Inorg. Chem. **1993**, *32*, 124–126

(1,4,7-Tris(4-*tert*-butyl-2-mercaptobenzyl)-1,4,7-triazacyclononane)iron(III): A Model for the Iron–Sulfur Center in Nitrile Hydratase from *Brevibacterium*, sp.

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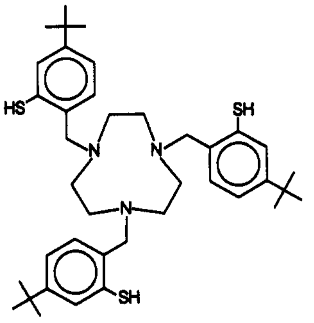
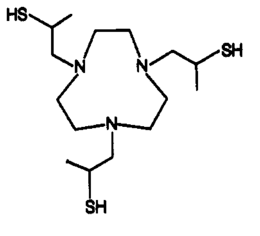
Received May 28, 1992

Nelson, Scarrow, Que, et al.[1] have recently presented strong UV–vis, EPR, resonance Raman, and EXAFS spectroscopic evidence that the bacterial enzyme nitrile hydratase from *Brevibacterium*, sp., strain R312,[2] contains a low-spin ferric ion in an octahedral ligand environment consisting of 2.5 (±1) oxygen or nitrogen donor atoms at 1.98 Å and 2.5 (±1) sulfur atoms at 2.21 Å. These authors point out that there are three cysteines present in a Cys–X–Y–Cys–Z–Cys motif at residues 110–115 of the α-chain, which they tentatively have assigned to the metal binding site. This is the first reported example of a protein-bound non-heme iron with a mixed sulfur and nitrogen or oxygen coordination sphere. We report here the synthesis of the novel hexadentate ligand 1,4,7-tris(4-*tert*-butyl-2-mercaptobenzyl)-1,4,7-triazacyclononane, LH$_3$, and its iron(III) complex [LFe^{III}] (**1**), which contains an N$_3$S$_3$ donor set. The ligand 1,4,7-tris(2-mercaptopropyl)-1,4,7-triazacyclononane (L^{2}H$_3$) also forms a stable complex [L^{2}Fe^{III}] (**2**) with an N$_3$S$_3$ donor set. The spectroscopic properties of **1** and **2** are compared with those reported for the iron–sulfur site in nitrile hydratase.



