Labilization of the 3-Carbon Hydrogens in Chelated (2*S*)-Aspartic Acid and *erythro*- and *threo*-(2*S*)-3-Methylaspartic Acid

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Abstract: Aspartic acid chelated to cobalt(III) as a tridentate ligand in *s-cis*-aspartato(triamine)cobalt(III) will selectively deuterate with retention of configuration to give 3-deuterioaspartic acid as established by proton NMR spectroscopy. The deuterated amino acid was characterized after removal from the complex. The position of deuteration was confirmed by the preparation and deuteration of the erythro and threo isomers of *s-cis*-(2*S*)-3-methylaspartato(diethylenetriamine)cobalt(III). The deuterium exchange studies revealed that the labile proton is the erythro proton. This study demonstrates that alkyl protons adjacent only to a ligated carboxylate can be labilized and supports previous studies which suggest that ring flexibility is an important factor in determining lability.

It has been known for some time that metal ions increase the reactivity of the 2-methylene hydrogens of amino acids.[2] Williams and Busch were the first to demonstrate the magnitude and specificity of the effect through their proton NMR study of the deuteration of Co(III)-chelated glycine, alanine, and EDTA.[2b] The lability of protons at the 2 carbon of certain amino acids and peptides was subsequently demonstrated for a series of glycine-like Co(III) chelates.[3] The methylene protons of malonate will also exchange with deuterium when the malonate is chelated to Co(III).[3d,4] In each case deuterium exchange occurs at an alkyl group located between two electron withdrawing groups. The proposed mechanism of deuterium exchange in basic solution involves abstraction of the proton by OD^- to form a carbanion intermediate which is stabilized by resonance to the enolate form. The intermediate is attacked by D_2O and OD^- is regenerated.[3b,c] Although a racemic product would be expected in the deuteration of a chelated amino acid, due to the planar enolate intermediate, kinetic stereoselectivity has been demonstrated in the preparation of 2-deuterioaspartic acid from aspartatobis(ethylenediamine)cobalt(III).[5]

2-Deuterio amino acids are usually prepared enzymatically. For example, glutamate-oxaloacetate transaminase can be used to prepare 2-deuterioaspartic acid.[6] It is also possible to prepare enzymatically the erythro[7] isomer of 3-deuterioaspartic acid. Methyl aspartate ammonia-lyase will produce *erythro*-(2*S*)-3-deuterioaspartic acid by adding ammonia to fumarate in D_2O.[8] In this study we have demonstrated that *erythro*-(2*S*)-3-deuterioaspartic acid can also be prepared from Co(III) complexes in which aspartic acid forms a tridentate chelate.

Experimental Section

Materials. (*S*)-Aspartic acid was purchased from Aldrich Chemical Co., 3-methylaspartic acid from Sigma Chemical Corporation, diethylenetriamine from Matheson Coleman and Bell, and acid washed blood charcoal from Eimer and Amend. Aspartato(1,*cis*-3,*cis*-5-triaminocyclohexane)cobalt(III) was kindly provided by Dr. W. T. Jordan,[9] and *s-cis*-aspartato(diethylenetriamine)cobalt(III) was prepared according to the method of Legg and Cooke.[10] The purity of the complexes was determined by comparison of the $\Delta\epsilon$ from circular dichroism spectra to the known values.

Dowex 50W-X8 cation exchange resin (100–200 mesh, hydrogen form) was purchased from Bio-Rad Laboratories and Sephadex G-10 gel for desalting was purchased from Pharmacia Fine Chemicals Incorporated.

Separation of 3-Methylaspartic Acid into *erythro*- and *threo*-3-Methylaspartic Acid. The commercial preparation of 3-methylaspartic acid was racemic at both the 2 and 3 carbons. The erythro isomers of 3-methylaspartic acid are much more soluble than the threo isomers,[12] and the two sets of isomers were separated by stirring 25 g of 3-methylaspartic acid in 250 ml of water for 1 h. The insoluble threo isomers were recovered by filtration. The filtrate was evaporated to dryness to recover the erythro isomers. Analysis by proton NMR spectroscopy showed the isomers to be 90–95% pure.

