Enhancement of Iron Excretion via Monoanionic 3-Hydroxypyrid-4-ones

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The ability of 3-hydroxypyrid-4-ones bearing either a carboxylic acid or sulfonic acid group to mobilize iron into the bile and urine of normal rats has been examined and compared with that produced by 1,2-dimethyl-3-hydroxypyrid-4-one (L1). The compounds tested were 3-hydroxy-1-methyl-4-oxopyridine-6-carboxylic acid and 1-[3-hydroxy-6-(hydroxymethyl)-4-oxopyridyl]-2-ethanesulfonic acid, whose synthesis, biological activity, and X-ray crystallographic properties are described. Although estimates of activity, based on polarity and membrane permeability, predict such compounds to be ineffective, they were found to have an iron-mobilizing ability similar to that of the compounds which do not bear any charge at physiological pH when given parenterally. When given orally, the 3-hydroxypyrid-4-one containing a carboxylate group enhanced the urinary excretion of iron, while the sulfonate analog did not substantially increase the excretion of iron in the urine relative to the controls. The results obtained here suggest that the previous emphasis on the preparation of 3-hydroxypyrid-4-ones that are electrically neutral at physiological pH is unnecessarily restrictive and that the presence of an appropriate group bearing a single negative charge is consistent with a high level of activity. It is proposed that such negatively charged molecules may gain access to the interior of cells in both the kidney and the liver via monoanionic transport systems. Such compounds may prove to be less toxic than the neutral 3-hydroxypyrid-4-ones.

Introduction

The introduction of the 3-hydroxypyrid-4-one iron chelators by Hider et al., Kontoghiorghes et al., Silver et al., and other researchers[1-9] has been a major advance in the quest to find oral, nontoxic chelators for the removal of iron. While the use of the current chelator, desferrioxamine, has significantly extended the life of individuals with hereditary disorders such as thalassemia,[10-12] it is unfortunately not effective when given orally, due to low bioavailability. Thus, it must be given by repeated subcutaneous injection over long periods, resulting in low patient compliance. As the 3-hydroxypyrid-4-ones can be administered orally, are relatively easy to prepare, are water-soluble, and are capable of enhancing both the urinary and fecal excretion of iron in animal models and humans, they represent a potentially ideal solution to the problem of iron overload.

While numerous preliminary studies have been performed on 1,2-dimethyl-3-hydroxypyrid-4-one, L1 (2), and related 3-hydroxypyrid-4-ones,[13-16] the use of these compounds has been recently called into question by toxicity studies[17] and previous data linking L1 to the production of autoantibodies such as antinuclear antibodies (ANA)[18,19] and the ability of many 3-hydroxypyrid-4-ones to inhibit the enzyme tyrosine hydroxylase.[3,20] Despite some evidence to the contrary,[21,22] current data would suggest that the most promising of these comopunds, L1, would be too toxic for humans due to its lipophilic character,[17] despite a $K_{par} = 0.21$.[23] Further studies by Kontoghiorghes et al.[24] have also shown that increasing the lipophilic nature of these compounds, while facilitating the passage of the chelators through cellular membranes, increases their toxicity.

While the design criteria which lead to more effective iron-mobilizing agents are known in part,[23,11,25-27] such treatments either concentrate on methods for the enhancement of the conditional stability constant[23,28,29] or assume that the chelating agent must pass through the lipid portion of the cellular membrane in order to gain access to intracellular deposits of a toxic metal ion. Recently, we have collected evidence that indicates that chelating agents bearing only a single negative charge may gain access to certain intracellular sites when the cell membrane contains anion transport systems,[30,31] and this may provide a way to significantly enhance chelator organ specificity. These studies showed that the removal of cadmium from intracellular sites in the kidney and liver by certain dithiocarbamates and monoesters of *meso*-2,3-dimercaptosuccinic acid could be suppressed by the coadministration of compounds known to interfere with monoanion-transport systems in the cellular membranes of these organs. The demonstrated ability of desferrithiocin, a monocarboxylic acid, to mobilize iron from intracellular sites encouraged us in this search, and the structural features which govern the ability of desferrithiocin to mobilize iron have been fully delineated by Bergeron and co-workers.[26] These investigators clearly demonstrated that desferrithiocin and a number of its analogs which contained a carboxyl group were effective in enhancing the biliary excretion of iron in the rat. This led us to question whether iron-chelating agents of the 3-hydroxypyrid-4-one-type bearing a single negative charge would be capable of enhancing the excretion of iron. The following study examines the ability of these monoanionic chelators to mobilize iron in comparison with the neutral standard, L1.