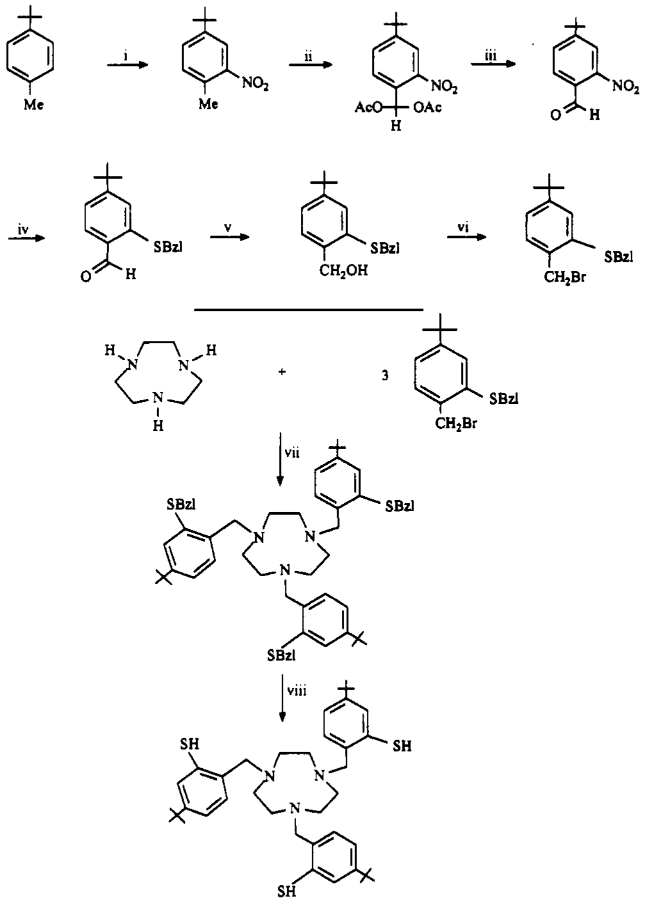
LH$_3$



L^{2}H$_3$

The ligand LH$_3$ containing an 1,4,7-triazacyclononane backbone and three thiophenolate pendant arms was prepared in good yields according to the route depicted in Scheme I, whereas L^{2}H$_3$ was prepared by reaction of propylenesulfide and 1,4,7-triazacyclononane in CHCl$_3$. When a solution of the ligand LH$_3$ and Fe(acac)$_3$ (1:1) in methanol was heated to reflux for 1 h under an argon blanketing atmosphere, a green-black precipitate of [LFe^{III}] (**1**) formed upon cooling to room temperature. We have also prepared colorless diamagnetic [LIn^{III}] from a refluxing methanolic solution of InCl$_3$ and LH$_3$ to which a few drops of N(Et)$_3$ have been added. Brown violet microcrystals of [L^{3}Fe^{III}] (**2**) were obtained from a warm methanolic solution of Fe^{II}(ac)$_2$ (ac = acetate) and L^{2}H$_3$ (1:1) in the presence of air. Solid **1** and **2** are stable in air both in the solid state and in solution (MeCN, CH$_2$Cl$_2$, CHCl$_3$). The tris(phenolato) analogue of **1** has been prepared previously.[3,4] Ligands of this type have been shown to form extremely stable ferric complexes with stability constants exceeding those of natural siderophores.[5]

Scheme I. Synthesis of the Ligand LH$_3$^{a}



^{a} Reagents; i, HNO$_3$/H$_2$SO$_4$; ii, CrO$_3$/Ac$_2$O; iii, HCl/H$_2$O/EtOH; iv, BzlSH/K$_2$CO$_3$/dmf; v, NaBH$_4$/MeOH; vi, PBr$_3$/CHCl$_3$; vii, KOH/toluene; viii, (a) Na/NH$_3$, (b) HCl under an argon atmosphere. Bzl = benzyl; Ac = acetyl.

Single crystals of **1** suitable for X-ray crystallography[6] were obtained by slow evaporation of an acetone solution in an argon gas stream. Figure 1shows the structure of the neutral molecule in crystals of **1** at 298 K. The ferric ion is in a pseudooctahedral environment consisting of three amine nitrogen atoms of the 1,4,7-triazacyclononane backbone and three thiophenolato sulfur atoms (*fac*-N$_3$S$_3$ donor set). The average Fe–S bond length of 2.28 Å is longer than in [bis((2-aminoethyl)thiosalicyclidenaminato)-iron(III)]chloride[7] at 2.21 Å.

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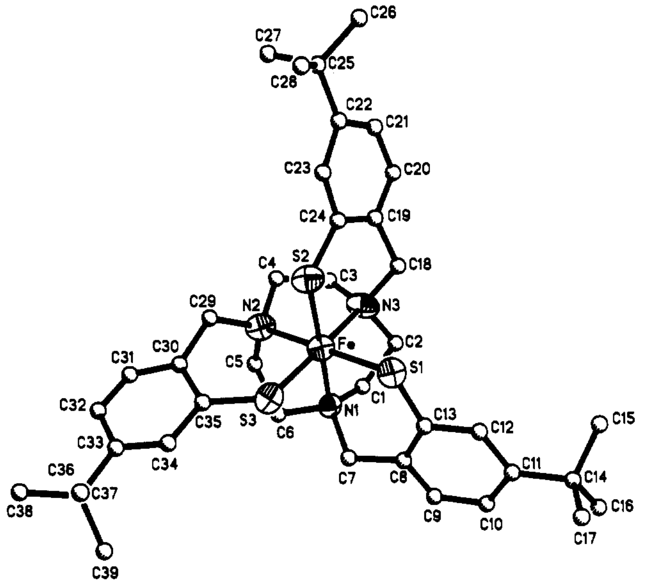

Figure 1. Structure of the neutral molecule in crystals of **1**. Important bond lengths (Å) and angles (deg): Fe-N(1) = 2.08 (1), Fe-N(2) = 2.08 (2), Fe-N(3) = 2.06 (2), Fe-S(1) = 2.269 (7), Fe-S(2) = 2.283 (5), Fe-S(3) = 2.288 (6); S(1)-Fe-S(2) = 86.5 (2), S(2)-Fe-S(3) = 85.2 (2), S(2)-Fe-N(1) = 178.1 (4), S(1)-Fe-N(2) = 179.3 (4), S(3)-Fe-N(2) = 94.3, S(1)-Fe-N(3) = 95.5 (5), S(3)-Fe-N(3) = 178.4 (5), N(2)-Fe-N(3) = 84.2 (6), S(1)-Fe-S(3) = 86.1 (2), S(1)-Fe-N(1) = 95.4 (4), S(3)-Fe-N(1) = 95.2 (4), S(2)-Fe-N(2) = 94.1 (4), N(1)-Fe-N(2) = 84.0 (5), S(2)-Fe-N(3) = 95.1 (5), N(1)-Fe-N(3) = 84.5 (6).

