populations that the D_{3d} hexamer exists as two interacting distorted D_{3h} trimers.

The bonding in the ethyllithium monomer and dimer is similar to that of CH_3Li and $(CH_3Li)_2$, respectively. In C_2H_5Li the methyl substituent about the central ethyl carbon causes the Li–C centroid of charge to be shifted from the Li–C axis while the Li substituent has a similar effect on the C–C bond. For the dimer only a small effect due to the presence of the methyl substituent is seen on the Li–C–Li bonds while the lithiums have little effect on the bonding in the ethyl group.

Conclusions

We have shown that there exist stable oligomers of $(CH_3Li)_n$ for n = 1–6 with significant binding energies and that for n = 4 and n = 6 there are at least two different geometries that yield stable clusters. The calculated ionization potentials and various structural predictions have been used to provide a new interpretation of previous mass-spectral studies. We have investigated the amount of metal–metal bonding through the use of overlap populations and find significant metal–metal interactions in the planar forms, but little intermetallic bonding in the condensed aggregates. The bonding in all of the alkyllithium clusters is interpreted in terms of localized molecular orbitals. The planar forms are bound by a network of closed three-center Li–C–Li bonds. The hybridization on carbon is normal, but the hybridization on Li for $n > 1$ usually has significantly more Li(2p) than Li(2s) character. This shows the large amount of promotion on Li needed for bonding in these clusters. The heavy atoms of the T_d tetramer are connected by closed four-centered bonds, each involving three lithiums and a carbon. The bonding in the D_{3d} hexamer consists of closed three-center Li–C–Li bonds with significant donation from a third lithium. This leads to the conclusion that the hexamer is composed of weakly interacting distorted D_{3h} trimers. Calculations on the monomer and dimer of ethyllithium show that the extra CH_3 substituent on the carbon has very little effect.

Acknowledgments. We thank the Alfred P. Sloan Foundation and Du Pont for support of this research. We also thank the University of Minnesota Computing Center for a grant of computer time.

Supplementary Material Available: Molecular coordinates (5 pages). Ordering information is given on any current masthead page.

Circular Dichroism, Crystal Structure, and Absolute Configuration of the Siderophore Ferric N,N',N''-Triacetylfusarinine, $FeC_{39}H_{57}N_6O_{15}$

M. B. Hossain, D. L. Eng-Wilmot, R. A. Loghry, and Dick van der Helm*

Contribution from the Department of Chemistry, The University of Oklahoma, Norman, Oklahoma 73019. *Received February 4, 1980*

Abstract: The siderophore ferric N,N',N''-triacetylfusarinine ($FeC_{39}H_{57}N_6O_{15}$) was isolated from iron-deficient cultures of an unidentified *Penicillium* species and purified by anion exchange, adsorption, and gel-filtration chromatographies. The chelate was crystallized as the benzene solvate (six to seven molecules of benzene per asymmetric unit) in the orthorhombic space group $P2_12_12_1$ with four molecules per unit cell of the dimensions a = 21.82 (3), b 23.89 (3), and c = 14.14 (1) Å at −135 (2) °C. The intensity data (5669) were collected on a CAD-4 automatic diffractometer, using Mo $K\bar{\alpha}$ radiation at −135 (2) °C. The structure was solved by a Patterson synthesis and successive difference Fourier syntheses, and refined by least-squares methods. The final R values were 0.141 for all data and 0.108 for 4566 observed data. The Fe^{3+} ion is coordinated by the oxygen atoms of the three hydroxamate groups in a distorted octahedron. The molecule is relatively flat with a total thickness of about 4.5 Å; three of the benzene solvent molecules are tucked between the macrocyclic rings. In the crystals obtained from ethanol/benzene, the molecules assume the Λ-cis absolute configuration, as determined from anomalous dispersion and circular dichroism on the solid state; however, in solution, and in the morphologically different crystals from chloroform, the molecule exists predominantly as the Δ-cis diastereoisomer, as determined by circular dichroism.

Introduction

It is well known under iron-limiting conditions (ca. 10^{-7} M Fe) that aerobic microbial growth is facilitated by the elaboration of low molecular weight, Fe(III)-specific sesquestering agents, termed siderophores, responsible for the acquisition and assimilation of iron.[1-3] Seven families of siderophores have been identified, including ferrichromes, ferrioxamines, fusarinines, rhodotorulic acids, aerobactins, enterobactins, and mycobactins. Two types of ligating groups prevail among these families, the hydroxamates and the catecholates, both of which form stable octahedral complexes with iron, where the iron atom is d^5, high spin,[4,5] and readily exchangeable.[6]

The fusarinines are members of a unique class of amino acid esters isolated from various species of fungi.[7-11] The monomeric unit, fusarinine, or N^{δ}-(*cis*-5-hydroxy-3-methylpent-2-enoyl)-N^{δ}-hydroxy-L-ornithine, has been isolated from *Fusarium roseum*.[7] Fusarinine undergoes polymerization via the formation of head-to-tail ester linkages (aminoacyl bonds) rather than the peptide bonds more common to other siderophores. Fusarinine C[7] or fusigen,[9] the cyclic triester of fusarinine, is thought to be the ferric ionophore for *F. roseum*. However, owing to the extreme lability of the aminoacyl bonds of fusarinine C, efforts to characterize this siderophore have been limited.

The N-acetyl analogue of fusarinine, or N^{α}-acetyl-N^{δ}-(*cis*-5-hydroxy-3-methylpent-2-enoyl) N^{δ}-hydroxy-L-ornithine, has been

---

(1) Neilands, J. B. "Inorganic Biochemistry", Eichhorn, G. L., Ed.; American Elsevier: New York, 1973; Vol. I, pp 167–202.

(2) Emery, T. F. *Adv. Enzymol.* **1971**, *35*, 135–185.

(3) Raymond, K. N.; Carrano, C. J. *Acc. Chem. Res.* **1979**, *12*, 183–190.

(4) Ehrenberg, A. *Nature (London)* **1956**, *178*, 379–380.

(5) Epstein, L. M.; Straub, D. K. *Inorg. Chem.* **1969**, *8*, 453–457.

(6) Lorenberg, W.; Buchanan, B. B.; Rabinowitz, J. C. *J. Biol. Chem.* **1963**, *238*, 3899–3913.

(7) Sayer, J. M.; Emery, T. F. *Biochemistry* **1968**, *7*, 184–190.

(8) Moore, R. E.; Emery, T. F. *Biochemistry* **1976**, *15*, 2719–2723.

(9) Diekmann, H.; Zähner, H. *Eur. J. Biochem.* **1967**, *3*, 213–218.

(10) Anke, H. *J. Antibiot.* **1977**, *30*, 125–128.

(11) Middleton, A. J.; Cole, D. S.; MacDonald, K. D. *J. Antibiot.* **1978**, *31*, 1110–1115.

0002-7863/80/1502-5766$01.00/0

isolated from cultures of *Penicillium*[8] and *Aspergillus*[9–11] species, and the chemical structure has been elucidated.[8] *N*-Acetylfusarinine also undergoes esterification, forming both linear and cyclic ligands capable of coordinating iron. These esters by virtue of the *N*-acylation of the α-amino group are considerably more stable than the corresponding fusarinines. The cyclic triester *N,N′,N″*-triacetylfusarinine is thought to be the siderophore for an unidentified *Penicillium* species, although the tris monomer complex has also been reported to transport iron.[12] In addition, this siderophore was reported to exhibit strong antibiotic activity toward various *Proteus* species[11] and moderate activity toward *Bacillus brevis*[10,11] and *Staphylococcus aureus*.[11] The specificity of iron transport by siderophores in fungi was demonstrated in *Neurospora crassa*[12] and several *Aspergillus* species.[13]

The mechanism of iron transport by the cyclic fusarinines has been investigated by Emery,[14,15] who isolated esterases involved in the iron release from the cyclic triesters. It was proposed that aminoacyl bond hydrolysis occurred concurrently with ferrous ion release; reduction of ferric ion seems a prerequisite for hydrolysis, since the esterase was completely inactive toward the aluminum chelate, although such a reduction was not experimentally supported. This mechanism is similar, but not analogous, to that proposed[16] for the release of ferric ion from enterobactin, the cyclic triester of 2,3-dihydroxybenzoylserine, a siderophore elaborated by *Escherichia coli*[17] and other enteric bacteria.[18]

The stereochemical and conformational role of ferric–hydroxamate coordination in microbial iron transport has been and continues to be of considerable interest for two reasons. First, it has been demonstrated that the ligand, whether linear or cyclic, must undergo a significant conformational change upon coordination of ferric ion. This was documented by Emery[19] in exchange experiments with ferrichrome and ferrioxamine chelates and with NMR studies[20] comparing the isomorphous aluminum chelates of ferrichromes and deferriferrichromes. Secondly, stereochemical specificity of the coordination isomer transported across fungal membranes has only recently been documented. The synthesis of *enantio*-ferrichrome was reported,[21] and it was shown that, while the natural Λ-cis isomer was actively transported in *N. crassa*, the Δ-cis isomer was assimilated by a diffusion-controlled process.[22] In *Aspergillus quadricinctus*, although ferrichrome was assimilated more effectively than *enantio*-ferrichrome, the mechanistic aspects of the kinetic results were inconclusive.[22] These findings suggest a conformational and stereospecific dependence of the membrane receptor involved in active iron transport by siderophores.