Design and Synthesis

Design. Preliminary toxicity studies with the orally available L1 have suggested that it may be unsuitable for use in humans, and this has prompted a reevaluation of the design of 3-hydroxypyrid-4-one iron chelators, which has traditionally focused on two structural parameters: lipophilicity and charge. The work of Hider and coworkers[27] has focused on improving the lipophilic balance of *neutral* chelators in order to facilitate their passage through the membrane. Such work yielded the most

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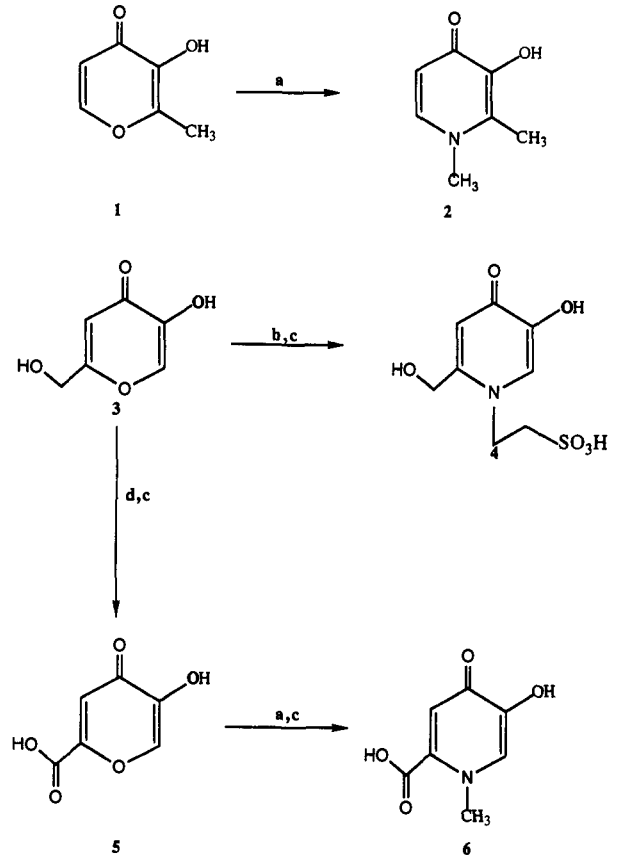
effective pyridinone chelator to date, the ethyl derivative of ethyl maltol, 1,2-diethyl-3-hydroxypyrid-4-one.[27] While the results of this work showed that the excretion of iron was significantly enhanced relative to L1, Kontoghiorghes et al.[24] found that among the iron chelators examined, this compound was also the most toxic. This led us to question whether these compounds, through their lipophilic nature, penetrate numerous other membranes, after which they participate in undesired metabolic interactions.

Problems of organ specificity and toxicity were compounded by the results of an iron-binding competition conducted between 3-hydroxypyrid-4-ones derived from the hydrophilic kojic acid and EDTA;[32] it became apparent that the removal of Fe from EDTA is a relatively slow process. As 3-hydroxypyrid-4-one chelators are excreted quickly *in vivo* and their attainment of equilibrium with endogenous iron compounds is slow, it seemed clear that other design factors must be considered which enhance the speed of the chelator reaching the iron-storage site. To this point, while neutral, lipophilic chelators that can quickly penetrate the cell membrane may function as superior iron chelators, their specificity has been sacrificed in the process. If organ specificity could indeed be achieved in conjunction with rapid chelate transport, this would reduce toxicity in two major ways: first, by reducing substantially the enormous molar ratio of chelator that must be administered to compensate for the slow attainment of equilibrium and high rate of excretion and, second, by preventing the chelators from interfering with metabolism in unaffected tissues which do not contain appreci able stores of iron.

On examination of previous work regarding organ specific, monoanionic transport systems,[33-36] it appeared that, fortuitously, these systems offered a way to reconcile problems regarding organ specificity, toxicity, and equilibria. Numerous reports which emphasize using a chelating agent which is electrically neutral at physiological pH,[10,11,14,23,26-28] assume that the pyridinones must diffuse through the lipophilic portion of the membrane. However, convincing evidence to the contrary has been collected in chelation studies with cadmium,[31] and the presence of organic anion-transport systems in both the liver[34] and the kidney[33,35,36] have been thoroughly documented. As the liver is one of the major storage sites for iron, the incorporation of appropriate negatively charged groups into a 3-hydroxypyrid-4-one moiety should then facilitate the transport of the chelating agent into hepatic intracellular deposits of iron. Additional evidence suggests that some of the anion-transport systems in the kidney are closely related to, or identical with, some of those in the liver, and this raises the possibility that suitable anionic chelating agents may be capable of enhancing both the biliary and renal excretion of iron.

