thermic by 33 kcal/mol.[11] Our failure to observe stable b ions from methoxy precursors such as CH_3OCH_3 (Table I) is consistent with a low activation energy for this dissociation and with the unfavorable steric requirements for the exothermic rearrangement of such precursor ions to form the marginally stable isomer $H_2 \cdots HCO^+$.

Charge Stabilization, O vs. S. In the previous paper[2] it was demonstrated that π bonding in $H_2C{=}SH$ is slightly more effective than that in a. A similar comparison of triplet b and $H_3C\text{-}S^+$ (ΔH_f = 215 kcal/mol)[2] gives ΔH(eq 3) = −59 kcal/mol. Clearly, a sulfur atom is much better suited to accommodating positive charge than oxygen, owing to the larger, more polarizable orbitals on sulfur. This appears to be the major factor responsible for the observed differences between gas-phase organosulfur and -oxygen cations.

$$H_3C{-}O^+ + CH_3SH \rightarrow CH_3OH + H_3C{-}S^+ \quad (3)$$

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References and Notes

(1) Collisional Activation and Metastable Ion Characteristics. 66. Part 65: ref 2.
(2) J. D. Dill and F. W. McLafferty, *J. Am. Chem. Soc.*, preceding paper in this issue.
(3) F. W. McLafferty, R. Kornfeld, W. F. Haddon, K. Levsen, I. Sakai, P. F. Bente, III, S.-C. Tsai, and H. D. R. Schuddemage, *J. Am. Chem. Soc.*, **95**, 3886 (1973).
(4) F. W. McLafferty and I. Sakai, *Org. Mass Spectrom.*, **7**, 971 (1973).
(5) B. van de Graaf and F. W. McLafferty, *J. Am. Chem. Soc.*, **99**, 6806, 6810 (1977).
(6) C. L. Brion and M. J. Dunning, *Trans. Faraday Soc.*, **59**, 647 (1963).
(7) A. G. Harrison, A. Ivko, and D. Van Raalte, *Can. J. Chem.*, **44**, 1625 (1966); K. M. A. Refaey and W. A. Chupka, *J. Chem. Phys.*, **48**, 5205 (1968).
(8) F. P. Lossing, *J. Am. Chem. Soc.*, **99**, 7526 (1977), and references cited therein.
(9) J. F. Wolf, R. H. Staley, I. Koppel, M. Taagepera, R. T. McIver, Jr., J. L. Beauchamp, and R. W. Taft, *J. Am. Chem. Soc.*, **99**, 5417 (1977).
(10) K. Hiraoka and P. Kebarle, *J. Chem. Phys.*, **63**, 1688 (1975).
(11) From ΔH(eq 1) = 3.9 kcal/mol[10] and $\Delta H_f(HCO^+)$ = 198: P. M. Guyon, W. A. Chupka, and J. Berkowitz, *J. Chem. Phys.*, **64**, 1419 (1976).
(12) K. Hiraoka and P. Kebarle, *J. Am. Chem. Soc.*, **99**, 366 (1977).
(13) R. D. Bowen and D. H. Williams, *J. Chem. Soc., Chem. Commun.*, 378 (1977).
(14) P. v. R. Schleyer, E. D. Jemmis, and J. A. Pople, *J. Chem. Soc., Chem. Commun.*, 190 (1978).
(15) H. M. Rosenstock, K. Draxl, B. W. Steiner, and J. T. Herron, *J. Phys. Chem. Ref. Data, Suppl. 1*, **6**, 1977.
(16) F. Bernardi, I. G. Csizmadia, H. B. Schlegel, and S. Wolfe, *Can. J. Chem.*, **53**, 1144 (1975); P. Ros, *J. Chem. Phys.*, **49**, 4902 (1968).
(17) P. A. Kollman, W. F. Trager, S. Rothenberg, and J. E. Williams, *J. Am. Chem. Soc.*, **95**, 458 (1973).
(18) S. Wolfe, H. B. Schlegel, and M.-H. Whangbo, *Can. J. Chem.*, **54**, 795 (1976).
(19) A. C. Hopkinson, N. K. Holbrook, K. Yates, and I. G. Csizmadia, *J. Chem. Phys.*, **49**, 3596 (1968).
(20) S. Saebo, *Chem. Phys. Lett.*, **40**, 462 (1976).
(21) M. J. S. Dewar and H. S. Rzepa, *J. Am. Chem. Soc.*, **99**, 7432 (1977).
(22) M. A. Haney, J. C. Patel, and E. F. Hayes, *J. Chem. Phys.*, **53**, 4105 (1970).
(23) W. A. Lathan, L. A. Curtiss, W. J. Hehre, J. B. Lisle, and J. A. Pople, *Prog. Phys. Org. Chem.*, **11**, 175 (1975).
(24) See, for example, (a) J. B. Collins, P. v. R. Schleyer, J. S. Binkley, J. A. Pople, and L. Radom, *J. Am. Chem. Soc.*, **98**, 3436 (1976); (b) J. D. Dill, L. C. Allen, W. C. Topp, and J. A. Pople, *ibid.*, **97**, 7220 (1975).

Ferric Ion Sequestering Agents. 3. The Spectrophotometric and Potentiometric Evaluation of Two New Enterobactin Analogues: 1,5,9-*N,N′,N″*-Tris(2,3-dihydroxybenzoyl)-cyclotriazatridecane and 1,3,5-*N,N′,N″*-Tris(2,3-dihydroxybenzoyl)triaminomethylbenzene[1]

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Abstract: The complexation and protonation equilibria of the ferric complexes of 1,5,9-*N,N′,N″*-tris(2,3-dihydroxybenzoyl)-cyclotriazatridecane (3,3,4-CYCAM) and 1,3,5-*N,N′,N″*-tris(2,3-dihydroxybenzoyl)triaminomethylbenzene (MECAM) have been investigated by potentiometric and spectrophotometric techniques. Proton dependent metal-ligand equilibrium constants $[K^* = ([ML][H]^3)/([M][H_3L])]$ have been determined to be log K^* = 9.5 and 3.4 for the ferric complexes of MECAM and CYCAM, respectively. These results have been used to estimate the normal formation constants as 10^{46} for ferric MECAM and 10^{40} for ferric CYCAM. The MECAM value is the largest formation constant of any synthetic iron chelator. Both complexes undergo a series of protonations which shift the mode of bonding from one involving coordination through the two phenolic oxygens of the dihydroxybenzoyl group (catecholate mode) to one in which the iron is coordinated to the carbonyl oxygen and the ortho phenolate group (salicylate mode). The results are discussed in relation to the chelation therapy of chronic iron overload, as occurs in the treatment of Cooley's anemia.

