have been seen previously in the complexes of 12-crown-4[42,48] and other systems.[45,46] Similar yet disordered hydrophilic "channels" have been reported recently as well.[49] With regards to the relation between this crystal structure and the structure of the complex in solution, we feel that the crystal structure should resemble fairly closely that in aqueous solution because of the large number of water molecules present and the "aqueous-like" environment in the crystal. However, the structure of the complex in organic solvents or synthetic membranes is probably quite different.

Attempts to isolate suitable single crystals of un-ionized **2** with other lithium salts at neutral pH or in water-free solutions have been unsuccessful. A pH dependence of complexation tendencies is implied a priori by the presence of an ionizable carboxylic acid. It is most reasonable that at alkaline pH, the complexation of lithium cations by **2** will be independent of the counterion that is present in the aqueous solution.

Of primary concern for the ability of **2** to function as an effective phase-transport reagent for lithium ions is the apical water molecule which is required to complete the coordination polyhedron of the metal cation. This "exposed" water molecule would certainly affect the stability of the complex in an organic phase, and the results of preliminary solvent polymeric membrane transport studies with **2** indicate that Na^+ and K^+ (and in some conditions Rb^+ and Cs^+) are transported more readily than Li^+.[50] Perhaps these larger cations (effective diameters[31] in the range of 1.98 Å (Na^+) to 3.56 Å (Cs^+)) do not require an intervening water molecule for favorable pentavalent coordination to **2**. If one constructs a three-dimensional "cavity" from the four ethereal oxygens (O1–O4) and O7 of the carboxylate group (and a possible solvent molecule, or two, to complete a 6- or 7-coordination shell), the adjusted diameter of this "cavity"[32] is ≈2.8 Å (O1–O7, 5.72 Å; O2–O7, 5.55 Å). This diameter compares rather well with the effective diameter of K^+ (2.74–2.92 Å for 4–7-coordinated K^+).[31] The assumption of this argument, that the conformation of the molecule remains similar to the one described for **2a**, requires further study. An additional factor which may relatively disfavor Li^+ complexation may be the differing nature of interaction in an organic environment (such as that in the synthetic polymeric membranes) between the coronand, the cation, and the surroundings. Thus, even the structure may be modified by the organic environment.

It does seem likely that a longer, and perhaps less flexible, pendant moiety would allow for more favorable Li^+ complexation relative to the other alkali metal cations. The binding of larger divalent metals to **2** is strongly disfavored,[50] as has been described previously for the DB-14-C-4 system.[12,22] This is an important quality in the molecular design of lithium-selective compounds for industrial and medical applications.[51,52]

Synthetic attempts are now underway to create a coronand which has a longer pendant carboxylate arm so that its complexation with Li^+ can be studied. Examination of molecular models suggests that some oxy-benzoic acid derivatives of DB-14-C-4 would also meet the structural requirements outlined above, and this may allow a more favorable syn orientation of the lithium cation to the carboxylate.

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Registry No. 2, 80186-73-8; **2a**, 88271-61-8; *sym*-hydroxydibenzo-14-crown-4, 42397-72-8; *sym*-hydroxydibenzo-14-crown-4, 78328-81-1; catechol, 120-80-9; 1,3-dibromopropane, 109-64-8; epichlorohydrin, 106-89-8; bromoacetic acid, 79-08-3.

Supplementary Material Available: A list of observed and calculated structure factors (Table S1), anisotropic temperature factors for non-hydrogen atoms (Table S2), and coordinates and temperature factors for all hydrogen atoms (Table S3) (21 pages). Ordering information is given on any current masthead page.

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Molecular Structure of Ferric Neurosporin, a Minor Siderophore-like Compound Containing N^{δ}-Hydroxy-D-ornithine

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Abstract: Ferric neurosporin, $FeC_{33}H_{51}N_6O_{15}\cdot 6CH_3CN$, or the ferric chelate of the cyclic triester of N^{α}-acetyl-N^{δ}-hydroxy-N^{δ}[(*R*)-3-hydroxybutyryl]-D-ornithine, is a new minor fusarinine-type siderophore-like compound isolated from cultures of *Neurospora crassa* (ATCC 10816). The compound was characterized chemically and spectroscopically and crystallized from ethanol equilibrated with acetonitrile at 0 °C in the rhombohedral space group *R*3 of the dimensions, hexagonal setting, $Z = 3$, $a = b = 19.774$ (18) Å, $c = 12.017$ (17) Å, $V = 4069.3$ Å^3, at −135 (2) °C. The structure was solved from a three-dimensional Patterson map and direct methods and refined to an *R* factor of 0.058. The molecule is flat (2.90-Å thickness) and assumes the Λ-cis absolute configuration about the central ferric ion, as determined from anomalous dispersion and circular dichroism measurements; the D configuration of ornithine was also proved by nonreductive hydrolysis–CD experiments. Conformationally, the N^{α}-acetylornithinyl groups are similar to those in ferric *N*,*N*′,*N*″-triacetylfusarinine, indicating that the stereochemistry of the ornithine residue asymmetrically induces the chirality at the metal ion in the fusarinines.

The N^{δ}-hydroxy analogue of the amino acid ornithine, $HON(H)CH_2CH_2CH_2CH(NH_3^+)COO^-$, is the fundamental structural unit for two classes of fungal cyclic trihydroxamate-type siderophores (the ferrichromes and fusarinines), or low molecular weight, high affinity Fe(III)-selective chelating agents responsible for the acquisition and active translocation of ferric ion across the fungal

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cell membrane.[1-3] It is thought that during "low-iron" stress fungal amino acid and lipid metabolic pathways are genetically diverted for the biosynthesis of ornithine and coenzyme A derivatives of specific fatty acids (R) for the enzymatic synthesis of N^{δ}-acyl-N^{δ}-hydroxyornithine (orn), $R-C(O)N(OH)-CH_2CH_2CH_2CH(NH_3^+)COO^-$.[4,5] Species specific, three molecules of N^{δ}-acyl-N^{δ}-hydroxyornithine are joined *either* with three additional amino acid residues (gly, ser, or ala) through their α-carboxyl and α-amino groups to form the homodetic (amide bond) cyclic hexapeptide backbone characteristic of the ferrichromes *or* by head-to-tail ester bonds between the α-carboxyl of one molecule and the primary hydroxyl of the acyl group of a second molecule to form the cyclic triester trihydroxamates (fusarinines).

