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Ferric Ion Sequestering Agents. 21.[1] Synthesis and Spectrophotometric and Potentiometric Evaluation of Trihydroxamate Analogues of Ferrichrome

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Two trihydroxamate analogues of the microbial iron chelator ferrichrome have been synthesized by attachment of three succinylmonohydroxamate [3-[(hydroxyamino)carbonyl]propanoyl] moieties to triamine backbones. The ferric ion complexation equilibria have been evaluated for the more soluble ligand, TRENDROX. The protonation constants of this ligand and formation and protonation constants of its Fe(III) complex have been determined by potentiometric and spectrophotometric techniques. The protonation constants [log K (esd)] of TRENDROX are 10.30 (1), 9.33 (1), 8.58 (2), and 6.51 (5). The last of these represents protonation of the tertiary amine; the others are the hydroxamate protonation constants. The ligand is a slightly stronger chelating agent than the natural trihydroxamate siderophores; the formation constant is log K = 32.9 (2) for the FeL ferric complex. The protonation constants for the reactions $FeL + H^+ = Fe(HL)^+$ and $Fe(HL)^+ + H^+ = Fe(H_2L)^{2+}$ are log K = 2.3 and 1.4, respectively. These reactions represent sequential protonations of the hydroxamate groups to give the bis(hydroxamate) and mono(hydroxamate) complexes, respectively.

Introduction

Siderophores[2-4] are microbially produced iron chelators whose mode of action and biomedical importance have emerged in recent years. Synthetic analogues incorporating certain key features of the siderophores can serve as systematic probes of the structural requirements of iron transport processes in vivo.[4] Siderophores have also served as important models for the biomimetic design of drugs to decorporate iron from iron-overloaded patients. The expense and therapeutic limitations of the current drug of choice for treatment of iron overload, the trihydroxamate siderophore desferrioxamine B (DFO) (Figure 1), has led to the search for analogues of increased clinical effectiveness.[5]

A few retrohydroxamate siderophores (ligands in which the positions of the hydroxamate carbon and the nitrogen have been interchanged relative to the positions in the natural siderophore ligands) have been synthesized,[6,7] and several totally synthetic hydroxamate ligands,[8] including some trihydroxamate ligands, are known[9] that could be used either as iron transport probes or as iron removal agents. This communication describes the synthesis and iron-binding properties of two synthetic trihydroxamate analogues of the siderophore desferrichrome (Figure 1), where desferrichrome's essential components (three hydroxamates appended to one central platform) have been retained. The synthesis of a linear tetrahydroxamate DFO derivative, suitable for the binding of tetravalent actinide metal ions, is also described.

Experimental Section

Synthesis. Previous synthetic strategies aimed at retro- and synthetic polyhydroxamates have centered on the use of a backbone containing multiple carboxylate moieties, which are then activated and conjugated with hydroxylamine to form the hydroxamate moiety in the last step. The synthesis to be described here (Figure 1) employs an alternative approach, whereby a monohydroxamate with an appended carboxylate group is first synthesized and then three such moieties are attached via amide linkages to a polyamine backbone. This approach, first employed by Prelog in the total synthesis of desferrioxamine B,[10] makes effective use of the natural carbonyl-activating properties of hydroxamate esters, thereby eliminating a number of activation, protection, and deprotection steps.

Succinic anhydride was combined with *p*-tolylhydroxylamine (R = $C_6H_4CH_3$) to give the succinylmonohydroxamate [3-[(hydroxyamino)-carbonyl]propanoyl] **1**, which was then cyclized with DCC (dicyclohexylcarbodiimide) to give the active hydroxamate ester **2**, simultaneously protecting the hydroxamate oxygen and activating the carboxylate group. Without isolation, 3 equiv of this active ester were coupled with 1,3,5-tris(aminomethyl)benzene (**3**) to yield the desired trihydroxamate product **4** (MEDROX). A similar reaction with TREN (**5**) yielded the trihydroxamate **6** (TRENDROX). The active ester **2** was found to be selective for primary amines; reaction with dipropylenetriamine (**7**) with 3 equiv of **2** gave only the dihydroxamate **8**. One equivalent of **2** also reacted with the terminal amine of desferrioxamine B (Desferal) to give the semisynthetic tetrahydroxamate derivative **9**.

Thin-layer chromatography was performed on precoated Analtech GHLF silica gel sheets and visualized by UV and Fe(III)/MeOH spray. The ^1H NMR spectra were recorded on a Varian EM-390 spectrometer with Me_4Si as internal reference. Microanalyses and positive-ion FAB mass spectra (glycerol matrix) were performed by the Analytical Service, Chemistry Department, University of California, Berkeley, CA. The THF and dioxane were distilled from sodium benzophenone ketyl; all other solvents were reagent grade.

