The Importance of Side Chain Intramolecular Interaction on the Stereochemical Course of the 2-Carbon Deuteration of Amino Acids Complexed to Cobalt(III)

William E. Keyes, Ruth E. Caputo, Roger D. Willett,* and J. Ivan Legg*

Contribution from the Department of Chemistry, Washington State University, Pullman, Washington 99163. *Received June 1, 1976*

Abstract: The complexes $[Co(en)_2(L\text{-}aa)]^{n+}$ where aa = aspartate, glutamate, asparagine, or glutamine and $[Co(NH_3)_4(L\text{-}asp)]^+$ were selectively deuterated at the amino acid 2-carbon, to give the 2-^{2}H amino acids. The deuteration, using mild base, racemized the 2-carbon of asparagine and glutamine but proceeded with kinetically controlled retention of configuration in each of the aspartate and glutamate complexes. Hydrogen bonding between the side chain carboxyl group and one of the (di)amine nitrogens explains the partial retention of configuration found for the aspartate and glutamate complexes. The crystal structure of $\Lambda[Co(en)_2(L\text{-}glu)]^+$ shows an unusual conformation for the glutamate side chain, in which the side chain is held closely associated with the complex, presumably due to hydrogen bonding to the diamine. The present deuterium exchange results suggested that this interaction and the unusual conformation exhibited would be absent for the asparagine and glutamine complexes. In order to investigate this proposition, the crystal and molecular structure of $\Lambda[Co(en)_2(L\text{-}asn)]I_{1.5}(NO_3)_{0.5}$ was determined from three-dimensional x-ray counter data. The complex crystallizes in the orthorhombic system, space group $P2_12_12_1$ with a = 15.964 (12), b = 8.282 (5), and c = 12.993 (8) Å. With Z = 4, the observed and calculated densities are 2.08 (3) and 2.080 g cm^{-3}, respectively. The structure was refined by full-matrix least-squares methods to a final value of R_1 = 0.071 for 1723 independent reflections. The geometry about the cobalt atom is distorted octahedral with asparagine coordinated through the amine and carboxylate to give the expected five-membered chelate ring. The absolute configuration of the complex, assigned from the known absolute configuration of L-asparagine as an internal reference, is Λ, in agreement with the assignment made by analysis of the circular dichroism spectrum of the complex. As predicted, the asparagine side chain is found in the more commonly encountered extended conformation in which there is no intramolecular interaction between the side chain amide group and any of the diamine nitrogens.

It has been known for some time that metal ions increase the reactivity of the 2-C methylene hydrogens of amino acids. Williams and Busch were the first to demonstrate the magnitude and specificity of the effect through their ^{1}H NMR study of the deuteration of Co(III)-chelated glycine, alanine, and EDTA.[1] Since this investigation, a series of studies involving the effect has been undertaken.[2-4] We have been investigating the various factors which govern the stereochemical course of the C-deuteration of chelated, naturally occurring amino acids.[3] In the case of aspartate chelated as a bidentate ligand, we have shown that the deuteration proceeds in mild base with 77 ± 2% retention of configuration.[3a]

Figure 1 summarizes the proposed mechanism for deuteration in basic solution of an amino acid coordinated as a bidentate ligand in $[Co(en)_2(\text{amino acid})]^n$.[4] The formation of an enolate anion (a resonance hybrid involving a planar 2-carbon) during deuteration would bring about racemization unless the ^2H_2O molecule attacked the 2-carbon preferentially from one side. Preferential attack could arise through a dissymmetric template, the bulk of the side chain, or side chain hydrogen bonding.

To gain insight into the mechanism involved in the preferential retention of configuration for deuteration at the 2-carbon in aspartate, a series of related amino acid Co(III) complexes was investigated.

Experimental Section

Analytical results are recorded in Table I. Yields were of the order of 50%.

Synthesis and Separation of the Isomers of Aspartatobis(ethylenediamine)cobalt(III) Chloride. Carbonatobis(ethylenediamine) cobalt(III) chloride (5.5 g, 0.02 mol) was dissolved in 50 ml of water; 4 g of activated charcoal (Norit-A, Alkaline) was added, and the mixture was kept at 40 °C with stirring for 20 min. L-Aspartic acid (2.66 g, 0.02 mol) was added to the mixture, and the temperature was raised to 70 °C. After the evolution of CO_2 ceased, the reaction mixture was allowed to stir an additional 30 min at 70 °C. The mixture was cooled to room temperature, and the carbon was removed by filtration through Whatman 2V filter paper. The filtrate was diluted to 1 l. 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 0.5 ml/min. The complex was eluted (flow rate ca. ½ ml/min) with 0.2 M sodium chloride, adjusted to a pH of 7.0 with $NaHCO_3$. The complex separated cleanly into two red-orange bands. Each band was collected in fractions. Circular dichroism data of the fractions showed each band to consist of one isomer. The fractions were combined for each band and evaporated to near dryness in an air 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 saturated salt/complex solution (40 ml) was passed down the column at a rate of 0.5 ml/min

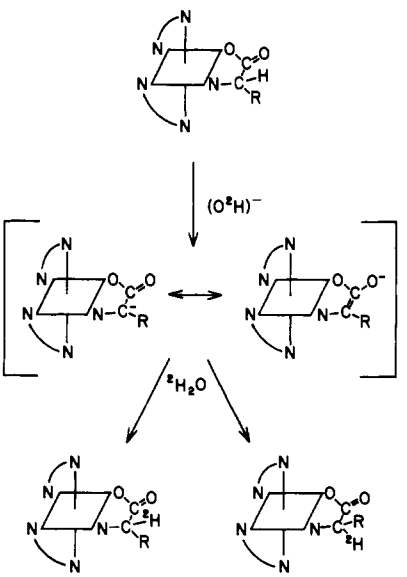

Figure 1. Proposed mechanism for the deuteration of a chelated amino acid in basic solution.

