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Three-Point Attachment of Citrate to a Cobalt(III) Tetraamine Complex

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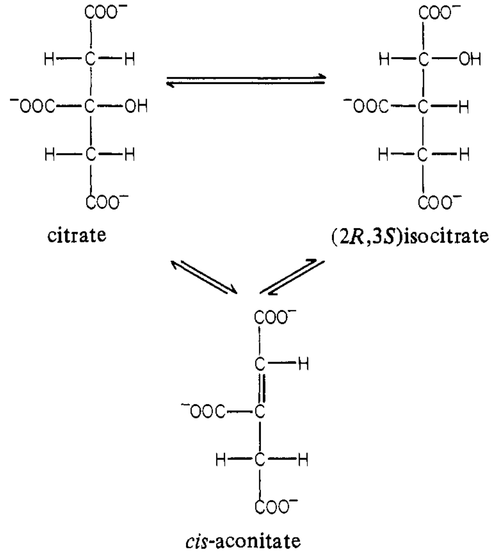
So that the possible modes of complexation of citrate to a metal-containing enzyme might be studied, the structure of cobalt(III) citrate complexed with triethylenetetramine, (citrato)(triethylenetetramine)cobalt(III) hydrate, $Co^{III}(C_6H_5O_7)(C_6H_{18}N_4)\cdot 5H_2O$, was determined by X-ray crystallographic techniques. The complex crystallizes in the triclinic space group $P\bar{1}$ with unit cell dimensions $a = 14.762$ (5) Å, $b = 17.498$ (5) Å, $c = 8.683$ (3) Å, $\alpha = 98.39$ (2)°, $\beta = 108.94$ (3)°, and $\gamma = 89.27$ (3)° with two complexes in the asymmetric unit. The tetraamine ligand has the conformation $\Lambda(\delta\lambda\lambda)$ or $\Delta(\lambda\delta\delta)$ (mirror-image forms). It coordinates to four octahedral positions around the Co(III) cation, leaving only two positions available for citrate coordination. The citrate ion is found to coordinate via the hydroxyl group and one of the oxygen atoms of the central carboxyl group, demonstrating the high affinity of the α-hydroxycarboxylate group for this metal cation. The complex is monomeric; that is, neither the tetraamine nor the citrate ion coordinates to more than one metal ion. Terminal carboxyl groups of the citrate ion also are involved in interactions; one forms an internal hydrogen bond to the tetraamine ligand, and in one of the two citrate ions in the asymmetric unit, there is an internal hydrogen bond between the hydroxyl group and the second terminal carboxyl group. The structure is heavily hydrated with approximately 10 water molecules in the asymmetric unit, and many of these are at disordered sites in channels that run through the crystal in the direction of the c axis. The structure described presents an instance of three-point attachment of citrate to an asymmetric molecule.

Introduction

Studies carried out in our laboratories have been aimed at the elucidation of mechanisms involving enzymes that utilize citrate as a substrate. One such example is the iron(II) activated enzyme aconitase, which is a member of the Krebs cycle system of enzymes and mediates the interconversion of the anions of the three tricarboxylic acids: citric, *cis*-aconitic and (+)-isocitric (Scheme I). The participation of citrate in the Krebs cycle was a puzzle to biochemists for some time since radiolabeling experiments had showed that the two carboxymethyl groups of citrate are distinguished in the aconitase reaction. Only one of the apparently equivalent groups is acted on by the enzyme aconitase. Ogston[2a] showed that this phenomenon could be explained by three-point attachment of the substrate to enzyme. In this case the two apparently equivalent groups would have nonequivalent surroundings. This is often referred to as chiral recognition of a prochiral center. As a result of the enzyme action, the symmetrical, prochiral citrate molecule is converted exclusively into the (2*R*,3*S*)-isocitrate molecule (one of four possible isomers). Proposed mechanisms based on stereochemistry surrounding the transition-metal ion include the "ferrous wheel" mechanism,[2b] trigonal twist mechanisms,[3] and rotation mechanisms.[4] Inactivation by (2*R*,3*R*)-fluorocitrate[5,6] has been proposed to occur as a result of formation of an inhibitory fluorocitrate adduct with a strategic base in the active site of the enzyme. In so doing, it is suggested that the fluorine atom in fluorocitrate coordinates to the metal.[5]

Scheme I



For the most part present arguments supporting such conjectures involving citrate enzymes are based on the crystal structures of metal citrate salts.[7] In an effort to establish binding conditions more nearly like those that are presumed to exist in the enzyme, salts of iron and nickel have been prepared.[8,9] However, these complexes have been shown to be polymeric and tetrameric, respectively, in the sense that one metal ion is coordinated to several citrate anions.