1. Succinic anhydride (8.00 g, 79.9 mmol) and *p*-tolylhydroxylamine (9.85 g, 80.0 mmol) were dissolved in 200 mL of ethyl ether (anhydrous). After 3 h, the product was collected by filtration and washed with ether, yielding 15.14 g (85%) of a white solid: R_f (10% MeOH/CH_2Cl_2) 0.34; ^1H NMR (CD_3OD) δ 2.3 (s, 3 H), 2.5-3.1 (m, 4 H), 7.1-7.5 (qt, 4 H). Anal. Calcd (found) for $C_{11}H_{13}NO_4$: C, 59.17 (58.95); H, 5.87 (5.89); N, 6.28 (6.18).

MEDROX (4). Compound **1** (0.252 g, 1.13 mmol), DCC (dicyclohexylcarbodiimide, 0.240 g, 1.17 mmol), and a catalytic amount of 4-(dimethylamino)pyridine were dissolved in THF (20 mL). After the mixture was stirred for 24 h at room temperature, the solution of **2** was filtered to remove DCU (dicyclohexylurea).

To a suspension of 1,3,5-tris(aminomethyl)benzene trihydrochloride (**3**) (0.060 g, 0.36 mmol) in EtOH (10 mL, anhydous) was added a minute amount of phenolphthalein and then enough NaOEt (1.0 M in EtOH) to turn the suspension pink. After evaporation of the solvent, the THF solution of compound **2** was added, heated slightly and then stirred at room temperature for 5 h. The solvent was evaporated and the product recrystallized (MeOH/ether), yielding 0.143 g (50%) of an off-white

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† Deceased.

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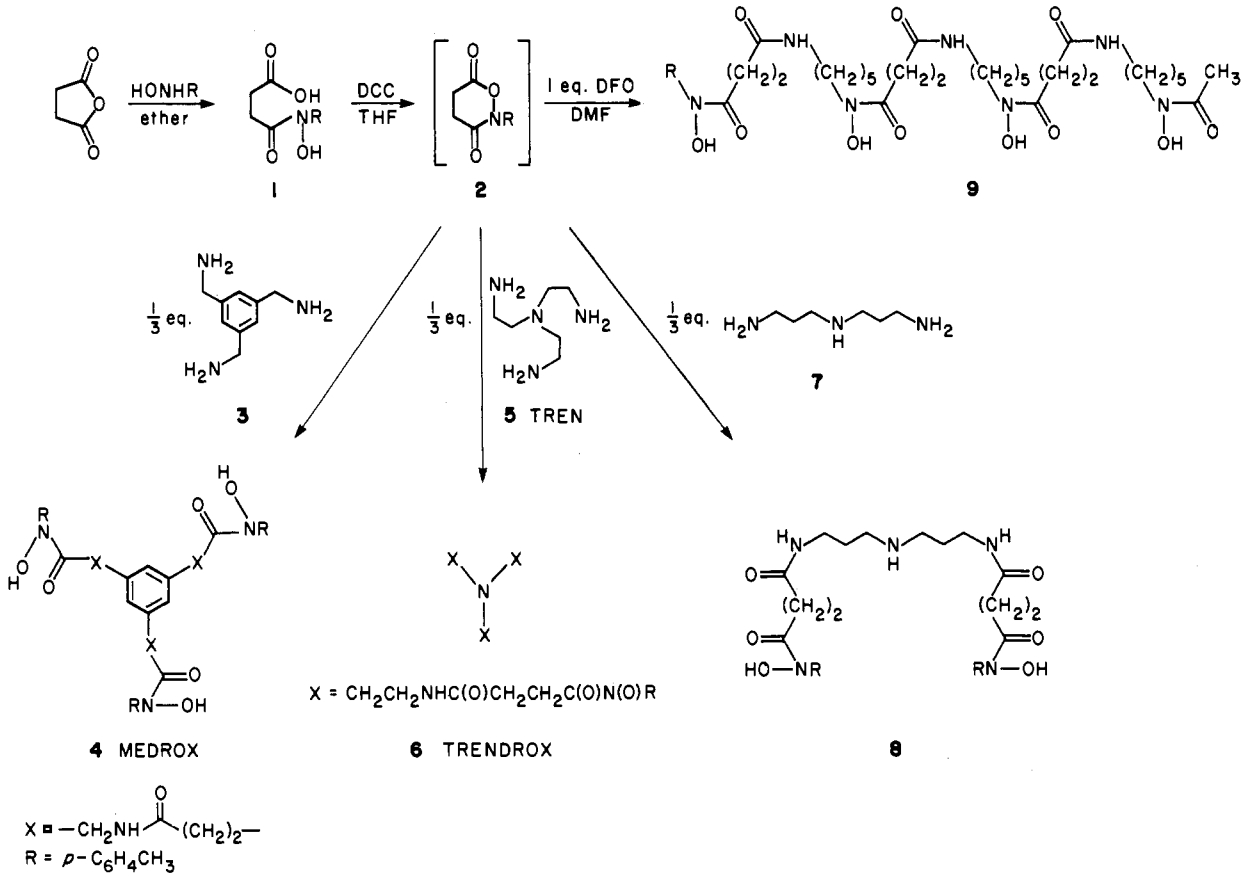

