Communications to the Editor

O-TRENSOX: A Promising Water-Soluble Iron Chelator (Both Fe^{III} and Fe^{II}) Potentially Suitable for Plant Nutrition and Iron Chelation Therapy

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Received April 14, 1995

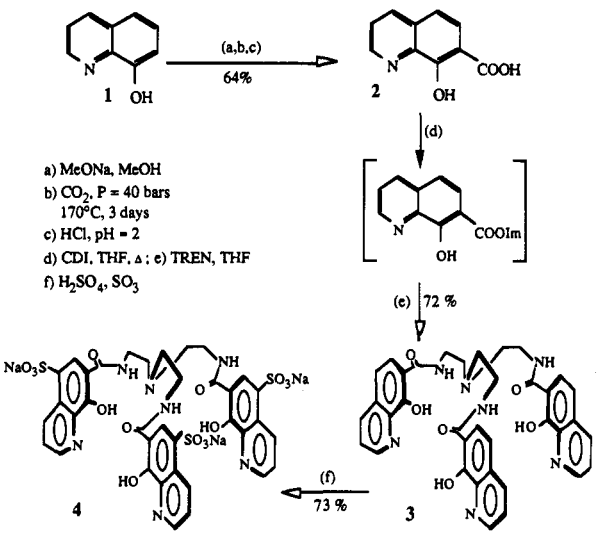
Although iron is one of the most common elements on Earth, its availability to living organisms is limited because of its extremely low solubility.[1] Microorganisms have evolved low-molecular-weight chelating agents called siderophores, which solubilize iron and facilitate its transport into the cell.[2] Plants have developed different strategies for enhancing iron acquisition, one of them using phytosiderophores.[3] Nevertheless, plants can develop syndromes of iron deficiency such as iron chlorosis, especially in calcareous soils, and solving this problem is fundamentally important for higher food production. Furthermore, iron overloading is a common and serious disease which needs iron chelation therapy.[4] A number of limitations to the use of desferrioxamine, the only approved drug for treatment of iron overload (need of parenteral administration, prohibitive price, short duration of action) underline the need for development of a truly orally effective drug.

We present here a preliminary report on O-TRENSOX (**4**, Scheme 1), a synthetic water-soluble tripodal iron sequestering agent, based on three 8-hydroxyquinoline subunits. The ferric complex exhibits highly promising properties for plant nutrition, and the free ligand seems to be well-suited for iron chelation therapy.

The synthetic pathway in Scheme 1 starts from 8-hydroxyquinoline, a very cheap precursor, which is easily transformed into a 7-carboxylated derivative, then activated (coupling of the carboxylate moieties was most conveniently accomplished using *N,N'*-carbonyldiimidazole, CDI), conjugated with a tripodal tetraamine [tris(2-aminoethyl)amine: TREN], and finally, regiospecifically sulfonated in position 5 in order to increase the acidity and the aqueous solubility.

Compounds **3** and **4** have been characterized by mass spectral data and ^1H and ^{13}C NMR spectroscopy.[5] The ligand **4** protonation constants were obtained by potentiometric titration; analysis of the data was performed using the SUPERQUAD program.[6] The complexation of iron with O-TRENSOX was investigated by potentiometric and spectrophotometric measurements. The spectrophotometric behavior of the ferric complex was studied as a function of pH in the range 0−12. This allowed us to determine the stepwise deprotonation equilibria in these complexes upon increasing pH in accordance with the potentiometric titration. The refinement of the data was processed with the LETAGROP program,[7] and the global

Scheme 1



thermodynamic constant relative to the deprotonated complex was determined: $\log \beta_{110} = 30.9$. This value of $\log \beta_{110}$ was confirmed from the spectrophotometric competition with EDTA done at pH 2.0−3.0. The electronic spectrum of Fe^{III}−O-TRENSOX (green complex) in distilled water at pH 7.5 (tris maleate buffer 50 mM) exhibits two absorption bands at 440 nm ($\epsilon = 5200\ M^{-1}\ cm^{-1}$) and 595 nm ($\epsilon = 5200\ M^{-1}\ cm^{-1}$) while the free ligand O-TRENSOX shows a maximum absorbance at 343 nm ($\epsilon = 11500\ M^{-1}\ cm^{-1}$) under the same conditions. The ferrous complex presents one absorption band at 576 nm with $\epsilon = 1700\ M^{-1}\ cm^{-1}$.

