(m, 8 H, CH_2OCH_2), 4.05–4.30 (m, 4 H, $ArOCH_2$), 7.48–7.70 and 6.80–7.00 (m, 3 H, aromatic H); MS, *m/e* (% relative intensity) 504 (M^+, 89), 280 (100). Anal. Calcd for $C_{31}H_{52}O_5$: C, 73.77; H, 10.38. Found: C, 73.51; H, 10.41.

Evaluation of Lithium Selectivity as a Potentiometric Selectivity Coefficient. Membrane Electrode. The typical procedure for membrane preparation is as follows: PVC (100 mg), NPOE (250 mg), crown ether (3.6 mg, 1 wt %), and KTpClPB (about 2.4 mg, 50 mol % to the crown ether) were dissolved in 3–4 mL of THF. This solution was then poured into a flat Petri dish of 34-mm inner diameter. Gradual evaporation of the solvent at room temperature gave a transparent, flexible membrane of about 0.15 mm in thickness. A disk of 7 mm in diameter was cut from the PVC membrane using a cork borer and incorporated into an electrode body of Orion Model 92. The diameter of the exposed membrane was about 2 mm. After injection of 1 M LiCl aqueous solution as the internal solution, the electrode was conditioned by soaking into 1 M LiCl aqueous solution overnight. The external reference electrode is a double junction type Ag/AgCl glass electrode. The composition of electrochemical cell is given as Ag·AgCl|1 M LiCl|PVC membrane|sample solution|0.1 M NH_4NO_3|4 M KCl|AgCl·Ag.

EMF Measurements. All EMF measurements were made at 25 °C, using a pH/mV meter of high input impedance in combination with a recorder. Sample solutions were magnetically stirred and kept in a double-wall glass container connected with a circulating bath. The electrode systems and the mV meter were contained in a Faraday cage to cut off any electrical noise. The EMF values were corrected by subtracting a liquid-junction potential between the external rederence electrode and the sample solution in the high Li^+ concentrations. The liquid-junction potentials were computed according to Henderson's equation.[47]

Selectivity Coefficients. The potentiometric selectivity coefficients k_{LiM}^{Pot} determined here are defined in the Nicolsky–Eisenman equation:[48]

$$E = \text{constant} + \frac{2.303RT}{F} \log [a_{Li} + k_{LiM}^{Pot}(a_M)^{1/z_M}]$$

where E = the experimentally observed potential, R = the gas constant, T = the thermodynamic temperature, F = the Faraday constant, a_{Li} = the Li^+ activity, a_M = the activity of the foreign cation, and z_M = the charge of the foreign cation. The selectivity coefficients were determined by a mixed solution method (fixed interference method) according to IUPAC recommendations.[20] The EMF of the electrochemical cell was measured with solutions of a constant level of foreign cation and varying Li^+ activity. The constant concentrations for the foreign cations were 5×10^{-2} M for alkali metal ions and H^+, and 5×10^{-1} M for alkaline-earth metal ions and NH_4^+ in the case of **2** through **4**. In the case of the other crown ethers, they were 5×10^{-5} M for alkali metal ions, 5×10^{-2} M for Mg^{2+} and H^+, and 5×10^{-3} M for the other foreign cations. When the EMF values obtained are plotted against the Li^+ activity, the intersection of the extrapolation of the linear portions of this plots gives the values of a which are to be used to compute k_{LiM}^{Pot} from the equation

$$k_{LiM}^{Pot} = a/(a_M)^{1/z_M}$$

In the Li^+ concentrations of less than 0.1 M the activity coefficients γ were calculated by using the following equation based on the Debye–Hückel theory:

$$\log \gamma = -0.509(I)^{1/2}/(1 + I^{1/2})$$

where I denotes the ionic strength. Some of the experimental values of activity coefficients in the literature[49] were also employed in the higher Li^+ concentrations.

Conductivity Measurements. The conductivity measurements were carried out at 25 ± 0.1 °C with use of a conductivity cell with a cell constant of 0.479 cm^{-1}. The procedure is as follows: An acetonitrile solutions of the alkali metal perchlorate (5×10^{-4} M, 10 mL) was placed in the cell and the resistance of the solution measured. A stepwise increase in the crown ether concentration was effected by addition of a crown ether solution (2×10^{-2} M) to the cell with use of microsyringe. In order to keep the salt concentration constant during the titration, the crown ether solution contained the same concentration of salt as the initial salt solution. The resistance of the crown ether–salt solution was measured on each addition and corrected by the conductivity of pure acetonitrile. The molar conductivity of each addition of the crown ether solution was computed from the corrected value of the resistance. According to the procedure in the literature,[50,51] the complex formation constants for the 1:1 complex were calculated by least-square methods using a microcomputer.