Table I. Elemental Analyses

Complex	% C		% H		% N	
	Calcd	Found	Calcd	Found	Calcd	Found
Λ[Co(en)$_2$(L-asp)]Cl·2H$_2$O	25.17	25.08	6.60	6.89	18.35	18.18
Δ[Co(en)$_2$(L-asp)]Cl·3½H$_2$O	23.51	23.30	6.91	7.01	17.14	17.23
Λ[Co(en)$_2$(L-glu)]Cl·2½H$_2$O	26.71	26.96	6.97	6.92	17.30	17.47
Δ[Co(en)$_2$(L-glu)]Cl·1½H$_2$O	27.95	28.06	6.78	6.86	18.11	18.06
Λ[Co(en)$_2$(L-gln)]Cl$_2$	27.35	27.12	6.38	6.36	21.27	21.24
Δ[Co(en)$_2$(L-gln)]Cl$_2$·H$_2$O	26.16	26.11	6.59	6.60	20.34	20.26
Λ[Co(en)$_2$(L-asn)]I$_{1.6}$(NO$_3$)$_{0.4}$	17.86	17.98	4.30	4.34	16.66	16.90
Δ[Co(en)$_2$(L-asn)]I$_2$·½H$_2$O	16.77	16.89	4.22	4.24	14.67	14.67
[Co(NH$_3$)$_4$(L-asp)]Cl	16.36	16.20	5.84	5.96	23.85	23.80

and collected in fractions. Testing of the fractions for NaCl with $AgNo_3$ showed a clean separation of the complex from NaCl. Solid [Co(en)$_2$(L-asp)]Cl was isolated by evaporation of the resulting solution. Each isomer was dissolved in a minimum amount of water, and ethanol was added until the solution became cloudy. A few drops of water were added until the solution became clear, and then the solution was set aside overnight to crystallize. The crystals were filtered, washed with 50% ethanol–water, then absolute ethanol, and air-dried.

Synthesis and Separation of the Isomers of Glutamatobis(ethylenediamine)cobalt(III) Chloride. The glutamate complex was synthesized using a procedure analogous to that employed for the aspartate complex except that 2.96 g (0.02 mol) of L-glutamic acid was used. The Δ isomer was recrystallized by dissolving the sample in 4 ml of water and adding it dropwise to 35 ml of vigorously stirred methanol. The solid Δ[Co(en)$_2$(L-glu)]Cl was collected by filtration and air-dried. The Λ isomer was recrystallized in the manner described for [Co(en)$_2$(L-asp)]Cl.

Synthesis and Separation of the Isomers of Glutaminatobis(ethylenediamine)cobalt(III) Chloride. Carbonatobis(ethylenediamine)cobalt(III) chloride (5.5 g, 0.02 mol) was dissolved in 50 ml of water, 4 g of activated charcoal (Norit-A, alkaline) was added, and the mixture was stirred at 40 °C for 20 min. L-Glutamine (2.92 g, 0.02 mol) was added to the stirred mixture, and the temperature was raised to 70°C. The reaction was maintained at pH 6 by addition of 6 N HCl. After the addition of approximately 4 ml of 6 N HCl, the pH no longer increased with time and the reaction mixture was allowed to stir an additional 30 min at 70 °C. The reaction mixture was allowed to cool to room temperature and the carbon was removed by filtration. Column elution, desalting, and recrystallization procedures were the same as the procedure used for the aspartate complex except that 1.0 M NaCl was used for the elution from the ion-exchange column, and methanol was used instead of ethanol for recrystallization.

Synthesis and Separation of the Isomers of Asparaginatobis(ethylenediamine)cobalt(III) Iodide/Nitrate. The asparagine complex was prepared using the procedure described for the aspartate complex except that 3.0 g (0.02 mol) of L-asparagine was used. After desalting in the described manner, the complex [Co(en)$_2$(L-asn)]Cl$_2$, dissolved in 50 ml of water, was 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 0.5 ml/min. The complex, now as the nitrate salt, was again passed down Sephadex. The solid [Co(en)$_2$(L-asn)](NO$_3$)$_2$ was dissolved in a minimum amount of water, and sodium iodide (22 g), dissolved in 20 ml of water, was added to the complex solution. The red-orange crystals were collected the following day. Recrystallization from an excess of NaI was repeated. The resulting red-orange crystals were recrystallized three times from hot (70 °C) water, filtered, washed with 95% ethanol, and air-dried. Potentiometric determination of I^- showed 1.66 ± 0.03 I/Co corresponding to an occupany factor of 0.83. The occupancy factor determined crystallographically (vide infra) was 0.803. The small discrepancy between the two occupancy factors is most likely attributable to the selection of a single crystal for the crystallographic study which was not necessarily representative of the sample in terms of I^-/NO_3^- composition.

Synthesis of Aspartatotetraamminecobalt(III) Chloride. The complex was prepared by modification of the method described by Kojima and Shibata.[5] Hexaamminecobalt(III) chloride (34 g) and L-aspartic acid (13 g) were added to a solution of 20 ml of concentrated NH_3 in 100 ml of H_2O. Activated charcoal (1 g) was added, and the mixture was stirred at 50 °C for 1 h; the solution became red-brown. After removal of the charcoal by hot filtration, concentrated HCl (16 ml) was added to the filtrate. The filtrate 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 0.5 M NaCl, the red-orange complex separated cleanly from the other species present. The same procedure for desalting and recrystallization was followed as was described for the aspartatobis(ethylenediamine) complex.

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 (a triplet) of the methine proton of the coordinated amino acid was no longer detectable (ca. 5–10 days).

Spectra. The absorption spectra were measured on a Cary 14 spectrophotometer using a tungsten lamp. 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 $\Delta\epsilon_{290} = +2.34$.[6] 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 JEOL MH-100 NMR spectrometer at a concentration of ca. 0.3 M and a pH of 9.6.

Crystal Data for Λ[Co(en)$_2$(L-asn)]I$_{1.6}$(NO$_3$)$_{0.4}$. Weissenberg and precession photographs of the deep red-orange needles indicated an orthorhombic unit cell with systematic extinctions for $h00$, $0k0$, $00l$, where $h, k, l = 2n + 1$ (odd), respectively. These conditions define the space group as $P2_12_12_1$. The unit cell dimensions were obtained by least-squares refinement[7] of 12 high angle reflections centered on a Picker four-circle automated diffractometer using Mo Kα radiation (λ 0.7107 Å) and are: a = 15.964 (12), b = 8.282 (5), and c = 12.993 (8) Å. With $Z = 4$ and a molecular weight of 531.6 amu, the calculated density is 2.080 g cm^{-3} compared with a measured density of 2.08(3) g cm^{-3} obtained by flotation in a 1,1,2,2-tetrabromoethane–dichloromethane mixture.