The electrochemical behavior of the ferric O-TRENSOX was studied in aqueous solution. Cyclic voltammetry experiments have shown that the system is rapid, quasi-reversible, and monoelectronic. A redox potential of +0.087 V vs NHE was measured and was found to be stable over the pH range 4.2−10.5. Taking into account the redox potentials E_c and E_f for the Fe−O-TRENSOX system and $Fe^{III}_{aq}/Fe^{II}_{aq}$ ($E_f = 0.77$ V vs NHE), respectively, the β_{110} stability constant of

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(5) Relevant physical data are as follows. **3**: fp = 101 °C. IR (KBr) 3500−2970 cm^{-1} ν(O−H), ν(N−H), $\nu(C_{sp^2}-H)$; 1620 cm^{-1} ν(C=O); 1610−1550−1461 cm^{-1} ν(C=C). ^1H NMR (DMSO-d_6, 400 MHz): δ 2.87 (br s, 6H, CH_2); 3.55 (dt, $J = 5.6$ Hz, 6H, CH_2); 7.20 (d_{AB}, $J_{AB} = 8.8$ Hz, 3H, H_6); 7.56 (dd, $J_1 = 8.3$ Hz, $J_2 = 4.1$ Hz, 3H, H_3); 7.87 (d_{AB}, $J_{AB} = 8.8$ Hz, 3H, H_5); 8.19 (dd, $J_1 = 8.3$ Hz, $J_2 = 1.4$ Hz, 3H, H_4); 8.79 (dd, $J_1 = 4.1$ Hz, $J_2 = 1.4$ Hz, 3H, H_2); 8.86 (t, $J \approx 5.0$ Hz, 3H, NH). ^{13}C NMR (acetone-d_6 + DMSO-d_6, 200 MHz, 2D "Delta hetero"): δ 38.71 (CH_2); 54.32 (CH_2); 117.62 (C_6), 123.87 (C_3), 126.74 (C_5), 136.55 (C_4); 114.11, 131.01, 139.70 ($C_{quatcycl}$); 149.22 (C_2H=N); 155.50 (C_8-OH); 167.88 (C=O). FAB^+ MS (NBA): $(M + H)^+$ 660, 515, 489, 471, 458, 446, 215, 172. FAB^- MS (NBA): $(M-H)^-$ 658, 513, 487, 470, 458, 444. HRMS FAB-(+): calcd for $C_{36}H_{34}N_7O_6$ $(M + H)^+$ 660.2570; found 660.2579. **4**: fp > 270 °C. IR (KBr): 3600−3000 cm^{-1} ν(O−H), ν(N−H), $\nu(C_{sp^2}-H)$; 1625−1546 cm^{-1} ν(C=O); 1608−1492 cm^{-1} ν(C=C); 1203 cm^{-1} ν(S=O). ^1H NMR (D_2O, pH 7.3, 200 MHz): δ (TSP) 3.16 (br.S, 6H, CH_2); 3.73 (br.s, 6H, CH_2); 7.58 (dd, $J_1 = 8.6$Hz, $J_2 = 4.3$ Hz, 3H, H_3); 8.33 (s, 3H, H_6); 8.46 (dd, $J_1 = 4.7$ Hz, $J_2 = 1.1$ Hz, 3H, H_2); 8.68 (dd, $J_1 = 8.6$ Hz, $J_2 = 1.1$ Hz, 3H, H_4). ^{13}C NMR (D_2O, pH 7.3, 400 MHz): δ 39.91 (CH_2); 55.45 (CH_2); 126.33 (C_3); 131.93 (C_6); 141.50 (C_4); 146.28 (C_2); 115.54, 123.58, 129.03, 139.12 ($C_{quatcycl}$); 164.80 (C_8-OH); 171.72 (C=O). FAB^+ MS (thioglycerol) of the sodium salt $C_{36}H_{27}N_7O_{15}S_3Na_6$: 1032 $(M + H)^+$; 1010 $(M - Na + 2H)^+$; 966 $(M - 3Na + 4H)^+$; 944 $(M - 4Na + 5H)^+$; 922 $(M - 5Na + 6H)^+$; 900 $(M - 6Na + 7H)^+$. Anal. (acidic form). Calcd (found) for $C_{36}H_{33}N_7O_{15}S_3{\cdot}4H_2O$: C, 44.48 (44.80); H, 4.25 (4.36); N, 10.09 (10.27); S, 9.88 (10.30).
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0002-7863/95/1517-9760$09.00/0

[Fe^{II}–O-TRENSOX] could be calculated at 298 K from the expression $E_f = E_c + 0.059 \log(\beta_{110}[Fe^{III}–L]/\beta_{110}[Fe^{II}–L])$, yielding $\log(\beta_{110}[Fe^{II}–L]) = 19.3$.