As a consequence of these observations, the conformation and absolute configuration of siderophores and deferrisiderophores are of interest. The crystal structures of six hydroxamate-type siderophores have previously been determined. These include ferrichrome (Λ-cis),[23] ferrichrome A[24] and its isomorphous Al(III) analogue, aluminochrome A (Λ-cis),[25] ferrioxamine E (racemic),[26] ferrichrysin (Λ-cis),[27] ferrimycobactin P (Δ-cis),[28] and tris(*N*-methylformothiohydroxamato)iron(III).[29] In addition, the structures of several synthetic model compounds have been reported, including tris(benzohydroxamato)iron(III),[30] and the potassium salt of tris(catecholato)ferrate(III).[31] We have determined and report here the molecular and crystal structure of the siderophore ferric *N,N′,N″*-triacetylfusarinine ($FeC_{39}H_{57}N_6O_{15}$) and its absolute configuration in the crystalline state and in solution.

Experimental Section

Organism Source and Culture. The organism, an unidentified *Penicillium* species, was obtained through the courtesy of Dr. Thomas Emery, Department of Chemistry and Biochemistry, Utah State University, Logan.

Stock cultures (50 mL) were maintained in modified Grimm–Allen medium,[32] pH 6.8, consisting of (per liter of doubly distilled, deionized water) 1.0 g of K_2SO_4, 3.0 g of K_2HPO_4, 3.0 g of ammonium acetate, 20 g of sucrose, and 1.0 g of citric acid. Metal enrichments, added as hydrated sulfate salts, included Mg^{2+}, 80 mg; Cu^{2+}, 5 μg; Zn^{2+}, 2 mg; Mn^{2+}, 25 μg (final concentration per liter). Cells were periodically incubated on agar plates prepared with this medium to check culture purity.

Organisms brought into culture for siderophore production were grown in the medium described above; however, prior to metal ion supplements and sterilization, the medium was passed through a bed of Chelex-100 resin (Na^+ form, Bio-Rad Laboratories) to remove residual amounts of iron. The average iron concentration of the production medium, determined spectrophotometrically as total iron,[33] was less than 1 μg/L (10^{-7} M Fe). Upon metal supplementation and pH adjustment, the medium was dispensed in 600-mL aliquots to acid-cleaned, sterile 2.8-L Fernbach flasks and autoclaved at 121 °C and 15 psi for 15 min. Cultures were grown at 24 °C in an controlled environment incubator (New Brunswick Scientific) with constant agitation (100 rpm).

Isolation and Purification of Ferric *N,N′,N″*-Triacetylfusarinine. Cultures were harvested after 7–14 days of incubation by filtration of the mycelia, and the cell-free filtrate was concentrated (ten times) by flash evaporation at 35 °C. The ferric complexes of the cyclic and linear ligands were prepared by addition of 0.7 g of $FeSO_4$/100 mL of concentrate and aerated for 2 h. Following centrifugation, the chelates were isolated by repeated extraction with phenol–chloroform (1:1 v/v), and then with 1-butanol, as described in detail elsewhere.[8]

Purification of the ferric chelate of the cyclic triester was accomplished in three steps. Initial separation of the cyclic siderophore from its linear analogues was achieved by anion exchange chromatography on DEAE-Sepadex (Cl^- form, Pharamacia, 2.2 × 20 cm). The neutral cyclic trimer was eluted with water, while the linear anionic complexes remained on the column.

Fractions containing the cyclic triester were pooled, evaporated to dryness, dissolved in 2 mL of redistilled chloroform (water saturated), and applied to a 3.5 × 17 cm bed of silica gel H (Type 60, TLC grade, E. Merck), previously washed with 1 L of water-saturated chloroform. The sample was eluted by using a stepwise gradient of water-saturated chloroform and methanol. Silica gel chromatography allowed the final separation of several neutral, nonpolar components, as well as traces of the complex of the linear trimer. The 90:10 v/v fractions containing the cyclic chelate were pooled, evaporated to dryness, and dissolved in a small amount of water.

Removal of residual amounts of dissolved silica was accomplished by gel filtration chromatography on a 1.5 × 75 cm bed of Bio Gel P-2 (100–200 mesh, Bio-Rad Laboratories) developed with water. Following purity assays (see below), the sample was filtered (0.2-μm Millipore membrane), lyophilized, dried in an oven (110 °C), and stored over P_2O_5 in a desiccator.

Sample homogeneity was evaluated during purification by using thin layer chromatography and high-voltage paper electrophoresis. Seven elution systems were employed to assess final purity, including (with

---

[12] Winkelmann, G.; Zähner, H. *Arch. Mikrobiol.* **1973**, *88*, 49–60.

[13] Wiebe, C.; Winkelmann, G. *J. Bacteriol.* **1975**, *123*, 837–842.

[14] Emery, T. *Biochemistry* **1976**, *15*, 2723–2728.

[15] Emery, T. *Anal. Biochem.* **1976**, *71*, 294–299.

[16] O'Brien, I. G.; Cox, G. B.; Gibson, F. *Biochim. Biophys. Acta* **1971**, *237*, 537–549.

[17] O'Brien, I. G.; Gibson, F. *Biochim. Biophys. Acta* **1970**, *215*, 393–402.

[18] Pollack, J. R.; Nielands, J. B. *Biochem. Res. Commun.* **1970**, 38, 989–992.

[19] Emery, T. F. *Biochemistry* **1967**, *6*, 3858–3866.

[20] Llinâs, M.; Klein, M. P.; Neilands, J. B. *J. Mol. Biol.* **1970**, *52*, 399–414.

[21] Naegeli, H.-U.; Keller-Schierlein, W. *Helv. Chim. Acta* **1978**, *61*, 2088–2095.

[22] Winkelmann, G. *FEBS Lett.* **1979**, *97*, 43–46.

[23] van der Helm, D.; Baker, J. R.; Eng-Wilmot, D. L.; Hossain, M. B.; Loghry, R. A. *J. Am. Chem. Soc.* **1980**, *102*, 4224–4231.

[24] Zalkin, A.; Forrester, J. D.; Templeton, D. H. *J. Am. Chem. Soc.* **1966**, *88*, 1810–1814.

[25] van der Helm, D.; Baker, J. R.; Loghry, R. A., submitted for publication in *Acta Crystallogr.*

[26] van der Helm, D.; Poling, M. *J. Am. Chem. Soc.* **1976**, *98*, 82–86.

[27] Norrestam, R.; Stensland, B.; Brändên, C.-I. *J. Mol. Biol.* **1975**, *99*, 501–506.

[28] Hough, E.; Rogers, D. *Biochem. Biophys. Res. Commun.* **1974**, *57*, 73–77.

[29] Murray, K. S.; Neuman, P. J.; Gatehouse, B. M.; Taylor, D. *Aust. J. Chem.* **1972**, *31*, 983–992.

[30] Lindner, J. J.; Göttlicher, S. *Acta Crystallogr., Sect. B* **1969**, *25*, 832–842.

[31] Raymond, K. N.; Isied, S. S.; Brown, C. D.; Fronczek, F. R.; Nibert, J. H. *J. Am. Chem. Soc.* **1976**, *98*, 1767–1774.

[32] Grimm, P. W.; Allen, P. *Plant Physiol.* **1954**, *29*, 369–377.

