Brief Articles

3-Hydroxy-2-(5-hydroxypentyl)-4*H*-chromen-4-one: A Bidentate or Tridentate Iron(III) Ligand?

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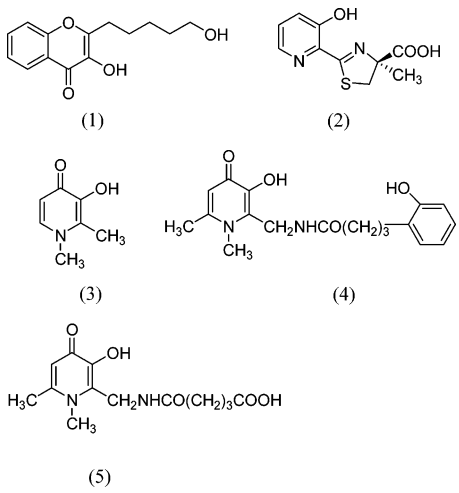
The pK_a value and iron affinity constants for 3-hydroxy-2-(5-hydroxypentyl)-4*H*-chromen-4-one **1** have been determined by spectrophotometry using aqueous methanol solutions. The extrapolated affinity constants β_1, β_2, and β_3 for iron(III) in aqueous solution were 9.95, 18.69, and 26.02, respectively, with a corresponding pFe^{3+} value of 14.64. Job plot and MS spectra data demonstrated that the 3:1 species is favored at pH 7.0. These results indicate that **1** acts as a bidentate ligand when coordinated to iron(III).

Introduction

There is considerable interest in the design of orally active iron chelators for application in the treatment of iron overload associated with thalassaemia and related diseases.[1] Medicinal chemists have been searching for suitable iron-selective orally active agents for over 30 years. Both bidentate and tridentate ligands are deemed to be prime candidates, due to their low molecular weight and associated ability to penetrate cells.[2] Tridentate ligands are considered to be superior scavengers in comparison to bidentate ligands, as they are less likely to form incompletely coordinated iron(III) complexes at pH 7.4. Tridentate ligands such as PIH,[3] DFT **2**,[4] and ICL670[5] have been extensively studied. These tridentate ligands all utilize both oxygen and nitrogen atoms to coordinate iron(III). In comparison with "hard" oxygen atoms (oxygen atoms possessing a high charge density), nitrogen atoms have a lower ability to bind high spin iron(III).[6] Ligands with appreciable ability to bind both iron(II) and iron(III) are potentially toxic due to redox cycling, resulting in the production of the toxic hydroxyl radical.[7] Furthermore, a ligand containing both oxygen and nitrogen will possess decreased selectivity for iron and will bind other metal ions such as zinc and nickel under physiological conditions.

In principle, optimal iron(III) selectivity could be achieved using a tridentate ligand containing three "hard" donating oxygen atoms. Using these guidelines, attempts were made to design such a compound based on 1,2-dimethyl-3-hydroxypyridin-4-one **3** (deferiprone) with three potential chelating oxygen atoms (**4**, **5**).[8] Although these chelators are in principle able to bind iron(III) in tridentate mode, the use of pH-dependent UV spectrophotometric titrations failed to provide any evidence for tridentate chelation and instead confirmed bidentate coordination. It was concluded that the loss in entropy associated with the involvement of the third ligand more than outweighs the enthalpy gained with coordinating iron(III) in the tridentate mode. These investigations were based on compounds containing hydroxypyridinones together with either a phenolic oxygen **4** or carboxylate oxygen **5** located at ring position 2. It was therefore surprising to find that recently 3-hydroxy-2-(5-hydroxypentyl)-4*H*-chromen-4-one **1** was reported to bind iron(III) in tridentate mode[9]—particularly as the proposed ligand has a terminal aliphatic hydroxyl group, which is a relatively poor iron(III) chelator. To further investigate this report, we decided to monitor the iron(III) chelation properties of 3-hydroxy-2-(5-hydroxypentyl)-4*H*-chromen-4-one **1**.



