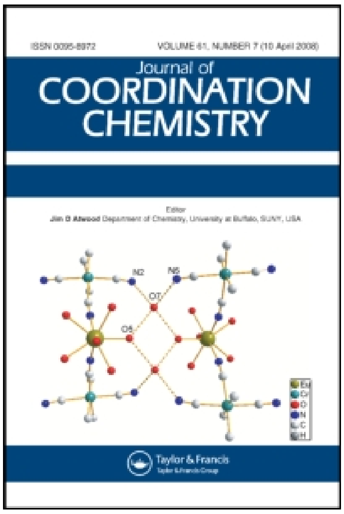

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Hydrogen Bonding in Catechoylamides

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HYDROGEN BONDING IN CATECHOYLAMIDES

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The siderophore enterobactin has an extraordinary affinity for ferric ion. Hydrogen bonding has been proposed to play a significant role in the extra binding affinity enterobactin shows relative to synthetic analogues. These analogues contain 2,3-dihydroxybenzoyl subunits or, as in the present study, 2,3-dihydroxyterephthalamide ligand groups. The bisethyl amide of 2,3-dihydroxybenzoic acid (**1**) crystallizes in space group $P2_1/c$ with $a = 7.387(2)$, $b = 25.068(4)$, $c = 7.198(2)$ Å, $\beta = 100.42(2)°$ with $z = 4$. The structure of **1** shows that the conformation of the acid form of these ligands has the amide carbonyl hydrogen bonded to the catechol proton. The cyclic trimer diamide of ethylenediamine with 2,3-dimethoxyterephthalic acid (**2**) crystallizes in *Pbca* with $a = 25.891(2)$, $b = 17.361(3)$, $c = 33.665(2)$ Å, $z = 16$. The two independent molecules of **2** have slightly different conformations largely due to the effects of hydrogen bonding from the amide proton to the catechol oxygen. This is the hydrogen bond structure found in the metal complexes of these ligands and represents a 180° rotation around the amide C–N bond from the free ligand to the metal complex forms of these compounds—a dominant feature of the metal complexation process.

Keywords: Siderophore, enterobactin, hydrogen-bonding, conformation, X-ray structures.

INTRODUCTION

Enterobactin (Figure 1) is a naturally-occurring iron(III) sequestering agent (siderophore) secreted by *E. coli* to bind and assimilate the iron needed for growth.[2-4] The formation constant for the binding of the ferric ion is extremely large (log K $\approx$ 50)[1,5] and the reasons for this are not yet fully understood, although the preorganization due to the triserine ring is a large component.[1] Analogous tris(catechoylamide) compounds of similar topology fail to yield similar equilibrium constants.[6-9] Shanzer and coworkers have asserted that extensive hydrogen bonding and preorganization of the ligand may lead to an unusually small reorganization entropy upon metal complexation.[10] They proposed that in the ferric complex the catechoylamide binding subunit will adopt conformation **A** (Figure 1) and that in the free ligand conformation **B** is of lower energy than **C**, preorganizing enterobactin for complexation. We have begun studying the effect of hydrogen bonding on the structure and stability of tris(catechoylamide) analogues of enterobactin and have recently reported the first structural characterization of any ferric tris(catechoylamide) complex.[7,8] To date, no corresponding structure of a free ligand tris(catechoylamide) has been available. We report here the first structural characterization of the catechoylamide binding subunit, in the class of compounds having enterobactin as

