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THE REACTION OF ACETALDEHYDE WITH SOME
COBALT(III) COMPLEXES CONTAINING COORDINATED GLYCINE

by
James C. Dabrowiak

A Dissertation
Submitted to the
Faculty of the School of Graduate
Studies in partial fulfillment
of the
Degree of Doctor of Philosophy

Western Michigan University
Kalamazoo, Michigan
August, 1970

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James C. Dabrowiak

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To
my wife, Tina
and
son, Robert

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LIST OF ABBREVIATIONS

A/C	Mole ratio of aldehyde to complexes
ala	Alaninate
ALLOTHR-Si	Allothreonine (silylated)
BSTFA	Bis-N(trimethylsilyl)trifluoroacetamide
cd	Circular dichroism
c-t	Charge transfer band
DEGS	Diethyleneglycolsuccinate
DSS	2,2 - Dimethyl-2-silapentane-5-sulfonate sodium salt
en	Ethylenediamine
glc	Gas-liquid chromatography
gly	Glycinate
GLY-Si	Glycine (silylated)
Hz	Hertz (cycles per second)
m	Molecular ion (mass spectrometry)
ord	Optical rotatory dispersion
ox	Oxalate
pmr	Proton magnetic resonance spectroscopy
pn	Propylenediamine (1,2 propanediamine)
Ra	R-allothreonine
RRa	R-proline-R-allothreonine dipeptide
RRT	R-proline-R-threonine dipeptide
RSa	R-proline-S-allothreonine dipeptide
RSt	R-proline-S-threonine dipeptide

Rt	R-threonine
Sa	S-allothreonine
ser	Serinate
SRa	S-proline-R-allothreonine dipeptide
SRT	S-proline-R-threonine dipeptide
SSa	S-proline-S-allothreonine dipeptide
SSt	S-proline-S-threonine dipeptide
St	S-threonine
TFA	Trifluoroacetic anhydride
thr	Threoninate
THR-Si	Threonine (silylated)
TMS	Trimethylsilyl group
uv	Ultraviolet spectroscopy

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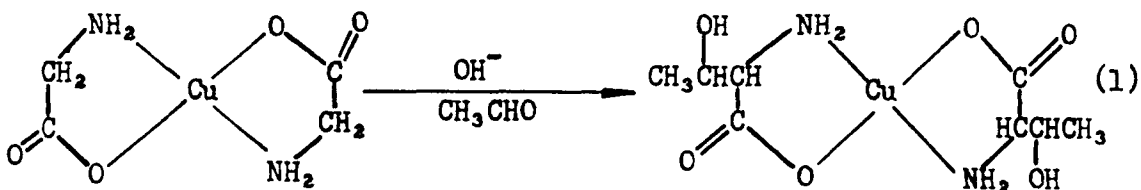
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I. INTRODUCTION

One of the most significant developments in recent years in the field of transition metal chemistry has been the utilization of metal complexes as catalysts in organic reactions. When an organic molecule is bound to a positively charged metal ion its reactivity may be drastically altered compared to the unbound ligand. The metal ion acts as a Lewis acid reducing the electron density of the donor atoms which in turn affect the electronic distribution about the remainder of the ligand atoms. There are many examples in the literature¹ of reactions of coordinated ligands which would not be possible were it not for the presence of the metal ion. Of these only a relatively small number have associated with them the possibility of formation of an asymmetric carbon atom and generation of optical activity in the product.

In 1957, S. Akabori² and coworkers demonstrated that the amino acid glycine in the bis(glycinato)copper(II) ion reacts with acetaldehyde in basic media to produce the hydroxy-amino acids threonine and allothreonine. In this reaction the metal ion reduces the electron density of the alpha carbon atom which makes it favorable for attack by the aldehyde to form amino acid products (Equation 1).



The resulting amino acids possess two asymmetric carbon atoms giving rise to four possible isomers (Figure 1).

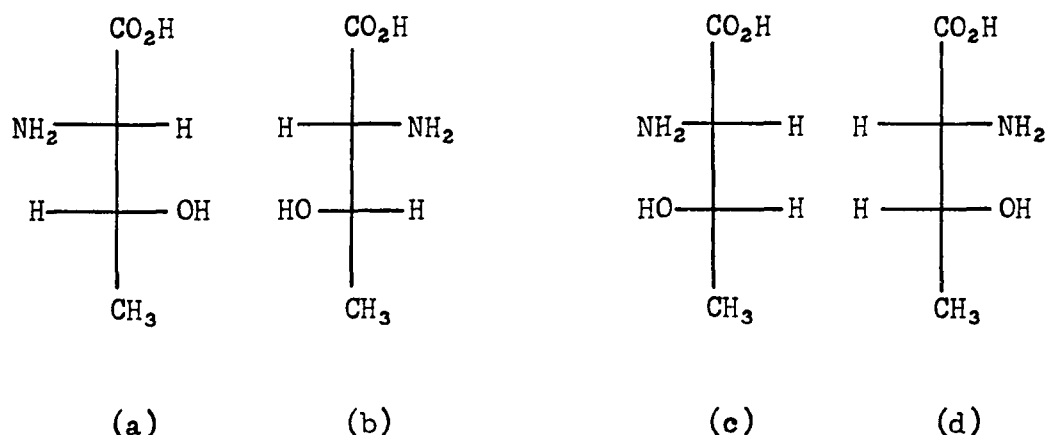


Figure 1. The optical isomers of threonine and allothreonine. (a) and (b) are S- and R-threonine respectively where S and R refer to the arrangement of groups about the carbon atom alpha to the carboxylic acid function. (c) and (d) are S- and R-allothreonine respectively.

Since the copper-glycine complex is optically inactive, no optical activity can exist in the products. However, the distribution of diastereoisomers was found to be 2:1 in favor of threonine. Various other compounds such as formaldehyde³, benzaldehyde and pyruvic acid⁴ have been used with success yielding serine, β -phenylserine and β -hydroxy- β -methyl aspartic acid respectively.

The versatility of this reaction was further demonstrated by Akabori⁵ with the condensation of coordinated glycine in tris(glycinato)cobalt(III) with acetaldehyde. Figure 2 shows the two geometric forms of this complex. (For simplicity only the coordinated atoms are shown.)



Figure 2. The geometric isomers of tris(glycinato)cobalt(III). (a) α -tris(glycinato)cobalt(III). (b) β -tris(glycinato)cobalt(III). Where $\text{O}-\text{N} = \text{NH}_2\text{CH}_2\text{CO}_2^-$.

Since the α isomer is dissymmetric and the β compound asymmetric, both have optically active forms. Unfortunately the compounds possess no formal charge which makes resolution by standard methods difficult and reactions were carried out using racemic compounds. In general the reactions of acetaldehyde with either racemic isomer were slower than with the copper(II) analogue. The α isomer was found to produce more threonine than allothreonine, about 7:1 versus 3:1 for the β isomer.

The possibility of obtaining optical activity in the products from such a reaction was demonstrated by M. Murakami and K. Takahashi⁶ who condensed acetaldehyde with the $(-)_58$, glycinatobis(ethylenediamine)cobalt(III) and $(-)_58$, glycinatobis(1-propylenediamine)cobalt(III) cations (Figure 3).

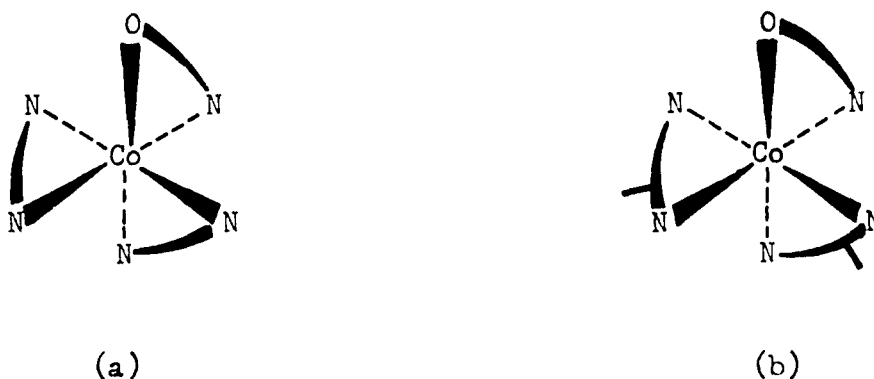
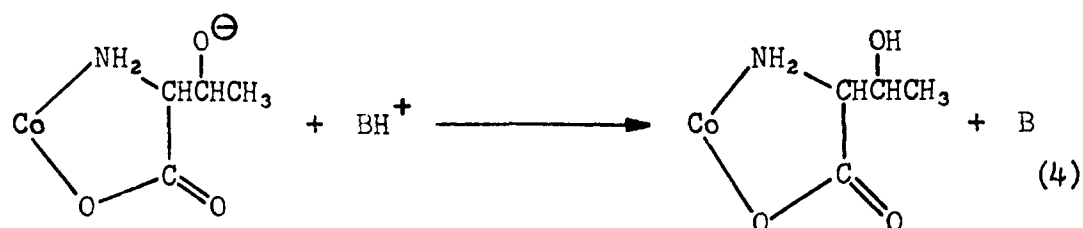
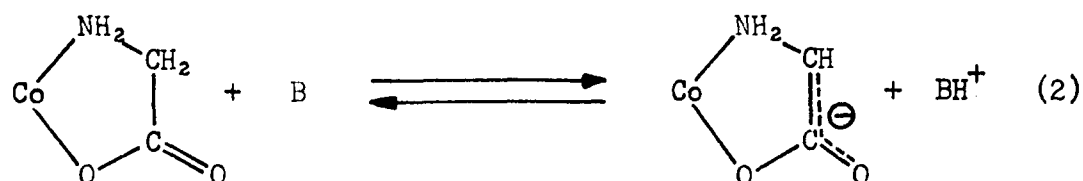


Figure 3. Complexes studied by M. Murakami and K. Takahashi. (a) $\Lambda(C_3)$ $(-)_58$, glycinatobis(ethylenediamine)cobalt(III). (b) $\Lambda(C_3)$ $(-)_58$, glycinatobis(1-propylenediamine)cobalt(III).

The reaction with the glycinatobis(ethylenediamine) compound gave 80% of the expected yield of hydroxy-amino acids with a ratio of threonine, allothreonine and glycine of 7:2:1. Further examination of the threonine showed that about 8% asymmetric synthesis occurred in favor of the R-isomer.

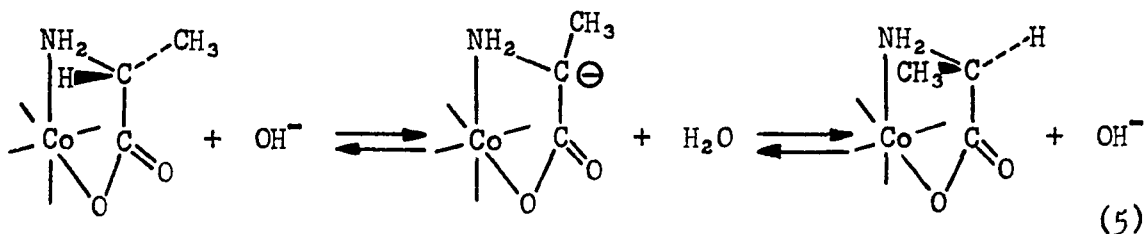
The results of the condensation involving the glycinatobis(1-propylenediamine) compound⁶ was less satisfying than its ethylenediamine analogue. The ratio of threonine, allothreonine and glycine was 6.5:3:0.5 with 1% asymmetric synthesis found in the threonine as the R-isomer.

The mechanism of this reaction has been investigated by D.C. Berndt⁷ using racemic glycinatobis(ethylenediamine)cobalt(III) chloride and found to be consistent with Equations 2-4. (For clarity the ethylenediamine ligands have been omitted.)



The aforementioned compounds can be thought of as molecular aggregates in which the coordinated molecules or ligands are arranged in a rigid, well-defined manner forming a "template" on which the reaction proceeds. If the arrangement of the members forming the "template" is known with certainty, valuable stereochemical information can be obtained by examining the reaction products, in this case threonine and allo-threonine. Analysis of the amounts of R- and S- threonine and R- and S- allothreonine produced in the reaction of M. Murakami and K. Takahashi⁶ above would show from which "side" of the coordinated glycine molecule the aldehyde entered. However, there is now strong evidence to show that such an analysis on the products of the above reactions would not necessarily yield the direction of the attacking aldehyde because of rearrangements which may occur.

Recent studies by A.M. Sargeson *et al.*⁸ on the $\Delta(C_3) (-)_{589}$ S-alaninatobis(ethylenediamine)cobalt(III) ion show that the arrangement of groups about the α carbon atom of the coordinated amino acid is subject to inversion in basic media. In Equation 5 this inversion is shown. (The ethylenediamine groups have been omitted for clarity.)



When this process reached equilibrium the amino acid was completely racemized indicating that the methyl group preferred both "sides" of the plane containing the amino acid and the cobalt ion equally well. It is evident from this that the arrangement of the ethylenediamine ligands about the asymmetric octahedron had no influence on the position of the methyl group. Inversion was also seen with $(-)_{589}$ S-leucinatobis(ethylenediamine) analogue although in this case the side chain was bulkier and the amino acid was not completely racemized. Since the products formed in the reaction between acetaldehyde and coordinated glycine are present in basic media, inversion may be occurring here also which could account for the low amounts of asymmetric synthesis obtained by M. Murakami and K. Takahashi⁶.

II. STRUCTURE OF SOME COBALT(III) COMPLEXES AND AMINO ACIDS USED IN THIS WORK

The geometric isomers of the bis(glycinato)ethylenediamine cobalt(III) ion and the structure of the glycinatobis(ethylenediamine) cobalt(III) ion are depicted in Figure 4. Assignments of absolute configuration for the complexes are defended in detail in the section on "The Absolute Configuration of the Complexes" (page 43). The compounds are named by the position of the oxygen atoms and the highest order axis of symmetry of the molecule.

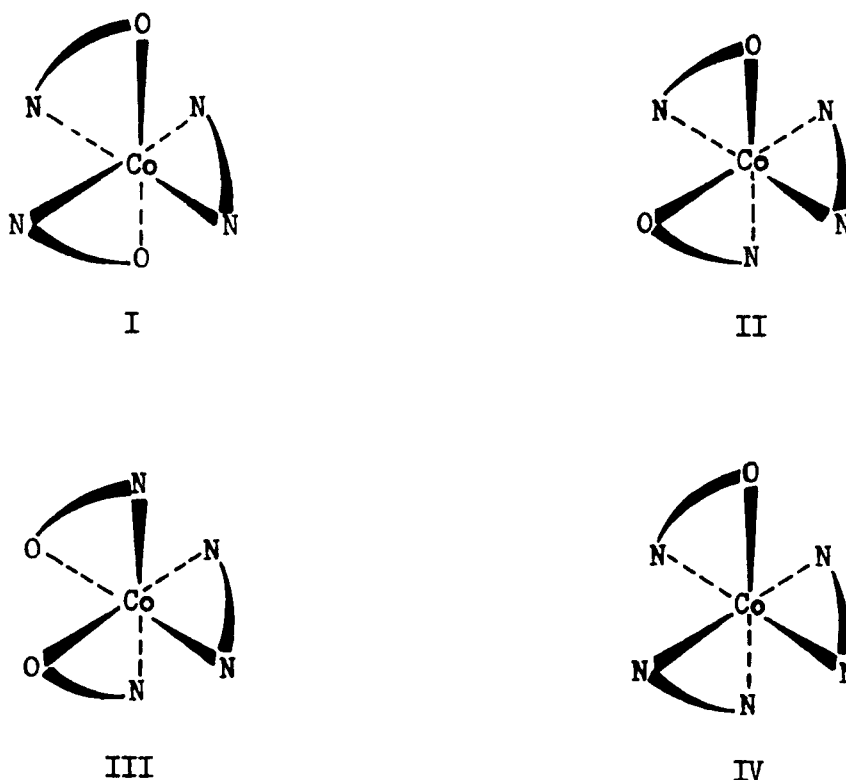


Figure 4. The structure of the complexes studied in this work. The bis(glycinato)ethylenediamine and glycinatobis(ethylenediamine) cobalt(III) cations. I, $\Lambda(C_3) (+)_{ss}$, $\text{trans}(O)C_2$ $[\text{Co}(\text{gly})_2(\text{en})]^+$; II, $\Lambda(C_3) (+)_{ss}$, $\beta\text{cis}(O)C_1$ $[\text{Co}(\text{gly})_2(\text{en})]^+$; III, $\Lambda(C_3) (+)_{ss}$, $\alpha\text{cis}(O)C_2$ $[\text{Co}(\text{gly})_2(\text{en})]^+$; and IV, $\Lambda(C_3) (+)_{ss}$, $[\text{Co}(\text{gly})(\text{en})_2]^{++}$ where gly = $\text{NH}_2\text{CH}_2\text{CO}_2^-$, (N—O) and en = $\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2$, (N—N). The molecular projection is the pseudo C_3 axis of the compounds.

The structures of some of the amino acids used in this work are shown in Figure 5.

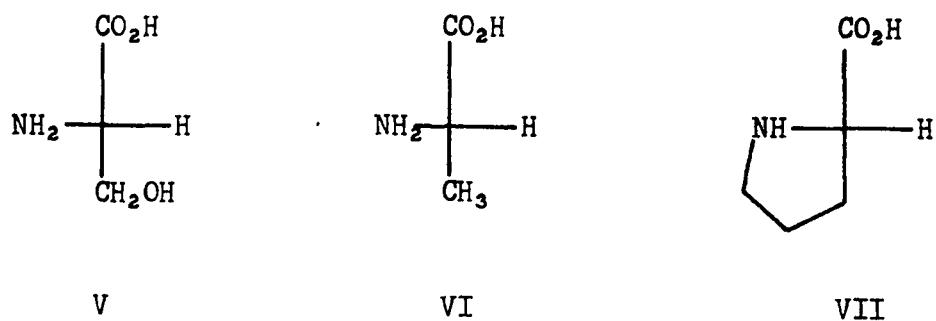


Figure 5. The structure of some amino acids used in this work. V, S-serine; VI, S-alanine; VII, S-proline.

III. STATEMENT OF PROBLEM

The purpose of the present work was to carry out reactions of acetaldehyde with the optically active complexes of bis(glycinato)ethylenediaminecobalt(III) to determine what effect the arrangement of ligands would have on the stereochemistry of the resulting amino acids threonine and allothreonine. The three geometric isomers of bis(glycinato)ethylenediaminecobalt(III) were to be synthesized and resolved into optical isomers. The absolute configuration of each enantiomer was to be determined using circular dichroism and proton magnetic resonance spectroscopy. The earlier Japanese work⁶ was to be repeated on the optically active glycinatobis(ethylenediamine)cobalt(III) cation and the products compared with those obtained from the bis(glycinato) series.

IV. EXPERIMENTAL

A. Reagents and Equipment

All chemicals were Reagent Grade unless otherwise noted. The amino acids glycine, S-serine, S-threonine and S-alanine were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. The racemic allothreonine was obtained from the same source but was crystallized twice from 15% ethanol to remove threonine. R-threonine and S-proline were obtained from Sigma Chemical Co., St. Louis, Missouri. The R-proline was purchased from Calbiochem, Los Angeles, California and a sample of R-allothreonine was generously supplied by Dr. Calvin Stevens, Wayne State University, Detroit, Michigan. The NaOH used for the reaction was purchased from Hartman-Leddon Co., Philadelphia, Pennsylvania and was CO₂ free. The silylating agent bis-N(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from the Regis Chemical Co., Chicago, Illinois under the name of "Regisil". The deuterium oxide and deuterated acids were purchased from Merck, Sharp and Dohme, Montreal, Quebec and Fluka AG Chemische Fabrick, Buchs, Switzerland.

All uv-visible spectra were obtained using a Cary 14 spectrophotometer. The optical rotatory dispersion (ord) spectra were obtained with a Beckman-DU, fitted with a Keston polarimeter attachment. A Sargent pH-stat fitted with a Sargent miniature combination electrode was used for the reactions. The pH-stat was standardized at pH's of 7.00 and 10.00 with standard buffers just prior to each run.

The proton magnetic resonance (pmr) spectra were recorded using a Varian A-60 spectrometer. An F and M (Hewlett-Packard, Skokie, Illinois) model 402 gas-liquid chromatograph (glc) with dual column flame ionization detectors was used for the amino acid analyses. Gas flows were: nitrogen 60 ml per min; hydrogen 25 ml per min; and air 300 ml per min. The mass spectra were obtained using an LKB-9000 gas chromatograph-mass spectrometer (LKB Instruments, Inc., Stockholm, Sweden) with a SE-30 column, 0.62 m x 4 mm I.D. glass.

B. Preparation of Complexes

1. Preparation and Isolation of the $\text{trans}(\text{O})\text{C}_2$ and $\beta\text{cis}(\text{O})\text{C}_1$ Isomers of Bisglycinato(ethylenediamine)cobalt(III) Chloride

The following is a modification of a method used by M. Matsuoka et al⁹. To 80 ml of 10 N NaOH was added 46.0 g (0.163 mol) of $[\text{CoCl}(\text{gly})\text{H}_2\text{O}(\text{en})]\text{Cl}$ and 60.0 g (0.810 mol) of glycine. Two hundred ml of water was added and the solution stirred for three days at room temperature. The solution was concentrated to 200 ml in a stream of air, cooled in an ice bath, and the pH of the dark red solution adjusted to 7 by careful addition of concentrated perchloric acid. Upon standing in a refrigerator for 1 hr, 15 g of the crude dark red $\text{trans}(\text{O})\text{C}_2$ isomer precipitated and was removed by filtration. Addition of 50 ml of methanol to the filtrate and refrigeration for 10 hr yielded 18.3 g of a pink precipitate containing principally the $\beta\text{cis}(\text{O})\text{C}_1$ isomer.

The crude $\text{trans}(\text{O})\text{C}_2$ precipitate was washed with several 50 ml portions of a 3:1 water:ethanol solution, dissolved in water and converted to the chloride salt by passing the solution through a Dowex 1-X8 (100-200 mesh) anion-exchange resin. The yield was 11 g. The structure was assigned on the basis of its visible absorption and pmr spectra.

A 6.0 g portion of the impure $\beta\text{cis}(\text{O})\text{C}_1$ isomer was separated from small amounts of the $\text{trans}(\text{O})\text{C}_2$ and $\alpha\text{cis}(\text{O})\text{C}_2$ isomers using Dowex 50W-X8 cation-exchange resin (100-200 mesh), and eluting with 0.3 N NaClO_4 . The eluting agent was removed from the pure $\beta\text{cis}(\text{O})\text{C}_1$ isomer by washing with absolute ethanol. The perchlorate salt was converted to the chloride salt using an anion exchange resin just like the $\text{trans}(\text{O})\text{C}_2$ isomer. The yield was about 3 g. The structure was assigned on the basis of its visible and pmr spectra.

2. Preparation and Isolation of $\alpha\text{cis}(\text{O})\text{C}_2$ Bisglycinato(ethylenediamine)cobalt(III) Chloride

Only a trace of the $\alpha\text{cis}(\text{O})\text{C}_2$ isomer was obtained using the procedure described for the $\text{trans}(\text{O})\text{C}_2$ and $\beta\text{cis}(\text{O})\text{C}_1$ isomers and the following approach was taken. The synthesis of these isomers was

repeated but after adjustment of the pH to 7, the solution was refrigerated for 10 hr yielding 30.2 g of the mixed isomers. This mixture was triturated with 47 g (0.63 mol) of KCl and 200 ml of water for 2 min and the KClO_4 which formed was collected by filtration and washed with two 5 ml portions of iced water. After combining the washings with the filtrate, 5.0 g of activated charcoal was added and the suspension was stirred at room temperature for 15 min. All traces of the charcoal were removed by filtration first through a "Whatman-42" filter paper and then through a "Celite" filter. The red solution was triturated with 142 g (0.63 mol) of $\text{AgClO}_4 \cdot \text{H}_2\text{O}$ for 2 min and immediately filtered to remove the silver halide and a small amount of the sparingly soluble trans(O) C_2 perchlorate. This isomer was recovered from the silver salt by washing with 500 ml of water. The volume was reduced to 100 ml by evaporation at reduced pressure at 40° and then placed in an ice bath for 1 hr. About 15 g of the trans(O) C_2 and β cis(O) C_1 isomers were recovered from the dark red solution. The α cis(O) C_2 isomer was isolated by placing the filtrate on a 5.0 cm I.D. ion exchange column containing 1.5 liters of 100-200 mesh Dowex 50W-X8 cation exchange resin in the sodium form and eluting with 0.3 N NaClO_4 . The work up and assignment of structure was the same as that used for the β cis(O) C_1 isomer. The yield was 1.6 g. This procedure was repeated 3 times until a sufficient quantity of material was available for resolution.