While iron is an essential element for growth in all living things and is needed in relatively large amounts in man (approximately 4 g of the element are in the average adult),[2] it is also very toxic when present in excess. In its acute form, iron overload (usually from the ingestion of iron supplement preparations by infants) constitutes one of the most common types of accidental poisoning.[3] In its chronic form, iron overload results as a side effect of the transfusion therapy of genetic disorders such as β-thalassemia major (Cooley's anemia) and related diseases.[3]

In searching for new ligands to be used in iron chelation therapy, one initial approach has been the examination of naturally occurring iron chelating agents. In microorganisms, the acquisition of iron usually involves the synthesis and excretion of low-molecular-weight ligands which show both a high affinity and a high specificity for ferric ion.[4,5] These li-

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ENTEROBACTIN

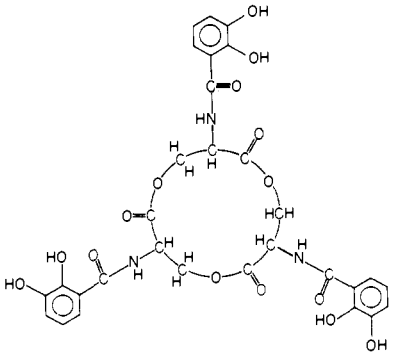


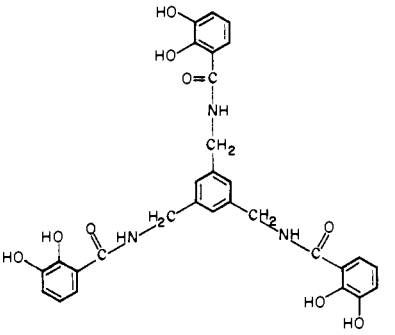
Figure 1. Structural formula of enterobactin.

gands, collectively referred to as siderophores, utilize primarily hydroxamate and catecholate functional groups to bind iron.[6] Examples of the former type include desferrichrome, desferrioxamine B, aerobactin, and rhodotorulic acid. The best-known catechol-based siderophore is enterobactin (H_6ent), shown in Figure 1, which is a cyclic triester of 2,3-dihydroxybenzoyl-l-serine. It is produced by several species of enteric bacteria and has been shown to form exceptionally stable, high-spin ferric complexes via coordination of the six phenolic oxygens.[4,7-9]

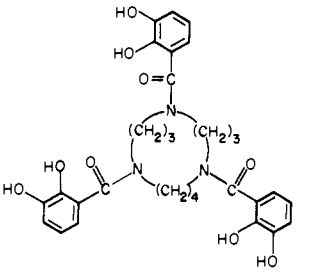
One of the trihydroxamate siderophores, desferrioxamine B, is currently used as a drug for the treatment of both acute and chronic iron overload.[3] Another siderophore, rhodotorulic acid, has entered clinical trials to determine its effectiveness in chelation therapy.[10] However, both of these ligands have fundamental limitations to their potential as iron-removal agents in man.

Recent thermodynamic data from our laboratory have shown that enterobactin forms the most stable iron complex ever characterized.[4,7] Specifically, the log K_{ML} of ferric enterobactin is 20 log units greater than that of ferrioxamine B, and, at pH 7.4, 10 μM ligand concentration and 1 μM total iron, the concentration of free Fe^{3+} is 10 log units lower for enterobactin than for desferrioxamine B.[4] Furthermore, we have shown that the catechol-based ligands—but not the hydroxamates—are kinetically able to remove iron from human transferrin.[1] These results indicate that catecholate compounds such as enterobactin might be substantially more effective than hydroxamates in the clinical removal of iron. However, enterobactin itself is poorly suited for chelation therapy due to the rapid hydrolysis of the free ligand at physiological pH.[11] We have begun, therefore, a program to design and evaluate hydrolytically stable synthetic iron chelators which are based on the enterobactin structure.

This paper reports the characterization of the equilibria and equilibrium constants from spectroscopic and potentiometric data for the ferric complexes of two new enterobactin analogues, 1,5,9-*N,N',N''*-tris(2,3-dihydroxybenzoyl)triazacyclotridecane [3,3,4-CYCAM] and 1,3,5-*N,N',N''*-tris(2,3-dihydroxybenzoyl)triaminomethylbenzene [MECAM], both of which are shown in Figure 2. Some preliminary results for the monomeric analogue of these compounds, 2,3-dihydroxy-*N,N*-dimethylbenzamide, are also included. Like enterobactin, these tricatecholate ligands are also expected to bind iron through the six phenolic oxygens and to have the same basic geometry and high affinity for iron that enterobactin has. Unlike enterobactin, the hydrolytic stability of the free ligands makes them viable candidates for use in the treatment of iron overload in humans. Preliminary accounts of some of these results have been reported.[12,13]



MECAM



3,3,4-CYCAM

Figure 2. Structural formulas of MECAM and CYCAM.

Experimental Section

Reagents. The syntheses of CYCAM and MECAM have been previously reported.[14,15] Samples of both compounds were kindly provided by Dr. Fred Weitl of Lawrence Berkeley Laboratory. The molecular weight of CYCAM was determined by potentiometric titration with KOH. The MECAM was not sufficiently soluble for such an analysis, but its molecular weight has been previously determined by osmometry.[14]

Stock solutions of Fe^{3+} were prepared by the dissolution of $Fe(NO_3)_3 \cdot 7(H_2O)$ into standardized nitric acid, followed by quantitative dilution to give a final solution which was typically 0.1 M in iron and 0.02 M in acid. The actual Fe^{3+} concentration was determined by the addition of a measured excess of EDTA (ethylenediaminetetraacetic acid) to an aliquot of the iron solution, followed by back-titration with zinc using Eriochrome black T as an indicator.[16]

Carbonate-free 0.1 M KOH solutions were prepared from Baker Dilut-It ampules using freshly boiled, doubly distilled water, and were stored under an atmosphere of ascarite-scrubbed argon. The absence of carbonate was confirmed by Gran's plots.[17]

Potentiometric Measurements. Samples (40 mL) were placed in a double-walled, capped titration cell maintained at 25 ± 0.05 °C by a circulating constant-temperature water bath. Solutions were adjusted to 0.10 M ionic strength by the addition of 1.0 M KNO_3 and kept under a positive pressure of argon, which was passed through ascarite and 0.10 M KNO_3.

Measurements were made with a Corning Model 130 digital pH meter equipped with Corning glass and saturated calomel electrodes. The apparatus was standardized by titrations of nitric and acetic acid solutions, such that the actual hydrogen ion *concentration*, not activity, could be calculated from the linear equation

$$p[H] = \alpha pH_{obsd} + \beta \quad (1)$$

where typically $0.99 < \alpha < 1.01$ and $-0.01 < \beta < 0.01$. The average deviation between the p[H] calculated from the known acid association constant of acetic acid[18] and the p[H] given by eq 1 was 0.002 over the pH range of 2-5. It was assumed that the pH electrodes responded linearly over the pH range 2-10.