[33] Collins, P. F.; Diehl, H.; Smith, G. F. *Anal. Chem.* **1959**, *31*, 1862–1867.

Table I. Crystal Data

ferric *N,N′,N″*-triacetylfusarinine-benzene solvate
$FeC_{39}H_{57}N_6O_{15}\cdot 7C_6H_6$
mol wt 1452.6
orthorhombic, $P2_12_12_1$
$a = 21.82\ (3)$ Å
$b = 23.89\ (3)$ Å
$c = 14.14\ (1)$ Å
$V = 7371$ Å at -135 ± 2 °C
$Z = 4$
$\rho_m = 1.29$ g/cm^3 at 22 °C
$\rho_c = 1.31$ g/cm^3
$\mu = 2.8$ cm^{-1} for Mo radiation

mean R_f values) (a) 3:1 $CHCl_3$–CH_3OH (0.65); (b) 12:3:5 1-butanol-acetic acid-water (0.51); (c) 4:1:1 1-butanol-acetic acid-water (0.28); (d) 3:1 $CHCl_3$–ethanol (0.45); (e) 65:25:4 $CHCl_3$–CH_3OH–H_2O (0.73); (f) 4:1:1 1-propanol-acetic acid-water (0.37); (g) 2:1:1 $CHCl_3$–benzyl alcohol–CH_3OH (0.70). Detection systems used included iodine vapors, 5% $FeCl_3$ in 0.5 N HCl, and UV light. High-voltage paper electrophoresis was performed on Whatman 3MM paper, using a pyridine-acetic acid buffer (14:10:930 mL of water, pH 4.9) at a constant voltage of 1000 V for 1.5 h in a water-cooled enclosed chamber. Aqueous sucrose was used as the neutral standard.

Reductive hydrolysis of 3–5-mg samples of the chelate and the metal-free ligand to ornithine with 55% hydriodic acid at 110 °C for 17 h was carried out by using established procedures.[8] The ornithine-containing residues were purified by anion exchange chromatography (DEAE-Sephadex, Cl^- form, pH 6) and purity was assessed by TLC and electrophoresis. The L stereoisomer was identified by circular dichroism spectra compared to the identical spectra of authentic L-ornithine monohydrochloride. The observed ellipticity (+) at 200 nm was linear in the range 10–1000 μg/mL.

Spectroscopic Measurements. Ultraviolet-visible spectra were measured on a Cary Model 118 recording spectrophotometer. Mass-spectrum analysis of the crystalline material was performed on a Hitachi Model RMU-6 spectrometer. Circular dichroism spectra were measured on a Cary Model 61 recording spectropolarimeter. Difference CD spectra were measured for aqueous and nonaqueous solutions of crystalline samples, as well as of single crystals randomly suspended in an anhydrous inert matrix (KBr). The KBr disks (13-mm diameter, less than 1-mm thickness) were prepared following a procedure reported previously.[34] The KBr disks were covered with a thin film of Nujol and stored over activated silica gel in a desiccator over P_2O_5. For the solution CD spectra, calculations of $\Delta\epsilon$ values were made by using the standard expression,[35] while for the crystalline CD measurements molar ellipticities $[\theta]$ were obtained by using the expression of Ho and Takagi.[34]

Crystallization and Data Collection. Ferric *N,N′,N″*-triacetylfusarinine was crystallized from an ethanol solution equilibrated with benzene at 0 °C. Large, blocky crystals appeared within 36–48 h along with some small, well-formed plates and rods. When dry, the crystals were extremely unstable at room temperature and turned to powder within 15–20 s. The crystals were, however, stable at low temperature (~−135 °C). A crystal of size 0.80 × 0.50 × 0.45 mm was used for all X-ray measurements. The crystal was mounted while still under benzene and quickly transferred into a beam of cold nitrogen.

Crystal data for the compound are listed in Table I. The cell parameters were obtained by least-squares fit to 2θ values of 18 reflections. The density was measured by the method of flotation, using a mixture of benzene and bromobenzene.

The intensities of all 5669 unique reflections with $2\theta \leq 46°$ were measured at −135 (2) °C on a Nonius CAD-4 automatic diffractometer, using a graphite monochromator and Mo $K\bar{\alpha}$ radiation and employing the θ–2θ scan technique. The scan width was calculated as $(1.0 + 0.2 \tan\theta)°$. A horizontal receiving aperture with a variable width of $(5.0 + 0.86 \tan\theta)$ mm, and a constant height of 6 mm, was located 173 mm from the crystal. A reflection was scanned for a maximum time of 60 s, with two-thirds of the time spent scanning the peak (P) and one-sixth of the time spent on each of the left and right backgrounds (LB and RB). The unscaled intensities were calculated as $I = P - 2(\mathrm{LB} + \mathrm{RB})$. A standard reflection was monitored after every 40 min of X-ray exposure. The intensity of the monitor refection decreased about 9% during the 7-day period required for data collection. Three orientation control reflections were centered after every 200 observations. In case of angular change greater than 0.1°, a new orientation matrix was automatically determined from a list of 15 reflections. Of the total number of reflections measured, 945 were considered "unobserved" on the basis that $I < 2\sigma(I)$, and these were not included in the least-squares calculations. Each structure amplitude was assigned a weight $w_F\ (=1/\sigma_F^2)$, where σ_F was obtained from counting statistics.[36] The data were corrected for Lorentz and polarization factors. No absorption correction was applied.

Structure Determination and Refinements. The position of the Fe atom was located from a three-dimensional Patterson map. Further examination yielded the positions of four of the six oxygen atoms of the coordination octahedron. Structure-factor calculations with these atoms gave an R factor of 45%. The atom positions were refined until the R factor was 37%. A difference Fourier synthesis calculated at this point contained a large number of peaks. Atom positions were carefully selected from the difference map accepting only those peaks which made chemically recognizable molecular fragments. The complete molecular structure was obtained from four successive difference Fourier maps.

The measured density suggested the presence of a substantial number of solvate molecules in the crystal. Mass-spectral analysis showed the presence of only benzene, with no traces of ethanol or water. It is estimated that there are probably six to seven benzene molecules per asymmetric unit. From the next difference Fourier map, the positions of three benzene molecules were easily identified. The structure was further refined with isotropic thermal parameters for the nonhydrogen atoms of the molecule and isotropic thermal parameters for the benzene molecules. A successive difference Fourier map showed several (12) peaks of appreciable height at positions larger than the van der Waals distance from the molecule. These peaks were all given the full occupancy of a carbon atom and their contributions were included in the structure-factor calculation. The methylene hydrogen positions were calculated and included in these computations. Anomalous dispersion effects for the ferric ion were also taken into account.

The refinements were discontinued when parameter shifts were less than 40% of their corresponding standard deviations. The final R factor for 4566 reflections included in the least-squares calculations was 10.8% and was 14.1% for all 5669 reflections. Several factors may account in part for the rather high discrepancy factor. In spite of our best effort, the crystal was exposed to air for a brief period (7–10 s) during the mounting procedure, which no doubt is responsible for some disorder. Only three of the six to seven benzene molecules were located. The 12 extra peaks were regarded as fragments of the other benzene molecules. Obviously, these molecules are disordered and no attempt was made to determine the degree or nature of the disorder. Finally, the size of the data crystal made an absorption correction imperative. Unfortunately, during deicing, the crystal decomposed before the faces could be measured.

In an effort to overcome some of the limitations of the present structure and to improve structure refinement and precision of these results, a second set of diffraction data was collected with a smaller, well-shaped crystal of the chelate. The intensity data were collected under the same experimental conditions described above. The structure solution was identical with that reported here and provided no further insight into the problem of the disorder of the four solvate molecules. In addition, there was no improvement in the precison neither for the bond lengths and angles nor for the final R values (11.1% for the 4056 reflections compared to 10.8% for 4566, originally).

All least-squares refinements were carried out by a block-diagonal least-squares program[37] in which the quantity $\sum w_F(|F_o| - |F_c|)^2$ was minimized. The scattering factors for Fe, O, N, and C atoms were taken from the International Tables for X-ray Crystallography,[38] and those of hyrogen from Stewart, Davidson, and Simpson.[39] The values of $\Delta f'$ and $\Delta f''$ for Fe were taken from International Tables for X-ray Crystallography.[38] The final positional parameters for all nonhydrogen atoms of the molecule are listed in Table II. The atom numbering scheme is shown in Figure 1.

