Coordination Isomers of Biological Iron Transport Compounds. V. The Preparation and Chirality of the Chromium(III) Enterobactin Complex and Model Tris(catechol)chromium(III) Analogues[1]

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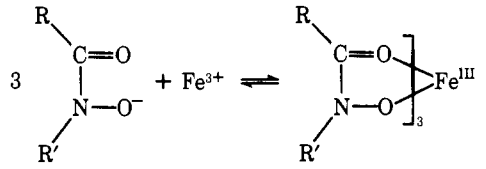
Abstract: Enterobactin (the cyclic triester of 2,3-dihydroxy-N-benzoyl-*l*-serine) is a microbial iron transport agent produced by true bacteria such as *E. coli* and *S. typhimurium*. The three side chains which terminate in catechol rings readily lose six protons and coordinate to ferric ion or similar cations to form stable octahedral complexes. The mode of bonding and absolute configuration of the most stable isomer of ferric enterobactin have been determined by preparing the corresponding chromic complex and comparing its visible absorption and circular dichroism spectra with those of a simple tris(catechol)chromate(III) model compound. The latter, $[Cr(cat)_3]^{3-}$, has been resolved, and the absolute configurations of its optical isomers have been assigned. The visible spectrum of $[Cr(cat)_3]^{3-}$ in basic aqueous solutions has λ_{max}, nm (ϵ, l. mol^{-1} cm^{-1}), at 592 (78) and 425 (104). The CD transitions of Δ-$[Cr(cat)_3]^{3-}$ occur at λ ($\Delta\epsilon$), 663 (+0.43), 582 (−2.0), and 435 (+0.80). The half-life for racemization of $[Cr(cat)_3]^{3-}$ in aqueous solution is 100 min at pH 9.5 and shows a dependence on $[H^+]$ which is approximately −0.3. The complex is sensitive to air oxidation but is otherwise indefinitely stable in basic aqueous solutions. The chromic enterobactin complex shows similar behavior but is sensitive to strongly basic conditions due to the ready hydrolysis of the cyclic triester moiety. The chromic complex of enterobactin has been purified by chromatographic techniques and occurs as only one diastereoisomer. The complex $[Cr(enterobactin)]^{3-}$ shows visible-uv maxima, λ (ϵ), at 586 (80) and 425 (600). The CD spectrum has transitions, λ ($\Delta\epsilon$), at 660 (+1.3), 574 (−7.0), and 420 (+1.6). The similarity of the CD spectrum to Δ-$[Cr(cat)_3]^{3-}$ establishes that $[Cr(enterobactin)]^{3-}$ occurs as the Δ-cis isomer of the octahedral complex formed by the three catechol rings. This is the first example of a preferred diastereoisomer of the siderochromes with Δ absolute configuration and establishes that the activity of these cell permeases is not dependent on having the Λ absolute configuration found in the ferrichromes, although specific recognition of chirality may be operating in individual microbial-ligand systems.

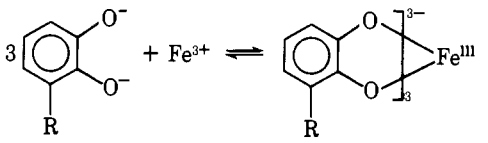
The siderochromes are low-molecular-weight compounds which are manufactured by microbes and are involved in their cellular iron transport.[2-4] The siderochromes are all chelating ligands which form extremely stable octahedral complexes with high-spin ferric ion. Two important classes of siderochromes, the ferrichromes and ferrioxamines, are trihydroxamic acids which (except for those containing charged substituents) form neutral complexes using three bidentate hydroxamate monoanions:



These complexes of Fe(III) are all kinetically labile. In contrast, the complexes in which chromic ion is substituted for ferric ion, although structurally the same, are kinetically inert. Thus coordination isomers can be isolated and this has been demonstrated for model hydroxamate complexes,[5] desferriferrichromes[6] and ferrioxamines.[1] The specific complex characterized as Λ-cis chromic desferriferrichrome is taken up into cells of the smut fungus *Ustilago sphaerogena* as rapidly as the native ferric complex.[7]