Figure 1. Synthesis off polyhydroxamate ligands and their abbreviated names.

solid: R_f (5% MeOH/CH_2Cl_2) 0.30; ^{1}H NMR (CD_3OD) δ 2.3 (s, 9 H), 2.4–3.1 (m, 12 H), 4.3 (s, 6 H), 7.1–7.5 (m, 15 H). Anal. Calcd (found) for $C_{42}H_{48}N_6O_9$: C, 64.57 (64.23); H, 6.21 (6.33); N, 10.76 (10.76). FAB-MS: m/e (M + Na) 803, (M + Na − xO) 787, 771, 755.

TRENDROX (6). Compound **1** (5.93 g, 26.5 mmol), DCC (5.48 g, 26.5 mmol), and a catalytic amount of 4-(dimethylamino)pyridine were suspended in 150 mL of dioxane and stirred for 24 h at room temperature. The DCU was filtered off and TREN (**5**) (1.18 g, 8.08 mmol) was added. The reaction mixture was heated slightly until all the solids dissolved and then stirred at room temperature for 48 h. After the solvent was evaporated, the residue was dissolved in CH_2Cl_2 (200 mL), washed with H_2O (3×, 100 mL) and extracted with 0.5 M HCl (2×, 100 mL). The aqueous layer was brought to pH 7 with 0.5 M NaOH, saturated with NaCl, extracted with CH_2Cl_2 (3×, 200 mL), and dried (Na_2SO_4). Removal of the solvent under vacuum gave 4.17 g (68%) of a tan solid: R_f (5% MeOH/CH_2Cl_2) 0.44; ^{1}H NMR (CD_3OD) δ 2.3 (s, 9 H), 2.4–2.6 (br m, 12 H), 2.6–3.0 (brm, 6 H), 3.0–3.3 (br m, 6 H) 7.1 (d, 6 H) 7.3 (d, 6 H). Anal. Calcd (found) for $C_{39}H_{51}N_7O_9 \cdot 0.5\ CH_2Cl_2$: C, 58.96 (58.69); H, 6.53 (6.49); N, 12.19 (12.00). FAB-MS: m/e (M + H) 762, (M + H − xO) 746, 730, 714.

8. A solution of compound **1** (1.22 g, 5.47 mmol), DCC (1.22 g, 5.91 mmol), and a catalytic amount of 4-(dimethylamino)pyridine were stirred in THF (50 mL) at room temperature for 24 h. After the DCU was filtered off, dipropylenetriamine (0.25 mL, 1.76 mmol) was added and the reaction mixture was heated briefly. After the reaction mixture was stirred at room temperature for 2 h, ether was added, precipitating 0.79 g (83%) of an off-white solid: R_f (5% MeOH/CH_2Cl_2) 0.07; ^{1}H NMR (CD_3OD) δ 1.4–1.8 (m, 4 H), 2.2 (s, 6 H), 2.3–3.3 (m, 16 H), 7.0–7.5 (qt, 8 H). Anal. Calcd (found) for $C_{28}H_{39}N_5O_6$: C, 62.06 (61.79); H, 7.28 (7.34); N, 12.93 (12.80). FAB-MS: m/e (M + H) 542, (M + H − xO) 526, 510.

9. Compound **1** (0.112 g, 0.50 mmol), DCC (0.104 g, 0.50 mmol), and a catalytic amount of 4-(dimethylamino)pyridine were stirred in 20 mL THF for 12 h. After filtration and evaporation of the solvent, desferrioxamine B (0.300 g, 0.46 mmol), NEt_3 (0.32 mL, 2.3 mmol), and 20 mL of DMF (distilled) were added. The reaction mixture was heated slightly until all the solids dissolved and then stirred at room temperature for 12 h. After evaporation of the solvent, the residue was washed with H_2O and recrystallized from MeOH to give 0.279 g (77%) of a white solid: ^{1}H NMR (DMSO-d_6) δ 1.0–1.8 (br m, 18 H), 1.95 (s, 3 H), 2.25 (s, 3 H), 2.3–2.8 (m, 12 H), 2.8–3.2 (m, 6 H), 3.2–3.6 (m, 10 H, MeOH), 7.1 (d, 2 H), 7.5 (d, 2 H), 7.7 (br m, 2 H), 9.6 (br m, 1 H). Anal. Calcd (found) for $C_{36}H_{59}N_7O_{11} \cdot MeOH$: C, 55.67 (55.50); H, 7.97 (7.76); N, 12.29 (12.53). FAB-MS: m/e (M + H) 766, (M + H − xO) 750, 734.