Registry No. 1a, 92144-64-4; **1b**, 92144-65-5; **2a**, 91539-73-0; **2b**, 91539-72-9; **2c**, 92144-66-6; **2d**, 92144-67-7; **3a**, 92144-68-8; **3b**, 92144-69-9; **4a**, 92144-70-2; **4b**, 92144-71-3; **5a**, 92144-72-4; **5b**, 92144-73-5; **6a**, 62150-58-7; **6b**, 92144-74-6; **7** (R = H), 10395-09-2; **7** (R = CH_3), 13064-31-8; **8** (*m* = 0; *n* = 1), 92144-75-7; **8** (*m* = 1; *n* = 0), 92144-76-8; **8** (*m* = 1; *n* = 1), 92144-77-9; **9**, 92144-78-0; diethyl dodecylmalonate, 7252-87-1; 5,5-dimethyl-3,7-dioxa-1,9-nonandioic acid, 65115-11-9; diethyl 5,5-dimethyl-3,7-dioxa-1,9-nonandioate, 65115-08-4; 5,5-dimethyl-3,7-dioxanonane-1,9-diol, 92144-79-1; 1,8-dicyano-3,6-dioxaoctane, 3386-87-6; dimethyl 4,7-dioxadecane-1,10-dioate, 19364-66-0; 4,7-dioxadecane-1,10-diol, 92144-80-4; 2-(hydroxymethyl)-12-crown-4, 75507-26-5; triethylene glycol ditosylate, 19249-03-7; 2,2-dimethyl-1,3-propanediol, 126-30-7; chloroacetic acid, 79-11-8; ethylene glycol, 107-21-1; acrylonitrile, 107-13-1; 1,3-dibromopropane, 109-64-8; 1,3-propanediol, 504-63-2; 1,2-bis(2-chloroethoxy)ethane, 112-26-5; octadecyl bromide, 112-89-0; octadecanoic acid, 57-11-4; Li^+, 17341-24-1; PVC, 9002-86-2.

(47) Henderson, P. *Z. Phys. Chem.* **1907**, *59*, 118–127; **1908**, *63*, 325–345.
(48) Koryta, J. "Ion-selective Electrodes"; Cambridge University Press: Cambridge, 1975; pp 64–67.
(49) Parsons, R. "Handbook of Electrochemical Constants"; Butterworths; London, 1959; pp 20–29.
(50) Takeda, Y.; Yano, H.; Ishibashi, M.; Isozumi, H. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 72–76.
(51) Kulstad, S.; Malmsten, L. Å. *J. Inorg. Nucl. Chem.* **1980**, *42*, 573–578.

Siderophore Iron-Release Mechanisms

Robert C. Hider,* David Bickar, Ian E. G. Morrison, and Jack Silver*

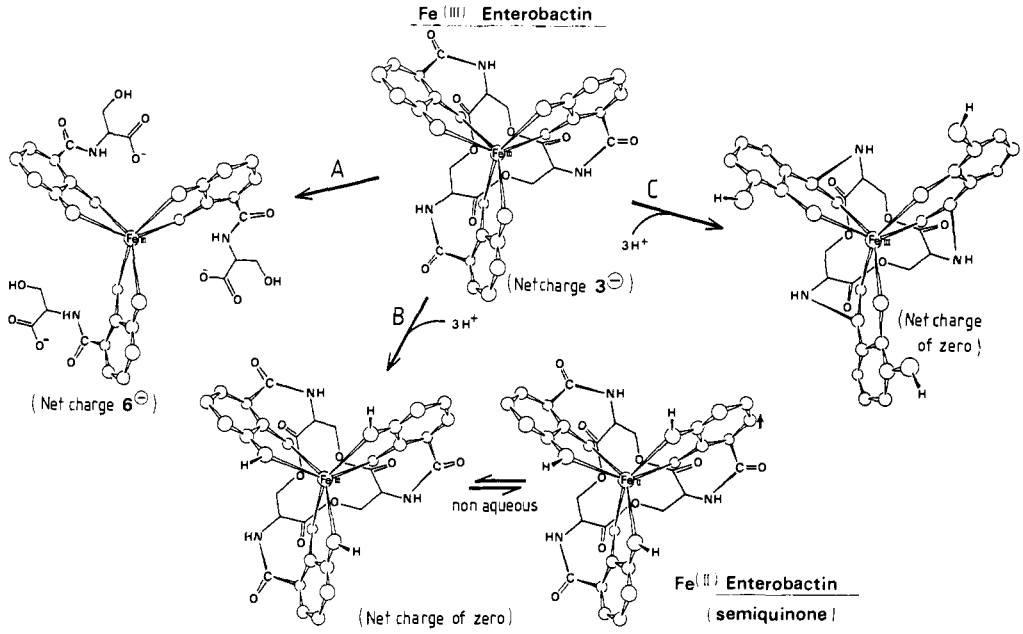
Contribution from the Department of Chemistry, University of Essex, Colchester, Essex, United Kingdom. *Received March 30, 1984*

Abstract: The structures of the iron complexes of catechol, enterobactin, and the synthetic siderophore *N,N′,N″*-tris(2,3-dihydroxybenzoyl)-1,3,5-tris(aminoethyl)benzene (MECAM) are discussed. The recent proposal for a "tris(salicylate)" mode of coordination involving the ortho carbonyl of the 2,3-dihydroxybenzoyl rings for the triprotonated complex $[Fe(H_3MECAM)]^0$ (Pecoraro et al. *J. Am. Chem. Soc.* **1983**, *105*, 4617) is questioned, since this complex would require considerable compression for each of the three carbonyl oxygens. We propose an alternative structure involving catecholato coordination and support this suggestion with cyclic voltammetry data. $[Fe(MECAM)]^{3-}$ is capable of donating iron to *Escherichia coli* yet cannot be hydrolyzed by enterobactin esterase. These observations are critical to the development of theories for the intracellular iron-release mechanism of catecholato siderophores.