Absolute Configuration. The absolute configuration of the chelate was determined by the Bijvoet method, using anomalous dispersion of Cu $K\alpha$ radiation by the iron atom. Structure factors for both (hkl) and $(\bar{h}\bar{k}\bar{l})$ were calculated for each reflection, using the anomalous dispersion of the Fe scattering factor due to Cu radiation. A set of 17 Friedel pairs with highest $|F|^2$ differences was chosen. A crystal selected from the same batch as the data crystal was mounted and intensities of both hkl and

---

(34) Ho, N.; Takagi, T. *Biochim. Biophys. Acta* **1970**, *221*, 430–441.

(35) Bunnenberg, E.; Djerassi, C.; Mislow, K.; Moscowitz, A. *J. Am. Chem. Soc.* **1962**, *84*, 2823–2826.

(36) Ealick, S. E.; van der Helm, D.; Weinheimer, A. J. *Acta Crystallogr., Sect. B* **1975**, *31*, 1618–1626.

(37) Ahmed, F. R. SFLS Program, NRC-10, National Research Council of Canada, Ottawa, 1966.

(38) "International Tables for X-ray Crystallography", Vol. IV; Kynoch Press: Birmingham, England, 1974; p 73.

(39) Stewart, R. F.; Davidson, E. R.; Simpson, W. T. *J. Chem. Phys.* **1965**, *42*, 3175–3187.

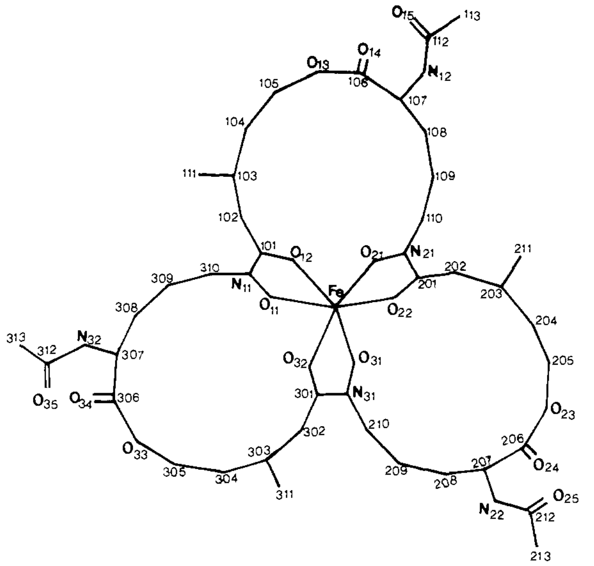

Figure 1. A schematic drawing of ferric N,N',N''-triacetylfusarinine showing the atom numbering scheme followed in the text.

$\bar{h}\bar{k}\bar{l}$ reflections for all 17 pairs were measured at -135 (2) °C.

Discussion

Description of the Crystal Structure. A stereoview of the single molecule is shown in Figure 2. The X-ray structure of ferric N,N',N''-triacetylfusarinine confirmed the postulated cyclic triester structure proposed earlier.[8] The molecule possesses three central five-membered chelate rings formed by hydroxamate groups of three N^{α}-acetyl-N^{δ}-hydroxy-L-ornithines, and these in turn are surrounded by three macrocycles of N^{δ}-(*cis*-5-hydroxy-3-methylpent-2-enoyl) units. In the crystalline state, the molecule assumes the Λ-cis configuration (vide infra) similar to that observed in the ferrichrome family.[23-25,27]

The bond lengths, bond angles, and torsion angles in the three macrocylic rings are listed in Tables III, IV, and V, respectively. Except for some differences in the bond lengths C(*i*01)–C(*i*02) and N(*i*2)–C(*i*07) and the bond angle O(*i*2)–C(*i*01)–C(*i*02), the three *N*-acetylfusarinine units are dimensionally equivalent and assume identical conformations leading to a pseudo-threefold symmetry for the overall molecule. All three ester bonds are situated about 5.2 Å from the central metal atom.

The molecule possesses a certain flatness; the maximum thickness at any point is 4.5 Å. A side view of the molecule is given in Figure 3. Individually, the three 15-membered macrocycles are surprisingly flat; the maximum deviation of a ring atom from the least-squares plane through the 15 atoms is less than 1.1 Å in all three rings. There is no unusual fold in the macrocycles and the *N*-acetyl groups are within the plane of the ring system.

A stereoview of the molecular packing, including the three benzene solvate molecules, is presented in Figure 4. The benzene molecules take up positions between the macrocyclic rings of the chelate such that their midpoints are nearly equidistant from the Fe atom (5.8, 5.9, and 6.2 Å, respectively). The closest Fe–Fe distances are 12.1, 13.4, and 14.4 Å. It is interesting to note that the benzene molecules lie almost along the lines between any two neighboring iron atoms. The overall packing indicates that there are no apparent cavities or channels through which solvate molecules might transverse the crystal, as might be expected from the extreme instability of the crystal. The presence of benzene molecules as solvate is unusual, and suggests a general lipophilic character of the chelate, an observation made previously in chemical studies.[11]

The geometry and dimensions of the coordination octahedron are given in Figure 5. The six hydroxamate oxygen donor atoms form the familiar trigonally distorted octahedral structure around the metal atom. The deviation from the perfect octahedral symmetry is reflected in the angles (O–Fe–O) in the chelate rings. The metal to *N*-hydroxyl oxygen distances are systematically shorter than the metal to carbonyl oxygen distances. The average Fe–O(N) distance of 1.973 (15) Å and Fe–O(C) distance of 2.037 (3) Å are quite similar to those observed in other iron trihydroxamates like ferrichrome[23] (1.983, 2.034 Å), ferrichrome A[24,25] (1.980, 2.033 Å), and ferrichrome E[26] (1.953, 2.005 Å).

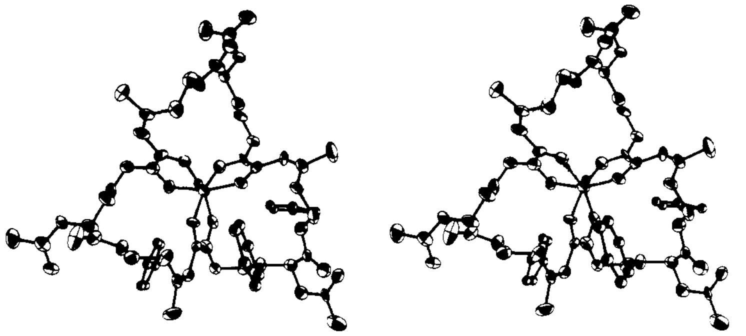

Figure 2. A stereoview of a single molecule of ferric N,N',N''-triacetylfusarinine along with the three benzene solvate molecules.

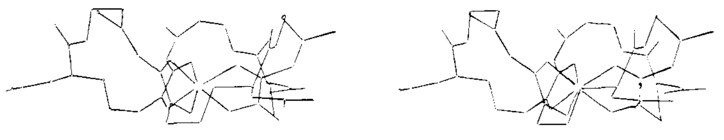

Figure 3. A side view of ferric N,N',N''-triacetylfusarinine showing the flatness of the molecule.

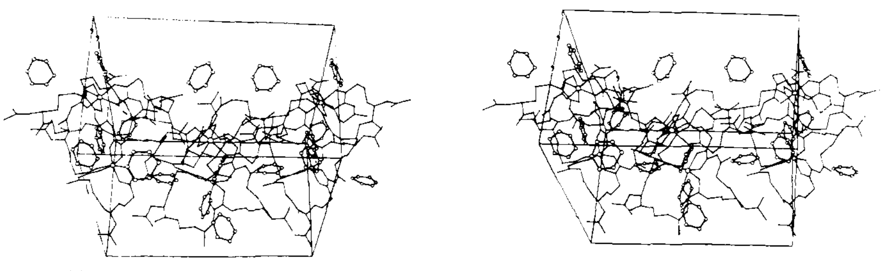

Figure 4. Stereoview of the packing scheme in the crystal structure of ferric triacetylfusarinine–benzene solvate.