The potentiometric data were refined by nonlinear least-squares analysis in which the appropriate equilibrium constants were varied to minimize the value of the residual function

$$R = \sum w^2(pH_{obsd} - pH_{calcd})^2 \quad (2)$$

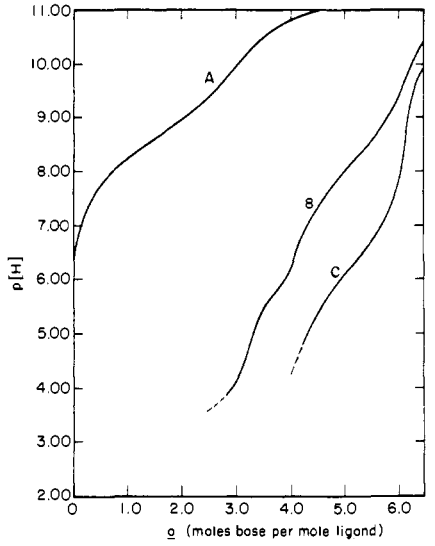


Figure 3. Potentiometric equilibrium curves for (A) 8.6×10^{-4} M CYCAM; (B) CYCAM + Fe^{3+}, 1:1, 6.7×10^{-4} M; (C) MECAM + Fe^{3+}, 1:1, 1.3×10^{-3} M. Dashed lines indicate precipitation. All solutions at 25 °C; $\mu = 0.10$ (KNO_3).

where w is the weighting factor defined by the function

$$\left(\frac{1}{w}\right)^2 = [\sigma(\mathrm{pH})]^2 = \sigma_c^2 + \sigma_v^2\left(\frac{\partial \mathrm{pH}}{\partial V}\right)^2 \quad (3)$$

The estimated uncertainty in the value of pH_{obsd}, σ(pH), is expressed as the sum of two terms: the intrinsic error in any given pH measurement (set to 0.003 in this work) and a second term representing the uncertainty in the volume of titrant delivered (σ_v, set to 0.002 mL) which is multiplied by the slope of the titration curve at pH_{obsd}.

Spectrophotometric Measurements. Visible spectra were recorded on a Cary Model 118 spectrophotometer. All solutions were adjusted to 0.10 M ionic strength by the addition of KNO_3. The visible spectra of ferric MECAM as a function of pH were obtained from a single solution prepared in the titration vessel described above. After each adjustment of the pH, an aliquot was removed and its visible spectrum recorded. Since equilibrium in the ferric CYCAM system was attained quite slowly, individual 10-mL samples were prepared and incubated overnight at 25 °C. In spectrophotometric competition experiments with EDTA or DTPA (diethylenetriaminepentaacetic acid), 10-mL samples were allowed to equilibrate overnight at 25 °C. Typical solutions were 5×10^{-4} M in ferric ion and either MECAM or CYCAM, with up to a sixfold excess of the reference ligand. The appropriate protonation and ferric ion formation complexes for EDTA and DTPA were taken from the critical compilation of Martell and Smith.[19]

Infrared Spectra. Infrared spectra of solid samples were recorded by using KBr pellets on a Perkin-Elmer Model 597 spectrometer.

Results

Ferric MECAM. The compound MECAM is not sufficiently soluble in neutral solution to permit the determination of the ligand protonation constants by potentiometric titration, although fairly concentrated samples can be prepared when the pH is raised above 10. The potentiometric equilibrium curve of an equimolar solution of MECAM and ferric ion is shown in Figure 3, in which MECAM has been treated as an H_6L ligand and a is the number of moles of base per mole of metal. There is a clear inflection at $a = 6$, and, at this point in the titration, the solution is deep red with λ_{max} 492 nm (ϵ 4700). These parameters are very similar to those of the tris complexes of 2,3-dihydroxy-*N*,*N*-dimethylbenzamide, λ_{max} 487 (ϵ 4910) and catechol, λ_{max} 490 (ϵ 4190).[20]

The inflection at $a = 6$ indicates that a single species has been formed in which 6 protons have been displaced from MECAM by the ferric ion, and the visible spectrum of this complex is very similar to those of the tris(bidentate) model

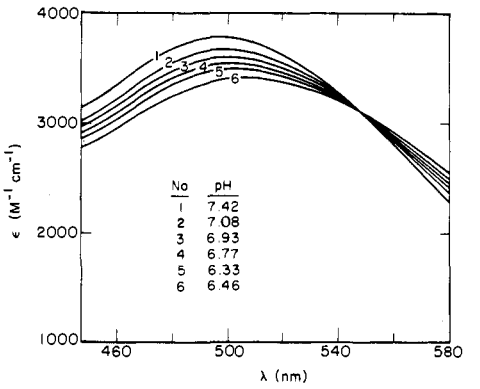


Figure 4. Visible spectra of ferric MECAM as a function of pH from pH 6.5 to 7.5. [Fe(MECAM)] = 2×10^{-4}; $\mu = 0.10$ (KNO_3); $t = 25$ °C.

compounds in which it is known that the iron is coordinated to six phenolic oxygens.[8] Therefore, the red complex reported here can be identified as the $[Fe(MECAM)]^{3-}$ species, in which the iron is coordinated through the six phenolic oxygens of the three dihydroxybenzoyl (DHB) side groups.

The titration curve of ferric MECAM has a two-proton buffer region from $a = 4$ to 6, which spans the pH range 4.5 to 8.5. Below pH 4, a dark purple material begins to precipitate, and at pH 3 this precipitation is essentially quantitative. This purple material is not a degradation product, since it can be redissolved in pH 10 buffer to regenerate the original spectrum of the red $[Fe(MECAM)]^{3-}$ species.

Because of the two-proton stoichiometry of the ferric MECAM buffer region, it was originally assumed that the reaction involved was the simultaneous, one-step protonation and dissociation of one of the DHB side groups. Such a reaction would be strictly analogous to the dissociation of a bidentate ligand such as catechol or DMB (i.e., eq 20). However, the changes in the visible spectra of ferric MECAM from pH 4.5 to 8 eliminate such a model. In the ferric DMB system, the spectra between pH 4 and 8, which reflect the bis to tris equilibrium, form a single, sharp isosbestic point at 542 nm. While the spectra of ferric MECAM also form an isosbestic point at 542 nm over the pH range 6.4 to 8 (Figure 4), the titration curve indicates that this pH range represents the addition of less than 1 equiv of hydrogen ion to the $[Fe(MECAM)]^{3-}$ complex. From pH 6.4 to 5.4, no isosbestic point is observed. Starting at pH 5.4, a second isosbestic point develops at 588 nm, that remains until pH 4.5, which corresponds to the addition of 2 equiv of hydrogen ion to the original $[Fe(MECAM)]^{3-}$ complex. These results indicate that the $[Fe(MECAM)]^{3-}$ is protonated in two *sequential one-proton* steps. The 542-nm isosbestic point reflects the initial reaction:

$$Fe(MECAM)^{3-} + H^+ \rightleftarrows Fe(HMECAM)^{2-} \quad (4)$$

$$K_{MHL} = \frac{[MHL]}{[ML][H]} \quad (5)$$

The 588-nm isosbestic point is present during the latter portion of the second chelate protonation:

$$Fe(HMECAM)^{2-} + H^+ \rightleftarrows Fe(H_2MECAM)^- \quad (6)$$

$$K_{MH_2L} = \frac{[MH_2L]}{[MHL][H]} \quad (7)$$

In the middle pH region from 5.5 to 6.5, these two equilibria overlap, so that no isosbestic point is observed.

