Copper Complexation by 3-Hydroxypyridin-4-one Iron Chelators: Structural and Iron Competition Studies

Ali El-Jammal,[†] P. Lynne Howell,[‡] Mary A. Turner,[‡] Naiyin Li,[§] and Douglas M. Templeton*[,†,‡]

Department of Clinical Biochemistry, University of Toronto, 100 College Street, Toronto, Canada M5G 1L5, Department of Biochemistry Research, Hospital for Sick Children, 555 University Avenue, Toronto, Canada M5G 1X8, and Medical Foundation of Buffalo, Incorporated, 73 High Street, Buffalo, New York 14203

Received September 28, 1993[•]

Clinical trials of 1,2-dimethyl-3-hydroxypyridin-4-one (1) as an orally available iron chelator are presently underway in several centers. Discrepant reports of toxicity in human and animal studies have stimulated debate on the role of iron status and the availability of iron for chelation relative to other essential elements like copper in determining the clinical effects of 1. Therefore, we investigated the ability of 1, its 1,2-diethyl analog 2, and their iron chelates to complex copper. Both compounds formed tetracoordinate 2:1 Cu(II) complexes which X-ray structure analysis showed to be planar and coordinated through the oxygen atoms of the hydroxy ketone functionality. Potentiometric analysis revealed that these complexes dominated at physiological pH, although between pH 6 and 7 approximately equal amounts of the mono and bis complexes of Cu with 1 were present at equilibrium. Comparing the stepwise formation constants deduced from the stability constants of these complexes (log β_2 = 21.7 ± 0.8 (1) and 20.2 ± 2.0 (2)) with those of their Fe(III) complexes (Motekaitis, R. J.; Martell, A. E. *Inorg. Chim. Acta* **1991**, *183*, 71-80) leads to a prediction of insignificant copper complexation when equimolar iron is present and dissociation products are thermodynamically unimportant. However, displacement of Fe^{3+} occurred from both complexes with stoichiometric amounts of Cu^{2+}, implicating the participation of metal hydrolysis products in the equilibria. We conclude that Cu(II) complexes of the 3-hydroxypyridin-4-one chelators are stable under physiological conditions and that copper can effect displacement of iron by these agents under circumstances where hydrolysis of the metals is important.

Introduction

Iron chelation therapy for Fe-loading anemias presently relies on desferrioxamine, the only chelating agent approved for this purpose. The need to administer desferrioxamine by continuous parenteral infusion, together with its high cost,[1] has stimulated interest in developing orally available Fe chelators.[2,3] The 3-hydroxypyridin-4-one derivatives, introduced by Hider and co-workers,[4,5] have come to clinical trial in several centers and have been found effective in enhancing urinary Fe excretion in overloaded patients.[6-9] We have shown that the 1,2-dimethyl derivative 1 is capable of normalizing tissue Fe stores in certain patients.[10] The apparent clinical success of 1 has prompted many investigations of its mode of action[11,12] and its toxicity and efficacy in comparison to those of desferrioxamine.[6,13-15] A number of other derivatives of 3-hydroxypyridin-4-one have also been synthesized[16,17] and tested for their Fe-binding properties[16,18-20] and pharmacological behavior,[21] and several of their metabolites have been characterized.[22,23]

Studies on the coordination chemistry of these chelators have focused on their complexes with some group III metals, mainly with Fe,[24] Al and Ga,[25,26] and In.[27] Crystallographic parameters of these complexes have been determined,[24-27] and their stability constants have been investigated by potentiometric titration.[28-32] Although 3-hydroxypyridin-4-ones posess a high affinity for Fe(III), these bidentate ligands are not Fe-selective chelators since they bind other group III metals, albeit with affinities lower than that for Fe. Moreover, these chelators bind divalent transition metals like Cu and Zn.[3]

Recent discussions over the interpretation of toxicological data with 1[33-37] are presently complicating the development of these promising agents. It is not clear if the reported adverse effects—which include thymic atrophy and marrow suppression—are inherent in the hydroxypyridinones and (or) their Fe chelates, result from the strategic sequestration of Fe from sensitive sites, or reflect the chelation of other essential elements. Previous studies with related structures have indicated interactions with Cu(II),[38,39] and exchangeable Cu is present in biological fluids in concentrations[40] significant with respect to non-transferrin-bound Fe[41] and therapeutic concentrations of 1. Therefore, we have investigated the ability of 3-hydroxypyridin-4-ones to form stable complexes with Cu(II) and studied the competition of Cu(II) and Fe(III) for these ligands. We report here the solution and crystal structurtes of the Cu complexes and demonstrate that Cu at equimolar concentrations displaces Fe from its hydroxypyridinone coordination sites in buffered aqueous solution, probably because of the greater stability of Fe hydrolysis products relative to those of Cu.

Results

(a) Potentiometric Analysis. Titration of 1 showed two pK_a values of 3.84 ± 0.02 and 9.86 ± 0.07, in good agreement with those determined voltammetrically[42] and close to the values reported previously.[28,29] Compound 2 had comparable pK_a values of 3.92 ± 0.11 and 10.09 ± 0.04. The stability constants β for the Cu(II) complexes of both ligands are given in Table 1. Species are designated by the set of stoichiometric coefficients (*mhl*) for the

* Correspondence to: D. M. Templeton, Dept. of Clinical Biochemistry, University of Toronto, 100 College St., Toronto, Canada M5G 1L5. Tel: 416-978-3972. Fax: 416-978-5650.
† University of Toronto.
‡ Hospital for Sick Children.
§ Medical Foundation of Buffalo.
• Abstract published in *Advance ACS Abstracts*, January 15, 1994.

