 4.7 \times 10^{-3}}.$$

	<u>(ii)</u>	<u>(iii)</u>
Acid Normality	.00346	.01921
Calculated $[D^+]$	.00283	.0127
pD	2.55	1.90
$[OD^-]$	$5.50 \times 10^{-13}$	$1.23 \times 10^{-13}$

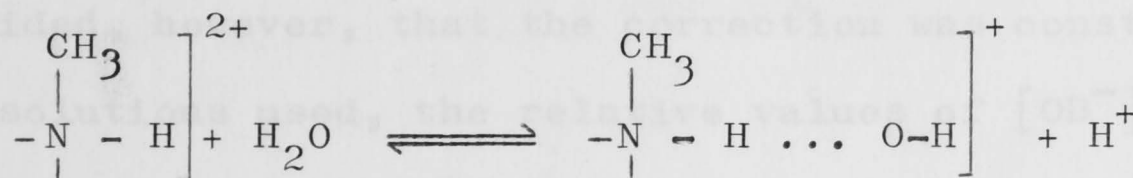
A comparison of these figures with those obtained from pD measurement of the acid shows that for solution (ii) there is now excellent agreement; for solution (iii) the agreement is not as good but is much improved.

However, Baker and Newton<sup>(64)</sup> found that  $K_2$  for the dissociation of  $D_2SO_4$  in  $NaClO_4$  solution ( $\mu = 1$ ) was  $5.1 \times 10^{-2}$ . The addition of the complex (10% concentration,



0.35M) to the acid solutions would be expected therefore, to increase the degree of ionization of the acid and consequently to lower pD. For solution (iii) there was a negligible change in pD on addition of the complex (2.04  $\rightarrow$  2.02), and for solution (i), pD increased from 3.09 to 3.25.

Titration of a solution of  $[\text{Co}(\text{NH}_3)_4\text{sarc}]\text{Cl}_2$  in  $\text{H}_2\text{O}$  against standard NaOH and HCl solutions gave no detectable endpoint. The complex ion therefore has no measurable pK by this method, and the equilibrium



would have no effect on the pH (or pD) of a solution.

The use of titration figures to calculate the actual  $[\text{OD}^-]$  in reaction solutions must be regarded with some reservation.

#### pD measurement:

Lumry, Smith and Glantz<sup>(65)</sup> used a pH meter with glass electrode to measure the pD of solutions of acetate buffers in  $\text{D}_2\text{O}$  and found that the "measured pD" was 0.4 less than the calculated pD. Their observation was confirmed by Glasoe and Long<sup>(66)</sup> who showed that over a wide range of pD(2-12),

$$\text{pD} = \text{pH meter reading} + 0.4.$$

(The meter was standardized with buffer solutions in  $\text{H}_2\text{O}$ ).

This correction was applied to all measurements made in

the present work. The measurement of pH generally does not give an accurate measure of  $[H^+]$  or  $[OH^-]$ , as an error of only 0.02 in pH (or pD) produces an error of 5% in the value of  $[OH^-]$  (or  $[OD^-]$ ). Another possible source of error, here, may exist in the correction term, 0.4. The measurements of Glasoe and Long were made on solutions having ionic strengths of 0.1 or less, whereas in the reaction solutions containing the complex the ionic strengths were approximately unity, and it may be that the correction term is different in these solutions.

Provided, however, that the correction was constant for the four solutions used, the relative values of  $[OD^-]$  would not be affected and these could be used effectively to confirm or disprove the first order dependence upon  $[OD^-]$ .

The values of the second order rate constant  $k$ , calculated using  $[OD^-]$  values from pD measurements, (Table 11), show that  $k$  may be regarded as being constant, when allowance is made for the lack of precision in the values of  $k'$  and  $[OD^-]$ , and thus the rate law

$$-\frac{d[N-H]}{dt} = k [OD^-][N-H]$$

may be considered valid.

The value of  $k$  for the deuteration of the sarcosine nitrogen at  $33.3^\circ$  is  $(7 \pm 2) \times 10^9 \text{ M}^{-1} \text{ min}^{-1}$ . It was not possible to measure the rate of deuteration at other temperatures.

From the plot of  $\log k'$  against  $\frac{1}{T}$  (Fig. 5) for the race-

mization of the optically active complex (acetate buffer), the racemization rate constant at 33.3° was calculated to be  $k = 2.01 \times 10^6 \text{ M}^{-1} \text{ min}^{-1}$ , which is significantly smaller than the deuteration rate constant.

Rates of Racemization of d- or l-[Co(NH<sub>3</sub>)<sub>4</sub>sarc](NO<sub>3</sub>)<sub>2</sub> in D<sub>2</sub>O solutions:

Because the exchange reaction was studied in D<sub>2</sub>O and the racemization was followed in H<sub>2</sub>O, several racemization rates were measured in buffered D<sub>2</sub>O solutions at 30.3°. [OD<sup>-</sup>] values were obtained from pD measurement of the reaction solution, using the correction,

$$\text{pD} = \text{pH meter reading} + 0.4.$$

The results are given in Table 13, and it is apparent that here, as in the H<sub>2</sub>O solutions, the derived rate constant (k) in phosphate buffer is less than that in acetate buffer; also in phosphate, k decreases with increasing pD.

TABLE 13

Rate Constants for the Racemization of d- or l-[Co(NH<sub>3</sub>)<sub>4</sub>sarc](NO<sub>3</sub>)<sub>2</sub> in D<sub>2</sub>O solutions at 30.3°

Buffer	pD	[OD <sup>-</sup> ]	k' (min <sup>-1</sup> )	$k = \frac{k'}{[\text{OD}^-]}$ (M <sup>-1</sup> min <sup>-1</sup> )
0.1M acetate	7.24	$2.69 \times 10^{-8}$	$4.03 \times 10^{-2}$	$15.0 \times 10^5$
0.1M phosphate	6.86	$1.12 \times 10^{-8}$	$7.07 \times 10^{-3}$	$6.31 \times 10^5$
0.1M phosphate	7.56	$5.62 \times 10^{-8}$	$2.68 \times 10^{-2}$	$4.76 \times 10^5$

In the phosphate system therefore, to compare the rate constants  $k_{\text{D}_2\text{O}}$  and  $k_{\text{H}_2\text{O}}$  it is necessary to make the comparison

at a value of pH and pD such that  $[\text{OH}^-] = [\text{OD}^-]$ . From the plot of  $\log k'$  against pH in 0.1M phosphate (Fig.6) the appropriate values of  $k_{\text{H}_2\text{O}}$  were obtained. These are shown in Table 14 and the ratios  $\frac{k_{\text{D}_2\text{O}}}{k_{\text{H}_2\text{O}}}$  are given also.

TABLE 14

Rate Constants for the Racemization of d- or l-[Co(NH<sub>3</sub>)<sub>4</sub>sarc]

Buffer	<u>(NO<sub>3</sub>)<sub>2</sub> in H<sub>2</sub>O and D<sub>2</sub>O at 30.3°</u>			
	OH <sup>-</sup>	$k_{\text{H}_2\text{O}}$ (M <sup>-1</sup> min <sup>-1</sup> )	$k_{\text{D}_2\text{O}}$ (M <sup>-1</sup> min <sup>-1</sup> )	$\frac{k_{\text{D}_2\text{O}}}{k_{\text{H}_2\text{O}}}$
0.1M acetate	$4.17 \times 10^{-8}$	$11.2 \times 10^5$	$15.0 \times 10^5$	1.3
0.1M phosphate	$1.12 \times 10^{-8}$	$5.5 \times 10^5$	$6.31 \times 10^5$	1.2
0.1M phosphate	$5.62 \times 10^{-8}$	$4.2 \times 10^5$	$4.76 \times 10^5$	1.1

From the racemization rate constant in H<sub>2</sub>O (acetate) at 33.3°,  $k = 2.01 \times 10^6 \text{ M}^{-1} \text{ min}^{-1}$  and the ratio

$$\frac{k_{\text{D}_2\text{O}}}{k_{\text{H}_2\text{O}}} = 1.3,$$

the calculated rate constant for racemization in D<sub>2</sub>O at 33.3° is  $k = 2.4 \times 10^6 \text{ M}^{-1} \text{ min}^{-1}$ .

Comparison of this value with the rate constant for deuteration of the sarcosine nitrogen at 33.3°,  $k = (7 \pm 2) \times 10^9 \text{ M}^{-1} \text{ min}^{-1}$ , shows that the exchange rate is some 2,000 to 4,000 times faster than the racemization rate. This means that if the racemization process occurs through hydrogen exchange on the sarcosine nitrogen only one exchange in 2,000 or more takes place with an inversion of configuration.

Deuteration without loss of Optical Activity:

To confirm that the rate of deuteration was much faster than the rate of racemization at the same pH, a sample of optically pure 1-[Co(NH<sub>3</sub>)<sub>4</sub>sarc](NO<sub>3</sub>)<sub>2</sub> (A) was deuterated in conditions such that negligible racemization was expected. The deuterated complex (B) was isolated and its I.R. and NMR spectra and optical activity were measured. It was then re-protonated under similar conditions and the spectra and optical activity of the isolated product (C) were determined.

The results of these measurements are given in Table 15 and the spectra are shown in Figs. 15, 16.

TABLE 15

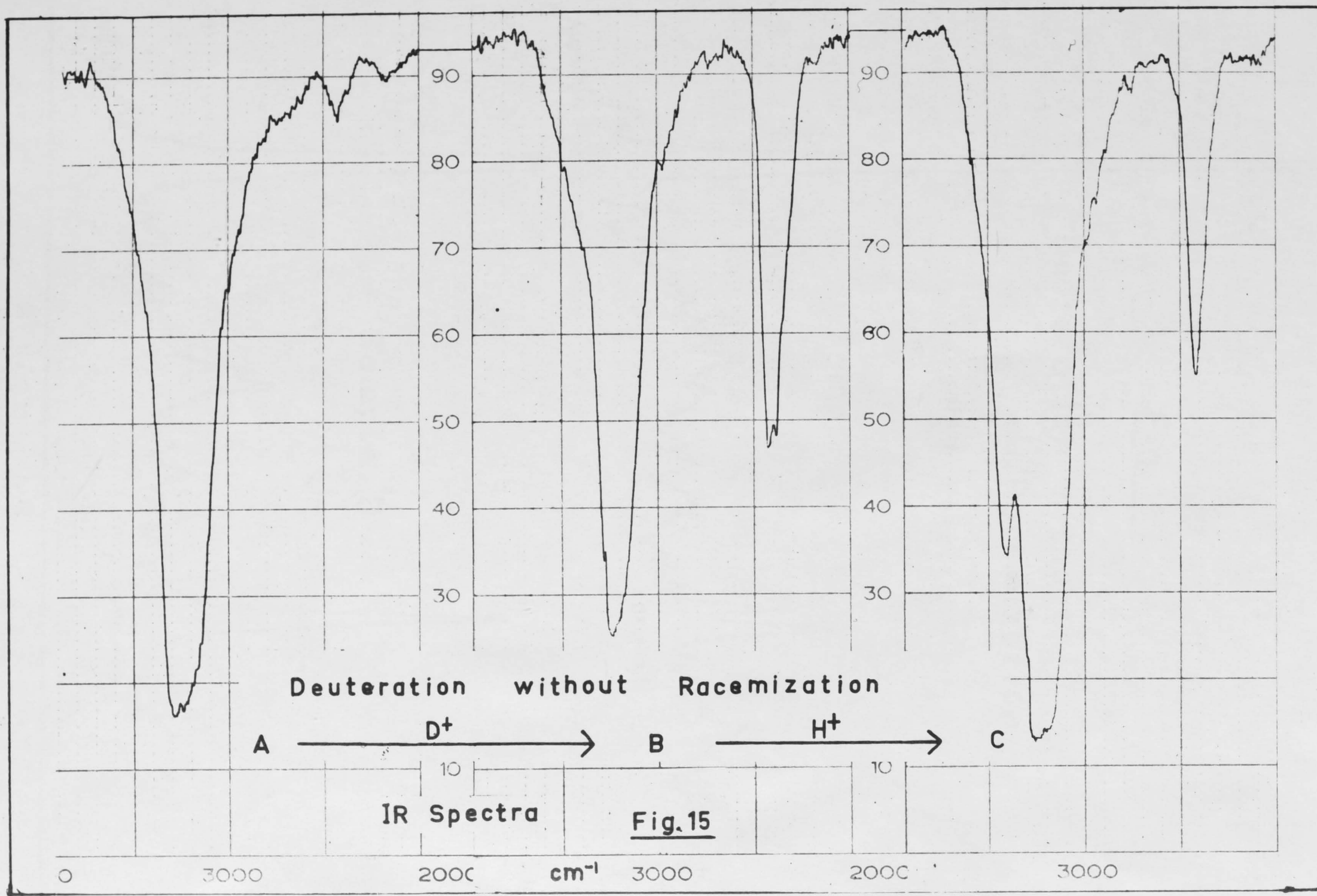
Deuteration and Reprotonation of

1-[Co(NH<sub>3</sub>)<sub>4</sub>sarc](NO<sub>3</sub>)<sub>2</sub> at 33.3°.

	A	$\xrightarrow[\sim 9(t_{1/2})]{D^+/D_2O}$	B	$\xrightarrow[\sim 20(t_{1/2})]{H^+/H_2O}$	C
$[\alpha]_{436}$	-221°		-211°		-221°
NMR signal at 2.4 p.p.m.	doublet		singlet		doublet
*I.R. (% transmittance)					
3250 cm <sup>-1</sup>	16		25		13
2400 cm <sup>-1</sup>			47		55

\* I.R. spectra were obtained using equal quantities of complexes A, B and C in KBr discs.

The singlet in the NMR spectrum for sample B showed that the sarcosine N was fully deuterated, and the doublet for sample C confirmed that this N had been reprotonated. The



difference between the specific rotations of the protonated (A,C) and deuterated (B) forms appears to be meaningful, as a similar increase in  $[\alpha]_{436}$  was found in another experiment where the deuterated complex was exchanged with hydrogen.

For each step A  $\rightarrow$  B and B  $\rightarrow$  C, the recovery of the product (by precipitation from solution) was  $\sim 90\%$ , and since the optically pure complex is more stable than the impure or racemic forms, the specific rotation figures show that both the deuteration and reprecipitation occurred with full retention of configuration.

The IR spectrum of Sample C showed absorptions due to N-D stretching vibrations ( $2400\text{ cm}^{-1}$ ) as well as N-H stretching vibrations ( $3250\text{ cm}^{-1}$ ) and the strong intensity of the N-D absorptions indicated that the sarcosine had been deuterated well as the sarcosine.

However the spectrum of C (Fig.15) also indicates that the ammonia ligands were not fully reprotiated although the sarcosine N-H had completely exchanged.

The rates of deuteration in cobalt(III) ammine complexes is relevant at this point. Palmer and Basile on doubly charged cations of the type  $[\text{Co}(\text{NH}_3)_5\text{X}]^{2+}$  (55) (where  $\text{X}^- = \text{Cl}^-, \text{Br}^-, \text{NO}_2^-$ ) showed that the rate of proton exchange was  $k_1$  at  $25^\circ\text{C}$  where  $k_1 = 0.11 \times 10^6 \text{ s}^{-1}$  and  $k_2 = 0.38 \times 10^6 \text{ s}^{-1}$  and the activation energy of

From these figures it might be expected that the NH groups in  $[\text{Co}(\text{NH}_3)_5\text{NH}_2]^{2+}$  change their protons at

Fig. 16

Deuteration without  
Racemization

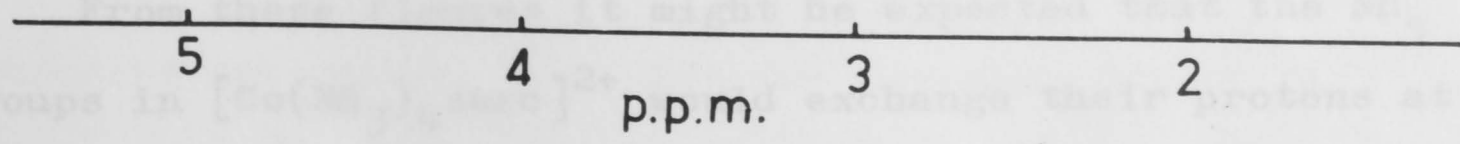
NMR Spectra

Sample C

sarc-NH

Sample B

sarc-ND



difference between the specific rotations of the protonated (A,C) and deuterated (B) forms appears to be meaningful, as a similar increase in  $[\alpha]_{436}$  was found in another experiment where the deuterated complex was exchanged with hydrogen.

For each step  $A \rightarrow B$  and  $B \rightarrow C$ , the recovery of the product (by precipitation from solution) was  $\sim 90\%$ , and since the optically pure complex is more soluble than the impure or racemic forms, the specific rotation figures show that both the deuteration and reprotonation occurred with full retention of configuration.

The IR spectrum of B (Fig.15) showed absorptions due to N-D stretching vibrations ( $2400 \text{ cm}^{-1}$ ) as well as N-H stretching vibrations ( $3250 \text{ cm}^{-1}$ ) and the strong intensity of the N-D absorption indicated that some of the ammonia ligands had been deuterated as well as the sarcosine -NH.

Moreover the spectrum of C (Fig.15) also indicates that the ammonia ligands were not fully reprotonated although the sarcosine N-D had completely exchanged. Some discussion of the rates of deuteration in cobalt(III) ammine complexes is relevant at this point. Investigations by Palmer and Basolo on doubly charged cations of the type  $[\text{Co}(\text{NH}_3)_5\text{X}]^{2+}$  (55) (where  $\text{X}^- = \text{F}^-, \text{Cl}^-, \text{Br}^-, \text{NO}_2^-$ ) showed that the rates of proton exchange for  $\text{D}^+$  at  $25^\circ$  were in the range  $k = 0.11 \times 10^6 - 0.38 \times 10^6 \text{ M}^{-1} \text{ sec}^{-1}$  with an observed activation energy of  $26-30 \text{ k.cal.mole}^{-1}$ .

From these figures it might be expected that the  $\text{NH}_3$  groups in  $[\text{Co}(\text{NH}_3)_4\text{sarc}]^{2+}$  would exchange their protons at

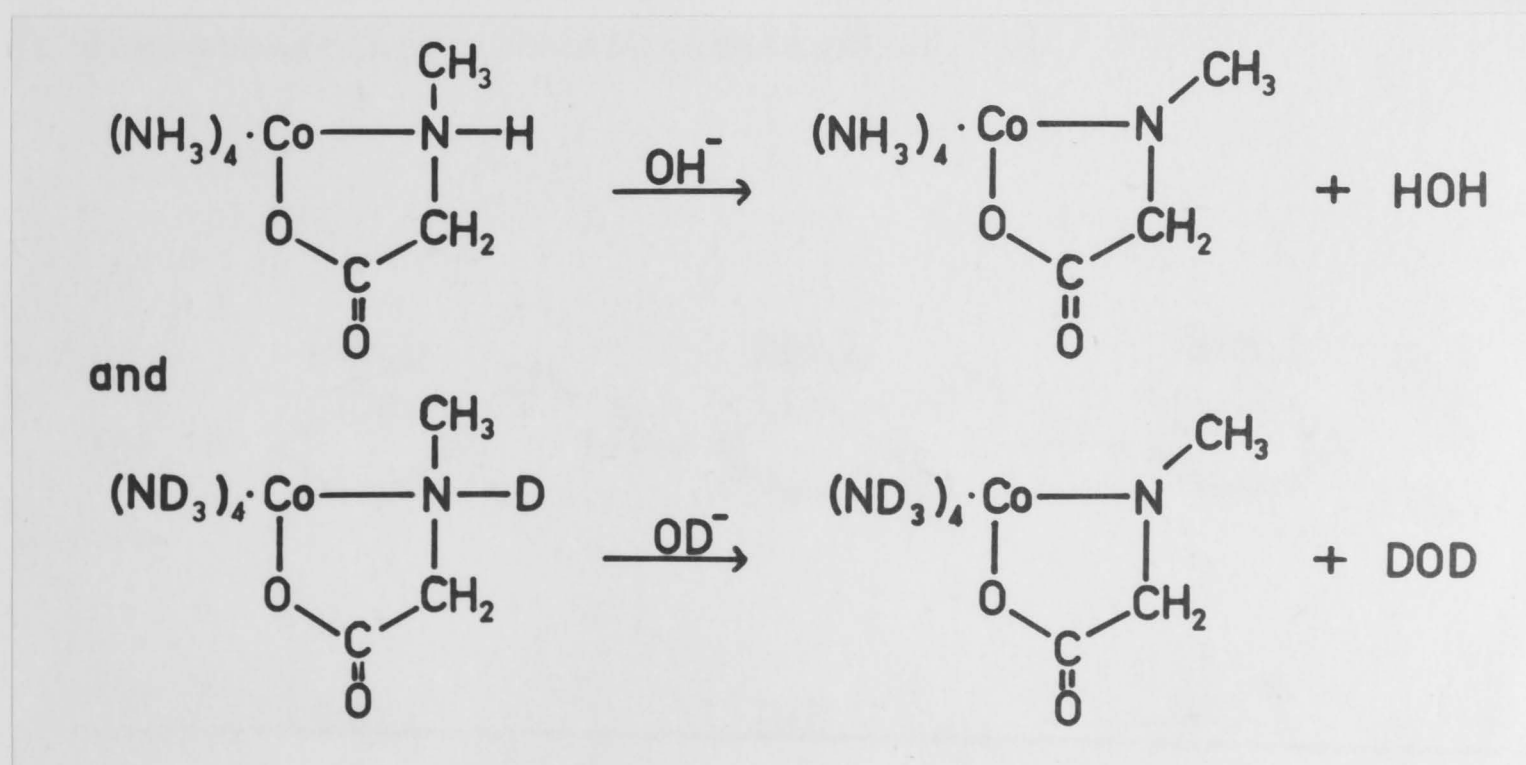


33.3° with rate constants of the order  $k = (5 - 10) \times 10^7$   $M^{-1} \text{ min}^{-1}$ ; that is the ammine H exchange would be slower than the observed sarcosine N-H exchange ( $k = 7 \pm 2 \times 10^9 M^{-1} \text{ min}^{-1}$ ) but appreciably faster than the racemization rate ( $k = 2 \times 10^6 M^{-1} \text{ min}^{-1}$ ).

The IR spectra of B and C qualitatively confirm this estimate of the rate of ammine -H exchange.

#### Isotope Effects:

Since the exchange rate of the sarcosine N-H is 2,000 to 4,000 times greater than the racemization rate, the actual processes observed when the racemization was followed in  $H_2O$  and  $D_2O$  probably were:

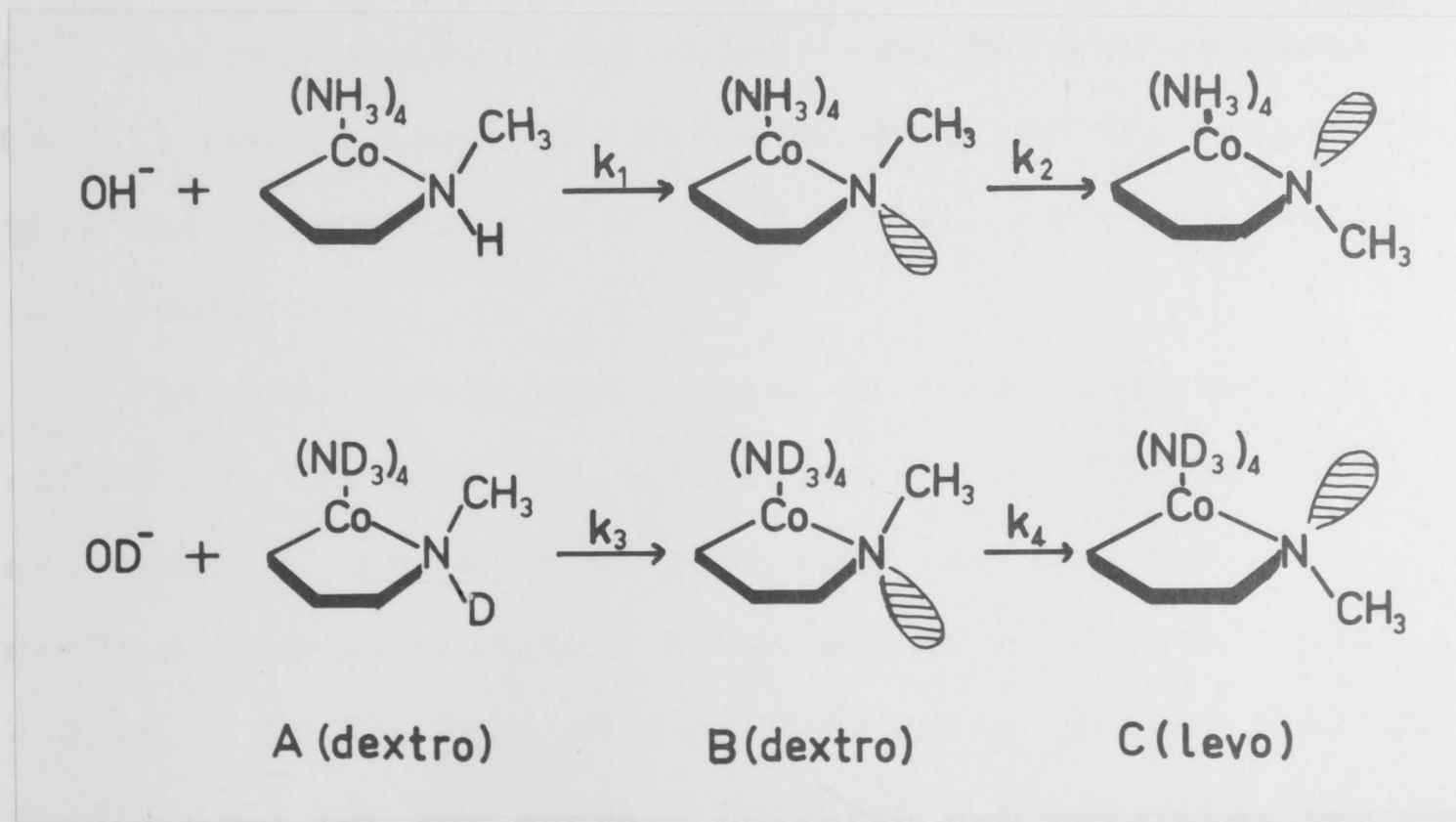


(It seems unreasonable that the abstraction of a proton from the co-ordinated ammonias could lead to racemization of the co-ordinated sarcosine.) Following these considerations the

rate constant ratio  $\frac{k_{D_2O}}{k_{H_2O}} = 1.3$  for racemization at 30.3°

in acetate buffer is unexpected. Normally because of its lower zero point energy the rate of abstraction of  $D^+$  should be up to an order of magnitude slower than for the abstraction of  $H^+$ . The contribution of  $OH^-$  relative to  $OD^-$  to the isotope effect should be negligible as their relative masses are 17:18 compared with  $H:D = 1:2$ . Similarly deuteration of the ammonia groups should give only a small secondary isotope effect and the contribution by the solvent should also be of this order.