The initial protonation of ferric MECAM can be described by the set of equations

$$\mathrm{Abs} = \epsilon_{ML}[ML] + \epsilon_{MHL}[MHL] \quad (8)$$

Table I. Chelate Protonation and Proton-Dependent Stability Constants for Ferric MECAM and CYCAM

	MECAM		CYCAM	
	spectr	potent.	spectr	potent.
log K_{MHL}[a]	7.08 (5)	6.9 (1)		9.0 (4)
log K_{MH_2L}	5.6 (1)	5.7 (1)	7.8	7.6 (1)
log K_{MH_3L}			5.6	5.9 (1)
log K^*[b]	9.5 (3)		1.5 (1)	

[a] $K_{MH_nL} = [FeH_nL]/([FeH_{n-1}L][H])$. [b] $K^* = ([FeL][H]^3)/([Fe^{3+}][H_3L])$.

$$[Fe]_{tot} = [ML] + [MHL] \quad (9)$$

which can be combined with eq 5 and rearranged to give the relation

$$\epsilon_{obsd} = \epsilon_{MHL} + \frac{(\epsilon_{ML} - \epsilon_{obsd})}{K_{MHL}[H]} \quad (10)$$

where $\epsilon_{obsd} = Abs/[Fe]_{tot}$. The data from pH 6.5 to 8 have been plotted according to eq 10 to give the straight line graph shown in Figure 5. From the slope of this line, log K_{MHL} has been calculated to be 7.08 (5). A similar set of equations describing the ferric MECAM system from pH 4.5 to 5.5 have been combined to give the function

$$\epsilon_{obsd} = \epsilon_{MHL} + K_{MH_2L}(\epsilon_{MH_2L} - \epsilon_{obsd})[H] \quad (11)$$

From a plot of ϵ_{obsd} vs. $(\epsilon_{MH_2L} - \epsilon_{obsd})[H]$, the value of log K_{MH_2L} has been determined to be 5.63 (14). The intercept of both eq 10 and 11 is ϵ_{MHL}, and the average value from both plots for two separate sets of spectra is 3900 ± 200 M^{-1} cm^{-1}.

Based on the spectrophotometric results, the potentiometric data from pH 4.5 to 8 have been refined with K_{MHL} and K_{MH_2L} as the only variables. The results, listed in Table I, are in good agreement with the spectrophotometric values.

The quantitative precipitation of a purple iron complex at pH 3 is consistent with the addition of a third proton to the $[Fe(H_2MECAM)]^-$ complex to form the neutral $Fe(H_3MECAM)$ species. Essentially identical results have been observed in the ferric enterobactin system.[4] Presumably such a complex would redissolve in stronger acid due to the formation of cationic complexes and eventual dissociation of the ferric ion. However, the tendency of ferric catecholate complexes to undergo internal redox reactions at low pH[20,21] prevented any studies of strongly acidic solutions.

Ferric CYCAM. The potentiometric equilibrium curves of CYCAM alone and in the presence of equimolar ferric ion are shown in Figure 3. The six dissociable protons of CYCAM split into two groups of three. The first group is titrated by KOH between $a = 0$ and $a = 3$, and the three ligand acid association constants have been calculated to be log $K_4^H = 9.26$ (13), log $K_5^H = 8.65$ (8), and log $K_6^H = 7.86$ (7), where K_n^H is defined by eq 12.

$$K_n^H = \frac{[H_nL]}{[H][H_{n-1}L]} \quad (12)$$

The average of these three constants is 8.6, which is fairly close to the DMB acid association constant of 8.4.[4] In addition, the differences between successive constants are 0.8 and 0.6 log unit, compared with a value of 0.5 log unit predicted on a purely statistical basis. Thus, there do not appear to be any significant intramolecular interactions between the three DHB side groups of CYCAM that affect the sequential deprotonation of the phenolic groups.

The potentiometric data past $a = 3$ form a very flat, high pH buffer region. It is very difficult to obtain accurate protonation constants from such data due to the deterioration in the performance of glass electrodes at high pH and to problems in the refinement of potentiometric data under conditions where $[OH] > [Fe]_{tot}$. Therefore, rather than attempt to refine three overlapping constants from these data, it has been assumed that the average of the three most basic MECAM and CYCAM protonation constants is equal to the higher protonation constant of DMB, log $K = 12.1$.[4]

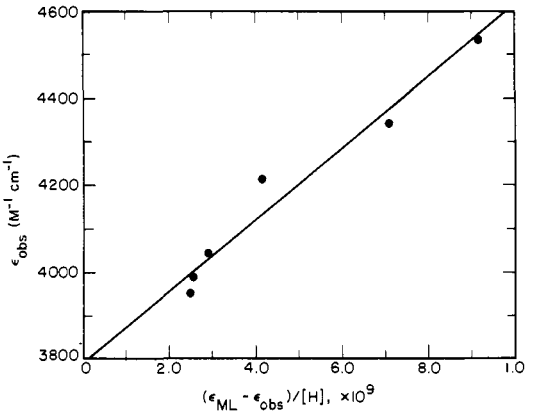


Figure 5. Plot of ϵ_{obsd} vs. $(\epsilon_{ML} - \epsilon_{obsd})/[H]$ for ferric MECAM from pH 6.5 to 8.0, where ϵ_{obsd} = absorbance/$[Fe]_{tot}$. Slope = $(8.2 \pm 0.8) \times 10^{-8}$; from $K_{MHL} = 1/\text{slope}$, log $K_{MHL} = 7.09$ (5). $[Fe]_{tot} = [MECAM]_{tot} = 1.85 \times 10^{-4}$; $\mu = 0.10$ (KNO_3).

As in the MECAM system, the addition of equimolar ferric ion to an alkaline CYCAM solution results in a deep red solution with λ_{max} 480, ϵ 4000. The spectra of solutions above pH 10 change slowly over a period of many hours, possibly due to the formation of hydroxo complexes. Thus the high pH data for ferric CYCAM are somewhat unreliable. The titration curve of ferric CYCAM has an inflection at $a = 3$, due to the addition of three protons to the $[Fe(CYCAM)]^{3-}$ complex to form the $Fe(H_3CYCAM)$ species, which precipitates as a blue solid around pH 4. The titration solutions were so intensely colored that it was not obvious exactly when this precipitation began, so the potentiometric data from $a = 3$ to 4 should be weighed less heavily than the spectrophotometric results.

The visible spectra of ferric CYCAM as a function of pH also form two sequential isosbestic points over the pH ranges 4.8 to 6.3 and 7.6 to 8.6, as shown in Figure 6. Based on the ferric CYCAM titration curve, it appears that these two pH ranges correspond primarily to the K_{MH_2L} and K_{MH_3L} equilibria (cf. eq 4 and 6). These data were fit to equations analogous to eq 10 and 11 to give the chelate protonation constants listed in Table I. Above pH 8.6, the visible spectra change relatively little with increasing pH, so that it was not possible to calculate a reliable value of K_{MHL}. It is clear, however, that the ferric CYCAM also reacts with hydrogen ion in sequential 1:1 protonation equilibria.

The ferric CYCAM potentiometric data have been refined with the three chelate protonation constants as the only variables. Although the fit was rather poor, the values of K_{MH_2L} and K_{MH_3L} are in basic agreement with the spectrophotometric results. An approximate value of log $K_{MHL} = 9.0$ (4) has also been calculated from the titration data. All potentiometric results are listed in Table I.