0022-2623/94/1837-0461$04.50/0

Table 1. Stability Constants of the Cu(II) Systems with Compounds 1 and 2

species (*mhl*)	log β ± sd	
	compound 1	compound **2**
101	11.5 ± 1.5	13.2 ± 0.2
102	21.7 ± 0.8	20.2 ± 2.0
103	27.3 ± 2.4	22.8 ± 0.6
111	12.9 ± 0.3	12.9 ± 0.2
122	27.7 ± 0.3	28.9 ± 0.9
1-21	-4.2 ± 2.1	-2.7 ± 0.3
1-22	1.5 ± 3.9	-0.40 ± 0.29
202	27.7 ± 1.1	28.6 ± 0.2
222	32.8 ± 0.4	32.6 ± 0.1
2-22	16.6 ± 1.6	18.7 ± 1.5
2-42	-2.8 ± 4.4	-0.40 ± 0.30
2-44	7.6 ± 5.9	4.4 ± 0.9

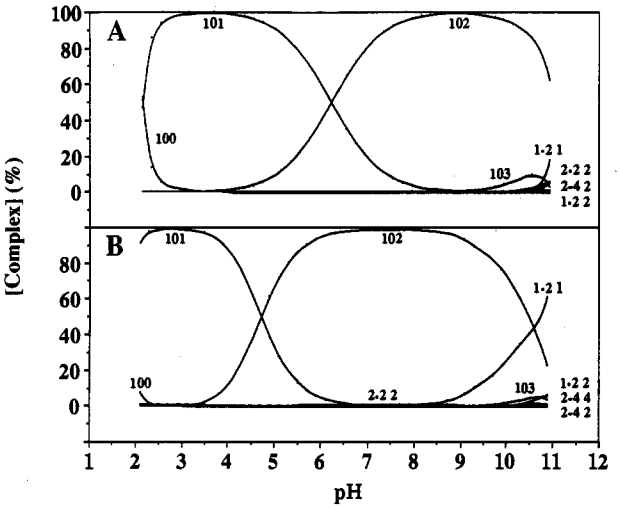


Figure 1. Species distribution of the Cu(II)–compound 1 system (A) and the Cu(II)–compound **2** system (B) as a function of pH. Conditions are given in the Experimental Section. [Complex] refers to the total Cu concentration for all species together. Species are designated (*mhl*) referring to the stoichiometric coefficients of metal, proton, and ligand, with 100 therefore denoting free (aquated) Cu^{2+}.

complex $Cu_mH_h(Ligand)_l$. The unprotonated species 101, 102, and 103 alone gave a squared residual of 7×10^{-7} when the ligand was 1. Inclusion of the protonated species 111, 122, and 133 did not improve the fit. Introducing Cu(II) hdyroxo species, with or without the protonated species, improved the fit and greatly diminished the contribution of the 103 complex at alkaline pH. Mixed ligand–hydroxo complexes or binuclear species were accomodated in the data modeling but did not significantly improve the fit. Their inclusion in the species distribution profile resulted in a very minor contribution. Identical reasoning describes the compound **2**–Cu(II) system, which has a squared residual of 4×10^{-7} when 101, 102, and 103 are considered alone. The species distributions for both systems, based on the best set of stability constants for the deduced species, are shown in Figure 1. The 101 complexes grow in below pH 2 and dominate up to about pH 5, from whence the 102 complexes predominate until very alkaline pH. Numerous minor species, although chemically feasible and accomodated by the data set, nevertheless make a negligible contribution to the Cu speciation.

(b) Crystal Structures. Figure 2 shows ORTEP diagrams for 1 and **2** and gives the atomic numbering scheme used to report the crystallographic data (see the supplementary material). During the X-ray analysis of both complexes, it was found that the copper ion sits at

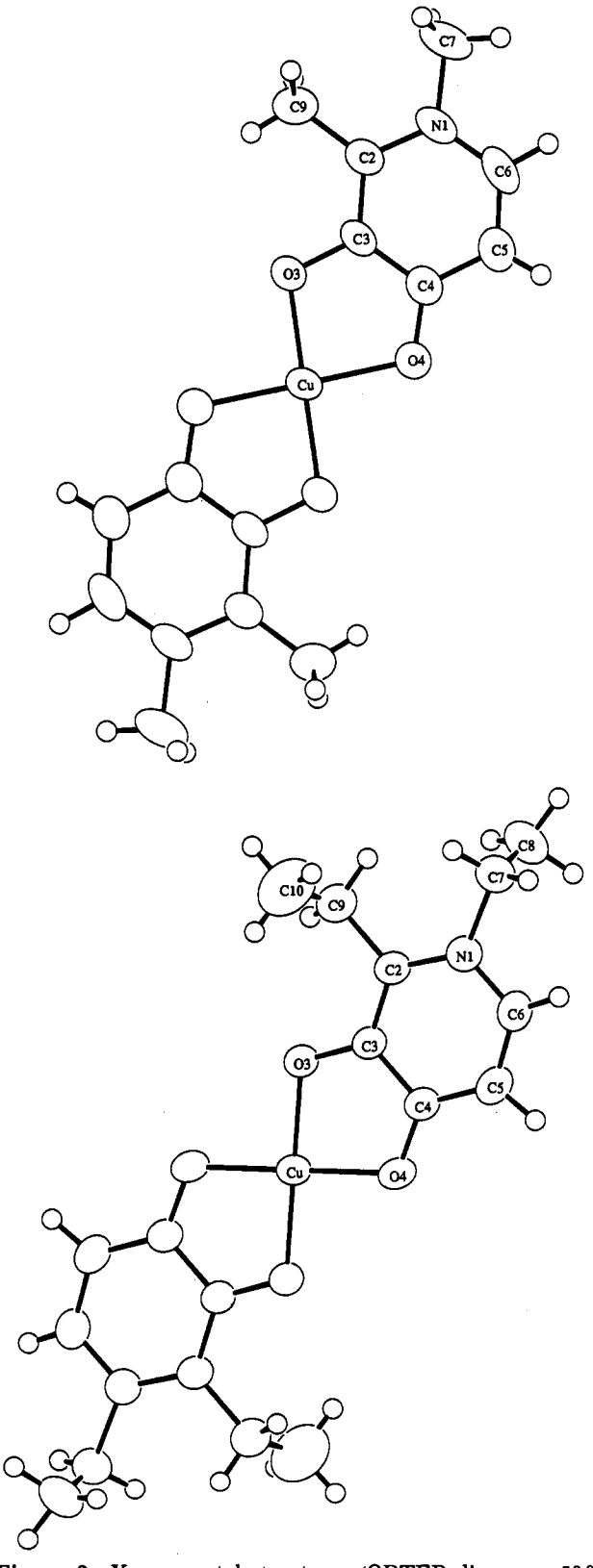


Figure 2. X-ray crystal structures (ORTEP diagrams, 50% probability elipsoids) for (top) 1 and (bottom) **2** showing the atom-numbering scheme used for reporting the crystallographic data. Hydrogen atoms are represented by their spherical isotropic temperature factor.

