C, 59.50; H, 7.08; N, 9.92. Found: C, 59.47; H, 6.99; N, 9.90.

(±)-Porantherine (1). (Phenylsulfonyl)hydrazone hydrochloride **29a** (110.0 mg, 0260 mmol) was suspended in 5 mL of diethyl ether contained in a 25-mL round-bottom flask equipped with a magnetic stirring bar. When the mixture cooled to −78 °C, *tert*-butyllithium (1.45 M, 1.21 mL, 1.76 mmol) was added by syringe in small portions over 5 min. After 1 h of stirring at 0 °C all the solid had dissolved, giving a yellow solution. Stirring was continued for 10 h as the solution was slowly warmed to room temperature. Over this period the solution gradually turned red. Subsequently, the reaction mixture was diluted with ether (10 mL) and quenched with a small amount of water (2.0 mL). The aqueous layer was extracted with ether (3 × 5 mL). The combined organic layers were dried (Na_2CO_3), filtered, and concentrated under reduced pressure to give a yellow oil. After flash chromatography (silica, 9:1 hexane–ethyl acetate with 5% added triethylamine), (±)-porantherine (**1**) was obtained as a waxy solid (52.5 mg, 0.241 mmol, 93.0%): IR (CCl_4) 2950, 2890, 1605, 1490, 1458, 1123, 1048, 1001, 992, 952, 855 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 5.69 (m, 2 H), 3.72 (br, 1 H) 2.39 (m, 1 h), 1.92–1.18 (m, 15 H), 1.14 (s, 3 H, CH_3), 0.98–0.85 (m, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) 131.94, 131.62, 53.33, 52.93, 50.72, 43.32, 39.62, 37.64, 35.76, 31.07, 28.18, 25.46, 23.51, 21.75, 16.94; high-resolution mass spectrum, calcd for $C_{15}H_{23}N$ 217.1830, found 217.1841; mass spectrum, (M^+) 217 (61.7), 202 (21.4), 189 (58.1), 174 (81.7), 167 (32.2), 165 (31.4), 83 (28.5), 69 (35.4), 67 (32.2), 57 (61.7), 43 (69.3), 41 (100).

Synthetic porantherine hydrochloride (1a) precipitated from an ether solution of the free base on acidification with ethereal HCl (~5 N): mp >200 °C dec; IR ($CHCl_3$) 3700, 2960, 2440, 1605, 1460, 1120 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 11.2 (br, 1 H NH), 5.72–5.80 (m, 2 H, CH=CH), 4.30 (m, 1 H, NC*H*), 2.73–1.73 (m, 16 H), 1.56 (s, 3 H CH_3), 1.40 (m, 1 H); mass spectrum, (M^+ − Cl) 218 (23.0), 217 (93.3), 202 (69.2), 189 (86.6), 188 (62.5), 175 (36.3), 174 (100), 148 (31.3), 109 (44.5), 41 (44.1), 36 (63.1).

Synthetic porantherine picrate (1b) precipitated slowly from an alcoholic solution of the free base after the addition of a saturated solution (ethanol) of picric acid. The picrate so obtained was dried in vacuo at 105 °C for 12 h. Anal. Calcd for $C_{21}H_{26}N_4O_7$: C, 56.50; H, 5.83; N, 12.55. Found: C, 56.34; H, 5.75; N, 12.33.

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Registry No. (±)-**1**, 54382-19-3; (±)-**1a**, 108818-56-0; (±)-**1b**, 108818-57-1; (±)-**13**, 54312-69-5; **18**, 88128-57-8; **18** (X = H), 5421-99-8; **18** (X = OH), 108818-49-1; **18** (Hg derivative), 108818-58-2; **19a**, 108818-50-4; **20**, 108818-51-5; **20a**, 108818-52-6; (±)-**22**, 108818-53-7; (±)-**25**, 108818-54-8; (±)-**26**, 54312-76-4; (±)-**29a**, 108818-55-9; CH_2=$CH(CH_2)_3Li$, 54313-25-6; $CH_3CO(CH_2)_3Cl$, 5891-21-4; $HOCH_2CMe_2CH_2OH$, 126-30-7; CH_2=$C(OAc)CH_3$, 108-22-5; *o*-methylvalerolactim, 5693-62-9; 2,2-di(4-pentenyl)piperidine, 108818-48-0; 2,2-dibutylpiperidine, 92144-58-6.

Structure and Conformation of Two Coprogen-Type Siderophores: Neocoprogen I and Neocoprogen II