The ferrichromes, which have a natural optical activity associated with the ligand, have been studied using several techniques. The NMR spectra of Al(III) and Ga(III) derivatives, as compared with the free ligand, have shown that a profound conformational change accompanies complex formation.[8] Except in those cases where the ligand was optically inactive (in which case the complexes are racemic mixtures), the previous siderochrome complexes have been found to have a Λ absolute configuration. Thus, while ferrioxamine E is racemic,[9] x-ray structure analyses of ferrichrome A[10] and ferrichrysin[11] have shown both to be Λ-cis isomers. A recent structure analysis has shown that ferric mycobactin P also has a Λ absolute configuration.[12]

Another common ligand functional group found in the siderochromes is catechol (*o*-dihydroxybenzene). Catechol is similar to hydroxamates in being a bidentate ligand which coordinates through two oxygen atoms, but is a dianion:



The isolation and characterization of the cyclic triester trimer of 2,3-dihydroxy-*N*-benzoyl-*l*-serine, a tricatechol siderochrome (Figure 1), was independently reported by both Pollack and Neilands[13] and O'Brien and Gibson.[14] The ligand was isolated from cultures of *Salmonella typhimurium* and *Escherichia coli* and given the names enterobactin and enterochelin, respectively.

Enterobactin and the other siderochromes are of medical importance, since iron has been tied to the establishment of bacterial infections. Direct links between iron binding and pathogenicity have been established for infections of *E. coli* and *S. typhimurium*. Both of these organisms produce enterobactin as an iron uptake and transport agent and enterobactin has been shown to affect the onset of certain of their infections.[2] In *E. coli* it has been shown that enterobactin is produced in response to a need for iron and the ferric complex is transported from solution into the cell against a very large concentration gradient. Once inside the cell, the complex does not dissociate to release ferric ion (as does ferrichrome). Instead, an enzyme, which acts on only the complex and not free enterobactin as a substrate, hydroly-

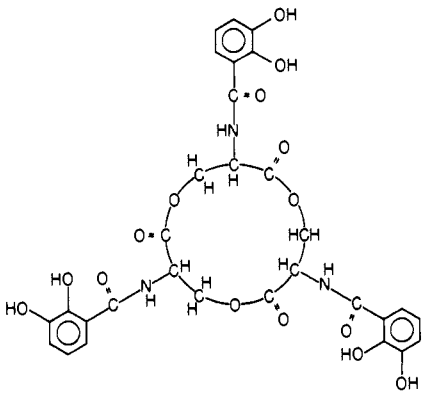


Figure 1. Structural diagram of enterobactin.

zes ferric enterobactin to a complex of the monomer, 2,3-dihydroxy-*N*-benzoyl-*l*-serine. This complex apparently dissociates rapidly and the free monomer is excreted from the cell without being reutilized to prepare new enterobactin.

The highly specific nature of the cellular transport of at least some of the siderochromes has led us to consider the possible role played by coordination isomers of the siderochrome complexes. It can be seen from molecular models that two diastereoisomers are possible for the ferric enterobactin complex, Λ-cis and Δ-cis. These are not mirror images because of the optical activity of the ligand. The similarity of the roles played by the ferrichromes and enterobactin lends additional speculative interest to the preferred absolute configuration of the iron complex.[15]

Before attempting the preparation of the chromic enterobactin complex, simple catechol complexes were prepared to perfect synthetic and separation techniques to be used with the small amounts of enterobactin available, and to use as simple models in comparing spectroscopic properties. The chemical literature of tris(catechol) complexes of transition metal ions is sparse. The one reference to a chromic complex reported that it was rapidly hydrolyzed in dilute aqueous solution.[16] This would of course preclude separation of optical isomers of the trischelates. Nevertheless, these complexes were reinvestigated as a prelude to preparing the chromic enterobactin complex. This paper describes the preparation and purification of the chromic enterobactin complex and the resolution and absolute configuration assignment of the $[Cr(cat)_3]^{3-}$ anion. This is the first resolution of a transition metal catechol complex.[17]

Experimental Section

Catechol, 99+% purity (Aldrich), was used as supplied. Chromium(III) acetate, chromic chloride hexahydrate (Baker Analyzed Reagent), and chromous chloride (Fisher Scientific Co.) were used without further purification. The complexes, Λ- and Δ-tris(ethylenediamine)chromium(III) chloride and Λ- and Δ-tris(ethylenediamine)cobalt(III) chloride were prepared and resolved using literature procedures.[18] The resolved compounds were repeatedly recrystallized until the measured optical purity agreed with the reported values.

