# Coordination Isomers of Biological Iron Transport Compounds. I. Models for the Siderochromes. The Geometrical and Optical Isomers of Tris(*N*-methyl-*l*-menthoxyacethydroxamato)chromium(III)

**John Leong and Kenneth N. Raymond*[1]**

*Contribution from the Department of Chemistry, University of California, Berkeley, California 94720.* *Received October 17, 1973*

**Abstract:** A number of microbial iron sequestering and transport agents (the siderochromes) are polyhydroxamic acids. The coordination properties of these compounds are similar to simple model hydroxamic acids of synthetic origin. The preparation of complexes of two such model ligands is reported. Ferric ion has been replaced by chromic ion to induce kinetic inertness in the complexes and allow the separation of coordination isomers. The preparation of tris(*N*-methyl-*l*-menthoxyacethydroxamato)chromium(III) and the separation and assignment of absolute configuration of the Λ-cis, Δ-cis, and (Λ,Δ)-trans isomers are reported. The Λ-cis isomer has CD maxima at 429 (−2.82), 573 (7.83), and 670 (−1.85) nm (Δε). The Δ-cis isomer has corresponding maxima at 425 (2.95), 574 (−8.26), and 671 (1.91) nm. The trans isomer is obtained as an unresolved mixture of the Δ and Λ isomers in which the Δ isomer predominates, with observed CD bands for the mixture at 395 (0.21), 461 (0.21), 574 (−0.62), and 678 (0.17) nm. Cis and trans isomers of tris(benzhydroxamato)chromium(III) also have been separated and characterized. The cis complex has visible absorption maxima at 415 (121) and 596 (82) nm (ε). The trans complex has corresponding bands at 400 (117) and 600 (83) nm. The complexes of both hydroxamic acids isomerize at room temperature with half-lives of several hours. The corresponding cobaltic complexes are unstable because of oxidation of the ligands by the cobaltic ion.

The profound insolubility of ferric ion at physiological pH engendered the evolution of special ligands which can dissolve, transport, and make available the element for aerobic organisms. Most of the microbial iron transport compounds characterized to date contain three hydroxamate groups which coordinate ferric ion octahedrally. These low molecular weight compounds of natural origin are called siderochromes.[2] Two important classes of microbial iron transport compounds are the ferrichromes and the ferrioxamines. In the ferrichrome series the basic structural feature is a cyclic hexapeptide with the hydroxamic acid linkages provided by $N^{\delta}$-acyl-$N^{\delta}$-hydroxy-*l*-ornithine. The ferrioxamines are made up of repeating units of 1-amino-ω-hydroxyaminoalkane and succinic or acetic acid.

The first of these naturally occurring compounds to be isolated was ferrichrome, which Neilands found was produced by the smut fungus *Ustilago sphaerogena*.[3] It was found that ferrichrome shows a potent growth factor activity for several microorganisms.[4,5] However, the most curious finding was that this substance is only produced in high yield by *Ustilago* when the organism is grown under iron-deficient conditions.[6,7] These observations led Neilands to suggest that ferrichrome acts as a cellular transport cofactor.[8] As research in this area has continued, compounds containing hydroxamic acid groups have been found in an increasingly broad range of living systems. Various of these compounds are potent growth factors, growth inhibitors, antibiotics, etc.[9,10] In all cases, however, the biological activity of these compounds centers on their unique ability to chelate iron and, following chelation, carry the iron *via* a cellular membrane transport system which is specific for the metal-bound hydroxamic acid.[11] In this regard they parallel the behavior of the alkali metal transport agents, such as valinomycin,[12-14] which can be deadly poisons or antibiotics and for which a number of synthetic analogs have been prepared and studied.[15-19]

The general chemistry of the hydroxamic acids forms a part of classical organic chemistry. The reaction with ferric ion is a standard test for the hydroxamic acid functional group. Simple hydroxamic acids have $pK_a$'s on the order of 9 and act as bidentate ligands in forming neutral tris(hydroxamate) complexes of ferric ion at neutral pH.[20] Simple hydroxamic acids and desferrisiderochromes exhibit remarkable affinity for ferric ion, little affinity for other ions which differ in

(1) Alfred P. Sloan Fellow, 1971–1973.
(2) Three recent reviews of the chemistry of the siderochromes are (a) J. B. Neilands, *Struct. Bonding (Berlin)*, 1, 59 (1966); (b) J. B. Neilands, "Inorganic Biochemistry," G. Eichhorn, Ed., Elsevier, New York, N. Y., 1973, p 167; (c) T. Emery, *Advan. Enzymol. Relat. Areas Mol. Biol.*, **33**, 135 (1971).
(3) J. B. Neilands, *J. Amer. Chem. Soc.*, **74**, 4846 (1952).
(4) R. M. Page, *Amer. J. Bot.*, **39**, 371 (1952).
(5) K. Nakayama, Z. Sato, and S. Kinoshita, *J. Gen. Appl. Microbiol.*, 10, 143 (1964).
(6) J. A. Garibaldi and J. B. Neilands, *Nature (London)*, **177**, 526 (1956).
(7) V. Prelog, *Pure Appl. Chem.*, **6**, 327 (1963).
(8) J. B. Neilands, *Bacteriol. Rev.*, **21**, 101 (1957).
(9) W. Keller-Schierlein and H. Zahner, *Progr. Chem. Org. Natur. Prod.*, 22, 279 (1964).
(10) J. B. Neilands, *Experentia, Suppl.*, **No. 9**, 22 (1964).
(11) T. F. Emery, *Biochemistry*, 10, 1483 (1971).
(12) B. C. Pressman, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **27**, 1283 (1968).
(13) B. C. Pressman, *Proc. Nat. Acad. Sci. U. S.*, **53**, 1076 (1965).
(14) E. J. Harris and B. C. Pressman, *Nature (London)*, **216**, 918 (1967).
(15) R. M. Izatt, D. P. Nelson, J. H. Rytting, B. L. Haymore, and J. J. Christensen, *J. Amer. Chem. Soc.*, **93**, 1619 (1971).
(16) U. Takaki, T. E. H. Esch, and J. Smid, *J. Amer. Chem. Soc.*, **93**, 6760 (1971).
(17) B. Metz, D. Moras, and R. Weiss, *J. Amer. Chem. Soc.*, **93**, 1806 (1971).
(18) B. Metz, D. Moras, and R. Weiss, *Chem. Commun.*, 444 (1971).
(19) B. Metz, D. Moras, and R. Weiss, *Chem. Commun.*, 217 (1970).
(20) G. Schwarzenbach and K. Schwarzenbach, *Helv. Chim. Acta*, **46**, 1390 (1963).

charge or size, and, in particular, little or no affinity for ferrous ion.[20,21] The ferric ion of water-soluble neutral siderochromes is fairly rapidly exchangeable[22] and can be removed by treatment with dilute base or reduction of ferric ion to ferrous.