a special crystallographic position in the unit cell and both complexes are symmetrical about the copper. Least-squares plane calculations have been performed for the copper, ligand ring, and substitutent atoms. Both structures are very nearly planar, mean deviations from the plane being 0.0233 Å for 1 and 0.0231 Å for **2**. Comparison

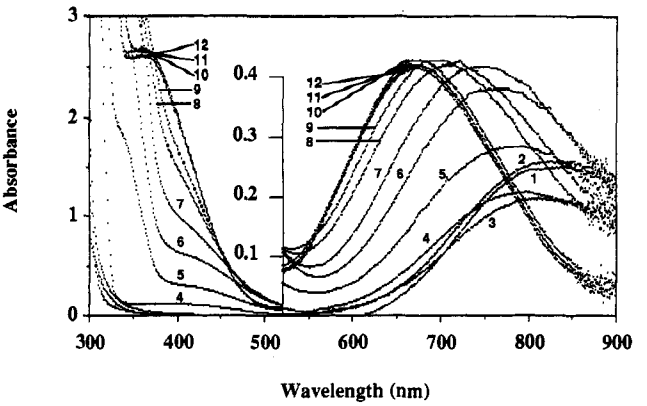


Figure 3. Dependence on pH of the spectral properties of the Cu(II)–compound 1 system. T 25 °C, μ = 0.15 M NaCl, $[Cu]_{total}$ = 17.23 mM, [1] = 34.46 mM. Spectra 2–12 are at pH 0.53, 1.20, 2.03, 2.20, 2.57, 3.00, 3.49, 3.74, 5.57, 7.85, and 11.21, respectively. Spectrum 1 is of a 17.23 M solution of $CuCl_2$ in the absence of ligand at pH 0.67.

of these complexes with 1 ligated to Fe^{3+} [24] shows no significant difference in the bond lengths and angles of the ligand.

(c) Spectrophotometric Analysis. One objective of this study was to investigate the effects of the presence of aqueous Cu(II) on Fe(III) coordination by the hydroxypyridinone Fe chelators. Because of the prohibitive complexity of analysis of mixed-metal systems by either potentiometric or electrochemical methods, we have investigated the electronic spectra of the systems defined structurally above as a useful basis for analysis of the perturbations caused by Fe–Cu mixtures (see below).

Solutions prepared by mixing 1 and Cu in stoichiometric ratios gave identical spectra to those obtained by dissolving single crystals of the complex. At pH less than 1, the spectrum of hexaquo-Cu(II) is obtained in the presence of ligand, but coordination starts as early as pH 1.2 and is considerable by pH 2 as seen by the appearance of a shoulder at 380 nm (Figure 3). The extinction coefficient of this absorption is about an order of magnitude higher than the electronic d–d transitions seen in the visible, consistent with its nature as a charge-transfer band.[43] It increases in intensity until the first ligand pK_a is passed; after pH 4, there is no further change in this region of the spectum. The visible absorptions are also unchanging above pH 4, consistent with complete complexation of Cu(II). Comparing these spectra with the pH dependence of the species distribution (Figure 1) indicates that the spectral features in the visible range (λ_{max} 675 and 750 nm) arise from the 101 and 102 complexes, respectively. The pH dependence of the compound 2–Cu spectrum is similar with the same λ_{max} values for both complexes (not shown). However, at a pH above 10, green crystals of $(2)_2Cu(II)$ begin to precipitate causing a decrease in the band intensity at 675 nm. The spectral interpretation indicates formation of the bis complexes at about pH 3.5 and narrowing of the pH range over which the mono species exist, relative to the conditions used for the potentiometric study. This can be attributed to the higher concentrations (86 times higher [Cu]) used for the spectroscopic study. Motekaitis and Martell[28] noted a decrease with concentration of more than 3 pH units in the transition between bis and tris complexes of 1 with Fe(III).

(d) Replacement of Fe(III) by Cu(II). The difference in the extinction coefficients of Fe and Cu d–d transitions ($\epsilon_{Fe}/\epsilon_{Cu}$ = 170 at λ_{max} for the hydroxypyridinones) means

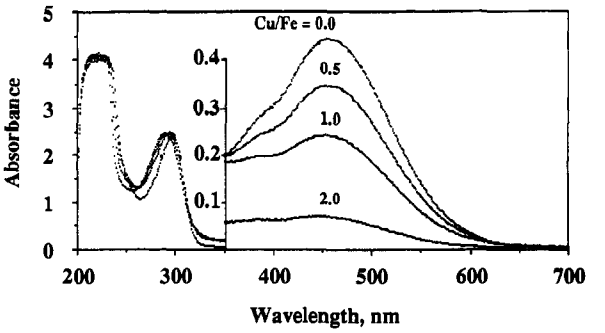


Figure 4. Spectrophotometric analysis of the competition between 0.1 mM Fe(III) and different concentrations of Cu(II) in the presence of 0.3 mM 1. T = 25 °C, μ = 0.15 mM NaCl, pH = 7, and Cu = 0.0, 0.05, 0.1, or 0.2 mM to give the Cu/Fe ratios indicated on each spectrum.