M. B. Hossain,[†] M. A. F. Jalal,[†] B. A. Benson,[†] C. L. Barnes,[‡] and D. van der Helm*[†]

Contribution from the Departments of Chemistry, The University of Oklahoma, Norman, Oklahoma 73019, and The University of Puerto Rico, Rio Piedras, Puerto Rico 00931.
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Abstract: Two coprogen-type siderophores, neocoprogen I (**4**), $C_{31}H_{47}N_6O_{12}Fe$, and neocoprogen II (**5**), $C_{27}H_{41}N_6O_{11}Fe$, have been isolated from the fungus *Curvularia lunata*, and their structures and conformations have been elucidated. The coprogens have a linear trimeric skeleton made from three N^{δ}-hydroxy-N^{δ}-acylated ornithines, two of which form a diketopiperazine ring while the third forms an ester linkage to the dimer. In **4**, one of the terminal N^{δ}-acyl groups is an acetyl group, while in **5**, both of these are acetyl groups. The structure of neocoprogen I (**4**) [identified as the ferric complex of isotriornicin (**7**)] has been determined by single-crystal X-ray diffraction at 138 K with crystals grown from ethanol/acetonitrile. The crystals are orthorhombic: $P22_12_1$, a = 8.683 (13) Å, b = 28.33 (4) Å, c = 36.84 (4) Å, V = 9062.2 Å^3, Z = 8. The structure has been determined from 2756 diffractometer data and refined to a final R = 0.114. The distinct feature of neocoprogen I (**4**) structure is the Δ-trans geometry of its iron coordination. This is the first example of a trans isomer of an iron trihydroxamate siderophore in the solid state. The structure of neocoprogen II (**5**), a novel siderophore, has been elucidated on the basis of chemical degradation and spectroscopic studies including ^1H and ^{13}C NMR spectroscopy. CD spectra show that both neocoprogen I (**4**) and neocoprogen II (**5**), like coprogen (**1**), exist predominantly as Δ optical isomers in aqueous solutions at neutral pH. Stereoisomeric possibilities of the ferric complexes of the linear trihydroxamates are discussed, and their structural features are related to their role in microbial iron transport.

Under iron-deficient growth conditions most microorganisms produce low molecular weight iron chelating agents called siderophores. It is now generally accepted that these siderophores are responsible for microbial iron transport. Several families of siderophores have been identified, including ferrichromes, ferrioxamines, fusarinines, coprogens, catecholamides, pseudobactins, and mycobactins.[1-3] Two types of ligating groups are prevalent among these families, the hydroxamates and the catecholates, both of which form stable octahedral complexes with iron.

Among the hydroxamate siderophores, the coprogen family is structurally characterized by the presence of a diketopiperazine ring. Although they are built up of typical units (δ-*N*-hydroxyornithine, *trans*-anhydromevalonic acid, and acetic acid) as in other fungal hydroxamate siderophores, the manner in which these building blocks are arranged in the coprogen family is completely different. Two of the amino acid components are linked together, head-to-head through a diketopiperazine ring, and the third component is linked to the rest of the molecule via an ester linkage. As a result, in the coprogen family the hydroxamate groups become parts of a long chain. The trihydroxamate members of the coprogen family are shown in Chart I. Dimerum acid[4] and

[†] The University of Oklahoma.
[‡] The University of Puerto Rico

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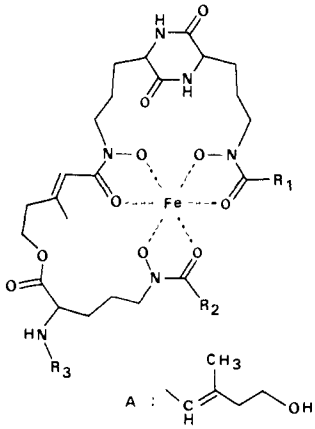
Chart I



1, $R_1 = R_2 = A$; $R_3 = CO\text{-}CH_3$
2, $R_1 = R_2 = A$; $R_3 = H$
3, $R_1 = A$; $R_2 = CH_3$; $R_3 = CO\text{-}CH_3$
4, $R_1 = CH_3$; $R_2 = A$; $R_3 = CO\text{-}CH_3$
5, $R_1 = R_2 = CH_3$; $R_3 = CO\text{-}CH_3$

rhodotorulic acid[5] are dihydroxamates and constitute a subgroup within the family. Coprogens are produced by a number of fungal species. Coprogen (**1**) was first isolated from *Neurospora crassa*[6] and coprogen B (**2**) from *Fusarium dimerum*.[4] Triornicin and isotriornicin (deferri forms of **3** and **4**, respectively) were obtained from the soil fungus *Epicoccum purpurascens*.[7,8]

The coprogens show various types of biological activity. It has been demonstrated that **1** can supply the iron in heme syntheses in vivo,[9] **2** can antagonize the actions of ferrimycin and albomycin,[4] and triornicin acts as a mild antitumor agent.[7] However, the primary interest in these compounds has been their role in microbial iron transport. A coprogen transport system has been studied in *N. crassa* by Winkelmann and co-workers.[10-12] Recently, Mössbauer studies have shown that coprogen also acts as an initial iron storage compound inside the cell, soon after the transport process.[13] Coprogen mediated iron uptake in *E. coli* has also been reported.[14,15]

Since the structure of **1** was first established by Keller-Schierlein and Diekmann (1973),[16] there has been considerable interest in the structural studies of coprogen-type siderophores. Of particular interest is the ability of these linear trihydroxamates to form various stereoisomers on complexation and the role of diketopiperazine ring in determining the chirality at the metal center. Circular dichroism (CD) studies have shown that **1** adopts primarily a Δ-absolute configuration around the iron.[17] There is, however, no report of any crystal structure for any of the coprogens. In this investigation, the molecular structure of neocoprogen I (ferric isotriornicin) (**4**) has been determined by single-crystal X-ray diffraction, and the structure of a novel member of the coprogen family, neocoprogen II (**5**), has been determined by ^1H and ^{13}C NMR spectroscopy. Both of these compounds have been isolated from iron-deficient cultures of *Curvularia lunata*, which also produce coprogen, ferricrocin, and minor amounts of a few other siderophores.