To examine the potential of exploiting monoanionic transport systems for the removal of iron, two 3-hydroxypyrid-4-ones were synthesized which bear a single negative charge at physiological pH. As shown in Scheme 1, we first synthesized the taurine (2-aminoethanesulfonic acid) derivative **4** of kojic acid (**3**). By oxidation of **3**, comenic acid (**5**) was obtained and then the methyl derivative **6**. Structurally, **6** is similar to L1, (**2**), as both possess a methyl substituent attached to the ring nitrogen. The compounds differ only in that the neutral **2** contains an additional methyl substituent at ring position 2, while ring position 6 of **6** is occupied by a carboxylic acid moiety. Thus, the effect of the negative charge on chelator effectiveness

Scheme 1[a]



[a] (a) CH_3NH_2, H_2O, N_2, 100 °C; (b) $NH_2(CH_2)_2SO_3H$, H_2O, $NaHCO_3$, N_2, 100 °C; (c) concentrated HCl; (d) O_2, Pd/C 5%, NaOH, H_2O, 10 °C.

relative to **2** could be examined, as other structural components remained nearly constant between the two molecules. Additionally, the *in vivo* effect of the sulfonate versus the carboxylate group could also be observed, though other factors such as differences in lipophilicity would make precise comparisons difficult.

Synthesis and Characterization. The synthesis of 3-hydroxypyrid-4-ones, using the one-step method first reported by Kleipool and Weibaut[37] and later by Kontoghiorghes,[38] is accomplished in a facile manner as shown in Scheme 1. Mechanistically, these reactions occur through a double Michael reaction, utilizing nitrogen as a nucleophile. The free nitrogen of the amine first attacks the pyran, resulting in ring opening, followed by a second attack by the nitrogen which closes the ring and liberates H_2O. The syntheses of **2**, **5**, and **6** were all run as one-step reactions in water followed by acidification in the case of **5** and **6**. In synthesizing derivative **4**, taurine was first converted into the sodium sulfonate salt and then reacted to circumvent formation of the zwitterion. For **6**, a large excess of methylamine was used, which was sufficient to form the carboxylic acid salt as well as to conduct the Michael reaction. Thus, for convenience, the reaction was performed in one step, and the mixture was then acidified. As **5** was not commercially available, it was obtained directly from **3**.

In synthesizing numerous pyridinone derivatives, it was observed that some of these compounds have a tendency to form the Schiff base, as reversible Schiff base formation readily competes with the Michael reaction.[32] Using solvent systems in which water is either absent or a minor component will frequently result in the formation of the Schiff base, and thus, all reactions were performed in solely

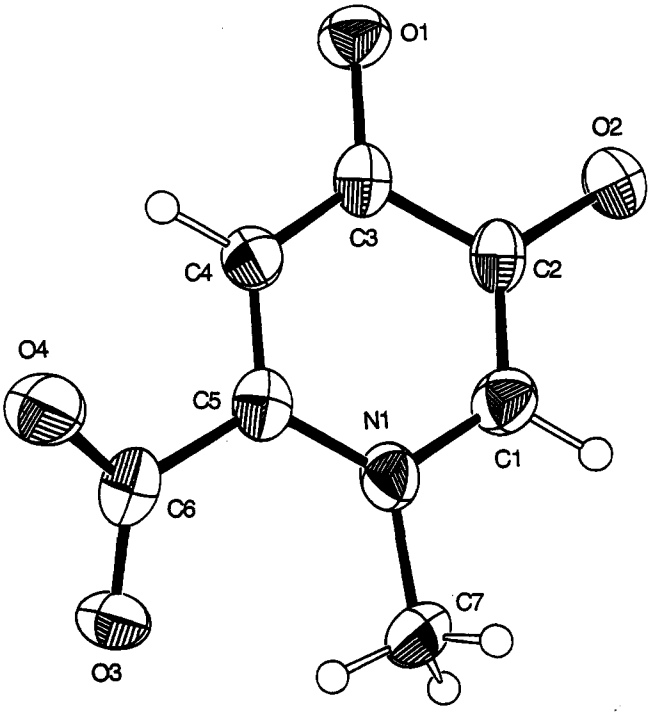


Figure 1. ORTEP diagram of 3-hydroxy-1-methyl-4-oxopyridine-6-carboxylic acid (**6**).