Iron is essential for virtually all life forms, having a critical role in the action of many enzymes and redox proteins. Animals obtain iron in their diet, but for plants and unicellular organisms iron is relatively inaccessible since it forms insoluble hydroxides. Many microorganisms have developed the ability to synthesize low molecular weight polydentate ligands that possess a high affinity

0002-7863/84/1506-6983$01.50/0

Scheme I



for iron(III).[1,2] There are two major groups of these siderophores, the hydroxamate class, as characterized by ferrichrome,[3] and the catecholate class, as characterized by enterobactin(I).[4] At low iron levels ([iron] $< 10^{-6}$ M) the cells secrete siderophores. The resulting iron complexes either are reabsorbed via specific receptors and donate iron within the organism or donate iron via a specific cell surface mechanism. For hydroxamate siderophores, the donation of iron is simple: the coordinated iron can be reduced to iron(II), which has a much lower affinity for the siderophore. This mechanism has been adequately demonstrated for a number of organisms.[5-8] Indeed, iron-free ferrichrome is recycled during iron absorption.[5,9] With catecholato siderophores, the mechanism of iron release is more complex and consequently more difficult to elucidate. Enterobactin possesses the largest formation constant ($K_f = 10^{52}$) of any known complex.

$$K_f = [\mathrm{Fe(ent)}]^{3-}/[\mathrm{Fe}^{3+}][\mathrm{ent}^{6-}]$$

At first sight, it seems difficult to envisage a chemical process capable of removing iron from enterobactin. However the formation constant takes no account of the hydrogen ion concentration, an important term in eq 1.

$$\mathrm{H_6(ent)} + \mathrm{Fe}^{3+} \rightleftharpoons \mathrm{Fe(ent)}^{3-} + 6\mathrm{H}^+ \qquad (1)$$

Indeed, the value 10^{52} is only valid at pH $\sim$14. The effective equilibrium constant, K', of the reaction depicted by eq 1 is given by

$$K' = [\mathrm{Fe(ent)}^{3-}][\mathrm{H}^+]^6/[\mathrm{Fe}^{3+}][\mathrm{H_6(ent)}]$$

and is found to be 10^{-10} over the pH range 4-6.[10] Thus at pH 6 the value of the ratio $[\mathrm{Fe(ent)}^{3-}]/[\mathrm{Fe}^{3+}][\mathrm{H_6(ent)}]$ is 10^{26}. This value, although large, indicates that the removal of iron may not be as difficult as is implied by a formation constant of 10^{52}.

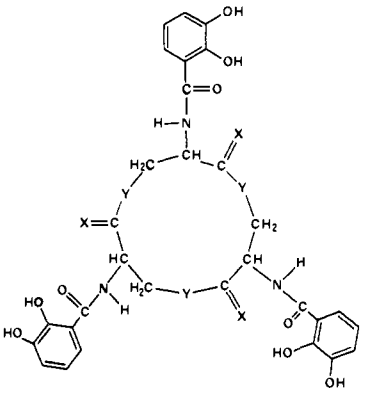
Three mechanisms have been proposed for the release of iron from enterobactin (Scheme I): (a) iron enterobactin hydrolysis yielding iron(III) coordinated by bidentate ligands; (b) protonation of iron enterobactin in a medium of low dielectric constant followed by internal electron transfer to give iron(II); (c) protonation of iron enterobactin in aqueous media generating iron(III) coordinated to enterobactin in a salicylato mode. In each of the three mechanisms the reduced affinity of the modified enterobactin could release iron to other cytoplasmic ligands, possibly via a physiological reductant. The hydrolytic cleavage mechanism (Scheme IA) originated with a cyclic voltammetry study that clearly demonstrated that at neutral pH in aqueous solution, enzymic hydrolysis to (2,3-dihydroxybenzoyl)serine was required in order to generate a complex that could be reduced.[11] Iron(III) enterobactin was subsequently found to be a substrate for an esterase found in *E. coli*.[12]

Additional support for this concept came with the determination of the extremely high formation constant (K_f) and the estimated redox potential of iron enterobactin, namely, -750 mV at pH 7.0.[10,13] Raymond and co-workers point out that this is well below the range of physiological reducing agents and conclude that the observed hydrolysis of enterobactin is a necessary prerequisite to in vivo release of iron from the siderophore via ferric ion reduction.[13]

The internal electron-transfer mechanism for iron release (Scheme IB) was first suggested by Hider, Silver, and co-workers.[14] This proposal is based on the known ability of phenols and catechols to reduce iron(III) under acid conditions.[15,16] An attractive feature of the mechanism is that microorganisms should not have to destroy enterobactin in order to gain access to the coordinated iron. This has subsequently been demonstrated for

