the α-keto group to generate the carbinolamine, and an even faster base-catalyzed elimination of water to give the imine.

The rate constants are in the vicinity of 10^6 M^{-1} s^{-1} at 25 °C and almost every deprotonation of the coordination ammonia leads to capture of the carbonyl group. Once the imine is formed, it is extremely stable even to strong acid, and that arises because Co(III) occupies the site for protonation to yield the iminium ion and thence prevents decomposition.

Cyclization of the imine complex to the pyrroline also occurs almost quantitatively.

The limiting rate constant (0.93 s^{-1} at 25 °C) indicates that the deprotonated form of the imine reacts rapidly with the brominated side chain by a nucleophilic intramolecular path displacing the bromide ion (Scheme II). A comparable intramolecular organic cyclization to produce a five-membered ring such as that of Br $CH_2CH_2CH_2CH_2NH_2$ (0.5 s^{-1} in H_2O at 25 °C)[23] shows much the same rate behavior for an amine of comparable basicity to the present example. In this respect the chemistry is not surprising.

The influence of the coordinated Co(III) ion in increasing the acidity of the imine proton is critical, however. The imine deprotonates at a much lower pH than the coordinated ammonias ($pK_a \sim 16$),[22] which makes the latter a less effective competitor for the nucleophilic reaction on a concentration basis. Other experiments where deprotonated imine and deprotonated ammonia can be compared indicate they are roughly equally good nucleophilic reagents despite the basicity difference.[24]

Finally, reduction of the pyrroline to the proline is also a facile and high-yield process. The use of $B_3H_4^-$ or $B^2H_4^-$ would regiospecifically label the proline methine hydrogen.

Exchange with labeled water at earlier stages in the synthesis would also allow labeling of the β protons. Opportunities for labeling the C atoms are also evident from the earlier discussion.

There are possibilities for extending the synthetic strategy to dehydropiperidine and azetine-2-carboxylic acids as well as substituted cyclopropyl and prolyl amino acids, but these avenues have not yet been explored.

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Cobalt(III)-Promoted Synthesis of β-Carboxyaspartic Acid. Intramolecular Addition of Coordinated Amide Ion to the Olefin Center in the (3,3-Bis(ethoxycarbonyl)-2-propenoato)pentaamminecobalt(III) Ion[1]

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Abstract: The condensation of $[(NH_3)_5CoO_2CCHO]^{2+}$ ion (**1**) with $CH^-(COOEt)_2$ in Me_2SO rapidly produces a β-substituted malate ester complex (**2**) which in turn is readily dehydrated to the $[(NH_3)_5CoO_2CCH{=}C(COOEt)_2]^{2+}$ ion (**3**). In aqueous base, an intramolecular addition of a *cis*-aminate ion at the olefin center yields the N,O-chelated diester of β-carboxyaspartic acid (Asa) (**4**). Alternatively, liquid NH_3 adds to the olefin to produce the Asa ester bound monodentate $[(NH_3)_5CoO_2CCH(NH_3)CH(COOEt)_2]^{3+}$ ion (**5**). Asa is recovered from **4** or **5** as the Ca salt by reduction of Co(III) followed by recovery and hydrolysis of the diethyl ester. The resolution of **4** is also described. The kinetics and mechanisms of the cyclization of **3** → **4** and of the ester hydrolysis of **4** are discussed. The latter reaction appears to proceed through an intermediate tetrahedral *gem*-diol species from which the rates of solvent exchange and hydrolysis are comparable.

γ-Carboxyglutamic acid (Gla) was discovered as a constituent of prothrombin in 1974.[2] It has since been found in a number of other proteins involved in the blood clotting process[3] and in small proteins which have been isolated from calcified tissues, including bone,[4] teeth and coral.[5] It is entirely possible that the aspartic acid analogue β-carboxyaspartic acid (Asa) may also be a constituent of mineralized tissue.[5,6] Alkaline hydrolysates of the EDTA-extracted proteins of both human teeth and hermatypic corals contain highly acidic peptide(s) that after treatment with strong acid yield largely aspartic and glutamic acids. The possibility that these peptides are rich in Asa and Gla, and therefore resistant to alkaline hydrolysis, is being investigated.

Confirmation of the identity of the unidentified material with Asa required synthesis of authentic samples of this amino acid. Since its preparation had not been previously reported, and since regular organic syntheses proved intractable,[5,7] we sought to make use of chemistry organized about a Co(III) center to prepare Asa

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(7) Since this project was commenced, it has been reported that dimethyl malonate ion condenses with ethyl cyanoformate in the presence of Zn(II) to produce ethyl 2-amino-3,3-bis(methoxycarbonyl)propenoate [Iimori, T.; Nii, Y.; Izawa, T.; Kobayashi, S.; Ohno, M. *Tetrahedron Lett.* **1979**, 2525]. We have attempted unsuccessfully to reduce the corresponding triethyl ester, similarly prepared, to the triethyl ester of Asa using $NaBH_3CN$ [Hutchins, R. O.; Rotstein, D.; Natale, N.; Fanelli, J. *J. Org. Chem.* **1976**, *41*, 3328].

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as a ligand in a complex of this metal ion. The syntheses described in this paper provide simple high-yielding routes to the pentaammine- and tetraamminecobalt(III) complexes of the diethyl ester of Asa, from which the free amino acid may be readily recovered.

Subsequent to our first report,[1] the synthesis of β-carboxyaspartic acid by regular organic routes[8,9] and the kinetics of its decarboxylation in acid to produce aspartic acid[10,11] have been reported. It has also been shown, based on a comparison of properties of isolated material with those of authentic Asa, that β-carboxyaspartic acid is a constituent of the ribosomal proteins of *Escherichia coli*.

Experimental Section

Visible (ϵ_λ^{max}, M^{-1} cm^{-1}) and rotatory dispersion ($[M]_\lambda$, deg M^{-1} m^{-1} at 25 °C) spectra were recorded in duplicate on a Cary 14 spectrophotometer and a Perkin-Elmer P22 spectropolarimeter, respectively. NMR spectra were measured with JEOL "Minimar" MH 100 (^1H) and FX-60 (^{13}C, proton decoupled) instruments using solutions in DCl/D_2O or Me_2SO-d_6, with sodium 4,4-dimethyl-4-silapentanesulfonate (DSS) for ^1H spectra and 1,4-dioxane for ^{13}C spectra as internal references unless otherwise specified. Reported chemical shifts (δ, ppm) are relative to these references; downfield shifts are positive. Measurements were made at ~25 °C (^{13}C) or ~30 °C (^1H) unless specified otherwise. For some ^{13}C experiments for which HCl or CF_3SO_3H/H_2O was used as solvent, an external deuterium lock was used.

Measurements of pH were made under nitrogen at 25 °C on a Radiometer PHM 26 meter and G202B glass or GK2401B combination glass electrode standardized at two pH values as described by Bates.[12] All evaporations were carried out at reduced pressure on Büchi rotary evaporators, such that the temperature of the solutions did not exceed 25 °C.

Syntheses. Hydrated (Glyoxylato)pentaamminecobalt(III) (1) Perchlorate. $[(NH_3)_5CoO_2CCH(OH)_2](ClO_4)_2$ was prepared from $[(NH_3)_5CoOH_2](ClO_4)_3$ (80 g) as described,[13] except that a 3-h reaction time in 0.4 L of 2.0 M sodium glyoxylate/glyoxylic acid (9:1) buffer was found sufficient. The product was recrystallized from warm water on addition of a little $HClO_4$ (70%, w/w) and cooling (63 g, 84% yield). Anal. Calcd for $[(NH_3)_5CoO_2CCH(OH)_2](ClO_4)_2$: C, 5.53; H, 4.18; N, 16.14; Cl, 16.34; Co, 13.58. Found: C, 5.8; H, 4.5; N, 16.0; Cl, 16.7; Co, 13.8. ^1H NMR: δ 2.90 (3H, NH_3 trans to O), 3.90 (12 H, NH_3 cis to O), 5.07 (1 H, $-CH(OD)_2$) in 0.1 M DCl; δ 2.74 (3H, trans NH_3), 3.76 (12 H, cis NH_3), 4.63 (~0.8 H, hydrated aldehyde $-CH<$), 9.02 (~0.2 H, CHO) in Me_2SO-d_6. ^{13}C NMR: δ 114.0 ($Co-O_2C-$), 20.3 ($-CH(OD)_2$) in D_2O. Visible spectrum: $\epsilon_{502.5}^{max}$ 70.3, ϵ_{347}^{max} 61.5, in 0.1 M HCl.

(3,3-Bis(ethoxycarbonyl)-2-hydroxypropanoato)pentaamminecobalt(III) (2) Trifluoromethanesulfonate Monohydrate. Triethylamine was freshly redistilled from KOH pellets. Reagent grade dimethyl sulfoxide (Me_2SO) and diethyl malonate were used without further purification. A solution of the perchlorate salt of **1** (60.8 g, 0.14 mol) in Me_2SO (0.4 L) was stirred vigorously as diethyl malonate (44.8 g, 0.28 mol) and triethylamine (14.2 g, 0.14 mol) were added. After stirring for 5 min at room temperature (~20 °C),[14] the mixture was slowly poured into a vigorously stirred solution of acetic acid (16.8 g, 0.28 mol) in water (3 L). After further dilution to 12 L, the products were sorbed on a column (10 × 45 cm) of SP-Sephadex C-25 (Na^+ form, Pharmacia). Elution with 0.3 M NaCl resulted in the separation of a major red 2+ band (>90%) from a number of minor byproducts. The eluent containing the major product was subjected to cycles of concentration and treatment with ethanol until the bulk of the NaCl had been removed. The filtrate was concentrated almost to dryness, redissolved in water (0.2 L), and filtered. The filtrate was stirred as solid $NaCF_3SO_3 \cdot H_2O$[15] (~30 g) was added and cooled at 4 °C overnight. The red crystalline product was collected and recrystallized from a minimum of 0.1 M $NaCF_3SO_3$ on cooling from 50 °C and dried in vacuo over P_2O_5 (76 g, 78% yield). Anal. Calcd for $[(NH_3)_5CoO_2CCH(OH)CH(COOCH_2CH_3)_2](CF_3SO_3)_2 \cdot H_2O$: C, 19.05; H, 4.36; N, 10.10; F, 16.44; S, 9.25; Co, 8.50. Found: C, 19.2; H, 4.3; N, 10.1; F, 16.6; S, 9.5; Co, 8.6. ^1H NMR: δ 1.28 (6 H, triplet, J_H = 7 Hz, ester CH_3), 2.95 (3 H, broad, trans NH_3), 3.91 (12 H, broad, cis NH_3), 4.09 (1 H, doublet, J_H = 4 Hz, β $-CH<$), 4.27 (4 H, quartet, J_H = 7 Hz, ester CH_2), 4.66 (1 H, doublet, J_H = 4 Hz, α-CH(OD)–) in 0.1 M DCl. ^{13}C NMR: δ 115.1 ($Co-O_2C-$), 102.6 (ester CO_2), 42.5, 63.6 (central peaks of the $CF_3SO_3^-$ quartet[15]), 3.3 (α-CH(OD)–), −3.5 (ester CH_2), −10.5 (β $-CH<$), −53.2 (ester CH_3) in 10^{-2} M DCl. Visible spectrum: $\epsilon_{502.5}^{max}$ 70.6, ϵ_{350}^{max} 58.5 in H_2O.

