Cobalt(III)-Promoted Syntheses of the Amino Acids (*RS*)-2-Cyclopropylglycine and (*R*)- and (*S*)-Proline

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Abstract: The intramolecular imine formation between a coordinated aminate ion and a 2-keto acid has been utilized to synthesize the two racemic amino acids 2-cyclopropylglycine and proline. The anation of the aquapentaamminecobalt(III) ion by the carboxylate of 5-bromo-2-oxopentanoic acid at pH 5 results in two major products, in comparable yield, the 2-oxo-2-cyclopropylethanoato complex I and the 5-hydroxy-2-oxopentanoato complex II. Both are converted to tetraammine–iminocarboxylato chelates by attack of an adjacent deprotonated ammonia. The cyclopropylimine complex was reduced to its corresponding amino acid complex by alkaline borohydride ion. The hydroxypentanoatoimine complex was rebrominated at position 5 and then rapidly cyclized to a tetraammine–pyrrolinecarboxylate complex that was reduced also by alkaline borohyride to the prolinato complex. The kinetics of cyclization of the tetraammine(5-bromo-2-iminopentanoato)cobalt(III) ion to the corresponding pyrroline complex were followed in the range pH 9–12.5. The chelated proline complex was also resolved by using the antimony tartrate ion (+)-$[(SbC_4H_4O_6)_2]^{2-}$.

Introduction

In previous publications,[2] investigations of synthetic utility were foreshadowed arising from intramolecular imine formation reactions between an ammonia or an amine and a 2-keto carboxylate mutually coordinated to cobalt(III). Other papers have illustrated some related condensations.[3] In this paper, the syntheses of the racemic amino acids (*RS*)-proline and (*RS*)-2-cyclopropylglycine via such intramolecular imine condensations are described along with the resolution of the proline complex.

A considerable number of routes for the synthesis of proline have been reported,[4] including a synthesis of the chiral form,[5] by reducing the inexpensive pyro-(*S*)-glutamic acid to optically pure (*S*)-proline in good yield. A simple synthesis of the racemate has also been reported recently.[6] Various synthetic routes to substituted prolines by the use of Michael additions of acrylic compounds to (*N*-pyruvylideneglycinato)copper(II) have also been described.[7]

The present work illustrates the ability of complexes of 2-keto carboxylates to influence the reactivity and thereby direct the syntheses of the ligands. The kinetic robustness of the complexes of cobalt(III) with respect to exchange of ligand ammines and coordinated carboxylate imines is also a key factor in the syntheses.

Experimental Section

Reagent grade reagents were used for all syntheses generally without further purification. Diethyl oxalate and butyrolactone were obtained from May & Baker and EGA, respectively. Visible spectra (ϵ, M^{-1} cm^{-1}) were recorded by using Cary 14 or 118C recording spectrophotometers. The ^1H NMR spectra were recorded at 100 MHz by using a JEOL JNM-MH-100 Minimar spectrometer at 25 °C with an external lock in D_2O or dimethyl-d_6 sulfoxide (Me_2SO-d_6) with sodium 4,4-dimethyl-4-silapentanesulfonate (DSS) as the internal standard. The ^{13}C NMR spectra were recorded at 25 °C in H_2O by using a JEOL FX-60 spectrometer at 15.04 MHz with an external lock (D_2O) and 1,4-dioxane as internal standard. All signals are referenced to tetramethylsilane with δ 67.4 for dioxane, and the chemical shifts (δ) are in ppm. The signals are recorded as singlets (s), doublets (d), triplets (t), or multiplets (m). Rotatory dispersion measurements were made with a Perkin-Elmer P22 spectrometer.

All pH measurements were made at 25.0 ± 0.05 °C by using a Radiometer pH meter 26 with a G 202 B electrode and a saturated calomel electrode connected by a salt bridge 1.6 M NH_4NO_3 + 0.2 M $NaNO_3$ at pH 7.00.

All evaporations were carried out in Büchi evaporators under reduced pressure (~20 mmHg) with a solution temperature ≤25 °C.

Kinetic measurements were made in aqueous solution at 25.0 ± 0.05 °C and an ionic strength of 1.0 M with $NaClO_4$ as background electrolyte. The spectrophotometric data were obtained by using either a Cary 118C spectrometer coupled with a hand-driven stopped-flow reactor ($t_{1/2}$ down to 1.5 s) or a Durrum-Gibson stopped-flow spectrophotometer for shorter half-lives. At 400 nm deprotonated imines have a high absorptivity (ϵ ~600), whereas the pyrroline complex has an ϵ of ~13 in 1 M $NaClO_4$. Solutions of $[(NH_3)_4\overline{CoO_2C(C{=}NH)}(CH_2)_3Br]Cl_2$ were freshly prepared for each kinetic determination by dissolution in 1 M $NaClO_4$ solution. Within 20 min this solution was mixed by the stopped-flow technique with an equal volume of buffer containing background electrolyte. The buffers used are listed at the base of Figure 3. Pseudo-first-order rate plots (log ($A_\infty - A_t$) vs. t) were linear for at least 3 half-lives. The values of A_∞ were taken after the reaction had proceeded for 7 or 8 half-lives. Above pH 12.0, there was some evidence of a concurrent decomposition reaction, but it was much slower and did not significantly affect the A_∞ values.

5-Bromo-2-oxopentanoic acid was prepared from α-ethoxalylbutyrolactone by the method of Öhler and Schmidt.[8] The α-ethoxalylbutyrolactone was prepared by the methods of Plieninger[9] and of Ksander et al.,[10] the latter being superior and yielding a product that slowly crystallized at room temperature. The hygroscopic 5-bromo-2-oxopentanoic acid (BKPA) was more conveniently handled as its lithium salt (LiBKPA), prepared by adding LiOH (1 M) dropwise to an aqueous solution of BKPA (6.5 g in 20 mL of H_2O) with stirring until a pH of 3.0 was achieved. A white bulky precipitate formed, which was collected and washed with ice cold water then several times with methanol. The off-white semicrystalline product analyzed as a monohydrate. Anal. Calcd for $C_5H_6BrLiO_3 \cdot H_2O$: C, 27.4; H, 3.7; Br, 36.5. Found: C, 27.6; H, 3.9; Br, 35.9.

Pentaammine(2-cyclopropyl-2-oxoethanoato)cobalt(III) perchlorate (I) and pentaammine(5-hydroxy-2-oxopentanoato)cobalt(III) perchlorate (II) were synthesized from $[(NH_3)_5CoOH_2](ClO_4)_3$ and 5-bromo-2-oxopentanoate ion in aqueous solution at an elevated temperature. The pH of the reaction mixture markedly influenced the product distribution (vide infra).

$[(NH_3)_5CoOH_2](ClO_4)_3$ (46 g) and LiBKPA (30 g) were suspended in water (600 mL) and warmed with stirring to 50–60 °C. The hydrophobic LiBKPA crystals were "wet" by the addition of several aliquots of ethanol (~25 mL total). When the ligand had mostly dissolved, solid

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Li_2CO_3 was added until the pH reached 5.0. The solution was heated and stirred at 50–60 °C a further 90 min (i.e., until visible spectral shifts had ceased, λ_{max} now being 501 nm). During this time some plates of $[(NH_3)_5CoO_2CC{=}OCH(CH_2)_2](ClO_4)_2$ crystallized. The solution was acidified to pH 2.0 with $HClO_4$ and cooled in ice overnight. The red plates resulting were collected and washed with ethanol and ether (I, 14.8 g).