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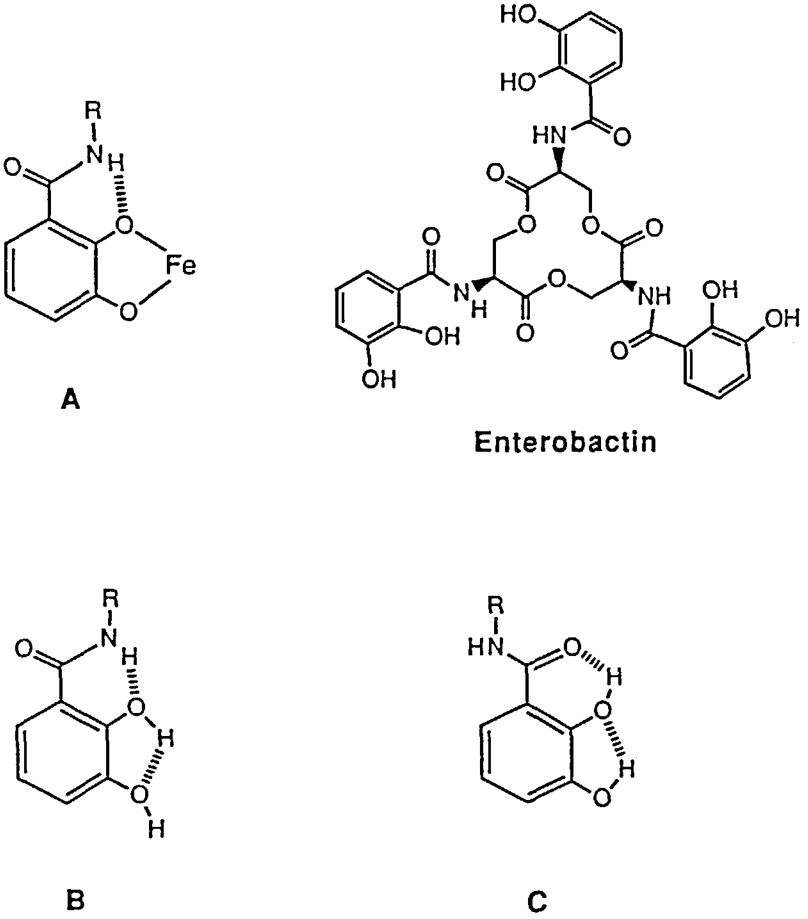

FIGURE 1 (Upper right) Molecular structure of the siderophore enterobactin and the conformations and hydrogen bonding schemes available to the 2,3-dihydroxybenzamide binding subunit of enterobactin and its analogues. A represents the ferric complex, B and C are the two possible free ligand conformations.

their archetype. Knowledge of the conformation of the ligand prior to iron binding is essential for evaluation of the contributions of preorganization of the binding sites to the stability of siderophore complexes and their analogues.

EXPERIMENTAL

Synthesis

The H_2ETA ligand and the Me_6 ethane trimer ligand precursor were synthesized as described earlier.[11,12] The compounds examined in this study all possess the 2,3 dihydroxyterephthalamide binding subunit (Figure 2).