Table I. Crystal Data of Neocoprogen I

compd	neocoprogen I (ferric isotriornicin)
formula	$C_{31}H_{47}N_6O_{12}Fe$
asymmetric unit	$2(C_{31}H_{47}N_6O_{12}Fe) + 1.5CH_3CN + 14H_2O$
fw	1817.0
cryst system	orthorhombic
space group	$P22_12_1$
cell params	
a, Å	8.683 (12)
b, Å	28.33 (4)
c, Å	36.84 (4)
V, Å^3	9062.2
Z	8
D_x, gm cm^{-1}	1.33
μ (Mo K$\bar{\alpha}$), cm^{-1}	3.7
F (000)	3844

Experimental Section

Organism Source and Culture. The fungus was isolated in this laboratory as a contaminant from air. A specimen culture was deposited with the Centraalbureau voor Schimmelcultures, Baarn, the Netherlands who identified it as *Curvularia lunata* (Wakker) Boedijn (previously reported as *C. subulata*[12,18]). Methods for the growth of cultures and production of siderophores have been described earlier.[19]

Extraction and Purification of Siderophores. After removing the cells by filtration the ferric complexes of the siderophores were prepared by addition of an aqueous $FeCl_3$ solution to the medium. Ferric siderophores were extracted with the chloroform–phenol–ether–water method[19] and initially purified by passing through a Biogel P2 column (100–200 mesh, 2.5 × 36.2 cm). **5** was separated from **1** and **4** on a silica gel column (60 H, TLC grade, 3.5 × 65 cm) with chloroform–methanol–water, 35:12:2, solvent system. **1** and **4** were separated from one another by preparative chromatography through a reversed phase column (2.2 × 30 cm) by using water–methanol gradient as described previously.[18] **5** was further purified by reversed-phase chromatography with the above system. All isolated compounds were finally passed through a Biogel P2 column followed by lyophilization. Yield of ferric siderophores per L of culture filtrate: **1**, 63 mg; **4**, 51 mg; and **5**, 55 mg.

Chromatography and Electrophoresis. Isolated siderophores produced single spots on silica gel thin layers (E. Merck) in the following solvent systems: (A) 35:12:2 $CHCl_3$:CH_3OH:H_2O, (B) 3:1 $CHCl_3$:CH_3OH, (C) 1:3 $CHCl_3$:CH_3OH, (D) 1:1:1 $CHCl_3$:CH_3OH:hexane, (E) 4:1:5 1-butanol:acetic acid:H_2O, upper phase. Relative mobilities (R_f) for **1**, **4**, and **5** in the above solvent systems (freshly prepared) were A) 0.28 0.29, 0.34, (B) 0.21, 0.24, 0.28, (C) 0.54, 0.47, 0.42, (D) 0.13, 0.13, 0.13, and (E) 0.17, 0.15, 0.13, respectively. All three compounds were neutral at pH 2.0 and 5.0 on high voltage paper eloctrophoresis, performed as described previously.[20]

Reductive Hydrolysis. Samples (3.0 mg) of **4** and **5** (in triplicate) were dissolved in 0.4 mL of 55% HI and kept at 110 °C for 18 h.[21] The ornithine produced was identified by chromatography with an authentic sample and quantitatively determined by spectrophotometric methods.[22] L-Ornithine produced 2.92, 2.84, and 2.84 μmol per μmol of **4** and 2.94, 3.01, and 2.96 μmol per μmol of **5**.

Absolute Configuration. The absolute configuration of ornithine produced after reductive hydrolysis of **4** and **5** was determined by polarimetry against an authentic sample of L-ornithine. The geometry around the iron atom in the ferric siderophores in aqueous solutions was determined by CD spectroscopy by a procedure reported earlier[20] in a Jasco J-500A spectropolarimeter fitted with a DP-500N data processor.

Spectroscopic Measurements. Electronic (visible) absorption spectra were recorded on a Lambda 3 UV-vis spectrophotometer. Lyophilized

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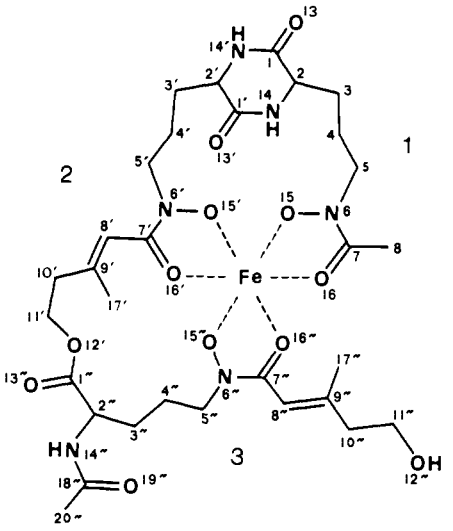


Figure 1. Atom numbering scheme in **4** used in the text. Bold numerals 1, 2, 3 indicate three units of the coprogen chain.

samples of the ferric siderophore, dried in a vacuum pistol at 50 °C for 72 h, were dissolved in known volumes of water for the visible and CD spectral measurements. The concentration of the ferric siderophore solution was accurately measured by spectrophotometric determination of ornithine produced from a known aliquot of the solution after reductive hydrolysis.