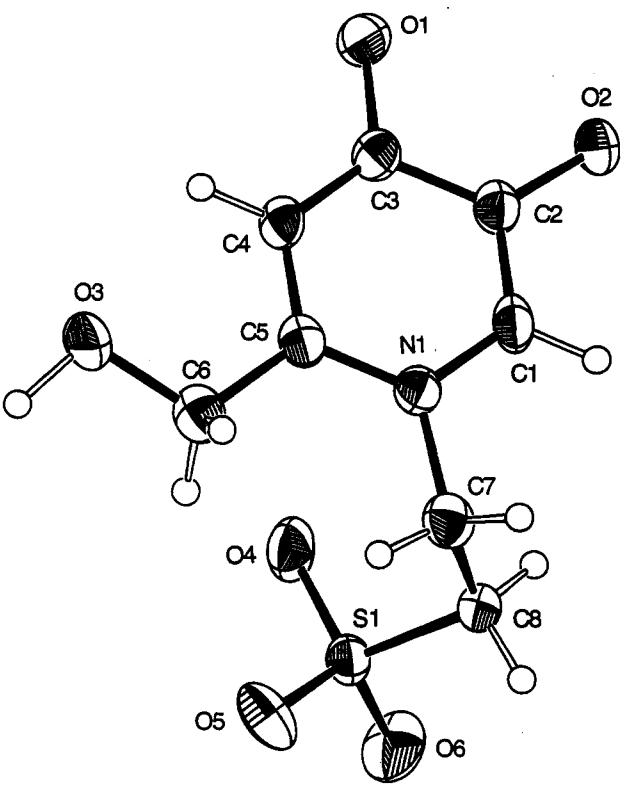


Figure 2. ORTEP diagram of 1-[3-hydroxy-6-(hydroxymethyl)-4-oxopyridyl]-2-ethanesulfonic acid (**4**).

aqueous media. As all of the starting amines and pyrans were soluble in water or converted into water-soluble acid salts, solvent constraints posed little difficulty.

Concern over Schiff base formation prompted us to search for a definitive way to distinguish between the imine and the substituted pyridinone, for the imine structure is expected to undergo extensive hydrolysis following oral administration. Spectroscopic techniques such as IR and proton NMR provide little aid in differentiating between Schiff bases and substituted pyridinones; indeed, the low melting points of the Schiff bases have been the primary means of characterizing the isomers. Unequivocal structural determination was finally accomplished through X-ray diffraction to give the structures in Figures 1 and 2, and these confirmed our predictions.

The structures of **4** and **6** reveal substitution of the nitrogen in the ring yielding the corresponding pyridinone from the parent pyran. While the 3:1 Fe(III) chelate structures of **1**, **2**, and related pyridinone-chelating agents have already been determined by X-ray diffraction,[32,39-41] this study examined the novel pyridinones uncomplexed to iron. It is interesting to note that the bond distances of the ring atoms within the molecules were not distinguishable at the 3σ level despite the potential differences due to substituent effects. Charalambous et al.[40] have placed considerable emphasis on the similarity of the C–O bond distances of the ligands in the iron complex with **2**, as the C–O distances are between what one would normally predict for C–O single and double bonds. Such data suggests that the ligand displays a "partially" aromatic character when chelated to iron, and this was accounted for by resonance structures that involve an aromatic pyridinone ring. However, the ligands still retained some ketonic character, as indicated by the variation in the C–O bond lengths. Significantly, the same distances in these two free ligands are actually *equivalent*, even between the two molecules. These C–O distances were identical at 1.34(1) Å; this equivalance, together with the similarity in bond distances between atoms within the ring, suggests that the free ligand exists in a nearly aromatic conformation.

The purification of the pyridinones remained the primary obstacle in these syntheses, though low yields are not uncommon for these one-step reactions.[38] Decomposition products formed during reflux and acidification substantially reduce the yield after the necessary recrystallization and/or charcoal filtration. While analysis of **5** showed it to be of nearly theoretical composition, it displayed a characteristic orange color that has been previously noted in the literature as being exceedingly difficult to remove, as comenic acid binds trace amounts of iron avidly.[42] The compound was found to contain 57 ppm of Fe, which resulted in the formation of a small amount of the highly colored iron complex. As the commercially available **3** contained 73 ppm of Fe, it seemed likely that this was the source of trace iron in the comenic acid. Further purification of the comenic acid was not attempted due to the considerable difficulty in removing the minute amount of iron.

