Coordination Chemistry of Microbial Iron Transport Compounds. 11.[1] Solution Equilibria and Electrochemistry of Ferric Rhodotorulate Complexes

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Abstract: Rhodotorulic acid (H_2RA) is a dihydroxamic acid which is produced in high yields by yeast as an iron transport agent (siderophore). The stability constants of the ferric complexes of RA have been determined potentiometrically and spectrophotometrically at 25 °C. The predominant species at neutral pH is the dimer, Fe_2RA_3, in which each iron is bound to three hydroxamate groups. Below pH ~3 this complex dissociates into the monomer, $FeRA^+$, in which each iron is bound to two hydroxamates. The formation constants for Fe_2RA_3 and $FeRA^+$ from Fe^{3+} and RA^{2-} are $\log \beta = 62.2$ (1) and 21.99 (6), respectively. The values of the acid dissociation constants are moderately correlated with $pK_{a1} = 8.49$ (3) and $pK_{a2} = 9.44$ (3). The electrochemical behavior of the ferric RA complexes was examined via cyclic voltammetry, and the pH dependence of the redox potentials determined. The reduction of Fe_2RA_3 is reversible and involves one electron per iron. That the two Fe(III) centers (9–10 Å apart) do not affect the redox behavior is consistent with ESR evidence which shows no interaction of the two ions. At pH 7 and millimolar concentration of free ligand, ferrous, and ferric species the formal potential $\mathcal{E}^0 = -0.279$ V vs. NHE. The numerical values of the formation constants of polyhydroxamate siderophores show little if any chelate effect. This observation and its implications are discussed.

Introduction

Low molecular weight microbial iron sequestering agents known as siderophores have been found to be among the strongest chelators of ferric ion known.[2] These natural products have evolved as transport agents and scavengers for iron, an essential element for growth, in a host of bacteria, fungi, and molds.[3–5] Siderophores fall generally into two groups, either hydroxamates or catecholates, both of which form exceedingly stable octahedral, high-spin complexes of iron(III).

Because of their great ability to sequester and hold iron (many are thermodynamically able to remove iron from the mammalian iron transport protein transferrin), some of the siderophores have found use in the treatment of acute iron poisoning and chronic iron poisoning such as occurs in the transfusion therapy of Cooley's anemia.[6,7] The most used clinical agent in such poisoning cases is Desferal (deferriferrioxamine B). This agent binds iron with an overall stability constant of 30.6,[8] and has recently been licensed for medical use in the U.S.A.

Although other factors come into play, it is clear that the stability constant is important in determining which chelates will be useful drugs. The stability constants for several of the siderophores are known, primarily the ferrioxamines and ferrichromes.[2] One of the general types about which little has been known is rhodotorulic acid, isolated from *Rhodotorula pilimanae* and related yeasts (Figure 1).[9] This dihydroxamate diketopiperazine has already been found to be more effective than Desferal as an iron chelating drug and has entered limited clinical trials,[10] although knowledge of even the composition of its iron complex has not been obtained until recently.[1] Owing to its potential usefulness as a drug and in the course of our investigation into the coordination chemistry of this novel siderophore, we have examined the equilibrium chemistry of rhodotorulic acid via potentiometric titration, spectrophotometric titration, and electrochemistry. The results of these investigations are reported here.

Experimental Section

Ferric stock solutions (approximately 0.1 M in 0.1 M HCl) were prepared from ferric chloride hexahydrate or ferric nitrate (0.1 M in 0.1 M HNO_3) and standardized both spectrophotometrically and by ignition to the oxide (900 °C, 12 h).

Rhodotorulic acid was prepared as described by Neilands et al.[9] and recrystallized at least six times before use. The product had IR, NMR, and melting point identical with the reported values. The purity of the ligand was also established by titration with standard KOH.

Potentiometric Titrations. The potentiometric titrations were performed as described by Raymond et al.[11]

Spectroscopic Measurements. Visible absorption spectra were measured using a Cary Model 118 UV–vis spectrophotometer. Solutions were prepared by mixing ligand and metal at molar ratios of 5:1 in individual portions. Small amounts of acid or base titrant were added and the pH and spectra were recorded. The system was kept at 1.0 M ionic strength via addition of KNO_3. In order to determine the equilibrium constant for the reaction

$$Fe^{3+} + H_2L \rightleftharpoons FeL^+ + 2H^+$$

it was necessary to adjust the acidity to pH values near zero decrease the L/M ratio ($[Fe]_T \approx 4 \times 10^{-4}$ M) to 1. At such low values of pH the glass electrode response is erratic and it was necessary to resort to calculations of $[H^+]$ based on total acid and base in the system. In such cases the reactions were carried out in 0.1 or 1 M HCl and the $[H^+]$ was calculated after additions of standard base.