Spectrophotometric Competition. Because precipitation of $Fe(H_3L)$ complexes and semiquinone formation limit the pH range over which reliable equilibrium measurements can be obtained, there is never any measurable amount of free ferric ion present during either potentiometric or spectrophotometric titrations. This condition allows these data to be refined in terms of chelate protonation constants, but it also rules out the calculation of overall stability constants for these complexes from titration data alone. Therefore, competition reactions

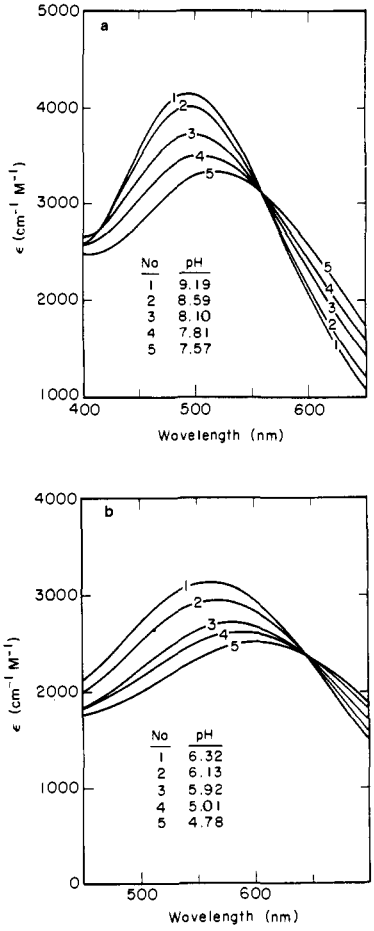


Figure 6. (a) Visible spectra of ferric CYCAM as a function of pH. (1) pH 9.19; (2) 8.59; (3) 8.10; (4) 7.81; (5) 7.57. $[Fe]_{tot} = [CYCAM]_{tot} = 1.7 \times 10^{-4}$; $\mu = 0.10$ M (KNO_3); $T = 25$ °C; path length = 1 cm. (b) Visible spectra of ferric CYCAM as a function of pH. (1) pH 6.32; (2) 6.13; (3) 5.92; (4) 5.01; (5) 4.78. $[Fe]_{tot} = [CYCAM]_{tot} = 1.7 \times 10^{-4}$ M; $\mu = 0.10$ (KNO_3); $T = 25$ °C; path length = 1 cm.

have been run with either EDTA or DTPA as reference ligands. Mixtures of ferric ion with two competing ligands are described by the following set of equations.

$$[Fe]_{tot} = \alpha[FeL] + \alpha'[FeL'] + \alpha_M[Fe^{3+}] \quad (13)$$

$$Abs = [FeL](\epsilon_{ML} + K_{MHL}[H]\epsilon_{MHL} + K_{MHL}K_{MH_2L}[H]^2\epsilon_{MH_2L}) \quad (14)$$

$$[L]_{tot} = \alpha[FeL] + \alpha_L[H_3L] \quad (15)$$

$$[L']_{tot} = \alpha'[FeL'] + \alpha_{L'}[L'] \quad (16)$$

The α's are the usual functions of ligand and chelate protonation constants, e.g., for ferric MECAM

$$\alpha = 1 + K_{MHL}[H] + K_{MHL}K_{MH_2L}[H]^2$$

Usually such competition equilibria are expressed in terms of the fully deprotonated forms of the ligands, so that L′ represents the tetraanion of EDTA. However, the first three ligand protonation constants of MECAM and CYCAM are not known, and thus it is not possible to calculate the concentration of the hexaanionic species. One way to treat such systems is to write the competition equilibria in terms of a different ligand species. Any ligand species can be used; the only requirement is that the α_L function in eq 15 must contain a term representing each individual ligand species which is actually present under experimental conditions. Since the first three ligand protonation constants are very large (probably on the order of 10^{12}), the free ligand will not be more than triply deprotonated under most conditions. Therefore, the equilibria have been expressed in terms of the H_3L^{3-} ligand species. The CYCAM ligand protonation constants have been used to calculate α_L for both CYCAM and MECAM.

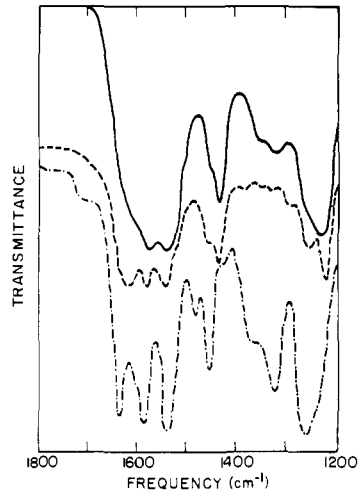


Figure 7. Infrared spectra of KBr pellets of MECAM (—•—), FeMECAM^{3-} (- - -), and FeH_3MECAM (—).

The exchange equilibrium may be expressed as

$$FeL' + H_3L \rightleftarrows FeL + L' + 3H^+ \quad (17)$$

and the distribution coefficient K_X is thus defined as

$$K_X = \frac{[FeL^3][L'][H^+]^3}{[FeL'][H_3L^{3-}]} = \frac{K^*}{K_{ML'}} \quad (18)$$

Since $K_{ML'}$ is the normal formation constant for either ferric EDTA or DTPA, K^* is defined as

$$K^* = \frac{[FeL^{3-}][H^+]^3}{[Fe^{3+}][H_3L^{3-}]} \quad (19)$$

Two assumptions have been made in the calculation of K^*. First, due to the excess ligand present, the $[Fe^{3+}]$ in eq 13 has been neglected. In addition, because the charge-transfer bands of the ferric catecholate complexes are so much more intense than the spin-forbidden d-d bands of the ferric EDTA and DTPA, the total absorbance has been expressed in terms of the catecholate complexes only.

Infrared Spectra. The frequencies and band assignments for the spectra of MECAM, DMB, the red ferric MECAM complex, and the purple FeH_3MECAM complex are listed in Table II, along with literature data for catechol.[22] The catechol frequencies are from Wilson's gas phase spectrum and can be expected to shift 10–15 cm^{-1} in a pellet spectrum.[22] The assignments are essentially those of Wilson, although that of the 1365-cm^{-1} band has been changed to conform to other reports.[23,24] The spectra of the MECAM species are shown in Figure 7. The most significant difference between the spectrum of $[Fe(MECAM)]^{3-}$ and $Fe(H_3MECAM)$ is the absence of a 1610-cm^{-1} band for the protonated species. This band has been assigned to the $\nu_{(C=O)}$ amide stretch, and in the $Fe(H_3MECAM)$ spectrum it has apparently shifted underneath the ring C=C bands at 1585 and 1540 cm^{-1}. The shoulder remaining above 1600 cm^{-1} is probably due to an underlying H–O–H bending mode of incidental water. Thus there appears to be a significant change in the environment of the amide carbonyl group upon protonation of the ferric MECAM complex.

