droxy-4,5-dihydropyrene, respectively.
(24) Variable-temperature NMR studies[25] have established the 9,10-dihydrophenanthrene ring system to exist in a flattened boat structure with groups in the 9,10 positions occupying preferentially the axial position. The cis and trans 9,10-disubstituted derivatives exist as pairs of conformers in dynamic equilibrium through ring inversion, the former between equivalent axial–equatorial forms, the latter between diaxial and diequatorial forms.
(25) P. W. Rabideau, R. G. Harvey, and J. B. Stothers, *J. Chem. Soc., Chem. Commun.*, 1005 (1969); P. P. Fu, R. G. Harvey, J. W. Paschal, and P. W. Rabideau, *J. Am. Chem. Soc.*, submitted for publication.
(26) A. F. Cockerill, G. L. O. Davies, R. C. Hardin, and D. M. Rackham, *Chem. Rev.*, **73**, 553 (1973).
(27) A. M. Jeffrey and D. M. Jerina, *J. Am. Chem. Soc.*, **97**, 4427 (1975).
(28) M. J. S. Dewar, "The Molecular Orbital Theory of Organic Chemistry", McGraw-Hill, New York, N.Y. 1969, p 295.
(29) Reaction of naphthalene 1,2-oxide with thioethanol has been shown to provide *trans*-1-hydroxy-2-ethylthio-1,2-dihydronaphthalene,[18] which is also in agreement with this prediction.
(30) G. J. Kasperek and T. C. Bruice, *J. Am. Chem. Soc.*, **94**, 198 (1972).
(31) Since the minor products were not characterized, it is possible that phenols, diols, and the products of distal attack[16] may be formed in trace quantities.
(32) Reactions of benzene oxide with glutathione have been shown to follow an SN1 path involving initial ionization of the oxide followed by relatively rapid trapping of the zwitterionic intermediate by the thiol.[33]
(33) D. M. Reuben and T. C. Bruice, *J. Chem. Soc., Chem. Commun.*, 113 (1974).
(34) H. C. Longuet-Higgins, *J. Chem. Phys.*, **18**, 265, 275, 283 (1950).
(35) J. P. Glusker, H. L. Carrell, D. E. Zacharias, and R. G. Harvey, *Cancer Biochem. Biophys.*, 1, 43 (1970).
(36) A cyclic sulfonium ion was suggested by Jeffrey and Jerina[27] to be intermediate in the rearrangement of *trans*-1-hydroxy-2-ethylthio-1,2-dihydronaphthalene.
(37) C. B. Huggins, J. Pataki, and R. Harvey, *Proc. Natl. Acad. Sci. U.S.A.*, **58**, 2253 (1967).
(38) E. T. Buchovaz, J. C. Morrison, H. L. James, C. F. Dais, and J. L. Wood, *Cancer Res.*, **30**, 155 (1970).
(39) A. M. Jeffrey, S. H. Blobstein, I. B. Weinstein, F. A. Beland, R. G. Harvey, H. Kasai, and K. Nakanishi, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
(40) R. G. Harvey, S. H. Goh, and C. Cortez, *J. Am. Chem. Soc.*, **97**, 3468 (1975).

Synthesis and Isolation of Optically Pure L- and D-2-^{2}H Amino Acids via Cobalt(III) Chelates

William E. Keyes[1] and J. Ivan Legg*

Contribution from the Department of Chemistry, Washington State University, Pullman, Washington 99163. *Received December 30, 1975*

Abstract: A nonenzymatic procedure for the facile preparation of enantiomorphically pure 2-^{2}H amino acids is described. The complexes $[Co(en)_2(L\text{-aa})]X_n$ ($X^- = Cl^-$, I^-, or NO_3^-), where aa = aspartate, glutamate, asparaginate, glutaminate, homoserinate, alaninate, leucinate, prolinate, or *S*-methylcysteinate, chelated through their five-membered glycinate rings, were synthesized and the diastereomers were separated by ion-exchange chromatography. At pH 9.6 and 37 °C each of the chelated amino acids exchanged deuterium at the 2-carbon with varying degrees of racemization, producing another set of diastereomers since configurational racemization did not occur under these conditions. Chromatography of the diastereomers produced in the deuteration of Λ-$[Co(en)_2(L\text{-Asp})]Cl$ separated the optically pure amino acid complexes Λ-$[Co(en)_2(L\text{-2-}^2H\text{-Asp})]Cl$ and Λ-$[Co(en)_2(D\text{-2-}^2H\text{-Asp})]Cl$. Sodium borohydride reduction of these isomers and of the complexes containing deuterated glutamate, asparaginate, and prolinate removed the amino acids from each complex with no loss of optical activity or deuterium at the 2-carbon. Sodium borohydride reduction of the *S*-methylcysteinate complex decomposed the amino acid, with alanine the major decomposition product. The complexes $[Co(en)_2(L\text{-Cys})]I$ (nitrogen–sulfur five-membered chelate ring), $[Co(en)_2(\beta Ala)]Cl_2$ (nitrogen–oxygen six-membered chelate ring), and [Co(EDDA)(L-Asp)] (anion under basic conditions) were synthesized and were found not to exchange deuterium at the amino acid 2-carbon under basic conditions.

Introduction

There exist a number of nonenzymatic methods for preparing deuterated amino acids. These include multistep organic procedures,[2] pyridoxal–metal ion catalyzed deuterium-exchange reactions,[3] and azlactonization by acetic anhydride[4] in deuterated solvents. Although of fairly general applicability, each of these procedures has limitations as to the type of amino acid that can be labeled and the ease of synthesis. Notably, all the methods produce racemic mixtures.

Enzymes which catalyze the racemization of amino acids have been used to isotopically label the 2-carbon during racemization.[5] Each enzyme, however, is specific for a particular amino acid, and, at present, very few have been identified or are available.

Reported here is an alternate nonenzymatic procedure for the facile preparation of enantiomorphically pure 2-^{2}H amino acids. The method is based on the metal ion catalyzed exchange of alkyl protons of chelated amino acids as first detailed by Williams and Busch[6] and the chromatographic separation of diastereomers.