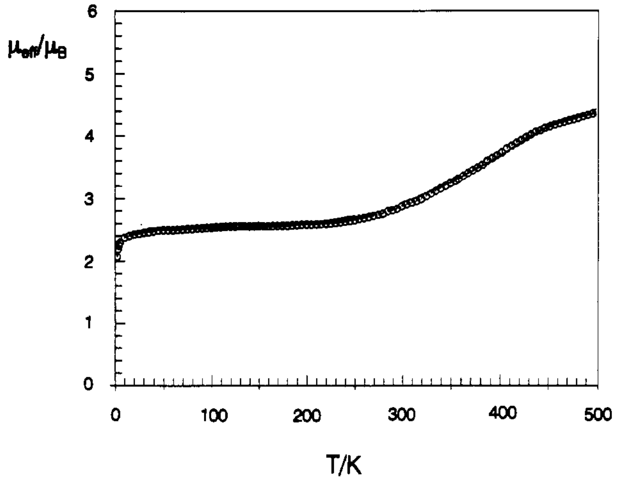

Figure 2. Temperature-dependent magnetic moments of solid **1**.

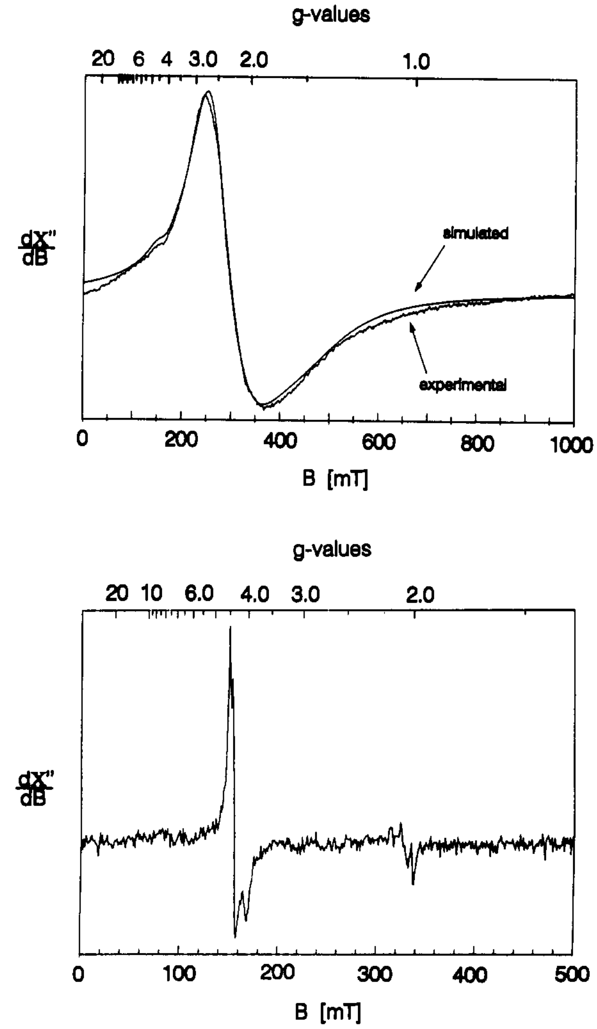

Figure 3. X-Band EPR spectra. Top: solid sample of crystalline **1** at 10.3 K. Bottom: solid sample of cocrystallized LIn/(**1**) (100/1) at 10 K.

Temperature-dependent susceptibility measurements on a solid sample (ground crystals) of **1** (Figure 2) in the range 2-500 K using a SQUID magnetometer revealed temperature dependent magnetic moments ranging from 2.4 μ_B/Fe at 77.0 K to 2.9 μ_B/Fe at 300 K to 3.7 μ_B/Fe at 398 K and to 4.36 μ_B/Fe at 500 K. This is typical for an electronic spin equilibrium between the high-spin $^6A_1$ state ($S = {}^5/_2$) and $^2T_2$ state ($S = {}^1/_2$) of an octahedral Fe^{III} complex. At ambient temperature solid **1** is predominantly low spin. In contrast, the magnetic moment of **2** was found to be temperature-independent, $\mu_{eff} = 5.6\ \mu_B$ (77-298 K), which is indicative of a predominantly high spin ferric ion in **2**.