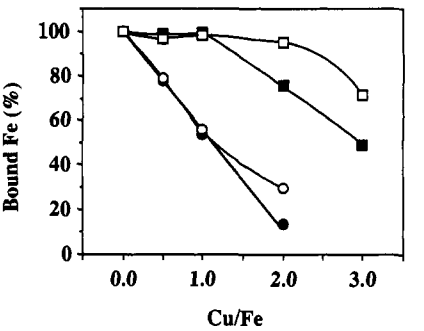


Figure 5. Effect of Cu(II) on displacement of Fe from $(1)_3Fe$ (open symbols) and $(2)_3Fe$ (closed symbols). Conditions are as in Figure 4, with 0.1 mM Fe and a ligand:Fe ratio of 3:1 (circles) or 6:1 (squares).

that Fe coordination can be monitored in the presence of Cu. The deep red $(1)_3Fe$ and $(2)_3Fe$ complexes were observed at 457 nm (Figure 4) in solutions either 10 or 100 μM in Fe. The higher concentration corresponds to the potentiometric studies whereas the lower is comparable to concentrations encountered *in vivo*. Addition of 2 equiv of Cu to a solution of 0.1 mM Fe^{3+} and 0.3 mM 1 at neutral pH results in displacement of the Fe with bleaching of the spectrum (Figure 4). Under these conditions in the ligand–single-metal systems, the neutral species CuL_2 and FeL_3 account for all the metal (Figure 3 and ref 28). On the basis of their stability constants (log β_2 for $Cu(1)_2$ = 21.7 (Table 1) and log β_3 for $Fe(1)_3$ = 35.9[28]), the Cu species should be a minor component in the presence of Fe when ligand concentration is limiting. However, quantitative precipitation of Fe hydrolysis products is observed when coordination is disrupted, effectively causing irreversible dissociation and leaving the hydroxypyridinone free to bind Cu. Loss of Fe coordination does not proceed by a stepwise path. As the spectrum of the Fe–tris complex bleaches, there is no shift in the band position of the 457-nm absorption, and no evidence of the maroon and violet bis and mono complexes (λ_{max} = 510 and 560 nm, respectively) is seen. With ligand limiting (Fe:ligand = 1:3), displacement of Fe begins upon addition of Cu (Figure 5, circles). When ligand is present in excess, the Fe species are less prone to dissociation, and the first equivalent of Cu has no effect (Figure 5, squares). With the second equivalent, 1 released 25% of its Fe whereas the $(2)_3Fe$ complex remains intact. Spectral measurements performed 2 and 24 h after the addition of Cu lead to the same conclusion. Nor is there an effect of dilution; Fe displacement proceeds to the same extent when the metals are present at 100 μM (Figure 4) or 10 μM (not shown).

Table 2. Effect of N- and C2-Substitution on pK_a and Stability Constants of Cu(II) Complexes of Some Hydroxypyridinones

ligand[a]	pK_{a1}	pK_{a2}	log K_1	log K_2	log K_3
ahpo[b]	3.36	9.01	9.49	7.64	
mhpo[b]	3.35	8.80	9.35	7.58	
L-mimosine[c]	2.62	8.86	9.48	7.33	
mimosinic acid[c]	2.92	8.91	9.48	7.48	
1[d]	3.84	9.86	11.48	10.22	5.64
2[d]	3.93	10.09	13.18	7.03	2.65
3[d]	3.79	9.79			
4[d]	3.74	9.70			

[a] ahpo: 3-hydroxypyridin-4-one; mhpo: 1-methylahpo; L-mimosine: L-α-amino-β-(3-hydroxy-4-oxo-1,4-dihydropyridin-1-yl)propanoic acid; mimosinic acid: β-(3-hydroxy-4-oxo-1,4-dihydropyridin-1-yl)propanoic acid; 1: 1,2-dimethylahpo; 2: 1,2-diethylahpo; 3: 1-ethyl-2-methylahpo; 4: 1-methyl-2-ethylahpo. [b] Values from ref 39. [c] Values from ref 38. [d] pK_a values from ref 42.

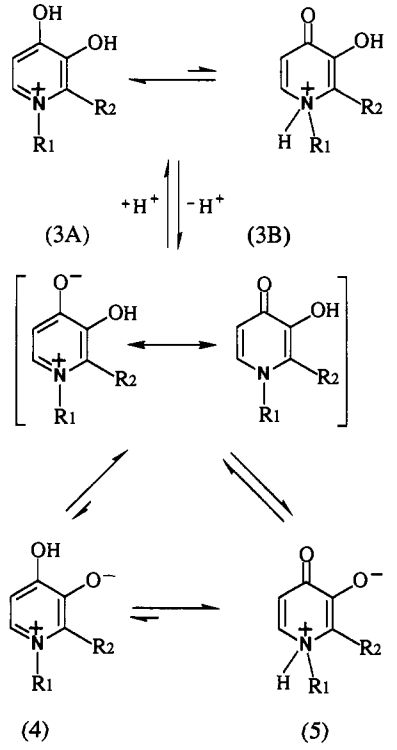
Discussion

Like the parent 3-hydroxypyridin-4-one and its *N*-substituted derivatives,[38,39] the compounds of the 3-hydroxypyridin-4-one series form stable complexes with Cu(II) coordinating through the hydroxypyridinone group. Comparing these complexes affords the opportunity to examine the effects of N- vs C_2-substituents on complexation; stabilities of the 101 complexes increase in the order X = 1-methyl-X < 1,2-dimethyl-X < 1,2-diethyl-X, where X is 3-hydroxypyridin-4-one (Table 2). This can be attributed to an inductive effect of the substituent ortho to the coordinating hydroxy group, also seen as an increase in the phenolic pK_a in the same series. Copper complexes of mimosine and mimosinic acid, on the other hand, illustrate the relative unimportance for complexation of proton and metal of the substituents at nitrogen. Comparison of these propanoic acid derivatives with 3-hydroxypyridin-4-one and its *N*-methyl derivative (Table 2) shows similar pK_{a2} values and stepwise formation constants for the mono and bis complexes with Cu(II). This may be of importance for the design of chelating agents since the aza substituents may profoundly affect metabolism without having much impact on chelation. For instance, it has been found that whereas 1 and 2 are metabolized at the hydroxy ketone functionality and thus lose chelating ability, this does not occur with *N*-propyl-3-hydroxypyridin-4-one (R. C. Hider, personal communication).