Experimental Section

Analytical results are recorded in Table I. Yields of the diastereomers were of the order of 50%. The complex cysteinatobis(ethylenediamine)cobalt(III) iodide was prepared by the method of Kothari and Busch.[7] The syntheses and separations of the isomers of $[Co(en)_2(L\text{-aa})]X_n$, where aa = aspartate, glutamate, asparaginate, or glutaminate ($X^- = Cl^-$, I^-, or NO_3^-), have been reported previously.[8]

Synthesis and Separation of the Isomers of $[Co(en)_2(L\text{-aa})]X_2$ Where aa = Homoserinate, Alaninate, Leucinate, Prolinate, or *S*-Methylcysteinate ($X^- = Cl^-$ or NO_3^-). The following procedure describes the synthesis and separation of the isomers of homoserinatobis(ethylenediamine)cobalt(III) chloride. The syntheses and separations of the isomers of the related complexes containing alanine, leucine, proline, or *S*-methylcysteine were identical with that of the homoserine complex except that the weight of amino acid used (0.01 mol) varied accordingly with its molecular weight.

A mixture of L-homoserine (L-Hse) (1.19 g, 0.01 mol) and NaOH (0.4 g, 0.01 mol) in 60 ml of water was heated to 40 °C. Solid *trans*-$[Co(en)_2Cl_2]Cl$[9] (2.86 g, 0.01 mol) was added to the warm solution, and the temperature was increased to 70 °C with stirring for 10 min. After cooling to room temperature, the orange solution was diluted to 500 ml with water and loaded on a Dowex 50W-X8 cation-exchange column (4 × 40 cm, 200–400 mesh, 1100 mequiv capacity, Na^+ form) at a rate of ½ ml/min. (A much longer column [4 × 150 cm] was employed for the separation of the isomers of $[Co(en)_2(L\text{-Ala})]^{2+}$.) Upon elution with 1 M NaCl (flow rate ½ ml/min) the complex separated cleanly into two orange bands. Circular dichroism spectra of fractions showed each band to consist of one isomer. The fractions were combined for each band, evaporated to near dryness in an air

Table I. Elemental Analyses

Complex	% C		% H		% N	
	Calcd	Found	Calcd	Found	Calcd	Found
Λ-[Co(en)$_2$(L-Hse)]Cl$_2$·½C$_2$H$_5$OH,H$_2$O	26.42	26.39	7.14	7.10	17.11	17.04
Δ-[Co(en)$_2$(L-Hse)]Cl$_2$·2H$_2$O	23.77	23.93	6.98	7.00	17.33	17.53
Λ[Co(en)$_2$(L-Ala)]Cl$_2$·2H$_2$O	22.47	22.32	7.00	7.02	18.72	18.68
Δ-[Co(en)$_2$(L-Ala)]Cl$_2$·½H$_2$O	24.22	24.50	6.68	6.72	20.18	20.01
Λ-[Co(en)$_2$(L-Leu)](NO$_3$)$_2$	27.72	27.56	6.51	6.58	22.63	22.46
Δ-[Co(en)$_2$(L-Leu)]Cl$_2$·1½H$_2$O	29.49	29.72	7.67	7.45	17.20	17.14
Λ-[Co(en)$_2$(L-Pro)]Cl$_2$·½C$_2$H$_5$OH,2H$_2$O	28.38	28.54	7.38	7.31	16.55	16.58
Δ-[Co(en)$_2$(L-Pro)]Cl$_2$·H$_2$O	28.28	28.15	6.86	6.96	18.32	18.32
Λ-[Co(en)$_2$(L-Cys(Me))]Cl$_2$·H$_2$O	23.89	23.88	6.52	6.47	17.41	17.35
Δ-[Co(en)$_2$(L-Cys(Me))]Cl$_2$·H$_2$O	23.89	23.75	6.52	6.51	17.41	17.33
[Co(en)$_2$(βAla)]Cl$_2$·2H$_2$O	22.47	22.40	7.00	6.96	18.72	18.68
Λ-*s*-*cis*-[Co(EDDA)(L-HAsp)]·2H$_2$O	29.94	29.90	5.02	5.02	10.47	10.34
Δ-*s*-*cis*-Na[Co(EDDA)(L-Asp)]·2H$_2$O	28.38	28.35	4.53	4.33	9.93	10.02
L-2-^{2}H-Asp	35.82	35.69	5.26	5.26	10.44	10.19

stream, and excess NaCl was filtered off. Final desalting was accomplished by gel permeation chromatography on a column (4 × 95 cm) of Sephadex G-10 (700 g dry wt). The solution (40 ml) was passed down the column at a rate of ½ ml/min. Testing for Cl^- with $AgNO_3$ showed a clean separation of the complex from NaCl. Solid [Co(en)$_2$(L-Hse)]Cl$_2$ was isolated by evaporation of the resulting solution.

Recrystallization of Λ-[Co(en)$_2$(L-Hse)]Cl$_2$, Δ-[Co(en)$_2$(L-Ala)]Cl$_2$, and Λ-[Co(en)$_2$(L-Pro)]Cl$_2$ was accomplished by dissolving each isomer in a minimum amount of water (~4 ml) and adding this solution dropwise to 35 ml of a 50/50 mixture of methanol/ethanol while vigorously stirring. The resulting slurry was stirred overnight. The orange solid was then collected by filtration, washed with absolute ethanol, and dried by drawing dry, filtered air through the solid overnight. Λ-[Co(en)$_2$(L-Pro)]Cl$_2$ was dried under vacuum at 100 °C for 24 h.

The Δ isomer of [Co(en)$_2$(L-Hse)]Cl$_2$ was dissolved in 4 ml of water, and the solution was added dropwise to 30 ml of vigorously stirred absolute ethanol. After stirring overnight, the solid was collected, washed, and dried using the procedure described for the Λ isomer.

To recrystallize the isomers Λ-[Co(en)$_2$(L-Ala)]Cl$_2$, Δ-[Co(en)$_2$(L-Pro)]Cl$_2$, and Δ-[Co(en)$_2$(L-Cys(Me))]Cl$_2$, each was dissolved in a minimum quantity of water (~4 ml), and 18 ml of methanol was added dropwise. After stirring overnight, the solid was collected by filtration, washed with methanol, and air-dried as described above.