(3,3-Bis(ethoxycarbonyl)-2-propenoato)pentaamminecobalt(III) (3) Hexafluorophosphate. To the trifluoromethanesulfonate salt of **2** (15 g) was added anhydrous sodium acetate (10 g) followed by 50 mL each of glacial acetic acid and acetic anhydride. The solution was heated with stirring at 80 °C for 90 min, and then the bulk of the solvent was distilled off at reduced pressure. The oily residue was repeatedly triturated with diethyl ether until it solidified. The solid was redissolved in acetone (0.2 L) and the solution filtered, leaving a residue of sodium acetate. The filtrate was concentrated to a viscous oil which was redissolved in ice-cold water (150 mL) and filtered into a receiver cooled in an ice-water bath. The solution was stirred as finely divided recrystallized[16] $NaPF_6$ was added. After 20 min, the pink crystals were collected, washed with ether, and dried in vacuo over P_2O_5. The dried material was washed with a little anhydrous ethanol, followed by ether, and redried (12.4 g, 88% yield). Anal. Calcd for $[(NH_3)_5CoO_2CCH{=}C(COOCH_2CH_3)_2](PF_6)_2$: C, 16.65; H, 4.04; N, 10.79; F, 35.12; P, 9.54; Co, 9.08. Found: C, 16.7; H, 4.0; N, 10.6; F, 33.6; P, 9.4; Co, 9.1. ^1H NMR: δ 1.20, 1.22 (6 H, overlapping triplets, J_H = 7 Hz, ester CH_3), 2.73 (3 H, broad, trans NH_3), 3.76 (12 H, broad, cis NH_3), 4.20 (4 H, broadened quartet, J_H = 7 Hz, ester CH_2), 6.71 (1 H, α-CH=) in Me_2SO-d_6. ^{13}C NMR: δ 105.5 ($Co-O_2C-$), 96.6, 98.2 (ester CO_2), 69.8 (α-CH=), 66.1 (β=C<), −4.5, −5.3 (ester CH_2), −52.6 (ester CH_3) in Me_2SO-d_6. Visible spectrum: $\epsilon_{502.5}^{max}$ 76.3, ϵ_{345}^{max} 75.7, in 0.3 M NaCl.

Diethyl (3-Carboxyaspartato)tetraamminecobalt(III) (4) Dithionate. The hexafluorophosphate salt of **3** (1 g) was dissolved in water (150 mL, 25.0 °C), and the pH adjusted to and maintained at 11.5 ± 0.05 by dropwise addition of 1 M NaOH. After 100 s, 5 M HCl was added cautiously to give pH ~6. The orange solutions from eight such reactions were pooled and, after dilution to 12 L, were sorbed onto a column (7 × 29 cm) of SP-Sephadex C-25 (Na^+ form). Elution with 0.2 M NaCl resolved from a number of byproducts (see Product Analyses) a major orange 2+ product which was collected. The solvent was removed, and the bulk of the NaCl crystallized out with ethanol as described above. The filtrate was concentrated; the residue was redissolved in water (50 mL) and cooled as a saturated aqueous solution of $Na_2S_2O_6$ was slowly added. The sparingly soluble orange crystals were collected, washed with a little cold water, and dried in vacuo over P_2O_5 (3.9 g, 61% yield). Anal. Calcd for $[(NH_3)_4CoO_2CCH(NH_2)CH(COOCH_2CH_3)_2](S_2O_6)$: C, 20.81; H, 5.05; N, 13.48; S, 12.35; Co, 11.35. Found: C, 20.8; H, 5.1; N, 13.6; S, 12.4; Co, 11.3. This reaction also occurred smoothly in saturated solutions of triethylamine (7 h) or KF (1.25 h) in Me_2SO at ~20 °C. In neither case was the yield of **4**, isolated as above, greater than that obtained in aqueous solution.

Diethyl (3-Carboxyaspartato)tetraamminecobalt(III) (4) Perchlorate Monohydrate. Since **4** could not be adequately characterized as the insoluble dithionate salt, it was converted to the perchlorate salt as follows. A stirred aqueous suspension of the dithionate salt (~10 g/L) was treated with sufficient Dowex AG 1-X4 (Cl^- form) anion-exchange resin to produce an orange solution of the chloride salt. The mixture was poured on a small column of the same resin, and the complex was washed through with water. The eluent was concentrated and treated with saturated aqueous $NaClO_4$ until crystallization commenced. The product obtained on cooling (4 °C, ~15 h) was recrystallized from warm (45 °C) water. Anal. Calcd for $[(NH_3)_4CoO_2CCH(NH_2)CH(COOCH_2CH_3)_2](ClO_4)_2 \cdot H_2O$: C, 18.76; H, 4.90; N, 12.15; Cl, 12.31; Co, 10.23. Found: C, 18.7; H, 4.8; N, 12.1; Cl, 12.3; Co, 10.1. ^1H NMR: δ 1.22, 1.26 (6 H, overlapping triplets, J_H = 7 Hz, ester CH_3), 2.75 (3 H, broad, NH_3 trans to O), 3.32 (5 H, broad, H_2O and cis NH_3), 3.44 (3 H, broad, cis NH_3), 3.74 (3 H, broad, NH_3 trans to H_2N), 4.1–4.4 (6 H, complex multiplet, α and β CH and ester CH_2), 7.2, 7.7[5] (each 1 H, very broad, $-NH_2Co$), relative to Me_4Si in Me_2SO-d_6 (Varian HA-100 spectrometer). The existence of four distinct ammine resonances (each 3 H) was confirmed by spectra in 0.1 M DCl, in which the HOD

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peak appeared downfield from the multiplet. The assignment of signals at δ 3.32, 3.44, and 3.74 ppm to specific ammines is tentative. ^{13}C NMR: δ 112.9 (CoO_2C–), 100.4, 101.6 (ester CO_2), −4.4 (ester CH_2), −11.4 (α-CH<), −13.1 (β-CH<), −52.6 (ester CH_3) in Me_2SO-d_6. Visible spectrum: $\epsilon_{493.5}^{max}$ 77.8, $\epsilon_{348.5}^{max}$ 95.2 in 0.3 M NaCl.

(3-(Ethoxycarbonyl)aspartato)tetraamminecobalt(III) (8) Chloride Sesquihydrate. A solution (150 mL) of the chloride salt of **4** (3.85 mmol) prepared as above was reacted under N_2 for 10 h at pH 9.0 and 25.0 °C, using a Radiometer (Copenhagen) pH-stat assembly (pH Meter 26 with a GK2401B combination electrode, Titrator 11, Autoburette ABU 12 with a 2.5-mL syringe, and SBR 2c recorder). The uptake of base (2.0 M LiOH, 3.62 mmol) followed a first-order rate law (k_{obsd} = 2.4 × $10^{-4}s^{-1}$). After the reaction had been quenched by addition of HCl to give pH 6, the mixture was diluted (3 L) and sorbed on a column (7 × 23 cm) of SP-Sephadex C-25 (Li^+ form). Elution with 0.1 M LiCl separated a major orange 1+ product (>95%) from a trace of the orange 2+ starting material (**4**). No other product was observed. The eluate was concentrated to ~10 mL and then added to anhydrous ethanol (1 L). The precipitate was collected and washed thoroughly with ethanol and ether and dried. It was redissolved in a minimum of water, which was allowed to evaporate slowly (~36 h) to give orange crystals. These were washed thoroughly with ethanol and dried in vacuo over P_2O_5 (1.1 g, 72% yield). Anal. Calcd for $[(NH_3)_4\overline{CoO_2CCH(NH_2)}CH(COO^-)(COOCH_2CH_3)]Cl\cdot{}^3/_2H_2O$: C, 21.41; H, 6.16; N, 17.84; Cl, 9.03; Co, 15.01. Found: C, 21.6; H, 6.0; N, 17.9; Cl, 9.2; Co, 14.2. ^1H NMR: δ 1.27, 1.32 (3 H, overlapping triplets, J_H = 7 Hz, ester CH_3), 3.14 (3 H, broad, NH_3 trans to O), 3.68, 3.84 (each 3 H, broad, cis NH_3), 3.99 (3 H, broad, NH_3 trans to H_2N), 4.2 to 4.5 (4 H, complex multiplet, α and β CH and ester CH_2), in ~2 M DCl. ^{13}C NMR: δ 116.0 (Co–O_2C–), 101.6, 102.5, 102.9, 103.8 (COOH and ester CO_2), −2.7 (ester CH_2), −10.5 (α-CH<), −13.0 (β-CH<), −53.3 (ester CH_3), in 1 M CF_3SO_3H (external lock). Visible spectrum: $\epsilon_{492.5}^{max}$ 83.7, ϵ_{348}^{max} 101.3 in water.

(3-Carboxyaspartato)tetraamminecobalt(III) (9). The dithionate salt of **4** (2.0 g) was suspended in water (150 mL, 25.0 °C). The pH of the suspension was adjusted to and maintained at 12.0 ± 0.05 with 1 M NaOH. After 45 min, 5 M HCl was added to give pH 7. The brown precipitate was collected by filtration and washed with water. Cobalt oxide was removed and the product recrystallized (twice) by neutralization (with NaOH) of a cooled solution in dilute HCl. The sparingly soluble orange crystals were washed with water and ethanol and dried in vacuo over P_2O_5 (1.0 g, 83% yield). The freshly dried product was apparently anhydrous, but regained two molecules of water of crystallization over a few days when stored in contact with the atmosphere. Anal. Calcd for $(NH_3)_4\overline{CoO_2CCH(NH_2)}CH(COO^-)_2\cdot 2H_2O$: C, 17.81; H, 5.98; N, 20.77; Co, 17.48. Found: C, 17.8: H, 5.8; N, 20.9; Co, 17.5. ^{13}C NMR (external deuterium lock in 1 M CF_3SO_3H): δ 116.1 (Co–O_2C–), 103.1, 104.0 (COOH), −10.6 (α-CH<), −13.2 (β-CH<). In 2 M $DClO_4$, the peak tentatively assigned to the β-methyne carbon appeared as a triplet at δ −0.0$_4$ ppm (J_{CD} = 14 Hz), consistently with rapid exchange of the β-CH proton under these conditions. Less than 20% of protons on the α-methyne carbon (δ −10.6 ppm in 2 M $DClO_4$) had exchanged during recording of the spectrum (~1 h at 25 °C).

(3-Carboxyaspartato)tetraamminecobalt(III) (9) Dichloride Monohydrate. The doubly protonated form of **9** crystallized as the dichloride salt from a solution of **9** (0.5 g) in dilute HCl (25 mL) on slow addition of concentrated HCl (25 mL) and cooling. The orange crystals (95% yield) were collected, washed with ethanol, and dried in vacuo over P_2O_5. Anal. Calcd for $[(NH_3)_4\overline{CoO_2CCH(NH_2)}CH(COOH)_2]Cl_2\cdot H_2O$: C, 15.03; H, 5.14; N, 17.86; Cl, 18.08; Co, 15.03. Found: C, 15.2; H, 5.2; N, 17.8; Cl, 17.7; Co, 15.2. Visible spectrum: $\epsilon_{492.5}^{max}$ 78.9, ϵ_{348}^{max} 92.6 in water.