Because the citrate molecule possesses four potential ligating groups (three carboxyl groups and one hydroxyl group), it is difficult to suppress the propensity toward oligomerization caused by coordination of these groups on each citrate ion to more than one metal ion. Therefore, if we wish to reproduce some of the stereochemical conditions found in the active site of the enzyme, it is necessary to tie up some of the metal coordination sites with another ligand (as is undoubtedly the case for the enzyme itself). We chose to study a Co(III) tetraamine complex of citrate because this would offer only two coordination sites for the citrate, thereby forcing the substrate to be bidentate and precluding oligomer formation. In addition, Co(III) complexes are substitutionally "inert" and

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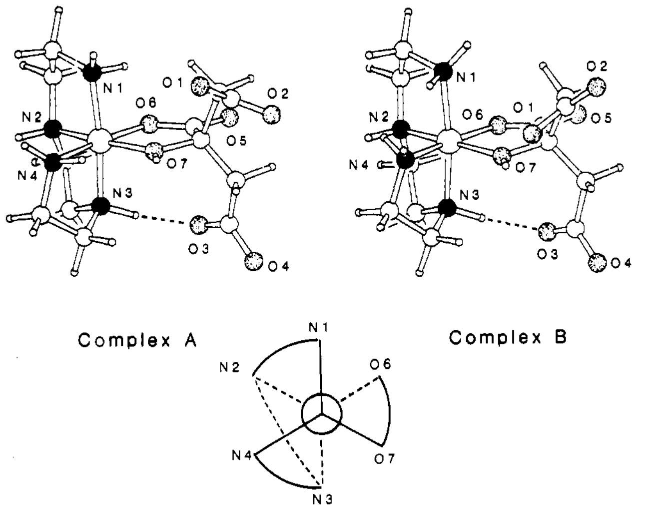
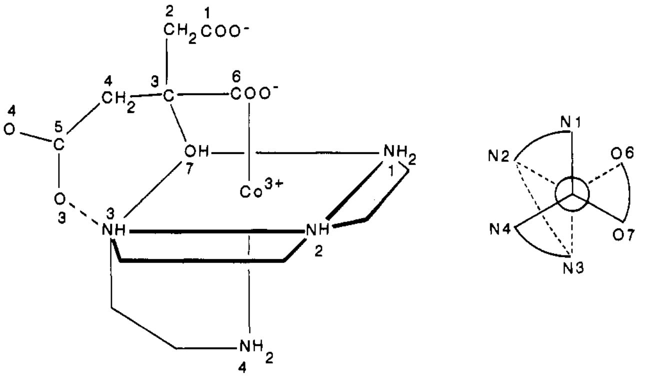


Figure 1. View of the two complexes in the asymmetric unit. In this and the following diagrams nitrogen atoms are black, oxygen atoms are stippled, and hydrogen atoms are represented by smaller circles. Since the crystal contains a racemate, each complex also exists in the mirror-image form. Note the similarities of the two complexes except for the torsion angle for the carboxyl groups involving O(1) and O(2).

as a result provide a stable complex in solution which can be expected to retain essential stereochemical aspects throughout mild chemical transformations. We report here the structure of (citrato)(triethylenetetramine)cobalt(III), which is shown, by an X-ray structural analysis, to be a monomeric citrate complex (I) that contains the citrate bound in a bidentate fashion (Figure 1).



I

Experimental Section

Synthesis of β-[(Citrato)(triethylenetetramine)cobalt(III)] Hydrate (I). Trisodium citrate dihydrate (4.73 g, 16.1 mmol), α-[dichloro(triethylenetetramine)cobalt(III)] chloride (5.0 g, 16.1 mmol), and 1 mL of triethylamine were refluxed for 40 h in 150 mL of methanol to yield a deep red solution. After the solvent was removed under reduced pressure, the solids were dissolved in a minimum volume of water and adsorbed onto a 4 cm × 25 cm cation-exchange column (Bio-Rad AG50WX2, H$^+$ form). Elution with 0.1 M NH$_3$ produced one red band from which the solvent was removed, under reduced pressure, to yield a red glass (4.26 g, 55.4%). Thermogravimetric analysis of the product, I, revealed a weight loss of about 16%, implying 4–5 molecules of water of hydration at this point. A 0.5-g quantity of I was dissolved in 1.5 mL of water at 70 °C and 9 mL of hot methanol added. When the solution cooled, well-formed red crystals were precipitated. The recrystallization conditions were critical. Too much water resulted in the separation of a red oil from the methanolic solution. Insufficient water resulted in a precipitation of a pink powder. The crystals were efflorescent.

Elemental Analysis. The material was dried at 100 °C overnight. Anal. Calcd: C, 6.22; H, 1.0; N/N, 2.42. Found: C, 6.07; H, 1.0; N/N, 2.45 (normalized to H).