The isomers Δ-[Co(en)$_2$(L-Leu)]Cl$_2$ and Λ-[Co(en)$_2$(L-Cys(Me))]Cl$_2$ were recrystallized in the same manner as Δ-[Co(en)$_2$(L-Cys(Me))]Cl$_2$ except that 14 ml of absolute ethanol was used instead of methanol.

The Λ isomer of [Co(en)$_2$(L-Leu)]Cl$_2$ was dissolved in 50 ml of water and passed down a Dowex 1-X8 anion-exchange column (4 × 28 cm, 200–400 mesh, 545 mequiv capacity, NO_3^- form) at a rate of ½ ml/min. The solid Λ-[Co(en)$_2$(L-Leu)](NO$_3$)$_2$ obtained by evaporation of the eluent was recrystallized using the same procedure as that employed for Δ-[Co(en)$_2$(L-Leu)]Cl$_2$.

Synthesis of β-Alaninatobis(ethylenediamine)cobalt(III) Chloride. A mixture of β-alanine (0.9 g, 0.01 mol) and NaOH (0.4 g, 0.01 mol) in 50 ml of water was heated to 40 °C with stirring. Solid *trans*-[Co(en)$_2$Cl$_2$]Cl[9] (2.86 g, 0.01 mol) and activated charcoal (3 g, Norit-A, Alkaline) were added to the warm solution. The mixture was heated at 70 °C with stirring for 15 min. After removal of the charcoal by hot filtration, the orange solution was diluted to 500 ml and loaded on a Dowex 50W-X4 cation-exchange column (4 × 30 cm, 200–400 mesh, 580 mequiv capacity, Na^+ form). Upon elution with 1 M NaCl, the orange complex separated cleanly from the other species present. Desalting was partially achieved using the procedure described for [Co(en)$_2$(L-Hse)]Cl$_2$. The complex was recrystallized twice by dissolving it in a minimum amount of hot (70 °C) water, followed by cooling to 5 °C with scratching. The red-orange solid was collected by filtration, washed with a small amount of ice-cold water, then ethyl ether, and air-dried as described for [Co(en)$_2$(L-Hse)]Cl$_2$.

Synthesis and Separation of the Isomers of *cis*-Ethylenediamine-*N,N'*-diacetato(L-aspartato)cobalt(III). A mixture of cobalt carbonate (2.62 g, 0.022 mol) and ethylenediamine-*N,N'*-diacetic acid (3.52 g, 0.02 mol) was added to 50 ml of water and heated at 60 °C with occasional stirring until carbon dioxide evolution ceased (about 45 min). The violet-pink solution was filtered through a medium fritted glass filter, then diluted to 250 ml with water. Activated charcoal (3 g, Norit-A, Alkaline) and L-aspartic acid (2.66 g, 0.02 mol) were added, and a stream of air was drawn through the suspension for 24 h. The mixture was heated at 60 °C with stirring for 30 min and then filtered to remove charcoal. The pH of the red-violet solution was adjusted to 7 with NaOH (1 M), and the volume was reduced to 120 ml by roto-evaporation. The pH was then brought to 4.7 by dropwise addition of HCl (6 N). The resulting suspension was heated (70 °C) with stirring to dissolve all solid material and then allowed to cool slowly. After 24 h, violet needle crystals were collected by filtration, washed with absolute ethanol, and air-dried as described for [Co(en)$_2$(L-Hse)]Cl$_2$. The pH of the filtrate had increased to 5.5. A second crop of crystals was obtained by lowering the pH to 4.7 with HCl and repeating the previously described steps. The CD spectra for the two crops were identical ($\Delta\epsilon_{540}$ = 4.16), and further recrystallization did not change the $\Delta\epsilon_{540}$. The pH was then brought to and maintained at 4.6, and all precipitate that formed (shown to be racemic by CD) was removed by filtration and discarded. The solution was then brought to pH 7.0 by addition of NaOH (1 M) and evaporated to a volume of 8 ml. This solution was added dropwise to 50 ml of rapidly stirred methanol. The resulting purple precipitate (~25% pure Δ) was filtered, washed with methanol, and air-dried. No further purification was attempted.

Deuteration. Deuterations were carried out in D_2O with the pH adjusted to 9.6 by addition of a small quantity of Na_2CO_3. The solutions were allowed to stand at 37 °C until the ^1H NMR signal of the 2-carbon proton of the coordinated amino acid was no longer detectable (1–30 days).

Separation of the Isomers of Λ-[Co(en)$_2$(2-^{2}H-Asp)]Cl. The deuterated complex mixture was diluted to 500 ml with water and loaded on a Dowex 50W-X8 cation-exchange column (4 × 40 cm, 200–400 mesh, 1100 mequiv capacity, Na^+ form) at a rate of ½ ml/min. Column elution and desalting of the two orange bands (Λ-[Co(en)$_2$(L-2-^{2}H-Asp)]Cl and Λ-[Co(en)$_2$(D-2-^{2}H-Asp)]Cl) were identical with the procedures used for the separation of the isomers of [Co(en)$_2$(L-Asp)]Cl.

Sodium Borohydride Reduction of Amino Acid Complexes. The following isolation of L-[2-^{2}H]aspartic acid from Λ-[Co(en)$_2$(L-2-^{2}H-Asp)] Cl is a typical example of the procedure followed for amino acid isolation from its corresponding complex. Λ-[Co(en)$_2$(L-2-^{2}H-Asp)]Cl (0.76 g, 2.0 mmol) was added to 50 ml of 0.1 N HCl, and a solution of 2 g of $NaBH_4$ in 15 ml of water was added dropwise until a pH of 7 was attained. The black precipitate was immediately removed by filtration using Celite Filter Aid and washed with a small amount of water. The combined filtrate was diluted to 100 ml with water and loaded on a Bio-Rad AG 1-X4 anion-exchange column (2 × 30 cm, 100–200 mesh, 134 mequiv ca-