Diethyl (3-Carboxyaspartato)pentaamminecobalt(III) (5) Chloride Tetrachlorozincate. The hexafluorophosphate salt of **3** (5.0 g) was added in small portions over 2 min to a solution of ammonium acetate (5 g) in liquid ammonia (~0.4 L, −33 °C). The solvent was immediately distilled off at reduced pressure (~5 min). The residue was redissolved in 0.2 M HCl (0.2 L), and further HCl (5 M) was added to give pH 3.5. After dilution to 12 L, the complex was sorbed on a column (4 × 30 cm) of SP-Sephadex C-25 (Na^+ form). After traces of red 2+ cations had been eluted with 0.2 M NaCl (3 L), the major (>80%) red product was eluted with 0.5 M NaCl. The bulk of NaCl was removed from the eluate by concentration and crystallization with ethanol as described above. The filtrate was concentrated to dryness. An aqueous solution (~100 mL) of the residue was cooled as a concentrated solution of $ZnCl_2$ in 1 M HCl was slowly added. The product that crystallized was collected, washed with ethanol, and dried (3.2 g, 66% yield). Recrystallization from warm water (100 mL) on cooling and addition of $ZnCl_2$/HCl solution (15 mL) followed by concentrated HCl (dropwise) gave pure **5** as the chloride tetrachlorozincate mixed salt. Anal. Calcd for $[(NH_3)_5CoO_2CCH(NH_3^+)CH(COOCH_2CH_3)_2](ZnCl_4)Cl$: C, 17.44; H, 4.88; N, 13.56; Cl, 28.59; Co, 9.51; Zn, 10.55. Found: C, 17.7; H, 4.9; N, 13.5; Cl, 28.7; Co, 9.5; Zn, 10.8. ^1H NMR: δ 1.28 (6 H, triplet, J_H = 7 Hz, ester CH_3), 2.98 (3 H, broad, trans NH_3), 3.95 (12 H, broad, cis NH_3), 4.25, 4.26 (4 H, pair of quartets, J_H = 7 Hz, ester CH_2), 4.32 (1 H, doublet, J_H = 4 Hz, β-CH<), 4.49 (1 H, doublet, J_H = 4 Hz, α-CH<) in D_2O. In D_2O, the doublets at δ 4.32 and 4.49 collapsed with a half-life of 10–15 min to a singlet (1 H) at δ 4.50. consistent with exchange of the β-CH proton. In 0.01 M DCl, this exchange occurred on a much shorter time scale. In Me_2SO-d_6, the ^1H NMR spectrum also showed a broad signal at δ 8.24 (3 H, NH_3^+). ^{13}C NMR: δ 107.3 ($Co–O_2C$–), 99.6 (ester CO_2), −4.4, −4.7 (ester CH_2), −14.3, −14.9 (α- and β-CH<), −52.6 (ester CH_3) in Me_2SO-d_6. Visible spectrum: $\epsilon_{501.5}^{max}$ 67.2, ϵ_{351}^{max} 54.0 in water.

Calcium 3-Carboxyaspartate (6) Sesquihydrate. The dithionate salt of **4** (10.0 g) was suspended in water (0.6 L). The pH of the suspension was maintained at 6.0 ± 0.1 with 1 M HCl as Zn powder (2.5 g) was added, and for 45 min thereafter. Excess Zn was filtered off and the pH of the filtrate brought to 1.5 with concentrated HCl. After dilution to 3 L, the solution was sorbed on a column (5 × 40 cm) of Dowex 50W-X2 (H^+ form) cation-exchange resin. After thorough washing with water, the column was eluted with 1 M aqueous ammonia. Fractions containing diethyl 3-carboxyaspartate were pooled and concentrated to dryness. The glassy residue was heated with 1 M NaOH (80 mL, ~2-fold excess) on a steam bath (80–90 °C, 1 h). The solution was filtered and then neutralized with 1 M HCl (~40 mL). Dropwise addition of saturated aqueous $CaCl_2$ gave white crystals which were collected and washed with water. This crude product was twice recrystallized from aqueous solutions (100 mL) containing a slight excess of HCl by neutralization with NaOH (2.9 g, 62% yield). Anal. Calcd for $Ca^{2+}[^-OOCCH(NH_3^+)CH(COO^-)_2]\cdot 1.5H_2O$: C, 24.80; H, 3.32; N, 5.78; Ca, 16.55. Found: C, 24.8; H, 3.3; N, 5.6; Ca, 16.6. The same product was similarly prepared by Zn reduction of the chloride tetrachlorozincate mixed salt of **5** (2.0 g) in water (80 mL) at pH 6.0 (30 min), followed by ion-exchange chromatography, saponification, and crystallization as above (0.65 g, 83% yield). Anal. Found: C, 25.0; H, 3.0; N, 5.7; Ca, 16.4. The product from both sources gave identical ^{13}C NMR spectra recorded as follows. The calcium salt of **6** (2.67 mmol) was dissolved in 5.0 M HCl (2.0 mL) to give solutions 1.33 M in **6**·HCl, 1.33 M in $CaCl_2$ and 1.0 M in HCl. Spectra were recorded at 2 °C using an external deuterium lock. In one experiment, the solution was heated for various times at 80.0 ± 0.5 °C, and spectra were recorded under the same conditions. A reference spectrum of L-aspartic acid hydrochloride (1.33 M) in 1.33 M $CaCl_2$–1.0 M HCl was similarly recorded.

A large-scale synthesis of **6** from $[(NH_3)_5CoOH_2](ClO_4)_3$ (80 g) was accomplished by way of complexes **1**, **2**, **3**, and **5** essentially as described above, except that the intermediate products were not recrystallized. It was not necessary to isolate **3** in solid form; the oily residue remaining after removal of the bulk of the solvent (by distillation) and sodium acetate (with acetone) was dissolved directly in liquid ammonia (~1 L). Isolation of **5** as above gave 60 g of the chloride tetrachlorozincate mixed salt (56% yield from $[(NH_3)_5CoOH_2](ClO_4)_3$). Reduction of this product followed by isolation and saponification as above gave 11.3 g of pure calcium 3-carboxyaspartate (**6**) sesquihydrate (48% yield from **5**, 27% from $[(NH_3)_5CoOH_2](ClO_4)_3$).

Resolution of the $[(NH_3)_4\overline{CoOOCCH(NH_2)}CH(COOCH_2CH_3)_2]^{2+}$ Ion (4). Method A. To a solution (150 mL) of the chloride salt of **4** (8.0 mmol) prepared from the dithionate salt as described above was added sodium (+)$_D$-antimony tartrate (2.47 g). The flocculent orange crystals that separated on concentration of the solution to 50 mL and cooling were collected by filtration, washed with ice-cold water, and dried in vacuo over P_2O_5 (3.5 g, 3.75 mmol). The product was recrystallized from warm (55 °C) water (~ 4 L) containing sodium (+)$_D$-antimony tartrate (2.47 g) by cooling and concentration. Three fractions (totalling 3.2 g) were collected, all of which gave satisfactory elemental analyses. Anal. (fraction 3) Calcd for $(-)_D$-$[(NH_3)_4\overline{CoOOCCH(NH_2)}CH(COOCH_2CH_3)_2](+)_D$-$(C_4H_4O_7Sb)_2$: C, 21.93; H, 3.68; N, 7.52. Found: C, 22.0; H, 3.5; N, 7.5. The fractions were separately converted to the dithionate salt by treatment of suspensions in water (300 mL) with sufficient Dowex AG1-X4 (Cl^- form) resin to produce solutions of the chloride salts. The mixtures were loaded onto columns (2 × 5 cm) of the same resin. The eluents were collected, concentrated (10 mL), filtered, and then treated with saturated aqueous $Na_2S_2O_6$. The crystals were collected, washed with cold water, ethanol, and ether, and dried in vacuo over P_2O_5 (combined yield 1.6 g, 3.06 mmol). All three fractions gave satisfactory microanalyses. Anal. (fraction 3) Calcd for $(-)_D$-$[(NH_3)_4\overline{CoOOCCH(NH_2)}CH(COOCH_2CH_3)_2](S_2O_6)$: C, 20.81; H, 5.05; N, 13.48; S, 12.35; Co, 11.35. Found: C, 21.1; H, 5.0; N, 13.5; S, 12.1; Co, 11.5. Molar rotations (fractions 1–3 respectively): $[M]_{589}$

−240, −244, −251; $[M]_{486}^{max}$ +1560, +1580, +1540, in water ([Co] = 2 mM) at 25 °C.

Method B. The mother liquors from the isolation and recrystallization of the $(-)_D$ isomer were combined and diluted with water to 4 L. Equal portions were separately sorbed on three columns (7 × 29 cm) of CM-Sephadex C-25 resin. Elution with 0.2 M sodium $(+)_D$-antimony tartrate separated two orange bands (fractions I and II). Corresponding fractions from the three separations were pooled, sorbed on, and eluted from two small columns of Dowex 50W-X2 (Na^+ form). The eluates (2 M NaCl) were concentrated and treated with ethanol to remove NaCl. The two fractions were separately rechromatographed on CM-Sephadex C-25 as above, in each case giving a single orange band. After removal of antimony tartrate by chromatography on Dowex 50W-X2 and removal of NaCl with ethanol, the isomers were separately crystallized as the dithionate salts from water by addition of saturated aqueous $Na_2S_2O_6$ (yields: fraction I, 0.21 g, 0.40 mmol; fraction II, 1.75 g, 3.37 mmol). Both fractions gave satisfactory elemental analyses (C, H, N, S, Co) for $(\pm)_D$-$[(NH_3)_4\overline{CoOOCCH(NH_2)CH(COOCH_2CH_3)_2}](S_2O_6)$. Molar rotations (fractions I and II, respectively): $[M]_{589}$ −239, +224; $[M]_{486}^{max/min}$ +1590, −1570, in water ([Co] = 2 mM) at 25 °C.

Kinetic Studies. Buffers were prepared from 1.00 N HCl or 1.00 N NaOH (Volucon) and reagent grade or redistilled bases in CO_2-free water, and made up to μ = 1.00 M with KCl. Absorbance changes were followed with a Cary 118C spectrophotometer with the sample compartment equilibrated at 25.0 ± 0.1 °C. Reactions were initiated by mixing equal volumes of buffers at twice the final concentration (μ = 1.00 M) and solutions of substrates in 1.00 M KCl, using a temperature-equilibrated stopped-flow mixer.[17] Reactions of **3**-$(PF_6)_2$ and **4**-$(ClO_4)_2 \cdot H_2O$ (0.4–1.2 mM) in buffers (pH 8.3–13.2) were monitored at 360 nm.

The decrease in A_{360} with **4** under basic conditions followed strictly first-order kinetics. Values of first-order rate constants (k_{obsd}) were evaluated by fitting absorbance vs. time data to a single-exponential function using a least-squares computer program, which also gave values of the A_{360} of solutions of **4** (zero time intercept). For the reactions of **3** in $Et_3N \cdot HCl$ buffers (pH 10.2–11.7), A_{360} increased rapidly to a maximum value and then decreased (Figure 5), indicating two sequential first-order reactions (eq 1).[18] For such a system, ϵ_t ($=A_t/[A]_0$) vs. time

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \qquad (1)$$

data obey an equation represented by the sum of two exponential functions (eq 2), in which k_f is the greater of the two rate constants and k_s

$$\epsilon_t = \alpha e^{-k_f t} + \beta e^{-k_s t} + \gamma \qquad (2)$$

is the lesser, $\alpha = (\epsilon_A - \epsilon_C) + k_1(\epsilon_B - \epsilon_C)/(k_2 - k_1)$, $\beta = -k_1(\epsilon_B - \epsilon_C)/(k_2 - k_1)$, and $\gamma = \epsilon_c$.[18] Values of k_f, k_s, α, β, and γ were evaluated by least-squares computer fitting of ϵ_t vs. time data to eq 2. Data points were carefully spaced with time such that similar numbers of points controlled the fit to each of the two phases of the reactions (Figure 5), giving similar estimates of the percent uncertainty in each of the rate constants. It should be noted that k_f and k_s (eq 2) cannot be assigned to k_1 and k_2 (eq 1) on the basis of curve fitting alone. In the present case, this assignment was made on the basis of the results of product distribution studies and independent study of the isolated intermediate species (see Results and Discussion). From the values of the parameters α, β, and γ and the assigned values of k_1 and k_2 were calculated values of ϵ_A, ϵ_B, and ϵ_C (eq 1). The values obtained for the rate constants and other parameters in each of these studies were derived as the mean of three or more determinations.