Computational Techniques. A computer program, STBLTY, was used for computation and refinement of acid dissociation and stability constants as previously described.[11]

Electrochemistry. Cyclic voltammograms were recorded using three-electrode circuitry with a hanging mercury drop working electrode, platinum wire auxiliary, and saturated calomel reference electrode (SCE) and are uncorrected for liquid junction potentials. All solutions for electrochemical examination were 1.0 M in KCl and 0.05 M each in potassium phosphate and sodium borate. Triangular waves were generated by the Princeton Applied Research (PAR) 175 programmer in conjunction with the PAR 380 coulometry system, which includes the PAR 173 potentiostat. Controlled potential electrolyses were conducted on a stirred mercury pool. Iron electrodes for potentiometric measurements were prepared by electroplating iron onto a copper wire from a stirred solution of ferrous chloride (28 g/100 mL of 0.01 M HCl) at 70 °C, using an analytically pure iron wire electrode. Potentiometric measurements were made with a Brinkmann pH-102 pH meter in the mV mode.

Results

Ligand Titration. The pK_a of the hydroxamate portion of free rhodotorulic acid was originally reported by Neilands et al.,[9] who found a value of ~9.3 for the pK_a but reported that the titration curve was skewed and mathematically incompatible with a single pK_a. In our examination of the ligand-only titration data, both hydroxamate groups and the deprotonation

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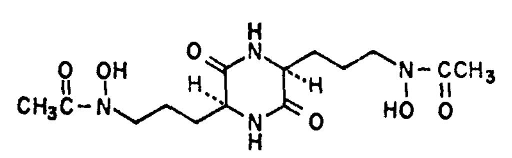


Figure 1. Structure of rhodotorulic acid.

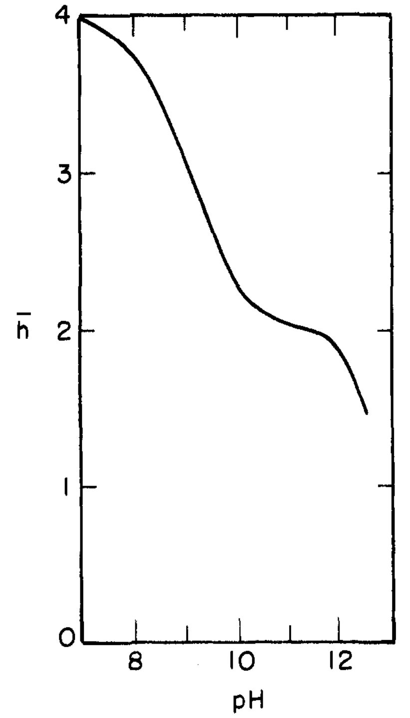


Figure 2. Ligand deprotonation curve of free rhodotorulic acid, with $\bar{h}$ representing average number of protons bound per ligand.

of the ring amide nitrogens at high pH were considered. As expected, the hydroxamate protons titrated in the region between pH 8 and 10. The data could be fitted equally well by assuming that the two hydroxamate protons had similar but slightly different pK_as or that they both had a single pK_a, the average of the two above. We chose the former case and obtained values of 8.49 (3) and 9.44 (3). By examination of Bjerrum[12] plots the deprotonation of the ring amide nitrogens was seen as the pH was raised above 10 (Figure 2). The values calculated for these constants were 12.7 (1) and 13.2 (est); the latter value, an estimate, was not refined. The overall fit of the experimental data to the refined constants is excellent—the goodness of fit is 0.72.[13]

Ferric Rhodotorulate. Potentiometric titration data at several ligand to metal ratios and total metal concentrations revealed a sharp break at 3 equiv of base per mol of iron (Figure 3). This indicates that each iron is being coordinated by three hydroxamate groups with the concomitant release of three protons. The formation curves were surprisingly simple with $\bar{n}$ (the average number of ligands bound per Fe) ~ 1.5 over a wide range of pH values.[11] This confirms our original formulation of the complex as Fe_2RA_3 throughout most of the pH range.[1] The stepwise stability constants of interest, K_1 and K_2, are defined as follows:

$$Fe^{3+} + RA^{2-} \overset{K_1}{\rightleftharpoons} FeRA^{+} \tag{1}$$

$$2FeRA^{+} + RA^{2-} \overset{K_2}{\rightleftharpoons} Fe_2RA_3 \tag{2}$$

Unfortunately it proved to be impossible to refine or deduce log K_1 from the potentiometric data, since $\bar{n}$ never became less than one in the pH range accessible by this technique. In fact the simple 1:1 complex detected spectrophotometrically proved to be stable down to a pH of 0 and below. Alternative ways to determine initial values of this constant were sought for use in calculation of the titration results. It proved possible to determine these spectrophotometrically.