pacity, Cl^- form) at a rate of 2–3 ml/min. After rinsing with 1.5 l. of distilled water, the eluting solution was changed to 0.1 N HCl. The fractions beginning 25 ml before the eluent became acidic were combined to give a total of 150 ml of solution and evaporated to dryness. The solid L-[2-^{2}H]aspartic acid was dissolved in 7 ml of 0.1 N HCl and filtered through a fritted glass filter funnel. Several drops of 1 M NaOH were added to the solution to bring the pH to 3.0 (isoelectric point). The resulting slurry was cooled overnight at 5 °C and then filtered cold. The white crystalline material was washed with a small amount of ice-cold water, then absolute ethanol, and dried under vacuum at 100 °C for 24 h. A second crop of material was obtained by addition of 20 ml of absolute ethanol to the filtrate. The crystals were collected and dried as described above. A quantitative amino acid analysis (Beckman 121 C automatic amino acid analyzer) showed that the combined deuterated sample consisted of 0.17 g (1.3 mmol) of pure aspartic acid, a 65% yield. Its ORD spectrum was identical within experimental error (±3%) to that of L-aspartic acid. The isolations for asparagine and proline differed from the above example in that the neutral amino acid solutions (after the filtration with Celite Filter Aid) were passed through both an anion- and cation-exchange column (Dowex 50W-X8, 2 × 30 cm, 100–200 mesh, 200 mequiv capacity, Na^+ form). At pH 7 asparagine and proline are neutral and pass through the exchange column with the void volume. The samples were then passed down Sephadex G-10 and evaporated to dryness.

Spectra. The absorption spectra were measured on a Cary 14 spectrophotometer. The CD and ORD spectra were recorded on a JASCO ORD/UV5 with an SS 20 CD modification by Sproul Scientific. The CD instrument was calibrated with purified *d*-10-camphorsulfonic acid using the value $\Delta\epsilon_{290} = +2.34$.[10] The absorption and CD spectra were recorded at room temperature at concentrations of ca. 10^{-3} M and at a pH of ca. 8.3. The amino acid samples were diluted with 3 N HCl to a concentration of ca. 0.1 M prior to obtaining ORD spectra.

Proton magnetic resonance spectra were recorded on a Varian A-60, Varian T-60, and a JEOL MH-100 NMR spectrometer at concentrations of ca. 0.3 M and a pH of ca. 9.6.

Results and Discussion

Outline of Procedure. The experimental procedure employed for the preparation of L- or D-2-^{2}H amino acid is shown schematically below.

(1) Synthesis of complex

$$[Co(en)_2CO_3]^+ + \text{L-aa} \rightarrow \Lambda\text{-}[Co(en)_2(\text{L-aa})]^{n+}$$
$$(\text{or } [Co(en)_2Cl_2]^+) \qquad \Delta\text{-}[Co(en)_2(\text{L-aa})]^{n+}$$

(2) Separation of diastereomers by ion-exchange chromatography

$$\begin{matrix}\Lambda(\text{L})\\ \Delta(\text{L})\end{matrix} \rightarrow \Lambda(\text{L}) + \Delta(\text{L})$$

(3) Deuteration plus racemization of amino acid

$$\Lambda\text{-}[Co(en)_2(\text{L-aa})]^{n+} \xrightarrow{(O^2H)^-} \Lambda\text{-}[Co(en)_2(\text{L-2-}^2\text{H-aa})]^{n+}$$
$$(\text{or } \Delta) \qquad\qquad \Lambda\text{-}[Co(en)_2(\text{D-2-}^2\text{H-aa})]^{n+}$$

(4) Separation of diastereomers by ion-exchange chromatography

$$\begin{matrix}\Lambda(\text{L})\\ \Lambda(\text{D})\end{matrix} \rightarrow \Lambda(\text{L}) + \Lambda(\text{D})$$

(5) Removal of amino acid from complex

$$\Lambda\text{-}[Co(en)_2(\text{D-2-}^2\text{H-aa})]^{n+} \xrightarrow{NaBH_4} \xrightarrow{\text{chromatography}} \text{D-2-}^2\text{H-aa}$$

The Λ(L) isomer isolated in step 4 can be treated as in step 5 to obtain the corresponding L-2-^{2}H amino acid. The method involves the following critical steps. The L-amino acid to be stereospecifically deuterated is chelated by a complex which itself consists of optical antipodes, thereby producing a set of diastereomers (step 1). These diastereomers are then separated chromatographically (step 2). The subsequent deuteration step which racemizes the amino acid over an extended period of time does not racemize the complex, and diastereomers are again obtained (step 3). Chromatography separates the L-deuterio from the D-deuterio amino acid complex (step 4), and the 2-C deuterated amino acid (step 5) is then removed from the complex 100% optically pure.

Synthesis and Isolation of Isomers. The bis(ethylenediamine) amino acid complexes were synthesized by either displacement of carbonate with the appropriate amino acid from $[Co(en)_2CO_3]^+$ in the presence of charcoal at 70 °C, or by reaction of the amino acid with *trans*-$[Co(en)_2Cl_2]^+$ at 70 °C without charcoal. Both methods were used for several of the syntheses and were found to give similar yields of the desired complex, although a different product distribution for the remainder of the reaction mixture was obtained. The iodide salts of the diastereomers of $[Co(en)_2(\text{L-aa})]^{2+}$, where aa = alaninate,[11] leucinate,[11] or prolinate,[12,13] and the complex $[Co(en)_2(\beta\text{ala})]Cl_2$[14] have been made before. The complex $[Co(en)_2(\text{L-Cys(Me)})]I_2$ has been synthesized previously but the diastereomers were not separated.[7] The diastereomers of *cis*-[Co(EDDA)(L-Asp)] were synthesized by oxidation of Co(II)EDDA in the presence of L-aspartic acid and charcoal at 55 °C.