It is conceivable that the observed isotope effect is decided by the relative rates of inversion of the deprotonated or dedeuterated intermediates (B), not the relative rates of deprotonation or dedeuteration of (A).



The observed rate law would still be obeyed, since

$$\text{Rate}_{\text{H}_2\text{O}} = k_1 k_2 [\text{d-Co}] [\text{OH}^-]$$

and  $\text{Rate}_{\text{D}_2\text{O}} = k_3 k_4 [\text{d-Co}] [\text{OD}^-]$ , but for

$$\frac{\text{Rate}_{\text{D}_2\text{O}}}{\text{Rate}_{\text{H}_2\text{O}}} > 1, \frac{k_3 k_4}{k_1 k_2} \text{ would have to be } > 1.$$

If  $k_3$  were less than  $k_1$  (as expected), then  $k_4$  would need to be greater than  $k_2$  by a much greater factor, but it is difficult to see any reason for a significant difference between  $k_2$  and  $k_4$ . It is therefore unlikely that the isotope effect arises from the inversion step. Subsequent reaction of the intermediates (B) and (C) with HOH or DOD should give only a small isotope effect and thus should not lead to any significant difference between retention and inversion.

Also the similarity between the rate parameters ( $E_a$  and  $\Delta S^\ddagger$ ) for racemization and those found for proton exchange with Co(III) ammine complexes strongly supports the proposition that the abstraction of  $\text{H}^+$  or  $\text{D}^+$  is the rate determining step in racemization.

The most likely explanation of this "inverse" isotope effect resides with the difficulty in determining  $[\text{OD}^-]$  accurately. As has been explained earlier,  $[\text{OD}^-]$  was derived from an empirical relation,  $\text{pD} = \text{pH meter reading} + 0.40$ . If the true pD were 0.20 units greater than the figure used for the acetate buffered  $\text{D}_2\text{O}$  solution, the derived rate constant would be  $k = 9.43 \times 10^5$  instead of  $15.0 \times 10^5$

$M^{-1} \text{ min}^{-1}$ ; the ratio  $\frac{k_{D_2O}}{k_{H_2O}}$  would be 0.84 instead of 1.3,

which is a complete reversal of the isotope effect. This empirical method may be unsatisfactory, and the problem might be clarified by examining the exchange of the deuterated sarcosine complex in  $H_2O$  containing a known amount of acid, although kinetic studies of exchange (NMR measurements) are less precise than those of racemization (rotation measurements).

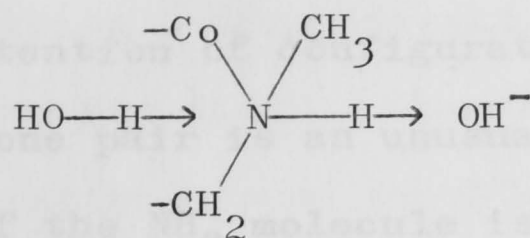
#### Mechanism:

The rate law for the racemization and deuteration processes is the same (within the limits of experimental error for the latter), i.e.

$$-\frac{d[Co]}{dt} = k[Co][OH^-]$$

and is consistent with the abstraction of the sarcosine N-H proton by  $OH^-$  or  $OD^-$  to give an intermediate (C) as shown in Fig.17.

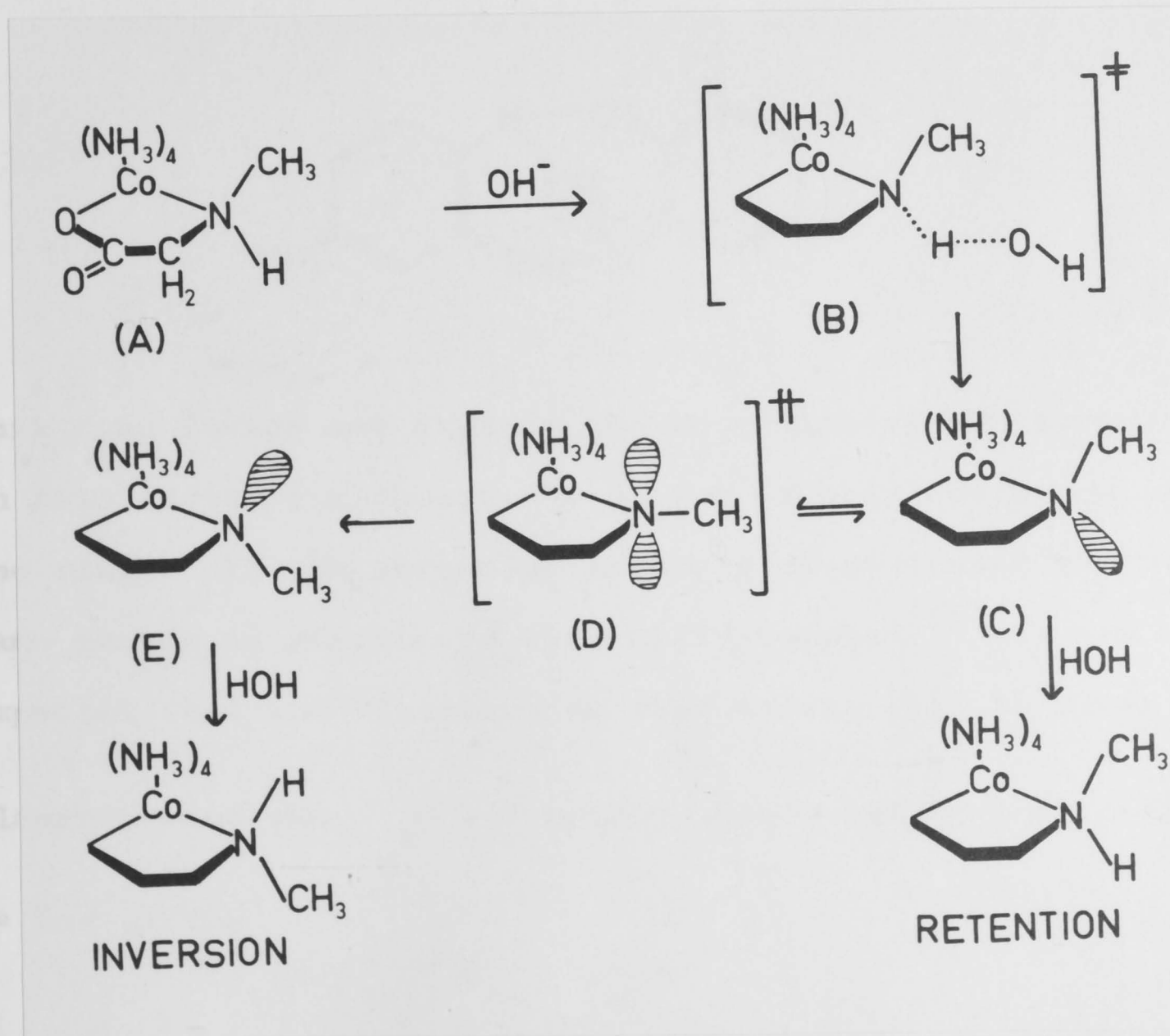
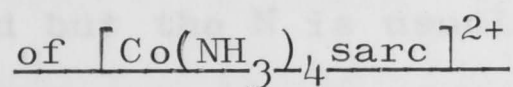
The interesting and dominating fact in this investigation is the enormous difference between the actual rate of racemization and the rate of deuteration, estimated to be a factor of 2,000 to 4,000. The immediate implication of this result is that the intermediate C in Fig.17 preserves its original configuration for most of its subsequent reactions. It is clear, of course, from observed optical rotations that under suitable conditions, deuteration does take place with full retention of configuration and this excludes attack by  $OH^-$  at the sarcosine N or the side opposite to the proton and a synchronous process such as:



both of which would conform with the rate law but conflict with the rotational evidence, in that they lead to inversion.

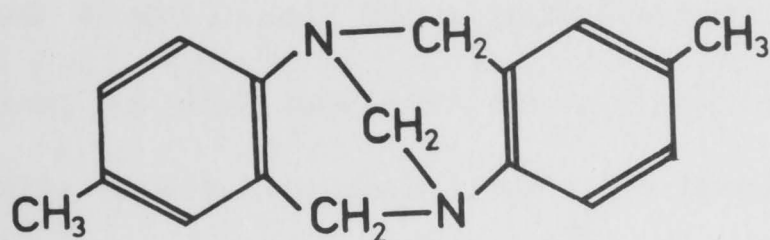
FIG. 17

Mechanism for the Proton Exchange and Racemization

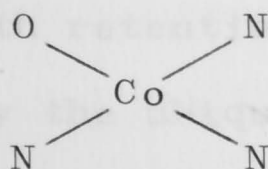


The retention of configuration around the N atom containing a lone pair is an unusual result. The frequency of inversion of the  $\text{NH}_3$  molecule is enormously fast,  $2.3 \times 10^{10}$  per second, and although a tertiary amine should have a lower frequency of inversion, many attempts to resolve unsymmetrical tertiary amines have been unsuccessful.

A few organic compounds containing tertiary N atoms have been resolved but the N is usually constrained in a particular configuration by fused ring systems, for example Tröger's base:



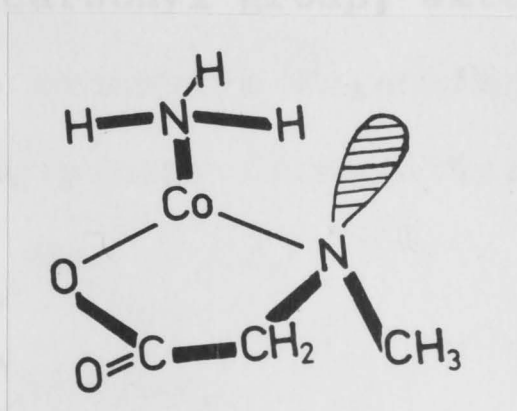
This ring factor may explain the retention of configuration in this instance although the N atom is only contained in one ring. If the sarcosine molecule co-ordinates in the same manner as glycine in the  $\text{Cu}(\text{II})$  complex<sup>(67)</sup> it can be expected that the Co-sarcosine ring system will be close to planarity and the  $\begin{array}{c} \text{O} \\ \diagdown \\ \text{C} \\ \diagup \\ \text{O} \end{array} - \text{C}$  moiety should not be much more than  $6^\circ$  out of the



plane. It is difficult to imagine that this small and almost

certainly labile conformational effect could affect that course of the reaction. However if it is required that the configuration around the N atom acquire the normal  $sp^2$  planar state before racemization can occur, then the ring system may actively hinder this process. The Co-N-C angle should be  $\sim 109^\circ$  by analogy with  $Cu(gly)_2$ . For the  $sp^2$  angle ( $120^\circ$ ) to be attained, the other angles and atomic positions in the ring system must undergo a fairly extensive rearrangement before the second transition state D is reached, and therefore retention is preferred.

Alternatively the lone pair, formed when the proton is removed, may be stabilised in a particular configuration by hydrogen bonding to the nearest co-ordinated ammonia, despite repulsion by the nearby nonbonding electrons in the  $d_{xy}$ ,  $d_{xz}$ ,  $d_{yz}$  orbitals, e.g.

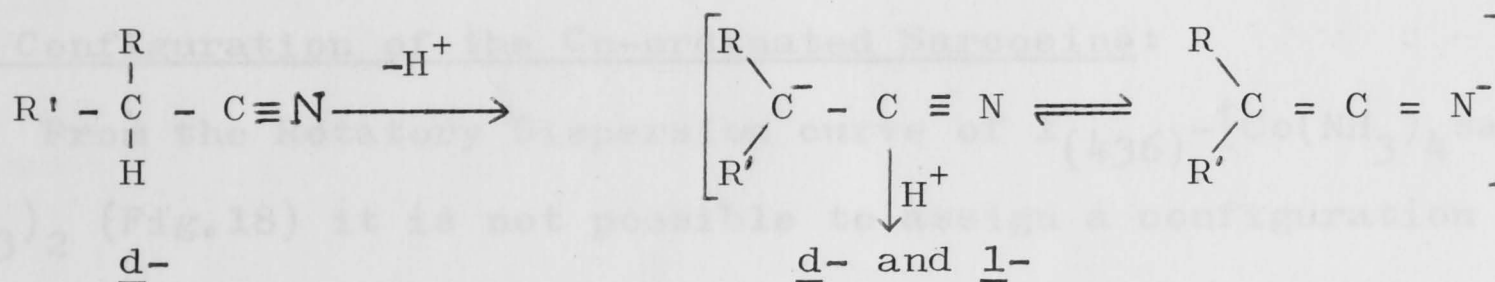


Space filled Leybold Molecular models show that the ammonia molecules are sufficiently close for such a tenuous attachment and this would allow the incoming water molecule to react predominantly with retention.

Finally, attack by the ubiquitous water immediately after formation of the intermediate might be expected to take place predominantly with retention of the original configura-

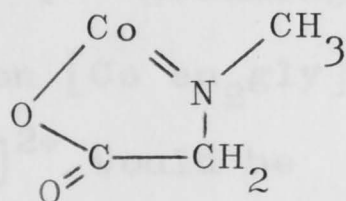
tion. The lone pair, surrounded by the solvent cage, is immediately exposed while attack by water from the rear is hindered by both the methyl group and the co-ordinated ammonia.

Some observations in the analogous field of carbanion stereochemistry suggest that racemization may be favoured if the planar  $sp^2$  state can be stabilized by resonance (68), e.g.



whereas carbanions that are less stabilized by resonance however, tend to retain their configuration (69).

In the present system, while there is delocalization in the vicinity of the carbonyl group, extending perhaps to the Co atom, there is no extensive delocalization in the region of the N atom and the planar formulation -



is unlikely to be achieved. From comparison with carbanions, therefore, the lack of stabilization for the  $sp^2$  transition state (D) (Fig. 17) is consistent with the observed high degree of retention accompanying exchange.

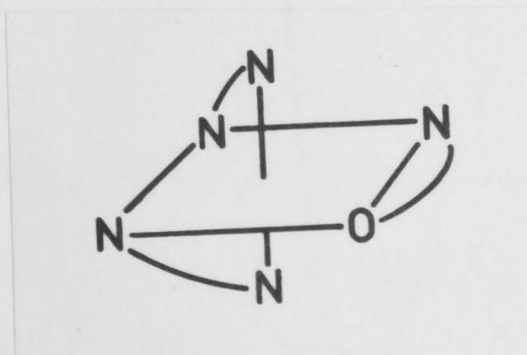
A factor which is interesting in this compound is why Co is so different from an alkyl substituent especially when the charge on the overall complex is larger than for the sub-



stituted ammonium salts, as the greater charge should favour the release of a proton. It would be of great interest to carry out the same experiments using (a) a monodentate ligand capable of being resolved, i.e. where there are no ring effects, or (b) similar bidentate ligands where the conformational effect may be significant and where the  $sp^2$  state can be achieved easily. These experiments could decide which of the possible mechanisms posed are significant.

The Configuration of the Co-ordinated Sarcosine:

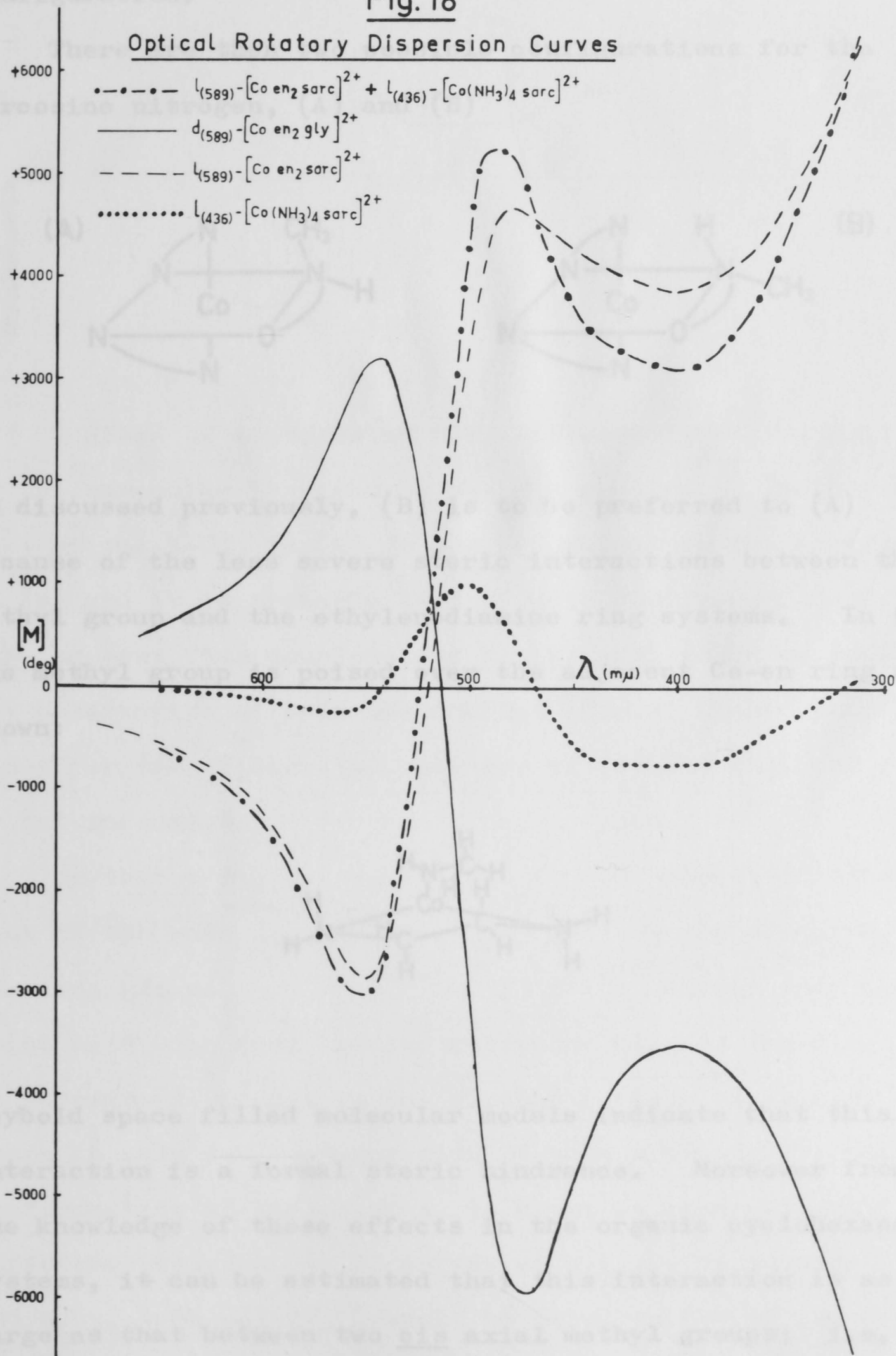
From the Rotatory Dispersion curve of  $1_{(436)}-[Co(NH_3)_4sarc](NO_3)_2$  (Fig.18) it is not possible to assign a configuration to the sarcosine nitrogen. Some conclusions may be made however, from a consideration of the stereochemistry and conformational effects in the ions,  $d_{(589)}-[Co en_2sarc]^{2+}$  and  $d_{(589)}-[Co en_2gly]^{2+}$ . MacDermott and Sargeson have shown that complexes of the type  $[Co en_2X_2]^+$  can be related to the configuration of  $d_{(589)}-[Co en_3]^{3+}$  through the similarity of their RD curves<sup>(70)</sup>. Assuming that a similar comparison may be made for the ion  $[Co en_2gly]^{2+}$ , the configuration of  $d_{(589)}-[Co en_2gly]^{2+}$  would be



Since the RD curve of  $d_{(589)}-[Co en_2sarc]^{2+}$  is very similar to that of  $d_{(589)}-[Co en_2gly]^{2+}$ , it also would have the above

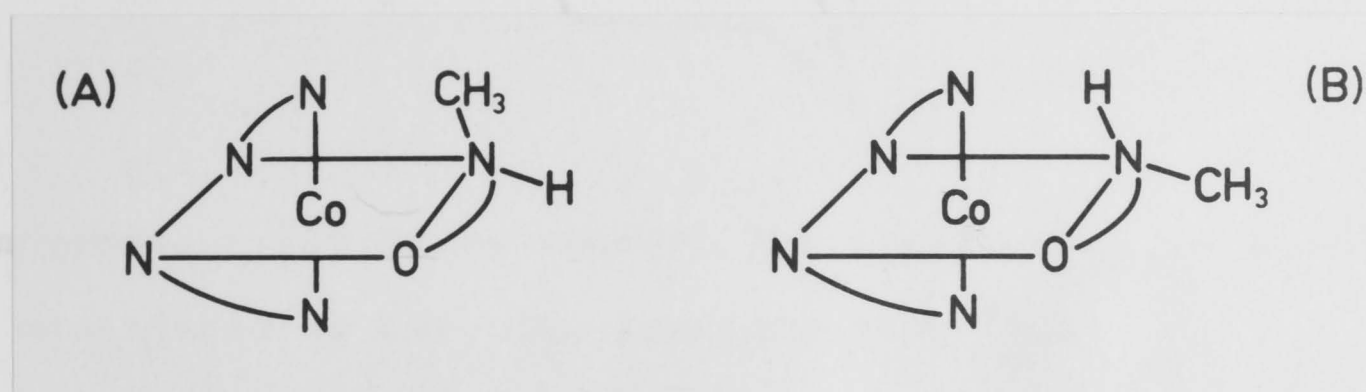
Fig. 18

Optical Rotatory Dispersion Curves

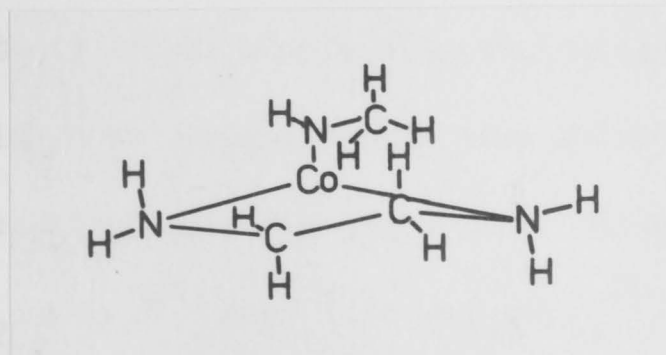


configuration.

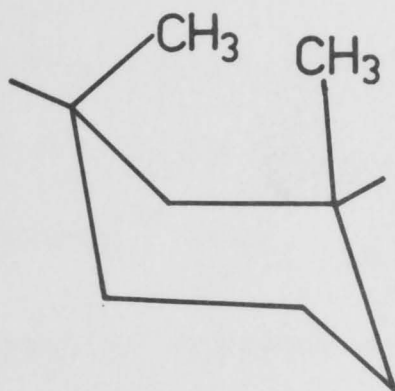
There are then two possible configurations for the sarcosine nitrogen, (A) and (B)



As discussed previously, (B) is to be preferred to (A) because of the less severe steric interactions between the methyl group and the ethylenediamine ring systems. In (A) the methyl group is poised over the adjacent Co-en ring as shown:



Leybold space filled molecular models indicate that this interaction is a formal steric hindrance. Moreover from the knowledge of these effects in the organic cyclohexane systems, it can be estimated that this interaction is as large as that between two cis axial methyl groups; i.e., approximately 5 k.cals/mole less stable than the equatorial

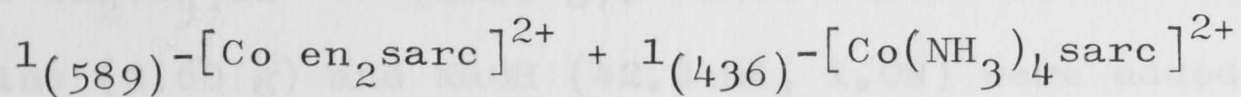


conformer. For these reasons the isomer  $d_{(589)}-[Co en_2 sarc]^{2+}$  is considered to have the configuration (B).