X-Ray crystallographic investigations have shown that ferrichrome A has the Λ-cis configuration,[23] while tris(benzhydroxamato)iron(III) crystallizes in a racemic cis form.[24,25] The observations that the ferric ion in siderochromes is ionic, high spin, and fairly rapidly exchangeable[22] are consistent with magnetic susceptibility,[26] Mössbauer,[27,28] and esr[28] data. Proton nmr data indicate that the desferriferrichromes undergo a dramatic conformational change upon complexation with diamagnetic trivalent metal ions such as aluminum(III) and gallium(III).[29] That a similar conformational change upon complexation with ferric ion takes place for desferriferrioxamine B was concluded from proton exchange rates.[30]

Many of the questions regarding the structure–function relationship of the siderochromes cannot be answered because of the kinetic lability of these high-spin iron(III) complexes. Surprisingly, the coordination chemistry of desferrisiderochromes with metal ions other than ferric is largely unknown.[31] We have begun a program to investigate the coordination geometries of desferrisiderochromes with kinetically inert trivalent metal ions such as cobalt(III) and chromium(III). Since hydroxamic acids are unsymmetrical bidentate ligands, there are both geometric and optical isomers in tris(hydroxamate) complexes. For an octahedral complex formed with three equivalent optically active hydroxamate anions there are two geometric isomers possible, the trans and cis. Each geometric isomer consists of Δ and Λ optical isomers.[25] These are diastereoisomers because of the ligand optical activity and so there is a total of four possible isomers: Λ-cis, Λ-trans, Δ-cis, Δ-trans.

Preliminary exploratory research has been directed toward the preparation and characterization of simple model tris(hydroxamate) complexes of cobalt(III) and chromium(III). Attempts to prepare tris(hydroxamate) complexes of cobalt(III) with benzhydroxamic acid or its *N*-methyl derivative result in oxidation of the ligand with concomitant reduction of cobalt(III) to cobalt(II). We report here the preparation of tris(benzhydroxamato)chromium(III), $Cr(benz)_3$, and the separation and characterization of its two geometric isomers.[32] To facilitate the separation of the four optical isomers of a simple model tris(hydroxamate)-chromium(III) complex, we have prepared the optically active hydroxamic acid, *N*-methyl-*l*-menthoxyacethydroxamic acid, men. A secondary (*N*-alkyl substituted) hydroxamic acid was chosen because: (i) all naturally occurring siderochromes contain secondary hydroxamic acids and (ii) it was expected that this might retard the racemization and isomerization of the tris(hydroxamate) complex. We report here the preparation of tris(*N*-methyl-*l*-menthoxyacethydroxamato)-chromium(III), the separation of the two cis diastereoisomers from the trans diastereoisomers,[32] and their characterization by electronic absorption and circular dichroism spectra.

## Experimental Section

Infrared spectra were recorded with a Perkin-Elmer 337 spectrophotometer; proton nmr spectra were obtained on a Varian T-60 spectrometer using tetramethylsilane as an internal standard; ultraviolet–visible spectra were measured with a Cary Model 118 spectrophotometer; CD spectra were measured with a Jasco J-20 automatic recording spectropolarimeter. Chemical analyses were performed by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley.

**Materials.** Reagent grade chemicals were used throughout. Benzhydroxamic acid[33] and its potassium salt,[33] *N*-methylhydroxylamine hydrochloride,[34] *N*-methylbenzhydroxamic acid,[34-38] *l*-menthoxyacetyl chloride,[39] $Na_3[Co(CO_3)_3]\cdot 3H_2O$,[40] and $Co(OH)_3$[41,42] were prepared by literature procedures. The $CrCl_3\cdot 3THF$ was prepared and stored under a purified nitrogen atmosphere.[43] Tris(benzhydroxamato)iron(III) was prepared by the method of Epstein and Straub.[27] Tetrahydrofuran was dried by distillation from sodium, while pyridine was distilled from barium oxide and stored over Linde 3A molecular sieve.

**Thin-Layer Chromatography.** Camag Kieselgel D-O silica gel was used for thin-layer and column chromatography. Tlc on Kieselgel coated glass plates was performed on all metal complexes. Solvent systems were 15% $CH_3OH$–$CHCl_3$ for tris(benzhydroxamato)iron(III) and chromium(III) and 3% $CH_3OH$–$CHCl_3$ for the corresponding tris(*N*-methyl-*l*-menthoxyacethydroxamate) complexes. Spots were detected visually or stained with iodine vapor.

**Cobalt Hydroxamates.** (i) An aqueous mixture of freshly prepared $Co(OH)_3$ and an excess of benzhydroxamic acid afforded after 12 hr at room temperature the pink cobalt(II) complex, $Co(benz)_2$, and benzoic acid, an oxidation product of the ligand.

(ii) Oxygen was bubbled through an ethanolic solution of $CoCl_2$ and an excess of potassium benzhydroxamate for 12 hr at room temperature. Pink $Co(benz)_2$ was isolated.

(iii) An ethanolic mixture of $Na_3[Co(CO_3)_3]\cdot 3H_2O$ and an excess of benzhydroxamic acid afforded after 1.5 hr of reflux pink $Co(benz)_2$.

