(6)-(4*R*-APA)-distamycin as a colorless amorphous material (9.5 mg, 30%): $[\alpha]^{22.5}_D$ +7.9° (c = 0.365, MeOH); IR (KBr) 3446, 1654, 1647 cm^{-1}; NMR (CD_3OD) δ 1.16 (9 H, s, $CH_3C \times 3$), 1.24 (3 H, d, J = 6.6 Hz, CH_3CH), 1.65-1.90 (4 H, m), 1.90-2.10 (2 H, m), 2.15-2.40 (4 H, m), 2.92 (6 H, s, $NCH_3 \times 2$), 3.00-3.30 (4 H, m), 3.35-3.45 (2 H, m), 3.80-4.20 (6 H, m), 3.87, 3.886, 3.894, 3.91 (each 3 H, s, $ArOCH_3$ and $NCH_3 \times 3$), 4.60-4.70 (1 H, m), 5.26 (1 H, d, J = 9.2 Hz), 6.79 (1 H, d, J = 1.8 Hz), 6.90 (1 H, d, J = 1.8 Hz), 6.95 (1 H, d, J = 1.8 Hz), 7.06 (1 H, d, J = 2.2 Hz), 7.13 (1 H, d, J = 1.8 Hz), 7.17 (2 H, distorted s), 7.35-7.45 (1 H, m), 7.38 (1 H, d, J = 2.6 Hz), 7.44 (1 H, distorted s); FABMS m/z 1112 (MH^+).

DNA-Cleavage Experiment. Nucleotide sequence cleavage was investigated on the 5'- and 3'-end-labeled strands of a 100-base-pair DNA restriction fragment (*Alu*I-*Hae*III) from the phage R199/G4ori. The reaction mixtures contained 10 mM Tris-HCl buffer (pH 7.4), the 5'- or 3'-end ^{32}P-labeled G4 phage DNA fragment, 1 μg of carrier calf thymus DNA, 1 mM dithiothreitol, and 1 μM PYML(6)-(4*R*-APA)-distamycin (or natural BLM)-iron complex. After the reaction solutions were incubated at 37 °C for 10 min, the DNA samples were subjected to electrophoresis on a 10% polyacrylamide/7 M urea slab gel. The autoradiogram was scanned with a microdensitometer.

Acknowledgment. We are particularly grateful to the late Professor Hamao Umezawa for bringing our attention to this fascinating field. This study was financially supported in part by Grant-in-Aid (No. 62114006) for Special Project Research and Grant-in-Aid for Scientific Research on Priority Areas (Multiplex Organic Systems) from the Ministry of Education, Science, and Culture, Japan, the Uehara Memorial Foundation, and the Mitsubishi Foundation. A.H. thanks Alexander von Humboldt-Stiftung for support.

Registry No. **1**, 98-79-3; **2**, 4931-66-2; **3**, 17342-08-4; **4**, 72479-05-1; **5**, 21395-93-7; **6**, 5937-83-7; (±)-**6**, 627-61-2; **7**, 123993-04-4; (*R*,*S*)-**8**, 123993-05-5; (*S*,*S*)-**8**, 123993-18-0; **9**, 57294-38-9; **10**, 2853-29-4; **11**, 40889-84-7; **12**, 123993-06-6; **13**, 123993-07-7; **14**, 123993-08-8; **15**, 123993-09-9; **16**, 13138-78-8; **17**, 28494-51-1; **18**, 109-55-7; **19**, 65361-30-0; **20**, 78486-14-3; **21**, 123993-10-2; **22**, 41215-80-9; **23**, 82692-03-3; **24**, 123993-11-3; **25**, 123993-12-4; **26**, 123993-13-5; **27**·HCl, 124020-63-9; **28**, 108998-85-2; **29**, 124020-64-0; **30**, 123993-14-6; **31**·TFA, 123993-15-7; **32**, 123993-16-8; (*R*)-MTPA-Cl, 39637-99-5; PYML-(6)-(4*R*-APA)-distamycin, 123993-17-9.

Proton Exchange and Epimerization of Co(III) Chelated Amino Acids via Carbanion Intermediates

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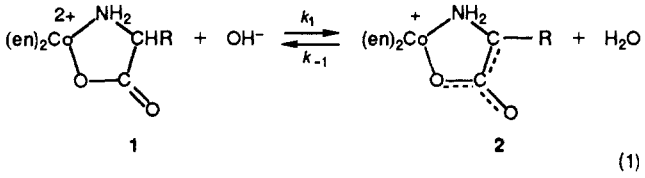
Abstract: An earlier investigation into proton exchange and epimerization of amino acids chelated to Co(III)[1] has been improved and extended to include the amino acids (AA) Phe, Val, Ala, Gly, Glu, and Asp in the complex cations Δ,Λ-$[Co(en)_2(S\text{-AA})]^{2+,+}$. Equilibrium concentrations of the diastereomers measured in H_2O (K_C(Δ-*S*/Λ-*S*) = 1.15 (Phe), 2.0 (Val), 1.0 (Ala, Gly), 0.85 (Glu), 0.67 (Asp)) vary little with ionic strength and are the same in D_2O. Rate constants for OD^--catalyzed proton exchange at the 2-CH centers differ for the Δ-*S* and Λ-*S* diastereomers and can be related to the rate constant for epimerization provided the concept of a common carbanion intermediate is used. There is no correlation between the rate data and the overall charge on the complex. Selectivity differences are demonstrated in the reprotonation process (Λ-*R*/Λ-*S* = 1.6 (Phe), 1.6 (Val), 0.9 (Ala), 0.8 (Gly), 0.75 (Glu), 0.5 (Asp)), and these are shown to be thermodynamically driven. This corrects previous investigations on the AA = Asp and Glu complexes. ^3H rate studies show a kinetic isotope difference of ~8 for reprotonation in favor of ^1H, but no selectivity difference between ^3H and ^1H in forming the Λ-*R*, Λ-*S* epimers.

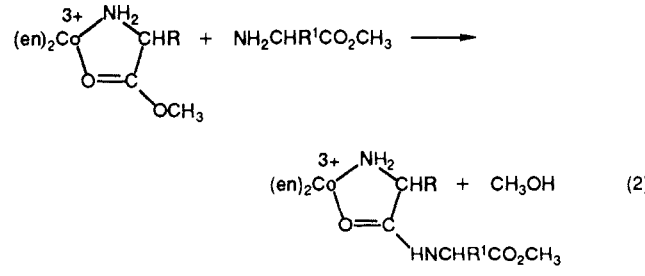
This paper extends our earlier investigation into proton exchange and epimerization of chelated amino acid anions of type **1**[1] by examining in greater detail the properties of carbanion **2** generated by deprotonation of **1** by OH^- ions in aqueous solution (eq 1).



(1)

It is well-known that metal ions enhance the carbon acidity of chelated amino acids,[2] but the influence of the additional asymmetric metal center (structures such as **1** are diastereotopic) on the thermodynamic and kinetic stereochemical preferences for electrophilic addition to the resulting prochiral carbanion **2** is little investigated or understood.

This new study came about for two reasons. First, an investigation of epimerization during peptide synthesis using Co(III)-activated amino acid esters[3] (eq 2) has revealed that carbanions **3**, generated under the conditions of the coupling reaction, have decided diastereomeric preferences for reprotonation, and it was of interest to compare these preferences with those of the somewhat more conjugated amino acid carbanions **2**. Such information could lead to an appreciation of the steric properties, and possibly lifetimes, of such intermediates. Second, subsequent



(2)

(1) Buckingham, D. A.; Marzilli, L. G.; Sargeson, A. M. *J. Am. Chem. Soc.* **1967**, *89*, 5133.

(2) (a) Sato, M.; Okawa, K.; Akabori, S. *Bull. Chem. Soc. Jpn.* **1957**, *30*, 937. (b) Williams, D. H.; Busch, D. H. *J. Am. Chem. Soc.* **1965**, *87*, 4644. (c) Yoshikawa, S.; Saburi, M.; Yamaguchi, M. *Pure Appl. Chem.* **1978**, *50*, 915. (d) Pasini, A.; Casella, L. *J. Inorg. Nucl. Chem.* **1974**, *36*, 2133.

(3) Buckingham, D. A.; Sutton, P. A. *Acc. Chem. Res.* **1987**, 20, 357. A detailed account of the epimerization during the peptide synthesis by the Co(III)-active ester method is being prepared for publication.

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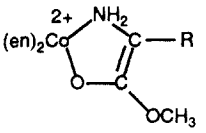


3

to our earlier study, Erickson,[4] and then Legg and co-workers in a series of publications,[5,7,8] suggested that carbanions **2**, generated from the chelates of aspartic and glutamic acids, had chiral properties for reprotonation that were not entirely thermodynamically controlled, since reprotonation appeared to preserve to some extent the original configuration of the asymmetric 2-C center; i.e., it appeared that there was an additional barrier to inversion over and above that necessary for proton loss. This was of considerable interest to us since chiral stability of a carbanion center adjacent to a carbonyl carbon (or sp^2 nitrogen) would be most unusual, and of considerable synthetic utility.

This paper describes results we have obtained on this problem for chelates **1** containing amino acid anions derived from valine, phenylalanine, alanine, glycine, and aspartic and glutamic acids.

Experimental Section

All chemicals were of LR or AR grade. Optical rotations and polarimetric rate data were obtained by using a thermostated 1-dm cell and a Perkin-Elmer 141 polarimeter. ^1H NMR data (equilibrium and rate data) were obtained by using 5-mm tubes and a Varian VXR 300 spectrometer at 25.0 °C or at other preset temperatures. Deuterated (trimethylsilyl)propanesulfonate (or *tert*-butyl alcohol) was used as an internal reference (and/or standard signal). Visible spectra were obtained with a Cary 219 spectrometer, and ^3H scintillation counting was carried out on a LKB 1217 Rackbeta liquid scintillation counter.

Preparations. The amino acid chelates Δ,Λ-$[Co(en)_2(S\text{-}AA)]X_2$ (AA = Gly,[9] Ala,[10] Phe,[9,10] Leu,[9] Val[1,9]; X = I^-, Br^-, Cl^-) were prepared and the Δ-*S* and Λ-*S* diastereomers separated and isolated as previously described. Δ,Λ-$[Co(en)_2(S\text{-}AAH)]X_2$ (AA = Glu, Asp; X = ClO_4^-, Cl^-, $CF_3SO_3^-$) were prepared as described by Legg and Steele.[11] For these two complexes we found that diastereomer separation was easily achieved using 0.5–1.0 M phosphate buffer (pH 6.9) and Dowex 50W-X2 cation-exchange resin, with the Λ-*S* (faster moving) and Δ-*S* (slower moving) diastereomers being finally removed with 2–3 M HCl. Λ- and Δ-$[Co(en)_2(AA)]ClO_4$ salts (AA = Glu, Asp) were isolated following neutralization with LiOH (to pH 7) of an aqueous solution of the recovered solid (rotary evaporation) and addition of excess $LiClO_4$ followed by MeOH (Λ-*S*) and PrOH (Δ-*S*). Crystallization was induced by cooling and scratching in an ice bath.