The filtrate was evaporated to ~250 mL, and fractions (125 mL) were sorbed on a BIO-GEL P2 column (200–400 mesh, 9 × 24 cm prewashed with 0.01 M $HClO_4$). The column was eluted with 0.01 M $HClO_4$, and at least five fractions separated. The first two were very faint and were assigned spectroscopically as $[(NH_3)_5CoCO_3]^+$ and $[(NH_3)_5CoO_2CC{=}O(CH_2)_3Br]^{2+}$ ions. They also eluted with excess ligand. The third fraction was very intensely colored and NMR spectroscopy identified it as a mixture of $[(NH_3)_5CoO_2CC{=}O(CH_2)_3OH]^{2+}$ and $[(NH_3)_5CoO_2CC{=}OCH(CH_2)_2]^{2+}$ (minor component). The fourth fraction was $[(NH_3)_5CoBr]^{2+}$ and the fifth $[(NH_3)_5CoOH_2]^{3+}$.

The third fraction (F_3) was evaporated to 80 mL and rechromatographed on BIO-GEL P2 (9 × 24 cm) with 0.01 M $HClO_4$ as eluent. It separated into two distinct fractions, the first of which was $[(NH_3)_5CoO_2CC{=}O(CH_2)_3OH]^{2+}$ and the second the cyclopropyl complex. The former complex was very soluble and was therefore difficult to isolate quantitatively. Thus the bulk of F_3 was kept for the imine synthesis in situ. A small portion was, however, concentrated by evaporation and cooled in ice. The resulting fine red crystals were collected and washed with ethanol and ether. They were recrystallized from a minimum volume of water by adding ethanol to the filtrate and cooling the solution slowly. The crystals were collected and washed with methanol, ethanol, and ether and dried in vacuo over P_2O_5.

The crude compound I isolated above was dissolved in warm water (300 mL) and chromatographed on BIO-GEL P2 (150 mL per 9 × 24 cm column) with 0.01 M $HClO_4$ as eluent. The major fraction (I) was preceded by a very minor fraction of II and followed by a small fraction of bromopentaamminecobalt(III). The major fraction was evaporated, and copious crystallization of red plates occurred (yield 10.5 g) on cooling and adding $NaClO_4$. The complex was recrystallized from warm 0.01 M $HClO_4$ by cooling and adding $NaClO_4$. The resulting crystals were washed with methanol and ether and dried in vacuo.

Anal. Calcd for $[CoC_5H_{20}N_5O_3](ClO_4)_2$ (I): Co, 12.92; C, 13.16; H, 4.42; N, 15.36; Cl, 15.55. Found: Co, 12.8; C, 13.1; H, 4.6; N, 15.1; Cl, 15.6. UV–visible (λ_{max}, ϵ_{max} in H_2O) 500 nm (72), 286 (sh, 82); ^1H NMR in ~1 M D_2SO_4) δ 1.12 (m, cyclopropyl CH_2's, 4 H), 2.6 (m, cyclopropyl CH, 1 H), 2.86 (br, trans NH_3, 3 H), 3.88 (br, cis NH_3, 12 H); ^{13}C NMR δ 15.07 (cyclopropyl CH_2, 2 C), 18.83 (cyclopropyl CH), 86.24 (α-$C(OH)_2$), 173.31 (COOH).

Anal. Calcd for $[CoC_5H_{22}N_5O_4](ClO_4)_2{\cdot}H_2O$ (II): Co, 11.98; C, 12.20; H, 4.92; N, 14.23; Cl, 14.41. Found: Co, 12.1; C, 12.1; H, 4.7; N, 14.3; Cl, 14.8. UV–visible (λ_{max}, ϵ_{max} in H_2O) 500 nm (71), 230 (sh, 4.20×10^4); ^1H NMR (in D_2O) δ 2.04 (m, γ-CH_2, 2 H), 2.84 (rough t, β-CH_2, 2 H), 3.90 (t, δ-CH_2, 2 H) (in this case the trans-NH_3 deuterates rapidly in D_2O, allowing the CH_2 signal to be clearly seen. The cis-NH_3 groups deuterate more slowly in D_2O, and during this process the β-CH_2 groups also deuterate); ^{13}C NMR δ 25.07 (γ-CH_2), 36.75 (β-CH_2), 70.26 (δ-CH_2), 182.07 (COOH), 103.63 (α-$C(OH)_2$).

$[(NH_3)_4\overline{CoO_2CC(=NH)}CH(CH_2)_2]^{2+}$ (III). $[(NH_3)_5CoO_2CC{=}OCH(CH_2)_2](ClO_4)_2$ (3 g) was dissolved in warm water (45–50 °C, 120 mL), and to this vigorously stirred solution was added NaOH (1 M, 15 mL) in a fine stream from a pipette over a period of 20 s (any longer period resulted in substantial complex decomposition). An excess of 30% $HClO_4$ was then rapidly added, and crystals appeared. $NaClO_4$ was then added and the mixture cooled. The orange crystals were washed with ethanol and then ether (2.6 g).

Recrystallization (94%) was effected from warm water (50 °C, 90 mL) by using $NaClO_4$ and a few drops of 30% $HClO_4$. The orange crystals were washed as above and dried in vacuo. Anal. Calcd for $[CoC_5H_{18}N_5O_2](ClO_4)_2$: Co, 13.45; C, 13.71; H, 4.14; N, 15.99; Cl, 16.19. Found: Co, 13.7; C, 13.9; H, 4.3; N, 16.1; Cl, 16.3.

So that sufficient solubility for the recording of a ^{13}C spectrum could be obtained, the perchlorate complex (1.25 g) was converted to the chloride salt by passing it through a BIO-RAD AG 1-X8 resin (Cl-form, 10 cm × 3 cm). The column was eluted with warm water and the eluate (~150 mL) acidified (3 M HCl, 3 drops) and evaporated to 10 mL. On cooling this in ice the compound crystallized in needles (0.68 g). Anal. Calcd for $[CoC_5H_{18}N_5O_2]Cl_2{\cdot}H_2O$: C, 18.30; H, 6.15; N, 21.34; Cl 21.61. Found: C, 18.4; H, 6.4; N, 21.6; Cl, 21.8. UV–visible (λ_{max}, ϵ_{max} in 0.01 M $HClO_4$) 484 nm (87), 289 (1.49×10^3); ^1H NMR (in Me_2SO-d_6) δ 1.28 (m, cyclopropyl CH_2, 4 H), 2.26 (m, cyclopropyl CH, 1 H), 2.82 (br s, NH_3 trans to carboxyl, 3 H), 3.26 (br s, NH_3's axial to chelate, 6 H), 3.86 (br s, NH_3 trans to imine, 3 H), 11.43 (s, imine NH, 1 H); ^{13}C NMR δ 14.42 (cyclopropyl $(CH_2)_2$, m), 17.40 (cyclopropyl CH), 172.98 (C=N), 188.30 (COOH); IR $\nu_{C=N}$ 1682 cm^{-1}.