Enterobactin was extracted from culture media of *Aerobacter aerogenes*, grown under low iron concentrations as described by Neilands et al.[13] Yields between 20 and 30 mg/l. of culture reproducibly were obtained. It was found necessary to store the white solid in a vacuum desiccator to avoid slow air oxidation.

Preparation of $K_3[Cr(cat)_3]$. The preparation of $K_3[Cr(cat)_3]$ (cat = *o*-dihydroxybenzene dianion) was carried out using degassed solvents in an atmosphere of oxygen-free nitrogen in a glass Schlenk apparatus. Chromium(III) acetate, 7.4 g (0.03 mol), and catechol, 10 g (0.09 mol), were dissolved in 40 ml of water and warmed on a hot water bath until the solids dissolved. To this was added dropwise 20 g (0.35 mol) of potassium hydroxide dissolved in 15 ml of water. The solution was stirred for 15 min and then 2-3 vol of degassed ethanol was added. After cooling at 0° a green solid deposited, which was washed several times with 95% ethanol, followed by ether. It was dried in a vacuum desiccator and stored in a dark bottle (yield 75-80%). Purification was achieved by reducing to half volume a saturated solution of $K_3[Cr(cat)_3]$ over P_2O_5 in a desiccator under nitrogen atomosphere. The crystalline solid formed was washed and treated again as above. Anal. Calcd for $[Cr(C_6H_4O_2)_3]\cdot 2H_2O$ (mol wt 529.6): C, 40.82; H, 3.05; Cr, 9.81. Found: C, 40.76; H, 2.99; Cr, 9.41. The ammonium salt was also prepared by this method.[16]

Resolution of Λ-$K_3[Cr(cat)_3]$. To a solution of 0.085 g (0.16 mmol) of $K_3[Cr(cat)_3]\cdot 2H_2O$ in 5 ml of degassed 0.1 *M* KOH was added a solution of 0.051 g (0.08 mmol) of Δ-$[Co(en)_3]I_3\cdot H_2O$ in 3 ml of water. The resultant solution was stirred in an insulated ice-bath at 0-5°. The solid $[Co(en)_3][Cr(cat)_3]$ was filtered out and 0.04 mmol of Δ-$[Co(en)_3]I_3\cdot H_2O$ in about 2 ml of water was added dropwise to the filtrate. Again the precipitated $[Co(en)_3][Cr(cat)_3]$ was filtered off and the CD spectrum of the second filtrate was measured. No further improvement in resolution was obtained by adding successive stoichiometric amounts of Δ-$[Co(en)_3]I_3\cdot H_2O$ in repeating the above procedures. This resolution represents the limit of solubility differences between Λ- and Δ-$K_3[Cr(cat)_3]$ salts of Δ-$[Co(en)_3]$, which may not correspond to complete resolution. Crystalline green solids of the resolved compounds were obtained by slow evaporation of the above solutions to dryness in an inert atmosphere. Partial racemization usually occurred during evaporation, so no optically pure solids were obtained. Similar procedures using Λ-$[Co(en)_3]I_3\cdot H_2O$ were used to prepare Δ-$K_3[Cr(cat)_3]$.