Results and Discussion

The spectrophotometric results with 3-hydroxy-2-(5-hydroxypentyl)-4*H*-chromen-4-one **1** are presented in Table 1. The free ligand spectra recorded at different pH values indicate that **1** exists predominantly in the form LH at pH 5 and, as the pH rises, L^- becomes more dominant. This leads to a shift in λ_{max} from 320 to 380 nm. An isobestic point is located at 340 nm. The titration end point was pH 11. The refined molar extinction coefficient spectra of the free ligand L^- and protonated ligand LH are presented in the Supporting Information. The extrapolated aqueous pK_a value 8.908 agrees well with the previously reported value of 8.88 ± 0.04.[9]

The complex of **1** with iron(III) has an absorbance in the range 400−800 nm whereas the free ligand is colorless. The

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Table 1. Formation Constants (log β) of Iron(III) Complexes with **1** (0.1 M KCl, 25 °C) in Different Volume Ratios of Water/Methanol Mixtures

	water/methanol ratios			
	1/0	6/4	5/5	0.34/0.66
pK_a	8.932 (±0.006)	9.751 (±0.008)	9.971 (±0.013)	10.298[c]
log β_{11}[a]	9.95[b]	11.819 (±0.015)	11.542 (±0.027)	12.765 (±0.012)
log β_{12}[a]	18.69[b]	21.937 (±0.026)	21.843 (±0.062)	23.721 (±0.018)
log β_{13}[a]	26.02[b]	30.466 (±0.033)	30.506 (±0.082)	32.973 (±0.021)

[a] $\beta_{ML} = [Fe_M\mathbf{1}_L]/[Fe]^M[\mathbf{1}]^L$. [b] Extrapolated result in aqueous solution. [c] Extrapolated from the pK_a best fit equation.

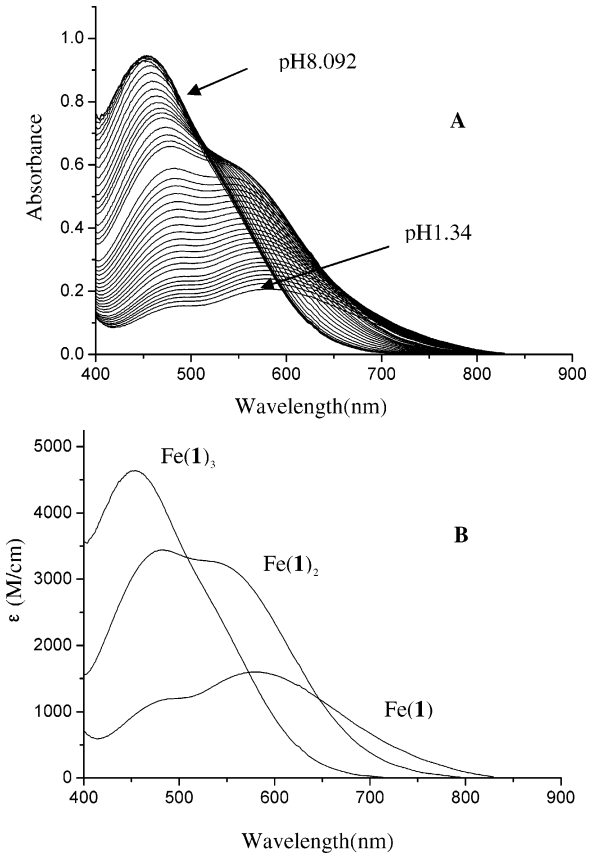

Figure 1. (A) Visible spectra of iron−**1** complex: pH 1.34−8.09, [**1**] = 261.6 μM, [Fe^{3+}] = 52.4 μM, 25 °C, μ = 0.1 M (KCl). (B) Molar extinction coefficients spectra of iron−**1** complexes: ML, ML_2, ML_3.

raw experimental spectra of the complex in 0.4 volume ratio methanol:water mixture is presented in Figure 1A. The molar extinction coefficient spectra of each species (Figure 1B) shows two isobestic points (520 and 650 nm). These isobestic points indicate the existence of three colored species: ML, ML_2, and ML_3 over the pH range 1−8. The absolute stability constants of **1** with iron(III) in different methanol:water mixtures, together with the extrapolated result for aqueous solution, are presented in Table 1.