3. Preparation of (+)_{5A9} and (-)_{5A9} trans(O) C_2 Bisglycinato)ethylene-diamine)cobalt(III) Ions

To a flask shielded from the light containing a suspension of 2.21 g (0.015 mol) of d-tartaric acid and 5.35 g (0.015 mol) of silver d-tartrate in 140 ml of water was added 100 ml of a solution containing 9.35 g (0.039 mol) of the racemic trans(O) C_2 $[\text{Co}(\text{gly})_2(\text{en})]\text{Cl} \cdot 2\text{H}_2\text{O}$. After stirring for 30 min, the silver chloride was removed by filtration, washed once with 10 ml of water and the volume of the filtrate and washing reduced under vacuum at 40° to 80 ml. While at 40° the volume was further reduced to 60 ml under a stream of air until the first crystals appeared. The solution was heated momentarily to 50° to redissolve the red solid and on standing at room temperature for 1.5 hr,

produced about 0.1 g of impure racemate. The small amount of red precipitate was removed and the solution kept at room temperature for one day producing 2.2 g of the impure (+)₅₈, trans(O)C₂ diastereoisomer. The material was washed once with 5 ml of ice water and then recrystallized from about 20 ml of water taking care so as to not heat above 40°; yield, 0.96 g ([M]₅₈, = +3,430°). Further recrystallization did not change the rotation.

After recovery of the (+)₅₈, diastereoisomer, the volume was reduced to 40 ml under a stream of air at 40° and the red solution kept at room temperature for 4 hr producing 1.35 g of the impure (-)₅₈, diastereoisomer; yield, 0.60 g, ([M]₅₈, = -3,269°).

The (+)₅₈, diastereoisomer, 0.96 g, was ground in a mortar with 10 ml of water. After addition of 1.7 g of sodium iodide the mixture was triturated for 2 min. The iodide salt of the complex was removed by filtration and washed once with 3 ml each of water, ethanol and finally acetone; yield of (+)₅₈, trans(O)C₂ [Co(gly)₂(en)] I·H₂O, 0.65 g.

The (-)₅₈, diastereoisomer was similarly converted to the iodide salt using 6 ml of water and 1.1 g of sodium iodide; yield of (-)₅₈, trans(O)C₂ [Co(gly)₂(en)] I·H₂O, 0.40 g.

The (+)₅₈, trans(O)C₂ [Co(gly)₂(en)] I·H₂O was suspended in 30 ml of water, and converted to the chloride salt by addition of 1.2 g of freshly prepared silver chloride. The suspension was stirred for 10 min in a flask shielded from the light and the silver chloride removed by filtration. The filtrate was evaporated to dryness at reduced pressure and at a temperature of 40°. A check of the rotation gave the same value as for the iodide salt.

4. Preparation of the (+)₅₈, and (-)₅₈, βcis(O)C₁ Bisglycinato(ethylene-diamine)cobalt(III) Ions

The resolution of this geometric isomer was similar to that of the trans(O)C₂ isomer except that crystals first appeared when the solution volume was reduced to 20 ml. When 4.76 g (0.0134 mol) of the βcis(O)C₁ compound was employed, the (+)₅₈, diastereoisomer ([M]₄₇₀ = +5,000°) crystallized first and after conversion to the iodide salt yielded 0.50 g of (+)₅₈, βcis(O)C₁ [Co(gly)₂(en)] I·2H₂O. The rotation

of the $(-)_58$, diastereoisomer was $[M]_{470} = +5,850^\circ$, and yielded 0.40 g as the iodide salt.

5. Preparation of the $(+)_58$, and $(-)_58$, \propto cis(0)C₂ Bisglycinato(ethylene-diamine)cobalt(III) Ions

Only a partial resolution of this isomer was accomplished with the mono-silver salt of d-tartaric acid and silver antimonyl-d-tartrate was used in the resolution.

The resolving agent was prepared by the dropwise addition of a solution containing 17.0 g (0.10 mol) of silver nitrate in 20 ml of water to 16.2 g (0.05 mol) of potassium antimonyl-d-tartrate dissolved in 150 ml of water. During the synthesis the reaction mixture was shielded from the light. The mixture was stirred at room temperature for 30 min, the silver antimonyl d-tartrate was removed and washed with 30 ml of water, acetone and ether and stored in a brown bottle until needed.

A solution of the complex, 4.44 g (0.139 mol) in 100 ml of water, was added dropwise to 5.45 g (0.0139 mol) of silver antimonyl-d-tartrate which was suspended in 250 ml of water in a darkened flask. The silver chloride which formed was removed by filtration and the red solution condensed under a stream of air to 70 ml followed by the addition of 40 ml of absolute ethanol. After standing in the refrigerator ($5-10^\circ$) for several days a very hard red tar formed which was coated with 2.0 g of the nearly pure $(+)_58$ isomer. The compound was removed from the tar and purified by heating momentarily at 30° in about 20 ml of water and then cooling and slow evaporation under a stream of air until crystallization occurred. After two recrystallizations the rotation did not change, $[M]_{480} = -10,400^\circ$. The yield was 1.01 g.

The $(+)_58$, diastereoisomer was converted to the chloride salt by suspending it in 11 ml of water and slowly adding concentrated hydrochloric acid until the initially formed $SbOCl$ dissolved. An equal volume of water was added, the $SbOCl$ was removed and the filtrate evaporated to dryness at reduced pressure at 30° . The red material was purified by dissolving in 5 ml of water and adding acetone until

precipitation occurred. This procedure was repeated twice to yield 0.50 g of (+)₅₈₉, \propto -cis(O)C₂[Co(gly)₂(en)]Cl·H₂O.

All efforts to isolate the (-)₅₈₉ isomer from the red tar failed, and silver antimonyl-l-tartrate¹⁰ was synthesized and used as the resolving agent. Thus the red tar, [M]₄₈₀ = +1,770°, was dissolved in 50 ml of water and converted to the chloride salt by passage through a column containing Dowex 1-X8 (100-200 mesh) in the chloride form. Isolation and purification for the (-)₅₈₉ isomer as the antimonyl-l-tartrate was the same as before. The yield was 0.77 g as the chloride salt.

6. Preparation and Isolation of the (+)₅₈₉ and (-)₅₈₉ trans(O) Glycinato-S-threoninatoethylenediamine and Bis(S-threoninato)ethylene-diaminecobalt(III) Ions

The procedure described for the preparation of the trans(O)C₂ and β -cis(O)C₁ glycine analogues was followed by reacting S-threonine (S-thr) with [CoCl(gly)H₂O(en)]Cl. Potassium hydroxide was used as the base and a 5-fold molar excess of the amino acid was employed instead of an 8-fold one. However in attempting to precipitate the desired complexes from the reaction mixture as their perchlorate salts only a cream-white solid was obtained (possibly KClO₄). This material was removed by filtration and the filtrate placed on a 5.0 cm I.D. chromatography column containing 1.5 liters of Dowex 50W-X8 resin (100-200 mesh) in the H⁺ form. Because of the large concentration of other ions present, the positively charged cobalt complexes did not form a tight band at the top of the resin but streaked badly making it impossible to do an effective separation of the desired compounds. Upon elution with 0.5 N HCl, the amino acid complexes which carried a +1 charge and had oxygen atoms in the trans position easily separated from their cis-oxygen counterparts and from compounds having higher positive charge. The acidic solution containing the trans(O) isomers was collected and evaporated to dryness at reduced pressure (40°). The residue was dissolved in 75 ml of water and KCl was removed by the dropwise addition of a 3.0 N AgClO₄ solution to the chilled (0-5°) deep red liquid until precipitation ceased. After removing the AgCl and KClO₄ by filtration, a

careful separation was performed on the filtrate using Dowex 50W-X8 ion-exchange resin in the H^+ form and eluting with 0.3 N HCl at a rate of about 0.3-0.5 ml-min. Within 40-50 days 4 bands were clearly separated. The third band from the bottom of the column, which was the broadest and most intense, contained the mixed glycinato-S-threoninato compounds. Using a fraction collector, 322-15 ml fractions were collected and several tubes checked for optical purity by recording the ratios of the observed rotation, $[M]_{480}$, to the absorbance at 530 m μ . The $(+)_58$ isomer eluted first (tubes 1-117) followed by 1,970 ml of essentially racemic material (tubes 118-249) and finally the $(-)_58$ isomer.

Evaporation of the solution containing the $(+)_58$ isomer to dryness at reduced pressure (40°) yielded a red solid which was very soluble in water. This material was redissolved in 50 ml of water and converted to the iodide salt by passing it over Dowex 1-X8 anion exchange resin in the iodide form. Evaporation as before and crystallization from about 20 ml of water afforded 1.2 g. Recovery and purification of the less soluble $(-)_58$ isomer was as before but conversion to the iodide salt was unnecessary. The yield was 0.7 g.

Proton magnetic resonance spectroscopy, elemental analysis, and optical rotatory dispersion identified the second band as the $(-)_58$ isomer of $\text{trans}(O)[\text{Co}(\text{S-thr})_2(\text{en})]\text{Cl}$. After removal of HCl and water as before the material was converted to the perchlorate salt by redissolving the chloride in 5 ml of water and adding a 1.0 N AgClO_4 solution dropwise until precipitation ceased. The AgCl was removed by filtration and absolute ethanol added to the filtrate until precipitation occurred. The yield was about 0.2 g. The solution containing the first band was evaporated to dryness and the residue washed with 100 ml of absolute ethanol. Optical rotatory dispersion and pmr establish this as the $(+)_58$, trans(O) bis(S-threoninato) compound - yield about 0.05 g. The fourth band, obtained only in trace amounts, could not be identified.

7. Preparation and Isolation of the $(+)_58$ and $(-)_58$, trans(O) Glycinato-S-serinatoethylenediamine and the $(+)_58$, Bis(S-serinato) ethylenediaminecobalt(III) Ions.

The procedure described for the S-threoninato analogues was followed, however the fourth band was not observed for this series. The order of elution from the column was the same as before with the exception of the second band which was established by pmr and ord as the (+)₅₈, bis-(S-serinato) compound. Yields for the chloride salts of the (-)₅₈, glycinato-(S-serinato) and (+)₅₈, bis-(S-serinato) compounds were 1.1 g and 0.2 g respectively. One gram of the (+)₅₈, glycinato-(S-serinato) isomer was recovered as the iodide salt.

8. The Preparation and Isolation of the Isomers of the Bis(S-alaninato) ethylenediamine cobalt(III) Ion.

Using the method of M. Matsuoka *et al.*⁹, [CoCl(S-ala)H₂O(en)]Cl was synthesized in a manner analogous to the glycine compound. The same procedure and scale as was used for the synthesis of the trans(O)C₂ and βcis(O)C₁ bis(glycinato) compounds was used for the S-alanine analogues except that the entire reaction mixture containing an excess of S-alanine was placed on a 7.0 cm I.D. column containing 2 liters of Dowex 50-X8 (100-200 mesh) in the Na⁺ form. The material "streaked" badly but a separation was made by eluting with 0.3 N NaClO₄ which after several days revealed the presence of three bands. The first two bands closest to the bottom of the column were clearly separated from each other and from the very broad diffuse band above them. The most rapidly eluting band was the (+)₅₈, trans(O)C₂ isomer followed by the (-)₅₈, trans(O)C₂ isomer. Recovery of the complexes was accomplished by evaporating the solution containing the complex and eluting agent to dryness at reduced pressure (40°) and taking up the NaClO₄ in several liters of absolute ethanol. The yield of both isomers was about 1 g as the perchlorate salts.

The broad band containing the cis(O) isomers partially separated into three bands. The fastest and slowest eluting materials were the (-)₅₈, βcis(O)C₁ and the (+)₅₈, αcis(O)C₂ isomers respectively and were recovered from the eluting agent in the same manner as the trans(O)C₂ isomers. The yields were 0.5 g and 1.5 g for the αcis(O)C₂ and βcis(O)C₁ isomers, respectively. The middle cis(O) band failed to separate and was recovered in the manner used for the other isomers

and placed on 5.0 cm I.D. ion exchange column containing 1 liter of Dowex 50-X8 in the H^+ form. After eluting for several days with 0.6 N HCl three bands separated, the fastest moving of which was the $(+)_{38}$, β -cis(0) C_1 isomer. Evaporation to dryness at reduced pressure (40°) yielded 1.3 g as the chloride salt. The other two bands remained unidentified. The identification of all the complexes was made using ord, pmr and uv-visible spectroscopy.

9. Preparation and Resolution of the Glycinatobis(ethylenediamine)cobalt(III) Ion

The procedure for preparation and resolution was the same as that described in reference 11.

C. Reactions of Cobalt Complexes

1. trans(0) C_2 Bisglycinato(ethylenediamine)cobalt(III) Chloride Dihydrate Reaction with Acetaldehyde

Ten ml of doubly distilled water (O_2 and CO_2 free) was added to 0.101 g (0.3 mmol) of the $(+)_{38}$ complex and the solution temperature adjusted to $25^\circ \pm 0.5^\circ$ in a pH-stat. To this solution was added 1.0 ml of the freshly prepared aldehyde solution and the pH automatically adjusted to 9.5 by the addition of about 0.1 ml of 0.08 N NaOH (CO_2 free). (The aldehyde solution was prepared just prior to initiating the reaction by adding 2 drops of concentrated H_3PO_4 to 10 ml of acetaldehyde and distilling the monomer in an N_2 atmosphere.) The distillate, 33 μ l (0.6 mmol), was added to 2.0 ml of chilled ($0-5^\circ$) doubly distilled O_2 and CO_2 free water. The reaction proceeded smoothly and was terminated after 3.0 hr by the addition of 1 drop of concentrated acetic acid.

Separation of the Product Complexes

The condensation product complexes were separated from the unreacted bisglycinato- compound by placing the acidified reaction mixture on a small (200 ml, 2.8 cm I.D.) chromatography column of Dowex 50W-X8 cation exchange resin (100-200 mesh) in the H^+ form. After eluting for 5 days with 0.1 N HCl at a rate of 0.2-0.4 ml/min, 3 bands separated.

Numbering from the bottom of the column the first two and least intense bands contained 18-20% of the material. Using a suction technique involving removal of the resin using a water aspirator, the third band was separated from the first two bands and the complexes removed from the resin using 0.3 N HCl. Absorption and ord spectra of the complexes in addition to glc analysis of the ligands establish that the combined first two bands contained the monocondensation products - complexes in which only one glycine molecule had reacted with the aldehyde. The third band contained the unreacted starting complex.

Isolation of Amino Acids

The residue containing the monocondensation products was taken up in 5 ml of water, acidified with 2 drops of concentrated acetic acid and purged with H₂S gas for 10 min. Slow addition of 5 drops of concentrated aqueous NH₃ usually caused the immediate precipitation of CoS. In some cases precipitation did not occur until air was bubbled through the solution for a few seconds. The CoS was removed by filtration through a "Celite" filter, washed twice with 2 ml portions of water, and the clear or very light brown filtrate evaporated to dryness at water aspirator pressure (50°). The residue was taken up in 3-5 ml of water and the solution placed on a chromatography column (1.4 cm I.D.) containing 12 ml of Dowex 1-X8 in the OH⁻ form. After washing with 30 ml of water to remove the ethylenediamine, the amino acids were released from the resin by addition of 50 ml of 1.0 N HCl. The acidic solution was evaporated to dryness at reduced pressure (50°) leaving a mixture of amino acid hydrochloride salts which was analyzed for the optical isomers of threonine and allothreonine using glc.

The residue containing the unreacted starting material was taken up in a 40 ml of water, and 1.0 ml of the resulting solution containing about 2 mg of complex was removed and placed in a 3.0 ml centrifuge tube. One drop of concentrated acetic acid was added and the CoS precipitated as before with H₂S and NH₃. After centrifuging, the supernatant liquid was removed and evaporated to dryness by heating (100°) in a 2 ml screw top test tube under a stream of dry N₂ gas. The residue containing ethylenediamine and the amino acids was analyzed for

the relative amounts of glycine, threonine and allothreonine using glc.

Reactions of the Racemic Complex

The reaction of the optically active complex was repeated for the racemic material except that a ratio of 3:1 aldehyde to complex was employed (105 μ l of aldehyde in 2.0 ml of water) and the reaction was terminated after 20 min. After evaporation to dryness at reduced pressure (30°) the residue was taken up in 10 ml of water and a 2.0 ml portion removed for amino acid analysis. The cobalt was precipitated as the sulfide as described on page 18.

The reaction of M. Murakami and K. Takahashi⁶ of the $(-)_589$ glycinatobis(ethylenediamine)cobalt(III) ion with acetaldehyde was repeated with the racemic trans(O) C_2 isomer. Two grams (6.0 mmol) of the chloride salt was dissolved in 138 ml of a 0.43% Na_2CO_3 solution. The larger volume of water was necessary because of the lower solubility of this compound as compared to $(-)_589, [Co(gly)(en)_2]I_2$. After addition of 1.0 ml of acetaldehyde, the flask was sealed and allowed to stand for 90 hr at room temperature. The reaction was terminated by the addition of 5 drops of concentrated acetic acid and 15 ml sample removed and evaporated to dryness at reduced pressure (30°) to remove any excess aldehyde. The residue was redissolved in 5 ml of water and the CoS precipitated as described on page 18. The amino acid ligands were analyzed for the relative amounts of threonine, allothreonine and glycine using pmr¹².

2. Epimerization of S-Threonine in $(+)_589$ and $(-)_589$ trans(O) Glycinato-S-threoninato(ethylenediamine)cobalt(III) Chloride Monohydrate

To 20 mg of the $(+)_589$ isomer 10 ml of O_2 and CO_2 free water was added, the pH adjusted to 9.5 with 0.08 N NaOH and the solution allowed to stand for 3.0 hr at 25° as in the reaction of the bis(glycinato) compound with acetaldehyde (page 17). Recovery of the amino acids was the same as that used for the monocondensation product complexes. The procedure was repeated for the $(-)_589$ isomer.

3. Epimerization of Threonine and Allothreonine in the trans(0) Monocondensation Product Complexes

The procedure described for the reaction of (+)₅₈, trans(0) bis (glycinato)ethylenediaminecobalt(III) with acetaldehyde (page 17) was followed except that in this case the starting complex was racemic. After 5 days of eluting, the complexes were removed from the resin and recovered in the same manner as with the optically active complex. The residue containing the monocondensation products was taken up in 10 ml of O₂ and CO₂ free water and the pH adjusted to 9.5 for 3.0 hr. The pH was lowered by the addition of 1 drop of concentrated acetic acid and the solution evaporated to dryness (30°) at water aspirator pressure. The recovery of the amino acids was described earlier (page 18).

4. β cis(0)C₁ Bis(glycinato)ethylenediaminecobalt(III) Chloride Trihydrate

The reaction with acetaldehyde was carried out using 107 mg (0.3 mmol) of the (+)₅₈ complex following the procedure described for the trans(0)C₂ isomer (page 17). After terminating the reaction, a 2.0 ml portion of the acidified reaction mixture was removed for ord and uv-visible absorption spectra and the remaining solution evaporated to dryness at reduced pressure (30°) to remove the unreacted aldehyde. The residue containing a mixture of the product and starting complexes was redissolved in 5 ml of water, acidified with 2 drops of concentrated acetic acid and purged with H₂S to recover the amino acids as was the trans(0)C₂ isomer. The amino acid hydrochloride salts were redissolved in 10 ml of water and 0.5 ml of this solution containing about 2 mg of glycine, threonine and allothreonine was removed to determine the extent of the reaction using glc. The remaining portion was evaporated to dryness at water aspirator pressure (40°) and analyzed for the optical isomers of threonine and allothreonine.

Attempts to separate the product complexes as with the trans(0)C₂ compound failed. After 40 days of eluting only one band was present.

5. α cis(0)C₂ Bis(glycinato)ethylenediaminecobalt(III) Chloride Hydrate

The reaction with acetaldehyde was similar to that described for the trans(O)C₂ isomer except that 96 mg (0.3 mmol) of the (+)_{5,8} complex was treated with 0.9 mmol of aldehyde. The aldehyde solution was prepared by dissolving 105 μ l of freshly distilled aldehyde in 2.0 ml of chilled (0-5°) doubly distilled O₂ and CO₂ free water. Workup and recovery of the amino acids was the same as in the procedure used for the β cis(O)C₁ compound. The reaction was repeated for the racemic complex and the amino acids isolated as for the β cis(O)C₁ compound and analyzed by the TFA method, page 25.

Separation of the complexes prepared with the racemic α cis(O)C₂ isomer in the manner described for the trans(O)C₂ compound gave three bands. After 10 days of eluting, band 1, (numbering from the bottom of the column) which was present in trace amounts easily separated from the bulk of the material. Identification of this band was accomplished using racemic material by carrying out a series of reactions using a 1:1 molar ratio of aldehyde to complex for reaction times of 3, 12, and 24 hr. (The amino acids from the 3 hr reaction were isolated as described for the β cis(O)C₁ isomer, page 18, and analyzed by the TFA method, page 25.) Separation of the reaction mixtures revealed that increasing the reaction time increased the yield of this band which for the 24 hr reaction was resolved into three distinct components. The lower two bands (1-A and 1-B) from glc analysis and uv-visible absorption were the trans(O) mono-condensation product complexes. The top component, 1-C, identified in the same manner was the trans(O)C₂ bis (glycinato) compound.

For the combined band 2 and the lower portion of band 3, which was broad suggesting the presence of several components, a cis(O) Co(N₄O₂) uv-visible spectrum was obtained. Gas liquid chromatographic analysis gave 44% threonine-allothreonine and 56% glycine. The bulk of the material, the top portion of band 3, also gave a cis(O) Co(N₄O₂) uv-visible spectrum and contained 97% glycine and 3% threonine-allothreonine. Efforts to resolve any of the components of band 3 on separate but identical reaction mixtures failed after 30 days of eluting.

6. Glycinatobis(ethylenediamine)cobalt(III) Chloride

The procedure for the reaction with acetaldehyde was the same as that described for the trans(0) isomer except that 93 mg (0.3 mmol) of the (+)_{SS} complex was used. Three reactions with the optically active cation were carried out - two at pH 9.5 with aldehyde to complex ratios of 1:1 and 4:1 and one at pH 11 with a ratio of 0.5:1. The aldehyde solutions were prepared by adding 33, 141, and 16 μ l respectively of freshly distilled aldehyde to 2.0 ml of chilled O₂ and CO₂ free water.

Two reactions were examined using racemic complex. On the same scale as the above reactions racemic [Co(gly)(en)₂]Cl₂ was treated with a 4:1 molar ratio of aldehyde to complex at pH 9.5.

The reaction of M. Murakami and K. Takahashi of (-)_{SS}, [Co(gly)(en)₂]I₂ with acetaldehyde⁶ was repeated on the racemic chloride salt. Thus 0.585 g (2.0 mmol) of racemic glycinatobis(ethylenediamine)cobalt(III) chloride was dissolved in 5 ml of a 4% Na₂CO₃ solution followed by addition of 0.34 ml of acetaldehyde. The solution was sealed in a flask and allowed to stand at room temperature for 90 hr. The reaction was terminated by the addition of 5 drops of concentrated acetic acid and a small amount of solid material (probably CoO) was removed by filtration. A 0.5 ml sample of the filtrate was reserved for amino acid analysis.

Workup and recovery of the amino acids from all reactions was the same as that for the β cis(0)C₁ compound.

D. Preparation of Proton Magnetic Resonance Samples

The complex (80-129 mg) as the chloride, nitrate or acetate salt was dissolved in 0.5 ml of D₂O. Exchange of the NH₂ protons was accomplished by addition of 1.0 N NaOD until the solution turned basic (1-2 drops) followed by 1 drop of 6 N DCl, DNO₃ or CD₃CO₂D.

For steric compression analysis of two diastereomers a small amount of one, 10-25%, was mixed with the other and the pmr of the mixture obtained.