Equilibration Experiments. The complexes (0.5–1.0 g) were dissolved in the various solvent systems listed in Table I and held at 25 °C for 1–7 days. They were then adsorbed onto the eluted from Dowex 50W-X2 cation-exchange resin (3 M HCl), and the total band was taken to dryness by rotary evaporation. The residue was then twice dissolved in D_2O and taken to dryness (to remove residual H_2O) and the solid stored in an evacuated desiccator. The AA = Glu and Asp complexes were similarly stored after neutralization to pH ~7 (0.1 M NaOD). Samples were dissolved in D_2O and the ^1H NMR spectra recorded and integrated. Spectra of samples similarly equilibrated in D_2O/OD^- solutions were either recorded directly in the solvent system following equilibration or acidified (3 M HCl) and the solvent and electrolyte removed as detailed above.

H-Exchange Rate Data. Solutions of the complexes were made up in 0.1 M NaCl–D_2O solutions (5–10 mg cm^{-3} at pH 7), and the NMR spectrometer was tuned on the internal reference (700 μL of solution in a 5-mm tube). Then 10–75 μL of 0.500 M NaOD was injected into the tube, the contents quickly shaken, a final quick instrument shim undertaken, and data collection begun. Normally 36 transients were collected for each data points (5.4-μs pulse width, 1.560-s delay), and a time delay was chosen to give repetitions at 5-, 10-, 15-, or 30-min intervals. Conditions of data collection, and probe temperature, were varied as necessary. At the completion of a run the CH_3 (valine) or CH_2 (phenylalanine) region of the spectrum was integrated to give epimer distributions at equilibrium. H-exchange rates were determined by integrating the 2-CH absorptions of each epimer and plotting log $(I_t - I_\infty)$ vs time. A residual I_∞ value was always found (instrument noise) even though there was no obvious remaining signal.

Epimerization Rate Data. For Λ-$[Co(en)_2(S\text{-}Val)]Br_2$, data were collected by ^1H NMR integration by following development of the CH_3 absorption at 1.10 ppm and decay of the CH_3 absorption at 1.15 ppm. Samples were prepared and data collected as outlined above, with *t*-BuOH as an internal (integrated) standard. For Λ-$[Co(en)_2(S\text{-}AA)]I_2$ (AA = Val, Phe) and Δ-$[Co(en)_2(S\text{-}AA)]ClO_4$ (AA = Glu, Asp), data were collected polarimetrically (25.0 °C) by following changes in optical rotation at the 436-nm Hg line. Solutions (~10^{-2} M) in 0.1 M NaCl (D_2O) were mixed with standardized NaOD and rotations recorded as a function of time. Similar experiments were carried out with solutions in 0.1 M NaCl (H_2O/OH^-). The polarimeter was initially set at approximately the starting rotation by using blank solutions of the complex.

^3H-Exchange Experiments. 1. Aspartate Complex. Approximately 0.15 g of an equilibrated mixture of Δ,Λ-$[Co(en)_2(S,R\text{-}AspH)]Cl_2$ (see above) dissolved in 5.0 cm^3 of 0.1 M NaCl was adjusted to pH 6.9 (2 M NaOH) and 20 μL of ^3H_2O (5 Ci cm^{-3}) added, followed by 5.0 cm^3 of 0.1 M NaOH. A 100-μL aliquot of this solution was immediately removed, quenched with 100 μL of 0.1 M HCl, and made up to 50 cm^3 (solution enrichment: this solution was further diluted 100-fold with water before counting). Sample A: after 5 min at 25 °C, 5.0 cm^3 was removed, quenched with 5.0 cm^3 of 0.05 M HCl, diluted with water (10 cm^3), adjusted to pH 7, and adsorbed onto Dowex 50W-X2 cation-exchange resin (Na^+ form, 10 × 1 cm column). Sample B: the remainder of the solution was treated similarly after 15 min at 25 °C. Samples were washed with 0.05 M phosphate buffer for 2 h and then with 0.1 M HCl for 2 h (slowly eluting) and then removed from the resin (2–3 M HCl) and rotary-evaporated to dryness. Residues were taken up in water, the pH was adjusted to 7, and the complexes were readsorbed onto long (30 × 1 cm) Dowex 50W-X2 columns (Na^+ form) and washed with 0.02–0.033 M phosphate buffer (pH 6.8) for 48 h. The complex slowly eluted during this time and separated into two orange bands (larger, faster moving, Λ-*S*/Δ-*R*; smaller, slower moving, Λ-*R*/Δ-*S*). The eluate was then changed to 0.1 M HCl and washing continued for 2 days. The separate bands were removed from the column and the complexes re-

Table I. Concentration Equilibrium Constants for Λ,Δ-$[Co(en)_2(S,R\text{-}AA)]^{n+}$ Complexes (25 °C)[a]

complex	charge type $n+$	conditions of equilibria	K_C[b]
Λ,Δ-Val	2+	0.1 M NaCl/0.05 M NaOH	2.0 ± 0.1
		0.1 M NaCl/0.01 M NaOH	2.0
		1.0 M NaCl/0.05 M NaOH	1.9
		0.025 M NaOH	1.92, 2.05
Λ,Δ-Val[c]	2+	0.1 M NaCl/0.01 M NaOD	2.1
		1 M NaCl/0.05 M NaOD	2.05
			av 2.0 ± 0.1
Λ,Δ-Ala	2+	0.1 M NaCl/0.05 M NaOH	1.0 ± 0.02
Λ,Δ-Leu	2+	0.1 M NaCl/0.05 M NaOH	0.95 ± 0.02
Λ,Δ-Phe	2+	0.1 M NaCl/0.005 M NaOH	1.1
		1 M NaCl/0.01 M NaOH	1.2, 1.2
		~0.2 M Na_2CO_3 (pH ~11)	1.16
Λ,Δ-Phe[c]		0.1 M NaCl/0.005 M NaOH	1.2
		~0.2 M Na_2CO_3 (pH 11.13)	1.1
			av 1.15 ± 0.1
Λ,Δ-Ala[c]	2+	0.1 M NaCl/0.05 M NaOD	1.0 ± 0.05
Λ-Glu	1+	1 M NaCl/0.05 M NaOH	0.85 ± 0.03
		0.05 M NaOH	0.86, 0.87
		0.2 M NEt_3 (pH 11.8)	0.88
		0.2 M Na_2CO_3 (pH 12.1)	0.74
		0.1 M Na_3PO_4 (pH 12.1)	0.64
Λ-Asp	1+	1 M NaCl/0.05 M NaOH	0.67 ± 0.03
		0.05 M NaOH	0.68
Λ,Δ-Asp		0.2 M NEt_3 (pH 11.8)	0.70
		0.2 M Na_2CO_3 (pH 12.1)	0.77
		0.1 M Na_3PO_4 (pH 12.1)	0.42

[a] Integration of 2-CH proton resonances (cf. Figures 1–3); acidified D_2O. [b] $K_C = ([\Lambda\text{-}R] + [\Delta\text{-}S])/([\Lambda\text{-}S] + [\Delta\text{-}R])$ for mixtures of Λ- and Δ-$[Co(en)_2(AA)]^{n+}$ complexes; $K_C = [\Lambda\text{-}R]/[\Lambda\text{-}S]$ for equilibration of Λ-$[Co(en)_2(AA)]^{n+}$ complexes. Errors, when given, represent maximum deviations from four experiments. [c] Equilibrated in D_2O/OD^-; integration of diastereomeric CH_3 doublets at 1.10 and 1.15 ppm (AA = Val, Ala), and diastereomeric CH_2 singlets at ~3.25 ppm (AA = Phe).

(4) Erickson, L. E.; Dappen, A. J.; Uhlenhopp, J. C. *J. Am. Chem. Soc.* **1969**, *91*, 2510.
(5) Keyes, W. E.; Legg, J. I. *J. Am. Chem. Soc.* **1973**, *95*, 3431.
(6) McClarin, J. A.; Dressel, L. A.; Legg, J. I. *J. Am. Chem. Soc.* **1976**, *98*, 4150.
(7) Keyes, W. E.; Legg, J. I. *J. Am. Chem. Soc.* **1976**, *98*, 4970.
(8) Keyes, W. E.; Caputo, R. E.; Willett, R. D.; Legg, J. I. *J. Am. Chem. Soc.* **1976**, *98*, 6939.
(9) Buckingham, D. A.; Collman, J. P. *Inorg. Chem.* **1967**, *10*, 1803.
(10) Liu, C. T.; Douglas, B. E. *Inorg. Chem.* **1964**, *3*, 1356.
(11) Legg, J. I.; Steele, J. *Inorg. Chem.* **1971**, *10*, 2177.

covered from the resin with 3 M HCl and reduced to dryness. The residues were made up in water to about the same Co concentration (~3 mM, ϵ(487 nm) = 104). A total of 1.0 cm^3 of each was added to 9 cm^3 of Bray's scintillation fluid (after background counting) and counted for 1 or 3 min. Data were as follows (sample, total volume (cm^3), concentration (mM), cpm, cpm (background)): A (Λ-*R*/Δ-S), 23.5, 3.16, 2645, 47; A (Λ-*S*/Δ-*R*), 36.4, 3.08, 3167, 48; B (Λ-*R*/Δ-*S*), 26, 3.12, 7651, 53; B (Λ-*S*/Δ-*R*), 40.5, 3.17, 8572, 55. The solvent enrichment ((5 × 10^4)-fold dilution) gave 45 604 cpm (background, 52 cpm). Sample B (Λ-*S*/Δ-*R*) was then divided in two. Half was washed for a further 1 day with phosphate buffer and worked up as before (sample C). To the other half was added an equal volume of 0.1 M NaOH; the mixture was left for 15 min at 25 °C and again worked up as before (sample D). Counting data for these samples were as follows (sample, concentration (mM), cpm, cpm (background)): C, 0.702, 2006, 92; D, 0.388, 1072, 87.

In another experiment a mixture of 59% Λ-[Co(en)$_2$(*S*-Asp)]ClO$_4$ (100 mg) and 41% Δ-[Co(en)$_2$(*S*-Asp)]ClO$_4$ (69 mg) was treated identically as above. Samples were removed after 15 min, 90 min, and 16 h. The diastereomers were washed and separated as above except that washing was extended by readsorbing the final recovered complexes onto fresh Dowex resin and washing for a further 8 h with phosphate buffer. Samples were collected as before and counting was carried out at two or three dilutions. Data are given in Table V. A total of 1 cm^3 of a 5 × 10^4 dilution of a 100-μL sample of the original solution gave 29 196 cpm (background, 54 cpm).

2. Valine Complex. About 0.1 g of an equilibrated mixture of Δ,-Λ-[Co(en)$_2$(*R*,*S*-Val)]Cl$_2$ was dissolved in 1.5 cm^3 of 0.1 M NaCl at pH 7; 10 μL of ^3H_2O (5 Ci cm^{-3}) was added, followed by 1.5 cm^3 of 0.1 M NaOH. After 10 min at 25 °C, 200 μL of 1 M HCl was added (quench), and the solution was diluted with water (10 cm^3), and pH was adjusted to 7, and the washing and recovery of the complex from Dowex 50W-X2 cation-exchange resin were carried out exactly as described above for the aspartic acid complex. For the valine complex the smaller Λ-*S*/Δ-*R* band (faster moving) only just separated from the larger Λ-*R*/Δ-*S* band (slower moving) on a 30-cm column, and representative middle cuts were used for counting (3 min) purposes. Data obtained were as follows (sample, concentration (mM), cpm, cpm (background)): A (Λ-*R*/Δ-*S*), 0.396, 1147, 90; B (Λ-*S*/Δ-*R*), 0.386, 1388, 63.