Cryoscopy. Pure I was dried at 120 °C in vacuo. Depression of the Na$_2$SO$_4$/Na$_2$SO$_4$·10H$_2$O transition temperature, according to the technique of Lefebvre,[10] was used to determine the molecular weight of the complex in aqueous solution. Solutions (0.100 M) of Na$_2$Fe(CN)$_5$NO and Λ-β-[(dmt)Co(*S*-alanine)]Cl$_2$[11] were prepared as standards against which a 0.100 M solution of I was compared.

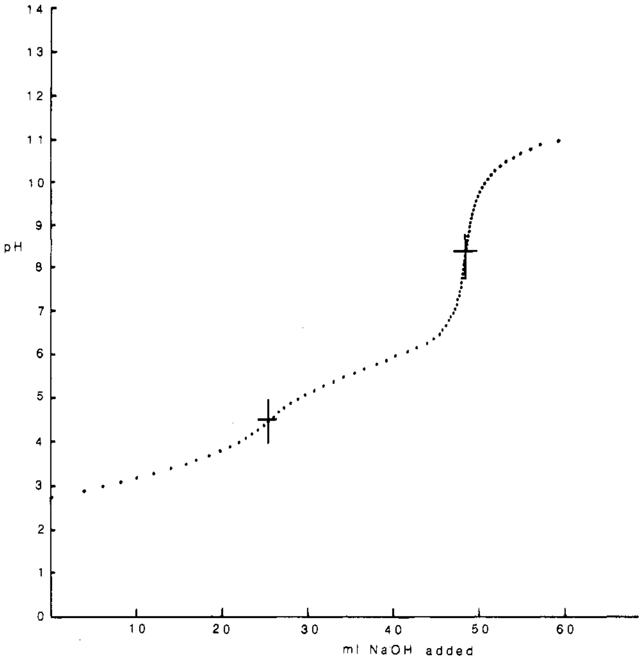


Figure 2. Titration curve. A 0.27-mmol quantity of cobalt complex was dissolved in 75 mL of water (3.64 × 10^{-3} M complex) and titrated with 1.1 × 10^{-2} M NaOH. Vertical lines indicate equivalence points (0.27 and 0.54 mM NaOH, respectively).

Titration. The titration of a 3.64 × 10^{-3} M solution of I, with 1.1 × 10^{-2} M NaOH, was monitored by a pH meter that had been standardized at pH 4.05 and 7.00. The titration curve is shown in Figure 2.

Spectrum. The major peak for I was at λ_{max} = 515 nm (ϵ 180); also a peak occurred at λ_{max} = 360 nm, both at pH 4.5. The spectrum is pH dependent. For the major peak λ_{max} = 495 nm at pH 1.0 and 527 nm at pH 13.0.

Diffraction Data Collection. The complex, Co^{III}(C$_6$H$_5$O$_7$)(C$_6$H$_{18}$N$_4$)·5H$_2$O, crystallized in the triclinic space group $P\bar{1}$, with unit cell dimensions (at room temperature) a = 14.762 (5) Å, b = 17.498 (5) Å, c = 8.683 (3) Å, α = 98.39 (2)°, β = 108.94 (3)°, γ = 89.27 (3)°, V = 2097 (1) Å^3, Z = 4 (two independent molecules per asymmetric unit), and λ = 0.71069 Å (Mo Kα radiation).

Three-dimensional data were collected on a Syntex P2$_1$ automated four-circle diffractometer with the θ–2θ variable scan technique and Mo Kα radiation using a highly oriented graphite-crystal monochromator. A total of 7835 independent reflections were scanned in the range (sin θ)/λ = 0 to 0.61 Å^{-1} of which 4092 had intensity I greater than the threshold of 2.33$\sigma(I)$, where $\sigma(I)$ was derived from counting statistics. Values of $\sigma(F)$ were determined as $\sigma(F) = (F/2)[(\sigma^2(I)/I^2) + \delta^2]^{1/2}$, where δ is an instrumental uncertainty determined from the variation of the measured intensities of four periodically monitored standard reflections (δ = 0.0505). There was no falloff in intensity as a function of time. The data were converted to structure amplitudes by application of Lorentz and polarization factors and an experimentally determined absorption correction and placed on an absolute scale with a Wilson plot.

Structure Determination and Refinement. The structure was solved by locating the cobalt atoms in a Patterson map and then using heavy-atom phasing methods to locate the other peaks. The complete structure was revealed in this way, including water molecules, some of which appear to be disordered. The atomic positions and anisotropic thermal parameters of all non-hydrogen atoms were refined by a full-matrix least-squares procedure. Hydrogen atoms were placed in computed positions and were included in these calculations. The quantity minimized was $\sum w[|F_o| - |F_c|]^2$, where the weights, w, were

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(10) J. Lefebvre, *J. Chim. Phys. Phys.-Chim. Biol.*, **54**, 581–600 (1957).