Biological Evaluation

To examine the iron-mobilizing activity of these compounds, we have determined the biliary excretion of iron in an unloaded rat model patterned after the model used by Pippard et al.[43] and Bergeron et al.,[26] and this is shown in Table 1. We have also determined the effect of these compounds on the urinary excretion of iron in an unloaded rat model with the animals serving as their own controls. The rats were placed in standard metabolic cages, and their urine was collected for 15 h (5 p.m.–8 a.m.) following the iv or po administration of 1 mL of distilled water, or 1 day later, an equal volume containing 0.2 mmol/kg for iv and 1 mmol/kg for po of the chelating agent under study. The urinary excretions following intravenous and oral administration of the chelating agents are shown in Tables 2 and 3, respectively.

Table 1. Biliary Excretion of Iron Induced by Pyridinones and Pyrans (μg of Fe/h)[a] Administered iv

compd	N	control period	treatment period
2	5	0.36 ± 0.07	4.78 ± 2.08[b,c]
3	3	0.31 ± 0.05	0.28 ± 0.10
4	5	0.35 ± 0.07	9.96 ± 3.97[b,c]
5	3	0.35 ± 0.09	0.46 ± 0.04
6	5	0.34 ± 0.04	5.87 ± 0.89[b,c]

[a] The bile ducts of normal rats were cannulated, and the bile was collected for 30 min to obtain the control biliary iron excretion rates for each rat. The animals were then given the indicated compound at 0.2 mmol/kg iv, and the bile was collected for a further 2 h. The mass of each bile sample was measured, and its iron content was determined by atomic absorption spectrometry. The results reflect the effect of pentobarbital or pentobarbital plus the chelating agent. The control animals received only the pentobarbital. [b] Significantly different from control values, $p \leq 0.05$. [c] Not significantly different from each other ($p \geq 0.05$).

Table 2. Urinary Iron Excretion Induced by iv Administration of the 3-Hydroxypyrid-4-ones (ng of Fe/15 h)[a]

compd	N	control period	treatment period
2	6	490 ± 170	8370 ± 3110[b]
4	3	504 ± 220	2200 ± 660[b,c]
6	3	410 ± 77	6830 ± 800[b]

[a] Each animal was given an initial iv injection of 1 mL of distilled water/200 g of body weight, and the urine was collected over a period of 15 h. The urine was then analyzed for iron. The next day, each animal was given an iv injection of 0.2 mmol/kg of the indicated compound in 1 mL of deionized H_2O, and the urine was again collected for 15 h followed by iron analysis. [b] Significantly different from control values, $p \leq 0.05$. [c] Significantly different from the value for compound 6, $p \leq 0.05$.

Table 3. Urinary Iron Excretion Induced by po Administration of the 3-Hydroxypyrid-4-ones (ng of Fe/15 h)[a]

compd	control period	treatment period
2	400 ± 110	17 400 ± 6400[b]
4	296 ± 110	350 ± 12[d]
6	497 ± 50	6740 ± 370[c]

[a] Rats were removed from their food supply at 5 p.m. and given 1 mL of distilled water po. Their urine was collected at 8 a.m. the next morning and analyzed for iron, at which time the animals were returned to their food supply. At 5 p.m., the animals were removed from their food supply and given 1 mL of distilled water containing 1 mmol/kg of the indicated chelating agent po. The urine was again collected at 8 a.m. the next morning and analyzed for iron, at which time the animals were returned to their food supply. [b] Significantly different from all other groups, $p \leq 0.05$. [c] Significantly different from all other groups, $p \leq 0.05$. [d] Not significantly different from control groups.

Biological Results. The initial biological studies on the monoanionic chelators suggest that the introduction of a negative charge does not interfere with the activity of the 3-hydroxypyrid-4-ones and may actually *enhance* the amount of iron excreted in the bile. The biliary excretion data in Table 1 show that both 4 and 6 are at least statistically equivalent in effectiveness to 2 when given iv and that in general, the N-substituted compounds increased the biliary iron excretion by a factor of at least 10-fold over the parent compounds 3 and 5. While the urinary excretion in Table 2 is again similar for compounds 2 and 6, the amount of iron excreted in the urine due to 4 drops significantly. However, when given orally, 2 is at least twice as effective as 6, while 4 shows almost no activity as seen in Table 3.