Preparation of $[NH_4]_3[Cr(enterobactin)]$. To a solution of 75 mg (0.12 mmol) of pure, dry enterobactin in 10 ml of pure degassed methanol was added 15.0 mg (0.12 mmol) of solid $CrCl_2$. While bubbling N_2 gas through the solution 50 mg (0.6 mmol) of solid $NaHCO_3$ was added with stirring. After 5 min the solution was opened to air for 5 min and then evaporated to dryness at room temperature. The crude solid $Na_3[Cr(ent)]$ (ent = enterobactin hexanion) was dissolved in degassed water and passed through a cation exchange column (Bio-Rad AG 50W-X2, 200-400 mesh) in the NH_4^+ form under a nitrogen atmosphere. This solution was taken to dryness as before and the solid was dissolved in the minimum amount of water and loaded on a Bio-Gel P-2 column (30x/cm). Elution with water separated a green band of the product from a brown impurity. The green solution was taken to dryness, dissolved in a minimum amount of methanol, and loaded on a 30x/cm Sephadex LH-20 column. When eluted with methanol, the green band of $[NH_4]_3[Cr(ent)]$ (which again slowly separated from a small brown band) was collected. Evaporation of the methanolic solution to dryness resulted in loss of ammonia, yielding the very hygroscopic solid $H_3[Cr(ent)]$. Anal. Calcd for $H_3[Cr(ent)]\cdot 9H_2O$: C, 40.91; H, 4.77; N, 4.77. Found: C, 41.14; H, 4.55; N, 4.31.

Thin-Layer Chromatography. Kieselgel D-O silica gel was used for thin-layer chromatography on glass coated plates. This was performed for the iron and chromium complexes of enterobactin in methanol-chloroform solvent systems. The best results were obtained using ammonium salts in 50% methanol-chloroform solutions. The spots were stained with iodine vapor.

Microanalyses. Microanalyses were performed by the microanalytical laboratory, Department of Chemistry, University of California, Berkeley.

Physical Measurements. Visible absorption spectra of the racemic and resolved catechol complexes were measured in water and in buffer solutions of pH 7-13. The spectrum of $[NH_4]_3[Cr(enterobactin)]$ was measured in water and methanol solutions from six different preparations. The total chromium concentration was measured by oxidation to chromium(VI) with basic hydrogen peroxide. Excess hydrogen peroxide was removed by boiling the solution for 30 min. The absorption at λ 372 nm (ϵ_{372} 4815 M^{-1} cm^{-1} for K_2CrO_4)[19] was measured after the solution was cooled to room temperature. Visible spectra were measured using a Cary Model 118 uv-visible spectrophotometer. Circular dichroism spectra were measured using a Jasco J-20 automatic recording spectropolarimeter.

Kinetic Studies. Rates of racemization were measured by monitoring the decrease with time of the CD band at λ 576 nm for Λ-$K_3[Cr(cat)_3]$ solutions (1×10^{-3} to 7×10^{-3} *M* concentration).

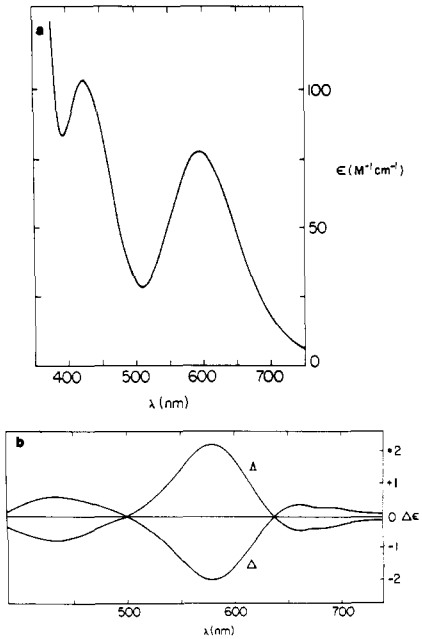


Figure 2. (a) Visible absorption spectrum of $K_3[Cr(cat)_3]$ in water. (b) Circular dichroism spectra of Δ- and Λ-$K_3[Cr(cat)_3]$ solutions.