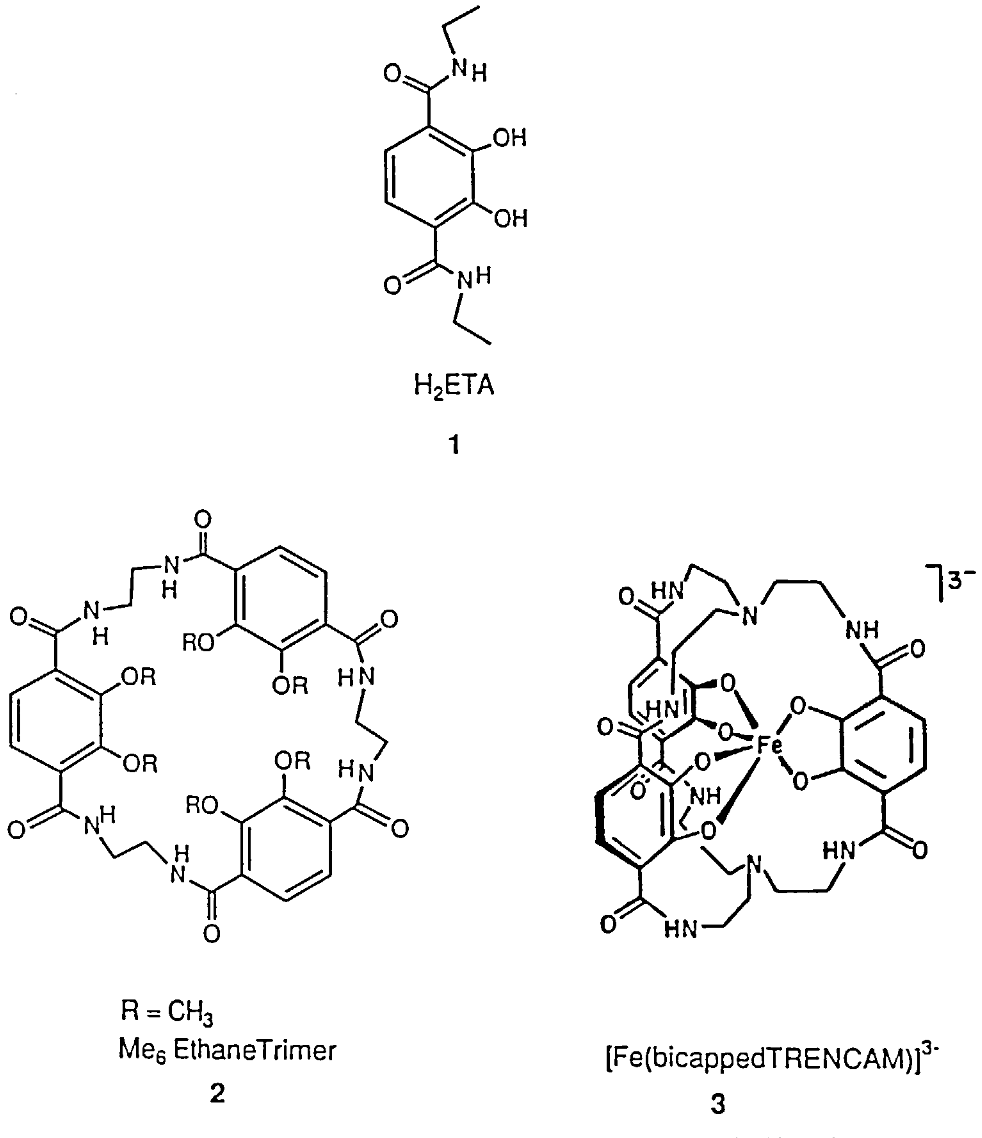

FIGURE 2 Catechoylamide analogues of enterobactin discussed in this study.

Structural Studies

2,3 dihydroxyethylterephthalamide (H_2ETA, 1)

X-ray quality crystals were obtained as clear thin plates by slow evaporation from aqueous ethanol; d_o by flotation ($CH_2Cl_2/CHCl_3$) = 1.34. The $2/m$ symmetry and systematic absences $h0l$, $l = 2n + 1$ and $0k0$, $k = 2n + 1$ are consistent only with the space group $P2_1/c$ (No. 14). Data were collected on an Enraf-Nonius CAD-4 diffractometer with MoKα radiation.[13,14] Crystal and data collection details are presented in Table I.

TABLE I
Crystal and data collection details for H_2ETA and Me_6 ethane trimer.

	Crystal parameters	
Compound (Figure 2)	H_2ETA	Me_6 ethane trimer
a, Å	7.387(2)	25.891(2)
b, Å	25.068(4)	17.361(3)
c, Å	7.198(2)	33.665(2)
β,°	100.42(2)	
space group	$P2_1/c$	$Pbca$
formula weight, amu	270.3	786.81
Z	4	16
d_c, g/cm^3	1.37	1.32
d_o, g/cm^3	1.34	1.34
μ, cm^{-1}	1.002	8.00
size of crystal, mm	0.11 × 0.25 × 0.328	0.23 × 0.24 × 0.43
	Data Collection Details	
radiation, Å	MoKα, 0.71073	CuKα, 1.5418
2θ range,°	$2 \leqslant 2\theta \leqslant 45$	$2 \leqslant 2\theta \leqslant 125$
reflections measured	$-7 \leqslant h \leqslant 7$	$-20 \leqslant h \leqslant 20$
	$0 \leqslant k \leqslant 27$	$0 \leqslant k \leqslant 30$
	$-7 \leqslant l \leqslant 7$	$0 \leqslant l \leqslant 39$
scan speed (θ–2θ), °/min	min 1.3, max 6.7	min 1.3, max 13.4
total data collected	3493	25260
no. of unique data, R_{avg}, %	1700, 2.3	12877, 2.2
no. of data, $F_o^2 > 3\sigma(F_o^2)$	980	7477
	Refinement Parameters	
R, %	3.3	5.3
R_w, %	4.4	7.8
p-factor[a]	0.04	0.05
G.O.F.	1.503	2.02
highest peak in final difference Fourier map, e-/Å3	0.135	0.499

[a] Least squares $\Sigma w(\Delta F)^2$ minimized, $w = 4F_o^2/\sigma^2(F_o)$, $\sigma^2(F_o) = [\sigma_o^2(F_o^2) + (pF^2)^2]$. See footnote 17 for other definitions.