(11) dmt = (2*S*,9*S*)-2,9-dimethyl-4,7-diazadecane.

Table I. Positional Parameters and Isotropic Temperature Factors for the Cobalt Citrate Complex[a]

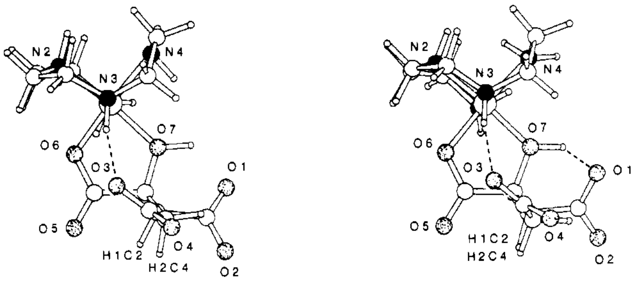
atom	*x*	*y*	*z*	⟨*B*⟩
Co(a)	0.05631 (9)	0.20915 (8)	0.3305 (2)	2.5 (1)
O(1)a	−0.0045 (5)	−0.0631 (4)	0.0998 (9)	3.7 (3)
O(2)a	0.1365 (5)	−0.1004 (4)	0.092 (1)	4.6 (4)
O(3)a	0.2829 (5)	0.1969 (4)	0.229 (1)	4.4 (4)
O(4)a	0.3223 (7)	0.1234 (5)	0.034 (1)	9.6 (5)
O(5)a	0.2940 (5)	0.1045 (4)	0.525 (1)	4.0 (4)
O(6)a	0.1726 (5)	0.1854 (4)	0.4827 (8)	2.9 (3)
O(7)a	0.0829 (4)	0.1186 (3)	0.1946 (8)	2.7 (3)
C(1)a	0.0855 (8)	−0.0589 (6)	0.154 (1)	3.0 (5)
C(2)a	0.1333 (7)	−0.0003 (6)	0.308 (1)	2.8 (5)
C(3)a	0.1644 (7)	0.0766 (5)	0.268 (1)	2.5 (4)
C(4)a	0.2288 (7)	0.0639 (6)	0.162 (1)	2.7 (5)
C(5)a	0.2817 (8)	0.1324 (7)	0.145 (2)	4.7 (6)
C(6)a	0.2175 (7)	0.1251 (6)	0.437 (1)	2.8 (5)
N(1)a	−0.0151 (6)	0.1509 (5)	0.433 (1)	3.5 (5)
N(2)a	0.0471 (6)	0.2974 (5)	0.485 (1)	3.3 (4)
N(3)a	0.1222 (6)	0.2789 (5)	0.246 (1)	3.5 (4)
N(4)a	−0.0578 (6)	0.2317 (4)	0.159 (1)	2.8 (4)
C(7)a	−0.0444 (8)	0.2053 (7)	0.556 (2)	5.1 (5)
C(8)a	0.0312 (9)	0.2675 (7)	0.625 (2)	5.0 (6)
C(9)a	0.1337 (8)	0.3476 (7)	0.519 (2)	4.1 (5)
C(10)a	0.1399 (8)	0.3577 (5)	0.356 (2)	4.4 (3)
C(11)a	0.0654 (8)	0.2830 (6)	0.075 (2)	4.0 (5)
C(12)a	−0.0368 (8)	0.2906 (6)	0.062 (2)	4.1 (5)
Co(b)	0.50639 (9)	0.31160 (8)	0.1482 (2)	2.58 (6)
O(1)b	0.6075 (5)	0.4779 (4)	0.5553 (9)	4.2 (3)
O(2)b	0.6226 (6)	0.6037 (4)	0.550 (1)	5.1 (4)
O(3)b	0.7698 (5)	0.3241 (4)	0.184 (1)	6.4 (4)
O(4)b	0.8831 (7)	0.3781 (7)	0.392 (1)	9.5 (7)
O(5)b	0.6305 (5)	0.4619 (4)	−0.0170 (9)	4.2 (3)
O(6)b	0.5437 (5)	0.3615 (4)	−0.0033 (9)	3.2 (3)
O(7)b	0.5986 (4)	0.3805 (4)	0.3152 (8)	2.8 (3)
C(1)b	0.6099 (7)	0.5357 (7)	0.481 (1)	3.4 (5)
C(2)b	0.5943 (7)	0.5179 (6)	0.297 (1)	3.0 (5)
C(3)b	0.6364 (6)	0.4398 (5)	0.250 (1)	2.2 (4)
C(4)b	0.7456 (7)	0.4474 (5)	0.320 (1)	2.9 (4)
C(5)b	0.8023 (8)	0.3766 (7)	0.292 (2)	4.3 (6)
C(6)b	0.6039 (7)	0.4212 (6)	0.065 (1)	3.2 (5)
N(1)b	0.3955 (6)	0.3791 (4)	0.118 (1)	3.1 (4)
N(2)b	0.4230 (6)	0.2415 (5)	−0.032 (1)	3.5 (5)
N(3)b	0.6031 (6)	0.2335 (5)	0.158 (1)	3.1 (4)
N(4)b	0.4784 (6)	0.2668 (5)	0.316 (1)	3.3 (4)
C(7)b	0.3100 (7)	0.3364 (7)	−0.013 (2)	4.1 (6)
C(8)b	0.3464 (9)	0.2838 (7)	−0.129 (2)	4.2 (6)
C(9)b	0.4862 (8)	0.1976 (7)	−0.117 (2)	4.2 (5)
C(10)b	0.5677 (8)	0.1694 (6)	0.014 (2)	4.2 (5)
C(11)b	0.6279 (8)	0.2070 (6)	0.318 (2)	3.9 (5)
C(12)b	0.5343 (8)	0.1943 (6)	0.350 (2)	4.2 (6)
OW(1)	0.7996 (7)	0.1055 (6)	0.185 (1)	7.3 (5)
OW(2)	0.8934 (8)	0.9404 (8)	0.318 (1)	11.0 (6)
OW(3)	0.3113 (6)	0.8486 (5)	0.283 (1)	10.1 (5)
OW(4)	0.6803 (7)	0.9932 (5)	0.193 (1)	13.3 (5)
OW(5)	0.3853 (9)	0.6819 (6)	0.314 (1)	17.9 (6)
OW(6)	0.5914 (7)	0.0171 (5)	0.500 (2)	15 (1)
OW(7)	0.156 (1)	0.625 (1)	0.293 (2)	28 (1)
OW(8)	0.2063 (9)	0.5079 (8)	0.111 (2)	23 (1)
OW(9)	0.001 (1)	0.506 (1)	0.381 (2)	19 (1)
OW(10)	0.939 (1)	0.5296 (9)	0.148 (3)	28 (2)
OW(11)	0.480 (2)	−0.002 (2)	0.186 (5)	14 (2)
OW(12)	0.724 (3)	0.295 (2)	0.824 (3)	23 (2)