Resolution of *erythro*- and *threo*-3-Methylaspartic Acid. The (2*S*)-optical isomers of both the erythro and threo isomer pairs were isolated by selective hydrolysis of the (*S*)-*N*-acetyl over the (*R*)-*N*-acetyl amino acid by hog kidney acylase. The general method of Greenstein et al.[13-15] was employed for both the erythro and threo isomers. The *N*-acetyl amino acid was prepared by the method of Barker.[16] Hog kidney acylase II was isolated according to the method of Greenstein[14,15] from the kidneys of freshly slaughtered hogs. The crude *threo*-(2*S*)-3-methylaspartic acid prepared in this manner was recrystallized from boiling water (5.3 g/200 ml), and the crystals were collected in three batches. The purity was checked by the $[\alpha]_D$ rotation. A 1% solution of the amino acid in 5 N HCl showed $[\alpha]_D$ optical rotations of +12.1° (fraction 1, 2.6 g), +11.8° (fraction 2, 0.8 g), and +9.16° (fraction 3, 0.9 g) as compared to a reported value of +13.4°.[15] Due to the high solubility of the erythro isomer, isolation of pure *erythro*-(2*S*)-3-methylaspartic acid proved difficult. The $[\alpha]_D$ optical rotation of a 1% solution in 3 N HCl was +32° as compared to a reported value of 42.5 ± 4°.[12] The proton NMR spectrum of the erythro isomer indicated the presence of ca. 5% *N*-acetyl amino acid.

Synthesis of *s-cis-erythro*-(2*S*)-3-Methylaspartato(diethylenetriamine)cobalt(III) Perchlorate. It was found that the desired product could be obtained only if the reaction mixture was kept oxygen free until all the necessary components had been added. Water (100 ml) was boiled in a 500-ml Erlenmeyer to drive off any dissolved oxygen and then cooled to ca. 90°. The water was stirred while freshly prepared $CoCO_3$[17] (0.81 g, 0.007 mol) and *erythro*-(2*S*)-3-MeAsp (1.1 g, 0.007 mol) were added. The flask was stoppered with a connecting rubber tube having its free end under water to allow the CO_2 to escape. The mixture was kept hot to prevent water from being drawn in and was stirred until evolution of CO_2 ceased (ca. 5 min). Concentrated hydrochloric acid (0.55 ml, 0.007 mol) was then added, the stopper replaced, and stirring continued with heating for an additional 5 min. The rubber tubing was then clamped, and the solution was cooled to room temperature. Diethylenetriamine (0.7 g, 0.007 mol), acid washed blood charcoal (0.8 g), and 30% H_2O_2 (3.5 ml) were then added, and the mixture was stirred exposed to the atmosphere for 12 h. The charcoal was filtered off through filter paper and washed with water until colorless washings were obtained. The filtrate and washings were combined and diluted to 2 l.

This solution was charged into a column (78.5 × 2.6 cm) of Dowex 50W-X8 resin (100–200 mesh, Na^+ form) at a rate of 1 drop/2 s. After washing the column with water to remove neutral and negatively charged species, the column was eluted with 0.05 M $NaClO_4$ at a rate of 1 drop/5 s for a period of 30 days. At this point the orange band due to the s-cis isomer was near the bottom and the two red bands (corresponding to the uns-cis isomers) had split. Elution was then continued with 0.5 M $NaClO_4$ at a rate of 1 drop/20–30 s. The s-cis and uns-cis isomers came off after 1 and 10 days, respectively.

The solution containing the s-cis isomer was collected in one fraction

Table I. Proton NMR and Circular Dichroism Data of the Complexes Investigated

	3-Me	2-H	$\bar{\gamma}_1{}^b$ ($\Delta\epsilon_1$)	$\bar{\gamma}_2$ ($\Delta\epsilon_2$)	$\bar{\gamma}_3$ ($\Delta\epsilon_3$)
s-cis-Co(dien)(*erythro*-(2*S*)-3-MeAsp)$^+$	1.47	3.80 (2 Hz)[a]	17.4 (−0.06)	20.3 (0.78)	27.6 (0.22)
s-cis-Co(dien)(*threo*-(2*S*)-3-MeAsp)$^+$	1.23	3.84 (5 Hz)[a]	17.5 (−0.09)	20.2 (0.61)	27.6 (0.17)
s-cis-Co(dien)((2*S*)-Asp)$^+$	—	4.05	—	20.4 (0.75)	27.7 (0.15)
Co(Tach)((2*S*)-Asp)$^+$	—	3.85	—	19.7 (0.35)	27.8 (0.13)

[a] 2-H, 3-H coupling constant. [b] $\bar{\gamma}$ in cm^{-1} × 10^{-3}.

and evaporated with removal of solid $NaClO_4$ as it formed. After the removal of most of the salt in this manner, the remainder of the $NaClO_4$ was removed by passing the solution through a Sephadex G-10 gel column (40 × 1.5 cm) at ca. 0.5 ml/min. The solution was evaporated to dryness, and the s-cis isomer was recrystallized by dissolution in a minimum amount of water followed by evaporation to a small volume in an air stream. After the fourth recrystallization no change in $\Delta\epsilon$ was noted. Yield 0.075 g. Anal. Calcd for $Co(C_9H_{20}N_4O_4)ClO_4{\cdot}H_2O$: C, 25.47; H, 5.22; N, 13.19. Found: C, 25.30; H, 5.06; N, 13.03.