NMR spectroscopy on the deferri forms of **1**, **4**, and **5** was carried out at 300 MHz for ^1H and 75.4 MHz for ^{13}C nuclei at 23 °C. The proton signal assignment and ^1H–^{13}C connectivities were established by selective decoupling and by correlation with the NMR spectra of other fungal siderophores containing similar structural units.[7,23-25] In addition, —CH_3, —CH_2—, —CH—, =C—, and —C=O carbon atoms were distinguished by an APT pulse sequence program.[26]

Crystallization and X-ray Diffraction Data Collection. **1** crystallized from a number of solvent systems such as ethanolic or methanolic solutions equilibrated with acetonitrile and slow evaporation of a chloroform–ethanol (1:1) solution. **5** crystallized as fibres from a solution of 3:1 chloroform–methanol saturated with water. None of these small crystals of **1** and **5** were suitable for single-crystal X-ray diffraction studies. However, **4** produced some large blocky crystals from an ethanolic solution equilibrated with acetonitrile at 4 °C. The crystals were unstable at room temperature, but stable at low temperature (approximately 138 K). Preliminary investigation showed that the crystals diffracted poorly and had rather large mosaic spread (approximately 1.4°). A crystal of dimensions 0.60 × 0.30 × 0.25 mm was selected for data collection. The crystal was mountd while still under mother liquor and quickly transferred into a beam of cold nitrogen gas. Crystal data of the compound are given in Table I.

The cell parameters were obtained by a least-squares fit to $\pm 2\theta$ values of 48 reflections measured in all octants at 138 K by using Mo $K\alpha_1$ radiation.

The intensities of all unique reflections with $2\theta < 40°$ were collected at 138 ± 2 K on an Enraf-Nonius CAD-4 automatic diffractometer by using Mo $K\bar{\alpha}$ radiation (graphite monochromator). A θ–2θ scan technique with a variable scan width of $(0.80 + 0.20 \tan \theta)°$ was employed. A horizontal receiving aperture with a variable width of $(4.00 + 0.86 \tan \theta)$ mm and a constant height of 6 mm was located at a distance of 173 mm from the crystal. A reflection was scanned for a maximum time of 90 s, with two-thirds of the time spent scanning the peak, and one-sixth of the time spent on each of the left and right backgrounds. The intensities of three standard reflections were monitored ever 2 h of X-ray exposure, and they showed no significant variation (≤2%). Of the 4798 reflections measured, 2042 were considered unobserved on the basis that $I < 2\sigma(I)$. The data were corrected for Lorentz and polarization factors,

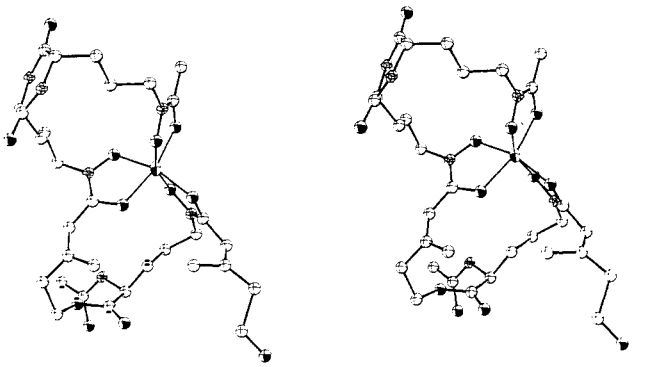
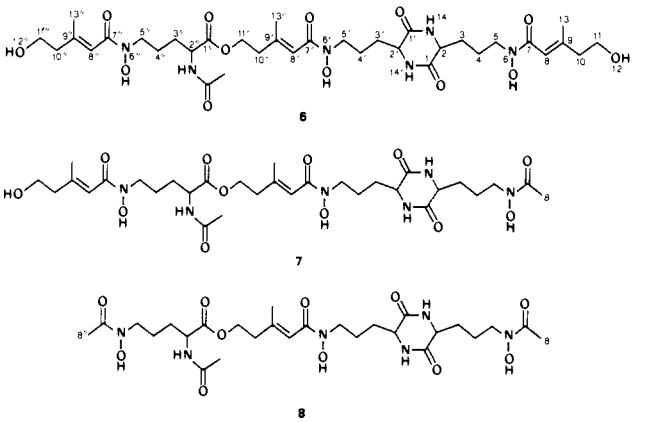


Figure 2. A stereoview of the single molecule of **4**.

Chart II



but no absorption correction was applied. Each structure amplitude was assigned a weight $w_F = 1/\sigma_F^2$, where σ_F was obtained from counting statistics.

Structure Determination. The positions of the two iron atoms in the two independent molecules in the asymmetric unit were located from a three-dimensional Patterson map. Refinements of iron coordinates and subsequent difference Fourier maps revealed very small fragments of the structure. The complete structure comprising two molecules of **4**, 14 water molecules, and 1.5 molecules of acetonitrile solvate, a total of 120 non-hydrogen atoms, was obtained after 15 successive difference Fourier syntheses. The structure was refined by blocked full-matrix least-squares methods by using isotropic temperature factors (only the iron atoms were anisotropic) to a final R of 0.114 and $R_w = 0.109$ for 2756 observed reflections. Atoms C(4) and O(12″) of molecule B were disordered. One of the acetonitrile molecules occupied a special position (twofold axis). The $(\text{shift/error})_{max}$ in the final cycle of refinement was 0.12 and S = 2.5 for 2756 observations and 470 variables. The final difference map contained peaks of height ±0.7 e/Å^3. The real and anomalous scattering factors were taken from the *International Tables for X-ray Crystallography* (1974).[27] All Fourier and least-squares calculations were preformed by using the SHELX76 program system.[28]

Results

(i) Crystal Structure of 4. The atom numbering scheme for **4** is shown in a schematic drawing in Figure 1. Figure 2 shows a stereoview of a single molecule (molecule A). The absolute configuration of the molecule is established on the basis of L-ornithine residues.