Stability Constant Measurements. Stock solutions of $Fe(NO_3)_3$ were prepared from $Fe(NO_3)_3 \cdot 9H_2O$ in nitric acid. The actual Fe(III) concentration was standardized with EDTA by using Varamine B as an indicator.[11] Concentration of nitric acid was determined by potentiometric titration with KOH. A carbonate-free 0.1 M KOH solution stored under ascarite-scrubbed argon atmosphere was prepared from Baker Dilut-It ampules by using freshly boiled doubly distilled water and was standardized with potassium acid phthalate. Standard 0.1 M HNO_3 and HCl were likewise prepared from Baker Dilut-It ampules.

Potentiometric Titration. Ten milligrams of ligand was added to 45.00 mL of freshly boiled doubly distilled water in a jacketed titration cell maintained at 25 ± 0.1 °C by a circulating temperature water bath. Solutions were adjusted to 0.100 M ionic strength by addition of 5.00 mL of 1.00 M KNO_3 and kept under an argon atmosphere that was first passed through a basic pyrogallol solution. Since the ligand was not very soluble at neutral pH, standard KOH was added to dissolve the ligand and the solution was titrated with HNO_3.

A motor-driven Metrom Dosimat 645 was used to deliver titrants. Measurements of pH were made with a Fisher Model 825 digital meter equipped with Sargent Welch glass and calomel reference electrodes. The electrodes were standardized to read hydrogen ion concentration directly.[12] In cases where standard buffer solutions were used, pH was corrected to concentration terms.

Both the Metrohm Dosimat and the Fisher pH meter were interfaced to a Commodore 64K microcomputer. Input data for the titration program specified number of titration points, titrant increment, stability margin, pH, and time limit. After each titration point, pH of the solution and volume of titrant added were recorded. Titration was terminated when either the pH or the total titrant added exceeded the preset limit.

Protonation and metal chelate stability constants were calculated by using the programs BEST[13] and PKAS.[14]

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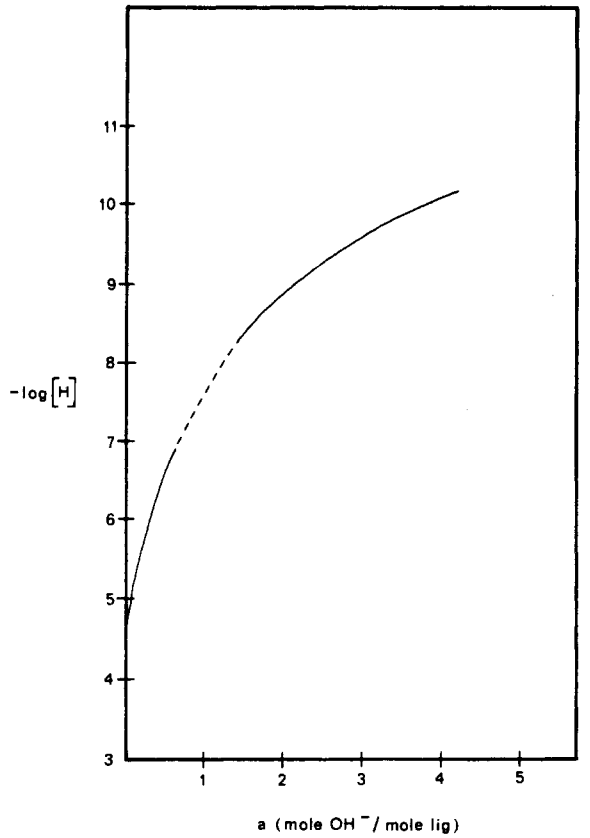

Figure 2. Potentiometric equilibrium curve of TRENDROX, H_4L, as a function of added base. [TRENDROX] = 2.48×10^{-4} M; μ = 0.100 M (KNO_3); T = 25.0 ± 0.5 °C. The dashed line indicates precipitation.

Table I. Protonation Constants of TRENDROX and Related Compounds at 25.0 °C, 0.100 M Ionic Strength

	TRENDROX	DFO[a]	BAMTPH[b]	compd 1
log K_0		>11 (RNH_2)		
log K_1	10.30 (1)	9.70	10.04	9.46 (4)
log K_2	9.33 (1)	9.03	9.33	4.63 (3) (COO^-)
log K_3	8.58 (2)	8.39	8.48	
log K_4	6.51 (5) (NR_3)			

[a] Reference 15. In order to compare the corresponding hydroxamate protonations, K_n is defined for the protonated amine form of DFO. [b] BAMTPH = *N,N',N''*-tris[2-(*N*-hydroxycarbamoyl)ethyl]-1,3,5-benzenetricarboxamide.[8]

Spectrophotometric Measurements. Visible and UV spectra were recorded on a HP 8450A spectrophotometer. The 1:1 ferric ion–TRENDROX solution in 0.100 M ionic strength was prepared in a jacketed cell fitted with a Trisma combination electrode. The visible spectrum of the ferric-TRENDROX complex was obtained as a function of pH by removing an aliquot of solution and recording its spectrum.