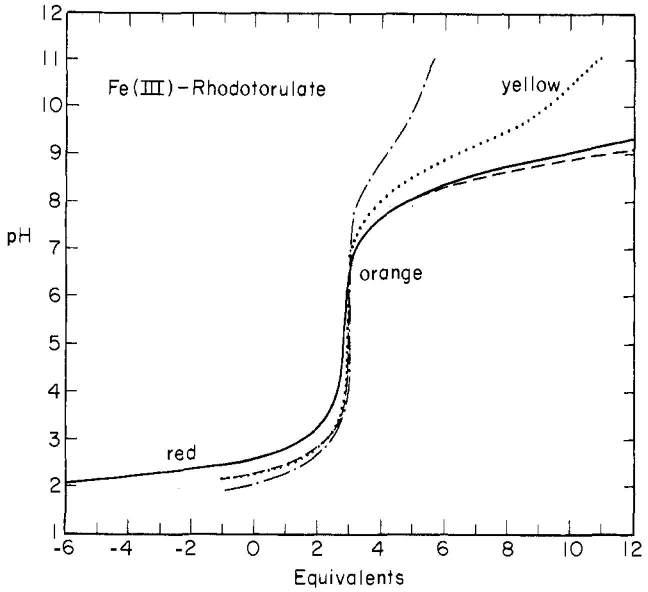


Figure 3. Metal-ligand titration curves for ferric rhodotorulate: (–), 1.0 mM [Fe^{3+}], L/M = 10.0; (- -), 3.7 mM [Fe^{3+}], L/M = 2.6; (– – –), 1.9 mM [Fe^{3+}], L/M = 10.0; (. . . .), 1.9 mM [Fe^{3+}], L/M = 5.2.

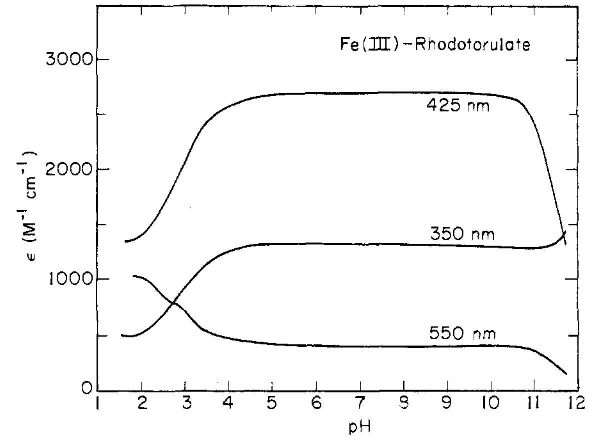


Figure 4. Plots of extinction coefficient of ferric rhodotorulate vs. pH at various wavelengths.

Spectrophotometric Titration. A spectrophotometric titration revealed changes in the visible absorption spectra with pH characteristic of only two species being present in solution. As the pH was lowered from 7.0 to 2.0 the λ_{max} shifted from 425 to 480 nm with an isosbestic point observed at 488 nm. Plotting the extinction coefficient vs. pH (Figure 4), as described by McBryde,[14] revealed two well-separated plateaus between pH 2.0 and 11.0 indicating that K_1 and K_2 differ sufficiently to allow their determination. Another plateau was found between pH 0 and 2 which allowed us to determine K_1. For the monomer-dimer equilibrium, eq 2, the data were analyzed for two wavelengths, 550 and 425 nm, at several pH values each. For the free metal-monomer equilibrium, eq 1, only a single wavelength, 480 nm, was analyzed at seven pH values.

The extinction coefficients for the pure monomer and dimer were found from the extinction coefficient vs. pH plots. Above pH 4.0 all the complex exists as the dimer (ϵ_{425} 2700 L cm^{-1}

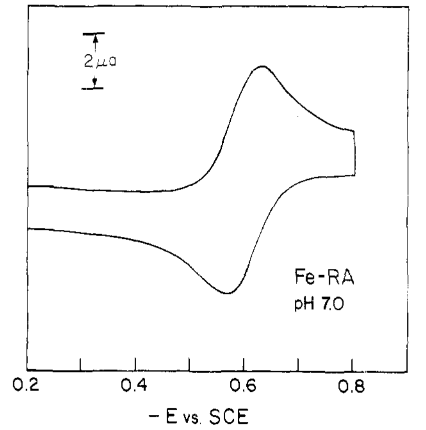


Figure 5. Cyclic voltammogram of $Fe_2^{III}RA_3$ on a hanging mercury drop electrode. [Fe(III)] = 1 mM, [RA] = 20 mM in 1 M KCl with 0.05 M potassium phosphate, 0.05 M sodium borate buffer, pH 7.0. Scan rate 100 mV/s.

mol^{-1}). Between pH 1.0 and 2.0 all the iron is present as the monomer (ϵ_{480} 1750 L cm^{-1} mol^{-1}). It was assumed that the extinction coefficient for free iron was zero in the wavelengths of interest. It was also necessary to correct for the equilibrium

$$Fe^{3+} + Cl^- \overset{K_1}{\rightleftharpoons} FeCl^{2+}$$

Since iron binding by chloride in 1 M HCl becomes significant, log K_1' = 0.63.[15]