Synthesis of *s-cis-threo*-(2*S*)-3-Methylaspartato(diethylenetriamine)cobalt(III) Perchlorate. A variation of the method of Legg and Cooke was employed.[10] A 50 ml solution of $Co(dien)Cl_3$ (1.82 g, 0.0068 mol) was heated to 40°, blood charcoal (1 g) was added, and the solution heated to 50–55°. *threo*-(2*S*)-3-MeAsp (1 g, 0.0068 mol) was added, followed by sodium hydroxide (0.55 g, 0.014 mol) and $AgNO_3$ (3.62 g, 0.021 mol). The solution was stirred for 20 min without heating as the temperature slowly dropped to 43°. The mixture was cooled to room temperature (1 h) and then was filtered through Celite filter aid on filter paper. The filtered solid was washed with water until the washings were colorless.

The filtrate and washings were diluted in 600 ml and chromatographed as described for the separation of the erythro isomers. The threo s-cis isomer was isolated and purified in the manner described for the erythro s-cis isomer. Anal. Calcd for $Co(C_9H_{20}N_4O_4)ClO_4{\cdot}H_2O$: C, 25.47; H, 5.22; N, 13.19. Found: C, 25.32; H, 5.04; N, 12.86. The uns-cis isomers of both the threo and erythro amino acid were also isolated[18] but are not reported here since they were not employed in the deuteration studies.

Deuterium Exchange Studies. (a) Co(Tach)(Asp)$^+$. D_2O solutions of Co(Tach)(Asp)$^+$ (0.5 M) adjusted to pD's of 9.4 and 10.4 with $NaHCO_3$ and Na_2CO_3 were heated at 40 ± 1°. The deuterium exchange was monitored by proton NMR and CD spectroscopy.

(b) *s-cis*-Co(dien)(Asp)$^+$. The complex was recrystallized from D_2O to replace the H_2O of crystallization[10] with D_2O. The solid complex was dissolved in a minimum amount of hot D_2O, ethanol was added slowly with stirring until a slight cloudiness persisted, and the solution was allowed to cool to room temperature. The complex was removed by filtration and air dried. Between 0.62 and 1.8 g of complex was dissolved in sufficient D_2O to make 0.25 M solutions. The solution was buffered with Na_2CO_3 (0.1 M) and $NaHCO_3$ (0.1 M) to give a pD of 10.0 and heated at 40 ± 1°. The deuterium exchange was monitored by NMR and CD spectroscopy. After 5 days the reaction mixture was applied to a strong acid ion-exchange column (1.7 × 1 cm) containing Dowex 50W-X8 (100–200 mesh, Na^+ form). A red-violet substance was eluted with water. The red-orange band which remained was eluted with 0.35 M NaCl. The band was collected, desalted by passing the solution through a Sephadex G-10 column (40 × 4.5 cm), and identified as *s-cis*-Co(dien)(3-D-asp)$^+$ by CD and NMR spectroscopy. The average yield of Co(dien)(3-D-asp)$^+$ was 58%. The CD spectrum of the red-violet substance was similar to aspartatotetra(ammine)cobalt(III) where aspartate functions as a bidentate ligand.[20]

(c) *s-cis*-Co(dien)(3-MeAsp)$^+$. Saturated solutions of the erythro and threo isomers of *s-cis*-Co(dien)(3-MeAsp)$^+$ in D_2O were adjusted to a pD of 10 with Na_2CO_3 (0.1 M) and $NaHCO_3$ (0.1 M) or a pD of 11 with Na_2CO_3 and heated at 40 ± 1°. The deuterium exchange was monitored by NMR and CD spectroscopy.

Recovery of *erythro*-(2*S*)-3-Deuterioaspartic Acid. 3-D-Asp was recovered from *s-cis*-Co(dien)(3-D-Asp)$^+$ by reduction of the complex with $NaBH_4$.[5] Quantitative amino acid analysis (Beckman 121 C automatic amino acid analyzer) showed that 27% of the aspartic acid was recovered based on the amount of *s-cis*-Co(dien)(Asp)$^+$ used. Comparison of the ORD curve of the recovered aspartic acid and a standard sample of (*S*)-aspartic acid showed the recovered 3-deuterioaspartic acid to be the pure *S* isomer.