Results

Ligand Protonation Constants. The compound TRENDROX (**6**; Figure 1) is not very soluble at neutral pH; however, when the pH is raised above 8 or lowered to 6, it dissolves readily. The potentiometric equilibrium curve of free TRENDROX ligand is shown in Figure 2. In contrast, the insolubility of MEDROX (**4**; Figure 1) precluded any evaluation of its protonation or formation constants, although it too forms a stable ferric complex.

The three highest protonation constants of TRENDROX were obtained by dissolving the ligand at high pH (>10.5) and titrating the solution with standard HNO_3. To characterize the fourth protonation reaction, a ligand solution was prepared at pH 3.5 and titrated with standardized KOH until precipitation occurred. In this way sufficient data were collected to permit the direct calculation from potentiometric data of the ligand protonation constants. The values of the proton association constants obtained,

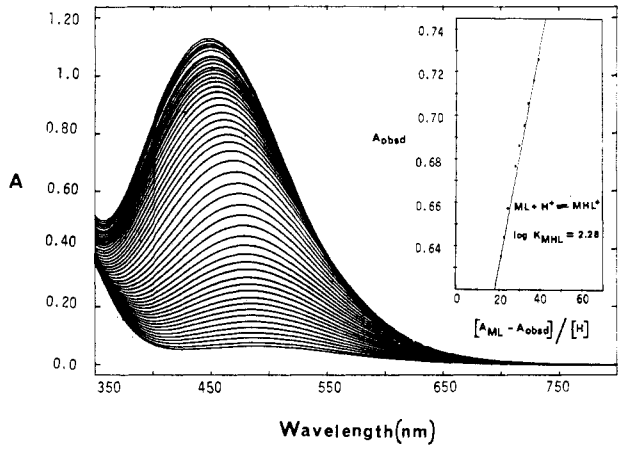

Figure 3. Visible spectra of a 1:1 solution of ferric ion and TRENDROX as a function of pH. [TRENDROX] = $[Fe]_{tot} = 4.12 \times 10^{-4}$ M; μ = 0.100 M (KNO_3); T = 25.0 ± 0.5 °C; pH 3.7–0.4. (Inset: Plot of A_{obsd} vs $(A_{ML} - A_{obsd})/[H]$ for ferric TRENDROX from pH 2.8 to 1.9, where A_{ML} is the average absorbance of [Fe(TRENDROX)] at λ = 440–470 nm, and A_{obsd} is the average apparent absorbance at λ = 440–480 nm at each pH value.)

defined by eq 1 and 2, are listed in Table I together with those of analogous compounds for comparison.[15]

$$H_{n-1}L + H \rightleftarrows H_nL \quad (1)$$

$$K_n^H = \frac{[H_nL]}{[H_{n-1}L][H]} \quad (2)$$

The hydroxamate moieties in TRENDROX are too widely separated for significant intergroup interaction. The protonation constants of the hydroxamate groups (log K values of 10.3, 9.31, and 8.85) reflect a slightly greater than statistical separation (log 3 or 0.48[16]). For comparison, protonation constants of the monomeric hydroxamate compound **1** were also determined. The value obtained for the hydroxamate group, log K = 9.46, is in excellent agreement with the average value of the protonation constants (log K) calculated for the hydroxamate groups of TRENDROX (9.4). The tertiary amine appears to exert little effect on the hydroxamates because of the long backbone chains.

The fourth observed protonation step is attributed to the protonation of the tertiary amine. The basicity of the amine is lowered by 3–4 pK_a units from that of a simple amine. This is ascribed to the formation of a five-membered ring resulting from hydrogen bonding between the amine and the N–H amide proton.