(21) G. Anderegg, F. L'Eplattenier, and G. Schwarzenbach, *Helv. Chim. Acta*, **46**, 1400 (1963).
(22) W. Lovenberg, B. B. Buchanan, and J. C. Rabinowitz, *J. Biol. Chem.*, **238**, 3899 (1963).
(23) A. Zalkin, J. D. Forrester, and D. H. Templeton, *J. Amer. Chem. Soc.*, **88**, 1810 (1966).
(24) Von H. J. Lindner and S. Gottlicher, *Acta Crystallogr., Sect. B*, **25**, 832 (1969).
(25) The convention for symbols of absolute configurations Δ and Λ are those of the IUPAC proposal (*Inorg. Chem.*, **9**, 1 (1970)). The cis isomer is defined as the isomer which has $C_3$ point symmetry.
(26) A. Ehrenberg, *Nature (London)*, **178**, 379 (1956).
(27) L. M. Epstein and D. K. Straub, *Inorg. Chem.*, **8**, 453 (1969).
(28) H. H. Wickman, M. P. Klein, and D. A. Shirley, *J. Chem. Phys.*, **42**, 2113 (1965).
(29) M. Llinas, M. P. Klein, and J. B. Neilands, *J. Mol. Biol.*, **68**, 265 (1972), and references therein.
(30) T. F. Emery, *Biochemistry*, **6**, 3858 (1967).
(31) A brief report of the CD spectrum of the chromium(III) complex of desferriferrichrysin has appeared (T. Buerer and E. Gulyas, Proceedings of the 9th International Conference on Coordination Chemistry, St. Moritz-Bad, 1966, p 512. However, the complex was apparently not isolated and the CD spectrum was not interpreted.
(32) Since all isolated isomers racemize and isomerize to a considerable extent upon concentration of their chromatographed fractions to dryness, all chromatographed fractions were stored in solution at −192° away from light. All isolated isomers racemize and isomerize even in solution at room temperature with half-lives of several hours. Their kinetics of interconversion are currently under investigation.
(33) C. R. Hauser and W. B. Renfrow, Jr., "Organic Syntheses," Collect. Vol. II, A. H. Blatt, Ed., Wiley, London, 1943, p 67.
(34) E. Beckmann, *Annalen*, **365**, 201 (1909).
(35) O. Exner and W. Simon, *Collect. Czech. Chem. Commun.*, 30, 4078 (1965).
(36) O. Exner and J. Holubek, *Collect. Czech. Chem. Commun.*, 30, 940 (1965).
(37) D. H. Moore, J. G. Cannon, W. M. McIsaac, and B. T. Ho, *J. Med. Chem.*, 12, 45 (1969).
(38) O. Exner and B. Kakac, *Collect. Czech. Chem. Commun.*, **28**, 1656 (1963).
(39) M. T. Leffler and A. E. Calkins, "Organic Syntheses," Collect. Vol. III, E. C. Horning, Ed., Wiley, New York, N. Y., 1955, p 547. An improved procedure for the carboxylic acid is given by T. G. Cochran and A. C. Huitric, *J. Org. Chem.*, **36**, 3046 (1971).
(40) H. F. Bauer and W. C. Drinkard, *Inorg. Syn.*, **8**, 202 (1966).
(41) H. Ley and H. Winkler, *Ber.*, **42**, 3894 (1909).
(42) R. G. Denning and T. S. Piper, *Inorg. Chem.*, **5**, 1056 (1966).
(43) J. P. Collman and E. T. Kittleman, *Inorg. Syn.*, **8**, 149 (1966).

(iv) An aqueous mixture of freshly prepared $Co(OH)_3$ and an excess of *N*-methylbenzhydroxamic acid afforded after 12 hr at room temperature a green solid, presumably the tris cobalt(III) complex, contaminated with a pink solid, the bis cobalt(II) complex. The contaminated green solid gradually turned completely pink over several hours.

(v) A methanolic mixture of $Na_3[Co(CO_3)_3]\cdot 3H_2O$ and an excess of *N*-methylbenzhydroxamic acid afforded after 2 days at room temperature a pink solid.

**Tris(benzhydroxamato)chromium(III).** This compound was prepared by the following two methods.

(i) A solution of 3.20 g (8.0 mmol) of $Cr(NO_3)_3\cdot 9H_2O$ in 30 ml of $H_2O$ was added in one portion to a rapidly stirred solution of 5.61 g (32.0 mmol) of potassium benzhydroxamate in 150 ml of $H_2O$. The resulting green slurry was stirred 12 hr at room temperature and suction filtered and the blue-green solid was washed well with $H_2O$ and then dry ethyl ether. The solid was dissolved in 200 ml of tetrahydrofuran, dried with $MgSO_4$, and concentrated to dryness *in vacuo* to afford 2.24 g (61%) of product, which was stored away from light: ir (neat) $cm^{-1}$ (relative intensity) 1661 (w), 1639 (w), 1600 (s), 1577 (m), 1572 (m), 1513 (s), 1484 (s), 1441 (m), 1359 (m), 1133 (s), 1042 (s), 1017 (s), 909 (s), 781 (m), 746 (w), 693 (s), 571 (s), 509 (m), 502 (m). The ir spectrum is identical with that of the corresponding Fe(III) complex. *Anal.* Calcd for $Cr(C_7H_6NO_2)_3\cdot H_2O$: C, 52.72; H, 4.21; Cr, 10.87; N, 8.78. Found: C, 52.35; H, 4.73, Cr, 10.8; N, 8.24.

(ii) A solution of 0.400 g (1.0 mmol) of $Cr(NO_3)_3\cdot 9H_2O$ in 2 ml of $H_2O$ was added in one portion to a rapidly stirred solution of 0.548 g (4.0 mmol) of benzhydroxamic acid in 60 ml of pH 4.7 buffer (0.1 *N* NaOAc–HOAc). The reaction mixture was stirred 12 hr at room temperature, filtered, and further treated as described above.