E. Racemization of the Complexes

To 0.3 mmol of each of the optically active complexes dissolved in 10 ml of water was added 0.08 N NaOH using a pH-stat until pH 9.5. After 3.0 hr the pH was lowered by the addition of 2 drops of concentrated acetic acid. The rotation of the resulting solution was measured at several wavelengths and compared with the values of the pure complexes dissolved in water. No racemization was noted.

The cis(O) C_2 isomer was also checked for a 24 hr period at pH 9.5. The material was placed on a Dowex 50-X8 column (H^+ form) and eluted with 0.1 N HCl for several days but no separation was observed. The optical rotation of the recovered material was identical to that of the authentic cis(O) C_2 isomer.

F. Gas-Liquid Chromatography of Amino Acids

1. Analysis of the Optical Isomers of Threonine and Allothreonine

Preparation of N-Trifluoroacetyl R- and S-prolyl Chloride

These compounds were prepared following a modification of Weygand¹³. Fifteen ml of trifluoroacetic anhydride was added with rapid stirring to 1.9 g (0.017 mol) of dry finely powdered R- or S-proline suspended in 20 ml of dry ether at dry ice-acetone temperature. (Before use the S-proline was twice recrystallized from boiling absolute ethanol, using "Norite-A" in the final recrystallization.) Immediately after this addition, the bath was removed and the stirred solution allowed to warm to room temperature. After one hour the ether and unreacted anhydride were removed by vacuum distillation at room temperature, leaving behind a faintly yellow oil. The material was converted to the acid chloride by the addition of 25 ml of a solution containing 15 ml of dry benzene and 10 ml of distilled thionyl chloride and stirring at room temperature for 2.5 hr. The thionyl chloride was distilled (bp 84°) from a 3:1 thionyl chloride-linseed oil solution and stored under N_2 at -20° until needed¹⁴. The benzene and unreacted thionyl chloride were removed by vacuum distillation at room temperature leaving a light yellow viscous oil. Working in a dry nitrogen atmosphere the oil was quantitatively taken up in 100 ml

of dry chloroform and the solution stored in 25-five ml vials with polyethylene push-on caps. Each vial was inserted into a larger bottle, sealed and stored in a freezer (-20°) until needed. Samples prepared and stored in this manner exhibited no loss of activity or detectable racemization after 6 months.

General Preparation of Amino Acid Derivatives

The amino acid mixture, containing threonine and/or allothreonine and/or glycine (30-50 mg) was esterified using 6 ml of a thionyl chloride-methanol solution (1:9) and refluxed for 4.5 hr¹⁵. (The solution was prepared by the dropwise addition of 10 ml of distilled SOCl_2 to 90 ml of dry methanol at dry ice-acetone temperature.) After cooling, a volume containing about 10 mg of the amino acids was placed in a 120 mm culture tube and the solvent removed by heating on a steam bath under a stream of dry nitrogen gas. One ml of methanol and 3-5 drops of triethylamine were added to the residual solid or oil which remained and the liquid again evaporated to dryness on a steam bath under nitrogen. Acetonitrile, 0.5 ml, was added to dissolve the material followed by addition of 0.5 ml of bis-N(trimethylsilyl)trifluoroacetamide (BSTFA). The sealed tube was placed on a steam bath for 2 min, then cooled under a stream of cold water for a few minutes. A 2 ml portion of N-trifluoroacetyl R- or S-prolyl chloride in chloroform was added followed by 10 drops of triethylamine. A 2-5 μl portion of this solution was injected into the gas chromatograph and the signals analyzed as shown in Table IX, page 58. When this solution was allowed to stand at room temperature, its color turned dark red-brown. However a check on the quantitative nature of the derivatives a day later showed only a slight loss of some of the signals.

2. Analysis of the Relative Amounts of Glycine, Threonine and Allothreonine

The Bis-N(trimethylsilyl)trifluoroacetamide (BSTFA) Method¹⁶

To 0.1-1.0 mg of amino acids in a 2 ml screw top test tube was added 10 μl of acetonitrile and 100 μl of BSTFA. The solution or

suspension was silylated in an oil bath at $125^{\circ} \pm 2^{\circ}$ for 15 min, cooled for 1 min under a cold water tap, and a 2-5 μ l portion injected into the gas chromatograph.

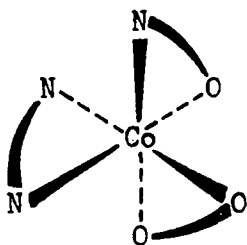
The Trifluoroacetic Anhydride (TFA) Method¹⁷

The amino acid mixture (0.1-1.0 mg) was esterified by adding 1 ml of a 1 N HCl-methanol solution and allowing it to stand at room temperature for 30 min. The solution was evaporated to dryness at 100° under a stream of N_2 gas and 1 ml of 1 N HCl-butanol was added. Trans-esterification of the methyl esters was accomplished in a 2 ml screw top test tube for 2.5 hr at 100° . The HCl-butanol was removed under N_2 as before and the residue taken up in 180 μ l of CH_2Cl_2 and 20 μ l of trifluoroacetic anhydride added. The amino acids were acetylated in an oil bath at 100° for 15 min, cooled under a cold water tap for 1 min, and a 1-5 μ l sample injected into the gas chromatograph.

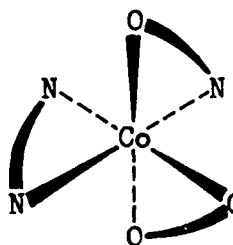
V. RESULTS AND DISCUSSION

A. Preparation and Characterization of the Cobalt(III) Complexes

The compounds used in this study were prepared by a modification of the method of M. Matsuoka et al.⁹ Their approach for synthesizing the bis(glycinato)ethylenediamine isomers was to react $[\text{CoCl}(\text{gly})\text{H}_2\text{O}(\text{en})]\text{Cl}$ with carbonate ion to form $[\text{CoCO}_3(\text{gly})(\text{en})]$ which exists in two geometric forms (VIII and IX). (For simplicity only the coordinated atoms are shown.) The desired products were then obtained by reacting the carbonate intermediates with glycine. For this study it was desirable to have sufficient amounts of the trans(O) C_2 and α cis(O) C_2 isomers for reaction.



VIII



IX

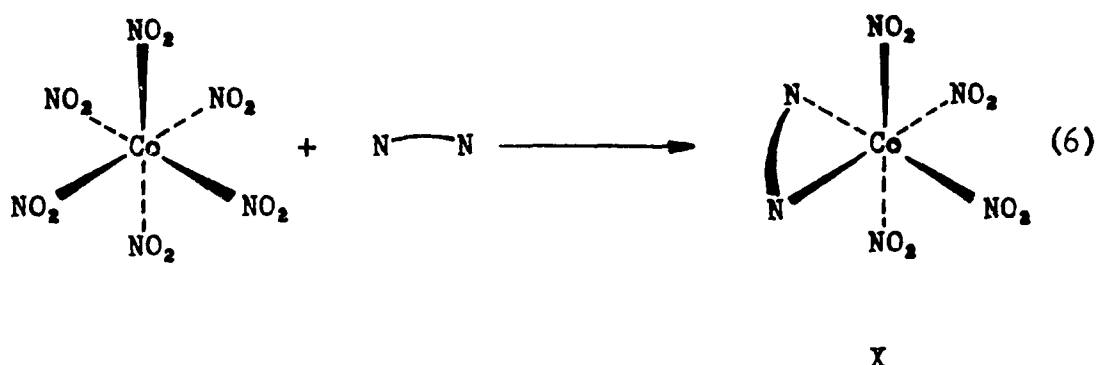
Replacement of the carbonate ligand ($\text{O}-\text{O}$) in VIII by glycinate should lead only to the β cis(O) C_1 (II racemate) and the α cis(O) C_2 (III racemate) isomers if no rearrangement occurs. The same reaction with IX would yield racemic trans(O) C_2 (I), and isomer II. In the subsequent workup of the products of the reaction of glycine with VIII and IX employing fractional recrystallization, they were able to isolate the trans(O) C_2 from IX and a mixture of the two cis(O) isomers from VIII. Unfortunately VIII, the only intermediate to give III, was formed in low yield. However rearrangement does occur, as was found in this laboratory and the reaction of either VIII or IX with glycinate produced all three geometric isomers in addition to complexes carrying charges other than +1. Furthermore separation using ion-exchange chromatography of the three geometric isomers of bis(glycinato)ethylenediaminecobalt(III)

obtained on rearrangement revealed that only a trace of isomer III was obtained from these reactions.

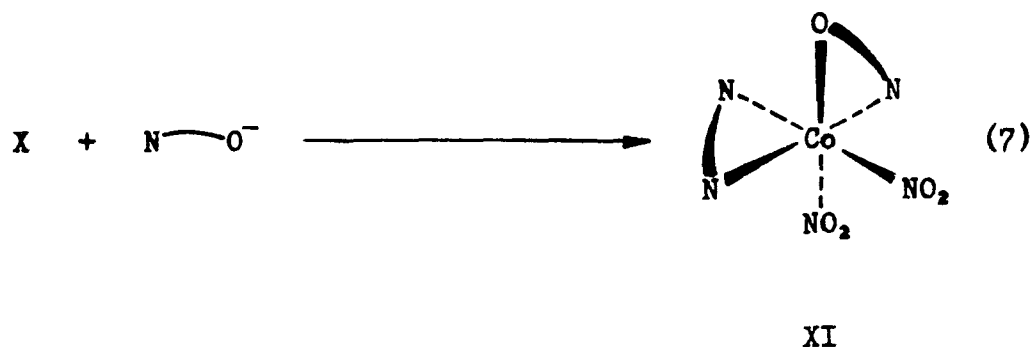
A second attempt by the Japanese to isolate the three isomers succeeded¹⁸. By air oxidizing a suspension composed of two equivalents of glycine and one each of ethylenediamine and cobalt(II) chloride the complexes were synthesized and then isolated using ion-exchange chromatography. Repeating this experiment showed that large amounts of the more stable tris(ethylenediamine)cobalt(III) and tris(glycinato)cobalt(III) compounds were formed and the desired complexes were only minor components.

The procedure finally chosen for the synthesis involved reacting $[\text{CoCl}(\text{gly})\text{H}_2\text{O}(\text{en})]\text{Cl}$ with an excess of glycine in a basic medium which resulted in good yields of two of the +1 complexes. Because of the mild conditions (3 days at room temperature) minimal amounts of the tris(ethylenediamine) and tris(amino acid) complexes were produced. These conditions were also particularly well suited for making the mixed amino acid complexes which would have been difficult to synthesize by other methods. However, even this approach produced only trace amounts (0.8-2.0%) of isomer III. This low yield of isomer III could be the result of a "trans effect" operating in the synthetic route.

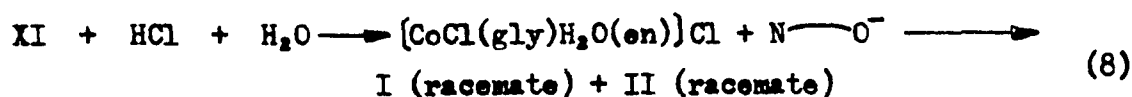
Although it is not as thoroughly documented as for platinum(II) systems, there is strong evidence to show that the "trans effect" may be applicable to low spin cobalt(III) systems as well¹⁹. In simple terms the "trans effect" is the ability of certain ligands within complexes to "weaken" the bonds which are trans to them. The first step in the synthesis of the bis(glycinato) compounds is assumed to be the formation of the hexanitrocobaltate(III) anion which then reacts with an equivalent of ethylenediamine (Equation 6).



Glycine exists as a zwitterion in aqueous solution and will probably coordinate first through the carbonyl function displacing any one of the four nitrite groups on the tetranitrito compound, X. However since NO₂⁻ is a stronger trans directing group than ethylenediamine, one of the nitrite groups which is trans to a second nitrite group would be the group to leave and be replaced by the carbonyl function of the amino acid (Equation 7).



If the arrangement of the two bidentate ligands remains unchanged, then subsequent reactions involving the replacement of the remaining NO₂⁻ groups by Cl⁻ and H₂O followed by addition of glycine would lead to only isomers I and II being formed (Equation 8).



The very low yields of isomer III that were obtained could be due to rearrangements or alternate reactions which were of minor importance.

The method finally chosen for the synthesis of III involved Equations 6 through 8, collecting the trans(O)C₂ and β cis(O)C₁ mixture and treating with activated charcoal. In a process which is not yet well understood for these systems²⁰, the charcoal allows the ligands to "rearrange" and in this case facilitates the production of the α cis(O)C₂ isomer. Unfortunately even with this rearrangement the yield was only about 5% based on the amount of the mixture of the other two isomers present before treatment with charcoal. Synthesis of the other compounds in the experimental section proceeded in a straightforward manner once a reasonable synthetic path was found for the bis(glycinato) complexes.

Resolution of the complexes presented no special difficulties with the possible exception of (-)_{5a}, α cis(O)C₂ [Co(gly)₂(en)] SbO-d-tartrate which was the most soluble diastereomer and failed to crystallize. This problem was solved by using the levo-tartrate thereby forming the compound which was enantiomeric to the least soluble diastereomer. The complexes containing optically active ligands were easily separated into distinct bands using ion-exchange chromatography except for the mixed glycinato(-S-serinato) and glycinato(S-threoninato) diastereoisomers which overlapped and had to be isolated using a fraction collector.

The assignment of the position of oxygen atoms in cis and trans Co(N₃O₂) systems from uv-visible spectroscopy is well established^{21,22}. Cobalt(III) in complexes containing six coordinated ammonia molecules exhibits octahedral symmetry and exists in a spin paired ground state (A_{1g}). Excitation of one electron gives rise to two triply degenerate excited states (T_{1g} and T_{2g}) resulting in two d-d absorption bands in the visible spectrum (Figure 6).

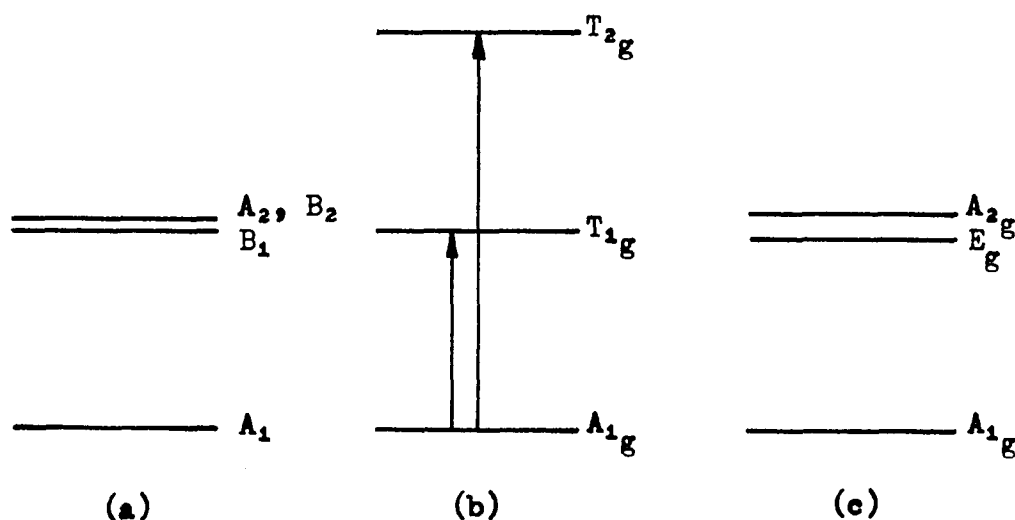


Figure 6. The energy levels of the first spin allowed d-d transition for Co(N₄O₂) and CoN₆. (a) cis(0) Co(N₄O₂); (b) octahedral CoN₆; (c) trans(0) Co(N₄O₂).

When two nitrogen donor groups are replaced by weaker oxygen donor groups the degeneracy of the excited states is reduced. The T_{1g} excited state splits into two excited states and the splitting of the first spin allowed d-d transition absorption band in Co(N₄O₂) systems is twice as great with the oxygen atoms in the trans position instead of the cis position. This produces a shoulder on the first absorption band of the trans(0) compound which is absent in the cis(0) case due to the smaller splitting. Figure 7 gives the electronic absorption spectra of the geometric isomers of [Co(gly)₂(en)]⁺ which are typical for the other compounds described in the experimental section. Distinction between the two cis(0) isomers was accomplished using pmr spectroscopy.

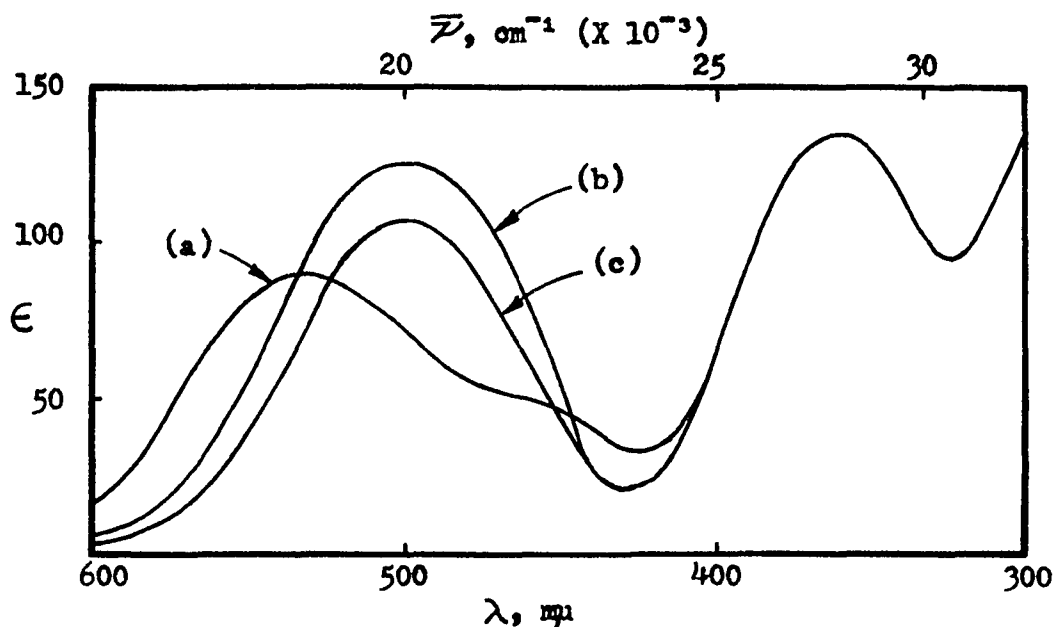


Figure 7. Electronic Absorption spectra of $[\text{Co}(\text{gly})_2(\text{en})]^+$. (a) $\text{trans}(\text{O})\text{C}_2$ $[\text{Co}(\text{gly})_2(\text{en})]^+$, (b) β $\text{cis}(\text{O})\text{C}_1$ $[\text{Co}(\text{gly})_2(\text{en})]^+$, (c) α $\text{cis}(\text{O})\text{C}_2$ $[\text{Co}(\text{gly})_2(\text{en})]^+$.

The analyses and some physical properties of the compounds synthesized in this work are contained in Tables I, II and III. In some cases nitrogen analyses deviate from the calculated value by 0.1-1.1% but there is little doubt about the composition of these materials on the basis of other measurements such as pmr and glc of the amino acid ligands. No analyses appear for the XV, (+)₅₈, $\text{trans}(\text{O})\text{C}_2$ $[\text{Co}(\text{S-thr})_2(\text{en})]^+$ or XXIII, (+)₅₈, α $\text{cis}(\text{O})\text{C}_2$ $[\text{Co}(\text{S-ala})_2(\text{en})]^+$ cations. Proton magnetic resonance spectroscopy uv-visible absorption of the complexes and in some cases glc of the amino acid ligands established that the structures are as assigned in the tables.

TABLE I

Analyses of Some Cobalt(III)-Amino Acid Complexes

Compound	Elemental Analysis %		
	C	H	N
I, ^a (+) ₅₈ , <u>trans</u> (O)C ₂ [Co(gly) ₂ (en)] I·H ₂ O [CoC ₈ H ₁₆ O ₄ N ₄] I·H ₂ O	Calcd. 17.49	4.40	13.60
	Found 17.58	4.69	13.49
II, ^a (+) ₅₈ , <u>β cis</u> (O)C ₁ [Co(gly) ₂ (en)] I·2H ₂ O [CoC ₈ H ₁₆ O ₄ N ₄] I·2H ₂ O	16.76	4.69	12.89
	16.42	4.68	12.89
III, ^a (+) ₅₈ , <u>α cis</u> (O)C ₂ [Co(gly) ₂ (en)] Cl·H ₂ O [CoC ₈ H ₁₆ O ₄ N ₄] Cl·H ₂ O	22.47	5.66	17.47
	22.47	5.37	17.54
IV, (+) ₅₈ , [Co(gly)(en) ₂] Cl ₂ [CoC ₈ H ₂₀ O ₄ N ₄] Cl ₂	22.30	6.18	21.70
	22.47	6.32	22.00
XII, (-) ₅₈ , <u>trans</u> (O) [Co(S-thr)(gly)(en)] Cl·H ₂ O [CoC ₈ H ₂₀ O ₄ N ₄] Cl·H ₂ O	26.30	6.04	15.39
	26.15	6.15	15.35
XIII, (+) ₅₈ , <u>trans</u> (O) [Co(S-thr)(gly)(en)] I [CoC ₈ H ₂₀ O ₄ N ₄] I	21.90	5.02	12.80
	21.90	5.41	13.31
XIV, (-) ₅₈ , <u>trans</u> (O) [Co(S-thr) ₂ (en)] ClO ₄ [CoC ₁₀ H ₂₄ O ₄ N ₄] ClO ₄	26.40	5.27	12.30
	26.44	5.35	11.70
XVI, (-) ₅₈ , <u>trans</u> (O) [Co(S-ser)(gly)(en)] Cl·H ₂ O [CoC ₇ H ₁₈ O ₄ N ₄] Cl·H ₂ O	24.00	5.73	16.02
	23.92	5.84	16.12
XVII, (+) ₅₈ , <u>trans</u> (O) [Co(S-ser)(gly)(en)] I·H ₂ O [CoC ₇ H ₁₈ O ₄ N ₄] I·H ₂ O	19.00	4.54	12.70
	19.17	4.63	13.89
XVIII, (+) ₅₈ , <u>trans</u> (O) [Co(S-ser) ₂ (en)] Cl [CoC ₈ H ₂₀ O ₄ N ₄] Cl	26.50	5.52	15.50
	26.89	5.61	14.60

TABLE I (continued)

Compound	Elemental Analysis %		
	C	H	N
XIX, (+) ₅₈ , <u>trans</u> (O) [Co(S-ala) ₂ (en)] ClO ₄ CoC ₈ H ₂₀ O ₄ N ₄ ClO ₄	Calcd. Found	24.39 24.46	5.07 5.27
			14.20 14.13
XX, (-) ₅₈ , <u>trans</u> (O) [Co(S-ala) ₂ (en)] ClO ₄ CoC ₈ H ₂₀ O ₄ N ₄ ClO ₄	24.39 24.41	5.07 5.32	14.20 14.21
XXI, (+) ₅₈ , <u>β-cis</u> (O) [Co(S-ala) ₂ (en)] Cl·0.5H ₂ O CoC ₈ H ₂₀ O ₄ N ₄ Cl·0.5H ₂ O	28.38 28.72	6.34 6.46	16.62 15.58
XXII, (-) ₅₈ , <u>β-cis</u> (O) [Co(S-ala) ₂ (en)] ClO ₄ ·0.5H ₂ O CoC ₈ H ₂₀ O ₄ N ₄ ClO ₄ ·0.5H ₂ O	23.75 23.73	5.20 5.02	13.89 13.91

^aAnalysis of the (-)₅₈ enantiomer is within acceptable limits ($\pm 0.3\%$).