The present structure determination shows that the ligand molecule in this ferric complex is isotriornicin, **7** (see Chart II). The terminal *trans*-anhydromevalonic acid moiety of deferricoprogen (**6**) nearest to the diketopiperazine ring is replaced by an acetyl group in this compound. In the isomeric triornicin, the acetyl group replaces the anhydromevalonic group at the other end of the trihydroxamate chain. In **5**, the novel compound described in this report, both terminals of the molecule contain

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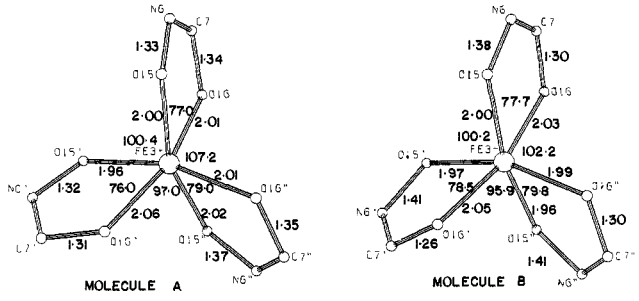

Figure 3. Selected bond distances (Å) and bond angles (°) in the iron coordination octahedron. Standard deviation range: bond distance, 0.02–0.04 Å; bond angles, 0.8–0.9°.

Table II. Average Values of Geometrical Parameters of Iron Coordination in Trihydroxamate Siderophores

	ferri-chromes[a]	ferri-oxamines[b]	fusarinine[c]	neoco-progen I
Fe–O(C)	2.035	2.053	2.04	2.03
Fe–O(N)	1.985	1.957	1.97	1.99
C–O	1.279	1.277	1.27	1.31
N–O	1.382	1.379	1.34	1.37
N–C	1.317	1.314	1.36	1.32
O–Fe–O	78.2	78.8	78.2	78.0
O···O	2.539	2.547	2.53	2.53
twist	42.1	43.9	42.5	40.7
bite	1.26	1.27	1.26	1.26

[a] Average of values observed in ferrichrome,[29] ferrichrome A,[30] ferricrocin,[31] and ferrirubin.[32] [b] Average of values observed in ferrioxamine E[33] and ferrioxamine D_1.[34] [c] Values observed in ferric triacetylfusarinine.[20]

acetyl groups. The three hydroxamic acid groups in their deprotonated form bind the iron in a familiar distorted octahedral complex. The configuration of the iron coordination is Δ-trans.

Due to the poor crystal quality, the intensity data are severely limited (only 57% of the data is observed with $2\theta_{max} = 40°$ or (sin $\theta/\lambda)_{max} = 0.48$ Å^{-1}) and only 2756 reflections were used to refine 120 non-hydrogen atoms. Consequently, the accuracy achieved in the present structure determination is limited. However, the average bond distances and bond angles in the present report compared well with those observed in other trihydroxamate siderophore structures. Selected bond distances and angles of the iron coordination for the two independent molecules are shown in Figure 3. The average geometrical parameters of the iron coordination of the present structure are compared in Table II with the corresponding values observed in other trihydroxamate siderophores.[29-34] Although there are individual differences, the average value of Fe–O(N) = 1.99 (2) Å, Fe–O(C) = 2.03 (2) Å, the chelate angle O–Fe–O = 78.0 (9)°, ligand bite of 1.26, and the twist angle of 41° generally are in good agreement. This shows that the difference in the geometrical isomerism (trans or cis) does not significantly affect the dimensions of the iron coordination octahedron.

The two independent molecules of **4** show some conformational differences. The major difference is in the unit 3 of the trimeric chain (Figure 1); torsion angle C(1″)–C(2″)–C(3″)–C(4″) is 179° in molecule A and −61° in molecule B, while torsion angle C(3″)–C(4″)–C(5″)–N(6″) is −51° in molecule A and 168° in molecule B. In molecule B, atom C(4) is disordered, which indicates conformational flexibility in the unit 1 of the trihydroxamate chain. There are some differences in the diketopiperazine (DKP) ring conformations. In both molecules, the DKP ring shows slight nonplanarity and assumes a flattened boat conformation. The deviations of the two C^{α} atoms from the plane defined by C(1), N(14), C(1′), and N(14′) are 0.09 and 0.17 Å in molecule A and 0.26 and 0.27 Å in molecule B. The peptide torsion angles (ϕ, ψ, ω) for the two ornithine residues are as follows: (3, −12, 5°) and (10, −18, 12°) in molecule A and (20, −21, −1°) and (23, −21, 1°) in molecule B. Both the C^{β} atoms occupy quasi-axial positins giving rise to a folding of the side chains. Such a folded conformation of the substituted DKP has been termed as the "flagpole boat"[35] and is reported to be a common feature of many cyclic dipeptide structures.[36-38] However, DKP, the simplest cyclic dipeptide, cyclo (gly-gly), is known for its conformational flexibility. It is planar in the crystalline state,[39] but replacement of one or both glycines may change the conformation greatly. Within the restriction of amide planarity, the DKP ring can be planar or assume two possible boat conformations, "flagpole" with cis C^{α}-carbons substituents quasi-axial and "bowsprit" with these substituents quasi-equitorial.[35] The quasi-equitorial substitution at C^{α} would lead to less folding of the molecule. The presence of a stretched out DKP system has been reported in the case of ferric complexes of rhodotorulic acid.[40,41]

Like many other trihydroxamate siderophores, the neocoprogen I structure contains a substantial solvent environment, mostly aqueous. The two independent molecules have different environments and slightly different hydrogen-bonding patterns. DKP in both molecules are involved in extensive hydrogen bonding where both the amides and carbonyl groups are utilized. Table III gives a list of intermolecular hydrogen bonds. In addition, both molecules form several hydrogen bonds with the water molecules.