With the exception of $[Co(en)_2(\text{L-Cys})]I$, all bis(ethylenediamine) amino acid complex diastereomers were separated by ion-exchange chromatography. In the majority of cases, the Λ[15] diastereomer eluted from the column before the Δ isomer; the reverse order was found for $[Co(en)_2(\text{L-Leu})]^{2+}$ and $[Co(en)_2(\text{L-Gln})]^{2+}$. Final desalting for all chromatographed isomers was achieved by gel permeation chromatography. Anion-exchange chromatography of the reaction mixture of $[Co(EDDA)(\text{L-Asp})]^-$ separated the uns-cis isomers from the s-cis isomers but did not separate the s-cis diastereomers.[16] Isolation of the pure Λ-s-cis diastereomer was achieved by fractional crystallization. Purification of the Δ-s-cis isomer beyond the separation obtained in initial crystallization (~25% pure Δ) was not attempted.

Spectral data for all complexes are recorded in Table II. The absolute configuration for each of the complexes was assigned by analysis of their circular dichroism spectra and reference to the absolute configuration of Λ-$[Co(en)_2(\text{asn})]^{2+}$ determined by x-ray crystallography.[8a]

Deuteration of Amino Acids at the 2-Carbon. Deuteration of the chelated amino acid at the 2-carbon was easily followed by ^{1}H NMR as exemplified by Λ-$[Co(en)_2(\text{L-Asn})]^{2+}$ in Figure 1. For the bis(ethylenediamine) complexes, all amino acids studied in which chelation was through their five-membered glycinate ring exchanged deuterium at the 2-carbon at pH 9.6 and 37 °C. Dipositive complex cations were found generally to exchange deuterium at the amino acid 2-carbon more rapidly than the monopositive cations. An exception to this rule was $[Co(en)_2(\text{L-Pro})]^{2+}$, in which the Λ isomer took 13 days to exchange completely and the Δ isomer required 34 days for deuteration, during which time some decomposition of the complex occurred. These rates were ~five-te times slower than the rate of deuteration observed for the average dipositive complex. The anion Λ-*s-cis*-$[Co(EDDA)(\text{L-Asp})]^-$ did not exchange deuterium at the amino acid 2-carbon at pH 9.8 at 40 °C (although the EDDA glycinate protons did exchange in ~2 days as previously observed for similar complexes[17,18]), and slow decomposition of the complex was observed. The fact that, in general, dipositive complexes deuterate more rapidly than monopositive complexes and negatively charged complexes are not observed to deuterate is consistent with the proposed mechanism for hydrogen exchange at the 2-carbon which involves initial dissociation of H^+ in the rate-controlling step.[18,19] Clearly other factors must also be involved since the EDDA glycinate protons do exchange in Λ-*s-cis*-$[Co(EDDA)(\text{L-Asp})]^-$. However, the rate of exchange in this negatively charged complex is much slower than in positively charged EDDA complexes.[18] A possible explanation for the

Table II. Spectral Data

Complex	Absorption maxima, nm (ϵ, M^{-1} cm^{-1})	Circular dichroism maxima, nm ($\Delta\epsilon$, M^{-1} cm^{-1})
Λ-[CO(en)$_2$(L-Asp)]Cl·2H$_2$O[a]	485 (101), 347 (109)	506 (2.45), 426 (−0.039), 370 (0.066), 348 (−0.024), 323 (0.082)
Δ-[Co(en)$_2$(L-Asp)]Cl·3½H$_2$O[a]	485 (100), 347 (103)	505 (−2.09), 370 (−0.101), 327 (−0.152)
Λ-[Co(en)$_2$(L-Glu)]Cl·2½H$_2$O[a]	488 (107), 349 (119)	504 (2.48), 431 (−0.111), 370 (0.049), 347 (−0.041), 322 (0,053)
Δ-[Co(en)$_2$(L-Glu]Cl·1½H$_2$O[a]	488 (105), 349 (116)	510 (−2.14), 365 (−0.151), 330 (−0.175)
Λ-[Co(en)$_2$(L-Gln)]Cl$_2$[a]	488 (105), 348 (116)	505 (2.24), 436 (−0.199), 370 (0.029), 348 (−0.040), 322 (0.053)
Δ-[Co(en)$_2$(L-Gln)]Cl$_2$·H$_2$O[a]	488 (105), 349 (112)	510 (−2.20), 367 (−0.168), 330 (−0.193)
Λ-[Co(en)$_2$(L-Asn)]I$_{1.5}$(NO$_3$)$_{0.5}$[a]	488 (100), 349 (109)	506 (2.39), 430 (−0.084), 369 (0.075), 323 (0.092)0
Δ-[Co(en)$_2$(L-Asn)]I$_2$·½H$_2$O[a]	486 (101), 348 (108)	506 (−2.09), 368 (−0.102), 327 (−0.149)
[Co(en)$_2$(L-Cys)]I·H$_2$O[b]	600sh (43), 490 (128)	
Λ-[Co(en)$_2$(L-Hse)]Cl$_2$·½C$_2$H$_5$OH,H$_2$O	488 (104), 348 (117)	504 (2.12), 435 (−0.135), 370 (0.033), 349 (−0.027), 321 (0.056)
Δ-[Co(en)$_2$(L-Hse)]Cl$_2$·2H$_2$O	488 (101), 348 (120)	510 (−1.78), 366 (−0.144), 332 (−0.158)
Λ-[Co(en)$_2$(L-Ala)]Cl$_2$·2H$_2$O	488 (108), 348 (115)	505 (2.46), 439 (−0.304), 367 (0.062), 324 (0.070)
Δ-[Co(en)$_2$(L-Ala)]Cl$_2$·½H$_2$O	488 (106), 348 (113)	510 (−1.87), 365 (−0.146), 330 (−0.167)
Λ-[Co(en)$_2$(L-Leu)](NO$_3$)$_2$	488 (103), 348 (115)	505 (2.36), 440 (−0.282), 368 (0.054), 348 (−0.015), 323 (0.061)
Δ-[Co(en)$_2$(L-Leu)]Cl$_2$·1½H$_2$O	487 (103), 348 (120)	508 (−2.28), 366 (−0.173), 330 (−0.194)
Λ-[Co(en)$_2$(L-Pro)]Cl$_2$·½C$_2$H$_5$OH,2H$_2$O	491 (117), 352 (136)	503 (2.53), 351 (−0.154), 320 (0.036)
Δ-[Co(en)$_2$(L-Pro)]Cl$_2$·H$_2$O	492 (103), 352 (114)	522 (−1.93), 450 (−0.263), 370 (−0.188), 335 (−0.236)
Λ-[Co(en)$_2$(L-Cys(Me))]Cl$_2$·H$_2$O	489 (102), 344 (131)	508 (2.19), 441 (−0.299), 368 (0.059), 327 (0.080)
Δ-[Co(en)$_2$(L-Cys(Me))]Cl$_2$·H$_2$O	488 (104), 344 (135)	507 (−1.97), 370 (−0.110), 330 (−0.124)
[Co(en)$_2$(βAla)]Cl$_2$·2H$_2$O	496 (126), 352 (87)	
Λ-*s*-*cis*-[Co(EDDA)(L-Asp)]·2H$_2$O	543 (99), 485sh (73), 372 (137)	540 (4.16), 464 (−1.83), 374 (0.446)
Δ-*s*-*cis*-Na[Co(EDDA)(L-Asp)]·2H$_2$O[c]	537, 495sh, 371	550 (−), 465 (+), 360 (−)