Table I. Band Maxima and Extinction Coefficients for the Visible Absorption and Circular Dichroism Spectra of $K_3[Cr(cat)_3]$ and $[NH_4]_3[Cr(ent)]$[a]

Assigned configuration	Visible spectrum band maxima,[b] nm (ε)	CD band maxima,[c] nm (Δε)
Λ-$K_3[Cr(cat)_3]$	592 (78) $^4A_{2g} \rightarrow {}^4T_{2g}$	663 (−0.49) $^4A_2 \rightarrow {}^4E_a$
	425 (104) $^4A_{2g} \rightarrow {}^4T_{1g}$	582 (+2.3) $^4A_2 \rightarrow {}^4A_1$
		435 (−0.91) $^4A_2 \rightarrow {}^4E_b$
Δ-$K_3[Cr(cat)_3]$	592 (78)	663 (+0.43)
	425 (104)	582 (−2.0)
		435 (+0.80)
Δ-*cis*-$[NH_4]_3$[Cr-(ent)]	586 (80)	660 (1.3)
	425 (600)	574 (−7.0)
		420 (1.6)

[a] Maxima units are nm, with ε and Δε (following in parentheses) in units of l. mol^{-1} cm^{-1}. [b] Assignment of transitions based on O_h symmetry. [c] Assignment of transitions based on D_3 symmetry.

Racemizations were followed for at least two half-lives. Linear plots of log ΔA_t vs. time were obtained in all cases, where ΔA_t is the difference in absorbance of left- and right-circularly polarized light at time t. The baseline of the instrument was established by setting ΔA_t at infinite time equal to zero.

Results and Discussion

Potassium Tris(catechol)chromate(III). The usual oxygen sensitivity of the catechol dianion was found to be substantially increased in the chromium complex.[20] It is this oxidation of the chromium complex that causes the green to red color changes reported previously as hydrolysis.[16] All preparations and handling of the chromium catechol complex were therefore carried out under oxygen-free nitrogen in a glove box which incorporates a recirculating gas purification system.

The visible spectrum of $[Cr(cat)_3]^{3-}$ anion is similar to other chromium(III) compounds with oxygen donor ligands. The bands at 592 (78) and 420 (104) nm (ε) are assigned to the spin allowed d-d transitions $^4A_{2g} \rightarrow {}^4T_{2g}$ and $^4A_{2g} \rightarrow {}^4T_{1g}$, respectively. These and other spectroscopic data are presented in Table I. No attempt was made to isolate any oxidized species; however, it was observed that a band around λ 520 nm gradually appeared when solutions of $[Cr(cat)_3]^{3-}$ were left in air for periods of minutes.

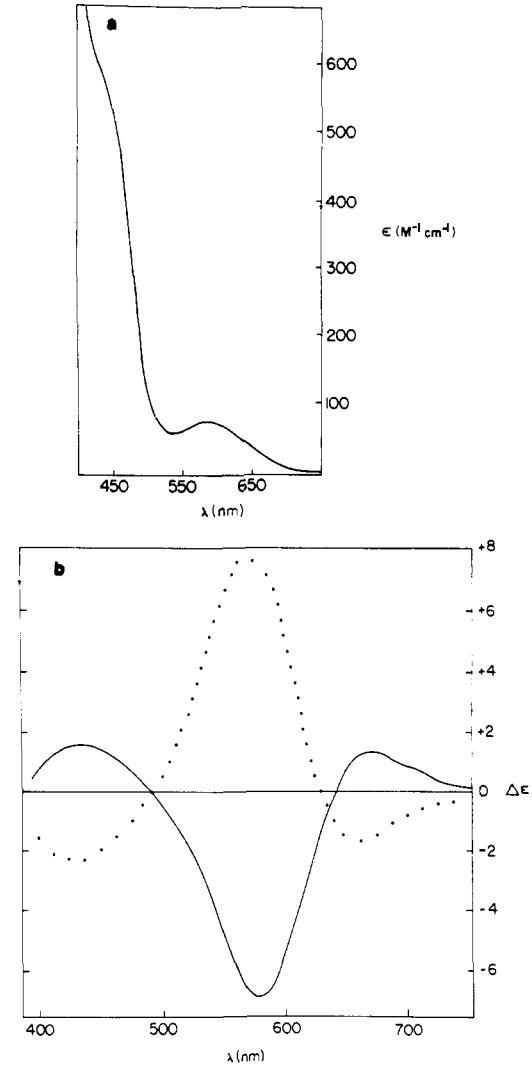


Figure 3. (a) Visible absorption spectrum of $[NH_4]_3[Cr(enterobactin)]$. (b) Circular dichroism spectra of Δ-$[NH_4]_3[Cr(enterobactin)]$ (—) and chromic desferriferrichrome (· · ·), ref 6.