Concentrations of OH^- in the various buffer solutions were determined from the measured values of pH, assuming pH = $-\log[H^+]$ and $K_w' = [H^+][OH^-] = 1.71 \times 10^{-14}$ (in 1.0 M KCl at 25.0 °C).[19]

Product Analyses. $[(NH_3)_5CoOOCCH{=}C(COOCH_2CH_3)_2](PF_6)_2$ in 10^{-2} M HCl. A solution of the hexafluorophosphate salt of **3** (2.0 g) in 10^{-2} M HCl (150 mL) was stirred for 4 h at 25.0 °C and then diluted to 5 L and sorbed on a column (4.5 × 23 cm) of SP-Sephadex C-25 (Na^+ form). Elution with 0.3 M NaCl gave a red 2+ ion as the sole product. The eluate was concentrated and NaCl removed with ethanol as above. To a cooled solution of the residue in water (40 mL) was added solid $NaCF_3SO_3 \cdot H_2O$ (5 g), resulting in the crystallization of the product (1.8 g, 84% yield). The time required from initiation of the reaction to crystallization was 24 h. This material was identified as $[(NH_3)_5CoO_2CCHOHCH(COOCH_2CH_3)_2](CF_3SO_3)_2 \cdot H_2O$ (**2**) by its elemental analysis and ^{13}C NMR spectrum. Anal. Found: C, 19.2; H, 4.5; N, 9.9; F, 16.9; S, 9.3; Co, 7.9. ^{13}C NMR: δ 115.1, 102.7, 3.2, −3.6, −10.7, −53.4 in 10^{-2} M DCl (assignments as for **2**, above).

$[(NH_3)_5CoOOCCH{=}C(COOCH_2CH_3)_2](PF_6)_2$ in Liquid NH_3 (−33 °C). Weighed samples of the hexafluorophosphate salt of **3** were dissolved in liquid ammonia alone or in liquid ammonia saturated with $NaNH_2$ (~0.02 M) or containing 0.1 M ammonium acetate (~100 mL). The reaction with $NaNH_2$ was quenched after 1 min with excess solid $(NH_4)_2SO_4$. In each case after 1 min, the solvent was removed at reduced pressure (~2 min) and the residue redissolved in 0.1 M HCl (100 mL). After neutralization (with NaOH to pH ~6) and dilution (to 0.5 L), the solutions were sorbed on columns (2 × 20 cm) of CM-Sephadex C-25 (Na^+ form). Three red 2+ bands (fractions I, ~2%; II, ~8%, and III, ~3%) and an orange 2+ band (fraction IV) separated completely on elution with 0.2 M $NaClO_4$. The major red 3+ product (fraction V) was eluted with 0.4 M $NaClO_4$, leaving a trace of red (polymeric) material at the top of the column. Concentrations of cobalt in the various fractions were determined by atomic absorption spectrometry. The total recovery of Co in fractions I to V was in each case 94–96% of that applied. The red 3+ product (fraction V) was identified as **5** by its isolation and characterization as the chloride tetrachlorozincate mixed salt from preparative scale experiments (see above). The orange 2+ product (fraction IV) was separated as above in a larger scale experiment (5 g of 3-$(PF_6)_2$ in ~0.5 L of liquid ammonia alone) by chromatography on CM-Sephadex C-25 (5 × 40 cm column, eluted with 0.2 M NaCl). After removal of NaCl with ethanol it was crystallized with $Na_2S_2O_6$ and then converted to the perchlorate salt and recrystallized as described for **4** above. It was identified as the monohydrate of the perchlorate salt of **4** by elemental analysis and by its visible spectrum. Anal. Found: C, 18.6; H, 5.1; N, 11.7; Cl, 12.1; Co, 10.2. Visible spectrum: $\epsilon_{493.5}^{max}$ 78.8, ϵ_{348}^{max} 98.0 in 0.3 M NaCl. The red 2+ products (fractions I to III) were not identified.

$[(NH_3)_4\overline{CoOOCCH(NH_2)CH(COOCH_2CH_3)_2}](ClO_4)_2 \cdot H_2O$ in Aqueous Base. Samples (0.1 g) of the perchlorate salt of **4** were reacted in water (50 mL) at 25 °C at pH 9 and 12 for ~10 half-lives under pH-stat conditions. After neutralization with HCl to pH 6, the undiluted solutions were sorbed on small columns of SP-Sephadex C-25, which were eluted with 0.1 M NaCl. The sole products, save for some cobalt oxides produced in the reaction at pH 12, were an orange nonelectrolyte (which passed unretarded through the column) and an orange 1+ cation. These were identified as **9** and **8**, respectively, by isolation and characterization on preparative scales as described above. The proportion of **9** increased from <10% at pH 9 to >90% at pH 12. No attempt was made to quantify the two products further.

$[(NH_3)_5CoOOCCH{=}C(COOCH_2CH_3)_2](PF_6)_2$ in Aqueous Base. A large-scale preparation of **4** from the hexafluorophosphate salt of **3** (23 × 1 g reactions) was carried out as above (pH 11.5, $\mu \approx 0$, 25.0 °C, 100 s). The products were chromatographed on SP-Sephadex C-25 (Na^+ form, 9 × 25 cm, eluted with 0.2 M NaCl), resulting in the resolution of a band of red-orange 1+ products (fraction I) from orange 2+ products (fraction II, predominantly **4**) and several minor unidentified byproducts. Fraction I (~1.5 L) was diluted (20 L) and sorbed on a column (7 × 25 cm) of Dowex 50W-X2 (Na^+ form). Elution with 0.5 M NaCl gave two well-separated bands, the first red (fraction Ia, $\epsilon_{501}^{max}/\epsilon_{350}^{max}$ = 1.05) and the other orange (fraction Ib, $\epsilon_{493}^{max}/\epsilon_{348}^{max}$ = 0.84). Quantification using ϵ_{501}^{max} = 71 M^{-1} cm^{-1} (Ia) and ϵ_{493}^{max} = 84 M^{-1} cm^{-1} (Ib) gave overall yields of 9% and 8%, respectively. Sodium chloride was removed from these solutions first by concentration and treatment with ethanol as above and then by chromatography on SP-Sephadex C-25 (Li^+ form, eluted with 0.05 M LiCl). The eluates were concentrated and the products precipitated with ethanol. The red product (Ia) was identified as the $[(NH_3)_5CoO_2CCH(OH)CH(COO^-)(COOCH_2CH_3)]^+$ ion (**7**) by ^{13}C NMR: δ 116.0 (Co–O_2C), 104.9, 105.1, 106.7, 107.1 (ester CO_2 and COO^-), 3.6, 4.2 (α-CH(OD)−), −3.5 (ester CH_2), −9.1, −10.0 (β-CH<), −52.4 (ester CH_3) in D_2O. The orange product (Ib) was identified as **8** by ^{13}C NMR (external deuterium lock in 1 M CF_3SO_3H): δ 116.0, 103.6, 102.9, 102.3, 101.5, −2.7, −10.7, −13.1, −53.3 from dioxane (assignments as for **8**, above).

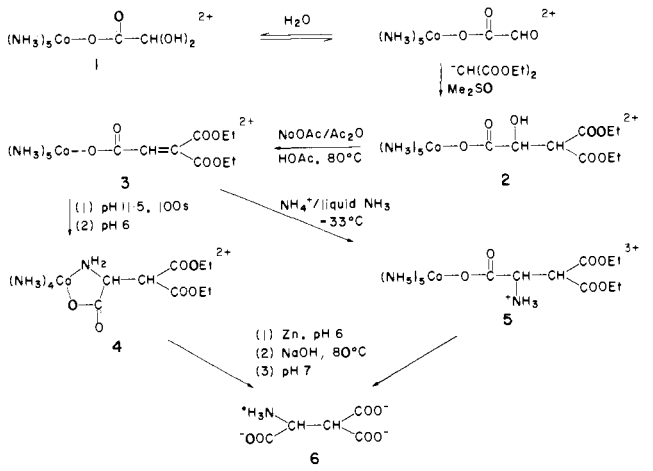
The eluate from SP-Sephadex containing the 2+ products (fraction II) was treated as described above to give the dithionate salt of **4** (10.0 g, 54% yield). The filtrate from crystallization, together with washings, was diluted (12 L) and sorbed on a column (7 × 25 cm) of Dowex 50W-X2 (Na^+ form). Elution with 2 M NaCl gave three well-separated bands: fractions IIa (red, $\epsilon_{502}^{max}/\epsilon_{348}^{max}$ = 1.09), IIb (orange, $\epsilon_{496}^{max}/\epsilon_{344}^{max}$ = 0.80), and IIc (orange, $\epsilon_{492}^{max}/\epsilon_{346}^{max}$ = 0.79). These products were quantified by using ϵ_{502}^{max} = 70.3 M^{-1} cm^{-1} (IIa, 2%), $\epsilon_{496}^{max} \approx$ 78 M^{-1} cm^{-1} (IIb, 1%), and ϵ_{492}^{max} = 77.8 M^{-1} cm^{-1} (IIc, 6%). Sodium chloride was removed from each of these fractions by concentration and treatment with ethanol as above. The red product (IIa) was crystallized

(17) A device similar to that described by: Inoue, Y.; Perrin, D. D. *J. Phys. Chem.* **1962**, *66*, 1689.

(18) Jackson, W. G.; Harrowfield, J. MacB.; Vowles, P. D. *Int. J. Chem. Kinet.* **1977**, *9*, 535.

(19) Harned, H. S.; Hamer, W. J. *J. Am. Chem. Soc.* **1933**, *55*, 2194.

Scheme I



as the trifluoromethanesulfonate salt by addition of $NaCF_3SO_3 \cdot H_2O$ and cooling overnight at 4 °C and was identified as the glyoxylatopentaamminecobalt(III) ion (**1**) by its NMR spectra. ^1H NMR: δ 2.89 (3 H, broad, trans NH_3), 3.87 (12 H, broad, cis NH_3), 5.05 (1 H, $-CH(OD)_2$) in 0.01 M DCl. ^{13}C NMR: δ 114.2 ($Co-O_2C$), 20.3 ($CH(OD)_2$) in 0.01 M DCl. Attempts to crystallize the minor product IIb failed, and this material was not further characterized. The orange 2+ cation IIc was crystallized as the dithionate salt, and then converted to the perchlorate salt as described for **4**, above. It was identified as **4** by its ^{13}C NMR spectrum: δ 113.2, 101.8, 100.6, −4.3, −11.3, −13.0, −52.5, in Me_2SO-d_6 (assignments as for **4**, above).