The structure was solved by direct methods (MULTAN/82).[15] The water oxygen and H1–H4 were found in a difference Fourier map. Refinement was performed with anisotropic temperature factors for all heavy atoms and isotropic temperature factors for H1–H4. The remaining H atoms were located in a difference Fourier map and placed at calculated positions but were not refined. A secondary extinction coefficient[16] of $7(2) \times 10^{-7}$ was applied. For the final least-squares cycle $R = 0.033$, $R_w = 0.044$, G.O.F. = 1.503, $(\Delta/\sigma)_{max} = 0.01$.[17] The highest peak in the final difference Fourier map was 0.135 e/Å^3. Atomic scattering factors for all atoms and f′ and f″ values for non-hydrogen atoms were taken from *International Tables for X-ray Crystallography*.[18] Tabulations of bond distances are shown in Table II, bond angles in Table III.[19]

TABLE II
Bond distances for H_2ETA 1.*

Atom 1	Bond distances (Angstroms) Atom 2	Distance
O1	C2	1.353(3)
O2	C3	1.354(3)
O3	C7	1.249(3)
O4	C10	1.259(3)
N1	C7	1.337(3)
N1	C8	1.469(3)
N2	C10	1.323(3)
N2	C11	1.458(3)
C1	C2	1.389(3)
C1	C6	1.400(3)
C1	C7	1.480(3)
C2	C3	1.391(3)
C3	C4	1.392(3)
C4	C5	1.404(3)
C4	C10	1.481(3)
C5	C6	1.369(3)
C8	C9	1.499(4)
C11	C12	1.426(4)
	Hydrogen bond distances (Angstroms)	
O1	O3	2.496(3)
O1	O5	2.961(3)
O2	O4	2.515(3)
O4	O5	2.822(3)
O5	N2	2.975(3)

* Although the H atom coordinates were refined, the H atom bond distances are not presented here since they are shorter than the true internuclear bond lengths. For a detailed explanation, see M.R. Churchill, *Inorg. Chem.*, **12**, 1213 (1973).

Me_6 ethane trimer (2)

X-ray quality crystals were obtained by slow evaporation from aqueous methanol as clear rectangular prisms; d_o by flotation (H_2O, KI) = 1.34. The mmm symmetry and systematic absences $0kl$, $k = 2n + 1$; $h0l$, $l = 2n + 1$; $hk0$, $h = 2n + 1$ are consistent only with the space group *Pbca*. The density and unit cell data showed that there are two formula units per cell, representing a relatively large "small molecule" problem. Data were collected on an Enraf-Nonius CAD-4 diffractometer with CuKα radiation.[13,20] Crystal and data collection details are shown in Table I.

As just described, the structure was solved by direct methods using procedures described previously.[15] Refinement was performed using anisotropic temperature factors. Hydrogen atom coordinates were not refined. A secondary extinction coefficient[16] of 6.3(1.1) × 10^{-8} was applied. For the final least-squares cycle $R = 0.053$, $R_w = 0.078$, G.O.F. = 2.02, $(\Delta/\sigma)_{max} = 0.3$. The highest peak in the final

TABLE III
Bond angles for H_2ETA 1.

Atom 1	Atom 2	Atom 3	Angle
		Bond angles (degrees)	
C7	N1	C8	120.99(21)
C10	N2	C11	122.34(24)
C2	C1	C6	118.38(23)
C2	C1	C7	118.27(21)
C6	C1	C7	123.35(21)
O1	C2	C1	122.66(24)
O1	C2	C3	116.64(22)
C1	C2	C3	120.70(23)
O2	C3	C2	116.46(23)
O2	C3	C4	122.81(24)
C2	C3	C4	120.73(22)
C3	C4	C5	118.26(22)
C3	C4	C10	119.07(21)
C5	C4	C10	122.65(23)
C4	C5	C6	120.83(22)
C1	C6	C5	121.09(21)
O3	C7	N1	120.42(24)
O3	C7	C1	120.07(22)
N1	C7	C1	119.51(22)
N1	C8	C9	112.51(22)
O4	C10	N2	121.1(3)
O4	C10	C4	119.4(3)
N2	C10	C4	119.46(22)
N2	C11	C12	113.2(3)
		Hydrogen bonding angles (degrees)	
O1	H3	O3	152.3(3)
O2	H4	O4	152.5(3)
O4	H13A	O5	170.1(3)
O1	H13B	O5	152.4(4)
O5	H2	N2	155.4(2)

difference Fourier map was 0.499 e/Å^3. Tabulations of bond distances are shown in Table IV, the bond angles are generally similar to those of H_2ETA and are in Table S6.[19]