[a] From ref 8a. [b] From ref 7. [c] Pure isomer not isolated, as discussed in text.

difference in rate between glycinate ring protons and other amino acid chelate rings is the presence of a sterically restricting side chain in the latter.

No change in the ^{1}H NMR spectrum of [Co(en)$_2$(L-Cys)]$^+$ (nitrogen–sulfur five-membered chelate ring[7]) and [Co(en)$_2$(βala)]$^{2+}$ (nitrogen–oxygen six-membered chelate ring) was observed after 30 days at pH 9.8 and 40 °C. These results suggest the necessity for a five-membered glycinate ring for 2-C deuterium exchange, although exchange at the 3-carbon takes place in a six-membered ring where aspartate is chelated as a tridentate.[20]

Racemization at the 2-carbon occurred at the same rate as deuteration for most of the amino acids studied. Racemization occurred more slowly than deuteration in the bis(ethylenediamine) complexes of L-aspartate and L-glutamate. The potential for intramolecular hydrogen bonding involving the amino acid side chain has been implicated as the factor controlling the stereochemical course of the deuteration in these two complexes.[8a,b] No racemization occurred during deuteration for Λ-[Co(en)$_2$(L-Pro)]$^{2+}$. Proline differs from the other amino acids in that inversion at the 2-carbon requires inversion of the coordinated amine.

In all cases, except Δ-[Co(en)$_2$(L-Pro)]$^{2+}$, the absolute configuration about the cobalt in [Co(en)$_2$(L-aa)]$^{n+}$ was preserved under the conditions necessary for the deuteration of the coordinated amino acid 2-carbon.

Separation of Diastereomers after Deuteration. Deuteration of Λ-[Co(en)$_2$(L-Asp)]Cl produced a mixture of isomers consisting of Λ-[Co(en)$_2$(L-2-^{2}H-Asp)]$^+$ and Λ-[Co(en)$_2$(D-2-^{2}H-Asp)]$^+$. The procedure for separation of these diastereomers (Λ(L), Λ(D)) was identical with the procedure for separation of the diastereomers produced in the synthesis of [Co(en)$_2$(L-Asp)]$^+$ (Λ(L), Δ(L)). The separation of isomers in the deuterated mixture is equivalent to the separation of isomers in the synthesis mixture since Λ(D) and Δ(L) are mirror images and therefore chemically equivalent (disregarding different hydrogen isotopes). It therefore follows that separation of the deuteration mixture should always be possible when separation of the synthesis mixture can be achieved.

Removal of Coordinated Amino Acids from the Complex by Sodium Borohydride Reduction. Aspartic acid, glutamic acid, asparagine, and proline were removed from their corresponding

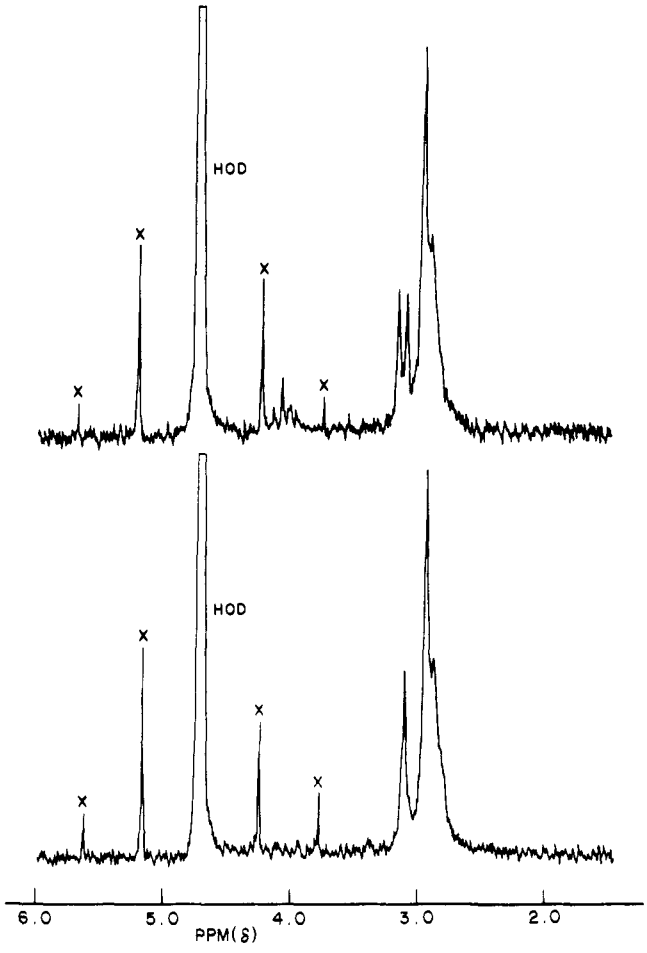


Figure 1. The 60 MHz ^{1}H NMR spectra of Λ-[Co(en)$_2$(2-^{2}H-Asn)]$^{2+}$ (lower) and Λ-[Co(en)$_2$(L-Asn)]$^{2+}$ (upper).