Although the hydroxypyridinones are proving useful in clinical trials in removing Fe from Fe-overloaded patients,[6-10] the removal of essential elements like Cu must also be considered when contemplating long-term chelating programs. Urinary Fe excretion increases with increasing dose of 1,[9] but a high ratio of 1:Fe will favor the chelation of other elements. Less obvious, low concentrations of ligand may also favor nonspecific chelation. In contrast to hexadentate Fe chelates, the speciation of the 1/Fe system is concentration dependent,[28] and in the presence of excess Fe, the 1:1 complex can be observed spectroscopically at neutral pH (El-Jammal and Templeton, unpublished). This mono complex also appears to occur during removal of Fe(III) from the core of ferritin by hydroxypyridinones.[12] Under these conditions, and because of the comparable stabilities of the mono complexes with Cu and Fe and of the bis complexes with both metals, Cu is a good candidate for competition with Fe.

Although the present study does not reproduce the complex environment of competing ligands present *in vivo*, competition experiments with both metals present in the same solution provide a more realistic picture than predictions based on comparison of the stability constants.

Scheme 1. Tautomeric Equilibria and Mesomerism of 1 (R_1,R_2 = $-CH_3$) and 2 (R_1,R_2 = $-CH_2CH_3$) at pK_{a1}[a]



[a] Structures are numbered for reference in the text.

Comparing stability constants of the major species Cu(L)$_2$ and Fe(L)$_3$ shows that the Fe complex should be at least an order of magnitude more stable than the Cu complex. However, at higher pH where deprotonation of the coordinating site favors complexation, there is competition with HO^- and the relative stabilities of the metal hydroxides also become important. The hydroxo species of Fe are more stable than those of Cu[44] and are favored; this would appear to explain the displacement of Fe seen in the presence of stoichiometric amounts of Cu. This replacement of Fe by Cu warrants careful consideration since the effects of releasing toxic Fe and removing essential Cu *in vivo* are undesirable.

The equilibrium constant for adding the second mole of ligand is decreased relative to the first in all the compounds of Table 2. The increasing difficulty of adding the third ligand is also observed in the Cu–EDTA system, (EDTA)$_3$Cu(II) occurring only at very high EDTA concentrations.[43] Jahn–Teller distortion of octahedral d^9 hexaquo-Cu(II) might account for this and contribute to the slow equilibrium in titration of the system between pH 7 and 10. Thus, a distorted symmetry of the Cu(II) complex with formation of the second five-membered ring may kinetically affect the formation of the bis from the mono complex. However, there is no evidence of distortion in the crystals, both structures being very planar. Kinetic restrictions may also arise from a slow tautomeric equilibrium, demonstrated previously for these compounds.[42] Below pK_{a1}, the dihydroxypyridinium form (Scheme 1, structure 1) is predominant and displacement of the hydroxyl protons by Cu should be facile. Above pK_{a1}, a tautomeric equilibrium between primarily structures 3 and 5 is established. These tautomers have distinct electroactivities and equilibrate slowly.[42] At neutral pH, the mono complex will react preferentially with one of these forms,

and readjustment of the equilibrium may become rate limiting. In keeping with this idea, increasing the ligand concentration to an excess causes equilibrium to be attained more rapidly during titration. This may in part be due to the availability of sufficient amounts of the preferred tautomer to accomodate the Cu.

Experimental Section

Synthesis of Hydroxypyridinones and Copper Complexes. All reagents were of analytical grade and used as received. Compounds 1 and 2 were synthesized as previously described.[42] The final products were recrystallized twice, and each compound was characterized by melting point and 500-MHz ^{1}H NMR. Reaction of an aqueous solution of 0.5 M cupric chloride with 2 or 3 equiv of 1 or 2 results in a light green acidic solution, neutralization of which leads to the precipitation of the dark green Cu complexes. These were filtered, washed with cold water, and air-dried, resulting in a yield of 50%, based on Cu. Starting with a methanolic solution increased this yield to 78%. In acetone, precipitation of the Cu complex of 1 occurs spontaneously after dissolving 1 in a solution of $CuCl_2$. Compound 2 fails to undergo a similar reaction in acetone.

Speciation Analysis. Potentiometric titrations for determining stability constants were carried out using an automated titration assembly (Radiometer, Copenhagen) consisting of an ABU 80 autoburette, a TTT80 titrator, and a pHM83 pH meter with a combined glass electrode (Radiometer GK2401) calibrated with buffers from the same supplier. Measurements were performed in carbonate-free, deionized water in a thermostated (25 °C), water-jacketed glass vessel under a purified nitrogen atmosphere in a volume of 40.00 mL. Standard Fe and Cu solutions were calibrated by graphite-furnace atomic absorption. HCl (0.025 M) was used to lower the starting pH, and NaCl (0.15 M) was the supporting electrolyte. Titration proceeded by adding CO_2-free NaOH (0.1957 ± 0.0001 M) in 0.005–0.1-mL increments in order to maintain a moderate increase in pH. The pK_w value was estimated to be 13.7 from titration of back-ground electrolyte. Ligand pK_a values were calculated by means of the program PKAMART,[45] employing the algorithm of Motekaitis and Martell.[46] Ligand concentrations of 0.2, 0.6, 2, and 6 mM were used in six different combinations for determining ligand pK_a. Potentiometric data for stability constant determinations were acquired by constructing two sets of pH titration curves for each complex in which both ligand and metal concentrations were systematically varied from 0.2 to 1 mM for the ligand and 0.05 to 0.33 mM for Cu. The common curve was 1 mM ligand and 0.2 mM metal. Solutions were allowed to equilibrate overnight before starting the titration, and during the titration, readings were considered stable if the change in pH was less than 0.01 unit in 5 min. Data analysis was performed using a series of programs written in Basic for an Apple Macintosh microcomputer, employing the Fletcher–Powell algorithm as previously described.[45] Equilibrium was attained slowly between pH 7 and 10, requiring about 1 h per incremental change in pH (about 0.3 pH unit in this range). Therefore, repetition of the experiments is time consuming. Instead, a set of nine titration curves was obtained for each system, and analyses were carried out on three subsets for each system, one of which contained all nine curves in order to examine self-consistancy within the data. The iterative algorithm used to calculate the β values[45] minimizes the summed squared residuals between the experimental data and those calculated from a guessed set of species. The choice of possible species was guided by the aqueous chemistry of Cu and the structures of some other Cu–pyridinones.[38,39] Hydrolysis constants (log β) taken from ref 44 were $Cu(OH)^+$, −8; $Cu(OH)_2$, −17.3; $Cu(OH)_3^-$, −27.8; $Cu(OH)_4^{2-}$, −39.6; and $Cu_2(OH)_2^{2+}$, −10.36. Only species for which stability constants could be refined (i.e., which converged under iteration) individually were included in collective minimization; combinations of up to 21 species were then tested.