Results

1. Equilibrium Distribution of Epimers. First, it was necessary to determine equilibrium distributions under conditions of complete proton exchange and epimerization as shown in eq 3, where K_C = ([Λ-*R*] + [Δ-*S*])/([Λ-*S*] + [Δ-*R*]). Previously such distri-

$$\Lambda,\Delta\text{-}[Co(en)_2(S\text{-}AA)]^{n+} + OH^- \underset{}{\overset{K_C}{\rightleftharpoons}} \Lambda,\Delta\text{-}[Co(en)_2(R\text{-}AA)]^{n+} + OH^- \quad (3)$$

butions had been found polarimetrically,[1,12-14] or from circular dichroism[8,15] measurements, by comparing rotations of the mutarotated mixtures (Λ-*S* + Λ-*R*, eq 4) with those of the optically

$$\Lambda\text{-}[Co(en)_2(S\text{-}AA)]^{2+} + OH^- \underset{}{\overset{K_C}{\rightleftharpoons}} \Lambda\text{-}[Co(en)_2(R\text{-}AA)]^{2+} + OH^- \quad (4)$$

pure isomers. This requires a very precise knowledge of the specific rotations of both optically pure forms under the conditions of the mutarotation experiment (Λ-*S*, and usually Δ-*S* (Λ-*R*), eq 4), and we know from bitter experience that this information is very difficult, if not impossible, to obtain.[16] Alternatively, equilibrium distributions have been found by separating the mutarotated mixture into its diastereomeric components by thin-layer,[17] ion-pair,[18] or ion-exchange[15,19] chromatography. However, such methods can be time consuming, and complete separations, or quantitative recoveries of the separated diastereomers, have in many cases proved difficult to achieve. Such methods also usually rely on the assumption that the two diastereomers have equal extinction coefficients. We chose to use the ^{1}H NMR method since it is easy to apply and quantitative information can be obtained by integrating the separate 2-CH signals. Also, it does not rely on any particular purity in the specimen, or electrolyte condition, and it can be applied to optically impure mixtures and to mixtures of more than one complex in nearly every instance.

Table II. H-Exchange Rate Data for Δ,Λ-[Co(en)$_2$(*S*,*R*-AA)]$^{2+,+}$ Complexes in D_2O/OD^- (0.1 mol dm^{-3} NaCl, 25 °C)[a]

complex	10^2[OD$^-$], mol dm^{-3}	10^4k_{obs}, s^{-1}		10^2k_H, dm^3 mol^{-1} s^{-1}	
		Λ-*S*/Δ-*R*[b]	Λ-*R*/Δ-*S*[c]	Λ-*S*/Δ-*R*	Λ-*R*/Δ-*S*
Ala	3.33	9.2	8.0	2.8	2.4
	1.72	4.7	4.1	2.7	2.4
	3.33[d]	6.1	5.25	1.8	1.6
	1.72[d]	3.0	2.6	1.8	1.5
Phe	1.39	16.3	22	11.7	15.8
	1.0	12.0	17	12	17
	0.50	6.5	9	13	18
	1.39[d]	9.05	12.7	6.5	9.1
	0.70[d]	4.55	6.4	6.5	9.1
Glu	4.84[d]	14.6	12.8	3.0	2.6
	2.70	15.6	13.6	5.8	5.0
	1.72	10.1	8.8	5.9	5.1
	1.05	6.0	5.25	5.7	5.0
Asp	3.33	13.2	10.5	4.0	3.15
	1.72	7.2	5.3	4.2	3.1
	4.84[d]	12.1	8.9	2.5	1.8
	2.70[d]	6.8	5.1	2.5	1.9
	1.72[d]	4.2	3.1	2.45	1.8

[a] ^{1}H NMR data. [b] Disappearance of high-field 2-CH signal. [c] Disappearance of low-field 2-CH signal. [d] 1.0 M NaCl.

Solutions were equilibrated for between 24 h and 4 weeks under conditions listed in Table I and the solvent and electrolyte removed. (This was unnecessary for the valine and phenylalanine complexes equilibrated in D_2O.) Spectra were run under acidic conditions in D_2O after the solutions had been allowed to stand at neutral pH (Na_2CO_3 added to pH ~7, 30 s) to remove unwanted N–H signals. Figures 1–3 give spectra of the fully equilibrated complexes, and the diastereomeric 2-CH resonances are clearly distinguished in each case. Table I lists K_C values obtained from instrument integration of these signals. Duplicate experiments were carried out, and it was shown that complete equilibration was achieved after 24 h in 0.01 M OH$^-$ although checks were made for up to 4 weeks. The presence or absence of NaCl does not affect the distribution, but equilibration in the presence of phosphate buffer (pH 6.88) clearly favors the Λ-*S* (Δ-*R*) diastereomer in the case of the Glu and Asp complexes. This latter aspect was not followed up but has been noted previously in related systems.[20] The presence of CO_3^{2-} also appears to affect the distribution but in opposite senses for the AA = Glu and Asp complexes. Equilibration of Λ,Δ-[Co(en)$_2$(*S*-Val)]$^{2+}$ in H_2O gave the same distribution as in D_2O, as demonstrated by integrating the diastereomeric CH_3 doublets at 1.10 ppm (Δ-*S*, Λ-*R*) and 1.15 ppm (Λ-*R*, Δ-*S*). This same result was found for Λ,Δ-[Co(en)$_2$(Phe)]$^{2+}$ by integrating the phenylalanine CH_2 signal at 3.25 ppm for a solution equilibrated in Na_2CO_3-buffered D_2O.[21] It can be concluded from these last two experiments that the solvent

(12) Buckingham, D. A.; Mason, S. F.; Sargeson, A. M.; Turnbull, K. R. *Inorg. Chem.* **1966**, *5*, 1649.
(13) Buckingham, D. A.; Dekkers, J.; Sargeson, A. M.; Wein, M. *Inorg. Chem.* **1973**, *12*, 2019.
(14) Buckingham, D. A.; Dekkers, J.; Sargeson, A. M.; Marzilli, L. G. *Inorg. Chem.* **1973**, *12*, 2019.
(15) Fujita, M.; Yoshikawa, Y.; Yamatera, H. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 3209.
(16) Very small, and varying, amounts of racemization often occur in the amino acid during synthesis of the [Co(en)$_2$(*S*-AA)]$^{2+}$ complexes (Tasker, R. F. Ph.D. Thesis, University of Otago, Dunedin, New Zealand, 1985), and this leads to varying specific rotations for the Δ-*S* and Λ-*S* diastereomers irrespective of the method of recovery. We are therefore not confident in using such rotations for quantitative comparative purposes.
(17) Warner, B. D.; Legg, J. I. *Inorg. Chem.* **1979**, *18*, 1839.
(18) Buckingham, D. A.; Clark, C. R.; Deva, M. M.; Tasker, R. F. *Inorg. Chem.* **1983**, *22*, 2754.
(19) Yamaguchi, M.; Yamamatsu, S.; Furusawa, T.; Yano, S.; Saburi, M.; Yoshikawa, S. *Inorg. Chem.* **1980**, *19*, 2010.
(20) Yamaguchi, M.; Masui, Y.; Saburi, M.; Yoshikawa, S. *Inorg. Chem.* **1982**, *21*, 4138.
(21) The CH_2 of the phenylalanine chelate is in reality two overlapping AB systems following deuteration at the 2-CH position, and only in Na_2CO_3-buffered D_2O does this signal appear as two A_2 systems (inset c to Figure 3). Other insets to this figure show coupling of the 2-CH proton to the one AB system in Δ-[Co(en)$_2$(*S*-Phe)]$^{2+}$ prior to epimerization in borax buffer (inset a) and the two AB systems present following equilibration at pH 9.7 in this same buffer for 1 week (inset b).

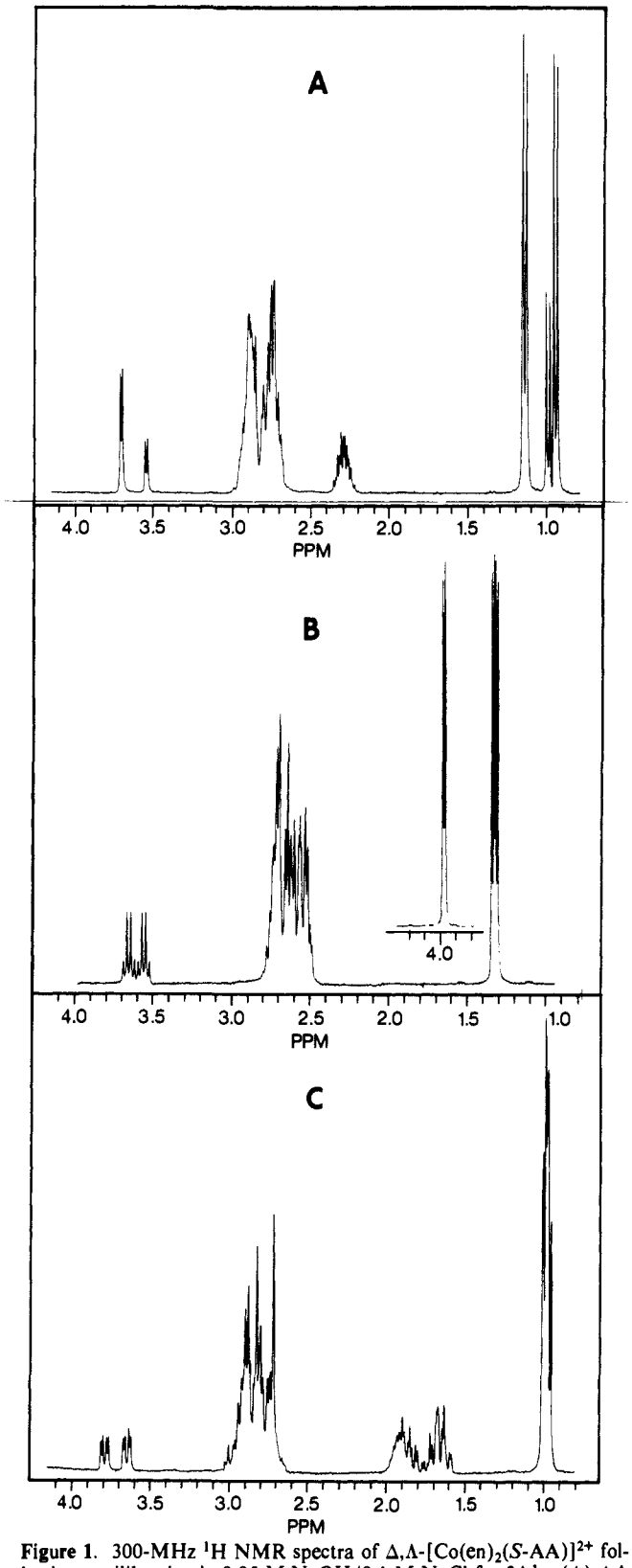


Figure 1. 300-MHz ^{1}H NMR spectra of Δ,Λ-$[Co(en)_2(S\text{-}AA)]^{2+}$ following equilibration in 0.05 M NaOH/0.1 M NaCl for 24 h: (A) AA = Val; (B) AA = Ala; (C) AA = Leu. In each case the 2-CH absorptions at lowest field, and the CH_3 absorption at highest field, are due to the Δ-*S* + Λ-*R* combination, and correspondingly the 2-CH absorption at higher field, and the CH_3 absorption at lower field, is due to the Δ-*R* + Λ-*S* enantiomers. The inset to (B) gives the two CH_3 singlets for the AA = Ala system following equilibration in 0.05 M $NaOD/D_2O$ solution for 24 h.