Table II. Positional Parameters of Nonhydrogen Atoms[a]

Chelate Molecule

atom	10^4x	10^4y	10^4z
Fe	76.1 (10)	4091.4 (9)	1934 (2)
O(11)	−576 (4)	3673 (4)	1280 (7)
O(12)	−616 (5)	4668 (4)	1910 (7)
O(21)	443 (5)	4264 (4)	672 (7)
O(22)	702 (5)	4689 (4)	2271 (6)
O(31)	576 (4)	3422 (4)	2093 (6)
O(32)	−123 (4)	3866 (4)	3290 (6)
O(13)	−817 (5)	5955 (4)	439 (7)
O(14)	−421 (5)	6768 (4)	−83 (8)
O(15)	−692 (6)	6295 (7)	−2374 (9)
O(23)	2076 (5)	4582 (4)	3603 (7)
O(24)	2626 (6)	4321 (4)	4850 (7)
O(25)	3587 (5)	3593 (5)	3566 (8)
O(33)	−1525 (4)	3390 (4)	4369 (7)
O(34)	−2521 (5)	3488 (6)	4595 (10)
O(35)	−2721 (6)	2185 (5)	4042 (9)
N(11)	−1085 (6)	3969 (5)	1115 (8)
N(12)	164 (6)	6199 (5)	−1544 (8)
N(21)	900 (6)	4629 (5)	713 (8)
N(22)	2752 (6)	3248 (5)	4246 (9)
N(31)	504 (6)	3150 (5)	2951 (9)
N(32)	−2759 (6)	2855 (6)	2939 (10)
C(101)	−1087 (8)	4488 (7)	1476 (10)
C(102)	−1661 (9)	4833 (7)	1386 (11)
C(103)	−1743 (8)	5367 (7)	1590 (12)
C(104)	−1263 (7)	5786 (7)	1951 (12)
C(105)	−1116 (8)	6220 (7)	1223 (12)
C(106)	−488 (8)	6267 (7)	−120 (11)
C(107)	−109 (7)	5896 (6)	−793 (10)
C(108)	356 (8)	5559 (7)	−241 (11)
C(109)	673 (8)	5099 (6)	−776 (10)
C(110)	1123 (7)	4790 (6)	−231 (11)
C(111)	−2372 (8)	5626 (9)	1450 (16)
C(112)	−140 (8)	6391 (8)	−2302 (12)
C(113)	215 (10)	6733 (8)	−3004 (13)
C(201)	1026 (7)	4878 (6)	1588 (9)
C(202)	1489 (6)	5299 (6)	1630 (10)
C(203)	1709 (7)	5554 (6)	2425 (10)
C(204)	1527 (7)	5430 (6)	3432 (10)
C(205)	2014 (7)	5133 (7)	3985 (11)
C(206)	2370 (6)	4214 (6)	4131 (9)
C(207)	2331 (7)	3637 (6)	3701 (11)
C(208)	1684 (7)	3428 (6)	3705 (11)
C(209)	1590 (8)	2904 (8)	3093 (12)
C(210)	922 (7)	2700 (6)	3127 (12)
C(211)	2172 (9)	6018 (9)	2327 (13)
C(212)	3346 (6)	3300 (6)	4185 (11)
C(213)	3711 (9)	3006 (9)	4899 (17)
C(301)	138 (6)	3416 (6)	3559 (9)
C(302)	35 (7)	3155 (6)	4539 (10)
C(303)	−302 (6)	3353 (6)	5213 (10)
C(304)	−741 (7)	3852 (6)	5179 (10)
C(305)	−1401 (7)	3690 (6)	5231 (11)
C(306)	−2117 (7)	3329 (8)	4139 (13)
C(307)	−2169 (9)	3045 (7)	3126 (13)
C(308)	−1964 (9)	3480 (7)	2387 (12)
C(309)	−1827 (7)	3230 (8)	1441 (12)
C(310)	−1596 (8)	3641 (8)	717 (12)
C(311)	−291 (8)	3040 (8)	6144 (11)
C(312)	−3026 (8)	2456 (7)	3473 (11)
C(313)	−3659 (9)	2314 (9)	3306 (15)

Three Benzene Molecules

atom	10^4x	10^4y	10^4z	B, Å^2
C(1)	−464 (9)	1498 (8)	2780 (13)	5.3 (4)
C(2)	−508 (9)	2056 (9)	2564 (14)	5.6 (4)
C(3)	−345 (9)	2274 (8)	1705 (13)	5.2 (4)
C(4)	−233 (9)	1142 (8)	2147 (14)	5.4 (4)
C(5)	−121 (12)	1885 (10)	1084 (16)	7.5 (6)
C(6)	−46 (12)	1323 (10)	1305 (17)	7.5 (6)
C(7)	2991 (11)	3274 (10)	−181 (17)	7.2 (6)
C(8)	2438 (16)	3225 (15)	−17 (25)	12.1 (10)
C(9)	3309 (13)	3616 (13)	379 (22)	10.1 (8)
C(10)	2066 (13)	3638 (13)	715 (21)	9.6 (7)
C(11)	2458 (12)	3968 (11)	1150 (18)	8.6 (7)
C(12)	3082 (15)	3997 (14)	1148 (22)	11.0 (9)
C(13)	348 (10)	4961 (9)	5054 (16)	6.1 (5)
C(14)	729 (10)	4561 (10)	5091 (17)	7.3 (6)
C(15)	1005 (11)	4367 (9)	5878 (16)	6.8 (5)
C(16)	734 (17)	4584 (16)	6761 (27)	13.2 (10)
C(17)	325 (13)	5014 (12)	6703 (21)	9.6 (7)
C(18)	90 (12)	5219 (10)	5837 (17)	7.8 (6)

Disordered Peaks

atom	10^4x	10^4y	10^4z
P1	−2955	4101	27
P2	−221	3852	−833
P3	−2355	3651	−1368
P4	−1217	4084	−1857
P5	1534	1675	−1410
P6	−3400	4560	−260
P7	−3250	4950	−880
P8	−1450	4890	−1280
P9	−1120	4610	−2000
P10	−1940	2700	−2000
P11	−2370	3030	−1600
P12	−150	3220	−800
P13	440	3110	−600

[a] Estimated standard deviation for the last digit in parentheses.

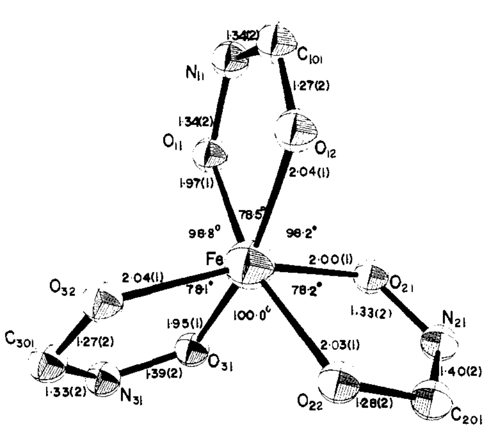

Figure 5. The geometry and dimensions of the iron coordination octahedron. The angles not shown here are listed in Table IV.

The difference between the two types of Fe–O bond lengths shows that the majority of negative charge resides on the *N*-hydroxy oxygen atoms. However, the planarity of the bonds around N(*i*1) atoms (sum of bond angles in all three cases is 359°) is an indication that some of the negative charge is located on the carbonyl oxygens. Further, the mean chelate ring O–Fe–O angle of 78.2° is quite similar to that found in the three hydroxamate structures referred to above. The hydroxamate groups are approximately planar (root mean square deviation of individual atoms in the three groups: 0.007, 0.016, and 0.010 Å). The metal atom deviations from the planes of the three hydroxamate groups are 0.087, 0.070, and 0.087 Å. The values are comparable to those observed in the case of ferrioxamine E.

The three five-membered chelate rings are nearly equidimensional. However, there are some differences in the ring dimensions, particularly in the N–O and N–C distances. In the present structure the average N–O and N–C distances within the chelate rings are nearly equal (1.35 and 1.36 Å, respectively), while in other ferric hydroxamates the N–O distances are systematicaly longer than the N–C distances.