There are two other significant differences between the infrared spectra of MECAM and the ferric MECAM complex. The strong bands in the ligand spectrum between 1300 and 1400 cm^{-1}, which have been assigned to OH deformations,

Table II. Infrared Band Assignments for Catechols and Their Ferric Complexes

assignment	catechol	DMB	MECAM	$FeMECAM^{3-}$	FeH_3MECAM
ν(C=O)		1600	1635	1620	
ν_1(C=O)	1616	1590	1585	1580	1580
ν_2(C=O)	1607	1560	1540	1540	1540
ν_3(C=C)	1504	1500	1485	1455	1455
ν_4(C=C)	1479	1455	1455	1435	1435
δ(O—H)	1365	1390	1370		1360
δ(O—H)	1324		1325		1325
α_1(C—H)	1275	1265	1260	1255	1260
ν_1(C—O)	1251	1235	1235	1225	1230
ν_2(C—O)	1195	1190	1175	1150	1170
α_2(C—H)	1151	1160			1155
α_3(C—H)	1092	1110	1075	1060	1070
α_4(C—H)	1035	1060	1015	1025	1025

disappear in the $[Fe(MECAM)]^{3-}$ spectrum. They reappear with much weaker intensity in the spectrum of Fe(H_3MECAM) due to protonation of half of the phenolic oxygens. In addition, there are substantial shifts in the 1260- and 1190-cm^{-1} bands assigned to C-O stretching modes.

One feature which is definitely absent from the spectrum of Fe(H_3MECAM) is any trace of a free ligand carbonyl stretch around 1630 cm^{-1}. Such a band would be expected if protonation of $[Fe(MECAM)]^{3-}$ were resulting in the dissociation of one of the DHB side groups.

Discussion

Protonation Equilibria. The most obvious difference between the sexidentate ligands used in this study are the simple bidentate ligands such as DMB and catechol is the tendency of ferric MECAM and CYCAM to react with hydrogen ion in a series of 1:1 protonations to form Fe(HL), Fe(H_2L), and Fe(H_3L) complexes. Although this difference is not always obvious from the titration curves, it is clearly evident in the visible spectra as a function of pH and is corroborated by the infrared spectrum of the neutral ferric MECAM complex. The spectra of ferric DMB form a single, sharp isosbestic point over the pH range 4 to 8, due to the presence of the single equilibrium:

$$Fe(DMB)_2^- + DMB^{2-} \overset{K_{ML_3}}{\rightleftharpoons} Fe(DMB)_3^{3-} \qquad (20)$$

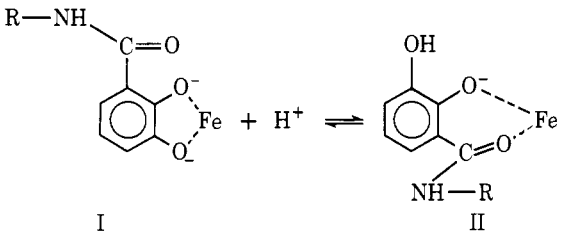
In contrast, as two protons are added to the ferric MECAM complex, the spectra form one isosbestic point from pH 8 to 6.4, and a second isosbestic point from pH 5.5 to 4.5. Thus the addition of two protons to ferric MECAM cannot be represented by an equation analogous to (20). This protonation must be occurring in a stepwise fashion.

$$Fe(MECAM)^{3-} + H^+ \rightleftarrows Fe(HMECAM)^{2-} \qquad (21)$$

$$Fe(HMECAM)^{2-} + H^+ \rightleftarrows Fe(H_2MECAM)^- \qquad (22)$$

The explanation for the sequential isosbestic points in the ferric CYCAM system is basically the same as that given for ferric MECAM, except that the high pH isosbestic point now corresponds to the reaction $Fe(HCYCAM)^{2-} + H^+ \rightleftarrows Fe(H_2CYCAM)^-$, while the low pH data are for $Fe(H_2CYCAM)^- + H^+ \rightleftarrows Fe(H_3CYCAM)$. The CYCAM system also differs from ferric MECAM in the region from $a = 5$ to 6. The $[Fe(HMECAM)]^{2-}$ complex has a λ_{max} at 515 nm with ϵ 3900. Deprotonation of this complex shifts λ_{max} slightly to 490 nm and increases ϵ to 4700. The $[Fe(HCYCAM)]^{2-}$ complex has its λ_{max} at 490 with ϵ 4300. However, deprotonation of this complex results in a decrease in the extinction coefficient to 4000. Since we know of no case where the coordination of a phenolate oxygen to ferric ion results in a decrease in ϵ, it appears that in the $[Fe(CYCAM)]^{3-}$ complex not all six phenolic oxygens are bound to the iron. The sixth equivalent of hydrogen ion detected in the titration curve could come either from the uncoordinated phenolic oxygen or from deprotonation of a coordinated water molecule to form an $[Fe(HCYCAM)(OH)]^{3-}$ complex.

Mode of Bonding. The observation of one-proton steps in the MECAM and CYCAM systems suggests the possibility of a shift in coordination from the high pH "catecholate" type bonding (I), to a "salicylate" mode of bonding shown in II.



I II

Such a scheme would explain the proton stoichiometry and still satisfy the coordination number for iron. The infrared spectra shown in Figure 7 support such a model. In the spectrum of $[Fe(MECAM)]^{3-}$, which is coordinated as a "tris catecholate" via the six phenolic oxygens, the carbonyl band is shifted 15 cm^{-1} to lower frequency from the free ligand (to 1620 cm^{-1}), presumably due to inductive effects of the ferric ion on the conjugation of the carbonyl group into the aromatic ring.[9] Nevertheless, the band is still clearly visible. In the spectrum of Fe(H_3MECAM), however, the carbonyl band has disappeared, with no appearance of a free ligand band at 1635 cm^{-1}. Since any band shifted to higher frequency would be clearly visible, it must be assumed that the 1620-cm^{-1} band has shifted to lower frequency and is responsible for the decrease in resolution of the 1585- and 1540-cm^{-1} bands. Such a decrease in the carbonyl stretching frequency is a strong indication of metal coordination to the carbonyl oxygen. We have also observed sequential 1:1 protonations in the ferric enterobactin system,[4] with infrared evidence supporting a shift to a salicylate mode of bonding.

There are two plausible models for the addition of three protons to ferric MECAM. These are outlined schematically below, where L-L-L represents the hexaanion of MECAM, and an * indicates an uncoordinated (dangling) DHB group. As noted above, the IR spectrum of Fe(H_3MECAM) does

$$Fe(L\text{-}L\text{-}L) + 3H^+ \rightleftarrows Fe(L\text{-}HL)\text{-}H_2L^* \qquad (23)$$

$$Fe(L\text{-}L\text{-}L) + 3H^+ \rightleftarrows Fe(HL\text{-}HL\text{-}HL) \qquad (24)$$

not contain any bands near the 1635-cm^{-1} frequency of the free carbonyl group. In addition the 1620-cm^{-1} band of Fe(MECAM) is also absent from the Fe(H_3MECAM) spectrum. These results are consistent with reaction 24, in which all three carbonyl groups are involved in salicylate bonding to

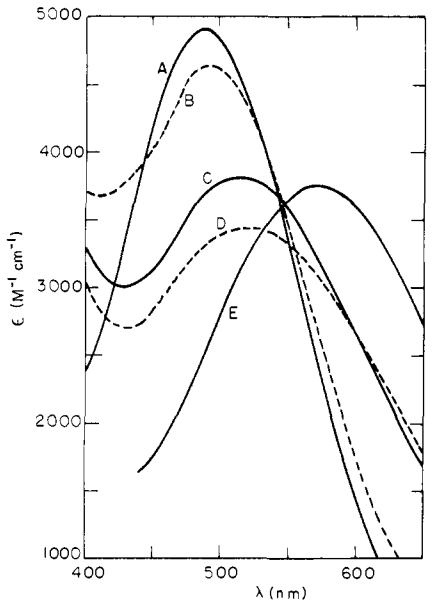


Figure 8. Visible spectra of aqueous solutions of $[Fe(DMB)_3]^{3-}$ (A), $[Fe(MECAM)]^{3-}$ (B), $[Fe(HMECAM)]^{2-}$ (C), $[Fe(H_2MECAM)]^-$ (D), and $[Fe(DMB)_2]^-$ (E).

the iron. Thus all the carbonyl bands are buried under the adjacent ring modes at 1540 and 1585 cm^{-1}.