(D_2O, H_2O) does not affect K_C.

2. H-Exchange Rate Data. These data were obtained from D_2O/OD^- mixtures by following decay of the 2-CH proton signal as a function of time. Since both diastereomeric protons were clearly distinguished, both exchanges could be followed in one experiment using mixtures. In this way small differences in rate became very apparent.

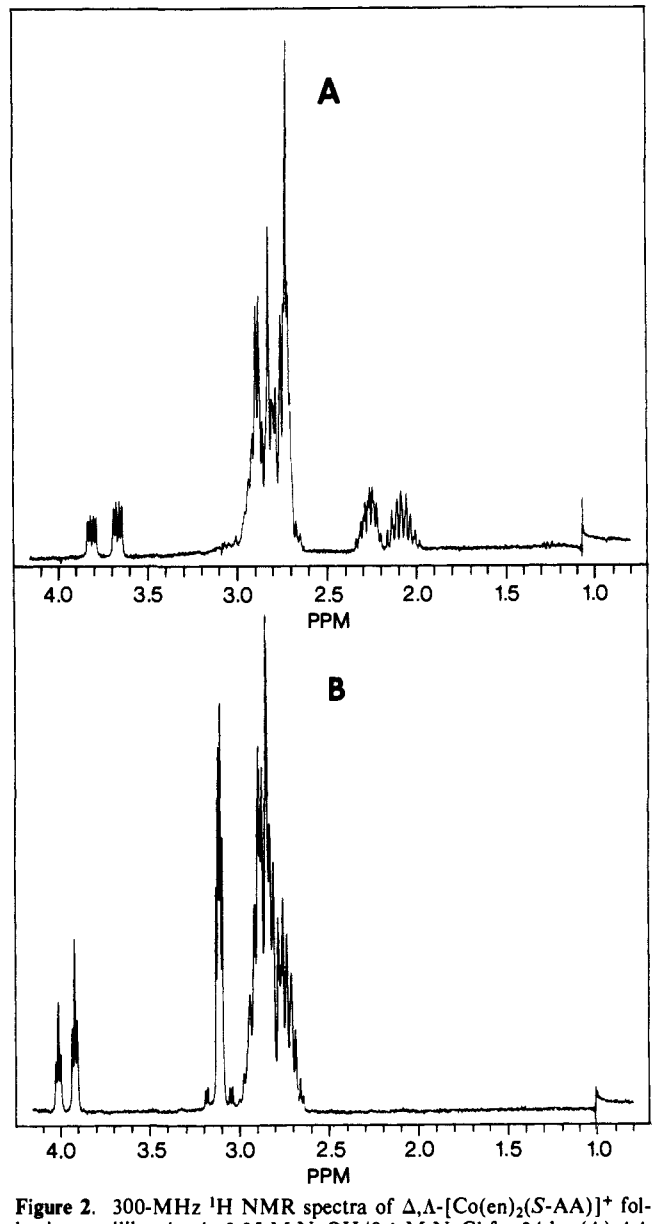


Figure 2. 300-MHz ^{1}H NMR spectra of Δ,Λ-$[Co(en)_2(S\text{-}AA)]^+$ following equilibration in 0.05 M NaOH/0.1 M NaCl for 24 h: (A) AA = Glu; (B) AA = Asp. In each case the downfield 2-CH multiplet is due to the Δ-*S* + Λ-*R* combination and the upfield multiplet to the Δ-*R* + Λ-*S* combination.

(a) Δ,Λ-$[Co(en)_2(S\text{-Ala})]^{2+}$. Data ($k_{obs}(H)$, k_H) are given in Table II. From the two experiments at different $[OD^-]$ the rate law $k_{obs}(H) = k_H[OD^-]$ is confirmed.[1] Figure 4A shows the results from a run using 33.3 mM $[OD^-]$. It can be seen that the initially equivalent signals corresponding to the Δ-*S* and Λ-*S* diastereomers decay at slightly different rates; the Λ-isomer is somewhat faster to exchange (6.08×10^{-4} s^{-1}) than the Δ-isomer (5.25×10^{-4} s^{-1}).

(b) Δ,Λ-$[Co(en)_2(S,R\text{-Val})]^{2+}$. In this case a previously equilibrated mixture of diastereomers was used as well as the separate isomers. Rate data are given in Table III (together with epimerization data; see below). Figure 4B shows decay of the two 2-CH resonances representing the Δ-*S*, Λ-*R* and Δ-*R*, Λ-*S* combinations in an experiment using 33.3 mM $[OD^-]$ in 1.0 M NaCl. The less abundant isomer (Δ-*R*, Λ-*S*) decays more rapidly (4.91×10^{-4} s^{-1}) than the more abundant isomer (Δ-*S*, Λ-*R*; 3.91×10^{-4} s^{-1}). The data for Λ-$[Co(en)_2(S\text{-Val})]^{2+}$ follow the rate law $k_{obs}(H) = k_H[OD^-]$. Second-order rate constants are 3.3×10^{-2} mol^{-1} dm^3 s^{-1} and 2.5×10^{-2} mol^{-1} dm^3 s^{-1} for the Λ-*S* (Δ-*R*)

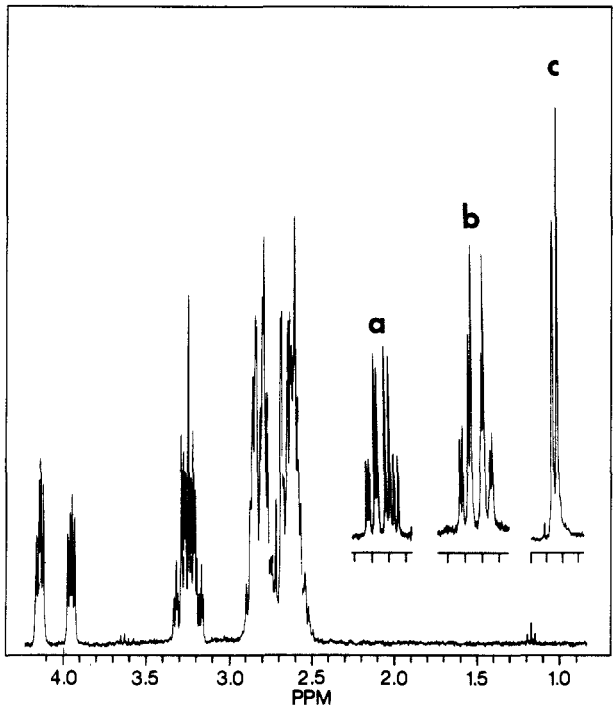


Figure 3. 300-MHz ^{1}H NMR spectrum of Δ,Λ-$[Co(en)_2(S\text{-Phe})]^{2+}$ following equilibration in 0.005 M NaOH/0.1 M NaCl for 24 h. The 2-CH multiplet at lowest field is due to the Δ-*S* + Λ-*R* enantiomers and the multiplet at slightly higher field to the Δ-*R* + Λ-*S* combination. The insets show the CH_2 multiplet of the phenylalanine chelate (3.25 ppm) for (a) Δ-$[Co(en)_2(S\text{-Phe})]^{2+}$ before equilibration (pH ~7), ABX system; (b) Δ-$[Co(en)_2(S\text{-Phe})]^{2+}$ following equilibration in borax buffer (pH ~9.7)/D_2O for 1 week; and (c) Δ-$[Co(en)_2(S\text{-Phe})]^{2+}$ following equilibration in ~0.2 M Na_2CO_3 (pH ~11)/D_2O for 4 days. In (b) and (c) the upfield of the two AB or A_2 systems is due to the Δ-*S* + Λ-*R* combination.

Table III. H-Exchange and Epimerization Rate Constants[a] for $[Co(en)_2(S\text{-Val})]^{2+}$ Complexes (0.1 mol dm^{-3} NaCl, 25 °C)

complex	10^2[OD$^-$], mol dm^{-3}	10^4k_{obs}(H), s^{-1}	10^4k_{obs}(E), s^{-1}	10^2k_H, mol^{-1} dm^3 s^{-1}	10^2k_E, mol^{-1} dm^3 s^{-1}
Λ-*S*	3.33	10.7, 10.5	9.9, 9.7	3.2	2.9
	2.0	6.0		3.0	
	1.72	5.8, 6.1	5.4, 5.6	3.4	3.2
	1.4	3.8		2.7	
	1.0	3.1		3.1	
	1.0[b]	4.3[b]		4.3[b]	
	3.33[c]	2.4	1.75	0.71	0.53
	3.33	11.3	9.6	3.4	2.9
	3.33[d]	40	33	12	9.9
	3.33[e]	4.91	4.53	1.47	1.36
	1.72[e]	2.7	2.5	1.6	1.45
Δ-*S*	3.33	8.5	10.0	2.6	3.1
	2.0	4.7		2.4	
	1.0	2.5		2.5	
	1.0[b]	3.9[b]		3.9[b]	
	3.33[e]	3.95	4.6	1.2	1.4
Λ-*S*/Δ-*R* +	3.33	10.7,[f] 8.5[g]		3.2,[f] 2.6[g]	
Λ-*R*/Δ-*S*	3.33[e]	4.9,[f] 3.9[g]		1.5,[f] 1.2[g]	

[a] D_2O–OD^- mixtures. [b] $I \sim 0$; no supporting electrolyte. [c] 10 °C. [d] 40° C. [e] I = 1.0 (NaCl). [f] Disappearnace of 3.75 ppm signal (Λ-*S*/Δ-*R*). [g] Disappearance of 3.90 ppm signal (Λ-*R*/Δ-*S*).

and Δ-*S* (Λ-*R*) diastereomers in 0.1 M NaCl. By following the decay of Λ-$[Co(en)_2(S\text{-Val})]^{2+}$ at three temperatures (40, 25, and 10 °C), an activation enthalpy for H-exchange of $\Delta H^{\ddagger}$ = 66 ± 5 kJ mol^{-1} was found.