**Separation of Geometric Isomers of Tris(benzhydroxamato)chromium(III).** The cis and trans isomers were separated by column chromatography as follows. Kieselgel (9.0 g) in 17% $CH_3OH$–$CHCl_3$ was packed under nitrogen pressure in a glass column with o.d. of 19 mm. After topping the column with washed Monterey sand, a solution of 100 mg of complex in 0.3 ml of eluent was applied to the column and eluted under nitrogen pressure. Two green fractions were collected within 80 min at 0° and stored in solution at −192° away from light until spectra and analyses were obtained. Cis and trans isomers were ascertained to be greater than 90% pure by thin-layer chromatography of the individual isomers.

***N*-Methyl-*l*-menthoxyacethydroxamic Acid.** To a rapidly stirred solution of 8.08 g (96.7 mmol) of *N*-methylhydroxylamine hydrochloride in 200 ml of dry pyridine at 0° under a nitrogen atmosphere was added dropwise 15.0 g (64.4 mmol) of *l*-menthoxyacetyl chloride over a 10-min period. The resulting white slurry was stirred at 0° for 6 hr and filtered and the filtrate was concentrated to an oil *in vacuo*. To this was added 100 ml of $H_2O$, and the resulting slurry was extracted with ethyl ether (3 × 100, 1 × 50 ml). The combined ether extracts were washed with 5% $NaHCO_3$ (3 × 100 ml), dried over $MgSO_4$, and concentrated *in vacuo* to afford 13.82 g (88%) of colorless viscous oil. The product gives a positive hydroxamic acid test with ferric chloride:[44] nmr ($CCl_4$) $\delta$ 1.6 (19 H, m), 3.2 (3 H, s), 4.4 (2 H, s), 9.5 (1 H, s); ir (neat) $cm^{-1}$ (relative intensity) 3145 (m), 2941 (s), 1639 (s), 1449 (m), 1383 (w), 1368 (w), 1332 (w), 1190 (s), 1124 (m), 1087 (s), 952 (w), 752 (w), 565 (w), 505 (w).

**Tris(*N*-methyl-*l*-menthoxyacethydroxamato)iron(III).** In one portion 0.191 g (1.17 mmol) of anhydrous ferric chloride was added to a rapidly stirred solution of 1.00 g (4.11 mmol) of *N*-methyl-*l*-menthoxyacethydroxamic acid in 50 ml of tetrahydrofuran containing a few milliliters of pyridine. The burgundy-colored solution was stirred at room temperature for 5 hr, and then concentrated to dryness *in vacuo*. The residue was washed with ethyl ether, dissolved in 100 ml of methylene chloride, washed with $H_2O$ (2 × 50 ml), dried with $MgSO_4$, and concentrated to dryness *in vacuo* to afford a reddish brown powder: ir (neat) $cm^{-1}$ (relative intensity) 2907 (s), 1603 (s), 1471 (s), 1393 (w), 1376 (w), 1353 (w), 1235 (m), 1087 (s), 990 (m), 752 (s), 662 (m), 606 (m), 546 (s). *Anal.* Calcd for $Fe(C_{13}H_{24}NO_3)_3$: C, 59.83; H, 9.27; Fe, 7.13; N, 5.37. Found: C, 59.6; H, 9.2; Fe, 7.14; N, 5.22.

**Tris(*N*-methyl-*l*-menthoxyacethydroxamato)chromium(III).** In one portion 1.28 g (3.43 mmol) of $CrCl_3\cdot 3THF$ was added to a rapidly stirred solution of 3.96 g (16.3 mmol) of *N*-methyl-*l*-menthoxyacethydroxamic acid in 100 ml of tetrahydrofuran under a nitrogen atmosphere. After approximately 5 ml of pyridine had been added, the reaction mixture was refluxed under dry air ($CaCl_2$ drying tube) for 14 hr. The reaction mixture was concentrated to dryness *in vacuo* and then dissolved in 250 ml of ethyl ether. The cold ether extract was washed with cold $H_2O$ (2 × 75 ml), cold 5% $NH_4OH$ (4 × 75 ml), and cold $H_2O$ (3 × 75 ml), dried with $MgSO_4$, and concentrated to dryness *in vacuo* to afford 2.62 g (98%) of green powder: ir (neat) $cm^{-1}$ (relative intensity) 3425 (w, impurity), 2933 (s), 2865 (s), 1672 (w, impurity), 1616 (s), 1453 (s), 1383 (m), 1366 (m), 1339 (m), 1259 (m), 1227 (m), 1176 (m), 1149 (m), 1087 (s), 1020 (s), 980 (s), 800 (m), 763 (s), 719 (s), 629 (m), 568 (s). *Anal.* Calcd for $Cr(C_{13}H_{24}NO_3)_3$: C, 60.13; H, 9.32; Cr, 6.67; N, 5.39. Found: C, 59.91; H, 9.16; Cr, 7.27; N, 5.10. The impurity is a chromium(III) hydroxo species which is separated from the desired diastereoisomers by the subsequent column chromatography.

**Separation of Diastereoisomers.** The two cis isomers were completely separated from the trans isomers by column chromatography. The complex (275 mg) was chromatographed on 23.4 g of Kieselgel with 3% $CH_3OH$–$CHCl_3$ as the eluent on a column with o.d. of 25 mm. Three bluish green fractions were collected within 90 min at 0° and stored in solution at −192° away from light until spectra and analyses were obtained. A light green band which did not elute contained the polar chromium(III) hydroxo species. The mixture of trans isomers and both cis isomers were ascertained to be greater than 95% pure by thin-layer chromatography of the individual isomers.