Physical Measurements. Proton NMR spectra were recorded on a Varian A-60 spectrometer in D_2O with sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an external reference. Optical rotatory dispersion and circular dichroism spectra were recorded on a Jasco Model ORD/UV-5 with CD attachment. Spectra of the deuterium exchange samples taken directly from the NMR tubes were recorded with a path length of 0.5 mm.

Results

Preparation of Compounds. Although *erythro*-(2*S*)-3-MeAsp isolated from the enzymatic resolution contained about 5% of the *N*-acetyl derivative, no further attempts were made to purify the amino acid since the *N*-acetyl amino acid was not expected to coordinate to the metal ion. The general method of Legg and Cooke[10] was employed to synthesize the [Co(dien or Tach)(amino acid)]$^+$ complexes with the exception of the *erythro*-3-Me-(*S*)-Asp complex where a new method involving direct synthesis from Co(II) and the ligand components was employed. Pertinent NMR and CD data for the complexes studied are presented in Table I. The structures of the isomers as assigned on the basis of their CD spectra[9,10,18] are shown in Figure 1. As expected the CD spectra of all s-cis isomers were similar to each other and distinct from the spectra of the uns-cis isomers.

Deuteration of Tridentate Aspartic Acid Cobalt(III) Chelates. Deuterium exchange on chelated aspartic acid was studied in the complexes Co(Tach)(Asp)$^+$ and *s-cis*-Co(dien)(Asp)$^+$ under basic conditions. The deuteration was monitored by proton NMR and CD spectroscopy. Figures 2 and 3 illustrate the changes in the NMR and CD spectra during the deuteration of Co(Tach)(Asp)$^+$ at a pD of 9.4 and 40°. Aspartic acid exhibits an ABX pattern in the NMR spectrum, Figure 2, for the methylene protons and methyne proton (Figure 1). The methylene resonances at ca. 2.8 ppm are partially obscured by the Tach backbone protons, but the methyne resonance is visible at 3.85 ppm. Over a period of 23 days the methylene resonances decrease in intensity and the methyne quartet is replaced by a doublet of approximately equal intensity. No further changes were observed for 9 days, at which time the pD was raised to 10.2. The methyne doublet collapsed and the intensity decreased to zero after 21 days. When the deuteration was repeated at an initial pD of 10.4 the methyne doublet formed in 11 days.

The CD spectrum, Figure 3, also changed during the deuteration. The CD spectrum of *s-cis*-Co(Tach)(Asp)$^+$ is essentially generated by the chelated (*S*)-aspartic acid.[19] Therefore any changes in the spectrum will reflect changes in the coordination or optical purity of the aspartic acid. During the period of time corresponding to the formation of the methyne doublet in the NMR, the CD maximum decreases and shifts slightly to higher wavelengths. After the pD of the solution was raised, the spectrum changed rapidly until it re-

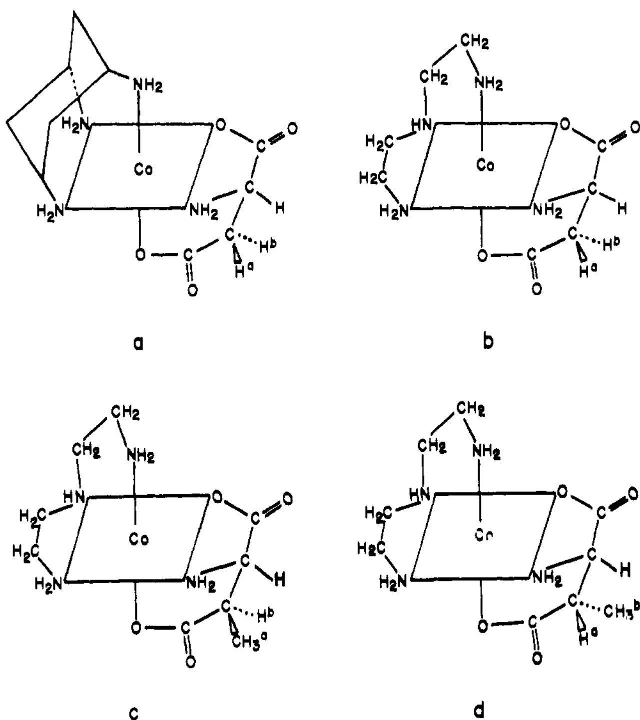

Figure 1. Stereochemistry of complexes investigated: (a) (*S*)-aspartato-(*cis,cis*-1,3,5-triaminocyclohexane)cobalt(III), (b) *s-cis*-(*S*)-aspartato(diethylenetriamine)cobalt(III), (c) *s-cis-erythro*-(2*S*)-3-methylaspartato(diethylenetriamine)cobalt(III), (d) *s-cis-threo*-(2*S*)-3-methylaspartato(diethylenetriamine)cobalt(III).