(c) Δ,Λ-$[Co(en)_2(S,R\text{-Phe})]^{2+}$. Data are given in Table II. Figure 4C shows decay of the two 2-CH absorptions for a nonequilibrium mixture of isomers. The more stable combination (Δ-*S*, Λ-*R*; downfield triplet) now exchanges more rapidly than the less stable combination (Δ-*R*, Λ-*S*; upfield triplet); k_{obs}(H)

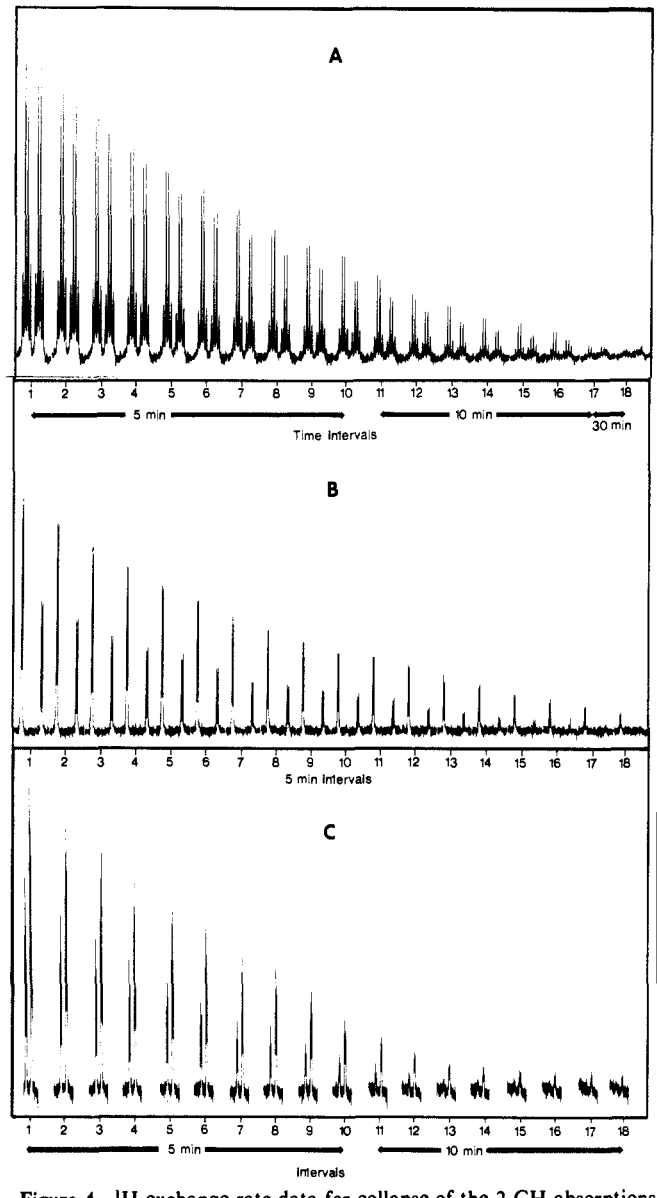


Figure 4. ^{1}H exchange rate data for collapse of the 2-CH absorptions in D_2O/OD^- solutions: (A) Δ,Λ-$[Co(en)_2(S\text{-Ala})]^{2+}$ (equilibrium mixture) in 33.3 mM NaOD/0.1 M NaCl, 25 °C, 80-s collection time; (B) Δ,Λ-$[Co(en)_2(S\text{-Val})]^{2+}$ (equilibrium mixture) in 1.0 M NaCl, 25 °C, 80 s; (C) Δ,Λ-$[Co(en)_2(S\text{-Phe})]^{2+}$ (nonequilibrium mixture) in 7.04 mM NaOD/0.1 M NaCl, 25 °C, 80 s.

= 6.42 × 10^{-4} s^{-1} and 4.55 × 10^{-4} s^{-1}, respectively, in 7.04 mM OD^-. This situation requires other factors, in addition to proton loss, to be involved in deciding equilibrium concentrations.

(d) Δ,Λ-$[Co(en)_2(S,R\text{-Glu})]^+$ and Δ,Λ-$[Co(en)_2(S,R\text{-Asp})]^+$. Rate data are given in Table II and the rate law $k_{obs}(H) = k_H[OD^-]$ again holds. Figure 5 shows typical decays for these two complexes. Again the more stable combination (Λ-*S*, Δ-*R*) exchanges more rapidly than the less stable combination (Λ-*R*, Δ-*S*), but now the relative stability order is reversed compared to the above Phe system. The decays shown give rate constants of 8.8 × 10^{-4} s^{-1} (Λ-*R*, Δ-*S*; downfield signal) and 10.1 × 10^{-4} s^{-1} (Λ-*S*, Δ-*R*; upfield signal) for the glutamate complex (Figure 5A) and 5.3 × 10^{-4} s^{-1} (Λ-*R*, Δ-*S*; downfield triplet) and 7.21 × 10^{-4} s^{-1} (Λ-*S*, Δ-*R*; upfield triplet) for the aspartate complex (Figure 5B). Second-order rate constants (k_H) are given in Table II.

(e) Δ,Λ-$[Co(en)_2(Gly)]^{2+}$. This is a most interesting system since the amino acid is achiral, but when coordinated to asymmetric Co(III), it becomes prochiral. At 60 MHz the complex displays a single resonance for the two 2-CH_2 protons, and there has been no previous report of these protons being distinguished

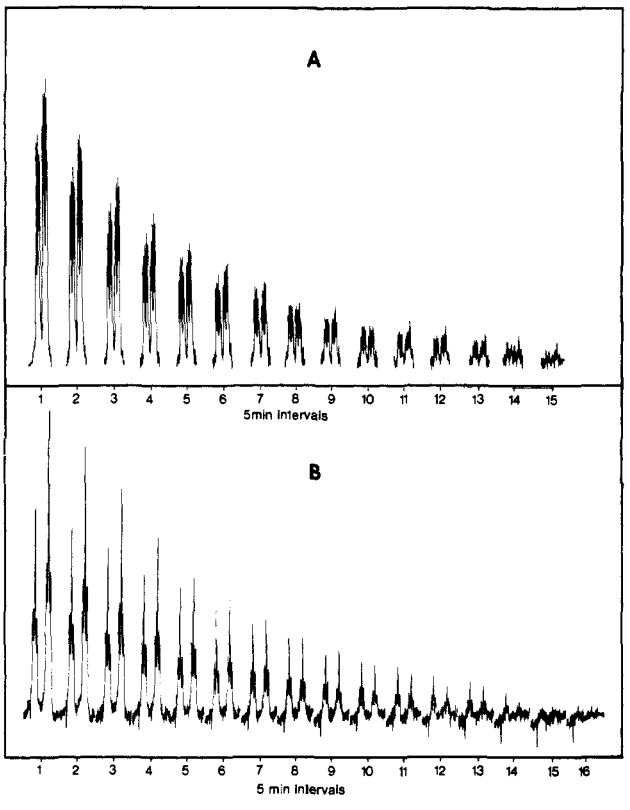


Figure 5. ^1H exchange rate data for collapse of the 2-CH absorptions in D_2O/17.2 mM OD^- solutions at 25.0 °C, equilibrium mixtures of diastereomers, and 80-s collection times: (A) Δ,Λ-$[Co(en)_2(S,R\text{-Glu})]^+$; (B) Δ,Λ-$[Co(en)_2(S,R\text{-Asp})]^+$.

by any physical technique. Indeed, other investigators have gone to varying lengths to develop observable chirality in chelated glycine by using more discriminating ligand systems[22] and/or by modifying the glycine chelate itself.[23,24]

At 300 MHz $[Co(en)_2(Gly)]^{2+}$ displays a clear AB pattern (Figure 6A; J_{AB} = 17.86 Hz). Furthermore, the two protons exchange at different rates. This is shown by the series of spectra given in Figure 6B. Initially the AB pattern ($\Delta\nu_{AB}$ = 14.0 Hz) collapses at a rate (6.80×10^{-4} s^{-1} in 13.9 mM OD^-) that was determined by following decay of either of the two AB satellites at 3.71 or 3.58 ppm. As exchange continues, a new singlet appears at 3.61 ppm. Another singlet, which is initially buried under the central portion of the AB system, is also produced at 3.66 ppm. The latter signal becomes obvious at longer times after the AB system has completely collapsed. However, its intensity at early times can be found by subtracting from the total signal that portion of the AB system which must still be present. The intensity ratio at zero time, $[AB_0]_{central}$:$[AB_0]_{satellites}$ = 7.63, was found by integrating the AB system in the absence of exchange,[25] and since this value must be time independent, the amount to be subtracted from the central portion is given by $[AB_t]_{satellites} \times 7.63$. In this way the intensity of the singlet at 3.66 ppm was found. Figure 7 plots intensities of the two singlets (3.66 and 3.61 ppm)[26] as a function of time. That at 3.66 ppm is clearly the more intense. This is assigned to the Λ-*R* (Δ-*S*) epimer containing the chiral

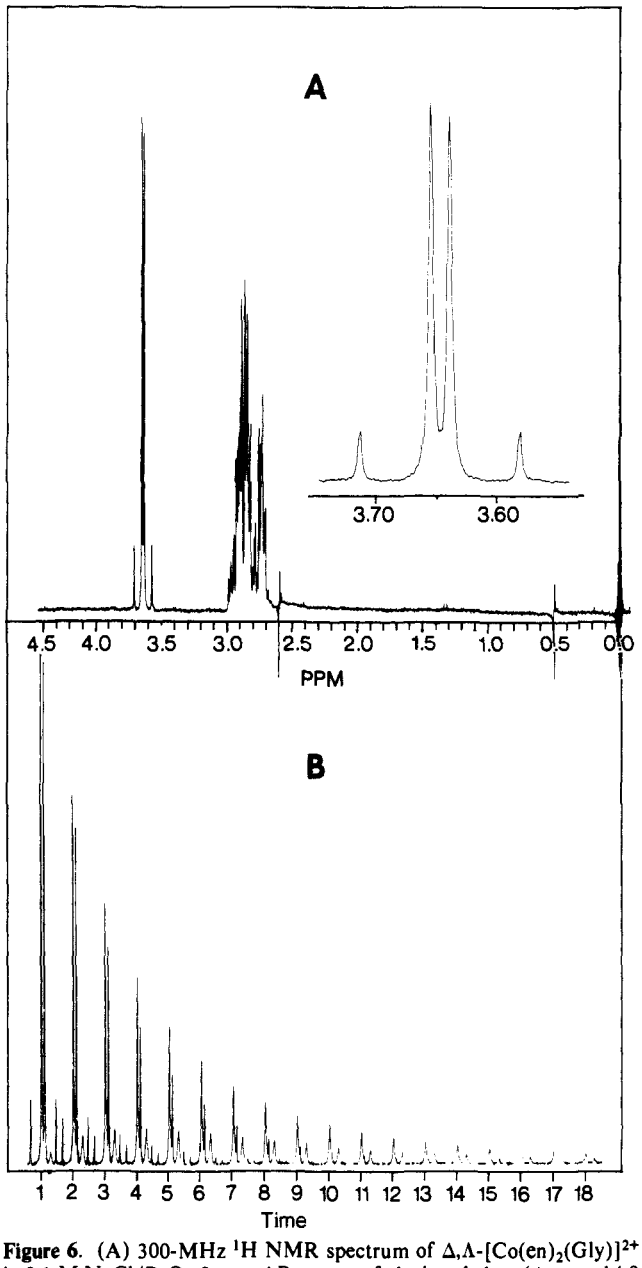
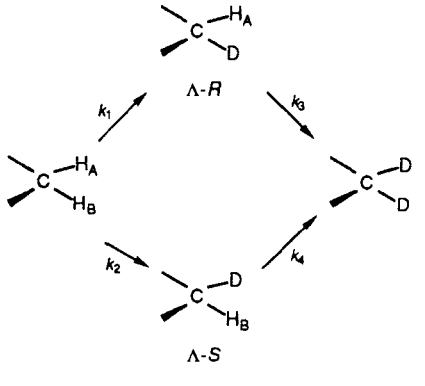


Figure 6. (A) 300-MHz ^1H NMR spectrum of Δ,Λ-$[Co(en)_2(Gly)]^{2+}$ in 0.1 M NaCl/D_2O. Inset: AB system of glycine chelate ($\Delta\nu_{AB}$ = 14.0 Hz; J_{AB} = 17.86 Hz). (B) Decay of AB system of glycine as a function of time in 1.39×10^{-2} M NaOD/0.1 M NaCl at 25.0 °C and 80-s collection time. The time interval between acquisitions was 10 min.