The results for K_1 were obtained a second time at 0.1 M ionic strength in 0.1 M $HClO_4$ by reducing the concentration of both metal and ligand. The value of log K_1 at 1 M ionic strength was 21.55 (8) and at 0.1 M ionic strengtth 21.99 (6). The value of log K_2 at 1 M ionic strength was determined to be 19.18 (3). The overall stability constants β_1 and $\beta_{3/2}$ are defined as follows:

$$Fe^{3+} + RA^{2-} \overset{\beta_1}{\rightleftharpoons} FeRA^+ \quad (3)$$

$$2Fe^{3+} + 3RA^{2-} \overset{\beta_{3/2}}{\rightleftharpoons} Fe_2RA_3 \quad (4)$$

Thus $\beta_1 = K_1$ and $\beta_{3/2} = K_1{}^2K_2$. This gives log β_1 = 21.55 (8) and log $\beta_{3/2}$ = 62.28 (12). The use of β_1 (at μ = 0.1 M) as a fixed parameter made possible refinement of $\beta_{3/2}$, using the STBLTY program, to yield a value of 62.2 (1). Using these two constants the overall fit of the potentiometric titration data with the calculated values was excellent, with a final goodness of fit of 1.34, which confirms our formulation of the $FeRA \rightleftharpoons Fe_2RA_3$ equilibrium.

Electrochemistry. Cyclic voltammetric (CV) examination of Fe_2RA_3 solutions on a hanging mercury drop electrode yielded reversible or quasi-reversible waves over the pH range 5.5-10.5 for an RA:Fe(III) ratio of 20:1 (Figure 5). The half-wave potential in this system was observed to become more negative with increasing pH until a limiting value of −656 mV vs. SCE was approached asymptotically (Figure 6). At lower ligand to metal ratios a sharp spike at −0.33 V was observed on anodic scans following the reduction wave. The spike, which had the sharpness characteristic of an adsorption process, decreased in current with increasing RA concentration, with concomitant increase in the reversible anodic wave. At 20:1 RA:Fe ratio no trace of the adsorption wave was present over the entire pH range.

Preparative reduction of Fe_2RA_3 on a stirred mercury pool electrode yielded a colorless solution of the ferrous complex, which could be reversibly reoxidized to the original orange solution of the ferric complex, and in which an iron wire exhibited a formal potential (corrected to unit concentrations of RA dianion and Fe_2RA_3) of −663 (10) mV vs. the normal hydrogen electrode (NHE).

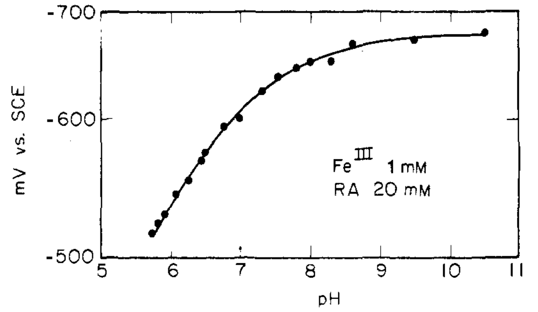


Figure 6. The pH dependence of $Fe_2^{III}RA_3$ reduction potential by cyclic voltammetry on a hanging mercury drop electrode. [Fe(III)] = 1 mM, [RA] = 20 mM in 1 M KCl, buffered with 0.05 M potassium phosphate, 0.05 M sodium borate. The solid line is the reduction potential calculated from the equilibrium constants reported here.

Discussion

Electrochemistry. The reduction waves observed for Fe_2RA_3 are consistent with simple reversible one-electron reduction of the ferric ion to the ferrous state. The preparative electrochemical reduction of Fe_2RA_3 to colorless solutions (lacking the characteristic ligand to metal charge transfer band of the ferric complex) demonstrates that simple reduction of the ferric ion is occurring. At 20:1 RA:Fe ratio the cyclic voltammogram exhibits a peak separation of 60 mV at pH 8.3, increasing to approximately 75 mV at the limits of the pH range 5.7-10.5. For an electrochemically reversible couple at 25 °C the predicted separation is $59/n$ mV, where n is the number of electrons transferred.[16] Since the separation was not observed to be scan rate dependent, we attribute the observed behavior to that of an electrochemically reversible one-electron couple. For such couples the observed half-wave potential may be identified with the thermodynamic reduction potential, to within an excellent approximation. It has been claimed previously that the cyclic voltammogram of a dimer ($n_1 = n_2 = 1$) should exhibit a 42-mV peak separation when the two electroactive centers have the same redox potential.[17] However, Anson and co-workers have recently shown that the analysis leading to the above prediction neglects statistical factors associated with the indistinguishability of electroactive sites. Inclusion of these considerations indicates that a peak separation of 60 mV is expected, just as in the single site case, but with twofold greater current.[18] Our CV results are in complete accord with the predictions of Anson and co-workers. Evidently the Fe(III) ions are sufficiently separated (molecular models show that the separation is 9-10 Å) that they do not interact and are reduced independently. In support of this explanation, our EPR studies revealed no difference between the spectrum of Fe_2RA_3 and $FeAlRA_3$ (produced by addition of a very small quantity of a ferric salt to a solution of Al_2RA_3).