TABLE II

Electronic Absorption and Molar Rotation of
Some Cobalt(III)-Amino Acid Complexes

Compound ^a	Absorption ^b		
	$\bar{\nu}$, cm ⁻¹	ϵ_{\max}	$[\text{M}]_{589}$
I ^c	18,800	90	+3,310
	22,200 sh	45	
	27,900	135	
II ^c	19,900	125	+2,550
	27,900	138	
III ^c	19,900	106	+2,730
	27,900	142	
IV	20,500	99 ^d	+3,290
	28,900	109 ^d	
XII	18,900	89	-3,990
	22,200 sh	48	
	27,600	140	
XIII	18,900	90	+3,100
	22,200 sh	46	
	27,600	142	
XIV	18,900	85	-4,150
	22,200 sh	51	
	27,600	161	
XV, (+) ₅₈₉ , trans(O)[Co(S-thr) ₂ (en)] ⁺	18,900		+
	22,200 sh		
	27,600		
XVI	18,900	90	-3,660
	22,200 sh	48	
	27,600	142	
XVII	18,900	90	-2,650
	22,200 sh	45	
	27,600	160	
XVIII	18,900	90	+2,810
	22,200 sh	45	
	27,600	153	
XIX	18,800	96	+3,010
	22,200 sh	51	
	27,900	153	

TABLE II (continued)

Compound	$\bar{\nu}$, cm^{-1}	Absorption ϵ_{max}	$[\text{M}]_{\text{ss}}$
XX	18,800	96	-3,140
	22,200	50	
	27,900	141	
XXI	19,900	138	+2,000
	27,900	142	
XXII	19,900	137	-2,940
	27,900	142	
XXIII, (+) _{ss} , \propto <u>cis</u> (O) [Co(S-ala) ₂ (en)] ⁺	19,900		+
	27,900		

^aStructures of the compounds are found in Table I, page 32. ^bWhere sh = shoulder. ^cRotation of the enantiomer is within $\pm 1\%$ of the value for this isomer. ^dAs tabulated in reference 11.

TABLE III

Electronic Absorption and Circular Dichroism Data
for the $[\text{Co}(\text{gly})_2(\text{en})]^+ \text{ Ion}$

Compound ^a	Absorption ^b $\bar{\nu}, \text{cm}^{-1}$	Circular Dichroism $\bar{\nu}, \text{cm}^{-1}$ $\Delta\epsilon_{\text{max}}$
I	18,800	+1.98
	22,200 sh	+0.66
	27,900	-0.41
II	19,900	+1.92
	27,900	+0.08
	27,800	-0.11
	30,400	+0.14
III	19,900	+2.90
	22,500	-0.30
	27,400	-0.07
	30,200	+0.10

^aStructures of the complexes are found in Figure 4, page 6 and Table I, page 32.^bThe $\Delta\epsilon$ values for the enantiomers are within $\pm 1\%$ of the values of the (+)₅₀ isomers tabulated. Where sh = shoulder.

The problem of distinguishing between the cis(O) isomers of the bis(glycinato)ethylenediaminecobalt(III) cation was accomplished using pmr. The presence of a C_2 symmetry axis in the α -cis(O) C_2 isomers makes each of the coordinated glycine molecules chemically identical. This is evident from the pmr spectrum of the complex which exhibits only two singlets - one for the methylene ($-CH_2-$) hydrogens of both the amino acids and the other for the ethylenediamine backbone, $-CH_2CH_2-$ (Table IV). However in the β -cis(O) C_1 isomer which is void of any elements of symmetry the amino acid molecules are not equivalent and two glycine methylene resonances are expected and found. The individual methylene units of the ethylenediamine backbone are also chemically different which is shown by the complicated resonance at 2.72 and 2.86 ppm.

The pmr data given in Tables IV-VIII were obtained in D_2O with DSS (2,2 dimethyl-2-silapentane-5-sulfonate sodium salt) as an internal standard. The NH_2 protons were exchanged to facilitate the spectral interpretation (see experimental section, page 22). Structures for these compounds appear in Table I (page 32) of this work. Values for the chemical shifts are in ppm from DSS followed by the splitting pattern, s = singlet, d = doublet, t = triplet, q = quartet, o = octet, m = multiplet, u = unsymmetrical.

TABLE IV

Proton Magnetic Resonance Spectra of Some Cobalt(III)-glycine Complexes

Complex	en- CH_2CH_2-	gly- CH_2-
I (racemate)	2.64s	3.61s
II "	2.72m; 2.86m	3.44s, 3.61s
III "	2.62s	3.53s
IV "	2.86m	3.63s

The pmr data for the complexes containing S-threonine and S-serine are given in Tables V and VI respectively. The chemical shifts are assigned on the basis of the integrated values of the peaks to-

gether with comparison of pmr spectra of known compounds. These compounds have trans oxygen atoms as is evident from their uv-visible absorption spectra. Three of the compounds have a C_2 symmetry axis making the coordinated amino acids identical. It is interesting to note that the methylene protons of the glycine are no longer equivalent in the mixed S-threoninato complexes and exhibit a doublet, which is probably the most intense portion of a simple AB pattern. However this is not the case for serine, where a singlet is observed for the methylene hydrogens in glycine.

TABLE V

Proton Magnetic Resonance Spectra of Some Cobalt(III)-S-threonine Complexes

Compound ^b	Chemical Shifts ^a			
	en-CH ₂ CH ₂ -	gly-CH ₂ - ^c	$\begin{array}{c} \diagup \text{c} \\ \text{-CH-} \\ \diagdown \end{array}$	S-threonine $\begin{array}{c} \text{-CH-} \\ \diagdown \text{e,d} \end{array}$ -CH ₃ ^d
XII	2.87s	3.64d	3.75d	1.44d
XIII	2.89s	3.63d	3.63d	1.47d
XIV	2.90s	-	3.77d	1.46d
XV ^e	2.94s	-	3.58d	1.43d

^aWhere s = singlet, d = doublet, o = octet. ^bThe structures of the complexes are found in Table I, page 32. ^cCoupling constant 1.0 Hz. ^dCoupling constant 7.0 Hz. ^eStructure of this compound is found in Table II, page 34.

TABLE VI

Proton Magnetic Resonance Spectra of Some Cobalt(III) S-serine Complexes

Compound ^b	Chemical shifts ^a			
	en-CH ₂ CH ₂	gly-CH ₂	S-serine	
			-CH	-CH ₂ -
XVI	2.84s	3.60s	4.03s	4.03s
XVII	2.84s	3.59s	3.85ut ^c	4.03ud ^c
XVIII	2.89s	-	3.82ut ^c	4.00ud ^c

^aWhere s = singlet, d = doublet, t = triplet, u = unsymmetrical. ^bThe structures of the compounds are found in Table I, page 32. ^cCoupling constant 2.0 Hz.

The pmr data for 5 of the 6 diastereomers of bis(S-alaninato) ethylenediaminecobalt(III) cation are contained in Table VI. Assignments of the α -cis(O)C₂ and β -cis(O)C₁ isomers were made by taking advantage of the presence of a C₂ symmetry axis in the former. The amino acids are chemically identical producing one methyl doublet and one methine (-CH) quartet. The β -cis(O)C₁ isomers give more complex spectra due to the non-equivalence of the amino acids.

TABLE VII

Proton Magnetic Resonance Spectra of the Isomers of the Bis(S-alaninato) ethylenediaminecobalt(III) Ion

Compound ^b	en-CH ₂ CH ₂ -	Chemical Shifts ^a	
		-CH ^c	S-alanine -CH ₃ ^c
XIX	2.83s	3.61q	1.55d
	2.85s	3.81q	1.53d
XX	2.71m	3.69q	1.50d
	2.82m	3.66q	1.48d
XXI	2.71m	3.68q	1.46d
	2.84m	3.61q	1.41d
XXII ^d	2.71s	3.86q	1.50d

^aWhere s = singlet, d = doublet, q = quartet, m = multiplet. ^bThe structures of the complexes are found in Table I, page 32. ^cCoupling constant = 7.0 Hz. ^dThe structure of this compound is found in Table II, page 34.

B. The Absolute Configuration of the Complexes

1. The Absolute Configuration as Determined by Circular Dichroism

The volume of material written on the subject of determining the absolute arrangement of ligands in coordination compounds using circular dichroism is substantial²³. Beginning with the discovery of quantum mechanics and later developments of the crystal-field and molecular orbital theories the number of theoretical approaches to the origins of optical activity in such systems rapidly grew. However, it was not until 1957 when x-ray diffraction methods unequivocally established the absolute configuration of the first coordination compound, (+)₈₈, tris(ethylenediamine)cobalt(III) chloride²⁴ that a standard was created on the basis of which theories could be accepted or rejected. Since then the number of x-ray determinations has increased and in the process reinforced the one-electron theory developed by E.U. Condon, W. Altar and H. Eyring²⁵. This theory employs a model composed of a single electron moving in a suitably dissymmetric field to produce optical rotatory power. With the aid of ligand-field theory to explain the origin of the absorption bands in metal complexes, the one-electron theory was modified by Moffitt²⁶ to predict the absolute configuration of ligands in certain cobalt(III) and chromium(III) metal complexes. In the past eight years S.F. Mason²³ using Moffitt's approach has assigned structures to a large number of coordination compounds.

Mason's analysis of the tris(ethylenediamine)cobalt(III) ion and other ions with D₃ or psuedo D₃ symmetry is well documented²⁷. As is discussed on page 30, the visible absorption spectra for low spin octahedral cobalt(III) complexes contains two spin allowed d-d transitions ($A_{1g} \rightarrow T_{1g}$ and $A_{1g} \rightarrow T_{2g}$). The first excited state for cobalt(III) in D₃ symmetry splits into E_g and A₂ symmetry states^{22, 28}. Ideally electronic transitions from the ground state, A_{1g} to these two excited states should result in an absorption band which should be unsymmetrical indicating the presence of two excited states under the curve envelope. However, it is evident from Figure 8 that the lowest energy absorption band (centered at 21,400 cm⁻¹) is quite symmetrical. This

is due to the small energy separation ($50\text{--}150\text{ cm}^{-1}$)²⁷ between the two states (E_g and A_g) which only results in broadening of the band. Fortunately resolution of the two states can be achieved using circular dichroism which mathematically is better suited for resolving closely lying energy levels than is unpolarized absorption²⁹.

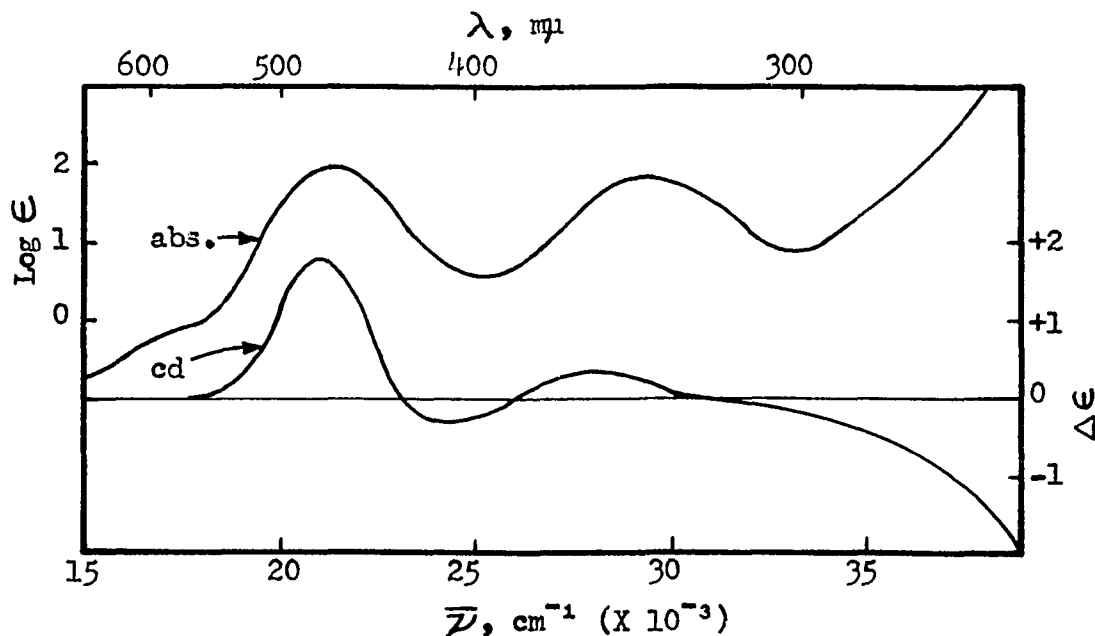


Figure 8. Absorption and cd spectra of $(+)_55$, $[\text{Co}(\text{en})_3]^{+++}$.

Mason's analysis of tris(bidentate)cobalt(III) molecules reduced to its simplest elements is concerned with locating the cd absorption band which is due to the $A_{1g} \rightarrow E_g$ electronic transition and determining its sign²⁷. The sign is then related to the absolute configuration of chelate rings of the complex. Circular dichroism spectra of a single crystal of this complex showed the lowest energy cd band to be the $A_{1g} \rightarrow E_g$ transition. This band is positive in sign and on this basis all complexes with positive $A_{1g} \rightarrow E_g$ transition are assigned the $\Lambda(C_3)$ configuration for their bidentate chelate rings. When this transition is negative the complex will have a $\Delta(C_3)$ arrangement of chelate rings. X-ray determination of the $(+)_55$, tris(ethylenediamine)cobalt(III) isomer²⁴ showed that it had the arrangement of rings shown in Figure 9 and was assigned the $\Lambda(C_3)$ absolute configuration.

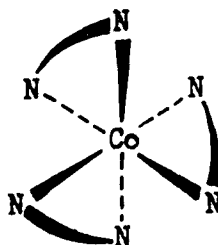


Figure 9. The absolute configuration of (+)₅₈, tris(ethylenediamine) cobalt(III).

Wentworth and Piper³⁰ have studied the trans $[\text{CoCl}_2(\text{R-pn})_2]^+$ and trans $[\text{Co}(\text{NO}_2)_2(\text{R-pn})_2]^+$ complexes (R-pn is R-propylenediamine). These complexes belong to the C_2 point group but the symmetry experienced by the cobalt(III) ion is D_{3h} if only the donor atoms are considered (chromophoric symmetry). This is reasonable since the atoms most directly affecting the electronic system of the metal are those of the donor atoms - ligand backbone atoms playing a minor role. The energy level diagrams for low spin cobalt(III) complexes in D_{3h} symmetry is given in Figure 10^{22, 23}.

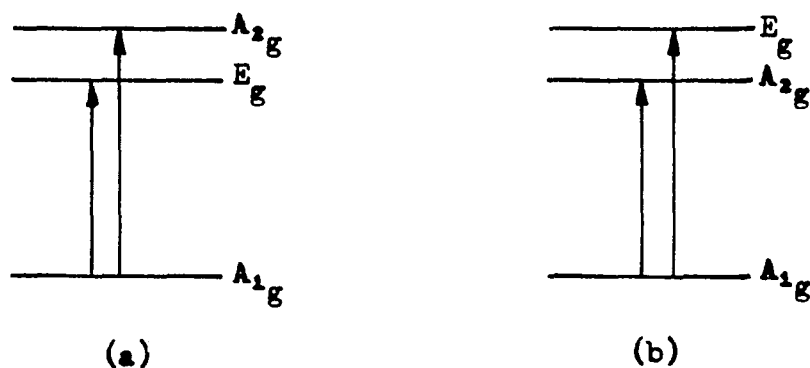


Figure 10. Energy levels of the first spin allowed d-d absorption band of cobalt(III) N_4X_2 for D_{3h} symmetry. (a) $Dq-X$ less than $Dq-N$. (b) $Dq-X$ greater than $Dq-N$.

In D_{3h} complexes two transitions in the first spin allowed region of the visible spectra should be present, $A_{1g} \rightarrow E_g$ and $A_{1g} \rightarrow A_{2g}$. In

trans $[\text{CoCl}_2(\text{R-pn})_2]^+$ Wentworth and Piper³⁰ observed two bands of opposite sign in the cd of the first absorption region. Because Dq-Cl is less than Dq-NH_2 , these workers have assigned the longer and shorter wavelength components to transition E_g and A_{2g} respectively. The sign of the two bands are reversed with trans $[\text{Co}(\text{NO}_2)_2(\text{R-pn})_2]^+$ which is in agreement with theory since Dq-NO_2 is greater than Dq-NH_2 .

The visible absorption and cd spectra for $(+)_\text{ss}$, trans(O) C_2 $[\text{Co}(\text{en})(\text{gly})_2]^+$ are shown in Figure 11.

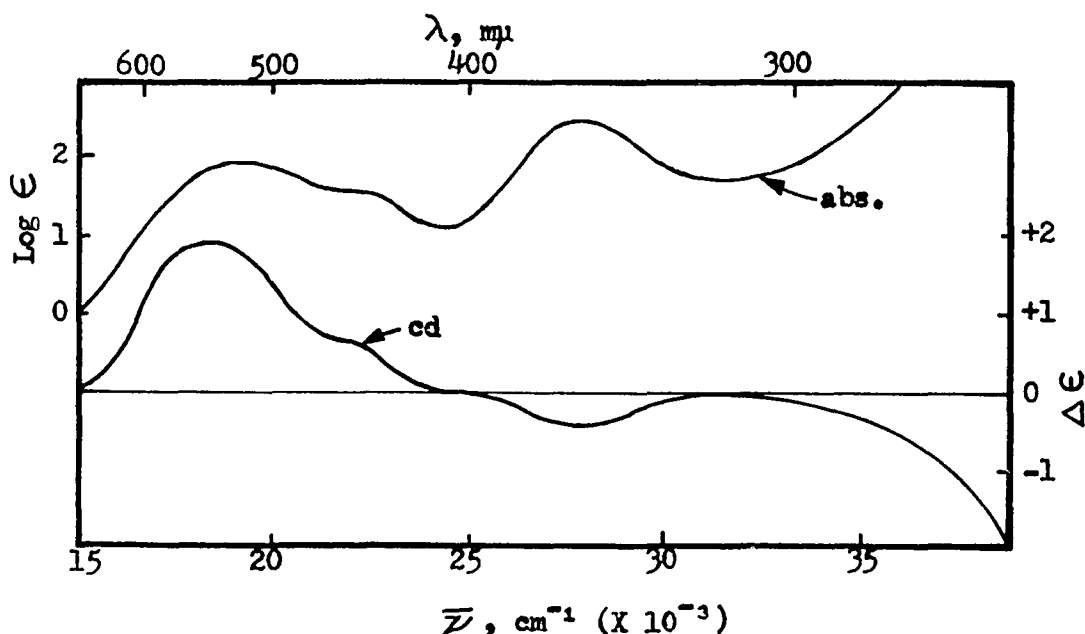


Figure 11. Absorption and cd spectrum of $(+)_\text{ss}$, trans(O) C_2 $[\text{Co}(\text{gly})_2(\text{en})]^+$

This complex is psuedo D_{4h} in symmetry and the absorption spectrum exhibits a transition with a shoulder in the first absorption region indicative of the $\text{A}_{1g} \rightarrow \text{E}_g$ and $\text{A}_{1g} \rightarrow \text{A}_{2g}$ transitions. Two transitions are also indicated in the same region of the cd spectra of the complex. In this case they both have a positive sign with the most dominant one centered at $18,300 \text{ cm}^{-1}$ probably corresponding to the E_g transition. The absolute configuration of this complex can be assigned using the reasoning of Mason²⁷. $\wedge(\text{C}_3) [\text{Co}(\text{en})_3]^+$ has the same configuration of the bidentate rings as $\wedge(\text{C}_3)$ trans(O) C_2 $[\text{Co}(\text{gly})_2(\text{en})]^+$ because the $\text{A} \rightarrow \text{E}_g$ transition is positive for both complexes.

Figure 11 shows that the cd maximum is shifted to lower wavelength than the absorption curve which is in tacit agreement with

W. Moffitt and A. Mowcowitz³¹ for systems of this type. Since the cd curves envelope contains the O-O transition band, whereas the absorption envelope begins with the I-O band, a shift of about 10 mμ to lower wavelength is expected for the latter.

Unfortunately the analysis of the $\beta_{\text{cis}}(0)C_1$ and $\alpha_{\text{cis}}(0)C_2$ isomers is not as clear as the previous case. Mason *et al.*³² studied a series of optically active cis $[\text{Co}(\text{X}_2)(\text{en})_2]^+$ cases where X was a monodentate ligand giving rise to a chromophoric symmetry of C_{2v} . If only the donor atoms are considered for the cis isomers of this work, II, III, page 6, the symmetry is also C_{2v} . Figure 12 shows the energy level diagram for this symmetry.

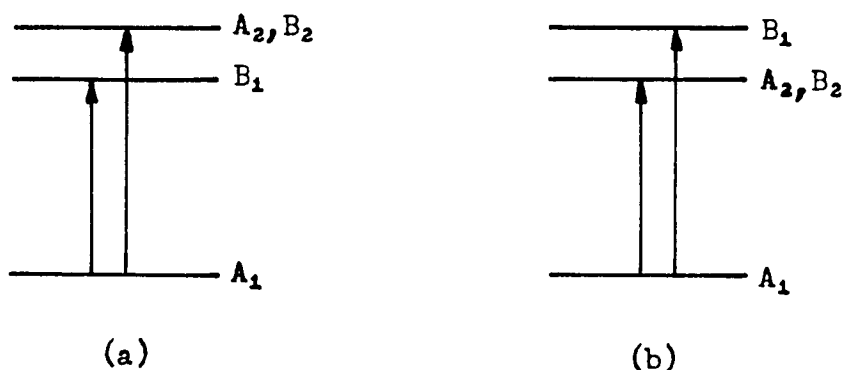


Figure 12. The energy levels of the first spin allowed d-d transition band of $\text{Co}(\text{N}_4\text{X}_2)$ for C_{2v} symmetry. (a) $Dq-X$ less than $Dq-N$. (b) $Dq-X$ greater than $Dq-N$.

Note that an excited state of E symmetry is not present in Figure 12 but group theoretical considerations indicate the A_2, B_2 states in C_{2v} symmetry correspond to the E_g level in D_{4h} . Therefore if this transition (A_2, B_2) is positive the complex has the $\wedge(C_3)$ configuration. Mason further observed that the area of the cd envelope corresponding to the transition to the A_2, B_2 states was greater than that for the B_1 state. On this basis it is evident from Figure 13 that the A_2, B_2 states are the lower and B_1 the higher energy transition state for the two cis isomers (considering only the absorption band centered at $20,000 \text{ cm}^{-1}$).

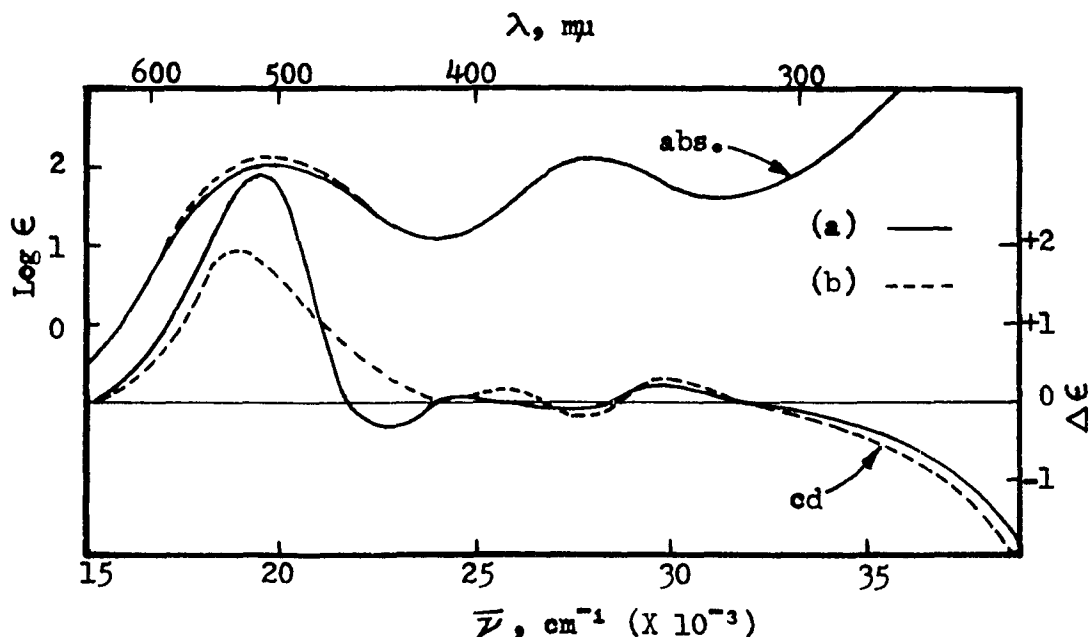


Figure 13. Absorption and cd spectra of the cis(O) isomers of $[\text{Co}(\text{gly})_2(\text{en})]^+$. (a) $(+)_\text{ss}$, α cis(O) C_2 $[\text{Co}(\text{gly})_2(\text{en})]^+$. (b) $(+)_\text{ss}$, β cis(O) C_1 $[\text{Co}(\text{gly})_2(\text{en})]^+$.