**Physical Measurements.** Visible spectra of the geometric isomers of tris(benzhydroxamato)chromium(III) were determined in 17% $CH_3OH$–$CHCl_3$ solution at room temperature. Visible and CD spectra of the diastereoisomers of tris(*N*-methyl-*l*-menthoxyacethydroxamato)chromium(III) were determined in 3% $CH_3OH$–$CHCl_3$ solution at room temperature. The desired concentrations for all isomers were obtained by either diluting the original chromatographed fraction with the appropriate eluent or concentrating the fraction *in vacuo* at 0°.[32] The concentrations of chromium(III) solutions were determined spectrophotometrically as $[CrO_4]^{2-}$ ($\epsilon_{372}^{max}$ 4815 l. $mol^{-1}$ $cm^{-1}$ [45]) after oxidation of an aliquot of the chromium-containing solution with alkaline hydrogen peroxide. Excess hydrogen peroxide was removed by boiling the solution for 0.5 hr. All coordination isomers were at least 90% pure after spectra had been obtained, as determined by tlc of the solutions.

## Results and Discussion

**Tris(benzhydroxamato)chromium(III).** Thin-layer chromatography of the complex results in two green bands, corresponding to the cis and trans isomers, whose elution $R_{st}$ values bracket that of the one broad reddish brown band of the iron(III) complex. (These and other properties of the tris(benzhydroxamate) and tris(*N*-methyl-*l*-menthoxyacethydroxamate) complexes are summarized in Table I.) The geometric isomers of the iron(III) complex are in rapid equilibrium in solution because of the lability caused by the high-spin $d^5$ electronic configuration of ferric ion. As a result, the mixture of these isomers elutes as one band with an $R_{st}$ value that is a weighted average of the $R_{st}$ elution values for the two individual isomers.

The assignment of geometric isomers to the two green bands of $Cr(benz)_3$ is based on three criteria. (1) The chromatographic behavior of geometric isomers of tris($\alpha$-amino acid) complexes of cobalt(III),[46–48] tris($\beta$-diketone) complexes of cobalt(III),[49–51] and chromium(III)[51] has been investigated extensively. In all

(44) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 5th ed, Wiley, New York, N. Y., 1964, p 133.

(45) G. W. Haupt, *J. Res. Nat. Bur. Stand.*, **48**, 414 (1952).

(46) F. Jursik, *J. Chromatogr.*, **35**, 126 (1968).

(47) F. Jursik, F. Petru, and B. Hajek, *J. Chromatogr.*, **45**, 319 (1969).

(48) G. B. Kauffman, R. P. Pinnell, and L. T. Takahashi, *Inorg. Chem.*, 1, 544 (1962), and references therein.

(49) R. A. Palmer, R. C. Fay, and T. S. Piper, *Inorg. Chem.*, **3**, 875 (1964).

(50) C. S. Springer, Jr., R. E. Sievers, and B. Feibush, *Inorg. Chem.*, 10, 1242 (1971).

(51) R. M. King and G. W. Everett, Jr., *Inorg. Chem.*, 10, 1237 (1971).

**Table I.** Characterization of Tris(benzhydroxamato)- and Tris(*N*-methyl-*l*-menthoxyacethydroxamato)iron(III) and -chromium(III) Complexes

| Assigned configuration[a] | $R_{st}$[b] | Abundance, % of total | Absorption max, nm ($\epsilon$)[c] | $CD_{max}$, nm ($\Delta\epsilon$)[c] |
|---|---|---|---|---|
| Fe(benz)$_3$ | 1.06 | | | |
| *trans*-Cr(benz)$_3$ | 1.48 | 68 | 400 (117.1), 600 (83.1) | |
| *cis*-Cr(benz)$_3$ | 0.92 | 32 | 415 (121.4), 596 (82.3) | |
| Fe(men)$_3$ | 1.23 | | | |
| *trans*-Cr(men)$_3$ | 2.05 | 69 | 416 (49.6), 596 (69.6) | 395 (0.21), 461 (0.21), 574 (−0.62), 678 (0.17) |
| Λ-*cis*-Cr(men)$_3$ | 1.26 | 10 | 425 (65.6), 591 (70.3) | 429 (−2.82), 573 (7.83), 670 (−1.85) |
| Δ-*cis*-Cr(men)$_3$ | 1.00 | 21 | 424 (66.7), 593 (70.2) | 425 (2.95), 574 (−8.26), 671 (1.91) |

[a] See text for discussion of these assignments. [b] $R_{st}$ = (distance of the complex from the starting point)/(distance of the standard from the starting point). The standard is the appropriate hydroxamic acid, *i.e.*, benzhydroxamic or *N*-methyl-*l*-menthoxyacethydroxamic acid. See Experimental Section for details of the chromatography. [c] Data refer to the visible region only. Units are l. mol$^{-1}$ cm$^{-1}$.

cases the cis isomers are more strongly bound by the sorbent because the dipole moments of the cis complexes are greater than those of the corresponding trans complexes. We similarly assign the cis isomer as the green band with the smaller $R_{st}$. (2) In the absence of any difference in energy, the trans isomer is expected to be more abundant, since the probability of forming the trans isomer is three times that of forming the cis isomer. The trans to cis ratio of 2.1 observed for Cr(benz)$_3$ differs slightly from the statistical value of 3.0. Since under the original preparative conditions there was sufficient time for isomer equilibration, this difference represents relative thermodynamic stabilities. (3) The cis isomers of tris($\alpha$-amino acid) complexes of cobalt(III) and chromium(III) have larger extinction coefficient maxima in their d–d electronic spectra than do the trans.[52-57] The difference in coordination environment between cis and trans isomers in the Cr(benz)$_3$ complexes is less than in the tris($\alpha$-amino acid) complexes, since both coordinating atoms of the hydroxamate ligand are oxygen. Nevertheless there is a small change in absorbance, and we assign the trans isomer to the complex with the spin-allowed d–d transitions $^4A_{2g} \rightarrow {}^4T_{1g}$ and $^4A_{2g} \rightarrow {}^4T_{2g}$ at 400 ($\epsilon$ 117) and 600 nm ($\epsilon$ 83), respectively, and the cis isomer to the complex with these transitions at 415 ($\epsilon$ 121) and 596 nm ($\epsilon$ 82).