The diversity of chemical structure and, therefore, of the chemistry and biological activity of members of the ferrichromes and fusarinines results, not unexpectedly, from differences in peptide backbone and acyl group composition of the former and acyl and α-amino group substituents in the latter. This has been demonstrated by comparison of the relative rates of uptake of the various ferric siderophores indigenous to the fungus *Neurospora crassa*.[6] Conformationally, the peptide backbones of the various ferrichromes are similar, as determined by X-ray diffraction studies of ferrichrome,[7] ferrichrome A,[8,9] ferrichrysin,[10] and ferricrocin.[11] In addition, there is stereochemical uniformity among these molecules. That is, to date, the L stereoisomer of ornithine, serine, and alanine and the Λ-C-cis,cis iron-coordination diastereoisomer prevail. The two known members of the fusarinine class, the cyclic triester of N^{δ}-(*cis*-5-hydroxy-3-methylpent-2-enoyl)-N^{δ}-hydroxyornithine and its N^{α}-acetyl analogue,[12-14] are also derived from L-ornithine, as determined by spectroscopic and biological assays, and X-ray diffraction analysis.[14,15] However, unlike the ferrichromes, the ferric chelate of the tri-N^{α}-acetyl derivative exists in a diastereoisomeric equilibrium, with the Δ-cis isomer predominant.[15] The predominance of the Λ-C-cis,cis absolute configuration of the ferrichromes appears to be a direct consequence of the L-ornithine-derived backbone, as supported in the enantiomorphic solution circular dichroism spectra of natural (L-orn, Λ-cis) and synthetic *enantio*-ferrichrome (D-orn, Δ-cis).[16]

The uniformity of the L (S) configuration of the asymmetric centers among ornithine-derived microbial iron transport compounds is not surprising and is a reflection of the genetic dictates imparted to the enzymatic pathways involved in siderophore and membrane-receptor protein biosynthesis and the stereospecificity of the membrane transport and iron removal mechanisms. It is, therefore, highly unusual to observe the D isomer of N^{δ}-hydroxyornithine in the peptide backbones of siderophore-like ferric ion chelating agents. Although this stereochemistry is often limited to peptide antiobiotics, such as bacitracin A[17] and enduracidin,[18] D-ornithine and other D-amino acids have been observed in several siderophore-like compounds, including ferribactin from *Pseudomonas fluorescens-migula*[19a-c] and pseudobactin and pseudobactin A from strains of *Pseudomonas fluroescens-putida*.[20,21] (Recent reports[19b,c] of the reinvestigation of ferribactin suggest that this dihydroxamate is a linear vis-ă-vis cyclic peptide of nine rather than ten amino acids, as originally reported;[19a] however, the configurations of the amino acids present in the new structure were not reported.) We report here the isolation, purification, and molecular structure determination of the ferric chelate of a new member of the fusarinine class of iron transport compounds, called neurosporin, a minor siderophore of *Neurospora crassa*. The compound is of interest because it is derived from D-vis-ă-vis L-hydroxyornithine, which is acylated by the hitherto unreported 3-hydroxybutyric acid group.

Experimental Section

Chemicals and Reagents. The siderophores ferricrocin (from *Aspergillus versicolor*) and ferric N,N',N''-triacetylfusarinine (from an unidentified *Penicillium* species) were purified and crystallized by previously described methods.[11,15] Ferric coprogen and the ferric chelate of dimerium acid were isolated and purified from cultures of *Neurospora crassa* (ATCC 10816) as described below. Crystalline samples of D- and L-ornithine monohydrochloride (Sigma) were used as obtained. All other chemicals and reagents were of analytical quality unless otherwise specified.

Organism Source and Culture. The various strains of *N. crassa* employed in these studies (ATCC 10816; Fungal Genetics No. 262, 353, 987, and 1945) were brought into culture in Vogel's Medium N,[22] pH 5.8 at 24 (2) °C. Once viable, cells were subcultured via asceptic conidal transfer into modified Medium N. Medium modification included $NaNO_3$ (2.0 gL^{-1}) for NH_4NO_3, $Na_3cit{\cdot}5H_2O$ (3.0 g L^{-1}) for $(NH_4)_3cit$ (cit = citrate), while $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ enrichments were omitted. This modification resulted in NO_3^- being the sole N source, invoking additional iron deficiency by stimulation of nitrate reductase biosynthesis.[23]

For large-scale siderophore experiments, cells were incubated via spore or conidal innocula in the modified Medium N described above that had been deferriated by treatment with Chelex-100 resin (Na^+ form, Bio-Rad Laboratories) prior to metal, biotin, and sucrose supplementation. Siderophore production was optimal for a surface-to-volume ratio of 35 cm^2/cm^3 (450 mL per 2.8-L Fernback flask).

Isolation and Purification of Ferric Neurosporin. Neurosporin, coprogen, and its degradation products (dimerium acid and N-acetylfusarinine) were isolated as their ferric chelates from 10–14-day cultures of *N. crassa* 10816 by using metal–chelate extraction procedure with 1:1 (v/v) phenol:chloroform, as reported earlier.[15] The red-brick chelates in aqueous solution were concentrated, applied to a gel-filtration column (3 × 30 cm, Bio-Gel P-2, Bio-Rad Laboratories), and eluted with water; fractions were immediately lyophilized to prevent additional ester hydrolysis of coprogen.