For examination of the extent of formation of **4** under the conditions of the kinetic studies, weighed samples of the hexafluorophosphate salt of **3** (0.2 g) were dissolved in 1.00 M KCl (100 mL, 25.0 °C). At zero time, 0.1 M triethylamine hydrochloride buffer (100 mL, 25.0 °C, pH 10.2–11.7, as used in the kinetic studies) was added to each, and the solutions were stirred at 25.0 °C for times corresponding to 6 half-lives of the first reaction (see Results and Discussion). Acetic acid (~2-fold excess) was added to quench the reactions, and then the pH of the solutions was cautiously readjusted to 6 with dilute NaOH. After dilution to 12 L, the mixtures of products were sorbed on columns (4.5 × 12 cm) of SP-Sephadex C-25 (Na^+ form). After traces of 1+ products (see above) had been eluted with 0.1 M NaCl (1.5 L), the 2+ cations were rapidly eluted with 0.3 M NaCl and collected in volumetric flasks (0.5 L). The major product thus collected was identified as **4** by the visible spectrum of these solutions (λ_{max} 494–494.5 nm, 348.5–349 nm), and by its isolation and characterization in preparative scale experiments (pH stat, pH 11.5, $\mu \approx 0$, 100 s) as described above. Other 2+ products (including unreacted **3**, see above) did not separate from **4** under these conditions. Concentrations of **4** in the eluates were determined spectrophotometrically in two ways. Direct spectra of the eluates gave values of A_{494} which were corrected for the calculated contribution of unreacted **3** (~ 1.5%, ϵ_{494} of **3** = 74.3 M^{-1} cm^{-1} in 0.3 M NaCl) and then divided by the value of the ϵ_{494} of **4** (77.8 M^{-1} cm^{-1}). Alternatively, the eluates (in 0.3 M NaCl) were mixed with equal volumes of 0.3 N NaOH using the stopped-flow mixing device,[17] and A_{360} was monitored over 30 s. The traces from five such determinations were extrapolated to the time of mixing and averaged (standard deviation ±1%). Under identical conditions, solutions of authentic **4** in 0.3 M NaCl gave ϵ_{360} = 2260 ± 20 M^{-1} cm^{-1}, from which the concentrations of **4** in the eluates were calculated.

Titration of $[(NH_3)_4\overline{CoOOCCH(NH_2)}CH(COOCH_2CH_3)_2](ClO_4)_2 \cdot H_2O$(4) with NaOH. The chelated amino acid diester complex **4** was not sufficiently stable in aqueous base to enable continuous titrations to be carried out. Therefore, points on the titration curve were obtained as follows. Solutions of the perchlorate salt of **4** (4.44 × 10^{-2} M, 9.0 mL) in 1.00 M KCl were equilibrated at 25.0 °C. Solutions of NaOH (1.00 mL, 0.1–0.4 M, μ = 1.00 M with KCl) were added and the resulting pH values measured immediately.

Results and Discussion

Syntheses. Procedures for the synthesis of the new amino acid, β-carboxyaspartic acid (Asa, **6**), from the glyoxylatopentaamminecobalt(III) ion (**I**) are summarized in Scheme I. The hydrated glyoxylato complex (**1**)[13] was prepared from $[(NH_3)_5CoOH_2](ClO_4)_3$. ^{13}C and ^1H NMR spectra of aqueous solutions of **1** confirm that the aldehyde is essentially fully hydrated in water. In Me_2SO-d_6 at room temperature, it exists to the extent of ~20% as the free aldehyde; it is undoubtedly this species that condenses with diethyl malonate under basic conditions (in Me_2SO at ~25 °C) to give the alcohol complex **2** in excellent yield. This (Knoevenagel) reaction in the present system occurs rapidly ($t_{1/2}$ ~ 30 s), and it may be compared with the rate of an analogous reaction. The condensation of *tert*-butyl glyoxylate with a 10-fold excess of dimethyl malonate apparently requires 72 h in refluxing (80 °C) benzene and yields, not unexpectedly, the α,β-dehydrated (olefin) product analogous to **3** in 47% yield.[20] The activation observed in the present system is almost certainly due to a favorable electrostatic interaction between the anionic nucleophile and the 2+ cation since the effect of coordination of the carboxylate to the strong electron-withdrawing group, the Co(III) center, should be similar to the effect of esterification.

Dehydration across the C_α–C_β bond in **2** to give the olefin **3** occurs smoothly and nearly quantitatively at 80 °C in a solution of sodium acetate in acetic acid/acetic anhydride. Both the preparation and isolation of **3** initially presented some difficulties. In acetic acid/acetic anhydride in the absence of acetate ion, the reaction produced **3** as the major product together with substantial amounts of the C_α–OH acetylated derivative of **2**. In the presence of excess base (sodium acetate), added to assist in the removal of the C_β–H proton, the rate of the dehydration and the yield and purity of the isolated hexafluorophosphate salt of **3** were increased. The only other product appeared to be a trace of Co(II) produced by decomposition of the complex(es). Initially, we attempted to isolate **3** free of excess solvent by ion-exchange chromatography of aqueous solutions of the reaction products (requiring ~12 h), but obtained (as the perchlorate salts) mixtures of **2** and **3**. The proportion of **2** (30–90%) was apparently related to the time the reaction products had been exposed to water. It was found that pure **3**-$(PF_6)_2$ reacted slowly (<24 h) in dilute aqueous HCl at 25 °C to re-form **2**, which could be isolated nearly quantitatively as the trifluoromethanesulfonate salt (the trifluoromethanesulfonate salt of **3** is exceedingly soluble in dilute $NaCF_3SO_3$ solution). Under the conditions described for isolation of the pure olefin complex (**3**) (Experimental Section), it was crystallized from cold aqueous solution with $NaPF_6$ in excellent yield. So long as exposure to water was not prolonged (<20 min at 0 °C), the product was free of contaminating **2**. For study of the reactions of **3** in aqueous solutions, and in the preparation of **4**, neutral solutions of **3** were prepared and used within 3 min.

Under basic conditions, the olefin **3** reacted rapidly to produce the diethyl ester of the chelated amino acid (**4**). The reaction proceeds by intramolecular attack of a cis-coordinated aminate ion (see below) on the α-carbon of the olefin to provide only the product with a five-membered chelate ring. Although this regiospecificity is expected in the present system where the C_α–C_β double bond is polarized by having two strongly electron-withdrawing substituents on the β-carbon, it nevertheless appears to be a general feature of these kinds of intramolecular reactions, and is apparent in a number of related systems.[21-23] Dreiding models indicate that the directions of nucleophilic attack by the *cis*-aminate ion at either the α- or β-carbons of the olefin could be equally favorable. The preference for attack at the α-carbon to produce a five-membered chelate ring presumably derives in part from probability factors related to the additional degrees of freedom of the six-membered-ring transition state coupled with the necessity in that case to displace a greater number of solvent molecules in the vicinity of the highly solvated cationic complex. In addition, the greater thermodynamic stability of the five-membered cyclic product resulting from the angle of the N–Co–O bond being constrained to near 90° favors formation of the five-membered chelate ring.

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The analogous reaction of the (methyl maleato)pentaamminecobalt(III) ion in base to produce (methyl aspartato)tetraamminecobalt(III) has been reported;[21] it occurs about 2 orders of magnitude more slowly than the conversion of **3** to **4** and is complicated by some decomposition to Co(II) and hydrolysis of the substrate ester function. The product of ester hydrolysis, the (maleato)pentaamminecobalt(III) ion, is not susceptible to attack by the intramolecular nucleophile.[21] Reaction of **3** at pH 11.5 (25 °C, 100 s) produces **4** in ~60% yield, together with significant amounts of four other products. Two 1+ products (~10% yield of each) were identified as the monoester analogues of **2** and **4** (i.e., **7** and **8**, respectively) by isolation and characterization. The complex **7** is presumed to be formed as the ultimate product of ester hydrolysis of **3**, followed by addition of water to the double bond during isolation (cf. the reaction of **3** in water to give **2**; see above). The product **8** is produced by hydrolysis of one of the ester functions of **4** subsequent to its formation (see below). Because of this subsequent reaction, the time that **3** is reacted at pH 11.5 must be carefully controlled in order to maximize the yield of **4**. Although the intramolecular cyclization also proceeds smoothly in Me_2SO in the presence of base (Et_3N or KF) without the problems of ester hydrolysis, polymeric products are formed even in dilute solution and the yields of **4** are no better than those from the reaction in aqueous base. Two 2+ products in addition to **4** were also produced in the reaction at pH 11.5. The orange product (~1% yield, λ_{max} 496 nm) was present in amounts too small to enable characterization. The other (red) 2+ byproduct (~2% yield) was isolated and identified as being **1**. Its origin is uncertain. It may be produced as the ultimate result of hydration of **3** to re-form **2** (see above), which has been independently observed to decompose rapidly in base to produce a number of unidentified Co(III) complexes and some free diethyl malonate. Thus, at least two minor pathways, ester hydrolysis (~10%) and hydration (≤5%), seem to be competitive with the intramolecular cyclization of the olefin complex **3** in aqueous base. Although the 2+ byproducts were not separated from **4** by chromatography on SP-Sephadex in preparative scale experiments, only **4** crystallized from the mixture as the sparingly soluble dithionate salt. All three 2+ products were readily separated by chromatography on CM-Sephadex C-25 or Dowex 50W-X2 (Na^+ form) resins.

The olefin **3** reacted in liquid ammonia (−33 °C) to produce, in addition to a number of unidentified byproducts, both the chelate (**4**) and monodentate (**5**) complexes of diethyl β-carboxyaspartate. Although solvent addition across the C_α–C_β double bond (to give **5**) predominated over the intramolecular reaction (to give **4**) under all conditions, the yield of **4** increased from 6% under acidic conditions (0.1 M ammonium ion) to 18% under neutral conditions to 23% in base (~0.02 M $NaNH_2$). Concomitantly, the yield of **5** decreased from 82% to 69% to 67%, respectively. This dependence of the products of reaction of **3** in liquid ammonia on basicity qualitatively parallels the pH dependence of the reaction in water. In both solvents under neutral conditions, solvent addition to the double bond predominates, the cyclization reaction becoming significant only in the presence of base. Since under acidic conditions **5** may be prepared in this manner in ~80% yield, it is the preferred intermediate for the preparation of the free amino acid (**6**).

Free β-carboxyaspartate was recovered from both of the diester complexes **4** and **5** in good yield. The complexes were first reduced with Zn at pH 6, and then diethyl β-carboxyaspartate was separated from anions, NH_4^+, Co^{2+}, and Zn^{2+} by ion-exchange chromatography. Following saponification with NaOH at ~80 °C, the amino acid was crystallized as the sparingly soluble calcium salt (sesquihydrate). A large-scale preparation from $[(NH_3)_5CoOH_2](ClO_4)_3$ (80 g) gave a 56% overall yield of **5** and a 27% overall yield of **6**. The low yield in the final step was unanticipated since **6** was recovered in much higher yield (~80%) in the small-scale experiments.

Characterization of Products. All of the cobalt(III) complexes and the final product gave correct elemental analyses and were further characterized by their ^1H and ^{13}C NMR and visible spectra. The monodentate (carboxylato)pentaamminecobalt(III) complexes (**1**, **2**, **3**, **5**, and **7**) were red (λ_{max} 502 ± 1 nm in H_2O) while the chelate (β-carboxyaspartato)tetraamminecobalt (III) derivatives (**4**, **8** and **9**) had the orange color (λ_{max} 493 ± 1 nm in H_2O) characteristic of such N,O-chelated amino acid complexes. The ^1H NMR spectra of the monodentate complexes showed two sets of ammine resonances at δ 2.7–3.0 (3 H, trans NH_3) and 3.7–4.0 (12 H, cis NH_3) from DSS, while the chelate complex **4** gave four discrete ammine resonances (each 3 H) at δ 2.75, 3.32, 3.44, and 3.74 from Me_4Si in Me_2SO-d_6. Since the α carbon of **4** is chiral, the cis NH_3 groups are diastereotopic, and the ^1H NMR spectrum shows two separate cis ammine signals in addition to the two expected for the inequivalent trans NH_3 groups.