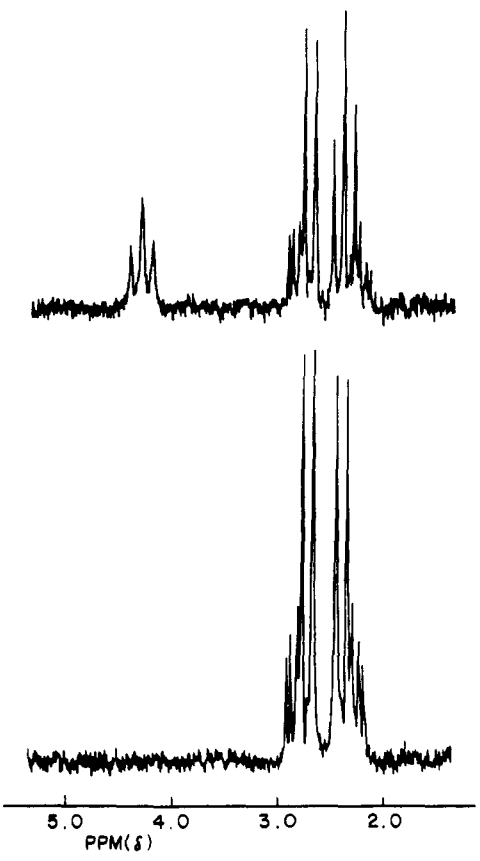


Figure 2. The 60 MHz ^{1}H NMR spectra of glutamic acid isolated from the reduction of Λ-[Co(en)$_2$(2-^{2}H-Glu)]$^+$ (lower) and undeuterated glutamic acid (upper).

bis(ethylenediamine) complexes by reduction of the cobalt(III) complex with $NaBH_4$ to give labile Co(II). Ion-exchange chromatography was used to separate the amino acids from the residual products, and yields after recrystallization were normally 60–90%. The reduction process does not exchange hydrogen (deuterium) at the 2-carbon as can be seen in the ^{1}H NMR spectra of [2-^{2}H]aspartic acid[8b] and [2-^{2}H]glutamic acid (Figure 2) in which the methine triplet of the nondeuterated acid located between 4 and 5 ppm is absent. The ORD spectra obtained for the $NaBH_4$ isolated L-aspartic acid (used as a control), L-[2-^{2}H]aspartic acid, and D-[2-^{2}H]aspartic acid confirmed that no loss in optical activity had occurred during reduction.

The $NaBH_4$ reduction process was not applicable to the isolation of *S*-methylcysteine. Decomposition of the amino acid occurred resulting in a mixture of products of which alanine was the major constituent.

Conclusion

Optically pure 2-C deuterium labeled amino acids can be synthesized employing chromatographically resolvable cobalt(III) diastereomers where the amino acid is chelated through the five-membered glycinate ring. Sodium borohydride reduction of the resolved complex was shown to be an effective means of removal of nonsulfur-containing amino acids from the cobalt complex.

The time-consuming procedure of crystallization of each of the diastereomers described above was followed in order to obtain complete characterization of each of the isomers. This procedure, which results in a decreased overall yield of the desired compound, would be unnecessary for the synthesis of deuterated amino acids.

Acknowledgments. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, the National Science Foundation (Grant GP-34490X), and the National Institutes of Health (Grant GM 18983) for support of this research. This investigation was supported in part by funds provided by the graduate School from the NIH Biomedical Sciences Support Grant.

References and Notes

(1) Oregon Graduate Center for Study and Research, 19600 N. W. Walker Road, Beaverton, Oregon 97005.
(2) A. T. Blomquist, R. J. Cedergren, B. F. Hiscock, S. L. Tripp, and D. N. Harpp, *Proc. Natl. Acad. Sci. U.S.A.*, **55**, 453 (1966); A. T. Blomquist, B. F. Hiscock, and D. N. Harpp, *J. Org. Chem.*, **31**, 4121 (1966).
(3) R. B. Johns and D. J. Whelan, *Aust. J. Chem.*, **19**, 2143 (1966).
(4) H. E. Carter, "Organic Reactions", Vol. 3, Wiley, New York, N.Y., 1946, p 198.
(5) K. Soda and T. Osumi, "Methods in Enzymology", Vol. 17, Academic Press, New York, N.Y., 1971, p 629.
(6) D. H. Williams and D. H. Busch, *J. Am. Chem. Soc.*, **87**, 4644 (1965).
(7) V. M. Kothari and D. H. Busch, *Inorg. Chem.*, **8**, 2276 (1969).
(8) (a) W. E. Keyes, R. E. Caputo, R. D. Willett, and J. I. Legg, *J. Am. Chem. Soc.*, in press; (b) W. E. Keyes and J. I. Legg, *ibid.*, **95**, 3431 (1973); (c) J. I. Legg and J. Steele, *Inorg. Chem.*, **10**, 2177 (1971).
(9) J. C. Bailar, Jr., "Inorganic Syntheses", Vol. 2, McGraw-Hill, New York, N.Y., 1946, p 222.
(10) W. C. Krueger and L. M. Pschigoda, *Anal. Chem.*, **43**, 675 (1971). d-10-Camphorsulfonic acid appears to be one of the most suitable standards for CD calibration since it is readily purified and gives a sharp CD maximum in the near-uv region. However, there is not, at this time, agreement as to the absolute circular dichroism of this compound. Therefore, the value used to calibrate the instrument is given here to facilitate accurate comparison of CD data.
(11) C. T. Liu and B. E. Douglas, *Inorg. Chem.*, **3**, 1356 (1964).
(12) D. A. Buckingham, J. Dekkers, A. M. Sargeson, and M. Wein, *Inorg. Chem.*, **12**, 2019 (1973).
(13) S. K. Hall and B. E. Douglas, *Inorg. Chem.*, **8**, 372 (1969).
(14) M. Ogawa, Y. Shimura, and R. Tsuchida, *Nippon Kagaku Zasshi*, **81**, 72 (1960).
(15) IUPAC, *Inorg. Chem.*, **9**, 1 (1970).