Spectroscopic Measurements. Optical spectra were recorded at 25 °C using a Perkin-Elmer Lambda 4C spectrometer equipped with a PE 7700 computer. Quartz cells of 1-cm light path were used. Sample pH was adjusted by addition of concentrated aqueous HCl or NaOH while maintaining an ionic strength of 0.15 M NaCl. Solutions were allowed to equilibrate for 2 h before recording the spectrum.

Table 3. Experimental Details of Crystallographic Analysis

parameter	$(1)_2Cu$	$(2)_2Cu$
chemical formula	$(C_7NO_2H_8)_2Cu$	$(C_9NO_2H_{12})_2Cu$
formula weight	243.54	395.54
lattice parameters		
a (Å)	7.628(1)	7.255(6)
b (Å)	13.888(1)	9.037(1)
c (Å)	7.157(1)	14.091(2)
β (°)	115.70(10	89.66(1)
volume (calculated, Å^3)	683.2(1)	923.9(1)
space group	$P2_1/c$	$P2_1/n$
Z value	2	2
calculated density (g cm^{-3})	1.66	1.42
total number of data	5443	6228
number of unique data	1363	2879
max sin (θ/λ)	0.705	0.809
R_{merge}[a] (%)	2.23	4.52
R[b]	0.042	0.055
R_w[c]	0.044	0.063
S[d]	2.67	2.48

[a] $R_{merge} = \sum|I - I_{mean}|/\sum|I|$. [b] $R = \sum||F_o| - |F_c||/\sum|F_o|$. [c] $R_w = [\sum w(|F_o| - |F_c|)^2/\sum wF_o^2]^{1/2}$, where w is a weighting scheme based on counting statistics. [d] $S = [\sum w(|F_o| - |F_c|)^2/(N_o - N_v)]^{1/2}$, where N_o is the number of observations and N_v is the number of variables.

X-ray Structural Analysis. Single dark green needle-shaped crystals of 2 complexed with copper were grown by slow evaporation of methanol/water (1:3, v/v). Single crystals of the Cu complex with 1 proved to be very difficult to grow from this solvent mixture. However, small green diamond-shaped crystals were grown by slow evaporation of a methanol/acetonitrile (3:1, v/v) mixture. For each complex, a single crystal was mounted and data were collected with Nb-filtered Mo Kα radiation (λ = 0.71069 Å) on a Nicolet P3 diffractometer using θ–2θ scans. Data were collected at room temperature. Table 3 lists the experimental details for both 1 and 2. The intensities measured were corrected for Lorentz and polarization factors (but not for absorption) to yield a total of 1363 and 2879 independent data for 1 and 2, respectively. The variance of each structure factor was calculated according to the method of Blessing.[47]

The structure of each complex was solved by direct methods.[48] All non-hydrogen atoms were refined anisotropically. All hydrogen atoms in the Cu complex of 1 were located by difference Fourier techniques and refined isotropically. In the Cu complex of 2, 7 of the 12 hydrogen atoms were located by difference Fourier techniques and refined isotropically. The rest were included at idealized positions. The supplementary material presents the results of the final full-matrix least-squares refinement for both structures for data $I > 3.00\sigma(I)$. For each structure at the completion of the refinement, a hydrogen atom was removed and a final difference Fourier calculated. The map was examined for the maximum positive and negative peaks. For 1, the largest peak was the hydrogen atom that had been removed (peak height 0.591 e/Å^3). The next peak was 91% of the hydrogen peak and 0.2 Å away from the Cu atom. This peak and the largest negative peak (−0.598 e/Å^3) also close to the copper arise from an inability to model the thermal motion of the Cu accurately. The next peak found in the difference Fourier was 78.5% of the hdyrogen peak (0.464 e/Å^3). Therefore, there are no solvent molecules in this structure. For 2, the difference Fourier revealed a possible alternative conformation for the methyl hydrogens around C10. We were unable to locate the hydrogen atoms in an earlier difference Fourier and included them in idealized positions. The higher thermal parameters for both C9 and C10 (see Supplementary Table 2 and Figure 2) reflect the conformational flexibility of this group. The next most significant peak (0.364 e/Å^3) was 66% of the removed hydrogen atom peak (0.546 e/Å^3), and the largest negative peak (−0.457 e/Å^3) was 83.6% of the hydrogen peak. No solvent molecules are present in this structure.

In order to assess the quality of the data and the accuracy of the structure, we have examined the root-mean-square amplitude of displacement along the principal axes of the trivariate normal probability density function, represented in Figure 2. Oblate spheroids lead to a small ratio for the maximum to minimum rmsd, indicating the presence of a systematic error in the data.

The maximum to minimum rmsd ratio for the Cu atom was 0.81 and 0.788 for 1 and 2, respectively. Typically, most values in both structures were found to be close to 0.70. The largest difference for 1 was found to reside on C7 and was 0.56. For 2, the largest ratio was 0.38 and was located on atom C9. This is probably a consequence of the potential alternative conformation available for the methyl group on C10. All calculations were performed using the teXsan Crystallographic software package (Molecular Structure Corporation, The Woodlands, TX).