A piece of evidence on the stereospecific attachment of the sarcosine is given by the NMR spectra. The environment of the  $N-CH_3$  in (A) is not equivalent to the environment in (B) and it is probable that if both configurations of N were present, two doublets due to the  $-CH_3$  would be observed. The observation of only one doublet (Fig.8) is not positive proof for the evidence of only one of (A) and (B), but it is not inconsistent with such a conclusion.

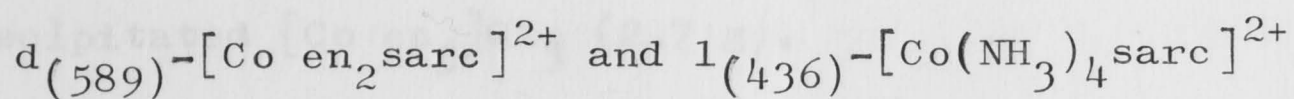
Further evidence supporting the stereospecific attachment of the sarcosine may be obtained from the RD curve. The ions  $[Co en_2 sarc]^{2+}$  and  $[Co en_2 gly]^{2+}$  differ only at the amino acid nitrogen; in the sarcosine complex the nitrogen is asymmetric whereas in the glycine complex it is not. If there is only one configuration of this nitrogen for each configuration of the complex, it might be expected that the difference between the RD curves of  $d_{(589)}-[Co en_2 sarc]^{2+}$  and  $d_{(589)}-[Co en_2 gly]^{2+}$  would be equal to the RD curves of one of the isomers of  $[Co(NH_3)_4 sarc]^{2+}$  in which the sarcosine

nitrogen is the only source of optical activity. Such a relation seems to exist, as is shown in Fig.18 where a composite curve of the rotations of

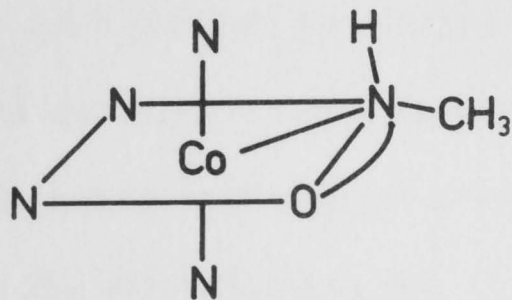


is plotted; this curve is almost a mirror image of the curve of  $d_{(589)}-[\text{Co en}_2\text{gly}]^{2+}$ .

It is concluded therefore that the ions,



have the same configuration about the sarcosine nitrogen, thus the latter may be written as



EXPERIMENTALd,1-[Co en<sub>2</sub>sarc]I<sub>2</sub>:

HCl (16.0 ml, 5.0N) was added with stirring to d,1-[Co en<sub>2</sub>CO<sub>3</sub>]Cl<sup>(71)</sup> (11.0 g). When effervescence ceased, sarcosine (3.60 g) and NaOH (42.0 ml, 1.0N) were added to the solution which was stirred and heated at 75° in an open beaker for 5 hours. The solution (30 ml) was cooled to room temperature and stood for 1 hour before filtering off the precipitated [Co en<sub>3</sub>]Cl<sub>3</sub> (2.7 g).

The precipitate was washed with 80% aqueous EtOH; the filtrate and washings were combined and treated with NaI (14 g). After standing overnight at room temperature the pink crystals were filtered, washed with 80% aqueous EtOH, EtOH and then acetone and dried in air. (12.4 g, 55% yield). The product was recrystallised from hot water to give [Co en<sub>2</sub>sarc]I<sub>2</sub> (dried under vacuum over silica gel).

Found: C, 15.95; H, 4.54; N, 13.30

[Co(C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>)<sub>2</sub>CH<sub>3</sub>·NH·CH<sub>2</sub>COO]I<sub>2</sub>; requires: C, 16.14; H, 4.26; N, 13.44.

Resolution of d,1-[Co en<sub>2</sub>sarc]I<sub>2</sub>:

d,1-[Co en<sub>2</sub>sarc]I<sub>2</sub> (13.03 g) in hot water (30 ml) was treated with a suspension of silver-d-Bromo camphor sulphonate monohydrate (Ag d-BCS·H<sub>2</sub>O) (21.70 g) in warm water (30 ml). After vigorous shaking in a stoppered flask for 15 minutes followed by standing at room temperature for one hour, the precipitated AgI was filtered off and washed with cold water.

The filtrate and washings were combined and evaporated in a rotary film evaporator to 30 ml and MeOH (90 ml) was added. After 2 hours a very fine flocculant precipitate was filtered off, washed with EtOH and acetone and dried under vacuum [I] (14.5 g) ( $[\alpha]_D^{20} = +31^\circ$ , 1.0% in  $H_2O$ ).

The sequence of recrystallization of diastereoisomer fractions and their  $[\alpha]_D^{20}$  values are shown in Fig. 19. Recrystallization of [I] from  $H_2O$ -EtOH three times and from 0.001M  $HClO_4$ -EtOH twice gave 1-[Co en<sub>2</sub>sarc]-d-[BCS]<sub>2</sub>·H<sub>2</sub>O [VI] (0.36 g)  $[\alpha]_D^{20} = -107^\circ$ , 1.0% in  $H_2O$  or 0.001M  $HClO_4$ , from which  $[M]_D^{20} = -970^\circ$ .

Found: C, 35.85; H, 5.66; N, 7.65;

$[Co(C_2H_8N_2)_2CH_3 \cdot NH \cdot CH_2COO][C_{10}H_{14}O_4BrS]_2 \cdot H_2O$ ; requires:  
C, 35.80; H, 5.79; N, 7.73.

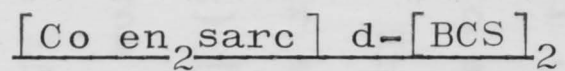
Evaporation to dryness of the mother liquor of the crude diastereoisomer yielded another fraction [I<sub>m</sub>] (6.8 g) ( $[\alpha]_D^{20} = +116^\circ$ , 1.0% in  $H_2O$ ). Recrystallization of this fraction from  $H_2O$ -EtOH twice and 0.001M  $HClO_4$ -EtOH once gave d-[Co en<sub>2</sub>sarc]-d-[BCS]<sub>2</sub>·H<sub>2</sub>O [I<sub>e</sub>] (0.44 g)  $[\alpha]_D^{20} = +227^\circ$ , 1.0% in  $H_2O$  or 0.001M  $HClO_4$ , from which  $[M]_D^{20} = +2050^\circ$ .

Found: C, 35.56; H, 5.89; N, 7.91;

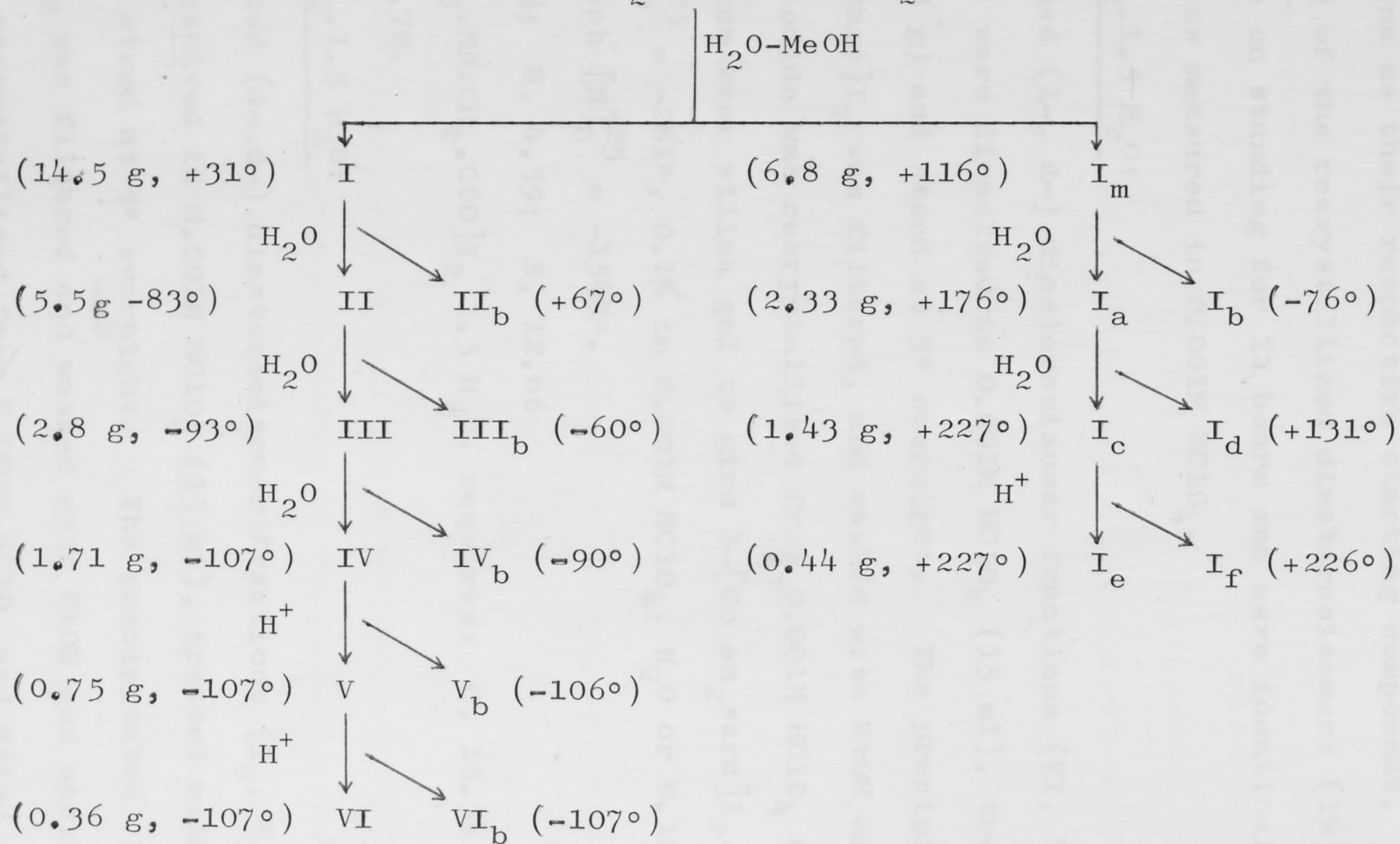
$[Co(C_2H_8N_2)_2CH_3 \cdot NH \cdot CH_2 \cdot COO][C_{10}H_{14}O_4BrS]_2 \cdot H_2O$ , requires:  
C, 35.80; H, 5.79; N, 7.73.

FIG. 19

Fractional Recrystallization of diastereoisomers of



← less soluble                      [Co en<sub>2</sub>sarc] d-[BCS]<sub>2</sub>                      more soluble →





Absence of Mutarotation:

Both the l-d- and d-d- diastereoisomers were recrystallised from 0.001M  $\text{HClO}_4$ -EtOH, but the fractions obtained showed the same rotations as their respective starting compounds. Solutions in  $\text{H}_2\text{O}$  of the recrystallized diastereoisomers (1%) showed no change on standing for 12 hours and were identical with the rotations measured in 0.001M  $\text{HClO}_4$ .

l-[Co en<sub>2</sub>sarc]I<sub>2</sub>·1.5 H<sub>2</sub>O:

The combined (l-, d-) diastereoisomer fractions ( $\text{VI}$ ,  $\text{V}_b$  and  $\text{VI}_b$ ) (1.4 g) were dissolved in 0.001M  $\text{HClO}_4$  (15 ml), treated with NaI (2.5 g) and stood at 5° overnight. The precipitated l-[Co en<sub>2</sub>sarc]I<sub>2</sub> was filtered, and washed with EtOH and acetone. The iodide was recrystallised from 0.001M  $\text{HClO}_4$  and dried under vacuum over silica gel to give l-[Co en<sub>2</sub>sarc]I<sub>2</sub>·1.5 H<sub>2</sub>O (0.3 g)  $[\alpha]_D^{25} = -281^\circ$ , 0.1% in 0.001M  $\text{HClO}_4$ ,  $\text{H}_2\text{O}$  or 0.1M  $\text{NaHCO}_3$ , from which  $[\text{M}]_D^{25} = -1540^\circ$ .

Found: C, 15.23; H, 4.59; N, 12.66,

$[\text{Co}(\text{C}_2\text{H}_8\text{N}_2)_2 \text{CH}_3\cdot\text{NH}\cdot\text{CH}_2\cdot\text{COO}] \text{I}_2 \cdot 1.5 \text{H}_2\text{O}$ , requires: C, 15.34; H, 4.60; N, 12.78.

d-[Co en<sub>2</sub>sarc]I<sub>2</sub>·1.5 H<sub>2</sub>O:

The combined (d-, d-) diastereoisomer fractions ( $\text{I}_e$ ,  $\text{I}_f$ ) (1.2 g) were dissolved in 0.001M  $\text{HClO}_4$  (15 ml), treated with NaI (2.5 g) and stood at 5° overnight. The precipitated d-[Co en<sub>2</sub>sarc]I<sub>2</sub> was filtered and washed with EtOH and acetone. The product was recrystallised from 0.001M  $\text{HClO}_4$  and dried under vacuum over silica gel to give d-[Co en<sub>2</sub>sarc]I<sub>2</sub>·1.5 H<sub>2</sub>O (0.3 g)  $[\alpha]_D^{25} = +275^\circ$ , 0.1% in 0.001M  $\text{HClO}_4$ ,  $\text{H}_2\text{O}$  or 0.1M

$\text{NaHCO}_3$ , from which  $[\text{M}]_D^{25} = +1510^\circ$ .

Found: C, 15.24; H, 4.74; N, 12.66,

$[\text{Co}(\text{C}_2\text{H}_8\text{N}_2)_2\text{CH}_3\cdot\text{NH}\cdot\text{CH}_2\text{COO}]\text{I}_2\cdot 1.5 \text{H}_2\text{O}$  requires: C, 15.34;

H, 4.60; N, 12.78.

$d,1-[\text{Co}(\text{NH}_3)_4\text{sarc}](\text{NO}_3)_2$

Sarcosine (3.6 g) was dissolved in NaOH (35 ml, 1.0N),  $[\text{Co}(\text{NH}_3)_4\text{Cl}\cdot\text{OH}_2]\text{SO}_4$  (72) (11.1 g) was added, followed by Ammonia (3 ml, 20N) and the solution was stirred and heated to  $70^\circ$ . After 90 mins. the solution was filtered, excess  $\text{NH}_4\text{NO}_3$  (10.0 g) was added and the solution was cooled overnight at  $5^\circ$ .  $[\text{Co}(\text{NH}_3)_4\text{sarc}](\text{NO}_3)_2$  was filtered off, washed with  $\text{H}_2\text{O}-\text{MeOH}$  (1:1) and MeOH (6.8 g, 50% yield).

The complex was recrystallised from warm water with  $\text{NH}_4\text{NO}_3$  and dried at room temperature under vacuum.

Found: C, 10.75; H, 5.79; N, 28.75.

$[\text{Co}(\text{NH}_3)_4\text{CH}_3\text{NHCH}_2\text{COO}](\text{NO}_3)_2$ , requires: C, 10.62; H, 5.35;

N, 28.91.

$d,1-[\text{Co}(\text{NH}_3)_4\text{sarc}]\text{Cl}_2$

$[\text{Co}(\text{NH}_3)_4\text{sarc}](\text{NO}_3)_2$  (5 g) was added to ice cold 5N HCl (50 ml) and stirred for several minutes. The solution was filtered and treated with MeOH (100 ml) and after 15 minutes the crude  $[\text{Co}(\text{NH}_3)_4\text{sarc}]\text{Cl}_2$  was filtered off, washed with MeOH and dried at the pump (3.2 g). This complex was recrystallized several times (to remove the less soluble nitrate) by dissolving in warm 1N HCl, adding  $\text{NH}_4\text{Cl}$ , and fractionally crystallizing by the addition of MeOH.

Found: C, 12.25; H, 6.48; N, 23.44;

$[\text{Co}(\text{NH}_3)_4\text{NH}\cdot\text{CH}_3\cdot\text{CH}_2\cdot\text{COO}]\text{Cl}_2\cdot 0.5 \text{H}_2\text{O}$ , requires: C, 12.21;  
H, 6.49; N, 23.74.

Resolution of  $[\text{Co}(\text{NH}_3)_4\text{sarc}]\text{Cl}_2$ :

All operations were carried out in 0.001M  $\text{HClO}_4$  solution. All rotations were measured in a 1 dm tube unless stated otherwise.  $[\text{Co}(\text{NH}_3)_4\text{sarc}]\text{Cl}_2\cdot 0.5 \text{H}_2\text{O}$  (5.72 g) was dissolved in 0.001M  $\text{HClO}_4$  (40 ml), d-Na $[\text{Co en ox}_2]\text{H}_2\text{O}$  <sup>(26)</sup> (3.36 g) was added, the solution was stirred well and stood for 30 minutes. The precipitated diastereoisomer 1- $[\text{Co}(\text{NH}_3)_4\text{sarc}]$  d- $[\text{Co en ox}_2]\text{Cl}\cdot\text{H}_2\text{O}$  was filtered, washed with MeOH and dried (4.0 g).

Found: C, 19.20; H, 5.40; N, 17.57.

$[\text{Co}(\text{NH}_3)_4\text{CH}_3\cdot\text{NH}\cdot\text{CH}_2\cdot\text{COO}][\text{Co}(\text{NH}_2\cdot\text{CH}_2)_2(\text{C}_2\text{O}_4)_2]\text{Cl}\cdot\text{H}_2\text{O}$  requires:  
C, 19.21; H, 5.02; N, 17.43.

1- $[\text{Co}(\text{NH}_3)_4\text{sarc}](\text{NO}_3)_2$ :

The diastereoisomer was ground in a mortar with  $\text{NH}_4\text{NO}_3$  (6.0 g) in 0.001M  $\text{HClO}_4$  (10 ml) and the 1- $[\text{Co}(\text{NH}_3)_4\text{sarc}](\text{NO}_3)_2$  was filtered off, washed with 50% aqueous MeOH and then MeOH. Fractional recrystallization from  $\text{HClO}_4$  solution with  $\text{NH}_4\text{NO}_3$  gave three fractions having  $[\alpha]_D^{25}$  values of  $-31^\circ$ ,  $-51^\circ$ ,  $-51^\circ$  (0.1% solutions in 0.001M  $\text{HClO}_4$ ). Fraction II was analysed.

Found: C, 10.80; H, 5.63; N, 29.20.

$[\text{Co}(\text{NH}_3)_4\text{CH}_3\cdot\text{NH}\cdot\text{CH}_2\cdot\text{COO}](\text{NO}_3)_2$  requires: C, 10.62; H, 5.35;  
N, 28.91.

Other rotations for the optically pure 1- isomer were quickly to a one dm. tube maintained at constant temperature

$[\alpha]_{578}^{25} = -67^\circ$ ,  $[\alpha]_{546}^{25} = -24^\circ$  and  $[\alpha]_{436}^{25} = -220^\circ$  (0.1% in  $\text{HClO}_4$  also).

$d\text{-}[\text{Co}(\text{NH}_3)_4\text{sarc}](\text{NO}_3)_2$ :

The filtrate from the diastereoisomer was treated with  $\text{NH}_4\text{NO}_3$  and six fractions of  $d\text{-}[\text{Co}(\text{NH}_3)_4\text{sarc}](\text{NO}_3)_2$  were collected, the latter fractions having greater optical purity. The  $[\alpha]_D^{25}$  values for 0.1% concentrations in  $\text{HClO}_4$  were:

<u>Fraction</u>	<u><math>[\alpha]_D^{25}</math></u>
II	+ 10°
III	+ 11°
IV	+ 17°
V	+ 41°
VI	+ 48°

Also for fraction VI  $[\alpha]_{578}^{25} = + 64^\circ$ ,  $[\alpha]_{546}^{25} = + 23^\circ$  and  $[\alpha]_{436}^{25} = + 206^\circ$ .

Found: C, 10.61; H, 5.41; N, 29.31,

$[\text{Co}(\text{NH}_3)_4\text{CH}_3\text{.NH.CH}_2\text{.COO}](\text{NO}_3)_2$ , requires: C, 10.62; H, 5.35; N, 28.91.

Rates of Racemization of  $d\text{-}$  or  $l\text{-}[\text{Co}(\text{NH}_3)_4\text{sarc}](\text{NO}_3)_2$ :

The racemization rates of the optically active nitrates were obtained over a range of pH (4.44 - 8.02) and temperature (20-40°) from measurement of rotations at 436 m $\mu$  in a PERKIN-ELMER MODEL 141 Polarimeter.

0.011 - 0.012 g. nitrate was dissolved in 10.0 ml of the appropriate buffer solution and the solution was transferred quickly to a one dm. tube maintained at constant temperature

( $\pm 0.1^\circ$ ). Initial rotations were  $0.20 - 0.25^\circ$  and readings were accurate to  $\pm 0.002^\circ$ . Reactions were followed for up to 2 half lives.

The buffers used were of 0.1M concentration in most cases, although several runs were made at other concentrations.

Three rates were measured at  $30.3^\circ$  in  $D_2O$  (Fluka, puriss  $\geq 99.8\%$ ) two of these in 0.1M phosphate buffer and the other in 0.1M acetate buffer.

Solutions in  $D_2O$  were prepared as follows:

	(i)	(ii)	(iii)
$NaH_2PO_4 \cdot 2H_2O$	.04670g	.10909g	
$Na_2HPO_4$	.09929g	.04246g	
$D_2O$	10.0 ml	10.0 ml	10.0 ml
$d-[Co(NH_3)_4sarc](NO_3)_2$	.0116g	.0115g	.0119g
$CH_3COONa$			.08117g
$CH_3COOH$ (0.20M)			.05 ml
pD	7.56	6.86	7.24

Rates of Deuteration of  $d,1-[Co(NH_3)_4sarc]Cl_2$ :

Rates of deuteration of the amino nitrogen of the sarcosine ligand were obtained at  $33.3^\circ$  from changes in the NMR spectra recorded by a PERKIN-ELMER R.10 Spectrometer.

0.1g  $[Co(NH_3)_4sarc]Cl_2$  was dissolved in 1.0 ml of each of the following solutions:

(a)	$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	$\text{Na}_2\text{HPO}_4$	$\text{D}_2\text{O}$
	0.01370g	0.00178g	1.0 ml
(b)	$\text{C}_6\text{H}_4\text{COOK} \cdot \text{COOH}$		1.0 ml
	0.01016g		
(c)	$\text{D}_2\text{SO}_4$		
	(i) ~ 0.0004g		10.0 ml
	(ii) ~ 0.0018g		10.0 ml
	(iii) 0.00962g		10.0 ml

Portion of the solution was transferred to the sample tube and the spectrum was recorded at suitable times.

Isolation of deuterated racemic and optically active samples:

Deuterated-d,1-[Co(NH<sub>3</sub>)<sub>4</sub>sarc]Cl<sub>2</sub>:

d,1-[Co(NH<sub>3</sub>)<sub>4</sub>sarc]Cl<sub>2</sub> (0.2 g) was dissolved in D<sub>2</sub>O (2.0 ml) containing NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O (0.02750 g) and Na<sub>2</sub>HPO<sub>4</sub> (0.00347 g). Estimated pD of the solution was 5.0 - 5.5. The solution was maintained at 33.3° for 10 minutes, before precipitating the complex by the addition of MeOH.