Figure 6 presents a view of the coordination octahedron down the pseudo-threefold axis along with all of the O–O distances outlining the octahedral edges. The short O–O distances, (O_{11}–O_{12}) 2.54, (O_{21}–O_{22}) 2.53, and (O_{31}–O_{32}) 2.51 Å, are in good

Table III. Interatomic Distances (Å) in the Three Macrocyclic Rings[a]

	i = 1	*i* = 2	*i* = 3
C(*i*01)-C(*i*02)	1.50 (3)	1.43 (2)	1.54 (2)
C(*i*02)-C(*i*03)	1.32 (2)	1.36 (2)	1.30 (2)
C(*i*03)-C(*i*04)	1.54 (2)	1.51 (2)	1.53 (2)
C(*i*04)-C(*i*05)	1.50 (2)	1.50 (2)	1.49 (2)
C(*i*05)-O(*i*3)	1.43 (2)	1.43 (2)	1.44 (2)
O(*i*3)-C(*i*06)	1.30 (2)	1.32 (2)	1.34 (2)
C(*i*06)-C(*i*07)	1.54 (2)	1.51 (2)	1.59 (3)
C(*i*07)-C(*i*08)	1.51 (2)	1.50 (2)	1.54 (2)
C(*i*08)-C(*i*09)	1.50 (2)	1.54 (2)	1.50 (2)
C(*i*09)-C(*i*010)	1.45 (2)	1.54 (2)	1.51 (2)
C(*i*010)-N(*i*+1,1)	1.47 (2)	1.43 (2)	1.47 (2)
C(*i*07)-N(*i*2)	1.42 (2)	1.52 (2)	1.39 (2)
N(*i*2)-C(*i*12)	1.34 (2)	1.30 (2)	1.35 (2)
C(*i*12)-C(*i*13)	1.50 (3)	1.47 (3)	1.44 (3)
C(*i*12)-O(*i*5)	1.23 (2)	1.24 (2)	1.23 (2)
C(*i*03)-C(*i*11)	1.52 (3)	1.51 (3)	1.51 (2)
C(*i*06)-O(*i*4)	1.21 (2)	1.19 (2)	1.16 (2)

[a] Estimated standard deviations are in parentheses.

Table IV. Bond Angles (Deg) in the Three Macrocyclic Rings[a]

	i = 1	*i* = 2	*i* = 3
Fe-O(*i*1)-N(*i*1)	114.4 (8)	113.4 (8)	114.9 (8)
Fe-O(*i*2)-C(*i*01)	112.1 (10)	116.2 (9)	114.2 (8)
O(*i*2)-C(*i*01)-C(*i*02)	122 (1)	127 (1)	123 (1)
O(*i*2)-C(*i*01)-N(*i*1)	120 (1)	114 (1)	118 (1)
N(*i*1)-C(*i*01)-C(*i*02)	118 (1)	118 (1)	118 (1)
O(*i*1)-N(*i*1)-C(*i*01)	115 (1)	118 (1)	114 (1)
O(*i*1)-N(*i*1)-C(*i*+2,10)	114 (1)	112 (1)	116 (1)
C(*i*01)-N(*i*1)-C(*i*+2,10)	129 (1)	129 (1)	129 (1)
C(*i*01)-C(*i*02)-C(*i*03)	129 (2)	127 (1)	127 (1)
C(*i*02)-C(*i*03)-C(*i*04)	128 (2)	127 (1)	128 (1)
C(*i*02)-C(*i*03)-C(*i*11)	119 (2)	119 (1)	117 (1)
C(*i*04)-C(*i*03)-C(*i*11)	113 (1)	114 (1)	115 (1)
C(*i*03)-C(*i*04)-C(*i*05)	112 (1)	113 (1)	114 (1)
C(*i*04)-C(*i*05)-O(*i*3)	109 (1)	108 (1)	106 (1)
C(*i*05)-O(*i*3)-C(*i*06)	118 (1)	116 (1)	116 (1)
O(*i*3)-C(*i*06)-C(*i*07)	110 (1)	111 (1)	110 (1)
O(*i*3)-C(*i*06)-O(*i*4)	127 (2)	125 (1)	124 (1)
O(*i*4)-C(*i*06)-C(*i*07)	122 (1)	125 (1)	126 (2)
C(*i*06)-C(*i*07)-C(*i*08)	110 (1)	111 (1)	107 (1)
C(*i*06)-C(*i*07)-N(*i*2)	113 (1)	109 (1)	112 (1)
C(*i*08)-C(*i*07)-N(*i*2)	112 (1)	111 (1)	111 (1)
C(*i*07)-C(*i*08)-C(*i*09)	116 (1)	113 (1)	113 (1)
C(*i*08)-C(*i*09)-C(*i*10)	115 (1)	112 (1)	115 (1)
C(*i*09)-(C*i*10)-N(*i*+1,1)	113 (1)	111 (1)	110 (1)
C(*i*07)-N(*i*2)-C(*i*12)	124 (1)	121 (1)	122 (1)
N(*i*2)-C(*i*12)-C(*i*13)	117 (2)	117 (1)	119 (1)
N(*i*2)-C(*i*12)-O(*i*5)	119 (2)	122 (1)	120 (2)
C(*i*13)-C(*i*12)-O(*i*5)	124 (2)	122 (1)	120 (2)
O(*i*1)-Fe-O(*i*+1,1)	88.6 (4)	92.7 (4)	92.4 (4)
O(*i*1)-Fe-O(*i*+1,2)	162.4 (4)	168.4 (4)	165.6 (4)
O(*i*2)-Fe-O(*i*+1,2)	91.5 (4)	96.2 (4)	92.1 (4)

[a] Estimated standard deviations for the last digit are given in parentheses.

agreement with the corresponding values observed in ferrichrome, ferrichrome A, and ferrioxamine E. The distorted octahedral geometry of the chelate is in fact an intermediate structure between a trigonal prism and an octahedron, with a twist angle of 42.5° and ligand bite of 1.26.[31]

Absolute Configuration of Ferric *N,N′,N″*-Triacetylfusarinine. Two geometric isomers, cis and trans, are possible for an octahedral complex formed by coordination of a ferric ion by three equivalent optically active hydroxamate groups. Each geometric isomer can exist as the Λ and Δ optical isomers. The iron-coordination geometry of ferric *N,N′,N″*-triacetylfusarinine is cis (Figure 6). The cis (fac) isomer appears to be the preferred isomer among the cyclic ferric trihydroxamates,[23-29] and its prevalence suggests that it is the thermodynamically stable isomer, despite the presence of significant negative charge (oxime group contributions) localized in one face of the octahedron. Examination of molecular models indicates that the trans isomers of cyclic siderophores are improbable for steric reasons, although both the cis and trans isomers of kinetically inert Cr(III) chelates of the linear trihydroxamates deferriferrioxamine B and deferriferrioxamine D$_1$ have been prepared and resolved chromatographically.[40]

Table V. Torsion Angles (Deg) in the Three Macrocyclic Rings

	i = 1	*i* = 2	*i* = 3
O(*i*+1,1)-Fe-O(*i*2)-C(*i*01)	84	89	88
Fe-O(*i*2)-C(*i*01)-C(*i*02)	178	179	−178
O(*i*2)-C(*i*01)-C(*i*02)-C(*i*03)	11	6	−2
C(*i*01)-C(*i*02)-C(*i*03)-C(*i*04)	2	2	8
C(*i*02)-C(*i*03)-C(*i*04)-C(*i*05)	111	108	110
C(*i*03)-C(*i*04)-C(*i*05)-O(*i*3)	−66	−68	−67
C(*i*04)-C(*i*05)-O(*i*3)-C(*i*06)	−159	−164	−163
C(*i*05)-O(*i*3)-C(*i*06)-C(*i*07)	169	173	174
O(*i*3)-C(*i*06)-C(*i*07)-C(*i*08)	−65	−66	−71
C(*i*06)-C(*i*07)-C(*i*08)-C(*i*09)	169	168	164
C(*i*07)-C(*i*08)-C(*i*09)-C(*i*10)	179	179	−177
C(*i*08)-C(*i*09)-C(*i*10)-N(*i*+1,1)	46	50	50
C(*i*09)-C(*i*10)-N(*i*+1,1)-O(*i*+1,1)	66	58	68
C(*i*10)-N(*i*+1,1)-O(*i*+1,1)-Fe	−174	−172	−174
N(*i*+1,1)-O(*i*+1,1)-Fe-O(*i*2)	94	98	94
C(*i*06)-C(*i*07)-N(*i*2)-C(*i*12)	−75	−70	−62
C(*i*07)-N(*i*2)-C(*i*12)-O(*i*5)	−2	−13	−12

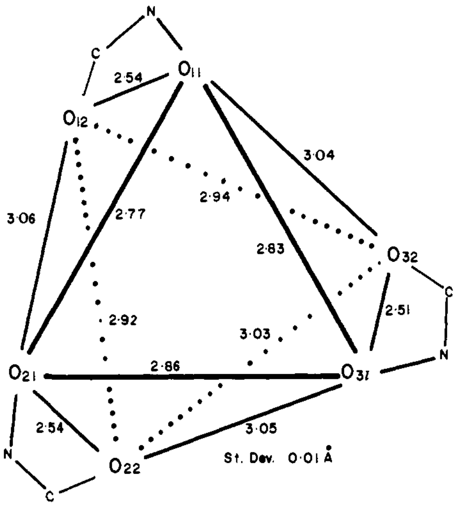

Figure 6. A view of the coordination octahedron down the pseudo-threefold axis, showing O-O distances of the octahedral edges.