Intensity Data Collection. Diffraction data were collected from a fragment of a much longer needle crystal with dimensions of 0.11 × 0.24 × 0.11 mm where the needle axis was designated as b^*. The data were collected on a Picker four-circle diffractometer automated with a PDP-8/L computer using programs of Busing et al.[7] The θ–2θ scan method was employed with a scanning rate of 1°/min. Stationary crystal, stationary counter background counts of 30 s were taken on each side of the reflection. All possible unique reflections with 2θ values between 5 and 50° were collected with a 2.2° scan using Zr-filtered Mo Kα radiation. Three standard reflections were monitored every 50 reflections and exhibited no systematic decrease in intensity. A total of 1723 reflections were measured and the standard Lorentz and polarization corrections applied. The standard error in the intensity of each measurement was calculated from the relationship: $\sigma^2(I) = \sigma_{stat}^2 + C^2I^2$ where I and σ_{stat} are the integrated intensity and the standard deviation calculated by the diffractometer programs[7] and $C = 0.05$. Absorption corrections (μ = 45.1 cm^{-1}) were applied by a modified version of ORABS.[8] The maximum and minimum transmission factors were 0.71 and 0.54.

Solution and Refinement of the Structure. A three-dimensional Patterson map was calculated, and the positions of the iodides and

Table II. Positional and Thermal Parameters[a] and Their Estimated Standard Deviations

Atom	X	Y	Z	B(1,1)	B(2,2)	B(3,3)	B(1,2)	B(1,3)	B(2,3)
I1	0.5885 (1)	0.3921 (2)	0.8486 (1)	0.0024 (1)	0.0154 (3)	0.0057 (1)	−0.0011 (1)	−0.0004 (1)	0.0024 (2)
I2	0.5717 (1)	0.3998 (2)	0.2099 (1)	0.0030 (1)	0.0136 (3)	0.0051 (1)	−0.0013 (1)	0.0007 (1)	−0.0011 (1)
Co	0.6472 (1)	0.6909 (2)	0.5198 (2)	0.0016 (1)	0.0066 (3)	0.0041 (1)	−0.0001 (1)	−0.0001 (1)	0.0001 (2)
O1	0.6850 (6)	0.4823 (13)	0.5537 (8)	0.0011 (4)	0.0101 (18)	0.0056 (8)	−0.0004 (7)	−0.0003 (4)	0.0031 (10)
N2	0.5379 (7)	0.5829 (15)	0.4922 (10)	0.0016 (5)	0.0073 (21)	0.0057 (9)	0.0002 (9)	0.0001 (5)	−0.0012 (12)
N3	0.7576 (8)	0.7771 (16)	0.5607 (9)	0.0021 (5)	0.0073 (22)	0.0038 (8)	−0.0004 (9)	−0.0008 (5)	−0.0009 (10)
N4	0.6133 (8)	0.7239 (17)	0.6652 (10)	0.0025 (5)	0.0097 (24)	0.0038 (8)	−0.0003 (10)	0.0003 (6)	−0.0000 (12)
N5	0.6872 (8)	0.6611 (15)	0.3798 (10)	0.0025 (5)	0.0079 (22)	0.0038 (8)	0.0010 (9)	0.0007 (6)	−0.0006 (11)
N6	0.6076 (7)	0.9045 (15)	0.4706 (9)	0.0021 (5)	0.0082 (19)	0.0034 (7)	0.0020 (9)	0.0000 (5)	−0.0014 (14)
O2	0.6485 (7)	0.2238 (12)	0.5567 (9)	0.0022 (5)	0.0045 (17)	0.0095 (11)	0.0004 (8)	−0.0003 (6)	−0.0003 (10)
C1	0.6306 (9)	0.3663 (18)	0.5491 (12)	0.0014 (6)	0.0060 (25)	0.0062 (12)	0.0000 (10)	0.0000 (7)	−0.0001 (14)
C2	0.5373 (10)	0.4284 (19)	0.5468 (13)	0.0022 (6)	0.0071 (27)	0.0068 (12)	0.0002 (11)	−0.0005 (7)	0.0010 (15)
C3	0.7483 (10)	0.8780 (26)	0.6561 (13)	0.0028 (7)	0.0217 (41)	0.0051 (11)	−0.0026 (16)	−0.0015 (8)	−0.0028 (20)
C4	0.6855 (13)	0.7856 (30)	0.7278 (14)	0.0056 (10)	0.0284 (49)	0.0045 (11)	0.0053 (21)	−0.0010 (9)	0.0066 (22)
C5	0.6641 (13)	0.8043 (26)	0.3120 (13)	0.0055 (11)	0.0151 (34)	0.0052 (12)	−0.0014 (18)	0.0021 (9)	0.0027 (19)
C6	0.6553 (11)	0.9502 (21)	0.3765 (14)	0.0040 (8)	0.0098 (30)	0.0051 (12)	−0.0022 (13)	0.0007 (9)	0.0007 (15)
C7	0.4837 (9)	0.2952 (20)	0.4929 (15)	0.0015 (6)	0.0084 (27)	0.0088 (15)	−0.0005 (11)	0.0002 (7)	−0.0007 (18)
C8	0.3907 (10)	0.3459 (21)	0.4969 (13)	0.0014 (6)	0.0115 (30)	0.0063 (12)	−0.0006 (11)	0.0000 (7)	0.0029 (15)
O3	0.3691 (7)	0.4744 (15)	0.5320 (11)	0.0026 (5)	0.0112 (19)	0.0114 (11)	0.0015 (9)	−0.0005 (7)	−0.0044 (14)
N1	0.3364 (9)	0.2329 (19)	0.4583 (13)	10.0029 (7)	0.0146 (28)	0.0085 (13)	−0.0016 (12)	−0.0004 (8)	0.0017 (17)

[a] The form of the anisotropic thermal ellipsoid is $\exp\{-(B_{11}h^2 \cdot b_{22}k^2 + B_{33}l^2 + 2B_{12}hk + 2B_{13}hl + 2B_{23}kl)\}$.

cobalt were determined. Least-squares refinement of these positions, including anisotropic thermal parameters for the iodides and isotropic thermal paramenters for cobalt, resulted in an R_1 value of 0.227. The remaining non-hydrogen atoms of the cation were successfully found from a succession of difference Fourier syntheses which were phased by the atoms already located.