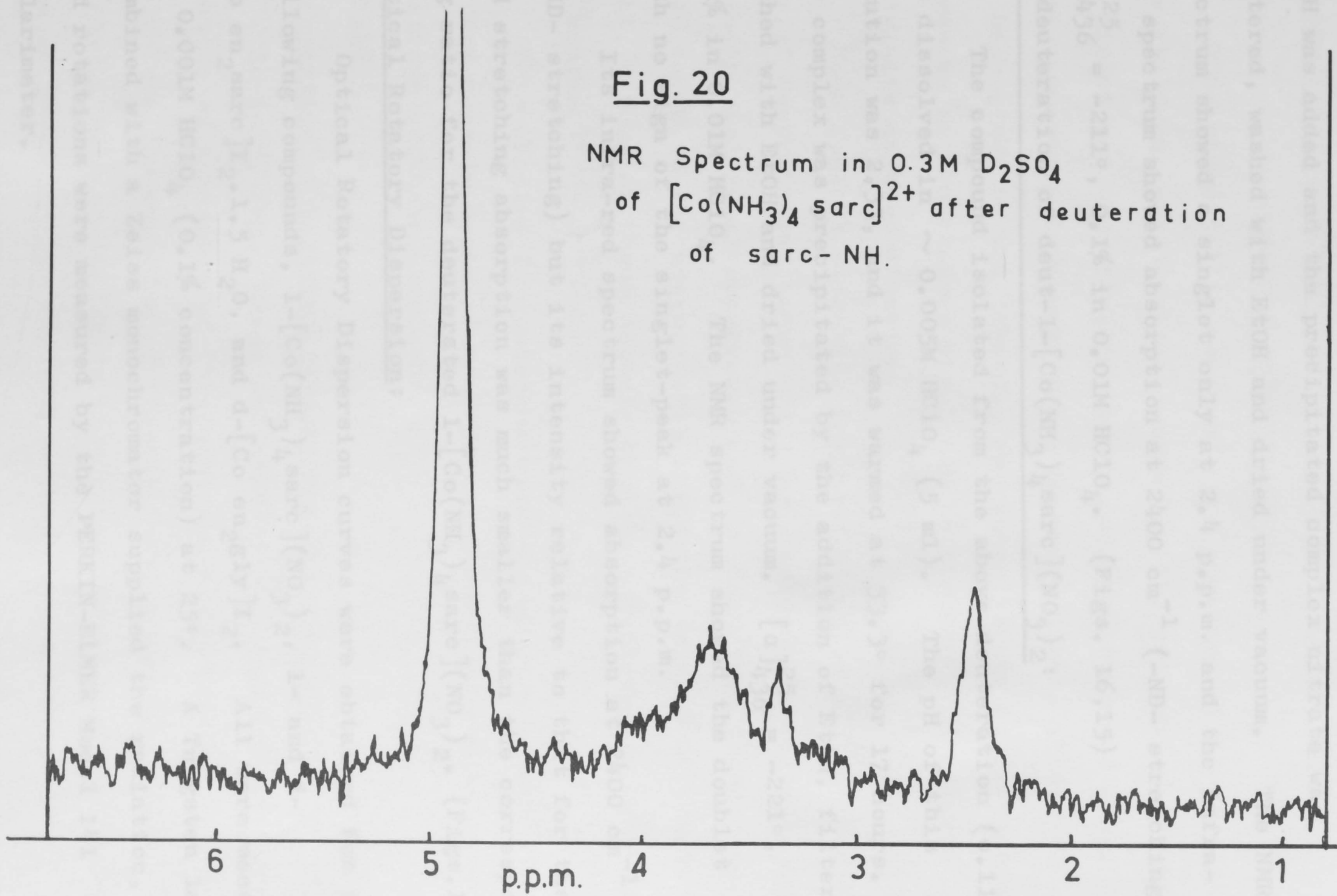
It was filtered quickly, washed with MeOH and dried under vacuum. Portion of this deuterated compound was dissolved in D<sub>2</sub>SO<sub>4</sub>-D<sub>2</sub>O (~0.3M) and its NMR spectrum was recorded. A singlet peak only was observed at 2.4 p.p.m. (Fig. 20).

Deuterated-1-[Co(NH<sub>3</sub>)<sub>4</sub>sarc](NO<sub>3</sub>)<sub>2</sub>:

1-[Co(NH<sub>3</sub>)<sub>4</sub>sarc](NO<sub>3</sub>)<sub>2</sub> ( $[\alpha]_{436}^{25} = -221^\circ$ , 0.1% in 0.01M HClO<sub>4</sub>) (0.22 g) was dissolved in 10.0 ml D<sub>2</sub>O containing 0.00315g HClO<sub>4</sub> (70%). The pD of this solution was 2.75.

Fig. 20

NMR Spectrum in 0.3M D<sub>2</sub>SO<sub>4</sub>  
of [Co(NH<sub>3</sub>)<sub>4</sub>sarc]<sup>2+</sup> after deuteration  
of sarc-NH.



The temperature was kept at  $33.3^\circ$  for 17 hours, after which EtOH was added and the precipitated complex nitrate was filtered, washed with EtOH and dried under vacuum. The NMR spectrum showed a singlet only at 2.4 p.p.m. and the infra-red spectrum showed absorption at  $2400\text{ cm}^{-1}$  (-ND- stretching).  $[\alpha]_{436}^{25} = -211^\circ$ , 0.1% in 0.01M  $\text{HClO}_4$ . (Figs. 16,15)

De-deuteration of deut-1-[Co(NH<sub>3</sub>)<sub>4</sub>sarc](NO<sub>3</sub>)<sub>2</sub>:

The compound isolated from the above deuteration (0.11 g) was dissolved in  $\sim 0.005\text{M}$   $\text{HClO}_4$  (5 ml). The pH of this solution was 2.33, and it was warmed at  $33.3^\circ$  for 17 hours. The complex was precipitated by the addition of EtOH, filtered, washed with EtOH and dried under vacuum.  $[\alpha]_{436}^{25} = -221^\circ$ , 0.1% in 0.01M  $\text{HClO}_4$ . The NMR spectrum showed the doublet with no sign of the singlet-peak at 2.4 p.p.m.

Its infra-red spectrum showed absorption at  $2400\text{ cm}^{-1}$  (-ND- stretching) but its intensity relative to that for the -NH stretching absorption was much smaller than the corresponding ratio for the deuterated 1-[Co(NH<sub>3</sub>)<sub>4</sub>sarc](NO<sub>3</sub>)<sub>2</sub>. (Figs.16,15)

Optical Rotatory Dispersion:

Optical Rotatory Dispersion curves were obtained for the following compounds, 1-[Co(NH<sub>3</sub>)<sub>4</sub>sarc](NO<sub>3</sub>)<sub>2</sub>, 1- and d-[Co en<sub>2</sub>sarc]I<sub>2</sub>·1.5 H<sub>2</sub>O, and d-[Co en<sub>2</sub>gly]I<sub>2</sub>. All were measured in 0.001M  $\text{HClO}_4$  (0.1% concentration) at  $25^\circ$ . A Tungsten lamp combined with a Zeiss monochromator supplied the radiation, and rotations were measured by the PERKIN-ELMER Model 141 Polarimeter.



### CHAPTER THREE

#### REACTIVITIES AND PROPERTIES OF SOME CO-ORDINATED

#### AMINO ACID DERIVATIVES

When non-metallic compounds are combined with metallic salts to form co-ordination compounds, the properties of both the ligand and the metallic ion are altered substantially. Until comparatively recent times however, little attention has been given to the ligands, but there is now an increasing awareness of the changes which can be induced in their reactivities on co-ordination. (73,74,75)

Some special attention has been directed at the reactivity of co-ordinated amino acids and their derivatives because of their biological occurrence and the fact that trace elements occur in the enzymes with which the amino acid substrates react. Some of these aspects have been reviewed by Greenstein and Winitz. (76)

This chapter is concerned with the ability of amino acids to form certain types of co-ordination compound and with some reactions of these compounds.

#### SECTION I

#### The reaction of Bis-glycinato copper(II) with aldehydes:

In 1957, Sato, Okawa and Akabori<sup>(77)</sup> reported the preparation of a mixture of threonine and allothreonine in 64% yield from the reaction of bis-glycinato copper(II), i.e.  $\text{Cu}(\text{gly})_2$ , with acetaldehyde in aqueous sodium carbonate solution at 50°. It was considered that the co-ordination of glycine favoured the reaction in two ways - (a) the  $\alpha$ -carbon

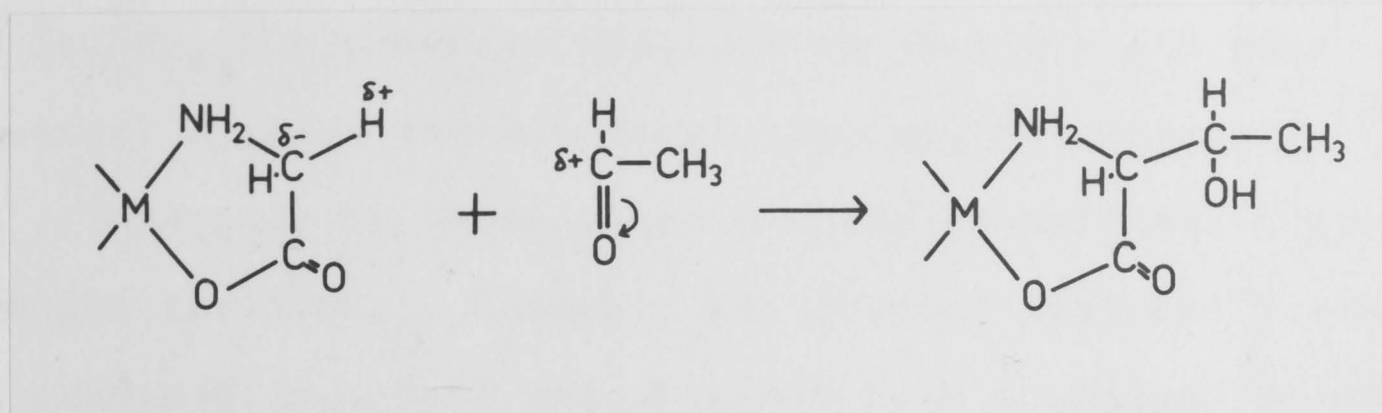
TABLE 16

Reactions of Cu(gly)<sub>2</sub> with carbonyl compounds

Carbonyl compound	Conditions of Reaction	Product	yield (%)	Ref.
formaldehyde	Na <sub>2</sub> CO <sub>3</sub> 95°, 1 hour.	*serine	30	78
sodium glyoxylate	1N NaOH 5°, overnight.	β-hydroxy aspartic acid.	64	79
pyruvic acid	1N NaOH 5° 12-18 hrs.	β-OH β-methyl aspartic acid.	60-70	80
iso-butyraldehyde	~3.6N NaOH or KOH 5°, overnight room temp., 5 hrs.	β-OH leucine	69-76	81
propionaldehyde	2N KOH	β-OH norvaline	58	82
n-butyraldehyde	cold, several days	β-OH norleucine	81	
n-heptaldehyde		β-OH α-amino pelargonic acid	61	
acetone		β-OH valine	46	
methyl ethyl ketone		β-OH isoleucine	3	

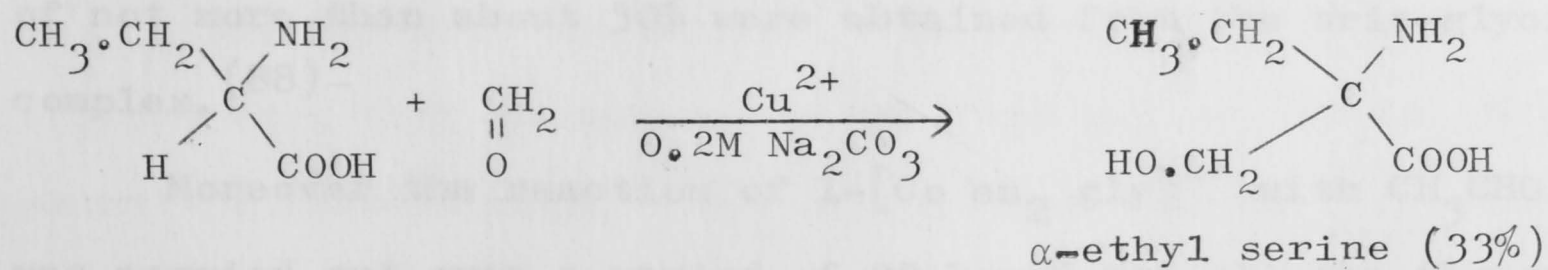
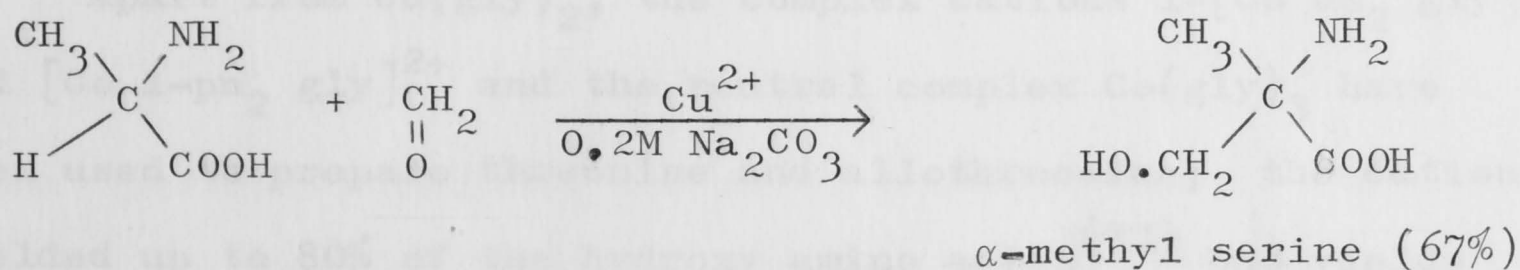
(\* see Page 102)

was activated towards electrophilic attack, and (b) the amino group was protected from unfavourable side reactions.



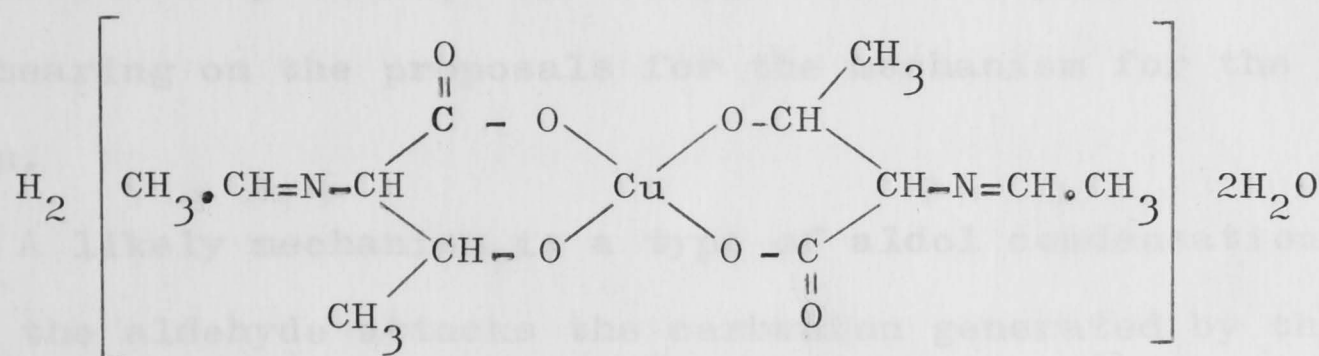
Subsequent reactions with other carbonyl compounds yielding  $\alpha$ -amino  $\beta$ -hydroxy acids were reported. Some of these are given in Table 16.

\* For the reaction with formaldehyde the best yields of serine were obtained using a Cu:glycine ratio of 1:100 or 1:200. With ratios of 1:10, 1:2, i.e.,  $\text{Cu}(\text{gly})_2$ , or 1:1 the predominant product was  $\alpha$ -hydroxymethyl serine formed by substitution of 2 moles of formaldehyde. Otani and Winitz also reacted formaldehyde with alanine and  $\alpha$ -amino butyric acid<sup>(83)</sup> thereby showing that the  $\alpha$ -carbon atoms of these amino acids were sufficiently activated for condensation.



The same type of condensation has been reported for the co-ordinated dipeptides  $\text{Cu}(\text{gly gly})$  and  $\text{Cu}(\text{gly ala})$ . After reaction with formalin solution containing pyridine and  $\text{Na}_2\text{CO}_3$ , the products obtained in 50-55% yield were D,L-seryl glycine and D,L-seryl alanine, respectively. (84)

Generally in these reactions the co-ordination products were not isolated. However, one product that was isolated and examined in detail was obtained from acetaldehyde and  $\text{Cu}(\text{gly})_2$ . (85) Elemental analysis of this compound fitted the formulation  $\text{Cu}(\text{threonine})_2 (\text{acetaldehyde})_2$  and from the crystallographic analysis the structure was written as:



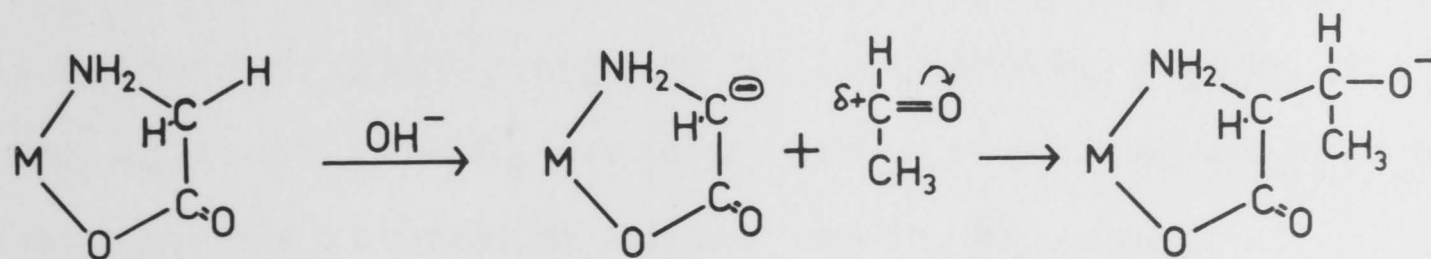
The complex of threonine was found to be much less water soluble than the complex of allothreonine, and the use of this property for the separation and purification of the threo- and allo- isomers is the subject of several patents. (86)

Apart from  $\text{Cu}(\text{gly})_2$ , the complex cations  $1-[\text{Co en}_2 \text{ gly}]^{2+}$  and  $[\text{Co 1-pn}_2 \text{ gly}]^{2+}$  and the neutral complex  $\text{Co}(\text{gly})_3$  have been used to prepare threonine and allothreonine; the cations yielded up to 80% of the hydroxy amino acids (87) but yields of not more than about 30% were obtained from the tris-glycinato complex. (88)

Moreover the reaction of  $1-[\text{Co en}_2 \text{ gly}]^{2+}$  with  $\text{CH}_3\text{CHO}$  was carried out over a period of 90 hours and all the threonine

isomers were produced. In the present investigation, attempts were made to isolate the threonine complexes without success, but the starting material and the isomeric mixture of products were separated on cellulose using n-Butanol, H<sub>2</sub>O, HCl (60:30:10) as eluent. By chromatographing the reaction mixture a qualitative assessment of the rate was obtained and it appeared that under the conditions used for the reactions described above, the condensation was complete with the [Co en<sub>2</sub>gly]<sup>2+</sup> ion in ~1 min, whereas for [Co gly<sub>3</sub>]<sup>0</sup> and the [Co ox<sub>2</sub> gly]<sup>2-</sup> ion the condensation rate was considerably less (~30 min). These experiments have some bearing on the proposals for the mechanism for the reaction.

A likely mechanism is a type of aldol condensation in which the aldehyde attacks the carbanion generated by the base.



Under these circumstances the metal protects the -NH<sub>2</sub> group and perhaps has an activating influence also. The experiments just described with the complexes of different charge are consistent with this mechanism, in that the cation [Co en<sub>2</sub>gly]<sup>2+</sup> shows the fastest rate. Not only is the rate of dissociation of the proton favoured in this instance but also the equili-

brium concentration of carbanion should be greatest (if this state is ever achieved). For the anion however, the overall negative charge reduces the ease of dissociation of  $H^+$  and a slower reaction rate follows.

For the mechanism to hold, the methylene protons on the co-ordinated glycine must exchange with the solvent protons in the presence of  $Na_2CO_3$ ; this exchange was studied by recording the n.m.r. spectrum of a glycine complex in  $D_2O$ .

Deuteration of Co-ordinated Glycine: The lability of the  $\alpha$ -amino hydrogen of co-ordinated glycine under mildly alkaline conditions has been demonstrated by the observation of its exchange with deuterium. The NMR spectra of  $[Co en_2 gly]^{2+}$  in acid, neutral and slightly alkaline solutions (Fig.21) showed differences which were assessed as follows:

In 1M  $D_2SO_4$  a broad absorption which centred around 5.2 p.p.m. (downfield relative to TPSNa) was assigned to the en- $NH_2$  and gly- $NH_2$  protons. At 3.8 - 3.5 p.p.m. there was a well defined triplet, assigned to the gly- $CH_2$  protons split by the adjacent gly- $NH_2$  protons, and a strong absorption at 2.8 p.p.m. was attributed to the en- $CH_2$  protons.

In  $D_2O$  there was no absorption around 5.2 p.p.m. and a sharp singlet at 3.6 p.p.m. had replaced the triplet; both changes were consistent with complete deuteration at the en- and gly- nitrogens. The absorption at 2.8 p.p.m. was unchanged.

In 0.1%  $Na_2CO_3$  solution the intensity of the singlet absorption at 3.6 p.p.m. steadily decreased with time; this

Fig. 21b

Fig. 21a

NMR Spectrum of  $[\text{Co en}_2 \text{ gly}]^{2+}$   
in  $1\text{M D}_2\text{SO}_4$

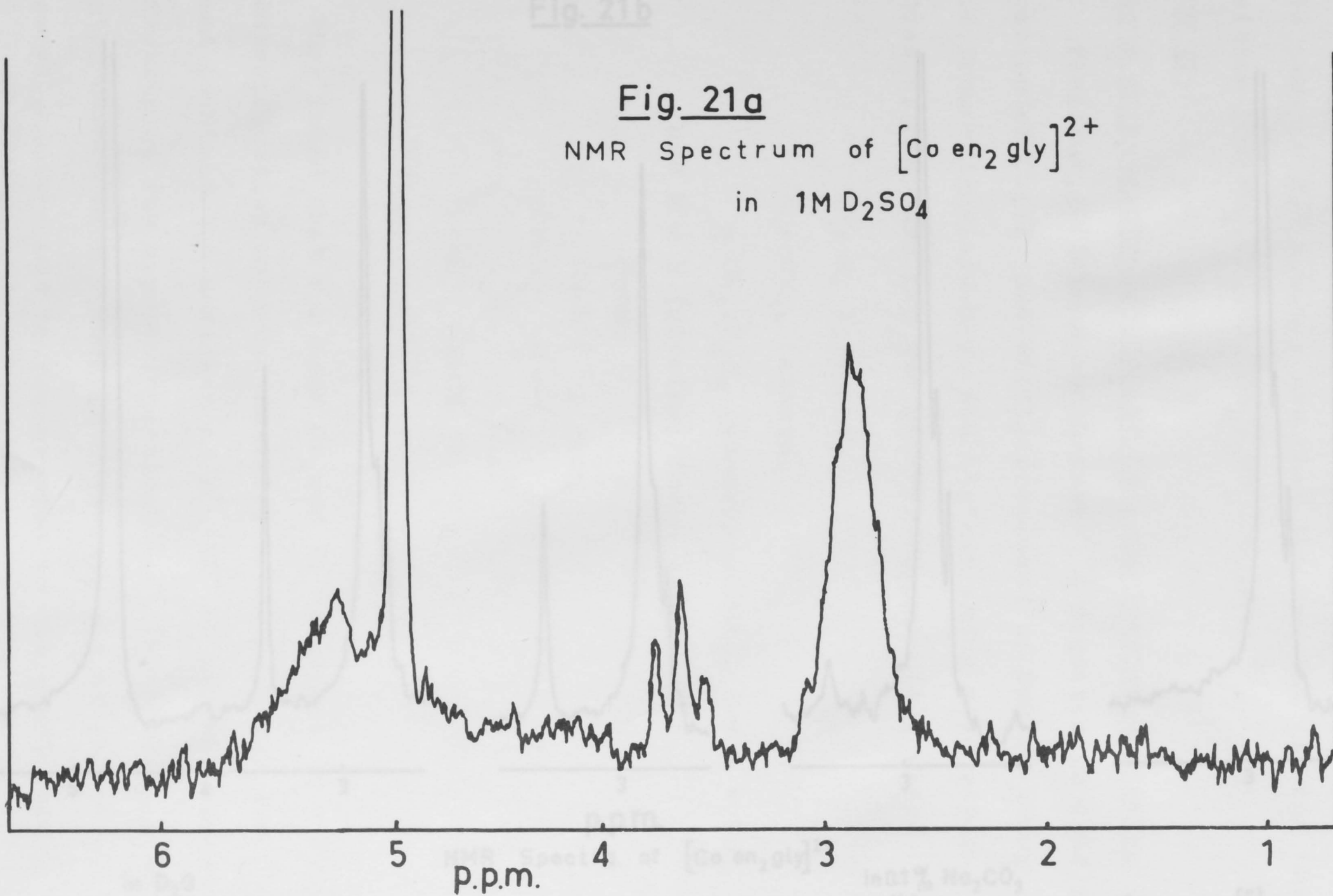
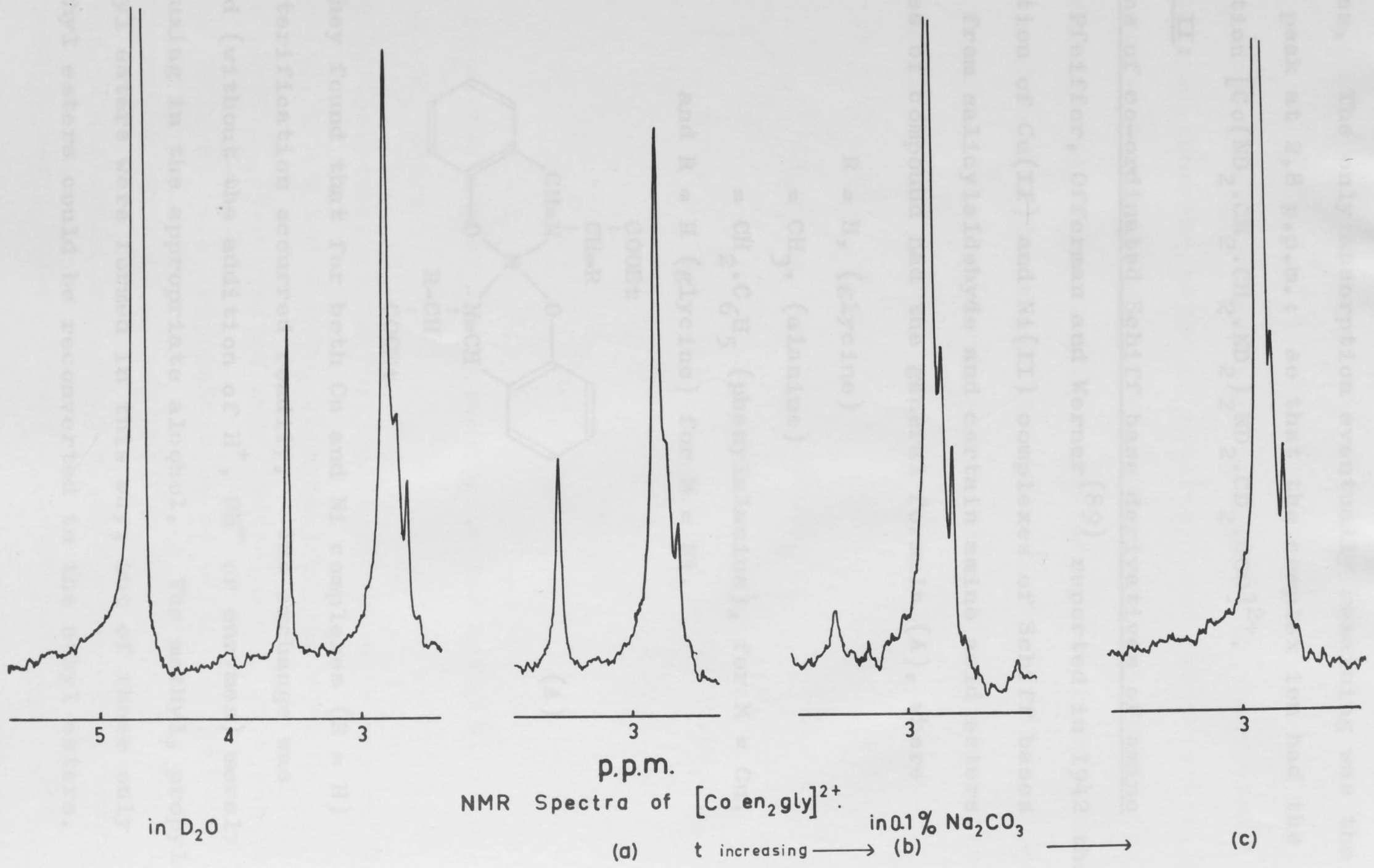