Reduction of $[(NH_3)_4\overline{CoO_2CC(=NH)}CH(CH_2)_2]^{2+}$ to $[(NH_3)_4CoO_2CCH(NH_2)CH(CH_2)_2]^{2+}$ (V). The imine perchlorate III (2.0 g) was dissolved in a minimum volume of water (150 mL, supersaturated at room temperature), and to this was added with stirring 1 M $Na_2CO_3/NaHCO_3$ buffer (1.5 mL, pH 10.0), whereupon the orange-red solution turned a deep red-brown. Finely ground $NaBH_4$ (BDH, LR, 1.0 g) was added with vigorous stirring. After 2 min, the red solution was diluted to 200 mL with water and rapidly sorbed with vacuum assistance on a short Dowex 50W × 2 column (Na^+ form, 5 × 3 cm). Excess BH_4^- was removed by copious washing with water (3 × 70-mL portions). Dilute hydrochloric acid (1 M, 3 × 50-mL portions) was passed through the column to remove Na^+, and no noticeable loss of complex occurred. The complex was then eluted with 3 M hydrochloric acid and the eluate evaporated to a small volume. Addition of ethanol until incipient crystallization and cooling gave the orange-red complex (1.1 g, 77%). This initial yield was slightly contaminated with the imine complex (shown by the UV–visible absorption spectrum and by NMR).

The complex was recrystallized by the same method, washed with ethanol and ether and dried in vacuo (V, 0.7 g). Anal. Calcd for $[CoC_5H_{20}N_5O_2]Cl_2$: Co, 18.88; C, 19.24; H, 6.96; N, 22.44; Cl, 22.72. Found: Co, 19.0; C, 19.5; H, 6.6; N, 22.5; Cl, 22.5.

The perchlorate salt was obtained by treating a dilute aqueous solution of the above complex with concentrated aqueous $AgClO_4$ until no further precipitation of AgCl occurred. Removal of AgCl, evaporation of the filtrate to a small volume, addition of ethanol, and cooling induced crystallization. The fine crystals (0.15 g) were recrystallized by dissolution in a minimum volume of water (5 mL) by adding ethanol (80 mL), some saturated aqueous $NaClO_4$ (~5 mL), and a drop of $HClO_4$. This cloudy suspension was warmed until clear and slowly cooled. The chunky red crystals that formed were washed with methanol and ethanol and dried in vacuo (0.11 g). Anal. Calcd for $[CoC_5H_{20}N_5O_2](ClO_4)_2$: Co, 13.39; C, 13.65; H, 4.58; N, 15.91; Cl, 16.11. Found: Co, 13.1; C, 13.8; H, 4.5; N, 16.2; Cl, 16.3. UV–visible (λ_{max}, ϵ_{max} in 0.01 M $HClO_4$) 491 nm (85), 347 (93); ^1H NMR (in D_2O (all NH deuterated)) δ 0.70 (br structured m, cyclopropyl CH_2, 4 H), 1.26 (br m, cyclopropyl CH, 1 H), 2.82 (d, α-CH); ^{13}C NMR δ 3.12 (cyclopropyl CH_2), 6.37 (cyclopropyl CH_2), 14.94 (cyclopropyl CH), 63.60 (α-CH), 185.70 (COOH).

$[(NH_3)_4\overline{CoO_2CC(=NH)}(CH_2)_3OH](ClO_4)_2$ (IV). To the vigorously stirred fraction (F_3 volume ~150 mL) from the previous preparation containing II was added NaOH (IM, 40 mL) from a pipette to pH 11.5–12.0. The solution, now a deep red-brown characteristic of the deprotonated imine, was stirred for 4 min and the reaction quenched by the addition of $HClO_4$ (30%) to pH 1.5 to give an orange solution. Upon the addition of excess $NaClO_4$, crystallization occurred slowly and was completed overnight in a refrigerator. The orange product was collected and washed with ethanol and ether and dried in air (yield 10.5 g). It was then dissolved in warm water and chromatographed on BIO-GEL P2 (20 cm × 7 cm) (0.01 M $HClO_4$ eluant). A minor red fraction followed the major orange complex fraction. The orange fraction was filtered, evaporated to ~7 mL, and cooled overnight in a refrigerator. Needlelike crystals grew in profusion with some clusters of rhombs also. The two forms were spectroscopically identical and behaved identically on SP Sephadex C-25 (Na^+ form, with 0.1 M $NaClO_4$ as eluant). The crystals were washed with methanol and ether. Evaporation of the filtrate to half its volume and seeding produced a second crop of crystals (also analytically pure), which were washed as above. Both crops were dried in vacuo over P_2O_5 (total yield 8.75 g). Anal. Calcd for $[CoC_5H_{20}N_5O_3](ClO_4)_2$: Co, 12.92; C, 13.17; H, 4.42; N, 15.36; Cl, 15.55. Found: Co, 12.8; C, 13.2; H, 4.7; N, 15.1; Cl 15.4. UV–visible (λ_{max}, ϵ_{max} in 0.01 M $HClO_4$) 485 nm (80), 347 (sh, 188×10^2), 292 (sh, 7.54×10^2); ^1H NMR (in Me_2SO-d_6) δ 1.84 (m, γ-CH_2, 2 H), 2.66 (t, β-CH_2, 2 H), 2.88 (br s, NH_3 trans O, 3 H), 3.24 (br s, 2 axial NH_3, 6 H), 3.44 (t, δ-CH_2, 2 H), 3.81 (br s, NH_3 trans imine, 3 H), 4.68 (br m, OH, 1 H), 11.92 (br s, imine NH, 1 H); ^{13}C NMR δ 28.31 (γ-CH_2), 33.38 (β-CH_2), 61.69 (δ-CH_2), 173.50 (C=N), 188.69 (COOH); IR $\nu_{C=N}$ 1668 cm^{-1}.

$[(NH_3)_4\overline{CoO_2CC(=NH)}(CH_2)_3Br]^{2+}$ (VI). $[(NH_3)_4\overline{CoO_2CC(=NH)}(CH_2)_3OH](ClO_4)_2$ (11.0 g) was dissolved with warming in HBr (55 mL, BDH, LR, 60%) and heated on a steam bath at 80–90 °C for 30 min. To the cooled solution, a large quantity of ethanol was added with trituration to yield a pink flocculent precipitate. This was collected, washed with ethanol and ether, and dried in air. The crude product was dissolved in warm water and passed through a freshly prepared BIO-RAD AG 1-X8 column (Cl^- form, 70 g dry weight, 17 × 3 cm) and the column eluted with warm water. The total eluate (~250 mL) was acidified by adding a few drops of HCl (3 M) and evaporated to about 50 mL. It was filtered, warmed, treated with ethanol until incipient precipitation, and cooled in ice. Crystallization was fairly slow, but

well-formed needles grew in clusters over a period of several hours. This compound was washed with ethanol and ether and dried in vacuo over P_2O_5 (yield 8.7 g). Anal. Calcd for $[CoC_5H_{19}N_5O_2Br]Cl_2 \cdot 0.5H_2O$: Co, 14.73; C, 15.01; H, 5.04; N, 17.50; Cl, 17.72; Br, 19.98. Found: Co, 15.2; C, 15.3; H, 5.1; N, 17.3; Cl, 17.8; Br, 19.7.