The absolute configuration of the complex was determined by reference to the known absolute configuration of the L-asparagine used in the synthesis of the complex. It was found that the complex structure as originally solved contained D-asn so the sign of the y-coordinate of all atoms was reversed to create the correct mirror image.[9] At this point, the R_w values for the two structures were $R_w = 0.147$ for the Δ (incorrect) isomer and $R_w = 0.129$ for the Λ (correct) isomer. In addition, it became apparent that the compound was nonstoichiometric, so the iodide occupancy parameters were varied.

Several cycles of full-matrix least-squares refinement with anisotropic thermal parameters for all located atoms including variation of the iodides' occupancy factors converged on $R_1 = 0.091$ and $R_w = 0.102$, where:

$$R_1 = \frac{\sum||F_o| - |F_c||}{\sum|F_o|}$$

$$R_w = \left[\frac{\sum w(|F_o| - |F_c|)^2}{\sum w|F_o|^2}\right]^{1/2}$$

The least-squares refinement minimized the function $\Sigma w(|F_o| - |F_c|)^2$ with $w = 1/\sigma^2(F)$. Attempts were made to locate the hydrogen atoms and the nitrate oxygen atoms from difference maps. The best refinement obtained from a structural model containing these oxygen and hydrogen atoms converged at $R = 0.078$ and $R_w = 0.086$. At this point, the structure factor list was scanned and it was noticed that an error had been made in calculating σ for the 555 reflection due to an error in reading the diffractometer paper tape output. This one reflection contributed about 80% of the residual. The σ was recalculated for this reflection, the hydrogen and nitrate oxygen atoms were removed from the model, and the structure refined again. Final refinement using all reflections converged at $R_1 = 0.071$, $R_w = 0.077$. The residual calculated, omitting reflections with $F_o < 3\sigma(F_o)$, was $R_w = 0.061$. The occupancy factors for the iodide positions converged at 0.815 (5) for I1 and 0.791 (5) for I2. Hydrogen positions were not located. The final positional and thermal parameters along with their standard deviations are listed in Table II. Final parameter shifts were less than half the estimated errors for each parameter. The largest peaks (1.5 e/A^3) on the final difference map were located about the iodide positions. These peaks could not be interpreted in terms of nitrate oxygen positions. Scattering factors and anomalous dispersion corrections for the iodides and the cobalt were those calculated by Cromer and Waber.[10] Scattering factors for the remaining atoms were taken from the "International Tables for X-Ray Crystallography".[11] A compilation of observed and calculated structure factors is available.[12] Computer programs used were from a local library containing modified versions of the following programs: ORFLS,[13] ORFFE,[14] ORTEP,[15] and ALFF.[16]

Results and Discussion

Synthesis and Isolation of Isomers. The bis(ethylenediamine) amino acid complexes were synthesized in good yield by displacement of carbonate with the appropriate amino acid from $[Co(en)_2(CO_3)]^+$ in the presence of charcoal at 70 °C. The aspartatotetraammine complex was synthesized by displacement of ammonia from the hexaammine complex.

All isomers were separated by ion-exchange chromatography. In all cases but one, $[Co(en)_2(L\text{-gln})]^{2+}$, the Λ[17] diastereomer eluted before the Δ isomer. Also, it was necessary to collect the glutaminato complex in fractions due to considerable overlapping of the diastereomer bands. The middle fractions were discarded. Final desalting for all isomers was achieved by gel permeation chromatography. Red-orange crystals were obtained in all cases except for Λ and Δ[Co(en)$_2$(L-glu)]Cl for which orange powders formed.

Visible absorption, circular dichroism, and nuclear magnetic resonance spectra of the aspartato and glutamato complexes agree well with previously reported data in which a different anion was used.[2,5,18,19] Spectral data for the two new complex cations $\Lambda[Co(en)_2(L\text{-asn})]^{2+}$ and $\Lambda[Co(en)_2(L\text{-gln})]^{2+}$ are recorded in Table III along with the data for the above previously reported cations. The absolute configuration for each of the complexes was tentatively assigned by analysis of their circular dichroism spectra.[3a,b] The crystal structure determination of $\Lambda[Co(en)_2(L\text{-asn})]^{2+}$ confirmed these assignments.

Determination of Degree of Retention of Configuration. Figure 2 summarizes the method employed for the determination of percent retention of configuration in the deuteration reactions. Shown in the figure are the circular dichroism (CD) spectra obtained before and after deuteration of $\Lambda[Co(en)_2(L\text{-Asp})]^+$. If complete retention of configuration had been obtained, the CD spectra should have been indistinguishable (assuming isotope effects to be negligible). However, the fact that partial retention of configuration has been obtained can be seen from the following argument. The mirror image of $\Delta[Co(en)_2(L\text{-Asp})]^+$ is $\Lambda[Co(en)_2(D\text{-Asp})]^+$. Its CD