Ferric neurosporin was purified by partition chromatography on silica gel 60 (Type H, TLC grade, E. Merck) developed with water-saturated chloroform. The column was eluted with a stepwise gradient of ethanol–water saturated chloroform. The title compound eluted in the 25% ethanol fractions, which were pooled and evaported to dryness. The residue was dissolved in distilled water, partitioned into phenol–chloroform solution and then back into aqueous solution with diethyl ether. The final ether-washed aqueous solution was chromatographed on Bio-Gel P-2. The sample was judged homogeneous on the basis of a single sharp spot on silica thin layers developed in a number of elution systems and detection systems (see below).

Chromatography and Electrophoresis. Thin-layer chromatography was performed on silica gel 60 plates (E. Merck) in vapor-saturated chambers by using the following elution systems (v/v): (A) 4:1 $CH_3OH:CHCl_3$;

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Table I. Crystallographic Data for Ferric Neurosporin

N^{α}-acetyl-N^{δ}-hydroxy-N^{δ}-[(*R*)-3-hydroxybutyryl]-D-ornithine, cyclic triester, iron(III) chelate-acetonitrile solvate	
molecular formula	$FeC_{33}H_{51}N_6O_{15}\cdot 6CH_3CN$
formula weight	1074.0
crystal system	rhombohedral
space group	*R*3 (No. 146)
unit cell dimensions	[−135 (2) °C]:
rhombohedral:	$a = b = c = 12.099$ (9) Å
	$\alpha = \beta = \gamma = 109.61$ (4)°; $Z = 1$
hexagonal:	$a = b = 19.774$ (18) Å; $c = 12.017$ (17) Å
	$\gamma = 120°$
	$V = 4069.3$ Å^3
	$Z = 3$
ρ_{calcd}, g cm^{-3}	1.315
radiation	Mo Kα_1 ($\lambda = 0.70926$ Å) for cell data
	Mo K$\bar{\alpha}$ ($\lambda = 0.71069$) for intensity data
μ, cm^{-1}	3.65

(B) 3:1 $CHCl_3$:CH_3OH; (C) 4:1:1 1-propanol:acetic acid:H_2O; (D) 2:1:1 $CHCl_3$:benzyl alcohol:CH_3OH; and (E) 65:25:4 $CHCl_3$:CH_3OH:H_2O. Relative migration values (R_f) for ferric neurosporin, ferric *N,N′,N″*-triacetylfusarinine, ferric coprogen, and ferricrocin in the above eluants were (system A) 0.65, 0.67, 0.46, 0.32; (system B) 0.61, 0.71, 0.31, 0.38; (system C) 0.56, 0.59, 0.38, 0.25; (system D) 0.58, 0.62, 0.31, 0.26; and (system E) 0.63, 0.72, 0.39, and 0.48, respectively. High-voltage paper electrophoresis was performed as described previously[15] by using a pyridine–acetic acid buffer (10:14:390 mL H_2O), pH 5.2, with aqueous sucrose as a neutral standard.

Detection systems included 0.2% ethanolic ninhydrin, 5% $FeCl_3$ in 0.5 N HCl, I_2 vapors, and UV.

Hydrolysis. Partial base hydrolysis of a 1-mg sample of the chelate was carried out with NaOH. The tris complex was prepared upon neutralization by the addition of excess $FeCl_3$ and was purified by paper electrophoresis. A sample of the hydrolysate of *N,N′,N″*-triacetylfusarinine was electrophoresised concurrently.

Reductive hydrolysis of a 2.5-mg sample of the chelate to ornithine was carried out in 0.3 mL of 55% HI at 110 °C for 18 h,[24] and ornithine was purified by ion-exchange chromatography at pH 6.0 (DEAE-Sephadex, Cl^- form, Pharmacia). A similar sample was prepared from ferric *N,N′,N″*-triacetylfusarinine. Ornithine was identified by thin-layer chromatography developed against authentic samples. The stereoisomer (D or L) present in the hydrolysate was identified quantitatively by circular dichroism spectroscopy.

Spectroscopic Measurements. Electronic absorption spectra were measured on a Cary Model 118 recording spectrophotometer. Infrared spectrum were measured on a Beckman IR-12 recording infrared spectrophotometer. The infrared spectrum of ferric neurosporin exhibited the following absorptions: ($\bar{\nu}$, cm^{-1}) 3470 (s, b), 3400 (s, b), 3282 (sh), 2990 (w), 2945 (w), 1740 (s), 1676 (s), 1648 (sh), 1582 (w), 1540 (m), 1456 (s), 1388 (m), 1324 (2), 1302 (w), 1276, 1260 (w), 1220, 1202 (m), 1136 (m), 1080, 1054, 1020, 952, 836 (w). Solution and single-crystal circular dichroism spectra were recorded with a Cary Model 61 spectropolarimeter by using procedures reported previously.[15]

Crystallization and X-ray Diffraction Data Collection. Solubility studies indicated that the title compound was soluble in water, chloroform, and lower alcohols; sparingly soluble in acetonitrile, 1-propanol, and ethyl acetate; and insoluble in benzene, hexane, acetone, and diethyl ether. Large deep-red prismatic crystals were obtained by using equilibration techniques from an ethanol–acetonitrile (OMNISOLV) system at 0 °C. The crystals were unstable at room temperature and lost crystallinity upon removal from the mother liquor. A suitable single crystal was selected, mounted directly from the mother liquor, and quickly transferred into the cold vapor stream (−135 °C) of a Nonius nitrogen cooling system fitted to a PDP-8/e controlled Enraf-Nonius CAD-4 automatic diffractometer.