However this result does not agree with the fact that Dq for acetate is less than Dq for the amine nitrogens and according to Figure 12, page 47, B_1 should be the lower energy state instead of A_2, B_2 if C_{2v} symmetry is to be considered.

A similar problem was encountered by Mason *et al.*³² when the X ligands in the cis $[\text{Co}(\text{X}_2)(\text{en})_2]^+$ compounds which they studied were bidentate instead of monodentate. Thus $(+)_\text{ss}$, $[\text{CoCO}_3(\text{en})_2]^+$ and $(+)_\text{ss}$, $[\text{Co}(\text{ox})(\text{en})_2]^+$ (where ox = oxalate) having positive unsymmetrical cd bands with a shoulder on the high energy side were assigned the $\Lambda(C_3)$ configuration. This band was assumed to be dominated by the more intense A_2, B_2 transitions for these assignments. Because of the similarity in Dq of the carbonate and oxalate to the carboxylate portion of the amino acid and also the failure of the previously mentioned monodentate analysis using C_{2v} symmetry, the $(+)_\text{ss}$, β cis(O) C_1 $[\text{Co}(\text{gly})_2(\text{en})]^+$ and $(+)_\text{ss}$, α cis(O) C_2 $[\text{Co}(\text{gly})_2(\text{en})]^+$ cations are assigned the $\Lambda(C_3)$ configuration II, III, page 6, on the basis of the sign of the dominant cd band for the first spin allowed d-d transition.

Further work by Mason and coworkers³³ has shown that the high energy

charge-transfer (c-t) band can be utilized to determine the absolute configuration of tris(bidentate) complexes. Complexes with $\wedge(C_3)$ configuration were found to have a negative cd absorption in the c-t region. The signs of the bands centered at $48,000\text{ cm}^{-1}$ for the bis(glycinato)ethylenediamine series are also in agreement with this assignment.

The assignment of absolute configuration of the remaining complexes in Table I, page 32, with the exception of IV was accomplished by comparing the ord curves with the corresponding curves for the bis(glycinato) compound. Table II gives the sign of rotation at the sodium D line which is also the sign of the dominant Cotton effect for the first spin allowed d-d transition. Thus all of the $(+)_{588}$, trans(O) bis(amino acid) complexes in Table I have the $\wedge(C_3)$ configuration. The same reasoning was applied to the cis(O) bis(S-alaninato) compounds which are also $\wedge(C_3)$ for the $(+)_{588}$ isomers. For all of these assignments the vicinal effect of the coordinated amino acid was found to be only a minor contribution to the total optical activity of the complex. The configuration of $(+)_{588}$, $[\text{Co}(\text{gly})(\text{en})_2]^{++}$ IV, page 6, was assigned by Douglas et al¹¹ using cd and is $\wedge(C_3)$.

2. The Absolute Configuration as Determined by Proton Magnetic Resonance Spectroscopy

The most definitive method of assigning the absolute configuration of a coordination compound is by x-ray diffraction³⁴. Historically, due to the relatively high cost of the equipment and the time consuming nature of the analysis, x-ray determinations of structures have not been widespread. More recently the use of computers has considerably shortened the time but the cost has continued to keep utility down. Circular dichroism offers relief from these disadvantage but often the interpretation of the data with regards to structure is more difficult and less certain than x-ray analysis. A recent and most important addition to structure elucidation of coordination compounds is proton magnetic resonance spectroscopy which when combined with cd can sometimes yield information leading to the assignment of absolute configuration^{35, 36}.

In coordination compounds containing organic ligands, there are several ways in which the electronic environment about a proton can be influenced by intramolecular interactions. These include anisotropy effects from bonds within the molecules³⁷, involvement of the d-electron system of the metal with ligand substituents³⁸, and van der Waals interactions or steric compressions³⁹. The last mentioned effect was very useful in assigning the absolute configurations of the trans(0) compound synthesized in this work³⁵.

Steric compression has been observed in certain organic compounds wherein hydrogen atoms within the molecule are forced, because of rigid secondary structure, into proximity of some other atom in the molecule⁴⁰. The affected proton experiences a deshielding effect which is manifested in a pmr chemical shift toward lower field than when the compression is absent. The reduction in field experienced by the two nuclei involved is proportional to the square of the electric fields, E , exerted by the two hydrogen atoms⁴¹. Furthermore E^2 is inversely proportional to the sixth power of the distance separating the two nuclei. Thus when the nuclei are separated by 2.4 and 2.0 and 1.7 Å the deshielding at both nuclei is 0.01, 0.2 and 0.5 ppm respectively.

In the trans(0) compounds prepared for this study certain diastereomeric pairs exist such that one member of the pair contains protons which exhibit compression, while with the other isomer no compression exists. The separation of the compression atoms varies and is dependent primarily on the conformations of the amino acid chelate rings. For the analysis, the R-group of the coordinated S-amino acids is assumed to form an angle of about 35° with the plane containing the metal atom and the two donor atoms of the amino acid. This forces the ring to be puckered with the large bulky R group located closer to the plane than the α proton. This seems reasonable since studies with the coordinated propylenediamine indicate that the methyl group is equatorially situated⁴². If the amino acid chelate rings were not puckered but instead planar, the R group would make an angle of 55° with the plane. The distances between the affected protons in the diastereomers exhibiting compression is of the order

of 2 Å as opposed to 2.5-3.0 Å for those isomers free of the interaction.

Figure 14, page 52, shows the structures of the trans(0) compounds of interest. For the (+)_{ss}, and (-)_{ss}, isomers of the bis(S-alaninato) ethylenediaminecobalt(III) ions the R groups of both amino acids in Figure 14 are methyl groups. The absolute configuration of S-alanine is known and thus the structure of the $\wedge(C_3)$ and $\Delta(C_3)$ isomers of the complex must correspond to the structures in Figure 14.

The α proton on the coordinated alanine is the group of interest in terms of the steric compression. In both the \wedge and Δ isomers this α proton is adjacent to an amine proton. It is true that these amines are different (in the \wedge complex this is an ethylenediamine amine while in the Δ complex it is an amino acid amine) but the arrangement of groups about the coordinated nitrogen atoms are similar. Molecular models show that in Figure 14 (b) the α proton of the coordinated alanine is closer to an amine hydrogen atom than is the same proton in Figure 14 (a). On this basis structure (b) should exhibit the steric compression or van der Waals interactions causing the α -proton to resonate at a lower field than the corresponding proton for structure (a).

TABLE VIII

Proton Magnetic Resonance Spectra of Some trans(0) -Bis(amino acid) Cobalt(III) Complexes

Compound	α -CH Signal of Complex With	
	(-) _{ss} , ^a	(+) _{ss}
<u>trans</u> (0) [Co(S-ala) ₂ (en)] ⁺	3.81	3.61
<u>trans</u> (0) [Co(S-thr)(gly)(en)] ⁺	3.75	3.63
<u>trans</u> (0) [Co(S-thr) ₂ (en)] ⁺	3.77	3.58
<u>trans</u> (0) [Co(S-ser)(gly)(en)] ⁺	4.03	3.85

^aAll complexes assigned $\Delta(C_3)$ configuration.

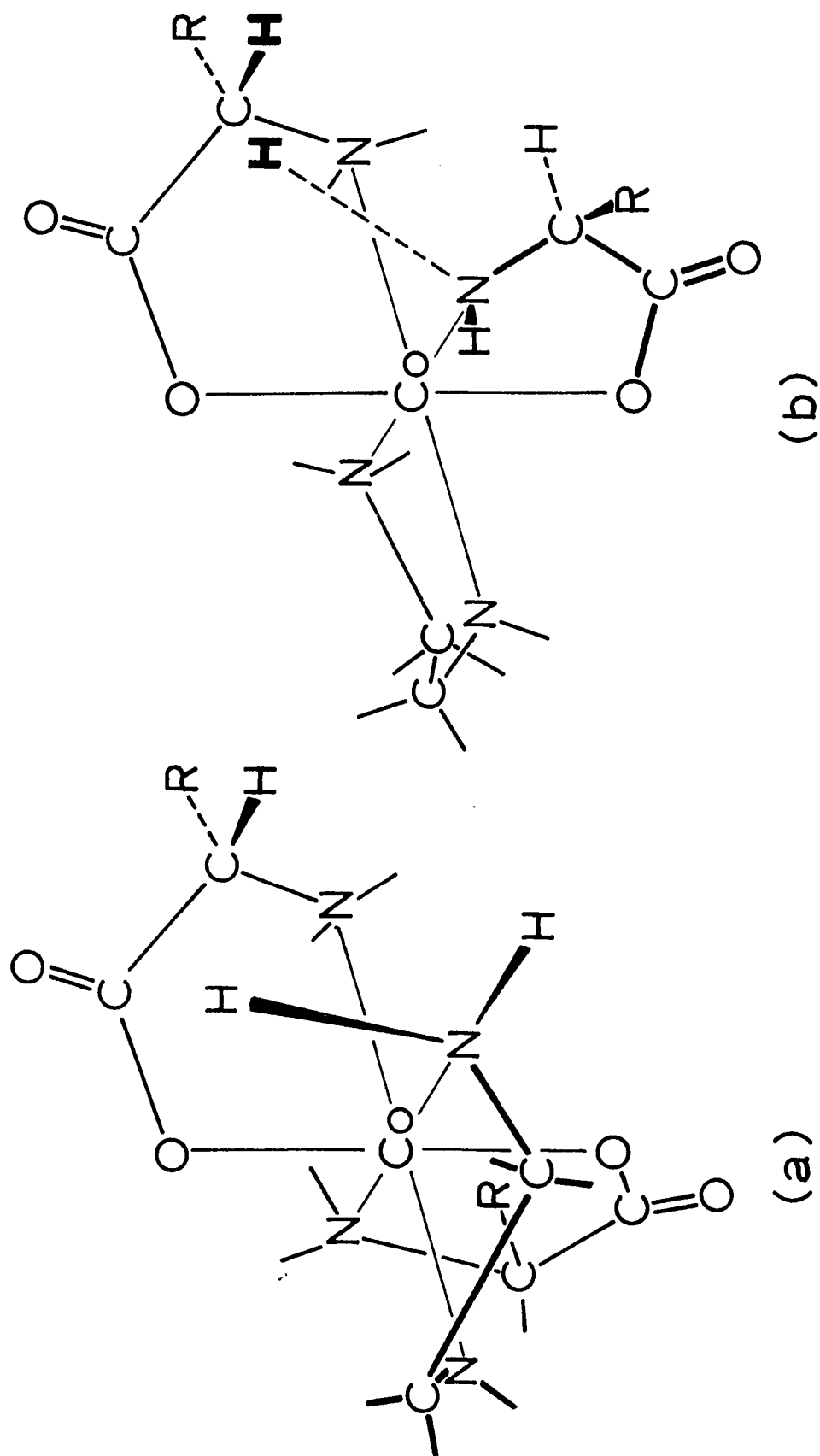


Figure 14. Structure of the trans(0) bis(amino acid)ethylenediaminecobalt(III) ions showing steric compression. (a) $\wedge(C_3)$, (b) $\Delta(C_3)$. Where R is $-CH_3$, $-CH_2OH$ or $-HCH_2OH$ depending on the complex. The bond lengths have been exaggerated to show the compression. The atoms involved in the compression are in bold type.

From Table VIII it can be seen that in the $(-)_58$, isomer the α proton resonates at a lower field, 3.81 ppm, than the $(+)_58$, isomer, 3.61 ppm, and therefore it is assigned structure (b) which is $\Delta(C_3)$. This isomer has a negative Cotton effect in the ord and a negative A \rightarrow E cd band. The $(+)_58$, isomer which has opposite effects and no compression must therefore have the $\Lambda(C_3)$ configuration. Once the absolute configuration and the sign of the Cotton effect for these compounds are known it is possible to assign the configuration of the other trans(0) bis(amino acid) complexes in this work on the basis of their Cotton effects alone. This assumes that the contribution to the total optical activity of the complex from the asymmetric center in the amino acid is minimal. Thus any trans(0) complex including the bis(glycinato) case which has a dominant (+) Cotton effect for the first spin allowed d-d transition region has a $\Lambda(C_3)$ configuration of ligands. It is satisfying to note that all the $(-)_58$, trans(0) compounds in Table VIII have α -proton resonances at lower field than their $(+)_58$, counterparts.

Application of steric compression analysis to the other geometric isomers of the bis(S-alaninato) series is more complex. The β cis(0) C_1 isomers are particularly difficult to analyze because of the non-equivalence of the coordinated amino acids. However assuming that the cd analysis of these compounds is correct and that the $(+)_58$, isomer has the $\Lambda(C_3)$ configuration some generalizations can be made. Molecular models show that in this isomer the α proton of both amino acids are in similar but not identical environments. Both are close to amine nitrogen terminal groups and therefore should have similar chemical environments. In the $\Delta(C_3)$ isomer on the other hand the α protons are in different environments, one being near an amine nitrogen atom while the other is closest to a carboxyl oxygen atom. The data in Table VII, page 42, shows there is greater difference in the chemical shifts of the α protons for the $\Delta(C_3)$ isomer, XXII than the $\Lambda(C_3)$ compound XXI which is in agreement with the above analysis.

Because of the failure to characterize the $(+)_58$, α cis(0) C_2 bis(S-alaninato) compound, steric compression analysis for these two diastereomers was not possible. In the α cis(0) C_2 geometry the α protons

of the amino acids are near the amino acid amine protons for the $\Delta(C_3)$ diastereomer and over carboxyl groups for the $\Lambda(C_3)$ isomer. Certainly the electric field of the one electron pairs of the coordinated oxygen atoms can exhibit a deshielding effect on nuclei near them³⁹. However this effect and its variance with distance is not yet well understood making the assignment of the two diastereomers using pmr steric compression difficult.

The utility of this type of pmr analysis to determine absolute configuration is dependent upon the presence of an optically active center in the molecule, the configuration of which is known with certainty. By measuring the chemical shifts of certain protons, provided these resonances are accessible and not obscured by other signals from the molecule, reconstruction of the ring system making up the optically active complex can be done. This approach has been applied to compounds in the literature with good results³⁵. It has even been used for conformational analysis of some trans trans diacetatobis(N-methylethylenediamine)cobalt(III) systems³⁵.

C. Gas-Liquid Chromatographic Analysis of the Amino Acids

1. The Optical Isomers of Threonine and Allothreonine

Because of the scarcity of some of the optically active starting complexes, (0.3-0.8 g) and also because it was desirable to terminate the reaction early to avoid additional side reactions it became apparent early in the study that a sensitive method must be developed to detect the relative amounts of R- and S-threonine and R- and S-allothreonine produced from the reaction of acetaldehyde and coordinated glycine. A rough calculation indicated that if 100 mg of any of the bis(glycinato) compounds was used in the reaction and the reaction terminated after 30% of the complex molecules had reacted with the aldehyde, only 6-10 mg of threonine and allothreonine would be synthesized. Clearly with any of the classical methods such as thin layer, paper or ion-exchange chromatography combined with polarimetry the task would have been very difficult at best.

In 1966 B. Halpern and J.W. Westley⁴³ using glc developed a rapid

5% RS dipeptides would be generated. Reaction with the remaining 75% of the molecules with the R configuration would give 60% SR and 15% RR. Since SS and RR are enantiomers as are RS and SR, they cannot be distinguished by the column and only two signals would appear with areas of $60 + 5 = 65\%$ and $15 + 20 = 35\%$ instead of 75% R and 25% S. If the amount of racemization of the acid chloride is known the isomeric composition of the original amino acid can with some effort be found. The analysis would require knowledge of the amount of each optical isomer present in the acid chloride. However, this calculation becomes more complex for the case of threonine-allothreonine mixtures.

The derivatization procedure for the diastereomeric hydroxy-amino acids threonine and allothreonine in addition to Equation 9 and 10 involved trimethyl silylating (TMS) the amino acid methyl esters with bis-N(trimethylsilyl)trifluoroacetamide (BSTFA) before reacting with S-XXV. A racemic mixture of R- and S-threonine and of R- and S-allothreonine gave two equal signals using glc when derivatized in the above manner confirming that the two diastereomers have identical response constants. When a mixture containing equal amounts of all four of the isomers, R- and S- threonine and R- and S-allothreonine is derivatized a chromatogram is obtained containing not four signals but three signals with ratios of 1:1:2 (Figure 15).

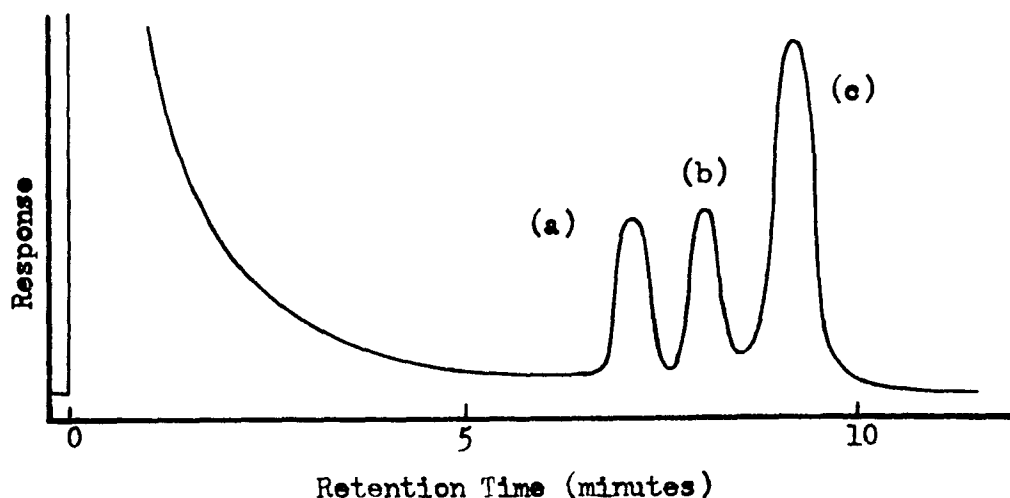


Figure 15. Chromatogram of a 1:1 racemic mixture of threonine and allothreonine using S-proline. (a), SRT; (b), SRa; (c), SSt + SSa. Column 10% w/w DEGS on chromosorb W/AW 80-100 mesh, 1.8 m x 4 mm I.D., glass, 200° isothermal.

The different signals were assigned using authentic samples of each of the amino acid isomers in separate derivatizations with the exception of the signal for S-allothreonine which was assigned by enrichment of racemic allothreonine with R-allothreonine. The signals correspond to the dipeptides S-proline-R-threonine (SRt), S-proline-R-allothreonine (SRa) and S-proline-S-threonine (SSt) plus S-proline-S-allothreonine (SSa) having retention times of 7.2, 8.1 and 9.2 min respectively.

Since the objective was to determine the relative amounts of the optical isomers of threonine and allothreonine it was disappointing that the last two compounds, SSt and SSa had identical retention times. Efforts to resolve these signals using SE-30 and propyleneglycoladipate²³ as liquid phases were unsuccessful. The relative amounts of St and Sa were eventually determined by synthesizing the R-enantiomer of S-XXV (R-XXV) and using it in the derivatization.

If a 1:1 racemic mixture of threonine and allothreonine was used in the R-proline derivatization, a chromatogram identical to that in Figure 15 was found except that all the compounds were enantiomeric to those in the figure. That is the derivatives in order of increasing retention times were R-proline-S-threonine (RSt), R-proline-S-allothreonine (RSa) and R-proline-R-threonine (RRt) plus R-proline-R-allothreonine (RRa). The signals representing S-threonine and S-allothreonine are now resolved into two distinct peaks. By derivatizing a portion of a mixture of unknown composition with S-XXV and a second portion with R-XXV the relative amounts of each optical isomer were found from the relative areas of the first two signals of each chromatogram. Furthermore, the combined relative percentages of the first two signals of the S-proline derivatization were always equal to (within $\pm 2\%$) the relative percent of the third composite signal of the R-proline chromatogram. The same was true of the first two signals of the R-proline chromatogram.

The importance of having optically pure R or S-proline derivatives is now evident. Unlike the previous case involving an amino acid with only one asymmetric center which generated four dipeptides and two signals the threonine-allothreonine mixtures produce 8 dipeptides

under three signals which would be more difficult to handle in a quantitative manner.

The purity of the N-trifluoroacetyl-R-prolyl chloride was determined using R-threonine. The R-threonine was optically pure and any signal besides the RRt signal had to be due to a small amount of the S-proline compound being present. This compound had a retention time identical to RSt but since only R-threonine was present in the sample it must be its mirror image, namely SRt. The same analysis was performed on the N-trifluoroacetyl-S-prolyl chloride. Table IX summarizes the results obtained on mixtures of varying composition of threonine and allothreonine.

TABLE IX

Gas-Liquid Chromatography of Threonine-Allothreonine
TMS-N-trifluoroacetyl R,S-prolyl Methyl Esters ^{a,b,c}

<u>Mole % Added</u>				<u>S-proline Mole % Found</u>			<u>R-proline Mole % Found</u>		
Rt	Ra	St	Sa	Rt	Ra	Sa+St	St	Sa	Rt+Ra
100	-	-	-	99.2	-	0.8 ^d	0.4 ^e	-	99.6
-	50	-	50	-	-	-	-	51	49
50	-	50	-	50	-	50	50	-	50
33	30	27	10	32	31	37	27	10	63
39	31	30	-	39	31	30	30	-	70

^aAll values are the average of at least two injections. For separation conditions see Figure 15, page 56. ^bRt = R-threonine, Ra = R-allothreonine, St = S-threonine, Sa = S-allothreonine. ^cThe areas of the signals were calculated from the product of the peak height and the peak width at half height. ^dAs R-proline-R-threonine. ^eAs S-proline-R-threonine.

The amino acid mixtures obtained from the reactions contained large amounts of glycine which was found not to affect the quantitative nature of the threonine-allothreonine signals. Efforts to check on the quantitative nature of the glycine derivative itself (retention time, 23.5 min) by using mixtures of known composition of threonine,

allothreonine and glycine indicated that the glycine derivative is not quantitative under the conditions used. The correct relative amounts for threonine and allothreonine were always found but not for the glycine signal which varied in a random fashion. This could be due either to the incomplete derivatization for this amino acid or simply due to the fact that it is degraded on the polyester column.

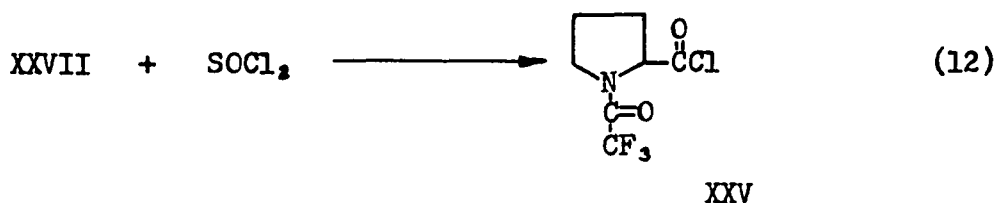
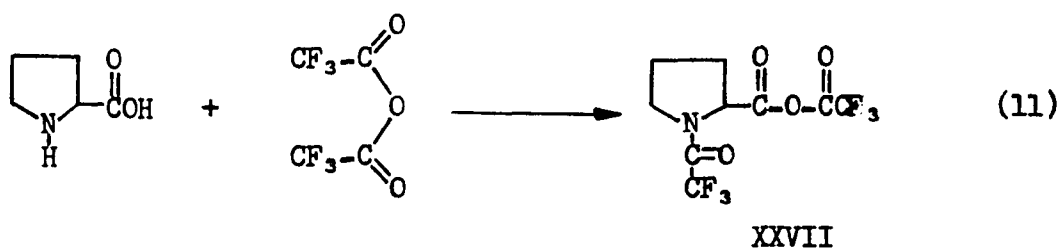
Glycine did cause a problem by "diluting" the amino acid sample to the extent that the amino acids of interest, threonine and allothreonine, were only a minor part of the reaction mixture (6-14%). This resulted in peaks which were about one order of magnitude less intense than those in Table IX. However, by reattenuating the signal coming from the detector the problem was solved at the expense of additional baseline noise.

Removal of the ethylenediamine from the reaction mixture before derivatization (see experimental section, page 18) is advantageous because it too reacts with the acid chloride having the effect of further "diluting" the amino acid sample. Attempts to concentrate the derivatization solution under dry N_2 gas so as to increase the concentration of the threonine-allothreonine derivatives produced a loss of intensity in some signals which could not be restored by adding additional amounts of BSTFA or the acid chloride.