Table III. Spectral Data and Deuteration Results

Complex	Absorption maxima, nm (ϵ, M^{-1} cm^{-1})	Circular dichroism maxima, nm ($\Delta\epsilon$, M^{-1} cm^{-1})	Amino acid optical ratio after deuteration (% L/% D)
Λ[Co(en)$_2$(L-asp)]Cl·2H$_2$O	485 (101), 347 (109)	506 (2.45), 426 (−0.039), 370 (0.066), 348 (−0.024), 323 (0.082)	77/23
Δ[Co(en)$_2$(L-asp)]Cl·3½H$_2$O	485 (100), 347 (103)	505 (−2.09), 370 (−0.101), 327 (−0.152)	89/11
Λ[Co(en)$_2$(L-glu)]Cl·2½H$_2$O	488 (107), 349 (119)	504 (2.48, 431 (−0.111), 370 (0.049), 347 (−0.041), 322 (0.053)	62/38
Δ[Co(en)$_2$(L-glu)]Cl·1½H$_2$O	488 (105), 349 (116)	510 (−2.14), 365 (−0.151), 330 (−0.175)	
Λ[Co(en)$_2$(L-gln)]Cl$_2$	488 (105), 348 (116)	505 (2.24), 436 (−0.199), 370 (0.029), 348 (−0.040), 322 (0.053)	50/50
Δ[Co(en)$_2$(L-gln)]Cl$_2$·H$_2$O	488 (105), 349 (112)	510 (−2.20), 367 (−0.168), 330 (−0.193)	
Λ[Co(en)$_2$(L-asn)]I$_{1.5}$(NO$_3$)$_{0.5}$	488 (100), 348 (109)	506 (2.39), 430 (−0.084), 369 (0.075), 323 (0.092)	50/50
Δ[Co(en)$_2$(L-asn)]I$_2$·½H$_2$O	486 (101), 348 (108)	506 (−2.09), 368 (−0.102), 327 (−0.149)	
[Co(NH$_3$)$_4$(L-asp)]Cl	493 (79), 349 (88)	516 (0.252), 458 (−0.324), 344 (−0.097)	60/40

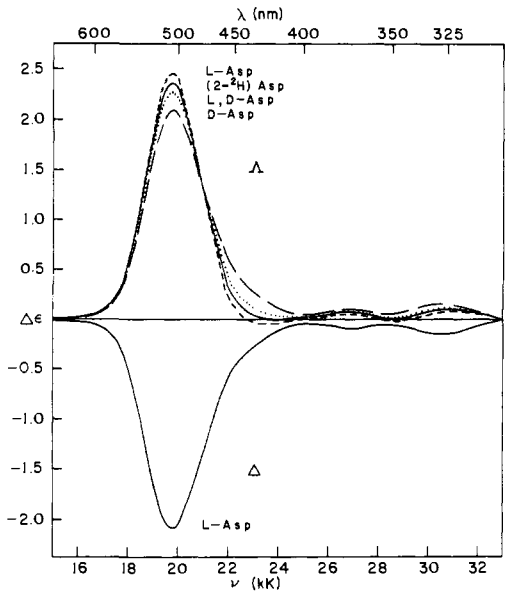

Figure 2. Circular dichroism spectra of various isomers of [Co(en)$_2$(asp)]$^+$. For spectra shown with positive maxima at 506 nm: (- - -) Λ(L) isomer before deuteration, (—) same isomer after deuteration, (· · ·) Λ(L,D) isomer, (– –) Λ(D) isomer.

spectrum can be obtained by mirroring the spectrum of the Δ isomer through the $\Delta\epsilon = 0$ axis as shown in Figure 2. The spectrum thus generated would be that obtained if the L-aspartic acid had inverted in configuration during the deuteration (Λ(L) → Λ(D)). If total racemization had occurred (Λ(L) → Λ(D,L)), a CD spectrum representing an average between that of Λ[Co(en)$_2$(L-Asp)]$^+$ and Λ[Co(en)$_2$(D-Asp)]$^+$ would have been obtained. As can be seen in Figure 2, this spectrum has a lower $\Delta\epsilon$ than the spectrum obtained after deuteration of the Λ(L) isomer. Thus, partial retention of configuration is strongly indicated. The optical ratio can be calculated from the relative $\Delta\epsilon$ values for the maxima at 507 nm to be 78% L- and 22% D-aspartic acid.

In order to check these observations the aspartic acid was removed from the complex by reduction with $NaBH_4$ to give labile Co(II).[3a,b] Ion-exchange chromatography was used to separate the aspartic acid from the residual products. The ^1H NMR spectrum of the aspartic acid thus obtained showed that the amino acid was still deuterated at the 2-carbon. It is important to note that the reduction method employed does not appreciably exchange hydrogen (deuterium) at the 2-carbon which strongly suggests that the configuration about that carbon is retained during this process. The ORD spectrum obtained from the 2-^2H amino acid revealed that a 77% L-/23 ± 2% D-amino acid mixture had been obtained, in excellent agreement with the CD results. Results of deuteration of all other complexes investigated are summarized in Table III.

Analysis of Factors Which Could Influence Degree of Racemization. A. Dissymmetric Template. Due to the presence of two chiral centers (dissymmetric arrangement of chelate rings and amino acid asymmetric carbon), the [Co(en)$_2$(L-aa)]^n complexes are diastereomeric. If equilibrium conditions obtain during the deuteration, a thermodynamic preference for one diastereomer over the other would explain the isomer distribution observed for deuteration of aspartate in Λ[Co(en)$_2$(L-asp)]$^+$. Alternatively, if equilibrium conditions do not obtain, attack on one side of the 2-carbon by the ^2H_2O molecule may occur at a greater rate due to the dissymmetry of the complex. The above arguments will be collectively referred to as the dissymmetric template effect.

Experimental evidence against the importance of employing a dissymmetric template has now been obtained. Both the Λ and Δ[Co(en)$_2$(L-asp)]$^+$ complexes were deuterated. If preferential retention of configuration for the Λ complex were the result of an equilibrium or kinetic selectivity in which Λ(L) is preferred to Λ(D), then in the deuteration of the Δ complex, Δ(D) would predominate over Δ(L) (note that Λ(L) and Δ(D) are optical isomers).

In actuality, deuteration of Δ[Co(en)$_2$(L-asp)]$^+$ results in an even greater ratio of L to D aspartate being formed than in the Λ complex (89% L, 11% D). This is in opposition, then, to what would be predicted by the dissymmetric template effect.

Furthermore, when the Λ[Co(en)$_2$(L-asp)]$^+$ complex is allowed to come to equilibrium by allowing the reaction to proceed much longer than is necessary for complete deuteration to take place (5×), racemization occurs. This demonstrates that there is no appreciable energy difference between the diastereomers. The observed partial retention of configuration, then, is not the result of a thermodynamic preference.