[a] Positional parameters are given as fractions of cell edges. Isotropic temperature factors are expressed as $\exp(-B(\sin^2 \theta)/\lambda^2)$ with *B* values given in Å^2. The standard deviations for each parameter, determined from the inverted full matrix, are given in parentheses and apply to the last specified digits.

Table II. Depression of the $Na_2SO_4/Na_2SO_4 \cdot 10H_2O$ Transition Temperature for Coordination Complexes

compd[a]	Δ*T*, °C[b]
$Na_2Fe(CN)_5NO$	0.190
Λ-β-[(dmt)Co(*S*-ala)]Cl_2	0.590
1	0.193

[a] Prepared at 0.100 M on the basis of formula weight. [b] Average of three determinations.



Complex A Complex B

Figure 3. Internal hydrogen bond from tetraamine to citrate (broken line). The view is onto the plane Co(III), O(6), O(7), C(3), and C(6).

$1/\sigma^2(F)$. Atomic scattering factors for non-hydrogen atoms were those listed by Cromer and Mann[12] and for hydrogen atoms those given by Stewart, Davidson, and Simpson.[13] A correction for anomalous scattering by the cobalt ion was applied.[14] The computer programs used are part of the Crystallographic Program Library written at the Institute for Cancer Research. The final value of $R = \sum(||F_o| - |F_c||)/\sum|F_o|$ was 0.098 (0.092 for the weighted *R* value). The fractional coordinates of the atoms are given in Table I (anisotropic factors in Table A, supplementary material).

Results

The synthesis of I (as the principal product) proceeded smoothly, under mildly basic conditions in methanol. This X-ray crystallographic analysis of I shows that the complex contains the citrate ion bound in a bidentate fashion via the hydroxyl group and one carboxyl group, in a five-membered chelate ring (Figure 1).

Using $Na_2Fe(CN)_5NO$ as a monomeric standard, we found the same molar transition depression as reported by Strouse (0.190 °C).[9] The 0.59 °C depression observed for Λ-β-[(dmt)Co(*S*-ala)]Cl_2 agreed well with 0.57 °C expected for a complex yielding three independent ions in solution. On the basis of the $Na_2SO_4/Na_2SO_4 \cdot 10H_2O$ transition temperature lowering (Table II) we conclude that complex I exists as a pure monomer in solution.

As would be expected for a monomeric species with two noncoordinated carboxyl groups, titration of I with NaOH revealed two titratable protons in the pH 2–11 range. Evaluation of the equivalence points led to estimated values of 3.43 and 5.72 for pK_1 and pK_2, respectively (Figure 2).