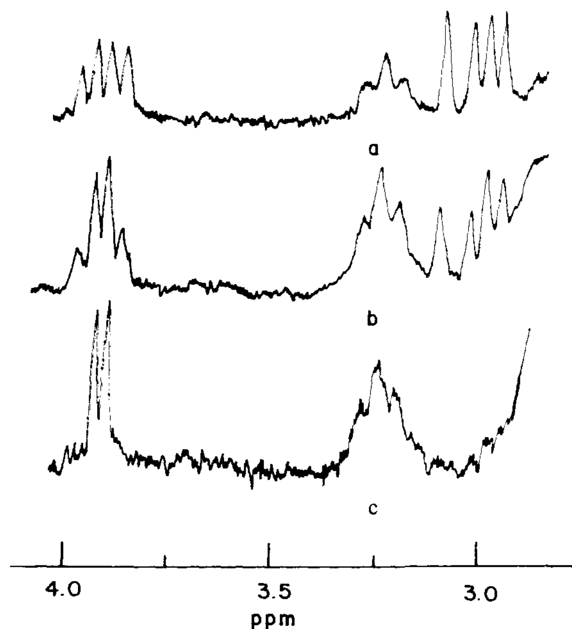

Figure 2. Proton NMR spectra of (*S*)-aspartato(*cis,cis*-1,3,5-triaminocyclohexane)cobalt(III) during deuterium exchange at a pD of 9.4 and at 40° (0.5 M in D_2O): (a) initial spectrum, (b) 7 days, (c) 23 days.

sembled the spectrum of aspartatotetra(amine)cobalt(III), where aspartic acid is chelated as a bidentate ligand.[3e,20]

During the deuteration of aspartic acid in Co(dien)(Asp)$^+$ analogous changes in both the NMR and CD spectra are observed, except the methylene resonances in the NMR spectra are completely obscured by the dien backbone protons. At a pD of 10 the methyne doublet replaces the quartet in 5 days. The coupling constant for the 2-methyne doublet was 2 Hz in

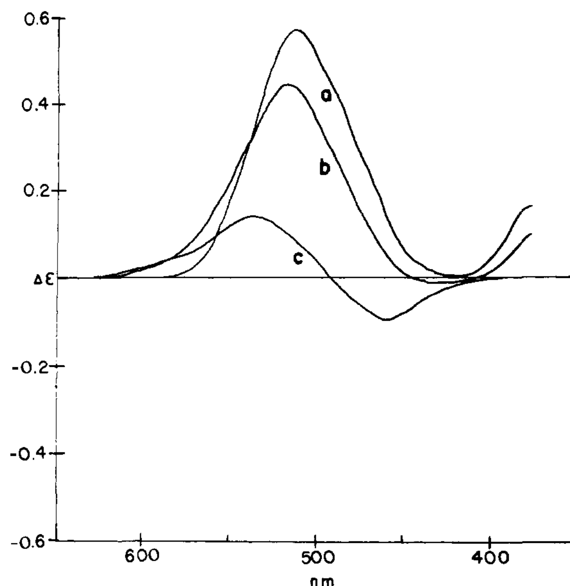

Figure 3. CD spectra of (*S*)-aspartato(*cis,cis*-1,3,5-triaminocyclohexane)cobalt(III) during deuterium exchange at 40° (0.5 M in D_2O): (a) initial spectrum (pD 9.4), (b) 23 days (pD 9.4), (c) 53 days (pD raised to 10.2 after 32 days).

both the deuterated Co(Tach)(Asp)$^+$ and *s-cis*-Co(dien)(Asp)$^+$.

Isolation of the Deuterated Aspartic Acid from *s-cis*-Co(dien)(Asp)$^+$. The complex Co(dien)(Asp)$^+$ was chosen for the preparation of deuterated aspartic acid because of the availability in large quantities of the dien ligand and the ease with which large yields of *s-cis*-Co(dien)(Asp)$^+$ can be obtained. *s-cis*-Co(dien)(Asp)$^+$ was heated (40°) at a pD of 10 for 5 days. The complex was separated from the red-violet side product by ion-exchange chromatography and the aspartic acid was removed from the complex by $NaBH_4$ treatment.[5] Comparison of the ORD of aspartic acid isolated from *s-cis*-Co(dien)(Asp)$^+$ to (*S*)-aspartic acid of known purity showed retention of the *S* configuration. The NMR spectrum of the deuterated aspartic acid is compared to nondeuterated aspartic acid in Figure 4.

