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Communications to the Editor

Sulfonated Catecholamide Analogues of Enterobactin as Iron Sequestering Agents

Sir:

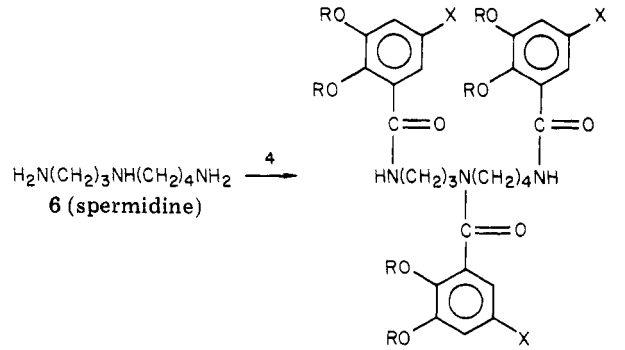
The treatment of certain genetic diseases, such as β-thalassemia major (Cooley's anemia), requires chronic transfusion therapy.[1,2] This produces a secondary hemochromatosis, which eventually results in the failure of vital organs due to buildup of iron in the tissues, and the death of most patients by early adulthood. Since there is no physiological mechanism in man for the removal of iron, the treatment of iron overload must rely on chelating agents which can effect iron excretion, the development of which is now the goal of a major program.[3] We have shown that the siderophore (microbial iron-transport agent[4,5]) enterobactin (1) is the most powerful iron(III) chelating agent known to date.[6,7] While enterobactin is itself not a suitable candidate as an iron-chelating drug, other 2,3-dihydroxybenzoylamides are good candidates, and we have previously reported the preparation of 1,5,9-*N,N',N''*-tris(2,3-dihydroxybenzoyl)cyclotriazatridecane (2) and 1,3,5-*N,N',N''*-tris(2,3-dihydroxybenzoyl)triaminomethylbenzene (3) (Chart I).[8-11] Thermodynamic[9] and biochemical[12] studies of these compounds have shown them to be very powerful chelating agents for ferric ion.

Sulfonation increases the acidity, resistance to air oxidation, and aqueous solubility of catechols. For these reasons, we expected that sulfonated catecholamide[8] ligands and related catechols would have chelating properties superior to those of the parent compounds. We report here the synthesis and preliminary evaluation of several sulfonated catechol chelating agents.

The synthesis of **9**[13] (see Scheme I) proceeds via the addition of 2,3-dimethoxybenzoyl chloride (**4**)[14] to the natural product 1 (spermidine[15]) in *N,N*-dimethylacetamide, followed by removal of the protecting methyl groups with BBr_3 in CH_2Cl_2[11] to give **8**: yield 85%; mp 267–270 °C dec; ^1H NMR (Me_2SO) δ 1.0–2.0 (br, 6 H, $-CH_2CH_2CH_2N<$), 2.7–3.7 (br, 8 H, $-CH_2N<$), 6.5–7.5 (br m, ~17 H, $-N(H)C(=O)C_6H_3(OH)_2$) (note absence of $-OCH_3$ peaks at δ 4.0); IR 3700–2700 (–OH, –NH–, –CH<), 1620 (–NHCO), 1588, 1540, 1460, 1244, 1065, 738 cm^{-1}. The sulfonation of **8**[8] proceeds smoothly in fuming H_2SO_4 to give **9**·$6H_2O$ or with $SOCl_2$ and H_2SO_4 to give **9**·1NaCl·$5H_2O$: yields 52 and 56%; ^1H NMR (D_2O) δ 1.3–2.3 (br, 6 H, $-CH_2CH_2CH_2N<$), 3.0–4.0 (br, 8 H, $-CH_2N<$), 7.38, 7.92 [d, 6 H, J_{AB} = 2 Hz, $-C_6H_AH_B(OH)_2(SO_3)$]; IR 3700–2800 (–OH, –NH–, –CH<), 1640–1600 (–NHCO), 1550–1460, 1223, 610 ($-SO_3^-$), 1095, 1040, 1008, 910 cm^{-1}.