Only partial resolution of solutions of $[Cr(cat)_3]^{3-}$ was achieved at neutral pH. The highest resolution was attained at pH 13 at 5°C. The rate of loss of optical activity for resolved $[Cr(cat)_3]^{3-}$ was found to be strongly dependent on hydrogen ion concentration, varying from half-times of several minutes to several hours between pH 7 and pH 13. The preliminary kinetic data are summarized by the following half-lives at 25°C (and at the corresponding pH): 32 (7.6), 68 (9.0), 102 (9.5), and greater than 400 min, at pH 11.5. The order of this rate dependence on $[H^+]$ is approximately −0.3. Similar behavior was observed in the racemization of $K[As(cat)_3]$, which shows a first-order rate dependence on $[H^+]$.[21]

The visible and circular dichroism spectra of $[Cr(cat)_3]^{3-}$ and $[Cr(ent)]^{3-}$ complexes are shown in Figures 2 and 3. The absorption spectra are similar except that the ligand transition occurs at lower energy in the enterobactin complex, thus masking the $^4A_{2g} \rightarrow {}^4T_{1g}$ d-d transition which appears as a shoulder on the edge of the more intense $\pi \rightarrow \pi^*$ ligand transitions. This is apparently due to the fact that enterobactin contains ortho-acyl-substituted catechol rings.

The assignment of absolute configuration for the Δ and Λ isomers of $[Cr(cat)_3]^{3-}$ is based on three points. (1) The

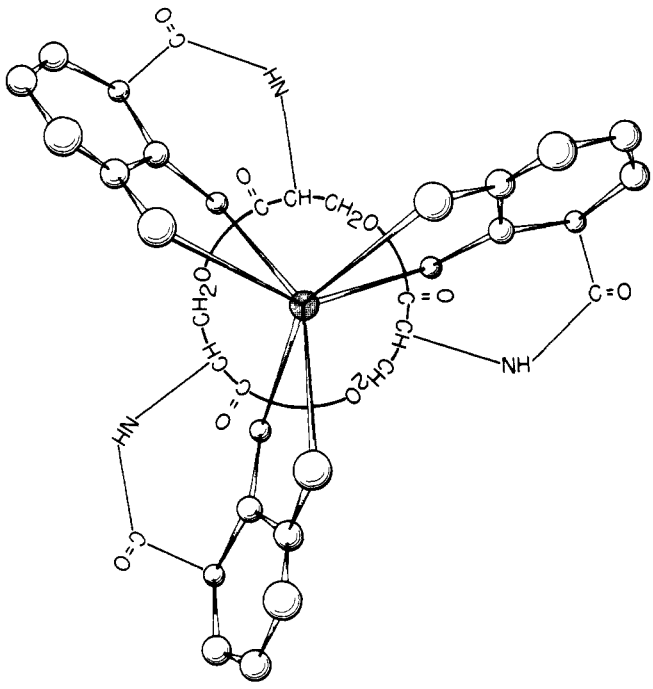


Figure 4. A diagram of the Δ-cis chromic enterobactin trianion.

CD spectra can be compared directly with complexes of known absolute configuration such as $[Cr(C_2O_4)_3]^{3-}$. This type of comparison was used previously in the assignment of absolute configurations of the hydroxamate complexes.[5] The CD spectra of the hydroxamate and catechol complexes are very similar. The close relationship in chelate ring size and electronic structure of the coordinating portions of the ligands allows direct comparison of their CD spectra in this case. (2) The empirical rule for assigning the absolute configurations of d^3 or d^6 metal complexes with D_3 point group symmetry can be used. This rule states that the low-energy transition with E symmetry will be positive for a Λ complex.[22] As can be seen from Figure 2, the assignments of Δ- and Λ-$[Cr(cat)_3]^{3-}$ CD spectra are consistent with this rule. (3) Finally, this assignment is also consistent with the principle of least soluble diastereoisomers, where salts in which the cation and anion have the same absolute configuration are less soluble than mixed Δ, Λ salts.[23] Thus in this case Δ-$[Co(en)_3]^{3+}$ or Δ-$[Cr(en)_3]^{3+}$ preferentially precipitate the isomer assigned as Δ-$[Cr(cat)_3]^{3-}$.