Fig. 21b

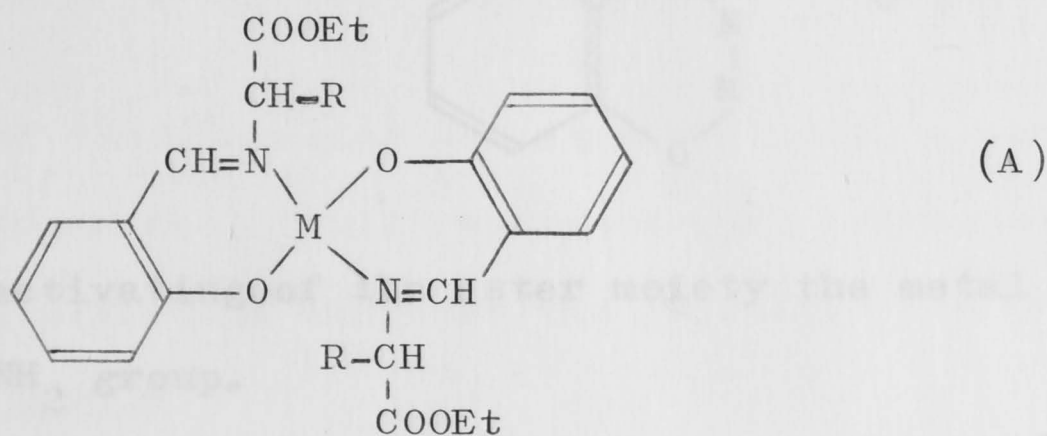
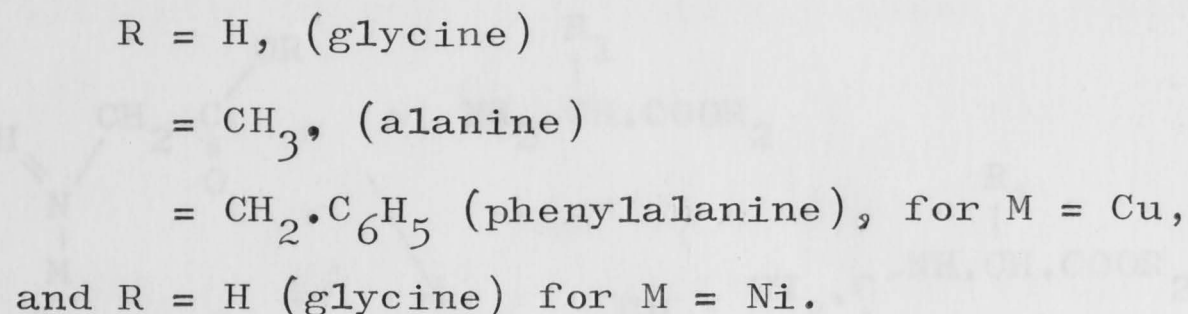




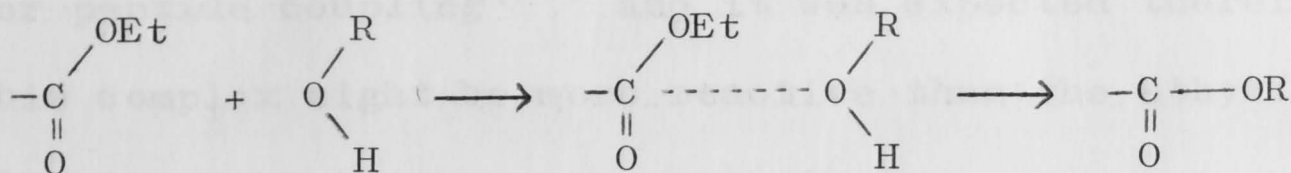
decrease was consistent with the replacement of both  $\alpha$ -carbon hydrogens. The only absorption eventually remaining was the single peak at 2.8 p.p.m.; so that the complex ion had the composition  $[\text{Co}(\text{ND}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{ND}_2)_2\text{ND}_2\cdot\text{CD}_2\cdot\text{COO}]^{2+}$ .

## SECTION II:

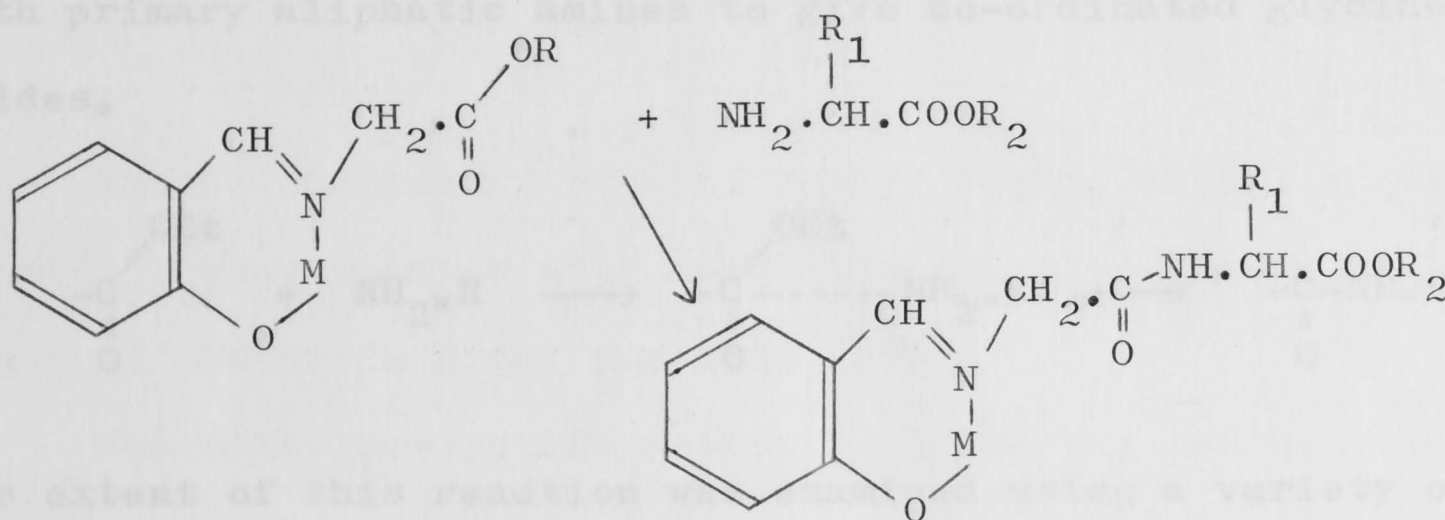
Reactions of co-ordinated Schiff base derivatives of amino acids: Pfeiffer, Offerman and Werner<sup>(89)</sup> reported in 1942 the preparation of Cu(II) and Ni(II) complexes of Schiff bases derived from salicylaldehyde and certain amino acid esters. One class of compound had the general formula (A), where



They found that for both Cu and Ni complexes ( $\text{R} = \text{H}$ ) transesterification occurred readily. The exchange was effected (without the addition of  $\text{H}^+$ ,  $\text{OH}^-$  or enzymes) merely by refluxing in the appropriate alcohol. The methyl, propyl and butyl esters were formed in this way, but of these only the methyl esters could be reconverted to the ethyl esters.



The fact that ester exchange occurred with these complexes implies that the ester is activated and the possibility then arises that the carbonyl carbon atom can be attacked by other nucleophilic reagents; in particular, that these complexes could be used for a peptide synthesis by reaction with another amino acid ester, leading to a salicylidene dipeptide ester complex.



As well as activating of the ester moiety the metal also protects the  $-\text{NH}_2$  group.

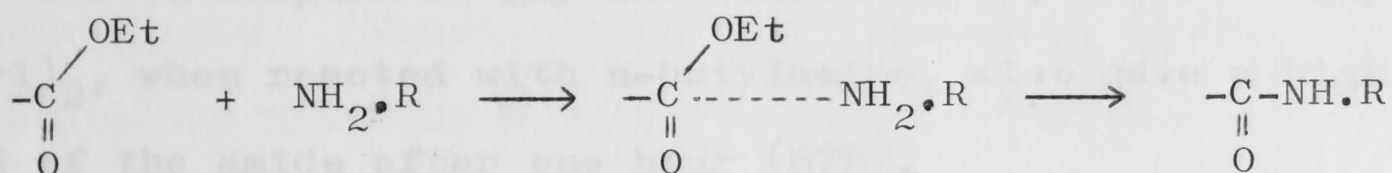
Bis[N-(ethoxycarbonylmethyl)salicylideneamino]copper(II), compound (A),  $\text{R} = \text{H}$  and  $\text{M} = \text{Cu}$ , alternatively denoted as  $\text{Cu}(\text{sal} = \text{glyOEt})_2$ , was reacted with several amino acid esters, either in  $\text{CHCl}_3$  at room temperature or by refluxing in  $\text{EtOH}$ , but there was no evidence of any peptide formation. These procedures were carried out also for the corresponding complex of the p-nitrophenyl ester,  $\text{Cu}(\text{sal} = \text{glyOPh-p-NO}_2)_2$ . The p-nitrophenyl esters of amino acids are normally very active

for peptide coupling<sup>(90)</sup> and it was expected therefore that this complex might be more reactive than the ethyl ester complex.

In fact condensation did take place to give some dipeptide but the reacting amino acid ester also replaced the less basic p-nitrophenyl glycine ester in both the Ni(II) and Cu(II) complex. Because of this complication this aspect of work was abandoned.

#### Reactions with Amines:

The complex  $\text{Cu}(\text{sal} = \text{glyOEt})_2$  was found to react readily with primary aliphatic amines to give co-ordinated glycine amides.



The extent of this reaction was examined using a variety of complexes and amines. All reactions were performed at room temperature, using the amine as the solvent.

#### (i) Glycine esters:

(a) primary aliphatic amines: For the Cu and Ni complexes of glycine ethyl ester reaction with allylamine and n-butylamine gave good yields of the glycine amides. As shown in Table 17 the reactions with the Cu complex were rapid and complete in less than one hour, whereas the Ni complex reacted much more slowly, although the final yield was approximately the same (80-85%).

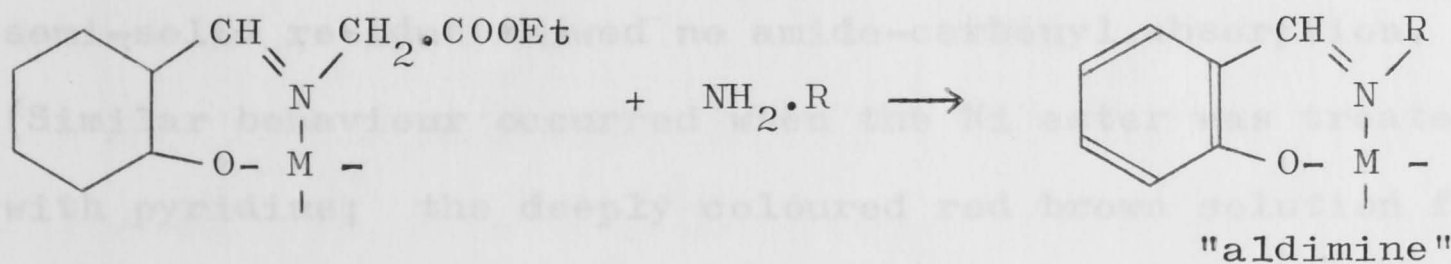
TABLE 17

Reaction of  $M(\text{sal} = \text{glyOEt})_2$  with  $R.NH_2$  to give  $M(\text{sal} = \text{glyNH.R})_2$

M	R	Reaction Time (hrs)	Yield (%)
Cu	allyl	1	85
		12	83
Ni	n-butyl	1	86
		14	77
	n-butyl	2	43
		$5\frac{1}{2}$	57
		14	81

The Cu complex of glycine benzyl ester,  $Cu(\text{sal} = \text{gly O Benzyl})_2$ , when reacted with n-butylamine, also gave a high yield of the amide after one hour (87%).

The other product from each reaction was the co-ordinated Schiff base of the amine,  $Cu(\text{sal} = N.R)_2$  (the "aldimine").



All aldimines were very soluble in benzene, which enabled their almost quantitative separation from the benzene insoluble amides. The course and extent of these reactions were confirmed by IR spectra. The ester, amide and aldimine absorbed strongly at  $1610-1630 \text{ cm}^{-1}$  ( $\text{>C=N<}$ ); in addition the ester absorbed at  $\sim 1730 \text{ cm}^{-1}$  ( $\begin{array}{c} \text{-C-OR} \\ || \\ \text{O} \end{array}$ ) and the amide absorbed at

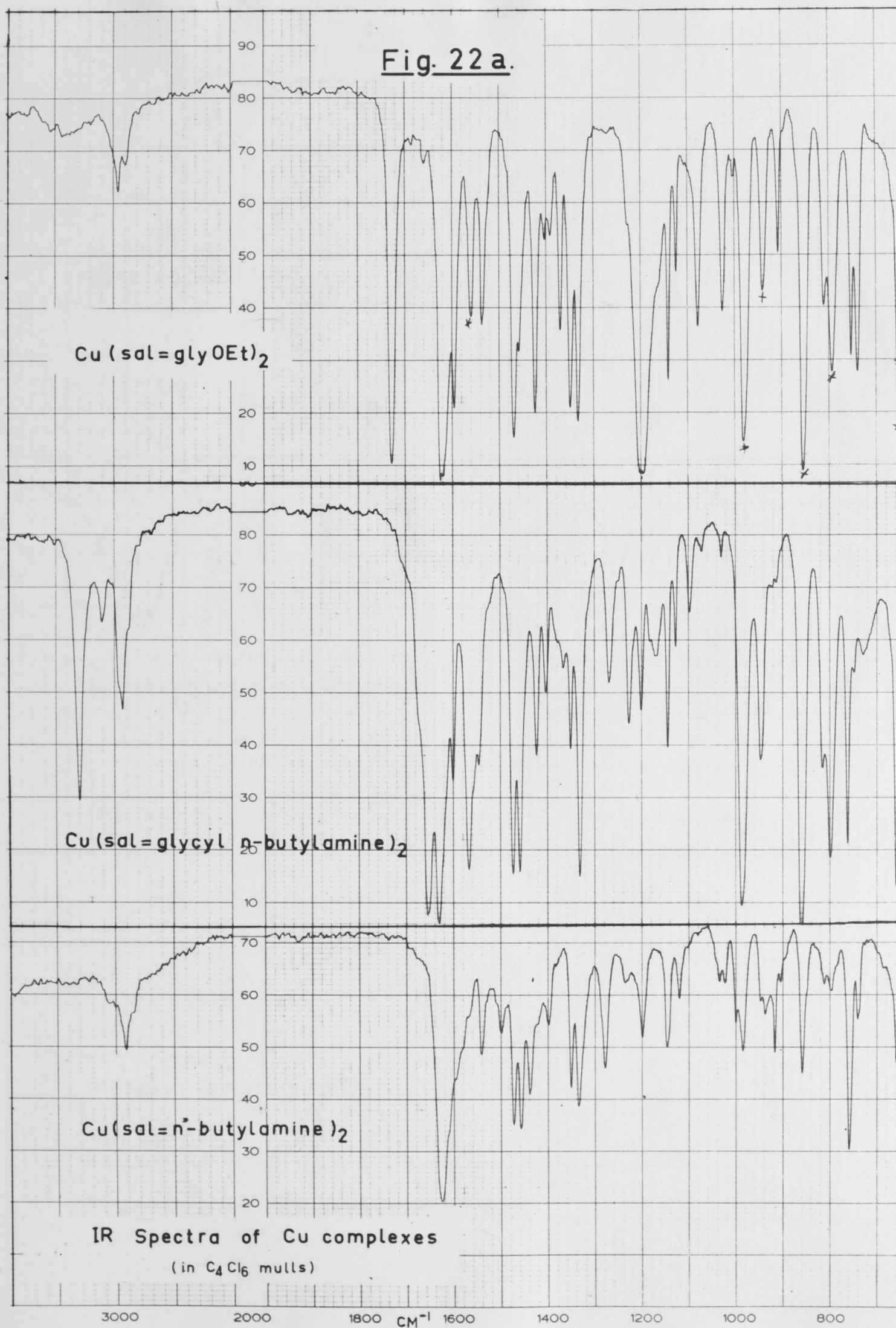
1650-1660  $\text{cm}^{-1}$  ( $\begin{array}{c} \text{-C-NH-} \\ \parallel \\ \text{O} \end{array}$ ), whereas the aldimine had no absorption in the range 1630-1800  $\text{cm}^{-1}$  (Fig.22). (The Cu-amides also gave a sharp absorption at  $\sim 3300 \text{ cm}^{-1}$  (-N-H), but the Ni amides showed only a very weak absorption in that region.)

In addition to analytical confirmation of the nature of the products, the Cu amides were decomposed and glycyll allylamine and glycyll n-butylamine were isolated as their picrates.

(b) secondary amines: It was thought that the reaction of a complex ester with a secondary amine might give more than 80% yield of amide, since the by-product obtained with a primary amine - the aldimine - could not be formed with a secondary amine. Reactions with the secondary amines diethylamine and piperidine were studied, but neither the Cu nor the Ni ester reacted with diethylamine. The Ni ester dissolved slowly in piperidine giving a red brown solution, and after evaporation of the piperidine an IR spectrum of the semi-solid residue showed no amide-carbonyl absorption. (Similar behaviour occurred when the Ni ester was treated with pyridine; the deeply coloured red brown solution formed much more quickly however, and solution was complete in two hours. Pfeiffer reported that a solution of the Ni ester in pyridine changed colour from green to orange after a few minutes.)

The Cu ester reacted with piperidine to form the amide but the yield was not high. The benzene soluble portion of the reaction mixture, a green powder, was not identified.

Fig. 22 a.



**Fig. 22b**

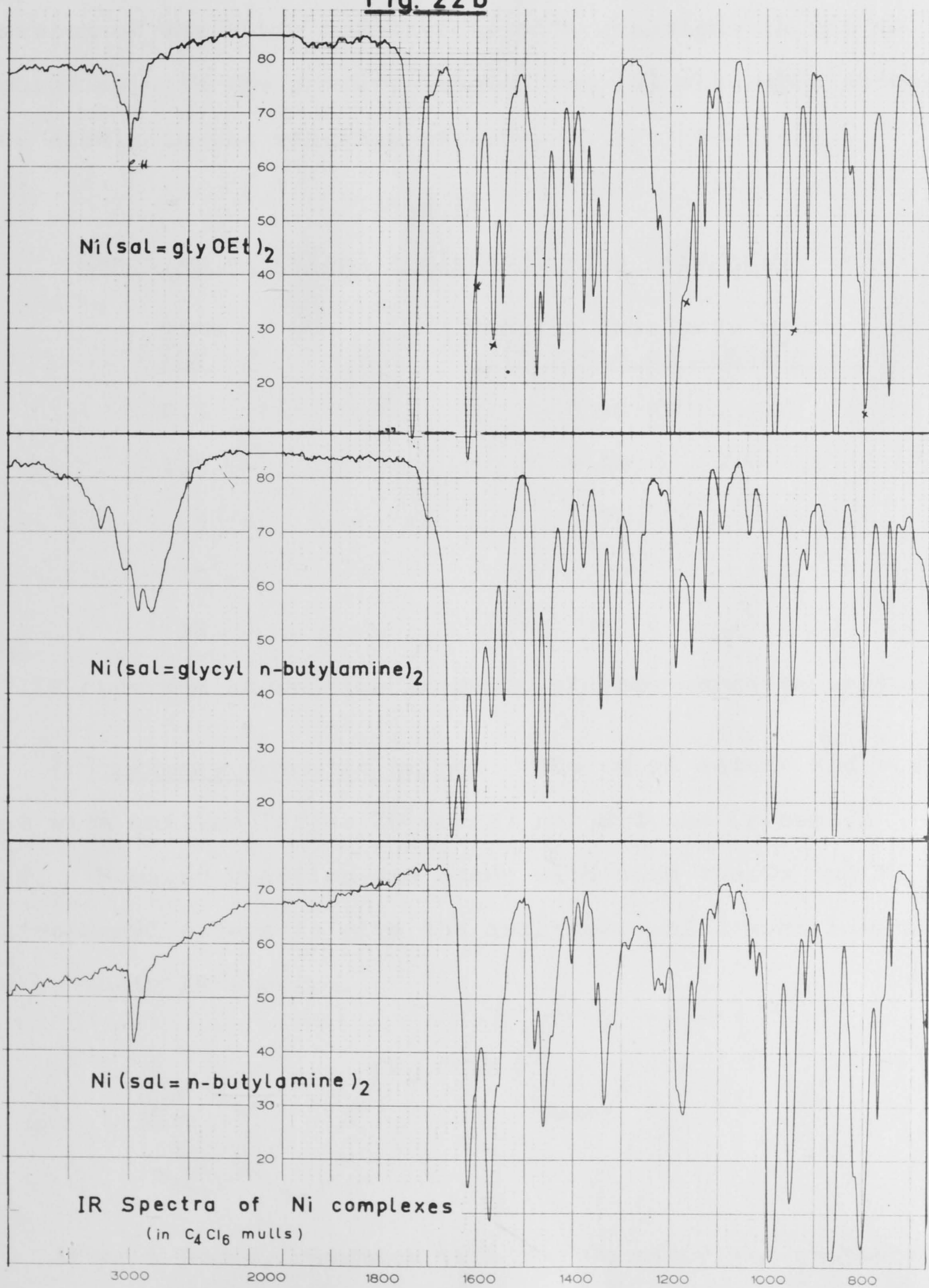


Table 18 shows the variations in yield of the Cu amide for different runs. From these figures it appears that (a) the formation of the amide relative to side reactions is not as favoured as with the primary amines, and (b) the amide decomposed slowly in the reaction mixture.

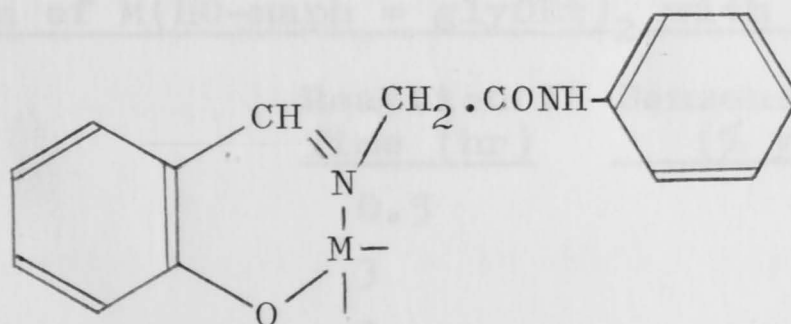
TABLE 18

Reaction of  $\text{Cu}(\text{sal} = \text{glyOEt})_2$  with piperidine

<u>Time (hr)</u>	<u>Benzene Insoluble product (% yield as amide)</u>
0.5	* 46
1.5	* 50
5	34
17	27
18	22

\* IR spectrum showed the presence of some unreacted ester.