Decreasing the pH below 7 causes the observed half-wave potential to become more positive at a rate of approximately 56 mV/pH unit. This observation is consistent with protonation of the reduction product (the ferrous complex) by one proton per electron added. Estimating the stepwise formation constants of the ferrous RA complex by those of acetohydroxamate reveals that the predominant form of the ferrous complex changes from the $Fe_2^{II}RA_3{}^{2-}$ dimer at high pH to the monomeric, "head to tail" $Fe^{II}RA$ complex at pH ~8 (Figure 7). Taking account of the reaction

$$Fe_2^{II}RA_3{}^{2-} \rightleftharpoons 2Fe^{II}RA + RA^{2-}$$

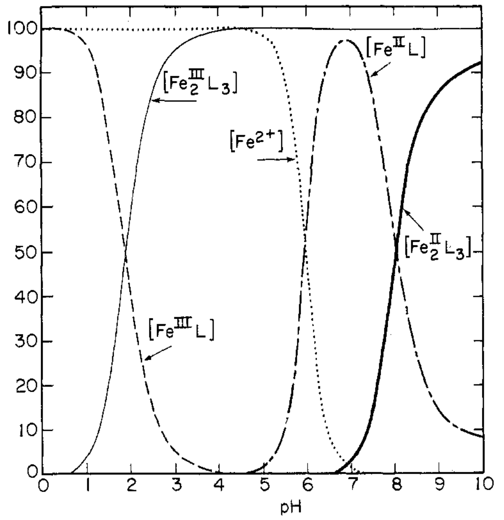


Figure 7. Distribution curve of the various species present at equilibrium following 50% reduction, as a function of pH ($[Fe^{2+}]_T = 5 \times 10^{-4}$ M, $[Fe^{3+}]_T = 5 \times 10^{-4}$ M, $[RA]_T = 20 \times 10^{-3}$ M).

Table I. pH Dependence of Ferric Rhodotorulate Cyclic Voltammetry[a]

pH	E_f^{calcd} (vs. SCE)	E_f^{obsd} (vs. SCE)	ΔE[b]
5.76	495	517	73
5.91	515	529	78
6.06	532	545	70
6.24	549	556	72
6.44	564	571	67
6.78	586	593	65
7.01	599	601	68
7.32	617	618	65
7.54	627	628	65
7.83	639	635	70
7.97	643	638	65
8.30	649	640	60
8.62	652	652	67
9.50	655	656	68
10.49	655	659	73

[a] [Fe(III)] = 1 mM; [RA] = 20 mM; scan rate 100 mV/s. [b] Peak separation, mV.

one can calculate the free ferric and ferrous ion concentrations from the distribution curves in Figure 7. By employing the Nernst equation, the pH dependence of the $Fe_2{}^{III}RA_3$ reduction potential can be calculated from the ratio of free Fe(III) to free Fe(II). The results of this calculation are summarized in Table I, with the CV data.

The limiting high pH reduction potential of −659 mV vs. SCE (−415 mV vs. NHE) corresponds to the reduction of the ferric dimer to the ferrous dimer, $Fe_2{}^{II}RA_3{}^{2-}$. This potential is related to the stability constants K_{ox} and K_{red} of the ferric and ferrous forms by

$$E_{1/2}{}^{complex} - E_{1/2}{}^{aquo} = \frac{59}{2} \log \frac{K_{ox}}{K_{red}}$$

Given K_{ox} (= $\beta_{3/2}$ from the titration results for Fe^{3+}), K_{red} (= $\beta_{3/2}$ for Fe^{2+}) is calculated to be $10^{21.1}$.

From the formal potential of an iron wire immersed in a ferrous-RA solution, an independent estimate of the stability constant of the ferric dimer can be made from the Fe(III)/Fe(II) potentials. With suitable signs and coefficients one obtains the free energy change for

$$2Fe(III) + 3RA^{2-} \rightleftharpoons Fe_3{}^{III}RA_3$$

Table II. Overall Stability Constants of Hydroxamate Siderophores

siderophores	log β_3	ref
ferrioxamine B (Desferal)	30.6	1
ferrioxamine E	32.5	1
ferrioxamine D_1	30.8	1
ferrichrome	29.1	1
ferrichrome A	32.0[d]	19
ferrichrysin	30.0	1
mycobactin[a]	31.5[d]	20
rhodotorulic acid	62.3 (31.2)[c]	this work
enterobactin[b]	52.0	21

[a] Mycobactin contains two hydroxamate groups; the third coordinating group is a phenolic hydroxyl and the nitrogen of an oxazoline ring. The stability constant is an estimate from the data given by Snow. [b] Enterobactin is not a hydroxamate siderophore but rather a catecholate; it is included as a comparison. [c] Estimate; see text. [d] Estimate.