Table III. Intermolecular Hydrogen Bonds in Neocoprogen I Structure

donor (D) acceptor (A)	D···A distance (Å)
(1) N(14)a[a]···O(15′)a[$x-1, y, z$]	2.96
(2) N(14′)a···O(13)b[$-x-1, {}^1/_2-y, z-{}^1/_2$]	2.86
(3) N(14″)a···O(13′)a[$x-1, y, z$]	2.83
(4) N(14)b···O(15″)b[$x-1, y, z$]	2.73
(5) N(14′)b···O(19″)a[$-x, {}^1/_2+y, {}^1/_2-z$]	2.83
(6) N(14″)b···O(13′)b[$x+1, y, z$]	2.78

[a] a and b refer to molecule A and molecule B, respectively.

Table IV. Visible and CD Spectral Characteristics of Coprogen (**1**), Neocoprogen I (**4**), and Neocoprogen II (**5**) in Aqueous Solutions

	visible		CD	
ferric siderophore	λ_{max}, nm	ϵ, M^{-1} cm^{-1}	Δ, nm	$\Delta\epsilon$, M^{-1} cm^{-1}
coprogen[a] (**1**)	434	2820	375	+2.10
			475	−1.26
neocoprogen I (**4**)	428	2730	365	+1.77
			461	−1.21
neocoprogen II (**5**)	422	2612	372	+1.82
			456	−1.16

[a] Data after ref 15.

(ii) Structure Determination of 5. Compound **5**, like a typical ferric hydroxamate complex,[42-43] shows an absorption maximum

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Table V. ^1H (300 MHz) Chemical Shifts of Deferricoprogen (**6**), Isotriornicin (**7**), and Deferrineocoprogen II (**8**)[a]

^1H chemical shift, δ			inventory	structrl	
6	**7**	**8**	of protons	group	position
		Diketopiperazine Ring			
3.82 (m)	3.82 (m)	3.82 (m)	2	>CH—	2,2′
8.14 (s)	8.14 (s)	8.14 (s)	2	—NH—	14,14′
		Ornithyl Residues			
4.18 (m)	4.18 (m)	4.18 (m)	1	>CH—	2″
8.27 (d, J = 7.3 Hz)	8.27 (d, J = 7.3 Hz)	8.27 (d, J = 7.3 Hz)	1	—NH—	14″
1.60 (m)	1.60 (m)	1.60 (m)	12	—CH_2—CH_2—	3,3′,3″ 4,4′,4″
3.49 (m)	3.49 (m)	3.49 (m)	6	—CH_2—	5,5′,5″
9.70 (s)	9.70 (s)	9.70 (s)	3	—NOH—	6,6′,6″
		N^α-Acetyl Residue			
1.84 (s)	1.84 (s)	1.84 (s)	3	—CH_3	16
		Middle N^δ-Anhydromevalonoyl Residue			
6.22 (s)	6.22 (s)	6.22 (s)	1	—CH=	8′
2.39 (t, J = 6.6 Hz)	2.39 (t, J = 6.6 Hz)	2.39 (t, J = 6.4 Hz)	2	—CH_2—	10′
4.18 (m)	4.18 (m)	4.18 (m)	2	—CH_2—	11′
2.03 (s)	2.03 (s)	2.03 (s)	3	—CH_3	13′
		Terminal N^δ-Anhydromevalonoyl Residues			
6.22 (s)			2	—CH=	8,8″
	6.22 (s)		1	—CH=	8″
2.25 (t, J = 6.7 Hz)			4	—CH_2—	10,10″
	2.25 (t, J = 6.7 Hz)		2	—CH_2	10″
3.53 (t, J = 6.7 Hz)			4	—CH_2—	11,11″
	3.53 (m)		2	—CH_2—	11″
4.59 (s)			2	—OH	12,12″
	4.56 (s)		1	—OH	12″
2.03 (s)			6	—CH_3	13,13″
	2.03 (s)		3	—CH_3	13″
		Terminal N^δ-Acetyl Residues			
	1.97 (s)		3	—CH_3	8
		1.97 (s)	6	—CH_3	8,8″

[a] Solvent $(CD_3)_2SO$.

at 422 nm. Its trihydroxamate nature (1:1 complex) is evident from the insensitivity of the absorption maximum to pH changes in the range of 8.0–2.0. When subjected to reductive hydrolysis, it produces L-ornithine (absolute configuration determined by polarimetry) as the only constituent amino acid. Hydroxamic groups in fungal siderophores are commonly formed by the N^δ-hydroxylation and N^δ-acylation of the ornithine residues. On the basis of its trihydroxamate nature, it has been proposed that **5** is made of three N^δ-hydroxylated N^δ-acylated ornithines, similar to **1** and **4**. The stoichiometry of reductive hydrolysis (each mol of **5** produces 3 mol of L-ornithine) also indicates the same conclusion.

Features of the CD spectrum (Table IV) show that the predominant iron coordination geometry in **5** is Δ. The same geometry is found for the solution structures of **1** and **4** and for the structure of **4** in the crystalline state. ^1H and ^{13}C NMR spectra of the deferri form of **5** have shown that the compound, indeed, is a novel member of the coprogen family with one N^δ-*trans*-anhydromevalonoyl group and two N^δ-acetyl groups in the three ornithyl residues.