In **2**, **4**, **5**, **6**, and **9** in which the α carbon is chiral, the similar substituents on the β-carbon are diastereotopic and would be expected to be inequivalent in NMR spectra. In the olefin **3**, the cis and trans β-ethoxycarbonyl groups are also inequivalent. Such inequivalence has been observed both in ^{13}C and ^1H NMR spectra of all of these products except the tricarboxy alcohol complex **2** (see Experimental Section). With the monoester complexes **7** and **8**, both the α-and β-carbons are chiral, and separate ^{13}C NMR signals due to each pair of enantiomers were apparent (see Experimental Section). In all of the complex ions, the coordinated carboxylate gave a single ^{13}C NMR resonance 7–13 ppm downfield from the signal(s) of the β-carboxyl groups, and its assignment is not ambiguous. The tentative assignments given in the Experimental Section for the α- and β-carbons (^{13}C) and protons (^1H) in some of the complexes are based on predicted chemical shifts and the effect of the variations in the α substituents.

Decarboxylation of β-Carboxyaspartic Acid. The decarboxylation of **6** in 1 M HCl was followed by ^{13}C NMR spectrometry (Figure 1[24]). The ^{13}C NMR spectrum of **6**, freshly dissolved in HCl, showed resonances at δ 102.4 102.7, 103.0 (inequivalent COOH), −15.0, and −15.9 (α- and β-CH) from dioxane (Figure 1A[24]). When this solution was heated at 80 °C, gas (CO_2) was slowly evolved over 3 h, and the final product had a spectrum (Figure 1C,[24] δ 104.2, 106.7, −17.1, −32.7) indistinguishable from that of authentic aspartic acid (Figure 1D,[24] δ 104.1, 106.6 (inequivalent −COOH), −17.1 (α-CH), −32.8 (β-CH_2)) under identical conditions. A spectrum recorded after 30 min at 80 °C (Figure 1B[24]) showed resonances assignable only to Asa or Asp and suggests that the decarboxylation of Asa has a half-life of ~20 min at 80 °C in 1 N HCl. Similar values have been reported by other workers.[10,11]

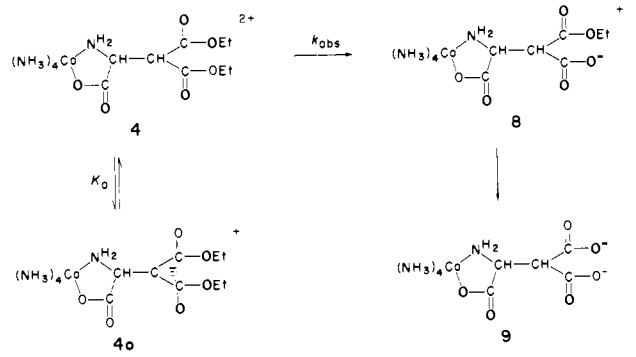
The product was further characterized by amino acid analysis and by high-voltage paper electrophoresis at pH 3.18.[6] Both techniques indicated the presence of a single ninhydrin-sensitive component, whose mobility was in each case consistent with its formulation as a tricarboxylic amino acid (by comparison with authentic γ-carboxyglutamic acid). Its mobility was also similar to that of the unidentified component of alkaline hydrolysates of the coral and teeth proteins.[6] Work to establish unambiguously the identity of the unknown amino acid (or dipeptide) is currently in progress.[5,6]

Resolution of the $[(NH_3)_4\overline{CoOOCCH(NH_2)}CH(COOCH_2CH_3)_2]^{2+}$ Ion. The enantiomers of the [(diethyl β-carboxyaspartato)tetraamminecobalt(III)]$^{2+}$ ion (**4**) were simply resolved by fractional crystallization of the diastereomeric salts with sodium $(+)_D$-antimony tartrate or by ion-exchange chromatography on CM-Sephadex C-25 using sodium $(+)_D$-antimony tartrate as eluant. The $(-)_D$-$[(NH_3)_4\overline{CoOOCH(NH_2)}CH(COOCH_2CH_3)_2]^{2+}$ ion crystallized as by far the least soluble isomer and could be isolated in optically and analytically pure condition very readily. The more soluble $(+)_D$ isomer was obtained by chromatography. The two enantiomers separated completely on short columns of CM-Sephadex, and each gave a single band under the same conditions on rechromatography.

The rotatory dispersion spectra of the two enantiomers (2 ×

(24) Supplementary material.

Scheme II



10^{-3} M as the dithionate salts in water, Figure 2[24]) resolved by the two separate methods showed equal and opposite rotations at all wavelengths. Extrema ($[M]_\lambda$, deg M^{-1} m^{-1}; for $(+)_D$ and $(-)_D$ isomers, respectively) occurred at 548 (+455, −463), 486 (−1530, +1530), 425 (+380, −380), and 370 nm (−21, +21).

The absolute configurations of the amino acid in these complexes have been tentatively assigned by comparison of the spectra in Figure 2[24] with similar reported spectra of (*R*)- and (*S*)-$[(NH_3)_4\overline{CoOOCCH(NH_2)}CH_3](SO_4)$[25] and of unresolved Δ,Λ-(*S*)-$[(en)_2\overline{CoOOCCH(NH_2)}CH_2C_6H_5]I_2$.[26] On the basis of the signs of the strong rotations in the region of the first ligand field band (~490 nm), the $(-)_D$-$[4]^{2+}$ ion has been assigned the *R*, and its enantiomer the *S* configuration.

We attempted to confirm these stereochemical assignments by examining the properties of derivatives of the enantiomers of **4**. The ester functions of a sample of the $(+)_D$ isomer were hydrolyzed at pH 12 (25 °C, 30 min) as described for the racemic complex (Experimental Section). The analytically pure dihydrate of **9** obtained after neutralization and recrystallization showed no optical activity (300–600 nm, using 2×10^{-3} M solutions in 0.1 M HCl and a 5-cm cell), indicating that racemization at the α carbon of **4** must occur at a rate at least comparable with that of ester hydrolysis at pH 12. Although proton exchange at the acidic α carbon of chelated amino acid complexes of Co(III) is well-known, we had anticipated that deprotonation of **4** at the more acidic β-carbon (see below) might have slowed it sufficiently to enable isolation of optically active (β-carboxyaspartato)tetraamminecobalt(III) (**9**).

A sample of the $(-)_D$ isomer of **4** was reduced with zinc at pH 6.0 ± 0.1, and the product was saponified as described for the isolation of racemic calcium β-carboxyaspartate (Experimental Section). The calcium salt of **6** obtained in good yield and analytically pure after two recrystallizations showed essentially no optical activity (300–600 nm, using a 0.1 M solution in 1M HCl and a 10-cm cell). After the solution had been heated at 80 °C for 2 h, during which time CO_2 was evolved, the aspartic acid produced showed no optical activity. Under the same conditions (0.1 M in 0.8 M HCl, 0.1 M $CaCl_2$), authentic (*S*)-aspartic acid (Sigma Chemical Co.) gave $[\alpha]_D$ +0.336°, corresponding to $[M]_D$ +336 (lit.[27] $[M]_D$ +338 in 5 M HCl). Apparently, the diethyl ester of Asa racemizes completely under the conditions used to reduce the complex **4** or in base during the subsequent ester hydrolysis. Although it may be possible to devise alternative routes to the optically active free amino acid, this aspect is yet to be pursued further.

Kinetic Studies. $[(NH_3)_4\overline{CoOOCCH(NH_2)}CH(COOCH_2CH_3)_2](ClO_4)_2 \cdot H_2O$ in Aqueous Base. The chelate complex of the diethyl ester of β-carboxyaspartic acid (**4**) reacted under basic conditions to produce only the two possible products of hydrolysis of its ester functions, the monoester **8** and the dicarboxylate **9** (Scheme II). Aqueous solutions of **4** underwent an immediately reversible color change from pale orange to intense yellow on treatment with NaOH. The electronic absorption spectrum of the immediate product had $\lambda_{max} \sim 360$nm, $\epsilon_{360} = 2.26 \times 10^3$ M^{-1} cm^{-1} (in 0.15 M NaOH). The color faded slowly with time as **4** reacted to give **8** and subsequently **9**. The intense absorption by **4** near 360 nm in base arises as a result of deprotonation, presumably at the most acidic β-CH group to produce the resonance-stabilized carbanion **4a** (Scheme II). The variation of ϵ_{360} of **4** over a range of pH values was obtained by extrapolation of kinetic data to zero time (Figure 3A). It showed a single sigmoidal curve which could be fitted to a dependence on a single acid dissociation, $pK_a' = 9.81 \pm 0.01$. The first product of ester hydrolysis **8** contains a free β-carboxylate substituent, the proximity of which to the β-CH group raises the pK_a' of the complex substantially above that of **4**. Since it does not deprotonate at pH values less than 13, **8** absorbs only weakly at 360 nm under basic conditions. The intense absorption by the conjugate base of **4** in contrast with that by all other substrate and product complexes (generally $\epsilon_{360} < 80$ M^{-1} cm^{-1}) greatly simplified the kinetic analyses.

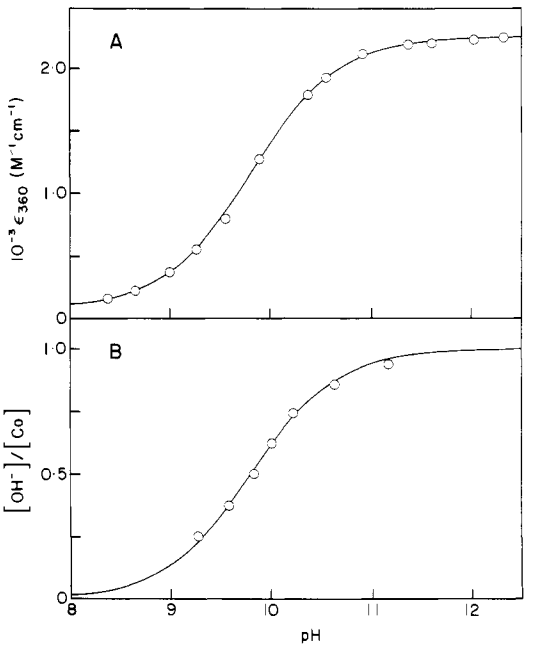


Figure 3. Titrations of the [(diethyl 3-carboxyaspartato)tetraamminecobalt(III)]$^{2+}$ ion at 25.0 °C, μ = 1.0 M (KCl). (A) Spectrophotometric titration. Values of ϵ_{360} in buffers (see Figure 4) were obtained by extrapolation of kinetic data to zero time. The solid curve was calculated assuming limiting values of ϵ_{360} of 81.4 (low pH) and 2260 M^{-1} cm^{-1} (high pH), and $pK_a' = 9.81$. (B) Titration with NaOH (see Experimental Section). The solid curve was calculated for the ultimate uptake of 1.0 equivalent of base, and $pK_a' = 9.80$.