Both the cis and trans isomers of Cr(benz)$_3$ are much more kinetically labile than most other complexes of chromium(III). We find the cis to trans isomerization to have a half-life of several hours in solution at room temperature.[32] However, the lability of Cr(benz)$_3$ is not surprising in view of the structural similarity between the hydroxamate and tropolonate ligands. Holm, *et al.*, have recently reported the fast isomerization kinetics of tris(tropolonato)cobalt(III) complexes.[58]

**Tris(*N*-methyl-*l*-menthoxyacethydroxamato)chromium(III).** The properties of the chromium(III) and iron(III) complexes, Cr(men)$_3$ and Fe(men)$_3$, are summarized in Table I. For the iron complex, thin-layer chromatography results in one broad reddish brown band whose elution $R_{st}$ value is bracketed by the bluish green bands of the cis and trans isomers of the chromium(III) complex. As with the tris(benzhydroxamate) complexes, this behavior is caused by the rapid equilibration of the kinetically labile ferric complex, which elutes at a rate which is a weighted average of the individual isomer elution rates.

The isomers of Cr(men)$_3$ isomerize with half-lives (several hours) similar to the Cr(benz)$_3$ complex. The rate of isomerization of the tris(hydroxamate) complexes is therefore not particularly sensitive to the substituent on the hydroxamate nitrogen atom, since the men ligand contains an alkylated nitrogen atom and the benz ligand contains an unsubstituted nitrogen atom.

Although four diastereoisomers (Λ-cis, Λ-trans, Δ-cis, and Δ-trans) are expected for Cr(men)$_3$, thin-layer chromatography of the complex yielded only three bluish green bands. Since an examination of molecular models shows all four diastereoisomers are possible, we conclude that two diastereoisomers are contained in one of the bluish green bands in the tlc. We assign the geometrical isomers to these three bands from the following considerations. (1) The Λ and Δ optical isomers of a given geometric isomer should have CD spectra which are mirror images, since the metal chromophores for the two optical isomers are mirror images. The vicinal effect of the *l*-menthoxy group is expected to be negligible since it is physically distant from the metal ion and itself does not absorb in this region of the spectrum. If the cis and trans diastereoisomers of Cr(men)$_3$ are assumed to have comparable CD intensities, $\Delta\epsilon$, as do the cis and trans diastereoisomers of tris($\alpha$-amino acid) complexes of cobalt(III)[42,59-62] and tris($\beta$-diketone) complexes of chromium(III),[51] then the fastest eluting band in the tlc must contain two diastereoisomers of opposite configuration from the CD data. (2) The electronic absorption spectra of the Λ-cis and Δ-cis isomers should be identical and distinguishable from the Λ-trans and Δ-trans isomers. Since the cis isomers are expected to have greater extinction coefficient maxima in their electronic absorption spectra than the trans isomers, the

(52) Y. Shimura and R. Tsuchida, *Bull. Chem. Soc. Jap.*, **29**, 311 (1956).
(53) F. Basolo, C. J. Ballhausen, and J. Bjerrum, *Acta Chem. Scand.*, **9**, 810 (1955).
(54) M. Mori, M. Shibata, E. Kyuno, and M. Kanaya, *Bull. Chem. Soc. Jap.*, **34**, 1837 (1961).
(55) J. H. Dunlop and R. D. Gillard, *J. Chem. Soc.*, 6531 (1965).
(56) G. Saraceno, I. Nakagawa, S. I. Mizushima, C. Curran, and J. V. Quagliano, *J. Amer. Chem. Soc.*, **80**, 5018 (1958).
(57) N. Israily, *C. R. Acad. Sci., Ser. C*, **262**, 1426 (1966).
(58) S. S. Eaton, J. R. Hutchison, R. H. Holm, and E. L. Meutterties, *J. Amer. Chem. Soc.*, **94**, 6411 (1972).
(59) M. Shibata, H. Nishikawa, and Y. Nishida, *Bull. Chem. Soc. Jap.*, **39**, 2310 (1966).
(60) B. E. Douglas and S. Yamada, *Inorg. Chem.*, **4**, 1561 (1965).
(61) R. D. Gillard and N. C. Payne, *J. Chem. Soc. A*, 1197 (1969).
(62) E. Larsen and S. F. Mason, *J. Chem. Soc. A*, 313 (1966).

fastest eluting band in the tlc must be a mixture of trans isomers[63] while the latter two bands with identical absorption spectra must be cis from the absorption data. (3) This assignment of geometric isomers is consistent with their polarity, *i.e.*, the more polar cis isomers elute slower in the tlc. Thus the bands with $R_{st}$ values of 1.00 and 1.26 are assigned as cis, and the band with an $R_{st}$ value of 2.05 must contain both trans isomers within it, unresolved. (4) The total ratio of trans to cis isomers as assigned of 2.2 is very similar to that observed for the tris(benzhydroxamate) complexes and near the statistical value of 3.0. If it is assumed that $\Delta\epsilon$ for the $E_a$ transition of the trans isomer is the same as the cis (8.0, *vide infra*), the relative abundance of the Δ-trans and Λ-trans isomers can be calculated as 38 and 31%, respectively.

The assignments of absolute configuration are based on the CD spectra shown in Figure 1 and summarized in Table I. These assignments are based on the following considerations. (1) Assuming $D_3$ coordination point symmetry for these complexes, the $^4A_{2g} \rightarrow {}^4T_{2g}$ octahedral transition factors into $^4A_2 \rightarrow {}^4A_1 + {}^4E$, which gives transitions of $A_2$ and E symmetry. The $A_2$ and low energy E (designated $E_a$) transitions should be opposite in sign and, if these transitions can be identified, the absolute configuration can be assigned from a widely used empirical rule. For $d^3$ and low-spin $d^6$ trigonal complexes, this rule predicts that if the low energy E band in the CD spectrum is positive, the complex has a Λ absolute configuration.[64] It has also been found that for most trigonal $d^3$ and low-spin $d^6$ complexes the stronger CD band from the $^4A_{2g} \rightarrow {}^4T_{2g}$ manifold is of $E_a$ rather than $A_2$ symmetry.[64] The relative energy of the $E_a$ and $A_2$ states is determined by the trigonal splitting constant $K$. For tris(β-diketone) cobalt(III)[50,51,64-67] and chromium(III)[51] complexes, $K$ has been found to be positive (*i.e.*, $E_a$ is of higher energy than $A_2$). The absolute configurations and CD band assignments of these complexes have been determined by X-ray diffraction[68] and single-crystal polarized CD spectra.[69] (2) Although the oxygen donor atom six-membered chelate rings of the β-diketones may be good electronic models for the hydroxamate complexes, they are not similar in geometry. However, the tris(oxalato) chromium(III) complex is both electronically and geometrically similar to the hydroxamates. The absolute configuration of the tris(oxalato) complex is known,[70-73] and $K$ is positive with the $E_a$ band both of higher energy and greater strength than the $A_2$ band. We therefore assign the isomer ($R_{st}$ = 1.26) with $\Delta\epsilon$ of 7.83 at 573 nm (the $E_a$ band) as the Λ-cis isomer and the isomer