This potential, −663 mV vs. NHE, was not highly reproducible, but gives a calculated value for log K_{ox} of ~57, which is consistent with the more precise potentiometric result of 62.2.

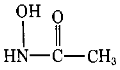
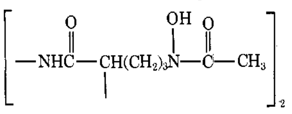
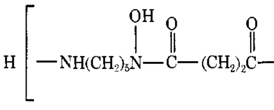
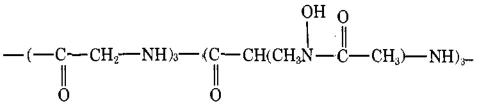
Equilibria. Using a variety of equilibrium techniques we have been able to determine the stepwise and overall stability constants for the formation of iron(III) rhodotorulic acid complexes. As expected this hydroxamate siderophore forms extremely stable complexes with ferric ion and only very weakly binds ferrous ion (as determined electrochemically). This study has confirmed that the predominant species at neutral pH can be formulated as Fe_2RA_3. This dimer exists over the range from pH 3 (where the monomer becomes predominant) to pH 12 (where iron precipitates as the hydroxide), and thus it is this complex that acts as the iron transport agent in the yeast *Rhodotorula pillimanae*.

The monomer, $FeRA^+$, is extremely stable toward acid dissociation; it is only about 50% dissociated at pH 0 when the ligand concentration is millimolar. The magnitude of log K_1, 21.55, indicates that both hydroxamate moieties in the single RA molecule are attached to the iron. For acetohydroxamate, log K_1 = 11.42, log K_2 = 9.68, and log K_3 = 7.23.[2] The value we report as log K_1 for RA is substantially higher than what one would expect for a single hydroxamate group attached to Fe(III) (expected to be ~10–12) but is exactly the value expected for two hydroxamate moieties attached to iron (β_2 for acetohydroxamic acid is 21.10). This supports the characterization of the monomeric FeRA complex as a monocation in which both hydroxamate residues of one RA bind to iron.[1] We were unable to observe a stepwise dissociation of the monomer to free iron in any experiments, although we did observe a slight pH dependence for the calculated values of K_1.

By way of comparison, several of the known values of the overall stability constants of the siderophores are listed in Table II. It is impossible to compare directly the values for rhodotorulic acid with those of the other siderophores, since the equilibrium constants have different units. However, a simple comparison is possible by determining the overall constant "per iron" for which log β is 1/2 the value of $\beta_{3/2}$ (vide infra).

Some General Comments on Hydroxamate Siderophore Equilibria. Table III summarizes equilibrium data for mono-, di-, and trihydroxamic acids. In Table III each hydroxamate functional group is represented as L, such that a dihydroxamate (e.g., rhodotorulic acid) is designated as H_2LL, while a trihydroxamic acid (for example, ferrichrome) is designated H_3LLL. Two interesting features follow: (1) The pK_a values are approximately the same for the first, second, or third proton being removed. (2) The magnitude of the formation constant for an Fe(hydroxamate)$_n$ complex is the same whether the n hydroxamate groups are separate or are part of one molecule.

Table III. A Comparison of Relative Stepwise Formation Constants for Hydroxamate Ligands

compd	pK_as	log β values FeL	 FeL_2	 FeL_3	ref
acetohydroxamic acid (HL)  $\overset{\mathrm{OH}}{\mathrm{HN}}-\overset{\mathrm{O}}{\overset{\Vert}{\mathrm{C}}}-\mathrm{CH_3}$	9.35	11.42	21.1	28.3	2
rhodotorulic acid (H$_2$LL)  $\left[-\mathrm{NH}\overset{\mathrm{O}}{\overset{\Vert}{\mathrm{C}}}-\mathrm{CH(CH_2)_3}\overset{\mathrm{OH}}{\mathrm{N}}-\overset{\mathrm{O}}{\overset{\Vert}{\mathrm{C}}}-\mathrm{CH_3}\right]_2$	8.49^a 9.44		21.6	31.1^b	this work
ferrioxamine B (H$_3$LLL)  $\mathrm{H}\left[-\mathrm{NH(CH_2)_5}\overset{\mathrm{OH}}{\mathrm{N}}-\overset{\mathrm{O}}{\overset{\Vert}{\mathrm{C}}}-\mathrm{(CH_2)_2}\overset{\mathrm{O}}{\overset{\Vert}{\mathrm{C}}}-\right]_3\mathrm{CH_3}$	8.39 9.03 9.70		21.8	30.6	22
ferrichrome (H$_3$LLL)  $-(-\underset{\mathrm{O}}{\underset{\Vert}{\mathrm{C}}}-\mathrm{CH_2}-\mathrm{NH})_3-(\underset{\mathrm{O}}{\underset{\Vert}{\mathrm{C}}}-\mathrm{CH(CH_3}\overset{\mathrm{OH}}{\mathrm{N}}-\overset{\mathrm{O}}{\overset{\Vert}{\mathrm{C}}}-\mathrm{CH_3})-\mathrm{NH})_3-$	8.11 9.00 9.83		20.7	29.1	8

^{a} Correlation coefficient, 0.66. ^{b} The formation constant for $\frac{1}{2}Fe_2RA_3$ ($= \beta_{3/2}{}^{1/2}$).