(c) primary aromatic amines: The ethyl esters did not react with aniline (after 20 hours) nor did the Cu-benzyl ester. However reaction did occur with both the Cu and Ni *p*-nitrophenyl esters to give the anilides, which were identified by their IR spectra.



After 6 hours, reaction with the Cu ester was complete (no ester-carbonyl at  $1760 \text{ cm}^{-1}$ ) and the Ni ester appeared to react at about the same rate.



Complexes derived from 2-hydroxy 1-naphthaldehyde:

Sheehan and Grenda<sup>(91)</sup> used 2-hydroxy 1-naphthaldehyde for the protection of an amino group during a peptide synthesis. They prepared the Schiff base of this aldehyde with the free amino acid, L-valine, and isolated it as a solid compound. This was then coupled with glycine ethyl ester or L-phenylalanine methyl ester by the carbodiimide method. The aldehyde protecting group was then easily removed by mild acidification.

The stability of the Schiff bases of 2-hydroxy 1-naphthaldehyde with the amino acids as observed by Sheehan and Grenda suggested that their complexes might be suitable for use in amidation reactions.

Cu and Ni (HO-naph = glyOEt)<sub>2</sub>: The Cu and Ni complexes of the Schiff base with glycine ethyl ester were readily prepared, but because of their poor solubility in organic solvents they were not purified by recrystallization.

Reaction with n-butylamine: Both esters reacted with n-butylamine and the yields of amide are given in Table 19.

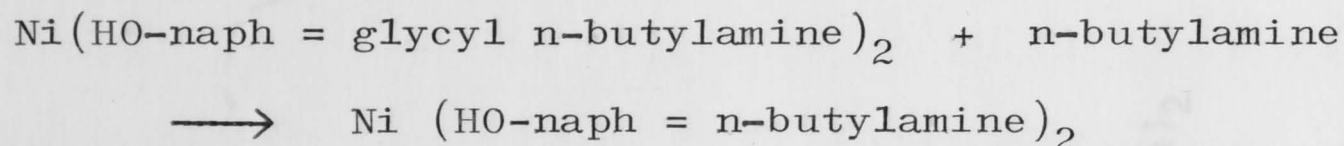
TABLE 19

Reaction of M(HO-naph = glyOEt)<sub>2</sub> with n-Butylamine

M	Reaction Time (hr)	Benzene Insoluble product (% yield as amide)
Cu	0.5	37
	3	38
Ni	2	13
	16	31
	17	14
	18	32
	24	32
	170	41

The major product at all times was the aldimine,  $M(\text{HO-naph} = n\text{-butylamine})_2$ . As was done for the salicylaldehyde system, the esters, amides and aldimines were identified from IR spectra. A point of difference here was that at  $3300\text{ cm}^{-1}$  ( $-\text{NH}$  stretching) the amide  $\text{Ni}(\text{HO-naph} = \text{glycyl } n\text{-butylamine})_2$  absorbed as strongly as the Cu amide (Fig.23).

The amides could not be recrystallized because of their insolubility in organic solvents. An attempt was made to recrystallize the Ni amide from  $n$ -butylamine in which it was readily soluble. The complex was dissolved at room temperature and ethanol was added immediately to the solution. After several minutes well formed green crystals appeared, but these were found to be the aldimine.



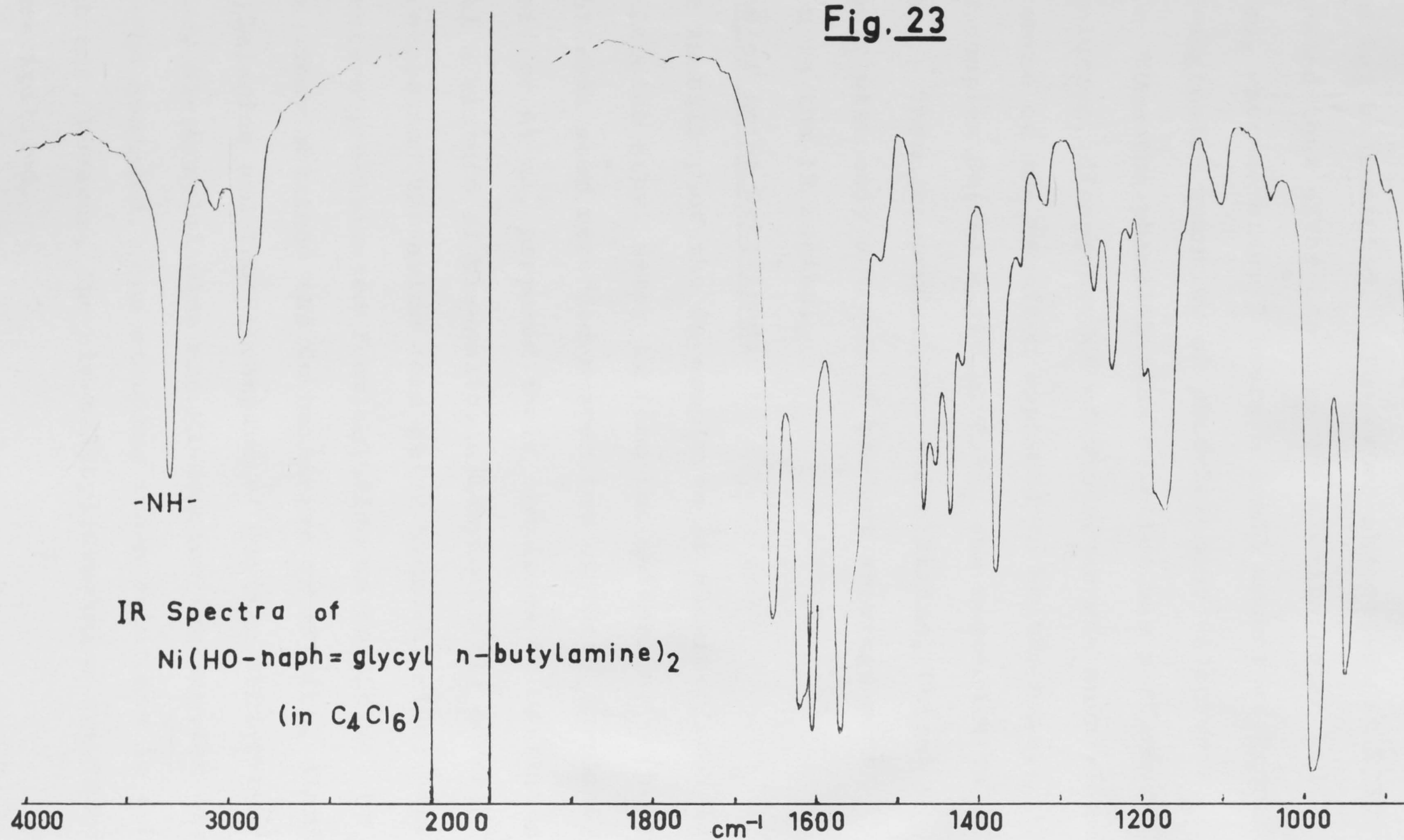
This rapid decomposition in the presence of the amine would account for the variable yields of amide obtained from the reaction (Table 19). For example, decomposition would be affected by the temperature at which the excess amine was evaporated.

The esters did not react with aniline.

(ii) Esters of alanine, phenylalanine, leucine and glycyl glycine:

The complexes  $\text{Cu}(\text{sal} = \text{AA OEt})_2$ , where AA = ala, phe, and leu, reacted with  $n$ -butylamine, but no amides were isolated from the reaction mixtures. In each case the product was a green sticky oil, very soluble in benzene and, although these

Fig. 23



IR Spectra of  
 $\text{Ni}(\text{HO-naph-glycyl-n-butylamine})_2$   
(in  $\text{C}_4\text{Cl}_6$ )

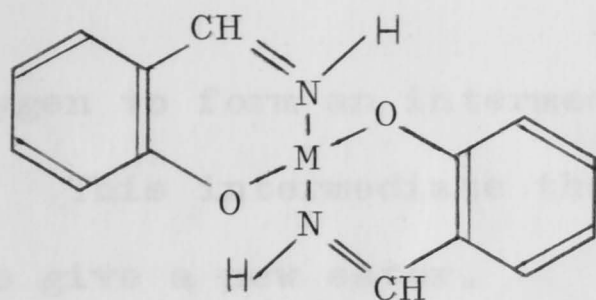
oils showed some IR absorption at 1660-1670 and 3300  $\text{cm}^{-1}$ , they could not be purified. The ester absorption at 1730  $\text{cm}^{-1}$  indicated that after one hour the alanine ester had disappeared, whereas after 3 hours a small amount of leucine ester and a greater quantity of phenylalanine ester were observed. When the phenylalanine reaction was performed in benzene solution, disappearance of the ester was much slower but the course of the reaction appeared to be the same.

The complex  $\text{Cu}(\text{sal} = \text{gly glyOEt})_2$  was reacted with allylamine. The major product was the aldimine,  $\text{Cu}(\text{sal} = \text{allylamine})_2$  with only a trace of product showing amide absorption in the IR region.

#### Discussion of Amine Reactions:

The inability of the Cu complexes of alanine, phenylalanine and leucine ethyl ester to form the amides is, perhaps, consistent with some recorded properties of these compounds.

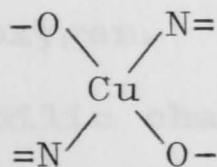
Pfeiffer et al. prepared their complexes by refluxing in ethanol a mixture of bis-salicylaldehyde Cu(II) or Ni(II), sodium acetate and the amino acid ester hydrochloride; the complex esters precipitated from solution on cooling. By this means they obtained the Cu complexes of glycine, alanine and phenylalanine and the Ni complex of glycine, but from Cu-leucine, Ni-phenylalanine and Ni-leucine the complex esters could not be isolated. On standing these solutions in contact with air, however, the bis-salicylideneimino complexes slowly precipitated.



In the present work the Schiff base was formed by the addition of one equivalent of NaOH to a solution of salicylaldehyde and the ester hydrochloride in ethanol. To this solution was then added an aqueous solution of the metal acetate and sodium acetate. The complex esters formed immediately at room temperature and precipitated out. The complexes obtained by Pfeiffer et al. were prepared in this way; in addition the Cu complex with leucine was isolated by this method and characterised by its I.R. absorption at  $1730\text{ cm}^{-1}$ , but it decomposed during the attempted recrystallization from ethanol.

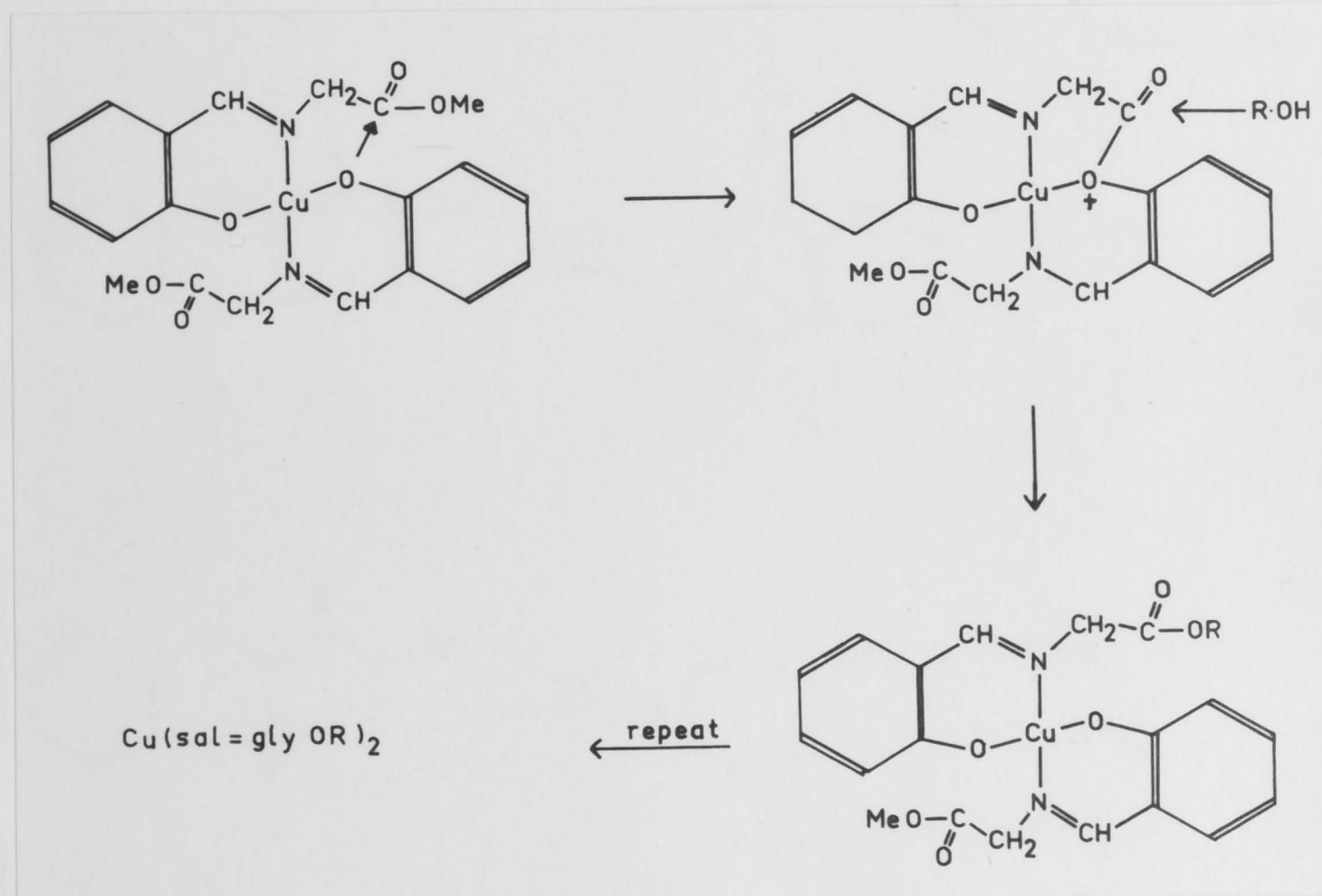
Preparation of the Ni complexes of alanine, phenylalanine and leucine was not achieved. The green solutions slowly deposited orange red bis-salicylideneimino Ni(II).

It was discovered subsequently that Verter and Frost<sup>(92)</sup> had prepared one of the complex amides,  $\text{Cu}(\text{sal} = \text{glycyl } n\text{-butylamine})_2$  from the ester,  $\text{Cu}(\text{sal} = \text{glyOMe})_2$ , in addition to studying ester exchange reactions. Their proposed mechanism for ester exchange required that the complex existed in the trans-configuration,



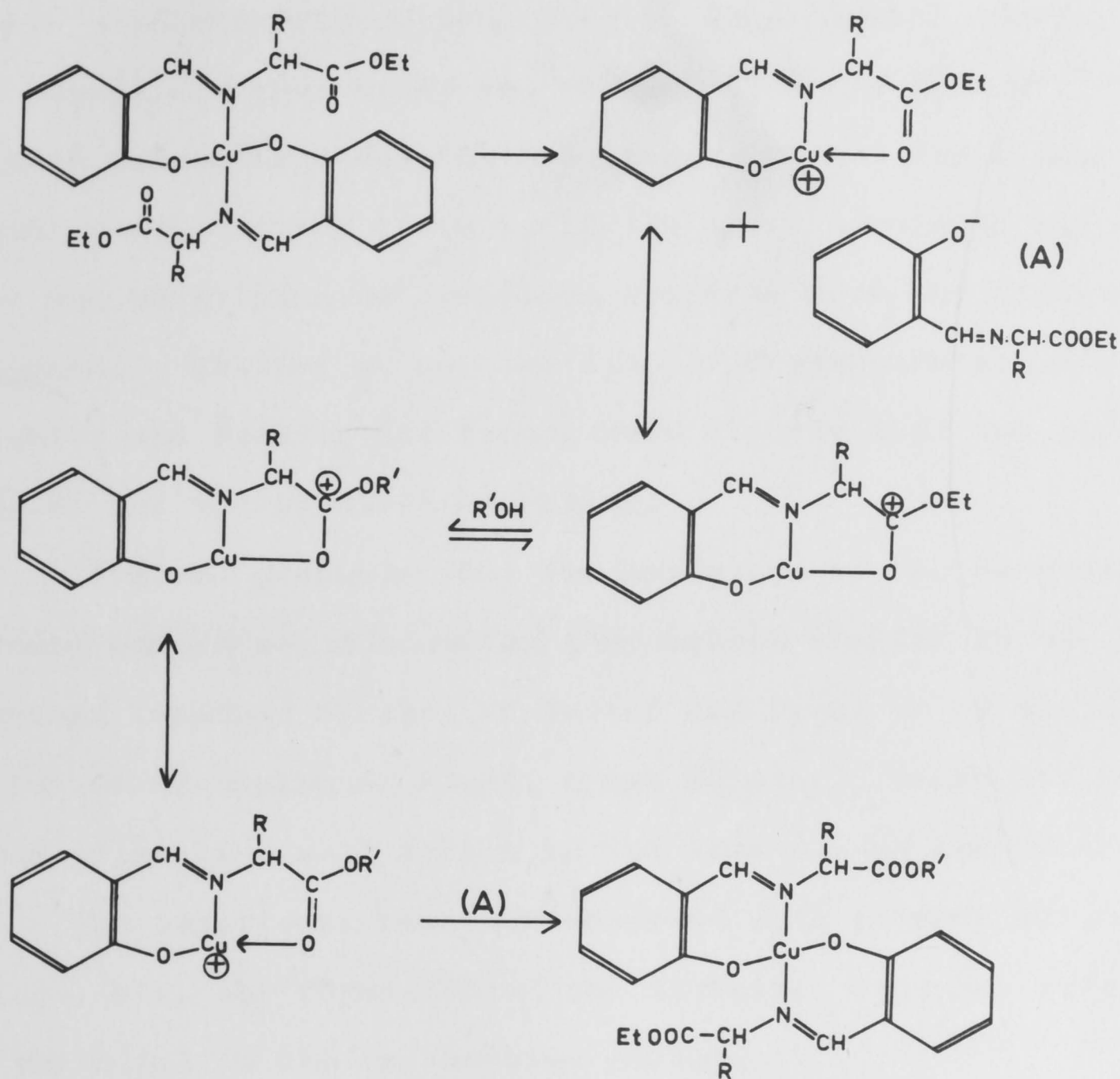
and that the carbonyl carbon was attacked by the adjacent

phenolic oxygen to form an intermediate lactone type structure. This intermediate then reacted with the alcohol in excess to give a new ester.

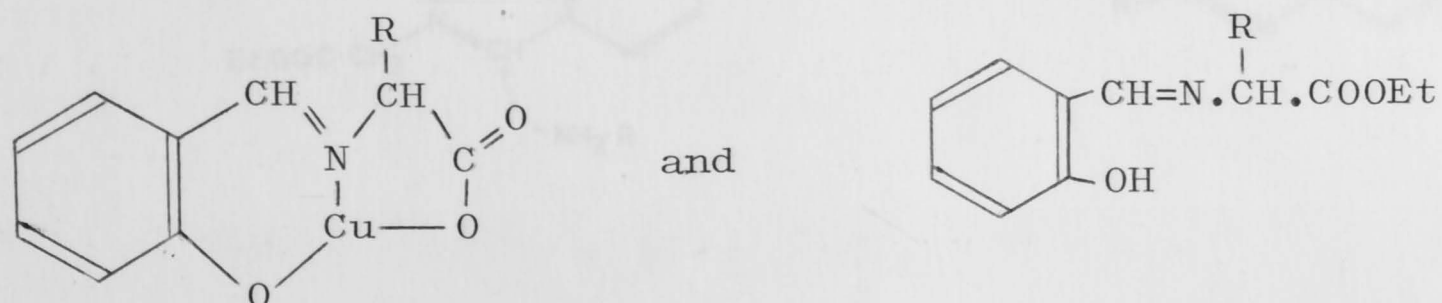


A mechanism for the amidation was not mentioned.

More recently, Houghton and Pointer (1964)<sup>(93)</sup> re-examined the ester exchange of some Cu-Schiff base complexes. They suggested that exchange occurred through the dissociation of one ligand, following which the ester group of the other ligand co-ordinated at a third position through the carbonyl oxygen. The co-ordination of this group enhanced the electrophilic character of the acyl carbon, thus facilitating removal and exchange of the alkoxy group.



Houghton and Pointer also observed that in hot alcohol solutions, the complexes of alanine and phenylalanine decomposed more readily than the glycine complex, forming the decomposition products:

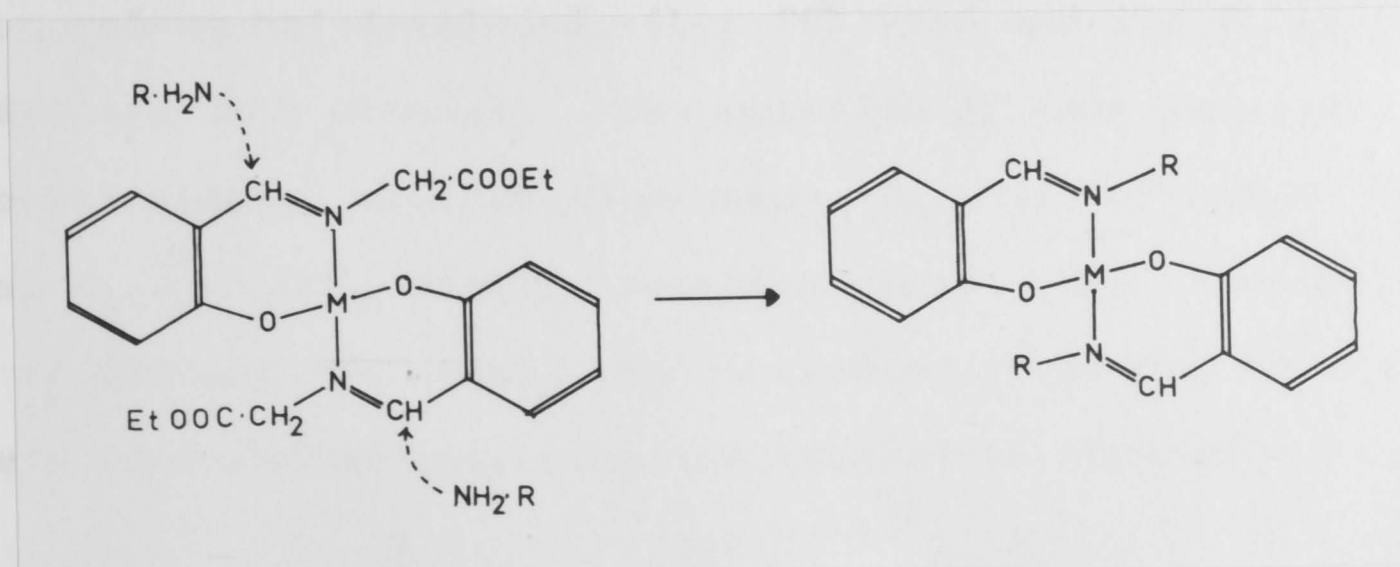


They suggested that this relative instability was probably due to steric effects, claiming "molecular models

indicate that these compounds are unable to assume the usual square planar configuration owing to considerable overlap of the phenolic oxygen atoms and the  $-\text{CH}(\text{R})\cdot\text{COOEt}$  groups". The Leybold molecular models however, indicate that for a square planar configuration there is little extra strain in the alanine and phenylalanine complexes compared with the glycine compound. Whether or not the steric effects are significant, Houghton and Pointer did demonstrate clearly that the glycine complex has the greatest stability.

It seems probable that the amidation of the co-ordinated glycine esters would occur by a mechanism similar to the ester exchange (whether by that of Verter and Frost or by the dissociation mechanism of Houghton and Pointer), since the amine would attack the acyl carbon in the same way as the alcohol.

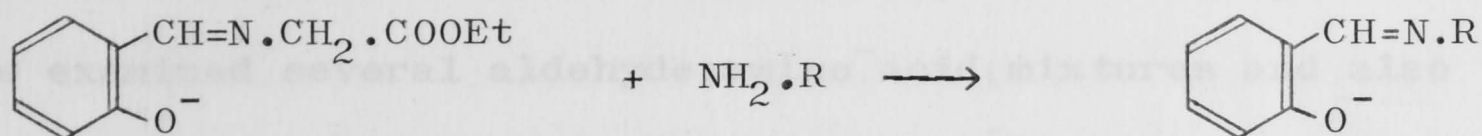
The additional reaction observed with primary aliphatic amines, viz. the formation of the aldimine, requires attack by the amine at the azo-methine carbon.



If the amidation did occur by the dissociation mechanism the amine could also attack the free ligand and replace the



amino acid ester, thus leading to the co-ordinated aldimine.