RESULTS AND DISCUSSION

The conformations available to the 2,3 dihydroxyterephthalamide ligands and their iron(III) complexes are shown in Figure 3. In the free ligand, where R = H, two hydrogen bonded conformations are available, E and F. Only E is preorganized for iron binding. The binding of iron at neutral pH is governed by the conditional stability constant K* where

$$K^* = [FeL][H^+]^n/[LH_n][Fe^{3+}] \qquad (1)$$

for the reaction

$$LH_n + Fe^{3+} \leftrightarrow FeL + nH^+ \quad (2)$$

If protonated forms of the ligand preorganize for metal binding (form E *versus* F) K* would be anticipated to increase.

TABLE IV
Bond distances for Me6 ethane trimer **2** (two independent molecules).

	Intramolecular distances			Intramolecular distances	
Atom 1	Atom 2	Distance	Atom 1	Atom 2	Distance
O1	C1	1.230(3)	O101	C101	1.220(4)
O2	C3	1.382(3)	O102	C103	1.383(3)
O2	C31	1.440(4)	O102	C131	1.464(4)
O3	C4	1.380(3)	O103	C104	1.375(3)
O3	C32	1.460(3)	O103	C132	1.455(4)
O4	C8	1.240(3)	O104	C108	1.208(3)
O5	C11	1.227(3)	O105	C111	1.232(3)
O6	C13	1.385(3)	O106	C113	1.374(3)
O6	C33	1.421(4)	O106	C133	1.442(4)
O7	C14	1.375(3)	O107	C114	1.380(3)
O7	C34	1.429(3)	O107	C134	1.413(5)
O8	C18	1.226(3)	O108	C118	1.220(3)
O9	C21	1.234(3)	O109	C121	1.223(3)
O10	C23	1.380(3)	O110	C123	1.371(3)
O10	C35	1.431(4)	O110	C135	1.440(3)
O11	C24	1.374(3)	O111	C124	1.381(3)
O11	C36	1.435(4)	O111	C136	1.432(4)
N1	C8	1.335(3)	N101	C108	1.321(3)
N1	C9	1.457(3)	N101	C109	1.456(4)
N2	C10	1.453(4)	N102	C110	1.457(4)
N2	C11	1.330(4)	N102	C111	1.331(4)
N3	C18	1.344(4)	N103	C118	1.337(4)
N3	C19	1.455(4)	N103	C119	1.455(3)
N4	C20	1.456(4)	N104	C120	1.446(4)
N4	C21	1.332(4)	N104	C121	1.313(4)
N5	C28	1.343(4)	N105	C128	1.337(3)
N5	C29	1.459(3)	N105	C129	1.448(4)
N6	C1	1.331(4)	N106	C101	1.269(4)
N6	C30	1.447(3)	N106	C130	1.447(4)
C1	C2	1.517(4)	C101	C102	1.496(4)
C2	C3	1.393(4)	C102	C103	1.392(4)
C2	C7	1.401(4)	C102	C107	1.397(4)
C3	C4	1.403(4)	C103	C104	1.400(4)
C4	C5	1.393(3)	C104	C105	1.400(4)
C5	C6	1.396(4)	C105	C106	1.390(4)
C5	C8	1.508(4)	C105	C108	1.486(4)
C6	C7	1.359(4)	C106	C107	1.375(4)
C9	C10	1.500(4)	C109	C110	1.510(4)
C11	C12	1.516(4)	C111	C112	1.517(4)

TABLE IV (continued)