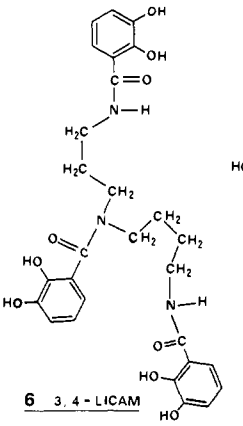
(1) Neilands, J. B.; Ratledge, C. In "Handbook of Microbiology"; Laskin, A. I., Lechevalier, H. A. Eds; CRC Press: Florida, 1982; Vol. IV, p 565.
(2) Neilands, J. B. *Annu. Rev. Microbiol.* **1982**, *36*, 285.
(3) Neilands, J. B. *J. Am. Chem. Soc.* **1952**, *74*, 4846.
(4) (a) Pollack, J. R.; Neilands, J. B. *Biochem. Biophys. Res. Commun.* **1970**, *38*, 989. (b) O'Brien, I. G.; Gibson, F. *Biochem. Biophys. Acta* **1970**, *215*, 393.
(5) (a) Emery, T. *Biochemistry* **1971**, *10*, 1483. (b) Emery, T. *Met. Ions Biol. Syst.* **1975**, *7*, 77.
(6) Leong, J.; Neilands, J. B. *J. Bacteriol.* **1976**, *126*, 823.
(7) Arceneaux, J. E. L.; Davis, W. B.; Downer, D. N.; Haydon, A. H.; Byers, B. R. *J. Bacteriol.* **1973**, *115*, 919.
(8) Brown, K. A.; Ratledge, C. *FEBS Lett.* **1975**, *53*, 262.
(9) Straka, J. G.; Emery, T. *Biochim. Biophys. Acta* **1979**, *569*, 277.
(10) Harris, W. R.; Carrano, C. J.; Cooper, S. R.; Sofen, S. R.; Avdeef, A. E.; McArdle, J. V.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 6097.
(11) O'Brien, I. G.; Cox, G. B.; Gibson, F. *Biochim. Biophys. Acta* **1971**, *237*, 537.
(12) Porra, R. J.; Langman, L.; Young, I. G.; Gibson, F. *Arch. Biochem. Biophys.* **1972**, *153*, 74.
(13) Cooper, S. R.; McArdle, J. V.; Raymond, K. N. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 3551.
(14) Hider, R. C.; Silver, J.; Neilands, J. B.; Morrison, I. E. G.; Rees, L. V. C. *FEBS Lett.* **1979**, *102*, 325.
(15) (a) Mentasti, E.; Pelizzetti, E. *J. Chem. Soc., Dalton Trans.* **1973**, 2305. (b) Silver, J.; Morrison, I. E. G.; Rees, L. V. C. *Inorg. Nucl. Chem. Lett.* **1979**, *15*, 433.
(16) (a) Hider, R. C.; Mohd-Nor, A. R.; Silver, J.; Morrison, I. E. G.; Rees, L. V. C. *J. Chem. Soc., Dalton Trans.* **1981**, 609. (b) Hider, R. C.; Howlin, B.; Miller, J. R.; Mohd-Nor, A. R.; Silver, J. *Inorg. Chim. Acta* **1983**, *80*, 51.

Chart I

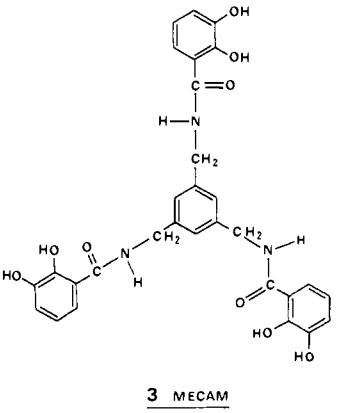


1 X = Y = O Enterobactin

2 X = H_2 Y = CH_2 Carbocyclic analogue of Enterobactin



6 3,4-LICAM



3 MECAM

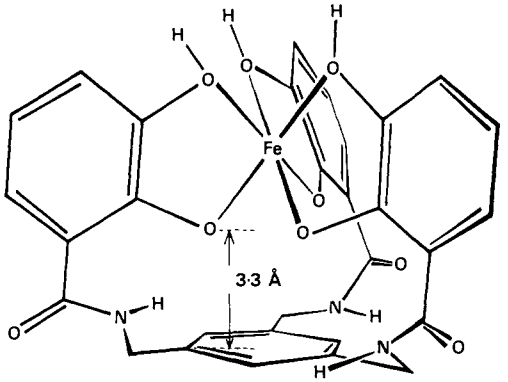
several enterobactin analogues that are incapable of cleavage by the enterobactin esterase. Both the carbocyclic analogue, 1,5,9-triaminocyclododecane fully acylated with dihydroxybenzoic acid (**2**)[17] and an aromatic analogue similarly derived from 1,3,5-tris(aminomethyl)benzene, MECAM (**3**)[18] support the growth of *E. coli* mutants (Chart I).