The structure revealed by the X-ray analysis shows that the citrate ion is coordinated to the cobalt(III) ion via one of the oxygen atoms of the central carboxyl group and the hydroxyl oxygen atom, O(6) and O(7), respectively. The tetraamine is coordinated to the Co(III) ion through its four nitrogen atoms. The two independent complexes have very similar structures as shown in Figure 1. In addition there is an intramolecular hydrogen bond from the amine to the citrate ion, as shown in Figure 3. The geometry of the coordination polyhedron is listed in Table III. Torsion angles are listed in Table B (supplementary material).

The main differences between the two independent complexes (molecules A and B) lie in the orientation of one terminal carboxyl group of the citrate ion (involving O(1), O(2), C(1), and C(2)). This is shown by the variation in torsion angles (marked with asterisks in Table B). Also in molecule A the hydrogen atom on O(7) takes part in a hydrogen bond

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(12) D. T. Cromer and J. B. Mann, *Acta Crystallogr., Sect. A*, **A24**, 321–324 (1968).

(13) R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, **42**, 3175–3187 (1965).

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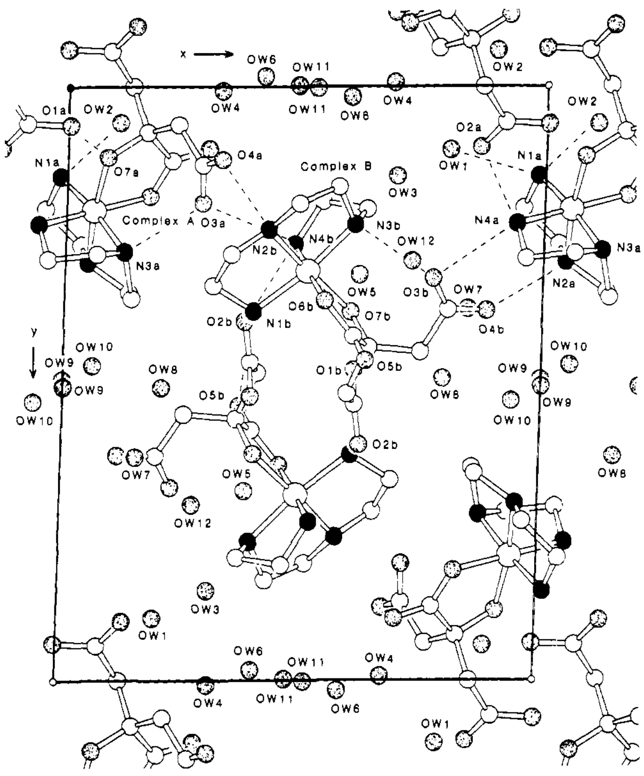

Figure 4. Packing in the unit cell. Hydrogen bonds involving the complex are indicated by broken lines.

Table III. Surroundings of Cobalt Ions

(a) Distances, A

Co(a)-O(6)a	1.881 (6)	Co(b)-O(6)b	1.901 (6)
-O(7)a	1.949 (5)	-O(7)b	1.913 (6)
-N(1)a	1.959 (7)	-N(1)b	1.977 (6)
-N(2)a	1.926 (8)	-N(2)b	1.924 (8)
-N(3)a	1.934 (7)	-N(3)b	1.954 (7)
-N(4)a	1.934 (7)	-N(4)b	1.911 (8)

(b) Angles, Deg[a]

	O(7)	N(1)	N(2)	N(3)	N(4)
O(6)	82.8 (2)	90.3 (3)	89.6 (3)	91.2 (3)	174.8 (3)
	85.5 (3)	88.9 (3)	89.8 (3)	92.9 (3)	174.7 (3)
O(7)		94.8 (3)	172.3 (3)	92.9 (3)	93.4 (3)
		97.7 (3)	174.0 (3)	90.3 (3)	89.2 (3)
N(1)			86.4 (3)	172.3 (3)	93.5 (3)
			86.0 (3)	171.9 (3)	92.5 (3)
N(2)				86.1 (3)	94.1 (3)
				86.2 (3)	95.4 (3)
N(3)					85.5 (3)
					86.5 (3)

[a] Values for complex A are above those for complex B.