The (ester) hydrolysis of **4** (monitored at 360 nm) in aqueous buffers followed strictly first-order kinetics over the pH range 8.3–12.2. Although the first product **8** subsequently hydrolyzed to **9** under these conditions, the change in A_{360} that accompanied this process was negligible. The pseudo-first-order rate constants (k_{obsd}) showed a first-order dependence on $[OH^-]$ at pH <8.5 but deviated from this behavior at higher pH (Figure 4). The simplest mechanism that accounts for an approach to a limiting rate at high pH is one in which only the protonated species (**4**, Scheme II) is reactive, undergoing hydrolysis by a reaction first order in $[OH^-]$. The rate law has the form of eq 3, where k_3 is a second-order rate constant, K'_w is the ion product of water, and K_a is the acid dissociation constant of **4**. Data from experiments at pH <10 fitted well to eq 3 with $k_3 = 7.5$ M^{-1} s^{-1}, $pK_a = 9.78$, but values of k_{obsd} at higher pH were substantially greater than those

$$k_{obsd} = \frac{k_3 K'_w [OH^-]}{K'_w + K_a [OH^-]} \quad (3)$$

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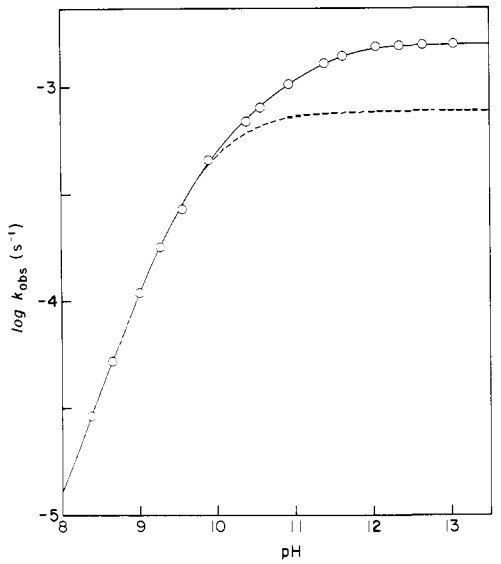


Figure 4. pH Dependence of k_{obsd} for the ester hydrolysis of the [(diethyl 3-carboxyaspartato)tetraamminecobalt(III)]$^{2+}$ ion at 25.0 °C, μ = 1.00 M (KCl). The buffers used were 0.05 M tris(hydroxymethyl)aminomethane–HCl (pH 8.37, 8.64), 0.05 M diethanolamine–HCl (pH 8.99, 9.26, 9.55, 9.89), 0.05 M triethylamine–HCl (pH 10.37, 10.55, 10.92, 11.38, 11.61), and 0.025, 0.05, 0.10 and 0.25 M NaOH. The broken curve was calculated by using eq 3, with k_3 = 7.51 M^{-1} s^{-1}, K_a = 1.66 × 10^{-10}, and K_w' = 1.71 × 10^{-14}. The solid curve was calculated by using eq 4, with α = 1.30 × 10^{-6}, β = 1.58 × 10^{-3}, γ = 1.76 × 10^{-7}, and δ = 1.92 × 10^{-3}, assuming K_w' = 1.71 × 10^{-14}.

calculated (Figure 4, broken curve).[28]

The simplest rate law that gives an adequate fit to the data has the form of eq 4. Data were fitted to eq 4 by computer with a

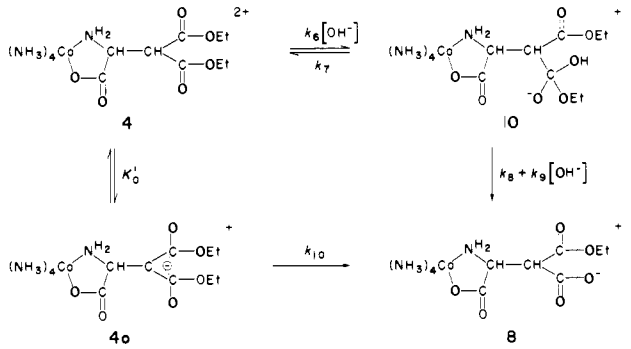
$$k_{obsd} = \frac{\alpha[OH^-] + \beta[OH^-]^2}{\gamma + \delta[OH^-] + [OH^-]^2} \quad (4)$$

least-squares program to give values of the four parameters: α = (1.30 ± 0.11) × 10^{-6}, β = (1.58 ± 0.01) × 10^{-3}, γ = (1.76 ± 0.18) × 10^{-7}, and δ = (1.92 ± 0.10) × 10^{-3} (Figure 4, solid curve). Mechanisms which give rise to rate laws of the form of eq 4 are discussed below.

The simplest model involves two sequential acid dissociations of **4** (pK_1, pK_2), where both the fully protonated and monodeprotonated forms undergo reactions first order in [OH^-] while the doubly deprotonated form is essentially unreactive. The derived rate law has the form of eq 4,[29] giving pK_1 = 9.73 and pK_2 = 11.05 (μ = 1.0 M, 25 °C). However, such a mechanism is not chemically reasonable. The substrate **4** has only two protons (the α- and β-methyne protons) that could conceivably dissociate in the pH range studied. Dissociation of either produces a carbanion, which ought to result in an increase in the pK_a' of the neighbouring methyne of 3 or more units. It is not possible, therefore, for the pK_a' values of **4** and its conjugate base to differ by as little as 1.3 units. Further, the data in Figure 3A would require that ϵ_{360} of the monodeprotonated species and its conjugate base be essentially identical, which would be a surprising coincidence. Finally, titration of **4** with NaOH (Figure 3B) clearly shows the uptake of only 1 equiv of base and a clean dependence on a single pK_a' of 9.8.

All of the data are consistent with the general mechanism shown in Scheme III, which involves a single acid dissociation of **4**, and the existence of an intermediate tetrahedral species **10**. Assuming

Scheme III



a steady-state concentration of **10** (i.e., k_7 and $k_8 + k_9[OH^-] >> k_6[OH^-]$), the derived rate law for the general mechanism has the form of eq 4, with $\alpha = [(k_6k_8K_w/K_a) + k_{10}(k_7 + k_8)]/k_9$, $\beta = k_{10} + (k_6K_w/K_a)$, $\gamma = (k_7 + k_8)K_w/k_9K_a$, and $\delta = K_w/K_a + (k_7 + k_8)/k_9$. Since it is sufficient that only one of the rate constants k_8 and k_{10} be greater than zero, the rate law can be simplified assuming either k_8 = 0 (mechanism 1) or k_{10} = 0 (mechanism 2).

Mechanism 1. For the mechanism given in Scheme III, assuming k_8 = 0, the parameters in eq 4 reduce to $\alpha = k_7k_{10}/k_9$, $\beta = k_{10} + (k_6K_w/K_a)$, $\gamma = k_7K_w/k_9K_a$, and $\delta = K_w/K_a + k_7/k_9$. Numerical substitution gives k_6 = 8.9 M^{-1} s^{-1}, k_{10} = 7.1 × 10^{-4} s^{-1}, k_7/k_9 = 1.82 × 10^{-3} M, and pK_a' = 9.76.

Mechanism 2. For the mechanism described by Scheme III, assuming k_{10} = 0, the parameters of eq 4 simplify to $\alpha = k_6k_8K_w/k_9K_a$, $\beta = k_6K_w/K_a$, $\gamma = (k_7 + k_8)K_w/k_9K_a$, and $\delta = K_w/K_a + (k_7 + k_8)/k_9$, whence k_6 = 16.3 M^{-1} s^{-1}, k_7/k_8 = 1.21, k_7/k_9 = 1.00 × 10^{-3} M, and pK_a' = 9.76.

Although both of the simpler mechanisms 1 and 2 are fully consistent with the kinetic data and both give values for the pK_a' of **4** in excellent agreement with those determined spectrophotometrically and by titration with NaOH (Figure 3), mechanism 2 appears to be the more chemically reasonable. Mechanism 1 requires that the pH-independent hydrolysis of the conjugate base of **4** be much faster than that of the parent acid. There is no simple reason why this should be so; the expectation would be that deprotonation deactivate the ester toward nucleophilic attack by H_2O. On the other hand, mechanism 2 requires only that a tetrahedral adduct (**10**) exist and that it collapse to products via two separate pathways, one independent of pH and one showing a first-order dependence on [OH^-] (i.e., second-order overall in [OH^-]). As pointed out by Jencks,[30] both the formation of the adduct and the appearance of a term second order in [OH^-] are expected in the hydrolysis of an ester containing a strongly electron-withdrawing acyl group and a poor leaving group (e.g., **4**). A further expectation in such a case is that in the limit at low pH where the second-order term for breakdown of the intermediate becomes insignificant (i.e., $k_8 >> k_9[OH^-]$), the rate of reversion of the tetrahedral species to reactants (resulting in water exchange) ought to become significant in comparison with its rate of collapse to products (resulting in hydrolysis). In the present case, the ratio of the rates of water exchange to hydrolysis approaches 1.2 in this limit (i.e., k_7/k_8 = 1.2). The only other example of exchange being faster than hydrolysis in an analogous reaction[30] is the alkaline hydrolysis of the $[(NH_3)_5CoO_2CCF_3]^{2+}$ ion,[31] in which the acyl moiety is activated by the CF_3 substituent, and the leaving group ($[(NH_3)_5CoO]^+$) is exceedingly poor. This reaction is also thought to proceed through a tetrahedral intermediate, and the rate law also shows a term second order in [OH^-].

One further aspect of the ester hydrolysis of **4** warrants comment. The distribution of the products (monoester **8** and dicarboxylate **9**) from reactions carried out under pH-stat conditions ($\mu \approx 0$, 25 °C, pH 9.0 and 12.0) for ~10 half-lives also showed

(28) This difference does not arise as a result of general base catalysis by the buffer since the value of k_{obsd} in 0.20 M triethylamine–HCl, pH 11.17, μ = 1.0 M (1.08 × 10^{-3} s^{-1}), was similar to that obtained in 0.05 M triethylamine buffer at the same pH.

(29) For this model the parameters of eq 4 are given by $\alpha = k_4K_w/K_1K_2$, $\beta = k_5K_w/K_2$, $\gamma = (K_w)^2/K_1K_2$, and $\delta = K_w/K_2$, where k_4 and k_5 are second-order rate constants for the OH^--catalyzed hydrolysis of a fully protonated and a monodeprotonated form of **4**, respectively.