The crystallographic data for ferric neurosporin are given in Table I. The chelate crystallized in the space group *R*3 (No. 146) with three molecules in the hexagonal unit cell. The molecule, therefore, has a 3-fold symmetry axis coincident with the crystallographic symmetry axis, and only two molecules of acetonitrile are independent. The unit cell parameters were determined by a least-squares fit of the ±2 θ values of 48 reflections measured throughout all octants of reciprocal space by using graphite monochromated Mo Kα_1 radiation (λ = 0.70926 Å).

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Table II. Atom Coordinates (×10^5) (Hexagonal Setting) of Non-Hydrogen Atoms in Ferric Neurosporin[a]

atom	*x*	*y*	*z*
Fe	0	0	0
O(1)	4651 (11)	9812 (11)	8439 (16)
O(2)	−2513 (11)	6846 (11)	−10153 (17)
O(3)	−13713 (11)	13719 (11)	−9775 (17)
O(4)	−19920 (13)	16224 (13)	3922 (17)
O(5)	−34377 (12)	7673 (12)	−15712 (17)
N(1)	4295 (13)	15654 (13)	2855 (20)
N(2)	−33223 (14)	1438 (14)	−897 (21)
C(1)	543 (15)	13843 (15)	−6702 (22)
C(2)	−234 (17)	19919 (17)	−13120 (24)
C(3)	−6617 (16)	21281 (16)	−8580 (26)
C(4)	−19608 (16)	11870 (17)	−2717 (22)
C(5)	−25450 (16)	3233 (16)	−3857 (22)
C(6)	−22664 (16)	−1178 (16)	3433 (23)
C(7)	−27324 (16)	−9939 (16)	1752 (24)
C(8)	−23487 (16)	−14115 (16)	7194 (23)
C(9)	−7270 (21)	27434 (20)	−15073 (39)
C(10)	−37170 (16)	3886 (17)	−7147 (23)
C(11)	−45169 (20)	1685 (24)	−2953 (27)
Acetonitrile Molecules			
N(21)	25555 (20)	5346 (22)	24779 (36)
N(21)′	9739 (36)	−3436 (39)	−48745 (53)
C(21)	15113 (23)	8906 (24)	30200 (31)
C(22)	20924 (21)	6844 (20)	27162 (28)
C(21)′	1245 (26)	−10973 (26)	−32302 (35)
C(22)′	6036 (28)	−6713 (27)	−41540 (41)

[a] Standard deviations for the least significant digit are given in parentheses.

The integrated intensities of 5653 triclinic reflections for $2\theta \leq 53°$ were measured at −135 (2) °C with Mo K$\bar{\alpha}$ radiation (λ = 0.71069 Å), using the θ–2θ scan technique. The set consisted of 1889 independent reflections. All reflections are indexed in the hexagonal system, and the intensities were not averaged. As determined from examination of the crystal mosaic, a variable scan width was used, as calculated from the expression $(1.0 + 0.2 \tan \theta)°$, while the horizontal receiving aperture, located 173 mm from the crystal at a constant height of 6 mm, varied as $(4.0 \pm 0.86 \tan \theta)$ mm. Each reflection was scanned for a maximum of 60 s with two-thirds of this period spent enumerating the peak and one-sixth of this time spent scanning the left and right backgrounds. Three intensity monitor reflections were measured after every 3600 s of X-ray exposure and showed a 6.1% maximum variation during data collection. Orientation control reflections were centered after every 200 measurements; a new orientation matrix was automatically calculated from a list of 16 reflections if an angular change of 0.1° was observed. A total of 234 data were considered indistinguishable from the background on the basis that $I \leq 2.0\sigma(I)$ and were not included in least-squares refinement. The data were corrected for Lorentz and polarization effects; no absorption correction was applied (μ = 3.65 cm^{-1}). Each structure amplitude was assigned an experimental weighing factor w_F ($=1/\sigma_F^2$, where σ_F was based on counting statistics).[25]

Structure Determination and Refinement. The structure was solved by direct methods, using the program MULTAN[26] and a Patterson synthesis. Subsequent difference Fourier syntheses revealed two molecules of acetonitrile.

The structure of ferric neurosporin was refined by using the SHELX system of programs,[27] which used the atomic scattering factors for the Fe, C, N, O, and H atoms and the anomalous dispersion of Mo K$\bar{\alpha}$ radiation by the Fe atom in structure factor computations. The block refinements were carried out by segregating the atomic positional parameters of the molecule from those of the solvate molecules, with the coordinates of the central Fe atom (0,0,0) constrained; the appropriate anisotropic thermal factors were used. The hydrogen atoms were refined isotropically. The final *R* factor was 0.058 for all data. The final positional parameters for all non-hydrogen and hydrogen atoms are given in Tables II and III.

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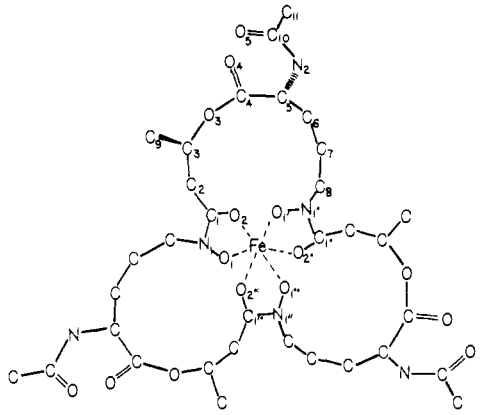

Figure 1. Chemical structure of ferric neurosporin.