Table IV. Hydrogen Bonding

(a) Citrate Complex Hydrogen-Bonding Distances (D-H···A), A[a]

D	H	A	D···A	symmetry operation for A
O(7)a	H(O7)a	O(1)a	2.49 (1)	$-x, -y, -z$
N(1)a	H(1N1)a	OW(1)	2.92 (1)	$x - 1, y, z$
N(1)a	H(2N1)a	OW(2)	2.97 (1)	$1 - x, 1 - y, 1 - z$
N(2)a	H(N2)a	O(4)b	2.74 (1)	$x - 1, y, z$
N(3)a	H(N3)a	O(3)a	2.79 (1)	x, y, z
N(4)a	H(1N4)a	O(3)b	3.05 (1)	$x - 1, y, z$
N(4)a	H(2N4)a	O(2)a	2.89 (1)	$-x, -y, -z$
O(7)b	H(O7)b	O(1)b	2.46 (1)	x, y, z
N(1)b	H(1N1)b	O(2)b	2.95 (1)	$1 - x, 1 - y, 1 - z$
N(1)b	H(2N1)b	O(5)b	3.02 (1)	$1 - x, 1 - y, -z$
N(2)b	H(N2)b	O(4)a	2.79 (1)	x, y, z
N(3)b	H(N3)b	O(3)b	2.87 (1)	x, y, z
N(4)b	H(1N4)b	O(2)b	3.00 (1)	$1 - x, 1 - y, 1 - z$
N(4)b	H(2N4)b	O(3)a	2.97 (1)	x, y, z

(b) Water Hydrogen-Bonding and Near-Neighbor Distances, A[b]

D-H	A or H-A	D···A	symmetry operation for A
OW(1)-H	O(2)a	2.84 (1)	$1 - x, -y, -z$
-H	OW(4)	2.68 (1)	$x, y - 1, z$
	H-N(1)a	2.91 (1)	$1 + x, y, z$
OW(2)-H	O(1)a	2.77 (1)	$1 + x, 1 + y, z$
-H	OW(4)	3.15 (2)	x, y, z
	H-N(1)a	2.97 (1)	$1 - x, 1 - y, 1 - z$
OW(3)-H	O(2)a	2.79 (1)	$x, 1 + y, z$
-H	OW(5)	3.12 (2)	x, y, z
	(or OW(12))	(or 2.55 (4))	
	H-OW(6)	2.86 (2)	$1 - x, 1 - y, 1 - z$
OW(4)*-H	O(4)a	2.62 (2)	$1 - x, 1 - y, -z$
-H	OW(11)	2.95 (4)	$x, 1 + y, z$
	H-OW(1)	2.68 (1)	$x, 1 + y, z$
	H-OW(2)	3.15 (2)	x, y, z
OW(5)*-H	O(1)b	3.14 (1)	$1 - x, 1 - y, 1 - z$
-H	O(6)b	3.19 (1)	$1 - x, 1 - y, -z$
	H-OW(3)	3.12 (1)	x, y, z
OW(6)-H	O(5)a	2.73 (1)	$1 - x, -y, 1 - z$
-H	OW(3)	2.86 (2)	$1 - x, 1 - y, 1 - z$
	H-OW(11)	2.66 (6)	x, y, z
	OW(6)	2.77 (1)	$1 - x, -y, 1 - z$
OW(7)*-H	O(4)b	2.98 (2)	$1 - x, 1 - y, 1 - z$
-H	OW(8)	2.65 (3)	x, y, z
	H-OW(10)	3.39 (2)	$x - 1, y, z$
OW(8)*-H	O(5)b	2.86 (2)	$1 - x, 1 - y, -z$
-H	O(1)b	3.26 (2)	$1 - x, 1 - y, 1 - z$
	H-OW(7)	2.65 (3)	x, y, z
	H-OW(10)	2.57 (2)	$1 - x, 1 - y, -z$
OW(9)*-H	O(4)b	2.86 (3)	$x - 1, y, z$
-H	O(4)b	2.75 (2)	$1 - x, 1 - y, 1 - z$
OW(10)*-H	OW(7)	3.39 (2)	$1 + x, y, z$
-H	OW(8)	2.57 (2)	$1 - x, 1 - y, -z$
OW(11)*-H	OW(6)	2.66 (6)	x, y, z
	OW(6)	3.27 (5)	$1 - x, -y, 1 - z$
	H-OW(4)	2.95 (4)	$x, y - 1, z$
OW(12)*-H	OW(7)	2.80 (4)	$1 - x, 1 - y, 1 - z$
-H	O(3)b	2.93 (4)	$x, y, 1 + z$
	H-OW(3)	2.55 (4)	$1 - x, 1 - y, 1 - z$

[a] Note that hydrogen positions are calculated and therefore only D···A is listed. [b] Note that hydrogen atoms on water molecules were not located, but their locations were inferred from distances and available hydrogen atoms for hydrogen bonding. Asterisks denote water molecules with high B values (see Table I). OW(9) and OW(10) occupy overlapping sites. OW(5) and OW(12) occupy overlapping sites.

to O(1) of another molecule, while in molecule B an intramolecular hydrogen bond is formed to O(1) of the same molecule (see Table IV, part a). In this same carboxyl group O(2) of molecule A is hydrogen bonded to N(4) of the amine, while O(2) of molecule B is hydrogen bonded to both N(4) and N(1) of the same amine. It is interesting that the temperature factors for O(4), not involved in this hydrogen bonding, are high in both molecules (Table I). The hydrogen bonding listed in Table IV shows that the carboxyl group (O(3), O(4), C(5)) is hydrogen bonded to N(2) and N(4) of another complex (carboxyl A to complex B and vice versa). The hydrogen bonding of the carboxyl group (O(1), O(2), C(1)) differs for molecules A and B but involves interactions with O(7) and with amine nitrogen atoms of the same type of complex (carboxyl A to complex A, etc.).

