Synthesis and Evaluation of an Enterobactin Model Compound. 1,3,5-Tris-(2,3-dihydroxybenzoylaminomethyl)benzene and its Iron(III) Complex

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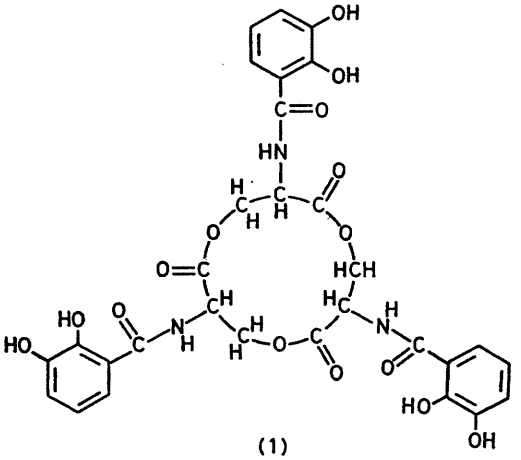
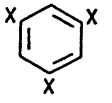
Summary The title compound, an analogue of the siderophore enterobactin, acts as a hexadentate ligand for Fe^{III} ion, co-ordinating *via* the six phenolic oxygens to give a complex whose overall formation constant is $10^{45\cdot8}$.

THE siderophores are a class of microbial iron chelating agents whose properties and function we have described previously.[1] In enterobactin (**1**), a siderophore produced by enteric and other bacteria, the metal co-ordination occurs *via* three catechol units. Although it has been shown that enterobactin forms very stable complexes with Fe^{III} ion,[2] quantitative thermodynamic data to identify the factors responsible for this have not been obtained. To explore this question and to develop new therapeutic iron chelating agents, we have prepared 1,3,5-tris(2,3-dihydroxybenzoylaminomethyl)benzene (**2**), and have determined its iron binding and metal chelate protonation constants by spectrophotometric and potentiometric techniques.

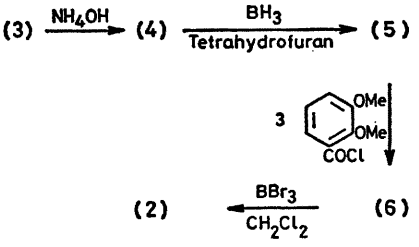


(1)



(2) X = $CH_2NHC(:O)C_6H_3(OH)_2$-*o*,*m*
(3) X = $C(:O)Cl$
(4) X = $C(:O)NH_2$
(5) X = $CH_2NH_2 \cdot HCl$
(6) X = $CH_2NHC(:O)C_6H_3(OMe)_2$-*o*,*m*

The ligand (**2**) was prepared *via* the reactions in the Scheme. The final product had m.p. of 130—135 °C, showed a single spot on t.l.c., and correctly analysed for C, H, N, and O (±0·1% of calculated values); M_{obs} 568, M_{calc} 573·6.



SCHEME

Potentiometric equilibrium curves for 1:1 mixtures of (**2**) with Fe^{III} ion display a 2 equiv. buffer region starting at 4 equiv. KOH (pH *ca.* 4·8) and extending to 6 equiv. KOH (pH 8·5). The visible spectrum at pH 8·5 contains a single broad charge-transfer band with λ_{max} 492 nm (ϵ 4700 cm^{-1} $l\ mol^{-1}$) which closely resembles the spectrum of tris(catecholato)iron(III), λ_{max} 490 nm (ϵ 4190).[3] Iron(III) enterobactin has λ_{max} 495 nm, with a slightly larger ϵ of 5600.[4] Further increase in pH past 8·5 causes no significant change in the visible spectrum of the iron complex with (**2**), indicating that at or above this pH the iron exists solely as the fully encapsulated, hexa-co-ordinate complex of (**2**).

Visible spectra recorded at small pH intervals from pH 5 to 8·5 form two sequential isosbestic points. One point, at λ 547 nm, exists in the pH range 8·1—6·5. There follows a region from pH 6·5 to 5·4 in which no isosbestic point is observed, but there is a shift in λ_{max} from 503 to 523 nm. A second isosbestic point develops at λ 588 nm, which exists over the pH range 5·40—4·80. These data clearly indicate that the two-proton buffer region (4—6 equiv.) observed in the titration curve actually represents two overlapping protonation reactions. Thus the spectral and potentiometric data have been refined based on the model of two monoprotic equilibria, defined as in equations (1) and (2). Values of these constants were obtained by

$$K^{H}_{MHL} = [FeHL]/([FeL]\ [H]) \quad (1)$$

$$K^{H}_{MH_2L} = [FeH_2L]/([FeHL]\ [H]) \quad (2)$$

least-squares fit of the absorbance data over the 8·1—6·5 and 5·4—4·8 pH regions, with log K^{H}_{MHL} = 7·08 and log $K^{H}_{MH_2L}$ = 5·47. They were also calculated from the titration data to be log K^{H}_{MHL} = 6·9(2) and log $K^{H}_{MH_2L}$ = 5·7(2).†

† It is not possible to extend the spectroscopic and potentiometric investigations to lower pH because of the precipitation of a purple solid at pH 3·8.

The overall formation constant of the FeL^{3-} species was determined spectrophotometrically by competition with diethylenetriaminepenta-acetic acid to be $\log \beta = 45{\cdot}8 \pm 0{\cdot}3$ for the reaction $Fe^{3+} + L^{6-} \rightleftharpoons FeL^{3-}$. With the exception of enterobactin itself,[2] this is the highest $\log \beta$ for any iron(III) complex, exceeding the value for *NN*-dimethyl-2,3-dihydroxybenzamide by five orders of magnitude. Thus the ligand (**2**) effectively mimics the extraordinary affinity enterobactin displays for Fe^{III} ion. Additionally, the lack of ester bonds in (**2**) obviates the problems of base-catalysed hydrolysis encountered with enterobactin. This combination of an exceptional affinity for iron and hydrolytic stability represents a significant improvement in the design of therapeutic reagents for the mobilization of iron in man.

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