The third mechanism, which involves conversion of the catecholato to salicylato mode of coordination (Scheme IC), was recently proposed by Raymond and co-workers[19,20] as a result of the above and related findings concerning the ability of microbial cells to acquire iron from enterobactin analogues lacking the triester ring. This now replaces their earlier proposal concerning ligand cleavage (Scheme IA),[13] which is now eliminated as a possible mechanism.

This paper presents an analysis of published results to determine which of the proposals shown as B and C in Scheme I represents the most likely mechanism.

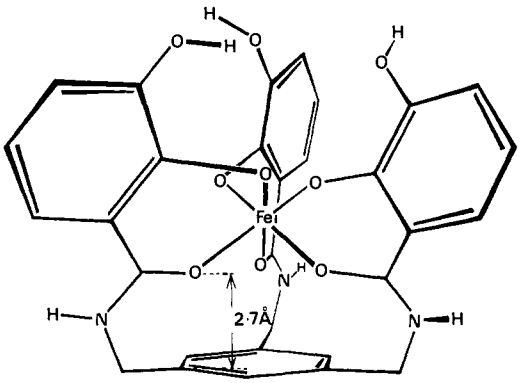
Results and Discussion

Structure of $[Fe^{III}(H_3MECAM)]^0$. In principle there would appear to be four possible structures for a hexacoordinated monomeric iron(III) complex of triprotonated MECAM (**3**). The catecholato mode of coordination may be retained, and the three hydroxyl groups meta to the carbonyl substituent protonated (**4**).



(4)

Alternatively, as proposed by Raymond and co-workers,[19] a salicylato mode of coordination occurs, with the three meta hydroxyl groups protonated but not coordinated to iron (**5**). There



(5)

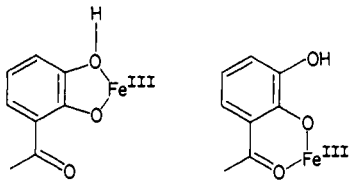
are two intermediate possibilities: two of the coordinating groups bind a catecholato mode and one in salicylato mode or one of these groups binds in catecholato mode and two in salicylato mode. In each of these four proposed complexes there is considerable stereochemical restriction since the planar aromatic ring and the hexadentate iron(III) are joined by three restrictive amide links. In the iron complexes the orientation and proximity of the oxygen and iron atoms to the symmetrically substituted aromatic ring can be used as an indicator of the constraints to formation of each of the complexes. The triprotonated catecholato structure **4** is capable of adopting a conformation without any prohibitive steric constraints, as demonstrated by the high affinity of MECAM for iron(III) at neutral pH values where it is completely deprotonated. However, the formation constant for iron(III) MECAM is 10^6-fold less than iron(III) enterobactin.[10] Since both ligands possess identical coordinating groups, some strain must be introduced into MECAM as a result of coordination with iron. This probably results from the rigid planarity of the central ring not permitting the α carbons to flex out of the plane toward the metal.[10] Nevertheless, iron(III) is tightly bound.

In contrast to the tris(catecholato) structure, the triprotonated salicylato structure **5** is incapable of adopting a conformation without severe steric constraints. In such structures there would be considerable compression between the aromatic ring and the three carbonyl oxygen atoms. The degree of compression associated with chelate formation was estimated by using normal bond lengths and angles[21] for this structure. The bond angles were

(17) Hollifield, W. C.; Neilands, J. B. *Biochemistry* **1978**, *17*, 1922.
(18) Venuti, M. C.; Rastetter, W. H.; Neilands, J. B. *J. Med. Chem.* **1979**, *22*, 123.
(19) Pecoraro, V. L.; Wong, G. B.; Kent, T. A.; Raymond, K. N. *J. Am. Chem. Soc.* **1983**, *105*, 4617.
(20) Pecoraro, V. L.; Harris, W. R.; Wong, G. B.; Carrano, C. J.; Raymond, K. N. *J. Am. Chem. Soc.* **1983**, *105*, 4623.

systematically varied up to ±20% in order to produce a structure with minimum overlap of nonbonded atoms. Because of the stereochemical constraints outlined above, the degrees of freedom associated with the three linking methylene amido segments are severly restricted. All possible conformations involve considerable compression between the three carbonyl oxygen atoms and the central aromatic ring. The oxygen–carbon distances fall between 2.4 and 2.7 Å and thus involve 0.7 and 0.4 Å of compression, assuming a van der Waal's C···O contact distance of 3.2 Å. [22] Although intramolecular nonbonding C···O distances less than 3.2 Å are frequently observed, for instance in phenols and amides, the oxygen lone pairs are not oriented along the line of closest approach. In contrast, structure **5** has at least one of the lone pairs on each amide oxygen oriented toward the π-cloud of the symmetrically substituted ring. Three such severely strained interactions existing simultaneously in a small structure is most improbable; an alternative structure (**4**) exists that lacks such severe steric constraints (the corresponding C···O distance in **4** is estimated to be 3.3 Å). Significantly, intermolecular potentials for the $H_2O\cdots C_6H_6$ system, calculated by using an ab initio SCF CI methodology, gives an oxygen to ring plane distance of 3.3 Å.[23] The same arguments hold for the two intermediate complexes mentioned above, which involve salicylato bonding, and for the equivalent complexes of 3,4-LICAM (**6**).