This structure is heavily hydrated with approximately 10 water molecules per asymmetric unit. Water does not take part in the first coordination sphere of the cobalt ion but serves to bind the structure together, and in certain areas this binding is quite loose. Essentially, there are channels of disordered water molecules running parallel to the c axis at $x = {}^1/_2$, $y = 0$ (OW(6) and OW(11)) and at $x = 0$, $y = {}^1/_2$ (OW(9) and OW(10)). Several water molecules were refined with partial occupancy, and so that the disorder might be understood further, highly anisotropic water molecules were represented

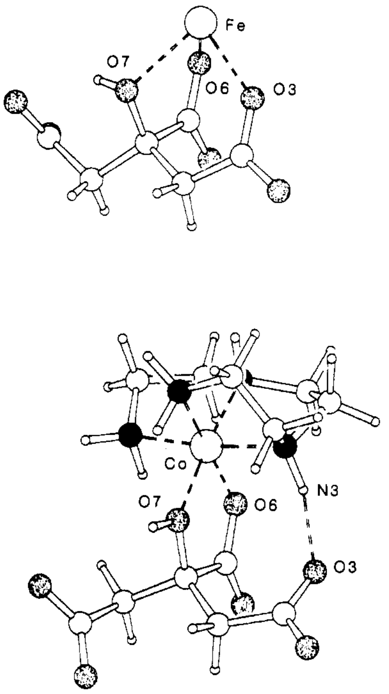

Figure 5. Comparative views of the cobalt complex and ferrous citrate.

as two isotropic molecules each at half-occupancy. The best analysis that could be arrived at is represented in Table IV. The hydrogen-bonding system is such that all water molecules except OW(9) are interconnected.

Discussion

This X-ray crystallographic analysis of (citrato)(triethylenetetramine)cobalt(III) has shown that the tetraamine ligand coordinates to four octahedral positions on the cobaltic ion and that the remaining two coordination positions of the metal ion are occupied by the central carboxyl group and the hydroxyl group in the position α to it in citrate. In addition, one terminal carboxyl group of citrate forms a hydrogen bond to the amine. Thus, although only two positions are available in the coordination octahedron of Co(III) for citrate to bind to the metal ion, a third point of attachment of the citrate ion to the cobalt tetraamine complex occurs as a result of this hydrogen bond. As a result, therefore, the two prochiral $-CH_2-COO^-$ groups should behave differently.[2] The tetraamine itself is not asymmetric, but its four-point attachment to the cobalt(III) ion, in the presence of citrate, results in an asymmetric complex in which two adjacent coordination sites are occupied by citrate. The cobalt tetraamine complex, with trans coordination positions available for further complexation, can be superimposed on its mirror image, but this is not true for the complex with cis coordination positions available. Citrate can coordinate, via the central α-hydroxycarboxylate group, to such a cis complex in two ways. It will, in fact, coordinate so that an additional interaction, that is, hydrogen bonding of a terminal carboxyl group to the tetraamine, can occur. This is "three-point attachment" and has been described in detail for an (aminomethyl)malonate complex of cobalt.[15]

The results shown here are consistent with the type of metal binding envisioned for the active site of the enzyme aconitase,[2b] although tridentate chelation such as is found in iron(II) citrate[9] was considered most likely.[2b] As shown in Figure 5, the only difference in the chelation of citrate in this Co(III) complex and in iron(II) citrate is the binding of a terminal carboxyl group which is hydrogen bonded to an NH group of the tetraamine in the Co(III) complex and lies on the Fe(II) coordination (inner) sphere in the iron(II) citrate complex. In both cases there is three-point attachment of citrate. An analysis of H–C–C(3)–O(7) torsion angles and C–C–C(3) bond angles indicated that both kinds of binding led to similar values for these structural parameters; that is, in neither type of binding is additional strain introduced into the citrate ion. Thus it is concluded that both types of binding are acceptable models for citrate binding to an enzyme.

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Registry No. $Co(C_6H_5O_7)(C_6H_{18}N_4)\cdot 5H_2O$, 82621-19-0; trisodium citrate dihydrate, 6132-04-3; α-[dichloro(triethylenetetramine)cobalt(III)] chloride, 15555-97-2; citric acid, 77-92-9.

Supplementary Material Available: Listings of anisotropic temperature factors, torsion angles, and observed and calculated structure factors (14 pages). Ordering information is given on any current masthead page.

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