Direct evidence for the formation of the FeL_3 complex was obtained from ESI mass spectra (Figure 2). The detection of noncovalent complexes by ESI mass spectrometry is strongly influenced by the temperature of the source capillary. In our hands, temperatures above 180 °C shifted the observed complexation pattern from the FeL_3 toward the FeL_2 species. In the studies reported herein, the complexation pattern was recorded at 175 °C. In Figure 3, the peaks at *m*/*z* 797.9 and *m*/*z* 820.1

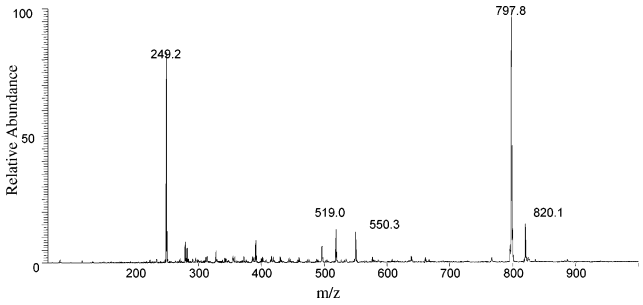

Figure 2. Positive ESI ion trap mass spectrum of an aqueous solution at pH 7.0 of a 1:3 molar mixture of iron(III) (33 μM) with **1** (100 μM) in pH 7 MOPS buffer (50% aqueous methanol, v/v); 797.8 registers the Fe(III)(**1**)$_3$ complex.

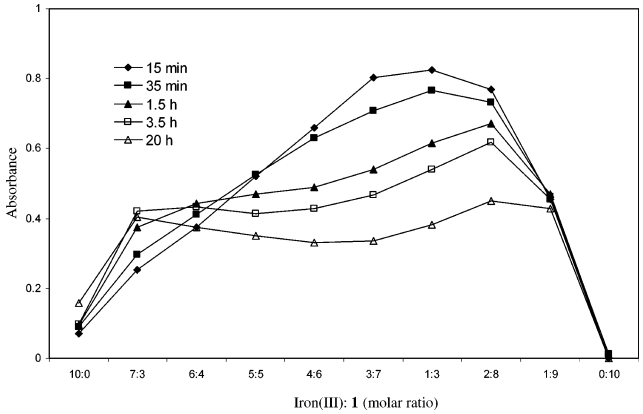

Figure 3. JOB plot of Fe:**1** mixtures monitored at 500 nm. Total concentration of iron(III) plus **1** is 0.2 mM.

correspond to the proton adduct of FeL_3 $[Fe+(L-H)_3+H]^+$ and the sodium adduct of FeL_3 $[Fe+(L-H)_3+Na]^+$, respectively. The presence of the FeL_2 complex was indicated by the peak at *m*/*z* 550.3, which corresponds to the proton adduct of FeL_2 $[Fe+(L-H)_2]^+$. This complex species already possesses a single positive charge and, unlike the FeL_3, does not require further protonation or cationization in order to be observed. In contrast, the FeL_3 complex possesses a net charge of zero and hence requires further ionization by a proton or cation. The relative abundance of the FeL_2 in the mass spectrum is likely to be overrepresented as a result of these differences. The strong peak at *m*/*z* 249.3 corresponds to the proton adduct of the free ligand $[L+H]^+$.

The 1:3 stiochiometry was further supported by a JOB plot (Figure 3). The maximum absorbance occurs when the Fe:**1** ratio is 1:3, indicating that the 1:3 complex is the most populated species at pH 7.0. Interestingly, the profile of the JOB plot changed with time, indicating that the iron(III)−**1** complex is relatively unstable at pH 7.0. Presumably, the hydroxyl anion slowly competes with **1**, leading to the formation of iron hydroxides. In contrast, 3-hydroxypyridinones, for instance deferiprone **3**, form stable complexes at pH 7.0.