The synthesis of **13** (see Scheme II) proceeds through the addition of 2,3-dimethoxybenzylamine (**5**)[15] to trimesoyl chloride (**10**)[15] in *N,N*-dimethylacetamide to give **11**: yield 76%; mp 241–243.5 °C. The removal of the methyl protecting groups with BBr_3 in CH_2Cl_2 gives **12**: yield 90%; mp 275–276 °C dec; ^1H NMR (Me_2SO–D_2O) δ 4.72 (br s, 6 H, $-NHCH_2-$), 7.07 (br s, 9 H, $-C_6(OH)_2H_3$), 8.97 (s, 3 H, $C_6(CO)_3H_3$); IR 3700–3500 (–OH, >CH), 1635 (–CONH–), 1590, 1535, 1480, 1280, 1255, 1215, 735 cm^{-1}. The sulfonation of **12** in fuming H_2SO_4 gives, following workup, the hygroscopic salt **13**·H_2O: yield 83%; ^1H NMR (D_2O) δ 4.90 (br s, 6 H, $-NHCH_2-$), 7.75, 7.78 (d, 6 H, $-C_6H_2(OH)_2$, J_{AB} = 3 Hz), 8.87 (s, 3 H, $C_6(CO)_3H_3$).

Scheme I. Synthesis of a Linear Trimeric Sulfocatecholate Ligand



7, R = CH_3; X = H
8, R = H; X = H
9, R = H; X = SO_3Na

We have previously reported the synthesis of **3** and some of its properties as an iron chelating agent.[8,9] The sulfonation of **3** in 30% fuming H_2SO_4 gives, following workup, the hygroscopic tripotassium salt of **14**·$7H_2O$: yield 50%; ^1H NMR (D_3O) δ 4.75 (br, 6 H, $-CH_2NH-$), 7.4–8.3 (br m, 9 H, Ar H); IR 3700–2500 (–OH, –NHCO–), 1640 (–NHCO), 1190, 1040, 618 ($-SO_3^-$), 1590, 1533, 1465, 1425, 1095 cm^{-1}. Ion exchange for H^+ with cation-exchange resin gives the hygroscopic acid **14**·$8H_2O$, yield 83%.

The addition of **4** to 1,4-bis(aminomethyl)benzene dihydrochloride (**15**)[16] in dimethylacetamide with NEt_3 as base gives (see Scheme III) good yields of **16**: yield 72%; mp 120–122 °C. The removal of the protecting methyl groups is again accomplished with BBr_3 in CH_2Cl_2 to give **17**: yield 91%; mp 247–250 °C dec; ^1H NMR (Me_2SO–D_2O) δ 4.60 (br s, 4 H, $-CH_2NH-$), 7.42 (s, 4 H, $-CH_2C_6H_4CH_2-$), 6.6–7.6 [complex m, 6 H, $-C_6H_3(OH)_2$].

The sulfonation of **17** in 20% fuming H_2SO_4 gives the tripotassium salt of **18**·H_2O: yield 50%; mp >300 °C. Cation exchange of this salt gave the hygroscopic acid **18**·$4H_2O$ (final based on **17**): yield 54%; mp 185–188 °C dec; IR 3700–2500 (–OH, –NH–), 1640 (–NHCO), 611 (SO_3^-), 1545, 1425, 1280, 1235, 1170 (m), 1037, 1020.

The sulfonated tricatecholamides react with ferric ion at pH 7–8 to give deep-red solutions with λ_{max} ~480–490

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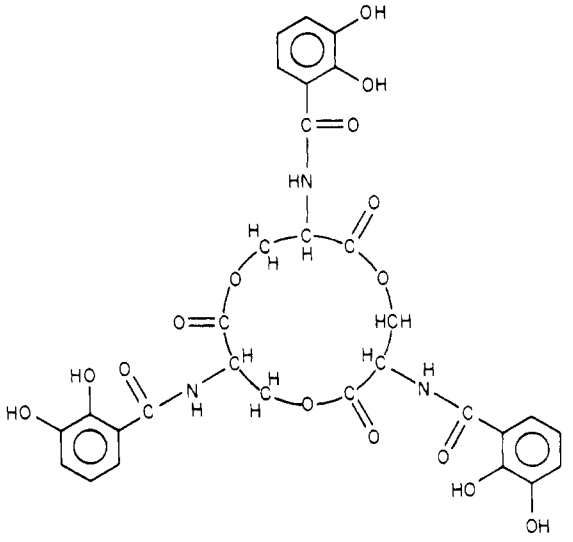
Chart I. Previous Tricatechol Ligands and Precursor Compounds