Since the β_{110} values do not reflect the competition of H^+ for the ligand, the pFe values provide a more direct comparison of the biological efficiency of ligands.[8] These values for O-TRENSOX are pFe(III) = 29.5 and pFe(II) = 17.9 under the conditions $[Fe]_T = 1\ \mu M$, $[L]_T = 10\ \mu M$, and pH = 7.4. Thus, O-TRENSOX is a strong complexing agent for both ferric and ferrous ions; nevertheless, the ferric oxidation state is stabilized, evidencing the preponderant role of the phenolic oxygens.

For comparison, we have synthesized the tripodal derivative (N-TRENOX) first described by Shrader[9] and its sulfonated derivative N-TRENSOX, an analog of O-TRENSOX in which the 8-hydroxyquinoline subunits are connected to the tripodal spacer at the 2-position. This ligand was found to be significantly less efficient than O-TRENSOX: a pFe(III) value of 21.6 has been obtained, which is 8 orders of magnitude lower than for O-TRENSOX. The explanation of the higher iron-sequestering properties of O-TRENSOX versus N-TRENSOX may reside in the well-characterized role of hydrogen bonding which stabilizes the ortho-amide-substituted phenolate ligands:[10] hydrogen bonding of the amide proton and the corresponding preorganization of the podand must occur with O-TRENSOX; it cannot occur with N-TRENSOX.

Contrary to the Fe–EDTA and Fe–citrate complexes, Fe^{III}–O-TRENSOX is not photoreducible: the photoreducibility of Fe(III) complexes was tested in the presence of ferrozine to allow Fe(II) quantification and to prevent its further oxidation. Under visible light, only Fe^{III}–citrate developed a fast photoreduction. The differential photosensitivity of Fe^{III}–citrate, Fe^{III}–EDTA, and Fe^{III}–O-TRENSOX was evidenced in UV light at 366 nm: Fe^{III}–TRENSOX does not generate Fe(II) when exposed to UV light. This was also controlled in the absence of ferrozine by absorption spectra of Fe–O-TRENSOX after irradiation.

In contrast to EDTA and citrate complexes, Fe^{III}–O-TRENSOX in the presence of ascorbate, or Fe^{II}–O-TRENSOX, does not induce radical damage in the presence of hydrogen peroxide as Fenton reagents (tested using supercoiled DNA as the target molecule[11]). It is very likely that the ligand provides a kinetic barrier to the electron transfer to hydrogen peroxide.

Fe^{III}–O-TRENSOX was found to be able to prevent and to reverse iron chlorosis in several plant species grown in axenic conditions.[12] The rate of iron metabolization was monitored by ^{59}Fe radio-iron. The first protein in which radio-iron was incorporated was ferritin, the iron storage protein. Using Fe^{III}–O-TRENSOX, the rate of iron incorporation was higher than when using Fe–EDTA.[13] The immediate precursor of ferritin iron is Fe^{II}; this implies that at least one reduction step is necessary for iron transfer from Fe^{III}–O-TRENSOX into ferritin.

A preliminary study of the inhibition of iron toxicity in rat hepatocyte cultures by O-TRENSOX has shown the protective effect of O-TRENSOX, which is effective by both decreasing iron uptake and increasing iron release by the cells.[14]

In conclusion, we emphasize the high complexing ability of O-TRENSOX for both the ferric and ferrous iron redox states, resulting in redox potential 400 mV higher in potential than the hydroxamate siderophores and higher still than the catecholates; this property is unique among the iron-sequestering agents described in the literature, and thus interesting biological properties may be expected. A full manuscript describing the physicochemical measurements is in preparation.

Acknowledgment. We are grateful to Dr. E. Saint-Aman for electrochemical measurements.

JA951195R

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