	Intramolecular distances			Intramolecular distances	
Atom 1	Atom 2	Distance	Atom 1	Atom 2	Distance
C12	C13	1.389(4)	C112	C113	1.387(4)
C12	C17	1.396(4)	C112	C117	1.399(4)
C13	C14	1.386(4)	C113	C114	1.397(4)
C14	C15	1.392(4)	C114	C115	1.399(4)
C15	C16	1.388(4)	C115	C116	1.384(4)
C15	C18	1.517(4)	C115	C118	1.509(4)
C16	C17	1.362(4)	C116	C117	1.376(4)
C19	C20	1.503(4)	C119	C120	1.524(4)
C21	C22	1.506(4)	C121	C122	1.518(4)
C22	C23	1.391(4)	C122	C123	1.394(3)
C23	C24	1.397(4)	C123	C124	1.398(4)
C24	C25	1.390(3)	C124	C125	1.400(4)
C25	C26	1.402(4)	C125	C126	1.384(4)
C25	C28	1.514(4)	C125	C128	1.514(4)
C26	C27	1.362(4)	C126	C127	1.383(4)
C29	C30	1.497(4)	C129	C130	1.509(4)
O201	O104	2.857(4)			
O201	O108	2.869(4)			
O202	O1	2.755(3)			
O202	O5	2.762(3)			
O203	O109	2.690(6)			
O203	O202	3.092(6)			

Prediction of which conformation is preferred by the molecule can be made by comparison of the strengths of the hydrogen bonds available to the two forms. If **A** is the acid and **B** the base, the strength of the hydrogen bond

$$\mathrm{A{-}H\cdots B} \qquad (3)$$

increases as the acid becomes more acidic and the base becomes more basic. For two conformations E and F (Figure 3) the most acidic proton is the phenolic proton (pK ≈ 7.0)[11] rather than the amide (pK ≈ 15);[21] hence the strongest hydrogen bond should occur in conformation F. This correlation between acidity and hydrogen bond strength is reported, for example, by Pimentel and McClellan,[22] who have listed representative ΔH values for hydrogen bonds in CCl_4 solutions. For N–H··O hydrogen bonds they give a value* of −3.9 kcal/mol, for O–H··O bonds a value of −6.4 kcal/mol. Although these measurements were performed in CCl_4, the O–H··O hydrogen bond should continue to be stronger than the N–H··O one in water. On the basis of these hydrogen bond strengths, form F is expected to be favoured for the case where R = H; form E should only exist in the case where the stronger phenolic hydrogen bond is not available.

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* 1 cal ≡ 4·184 J.

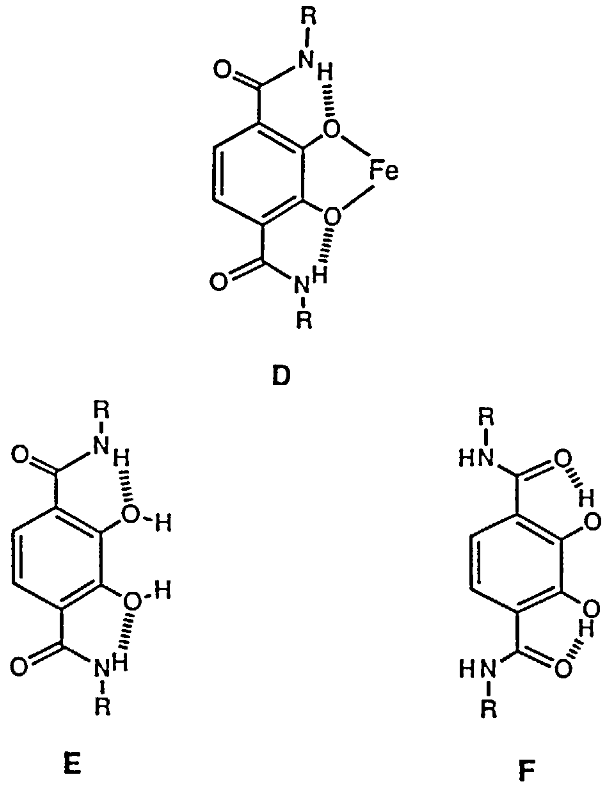

FIGURE 3 Conformations of the 2,3-dihydroxyterephthalamide binding subunits. Conformation D represents the ferric complex, E and F are the two possible ligand conformations.