These two statements are equivalent to saying that the free energy *per hydroxamate group* is constant for these polyhydroxamate chelating agents and their ferric complexes *at a standard state of 1 M.* This results in the complete absence of any apparent "chelate effect" in comparing formation constants for the poly- or monohydroxamate ligands.[23] The following thermodynamic cycle summarizes these results for the formation of tris complexes. Similar cycles for the formation of mono or bis complexes would have the same form, with ΔG for interconversion of related species also ~ 0:

$$\begin{tikzcd}
{}^3/_2H_2LL \arrow[r, leftrightharpoons] \arrow[d, Leftrightarrow, "\Delta G \approx 0"] & 3H^+ + {}^3/_2LL^{2-} \arrow[r, leftrightharpoons, "+Fe^{3+}"] \arrow[d, Leftrightarrow, "\Delta G \approx 0"] & {}^1/_2Fe_2(LL)_3 \arrow[d, Leftrightarrow, "\Delta G \approx 0"] \\
3HL \arrow[r, leftrightharpoons] \arrow[d, Leftrightarrow, "\Delta G \approx 0"] & 3H^+ + 3L \arrow[r, leftrightharpoons, "+Fe^{3+}"] \arrow[d, Leftrightarrow, "\Delta G \approx 0"] & FeL_3 \arrow[d, Leftrightarrow, "\Delta G \approx 0"] \\
H_3LLL \arrow[r, leftrightharpoons] & 3H^+ + LLL \arrow[r, leftrightharpoons, "+Fe^{3+}"] & Fe(LLL)
\end{tikzcd}$$

The small change in pK_a values with successive deprotonation keeps the equilibrium constants for ligand acid disproportionation within an order of magnitude of unity, e.g.

$$2HLL^- \rightleftharpoons H_2LL + LL^{2-} \qquad K \approx 1$$

Since the formation constant for ferric hydroxamate complexes depends only on the number of hydroxamate ligands bound to Fe and not on whether they are independent ligands or are part of a larger multichelate molecule, equilibria such as

$$2Fe(HLL)_3 \rightleftharpoons Fe_2(LL)_3 + 3H_2LL \qquad K \approx 1$$

also have equilibrium constants near unity. In the above case, when both $[Fe]_{total}$ and $[H_2LL]$ are about 10^{-3} M, the ratio $[Fe_2(LL)_3]/[Fe(HLL)_3]$ is about 10^3, since the reaction at low concentrations is strongly driven to the right. Thus for rhodotorulic acid: (1) *The only tris(hydroxamate) ferric complex present in significant concentration is the dimer* Fe_2RA_3. The lower the concentration of all species the more rigorous this conclusion becomes.

Similar considerations apply to bis(hydroxamate) complexes, such as a 2:1 complex, $Fe(HLL)_2$, or a bis complex dimer such as $Fe_2(LL)_2$:

$$Fe(HLL)_2 \rightleftharpoons Fe(LL) + H_2LL \qquad K \approx 1$$

$$Fe_2(LL)_2 \rightleftharpoons 2Fe(LL) \qquad K \approx 1$$

Both these reactions are strongly driven to the right at low concentrations and so for rhodotorulic acid: (2) *The only bis(hydroxamate) ferric complex of significance is the monomer Fe(RA) in which both hydroxamate groups are coordinated.*

Finally, examination of mono(hydroxamate) species and reactions such as the disproportionation

$$2Fe(HLL) \rightleftharpoons Fe(LL) + Fe^{3+} + H_2LL$$

$$K \approx \sqrt{K_2/K_1} \approx 1/10$$

is only weakly driven to the right, nevertheless. (3) *At low concentrations of rhodotorulic acid, no stable monohydroxamate exists in significant concentration.*

The phenomenal production of rhodotorulic acid by *R. pilimanae* can now be understood. In competition with other siderophores (e.g., ferrichrome), RA is only effective at high concentration. For typical physiological concentrations of RA and ferrichrome (1.5×10^{-2} and 5.5×10^{-5} M, respectively) and 10^{-6} M total iron, the RA complex is favored by a factor of 2-4. Thus the high concentrations of RA produced are needed to compete effectively for iron. The biosynthesis of RA is relatively simple, and a large output of metabolic energy is not required to achieve this end.