As conclusive evidence against the importance of employing a dissymmetric template, a Co(III) complex in which the metal ion is no longer a center of dissymmetry was used. Unlike the bis(ethylenediamine) complex, [Co(NH$_3$)$_4$(L-asp)]$^+$ cannot exist as diastereomers. As a result, the L- or D-amino acid complexes are identical in energy. Deuteration of this complex resulted in partial retention of configuration (60% L, 40% D) definitively ruling out the need for a dissymmetric template.

These experiments establish that the dissymmetric template

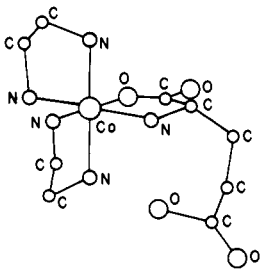

Figure 3. X-Ray crystal structure of $\Lambda[Co(en)_2(L\text{-glu})]^+$ (see ref 18).

afforded by the two chelated ethylenediamine rings is not involved either in a thermodynamic or kinetic sense in the partial retention of the configuration observed. Furthermore, the retention is kinetically but not thermodynamically controlled.

B. Mass of the Side Chain. The mass of the side chain may affect the rate of inversion about the 2-carbon which in turn could influence the stereospecificity of deuteration. An increase in the mass of the side chain then, would be expected to increase the degree of retention of configuration. However, deuteration of the glutamate complex $\Lambda[Co(en)_2(L\text{-glu})]^+$ (where the glutamate side chain contains one more $-CH_2-$ group than aspartate) results in *less* retention of configuration (62% L/38% D) than the aspartate complex (77% L/23% D). From these results it can be concluded that the mass of the side chain is not the major cause of the selectivity observed.

C. Side Chain Hydrogen Bonding. Interaction of the basic component on the side chain of aspartate and glutamate with the complex could account for the stereoselectivity observed in the deuteration of the amino acids. Models of $[Co(en)_2(L\text{-asp})]^+$ and $[Co(en)_2(L\text{-glu})]^+$ show that their free carboxylate group is capable of hydrogen bonding to one of the nitrogens of an ethylenediamine ring on the complex. A crystallographic study of $\Lambda[Co(en)_2(L\text{-glu})]^+$ by Gillard et al.[18] shows that the side chain of glutamate is indeed oriented in this manner (Figure 3) in the crystalline state, in contrast to the usual orientation of bidentate glutamate, and other amino acids, in which the side chain is extended from the complex.[20] This atypical conformation may be due, in part, to the interaction between the γ-carboxylate and the aforementioned diamine nitrogen hydrogens (N–O distance 3.2 Å). Kojima and Shibata[5] invoked hydrogen bonding between an ethylenediamine NH_2 group and the β-carboxylate of aspartate in $[Co(en)_2(L\text{-asp})]^+$ to explain the reduced rate of hydrogen exchange on that nitrogen.

Preferential attack on one side of the 2-carbon over the other would explain partial retention of configuration during deuteration. Limiting the accessibility of one side of the deprotonated 2-carbon compared to the other would accomplish this. This limitation could be provided by the interaction between the β- or γ-carboxylate and the diamine nitrogen in the following manner. Deprotonation of the 2-carbon results in a planar configuration about the 2-carbon (Figure 4). Models show that this results in a loss of interaction between the β-carboxylate and the diamine nitrogen for $\Lambda[Co(en)_2(L\text{-asp})]^+$ due to their increased separation. However, greater accessibility to attack at position A compared to position B (Figure 4) would be expected until *further* reorientation of the side chain occurred. If protonation occurred at a greater rate than reorientation, partial retention of configuration would result.

In the limiting case, accessibility would be totally blocked at position B (Figure 4) until reorientation of the side chain occurred. The percent retention of configuration would be determined by the percentage of complex ions whose side chains were oriented in the manner described above. This percentage would be largely dependent on the strength of the interaction between the side chain and the diamine nitrogen.

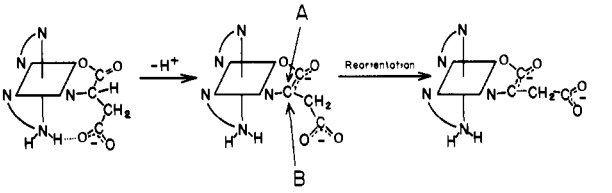

Figure 4. Proposed stereochemistry for the deuteration of $[Co(en)_2(L\text{-asp})]^+$ in basic solution.

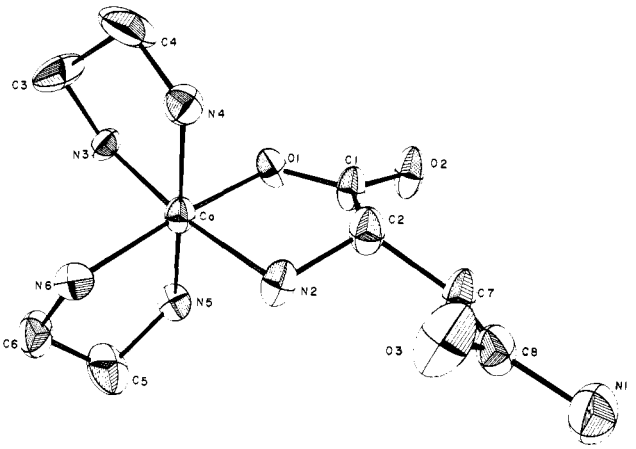

Figure 5. Molecular structure of $\Lambda[Co(en)_2(L\text{-asn})]^{2+}$. All hydrogen atoms have been omitted for clarity. Thermal ellipsoids are plotted at the 50% probability level.

An alternate possibility for providing a path for partial racemization in this limiting case would be for protonation and reorientation of the side chain to occur at similar rates. A means of distinguishing between these possibilities was not determined.