Acknowledgment. Supported by a grant from the Medical Research Council of Canada.

Supplementary Material Available: Atomic coordinates, bond lengths and bond angles, least-squares planes, anisotropic displacement factors, and the root-mean-square displacements along the principal axes of the probability density functions (12 pages). Ordering information is given on any current masthead page.

References

(1) Cohen, A. Current status of iron chelation therapy with deferoxamine. *Semin. Hematol.* **1990**, *27*, 86–90.
(2) Martell, A. E. In *The Design and Synthesis of Chelating Agents*; Martell, A. E., Anderson, W. F., Badman, D. G., Eds.; Elsevier North Holland: Amsterdam, 1981; pp 67–104.
(3) Hider, R. C.; Hall, A. D. Clincially useful chelators of tripositive elements. *Prog. Med. Chem.* **1991**, *28*, 43–173.
(4) Hider, R. C.; Kontoghiorges, G.; Silver, J. Pharmaceutical Compositions. UK Patent GB 2118176A, 1982.
(5) Huehns, E. R.; Porter, J. B.; Hider, R. C. Selection of hydroxypyridin-4-ones for the treatment of iron overload using in vitro and in vivo models. *Hemoglobin* **1988**, *12*, 593–600.
(6) Olivieri, N. F.; Koren, G.; Hermann, C.; Bentur, Y.; Chung, D.; Klein, J.; St. Louis, P.; Freedman, M. H.; McClelland, R. A.; Templeton, D. M. Comparison of oral iron chelator L1 and desferrioxamine in iron-loaded patients. *Lancet* **1990**, *336*, 1275–1279.
(7) Tondury, P.; Kontoghiorghes, G. J.; Ridolfi-Luthy, A.; Hirt, A.; Hoffbrand, A. V.; Lottenbach, A. M.; Sonderegger, T.; Wagner, H. P. L1 (1,2-dimethyl-3-hydroxypyrid-4-one) for oral iron chelation in patients with beta-thalassaemia major. *Br. J. Haematol.* **1990**, *76*, 550–553.
(8) Bartlett, A. N.; Hoffbrand, A. V.; Kontoghiorghes, G. J. Long-term trial with the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L1). *Br. J. Haematol.* **1990**, *76*, 301–304.
(9) Agarwal, M. B.; Gupte, S. S.; Viswanathan, C.; Vasandani, D.; Ramanathan, J.; Desai, N.; Puniyani, R. R.; Chhablani, A. T. Long-term assessment of efficacy of L1, an oral iron chelator, in transfusion dependent thalassaemia: Indian trial. *Br. J. Haematol.* **1992**, *82*, 460–466.
(10) Olivieri, N. F.; Koren, G.; Matsui, D.; Liu, P. P.; Blendis, L.; Cameron, R.; McClelland, R. A.; Templeton, D. M. Reduction of tissue iron stores and normalization of serum ferritin during treatment with the oral iron chelator L1 in thalassemia intermedia. *Blood* **1992**, *79*, 2741–2748.
(11) Kontoghiorghes, G. J. Structure/red blood cell permeability. Activity of iron(III) chelator complexes. *Inorg. Chim. Acta* **1988**, *151*, 101–106.
(12) Brady, M. C.; Lilley, K. S.; Treffry, A.; Harrison, P. M.; Hider, R. C.; Taylor, P. D. Release of iron from ferritin molecules and their iron-cores by 3-hydroxypyridinone chelators in vitro. *J. Inorg. Biochem.* **1989**, *35*, 9–22.
(13) Blatt, J.; Taylor, S. R.; Kontoghiorghes, G. J. Comparison of the activity of deferoxamine with that of oral iron chelators against human neuroblastoma cell lines. *Cancer Res.* **1989**, *49*, 2925–2927.
(14) Hewitt, S. D.; Hider, R. C.; Sarpong, P.; Morris, C. J.; Blake, D. R. Investigation of the anti-inflammatory properties of hydroxypyridinones. *Ann. Rheum. Dis.* **1989**, *48*, 382–388.
(15) Porter, J. B.; Hoyes, K. P.; Abeysinghe, R. D.; Brooks, P. N.; Huehns, E. R.; Hider, R. C. Comparison of the subacute toxicity and efficacy of 3-hydroxypyridin-4-one iron chelators in overloaded and non-overloaded mice. *Blood* **1991**, *78*, 2727–2734.
(16) Kontoghiorghes, G. J. Structure/iron binding activity of 1-hydroxypyrid-2-one chelators intended for clinical use. *Inorg. Chem. Acta* **1987**, *135*, 145–150.
(17) Kontoghiorghes, G. J.; Sheppard, L. Simple synthesis of the potent iron chelators 1-alkyl-3-hydroxy-2-methylpyrid-4-ones. *Inorg. Chim. Acta* **1987**, *136*, 11–12.
(18) Porter, J. B.; Hider, R. C.; Huehns, E. R. Update on the hydroxypyridinone oral iron-chelating agents. *Semin. Hematol.* **1990**, *27*, 95–100.
(19) Porter, J. B.; Morgan, J.; Hoyes, K. P.; Burke, L. C.; Huehns, E. R.; Hider, R. C. Relative oral efficacy and acute toxicity of hydroxypyridin-4-one iron chelators in mice. *Blood* **1990**, *76*, 2389–2396.
(20) Brock, J. H.; Liceaga, J.; Arthur, H. M. L.; Kontoghiorghes, G. J. Effect of novel 1-alkyl-3-hydroxy-2-methylpyrid-4-one chelators on uptake and release of iron from macrophages. *Am. J. Hematol.* **1990**, *34*, 21–25.
(21) Kontoghiorghes, G. J.; Goddard, J. G.; Barlett, A. N.; Sheppard, L. Pharmacokinetic studies in humans with the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one. *Clin. Pharmacol. Ther.* **1990**, *48*, 255–261.