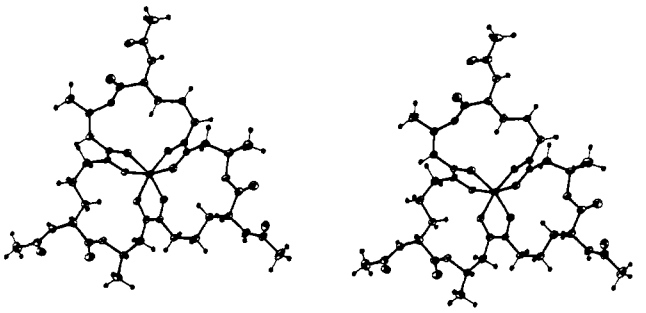

Figure 2. Stereoview of ferric neurosporin.

Determination of Absolute Configuration. The absolute configuration of ferric neurosporin was determined by the Bijvoet method using the anomalous scattering of Cu Kᾱ radiation (λ = 1.5418 Å) by the Fe atom. A set of 15 enantiomorphic-sensitive reflections were selected with the largest values of $(F_+^2 - F_-^2)/\sigma(F_o^2)$. The values of F_+^2 and F_-^2 were calculated according to the method of James.[28] The intensities of I_{hkl} and $I_{-h,-k,-l}$ for all Friedel pairs were measured at −135 (2) °C. Because of the unexpected configurations of the asymmetric carbon atoms, the experiment was performed several times by using different crystals.

Results and Discussion

Physical and Chemical Properties of Ferric Neurosporin. Although only about 18 mg of ferric neurosporin were isolated from the culture fluid of *N. crassa* ATCC 10816, considerable chemical information about the molecule was collected concurrently with the structure determination by X-ray diffraction.

Ferric neurosporin was electrophoretically neutral at pH 5.2, readily reduced by hydrosulfite, but only slowly decolorized by 0.2 M EDTA. The red-gold chelate exhibited a broad absorption at 440 nm characteristic of cyclic trihydroxamates with a molar absorptivity, $\epsilon_{1.0}^{M}$, of 2.71 × 10^3 L mol^{-1} (based on a molecular weight of 828, determined from the crystal structure); this charge-transfer band was pH invariant. The infrared spectrum exhibited strong C=O and C—O—C stretching vibrations characteristic of an ester at 1740 cm^{-1} and 1220 and 1202 cm^{-1}, respectively, which were also observed in the spectrum of the cyclic triester, ferric *N,N′,N″*-triacetylfusarinine, with N-substituted amide C=O stretching vibrations at 1676 and 1648 cm^{-1}, an N–H bend at 1540 cm^{-1}, and a $-CH_3$ bending vibration at 1388 cm^{-1}.

Partial hydrolysis in dilute base (1 h, room temperature) yielded a single $FeCl_3$-positive, ninhydrin-negative compound that behaved as a 1− anion at pH 5.2 with a relative electrophoretic mobility nearly identical with that of N^{α}-acetyl-N^{δ}-hydroxy-N^{δ}-(*cis*-5-hydroxy-3-methylpent-2-enoyl)-L-ornithine. Reductive hydrolysis with 55% HI yielded quantitatively (92%) 3 mol of ornithine per mol of chelate as the sole amino acid present.

Description of the Structure. The chemical structure, atom numbering scheme, and configuration of the asymmetric carbon atoms of ferric neurosporin, as determined by single-crystal X-ray diffraction, are shown in Figure 1; a stereoscopic view of the molecule is given in Figure 2. The molecule can be described as the ferric chelate of the cyclic triester having the fundamental repeat unit of N^{α}-acetyl-N^{δ}-hydroxy-N^{δ}-((*R*)-3-hydroxybutyryl)-D-ornithine. This siderophore-like compound (siderochrome) constitutes a new, third member of the triester family of cyclic trihydroxamate siderophores and contains the hitherto unreported acyl functionality, 3-hydroxybutyric acid. The configuration at the asymmetric carbon atom, C(3)*, of the acyl group is *R*, while that in the ornithinyl backbone, C(5)*, is D. In the crystalline state and in solution, the cyclic hexadentate ligand assumes the Λ-cis configuration about the central ferric ion (vide infra).

By virtue of the overall 3-fold symmetry, the three units of the molecule are dimensionally equivalent and assume identical conformations. The bond distances, bond angles, and torsion angles for ferric neurosporin are presented in Tables IV, V, and VI, respectively. There were no unusual or unexpected dimensional parameters observed in the molecule. Within the 13-membered macrocyclic rings, the conformations about the carbon–carbon bonds C(6)–C(7) and C(7)–C(8) of the ornithine backbone are anti and gauche, respectively, and that about the bond C(2)–C(3)* of the butyryl moiety is gauche. The ester linkage, including atoms C(5)*, C(4), O(3), and C(3)*, is nearly planar, with the carbonyl oxygen O(4) lying in the plane. Such a conformation, coupled with the configuration at C(5)*, gives rise to a large degree of planarity of the molecule, which has a maximum thickness at any point of 2.90 Å. The maximum deviation of any ring atom from the least-squares plane through the 13-membered macrocycles is −0.650 and 0.722 Å for O(2) and O(1)′. As observed in ferric *N,N′,N″*-triacetylfusarinine,[15] the N^{α}-acetyl group of ferric neurosporin lies in the plane of the macrocyclic ring, with the carbonyl group C(10)–O(5) and the exocyclic methyl C(9) directed toward the ester bond C(4)–O(3). Such an orientation no doubt imparts the reduced lability of the ester linkage toward hydrolysis.