An ORTEP diagram of the two conformers of Me_6 ethane trimer **2** is shown in Figure 4. Both independent molecules of the asymmetric unit assume a similar conformation for two of the three 2,3 dihydroxyterephthalamide moieties. The third ring (located in the upper right hand side of both diagrams) shows a conformational dissimilarity in the side relationship of the amide bonds to the aromatic ring. Both compounds exhibit hydrogen bonding between the amide hydrogen and the catecholate oxygen in five of the six possible locations. Apparently, the molecule is conformationally prohibited from hydrogen bonding in the sixth site thus giving rise to the two lowest energy conformations found in the X-ray structure. We expected ethane trimer to form conformation E, since the CH_3 groups preclude phenolic hydrogen bond formation. This is confirmed by the structure.

An ORTEP diagram of H_2ETA **1** is shown in Figure 5. There are strong hydrogen bonds between H3 and O3 (1.61 Å) and between H4 and O4 (1.60 Å) and the extended π network of the aromatic amide linkages hold the molecule in an almost perfect planar array. The dihedral angle between the plane defined by O2/O4/C3/C4/C10 and O1/O3/C1/C2/C7 is 3.2°. The torsion angle between C3/C4 and C10/O4 is 5.1°. Thus, the methyl and deprotected ligands are found to have their catecholate subunits in conformations related by a rotation of the amide group by 180°.

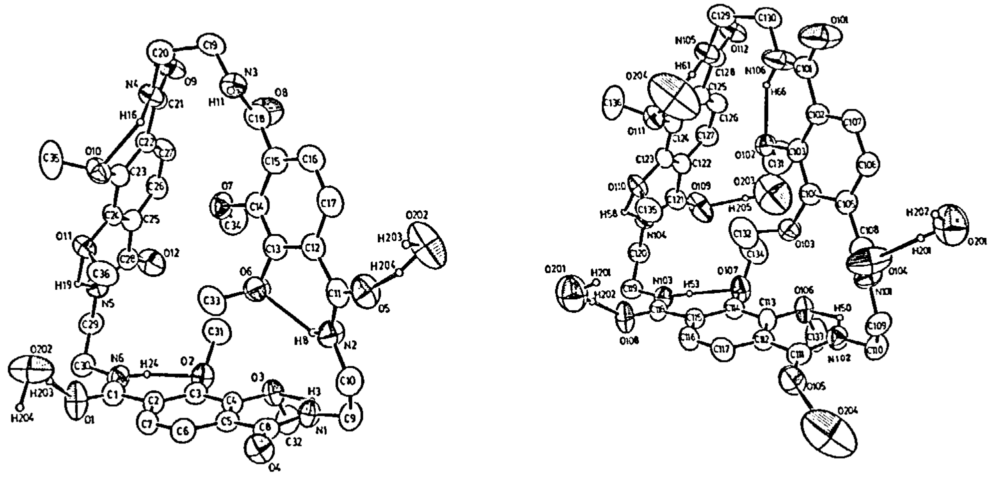

FIGURE 4 An ORTEP diagram of the two independent molecules of Me_6 ethane trimer **2** (see Figure 2 for the simplified structure). Molecule a (left panel) contains non-hydrogen atom numbers < 100, molecule b contains non-hydrogen atom numbers > 100. Note that with the exception of the amide bonds at C18 (molecule a) and C108 (molecule b) all amide groups are in the plane of the corresponding catechol groups; these structures contain N–H··O hydrogen bonds identical to those in Figure 4. The thermal ellipsoids are scaled to represent the 50% probability surface, hydrogen atoms have been given arbitrarily small spheres for clarity.

An ORTEP diagram of Fe(bicappedTRENCAM) **3** is shown in Figure 6. Strong hydrogen bonds between the amide hydrogen and the catecholate oxygens lock each binding subunit in a planar configuration. As for Me_6 ethane trimer, this compound cannot form the strong phenolic hydrogen bond. Thus this structure also has the opposite subunit conformation to that found in the ligand. We conclude therefore that the terephthalamide binding subunit is *not* preorganized for iron binding. At some point in binding of iron the ligand must undergo a conformational change to allow hydrogen bonding from the amide to the catecholate oxygen (180° rotation of the amide bond). Extrapolation of these results to the similar 2,3 dihydroxybenzamide subunit would predict that conformation **C** is of lower energy than **B** (Figure 1), since it can form the stronger O–H··O hydrogen bond at the expense of the weaker N–H··O hydrogen bond. By analogy to the Fe(bicappedTRENCAM) structure, conformation **A** is predicted for the ferric complex of enterobactin and other ligands containing the 2,3 dihydroxybenzamide subunit. Hence no preorganization of the binding subunit due to this hydrogen bonding is expected.