The species plot of the **1**−iron(III) system (Figure 4A), calculated by HYSS[10] and based on the affinity constants determined in this study, corresponds to the conditions [Fe^{3+}] = 10^{-6} M [**1**] = 10^{-4} M. The plot demonstrates that under these conditions the 3:1 iron complex predominates at pH 7.4 when the concentration of **1** is 100 times larger than that of iron(III). However at lower **1** concentrations, for instance 5×10^{-6} M (Figure 4B), it is difficult to detect the existence of the Fe(**1**)$_3$ species (the value falling below 1%). The pFe^{3+} value of

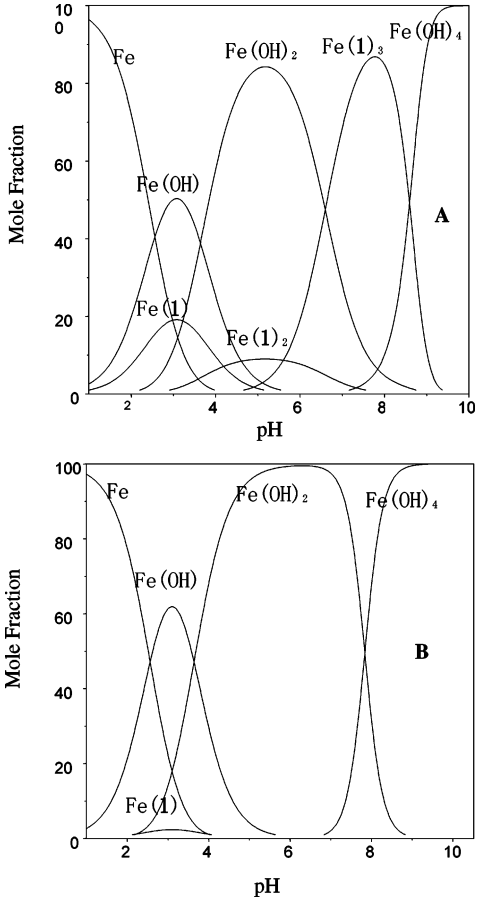

Figure 4. Speciation plots of iron−**1** complexes: (A) $[Fe^{3+}] = 10^{-6}$ M, [**1**] $= 10^{-4}$ M, 25 °C, $\mu = 0.1$ M (KCl); (B) $[Fe^{3+}] = 10^{-6}$ M, [**1**] $= 5 \times 10^{-6}$ M, 25 °C, $\mu = 0.1$ M (KCl).

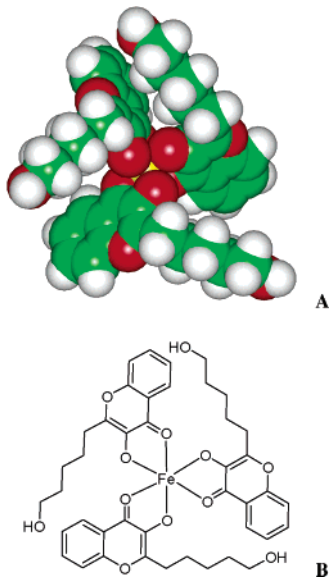

Figure 5. Simulated structure of the bidentate mode binding iron(III) complex of **1**: (A) space filling mode; (B) sticks mode.

compound **1** is 14.6, which is much lower than that of orally active iron chelator deferiprone **3** which possesses a pFe^{3+} value of 19.4.[11]

Conclusion

No evidence has been obtained for the tridentate mode of iron(III) coordination by **1** over the pH range 2−9. The most obvious reason for this observation is that the side chain aliphatic oxygen remains protonated over this pH range and is therefore an ineffective iron(III) ligand. In fact, **1** binds iron(III) in a bidentate mode (Figure 5) with relatively weak affinity.

Materials and Methods

The automatic titration system used in this study is comprised of an autoburet (Metrohm Dosimat 765 l mL syringe) and Mettler Toledo MP230 pH meter with Metrohm pH electrode (6.0133.100) and a reference electrode (6.0733.100). A 0.1 M KCl electrolyte solution was used to maintain the ionic strength. The temperature of the test solutions was maintained in a thermostatic jacketed titration vessel at 25 ± 0.1 °C using a Techne TE-8J temperature controller. The solution under investigation was stirred vigorously during the experiment. A Gilson Mini-plus#3 pump with speed capability (20 mL/min) was used to circulate the test solution through a Hellem quartz flow cuvette. For stability constant determinations a 50 mm path length cuvette was used, and for pK_a determinations a cuvette path length of 10 mm was used. The flow cuvette was mounted on a HP 8453 UV−visible spectrophotometer. All the instruments were interfaced to a computer and controlled by a Visual Basic program. Automatic titration and spectral scans adopted the following strategy: The pH of a solution was increased by 0.1 pH unit by the addition of KOH from the autoburet. When pH readings varied by <0.001 pH unit over a 3 s period, an incubation period was activated. For pK_a determinations, a period of 1 min was adopted; for stability constant determinations, a period of 5 min was adopted. At the end of the equilibrium period, the spectrum of the solution was then recorded. The cycle was repeated automatically until the defined end point pH value was achieved. All the titration data were analyzed by pHab.[12]