The enormous drop in urinary iron excretion for the oral administration of 4 can be explained by the poor absorption of sulfonates in the gastrointestinal tract. However, such data also point to shortcomings in the ability to measure iron chelator effectiveness by the oral method alone. Notably, in looking at chelator 4, one notes that the iron excreted is significantly higher in the bile as opposed to in the urine when the chelator is given iv. This trend is seen to an extreme degree in the analysis of the urine in the oral administration, and this data alone may lead one to conclude that such a chelator has almost no activity. It is therefore evident that studies which measure only the amount of iron excreted in the urine provide only partial data and that chelators which enhance biliary excretion may be overlooked.

Conclusion

The data collected here indicate that the presence of a group with a single negative charge from either a carboxylate or sulfonate group does not interfere with the ability of parenterally administered 3-hydroxypyrid-4-ones to enhance the biliary excretion of iron. Under these conditions, these compounds also enhance the urinary iron excretion *in vivo*. While the carboxylic acid analog 6 enhanced the urinary excretion of iron when given orally, the sulfonic acid analog 4 showed almost no oral activity. As these compounds generally displayed activity of the same sort as the neutral L1, the data support the hypothesis that the monoanionic iron chelators are actively transported to intracellular deposits of iron. The design of newer iron-chelating agents may incorporate features which facilitate passage to intracellular sites of iron via monoanionic transport systems, as the constraint regarding the necessity of neutrality would not appear critical. Through the manipulation of such factors as polarity and functional groups, a more appropriate lipophilic balance may be achieved which will further enhance the excretion of iron and at the same time reduce the toxicity of the pyridinones. Monoanionic iron-chelating agents may eventually be designed for uptake by a wide range of anionic transport systems in such organs as the liver, the kidney, and the heart. The hypothesized relationships between iron overload and cardiomyopathies,[44,45] in addition to diseases such as thalassemia and sickle cell anemia, whose treatment leads to toxic levels of iron, indicate that iron-chelating agents designed for uptake by the liver, kidney, and heart might have a wide range of practical clinical applications.

Experimental Section

Maltol was obtained from Aldrich Chemical Co., Milwaukee, WI, and kojic acid (99+%) was obtained from Tokyo Kasei Chemical Co., Portland, OR. Both of these were used without further purification. Sprague–Dawley rats were obtained from Sasco, Omaha, NB.

1,2-Dimethyl-3-hydroxypyrid-4-one (L1) (2) was prepared by a minor modification of a method published previously.[9]

3-Hydroxy-4-oxo-4*H*-pyran-6-carboxylic acid (comenic acid) (5) was prepared from kojic acid in 69% yield by the method of Tate et al.[42]

1-[3-Hydroxy-6-(hydroxymethyl)-4-oxopyridyl]-2-ethanesulfonic Acid (4). Taurine (22.53 g, 0.18 mol) was dissolved in H_2O (100 mL) with $NaHCO_3$ (18.75 g, 0.22 mol) and stirred until CO_2 was no longer released. The mixture was then added to a solution of 3 (30 g, 0.21 mol) in H_2O (300 mL) and refluxed under N_2 for 9 h. After cooling, the mixture was acidified with concentrated HCl to a pH of 1. The volume was reduced (150 mL), and the flask was placed in the refrigerator for 12 h. The crystals were filtered and washed with acetone and ether and then purified by charcoal filtration in H_2O (300 mL). Subsequent rinsing with boiling H_2O (50 mL) increased the volume to 350 mL. Following filtration, 8 g of 4 was isolated (15.3%) as golden brown crystals: ^1H NMR (D_2O) δ 8.2 (s, 1H), 7.3 (s, 1 H), 4.8 (s, 2 H), 4.6 (t, 2 H), 3.4 (t, 2 H). Anal. ($C_8H_{11}NO_6S$) C, H, N.