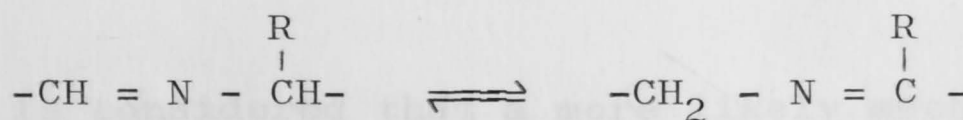


However, in the case of the complex,  $\text{Ni}(\text{sal} = \text{glyOEt})_2$  which required long reaction times ( $\sim 14$  hrs) to form the maximum quantities of the amides, a dissociation mechanism might be expected to produce very much greater quantities of aldimine than were actually found ( $\sim 20\%$ ).

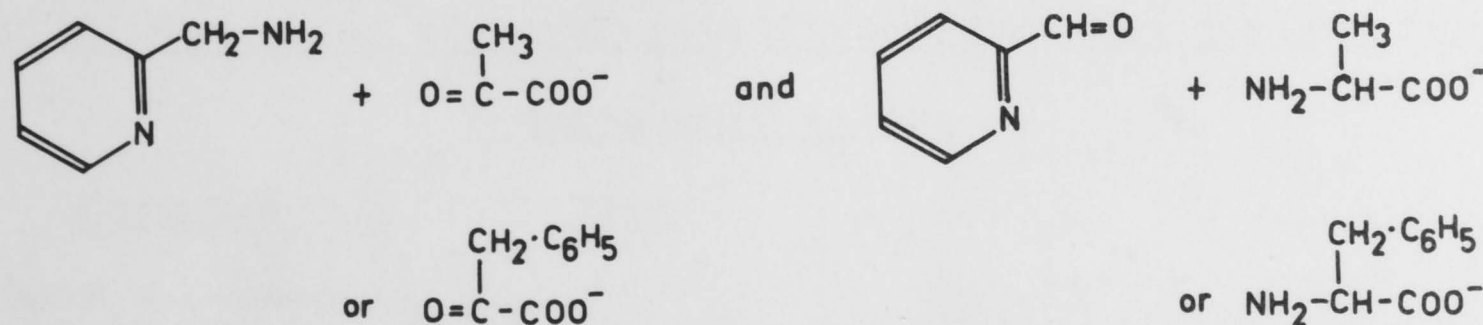
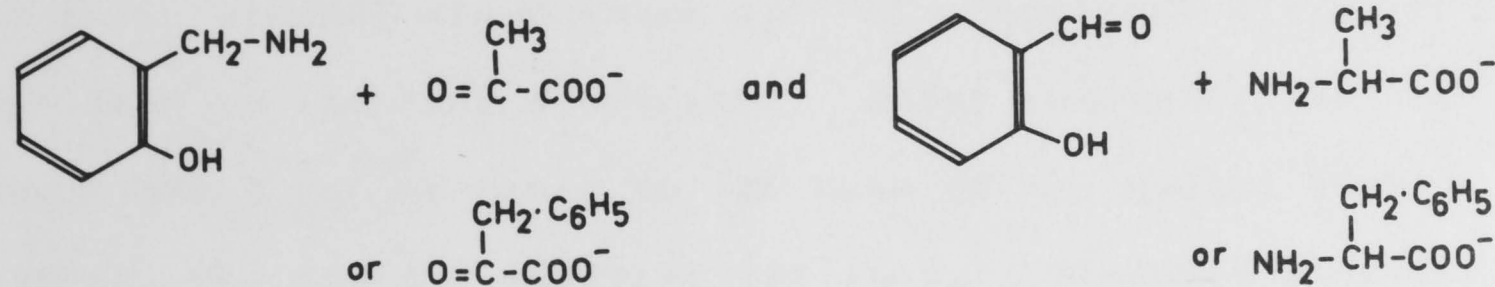
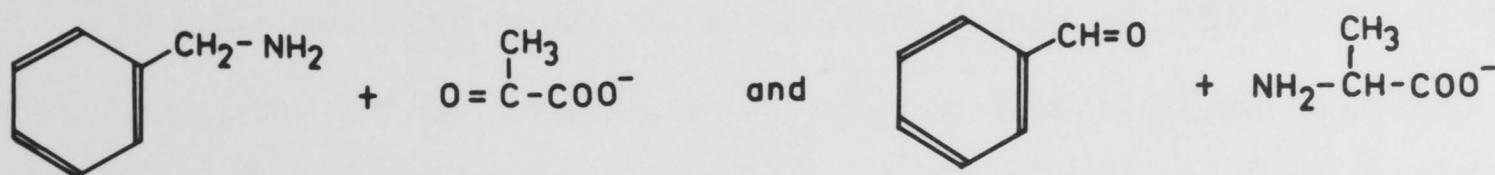
### SECTION III:

#### Complexes of Schiff bases derived from L-amino acids:

Pfeiffer et al. used the L-amino acid ester hydrochlorides in the preparation of the complexes  $\text{Cu}(\text{sal} = \text{alaOEt})_2$  and  $\text{Cu}(\text{sal} = \text{pheOEt})_2$ , but these products showed no measurable optical activity. When the latter compound was decomposed, only D,L-phenylalanine was recovered. In another experiment a mixture of salicylaldehyde, L-phenylalanine ethyl ester hydrochloride and sodium acetate in ethanol was heated until a yellow colour had developed, then filtered and the filtrate was diluted with ethanol. The rotation of this solution decreased slowly, e.g. at 15 minutes,  $\alpha_D = -2.46^\circ$  and at 40 hours,  $\alpha_D = -1.00^\circ$ , finally reaching zero. The racemization, with or without the metal, was considered to be due to a tautomeric equilibrium involving the asymmetric carbon:

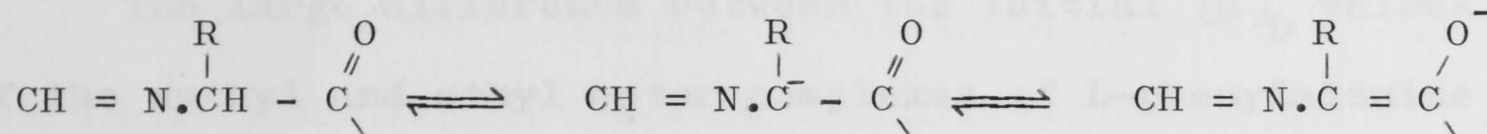


From our observations on these types of systems the transamination mechanism seems unlikely at room temperature. We examined several aldehyde amino acid mixtures and also the corresponding keto acid amine pairs in attempts to achieve the transamination from either direction, catalysed by metal ions. In particular the following pairs of compounds were examined in the presence of  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  ions.



At room temperature no evidence of transamination was obtained in several hours from the visible spectra of the aqueous solution.

It is considered that a more likely mechanism for racemisation in these complexes arises from a tautomeric shift involving the carbonyl oxygen, i.e.



In contrast to Pfeiffer's observations, Sheehan and Grenda (p.112) found that no racemization accompanied the formation of the Schiff base of L-valine, and the resolution of phenylalanine (Chapter I, Section III) was achieved via its Schiff base derivative with salicylaldehyde.

In the present work Cu complexes were prepared from the hydrochlorides of L-alanine ethyl ester and L-phenylalanine methyl and ethyl esters by the method used for the racemic amino acid esters. It was found that solutions of these complexes in ethanol did possess optical activities although they were lost on standing overnight. After recrystallization from ethanol (or methanol in the case of the methyl ester) however, the optical activity was lost. Observed rotations are recorded in Table 20.

TABLE 20

Optical Rotations of unrecrystallized complexes derived from

<u>L-amino acid esters</u>			
<u>Compound</u>	<u>Time</u>	<u><math>\alpha_D</math></u>	<u><math>[\alpha]_D</math></u>
Cu(sal = L-pheOMe) <sub>2</sub> .024% in EtOH	0	-0.25°	-1040°
	15 m.	-0.20°	
	32 m.	-0.14°	
	92 m.	-0.04°	
	17 hr.	zero	
Cu(sal = L-pheOEt) <sub>2</sub> .032% in EtOH	0	-0.08°	-250°
	17 hr.	zero	
Cu(sal = L-alaOEt) <sub>2</sub> .035% in EtOH	0	-0.04°	~ -110°
	17 hr.	zero	

The large difference between the initial  $[\alpha]_D$  values of the methyl and ethyl ester complexes of L-phenylalanine suggests that considerable racemization had occurred during the preparation of the ethyl ester complex. The rate at which the methyl ester complex lost its activity makes it seem likely that there was some racemization during its preparation also. The complete loss of activity which occurred during recrystallization from hot alcoholic solutions would account for the racemic products obtained by Pfeiffer et al. since their compounds were prepared by refluxing in ethanol.

The tridentate mono-complexes of salicylidene L-phenylalanine with Co(II), Ni(II) and Cu(II) were prepared by combining the sodium salt of the Schiff base with the metal acetate in a 1:1 molar ratio. The water insoluble products were hydrated - the Co and Cu salts contained  $2H_2O$  and the Ni salt contained  $3H_2O$ . The molecular weights of these compounds were determined in methanol solution and the results, shown below, clearly indicate that the complexes were mononuclear.

<u>Compound</u>	<u>Molecular Weight</u>	
	<u>found</u>	<u>calc.</u>
$[Co(sa1 = L-phe)2H_2O]$	371	362
$[Ni(sa1 = L-phe)3H_2O]$	376	380
$[Cu(sa1 = L-phe)2H_2O]$	388	367

Solutions of these complexes in methanol were optically active and they showed no decrease in rotation after several days. Values of  $[\alpha]_D$  are recorded in Table 21.

TABLE 21

Optical Rotations of the complexes M(sal = L-phe).xH<sub>2</sub>O

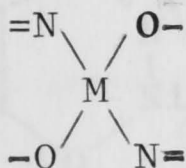
Solutions in Methanol, T = 20°

M	Co	Ni	Cu
conc. (%)	0.102	0.32	0.043
[ $\alpha$ ] <sub>D</sub>	-423°	-163°	
[ $\alpha$ ] <sub>578</sub>	-473°	-188°	
[ $\alpha$ ] <sub>546</sub>	-522°	-297°	~ -140°

The optical activity and the optical stability of the above complexes show that the co-ordination of a Schiff base derived from an optically active amino acid does not necessarily cause racemization.

SECTION IVStereochemistry of Schiff base complexes:

The complex esters M(sal = glyOEt)<sub>2</sub>, M = Cu or Ni, have been regarded as square planar trans complexes<sup>(92,93)</sup>,

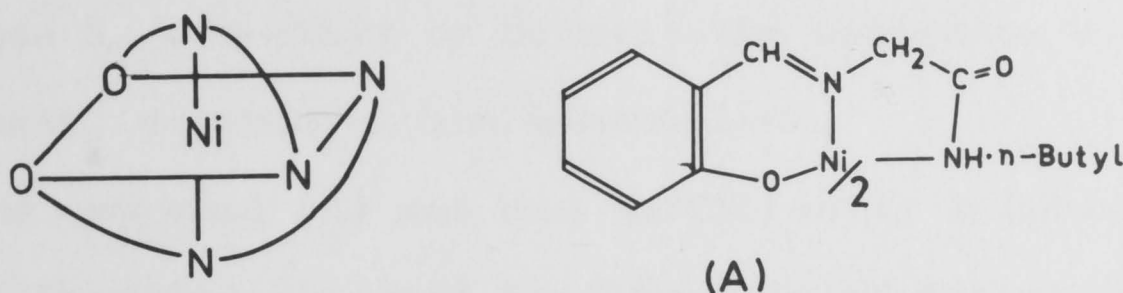


and it was assumed here that the complex amides would be the same.

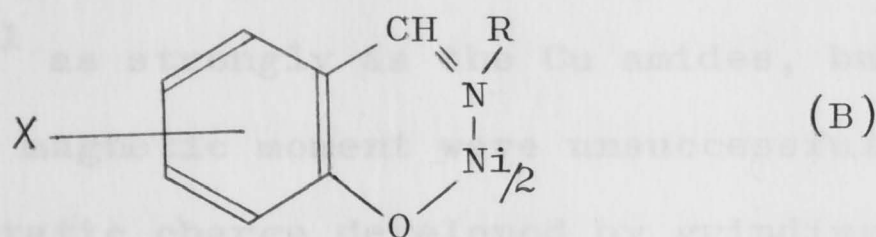
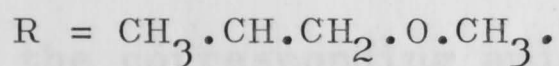
The Cu allyl and n-butyl amides were dull olive-green in colour similar to the Cu ester, but the Ni amides were bright light green, very different from the olive green of the Ni ester. The magnetic moments of the Ni ester and the n-butyl amide were measured, and whereas the ester was diamagnetic as expected for a square planar four co-ordinate configuration,

the amide was paramagnetic ( $\mu = 2.91$  B.M.) indicating an octahedral six co-ordinate structure.

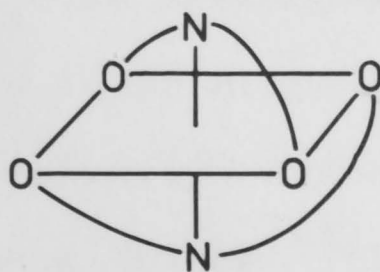
Leybold molecular models indicated that each amide ligand could function as a linear tridentate, with the amide nitrogen providing the extra bonding site, as shown below.



Holm and co-workers<sup>(94)</sup> have recently prepared Ni(II) Schiff base complexes of the general formula (B), where the side chain R contained a potential donor atom, e.g.



They found that with variation of R and X the structure varied from octahedral to planar or tetrahedral. The octahedral compounds were mononuclear and possessed magnetic moments,  $\mu \sim 3.2$  B.M. and were depicted as shown:



Spectral properties, both of the solid and its solutions, were also used in the assignment of structures.

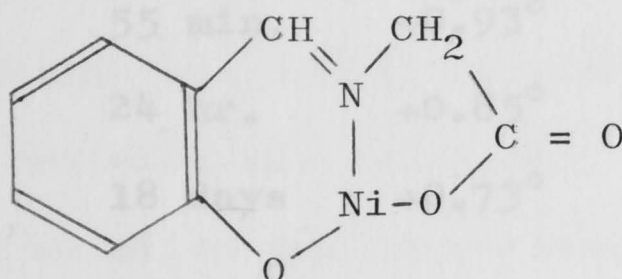
More recently again, Sacconi et al.<sup>(95)</sup> have reported a similar study of the complexes (B),  $R = -CH_2 \cdot CH_2 N \begin{matrix} R_1 \\ R_2 \end{matrix}$  and showed that with suitable combinations of  $R_1$ ,  $R_2$  and X (e.g.  $R_1 = H$  and  $R_2 = n\text{-alkyl}$  or benzyl) the compounds were six co-ordinate, octahedral and mononuclear.

The compound (A) was not sufficiently soluble in "inert" solvents to allow spectral confirmation of the proposed six co-ordinate structure. It is conceivable that the very marked weakness of its -NH stretching absorption at  $3300 \text{ cm}^{-1}$  is due to co-ordination of this nitrogen, although such an effect would not normally be expected. It was noted that the corresponding amide derived from 2-hydroxy 1-naphthaldehyde, i.e.  $Ni(\text{HO-naph} = \text{glycyl } n\text{-butylamine})_2$ , absorbed at  $3300 \text{ cm}^{-1}$  as strongly as the Cu amides, but attempts to determine its magnetic moment were unsuccessful because of extreme electrostatic charge developed by grinding.

Thus, although it is considered very probable that the Schiff base amides in complexes  $Ni(\text{sal} = \text{glycyl allylamine})_2$  and  $Ni(\text{sal} = \text{glycyl } n\text{-butylamine})_2$  are co-ordinated as tridentates, the evidence available is not conclusive. The magnetic moment indicates octahedral co-ordination in the solid state, but this could arise from polymer formation as has been shown for bis(acetylacetonato)nickel(II), which exists as a crystalline trimer<sup>(96)</sup>.

Ionic Complexes of Schiff bases:

Nickel (II): The Schiff base, salicylidene glycinate, is known to co-ordinate to metals as a tridentate ligand forming mono-complexes, e.g.  $\text{Ni}(\text{sal} = \text{gly})$ .<sup>(97)</sup>



An examination of Leybold molecular models indicated that the existence of a bis-complex, having the same configuration as the amide complex (A), should be sterically possible. The bis-complex of Ni(II) would be a doubly charged anion  $[\text{Ni}(\text{sal} = \text{gly})_2]^{2-}$ . This complex anion was readily formed in aqueous solution at room temperature by the mixing of salicylaldehyde and glycine (each 2 moles), nickel acetate, (1 mole) and sodium hydroxide (4 moles), and was isolated as its calcium or barium salt. The calcium salt  $\text{Ca}[\text{Ni}(\text{sal} = \text{gly})_2] \cdot 4\text{H}_2\text{O}$  was recrystallized from aqueous ethanol, but the barium salt,  $\text{Ba}[\text{Ni}(\text{sal} = \text{gly})_2] \cdot 4\text{H}_2\text{O}$  was only slightly soluble in water and could not be recrystallized. The magnetic moment of the barium salt was 3.0 B.M.

The analagous compound derived from D,L-alanine was prepared similarly, and isolated as its barium salt,  $\text{Ba}[\text{Ni}(\text{sal} = \text{ala})_2] \cdot 4\text{H}_2\text{O}$  which was recrystallized from aqueous ethanol. When L-alanine was used in the preparation, the complex was optically active. The aqueous solution however, lost some activity but did not racemize completely. For a 0.39%



solution in water, the rotations observed were:

<u>time</u>	$\alpha_D^{20}$	$[\alpha]_D^{20}$
0	+1.00°	+257°
25 min.	+0.97°	
55 min.	+0.93°	
24 hr.	+0.85°	
18 days	+0.73°	

Iron(II): A complex anion containing iron(II) was also prepared from D,L-alanine and was isolated as its sodium salt,  $\text{Na}_2[\text{Fe}(\text{sal} = \text{ala})_2] \cdot 2\text{H}_2\text{O}$ , which was recrystallized from aqueous acetone as red-brown crystals.

Cobalt(III): Solutions of the cobalt(III) complex anions,  $[\text{Co}(\text{sal} = \text{gly})_2]^-$  and  $[\text{Co}(\text{sal} = \text{ala})_2]^-$  were prepared by the formation of the cobalt(II) complex, which was then oxidised by hydrogen peroxide. The barium salt of the glycine complex was isolated and recrystallised from water as large shining black crystals  $\text{Ba}[\text{Co}(\text{sal} = \text{gly})_2]_2 \cdot 10\text{H}_2\text{O}$ . The compound was diamagnetic. When dried at  $110^\circ$ , analysis indicated the loss of 8 molecules of water, but on subsequent exposure to air at room temperature, 5 molecules of water were regained.

The complex anion was partially resolved using the optically active cation,  $d\text{-}[\text{Co en}_2 \text{ ox}]^+$ . Two fractions of diastereoisomer, ( $D_I$ ,  $D_{II}$ ) and the remaining mother liquor ( $F_{II}$ ) were treated with NaI to remove the resolving agent. The filtrates from  $D_I$  and  $D_{II}$  showed dextro rotations and the filtrate from  $F_{II}$  was levo rotatory, but the optically active anions could not be precipitated as their barium salts.

The alanine complex ion,  $[\text{Co}(\text{sal} = \text{ala})_2]^-$  could not be isolated as its sodium, potassium, calcium or barium salts, but a partial resolution was achieved with the cation,  $d\text{-}[\text{Co en}_2 \text{ ox}]^+$ . The first small fraction of diastereoisomer was khaki in colour, only slightly soluble in water and its aqueous solution was dextro rotatory,  $[\alpha]_D^{20} = +3400^\circ$ , whereas the second fraction was red-brown, very soluble in water and its aqueous solution was levo-rotatory,  $[\alpha]_D^{20} = -750^\circ$ .

Although the complex ions,  $[\text{Ni}(\text{sal} = \text{gly})_2]^{2-}$  and  $[\text{Ni}(\text{sal} = \text{ala})_2]^{2-}$  were isolated and the salt  $\text{Ba}[\text{Ni}(\text{sal} = \text{gly})_2] \cdot 4\text{H}_2\text{O}$  was found to be paramagnetic, there is no evidence that the Schiff bases were co-ordinated as tridentate ligands, particularly in aqueous solution. The partial resolution of the anions  $[\text{Co}(\text{sal} = \text{gly})_2]^-$  and  $[\text{Co}(\text{sal} = \text{ala})_2]^-$  however, gave positive confirmation that it is sterically possible to co-ordinate two of these Schiff bases around a metal ion, each being co-ordinated at three positions.

EXPERIMENTALDeuteration of co-ordinated glycine:

The deuteration of glycine in the complex  $[\text{Co en}_2 \text{ gly}] \text{Cl}_2$  was followed from changes in the NMR spectra recorded on a PERKIN-ELMER R.10 Spectrometer at  $33.3^\circ$ .

Spectra were obtained for solutions in

- (i) 1M  $\text{D}_2\text{SO}_4$  in  $\text{D}_2\text{O}$ .
- (ii)  $\text{D}_2\text{O}$  and
- (iii)  $\text{D}_2\text{O}$  containing 0.1%  $\text{Na}_2\text{CO}_3$ .

Preparation of methyl and ethyl ester hydrochlorides:

The ethyl esters of glycine, glycylic acid, D,L-alanine, L-alanine, D,L-phenylalanine and L-phenylalanine and the methyl ester of L-phenylalanine were prepared and isolated as their hydrochlorides, by a method similar to that reported for the preparation of methyl esters. (98,99)

Thionyl chloride (6 ml) was added slowly from a dropping funnel to the anhydrous alcohol (50 ml) maintained at  $-5^\circ$  in an ice-ethanol bath. The amino acid (0.10 mole) was added and the solution was heated under reflux. Boiling was continued for 10 minutes after the amino acid had dissolved. The volume of the solution was reduced on a rotary evaporator until precipitation commenced. Anhydrous ether was added to complete the precipitation. The product was filtered, washed with anhydrous ether and dried under vacuum over silica gel. Yields were 80-90%.

Preparation of Glycine benzyl ester hydrochloride:

From glycine, benzyl alcohol and p-toluene sulphonic acid, the compound glycine benzyl ester p-toluene sulphonate

was prepared using the method described by Greenstein and Winitz<sup>(100)</sup>.

The method given by these authors for conversion of this compound to the hydrochloride was found to be entirely unsatisfactory, but the following method gave up to 50% yields of the hydrochloride. To a suspension of glycine benzyl ester p-toluene sulphonate (10.1 g) in ether (50 ml) in a separatory funnel, aqueous NaOH solution (30 ml 1.0N) was added and the solutions were shaken vigorously for several minutes. The ethereal solution was dried over  $\text{Na}_2\text{SO}_4$  and then saturated with gaseous HCl. The hydrochloride, which precipitated from solution was filtered, washed with anhydrous ether and dried under vacuum over silica gel. (3.0 g).

Cu(II) and Ni(II) complexes of Schiff bases of amino acid esters:

The amino acid ester hydrochloride (0.01 mole) and the aldehyde (0.01 mole) were dissolved in ethanol (20 ml) and the Schiff base was formed after the addition of aqueous NaOH solution (10.0 ml 1.0N). To this yellow solution an aqueous solution of the metal acetate (0.005 mole) and hydrated sodium acetate (3 g) was added slowly with stirring. The green product sometimes crystallized readily from solution or sometimes separated as a gum which usually solidified after treatment with a small amount of ethanol. Yields were from 60 to 80%. The following compounds were prepared in this way.

Bis-[N-(ethoxycarbonylmethyl)salicylideneamino] copper(II),  
or  $\text{Cu}(\text{sal} = \text{glyOEt})_2$ , recrystallized from benzene.

Bis-[N-(ethoxycarbonylmethyl)salicylideneamino] nickel(II),  
or  $\text{Ni}(\text{sal} = \text{glyOEt})_2$ , recrystallized from benzene.

Bis-[N-(ethoxycarbonylmethylcarbamolymethyl)salicylidene-  
amino] copper(II) or  $\text{Cu}(\text{sal} = \text{glyglyOEt})_2$ , recrystallized  
from dimethyl formamide-water.

Found: C, 52.99; H, 5.21; N, 9.48.  $\text{C}_{26}\text{H}_{30}\text{O}_8\text{N}_4\text{Cu}$ , requires  
C, 52.91; H, 5.09; N, 9.50.

Bis-[N-(benzoxycarbonylmethyl)salicylideneamino] copper(II)  
or  $\text{Cu}(\text{sal} = \text{glyOBenzyl})_2$ , recrystallized from chloroform.

Found: C, 63.39; H, 4.92; N, 4.57.  $\text{C}_{32}\text{H}_{28}\text{O}_6\text{N}_2\text{Cu}$ , requires  
C, 64.04; H, 4.70; N, 4.67.

Bis-[N-(1-ethoxycarbonylmethyl)salicylideneamino] copper(II)  
or  $\text{Cu}(\text{sal} = \text{alaOEt})_2$ , recrystallized from ethanol.

Found: C, 56.77; H, 5.55; N, 5.51.  $\text{C}_{24}\text{H}_{28}\text{O}_6\text{N}_2\text{Cu}$ , requires  
C, 57.19; H, 5.60; N, 5.56.

Bis-[N-(1-ethoxycarbonyl 2-phenylethyl)salicylideneamino]  
copper(II), or  $\text{Cu}(\text{sal} = \text{pheOEt})_2$ , recrystallized from ethanol.