Deuteration of the Erythro and Threo Isomers of a Tridentate 3-Methylaspartic Acid Cobalt(III) Chelate. *s-cis*-Co(dien)(*erythro*-(2*S*)-3-MeAsp)$^+$ and *s-cis*-Co(dien)(*threo*-(2*S*)-3-MeASp)$^+$ were heated at pD's of 10 and 11 and a temperature of 40 °C, and the deuterium exchange was monitored by proton NMR spectroscopy. The region of interest is illustrated in Figure 5. The 3-methyl protons and the 2-methyne proton are split into doublets by the 3-methyne proton (see Figure 1). After 1 day at a pD of 11 the doublets in the threo complex collapsed to singlets, whereas no change occurred in the erythro complex. The same result was observed at pD 10 in 15 days. The 2-methyne proton eventually deuterates in both isomers, but the 2-methyne proton exchanges with deuterium before the 3-methyne proton in the erythro isomer.[18]

Discussion

Tridentate aspartic acid chelates of Co(III) have a labile proton at the 3 carbon and undergo deuterium exchange in basic solutions as illustrated by the decrease in the intensity of the 3-methylene resonances of Co(Tach)(Asp)$^+$, shown in Figure 2. Although the resonance is not visible, one of the 3-methylene protons is not exchanging with deuterium and is causing the 2-methyne proton to be split into a doublet. This is confirmed by the isolation of the deuterated aspartic acid. The spectrum of deuterated aspartic acid isolated from Co-

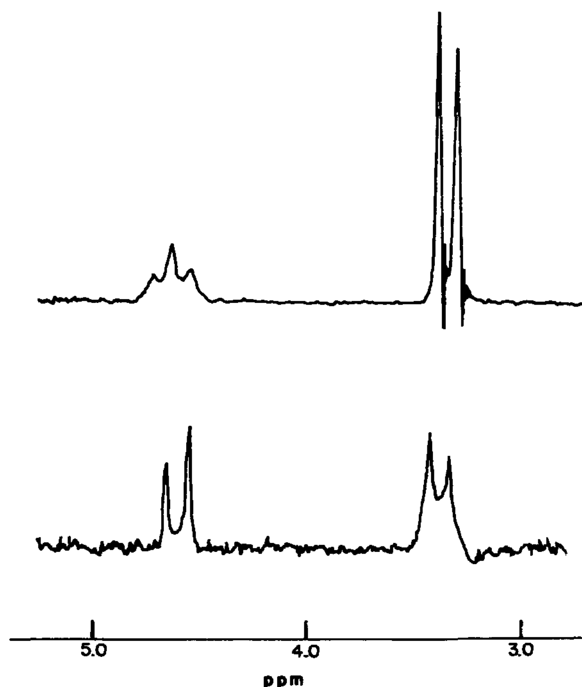

Figure 4. Proton NMR spectrum of *erythro*-3-deuterioaspartic acid (lower) and undeuterated aspartic acid (upper).