3-Hydroxy-1-methyl-4-oxopyridine-6-carboxylic Acid (6). A mixture of 40% methylamine (75 mL, 29.8 g, 0.96 mol) and 5

Table 4. Summary of Crystal Data and Intensity Collection

	3-hydroxy-1-methyl-4-oxopyridine-6-carboxylic acid	1-[3-hydroxy-6-(hydroxymethyl)-4-oxopyridyl]-2-ethane-sulfonic acid
formula	$C_7H_7NO_4$	$C_8H_{11}NO_6S$
formula weight	169.14	249.24
crystal system	orthorhombic	monoclinic
a (Å)	12.91(1)	5.707(5)
b (Å)	4.51(8)	15.914(5)
c (Å)	12.0(1)	10.879(3)
α (deg)	90	90
β (deg)	90	94.08(4)
γ (deg)	90	90
volume (Å^3)	698(3)	985.5(9)
$F(000)$	352	520
μ(cm^{-1})	11.06	3.27
space group	$Pca2_1$	$P2_1/n$
Z	4	4
crystal dimensions (mm)	0.225 × 0.550 × 0.125	0.200 × 0.240 × 0.530
temperature (°C)	20 ± 1	20 ± 1
radiation (Å)	Cu Kα (0.71073)	Mo Kα (1.5418)
data collection mode	ω-scan	ω-scan
scan speed (deg/min)	4	8
background counts	stationary counts; peak/background counting time = 2:1	stationary counts; peak/background counting time = 2:1
2θ limits (deg)	$6.0° \leq 2\theta \leq 119.6°$	$6.0° \leq 2\theta \leq 50.2°$
total reflections collected	654	2000
no. of unique intensities	654	1815
no. of intensities with $F > 3.00(F)$	461	1118
R_f/R_w	0.061, 0.076	0.041, 0.05

Table 5. Intramolecular Distances (Å) and Bond Angles (deg) Involving the Non-Hydrogen Atoms for 3-Hydroxy-1-methyl-4-oxopyridine-6-carboxylic Acid

Distances			
O(1)–C(3)	1.34(1)	N(1)–C(7)	1.49(1)
O(2)–C(2)	1.34(1)	C(1)–C(2)	1.35(1)
O(3)–C(6)	1.25(1)	C(2)–C(3)	1.42(1)
O(4)–C(6)	1.25(1)	C(3)–C(4)	1.37(1)
N(1)–C(1)	1.35(1)	C(4)–C(5)	1.36(1)
N(1)–C(5)	1.36(1)	C(5)–C(6)	1.53(1)
Angles			
C(1)–N(1)–C(5)	119.9(8)	C(2)–C(3)–C(4)	117.0(8)
C(1)–N(1)–C(7)	116.1(8)	C(3)–C(4)–C(5)	123.3(9)
C(5)–N(1)–C(7)	123.9(8)	N(1)–C(5)–C(4)	118.2(8)
N(1)–C(1)–C(2)	123.4(8)	N(1)–C(5)–C(6)	121.1(8)
O(2)–C(2)–C(1)	123.2(8)	C(4)–C(5)–C(6)	120.7(8)
O(2)–C(2)–C(3)	118.8(8)	O(3)–C(6)–O(4)	127.5(8)
C(1)–C(2)–C(3)	118.0(8)	O(3)–C(6)–C(5)	117.2(7)
O(1)–C(3)–C(2)	118.4(7)	O(4)–C(6)–C(5)	115.2(8)
O(1)–C(3)–C(4)	124.5(8)		

Table 6. Intramolecular Distances (Å) and Bond Angles (deg) Involving the Non-Hydrogen Atoms for 1-[3-Hydroxy-6-(hydroxymethyl)-4-oxopyridyl]-2-ethanesulfonic Acid

Distances			
S(1)–O(4)	1.448(3)	N(1)–C(5)	1.355(5)
S(1)–O(5)	1.459(3)	N(1)–C(7)	1.484(5)
S(1)–O(6)	1.432(3)	C(1)–C(2)	1.355(6)
S(1)–C(8)	1.774(4)	C(2)–C(3)	1.407(6)
O(1)–C(3)	1.329(5)	C(3)–C(4)	1.383(6)
O(2)–C(2)	1.349(5)	C(4)–C(5)	1.377(6)
O(3)–C(6)	1.408(5)	C(5)–C(6)	1.504(6)
N(1)–C(1)	1.356(5)	C(7)–C(8)	1.518(6)
Angles			
O(4)–S(1)–O(5)	111.5(2)	C(1)–C(2)–C(3)	118.7(4)
O(4)–S(1)–O(6)	113.1(2)	O(1)–C(3)–C(2)	116.7(4)
O(4)–S(1)–C(8)	107.2(2)	O(1)–C(3)–C(4)	125.0(4)
O(5)–S(1)–O(6)	113.4(2)	C(2)–C(3)–C(4)	118.3(4)
O(5)–S(1)–C(8)	104.3(2)	C(3)–C(4)–C(5)	121.3(4)
O(6)–S(1)–C(8)	106.7(2)	N(1)–C(5)–C(4)	118.8(4)
C(1)–N(1)–C(5)	120.8(4)	N(1)–C(5)–C(6)	119.8(4)
C(1)–N(1)–C(7)	115.8(3)	C(4)–C(5)–C(6)	121.4(4)
C(5)–N(1)–C(7)	123.3(3)	O(3)–C(6)–C(5)	108.1(4)
N(1)–C(1)–C(2)	122.0(4)	N(1)–C(7)–C(8)	113.0(3)
O(2)–C(2)–C(1)	119.1(4)	S(1)–C(8)–C(7)	114.6(3)
O(2)–C(2)–C(3)	122.2(4)		