Found: C, 66.24; H, 5.52; N, 4.12.  $\text{C}_{36}\text{H}_{36}\text{O}_6\text{N}_2\text{Cu}$ , requires  
C, 66.89; H, 5.53; N, 4.27.

Bis-[N-(1-ethoxycarbonyl 3-methylbutyl)salicylideneamino]  
copper(II), or  $\text{Cu}(\text{sal} = \text{leuOEt})_2$ , decomposed on attempted  
recrystallization from ethanol.

Bis-[N-(ethoxycarbonylmethyl) 2-oxo 1-naphthylideneamino]  
copper(II) or  $\text{Cu}(\text{HO-naph} = \text{glyOEt})_2$ .

Bis-[N-(ethoxycarbonylmethyl) 2-oxo 1-naphthylideneamino]  
nickel(II) or  $\text{Ni}(\text{HO-naph} = \text{glyOEt})_2$ .

Also prepared were complexes from copper(II), salicylaldehyde and the hydrochlorides of

- (i) L-alanine ethyl ester
- (ii) L-phenylalanine ethyl ester, and
- (iii) L-phenylalanine methyl ester.

Reactions with Amines:

The complex ester (0.2-0.5 g) was stirred with the amine (~5 ml) in a stoppered flask at room temperature. Except for reactions with aniline, the reaction was terminated by the addition of benzene and evaporation on the rotary evaporator. The addition of benzene and evaporation to dryness was repeated three times to remove the excess amine. When aniline was used, the addition of anhydrous ether precipitated the complex ester and/or the anilide from solution.

Cu and Ni (sal = glyOEt)<sub>2</sub> and Cu (sal = glyOBenzyl)<sub>2</sub>:

(a) primary aliphatic amines: These complex esters were reacted with allylamine and n-butylamine. The complex aldimines were easily soluble in benzene whereas the amides were not, so that the products were effectively separated by treatment of the solid residue from the reaction mixture with benzene at room temperature.

The compounds isolated were:

Bis-[N-(allylcarbamoylemethyl)salicylideneamino] copper(II)  
or Cu(sal = glycyl allylamine)<sub>2</sub>, recrystallized from CHCl<sub>3</sub>-MeOH.

Bis-[N-(n-butylcarbamoylemethyl)salicylideneamino] copper(II)  
or Cu(sal = glycyl n-butylamine)<sub>2</sub>, recrystallized from CHCl<sub>3</sub>-MeOH.

Found: C, 59.13; H, 6.20; N, 10.21.  $C_{26}H_{34}O_4N_4Cu$ , requires C, 58.98; H, 6.42; N, 10.57.

Bis-[N-(allylcarbamoylemethyl)salicylideneamino] nickel(II)  
or Ni(sal = glycyl allylamine)<sub>2</sub>, recrystallized from MeOH.

Found: C, 58.46; H, 5.43; N, 11.34.  $C_{24}H_{26}O_4N_4Ni$ , requires C, 58.45; H, 5.31; N, 11.36.

Bis-[N-(n-butylcarbamoylemethyl)salicylideneamino] nickel(II)  
or Ni(sal = glycyl n-butylamine)<sub>2</sub>, recrystallized from MeOH.

Found: C, 59.91; H, 6.57; N, 10.63.  $C_{26}H_{34}O_4N_4Ni$ , requires C, 59.45; H, 6.52; N, 10.67.

Bis-[N-allyl salicylideneamino] copper(II)  
or Cu(sal = allylamine)<sub>2</sub>.

Bis-[N-n-butyl salicylideneamino] copper(II)  
or Cu(sal = n-butylamine)<sub>2</sub>.

Bis-[N-allyl salicylideneamino] nickel(II)  
or Ni(sal = allylamine)<sub>2</sub>.

Bis-[N-n-butyl salicylideneamino] nickel(II)  
or Ni(sal = n-butylamine)<sub>2</sub>, recrystallized from MeOH.

Found: C, 64.23; H, 6.88; N, 6.83.  $C_{22}H_{28}O_2N_2Ni$ , requires C, 64.28; H, 6.82; N, 6.82.

Glycyl allylamine: Cu(sal = glycyl allylamine)<sub>2</sub> (1.0 g) was dissolved in HCl (20 ml 1N) and extracted twice with ether to remove salicylaldehyde. The aqueous phase was saturated with H<sub>2</sub>S and the precipitated CuS was filtered off. The filtrate was treated with EtOH (10 ml) and evaporated to dryness yielding colourless crystals of glycyl allylamine hydrochloride. A saturated aqueous solution of sodium picrate was added to these crystals which dissolved readily. After several minutes

yellow crystals of the picrate were filtered off, washed with a little ice water and recrystallized from hot water. M.Pt. 135°. Harries and Peterson reported M.Pt. 136-8°. (101)

Found: C, 38.10; H, 3.89; N, 20.20.  $C_{11}H_{13}O_8N_5$ , requires C, 38.49; H, 3.82; N, 20.40.

Glycyl n-butylamine: Glycyl n-butylamine was isolated as its picrate from  $Cu(sal = glycyl\ n\text{-butylamine})_2$  by the same method as was used for glycyl allylamine. M.Pt. 140-1°.

Found: C, 40.01; H, 5.04; N, 19.44.  $C_{12}H_{17}O_8N_5$ , requires C, 40.12; H, 4.77; N, 19.50.

(b) secondary amines: The complex ethyl esters were reacted with diethylamine and piperidine. Because of only slight solubility in diethylamine, benzene was added to some reaction mixtures to increase the amount of ester in solution, but both the Cu and Ni esters were recovered unchanged.

The benzene insoluble portion of the residue from the reaction of the Cu ester with piperidine was the complex amide, Bis-[N-(piperidino carbonylmethyl)salicylidineamino] copper(II) or  $Cu(sal = glycyl\ piperidine)_2$  recrystallized from  $CHCl_3$ -MeOH. Found: C, 60.52; H, 6.24; N, 9.85.  $C_{28}H_{34}O_4N_4Cu$ , requires C, 60.69; H, 6.18; N, 10.11.

The benzene soluble portion was a dull green amorphous powder, and neither recrystallization nor chromatography yielded identifiable products.

The Ni ester dissolved slowly in piperidine to give a red brown solution. Any benzene insoluble portion of the residue was unreacted ester. The benzene soluble red brown oil was not identified.



(c) primary aromatic amines: The esters were reacted with aniline, but were recovered unchanged after 20 hours. The Cu ethyl and benzyl esters dissolved fairly easily but the Ni ethyl ester was only slightly and slowly soluble, e.g. 0.1 g Ni ester had not completely dissolved in 15 ml aniline after 24 hours.

Bis-[N-(p-nitrophenoxycarbonylmethyl)salicylideneamino] copper(II) or  $\text{Cu}(\text{sal} = \text{glyOPhenyl-p-NO}_2)_2$ , after reaction with aniline for 6 hours gave the complex anilide. (I.R. absorption at  $3260 \text{ cm}^{-1}$  and  $1634 \text{ cm}^{-1}$ , no absorption at  $1760 \text{ cm}^{-1}$ ). Recrystallization from dimethylformamide-water gave

Bis-[N-(phenyl carbamoylmethyl)salicylideneamino] copper(II) or  $\text{Cu}(\text{sal} = \text{glycyl aniline})_2$ .

Found: N, 9.69.  $\text{C}_{30}\text{H}_{26}\text{O}_4\text{N}_4\text{Cu}$ , requires N, 9.83.

Bis-[N-(p-nitrophenoxycarbonylmethyl)salicylideneamino] nickel(II) or  $\text{Ni}(\text{sal} = \text{glyOPhenyl-p-NO}_2)_2$ , after reaction with aniline for  $3\frac{1}{2}$  hours gave a residue which was largely the anilide but contained some unreacted ester. (I.R. absorption at  $3300 \text{ cm}^{-1}$  and  $1635 \text{ cm}^{-1}$ , both broad, and at  $1765 \text{ cm}^{-1}$ , weak.)

Cu and Ni (HO-naph = gly OEt)<sub>2</sub>:

(a) primary aliphatic amine: The complex esters were reacted with n-butylamine and the amide and aldimine products were readily separated by treatment with cold benzene. The compounds isolated were:

Bis-[N-(n-butylcarbamoylmethyl)2-oxo-1-naphthylideneamino] copper(II) or  $\text{Cu}(\text{HO-naph} = \text{glycyl n-butylamine})_2$ .

Bis-[N-(n-butylcarbamoylmethyl)2-oxo-1-naphthylideneamino] nickel(II) or  $\text{Ni}(\text{HO-naph} = \text{glycyl n-butylamine})_2$ , purified by digestion with boiling EtOH.

Found: C, 65.28; H, 5.92; N, 8.73.  $\text{C}_{34}\text{H}_{38}\text{O}_4\text{N}_4\text{Ni}$  requires C, 65.30; H, 6.12; N, 8.96.

Bis-[N-n-butyl 2-oxo-1-naphthylideneamino] copper(II) or  $\text{Cu}(\text{HO-naph} = \text{n-butylamine})_2$ , recrystallized from MeOH.

Found: C, 69.80; H, 6.29; N, 5.58.  $\text{C}_{30}\text{H}_{32}\text{O}_2\text{N}_2\text{Cu}$  requires C, 69.81; H, 6.25; N, 5.43.

Bis-[N-n-butyl 2-oxo-1-naphthylideneamino] nickel(II) or  $\text{Ni}(\text{HO-naph} = \text{n-butylamine})_2$ , recrystallized from MeOH.

Found: C, 70.35; H, 6.38; N, 5.34.  $\text{C}_{30}\text{H}_{32}\text{O}_2\text{N}_2\text{Ni}$  requires C, 70.47; H, 6.31; N, 5.48.

(b) primary aromatic amine: The complex esters were reacted with aniline for 7 days but were recovered unchanged.  $\text{Cu}(\text{sal} = \text{amino acid ethyl ester})_2$  complexes of alanine, phenylalanine, leucine and glycyl glycine:

primary aliphatic amine:

(a) The three complex amino acid esters were reacted with n-butylamine. Green sticky oils were obtained in all cases and no identifiable products were isolated. I.R. spectra of these oils indicated that very little amide had been formed. The intensity of the carbonyl ester absorption at  $1730 \text{ cm}^{-1}$  was used as an approximate measure of the extent of these reactions.

(b) The complex dipeptide ester,  $\text{Cu}(\text{sal} = \text{gly glyOEt})_2$  was reacted with allylamine and the major product was the aldimine.

Bis-[N-allyl salicylideneamino] copper(II) or  $\text{Cu}(\text{sal} = \text{allylamine})_2$ , recrystallized from MeOH.

Found: C, 62.76; H, 5.40; N, 7.31.  $\text{C}_{20}\text{H}_{20}\text{O}_2\text{N}_2\text{Cu}$  requires C, 62.58; H, 5.22; N, 7.30.

Complexes derived from Optically Active Amino Acids:

(a) The optical rotations of alcoholic solutions of the unrecrystallized complexes  $\text{Cu}(\text{sal} = \text{L,alaOEt})_2$ ,  $\text{Cu}(\text{sal} = \text{L,PheOEt})_2$  and  $\text{Cu}(\text{sal} = \text{L,PheOMe})_2$  were measured and changes with time were noted. The recrystallized complexes were optically inactive.

(b) The 1:1 complexes of Co(II), Ni(II) and Cu(II) with the Schiff base from salicylaldehyde and L-phenylalanine were prepared by the following method:

L-phenylalanine (0.82 g, 0.005 mole) was dissolved in water (50 ml) and treated with salicylaldehyde (0.61 g, 0.005 mole) and NaOH (5.0 ml 1.0N). This yellow solution was added to a solution of the metal acetate (0.005 mole) and hydrated sodium acetate (3 g) in water (50 ml). The product crystallized from solution, was filtered off, washed well with water and dried under vacuum over silica gel. N-[2-(3-phenyl) propionato] salicylideneamino cobalt(II) dihydrate or  $\text{Co}(\text{sal} = \text{L-phe}) \cdot 2\text{H}_2\text{O}$ .

Found: C, 53.41; H, 5.17; N, 3.93.  $\text{C}_{16}\text{H}_{13}\text{O}_3\text{N Co} \cdot 2\text{H}_2\text{O}$  requires: C, 53.05; H, 4.73; N, 3.87.

N-[2-(3-phenyl)propionato] salicylideneamino nickel(II) trihydrate or  $\text{Ni}(\text{sal} = \text{L-phe}) \cdot 3\text{H}_2\text{O}$ .

Found: C, 50.70; H, 5.50; N, 3.84.  $\text{C}_{16}\text{H}_{13}\text{O}_3\text{N Ni} \cdot 3\text{H}_2\text{O}$  requires C, 50.57; H, 5.57; N, 3.69.

N-[2-(3-phenyl)propionato] salicylideneamino copper(II) dihydrate or  $\text{Cu}(\text{sal} = \text{L-phe}) \cdot 2\text{H}_2\text{O}$ .

Found: C, 53.09; H, 4.75; N, 3.95.  $\text{C}_{16}\text{H}_{13}\text{O}_3\text{N Cu} \cdot 2\text{H}_2\text{O}$  requires C, 52.38; H, 4.67; N, 3.82.

The optical rotations of solutions of these complexes in methanol were measured and they did not change over 3 days.

The molecular weights of these compounds were obtained in methanol solution at  $37^\circ$  using a Mechrolab Model 301A Osmometer. Tris-acetylacetonato cobalt(III) was used as calibrant.

#### Magnetic measurements:

Magnetic moments were determined by the Gouy method at  $20^\circ$ .  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  was used as the standard.

#### Preparation of Ionic Complexes of Schiff bases:

##### (i) Nickel(II) complexes:

Solutions of the anions  $[\text{Ni}(\text{sal} = \text{A})_2]^{2-}$ , where A = glycine, D,L-alanine or L-alanine, were prepared as follows:

The amino acid (0.01 mole) was dissolved in NaOH solution (10.0 ml 1.0N), salicylaldehyde (1.22 g) was added and the solution was stirred for 5 minutes. A solution of nickel acetate tetrahydrate (1.24 g) in water (20 ml) was added in portions (2 ml) alternately with (1 ml) portions of NaOH solution (10.0 ml 1.0N).

(a) Isolation of the glycine complex as the calcium salt: Calcium acetate (4.0 g) was added to the clear green solution followed by ethanol (100 ml). After standing at 5° overnight, fine light green crystals were filtered off and washed with ethanol.

Calcium Bis-[N-acetato salicylideneamino] nickelate(II) tetrahydrate or  $\text{Ca}[\text{Ni}(\text{sal} = \text{gly})_2] \cdot 4\text{H}_2\text{O}$ , recrystallized from aqueous ethanol.

Found: C, 40.45; H, 4.04; N, 5.13.  $\text{C}_{18}\text{H}_{14}\text{O}_6\text{N}_2\text{Ni Ca} \cdot 4\text{H}_2\text{O}$  requires C, 41.17; H, 4.22; N, 5.33.

(b) Isolation of the glycine complex as the barium salt: Barium acetate (3.0 g) was added to the green solution and, after standing overnight, the green crystals were filtered off and washed with cold water and ethanol.

Barium Bis-[N-acetato salicylideneamino] nickelate(II) tetrahydrate or  $\text{Ba}[\text{Ni}(\text{sal} = \text{gly})_2] \cdot 4\text{H}_2\text{O}$ .

Found: C, 34.22; H, 3.31; N, 4.41.  $\text{C}_{18}\text{H}_{14}\text{O}_6\text{N}_2\text{Ni Ba} \cdot 4\text{H}_2\text{O}$  requires C, 34.73; H, 3.56; N, 4.50.

(c) Isolation of D,L-alanine and L-alanine complexes as the barium salts: The green solution was evaporated under reduced pressure to 20 ml, barium acetate (3.0 g) and ethanol (150 ml) were added and the solution was stood overnight at 5°. Light green needles were filtered off and washed with ethanol. Barium Bis-[N-(2-propionato)salicylideneamino] nickelate(II) tetrahydrate or  $\text{Ba}[\text{Ni}(\text{sal} = \text{ala})_2] \cdot 4\text{H}_2\text{O}$ , recrystallized from aqueous ethanol.

Found: C, 37.25; H, 4.71; N, 4.39.  $\text{C}_{20}\text{H}_{18}\text{O}_6\text{N}_2\text{Ni Ba} \cdot 4\text{H}_2\text{O}$  requires C, 36.93; H, 4.03; N, 4.31.

The corresponding compound derived from L-alanine was not analysed. Its optical rotation in aqueous solution was measured.

(ii) The Iron(II) complex of salicylaldehyde and D,L-alanine:

D,L-alanine (0.89 g) was dissolved in NaOH solution (10.0 ml, 1.0N), salicylaldehyde (1.22 g) was added and the solution was stirred for 5 minutes. A solution of ferrous sulphate heptahydrate (1.39 g) in water (20 ml) was added in portions alternately with portions of NaOH solution (10.0 ml, 1.0N).

The blood red solution was evaporated under reduced pressure to 20 ml, and acetone (100 ml) was added slowly with constant stirring.

After several hours the crystalline precipitate was filtered off and washed with acetone.

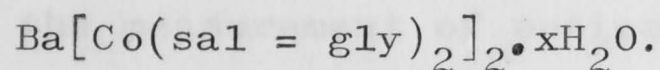
Sodium Bis-[N-(2-propionato)salicylideneamino] ferrate(II) dihydrate or  $\text{Na}_2[\text{Fe}(\text{sal} = \text{ala})_2] \cdot 2\text{H}_2\text{O}$ , recrystallized from aqueous acetone.

Found: C, 46.11; H, 4.72; N, 5.36.  $\text{C}_{20}\text{H}_{18}\text{O}_6\text{N}_2\text{FeNa}_2 \cdot 2\text{H}_2\text{O}$  requires, C, 46.18; H, 4.26; N, 5.39.

(iii) Cobalt(III) complexes:

(a) Glycine: Glycine (0.75 g, 0.01 mole) was dissolved in NaOH solution (10.0 ml, 1.0N) and salicylaldehyde (1.22 g) was added. After stirring for 5 minutes, a solution of cobalt acetate hexahydrate (1.24 g) in 50% aqueous methanol (20 ml) was added. After stirring for a further 10 minutes, barium acetate (1.0 g) was added, and this was followed after

5 minutes by hydrogen peroxide (5 ml, 100 vol). Stirring was continued until effervescence had ceased (~ 15 minutes). A small amount of insoluble material was collected and the solution was evaporated under reduced pressure. Brown crystals were obtained in several fractions after successive evaporations and filtrations. The total yield was 2.2 g, which was recrystallized from hot water. Slow cooling of the aqueous solution gave large black needles of Barium Bis-[N-acetato salicylideneamino] cobaltate(III) which were filtered and washed with cold water.



Dried in air:  $10\text{H}_2\text{O}$

Found: C, 37.96; H, 4.33; N, 5.00.  $\text{C}_{36}\text{H}_{12}\text{O}_{12}\text{N}_4\text{Co}_2\text{Ba} \cdot 10\text{H}_2\text{O}$  requires, C, 37.80; H, 4.23; N, 4.90.

Dried at  $110^\circ$ , then kept in vacuum:  $2\text{H}_2\text{O}$ .

Found: C, 43.16; H, 3.12; N, 5.50.  $2\text{H}_2\text{O}$  requires C, 43.24; H, 3.23; N, 5.60.

Dried at  $110^\circ$ , then exposed to air:  $7\text{H}_2\text{O}$ .

Found: C, 39.91; H, 4.08; N, 5.14.  $7\text{H}_2\text{O}$  requires C, 39.67; H, 3.88; N, 5.14.

Partial Resolution of the complex ion  $[\text{Co}(\text{sal} = \text{gly})_2]^-$ :

$\text{Ba}[\text{Co}(\text{sal} = \text{gly})_2]_2 \cdot 10\text{H}_2\text{O}$  (4.0 g) was suspended in water (50 ml) at  $70^\circ$  and treated with a solution of  $\text{K}_2\text{SO}_4$  (1.30 g) in water (15 ml). After maintaining at  $70^\circ$  for 30 minutes, the suspension was allowed to cool for 90 minutes before filtering off the precipitated  $\text{BaSO}_4$ . The filtrate was treated with a solution of d-[Co en<sub>2</sub> ox]OAc [prepared from

d-[Co en<sub>2</sub> ox]Br·H<sub>2</sub>O (2.60 g) and Ag OAc (1.19 g) in water (30 ml)] and evaporated under reduced pressure to 30 ml. After standing at 5° for several hours, red brown crystals (Diast<sub>I</sub>, 0.6 g) were filtered off. The volume of the filtrate was reduced to 20 ml, and after standing at 5° overnight further precipitate (Diast<sub>II</sub>, 0.7 g) was filtered off. The filtrate (F<sub>II</sub>) and the diastereoisomers, D<sub>I</sub> and D<sub>II</sub> were each treated with a solution of sodium iodide in water and the precipitated d-[Co en<sub>2</sub> ox]I was filtered off. Small portions of the filtrates were diluted sufficiently (100-200 times) for the measurement of optical activity in a one dm. tube and the following rotations were observed.

$$D_I \alpha_D = + 0.24^\circ, \quad \alpha_{578} = + 0.40^\circ, \quad \alpha_{546} = + 0.85^\circ$$

$$D_{II} \alpha_D = + 0.11^\circ, \quad \alpha_{578} = + 0.17^\circ, \quad \alpha_{546} = + 0.53^\circ$$

$$F_{II} \alpha_D = - 0.18^\circ, \quad \alpha_{578} = - 0.30^\circ.$$

(The rotations of an aqueous solution of the resolving agent, d-[Co en<sub>2</sub> ox]I were  $\alpha_D = + 0.50^\circ$ ,  $\alpha_{578} = + 0.55^\circ$ ,  $\alpha_{546} = + 0.55^\circ$ .)

Barium acetate was added to the remainder of the filtrates, and some brown crystals precipitated. These barium salts were optically inactive but the solutions remaining after their removal retained their optical activity.

(b) D,L-alanine: A solution of the complex ion [Co(sal = ala)<sub>2</sub>]<sup>-</sup> was prepared exactly as for the glycine complex, except that D,L-alanine (0.89 g, 0.01 mole) was used. A solid product was not obtained after evaporation, nor did the use of NaOAc or KOAc in place of Ba(OAc)<sub>2</sub> lead to crystalline complexes.



Partial Resolution of the complex ion  $[\text{Co}(\text{sal} = \text{ala})_2]^-$ :

A solution of the complex ion  $[\text{Co}(\text{sal} = \text{ala})_2]^-$  was prepared from D,L-alanine (1.78 g), NaOH solution (20.0 ml, 1.0N), salicylaldehyde (2.44 g),  $\text{Co}(\text{OAc})_2 \cdot 6\text{H}_2\text{O}$  (2.48 g) NaOAc  $\cdot 3\text{H}_2\text{O}$  (4.0 g) and  $\text{H}_2\text{O}_2$  (100 vol, 10 ml).

This solution was treated with a solution of d- $[\text{Co en}_2 \text{ ox}] \text{OAc}$  [prepared from d- $[\text{Co en}_2 \text{ ox}] \text{Br} \cdot \text{H}_2\text{O}$  (1.82 g) and AgOAc (0.83 g) in water (15 ml)] and evaporated under reduced pressure to 15 ml. After standing overnight at 5° a small quantity of khaki precipitate was filtered off (Diast<sub>I</sub>, 0.30 g). The volume of the filtrate was reduced to 10 ml and, after standing at 5° overnight, a red-brown precipitate (Diast<sub>II</sub>, 0.36 g) was filtered off. D<sub>I</sub> was recrystallized by dissolution in a large volume of hot water followed by evaporation.

Oxalato bis-ethylenediamine cobalt(III) bis-[N-(2-propionato) salicylideneamino] cobaltate(III) tetrahydrate, or d- $[\text{Co en}_2 \text{ ox}] \text{d-}[\text{Co}(\text{sal} = \text{ala})_2] \cdot 4\text{H}_2\text{O}$ .

Found: C, 40.15; H, 5.25; N, 10.92. ( $\text{C}_6\text{H}_{16}\text{O}_4\text{N}_4\text{Co}$ )

( $\text{C}_{20}\text{H}_{18}\text{O}_6\text{N}_2\text{Co}$ )  $\cdot 4\text{H}_2\text{O}$  requires C, 40.01; H, 5.42; N, 10.77.

For a 0.0493% solution in  $\text{H}_2\text{O}$ ,  $\alpha_D = + 1.68^\circ$ , giving

$$[\alpha]_D^{20} = + 3400^\circ.$$

D<sub>II</sub> was recrystallized by dissolution in a small volume of cold water followed by the addition of acetone. The analytical figures of C = 31.1, H = 5.6 and N = 13.9 suggest that D<sub>II</sub> was a mixture containing additional resolving agent. For a 0.0494%

solution in water,  $\alpha_D = -0.37^\circ$ , giving  $[\alpha]_D^{20} = -750^\circ$ . Portion of each diastereoisomer was stirred with NaI in water and the precipitated d-[Co en<sub>2</sub> ox]I was filtered off. The filtrate from D<sub>I</sub>, when diluted, gave a rotation  $\alpha_D = +2.7^\circ$ , and the filtrate from D<sub>II</sub>, when diluted, gave a rotation  $\alpha_D = -1.8^\circ$ .

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