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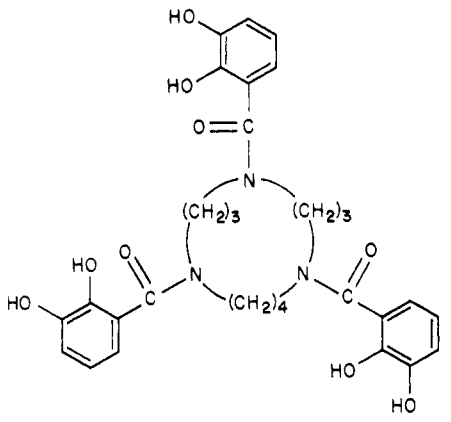

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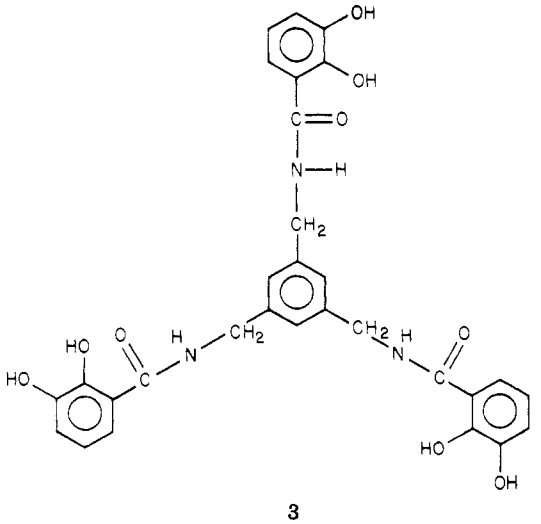

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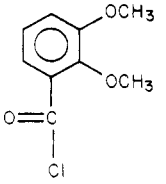

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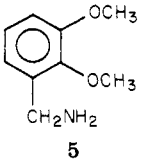

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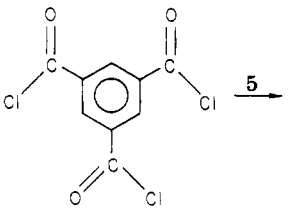
Scheme II. Synthesis of Trimeric Sulfocatecholate Platform Ligands



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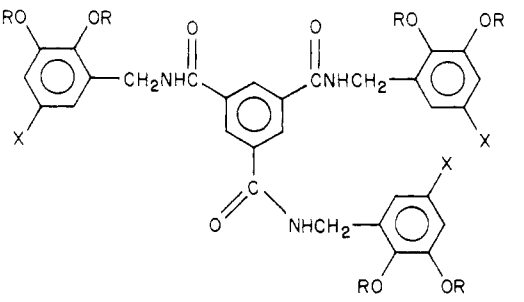
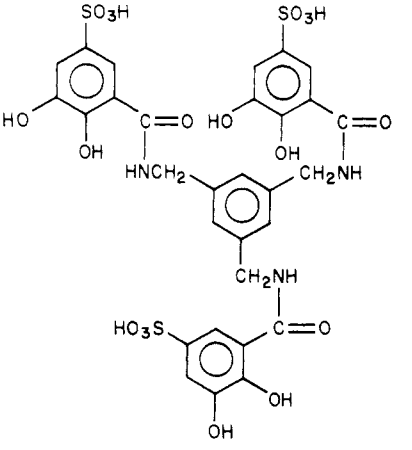


11, R = CH_3; X = H
12, R = H; X = H
13, R = H; X = SO_3Na



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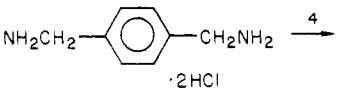
Scheme III. Synthesis of a Dimeric Linear Sulfocatecholate Platform Ligand



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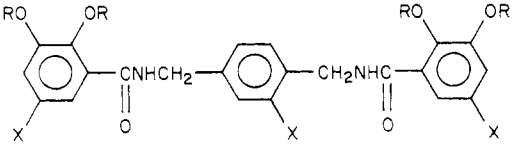

16, R = CH_3; X = H
17, R = H; X = H
18, R = H; X = SO_3H

and ϵ 5000–6000. These values strongly suggest that the ferric ion is coordinated to all six phenolic oxygens of the three dihydroxybenzoyl side groups. As the pH is lowered, the ferric complexes are protonated in discrete one-proton steps to form FeHL, FeH_2L, and FeH_3L complexes. Each successive protonation shifts λ_{max} to longer wavelengths and decreases ϵ. Similar results have been observed with 3 and 4 and with enterobactin (1).[6]