Infrared data on solutions of the iron complexes of the sulfonate derivative of MECAM and 3,4-LICAM are highly informative.[20] Free ligating arms only begin to make an appreciable contribution to the spectra of the iron complexes at pH values lower than 2.5. At pH 2.5 the triprotonated complexes predominate and the iron remains coordinated in a hexadentate fashion. Only when additional protons bind to the iron complex do the ligand arms dissociate. Over the pH range 7.0–2.0 both complexes become triprotonated, yet the carbonyl associated with the coordinated catechol remains in the 1605–1610 cm^{-1} region for Fe(MECAMS) and in the 1602–1605 cm^{-1} region for Fe(3,4-LICAMS).[20] If there were a transition from a catecholato (**7**) to a salicylato (**8**) mode



(7) (8)

of coordination, a large shift toward lower wavenumbers would be anticipated.[24] In fact there is a small migration in the opposite direction, toward higher wavenumbers. This is most readily explained by the complexes retaining a catecholato mode of coordination during protonation.

Intramolecular Electron Transfer between Iron(III) and Monoprotonated Catechol Derivatives. An internal redox reaction between iron(III) and catechol ligands is well documented.[15,16] Indeed, if the pH of the solution is not too acidic (>pH 2.0), the oxidized catechol moiety, a semiquinone, is capable of coordinating iron(II), albeit rather weakly. Thus, when the reactions are studied in the absence of oxygen, the semiquinone is not readily polymerized or disproportionated to the quinone, and the reaction is reversible. Under such conditions iron(III) is regenerated on

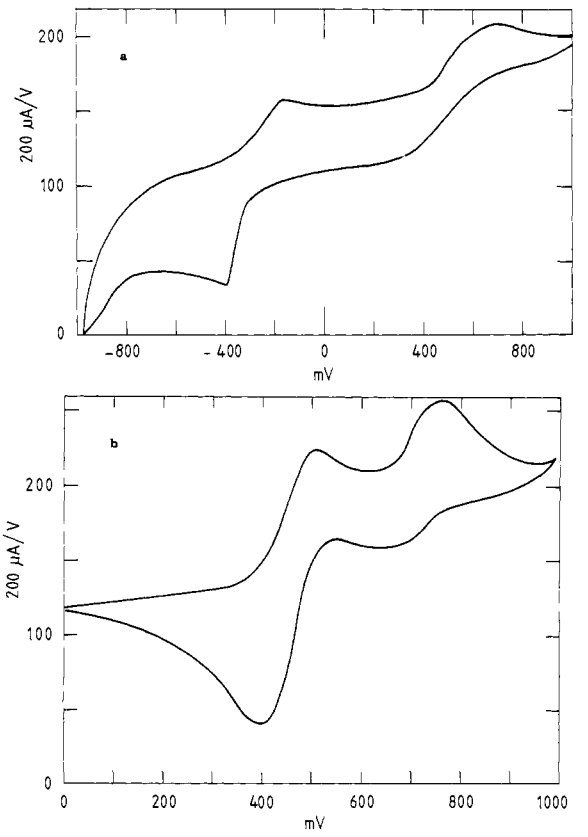


Figure 1. (a) 1 mM iron(III) catechol (pH 3.5). (b) 1 mM catechol (pH 3.5). Solutions: 1–5 mM $FeCl_3$, Fe(catechol), or $Fe(catechol)_3$ in 1 M glycine and 1 M NaCl, saturated with N_2. Immediately prior to assay the pH was checked and adjusted as necessary. The solutions were stored under N_2 and assayed under a stream of N_2. Cyclic voltammagrams were performed by using a Bioanalytical Systems CV-1A, modified to generate the dc ramps and to record and store the resultant voltages and currents using a Commodore 4000. The C–V cell was of all glass construction with a conventional three-electrode system. The working electrode was a 0.5-mm gold wire approximately 3 mm long, sealed in a glass pipette. The auxiliary electrode was a platinum wire 0.5 mm in diameter and 25 mm long. The reference electrode was an Ag/AgCl electrode in 2 M LiCl with 1.5% agar, connected to the solution by a Luggin capillary tip. Voltage potentials are reported as recorded and may be normalized to zero hydrogen potential by adding 220 mV to their values. The reference electrode was standardized against ferro/ferricyanide potential of 210 mV (430 mV vs. hydrogen electrode).

neutralization of the medium.[14,16] Hence the green iron catechol (1:1) complex, observed by both Avdeef et al.[25] and Hider et al.[16] is an iron(II) semiquinone complex. Cyclic voltammetry of iron(III) catechol solutions is consistent with this interpretation. Reversible voltammagrams are obtained with both catechol and iron(III) catechol at pH 3.5 (Figure 1). In the absence of iron, two potentials are observed (Figure 1b), 345 mV, corresponding to catechol + e ⇌ semiquinone, and 610 mV, corresponding to semiquinone + e ⇌ quinone. However, in the presence of iron(III), the higher potential is not observed (Figure 1a). The results are identical for iron/catechol ratios of 1:1 and 1:3. A similar intramolecular electron transfer has been reported for a cobalt(III) catechol complex,[26] but not for complexes of chromium(III).[27] Presumably the chromium(III)/chromium(II) redox potential is too low for chromium(III) to be reduced by catechol.