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$$\sqrt{\left[\sum \left(\frac{\Delta}{\sigma}\right)^2 (n_{\mathrm{obs}} - n_{\mathrm{var}})\right]}$$

where Δ is the observed deviation between observed and calculated values, σ is the standard deviation of the observation, n_{obs} is the number of independent observations, and n_{var} is the number of variables optimized in the refinement.
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Preparation and Characterization of Organoiron Secondary Alkoxycarbene Complexes

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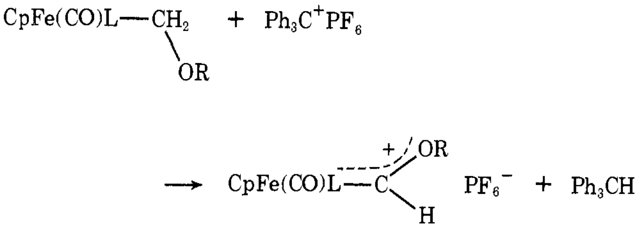
Abstract: A series of organoiron secondary alkoxycarbene salts, $CpFe(CO)L\{C(OR)H\}^+ PF_6^-$ (L = CO, Ph_3P; R = Me, Et) (**2a–d**) has been synthesized by hydride abstraction from the requisite alkoxymethyl complexes (**1a–d**) with a trityl salt. The dealkylation of **2** with iodide was investigated as a synthetic approach to η^1-formyl complexes, CpFe(CO)L(CHO) (**3**). One equivalent of iodide dealkylated **2a–c** to mixtures of $CpFe(CO)_2L^+$ (**6a,b**), **1a–c**, and alkyl iodide, by a reaction sequence involving hydride transfer from a transient **3** to **2**. The hydride acceptor properties of **2** were also investigated by their reactions with borohydride. The observed reduction of coordinated alkoxycarbenes to alkoxymethyl and methyl ligands is of possible relevance to the catalytic fixation of carbon monoxide (e.g., Fischer–Tropsch processes) to hydrocarbons, since surface-bound secondary hydroxycarbene ligands have been postulated in forming the initial C–H bonds.

The Fischer–Tropsch synthesis and related processes[1] for fixing CO/H_2 mixtures to methane, methanol, and higher homologues with transition metal containing heterogeneous catalysts are regaining importance as a major area of catalysis research.[2] Impetus for this research activity derives from the possible use of coal, a source of these CO/H_2 mixtures of "synthesis gas", as a future source of petrochemicals. Although little is known about the mechanism of CO fixation, plausible intermediates in forming initial C–H bonds often are represented as surface-bound secondary hydroxycarbene [M=CH(OH)] or formyl [M-C(O)H] ligands.

Development of homogeneous analogues to this synthesis and ascertaining the role of formyl and secondary hydroxycarbene complexes in the fixation of CO correspond to recent research directions. Homogeneous hydrogenation of coordinated CO with mononuclear organometallic compounds of early transition metals may involve transitory formyl intermediates.[3,4] Carbonyl ligands are also hydrogenated stoichiometrically with borohydride reagents. Thus, cationic carbonyl compounds reduce to neutral methyl[5a] and hydroxymethyl[5b] complexes, and neutral carbonyl compounds reduce to anionic η^1-formyl[6] complexes. Neutral η^1-formyl complexes may be intermediates in the former borohydride reactions, but such compounds[7] have not been detected as a result of intermolecular hydride transfer (or intramolecular hydride ligand transfer)[8] to a coordinated carbonyl. Secondary hydroxy- or alkoxycarbene complexes have not been reported, although many tertiary examples exist.[9] Information on the reactions of coordinated secondary hydroxy- and alkoxycarbene ligands clearly would be useful in probing the reaction paths available during CO fixation with homogeneous or heterogeneous catalysts.

We now report preparation of secondary organoiron alkoxycarbene complexes and present preliminary observations of their reactivity that may be relevant to CO fixation. Previous workers demonstrated that alkoxide abstraction from the methoxymethyl iron complexes, $CpFe(CO)L(CH_2OCH_3)$ (Cp = η^5-C_5H_5), generated transient methylene iron compounds, $CpFe(CO)L(CH_2)^+$ (L = CO, Ph_3P).[10,11] We find that $Ph_3C^+PF_6^-$ abstracts hydride quantitatively from alkoxymethyl iron complexes (**1a–d**) in methylene chloride to give the alkoxycarbene salts (**2a–d**).[12,13]



$$CpFe(CO)L{-}CH_2(OR) + Ph_3C^+PF_6 \longrightarrow [CpFe(CO)L{-}C(OR)(H)]^+ PF_6^- + Ph_3CH$$

2

a, L = CO; R = Me **b**, R = Et
c, L = Ph_3P; R = Me **d**, R = Et

All four products were isolated at room temperature in 80–90% yields as stable, yellowish solids, although **2a,b** were hydrolyzed slowly in air. Spectroscopic data, particularly the diagnostic ^1H NMR carbene proton absorptions at low downfield positions,[9a,11] and analytical evidence agree with