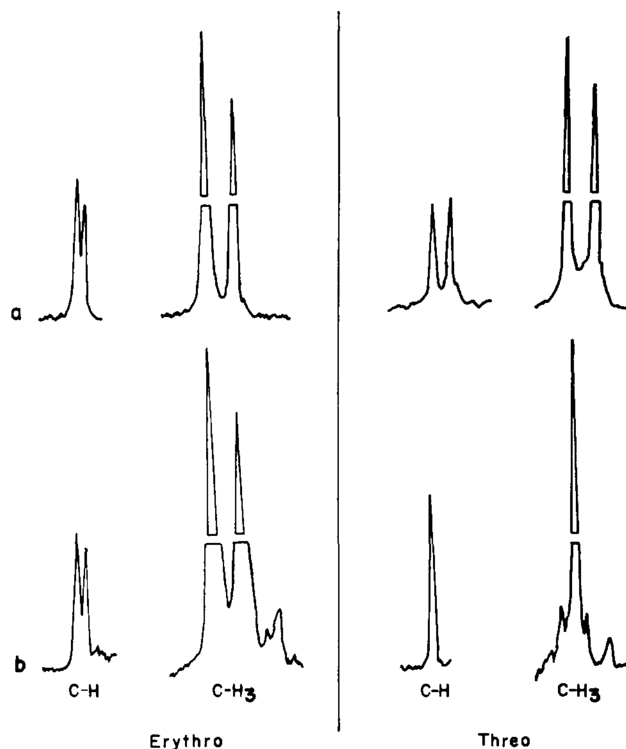

Figure 5. Proton NMR of *erythro*- and *threo*-(2*S*)-3-methylaspartato-(diethylenetriamine)cobalt(III) during deuterium exchange at a pD of 11 and a temperature of 40 °C (ca. 50 mg/0.5 ml of D_2O): (a) initial spectra, (b) 1 day.

(dien)(Asp)$^+$, Figure 4, shows a typical AX pattern arising from the protons on the 2 and 3 carbons. The doublet at ca. 3.2 ppm, corresponding to the remaining 3-methylene proton is broadened somewhat probably due to coupling with the deuterium located on the same carbon. This may also explain, in part, the fact that the doublet is not observed in the spectrum of deuterated Co(Tach)(Asp), although it is more likely that the center of that resonance is accidentally coincident with the Tach backbone protons.

In order to determine which of the 3-methylene protons of chelated (*S*)-aspartic acid is labile and exchanging with deuterium, the lability of *s-cis*-Co(dien)(*erythro*- and *threo*-(2*S*)-3-MeAsp)$^+$ toward deuterium exchange was investigated. The erythro isomer has a proton in position b, Figure 1, and the threo isomer has a proton in position a.[21] Rapid deuteration is observed for the threo isomer as evidenced by the collapse of the 3-methyl doublet and the 2-methyne doublet, Figure 5. That the proton in position a in (*S*)-aspartic acid (Figure 1) also rapidly exchanges with deuterium in the complexes Co(Tach)((2*S*)-Asp)$^+$ and *s-cis*-Co(dien)((2*S*)-Asp)$^+$, can be shown by a comparison of vicinal coupling constants. The observed splitting for *s-cis*-Co(dien)-(*erythro*-(2*S*)-3-MeAsp)$^+$ where a methyl group is in position a is the same as deuterated aspartic acid in the Co(Tach)-(Asp)$^+$ and *s-cis*-Co(dien)(Asp)$^+$ complexes where a deuterium is in position a.

Examination of models of tridentate aspartic acid complexes reveals a significant difference in the environments of a proton in position a and b, Figure 1. A proton in position b is shielded from the solution by the two carboxylate groups of the aspartic acid, whereas a proton in position a is exposed. It seems reasonable, therefore, to expect proton a to be more labile than proton b.

The ability of the chelated ring to assume a planar intermediate appears to be an important factor in determining which proton is labilized. The fact that the 2-methyne proton is not labile in tridentate aspartic acid chelates may be attributed to the loss of flexibility in the glycine-like chelate ring upon coordination of the side chain. Models show that a planar enolate intermediate for the glycine-like ring cannot be achieved easily. Similar results have been reported for the selective deuteration of acetate ring methylene protons in EDDA and EDTA-type cobalt(III) complexes. Deuterium exchange will not occur in the strained rings in the plane formed by the cobalt and two nitrogens.[2b,3a,c,d] However, the six-member ring is sufficiently flexible to accomodate a planar intermediate necessary for the exchange of a 3-methylene proton with deuterium.

It is interesting to note that the 2-methyne proton will exchange with deuterium after extended incubation at a high pH. This deuteration occurs concurrent with changes in the CD spectrum, Figure 3. The CD spectrum which results is very similar to that observed for $Co(NH_3)_4$((*S*)-Asp)$^+$ where Asp is chelated as a bidentate through the glycinate ring[3d,20] except that, as expected, the bands are shifted to lower energy due to the weaker ligand field (N_4O_2 as compared to N_5O for the tetraammine). It is probable that the six-member ring of aspartic acid is dissociating and therefore increasing the flexibility of the five-member chelate ring.

Previous studies have shown that alkyl protons are labilized only when located between two electron withdrawing groups.[2-5] In this study we have demonstrated that an alkyl proton adjacent to only one electron withdrawing group, a carboxylate, can be labilized. We are currently attempting to establish to what extent this observation can be generalized.