The perchlorate salt was also prepared by dissolving the halide salt (~0.1 g) in warm H_2O, adding $NaClO_4$, and allowing the solution to cool to 20 °C. The beautiful orange-red crystals that deposited were washed with methanol and ether and dried in vacuo at 60 °C. Anal. Calcd for $[CoC_5H_{19}N_5O_2Br](ClO_4)_2$: Co, 11.36; C, 11.57; H, 3.69; N, 13.49; Cl, 13.66; Br, 15.40. Found: Co, 11.1; C, 11.9; H, 3.9; N, 13.5; Cl, 13.7; Br, 15.4. UV–visible (chloride salt (λ_{max}, ϵ_{max} in H_2O)) 486 nm (80), 345 (sh, 2.21×10^2); ^1H NMR δ 2.22 (m, γ-CH_2, 2 H), 3.04 (t, β-CH_2, 2 H), 3.24 (br s, NH_3 trans O, 3 H), 3.53 (br s, 2 axial NH_3, 6 H), 5.61 (t, δ-CH_2, 2 H), 4.08 (br s, NH_3 trans to imine, 3 H); ^1H NMR (in Me_2SO_4-d_6) δ 12.78 (br s, imine NH, 1 H); ^{13}C NMR δ 28.31 (γ-CH_2), 34.16, 35.07 (β-CH_2, δ-CH_2), 173.37 (C=N), 187.39 (COOH).

Cyclization of VI to the Pyrroline $[(NH_3)_4\overline{CoO_2CC{=}N)\text{-}CH_2CH_2CH_2}]^{2+}$ (VII). $[(NH_3)_4\overline{CoO_2CC(=NH)(CH_2)_3Br}]Cl_2 \cdot H_2O$ (4.0 g) was dissolved and stirred vigorously in a minimum volume of water at room temperature (35 mL, 20 °C). Buffer (1 M $NaHCO_3/Na_2CO_3$, 20 mL, pH 10.0) was added. The solution immediately changed from the typical imine orange-red to the deep brown-red of a deprotonated imine complex and returned to an orange-red after about 20 s when the cyclization was nearing completion. After a total reaction time of 45 s, the reaction mixture was acidified with HCl (3 M) to pH 0–1 and until effervescence had almost ceased. Within a short time, fine orange-red crystals began to form. After 1 h at 0 °C, the product was collected, washed with methanol, ethanol, and ether, and air-dried (yield 2.46 g). On evaporation of the filtrate, a second crop was obtained (0.31 g). The combined product was recrystallized by dissolution in a minimum volume of warm water (~50 mL at 50 °C) and cooling in ice with a few drops of HCl (3 M) added to the filtrate. Well-formed orange-red needles grew slowly. The product was washed with methanol, ethanol, and ether, and dried in vacuo (1.45 g). Evaporation of the filtrate and cooling produced a second crop of beautifully formed crystals (0.61 g). Anal. Calcd for $[CoC_5H_{18}N_5O_2]Cl_2 \cdot 2H_2O$: Co, 17.03; C, 17.35; H, 6.41; N, 20.24; Cl, 20.49. Found: Co, 17.4; C, 17.3; H, 6.4; N, 20.0; Cl, 20.3.

The perchlorate salt was also obtained from a portion of the filtrate by dropwise addition of saturated aqueous $NaClO_4$. As the solution stood at room temperature, well-formed rose-red crystals grew. These were collected and washed with methanol, ethanol, and ether, and dried in vacuo over P_2O_5. Anal. Calcd for $[CoC_5H_{18}N_5O_2](ClO_4)_2$: Co, 13.45; C, 13.71; H, 4.14; N, 15.99; Cl, 16.19. Found: Co, 13.5; C, 14.0; H, 4.4; N, 16.1; Cl, 16.5. UV–visible (chloride salt (λ_{max}, ϵ_{max} in H_2O)) 486 nm (77), 338 (1.65×10^2), 284 (sh, 7.2×10^2); ^1H NMR (in D_2O (all NH sites deuterated), chloride salt) δ 2.49 (m, γ-CH_2, 2 H), 3.15 (m, β-CH_2, 2 H), 4.23 (m, δ-CH_2, 2 H); ^{13}C NMR δ 22.24 (γ-CH_2), 35.07 (β-CH_2), 58.86 (δ-CH_2), 171.03 (C=N), 186.48 (COOH).

Reduction of the Pyrroline Complex to $[(NH_3)_4Co(RS)\text{-Pro}]Cl_2$ (VIII). Compound VII (1.0 g) was dissolved in water (40 mL) and stirred vigorously. Buffer (1 M $Na_2CO_3/NaHCO_3$, 1 mL, pH 10.0) and $NaBH_4$ BDH, LR, 0.5 g) were added, and the stirring was continued for 5 min. During this time the orange-red pyrroline complex solution darkened somewhat to that of the rose-red proline complex. (The relatively long reduction time appears necessary in this case for high yield.) The solution was diluted to 120 mL with water and sorbed quickly on a short Dowex 50W-X2 resin column (Na^+ form, 5 × 3 cm) (note that if some residual H^+ is present on this resin, decomposition to green cobalt(II) species occurs rapidly in the presence of BH_4^-). The column was washed quickly with water (3 × 50-mL aliquots) to remove excess borohydride and a small amount of green decomposition product. It was then washed with 1.5 M HCl (100 mL) to convert any Na^+ resin to the H^+ form and remove traces of Co^{2+} species. The compound was then eluted with 3 M HCl as a single band. On addition of ethanol and ether to this eluate fine red crystals of $[(NH_3)_4Co(RS)\text{-Pro}]Cl_2$ formed. These were washed with methanol and ether (crude yield 0.85 g). The compound was recrystallized in >90% yield by dissolution in a minimum volume of warm water, by adding methanol and ethanol until incipient crystallization and cooling the solution in ice. The small red crystals were washed with methanol and ether and dried in vacuo. Anal. Calcd for $[CoC_5H_{20}N_5O_2]Cl_2$: Co, 18.88; C, 19.24; H, 6.46; N, 22.44;, Cl, 22.72. Found: Co, 18.9; C, 19.6; H, 6.5; N, 22.1; Cl, 22.6. UV–visible (λ_{max}, ϵ_{max} in H_2O) 495 nm (79), 350 (96); ^1H NMR δ 1.8–2.7 (complex m, β-CH_2 and γ-CH_2, 4 H), 3.34 (m, δ-CH_2, 2 H), 4.20 (t, α-CH, 1 H), 6.70 (br m, proline NH, 1 H) (the last signal was recorded in a D_2O solution freshly prepared. The other signals were recorded after all NH sites were deuterated (15 min in D_2O/Na_2CO_3, pH 10 and then acidified DCl)); ^{13}C NMR δ 26.88, 29.74 (γ- and β-CH_2), 50.26 (δ-CH_2), 65.71 (α-CH, low intensity), 186.87 (COOH).