Proton-dependent metal ligand formation constants

Table I. pM[a] Values for Synthetic Catecholamides and Pertinent Biological Iron Chelators

compound	pM
enterobactin (1)	35.5
14	29.4
3	29.1
9	28.5
desferrioxamine B	26.6
13	25.1
transferrin	23.6
N,N-dimethyl-2,3-dihydroxy-5-sulfobenzamide	19.2

[a] Total Fe concentration = 10^{-6} M; total ligand concentration = 10^{-5} M; pH 7.4.

have been determined spectrophotometrically by competition with ethylenediaminetetraacetic acid (eq 1).[6] At

$$Fe(EDTA)^- + H_6L^{3-} \rightleftharpoons Fe(L)^{6-} + EDTA^{4-} + 6H^+ \quad (1)$$

neutral or basic pH, complexation by the catecholamides is strong enough to remove essentially all the iron from $Fe(EDTA)^-$. Thus it was necessary to measure this equilibrium at pH ~5, in order to ensure a detectible distribution of iron between the two ligands.

The proton-dependent formation constants are not the best indicator of a ligand's ability to sequester ferric ion at physiological pH, since they do not reflect the competition from hydrogen ion for the very basic phenolic ligating groups. Therefore, we have calculated the equilibrium concentration of $[Fe(H_2O)_6]^{3+}$ in a solution which is 1 μM total iron and 10 μM total ligand at pH 7.4. The results, expressed as pM ($pM = -\log [Fe(H_2O)_6]^{3+}$), are shown in Table I. A comparison of various ligands is now straightforward; i.e., a larger pM denotes a more effective ligand. The pM values of all the tricatecholamides exceed the transferrin value, indicating that these ligands are *thermodynamically* capable of removing iron from transferrin. Furthermore, the pM values of **9** and **14** indicate that these ligands are several hundred times more effective at sequestering ferric ion than is desferrioxamine B, the current drug of choice for therapeutic iron removal in man.[3]

Thus, we have prepared a new class of ligands which combine several properties needed for an effective iron-removal agent. Like enterobactin, they have an exceptionally high affinity for iron, much higher than transferrin or desferrioxamine B. Unlike enterobactin, they are hydrolytically stable over a wide range of pH. In addition, sulfonation greatly increases the water solubility of these ligands and stabilizes the dihydroxybenzoyl groups toward air oxidation. Finally, recent studies in this laboratory have shown that, while the hydroxamate ligands such as desferrioxamine B are *kinetically* unable by themselves to remove iron from transferrin, the catecholate ligands reported here readily remove the iron from this iron-transport protein of human serum. The kinetics of this process have been investigated in detail at 25 °C and pH 7.4 using iron-saturated human transferrin (5 μM) and the free ligand **9** (0.2 mM). The kinetic behavior shows rapid formation of a ternary complex of the ferric transferrin (FeTr) with the ligand (L) followed by a slow step which gives apotransferrin and the ferric complex of **9** as shown in eq 2, where $K_{eq} = 4.1\ (6) \times 10^2\ M^{-1}$ and $k_2 = 0.066\ (4)$ min^{-1}. While toxicity studies with mice for related compounds support the expectation of low toxicity for these compounds,[17] their further biological properties must be determined by in vivo tests, which are in progress.

$$L + FeTr \overset{K_{eq}}{\rightleftharpoons} LFeTr \overset{k_2}{\rightarrow} FeL + Tr \quad (2)$$

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References and Notes

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(13) Total elemental analyses (excepting oxygen) were performed for all compounds. In general, observed and calculated analyses agree to less than 0.4%. The two most hygroscopic compounds have two analyses that differ by 0.6%.
(14) The precursor 2,3-dimethoxybenzoic acid was purchased from Aldrich Chemical Co. and converted to the acid chloride with $SOCl_2$.
(15) Purchased from Aldrich Chemical Co., Milwaukee, Wis.
(16) Prepared from terephthaloyl chloride (ref 15).
(17) Durbin, P. W.; Jones, E. S.; Weitl, F. L.; Raymond, K. N. *Health Phys.*, submitted for publication.

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