We find that over the pH range (2.8–4.5) the catechol ligand remains monoprotonated when bound to iron. Similar protonated catechol metal species have been found previously.[28,29] Such protonation necessitates the recalculation of the affinity constant

(21) C=O = 1.23 Å, C—N = 1.48 Å, C—C = 1.54 Å, and C=N = 1.27 Å: Lonsdale, K. "International Tables for X-ray Crystallography"; Kynoch Press: Birmingham, U.K., 1968. The oxygen–oxygen bite distance of the salicylato ligand was adjusted to 2.8 Å, and the oxygen–oxygen distances at the top and base of the propeller structure were adjusted at 2.9 Å. These values are close to those reported for tris(catecholato)ferrate(III). (Raymond, K. N., Isied, S. S., Brown, L. D., Fronczek, F. R.; Nibert, J. H. *J. Am. Chem. Soc.* **1976**, *98*, 1967) and tris(pentane-2,4-dionato)ferrate(III) (Iball, J.; Morgan, C. H. *Acta Crystallogr.* **1967**, *23*, 239 and Nassimbeni, L. R.; Thackeray, M. M. *Acta Crystallogr. Sect. B* **1974**, *B30*, 1072).

(22) van der Waals radii: carbon 1.7 Å, and oxygen, 1.5 Å.

(23) Karlström, G.; Linse, P.; Wallqvist, A.; Jönsson, B. *J. Am. Chem. Soc.* **1983**, *105*, 3777.

(24) Bellamy, L. J.; Branch, R. F. *J. Chem. Soc.* **1954**, 4491.

(25) Avdeef, A.; Sofen, S. R.; Bregante, T. L.; Raymond, K. N. *J. Am. Chem. Soc.* **1978**, *100*, 5362.

(26) Buchanan, R. M.; Pierpoint, C. G. *J. Am. Chem. Soc.* **1980**, *102*, 4951.

(27) Pierpoint, G. C.; Buchanan, R. M. *Coord. Chem. Rev.* **1981**, *38*, 45.

for the iron catechol (1:1) complex over this pH range. The corrected value of 10^9, in contrast to the earlier figure of 10^{20},[25,30] is compatible with ligand competition studies.[16] It appears likely that protonation of the iron catechol complex is a prerequisite for an internal redox reaction.

Hider, Silver, and co-workers, using Mössbauer spectroscopy, demonstrated that in methanolic solutions iron(III) enterobactin undergoes an internal redox reaction under acid conditions leading to the generation of iron(II) [ΔE_Q = 3.41 (2) mm s^{-1}, δ = 1.37 (1) mm s^{-1}.][14] Pecararo et al.[19] have confirmed these observations for iron enterobactin in acidic methanol (ΔE_Q = 3.44 mm s^{-1}, δ = 1.38 mm s^{-1}).[31]

Comparison of the Two Proposed Acid-Dependent Mechanisms for Iron Release from Catecholato Siderophores. Mechanism C (Scheme I). Raymond and co-workers have shown that in order for iron to dissociate from hexadentate siderophores in aqueous media, the complex must be triprotonated.[19] These triprotonated species begin to form at pH 5.0, and for [Fe(H_3MECAMS)]$^{3-}$ 50% formation occurs at pH 3.3. Iron enterobactin and iron MECAM are both insoluble at this pH. Bacteria, unlike eukaryotes, possess a single intracellular compartment, and it is extremely unlikely that a bacterium would permit the pH of its cytoplasm to fall to these acidic pH values and thereby expose all intracellular enzymes to such acidity. Even in eukaryotic tissue, where acid intracellular compartments allow the removal of iron from transferrin, the pH barely falls below pH 5.0.[32] Thus conditions favoring the mechanism proposed in Scheme IC are unlikely to be found in the cytoplasm of microorganisms. Furthermore, there is no conclusive evidence for the existence of the proposed tris(salicylato) [Fe(H_3 enterobactin)]^0 structure, and formation of such a complex is improbable from stereochemical grounds.

Mechanism B (Scheme I). Since enterobactin precipitates from aqueous acid solutions, Hider and co-workers considered the possible involvement of media having reduced dielectric constants.[14] In living systems, nonaqueous environments are common, for instance, the lipid assemblies associated with membranes and the active sites of many enzymes. Marked changes in pK values can occur in such microenvironments.[33] Thus the observation that protonated tris(catecholato)iron(III) complexes are capable of undergoing internal redox reactions in nonaqueous media may be relevant to the physiological situation. Scheme IB therefore offers a possible mechanism for the reductive release of iron from enterobactin in bacterial cytoplasm.[34-36]

Registry No. Fe, 7439-89-6.