A recent study of protonation of the ferric complex of enterobactin shows that similar amide conformational changes occur as the coordination changes from a catecholate to salicylate mode of bonding.[23] These results complement recent thermodynamic studies of enterobactin and its tris(catechoylamide) ligand analogues.[1,24]

ACKNOWLEDGEMENTS

We thank Professor S. Lifson for helpful discussions and Dr. F. J. Hollander for his help with the crystallography. We acknowledge the late Dr. S. J. Rodgers who

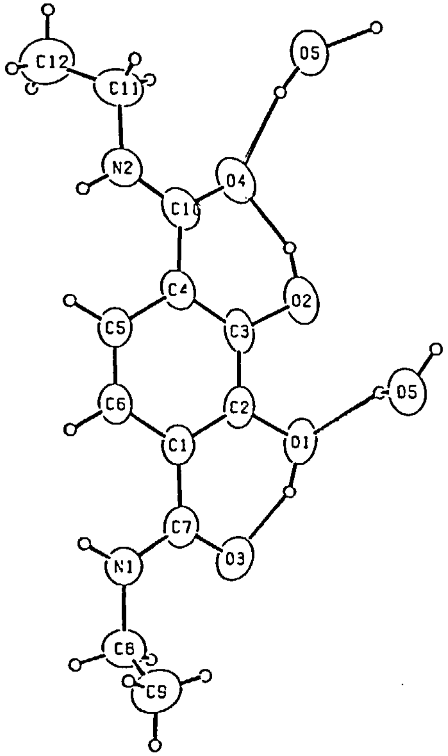

FIGURE 5 An ORTEP diagram of H_2ETA 1 (see Figure 2 for the simplified structure). The thermal ellipsoids are scaled to represent the 50% probability surface, hydrogen atoms have been given arbitrarily small spheres for clarity.

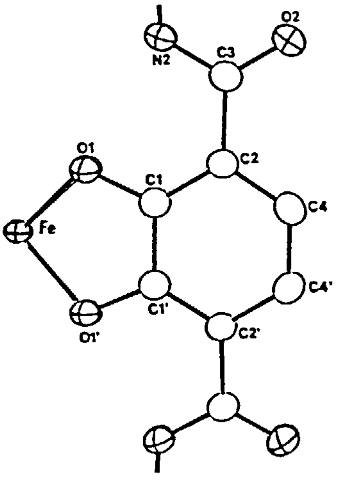

FIGURE 6 An ORTEP diagram of one catechol subunit of the Fe(bicappedTRENCAM) anion 3 (Figure 2). The thermal ellipsoids are scaled to represent the 50% probability surface.

provided the X-ray quality crystal of Me_6 ethane trimer. This research is supported by NIH grant AI11744.

SUPPLEMENTARY MATERIAL

Supplementary information, Tables: S1 (Positional Parameters and B Values for H_2ETA), S2 (Anisotropic Thermal Parameters for H_2ETA), S3 (Observed and Calculated Structure Factors for H_2ETA), S4 (Positional Parameters and B Values for Me_6 Ethane Trimer), S5 (Anisotropic Thermal Parameters for Me_6 Ethane Trimer), and S6 (Bond Angles for Me_6 Ethane Trimer **2** (Two Independent Molecules)) are available from the senior author.

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converted to structure factor amplitudes and their e.s.d.'s by correction for scan speed, background, Lorentz and polarization effects. No correction was applied for absorption. A small decay correction was applied (Max = 1.021, Avg = 1.010). Direct methods gave an initial model which determined the positions of 24 of 28 O atoms, 11 of 12 N atoms, and 66 of 72 C atoms. Full-matrix least-squares refinements and Fourier synthesis yielded the positions of the remaining non-hydrogen atoms. The majority of the H atoms were located in the difference Fourier map, 84 of 92 H atoms were placed at calculated positions. Two water H atoms were found in the difference Fourier map and four of the remaining six water H atoms were placed at calculated hydrogen bonding positions.

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