Resolution of $[(NH_3)_4Co(RS)\text{-Pro}]Cl_2$ (VIII) into Chiral Forms. The salt (1.0 g) was dissolved in water (15 mL), and $Na_2[(Sb_2(+)\text{-(tartrate)}_2]$ (~0.5 equiv, 1.0 g) in water (5 mL) was added. The solution volume was reduced to ~10 mL by rotary evaporation, and a fine pink flocculent powder began to precipitate. On standing, the precipitate was filtered, washed with water and methanol, and air-dried. The remaining filtrate and added washings were then reduced in volume to give more of the diastereoisomeric salt. The filtrate from this crystallization was evaporated to dryness and gave a water soluble, reddish powder. The less soluble diastereoisomeric salt (yield 0.75 g) was purified by successive recrystallizations and identified as $[(NH_3)_4Co(R)\text{-Pro}](Sb_2(+)\text{-(tartrate)}_2)$ (IX). Dissolution of crude IX (0.6 g) in water (500 mL) at 50 °C followed by concentration of the solution to ~25 mL and dropwise addition of methanol recrystallized the diastereoisomer. Successive fractions had the same rotatory power and were combined, washed with water and methanol, and dried once in vacuo (0.4 g). An 0.1% aqueous solution gave specific rotations of $[\alpha]^{25}_D$ +70° and $[\alpha]^{25}_{480}$ +310°. Anal. Calcd for $[CoC_5H_{20}N_5O_2](Sb_2(C_4H_4O_6)_2)$: Co, 7.55; C, 20.00; H, 3.61; N, 8.97. Found: Co, 7.6; C, 20.4; H, 4.4; N, 8.9.

The chloride salt of IX, $[(NH_3)_4Co(R)\text{-Pro}]Cl_2$ (X), was prepared by dissolution of IX (0.4 g) in water (300 mL) at 50 °C and removal of $[Sb_2(+)\text{-(tartrate)}_2]^{2-}$ by using an ion exchange resin, Dowex 1-X8 (6.5 × 2 cm, Cl^- form). The eluant volume was reduced to ~5 mL, and dropwise addition of methanol gave a rose red powder (X, 0.1 g). A 0.1% aqueous solution gave specific rotations of $[\alpha]^{25}_D$ −35°, $[\alpha]^{25}_{530}$ −353°, and $[\alpha]^{25}_{480}$ +641°. Anal. Calcd for $[CoC_5H_{20}N_5O_2]Cl_2$: C, 19.24; H, 6.46; N, 22.44. Found: C, 19.0; H, 6.5; N, 21.7.

$[(NH_3)_4Co(S)\text{-Pro}]Cl_2$ (XI). The water soluble fraction remaining from the resolution procedure was dissolved in water (~25 mL) and passed through a column of Dowex 1-X8 (4.5 × 2 cm, Cl^- form), which was then washed with water. The eluate and washings were reduced in volume to ~10 mL and on dropwise addition of methanol gave a pink solid (IX). Preferential crystallization allowed removal of IX by filtration. The filtrate remaining was then reduced considerably, and addition of methanol caused precipitation of a rose-red powder (XI, 0.08 g). A 0.1% aqueous solution gave specific rotations of $[\alpha]^{25}_D$ +33°, $[\alpha]^{25}_{530}$ +340°, and $[\alpha]^{25}_{480}$ −603°. Anal. Calcd for $[CoC_5H_{20}N_5O_2]Cl_2$: C, 19.24; H, 6.46; N, 22.44; Cl, 22.72; Co, 18.88. Found: C, 19.0; H, 6.8; N, 21.1; Cl, 21.3; Co, 18.1.

Ion Exchange Chromatography. Resolution of the $[(NH_3)_4Co(RS)\text{-Pro}]^{2+}$ complex by ion exchange chromatography was partially achieved by using SP Sephadex C-25 (Na^+ form, 20 × 2 cm) with 0.1 M $Na_2[Sb_2(+)\text{-(tartrate)}_2]$ eluant. Although no actual splitting into bands occurred, rotatory dispersion (RD) measurements showed Cotton effects of opposite sign for eluant fractions at the front ((*S*)-proline complex) and trailing edges of the sorbed complex. Samples were tested by removal of the resolving agent on Dowex 50W-X2 (Na^+ form), eluting the complex with 3 M HCl and recording visible absorption and RD spectra. Separation using a longer column (122 × 2 cm) and 0.15 M eluant was not successful, although further spreading of the band was observed.

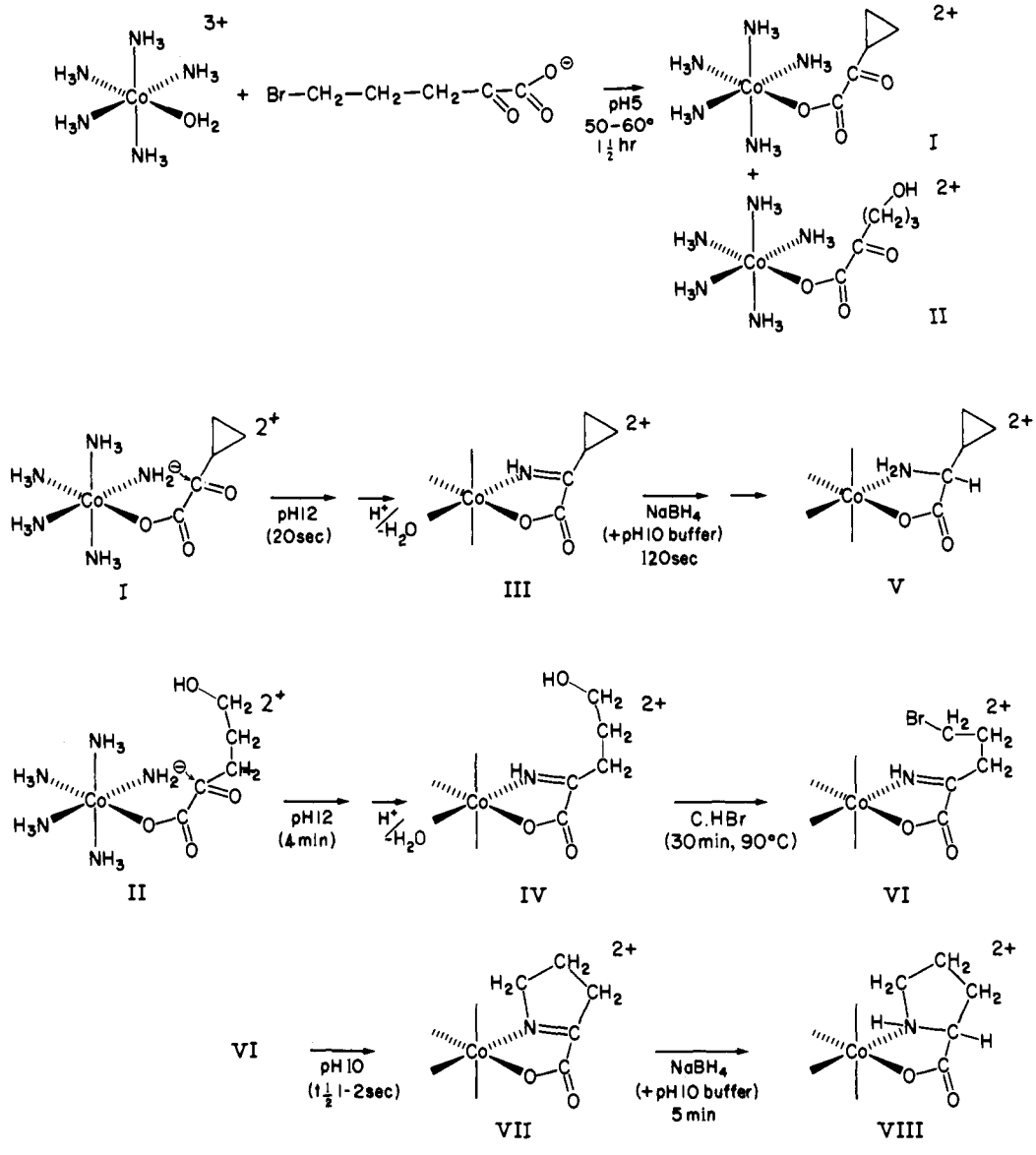
Results

Syntheses. An outline of the synthetic routes is illustrated in Scheme I. The synthesis of 5-bromo-2-oxopentanoic acid is briefly described in the Experimental Section. It is of passing interest that the ^1H NMR spectrum of the lithium salt showed two signals for the β-methylene protons of almost equal intensity, indicating approximately equal proportions of the α-keto acid salt and the α-diol salt.