Iron chloride (17.906 mM in 1% HCl, atomic absorption standard, Aldrich) was utilized in this study. 4-Morpholinepropanesulfonic acid (MOPS, pH 7.4) from BDH, analytical grade volumetric HCl (0.201 M) from Aldrich, analytical reagent grade HCl (37%) and KOH (10M) ampules from Fisher, and HPLC grade water and methanol (Fisher) were used in the preparation of all solutions.

3-Hydroxy-2-(5-hydroxypentyl)-4*H*-chromen-4-one **1** was synthesized as previously reported[9] and characterized by NMR, mass spectra, and elemental analysis (see Supporting Information).

pK_a Determination. The pH electrodes were calibrated by titrating a volumetric standard strong acid HCl (0.160 mL, 0.201 M) in KCl (20 mL 0.1 M) with KOH (0.1 M) under an argon gas atmosphere at 25 °C. The E_0, slope of electrode, and pK_w of the solution were both optimized by GLEE.[13] Following electrode calibration, 20.04 mL of 0.1 M KCl solution with 51.1 μM **1** at an initial pH value of 5.8 were alkalimetrically titrated to pH 11.1. The spectra of **1** and the titration experimental data at different pH values were analyzed by refining the extinction coefficient of the protonated and deprotonated species. Both the free ligand **1** and its iron(III) complex possess a low solubility in water. To extrapolate the observed values of the affinity constant measurement to aqueous solution, three experiments were performed in 0, 0.4, and 0.5 methanol:water mixtures (volume ratio).

pH-Dependent UV Spectrophotometric Titration of Iron(III)−Ligand Complexes. Methanol:water mixtures (0.4, 0.5, and 0.66 volume ratios) were used to guarantee that the complex did not precipitate during the experiment. Electrode calibration data and pK_a values of the corresponding solutions were adopted when refining the stability constants. All experiments were carried out under an argon gas atmosphere. To prepare 10 mL of a 0.4:0.6 methanol:water volume ratio mixture, methanol (8 mL) and water (12 mL) were mixed together; KCl (0.7455 g) was added to maintain the ionic strength at 0.1. Compound **1** and atomic absorption standard iron chloride were added to give an initial concentration of **1** of 261.6 μM and an initial iron(III) concentration of 52.4 μM. The solution was acidified with analytical reagent grade HCl (37%). The experiment start volume was 10.28 mL at pH 1.34. This solution was then alkalimetrically titrated to pH 8.092, which yielded 45 spectra for analysis.

Electrospray Ionization Mass Spectrometry. Samples were directly infused into a LCQ Deca XP ion trap mass spectrometer (ThermoFinnigan, San Jose, CA) using a 250 μL syringe at a flow rate of 5.0 μL/min. The instrument was operated in positive ion mode employing the following conditions: source voltage, 4.5 kV; capillary voltage, 25 V; capillary temperature, 100−300 °C; tube lens voltage, 10 V.

JOB Plot of 3-Hydroxy-2-(5-hydroxypentyl)-4*H*-chromen-4-one with Iron(III). Different ratios of iron(III) and **1** were prepared while keeping the total concentration of metal plus ligand at 0.2 mM. A 25 mM MOPS buffer with 50% methanol (v/v) was used to maintain pH at 7.4. The solution in the UV cuvette was mixed by pipet several times and then sealed in order to prevent evaporation. The spectrum of each solution was recorded at 15 min, 35 min, 1.5 h, 3.5 h, and 20 h. All experiments were carried out at 25 °C.

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Supporting Information Available: Elemental analysis, NMR, UV, molar extinction coefficients, pK_a, and stability constants data. This material is available free of charge via the Internet at http://pubs.acs.org.

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