Preparation and Absolute Configuration of the Chromic Enterobactin Complex. The ease of oxidation of the catechol portions of enterobactin and the ease of hydrolysis of the cyclic triester portion of the molecule make this compound extremely difficult to handle relative to siderochromes of the hydroxamate type. The sensitivity to hydrolysis precludes the use of the synthetic procedures used previously to prepare hydroxamate and catechol complexes. The use of chromous ion in the preparations avoids these difficulties as well as assuring that an equilibrium mixture is formed for the product. Although some formation of polymeric complexes of chromic enterobactin occurs in the preparation, the polymers and oxidation products were separated from the monomeric complex by ion exchange and gel filtration techniques. The movement of $[NH_4]_3[Cr(ent)]$ on silica gel TLC plates showed one sharp band with an R_f of 1.25, in 50% $CHCl_3$–CH_3OH solvent system, which is identical with the value observed for the ferric complex.

Since ferric ion is a high-spin d^5 complex it has no spin-allowed d → d transitions. Hence the CD spectra of the ferric siderochrome complexes are due to electronic transitions which directly involve the ligands. In contrast, the visible spectra of the chromium complexes are due to d → d transitions of the metal chromophore and are essentially independent of the ligands except for their ligand field effect and some ligand-to-metal charge transfer. The CD spectra of $[Cr(ent)]^{3-}$ and $[Cr(cat)_3]^{3-}$ are even more similar than their absorption spectra, since the ligand π to π^* transitions of $[Cr(ent)]^{3-}$ are not associated with the optical center of the metal chromophore and the isostructural ferric complex.

The structures of $K_3[Cr(cat)_3]\cdot 1.5H_2O$ have recently been determined;[24] the anions are found to be distorted octahedral complexes with D_3 coordination point symmetry and similar molecular parameters. Although in principle the 2-3-dihydroxy-*N*-benzoyl arms of enterobactin could coordinate through the carbonyl oxygen and the 2-hydroxy oxygen atom to give a β-diketone type of bonding, this can be rejected as a possibility since: (1) the CD and visible spectra are very similar to those of $[Cr(cat)_3]^{3-}$, and the structure of the latter is known; (2) inspection of molecular models shows coordination in the β-diketone fashion involves a prohibitively large amount of ring strain; (3) the ^{13}C and ^1H NMR spectra of the gallium enterobactin complex have been interpreted and catechol coordination was one conclusion of the derived conformation of the complex.[25]

Enterobactin is itself optically active, hence Λ- and Δ-$[Cr(ent)]^{3-}$ would be diastereoisomers and should be separable by chromatographic techniques. Since we observe only one chromic complex of enterobactin and since this complex has an optical activity which is even greater than resolved $[Cr(cat)_3]^{3-}$, we conclude that one isomer predominates to the exclusion of the other. By comparison of the $[Cr(ent)]^{3-}$ CD spectrum with the CD spectra of the Λ and Δ isomers of $[Cr(cat)_3]^{3-}$ and of chromic desferriferrichrome, we give the following assignment: *the predominant isomer of the chromic enterobactin monomeric complex has a Δ-cis absolute configuration* (*Figure 4*). The ferric complex is known to have a net optical activity, and hence one isomer also predominates in ferric enterobactin.[15] This isomer of ferric enterobactin can also be assigned as Δ since: (1) the ionic radii of Fe^{3+} and Cr^{3+} are equal to within 0.03 Å and the salts $K_3[M(cat)_3]\cdot 1.5H_2O$ (M = Fe, Cr) are isostructural;[24] (2) the identical chromatographic properties of $[Fe(ent)]^{3-}$ and $[Cr(ent)]^{3-}$ salts indicate identical structures. Confirmation of this absolute configuration by x-ray crystallographic techniques has as yet been precluded by the unavailability of suitable single crystals. The Δ chirality of $[Cr(ent)]^{3-}$ and $[Fe(ent)]^{3-}$ is opposite in absolute configuration to the other optically active siderochromes characterized to date. The role of the siderochromes as cellular permeases for ferric ion is therefore not dependent on the complex always having the Λ-cis configuration observed in all previous optically active siderochromes, although this configuration or other isomers may be specifically transported in individual microbial-ligand systems.