Chelate Protonation Constants. Spectrophotometric titrations of the ferric–TRENDROX complex were performed to measure the chelate protonation constants. The visible spectra of the ferric–TRENDROX complex as a function of pH are shown in Figure 3. The solution of ferric–TRENDROX complex is reddish orange with λ_{max} at ~445 nm, typical of a ferric trihydroxamate complex, in which the metal is coordinated to the six oxygens of the hydroxamate moieties. As the pH is lowered, absorbance of the ferric complex decreases and λ_{max} shifts to longer wavelength (488 nm). Isosbestic points were observed over narrow pH ranges; one is the isosbestic point at 428 nm between pH 2.5 and 1.9 in the first-derivative plot shown in Figure 4. Componentization of the visible (402–650 nm) spectral data by the method of Maeder and Gampp[17,18] indicates at least three colored species are present

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(16) For three equivalent, *noninteracting* protons with an intrinsic protonation constant K, the observed log K values will be log K and log K ± log 3. See: Adams, E. Q. *J. Am. Chem. Soc.* **1916**, *38*, 1503.
(17) Maeder, M.; Gampp, H. *Anal. Chim. Acta* **1980**, *122*, 303.
(18) The spectral componentization and least-squares refinements employed FORTRAN programs run on IBM PC or AT microcomputers with 256K RAM and floating point coprocessors. Details and code for these programs are given in: Scarrow, R. C. Ph.D. Thesis, University of California Berkeley, 1985, pp 225–291 (available from University Microfilms).

Table II. Comparison of Refined Values of K_{ML}, K_{MHL}, and K_{MH_2L} and Spectral Data for Models Describing the Low-pH Ferric–TRENDROX System[a]

fit	FeL			$FeLH^+$			$FeLH_2^{2+}$			
no.	log K_{ML}	λ_{max}, nm	ϵ, M^{-1} cm^{-1}	log K_{MHL}	λ_{max}, nm	ϵ, M^{-1} cm^{-1}	log K_{MH_2L}	λ_{max}, nm	ϵ, M^{-1} cm^{-1}	rms ΔA[b]
1	large[c]	444	2794	2.00 (2)	486	1789	0.86 (1)	479[d]	564	0.0033
2	34.324	444	2846	2.03 (2)	482	1710	0.80 (1)	462[d]	89	0.0020
								603	84	
3	34.16 (4)	448	2792	1.81 (3)	493	1210				0.0077
4	32.9[e]	442	2934	2.28 (3)	468	1960	1.36 (2)	488	1386	0.0026

[a] In fits 2–4, extinction spectra of Fe^{3+} and $Fe(OH)^{2+}$ were fixed as determined from the absorbance of dilute $Fe(ClO_4)_3$ solutions at pH 1.0, 2.0, and 2.64. The first hydrolysis constant of ferric ion was fixed at log $K_h = -2.61$.[21] The standard deviations of the last digits are given in parentheses; these values are determined by the least-squares fits. [b] The degree to which the various models fit the data is compared by rms $\Delta A = [\{\sum\sum(A_{obsd} - A_{calcd})^2\}/n_s n_\lambda]^{1/2}$, where n_s = number of spectra = 43 and n_λ = number of measured (and refined) absorbance = 125. For comparison, $[\{\sum\sum A_{obsd}^2\}/n_s n_\lambda] = 0.326$. [c] In model 1, log β was fixed at 50.0. This implies no dissociation of FeL into Fe^{3+} and H_4L^+. [d] Minimum in the extinction coefficient curve. [e] Fixed at a value determined by electrochemistry. The error limits given next to each protonation constant reflect the changes in refined values when the fixed value of K_{ML} was increased by its estimated error.

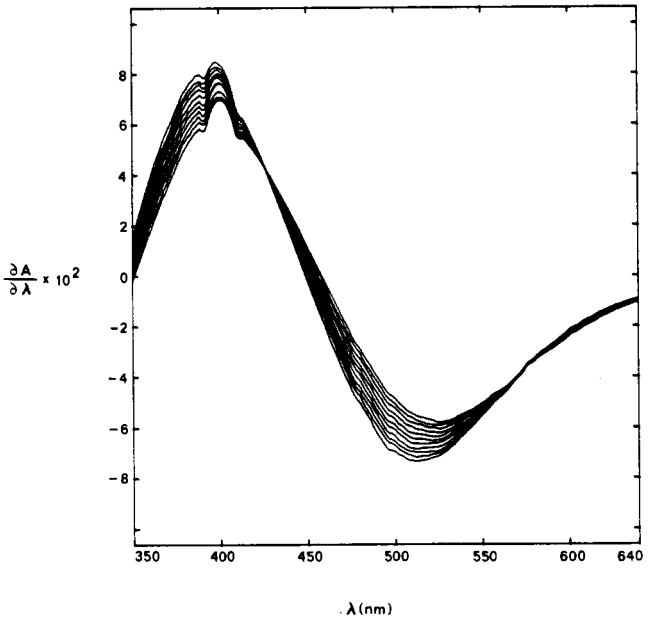

Figure 4. First-derivative plot, $\partial A/\partial\lambda$ vs λ, of the visible spectra of the 1:1 ferric ion–TRENDROX complex from pH 1.9 to 2.5. (The irregular shape of these curves at 400 nm is due to a small discontinuity in the absorbance plot created by the spectrometer, which is amplified in the derivative plot.)

between pH 0.5 and 2.5.[19] At the low pH end of this range, the solution becomes nearly colorless (with a 9.2 mM concentration of iron and TRENDROX).