A molecule of ferric *N,N′,N″*-triacetylfusarinine possesses four centers of chirality: an asymmetric carbon atom [C(*i*07), Figure 1] in each of the three substituted ornithinyl residues, and the ferric ion coordination sphere. Three independent determinations establish that *N*-hydroxyornithine is present in the title compound in solution and the crystalline state as the L stereoisomer. Reductive hydrolysis with 55% hydriodic acid of the chelate and ligand yielded L-ornithine. This was determined by comparison of circular dichroism spectra of the purified hydrolysis products to those of authentic samples of L-ornithine. Moore and Emery[8] also demonstrated the presence of L-ornithine in the title compound, using an L-ornithine-requiring mutant of *E. coli*. Finally, the absolute configuration of the molecule, crystallized from ethanol equilibrated with benzene, was determined by using anomalous dispersion. The results, presented in Table VI, show for 16 of the 17 reflections that the cyclic triester is indeed derived from L-ornithine and that the absolute configuration of the three ligating hydroxamate groups about the central ferric ion is Λ-cis. This absolute configuration is shown in all the figures.

A comparison was made of the absolute configuration of the molecule in the crystalline state and in solution by measuring circular dichroism of the chelate in proximity of the characteristic charge-transfer absorption at 440 nm (ϵ 2996 M^{-1} cm^{-1}, upper curve, Figure 7). The CD spectrum of single crystals dispersed

---

(40) Leong, J.; Raymond, K. N. *J. Am. Chem. Soc.* **1975**, *97*, 293–296.

Table VI. Comparison of Observed and Calculated Bijvoet Differences

			DEL[a]		SF[b]	
h	*k*	*l*	calcd	obsd	calcd	obsd
7	3	6	−156	−182	−55	−22
9	3	4	−70	−132	−46	−26
10	3	3	−161	−116	−56	−15
1	5	5	−58	−78	−46	−13
2	5	6	−109	−123	−65	−20
3	5	3	154	82	59	9
4	5	2	101	79	49	8
5	5	3	77	15	48	9
2	6	2	106	44	69	5
1	12	1	−85	−44	−48	−9
1	6	1	−44	−82	−45	−13
6	1	4	57	−10	44	−2
8	1	6	−74	−151	−45	−29
8	3	3	−72	−137	−45	−22
5	11	5	−144	−111	−70	−27
2	10	2	73	73	50	13
3	7	5	−104	−87	−45	−18

[a] DEL = $[F^2(+) - F^2(-)]/{}^1/_2[F^2(+) + F^2(-)] \times 100$. [b] SF = $[F^2(+) - F^2(-)]/\sigma(F^2)$, where $F^2(+) = F^2(hkl)$ and $F^2(-) = F^2 \cdot (\bar{h}\bar{k}\bar{l})$ and $\sigma(F^2)$ is obtained from the estimated error in a single observation for SF(obsd) and calculated from the root mean square error for SF(calcd).

in a KBr disk (solid curve A, Figure 7, where [θ] at 500 nm is +1.76 × 10^5 deg cm^2 dmol^{-1} and at 370 nm −10.64 × 10^5) is qualitatively the same as solution and crystalline CD spectra of ferrichrome.[23] The absolute configuration of ferrichrome, discussed elsewhere,[23] is Λ-cis, and these results are thus in agreement with the Λ-cis absolute configuration determination for ferric *N,N′,N″*-triacetylfusarinine in the crystalline state, by anomalous dispersion, reported here.

In aqueous and nonaqueous solutions, however, the circular dichroism spectra of the chelate (dashed curve C, Figure 7, Δε −2.04 M^{-1} cm^{-1} at 467 nm and +3.25 at 370 nm) indicate that molecules of the ferric chelate exist predominatly as the Δ-cis diastereoisomer in solution vis-à-vis the Λ-cis in the crystalline state. Although the complexation constants for ferric trihydroxamates are quite large (ca. 10^{30}), the d^5 electronic configuration of the Fe^{3+} ion renders the complexes kinetically labile. It is apparent then that, on dissolution of crystalline material, an equilibrium is established between the Λ-cis and Δ-cis isomers, in which the latter is predominant. This isomeric equilibrium is established rapidly in solution (less than 1 min) as evidenced by enantiomorphic CD curves obtained by measurement of the spectrum immediately upon dissolution of a KBr disk containing crystals. A similar rapid equilibrium was observed in the case of the tris(benzohydroxamato)iron(III) complex, for which the diastereoisomers were not resolvable.[41] The compound crystallized in a racemic cis form.[30]

Crystallization of ferric *N,N′,N″*-triacetylfursarinine from ethanol equilibrated with benzene at 0 °C resulted in the preferential crystallization of the minor and less soluble isomer (Λ-cis). It is obvious that the absoslute configuration of the other three chiral centers (L-ornithine) does not change. Consequently, if both diastereoisomers crystallized, the crystals could not be isomorphic. To validate the preferential crystallization of the Λ-cis isomer, 15 representative crystals were mounted and examined at −135 °C. The results were unequivocal in that all showed the same unit cell dimensions and the same Bijvoet sign differences (Table VI).

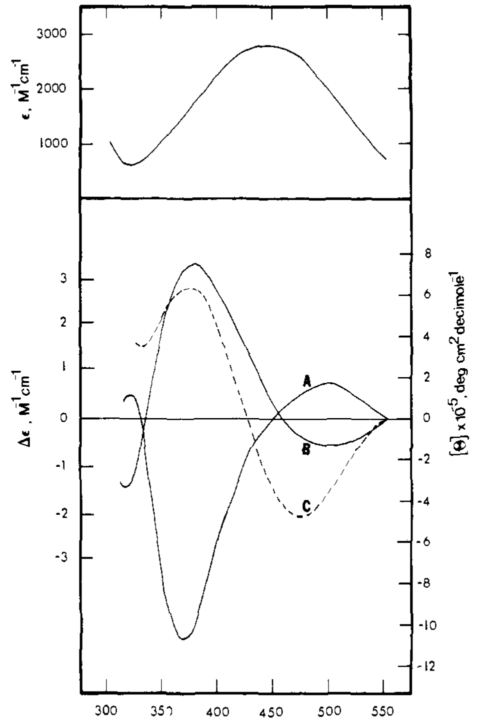

Figure 7. Visible (upper) and circular dichroism (lower) spectra of ferric *N,N′,N″*-triacetylfusarinine. CD spectra A and B are of crystals obtained from ethanol–benzene and chloroform, respectively, and curve C is a solution spectrum.

Crystals of the title compound were also obtained as long, thin needles by slow evaporation from chloroform. As yet, no crystals from chloroform have been observed that were suitable for X-ray diffraction data collection. Nonetheless, preliminary oscillation and Weissenberg photographs indicate that the cell dimensions differ from those reported here. The circular dichroism spectrum of these crystals dispersed in KBr (solid curve B, Figure 7, where [θ] is −1.27 × 10^5 at 500 nm and +7.48 × 10^5 at 380 nm) showed that this crystal form of the chelate was diastereomeric to crystals obtained from ethanol–benzene solution. Molecules in these crystals from chloroform therefore assume the same absolute configuration (Δ-cis) for the iron coordination as that observed for the predominant isomer in solution. The conclusion therefore has to be made that one should consider the possibility in the case of all siderophores that an equilibrium may exist between the Λ-cis and Δ-cis isomers of the ferric chelate of the siderophore in solution.