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Table II. Product Distributions and Second-Order Rate Constants for the Intramolecular Cyclization of $[(NH_3)_5CoOOCCH{=}C(COOCH_2CH_3)_2](PF_6)_2$ in 0.05 M Triethylamine-HCl Buffers, μ = 1.0 M (KCl) at 25.0 °C

pH	ϵ_B,[a] M^{-1} cm^{-1}	ϵ_4,[b] M^{-1} cm^{-1}	$[4]/[3]_0$[c]	10^2k_f,[d] s^{-1}	10^2k_{11},[e] s^{-1}	$k_{11}/[OH^-]$,[f] M^{-1} s^{-1}
10.27	1134	1654	0.670	0.29_7	0.19_9	6.25
10.58	1401	1925	0.716	0.56_7	0.40_6	6.24
10.98	1569	2120	0.730	1.44	1.05	6.45
11.36	1631	2188	0.736	3.3_7	2.4_8	6.33
11.64	1656	2226	0.734	6.4_3	4.7_2	6.32
						6.32 ± 0.08

[a] Collective ϵ_{360} of the intermediate species in the sequential reaction pathway (see Experimental Section). [b] ϵ_{360} Of authentic 4-$(ClO_4)_2\cdot H_2O$ under identical conditions. [c] Ratio of [4] produced in the first step to the initial [3], determined as described in the text. [d] Separated first-order rate constant for the faster step in the sequential reaction pathway of 3-$(PF_6)_2$ (see the text and Table I[24]). [e] Pseudo first-order rate constant for the intramolecular cyclization reaction (3 → 4). [f] Determined by using $K'_w = 1.71 \times 10^{-14}$ in 1 M KCl (see Experimental Section).

a dependence on pH. At the lower pH, the monoester **8** predominated, indicating that k_{obsd} for the second ester hydrolysis (i.e., **8** → **9**) was less than that for the first step under these conditions. At high pH, k_{obsd} for the reaction **4** → **8** becomes independent of pH (Figure 4), its value becomes less than that for the subsequent reaction (**8** → **9**), and **9** is the major product observed. Assuming a strict first-order dependence of k_{obsd} for the second reaction on $[OH^-]$ (expected since **8** does not deprotonate at pH <13), then the second-order rate constant (k_{OH}) for this process is thus estimated to be >0.05 M^{-1} s^{-1} (cf. k_6 = 16.3 $M^{-1}s^{-1}$, mechanism 2, Scheme III). In comparison, the ratio of the second-order rate constants for the two steps in the alkaline hydrolysis of unsubstituted diethyl malonate in 50% aqueous Me_2SO has been reported to be 47 at 35 °C.[32]

$[(NH_3)_5CoOOCCH{=}C(COOCH_2CH_3)_2](PF_6)_2$ in Aqueous Base. The monodentate olefin complex **3** reacted rapidly in aqueous base to produce, in addition to several weakly colored byproducts (see above), the chelated amino acid diester complex **4** (in equilibrium with its intensely absorbing conjugate base **4a**). The color of the product solution then faded more slowly as **4** hydrolyzed to **8** and **9** as described above. This behavior is illustrated by the variation in A_{360} during the course of such a reaction, showing a rapid increase to a maximum value followed by a slower decrease (Figure 5[24]). These kinetic data were consistent with consecutive first-order reactions (eq 1). Computer analysis (see Experimental Section) gave values of the two first-order rate constants (k_f and k_s, eq 2) at each of several pH values (in 0.05 M triethylamine–HCl buffers, μ = 1.0 M (KCl), 25.0 °C, Table I[24]). The values of the lesser rate constant (k_s) compared closely with the independently measured values of the first-order rate constants for the ester hydrolysis of authentic $[4](ClO_4)_2\cdot H_2O$ under identical conditions (k(**4**), Table I,[24] and Figure 5[24]). These comparisons, together with the consistent product distribution studies reported below, establish that k_f refers to the first reaction (intramolecular addition, **3** → **4** and byproducts) and k_s to the second (ester hydrolysis, **4** → **8**).

Computer analyses of the raw data gave, in addition to the rate constants, values for the collective molar absorption coefficients ϵ_B of the products of the first phase of the reactions of **3** (see Experimental Section, eq 1 and 2) and of authentic **4** (ϵ_4, zero-time intercept in the reactions of **4**) under identical conditions (Table II). Under all circumstances, since **4** was not the sole product of the first reaction ϵ_B was less then ϵ_4. Since **4** was the only intensely absorbing product, these data could be used to determine the distribution of products of the first reaction. Assuming all other such products had $\epsilon_{360} \approx 80$, then the concentration of **4** produced, [**4**], relative to the initial concentration of **3**, $[3]_0$ (Table II), is given by the relationship $[4]/[3]_0 = (\epsilon_B - 80)/(\epsilon_4 - 80)$. Assuming the first reaction involves parallel first-order processes, one producing **4** (k_{11}) and the other giving rise to the various byproducts (k_{12}), then the first-order rate constant for the cyclization reaction is given by $k_{11} = k_f[4]/[3]_0$ (Table II).

The values of k_{11} determined at the various pH values were directly proportional to the concentration of hydroxide ion, consistent with a rate law of the form of eq 5, where the second-order rate constant is k_{13} = 6.3 ± 0.1 M^{-1} s^{-1} (Table II).

$$k_{11} = k_{13}[OH^-] \quad (5)$$

Table III. Product Distributions for the Reactions of $[(NH_3)_5CoOOCCH{=}C(COOCH_2CH_3)_2](PF_6)_2$ in 0.05 M Triethylamine-HCl Buffers, μ = 1.0 M (KCl) at 25 °C[a]

		$([4]/[3]_0)_t$[c]		
pH	time, s[b]	obsd (NaCl)[b]	obsd (NaOH)[e]	calcd[f]
10.27	1400	0.47	0.41	0.37
10.58	730	0.53	0.48	0.46
10.98	290	0.62	0.59	0.57
11.36	120	0.69	0.64	0.65
11.64	65	0.70	0.67	0.68

[a] Products were separated chromatographically as described (see Experimental Section). [b] Time at which the reactions were quenched (~6 half-lives of first reaction). [c] Apparent concentrations of 4 at time of quenching relative to intial concentration of 3. [d] Deduced from ϵ_{494} of the fraction (in 0.3 M NaCl) containing 2+ products, corrected for residual (~1.5%) 3 (see Experimental Section). [e] Deduced from ϵ_{360} of a 1:2 dilution of the fraction (in 0.3 M NaCl) containing 2+ products with 0.3 N NaOH (see Experimental Section). [f] Calculated $([4]/[3]_0)_t$ at time of quenching using values of k_f and k_s (Table I[24]) and $[4]/[3]_0$ (Table II).

Since the kinetic analysis given above depends on the reliability of the estimates of $[4]/[3]_0$, product distribution studies were carried out. Reactions of the hexafluorophosphate salt of [**3**] in the buffers used for the kinetic studies were quenched after six half-lives of the first (k_f) step, and **4** and other 2+ products were separated from 1+ products by cation-exchange chromatography. The 2+ products were quantified spectrophotometrically with solutions in 0.3 M NaCl (approximating the total concentration of 2+ products, including **4**) or in 0.15 N NaOH–0.15 M NaCl (giving [**4**]). The values obtained (Table III) for the amount of **4** present at the time of quenching were in satisfactory agreement with those calculated from the values of k_f, k_s, and the ratio $[4]/[3]_0$ given in Tables I and II. The consistency of these results therefore indicates that the kinetic analyses given above are reliable.

Intramolecular nucleophilic addition of a cis-coordinated ammine to an olefin has previously been observed with coordinated maleate esters. Treatment of the $[(NH_3)_5CoOOCCH{=}CHCOOCH_3]^{2+}$ ion with aqueous base results in the formation of the chelate aspartatotetraammine complex in ~50% yield, the other products being the ester-hydrolyzed [(maleato)pentaamminecobalt(III)]$^+$ ion and cobalt oxides.[21] The observed first-order rate constant for the intramolecular reaction in 0.1 N NaOH at 25.0 °C was $\sim 4 \times 10^{-3}$ s^{-1}, about 2 orders of magnitude less than that calculated for the analogous reaction in the present system. This difference reflects the additional activation conferred by the extra alkoxycarbonyl substituent on the β-carbon.

Other examples of intramolecular cyclization reactions involving cis aminate ions as nucleophiles in pentaamminecobalt(III) complexes include carbinolamine and imine formation from (α-

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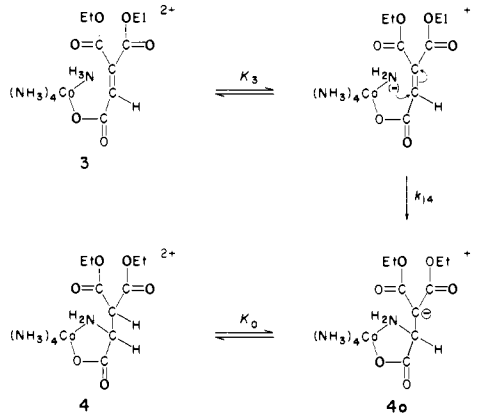
Scheme IV



ketocarboxylato)pentaamminecobalt(III) complexes[33,34] and the intramolecular ester aminolysis of the $[(NH_3)_5CoNH_2CH_2COOCH_2CH_3]^{3+}$ ion to produce the N,N′-chelated glycine amide complex.[35] Moreover, one of the reaction paths for the alkaline hydrolysis of the [(4-nitrophenyl phosphato)pentaamminecobalt(III)]$^+$ ion involves the intermediate formation of an N,O-chelated phosphoramidate complex by nucleophilic attack of a cis aminate ion on the phosphorus atom.[36]

In each of these cases, the intramolecular nucleophilic addition reaction shows a first-order dependence on [OH^-]. This has been invariably interpreted in terms of mechanisms involving a deprotonated cis ammine (i.e., a coordinated aminate ion) as the reactive nucleophile. In the present system, the detailed mechanism would be as given in Scheme IV, for which the rate law is given by eq 6. Values of the acid dissociation constant K_3 for

$$k_{obsd} = k_{11} = k_{14}K_3[OH^-]/(K_3[OH^-] + K_w) \qquad (6)$$

cis deprotonation in simple pentaamminecobalt(III) complexes are generally estimated to be $<10^{-16}$,[37] so that at pH <12, $K_w >> K_3[OH^-]$ and $k_{11} = k_{14}K_3[OH^-]/K_w$. This equation has the form of eq 5, with $k_{13} = k_{14}K_3/K_w$. Since $K_w/K_3 > 100$, k_{14} is estimated to have a value in excess of 600 s^{-1}.

In this paper we have presented novel synthetic routes to authentic β-carboxyaspartic acid (Scheme I), a new amino acid which is currently being examined as a possible constituent of various calcified tissues.[5,6] The key intermediate in the synthetic scheme is the olefin complex **3**, $[(NH_3)_5CoOOCCH{=}C(COOCH_2CH_3)_2]^{2+}$. It reacts with the solvent in liquid ammonia to form the monodentate amino acid complex **5**, and in aqueous base it produces the chelated amino acid complex **4**. The free amino acid can be readily recovered from either **4** or **5** by reduction, followed by saponification.

The kinetics of the intramolecular reaction of **3** in aqueous buffers show a first-order dependence on [OH^-] consistent with the cis-coordinated aminate ion being the reactive intramolecular nucleophile (Scheme IV). Under the basic conditions, **4** is susceptible to subsequent ester hydrolysis. This reaction, as expected for such an ester activated by a strongly electron-withdrawing acyl substituent,[30] proceeds through a tetrahedral *gem*-diol intermediate species (Scheme III) which can either collapse by elimination of ethanol (ester hydrolysis) or revert to reactants (water exchange). It is apparently only the second example of a reaction of this type (and the first of a true carboxylic ester hydrolysis) for which the rate of water exchange exceeds the rate of hydrolysis.

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Registry No. **1**-$(ClO_4)_2$, 43227-15-2; **2**-$(CF_3SO_3)_2$, 83311-83-5; **3**-$(PF_6)_2$, 83311-85-7; **4**-(S_2O_6), 83311-87-9; **4**-$(ClO_4)_2$, 83311-88-0; **4**-Cl_2, 83311-95-9; $(-)_D$-**4**-$((+)_D$-$(C_4H_2O_6Sb)_2)$, 83376-20-9; $(-)_D$-**4**-(S_2O_6), 83376-21-0; $(+)_D$-**4**-(S_2O_6), 83376-23-2; **5**-$(ZnCl_4)Cl$, 83311-90-4; **6**, 83292-16-4; **7**, 83311-91-5; **8**-Cl, 83311-92-6; **9**, 83311-93-7; **9**-$(HCl)_2$, 83311-94-8; $[(NH_3)_5CoOH_2](ClO_4)_3$, 13820-81-0; Co, 7440-48-4; sodium glyoxylate, 2706-75-4; glyoxylic acid, 298-12-4; diethyl malonate, 105-53-3; β-carboxyaspartic acid, 75898-26-9.

Supplementary Material Available: Table of kinetic data and spectra (Figures 1, 2, and 5) (5 pages). Ordering information is given on any current masthead page.

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