The 428-nm isosbestic point (Figure 4) reflects the following reaction:

$$FeL + H^+ \rightleftarrows FeHL \quad (3)$$

$$K_{MHL} = \frac{[MHL]}{[ML][H]} \quad (4)$$

From a Schwarzenbach plot[20] of A_{obsd} vs $(A_{ML} - A_{obsd})/[H]$ (inset to Figure 3) the value of log K_{MHL} is determined to be 2.38; this reaction is consistent with the protonation and subsequent dissociation of one hydroxamate group to give a bis(hydroxamate) complex. As the pH of the ferric complex solution is lowered below 1, the reddish color of the solution starts to fade and eventually disappears completely at pH 0. However, from pH 0.8 to 0.4, another isosbestic point is observed in the first-derivative plot $(\partial A/\partial\lambda)$, representing further protonation of the MHL complex.

$$FeHL + H^+ \rightleftarrows FeH_2L \quad (5)$$

$$K_{MH_2L} = \frac{[MH_2L]}{[MHL][H]} \quad (6)$$

A similar Schwarzenbach plot of A_{obsd} vs $(A_{MHL} - A_{obsd})/[H]$, where A_{MHL} is determined from the intercept of the previous plot, establishes the value of log K_{MH_2L} as 0.7. Due to the variation of the ionic strength in this pH range, this value is less accurate than the others reported here (Table II). The corresponding reaction is assigned as the generation of a mono(hydroxamate)–Fe(III) complex.

Metal–Ligand Formation Constant. The ferric–TRENDROX complex formation constant cannot be determined directly from potentiometric titration data since total complexation of Fe(III) ion with this ligand is essentially complete even at pH 1.5. Moreover, as the pH of the ferric complex solution is raised above 3, precipitation of the (presumably neutral) chelate, FeL, occurs; this severely limits the possibility of determining the formation constant by spectrophotometric observation of a competition reaction, since the most commonly used competing ligands such as EDTA or DTPA are not effective at this pH. For these reasons the overall formation constant (eq 7, 8) was determined by

$$Fe^{3+} + L^{3-} \rightleftarrows FeL \quad (7)$$

$$K_{ML} = \frac{[\mu L]}{[\mu][L]} \quad (8)$$

electrochemical methods to determine the chemical activity of the aqueous ferric ion. In a solution of constant ionic strength, containing Fe^{3+}, Fe^{2+}, and ligand, the potential E of the Fe^{3+}/Fe^{2+} couple at 25 °C is given by

$$E = E^\circ - (RT/\mathcal{F}) \ln [[Fe(III)]/[Fe(II)]] \quad (9)$$

where E° is the standard potential and the Fe(III):Fe(II) ratio varies due to complexation. In order to measure this couple, a platinum disk electrode (5 mm diameter), in addition to the glass and reference electrodes, was fitted into the titration cell. Various ratios of Fe(III) ion to ligand solutions were prepared and purged with O_2- and CO_2-free argon for 1–2 h. The redox potential and the pH of the solution were measured after capsizing a glass boat containing a measured amount of Fe(II) salt into the solution. The pH was kept between 1.2 and 1.8 to avoid precipitation of the neutral chelate and to ensure complete complexation.

To eliminate systematic errors, the potential of the Fe(III)/Fe(II) couple of solutions with different Fe(III):Fe(II) ratios under the same conditions was measured. With anion complexation taken into account, the mean value was found to be −0.4948 (8)

(19) The 43 spectra, each containing 125 absorbance values between 402 and 650 nm, could be presented as linear combinations of three "components" (or extinction coefficient) spectra within an rms tolerance of 0.00034 absorbance unit. For comparison, the rms absorbance is 0.326 and rms tolerances are 0.027, 0.0027, and 0.00013 for the best descriptions employing one-, two-, and four-component spectra. The rms noise of our spectrometer for base-line-corrected data in the visible region is usually on the order of 0.0001–0.0002, so the presence of a minor fourth spectral species is suggested. The least-squares modeling of the spectra (see text, below) includes a small contribution from absorption by $Fe(OH)^{2+}$ in addition to major contributions from three ferric TRENDROX species.

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Table III. Equilibrium Constants and Chelate Protonation Constants of Ferric Trihydroxamate Complexes

	TRENDROX	DFO	BAMTPH
log K_{FeL}	32.9 (2)	30.6	26.32
log K_{FeHL}	2.38 (3)	0.94	
log K_{FeH_2L}	0.7 (1)		
pM[a]	27.8[b]	26.6	21.5[b]

[a] Equilibrium free metal ion concentrations pM = −log [Fe^{3+}] calculated for 10 μM ligand, 1 μM Fe^{3+}, pH 7.4. [b] Assuming [FeL] is soluble at 1 μM concentration.