The dimensions of the five-membered hydroxamate chelate rings are given in Figure 3 and are compared to the corresponding mean values of the hydroxamate rings of ferrichrome A, the structure determination of which was nearly as accurate (R = 0.071)[9] as

Table IV. Bond Distances (Å) in Ferric Neurosporin[a]

bond	distance	bond	distance
Fe–O(1)	1.963 (2)	C(5)–C(6)	1.521 (4)
Fe–O(2)	2.059 (2)	C(6)–C(7)	1.515 (4)
O(1)–N(1)	1.368 (3)	C(7)–C(8)	1.520 (4)
N(1)–C(1)	1.316 (4)	C(3)–C(9)	1.504 (5)
O(2)–C(1)	1.271 (4)	C(4)–O(4)	1.198 (4)
C(1)–C(2)	1.499 (4)	C(5)–N(2)	1.439 (4)
C(2)–C(3)	1.518 (5)	N(2)–C(10)	1.336 (4)
C(3)–O(3)	1.459 (4)	C(10)–C(11)	1.502 (5)
O(3)–C(4)	1.336 (4)	C(10)–O(5)	1.229 (4)
C(4)–C(5)	1.516 (4)	C(8)–N(1)′	1.458 (4)
Acetonitrile Solvate Molecules (2)			
C(21)–C(22)	1.445 (6)	C(21)′–C(22)′	1.429 (7)
C(22)–N(21)	1.131 (6)	C(22)′–N(21)′	1.110 (9)

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

Table VI. Torsion Angles (deg) in Ferric Neurosporin

Fe–O(2)–C(1)–C(2)	177.1	Fe–O(1)–N(1)–C(1)	3.7
O(2)–C(1)–C(2)–C(3)	88.1	O(1)–N(1)–C(1)–O(2)	0.1
C(1)–C(2)–C(3)–O(3)	−59.8	N(1)–C(1)–O(2)–Fe	−4.5
C(2)–C(3)–O(3)–C(4)	151.1	C(1)–O(2)–Fe–O(1)	4.3
C(3)–O(3)–C(4)–C(5)	−170.3	O(2)–Fe–O(1)–N(1)	−5.3
O(3)–C(4)–C(5)–C(6)	101.8	O(4)–C(4)–C(5)–N(2)	33.5
C(4)–C(5)–C(6)–C(7)	−173.7	C(4)–C(5)–N(2)–C(10)	78.0
C(5)–C(6)–C(7)–C(8)	167.0	C(6)–C(5)–N(2)–C(10)	−170.4
C(6)–C(7)–C(8)–N(1)′	−46.5	C(5)–N(2)–C(10)–C(11)	−179.2
C(7)–C(8)–N(1)′–O(1)′	97.9	C(5)–N(2)–C(10)–O(5)	0.8
C(8)–N(1)′–O(1)′–Fe	−167.1	C(7)–C(8)–N(1)′–C(1)′	−72.4
N(1)′–O(1)′–Fe–O(2)	72.0	C(1)–C(2)–C(3)–C(9)	179.5
O(1)′–Fe–O(2)–C(1)	117.1	C(8)–N(1)′–C(1)′–C(2)′	−9.2

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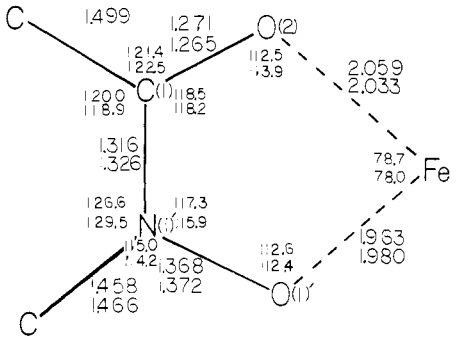

Figure 3. Comparison of the hydroxamate chelate rings of ferric neurosporin (upper values) and the corresponding mean values in ferrichrome A (lower values).

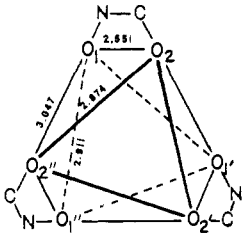

Figure 4. Dimensions of the coordination octahedron, viewed down the 3-fold axis.

the present structure ($R = 0.058$). Dimensionally, the two chelate rings are nearly equivalent, with the major difference occurring in the angles Fe–O(2)–C(1) and O(1)–N(1)–C(1), which differ by 1.4°, and in the Fe–O(1) and Fe–O(2) bond distances. These differences may be reflected in the 4° difference observed in the twist angle, or the relative orientation of the octahedral faces formed by the nitroso oxygen atoms O(1), O(1)′, and O(1)″ and that of the carbonyl oxygen atoms O(2), O(2)′, and O(2)″. For ferric neurosporin and ferrichrome A, the twist angles are 45.5 and 41.5°, respectively; the ligand bite for both chelates is 1.26.

The chelate rings in ferric neurosporin are nonplanar with a mean deviation of any atom in the ring from its least-squares plane of 0.029 Å. The conformational angles, defining the chelate ring, are presented in Table VII. The chelate ring exists in the "ideal" envelope form with the pseudorotation phase angle, Δ, of 36.8°.[29] The iron atom is displaced 0.112 Å from the chelate ring, a value which is larger, but in agreement with the displacement observed in ferric *N,N′,N″*-triacetylfusarinine (mean value 0.081 Å)[15] and ferrioxamine E (mean value 0.149 Å).[30] Although the Fe–O(N) distance (1.963 (2) Å) is significantly shorter than the Fe–O(C) distance (2.059 (2) Å), a characteristic of the ferric trihydroxamates determined to date,[7-11,15] the bonds to N(1) are planar (sum of the bond angles at N(1) is 358.9°), indicating that some negative charge resides on the carbonyl oxygen atom O(2). Iron chelation by hydroxamates leads to electron delocation and additional stabilization of the hydroxamate chelate ring. This is most evident in the systematic lengthening of the C=O bond and a shortening of the N—O and C—N bonds when the dimensional parameters of the chelates are compared with that of model hydroxamate ligands.[31] This translocation of the ferric ion is also evident by examination of the geometry and dimensions of the trigonally distorted coordination octahedron, shown in Figure 4. The Fe atom lies on the line joining the centers of the octahedral faces formed by O(1), O(1)′, and O(1)″ and O(2), O(2)′, and O(2)″. The distances of the Fe atom to these faces are 1.014 and 1.220 Å, respectively.