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(19) The dissymmetry of the s-cis isomer is due to the presence of Asp only. That is, if Asp were replaced by a nondissymmetric tridentate ligand such as iminodiacetate, the complex would possess a plane of symmetry (see ref 10).
(20) Y. Kohima and M. Shibata, *Inorg. Chem.*, **12**, 1009 (1973).
(21) The absolute configuration assignments to the two isomers of (2*S*)-3-MeAsp (ref 8 and 12) were confirmed by comparing the 2-H, 3-H coupling constants observed for the two *s-cis*-Co(dien)((2*S*)-3-MeAsp) isomers, Table I. Examination of molecular models of the two isomers, c and d in Figure 1, shows an H-C^1-C^2-H dihedral angle of 70–80° in isomer c and 40–50° in isomer d. The Karplus vicinal coupling constant relationship (*J. Am. Chem. Soc.*, **85**, 2870 (1963); *J. Phys. Chem.*, **30**, 11 (1959)) would then predict a larger 2-H, 3-H coupling constant for isomer d than for isomer c. These are therefore assigned erythro and threo, respectively, Table I, in agreement with assignments made for the free ligand (ref 8 and 12).

Rate-Determining Processes in the Hydrolysis of Maleanilinic Acids in Acidic Solutions[1]

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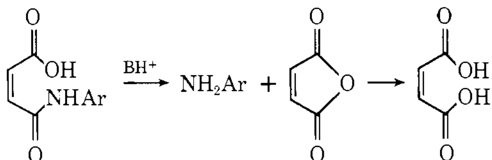
Abstract: Aryl-substituted maleanilinic acids hydrolyze via intramolecular participation of the carboxyl group. In dilute acid, the rate of hydrolysis increases with acidity but in concentrated acid ($H_0 > 3$), the rate decreases with acidity. In dilute acid, the rates of hydrolysis parallel the basicity of the leaving group, while in concentrated acid the order of reactivity is reversed. Quantitative evaluation of these observations permits the establishment of a set of rate equations which can explain these results. These equations can be related to a detailed mechanism describing the behavior of these compounds in acidic solutions in general. The observations are accommodated by equations incorporating these features: (1) specific acid-general base catalyzed decomposition of intermediates, (2) acidity of protonated substrates and intermediates, (3) activity of water in the reaction solutions.

Amides adjacent to undissociated carboxylic acids undergo extremely rapid hydrolysis in comparison to unsubstituted amides. Evidence has been accumulated that indicates this facilitation is due to nucleophilic catalysis by the carboxylic acid.[3-14] Reaction proceeds via an addition intermediate from which the amine is expelled, producing a cyclic anhydride as the second product. This route circumvents the high barrier to hydration of the amide.[15] The reaction is of particular interest because it can be compared with catalytic routes provided by many proteolytic enzymes since the enzymes also function by a nonhydrated addition intermediate.[16,17]

It has previously been shown that the hydrolysis of maleanilinic acids is general acid catalyzed.[14] By evaluating the behavior of these compounds in strongly acidic solutions,



we could expect protonation of the substrate and intermediate might lead to a change in observed kinetic behavior in which C–N bond strength (rather than a combination of basicity and bond strength[14]) would become the rate-controlling feature. We have observed that in strong acid the rate of hydrolysis of maleanilinic acids does depend directly on C–N bond strength, and as a result of effects related to a decrease in water activity, observed rates *decrease* with increasing acid strength. Rate equations can be derived which suggest that the breakdown of a protonated cyclic intermediate is concerted with loss of its hydroxylic proton in the rate-determining process in strong acid and in dilute acid. The implications of these results can be extended to other reactions involving nucleophilic assistance of amide hydrolysis (including enzymatic cases).

Experimental Section

Materials. Aryl-substituted maleanilinic acids were prepared and purified according to published procedures.[14] Reagent grade sulfuric acid was obtained from Corco Chemical Corp.

Kinetic Methods. Sulfuric acid solutions were prepared on a molarity basis (up to 10 M) and were standardized against sodium hydroxide. For sulfuric acid concentrations above 10 M, percentage-weight was used. The reagent was determined to contain 96.4% sulfuric acid. For more dilute solutions, a measured weight of the reagent acid was diluted with measured weight of distilled water. Values of H_0 and water activities were taken from literature compilations.[18,19]

The hydrolysis of maleanilinic acid was followed by monitoring the decrease in absorbance of the amide band in the uv. Wavelengths used are: 4′-nitromaleanilinic acid, 320 nm; 3′-nitromaleanilinic acid, 270 nm; 4′-chloromaleanilinic acid, 280 nm; maleanilinic acid, 275 nm; 4′-methoxymaleanilinic acid, 300 nm. Temperature was maintained at 30.0 ± 0.1 °C in the sample compartment of a Unicam SP 1800A