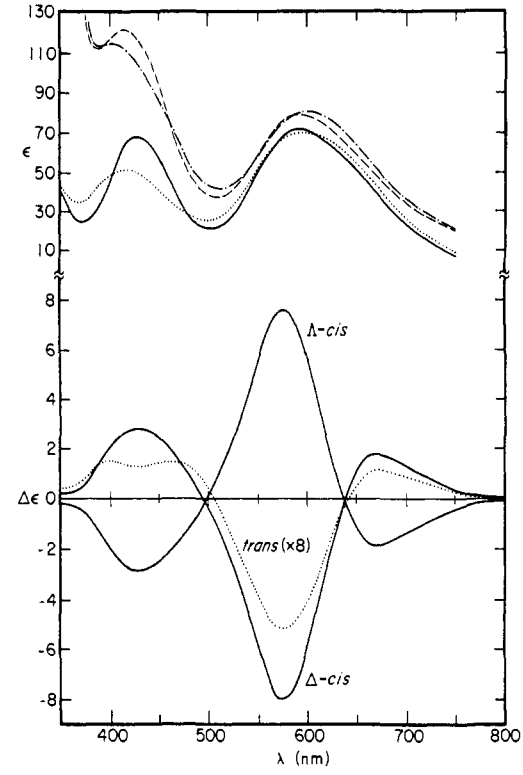


**Figure 1.** Absorption spectra of $Cr(benz)_3$ in 17% $CH_3OH$–$CHCl_3$ solution, and both absorption and CD spectra of $Cr(men)_3$ in 3% $CH_3OH$–$CHCl_3$ solution: *cis*-$Cr(benz)_3$ (— — —), *trans*-$Cr(benz)_3$ (— · —), *cis*-$Cr(men)_3$ (—), and *trans*-$Cr(men)_3$ (· · · · ·). The CD spectrum of the mixture of trans isomers has been multiplied by eight.

($R_{st}$ = 1.00) with the mirror image spectrum as Δ-cis. The isomer ($R_{st}$ = 2.05) with $\Delta\epsilon$ of −0.62 at 574 nm (the $E_a$ band) is assigned as a mixture of the trans Λ and Δ isomers in which the latter predominates. (3) The $^4A_{2g} \rightarrow {}^4T_{1g}$ octahedral transition at high energy factors, in point group $D_3$, into $^4A_2 \rightarrow {}^4A_2 + {}^4E$, which gives transitions of $A_1$ and E (designated $E_b$) symmetry. The $^4A_2 \rightarrow {}^4A_2$ transition is forbidden and so the high energy CD band has been assigned as $^4A_2 \rightarrow {}^4E_b$ in tris(oxalato)chromium(III)[73] and tris((+)-hydroxymethylenecamphorato)chromium(III).[64,67] The treatment of Piper and Karipides predicts that when $K$ is positive the signs of the $E_a$ and $E_b$ transitions will be opposite.[74] This is consistent with the assignments in Table I. Furthermore, the lower symmetry of the trans isomer further factors the E bands, which could explain the split $E_b$ band for the isomer with $R_{st}$ = 2.05.

In summary, tris(hydroxamate) complexes of chromium(III) have been prepared and resolved. The characterization and identification of the geometrical and optical isomers of these complexes have been made from their visible and circular dichroism spectra and their chromatographic properties. Since the visible and CD spectra of the isomers are wholly dominated by the metal complex chromophore, these data can be used in the characterization and identification of coordination isomers of complexes formed by the siderochromes. The characterization of such isomers is in progress and will be reported in subsequent papers of this series.

(63) Attempts to separate the two trans diastereoisomers by tlc with different solvent systems and sorbents such as cellulose powder and aluminum oxide were unsuccessful.
(64) A. F. McCaffery, S. F. Mason, and R. E. Ballard, *J. Chem. Soc.*, 2883 (1965).
(65) J. H. Dunlop, R. D. Gillard, and R. Ugo, *J. Chem. Soc. A*, 1540 (1966).
(66) G. W. Everett, Jr., and Y. T. Chen, *J. Amer. Chem. Soc.*, **92**, 508 (1970).
(67) S. F. Mason, *Quart. Rev., Chem. Soc.*, 17, 20 (1963).
(68) W. D. Horrocks, Jr., D. L. Johnston, and D. MacInnes, *J. Amer. Chem. Soc.*, **92**, 7620 (1970).
(69) T. S. Piper, *J. Chem. Phys.*, **35**, 1240 (1961).
(70) K. R. Butler and M. R. Snow, *Chem. Commun.*, 550 (1971).
(71) T. S. Piper, *J. Chem. Phys.*, **36**, 2224 (1962).
(72) T. S. Piper and R. L. Carlin, *J. Chem. Phys.*, **35**, 1809 (1961).
(73) A. J. McCaffery and S. F. Mason, *Trans. Faraday Soc.*, **59**, 1 (1963).
(74) A. G. Karipides and T. S. Piper, *J. Chem. Phys.*, 40, 674 (1964), and references therein.