(30 g, 0.192 mol) was prepared in H_2O (300 mL). The mixture was refluxed for 8.5 h, after which 100 mL of solvent and excess amine were removed. The pH was reduced to 1 with concentrated HCl, and acetone (10–15 mL) was added to induce crystallization. The flask was placed in the refrigerator for 12 h, and the resulting brown product was filtered and washed with acetone and ether. The crystals were dissolved in a 2:1 mixture of H_2O/MeOH (600 ML) and boiled for 20 min, and the undissolved solid was filtered as impurity. The solution was allowed to stand, and the resulting beige crystals weighted 6.5 g (20%): ^{1}H NMR (D_2O) δ 7.0 (s, 1 H), 6.4 (s, 1H), 3.4 (s, 3 H). Anal. ($C_7H_7NO_4$) C, H, N.

Animal Studies. Female Sprague–Dawley rats (190–220 g) from Sasco, Omaha, NB, were used in the animal studies. The animals were provided with food and water *ad libitum* except during the urine collection periods, in which they were allowed access to water only. The animals were housed in an AAALAC approved facility.

Solutions for injection were prepared just prior to administration in deionized water. Solution concentrations were adjusted so that 1 mL of injectate/kg of body weight was administered. Sodium bicarbonate was used to adjust the pH to approximately 7. All solutions given intravenously were administered via tail vein injections while the animals were under ether anesthesia.

Biliary Iron Excretion. Bile ducts of untreated rats were cannulated using PE-10 tubing while the animals were anesthetized. The cannulation tubing was then routed to a dorsal access point just behind the animal's head, and the incision used to gain access to the bile duct was sutured closed. The animal was fitted with a Velcro jacket, and bile samples were collected in 10- × 75-mm polystyrene tubes which were attached to the jackets after the animals recovered from the anesthesia.

Urinary Iron Excretion. Data on urinary iron excretion were collected for both intravenous and oral administration of the chelating agents. Distilled water (1 mL) was administered to previously untreated animals, and the animals were placed in Nalgene metabolic cages. Urine was collected for 15 h and analyzed for iron content. This data served to provide control levels of iron in the urine. The animals administered chelator iv were given 0.2 mmol/kg of the chelating agent in 1 mL of water, and po administration involved 1.0 mmol/kg of chelator in 1 mL of water. Urine was again collected for 15 h and then analyzed for iron. During urine collection periods, the animals were allowed access only to tap water.

Iron Analysis. Bile and urine samples were analyzed directly using a Perkin-Elmer 4000 atomic absorption spectrometer equipped with a Perkin-Elmer 400 graphite furnace. The instrument was operated using standard conditions with deuterium background correction.

X-ray Crystallography. All measurements were performed on Rigaku AFC6S diffractometers at Vanderbilt University with graphite monochromated Mo Kα radiation for 4 and Cu Kα radiation for 6. The structure of 6 was solved by direct methods (SIR) as was 4 (SHELX-86), though an empirical absorption correction (psi scan) was only applied to the latter. All non-hydrogen atoms were refined anisotropically. The ORTEP diagrams for both are shown in Figures 1 and 2. Summaries of the crystal data and intensity collection are shown in Table 4 for 4 and 6, and intramolecular distances and bond angles for 6 and 4 are shown in Tables 5 and 6, respectively.

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Supplementary Material Available: Tables 7–10 of the Uij and positional parameters for compounds 4 and 6 (4 pages). Ordering information is given on any current masthead page.

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