The X-band EPR spectrum of solid **1** corroborates nicely the notion of a spin equilibrium. At 10.3 K, a strong signal centered at $g_{av} = 2.1$ is observed, indicative of the low-spin form ($S = {}^1/_2$) (Figure 3, top). The anisotropic signal was successfully fitted by using the parameters $g_x = 2.54$, $g_y = 2.14$, and $g_z = 1.5$. In contrast, the EPR spectrum at 10 K of a cocrystallized solid sample of LIn/LFe (100:1) displays a tpyical high-spin ferric signal at $g = 4.3$ (Figure 3, bottom). Thus a magnetically diluted sample of **1** is high spin as are $CHCl_3$ solutions of **1** at 20° C as was determined by ^1H NMR spectroscopy (the Evans[8] method).

The Mössbauer spectrum of **1** shows a symmetrical doublet with the following isomer shift and quadrupole splitting parameters: at 4.2 K, $\delta = 0.37$ (2) mm s^{-1} and $\Delta E_Q = 1.05$ (1) mm s^{-1} and, at 77 K, $\delta = 0.37$ (1) mm s^{-1} and $\Delta E_Q = 0.97$ (1) mm s^{-1} whereas at room temperature (293 K) $\delta = 0.29(2)$ mm s^{-1} and $\Delta E_Q = 0.46$ (1) mm s^{-1} and at 400 K $\delta = 0.24(2)$ mm s^{-1} and $\Delta E_Q = 0.34$ (1) mm s^{-1}. Due to fast relaxation only one doublet is observed even at temperatures >300 K. The spin conversion time is shorter than the life time of the $I = {}^3/_2$ state of ^{57}Fe ($\tau \approx 10^{-7}$ s). For the corresponding spectrum of **2** at 100 K the following parameters have been established: $\delta = 0.43$ mm s^{-1}, and $\Delta E_Q = 0.64$ mm s^{-1}.

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(6) Crystal data for **1**: $[C_{39}H_{54}N_3S_3Fe]$, $M = 716.9$; orthorhombic, *Iba*2, $a = 26.087$ (5) Å, $b = 14.269$ (3) Å, $c = 22.572$ (5) Å, $V = 8402$ (3) Å^3, $Z = 8$, $D_c = 1.09$ g cm^{-3}, crystal dimensions 0.8 × 0.4 × 0.4 mm^3, Mo Kα radiation (0.710 73 Å), $T = 293$ K, Siemens P4 diffractometer. Data were reduced by using the SHELXTL PLUS program package, and the structure was solved using this program. Hydrogen atoms were included at calculated positions with fixed isotropic thermal parameters: $\mu = 0.53$ mm^{-1}; $3.0 < 2\theta < 50°$; 3780 unique reflections; 1963 observed reflections with $I > 2.0\sigma(I)$; 367 least-squares parameters; $R = 0.080$ ($R_w = 0.078$). The carbon atoms of the *tert*-butyl groups are disordered as is judged from unrealistically large thermal displacement parameters. The crystals are soft; relatively few reflections with $2\theta > 40°$ were deemed observed ($F > 4.0\sigma(F)$).

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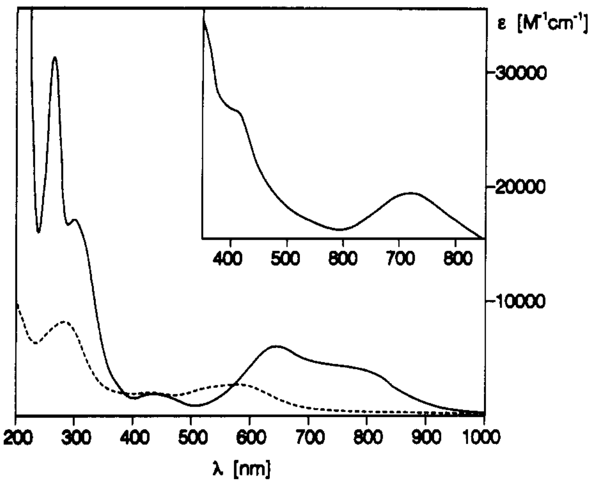

Figure 4. Electronic spectrum of **1** in MeCN (—) and of **2** in H_2O (- - -) at 295 K. The inset shows the spectrum of the enzyme nitrile hydratase from *Brevibacterium*, sp.[2]