Crystal Structure and Hydrogen Bonding. A stereoscopic view of the molecular packing is given in Figure 5. The six solvate molecules take up positions above and below the cavities of the macrocyclic rings with their methyl groups oriented in the direction of the coordination octahedron. The minimum Fe–Fe distance observed is 12.017 Å, and it is clear from Figure 5 that Fe–Fe channels are not formed in the crystal structure.

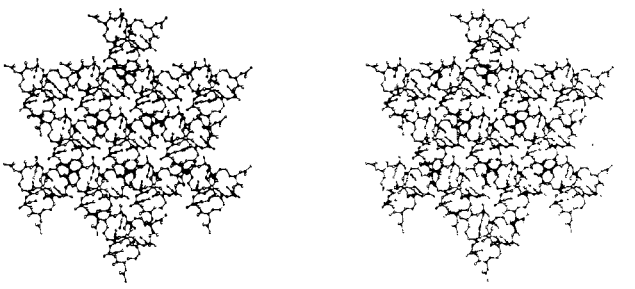

Figure 5. Stereoscopic view of the crystal packing in ferric neurosporin.

Although no intramolecular hydrogen bonds were observed in the molecule, the hydrogen atom of the α-amino nitrogen atoms, N(2), of the ornithinyl moiety makes intermolecular hydrogen bonds to the N^{α}-acetyl carbonyl oxygen, O(5)″, at $(x-1, y, z)$. The N(2)···O(5)″ and H···O(5)″ distances are 2.874 and 2.079 Å, with the N(2)–H···O(5)″ angle of 173.3°. The solvate molecules do not participate in any hydrogen-bonding scheme.

Absolute Configuration. Although there are four diastereoisomers possible (Λ-cis, Δ-cis, Λ-trans, and Δ-trans) for ferric neurosporin, it is apparent that only the Λ-cis diastereoisomer predominates in both the crystalline state and aqueous solution. This was proven and confirmed by a series of experiments that included both X-ray diffraction and chemical and spectroscopic techniques.

The absolute configuration of ferric neurosporin was determined by using the Bijvoet method of anomalous dispersion of Cu Kα radiation by the ferric ion. The results, presented in Table VIII, and repeated several times on different crystals, consistently indicated that the ligand assumes the Λ-cis configuration about the central metal ion; the configurations at the remaining six asymmetric carbon atoms, C(5)* of the ornithinyl groups and C(3)* of the butyryl groups, were D and R, respectively.

The observation (and occurrence) of D-ornithine in biological molecules and siderophores, or siderophore-like molecules, is indeed rare, and is limited to date (in siderophores) to two examples, ferribactin, from *Pseudomonas fluoresens migula*,[19a-c] and the new, unusual siderophore, ferric pseudobactin, from the plant growth promoting *Pseudomonas fluoresens-putida*.[20,21] As a second proof of its existence in neurosporin, a crystalline sample of the chelate was subjected to reductive hydrolysis with periodate and the isomer present identified by circular dichroism (CD) spectroscopy. Controls included purified L-ornithine from ferric *N,N′,N″*-triacetylfusarinine and D- and L-ornithine monohydrochloride. The results indicated that D-ornithine, which exhibited a negative ellipticity at 203 nm, was present in the ratio of 3 mol/mol of ligand.

Recently, we reported the existence of a diastereoisomeric equilibrium (Λ-cis ⇄ Δ-cis) for the siderophore ferric *N,N′,N″*-triacetylfusarinine;[15] a similar observation has been reported in ferric pseudobactin.[21] Although only one crystal form of ferric neurosporin has been observed, a comparison was made of the CD spectra of the chelate measured on single crystals dispersed in anhydrous KBr disks and of the disks dissolved in water to determine the predominate solution configuration of the chelate (Figure 6). The spectra of both samples were qualitatively identical and exhibited a strong positive Cotton effect in the region of the ligand charge-transfer absorption at 440 nm indicative of the Λ-cis stereochemistry.

It is quite evident that the predominance of a single coordination diastereoisomer for cyclic ferric siderophores is directly related to the stereochemistry of the ligand. This is supported by structural, synthetic, and biological evidence. A comparison of the observed conformation and stereochemistry in the known molecular structures of two members of the fusarinine family of

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(31) Eng-Wilmot, D. L.; Hossain, M. B.; van der Helm, D. *Acta Crystallogr., Sect. B* **1981**, *B37*, 1207-1214.

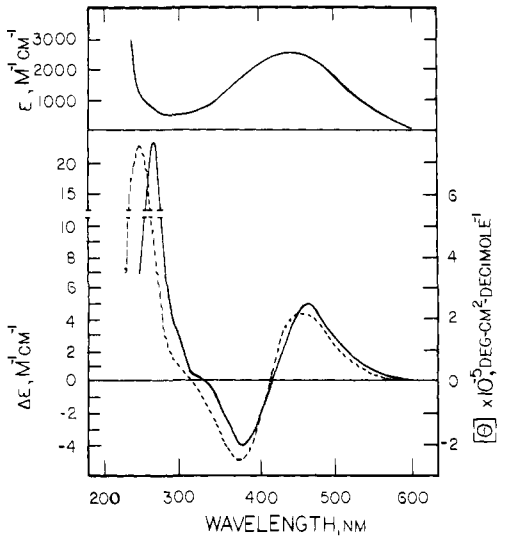

Figure 6. Absorption (upper) and circular dichrosim (lower) spectra of ferric neurosporin. Spectrum of single crystals dispersed in anhydrous KBr disk (solid curve) and disk dissolved in water (dashed curve).

cyclic triester trihydroxamate siderophores, ferric *N,N′,N″*-tricetylfusarinine [L-ornithine and Δ-cis (the predominant diastereoisomer in solution)] and ferric neurosporin (D-ornithine and Λ-cis), reveals that (a) there is a high degree of conformational similarity in the N^{α}-substituted ornithinyl portions of the macrocyclic backbones of the two ligands and (b) the configuration at the asymmetric carbon atom of the ornithinyl groups asymmetrically induces the chirality of the ferric coordination diastereoisomer in solutions of these chelates.