(22) Singh, S.; Epemolu, R. O.; Dobbin, P. S.; Tilbrook, G. S.; Ellis, B. L.; Damani, L. A.; Hider, R. C. Urinary metabolic profiles in human and rat of 1,2-dimethyl- and 1,2-diethyl-substituted 3-hydroxypyridin-4-ones. *Drug Metab. Dispos.* **1992**, *20*, 256–261.
(23) Epemolu, O. R.; Singh, S.; Hider, R. C.; Damani, L. A. The pharmacokinetics of 1,2-diethyl-3-hydroxypyridin-4-one. *Drug Metab. Dispos.* **1992**, *20*, 736–741.
(24) Charalambous, J.; Dodd, A.; McPartlin, M.; Matondo, S. O. C.; Pathirana, N. D.; Powell, H. R. Synthesis and x-ray crystal structure of tris (1,2-dimethyl-3-hydroxypyrid-4-onato) iron (III). *Polyhedron* **1988**, *7*, 2235–2237.
(25) Nelson, W. O.; Karpishin, T. B.; Rettig, S. J.; Orvig, C. Aluminum and gallium compounds of 3-hydroxy-4-pyridinones: Characterization, and crystallography of biologically active complexes with unusual hydrogen bonding. *Inorg. Chem.* **1988**, *27*, 1045–1051.
(26) Nelson, W. O.; Rettig, S. J.; Orvig, C. Aluminum and gallium complexes of 1-ethyl-3-hydroxy-2-methyl-4-pyridinone: a new exoclathrate matrix. *Inorg. Chem.* **1989**, *28*, 3153–3157.
(27) Matsuba, C. A.; Nelson, W. O.; Rettig, S. J.; Orvig, C. Neutral water-soluble indium complexes of 3-hydroxy-4-pyrones and 3-hydroxy-4-pyridinones. *Inorg. Chem.* **1988**, *27*, 3935–3959.
(28) Motekaitis, R. J.; Martell, A. E. Stabilities of the iron(III) chelates of 1,2-dimethyl-3-hydroxy-4-pyridinone and related ligands. *Inorg. Chim. Acta* **1991**, *183*, 71–80.
(29) Kline, M. A.; Orvig, C. Complexation of iron with the orally active decorporation drug L1 (3-hydroxy-1,2-dimethyl-4-pyridinone). *Clin. Chem.* **1992**, *38*, 562–565.
(30) Clevette, D. J.; Nelson, W. O.; Nordin, A.; Orvig, C.; Sjoeberg, S. The complexation of aluminum with N-substituted 3-hydroxy-4-pyridinones. *Inorg. Chem.* **1989**, *28*, 2079–2081.
(31) Clevette, D. J.; Lyster, D. M.; Nelson, W. O.; Rihela, T.; Webb, G. A.; Orvig, C. Solution chemistry of gallium and indium 3-hydroxy-4-pyridinone complexes in vitro and in vivo. *Inorg. Chem.* **1990**, *29*, 667–672.
(32) Zhang, Z.; Rettig, S. J.; Orvig, C. Lipophilic coordination compounds: aluminum, gallium, and indium complexes of 1-aryl-2-methyl-4-pyridinones. *Inorg. Chem.* **1991**, *30*, 509–515.
(33) Berdoukas, V.; Bentley, P.; Frost, H.; Schnebli, H. P. Toxicity of oral iron chelator L1. *Lancet* **1993**, *341*, 1088.
(34) Hershko, C. Development of oral iron chelator L1. *Lancet* **1993**, *341*, 1088–1089.
(35) Pfannkuch, F.; Bentley, P.; Schnebli, H. P. Future of oral iron chelator deferiprone (L1). *Lancet* **1993**, *341*, 1480.
(36) Mehta, J.; Singhal, S.; Mehta, B. C. Future of oral iron chelator deferiprone (L1). *Lancet* **1993**, *341*, 1480.
(37) Kontoghiorghes, G. J.; Agarwal, M. B.; Tondury, P.; Kersten, M. J.; Jaeger, M.; Vreugdenhil, G.; Vania, A.; Rahman, Y. E. Future of oral iron chelator deferiprone (L1). *Lancet* **1993**, *341*, 1479–1480.
(38) Stünzi, H.; Perrin, D. D.; Teitei, T.; Harris, R. L. N. Stability constants of some metal complexes formed by mimosine and related compounds. *Aust. J. Chem.* **1979**, *32*, 21–30.
(39) Stünzi, H.; Harris, R. L. N.; Perrin, D. D.; Teitei, T. Stability constants for metal complexation by isomers of mimosine and related compounds. *Aust. J. Chem.* **1980**, *33*, 2207–2220.
(40) Halliwell, B.; Gutteridge, J. M. C. Role of free radicals and catalytic metal ions in human disease: An overview. *Methods Enzymol.* **1990**, *186*, 1–88.
(41) Hershko, C.; Weatherall, D. J. Iron-chelating therapy. *CRC Crit. Rev. Lab. Sci.* **1988**, *26*, 303–346.
(42) El-Jammal, A.; Templeton, D. M. Electrochemical oxidation of some therapeutic 3-hydroxypyridin-4-one iron chelators. *Electrochim. Acta* **1993**, *38*, 2223–2230.
(43) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*; John Wiley & Sons: New York, 1972; pp 616, 914.
(44) Baes, C. F.; Mesmer, R. E. *The Hydrolysis of Cations*; Robert E. Krieger Publishing Co.: FL, 1986; pp 226–274.
(45) Templeton, D. M.; Sarkar, B. Fletcher-Powell minimization of analytical potentiometric data by microcomputer: Application to the Cu(II) complexes of biological polyamines. *Can. J. Chem.* **1985**, *63*, 3122–3128.
(46) Motekaitis, R. J.; Martell, A. E. Program PKAS: a novel algorithm for the computation of successive protonation constants. *Can. J. Chem.* **1981**, *60*, 168–173.
(47) Blessing, R. H. Data reduction and error analysis for accurate single crystal diffraction intensities. *Crystallogr. Rev.* **1987**, *1*, 3–58.
(48) Hai-Fu, F. *SAP190 Structure Analysis Programs with Intelligent Control*; Riguku Corp.: Tokyo, 1990.