**Acknowledgments.** We thank Mr. David Lee for experimental assistance, Professors Hubbell and Sauer for the use of their equipment, and Dr. Eileen N. Duesler for helpful discussions. This research was supported by the National Institutes of Health (Grant AI11744) and, in part, by the National Science Foundation.

# Heavy Metal–Nucleotide Interactions. Binding of Methylmercury(II) to Pyrimidine Nucleosides and Nucleotides. Studies by Raman Difference Spectroscopy[1]

**Samir Mansy, Thomas E. Wood, Jolyon C. Sprowles, and R. Stuart Tobias***

*Contribution from the Department of Chemistry, Purdue University, West Lafayette, Indiana 47907.* *Received October 12, 1973*

**Abstract:** Using data on the equilibrium constants for hydrolysis of $CH_3Hg^+$ and for its binding to uridine and cytidine, solutions in the 10–100 m*M* range have been prepared for which a particular complex should predominate. Raman spectra and particularly Raman difference spectra have been used to determine the perturbations of the cation and of the nucleoside ligand vibrations upon metal binding. The difference technique, applied here for the first time, is particularly effective for observing small spectral changes. Spectra have been obtained for the complex with polyuridilic acid to show that the perturbations are very similar with polynucleotides. A procedure for determining heavy metal binding sites on polynucleotides with two or more base moieties in aqueous solution is outlined. The methylmercury(II) ion binds to uridine (Urd) with displacement of a proton and coordination to $N_{(3)}$. Binding to cytidine also occurs at $N_{(3)}$, although, at pH 7, coordination to Urd is favored. The behavior of $CH_3Hg^+$ and $Hg^{2+}$ are compared.

The binding of metals by nucleosides and nucleotides has been investigated for a number of years. Because of recent observations that $CH_3Hg^+$ causes chromosome damage and consequently is mutagenic[2,3] and that certain platinum(II) compounds inhibit mitosis by selective inhibition of DNA synthesis,[4,5] there is renewed interest in the binding of heavy metals to polynucleotides.

In this work we have examined the interaction between $CH_3Hg^{II}$ and pyrimidine nucleosides and nucleotides. Equilibria of $CH_3Hg^+$ are relatively simple, since it is primarily a unifunctional electrophile. Consequently, the methylmercury cation should serve as a model for the binding of heavy metals to nucleosides, nucleotides, and polynucleotides. The mutagenic effect of the unifunctional $CH_3Hg^+$ electrophile compared to the antimitotic effect in tumor tissue of the (presumed) bifunctional $(NH_3)_2Pt^{II}$ parallels the activity of uni- and bifunctional organic alkylating agents.[6,7]

In 1961, Ferreira, *et al.*,[8] noted that $CH_3Hg^+$ and $C_2H_5Hg^+$ formed complexes with dThd,[9] and the competition reaction between $H^+$ and $CH_3Hg^+$ was studied. Because of the proton dependence, binding was assumed to occur at $N_{(3)}$. In 1966, Gruenwedel and Davidson[10] obtained a value for the equilibrium constant for dThd reacting with $CH_3HgOH$ by measuring the effect of dThd on the distribution between an aqueous and a toluene phase. Simpson[11] in 1964 used uv spectrophotometric measurements to obtain equilibrium constants for binding to Urd and to Cyd. Binding was assumed to occur at $N_{(3)}$ in both cases. With Cyd at high pH, a second reaction was observed, and this was assumed to be $CH_3Hg^+$ binding to the $C_{(4)}NH_2$ group.

Carrabine and Sundaralingham[12] recently determined the structure of the crystalline adduct $HgCl_2 \cdot 2Ura$ (Ura = uracil). This consists of a linear $HgCl_2$ molecule coordinated to one oxygen ($C_{(4)}=O$) from each of two Ura molecules with rather short Hg–O interactions of 2.71 (2) Å. In addition there are two chlorides from adjacent $HgCl_2$ molecules coordinated about mercury giving distorted octahedral coordination.

On the basis of the $HgCl_2 \cdot 2Ura$ structure, Carrabine and Sundaralingham suggested that reaction of mercury(II) with Ura, Urd, and dThd in aqueous solution occurs by coordination only with the oxygen of the $C_{(4)}=O$ group. The pH dependence of the binding was suggested to be caused by proton transfer from a water molecule in the first coordination sphere of the bound $Hg^{2+}$. This, however, does not account for the similar proton dependence observed for $CH_3Hg^+$ and $C_2H_5Hg^+$[8] where the second principal coordination site of mercury is blocked by the inert carbanion ligands.

Recently the usefulness of Raman spectroscopy in

(1) Work supported by Public Health Service Grant AM-16101 from the National Institute for Arthritis, Metabolism, and Digestive Diseases and by the National Science Foundation Grant GP-23208.

(2) G. Löfroth, "Methylmercury," Bulletin No. 4, Swedish National Science Research Council, Stockholm, 1969.

(3) J. J. Mulvihill, *Science*, **176**, 132 (1972).

(4) H. C. Harder and B. Rosenberg, *Int. J. Cancer*, **6**, 207 (1970).

(5) G. R. Gale J. A. Howle, and E. M. Walker, Jr., *Cancer Res.*, **31**, 950 (1971).

(6) W. C. J. Ross, "Biological Alkylating Agents," Butterworth, London, 1962.

(7) A. Haddow, *Advan. Cancer Res.*, **16**, 181 (1972).

(8) R. Ferreira, E. Ben-Zvi, T. Yamane, J. Vasilevskis, and N. Davidson, "Advances in the Chemistry of the Coordination Compounds," S. Kirschner, Ed., Macmillan, New York, 1961, p 457.

(9) The IUPAC–IUB abbreviations for nucleosides, etc., are employed throughout; see *Biochemistry*, **9**, 4022 (1970).

(10) D. W. Gruenwedel and N. Davidson, *J. Mol. Biol.*, 21, 129 (1966).

(11) R. B. Simpson, *J. Amer. Chem. Soc.*, **86**, 2059 (1964).

(12) J. A. Carrabine and M. Sundaralingham, *Biochemistry*, 10, 292 (1971).