The use of single-crtystal circular dichroism in an inert matrix such as KBr offers a unique opportunity to compare the absolute configuration of a molecule in both solution and in the crystalline state. However, two problem areas were recognized, using crystalline circular dichroism techniques. First, pellets should be isotropic. Microscopic examination of the disks with polarizing light indicated that the crystals were intact and randomly dispersed throughout the pellet and that there were no preferential orientations of individual crystals in the disk, as reported previously.[34] This, with the observation that the spectrum was unchanged upon rotation of the disk in either direction from the initial position, indicated that the pellets were isotropic. Perhaps the most serious problem and limitation encountered was control of moisture. It was important that the disks be isolated from air, inasmuch as minimal moisture resulted in the loss of disk transparency. Also, owing to the hygroscopic nature of the matrix and the crystals, the pellets must remain "anhydrous" during preparation and storage; otherwise, the dissolution of single crystals would alter the results significantly. These problems were solved by covering the disks with a thin layer of Nujol and storing them over activated silica gel.

In summary, we report the purification, crystal structure, and absolute configuration in the crystalline state and in solution of the ferric chelate of the siderophore *N,N′,N″*-triacetylfusarinine. This is one of the first reports on the resolution of an equilibrium mixture of diastereoisomers of a kinetically labile ferric chelate by preferential crystallization. More significant, however, is the approach taken in the evaluation of the absolute configuration

---

(41) Leong, J.; Raymond, K. N. *J. Am. Chem. Soc.* **1974**, *96*, 1757–1762.

of such a chelate, that is, the direct correlation of the crystalline and solution circular dichroism spectra with crystallographic results, rather than the more empirical assignments based on the use of model ligands, more kinetically inert metal-ion complexes, or closely related compounds relied on much in the past. Two additional conclusions can be drawn from our observations. In the case of ferric chelates of biological interest, it is necessary to compare the absolute configuration of the molecule in the solid state with that in solution. This communication represents such an example, and, in absence of an anomalous dispersion experiment, single-crystal and solution circular dichroism spectroscopy offers a seemingly satisfactory method. Our results can also be used to rationalize the apparent inactivity of the *Penicillium* esterase toward the aluminum chelate of *N,N′,N″*-triacetylfusarinine[14] by assuming stereochemical specificity of the esterase and a slow rate of equilibrium for the chelate. Finally, it is noteworthy that our findings do not allow the prediction of which diasteroisomer (Λ-cis or Δ-cis) of ferric *N,N′,N″*-triacetylfusarinine is actively transported across the microbial cell membrane. Experimentally, this question will be approached by performing the transport experiments with chromatographically resolved diastereoisomers of the chelate of a kinetically inert metal ion, such as Cr(III) or Co(III). This method was successfully used in probing the transport of ferrichrome in *Ustilago sphaerogena*, using resolved Λ-cis and Δ-cis chromic deferriferrichrome.[42]

Acknowledgments. We wish to express our gratitude to Dr. Neil Purdie, Department of Chemistry, Oklahoma State University, Stillwater, for the use of the spectropolarimeter, John M. Bowen for his expert technical assistance in obtaining the CD data, and Mr. Fred E. Dillon for his technical help in obtaining the mass-spectral data. One of the authors (D.v.d.H.) expresses his gratitude to Dr. T. Emery, Utah State University, for the help and hospitality he received while working in his laboratory. This research was sponsored by the National Institute of General Medical Sciences (GM 21822).

Supplementary Material Available: A listing of structure amplitudes, anisotropic thermal parameters of nonhydrogen atoms, and hydrogen atom parameters (33 pages). Ordering information is given on any current masthead page.

---

(42) Leong, J.; Neilands, J. B.; Raymond, K. N. *Biochem. Biophys. Res. Commun.* **1974**, *60*, 1066–1071.

Synthesis and Crystal and Molecular Structure of $[(Ph_3P)_2N^+][S_4N^-]$ and the Electronic Structure of the Planar Acyclic Anion, S_4N^- [1]

T. Chivers,* W. G. Laidlaw, R. T. Oakley, and M. Trsic

Contribution from the Department of Chemistry, University of Calgary, Calgary T2N 1N4, Alberta, Canada. *Received February 5, 1980*

Abstract: The thermal decomposition of $PPN^+S_4N_5^-$ [$PPN^+ = (Ph_3P)_2N^+$] in acetonitrile at 78 °C leads, sequentially, to the corresponding salts of the $S_3N_3^-$ and S_4N^- anions. The crystal and molecular structure of the dark blue salt, $PPN^+S_4N^-$, has been determined by X-ray crystallography. The compound crystallized in the space group $P\bar{1}$, triclinic, $a = 11.220$ (2) Å, $b = 16.860$ (3) Å, $c = 9.862$ (2) Å, $\alpha = 99.17$ (1)°, $\beta = 98.04$ (1)°, $\gamma = 71.56$ (1)°, $V = 1739.2$ Å^3, and $Z = 2$. The refined structure ($R_1 = 0.064$) shows that the S_4N^- anion is an essentially planar, cis,trans chain with nitrogen as the central atom. The terminal S–S bond distances are remarkably short (1.879 (3) and 1.943 (2) Å), while the S–N bond lengths show a pronounced inequality (1.667 (5) and 1.521 (5) Å). The bond angles at the internal sulfur atoms are ca. 110.5 (2)° and the angle at nitrogen is 120.8 (3)°. Infrared and Raman spectra of $PPN^+S_4N^-$ and $PPN^+S_4{}^*N^-$ (where $^*N = 30\%\ ^{15}N$) demonstrate that the vibrational frequencies at ca. 592 and 565 cm^{-1} can be assigned to the stretching modes of the unequal S–S bonds. Ab initio Hārtree–Fock–Slater SCF calculations have been carried out for S_4N^- and show that the calculated statistical energy of the experimental conformation is not substantially lower than that of the symmetrical cis,trans or trans,trans conformations of the anion. The ground-state electronic structure of S_4N^- is an 8π-electron system. The strong absorption in the visible spectrum at 582 nm appears to be the result of a $\pi^*(\text{HOMO}) \rightarrow \pi^*(\text{LUMO})$ transition. The S–N linkages can be described approximately as net $^2/_3\sigma$ plus $^1/_2\pi$ bonds, while the S–S bonds are approximately $^5/_4\sigma$ plus $^1/_4\pi$. The terminal sulfur atoms each carry two lone pairs of electrons and bear large negative charges, −0.42 and −0.44, as does the nitrogen, −0.35; the internal sulfur atoms have one lone pair of electrons and have small positive charges, +0.01 and +0.20. The experimental conformation may be the result of an electrostatic interaction between terminal and internal sulfur atoms.

Introduction

The formation of a blue color on deprotonation of cyclic sulfur imides, e.g., S_7NH, in basic media is used as a spot test in the chromatographic separation of these imides for *cyclo*-S_8.[2] The same deep blue color is also dramatically apparent during the preparation of sulfur imides from (a) sulfur monochloride and ammonia in DMF[2] or (b) sodium azide and *cyclo*-S_8 in HMPA.[3] A number of attempts to identify the species responsible for the blue color have been made. The suggestion that it is a neutral sulfur radical S_x ($x = 2$–4)[4] was shown to be incorrect by Chapman and Massey, who demonstrated that it was negatively charged and diamagnetic; they proposed it to be the S_7N^- anion.[5] Subsequent work by Olsen and Olsen established the presence of *cyclo*-S_7N^- in solutions resulting from the deprotonation of S_7NH with various bases.[6] On the basis of detailed studies of the alkylation of such solutions, these workers suggested that *cyclo*-S_7N^- was in equilibrium with other anions, including an "open-

---

(1) For a preliminary communication see: Chivers, T.; Oakley, R. T. *J. Chem. Soc., Chem. Commun.* **1979**, 752.

(2) Heal, H. G.; Kane, J. *Inorg. Synth.* **1968**, *11*, 184.

(3) (a) Bojes, J.; Chivers, T. *J. Chem. Soc., Dalton Trans.* **1975**, 1715. (b) Bojes, J.; Chivers, T.; Drummond, I. *Inorg. Synth.* **1978**, *18*, 203.

(4) Lux, H.; Anslinger, H. *Chem. Ber.* **1961**, *94*, 1161.

(5) Chapman, D.; Massey, A. G. *Trans. Faraday Soc.* **1962**, *58*, 1291.

(6) (a) Olsen, B. A.; Olsen, F. P. *Inorg. Chem.* **1969**, *8*, 1736. (b) Tingle, E. M.; Olsen, F. P. *Ibid.* **1969**, *8*, 1741.

0002-7863/80/1502-5773$01.00/0