(16) There are several nomenclatures used to describe the isomers of a linear tetradentate chelate. The one employed here was first used for EDDA chelates by Jordan and Douglas [*Inorg. Chem.*, **12**, 403 (1973)]. It is based on the arrangement of positions on the octahedron not occupied by the tetradentate.
(17) P. F. Coleman, J. I. Legg, and J. Steele, *Inorg. Chem.*, **9**, 937 (1970), and references therein.
(18) J. L. Sudmeier and G. Occupati, *Inorg. Chem.*, **7**, 2524 (1968).
(19) D. A. Buckingham, L. G. Marzilli, and A. M. Sargeson, *J. Am. Chem. Soc.*, **89**, 5133 (1967).
(20) J. A. McClarin, L. A. Dressel, and J. I. Legg, *J. Am. Chem. Soc.*, **98**, 4150 (1976).

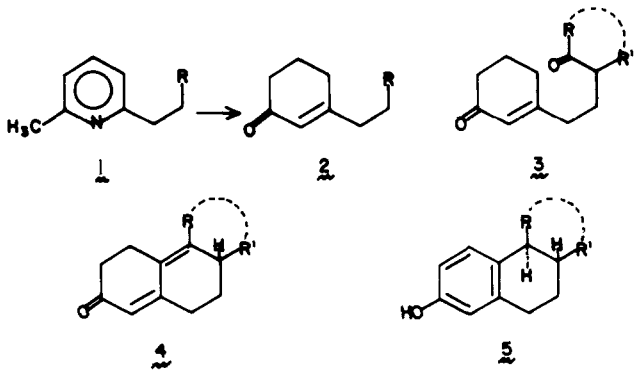
Optically Specific Synthesis of Estrone and 19-Norsteroids from 2,6-Lutidine[1]

Samuel Danishefsky* and Paul Cain

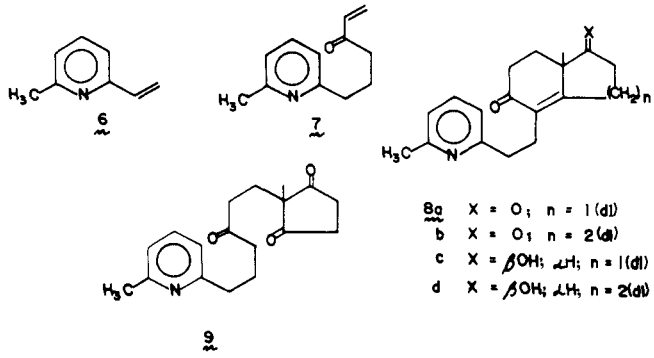
Contribution from the Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260. *Received December 26, 1975*

Abstract: Reaction of 2-methyl-2-[3-oxo-6-(6-methylpyrid-2-yl)hex-1-yl]cyclopentane-1,3-dione with L-phenylalanine and perchloric acid gives 4-[2-(6-methylpyrid-2-yl)ethyl]-7a-methyl-7,7a-dihydro-1,5(6*H*)-indandione with high (85 ± 1%) optical specificity of the 7a*S* enantiomer. The latter is converted to optically pure (+)-estrone and optically pure 19-norandrogens containing the 13*S* configuration, utilizing as key steps reductive hydrolytic cyclization and vinylogous aldolization.

In previous papers in this series, we have demonstrated the convertability of 6-substituted α-picolines of type **1** to 3-substituted cyclohexenones such as **2**.[2-5] The transformation is highly efficient in those cases where R represents a cycloalkanone ketal joined at its α position. The derived enedione **3** suffers smooth vinylogous aldolization to give dienone **4**, which can be isomerized to phenol **5**. This combination of reactions constitutes an attractive route to phenolic steroids such as estrone and its derived 19-norandrogens. The viability of this route to steroids was demonstrated in the context of the total synthesis of *dl*-*D*-homoestrone.[4,5]



In our early studies, systems of the type **1** were assembled by Michael additions to vinylpicoline **6**. An important improvement was achieved when it was found that the tris annelating agent **7** could be readily synthesized (57%) from 2,6-lutidine and that it reacted smoothly with both 2-methylcyclopentane-1,3-dione and 2-methylcyclohexane-1,3-dione to give, directly, 4-alkylated bicyclenones of the type **8**.[6] Moreover it was found that the Michael addition and cyclodehydration stages of the annelation can be decoupled. For instance, reaction of **7** with 2-methylcyclopentane-1,3-dione under the influence of triethylamine in ethyl acetate[7] gave a nearly quantitative yield of the seco system **9**. Experimental details for the synthesis of annelating agent **7**, *dl* systems **8**, and prochiral system **9** have been provided.[5,6]



In projecting a total synthesis of estrone from this prior art there remained two major obstacles. For achieving the required stereochemistry, it was crucial to generate the trans C:D junction of steroids by the reduction of **8a** or some suitable, easily accessible derivative. This appeared to be a more complicated proposition than the relatively simple elaboration of the trans C:D system in the *D*-homo series (**8d**). The difficulties associated with the construction of trans hydrindanones from progenitors bearing unsaturation at the junction have been an enduring problem in steroid total synthesis.[8]

The other objective was, of course, the synthesis of estrone in the required antipodal form. Our interest in preparing trione **9** stemmed from the hope that its prochiral nature could be exploited in a chirally specific synthesis of the 13*S*[9] configuration required in the final product. If this asymmetry were to be induced, the possibility of synthesizing optically active steroids without recourse to resolution and with nearly full utilization of intermediates would be within reach.

Of course, the feasibility of inducing asymmetry by amino acid promoted aldolization of prochiral precursors was demonstrated concurrently in the laboratories of Z. Hajos at Hoffmann-La Roche[10a] and U. Eder at Schering AG.[10b] Both groups realized high chiral specificity in the cyclization of **10** → **11a** by use of the cyclic amino acid, L-proline.

It should be noted that this easy access to the optically pure **11a** makes this compound per se an attractive intermediate for steroid synthesis. Such routes gain added desirability from the discovery that very high sterospecificities in favor of trans C:D stereochemistry are achieved in the catalytic hydrogenation of hydrindenones **12**[11] (which is prepared from **11a**) and **13**[12] (which is derived from the angular ethyl analogue of **11a**).

Our interest in preparing optically active **15** and **16** by