Scheme I



2-C^1H^2H glycine chelate, while that at 3.61 ppm is assigned to the Λ-*S* (Δ-*R*) epimer. This assignment is based on the order of chemical shifts found for the other chiral amino acid chelates (Figures 1–3). Analysis[27] of these time-dependent data (Figure

(22) Belokon, Y. N.; Melikyan, A. S.; Salel'eva, T. F.; Bakhmutov, V. I.; Vitt, S. V.; Belikov, V. M. *Tetrahedron* **1980**, *36*, 2327.
(23) Golding, B. T.; Gainsford, G. J.; Herlt, A. J.; Sargeson, A. M. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 495; *Tetrahedron* **1976**, *32*, 389.
(24) Dokuzovic, Z.; Roberts, N. K.; Sawyer, J. F.; Whelan, J.; Bosnich, B. *J. Am. Chem. Soc.* **1986**, *108*, 2034.
(25) A ratio (8.52) can also be calculated from the $\Delta\nu_{AB}$ (14.0 Hz) and J_{AB} (17.86 Hz) values (Mathieson, D. W. *NMR for Organic Chemists*; Academic Press: New York, 1967; p 69).
(26) $\Delta\nu \sim 15$ Hz for the two chiral monodeuterated glycine chelates. This is the same as that found for the prochiral protonated complex ($\Delta\nu_{AB}$ = 14.0 Hz).

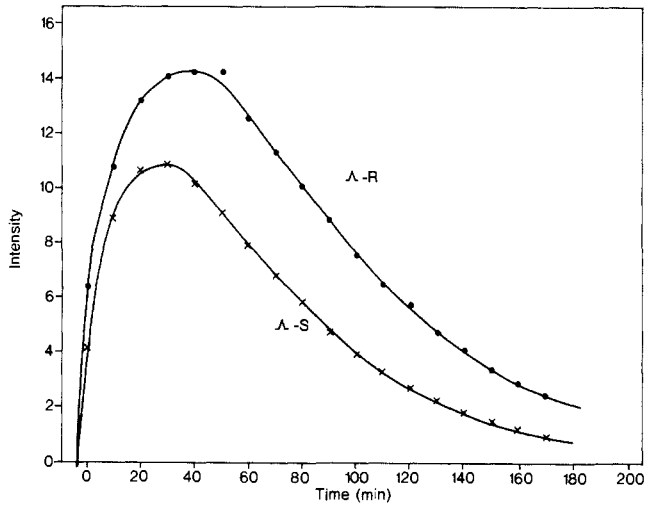


Figure 7. Appearance and disappearance of Λ-[Co(en)$_2$(*S*,*R*-Gly(H,D))]$^{2+}$ diastereomers on deuterating Λ-[Co(en)$_2$(Gly)]$^{2+}$ in 1.39×10^{-2} M NaOD/0.1 M NaCl at 25.0 °C.

7) according to Scheme I gives the following rate ratios and rate constants: $k_1/k_2 = 1.25$, $k_1 = 3.8 \times 10^{-4}$ s^{-1}, $k_2 = 3.02 \times 10^{-4}$ s^{-1}, $k_3 = 2.63 \times 10^{-4}$ s^{-1}, $k_4 = 3.26 \times 10^{-4}$ s^{-1}. Thus removal of the prochiral Λ-*S* (Δ-*R*) proton is some 1.25 times faster than removal of the prochiral Λ-*R* (Δ-*S*) proton, and the secondary isotope effect of replacing H with D in the two chiral glycine chelates gives a rate decrease of 15% for removing the remaining proton ((k_1/k_4) and $(k_2/k_3) = 1.15$).

3. Epimerization Rate Data. These data were obtained either by following ^{1}H NMR changes (AA = Val) or by measuring polarimetrically (AA = Val, Phe, Glu, Asp). In both methods, one epimer was used and changes were followed as a function of time until equilibrium was reached (eq 4). ^{1}H NMR data for Λ-[Co(en)$_2$(*S*-Val)]$^{2+}$ are given in Table III. Both the appearance of the upfield CH_3 doublet (1.10 ppm, Λ-*R*) and the decay of the downfield doublet (1.15 ppm, Λ-*S*) (Figure 8A) were followed. These changes are shown in Figure 8B.[28] Both signals gave linear log $(I_t - I_\infty)$ vs time plots, and the resulting k_{obs}(E) values at different [OD$^-$] follow the expression $k_{obs}(E) = k_E[OD^-]$, with $k_E = 3.1 \times 10^{-2}$ mol^{-1} dm^3 s^{-1}. Final intensities gave [Λ-*R*]/[Λ-*S*] = 2.0 ± 0.1, which is in excellent agreement with the equilibrium distribution found in H_2O. Data at 40, 25, and 10 °C gave $\Delta H^{\ddagger}(E) = 69 \pm 5$ kJ mol^{-1}. This experiment was also used to determine $\Delta H^{\ddagger}(H)$ for proton exchange by following decay of the 2-CH absorptions (see above).

Polarimetric rate data were obtained in both D_2O and H_2O and are given in Table IV. Mutarotation was followed at 436 nm (largest change with the Na or Hg lamps of the 141 polarimeter) and good first-order traces were obtained for all four complexes (AA = Val, Phe, Glu, Asp). The rate expression $k_{obs}(E) = k_E[OD^-]$, or $k_E[OH^-]$, is followed. Rates in H_2O are about twice as slow as in D_2O, which is consistent with earlier data.[1]

4. ^{3}H-Incorporation Experiments. These experiments were carried out for two reasons. First, we wanted to obtain a direct measure of the reprotonation rate ratio k_{-2}/k_{-1} to see if it was hydrogen isotope independent. This is important because the H-exchange experiments were (necessarily) carried out in D_2O where the reprotonating acid is D_2O rather than H_2O. Second, we wished to see if the reprotonation rate was also isotope independent (i.e., k_{-1}^H/k_{-1}^T or k_{-2}^H/k_{-2}^T). This aspect is important in evaluating the ^{3}H method of determining epimerization rates in such systems (see Discussion). For the first experiments, equilibrium mixtures of Δ,Λ-[Co(en)$_2$(*S*,*R*-Asp)]$^+$ and Δ,Λ-[Co(en)$_2$(*S*,*R*-Val)]$^{2+}$ were treated with 0.05 M NaOH containing a trace of ^3H_2O for times corresponding to somewhat less than 1 half-time for epimerization in H_2O. Following removal of extraneous exchange into the amine functions of the ethylenediamine and amino acid ligands (this exchange is fast and complete under alkaline conditions), residual activity in the 2-CH position

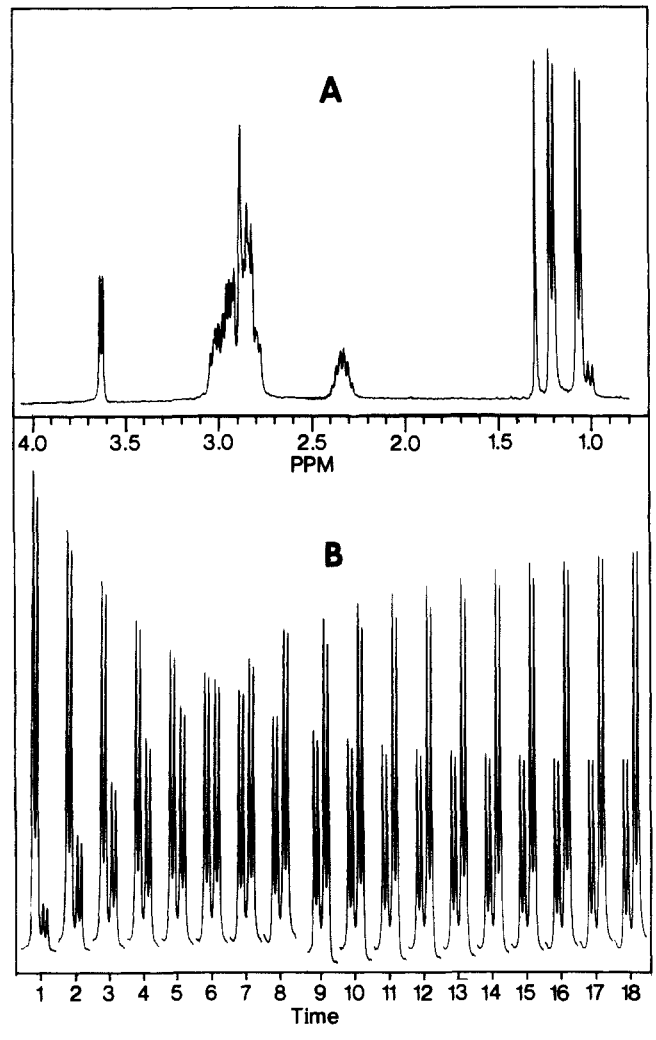


Figure 8. (A) Initial spectrum (after ~3 min) of Λ-[Co(en)$_2$(*S*-Val)]$^{2+}$ in 1.72×10^{-2} M NaOD/0.1 M NaCl/D_2O. (B) Time-dependent changes in highest field CH_3 resonances showing formation of the Λ-*R* diastereomer: 80-s collection time; 5-min intervals (1–7); 30-min intervals (8–18).

Table IV. Epimerization Rate Constants for [Co(en)$_2$(*S*-AA)]$^{2+}$ Complexes in D_2O/OD^- Solution (I = 0.1 mol dm^{-3} (NaCl), 25.0 °C)[a]

complex	10^2[OD$^-$], mol dm^{-3}	$10^4 k_{obs}$(E), s^{-1}	$10^2 k_E$, mol^{-1} dm^3 s^{-1}
Λ-[Co(en)$_2$(*S*-Val)]I$_2$	3.33	10.9	3.3
	1.65	4.95	3.0
	1.65	4.8	2.9
	0.825	2.4	2.9
Λ-[Co(en)$_2$(*S*-Phe)](ClO$_4$)$_2$	1.25	17.25	13.8
	0.63	9.06	14.5
Δ-[Co(en)$_2$(*S*-Phe)](ClO$_4$)$_2$	1.25	17.0	13.6
Λ-[Co(en)$_2$(*S*-Glu)]ClO$_4$	2.50	13.8	5.5
	1.43	7.73	5.4
	5.0[b]	14.1	2.8
Δ-[Co(en)$_2$(*S*-Asp)]ClO$_4$	3.33	9.79	3.0
	2.50	6.92	2.8
	1.43	4.58	3.2
	5.0[b]	8.0	1.6

[a] Measured polarimetrically. [b] In H_2O/OH^- solution, I = 0.1 (NaCl), 25 °C.

(27) Computer-assisted analysis was carried out for Scheme I with a conventional consecutive first-order rate program; $k_{obs}(1) = k_1 + k_2$ for exchange of the first proton and $k_{obs}(2)$ represents k_3 or k_4 for exchange of the remaining proton.

(28) Only one of the CH_3 doublets of chelated valine shows an appreciable chemical shift difference between the two diastereomers (cf. Figure 1A).