The visible spectra of the protonated complexes are also consistent with reaction 24. In the ferric MECAM system, the spectra of $[Fe(MECAM)]^{3-}$ and $[Fe(H_2MECAM)]^-$ can be measured directly. Given the extinction coefficient of the $[Fe(MECAM)]^{3-}$ complex at a series of wavelengths and the value of K_{MHL}, the extinction coefficients of $[Fe(HMECAM)]^{2-}$ can be calculated point by point to generate the spectrum shown in Figure 8. The spectra of $[Fe(MECAM)]^{3-}$ and $[Fe(DMB)_3]^{3-}$ are very similar, particularly with respect to λ_{max} (Figure 8, curves A and B). However, the λ_{max} of $[Fe(H_2MECAM)]^-$ has shifted to only 520 nm, whereas the $[Fe(DMB)_2]^-$ complex has a λ_{max} 570 nm. The large difference between the absorbance maxima of the bis(DMB) complex and $[Fe(H_2MECAM)^-$ (Figure 8, curves D and E) favors the formulation [Fe(HL-HL-L)] for the latter, in which each protonated DHB group has shifted to a salicylate mode of bonding. If the two protons were going onto the same DHB to form $[Fe(H_2L^*\text{-}L\text{-}L)]$, one would expect the visible spectrum of the diprotonated MECAM complex to resemble closely that of $[Fe(DMB)_2]^-$.

It is clear that the bidentate ligand DMB does not bind iron through the amide carbonyl oxygen, and this is readily understood on the basis of simple thermodynamic arguments. From the known formation constants of DMB and salicylamide, one can estimate the equilibrium constant for the addition of a monoprotonated DMB anion, coordinating in a salicylate mode, to a bis(DMB) complex.

$$Fe(DMB)_2^- + HDMB^- \rightleftarrows Fe(DMB)_2(HDMB)^{2-} \quad (25)$$

$$K_{sal} = 250$$

Given this value of K_{sal}, one can estimate the disproportionation constant for the reaction

$$2[Fe(DMB)_2(HDMB)] \rightleftarrows Fe(DMB)_3 + Fe(DMB)_2 + H_2DMB \quad (26)$$

$$K_D = \frac{K_{ML_3}K_1^H}{(K_{sal})^2K_2^H} = 1.6 \quad (27)$$

where K_{ML_3} is the third stepwise formation constant for DMB, defined by eq 20. Thus the formation of simple bis and tris DMB complexes is slightly favored thermodynamically. What actually drives this disproportionation of the salicylate complex is the concentration dependence of eq 26, which pushes the equilibrium to the right in dilute solutions. At the concentrations used in this study (approximately millimolar), only 5% of the iron would ever be present as the $[Fe(DMB)_2(HDMB)]$ mixed-mode complex. In contrast, a solution which was 1 M in $[Fe(DMB)_2]$ and $[Fe(DMB)_3]$ with a fivefold excess of DMB would contain about 60% of the iron as the mixed-mode species.

Table III. Formation Constants and pM Values for Ferric Siderophores and Related Complexes

ligand	log K_{ML} or log β_3	pM [a]
enterobactin	52	35.6
MECAM	46	29.1
desferrioxamine B	30.6	26.6
transferrin		23.6[b]
CYCAM	40	23.0
nitrocatechol	(43.3)	23.4
Tiron	(~45)	~20
catechol	(43.7)	15.8[c]
3,4-dihydroxyphenylacetic acid	(43.9)	15[c]
2,3-dihydroxy-N,N'-dimethylbenzamide	(39.8)	15[c]

[a] Calculated for pH 7.4, $[Fe]_{tot} = 10^{-6}$; $[L]_{tot} = 10^{-5}$. [b] Based on constants reported in ref 25. [c] pM is below lower limit set by the K_{sp} of ferric hydroxide, indicating precipitation of $Fe(OH)_3$ under these conditions.

The disproportionation equilibrium for ferric MECAM is defined as

$$2Fe(L\text{-}L\text{-}HL) \overset{K_D'}{\rightleftarrows} Fe(L\text{-}L\text{-}L) + Fe(L\text{-}L)\text{-}H_2L^* \quad (28)$$

where the * denotes an uncoordinated DHB side group. Unlike the DMB system, there is no concentration effect favoring disproportionation, since the number of molecules on each side of eq 28 is the same. To a crude first approximation the two disproportionation constants K_D and K_D' should be equal to one another. However, attachment of the DHB to the cyclic backbone should enhance both catecholate and salicylate bonding relative to the bidentate analogues due to standard chelate effects. This increases both K_{ML_3} and K_{sal} in eq 27, but since K_{sal} appears as a squared term in the denominator, the net result is to decrease K_D'; thus the value of 1.6 calculated above is an upper limit. In fact, K_D' is likely to be 3–4 orders of magnitude less than K_D, or about 10^{-3} to 10^{-4}. Given these more realistic values for K_D', we calculate that at millimolar concentration of $[Fe(HMECAM)]^{2-}$ over 96% of the iron will be present as the [Fe(HL-L-L)] species.

Formation Constants. The K^* values reported in Table I, while valid thermodynamic constants, are difficult to compare with normal formation constants tabulated for other metal complexes. Based on the DMB value of 12.1 as the log of the average protonation constant for the three very basic phenolic oxygens of CYCAM and MECAM, the K^* values have been converted to normal formation constants ($K_{ML} = [ML]/([M][L])$), with log K_{ML} = 46 for ferric MECAM and log K_{ML} = 40 for ferric CYCAM. These values are listed in Table III, along with the values for enterobactin and several simple catecholates.

The MECAM complex is one of the most stable iron complexes ever characterized, much more stable than most of the simple catecholates. Tiron (4,5-dihydroxy-1,3-benzenedisulfonic acid), which forms unusually stable complexes with iron, has a log β_3 on the order of 45–46.[19,20] When comparing K_{ML} values of sexidentate ligands with β_3 values of bidentate ligands, it must be remembered that these formation constants

reflect the relative effectiveness of the two ligands only in their standard state of 1 *m*. At more dilute concentrations, the effectiveness of the sexidentate ligand is greatly enhanced relative to that of a simple catecholate. This is shown by the p*M* values listed in Table III, where p*M* is $-\log [Fe^{3+}(H_2O)_6]$ of a pH 7.4 solution which is 10^{-6} M in iron and 10^{-5} M in ligand. Although MECAM and Tiron have similar formation constants, the p*M* value of MECAM is almost 10 log units higher than that of Tiron under the given conditions. This ability to sequester iron in very dilute solutions is critical in the evaluation of synthetic iron chelators for possible therapeutic use in the treatment of iron overload, since the in vivo concentration of the drug will necessarily be quite low.