Evidence for the importance of possessing a potential for side chain hydrogen bonding has been obtained through the deuteration of $\Lambda[Co(en)_2(L\text{-asn})]^{2+}$ and $\Lambda[Co(en)_2(L\text{-gln})]^{2+}$. Asparagine (asn) and glutamine (gln) each contain an amide in place of the β- and γ-carboxylate group of aspartate and glutamate, respectively. Hydrogen bond interaction between an amide and an amine should be much weaker than the interaction between a carboxylate and an amine. Lack of this interaction should result in the more usual extended orientation for the amino acid side chain, and deuteration of these complexes should racemize the 2-carbon. As predicted, the deuteration of $\Lambda[Co(en)_2(L\text{-asn})]^{2+}$ and $\Lambda[Co(en)_2(L\text{-gln})]^{2+}$ results in racemization of the 2-carbon of asparagine and glutamine.

An important assumption in the hydrogen bonding argument has been that the unusual geometry for the side chain of $\Lambda[Co(en)_2(L\text{-glu})]^+$ results from an interaction of the side chain carboxyl group and one of the amines. As stated above, removal of the potential for this interaction should result in the more commonly encountered extended position for the side chain. To lend support to this assumption, the crystal and molecular structure of the complex $\Lambda[Co(en)_2(L\text{-asn})]^{2+}$ was undertaken.

Description of the Complex Cation. The geometry of the complex cation assigned as $\Lambda[Co(en)_2(L\text{-asn})]^{2+}$ by chemical and spectroscopic means is confirmed by the structure determined, Figure 5. Asparagine coordinated as a bidentate through the amine and carboxylate forms a five-membered ring with cobalt. The remaining four coordination positions are taken up by the two ethylenediamines. The absolute configuration of the complex, assigned from the known absolute configuration of L-asn as an internal reference, is Λ, in agreement with the assignment made by analysis of the circular dichroism spectrum of the complex.

Table IV. Intramolecular Bond Distances (Å)

Atoms	Distances	Atoms	Distances
Co-O1	1.88 (1)	N1-C8	1.37 (2)
		N2-C2	1.46 (2)
Co-N2	1.99 (1)	N3-C3	1.50 (2)
Co-N3	1.97 (1)	N4-C4	1.50 (2)
Co-N4	1.98 (1)	N5-C5	1.52 (2)
Co-N5	1.94 (1)	N6-C6	1.48 (2)
Co-N6	1.98 (1)		
		C1-C2	1.58 (2)
O1-C1	1.30 (2)	C2-C7	1.56 (2)
O2-C1	1.22 (2)	C3-C4	1.57 (2)
O3-C8	1.21 (2)	C5-C6	1.47 (3)
		C7-C8	1.54 (2)

Table V. Bond Angles (deg)

Atoms	Angles	Atoms	Angles
O1 Co N2	84.9 (5)	O1 C1 O2	123.8 (14)
O1 Co N3	89.0 (5)	O1 C1 C2	113.1 (12)
O1 Co N4	89.5 (5)	O2 C1 C2	122.7 (13)
O1 Co N5	89.9 (5)	O3 C8 N1	124.0 (15)
O1 Co N6	174.7 (5)	O3 C8 C7	121.8 (15)
N2 Co N3	172.8 (5)	N1 C8 C7	114.2 (15)
N2 Co N4	89.7 (5)	N2 C2 C1	106.8 (12)
N2 Co N5	93.5 (5)	N3 C3 C4	106.4 (15)
N2 Co N6	93.6 (5)	N4 C4 C3	109.6 (13)
N3 Co N4	86.4 (5)	N5 C5 C6	109.4 (13)
N3 Co N5	90.3 (5)	N6 C6 C5	107.9 (13)
N3 Co N6	92.8 (5)	Co O1 C1	117.0 (9)
N4 Co N5	176.6 (5)	Co N2 C2	108.1 (9)
N4 Co N6	95.6 (5)	Co N3 C3	109.5 (9)
N5 Co N6	85.2 (5)	Co N4 C4	110.7 (10)
C1 C2 C7	107.2 (12)	Co N5 C5	111.3 (9)
C2 C7 C8	108.6 (13)	Co N6 C6	109.1 (9)

The intramolecular bond distances and bond angles are given in Tables IV and V.[14] The bond length distortions are minimal except for the Co-N2 bond length of 1.99 (1) Å, and the N5-C5 bond length of 1.52 (2) Å, both of which are significantly longer than the normal values of 1.92 and 1.47 Å, respectively.[21] The angular distortions about Co from the ideal octahedral geometry are significant. The five-membered glycinate ring introduces the largest distortion (O1-Co-N2 bond angle of 84.9 (5)°) followed by one of the diamine rings (N5-Co-N4 bond angle of 85.2 (6)°). For the other diamine ring, the angle is closer to 90° (N3-Co-N4 bond angle of 86.4 (6)°). The side chain is oriented in a relatively planar, extended conformation. The only intramolecular contact of the side chain with the remainder of the complex is between the amide oxygen and the amino acid amine (O3-N2 distance of 2.89 (2) Å).

Table VI. Close Intermolecular Distances (Å)

Atoms	Distance	Sum of the van der Waal's radii[a]
I1-N4	3.66 (1)	3.65
I1-N4[b]	3.51 (1)	3.65
I1-N5[c]	3.63 (1)	3.65
I2-N3[c]	3.65 (1)	3.65
I2-N5	3.60 (1)	3.65
I2-N6[b]	3.70 (1)	3.65
O1-N1[d]	3.01 (1)	2.90
O2-N1[d]	3.07 (2)	2.90
O2[e]-N6	2.94 (2)	2.90
O3[f]-N3	3.08 (2)	2.90

[a] L. Pauling, "Nature of the Chemical Bond", 3d ed, Cornell University Press, Ithaca, N.Y., 1960, pp 257-264. [b] Transformed, related to the coordinates in Table II by the transformation $-x$, $\frac{1}{2} + y$, $\frac{1}{2} - z$. [c] Transformation $\frac{1}{2} - x$, $-y$, $\frac{1}{2} + z$. [d] Transformation $\frac{1}{2} + x$, $\frac{1}{2} - y$, $-z$. [e] Transformation x, $1 + y$, z. [f] Transformation $\frac{1}{2} + x$, $\frac{3}{2} - y$, $-z$.