V vs a saturated calomel electrode at 25 °C.

Since ferrous ion is not complexed by hydroxamate ligands at these low pH values,[2] [Fe^{2+}] is equal to the analytical concentration of the ferrous salt added. Therefore, the concentration of free [Fe^{3+}] can be calculated directly from the potential measurement and the ferrous ion concentration as

$$[Fe^{3+}] = [Fe^{2+}]10^{(E-E^\circ)/0.059} \quad (10)$$

From the mass balance equations of ferric ion and the ligand TRENDROX

$$[Fe]_{tot} = [Fe^{3+}] + [FeHL] + [FeH_2L] + [FeL] + [FeX] + [FeX_2] + [Fe(OH)] + [Fe(OH)_2] \quad (11)$$

where X = NO_3^- or Cl^- and

$$[Ligand]_{tot} = [L] + [HL] + [H_2L] + [H_3L] + [H_4L] + [FeL] + [FeHL] + [FeH_2L] \quad (12)$$

[FeL] and [L] are determined by substituting in the known equilibrium constants (ligand and chelate protonations, and ferric hydrolysis[21] and anion binding constants). The overall formation constant, K_{ML} = [ML]/[M][L], was thus determined as log K = 32.9 (2).

Factor and Least-Squares Analysis. A set of 43 absorbance spectra from 402 to 650 nm of solutions 0.2–0.3 mM in Fe(TRENDROX) and pH 2.5–0.5 were componentized and used for least-squares refinement of extinction coefficient spectra and equilibrium constants. Several equilibrium models were compared for their ability to fit the experimental data. When K_{ML} was fixed at $10^{32.9}$, a good fit was obtained with λ_{max}/nm (ϵ/M^{-1} cm^{-1}) values for FeL, $FeLH^+$, and $FeLH_2^{2+}$ of 442 (2930), 468 (1960), and 488 (1390), respectively (fit 4 of Table II). The value of K_{MHL} determined by this procedure is sensitive to the value of K_{ML} chosen, but is close to the value determined graphically. On the other hand, the deviation of the least-squares (fit 4) value of K_{MH2L} from the graphical value ($10^{0.7}$) is greater than can be attributed to error in K_{ML}. This is because the dissociation of the metal–ligand complex renders the Schwarzenbach method invalid, despite the straight-line fit obtained from data at a single wavelength. For this reason and because all the visible spectral data are used, the values of K_{MHL} and K_{MH2L} from least-squares fit 4 are considered the most reliable and are included as the values we report in Table III.

The electrochemical value of K_{ML} was needed to determine K_{MHL} annd K_{MH2L} because of high correlation. As a control, the value of K_{ML} was recalculated using the new values of 2.28 and 1.36 for log K_{MHL} and log K_{MH2L}; no significant change occurred.

Discussion

There are two advantageous properties of TREN as the ligand backbone that emerge from this study. Protonation of the tertiary amine of TRENDROX enhances the solubility of the ligand over a wider pH range than was observed for MEDROX. More important, the conformational flexibility of TREN seems to afford the optimal encapsulation of the ferric ion by the hydroxamate moieties. In contrast, the rigidly planar benzene unit in BAMTPH[8] (defined in Table I) restrains the coordination of the hydroxamate groups around the ferric ion, resulting in greatly reduced stability of the complex.

Protonation of the tertiary amine of the ferric-TRENDROX complex might seem likely to increase its solubility over a wide range of pH, as in the case of desferrioxamine. However, the orange-red ferric–TRENDROX solution starts to precipitate above pH 3, forming a neutral species. In fact, spectrophotometric data indicate that protonation of the amine is insignificant even down to very low pH, which we ascribe to hydrogen bonding between the amine nitrogen and amide protons. This substantial lowering of the TREN nitrogen atom in other amide derivatives has been seen in some similar catechol derivatives.[22,23] The limited solubility of the ferric complex is probably also diminished by the large hydrophobic *p*-tolyl groups.

Although the overall formation constant is a strong indication of the stability of metal complexes, the value of K_{ML} does not necessarily directly reflect the *relative* binding effectiveness of a ligand at specific conditions. Ligand protonation as well as the number and nature of donor groups involved strongly influences the species formation at various pH. A more direct comparison of relative effectiveness of ligand in metal binding can be achieved by calculating the free hexaaquairon(III) concentrations in a solution at given conditions. The larger the pM value, where pM = −log [Fe(III)], the more effective the ligand will be under these conditions. The pM of TRENDROX (determined at 10 μM total ligand concentration, 1 μM total Fe^{3+} concentration, and pH 7.4) is 27.8, compared to 26.6 for DFO. It is clear that TRENDROX is a very effective chelating agent for ferric ion at physiological pH.

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