Conformationally, the N^{α}-substituted ornithinyl groups in both molecules are similar, as evident from comparison of the torsion angles along the backbone of their macrocyclic rings. For C(2)–C(3)–O(3)–C(4), C(3)–O(3)–C(4)–C(5)*, O(3)–C(4)–C(5)*–C(6), C(4)–C(5)*–C(6)–C(7), C(5)*–C(6)–C(7)–C(8), and C(6)–C(7)–C(8)–N(1)′, these angles are 151 (−162), −170 (172), 102 (−67), −174 (167), 167 (178), and −47 (49)°, respectively, with the corresponding mean torsion angles in the crystal structure of the Λ-cis diastereosiomer of ferric *N,N′,N″*-triacetylfusarinine given in parentheses. The major differences in the conformational angles appear in (a) the opposite signs of the angles, which is indicative of the D vs. L configuration at the chiral center, C(5)* of ornithine, and (b) the 35° difference about the bond C(5)*–C(4). (This latter difference is offset somewhat by the fact that the sum of the angles, neglecting sign, differs by only 16° for the two molecules.) In addition, the conformations about the bonds C(6)–C(7) and C(7)–C(8) are anti and gauche, respectively, in both chelates, and the N^{α}-acetyl groups take up the equatorial position with respect to the asymmetric carbon, C(5)*. It seems highly reasonable that the observed conformations of the chelate are the most energetically favorable, with the N^{α}-acetyl groups in the equatorial positions for maximum solute–solvent interaction; a different conformation of the backbone would place the N^{α}-acetyl groups in the axial position, which would both reduce solvation and increase steric interactions.

It is reasonable then that the conformation of the ornithinyl portions of the ligand and the chirality at its asymmetric centers asymmetrically induce the chirality of coordination octahedron and lead to the predominance of one coordination diastereoisomer in solution. These results suggest for the fusarinine family that L-ornithine induces the predominance of the Δ-cis diastereoisomer while D-ornithine directs the Λ-cis isomer. The observation of a diastereoisomeric equilibrium in the ferric *N,N′,N″*-triacetylfusarinine system, but not in ferric neurosporin, no doubt is caused in part by the increased dimensions of the coordination cavity made by the 36 macrocyclic ring atoms of the former ligand as compared to that of neurosporin, which is composed of 30 ring atoms.

Similar relationships between ligand configuration and the asymmetric induction of one ferric coordination diastereoisomer among other families of siderophores seem evident, based largely on some recent, elegant synthetic schemes for *enantio*-ferrichrome[16] and *enantio*-enterobactin,[32] prepared by using the antipodes of the natural amino acids. Both ligands assume the opposite absolute configuration for the iron coordination compared to the natural siderophores. The results of kinetic uptake studies of natural ferrichrome and enterobactin and their synthetic antipodes[32,33] strongly support (a) the hypothesis of stereochemical dependence of active iron transport on the chirality of the metal coordination center and (b) the chiral nature of the membrane (outer or plasma) receptor site, inasmuch as while the two natural siderophores were actively assimilated, their antipodes were not.[32,33] These observations lead to important questions concerning the biological activity and occurrence of ferric neurosporin in *N. crassa*.

Horowitz and co-workers[34] have isolated and identified from *N. crassa* wild-type strain 74A a number of siderophores, including coprogen from the culture filtrate, ferricrocin and ferrichrome C from conidia extracts, and ferricrocin, ferrichrome C, and a yet to be identified third, minor siderophore from mycelia extracts, all thought to be essential for germination. Unfortunately, the uptake and antibiotic screening experiments with neurosporin leading to answers concerning its biological activity have not been performed for lack of sufficient material; neurosporin did not correspond to the third minor siderophore isolated by Horowitz et al.[34] based on TLC.

Curiously, a major portion of the 18-mg sample of neurosporin isolated from *N. crassa* 10816 (ATCC) was obtained from initial cultures and was present as approximately 30% of the total siderophores produced; however, over a period of several months of culturing experiments, this value decreased to less than 1%, as estimated from TLC, and yet was observed after 1 year. Further, the compound was not detected in the isolates (both culture filtrates and mycelia extracts) from the large-scale screening of other strains of *N. crassa*, including strains F.G. 262, 353, 987, and 1945 (obtained from Fungal Genetics). Three possible explanations can be advanced to rationalize the production of neurosporin in cultures of *N. crassa* 10816, including contamination of the initial cultures, the presence of subsequent loss of a plasmid, or adaptation to unknown environmental stress, such as the lowering of water activity. Concerning contamination of cultures, it should be noted that no obvious contamination of fluid cultures or stock plates was observed.

In summary, we report here the molecular and crystal structures of ferric neurosporin, a new, minor siderophore-like compound of the fusarinine family that is derived from D-ornithine and contains the hitherto unreported 3-hydroxybutyryl acyl group; these structures represent the most accurate crystal structure of a siderophore to date.

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Registry No. Ferric neurosporin·acetonitrile, 88326-02-7; ferric neurosporin, 88326-01-6.

Supplementary Material Available: Hydrogen atom parameters (Table III), bond angles (Table V), conformational angles of chelate ring (Table VII), anomalous dispersion data (Table VIII), anisotropic thermal parameters (Table IX), and structure factors (Table X) (32 pages). Ordering information is given on any current masthead page.

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