(28) Tyson, C. A.; Martell, A. E. *J. Am. Chem. Soc.* **1968**, *90*, 3379.
(29) McBryde, W. A. E. *Can. J. Chem.* **1964**, *42*, 1917.
(30) Mentasti, E.; Pelizzetti, E.; Saini, G. *J. Inorg. Nucl. Chem.* **1976**, *38*, 785.
(31) Pecoraro et al.[19] suggested that only 30% of the iron is in the iron(II) state at pH 4 in their spectra. In their case of dilute solutions, base line curvature can lead to over estimation of the broadened iron(III) component. If the major iron(III) contribution lies in the sharp quadrupole pair rather than the slowly relaxing component, then the percentage of iron(II) has been underestimated. The spectra obtained in concentrated solutions by Hider et al. do not suffer from this problem, so that the iron(II) contribution cannot be derived from a minor impurity; it can in any case be quantitatively converted back to iron(III) enterobactin by neutralization.
(32) Tycho, B.; Maxfield, F. R. *Cell* **1982**, *28*, 643.
(33) Fersht, A. "Enzyme Structure and Mechanism"; W. H. Freeman: San Francisco, 1977.
(34) Tait, G. H. *Biochem. J.* **1975**, *146*, 191.
(35) Neilands, J. B. In "Iron in Biochemistry and Medicine", Jacobs, A., Worwood, M. Eds.; Academic Press: London, 1980; Vol. II, p 529.
(36) Lodge, J. S.; Gaines, C. Y.; Arcenaux, J. E. L.; Byers, B. R. *Biochem. Biophys. Res. Commun.* **1980**, *97*, 1291.

Reactions of Dinuclear Niobium(III) and Tantalum(III) Compounds with Alkyl Isocyanides To Give Dinuclear Products with Dimerized Isocyanides

F. Albert Cotton,* Stan A. Duraj, and Wieslaw J. Roth

Contribution from Department of Chemistry and Laboratory for Molecular Structure and Bonding, Texas A&M University, College Station, Texas 77843. *Received May 14, 1984*

Abstract: Reactions of $Nb_2Cl_6(SMe_2)_3$ and $Ta_2Cl_6(SMe_2)_3$ with the isocyanides Me_3CNC, Me_2CHNC, and c-$C_6H_{11}NC$ have been carried out under conditions where the products are all of the composition $M_2Cl_6(RNC)_6$. All products have been characterized by elemental analyses and IR spectroscopy, and two compounds, $Nb_2Cl_6(t\text{-BuNC})_6$ (**1**) and $Ta_2Cl_6(i\text{-PrNC})_6$ (**2**), have been further studied by X-ray crystallography. Each of these compounds contains a tetradentate ligand, RNCCNR, formed by dimerization of the isocyanide; this ligand is bonded by its two central carbon atoms to a $MCl_2(CNR)_4$ group and through its nitrogen atoms to a MCl_4 group. The first metal atom has roughly pentagonal bipyramidal coordination geometry with one Cl and the pair of carbon atoms at apical positions, while the second metal atom has essentially octahedral coordination geometry with the nitrogen atoms in cis positions. Crystallographic results are as follows. For **1**: *Pn*; a = 14.102 (2) Å, b = 13.486 (5) Å, c = 11.157 (4) Å, β = 93.26 (2)°, V = 2118 (1) Å^3, Z = 2, R_1 = 0.0543, R_2 = 0.0727. For **2**: $P2_1/c$; a = 18.014 (8) Å, b = 11.862 (4) Å, c = 17.948 (5) Å, β = 98.23 (3)°, V = 3796 (4) Å^3, Z = 4, R_1 = 0.0621, R_2 = 0.0741. The compounds are diamagnetic, and this is explained by using an MO scheme that leads to the occupation of a delocalized π MO by both of the metal d electrons.

The ability of the $M_2Cl_6(SR_2)_3$ molecules (M = Nb or Ta and SR_2 = SMe_2 or SC_4H_8) to react with C≡C and C≡N bonds has been well documented. Often these reactions have included dimerization of the organic ligand so as to form new C—C or C=C bonds.

With internal acetylenes, $R_1C{\equiv}CR_2$, where R_1 = R_2 = C_6H_5 or $C(CH_3)_3$ and R_1 = $C(CH_3)_3$, R_2 = CH_3, simple mono- or diacetylene adducts are formed,[1-4] while terminal acetylenes are catalytically cyclotrimerized or polymerized.[5] Nitriles[1,6] and

(1) Cotton, F. A.; Hall, W. T. *J. Am. Chem. Soc.* **1979**, *101*, 5094.
(2) Cotton, F. A.; Hall, W. T. *Inorg. Chem.* **1980**, *19*, 2352, 2354.
(3) Cotton, F. A.; Hall, W. T. *Inorg. Chem.* **1981**, *20*, 1285.
(4) Cotton, F. A.; Roth, W. J. *Inorg. Chim. Acta* **1984**, *85*, 17.

0002-7863/84/1506-6987$01.50/0