Description of the Unit Cell. The unit cell, Figure 6, consists of four discrete complex cations and the eight counterions. The counterion positions, I1 and I2, have occupancies of 0.815 (5) and 0.791 (5), respectively. The bonding in the unit cell appears to be mostly electrostatic with only weak hydrogen bonding, most distances being close to the sum of the van der Waal's radii. The relevant distances are given in Table VI. The only case in which strong hydrogen bonding is indicated is I1-N4.

Side Chain Orientation. As predicted from the chemical studies, the amino acid side chain in Λ[Co(en)$_2$(L-asn)]$^{2+}$ adopts the more commonly encountered extended conformation. Intermolecular and intramolecular hydrogen bonding of the amide group is significantly reduced compared to that found for the side chain carboxyl group in the glutamate complex Λ[Co(en)$_2$(L-glu)]$^+$.[18] Whereas the intermolecular hydrogen bonding for the glutamate side chain consists of several close interatomic contacts (N-O distances of 2.86, 2.88, 2.95, and 3.04 Å),[18] the side chain amide group in Λ[Co(en)$_2$(L-asn)]$^{2+}$ is involved in much weaker intermolecular interactions (O3-N3 distance of 3.08 (2) Å and N1-O1 distances of 3.02 (2) and 3.01 (2) Å). The intramolecular interactions of the glutamate side chain with the remainder of the complex consist of an O-N interatomic distance of 2.9 Å between the side chain carboxyl oxygen and the amino acid nitrogen, and an interaction between the side chain carboxyl oxygen and one of the diamine nitrogens (O-N distance of 3.2 Å). For Λ[Co(en)$_2$(L-asn)]$^{2+}$ hydrogen bonding between the side chain and the amino acid, nitrogen is also present (O3-N2 distance of 2.89 Å). Any intramolecular interaction between the side chain and a diamine nitrogen, however, is completely

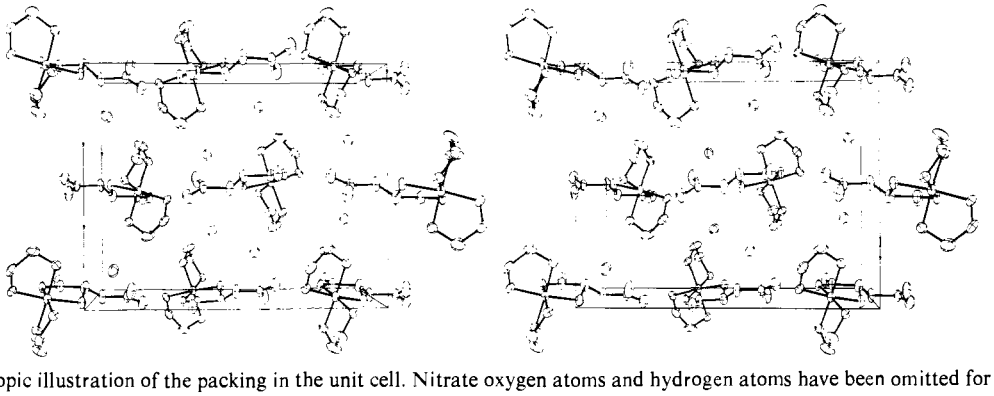

Figure 6. Stereoscopic illustration of the packing in the unit cell. Nitrate oxygen atoms and hydrogen atoms have been omitted for clarity.

absent (O3–N5 distance of 5.68 Å) as a result of the extended conformation for the side chain. These results agree with our original contention that in these systems varying the hydrogen bonding capability of the side chain will significantly alter that chain's conformation.

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Supplementary Material Available: A listing of structure factor amplitudes (2 pages). Ordering information is given on any current masthead page.

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Five-Coordinate Amino Acid Complexes. Synthesis and Characterization of Nickel(II), Copper(II), and Zinc(II) Complexes of 1,5-Diazacyclooctane-*N*-monoacetic Acid

Michael H. West and J. Ivan Legg*

Contribution from the Department of Chemistry, Washington State University, Pullman, Washington 99163. *Received September 19, 1975*

Abstract: A new tridentate ligand, 1,5-diazacyclooctane-*N*-monoacetic acid (dacoma), has been synthesized. By comparison with the closely related ligand 1,5-diazacyclooctane-*N,N'*-diacetic acid (dacoda), dacoma is expected to restrict metal ions to a square pyramidal five-coordinate geometry. A close resemblance of the electronic absorption spectrum of Ni(II)-substituted carboxypeptidase A in the presence of an inhibitor to that of $[Ni(dacoda)H_2O]$ has been demonstrated. Chelation of dacoma leads to a well-defined geometry in which the ligand occupies three planar sites with the remaining planar site and one apical site left free for additional coordination. The Ni(II), Cu(II), and Zn(II) complexes of dacoma were prepared and characterized by magnetic susceptibility measurements, x-ray powder-pattern analysis, visible absorption, and infrared spectroscopy as well as potentiometric pH titrations. By comparison with the solution spectrum of $[Ni(dacoda)H_2O]$, $[Ni(dacoma)(H_2O)_2]^+$ is shown to have a square pyramidal geometry. The Ni(II) and Zn(II) complexes of dacoma are isomorphous in the solid state, but the Cu(II) complex is not. Although Ni(II)- and Zn(II)-dacoma are not stable at basic pH's, $[Cu(dacoma)(H_2O)]^+$ is quite stable over a wide pH range. The interaction of optically active amino acids with $[Cu(dacoma)H_2O]^+$, which has two cis sites available for chelation, was examined as a function of pH by circular dichroism (CD) and visible absorption spectroscopy. Potentiometric pH measurements were used to determine formation constants for the ternary complexes. The L-amino acids were shown to chelate to $[Cu(dacoma)H_2O]^+$ through an equatorial amino nitrogen and an apical carboxylate group. This study supports the observation that a biphasal CD spectrum with a lower energy, positive band which is dominant or comparable in intensity to a higher energy negative band is characteristic of this mode of amino acid chelation to Cu(II).

Five-coordinate complexes have seldom been prepared and studied under aqueous conditions due to their inherent instability in this solvent.[1] Averill et al. reported the synthesis of a series of five-coordinate, square pyramidal complexes stable in aqueous solution involving the tetradentate ligand, 1,5-diazacyclooctane-*N,N'*-diacetic acid (dacoda), Figure 1.[2]