A comparison of the ^1H and the ^{13}C NMR signals of deferricoprogen (**6**), isotriornicin (**7**), and deferrineocoprogen II (**8**) are listed in Tables V and VI. The signals originating from the ornithyl residues, including the diketopiperazine ring and the N^α-acetyl group are common to all three compounds. The majority of the ^1H signals and all of the ^{13}C signals of the N^δ-*trans*-anhydromevalonoyl group of the middle ornithyl residue in **6** are distinct from the signals produced by the two terminal N^δ-*trans*-anhydromevalonoyl groups. This distinction, reported earlier,[24] arises from the fact that the hydroxyl group in the middle N^δ-acyl moiety forms an ester link with the adjacent ornithyl carboxyl group, whereas the terminal N^δ-acyl moieties have free hydroxyl groups. The signal originating from the middle N^δ-*trans*-anhydromevalonoyl moiety are common to **6**, **7**, and **8**. The presence of these common signals in **8** indicates that its three N^δ-hydroxy N^δ-acyl ornithine residues are joined in a manner similar to **6** and **7** (i.e., two ornithyl residues condense to form a diketopiperazine ring, and the third one is N^α-acetylated and ester linked to the others). Compared to **6**, the intensities of the NMR signals produced by the terminal N^δ-*trans*-anhydromevalonoyl group are reduced to half in **7**. In **8**, these signals are altogether absent suggesting the absence of the N^δ-*trans*-anhydromevalonoyl group in both the terminii. One of the terminal N^δ-anhydromevalonoyl groups of **6** is replaced by an N^δ-acetyl group in **7**. The carbonyl (^{13}C) signal of this N^δ-acetyl group is observed at δ 170.16 and its methyl signals at δ 1.97 (^1H) and 20.21 (^{13}C), respectively. **8** produces signals for two N^δ-acetyl groups [C=O, δ 170.15 and 170.20; —CH_3, δ 1.97 (^1H) and 20.22 (^{13}C), while the peak areas are doubled compared to the —CH_3 signals in **7**] indicating that both of the terminal N^δ-*trans*-anhydromevalonoyl groups found in **6** are replaced by the two N^δ-acetyl groups in **8**.

The replacement of the terminal N^δ-*trans*-anhydromevalonoyl groups by N^δ-acetyl groups, affects both the visible absorption maximum and the CD band. The visible absorption maximum shifts hypsochromically, and its molar absorptivity decreases gradually with a decreasing number of N^δ-*trans*-anhydromevalonoyl groups in the compound (Table IV). Similarly, the negative CD band in these compounds shifts toward lower wavelength and diminishes in intensity ($\Delta\epsilon$) with the gradual replacement of the N^δ-*trans*-anhydromevalonoyl groups by the N^δ-acetyl groups. These effects originate from the modification of the chromophore in these compounds. In **1**, the chromophore of the iron coordination is extended by conjugation with three double bonds in the N^δ-*trans*-anhydromevalonoyl residues. The number of these conjugated double bonds decreases to two in **4** and to one in **5**.

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Table VI. ^{13}C (75.4 MHz) Chemical Shifts of Deferricoprogen (**6**), Isotriornicin (**7**), and Deferrineocoprogen II (**8**)[c]

^{13}C chemical shift, δ			inventory	structrl	
6	**7**	**8**	of C atoms	group	position
		Diketopiperazine Ring			
167.78	167.76	167.74	2	>C=O	1,1′
53.73	53.73	53.71	2	>CH—	2,2′
		Ornithyl Residues			
169.54	169.52	169.48	1	>C=O	1″[a]
51.90	51.89	51.87	1	>CH—	2″
30.27	30.25	30.18	2	—CH_2—	3,3′
28.00	27.95	27.86	1	—CH_2—	3″
23.12	23.05	22.95	2	—CH_2—	4,4′
22.12	22.07	21.93	1	—CH_2—	4″
46.72	46.72	46.69	2	—CH_2—	5,5′
46.38	46.37	46.28	1	—CH_2—	5″
		N^{α}-Acetyl Residue			
172.05	172.00	172.00	1	>C=O	15[a]
22.00	22.07	22.06	1	—CH_3	16
		Middle N^{δ}-Anhydromevalonoyl Residue			
166.12	166.14	166.12	1	>C=O	7′
117.36	117.09	117.05	1	—CH=	8′
148.80	148.80	148.80	1	=C<	9′
40.00[b]	40.00[b]	40.00[b]	1	—CH_2—	10′
62.27	62.23	62.20	1	—CH_2—	11′
17.98	17.95	17.91	1	—CH_3	13
		Terminal N^{δ}-Anhydromevalonoyl Residues			
166.40			2	>C=O	7,7″
	166.40		1	>C=O	7″
116.16			2	—CH=	8,8″
	116.14		1	—CH=	8″
150.96			2	=C<	9,9″
	150.82		1	=C<	9″
43.75			2	—CH_2—	10,10″
	43.70		1	—CH_2—	10″
59.09			2	—CH_2—	11,11″
	59.09		1	—CH_2—	11″
18.20			2	—CH_3	13,13″
	18.17		1	—CH_3	13″
		Terminal N^{δ}-Acetyl Residues			
	170.16	170.15	1	>C=O	7
		170.20	1	>C=O	7″
	20.21		1	—CH_3	8
		20.22	2	—CH_3	8.8″

[a] May be vice-versa. [b] Underneath solvent peak. [c] Solvent $(CD_3)_2SO$.