The product distribution in the syntheses of the α-keto carboxylate complexes from aquapentaamminecobalt(III) and the buffered α-keto acid was markedly pH dependent. At a pH of 5, approximately equal yields of compounds I and II were obtained. In syntheses attempted at pH 2 or 3.5, II was the only significant product, but its overall yield was lower at pH 2 and comparable at pH 3.5 to that obtained at pH 5.

Attempts to separate the components in the (α-oxocarboxylato)pentaamminecobalt(III) preparations using Dowex 50W-X2 with HCl (0.5–1 M) as eluant were thwarted by anation from Cl^- ion, giving $[CoCl(NH_3)_5]^{2+}$, during evaporation of fractions. However, the separating power of BIO-GEL P2 polyacrylamide gel filtration for these various pentaamminecobalt(III) complexes solved this difficulty. Such experiments showed that syntheses at pH 5 produced I and II in comparable yield but also some $[CoCO_3(NH_3)_5]^+$, $[CoBr(NH_3)_5]^{2+}$, and $[Co(NH_3)_5H_2O]^{3+}$.

Scheme I. Outline of the Syntheses of $[(NH_3)_4\overline{CoO_2CC(NH_2)CH(CH_2)_2}]^{2+}$ and $[(NH_3)_4Co(RS)\text{-Pro}]^{2+}$



Compound II was extremely soluble and difficult to crystallize, so the fraction, from the gel filtration containing it, was treated directly with base to generate the desired imine. The formation of the imine complexes was conveniently followed visually by the color change from the red carboxylato pentaammine to the deep brown-red of the deprotonated imine in base and then to the orange of the protonated imine upon acidification.[2] As seen in Scheme I, the conditions for the preparation of the imines III and IV were quite different. The imine III formed much more rapidly (20 s) at pH 12 than did IV (4 min). Any longer time for the synthesis of III from I at pH 12 resulted in substantial complex decomposition. The imine IV was not very stable in basic aqueous solution. It decomposed to a brown compound on standing at 20 °C over 2 h or in minutes at 50–60 °C. The imines III and VI were much more stable.

The bromination of imine IV to give imine VI illustrates a synthetic advantage of the cobalt(III) ammine system. Several different conditions of bromination were attempted, including the traditional organic route using concentrated HBr/H_2SO_4, but the optimum method involved dissolution of IV in concentrated HBr and warming at 80–90 °C for 30 min. The extent of the conversion was conveniently followed by quenching portions with ethanol and recording NMR spectra as discussed below. The HBr/H_2SO_4 bromination always yielded mixtures, but the HBr medium gave a clean product in over 90% yield.

The cyclization of VI to the pyrroline complex VII at pH 10 was conveniently followed visually since the deprotonated imine of VI was deep brown and the pyrroline complex VII orange-red. The kinetics of this cyclization were also followed spectrophotometrically and are discussed separately below.

Alkaline borohydride reductions of compounds III and VII gave the respective amino acid complexes readily. The reduction of III was accompanied by a decomposition reaction. Optimal conditions at pH 10 in carbonate buffer with 0.33 M BH_4^- over 2 min of reaction time gave a 77% yield. The reduction of the pyrroline complex VII to the proline complex VIII was more successful. Reduction over 5 min at pH 10.0 gave little decomposition and a >90% yield. Note, it is necessary to avoid acid in the presence of BH_4^- and the Co(III) complexes. Co(III) ammine complexes rapidly reduce to Co(II) under these conditions.

Both of the racemic amino acids were freed from the metal ion by the addition of H_2S to remove cobalt as the sulfide.

The resolution of the proline complex on a SP Sephadex C-25 ion exchange column using sodium antimony-(+)-tartrate was only partially successful. However, resolution via the antimony tartrate salt was effected readily. Also, the resolution of free (*RS*)-proline has been reported by using natural tartaric acid[11] and by liquid

(11) Yamada, S.; Hongo, C.; Chibata, I. *Agric. Biol. Chem.* **1977**, *41*, 2413.

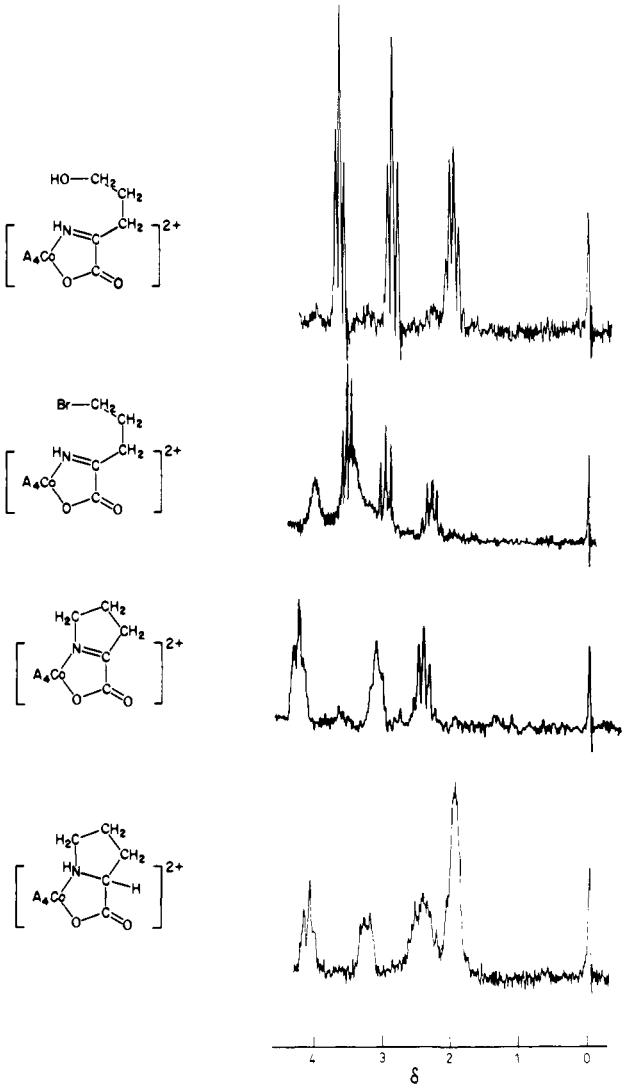


Figure 1. Methylene and methine proton resonances for complexes used in the synthesis of $[(NH_3)_4Co(RS)\text{-Pro}]^{2+}$ (spectra in D_2O vs. DSS).

chromatography.[12]

The possibility that the cyclopropyl 2-keto acid formed directly from LiBKPA under the reaction conditions used for the synthesis of the (2-oxocarboxylato)pentaamminecobalt(III) complex was investigated by NMR spectroscopy in the absence of the starting complex. Only a low percentage of the cyclopropyl species formed on heating at pH 5. After 4 h at 50–60 °C and pH 5.0, the NMR spectrum of the solution indicated the product was predominantly 2-oxopentyl-5-lactone with approximately 10% being the cyclopropyl 2-keto carboxylate and approximately 20% as the 5-hydroxy-2-oxopentanoic acid. Thus the cobalt(III) complex formation significantly influences the products by removing 2-keto carboxylates as they are formed, presumably by influencing the reactivity of the β carbon of the 2-keto acid.