To ensure that these compounds did not slowly racemize after preparation, the stock samples were checked periodically throughout the course of the investigation by their reaction with R-threonine and found to show no racemization. Mixtures of known composition were also derivatized at intervals but no suspicious behavior was noted. Also, the additive nature of the signals of the R- and S-proline chromatograms proved to be of value as a check on the acid chloride purity.

It was pointed out that a problem existed in obtaining optically pure S-XXV. This reagent is commercially available in 0.1 N chloroform solution from the Regis Chemical Company, Chicago, Illinois. The purity of several samples purchased from this source was checked using R-threonine and found to contain 88-96% S-isomer - the remainder being the R-isomer. These values were too large to produce satisfactory results from the threonine-allothreonine mixtures without numerous

correction factors in calculating actual signal areas. Repeating the procedure of Weygand *et al.*¹³ for the synthesis of the material resulted in compounds with optical purities no better than those of the commercial product. Conversations with J.W. Westley (1969, personal communication) also indicated that obtaining materials with good optical purity was a problem. On further investigation in our laboratory it was found that the critical step in the synthesis was the reaction of the amino acid and the trifluoroacetic anhydride (Equation 11).



Performing this step at ice bath temperature as Weygand had done or at room temperature produced significant amounts of racemization with optical purities of 52-92%. However, carrying out the addition at dry ice-acetone temperature and allowing the suspension to slowly warm to room temperature (see experimental section, page 23) resulted in essentially optically pure material (99%). Care was taken to carry out the remainder of the synthesis, Equation 12, at temperatures below 25°.

The procedure for the synthesis of the threonine-allothreonine derivatives involves a reaction with a silylating agent whose function is to convert the hydroxyl function to less polar TMS ether. This is commonly done for glc separations of polar materials which would otherwise move in the liquid phase of the column with very long retention times, or not move at all¹⁴. Mass spectral data of the SSt dipeptide using a combination gas-liquid chromatograph-mass spectrometer shows that this derivative has a molecular weight of 398 a.m.u., which

corresponds to a mono-silylated material. However, the fragmentation pattern suggests that the amide function carries the TMS group and not the hydroxyl as expected (Figure 16).

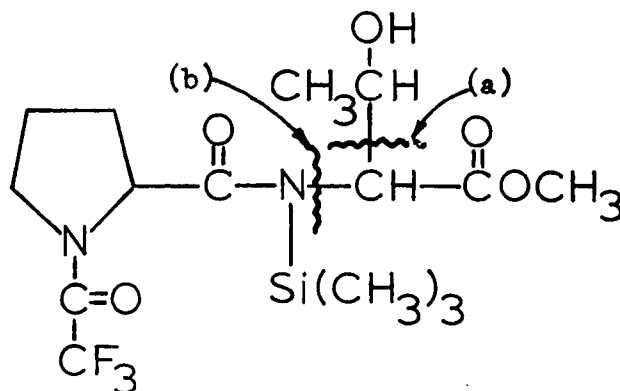


Figure 16. Structure of the SSt derivative from mass spectral data.

Strong intensities for $m-45$ and $m-117$ fragments (a) and (b) respectively of Figure 16 and lack of $m-89$ and $m-90$ fragments, $[\text{OSi}(\text{CH}_3)_3]^+$ and $[\text{HOSi}(\text{CH}_3)_3]^+$ respectively, indicate that the TMS group is on the amide function⁴⁵. This is very unusual because silyl ethers are usually much more stable than TMS amides⁴⁶.

2. Determination of the Relative Amounts of Product Amino Acids and Glycine

The bis-N(trimethylsilyl)trifluoroacetamide (BSTFA) Method

Since the method for determining the optical isomers of threonine and allothreonine was not quantitative for glycine an alternate approach using the method of G.W. Gehrke et al.¹⁶ was used. In the presence of BSTFA all the functional groups of all of the common amino acids can be silylated. The structures of the silylated amino acids used in this work are depicted in Figure 17.

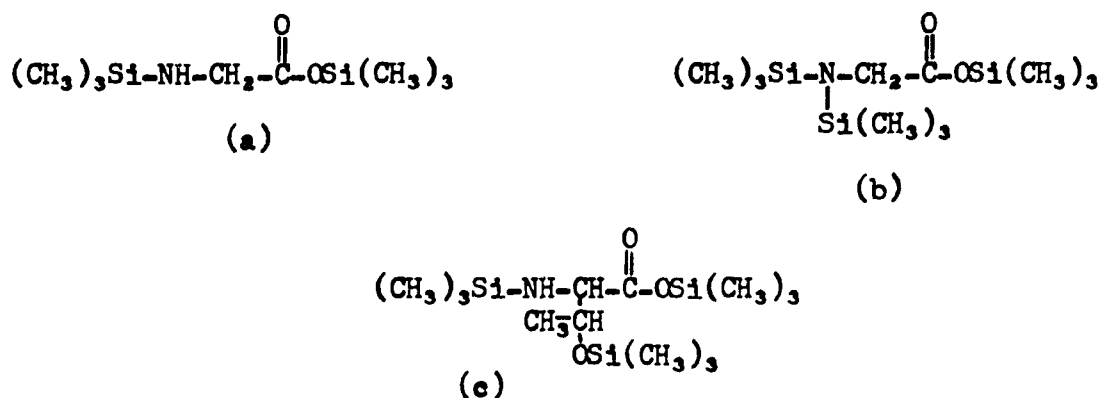
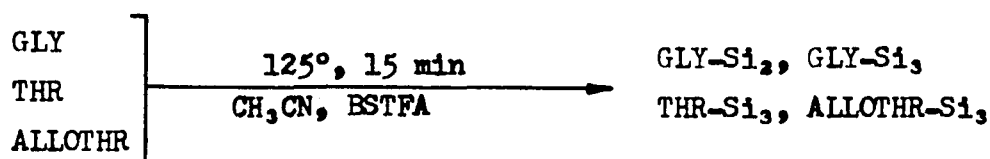


Figure 17. Silylated amino acids. (a) glycine, GLY-Si₂, (b) glycine, GLY-Si₃, (c) threonine or allothreonine, THR-Si₃ or ALLOTHR-Si₃.

The derivatization is simple and can be done on 1 mg samples (Equation 13).



In this derivatization the temperature and time are important as they regulate the amounts of GLY-Si₃ and GLY-Si₂ produced. The conditions indicated above resulted in quantitative determinations based on the GLY-Si₂ signal. A mixture containing 1:1:1 mole ratios of glycine, threonine and allothreonine gave the chromatogram shown in Figure 18.

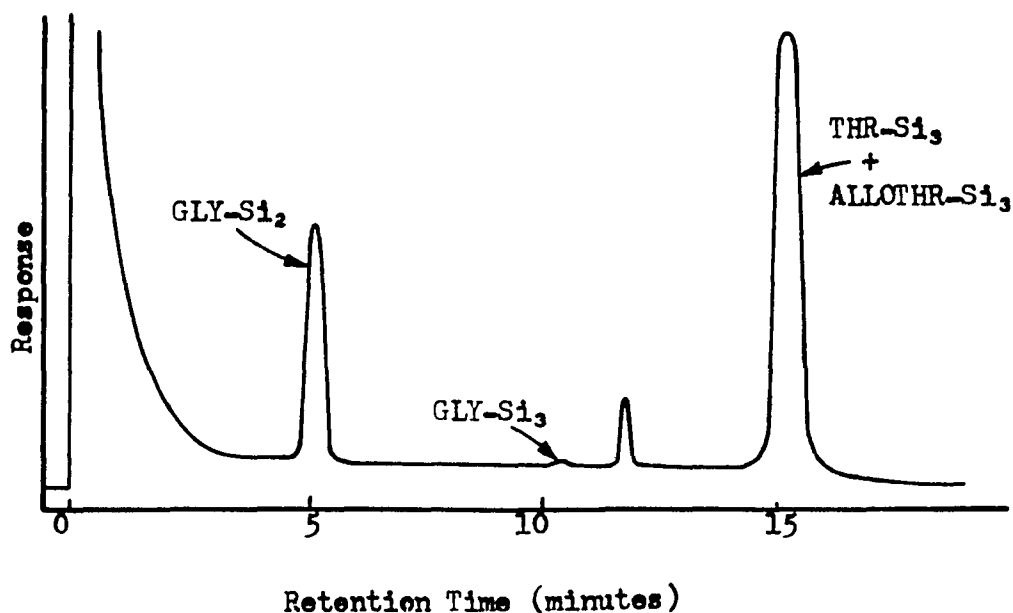


Figure 18. Gas-liquid chromatogram of TMS derivative of glycine, threonine and allothreonine 1:1:1. 1.0 μ l sample injected. Column SE-30 3% w/w on 80/100 mesh chromosorb W-AW, 1.3 m x 4mm I.D., glass. Initial temp 80°, 5 min hold, then 5°/min.

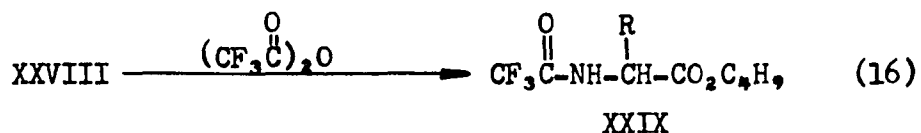
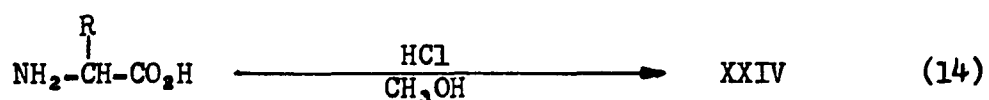
Threonine and allothreonine (THR-Si₃ and ALLOTHR-Si₃) had nearly identical retention times, 15.2 and 15.4 min respectively. Unfortunately, these compounds can be eluted quantitatively only from non-polar columns¹⁶ which severely limited the choices of liquid phases available for separation, and for this system resulted in one composite signal for these two amino acids. However, semi-quantitative information could be obtained from the SE-30 column using the shape of the threonine-allothreonine signal which if unsymmetrical on the low retention time side would indicate an excess of allothreonine. The high retention time side was affected if an excess of threonine was present. This provided a crude check on the results of the optical isomer determination.

The relative molar response of glycine and the composite threonine and allothreonine signal was checked using mixtures of known composition. To obtain the molar percent composition of a mixture containing the hydroxy amino acids and glycine, the latter signal must be multiplied by a factor of 1.85 which is in good agreement with the factor of 1.91 found by Gehrke¹⁶.

One of the peaks in the chromatogram in Figure 18 (retention time 12.0 min) is possibly due to the presence of small amounts of ethylenediamine which gave a signal in this area. Also when residue from the Dowex-1 column was treated with BSTFA a peak was observed at this retention time.

The Trifluoroacetic Anhydride (TFA) Method

This method was reported by Gehrke *et al.*¹⁷ for glc analysis of protein amino acids and is outlined by Equations 14-16.



The derivatives, XXIX, which for threonine and allothreonine also have the hydroxyl function trifluoroacetylated, are stable and sufficiently volatile to be easily separated using glc. A chromatogram of a mixture containing equal molar amounts of glycine, threonine and allothreonine produced signals with retention times of 2.4, 3.2 and 4.1 min and is shown in Figure 19.

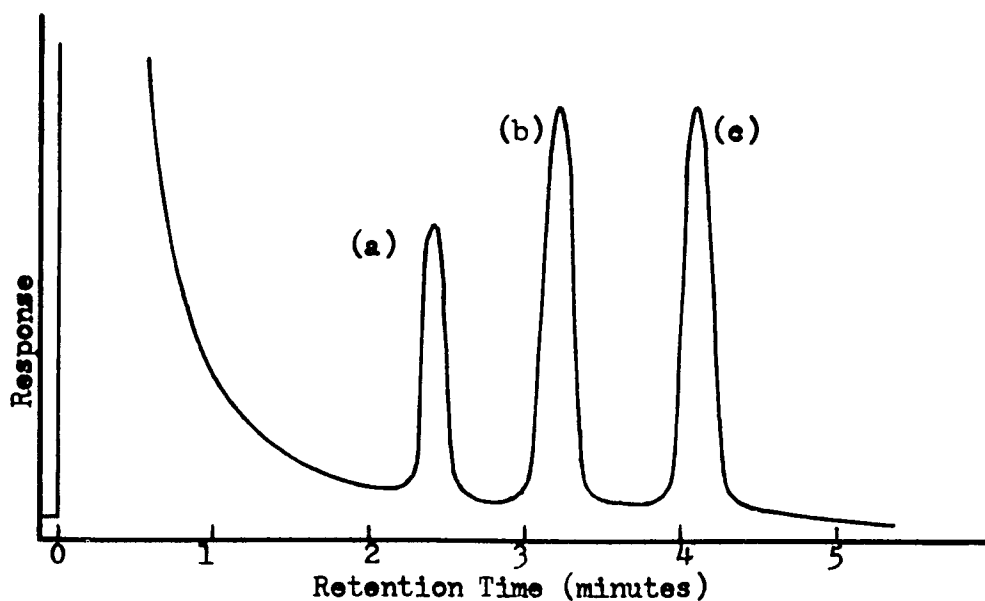


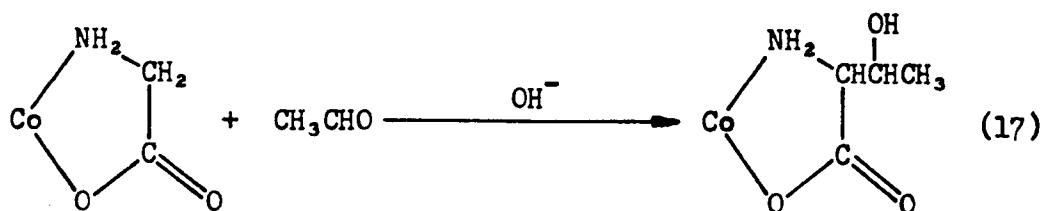
Figure 19. Gas-liquid chromatogram of glycine, threonine and allothreonine 1:1:1 using the TFA method. (a) glycine, (b) threonine, (c) allothreonine. Column SE-30, 3% w/w on 80-100 mesh chromosorb W-AW, 1.3 m x 4 mm I.D., glass, 100° isothermal.

Unfortunately the glycine signal did not prove to be as quantitative as with the BSTFA method. Thus the TFA method was used only to obtain information about the distribution of the diastereomers threonine and allothreonine. The quantitative nature of this method was confirmed using mixtures of known composition assuming equal response constants for the diastereomers.

D. The Reactions of the Complexes with Acetaldehyde

1. General Considerations

The reaction of coordinated glycine with acetaldehyde to form threonine and allothreonine proceeds as shown in Equation 17. For clarity only one amino acid is shown.



One purpose of this study was to determine if the template provided by the optically active complexes exerted any influence on the distribution of optical isomers in the resulting amino acids threonine and allothreonine. Figure 20 shows the environment about the coordinated glycine molecule in the cobalt(III) complexes studied.

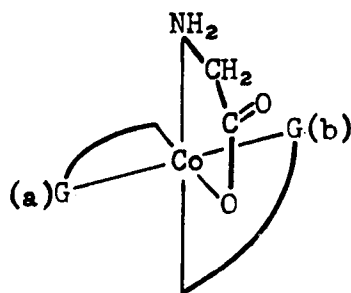


Figure 20. Model of the complexes showing the neighboring groups. Where (a) and (b) depend on the complex and may be the amine terminus of ethylenediamine or glycine or the carboxyl function of glycine.

Molecular models confirmed that the reaction site of the coordinated glycine molecule, the carbon atom of the $-\text{CH}_2$ groups, was about 3 \AA removed from terminal atoms of the other ligands making up the optically active coordination compound. Of particular interest in this investigation were steric and hydrogen bonding interactions which these groups might exert on the direction of the attacking aldehyde. The effect of various groups at positions (a) and (b) of Figure 20 on the direction of the attacking aldehyde could be obtained by quantitatively analyzing the optical isomers of threonine and allothreonine produced in the reaction. Knowing both the absolute configuration of the complex and the amounts and configurations of the hydroxy-amino acids, the direction of the attacking aldehyde could be deduced or more accurately the side of the glycine molecule on which it finally resided could be found.

In order to observe these subtle effects it was necessary to carry out the reaction using relatively mild conditions. To make a valid comparison between the product distribution from the various complexes it was also important to show that the newly synthesized hydroxy-amino acid while coordinated to the metal ion did not experience any inversion of its optically active centers between the time of synthesis and

analysis. There was reason to believe that this might occur since Buckingham et al.⁸ (see introduction, page 5) noted the racemization of S-alanine in (-)₈₈, S-alaninatobis(ethylenediamine)cobalt(III) in basic media. During the 3.0 hr reaction time the complexes were in a solution at pH 9.5 which could cause some epimerization of threonine and allothreonine. For these two amino acids, interconversion of diastereoisomers would be observed if the α proton was effected. That is, Rt would epimerize to Sa and vice versa and Ra would be converted to St and vice versa. Ideally for valid comparisons between the complexes the aldehyde should react with the glycine molecule, forming a particular threonine or allothreonine and not revert back to another isomer after the initial reaction.

To compare the product distribution from the glycinatobis(ethylenediamine)cobalt(III) with the bis(glycinato) series it was considered advisable to terminate the reactions early for the latter series. This was done to minimize the formation of complexes in which both glycine molecules had reacted with the aldehyde. The stereochemical effect of a complex which had reacted once on the second attacking aldehyde was difficult to predict and might have complicated the comparison of product distributions between the glycinato and bis(glycinato) complexes.

The reaction conditions necessary to ensure that no epimerization was taking place and that only monocondensation product complexes were being formed were established using the trans(O)C₂ isomer. It was convenient to use this compound because of its abundance and also the relative ease of separation of the dicondensation products from the monocondensation and starting complexes. After only a few hours of eluting, the dicondensation products rapidly separated from the tight band at the top of the ion exchange column. This investigation showed that if the reaction was terminated before 15% of the glycine in the system had reacted, only small amounts of the dicondensation product complexes would be synthesized. This number was used as a guide for the other geometric isomers where it was less convenient to do the same analysis because of the small amounts available or more difficult separations of the dicondensation complexes.

The handling of acetaldehyde was a problem because of its low boiling point (22°) and its ability to form cyclic trimers which may or may not react with the coordinated glycine. It has been shown that distillation of the aldehyde from a few drops of phosphoric acid under a blanket of N₂ gas results in monomeric acetaldehyde^{*7}. The appropriate volume (assuming a density of 0.8 g/ml) of the aldehyde was measured, using a prechilled (-20°) microliter syringe, and added to water at ice bath temperature. Some loss due to the volatility of the aldehyde in making up the aqueous solution was unavoidable but the extent of the reaction as measured by glc of the product amino acids was fairly reproducible for repetitive reactions with the same complex. The lowered vapor pressure of the aldehyde upon dilution and the use of a sealed reaction vessel for the pH-stat ensured only minimal loss of the aldehyde during the course of the reaction. Deoxygenated water was used to minimize the oxidation of the aldehyde to acetic acid.

Since the reaction was base catalyzed, a pH-stat was employed to maintain a constant pH. This was done to ensure that the product distribution of the amino acids was solely a function of the particular complex used since additional unnecessary ions such as those present in common buffer systems conceivably could form outer sphere complexes with the coordination compounds which could influence the product distribution^{*8}.

The only products isolated from the reaction of coordinated glycine with acetaldehyde were threonine and allothreonine. This was confirmed by paper chromatography of amino acids using tertiary-butyl alcohol-methylethyl ketone-water-concentrated aqueous NH₃ 4:3:2:1 as a solvent^{*9}. The R_f values were 0.30, 0.44, 0.54 for glycine, allothreonine and threonine respectively. A pmr spectrum also confirmed the presence of only these three amino acids¹².

The results of the reactions of the complexes are summarized in Table X. Agreement between duplicate determinations was within ⁺2%. In some cases the sum of the percentages of all isomers does not add to be 100% but is always within ⁺5% of this value.

TABLE X
Reaction of Some Glycine-Cobalt(III) Complexes with Acetaldehyde

	Mole % ^a				pH	A/C ^b	time ^c	Mole % ^d Reacted
	Rt	St	Ra	Sa				
I, $\wedge(C_3)$ <u>trans</u> (O)C ₂ [Co(gly) ₂ (en)] ^{+e}	10	22	23	44	9.5	1	3	11
I, racemate ^f	36		64		9.5	1	3	11
" ^f	46		54		9.5	3	0.3	6
" ^g	80		20		11.0	3	90	95
IV, $\wedge(C_3)$ [Co(gly)(en) ₂] ^{++e}	11	16	25	51	9.5	1	3	12
"	9	19	29	48	9.5	4	3	65
"	37	34	14	16	10.5	0.5	3	21
" ^h	36	42	-	-	11.0	3	90	80
IV, racemate	30		70		9.5	4	3	61
"	82		16		11.0	3	90	60
II, $\wedge(C_3)$ <u>β</u> cis(O)C ₁ [Co(gly) ₂ (en)] ⁺	10	16	24	45	9.5	1	3	6
III, $\wedge(C_3)$ <u>α</u> cis(O)C ₂ [Co(gly) ₂ (en)] ^{+e}	26	41	3	29	9.5	3	3	14
III, racemate ^f	62		38		9.5	3	3	14
" ^f	36		64		9.5	1	3	2

^aFor separation conditions of optical isomers using glc see Figure 15, page 56, unless otherwise noted. Entries are an average of at least two chromatograms from one reaction unless otherwise stated. ^bA/C = mole ratio of aldehyde to complex. ^cReaction time in hr. ^dMole percent of glycine reacted as determined by the BSTFA method, Figure 18, page 63. ^eThe average of two duplicate reactions rounded off to the nearest percent is given. ^fAmino acid distribution as determined by the TFA method, Figure 19, page 65. ^gAmino acid distribution as determined by pmr, reference 12. ^hData from reference 6.

2. The Reactions of $\Lambda(C_3)$ (+)_{ss}, and Racemic $\text{trans}(O)C_2$ Bis(glycinato)ethylenediaminecobalt(III) Chloride

When the reaction with acetaldehyde was carried out with the optically active isomer at pH 9.5 with an aldehyde to complex ratio (A/C) of 1:1, separation of all the complexes in the mixture produced three bands on the ion-exchange column. The two fastest moving bands contained the four monocondensation products pictured in Figure 21.