The electronic spectra of **1** in CH_3CN and of **2** in H_2O at ambient temperature are shown in Figure 4. The spectrum of the enzyme nitrile hydratase[2] is also displayed for comparison. At room temperature both **1** and **2** are high-spin. It is therefore significant that the intense low-energy sulfur-to-iron(III) charge transfer bands are shifted to higher energy on going from a tris(thiophenolato) (**1**: 651 nm (6.11×10^3 M^{-1} cm^{-1}), 775 nm (4.52×10^3 M^{-1} cm^{-1})) to a trimercapto ligand environment (**2**: 551 nm (2.37×10^3 M^{-1} cm^{-1}), 520 nm (sh)). The corresponding high-spin tris(phenolato) analogue of **1** $L'Fe^{III}$ ($L'H_3$ = 1,4,7-tris(5-*tert*-butyl-2-hydroxybenzyl)-1,4,7-triazacyclononane) exhibits a phenolate-to-iron CT band at 486 nm (4.9×10^3 M^{-1} cm^{-1}).[9]

Figure 5 shows the cyclic voltammogram (CV) of **1** in CH_2Cl_2 (0.1 M $[Bu_4{}^nN]PF_6$ at room temperature. Three electron transfer waves are clearly identified. The reversible one-electron transfer process at $E_{1/2} = -0.215$ V vs Fc^+/Fc[10] corresponds to an oxidation of **1** forming a monocation $[LFe]^+$ as was established by a coulometric measurement. We have not been able to isolate this species and, therefore, cannot distinguish between a metal-centered (Fe^{IV}) and a ligand oxidation yielding a species with a coordinated ligand radical. The quasi-reversible wave at $E_{1/2} = -1.03$ V Fc^+/Fc corresponds to the reduction of **1** with formation of $[LFe^{II}]^-$, which is not stable on the time scale of a CV experiment. Finally, an irreversible oxidation of **1** occurs at $E_{ox} = +0.56$ V vs Fc^+/Fc.

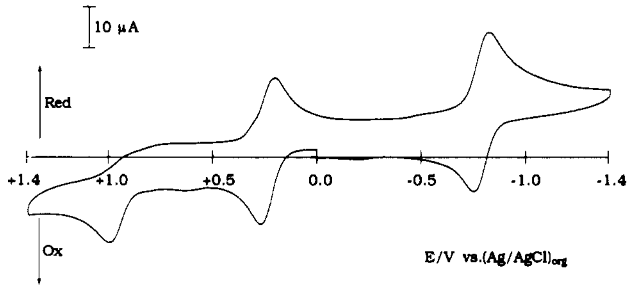

Figure 5. Cyclic voltammogram of **1** in CH_2Cl_2 (0.1 M $[Bu^n{}_4N]PF_6$ supporting electrolyte, glassy-carbon working electrode; ferrocene internal standard, scan rate 100 mV s^{-1}).

The CV of **2** in CH_3CN (0.1 M $[Bu_4{}^nN]PF_6$) is similar: Two reversible one-electron transfer waves are observed. At $E^1{}_{1/2} = -0.395$ V vs Fc^+/Fc, **2** is reversibly oxidized forming an Fe(IV) or ligand radical species, and at $E^2{}_{1/2} = -1.59$ V vs Fc^+/Fc, **2** is reversibly reduced forming an $[L^2Fe^{II}]^-$ species. Thus the trimercapto ligand L^2 stabilizes trivalent iron(III) more efficiently than the tris(thiophenolato) ligand L.

In conclusion, the spectroscopic properties of **1** and **2** reinforce the notion that the ferric ion in nitrile hydratase contains coordinated mercapto ligands, but it remains puzzling how such a strong ligand field is achieved in the enzyme to enforce a low-spin configuration. The similarity of the electronic spectra of the model complexes and the enzyme suggests to us that the ferric ion in the enzyme may also be partly high spin at room temperature.

Acknowledgment. We thank Professor K. Raymond and B. L. Hemming (UC Berkeley) for suggesting step viii in Scheme I.

Supplementary Material Available: Tables of crystallographic data, atom coordinates, bond distances and angles, anisotropic displacement parameters, and calculated H-atom coordinates (8 pages). Ordering information is given on any current masthead page.

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(10) Under our experimental conditions, the couple ferrocenium (Fc^+)/ferrocene (Fc) is at $E_{1/2} = 0.455$ V vs Ag/AgCl (saturated LiCl in MeOH). Ferrocene was used as internal standard.