The K_{ML} value for ferric CYCAM is lower than had been expected. The three DHB groups of CYCAM are attached directly to the central cyclotriazatridecane ring. Although the ring itself is flexible, the visible spectra indicate that all six of the phenolic oxygens do not coordinate simultaneously to the ferric ion. Thus it appears that this ligand is not capable of fully encapsulating ferric ion, and this is reflected in the lower formation constant.

In MECAM, the outer DHB groups are linked to the central ring by $-CH_2—NH-$ bridges. Even though the $-CH_2-$ group is not able to flex toward the metal because of the planar aromatic ring, positioning the amide nitrogen out of the central ring appears to permit the ligand to encapsulate ferric ion fully. This results in an increase in log K_{ML} of 6 log units over that for ferric CYCAM. The value of 46 found for ferric MECAM is still 5 log units lower than the value for enterobactin,[4] which combines a flexible center ring with exocyclic amide groups. It appears that both these factors are important in maximizing the iron binding ability of these types of ligands.

One factor widely thought to be critical in the evaluation of synthetic iron chelators for drug use is the ability of the ligand to remove iron from transferrin, the iron transport protein in humans. The p*M* value for transferrin has been calculated from literature equilibrium constants[25] to be 23.6. Desferrioxamine B, which is presently the most effective drug available for iron removal in man,[3] has a p*M* of 26.6, and thus meets this thermodynamic requirement. The p*M* value for CYCAM is 23, which is slightly below the transferrin value of 23.6. Thus CYCAM can remove only a fraction of transferrin-bound iron. However, MECAM, with a p*M* of 29.1, is capable of removing essentially all the iron from an equivalent concentration of ferric transferrin at pH 7.4. Thus it clearly meets the thermodynamic criterion for a successful drug. *In addition, recent results from this laboratory have demonstrated that these tricatecholate ligands (including enterobactin) are also kinetically capable of removing iron from transferrin under conditions at which the hydroxamates are totally ineffective.*[1]

Summary

We have characterized the iron binding abilities of two new tricatecholate ligands. At high pH, MECAM coordinates to ferric ion through six phenolic oxygens, whereas CYCAM coordinates through only five, presumably due to steric strain resulting from its endocyclic amide structure. Both complexes react with hydrogen ion in discrete one-proton steps with a concomitant shift in the mode of bonding to a salicylate-type structure with coordination via the orthophenolate and the amide carbonyl groups. Such protonations eventually lead to precipitation of the neutral FeH_3L species.

The formation constants of the ferric complexes are estimated to be log K_{ML} = 46 for MECAM and 40 for CYCAM. The calculation of free ferric ion concentrations present at equilibrium in metal–ligand solutions indicates that MECAM is thermodynamically capable of removing essentially all the iron from transferrin, while CYCAM can remove ≈20% of transferrin bound iron.

These compounds have now been shown to have three characteristics which are important in the design of an effective iron-removal agent: (1) an extremely high affinity for ferric ion; (2) total resistance to hydrolysis of the free lignad over the normal pH range; and (3) the ability to remove significant amounts of iron from transferrin under conditions where the hydroxamates are totally ineffective.[1] This combination of features represents a significant advance in the design of synthetic iron chelators for the treatment of iron overload in humans.

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References and Notes

(1) Previous paper in this series: Carrano, C. J.; Raymond, K. N. *J. Am. Chem. Soc.* **1979,** *101,* 5401–5404.
(2) Beck, W. S., Ed. "Hematology"; The MIT Press: Cambridge, Mass., 1973.
(3) Anderson, W. F.; Hiller, M. C., Eds. *DHEW Publ. (NIH) (U.S.), NIH 77-994 1977.*
(4) Harris, W. R.; Carrano, C. J.; Cooper, S. R.; Sofen, S. R.; Avdeef, A.; McArdle, J. V.; Raymond, K. N. *J. Am. Chem. Soc.* **1979,** *101,* 6097–6104.
(5) Neilands, J. B., Ed. "Microbial Iron Metabolism"; Academic Press: New York, 1974.
(6) Carrano, C. J.; Raymond, K. N. *Acc. Chem. Res.* **1979,** *12,* 183–190.
(7) Harris, W. R.; Carrano, C. J.; Raymond, K. N. *J. Am. Chem. Soc.* **1979,** *101,* 2213–2214.
(8) Isied, S. S.; Kuo, G.; Raymond, K. N. *J. Am. Chem. Soc.* **1976,** *98,* 1763–1767.
(9) Llinas, M.; Wilson, D. M.; Neilands, J. B. *Biochemistry* **1973,** *12,* 3836–3843.
(10) Rawls, R. L. *Chem. Eng. News* **1977,** *55,* 24–25.
(11) O'Brien, I. G.; Cox, G. B.; Gibson, F. *Biochim. Biophys. Acta* **1970,** *201,* 453.
(12) Harris, W. R.; Weitl, F. L.; Raymond, K. N. *J. Chem. Soc., Chem. Commun.,* in press.
(13) Harris, W. R.; Weitl, F. L.; Sofen, S. R.; Raymond, K. N. "Abstracts of Papers", 175th National Meeting of the American Chemical Society, Anaheim, Calif., March 1978; American Chemical Society: Washington, D.C., 1978; INOR 228.
(14) Weitl, F. L.; Raymond, K. N. *J. Am. Chem. Soc.* **1979,** *101,* 2728–2731.
(15) Subsequent to our original report of the preparation of MECAM, another, independent, synthesis has appeared: Venuti, M. C.; Rastetter, W. H.; Neilands, J. B. *J. Med. Chem.* **1979,** *22,* 123–124.
(16) Welcher, T. J. "The Analytical Uses of Ethylenediamine Tetraacetic Acid"; Van Nostrand: Princeton, N.J., 1958.
(17) Rossotti, F. J. C.; Rossotti, H. *J. Chem. Educ.* **1965,** *42,* 375–378.
(18) Harned, H. S.; Hickey, F. C. *J. Am. Chem. Soc.* **1937,** *59,* 1284–1289.
(19) Martell, A. E.; Smith, R. M. "Critical Stability Constants", Vol. 1, Plenum Press: New York, 1974.
(20) Avdeef, A.; Sofen, S. R.; Bregante, T. L.; Raymond, K. N. *J. Am. Chem. Soc.* **1978,** *100,* 5362–5370.
(21) Mentasi, E.; Pelizzetti, E.; Saini, F. *J. Chem. Soc. A* **1973,** 2609–2614.
(22) Wilson, H. W. *Spectrochim. Acta* **1974,** *30A,* 2141–2152.
(23) Katritzky, A. R.; Jones, R. A. *J. Chem. Soc.* **1959,** 3670–3674.
(24) Hildago, A.; Otero, C. *Spectrochim. Acta* **1960,** *16,* 528–539.
(25) Aisen, P.; Leibman, A.; Zweier, J. *J. Biol. Chem.* **1978,** *253,* 1930–1937.