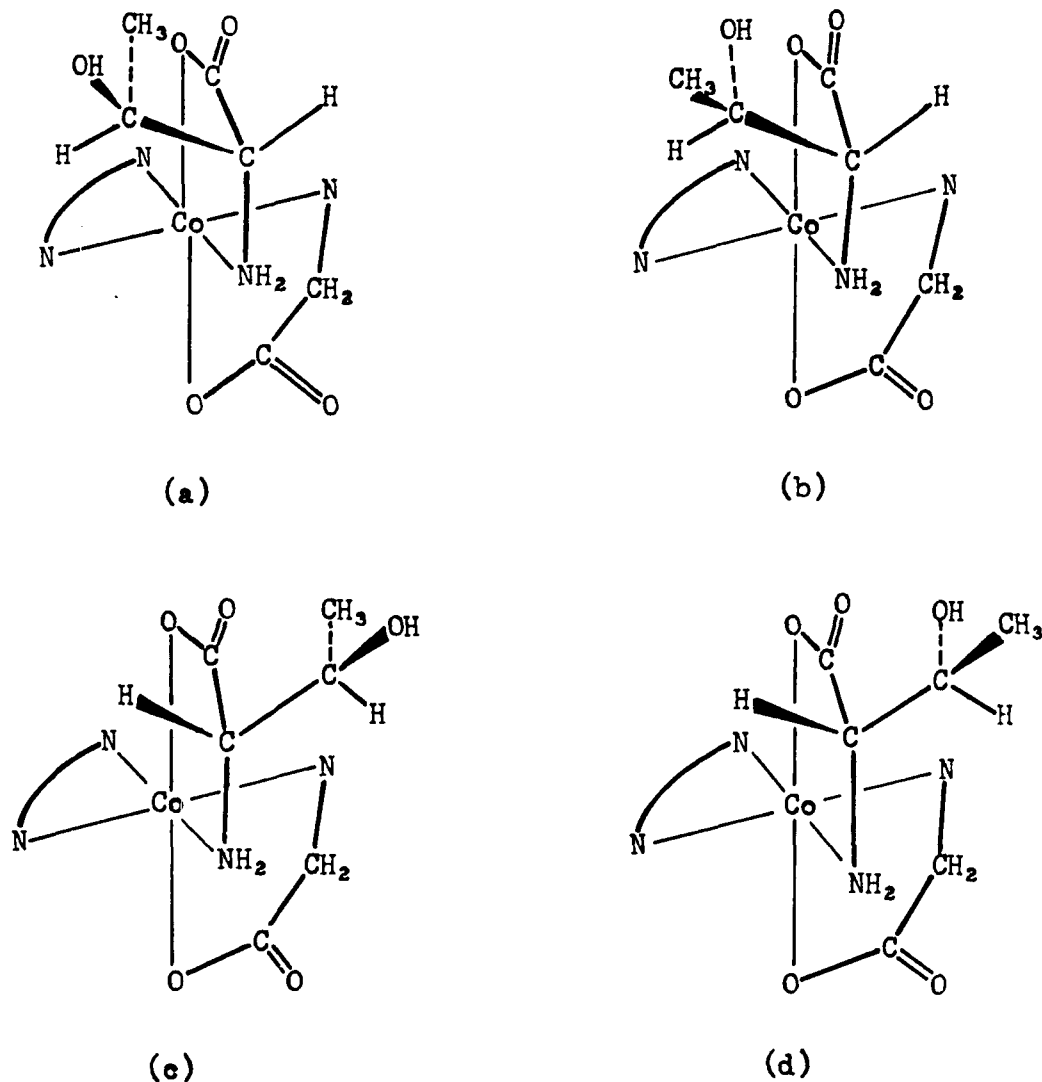


Figure 21. The four monocondensation products of $\Lambda(C_3)$ $\text{trans}(O)C_2$ bis(glycinato)ethylenediaminecobalt(III). (a) $\Lambda(C_3)Rt$, (b) $\Lambda(C_3)Ra$, (c) $\Lambda(C_3)St$, and (d) $\Lambda(C_3)Sa$.

Because of the C_2 symmetry axis in this compound each glycine molecule is identical and only four monocondensation products exist. The uv-visible spectrum of the lower two bands gave the typical trans(O) $\text{Co}(\text{N}_4\text{O}_2)$ spectrum as did the third and most abundant band. Gas-liquid chromatographic analysis of the combined first two bands showed that the ratio of threonine-allothreonine to glycine was 1:1. The same analysis of the third band showed that the only amino acid present was glycine thus excluding the presence of any dicondensation products present in the mixture and showing that all the monocondensation products were in the first two bands. Isomerization to other geometric isomers, the β cis(O) C_1 or α cis(O) C_2 isomers can also be ruled out by the absence of other bands in the separation.

No racemization of the trans(O) C_2 cation was observed at pH 9.5 during the course of the reaction. A check on the optical activity of the separated monocondensation product complexes showed that the reaction conditions probably did not effect the ring system in these compounds either (Figure 22).

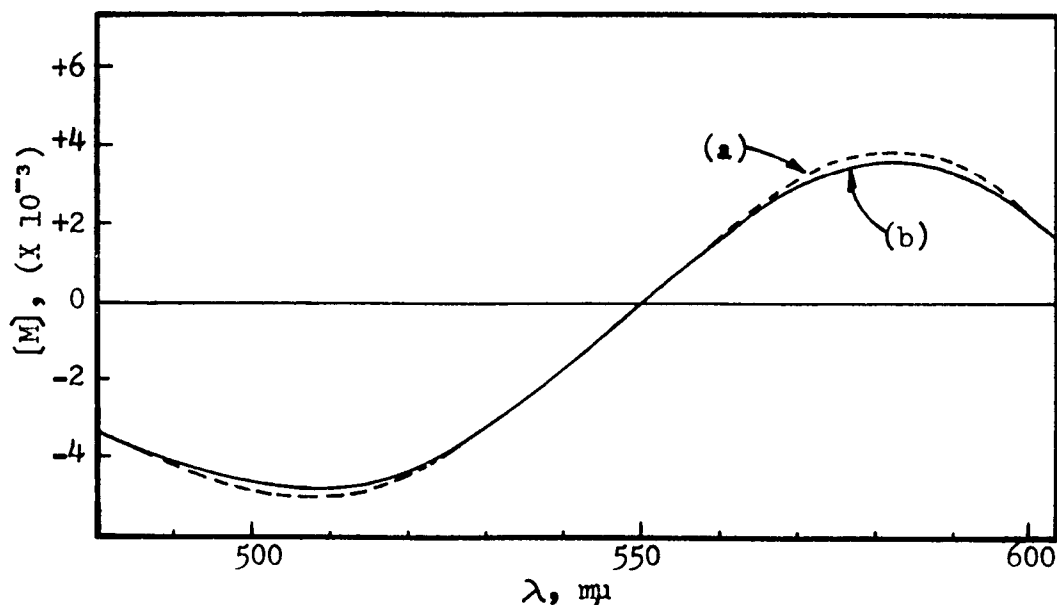


Figure 22. The ORD curves for the $\Lambda(\text{C}_3)$ trans(O) monocondensation products, (a); $\Lambda(\text{C}_3)$ trans(O) C_2 $[\text{Co}(\text{gly})_2(\text{en})]^+$, (b). The $\epsilon_{530\text{m}\mu}$ for the monocondensation complexes was assumed to be the same as the bis(glycinato) compound at the same wavelength.

The presence of all four monocondensation products in the first

two bands was confirmed by glc analysis of the optical isomers of threonine and allothreonine (Table X, page 69). Before detailed analysis of the significance of these data could be carried out it was necessary to show that no epimerization was occurring. That is, that the aldehyde upon reacting with the carbanion intermediate (see introduction, page 4) generated asymmetric centers which were not altered after the initial reaction. If this occurred then comparison of the products from each of the complexes studied on the basis of their different molecular geometry would be more difficult.

Two of the monocondensation products, $\wedge(C_3)$ (+)₅₈, trans(O) [Co(S-thr)(gly)(en)]⁺, XIII, and $\Delta(C_3)$ (-)₅₈, trans(O) [Co(S-thr-(gly)(en)]⁺, XII, were synthesized using authentic S-threonine. The latter compound is enantiomeric to compound (a), Figure 21, and therefore has the same chemical properties. The two allothreonine monocondensation products were more difficult to synthesize because of the unavailability of optically active allothreonine and also the high cost of the racemic amino acid. These compounds were finally synthesized by treating racemic trans(O)C₂ [Co(gly)₂(en)]⁺ with acetaldehyde and isolating the monocondensation products containing both the isomers of threonine and allothreonine. The possibility of epimerization of both the authentic threonine monocondensation products and the allothreonine compounds present in the monocondensation mixture was checked at pH 9.5 after 3.0 hr. Table XI summarizes the results of the study.

TABLE XI

Epimerization of trans(O) Monocondensation Product Complexes^a

		Mole %			
		Rt	Ra	St	Sa
$\wedge(C_3)$ <u>trans</u> (O) [Co(S-thr)(gly)(en)] ⁺	before	-	1	99	-
	after	-	2	98	-
$\wedge(C_3)$ <u>trans</u> (O) [Co(R-thr)(gly)(en)] ⁺ ^b	"	99	-	-	1
	"	98	-	-	2
Racemic Monocondensation Mixture	"	18	32	18	32 ^c
	"	18	32	18	32

^aDetermined using the S-proline derivative, S-XXV. For separation conditions see Figure 15, page 56. ^bThe enantiomer of this compound was used in the study. ^cAs tabulated in Table X, page 69 for racemic trans(O)C₂ [Co(gly)₂(en)]⁺.

The data in Table XI for the two threonine monocondensation products shows that very little or no epimerization took place at pH 9.5. The small amounts of epimerization product present (about the α carbon atom) before the pH was adjusted to 9.5 was probably due to the fact that these compounds were synthesized in basic media for 3 days (experimental section, page 14). It is safe to assume that the asymmetric centers of $\wedge(C_3)Rt$ and $\wedge(C_3)St$ once formed in the aldehyde reactions do not change. Since there was no change in the composition of the mixture containing both threonine and allothreonine products after standing for 3 hrs at pH 9.5, no change took place in the allothreonine asymmetric centers. It can be argued that the monocondensation mixture is the result of a dynamic equilibrium between the four coordinated amino acid isomers and that changing the pH to 9.5 would have no effect on the distribution of isomers. But if this were true then the authentic threonine compounds should have rapidly epimerized to give a mixture of products which did not occur.

It is now possible to examine the $\wedge(C_3)$ trans(O) C_2 reaction in Table X in detail. A respectable stereoselective synthesis of threonine (37%) and allothreonine (31%) was accomplished. This implied that the dissymmetric complex is able to influence the distribution of isomers of the product amino acids. Since the S-amino acids predominate over the R-isomers by 2:1, the attacking aldehyde favors the S-side of the coordinated amino acid by the same ratio, Figure 21 (c) and (d). This is also the steric compression side where the NH_2 protons of the amino acid are closer to the glycine methylene protons than the ethylenediamine amine protons on the R-side. If steric interactions were the most important consideration then the R-side would be more susceptible to attack than the S-side. However this was not observed. It is tempting to suggest that hydrogen bonding between the oxygen of the aldehyde and one of the amino acid NH_2 protons is occurring on the S-side thereby increasing the yield of the S-isomers. Unfortunately work on the other complexes designed to alter these effects is necessary in order to definitely tell what factors associated with the complex are influencing the stereochemistry of the products.

3. The Reactions of $\Lambda(C_3)$ (+)₅, Glycinatobis(ethylenediamine) cobalt(III) Chloride

Encouraged by the results of the trans(O)C₂ isomer with acetaldehyde, it was interest to see what effect the substitution of an ethylenediamine ligand for glycine would have on the product distribution (Figure 23).

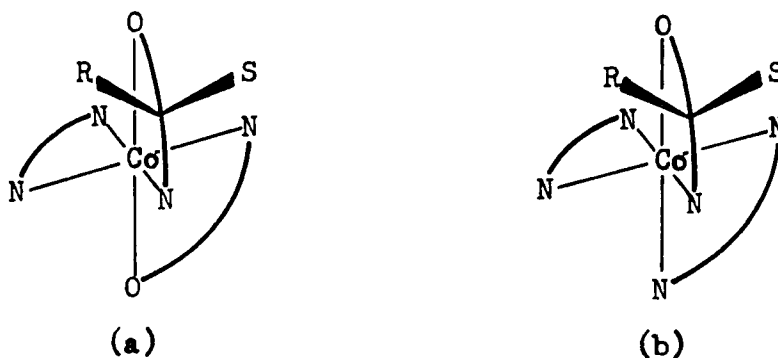


Figure 23. Structures of $\Lambda(C_3)$ trans(O)C₂ [Co(gly)₂(en)]⁺ (a) and $\Lambda(C_3)$ [Co(gly)(en)₂]⁺⁺ (b). R and S corresponds to the R- and S-sides of the coordinated amino acid.

Figure 23 shows that the R-side of the trans(O)C₂ compound is identical to the R-side of the bis(ethylenediamine) complex. If the same factors were operating in both complexes, the product distribution on the R-side of both molecules should be similar. Although their S-sides are not identical, a glycine molecule versus an ethylenediamine ligand, the groups closest to the reaction center are very similar in that they are both primary amines. To a first approximation the product distributions from the two molecules should be very similar.

The reaction was carried out at pH 9.5 with A/C of 1 but no attempt was made to separate the condensation products from the unreacted glycinato compound. Being +2 cations, separation using ion-exchange would be difficult at best and was not as critical as in the previous case where both mono- and dicondensation products were possible. The glycinato-bis(ethylenediamine) compound had only one glycine molecule and could only react once with the aldehyde.

Figure 24 shows the ord curves for the optically active glycinato compound and the mixture containing 12% condensation complexes. The

similarity of the two curves indicates that no racemization of the starting complex occurred during the reaction.

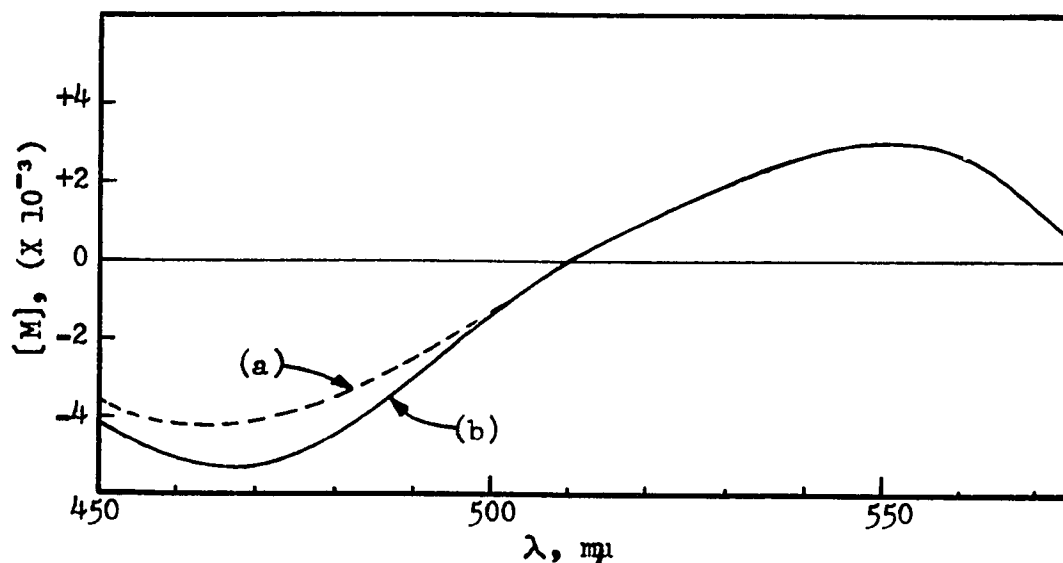


Figure 24. The ord curve for $\Lambda(C_3) [Co(gly)(en)_2]^{++}$ (a), and the reaction mixture containing 12% condensation products (b).

The possibility of epimerization again must be considered. Since this complex carried two positive charges versus one for the bis (glycinato) series, the ability of the metal ion to act as a Lewis acid should be greater than the +1 complex. This should have the effect of increasing the acidity of the α hydrogen atom of the newly formed amino acid thereby increasing the probability of epimerization. The first step to epimerization presumably is the abstraction of the α proton generating a planar carbanion which is then reprotonated from the opposite side and in the process inverting the α center. Buckingham *et. al.*⁸ have studied the kinetics of this process for the $(-)_S,S$, S-alaninatobis (ethylenediamine)cobalt(III) ion which is also a +2 cation. The methyl groups of alanine (and the isopropyl group of valine) probably have approximately the same inductive effect on the α center as the R group $(-CH\begin{smallmatrix} OH \\ \diagup \\ CH_3 \end{smallmatrix})$ of threonine or allothreonine. Using the numbers that Buckingham *et al.* obtained for this system, the following results were obtained. The reaction conditions for carrying out the optically active synthesis in the work involved a OH^- concentration of 3.16×10^{-5} (pH 9.5), complex concentration, 2.73×10^{-2} M, μ of 0.082 M and a

temperature of 25°. The rate constant for α -proton exchange, the first step of epimerization, for the coordinated alanine molecule which gives the best match to the above conditions is a k_2 of 5.4×10^{-2} mole⁻¹ liter sec⁻¹ for μ of 0.5 M and temperature 34.3°.

If pseudo first order kinetics are assumed then,

$$\frac{d[\text{complex}]}{dt} = k_1 [\text{complex}]$$

where, $k_1 = k_2 [\text{OH}^-]$

and $k_1 = 1.71 \times 10^{-6} \text{ sec}^{-1}$

then $t_{1/2} = \frac{0.69}{k_1} = 4.05 \times 10^{+5} \text{ sec}$

or 113 hr

This value probably represents a lower limit since the temperature was 10° less for the glycinatobis(ethylenediamine)-aldehyde reaction than the α -proton -S-alaninato exchange data. However it was found in the latter case that decrease in μ increased the value of k_2 .

The data for the $\Lambda(C_3)[\text{Co}(\text{gly})(\text{en})_2]^{++}$ reaction at pH 9.5 were analyzed assuming that no epimerization had occurred during synthesis of the amino acids. In this case as with the trans(O)₂ isomer fair amounts of stereoselective syntheses were found in the threonine (18%) and allothreonine (34%). Furthermore the R-side in the $\Lambda(C_3)$ trans(O)₂ isomer gave a 2:1 ratio of the hydroxy amino acids in favor of allothreonine. This was in good agreement with the amino acid distribution on the R-side of the $[\text{Co}(\text{gly})(\text{en})_2]^+$ compound. The data from the S-side showed that a slight change had occurred in the distribution of threonine and allothreonine from $\Lambda(C_3)$ trans(O)₂ $[\text{Co}(\text{gly})_2(\text{en})]^+$ (2:1) to $\Lambda(C_3)[\text{Co}(\text{gly})(\text{en})_2]^{++}$ (3:1). This may be due to the fact that the S-side of both complexes are not identical (an ethylenediamine ring versus an amino acid ligand) and the formation of the β center of the amino acid is influenced differently by the presence of the ethylenediamine ring. As with the trans(O)₂ isomer the aldehyde favors the S-side over the R-side by 2:1.

4. The Reactions of $\Lambda(C_3)$ (+)₅₈, β cis(O)₂ Bis(glycinato) ethylenediaminecobalt(III) Chloride

The interpretation of the results of the reaction of this compound with acetaldehyde is more complex than in the previous cases. Like the trans(O) C_2 compound the β cis(O) C_1 isomer possesses two glycine molecules but because of the lack of an axis of symmetry the amino acids are not equivalent. However, the complex does contain elements of the previous two complexes (Figure 25).

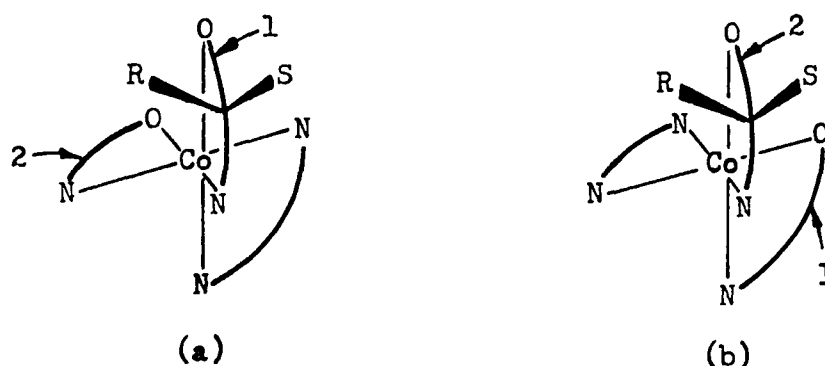


Figure 25. The projection of both glycine molecules in $\wedge(C_3)$ β cis(O) C_1 $[\text{Co}(\text{gly})_2(\text{en})]^+$. (a) View of R and S-sides of glycine 1. (b) View of R and S-sides of glycine 2.

As seen in the figure the S-side of (a) and the R-side of (b) are identical to the R- and S-sides respectively of the lone coordinated amino acid in $\wedge(C_3)$ $[\text{Co}(\text{gly})(\text{en})_2]^+$, Figure 23 (b). The R-side of (a) and the S-side of (b) are common only to the β cis(O) C_1 isomer but similarities between the R-side of Figure 23 (a) and the R-side of Figure 25 (a) can be noted. It was found in the case of the trans(O) C_2 $[\text{Co}(\text{gly})_2(\text{en})]^+ - [\text{Co}(\text{gly})(\text{en})_2]^{++}$ comparison that substitution of the ethylenediamine ring by a glycine ligand had little effect on the product distribution. Thus the R-side of (a) should be similar to that in the $\wedge(C_3)$ trans(O) C_2 isomer.

The reaction with aldehyde was carried out in the usual manner (pH 9.5, 3.0 hr, A/C of 1), and the ord curves for the reaction mixture and the starting complex were recorded.

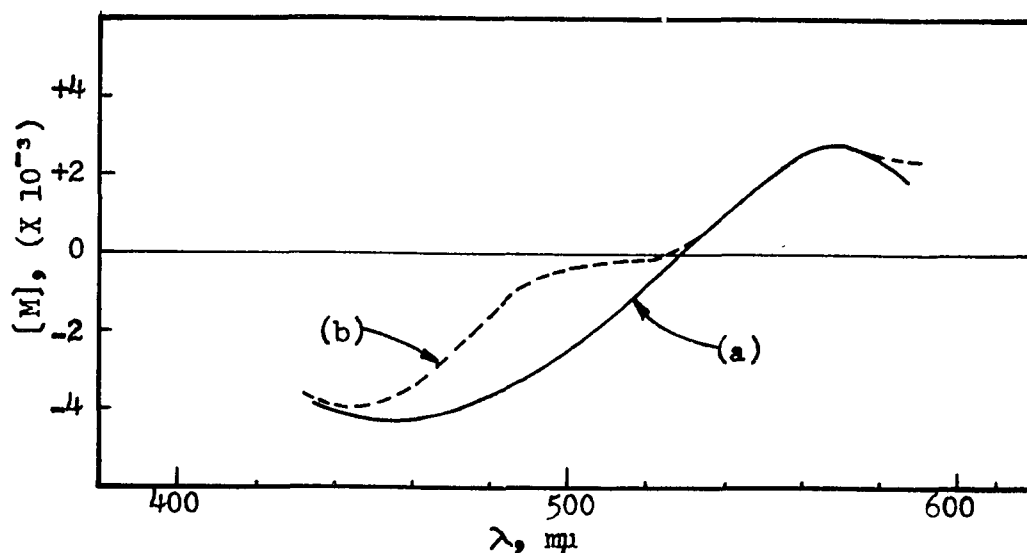


Figure 26. The ord curves of (a) $\Lambda(C_3) \beta \text{cis}(O)C_1 [\text{Co}(\text{gly})_2(\text{en})]^+$.
(b) The reaction mixture containing 6% monocondensation complexes.

Attempts to separate the monocondensation products using ion-exchange chromatography failed. If only monocondensation product complexes are considered, eight diastereomers containing coordinated threonine and allothreonine would be possible making separation by ion-exchange chromatography difficult. Only one band was noted after many days of eluting - presumably containing the starting material and condensation products (experimental section, page 20). This does not eliminate the presence of dicondensation products but they are probably absent due to the small amounts of product formed.

It was difficult to eliminate the possibility of epimerization in the $\beta \text{cis}(O)C_1$ reaction in the same manner used for the $\text{trans}(O)C_2$ isomer because of the large number of monocondensation products which would have to be examined. Significant importance was attached to the observation that this did not occur for the $\text{trans}(O)C_2$ isomer, also a +1 compound, and therefore would probably not occur for the $\beta \text{cis}(O)C_1$ compound using the same reaction conditions.

Table X, page 69 lists the product distribution from this compound. These values do not deserve as much confidence as the other values in the table because of the small amounts of product obtained. The signals of interest in the chromatogram were of low intensity and positioned on the side of a large "solvent" signal making quantitative evaluation difficult.

Essentially the same distribution was found for the $\beta\text{cis}(\text{O})\text{C}_1$ isomer as was determined for the two previous molecules. This was expected because of the similarity of this isomer to the first two compounds studied. The effect of the acetate group on the product distribution is either essentially the same as that of an amine function, or is different from an NH_2 function but cannot be detected because the data are weighted heavily in favor of the first two complexes studied.

5. The Reactions of $\alpha\text{cis}(\text{O})\text{C}_2$ Bisglycinato(ethylenediamine)cobalt(III) Chloride

This complex possesses a C_2 symmetry axis and it also has elements of some of the previously mentioned complexes (Figure 27).

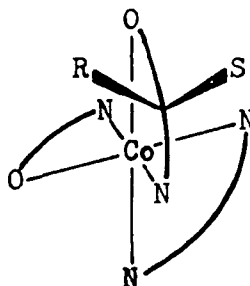


Figure 27. The $\Lambda(\text{C}_3) \alpha\text{cis}(\text{O})\text{C}_2 [\text{Co}(\text{gly})_2(\text{en})]^+$ ion.