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Coordination Isomers of Biological Iron Transport Compounds. VI. Models of the Enterobactin Coordination Site. A Crystal Field Effect in the Structure of Potassium Tris(catecholato)chromate(III) and -ferrate(III) Sesquihydrates, $K_3[M(O_2C_6H_4)_3]\cdot 1.5H_2O$, M = Cr, Fe[1]

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Abstract: The structures of the title compounds, $K_3[M(cat)_3]\cdot 1.5H_2O$, M = Cr, Fe, have been determined by single-crystal x-ray diffraction methods using counter data. These isostructural tris(catechol) complexes appear to be similar to the coordination sites of the ferric and chromic complexes of the microbial iron transport compound enterobactin (the cyclic triester of 2,3-dihydroxy-*N*-benzoyl-*l*-serine), which is itself a tricatechol. The complexes have approximate molecular D_3 point symmetry, the primary distortion being a bending of the catechol rings away from coplanarity with the O-M-O planes. The average M-O bond lengths are 1.986 (4) Å for Cr and 2.015 (6) Å for Fe. The average ring O-M-O bond angles are 83.56 (14)° for Cr and 81.26 (7)° for Fe. The structural parameters are compared with the $[P(cat)_3]^-$, $[Si(cat)_3]^{2-}$, and $[As(cat)_3]^-$ complexes. A significant increase in the trigonal twist angle of the chromic complex (50.5°) relative to the ferric complex (44.7°) is attributed to a crystal field effect. Dark green crystals of the chromic salt, obtained from basic aqueous solution conform to space group $C2/c$ with a = 20.796 (4), b = 15.847 (4), and c = 12.273 (3) Å with β = 91.84 (1)°. For eight molecules per unit cell the calculated density is 1.68 g/cm^3; the observed density is 1.69 g/cm^3. For 2315 independent data with $F_o^2 > 3\sigma(F_o^2)$ full-matrix least-squares refinement with anisotropic thermal parameters for all nonhydrogen atoms converged to unweighted and weighted R factors of 3.5 and 4.6%, respectively. The ferric compound was prepared from ferric oxide and catechol in excess aqueous base. Dark red-brown crystals of the ferric salt, obtained from basic aqueous solution, conform to space group $C2/c$ with a = 20.612 (7), b = 15.873 (5), and c = 12.307 (4) Å with β = 91.76 (1)°. For eight molecules per unit cell the calculated density is 1.73 g/cm^3; the observed density is 1.72 g/cm^3. For 1445 independent data with $F_o^2 > 3\sigma(F_o^2)$ full-matrix least-squares refinement with anisotropic thermal parameters for all nonhydrogen atoms converged to unweighted and weighted R factors of 4.6 and 4.7%, respectively.

Enterobactin (the cyclic triester of 2,3-dihydroxy-*N*-benzoyl-*l*-serine) is a microbial iron transport agent produced by true bacteria such as *E. coli* and *S. typhimurium*. We have recently reported the preparation of its chromic complex and the optical activity of this and the related model catechol complex.[1] Like all of the siderochromes, enterobactin is a low-molecular-weight compound which is manufactured by the producing organism to facilitate uptake and transport of ferric ion.[2-4] Unlike the ferrichrome[5] or ferrioxamine[6] complexes which contain hydroxamate functional groups, the chelating groups in enterobactin are catechol (1,2-dihydroxybenzene) moieties.

Catecholate dianion has long been known to form coordination compounds with a variety of transition and non-transition metals.[7] As the free anion and, especially, in complexes, catechol can undergo a series of stepwise oxidations. Several reactions of this type have been reported by Holm et al.[8] Recently complexes of the oxidized ligand tetrachloroquinone have been reported.[9] Although under very acid conditions ferric ion oxidizes catechol,[10] near neutral pH