Table V. ^3H Incorporation into $[Co(en)_2(Asp)]^+$ and $[Co(en)_2(Val)]^{2+}$ Complexes (0.05 M NaOH/0.05 M NaCl, 25 °C)

complex	time, min	cpm[c] ([Co], mM)	cpm/[Co] (×10⁶)	k_{-2}^T/k_{-1}^T[e]
Λ-*R*/Δ-*S*, Asp[a]	5	2598 (3.16)	0.8222	
Λ-*S*/Δ-*R*, Asp[a]	5	3119 (3.08)	1.0127	0.54, 0.52[f]
Λ-*R*/Δ-*S*, Asp[a]	15	7598 (3.12)	2.435	
Λ-*S*/Δ-*R*, Asp[a]	15	8517 (3.17)	2.687	0.61, 0.58[f]
Λ-*R*/Δ-*S*, Asp[b]	15	6240 (4.63)	1.348	
	15	1571 (0.926)	1.697	
	15		1.77 (0)[d]	
	90	31880 (4.79)	6.652	
	90	7525 (0.958)	7.855	
	90		8.13 (0)[d]	
	16 h	36842 (0.958)	7.975	
	16 h	8806 (0.924)	9.530	
	16 h		9.92 (0)[d]	0.58
Λ-*S*/Δ-*R*, Asp[b]	15	9526 (5.84)	1.631	
	15	5285 (2.92)	1.810	
	15	2310 (1.168)	1.978	
	15		2.05 (0)[d]	
	90	38730 (5.125)	7.557	
	90	20601 (2.56)	8.047	
Λ-*S*/Δ-*R*, Asp[b]	90	8875 (1.025)	8.659	
	90		8.89 (0)[d]	
	16 h	45480 (5.25)	8.663	
	16 h	23881 (2.62)	9.115	
	16 h	10532 (1.05)	10.030	
	16 h		10.32 (0)[d]	
Λ-*R*/Δ-*S*, Val[a]	10	1157 (0.396)	2.669	
Λ-*S*/Δ-*R*, Val[a]	10	1325 (0.388)	3.433	1.55

[a] An equilibrium mixture of all four diastereomers was used as the starting material in these experiments. [b] The starting mixture for this experiment consisted of 59% Λ-$[Co(en)_2(S\text{-Asp})](ClO_4)$ and 41% Δ-$[Co(en)_2(S\text{-Asp})]ClO_4$. [c] Corrected for background counts, typically 45–50 cpm. [d] Value from extrapolation of cpm/[Co] to [Co] = 0. [e] Calculated with K_C = 0.67 (Asp), 2.0 (Val). [f] Directly obtained from total collected band (cf. Experimental Section).

was counted. Results are given in Table V. For the aspartate complex, the k_{-2}^T/k_{-1}^T ratio was found both from the total counts incorporated into each diastereomer (footnote *f*) and from the cpm/[Co] values with K_C = 0.67. Both methods gave essentially the same result; $k_{-2}^T/k_{-1}^T = 0.55 \pm 0.03$. Removal of extraneous counts was shown to be complete (103% retention of activity) by extending the washing period (sample C, Experimental Section), and removal of ^3H from the complex once incorporated was shown to be slow under these conditions. (Sample D, Experimental Section, shows 94% retention of activity following treatment with 0.05 M NaOH for 15 min.) At long times (16 h), however, incorporation into the two diastereomers approaches the same limiting value (cpm/[Co]) as complete equilibration with ^3H takes over. For the valine complex, k_{-2}^T/k_{-1}^T was found to be 1.55 (single experiment).

The second experiment gives estimates of rates of ^3H incorporation into the aspartate complex. Data at 90 min give rate constants of 1.9×10^{-4} s^{-1} for the Λ-*R*/Δ-*S* pair of epimers and 2.2×10^{-4} s^{-1} for the Λ-*S*/Δ-*R* pair on the basis of complete equilibration after 16 h. Identical values would be expected. However, the experimental enrichments at 16 h are not quite the same (9.92×10^6 and 10.32×10^6 cpm/[Co]), probably because complete equilibration of the tritium label is very slow as we have shown above. Final enrichments represent 75–80% of the total available enrichment based on the solvent figure with the major part of the difference from 100% probably reflecting quenching by the metal. This aspect was not investigated further here, but quenching factors of ~70% are often observed in Co(III) systems. The averaged rate constant for ^3H incorporation of 2.0×10^{-4} s^{-1} is to be compared with an epimerization rate of $\sim 1.55 \times 10^{-3}$ s^{-1} under the same conditions, giving $k_{-1}^H/k_{-1}^T \sim 8$.

Discussion

Rate and equilibrium (concentration) constants are summarized in Table VI. The former are given as second-order values since the rate law $k_{obs} = k[OD^-]$ for epimerization ($k = k_E$) and exchange ($k = k_H$) is established, or verified, in sufficient detail to assume that it holds in all cases. All electrophilic substitutions in such systems appear to be base-catalyzed (in this case by OD^- or OH^-).

Table VI. Equilibrium Constants and H-Exchange and Epimerization Rate Constants for Λ-$[Co(en)_2(S,R\text{-AA})]^{2+,+}$ Complexes (25 °C, I = 0.1 mol dm^{-3})[a,b]

aa	K_C	10^2k_1	10^2k_2	10^2k_E	k_{-2}/k_{-1}, eq 5	k_{-2}/k_{-1}, eq 6
Phe	1.15 ± 0.1	12	17	14.0[d]	1.6	1.7
Val	2.0 ± 0.1	3.3	2.5	3.1,[c] 3.0[d]	1.5	1.7
Ala	1.0 ± 0.02	2.7_5	2.4		0.87	
Gly	1.0	2.7	2.16		0.80	
Glu	0.85 ± 0.03	5.8	5.05	5.45[d]	0.74	0.75
Asp	0.67 ± 0.03	4.1	3.1	3.0[d]	0.51	0.42

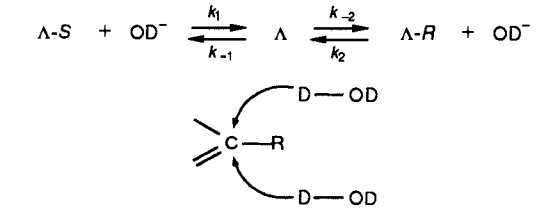
[a] K_C is a concentration constant and is dimensionless; k_1, k_2, and k_E are second-order rate constants (mol^{-1} dm^3 s^{-1}). [b] $K_C = [\Lambda\text{-}R]/[\Lambda\text{-}S] = k_1k_{-2}/k_2k_{-1}$ (mechanism I). [c] In D_2O; ^1H NMR data (Table III). [d] In D_2O; polarimetric data (Table IV).

In our earlier paper[1] we showed that for the AA = Ala and Val chelates H-exchange occurred at about the same rate as mutarotation when both experiments were carried out in D_2O/OD^-. The present study confirms this, but with the higher precision possible by using the 300-MHz NMR instrument, small differences now come to light. This is particularly so for the valine complex where H-exchange for both epimers, or H-exchange for one epimer and epimerization, was followed in one experiment. H-exchange for the two epimers was nearly always followed in this manner with mixtures. It is obvious from these k_H data (Table VI) that k_1/k_2 is not a measure of K_C, as was originally thought. Also, it can be seen from the table that the relative abilities of these complexes to undergo H-exchange (and epimerization) follow the order Phe > Glu > Asp > Val > Ala ~ Gly. This is not in agreement with previous ideas[29] that would have the less positively charged Glu and Asp complexes (1+) being less reactive than those of Phe, Val, Ala, and Gly (2+).

Differences between k_H and k_E have an important bearing on the mechanism. In particular, they allow previous suggestions of a chiral carbanion intermediate[4-7] to be examined. Three possible situations arise and we now briefly examine each of these. In what follows, k_E is the second-order rate constant for epimerization, and $k_H = k_1$ or k_2 is the second-order rate constant for H-exchange.

Stepwise (S_E1) Process with a Common (Planar) Carbanion Intermediate. This will occur when proton loss and reprotonation occur as separate steps via a common intermediate. This situation is likely since carbon acidity is aided by delocalization of the developing negative charge onto the electronegative oxygen atoms of the carboxyl group.[30] Co(III) will aid this process, and the extensive crystallographic data available on amino acid chelates[31] suggest that there should be no steric constraints preventing attainment of the planar structure in the carbanion. Mechanism I then holds, with $k_H = k_1$ (or k_2) for each epimer, $k_E = (k_1k_{-2}$

mechanism I



$+ k_2k_{-1})/(k_{-1} + k_{-2})$, and $K_C = k_1k_{-2}/k_2k_{-1}$. Furthermore, k_E

(29) (a) Norman, P. R.; Phipps, D. A. *Inorg. Chim. Acta* **1978**, *28*, L161. (b) Miyanaga, A.; Sakaguchi, U.; Morimoto, Y.; Kushi, Y.; Yoneda, H. *Inorg. Chem.* **1982**, *21*, 1387. (c) Ama, T.; Kawaguchi, H.; Kanekiyo, M.; Yashi, T. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 956.

(30) Cram, D. J. *Fundamentals of Carbanion Chemistry*; Academic Press: New York, 1965; p 52.

(31) Sigel, H.; Martin, R. B. *Chem. Rev.* **1982**, *82*, 385.

is directly related to K_C, and the experimental quantities may be compared by using expressions 5 and 6.

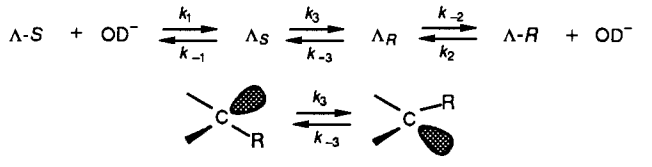
$$K_C k_2/k_1 = k_{-2}/k_{-1} \quad (5)$$

$$k_E/(k_1(1 + K_C^{-1}) - k_E) = k_{-2}/k_{-1} \quad (6)$$

Table VI (columns 6 and 7) compares such k_{-2}/k_{-1} ratios and their agreement supports the correctness of the model. However, it must be pointed out that the experimental quantities do not correspond exactly to the cyclic process required by mechanism I since H-exchange (or more correctly H-removal) was necessarily measured in D_2O, and k_{-1} and k_{-2} then represent deuteration rather than reprotonation rate constants. This is a common problem and has no effect on the final outcome provided k_{-2}/k_{-1} is independent of the reprotonating isotope. This is likely, because although D_2O is a weaker acid than H_2O (and therefore k_{-2} and k_{-1} are expected to have smaller values in D_2O), the effect should be the same for both. The tracer experiments using ^3H_2O as the reprotonating acid prove this to be so. After a short time in the presence of 0.05 M NaOH, the recovered Λ-*S*/Δ-*R* and Λ-*R*/Δ-*S* diastereomers show ^3H incorporations that directly give k_{-2}^T/k_{-1}^T. The agreement between this value and that found for k_{-2}/k_{-1} in D_2O (viz., 0.52, 0.54 (Table V) vs 0.42, 0.51 (Table VI) for the Asp complex and 1.55 (Table V) vs ~1.6 (Table VI) for the Val complex) shows this ratio to indeed be independent of the reprotonating isotope. It should also be pointed out in this context that the equilibrium constant K_C is also the same in H_2O and D_2O (Table I), and this together with the above result requires k_1/k_2 to also be independent of hydrogen isotope in the abstracting base (OH^- or OD^-).