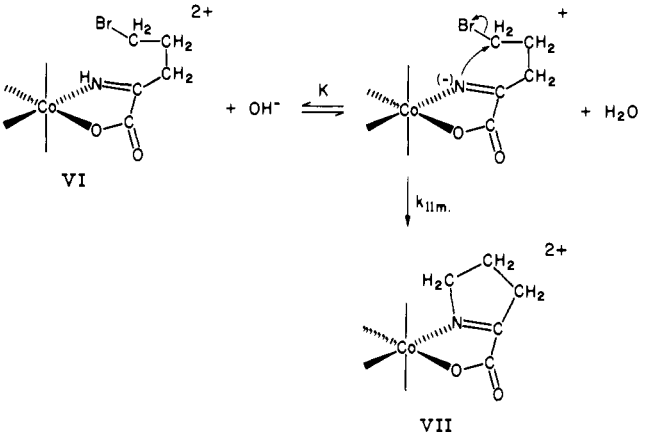
Characterization of Complexes. The synthesis of the ligand and all reaction steps illustrated in Scheme I were monitored by ^1H NMR spectroscopy. Figure 1, for example, illustrates the changes in methylene and methine resonances that occur during the synthesis of $[(NH_3)_5Co(RS)\text{-Pro}]^{2+}$. Where possible, reaction lifetimes were also determined by both NMR spectroscopy and visible absorption spectral shifts. Complex products were characterized by the above and by their chromatographic behavior on Bio-Gel, SP Sephadex C-25, or on Dowex 50W-X2. On the former gel, separation was largely consistent with charge, the tripositive species being held more strongly on the gel in 0.01 M $HClO_4$ than the di- or monopositive species.

Table I. Kinetic Data for the Cyclization of $[(NH_3)_4CoO_2CC(=NH)(CH_2)_3Br]^{2+}$ to the Pyrroline Complex in Aqueous Solution[a]

pH	buffer,[b] M	k_{obsd}, $s^{-1}(\times 10^{-1})$	$k_{(calcd)}$ $s^{-1}(\times 10^{1})$
9.00	ethanolamine, 0.1	0.60	0.50
9.02	ethanolamine, 0.2	0.59	
9.45	ethanolamine, 0.1	1.45	1.49
9.46	ethanolamine, 0.2	1.51	
9.80	ethanolamine, 0.1	2.76	2.78
9.81	ethanolamine, 0.2	2.73	
10.19	ethanolamine 0.1	4.92	4.76
10.21	ethanolamine, 0.2	4.83	
10.72	piperidine, 0.2	7.18	7.25
11.15	triethylamine, 0.05	8.78	8.42
11.41	triethylamine, 0.05	9.26	8.82
12.51	NaOH, 0 1	9.10	9.25

[a] 25.0 ± 0.05 °C, μ = 1.0 ($NaClO_4 \cdot H_2O$), complex 9.70 × 10^{-4} M. [b] Each buffer pH adjusted with $HClO_4$.

Scheme II. Intramolecular Cyclization of the Bromo–Imine Complex to the Pyrroline via the Deprotonated Imine



The ammine resonances in Me_2SO-d_6 were useful in characterizing imine complexes, the symmetry of the cobalt ion being reduced from C_{4v} in the pentaammine cases to C_s in the imine with loss of one ammine resonance. The very low field imine proton (HN=C) resonance in Me_2SO-d_6 occurred in the region 11–12-ppm downfield of DSS, and the ^{13}C spectra had two resonances in the 170–190-ppm region (downfield of Me_4Si), one a carboxyl and the other the imine carbon. Also the IR spectra of the imines exhibited C=N stretches at 1670–1680 cm^{-1}, characteristic of coordinated imines.[2]

The cyclopropyl moiety has characteristic high-field A_2B_2 multiplet resonances (δ 1.2 and 2.6) in its ^1H NMR spectrum and was thus easily recognizable in complexes. High-field ^{13}C signals were also observed for this moiety, and in these cases their position was a little more sensitive to changes in the ligand than were proton resonances (e.g., methylenes resonate at δ 15.1 in I and at δ 3.1 in V.

The $[(NH_3)_4Co(RS)\text{-Pro}]^{2+}$ complex synthesized in the present work was spectroscopically identical with the compound reported in the literature[13] (NMR and UV–visible). The chiral complex has also been synthesized from L-(*S*)-proline, and the CD and RD curves have been measured.[14] The optical rotatory power of the complexes synthesized here agrees with that reported previously. It follows that the absolute configuration of the proline complex synthesized can also be assigned by comparison of the sign of its rotatory power with that of the complex synthesized from the natural form of proline. In this respect, the less soluble dia-

(12) See, for example: Jozefonicz, J.; Muller, D.; Petit, M. A. *J. Chem. Soc., Dalton Trans.* **1980**, *76*.

(13) Hawkins, C. J.; Lawson, P. J. *Aust. J. Chem.* **1970**, *23*, 1735.

(14) Yasui, T.; Hidaka, J.; Shimura, Y. *Bull. Chem. Soc. Jpn.* **1966**, *39*, 2417.

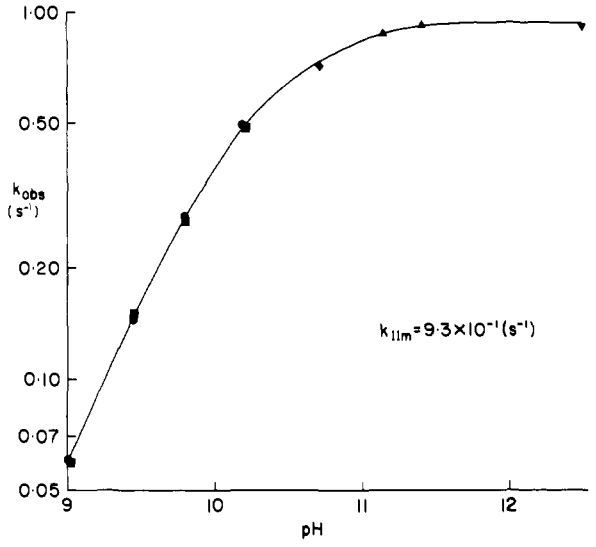


Figure 2. pH dependence of the rate constant (at 25.0 ± 0.05 °C) for the crystallization of $[(NH_3)_4\overline{CoO_2C(=NH)}(CH_2)_3Br]^{2+}$ to the pyrroline complex $[(NH_3)_4\overline{CoO_2CC(=N)}CH_2CH_2CH_2]^{2+}$ (complex 9.7 × 10^{-4} M; μ 1.0 M ($NaClO_4$); buffer pH adjusted with $HClO_4$): (■) 0.2 M ethanolamine; (●) 0.1 M ethanolamine; (♦) 0.2 M piperidine; (▲) 0.5 M triethylamine; (▼) 0.1 M NaOH.

stereoisomer contains D-(*R*)-proline, the "unnatural" form.