The S-side of the coordinated amino acid is identical to the S-side of the $\Lambda(\text{C}_3) [\text{Co}(\text{gly})(\text{en})_2]^{++}$ ion, Figure 23 (b), and similar to the S-side of $\Lambda(\text{C}_3) \text{trans}(\text{O})\text{C}_2 [\text{Co}(\text{gly})_2(\text{en})]^+$, Figure 23 (a). However the R-side is common only to the $\alpha\text{cis}(\text{O})\text{C}_2$ isomer but could be considered similar to the S-side of Figure 25 (b), page 77. Unlike the $\beta\text{cis}(\text{O})\text{C}_1$ isomer which has non-equivalent glycine molecules to complicate the product distribution, amino acids formed on the R-side of the $\Lambda(\text{C}_3) \alpha\text{cis}(\text{O})\text{C}_2$ compound should better demonstrate the effect of an acetate group on the synthesis of threonine and allothreonine.

When the reaction conditions of pH 9.5 and A/C of 1 were used on the racemic complex only 1-2% of the coordinated glycine reacted to form products. This amount would be insufficient for a meaningful

determination of the optical distribution of products had optically active complex been involved, so the relative amounts of threonine and allothreonine were determined by TFA method (page 25). The analysis showed that 64% allothreonine and 36% threonine were produced which was similar to that obtained for the other compounds.

Separation of the complexes from the 3 hr reaction mixture using ion-exchange chromatography revealed the presence of a small amount (1-3%) of a fast moving +1 complex. By increasing the reaction period to 24 hr the amount of this material increased and eventually separated into three bands which were subsequently identified as trans(O)C₂ [Co(gly)₂(en)]⁺ and its monocondensation product complexes. This proved that geometric isomerization was taking place during the reaction which was not observed for the other compounds using similar reaction conditions. Geometric isomerization was however observed for the other +1 complexes under conditions of higher pH and after longer reaction times.

Subjecting the $\wedge(C)_3 \propto \text{cis}(O)C_2$ isomer to pH 9.5 for as long as 24 hr showed no detectable loss in rotation. Furthermore analysis of the resulting solution using ion-exchange chromatography revealed that no geometric isomerization had taken place. Only one band was found which was identified by uv-visible absorption and glc of the ligands as authentic $\wedge(C)_3 \propto \text{cis}(O)C_2$ [Co(gly)₂(en)]⁺ thus proving that the isomerization must be occurring during or after the formation of the product complexes. Unfortunately the presence of unreacted trans(O)C₂ [Co(gly)₂(en)]⁺ is difficult to account for by using this explanation, unless isomerization takes place only in the presence of the aldehyde and is not related to the condensation reaction.

The impact of geometric isomerization during the synthesis had little effect on the distribution of threonine and allothreonine which was similar to the other isomers studied. If the isomerization takes place after the reaction of the complex with aldehyde and rearrangement of asymmetric centers in the products do not occur, it follows that the acetate group on one side of the coordinated amino acid does not noticeably affect the distribution of threonine and allothreonine.

A reaction involving the optically active complex at pH 9.5,

A/C of 1 was not attempted because of the small amount of product (2%) that would be produced. Experience with the β -cis(O) C_1 isomer which produced only 6% product amino acids showed that problems with the glc method developed at lower concentrations of threonine and allothreonine, page 78.

6. Further Reactions of the Complexes

The need for additional reactions of the complexes with acetaldehyde was underscored by the inability of the α -cis(O) C_2 isomer to synthesize a sufficient amount of amino acids at pH 9.5 and A/C of 1. By carrying out reactions under different conditions the effect of variables such as pH and the ratio of aldehyde to complex on the product distribution could be examined.

The analysis of the optical isomers produced from the reaction of the $\Lambda(C_3)$ α -cis(O) C_2 with acetaldehyde was finally accomplished using pH 9.5 and A/C of 3. Reasonable yields (13-14%) of amino acids were obtained. Furthermore in the subsequent separation of the reaction mixture using an ion-exchange column only traces of the trans(O) isomers were found. The other bands containing the bulk of the material gave typical $Co(N_4O_2)$ spectra but the absorption maximum of the first d-d band was shifted 5-8 m μ lower than that of authentic α -cis(O) C_2 bis(glycinato) compound (502 m μ). This change in the wavelength was easily visible by the change in color of the reaction mixture during the course of the reaction from a cherry red to an orange red solution. Similar shifts in the absorption maximum of the reaction mixtures for the other compounds were noted with all reaction conditions used but color changes were not as dramatic as with the α -cis(O) C_2 isomer. These small changes in spectra could be due to the replacement of glycine in the coordination sphere of the metal with threonine or allothreonine. Similar shifts were observed for the S-threonine and S-serine compounds listed in Table II, page 34.

During the course of the reaction the pH stat added 0.2-0.4 ml of the titrant in order to maintain the pH at 9.5. This behavior was noticed for all three bis(glycinato) isomers with A/C of 1 but the quantities of titrant were only 0.05-0.10 ml. No titrant was added

for the glycinatebis(ethylenediamine) compound with A/C of 1. A check on the reaction solution containing the same concentration of aldehyde as used in the α -cis(O) C_2 , A/C of 3 reaction, without the complex, showed that only 0.01-0.02 ml of base was needed to maintain pH 9.5 over a 3.0 hr period. This additional titrant was presumably due to the oxidation of the aldehyde or other side reactions that it might have undergone. Furthermore, the compounds which were isolated using ion-exchange chromatography from the $\Lambda(C_3)$ α -cis(O) C_2 with A/C of 3 could be converted into materials which gave the typical cis(O)

Co(N₄O₂) spectra ($\epsilon_{\text{max}} = 502 \text{ m}\mu$) by the addition of two drops of a 10 N NaOH solution, allowing it to stand at room temperature for a few minutes and then adding 12 N HCl until the solution was acidic.

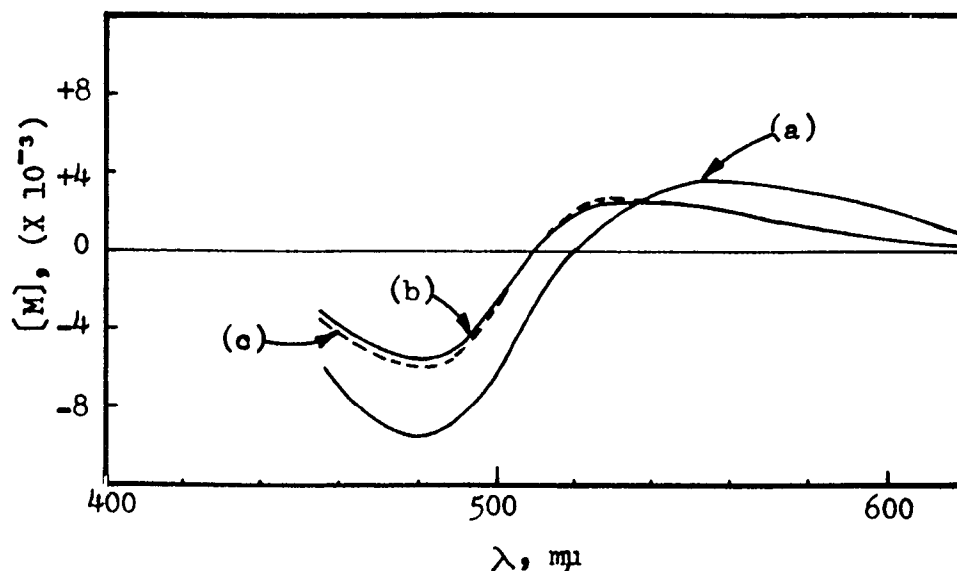


Figure 28. The ord curves of (a) $\Lambda(C_3)$ α -cis(O) C_2 $[\text{Co}(\text{gly})_2(\text{en})]^+$ and (b) reaction mixture containing 14% monocondensation products. (c) After treatment with 10 N NaOH.

After the reaction, a significant change in magnitude of the ord curve had occurred which was not evident for the trans(O) C_2 or $[\text{Co}(\text{gly})(\text{en})_2]^{++}$ compounds with A/C of 1. The β -cis(O) C_1 isomer also appears to have undergone a significant change during the reaction as evidenced by its ord curve. Upon subjecting the α -cis(O) C_2 reaction products to the above pH changes, a slight increase in the optical rotation was noted.

The analysis of the product distribution from the α -cis(O) C_2 , A/C of 3 reaction showed that the aldehyde still favored the S-side over the R-side (Figure 27, page 79, and Table X, page 69) by 2:1. However the distribution of threonine and allothreonine was distinctly different from the other isomers, in that threonine was favored over allothreonine, 2:1. Perhaps the most spectacular was the stereoselective synthesis of allothreonine which was about 80% in favor of the S isomer. Although the presence of epimerization was difficult to disprove it seems unlikely because of the similarity of the +1 compound to the trans(O) monocondensation isomers which did not epimerize under identical pH conditions.

It is now apparent that changing the aldehyde to complex ratio from 1:1 to 3:1 for the α -cis(O) C_2 isomer drastically changed the distribution of threonine and allothreonine. The increased aldehyde concentration also had a noticeable effect on the products from the reaction of the trans(O) C_2 isomer. The amount of allothreonine synthesized decreased from 67% for the A/C of 1 optically active synthesis to 54% for the A/C of 3 racemic reaction. (In order to synthesize only the monocondensation product complexes in the latter reaction it was necessary to stop the reaction after 20 min producing about 6% of the amino acids, Table X, page 69.)

The influence of increased aldehyde concentration, A/C of 4, on the $[Co(gly)(en)_2]^+$ reaction does not have much effect. The data in Table X for optically active and racemic synthesis gave 70-77% allothreonine which was comparable to the value for the A/C of optically active synthesis. Also of the four compounds studied this is the only one which required very little (0.03 ml) titrant for high aldehyde to complex ratios. The α -cis(O) C_2 and trans(O) C_2 reactions at A/C of 3 required about 10 times this amount of NaOH.

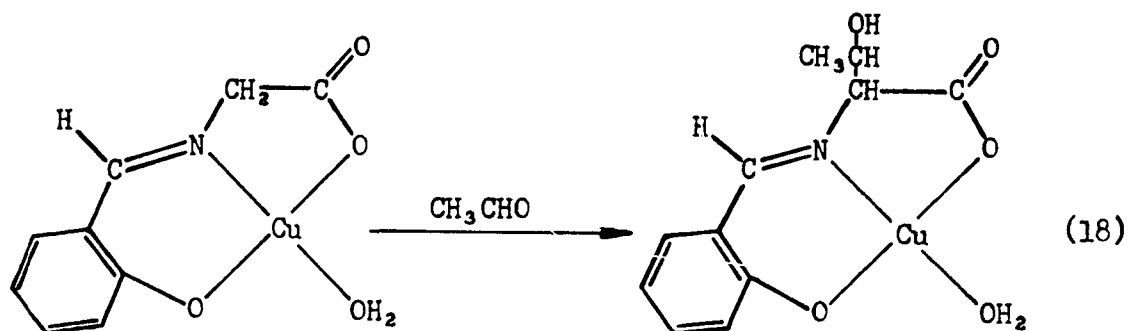
Finally the 90 hr reaction of $(-)_588$, $[Co(gly)(en)_2]I_2$ reported in the literature⁶ was repeated for racemic trans(O) C_2 $[Co(gly)_2(en)]Cl$ and the results are summarized on page 69. These values probably reflect the relative thermodynamic stability of coordinated threonine and allothreonine. The ratio of threonine to allothreonine was 4:1 which was in good agreement with the earlier results obtained for the reaction of acetaldehyde with the α and β isomers of tris(glycinato)

cobalt(III) (introduction, page 2) where reaction conditions were such that thermodynamic control should have been achieved. It is conceivable that high amounts of allothreonine were produced as with the reactions at lower pH and the initial products epimerized about the α carbon to generate high yields of threonine. Similarly the product distribution from the reaction of $\Lambda(C_3)$ $[\text{Co}(\text{gly})(\text{en})_2]^+$ at pH 10.5 probably was the result of an initial synthesis to give high amounts of allothreonine followed by partial epimerization about the α carbon atom to predominantly threonine.

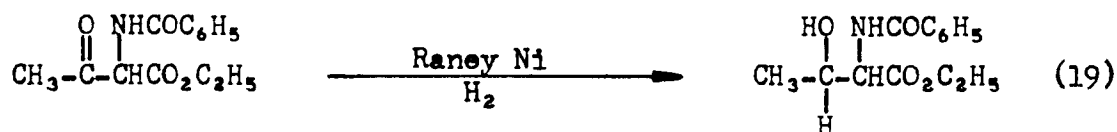
7. Final Observations

It is apparent from the data in Table X that regardless of the geometry of the complex undergoing the pH of 9.5, A/C of 1 reaction, the product distributions are essentially the same. Thus the effect of different neighboring groups (Figure 20, page 66) on the stereochemistry of the products formed is small or nonexistent. Yet the complex is able to influence stereoselective synthesis in the products which raises basic questions concerning the role played by the optically active template in the synthesis. What factors associated with the $\Lambda(C_3)$ complexes result in high yields of the S-amino acids? Why are the products obtained from bis(glycinato) complexes dependent on the aldehyde concentration while the products from the $[\text{Co}(\text{gly})(\text{en})_2]^+$ cation are not? Is this behavior related to the base requirement of the reactions?

An examination of the A/C of 1 optically active synthesis for the trans(O) C_2 , β cis(O) C_1 , and $[\text{Co}(\text{gly})(\text{en})_2]^+$ complexes showed that the arrangement of groups about the β center of the amino acid apparently is not affected by the presence of the dissymmetric complex. Regardless of the side from which the aldehyde enters, the formation of allothreonine is preferred over threonine by 67-77%. The same is probably true for the α cis(O) C_2 isomer but data on the optically active synthesis are not available. This observation compares favorably with the results found by K. Harada and J. Oh-nashi⁴ who studied the reaction of the N-salicylidene-glycinato-aquocopper(II) complex with acetaldehyde (Equation 18).

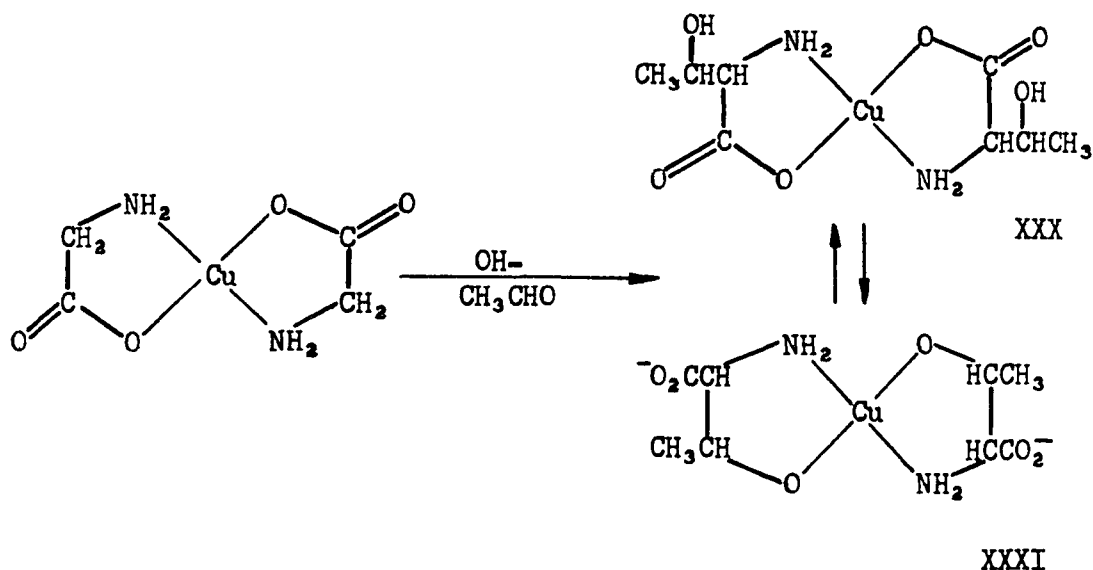


This reaction was carried out using mild conditions (pH 7-8 and 25°) which probably did not allow the α center to epimerize. Since the complex is not optically active, attack of the aldehyde from either side of the coordinated Schiff's base is equally likely and no activity was found in the product amino acids. However, the arrangement of groups about the β carbon atom 66-69% of the time formed allothreonine as opposed to threonine. Other workers have observed the same effect in the synthesis of the hydroxy-amino acids involving the reduction of ethyl α -benzamidoacetoacetate⁵⁰, (Equation 19).



Subsequent workup of the free amino acids revealed that the reduction of the center gave 70% allothreonine and 30% threonine. This evidence suggests that the arrangement about the β center of the amino acids is a property of the amino acids themselves and has little to do with the complex. However, this distribution is dependent on the concentration of the aldehyde or on the overall rate of the synthesis as is evidenced by the products obtained for the A/C of 3 reaction of the trans(O) C_2 and α cis(O) C_2 isomers. (A decrease in the amount of allothreonine was found as A/C increases.) No such dependence was observed for the $[\text{Co}(\text{gly})(\text{en})_2]^+$ case.

The increase in the amount of threonine produced from the bis(glycinato) isomers with increasing ratios of aldehyde to complex may be the result of the equilibrium observed by M. Sato, K. Okawa and S. Akabori for the bis(glycinato)copper(II)-acetaldehyde reaction² (Scheme 1).



The reaction conditions involved A/C of 3 for 1 hr at 50° and the products were isolated by fractional crystallization. Although the total product was not analyzed for the relative amounts of the diastereomers present, the ratio of threonine to allothreonine was about 3. Apparently for this system the newly synthesized amino acids can coordinate as bidentate ligands in two ways. Spectral evidence was given for Form XXXI predominating in the reaction conditions used and it was noted that ligand field about the copper ion had increased in going from XXX to XXXI. This type of rearrangement could explain the shift to lower wavelength observed for all the reaction mixtures studied in this work.

The extension of Scheme 1 to the trans(O)₂C₂ and α cis(O)₂C₂ cases suggests that large amounts of threonine should be synthesized. Molecular models showed that the substituted ethanolamine chelate ring in XXXI is puckered and the most favorable conformation exists when the -CH₃ and CO₂⁻ groups are equatorial as in threonine. In the case of allothreonine both groups are axial. Conceivably the reaction could

be giving a product distribution which is similar to the A/C of 1 reactions studied but a rearrangement takes place as shown in Scheme 1 followed by epimerization about the α carbon atom to give the most stable product threonine. Epimerization about the carbon cannot be ruled out but on the basis of the epimerization studies on the trans(0) isomers it is not likely to occur. One attractive feature of the coordinated ethanolamine type ligand is the fact that the pH-stat added titrant during the reaction, which is consistent with the release of the acidic carboxyl group. Unfortunately the effect of the aldehyde concentration on the reaction given in Scheme 1 is difficult to see.

Some evidence is available to discount the operation of the coordinated ethanolamine scheme in the reactions studied. The epimerization studies on the authentic trans(0) threonine monocondensation product complexes indicate that rearrangements of the type shown in Scheme 1 do not occur. In fact no epimerization occurred and no additional titrant was required after pH 9.5 was reached. It is possible that the presence of the aldehyde is necessary for rearrangement to a coordinated ethanolamine ligand but because of further reactions of the complex with aldehyde this would be difficult to show experimentally. Any change in the product distribution from such an experiment could be the result of either the second unreacted glycine molecule reacting to form product or the rearrangement of the coordinated threonine or allothreonine according to Scheme 1 or both.

In retrospect a more thorough understanding of the factors affecting the stereochemistry of the reactions studied could best be gained by studying the glycinatobis(ethylenediamine)cobalt(III) ion. This complex contains only one glycine molecule and apparently does not suffer from the complications associated with the +1 complexes. Also much stereochemical information can be gained by simply determining the relative amounts of threonine and allothreonine using a variety of reaction conditions before proceeding with the more involved and probably less definite analysis of the optical isomers from an optically active synthesis.

VI. SUMMARY

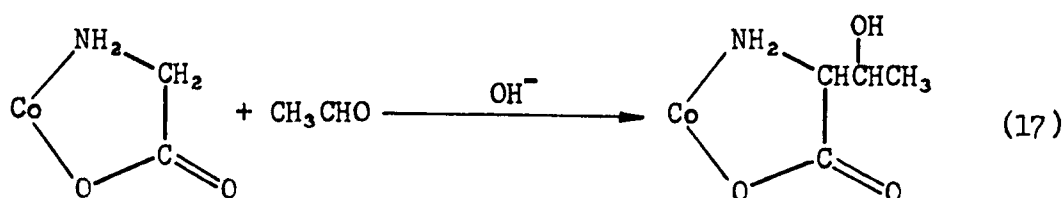
The three geometric isomers of the bis(glycinato)ethylenediamine cobalt(III) ion have been synthesized using a modification of the method of M. Matsuoka *et al.*⁹ and resolved into optical isomers. Several other bis(amino acid)cobalt(III) complexes containing S-threonine, S-serine and S-alanine have been synthesized and characterized.

The absolute configuration of all the complexes have been assigned using circular dichroism by relating the sign of the A→E electronic transition of the bis(amino acid) complexes to the sign of the same transition for the (+)_{ss}, tris(ethylenediamine)cobalt(III) ion²⁷. In addition, the absolute configuration of the trans(0) geometric isomers containing optically active amino acids have been confirmed using pmr steric compression analysis³⁵. For a diastereomeric pair the α methine ($-\overset{\text{H}}{\underset{|}{\text{C}}}$) proton of the amino acid in one isomer experiences a van der Waals interaction with a neighboring amine proton on an adjacent coordination site. This results in deshielding of the affected proton which causes it to resonate at a lower field than the corresponding proton in the isomer free of the compression. With the aid of molecular models and the known absolute configuration of the asymmetric center in the amino acid, the absolute configuration of the complex can be found. The configuration of the trans(0)C₂ bis(glycinato)ethylenediaminecobalt(III) ion which contains no optically active amino acids was assigned by comparing its cd curve with curves of compounds of similar known geometry already established using steric compression.

The gas-liquid chromatographic method of B. Halpern and J.W. Westley^{*3} for the analysis of optical isomers of amino acids was extended to the quantitative analysis of the optical isomers of threonine and allothreonine mixtures. The procedure involved converting the amino acids to dipeptides by treating them with N-trifluoroacetyl R- or S- prolyl chloride which were then separated by glc. Essential to obtaining good quantitative results was preparing the acid chloride in optically pure form which was accomplished using a modification of the method of Weygand¹³. The glc approach is very sensitive requiring only

6-10 mg of amino acids and was well suited for analysis of the amino acid distribution produced from the reaction of coordinated glycine with acetaldehyde. Two other glc methods involving the use of bis-N (trimethylsilyl)trifluoroacetamide¹⁶ and trifluoroacetic anhydride¹⁷ were also used in analyzing the amino acids synthesized in the reaction.

The reactions of acetaldehyde with the $\Lambda(C_3)$ trans(O)C₂, β cis(O)C₁ and α cis(O)C₂ bis(glycinato)ethylenediaminecobalt(III) ions in addition to the $\Lambda(C_3)$ glycinatobis(ethylenediamine)cobalt(III) ion were studied (Equation 17). The other ligands have been omitted for clarity.



The reactions were initially carried out at pH 9.5 for 3 hr with an aldehyde to complex (A/C) ratio of one and allothreonine was found to predominate over threonine by about 2:1. In the case of the bis (glycinato) complexes the reactions were terminated early and only monocondensation products were found. Values of stereoselectivity from the $\Lambda(C_3)$ complexes ranging from 20-37% were found in the amino acids synthesized with the S-isomers preferred over the R-isomers by 2:1. The distribution of optical isomers from the $\Lambda(C_3)$ α cis(O)C₂ [Co(gly)₂(en)]⁺ ion was not determined at A/C of 1 because of the small amount (2%) of amino acids produced. At increasing values of pH the products obtained from the trans(O)C₂ and glycinatobis(ethylenediamine) compounds were probably the result of epimerization about the α carbon atom of the coordinated amino acid to give threonine over allothreonine by about 4:1.

The reaction of the α cis(O)C₂ isomer at pH 9.5 with A/C of 3 gave a large value of stereoselective synthesis (80% S-allothreonine) and threonine was produced over allothreonine by 2:1. The distribution of the diastereomers threonine and allothreonine from the α cis(O)C₂

and trans(O)C₂ isomers was sensitive to the value of A/C but the glycinatobis(ethylenediamine) compound was not. This difference may be related to the observation that the former reactions are not base catalyzed.

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VII. VITA

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