Stepwise (S_E1) Chiral (Nonplanar) Carbanions. Previous studies on metal complexes of amino acids had suggested this situation.[4-7] It would arise if deprotonation (k_1 or k_2) resulted in separate sp^3 carbanions that had a significant lifetime before inverting or if steric factors (such as orientation of the amino acid side chain) controlled the rate of equilibration between two otherwise planar sp^2 carbanions. Mechanism II describes this situation and gives

mechanism II



$k_H = k_1$ (or k_2), $k_E = (k_1k_{-2}k_3 + k_{-1}k_2k_{-3})/(k_{-1}k_{-2} + k_{-1}k_{-3} + k_{-2}k_3)$, and $K_C = k_1k_3k_{-2}/k_{-1}k_{-3}k_2$. The experimental quantities then take the form

$$K_C k_2/k_1 = k_3k_{-2}/k_{-3}k_{-1} \quad (7)$$

and

$$k_E/(k_1(1 + K_C^{-1}) - k_E) = k_3k_{-2}/k_{-3}k_{-1} \quad (8)$$

or

$$k_E/(k_1(1 + K_C^{-1}) - k_E) = k_3/k_{-1} \quad (9)$$

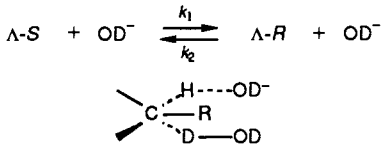
Expression 8 holds if inversion is fast compared to reprotonation ($k_3,k_{-3} \gg k_{-1},k_{-2}$), while expression 9 holds if this is not so ($k_3,k_{-3} \ll k_{-1},k_{-2}$).

The experimental data allow a clear choice. Expressions 7 and 8 are in agreement, but expressions 7 and 9 are not since $k_E/(k_1(1 + K_C^{-1}) - k_E)$ would need to be smaller than K_Ck_2/k_1 and this is not so (Table VI). Clearly, inversion within the carbanions must be fast relative to reprotonation (i.e., k_3,k_{-3} cannot be rate determining). The observed activation energies for proton loss and epimerization for the valine complex in D_2O ($\Delta H^*(H) = 66 \pm 5$ kJ mol^{-1}; $\Delta H^*(E) = 69 \pm 5$ kJ mol^{-1}) support this conclusion, since their agreement supports rate-determining proton loss.

Furthermore, if separate carbanion intermediates did exist for appreciable lengths of time but their interchange was still fast relative to reprotonation (eq 7 and 8), then the ^3H experiments would require the inversion ratio k_3/k_{-3} be unity since $k_{-2}^T/k_{-1}^T = k_{-2}/k_{-1} = k_3k_{-2}/k_{-3}k_{-1}$; that is, the two carbanion intermediates Λ_S and Λ_R would need to be equally stable. This possibility is considered unlikely in view of the significant differences in stability found for the parent (ground state) valine and aspartate complexes ($K_C = 2.0$ and 0.67, respectively).

Concerted (S_E2) Inversion and Exchange. Such a situation would arise if reprotonation accompanied proton loss without participation of a discrete carbanion intermediate. If the reprotonating acid (D_2O or H_2O) approached exclusively from the opposite face (i.e., with inversion), then mechanism III would hold

mechanism III



with $k_H = k_1$ (or k_2), $k_E = k_1 + k_2$, and $K_C = k_1/k_2$. Clearly this situation does not obtain since the data (Table VI) show that k_E is not equal to the sum $k_1 + k_2$, nor is K_C equal to k_1/k_2. If concerted reprotonation were to occur in part with retention (i.e., both front- and back-side attack), then k_E would be less than the sum $k_1 + k_2$ since k_1 and k_2 do not now measure only inversion. The observed k_E values are indeed less than the sum of $k_1 + k_2$ (Table VI), so this possibility cannot be excluded, but it is considered unlikely in view of the obvious acidity of these carbon acids. Such possibilities have been discussed in detail by Jencks.[32]

Mechanistic Conclusions. It can therefore be safely assumed that a single (planar) carbanion intermediate is formed from these complexes and that this controls the thermodynamic distribution of epimers (mechanism I). Thus the suggested noncorrespondence of deuteration and epimerization rates found by Legg and coworkers for the aspartate and glutamate complexes[5,7,8] must be in error. However, their undoubtedly correct demonstration of retention of asymmetry in tridentate aspartate complexes of Co(III)[6] presumably arises from a thermodynamically preferred direction for reprotonation. It is expected that thermodynamic control will always be maintained in these situations.

The molecular basis for this control is less easy to unravel. There have been several investigations in this area and some noteworthy demonstrations of selectivity have been found. For the systems studied here, some comment can be made. For $[Co(en)_2(Gly)]^{2+}$, where the amino acid is achiral, there is a slight preference for the electrophile (D_2O) to enter from that face resulting in the Λ-*R* epimer ($k_{-2}/k_{-1} = 0.80$). The same directional preference is found for Λ-$[Co(en)_2(Ala)]^{2+}$ ($k_{-2}/k_{-1} = 0.87$), in this case forming the Λ-*S* epimer. This selectivity presumably measures the dissymmetric environment provided by the ethylenediamine chelates, with reprotonation being slightly favored from one side of the carbanion face. An examination of molecular models shows no obvious reason why this should be so. For the glutamate and aspartate complexes this preference is increased ($k_{-2}/k_{-1} = 0.74$ and 0.4–0.50, respectively), and the increase could be attributed to H-bonding between the side chain of the amino acid and other amine functions in the manner suggested by Legg.[5-8] For the larger hydrophobic substituents of phenylalanine and valine the reverse holds, with the Λ-*R* epimer now being favored for reprotonation ($k_{-2}/k_{-1} = 1.62$ and 1.55, respectively). This could arise from steric factors and suggests that in the absence of specific interactions of the type mentioned above for aspartate and glutamate increasing size favors reprotonation from the carbanion face, giving rise to the Λ-*R* epimer.

This interpretation certainly requires further examination. But it should be pointed out that reprotonation of the carbanion produced from the amino acid ester chelate Λ-$[Co(en)_2(S\text{-AlaOMe})]^{3+}$ by $HAlaOMe^+$ in DMSO also prefers retention over inversion by a respectable margin ($k_{-2}/k_{-1} \sim 0.45$). The well-known stereoselective addition of acetaldehyde to the glycinate

(32) Jencks, W. P. *Chem. Soc. Rev.* **1981**, *10*, 345; *Acc. Chem. Res.* **1980**, *13*, 161.

carbanion to give the threonine and allothreonine chelates[33] provides a different, but related, example. In this case the electrophile adds preferentially to give the Λ-*S* epimer (16% isomer excess for threonine; 35% excess for allothreonine), whereas we have shown that D_2O deuterates preferentially from the opposite face to give the Λ-*R* epimer. The important point here is that kinetic selectivity for prochiral glycine in $[Co(en)_2(Gly)]^{2+}$ has been demonstrated without the need for a more elaborate process such as that involving prior stereoselective addition of the electrophile to an adjacent deprotonated amine center to generate a more asymmetric environment.[34] While the factors mentioned above require further examination for a range of electrophiles, the reprotonation selectivities given in Table VI provide a useful starting point.

Tritium Isotope Effect and Carbanion Stability. Finally, some comment is appropriate on the observation that the rate of ^3H incorporation into the aspartate carbanion is some 8 times slower than that for epimerization ($k^T \sim 4 \times 10^{-3}$ mol^{-1} dm^3 s^{-1} vs $k(E) = 3.0 \times 10^{-2}$ mol^{-1} dm^3 s^{-1}). Smith and Sivakau had previously found no isotope effect in reprotonating the carbanion produced from phenylglycine in phosphate buffer at elevated temperatures,[35] and they interpreted this in terms of diffusion-controlled reprotonation.[35,36] It might be inferred that this would be a general result for amino acids and peptides, and indeed we assumed this to be the case in designing our initial experiments on the epimerization of Co(III)–amino acid methyl ester complexes used in synthesizing small peptides.[37] However, others[38] have subsequently found epimerization in this system to be much faster than we expected from our tritium studies, and this suggested that the Co(III)-activated ester method was inappropriate for the retention of asymmetric centers. While proton exchange in the active ester is indeed generally faster than reaction to form the peptide bond, this can be controlled by using the correct asymmetry about the Co(III) center. We will return to this important problem when discussing our results on the peptide-forming reaction.[3]

However, it could be that the metal stabilizes the carbanion in such a way as to discriminate between potential electrophiles. This comes directly from our tritium results compared to those for the free amino acid.[35,36] Also, the metal–carbanion is under thermodynamic control so that it must at least be in equilibrium with its solvent cage. Whether it is more stable than this, and discriminates in an equilibrium sense between electrophiles from the bulk solution, is not yet certain.

Registry No. Λ-$[Co(en)_2(S\text{-Ala})]^{2+}$, 28459-63-4; Δ-$[Co(en)_2(S\text{-Ala})]^{2+}$, 28536-95-0; Ω-$[Co(en)_2(S\text{-Phe})]I_2$, 123881-50-5; Δ-$[Co(en)_2(S\text{-Phe})]I_2$, 123881-51-6; Ω-$[Co(en)_2(S\text{-Phe})](ClO_4)_2$, 123881-52-7; Δ-$[Co(en)_2(S\text{-Phe})](ClO_4)_2$, 39000-16-3; Ω-$[Co(en)_2(S\text{-Val})]I_2$, 123881-53-8; Δ-$[Co(en)_2(S\text{-Val})]I_2$, 123881-54-9; Λ-$[Co(en)_2(S\text{-Val})]Br_2$, 123881-55-0; Δ-$[Co(en)_2(S\text{-Val})]Br_2$, 123881-56-1; Λ-$[Co(en)_2(S\text{-Glu})]ClO_4$, 16040-63-4; Δ-$[Co(en)_2(S\text{-Glu})]ClO_4$, 33293-37-7; Λ-$[Co(en)_2(S\text{-Asp})]ClO_4$, 33864-49-2; Δ-$[Co(en)_2(S\text{-Asp})]ClO_4$, 33864-50-5; Δ,Λ-$[Co(en)_2(S,R\text{-AspH})]Cl_2$, 123932-07-0; Δ,Λ-$[Co(en)_2(R,S\text{-Val})]Cl_2$, 123881-57-2; Λ-$[Co(en)_2(Gly)]^{2+}$, 19657-80-8; Δ-$[Co(en)_2(Gly)]^{2+}$, 19657-79-5; H_2, 1333-74-0.

(33) Dabrowiak, J. C.; Cooke, D. W. *Inorg. Chem.* **1975**, *14*, 1305.
(34) Phipps, D. A. *Inorg. Chim. Acta* **1978**, *27*, L103.
(35) Smith, G. G.; Sivakua, T. *J. Org. Chem.* **1983**, *48*, 627.
(36) Straud, E. D.; Fife, D. J.; Smith, G. G. *J. Org. Chem.* **1983**, *48*, 5368.
(37) Clark, C. R.; Tasker, R. F.; Buckingham, D. A.; Knighton, D. R.; Holding, D. R. K.; Hancock, W. S. *J. Am. Chem. Soc.* **1981**, *103*, 7023.
(38) (a) Wautier, H.; Marchal, D.; Fastrez, J. *J. Chem. Soc., Dalton Trans.* **1981**, 2484. (b) Mensi, N.; Isied, S. S. *Inorg. Chem.* **1986**, *25*, 147.