Kinetics of Cyclization of VI to VII. The cyclization reaction of the imine VI to the pyrroline VII illustrated in Scheme I was followed under pseudo-first-order conditions. The observed rate constant k_{obsd} (Table I) showed a simple dependence on hydroxide ion concentration up to pH 10 and shortly thereafter reached a limiting value. The kinetics are thus consistent with a mechanism in which the approach of the rate to independence of base concentration involves a single OH^--dependent preequilibrium as illustrated in Scheme II. This reaction sequence is consistent with the rate law

$$k_{obsd} = k_{lim}K[OH^-]/(1 + K[OH^-])$$

where $k = 1/K_b$.

The observed and calculated rate constants from use of this rate law are compared in Table I for a pK_b value of 10.17.

The possibility of general base catalysis was eliminated by measuring the reaction rate of constant pH for two different buffer concentrations (over several pH values). No significant differences were observed as illustrated in Figure 2. Some evidence of a much slower concurrent decomposition reaction was observed at pH values above 12.0. However, it did not significantly affect the A_∞ values, and the near quantitative conversion of the imine to the pyrroline complex indicates the insignificance of the decomposition within the lifetime of the reaction.

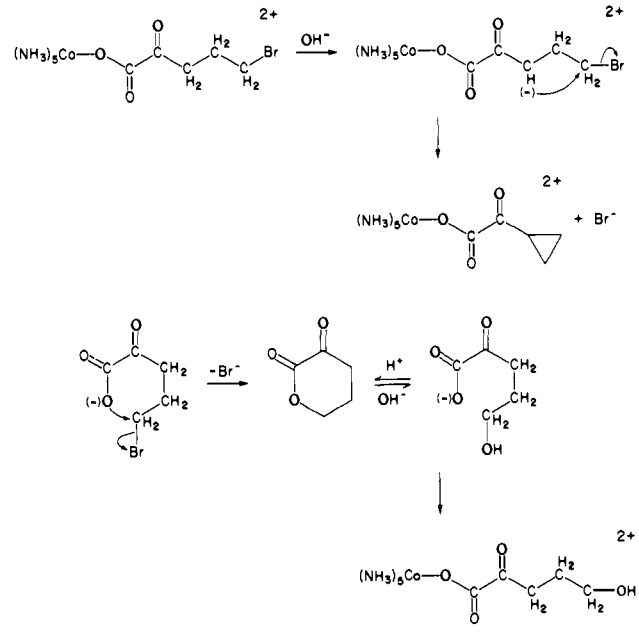
Discussion

The reaction of the 5-bromo-2-oxopentanoate ion with the aquapentaamminecobalt(III) ion leads directly to the cyclopropyl 2-keto acid and the 5-hydroxy-2-oxopentanoato complexes. The former does not arise from prior cyclization, and therefore it arises as a result of coordination of the bromo keto carboxylate ion to cobalt(III).

The β-carbon protons could be expected to be much more acidic when the carboxylate ion is bound to Co(III) than when it exists as the free ion. The β carbanion then becomes accessible in near neutral to basic conditions, and intramolecular elimination of Br^- by a nucleophilic path to yield the cyclopropyl group becomes relatively facile (Scheme III). There is also a competitive lactone formation and hydrolysis to yield the 2-oxo-5-hydroxypentanoic acid anion, which also coordinates to Co(III) (Scheme III).

Both the coordinated cyclopropyl keto acid and the 5-hydroxy-2-oxopentanoic acids would have labile protons on the C atom α to the keto group, which could be exchanged readily

Scheme III. Routes to the Cyclopropylketo Acid Complex and the the 5-Hydroxy-2-oxopentanoato Complex



with deuterium or tritium. After cyclization to the imines in base these same protons would also be exchangeable in basic conditions although they would probably be less labile than in the keto complex. Reduction to the chelated amino acids makes the β-carbon protons inert to exchange although the α-methine protons still have a degree of lability in strongly basic conditions.[15] Clearly, there are also possibilities for labeling carbon atoms during the synthesis of the α-ethoxalylbutyrolactone from butyrolactone and diethyl oxalate. Syntheses by these routes therefore lead readily to specific labeling possibilities for both C and H. Such compounds may be useful in biosynthesis or biodegradation studies of the amino acids.

The 2-cyclopropylglycine has been synthesized previously in low yield by Lowy[16] using a Strecker synthesis commencing with cyclopropyl cyanide. Interest in such cyclopropyl derivatives is developing since the plant amino acids *cis*- and *trans*-α-(carboxycyclopropyl)glycine have been isolated from *Aesculus parviflora* and *Blighia sapida*, respectively,[17] and an acetoxy cyclopropylglycine has been isolated also from Sapindaceae.[18] Harding and DeShazo[19] have reported that cyclopropylglycine is an inhibitory analogue of valine, participating in ATP–pyrophosphate exchange catalyzed by valyl RNA synthetase. They find that this amino acid inhibits the growth of *E. coli*, an effect that may be noncompetitively reversed by adding valine. The α-cyclopropylglycine also acts as an alternative substrate for valyl transfer RNA synthetases from *Aesculus* species.[20]

Given a facile and quantitative synthesis of the $(NH_3)_5CoOOC(CO)CH_2CH_2CH_2Br^{2+}$ ion, the proline synthesis would be near quantitative. The cyclization to the imine is fast and quantitative. Such reactions have been examined in some detail.[2,3,21] They rely on deprotonation of a coordinated NH_3 group[22] adjacent to the α-keto acid, attack of the aminate ion at

(15) Buckingham, D. A.; Marzilli, L. G.; Sargeson, A. M. *J. Am. Chem. Soc.* **1967**, *89*, 2772 and references therein.
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(17) Fowden, L.; Smith, A.; Millington, D. S.; Sheppard, R. C. *Phytochemistry* **1969**, *8*, 437.
(18) Fowden, L.; Macgibbon, C. M.; Mellon, F. A.; Sheppard, R. C. *Phytochemistry* **1972**, *11*, 1105.
(19) Harding, W. M.; DeShazo, M. L. *Arch. Biochem. Biophys.* **1967**, *118*, 23.
(20) Anderson, J. W.; Fowden, L. *Biochem. J.* **1970**, *119*, 691.
(21) Gainsford, A. R.; Pizer, R. D.; Sargeson, A. M.; Whimp, P. O. *J. Am. Chem. Soc.* **1981**, *103*, 792. Pearson, R. G.; Basolo, F. *Ibid.* **1956**, *78*, 4878.
(22) Wilinski, J.; Kurland, R. J. *Inorg. Chem.* **1973**, *12*, 2202. Bramley, R.; Creaser, I. I.; Mackey, D. F.; Sargeson, A. M. *Ibid.* **1978**, *17*, 244.

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.

Acknowledgment. We thank the Australian National University Microanalytical Service, Dr. Charles Clark, and Brian Fenning for technical assistance. P.J.L. thanks the Council, the Chemistry Staff of CIAE, and the RSC for creating the opportunity to do the work.

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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

(1) This synthesis was presented at the 9th National Meeting of the Division of Coordination and Metallo-organic Chemistry, The Royal Australian Chemical Institute, held at the University of New South Wales (Sydney, Australia) in February 1980.

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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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