Discussion

The structural studies of coprogens show that they have certain structural features which distinguish them from other known hydroxamate siderophores. The presence of a diketopiperzine ring, the head-to-head cyclic condensation of two of the amino acid components, linearity of the trihydroxamate chain, the preference of a Δ-configuration of the iron coordination, all of these contrast with the structural characteristics of other fungal siderophores, like the ferrichromes and fusarinines. Members of the coprogen family are further characterized by having heterogenous *N*-acyl groups, while most trihydroxamate siderophores have three identical *N*-acyl groups as in ferrichrome, ferrichrome A, ferricrocin, ferrirubin, etc. Only in the case of the asperchromes, a group of ferrichrome-type siderophores isolated from *Aspergillus ochraceous*, has the presence of different acyl groups in the same siderophore been reported.[18,19] The coprogen structures provide further evidence of the versatility in microbial biosyntheses of siderophores. The possible role of these closely related siderophores in the same organism is not yet clear. Recent studies[12] have revealed that the rate of iron transport and the degree of coprogen-uptake inhibition shown by these siderophores are different. In addition to the coprogens, this fungus produces a fourth siderophore, ferricrocin, which belongs to a different structural family (ferrichromes). Many fungi seem to produce a plethora of siderophores (e.g., *A. ochraceous*[19] produces as many as twelve),

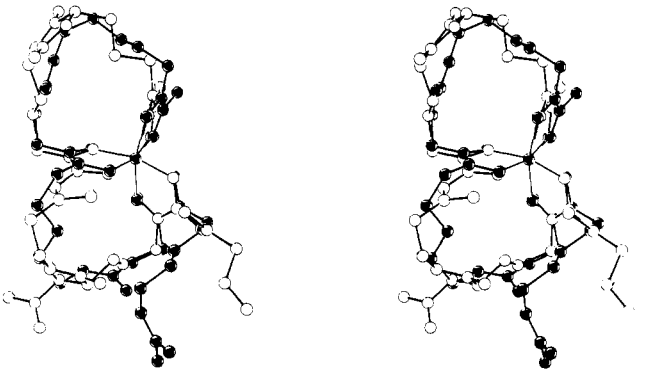

Figure 4. Stereoviews of the superimposed molecule of neocoprogen I (open circle) and ferrioxamine D_1 (dark circle).

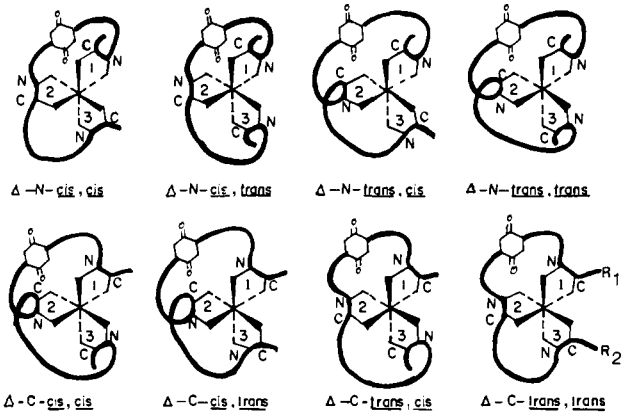

Figure 5. Eight distinct diastereomeric geometrical isomers of neocoprogen I, **4** (only the Δ optical isomers are shown). See ref 47 for the explanation.

to serve the same apparent function of iron transport.

Although coprogens are of fungal origin and are built up of typical units of other fungal siderophores, the general shape of the molecule resembles more closely that of the linear ferrioxamines, a group of bacterial siderophores. In both the coprogens and linear ferrioxamines, the three hydroxamate groups are members of a long chain. Figure 4 shows superimposed molecules of **4** and ferrioxamine D_1[34] obtained by a least-squares fit of 13 atoms. The two molecules show strikingly similar topographical features.

The distinct feature of the neocoprogen I structure is its trans geometry. It is the first example of a trans coordination of an iron trihydroxamate in the solid state. Although linear trihydroxamates have the greatest possibility to form a trans isomer compared to cyclic ones, the recent crystal structure of ferrioxamine D_1, a linear trihydroxamate, shows that it adopts a (Δ and Λ) cis configuration.[34] It has been reported[44] that the Cr^{3+} complex of deferrioxamine B (and also of D_1) exists as a mixture of two geometrical isomers in solution (cis and trans). The presence of only two distinct coordination isomers (one cis and one trans) of Ga^{3+} and Al^{3+} complexes has also been reported.[45] Theoretically there are eight possible geometrical isomers for each of the two optical isomers, Δ and Λ.[45,46] The eight possible geometrical isomers for coprogens[47] are shown in Figure 5. A simple model building exercise shows that out of the possible eight, four isomers, Δ-N-trans,cis, Δ-N-trans,trans, Δ-C-cis,cis, and Δ-C-cis,trans, are improbable because of severe steric interference caused by multiple twists in the trihydroxamate chain (shown by a thick line in Figure 5). Of the remaining four, only in Δ-C-trans,trans, is the trihydroxamate chain free of any twist, and this is the configuration adopted by isomer **4** in the crystal structure.

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Similar model building with ferrioxamine B and D_1 shows that only in the case of Δ(orΛ)-N-cis,cis the trihydroxamate chain is twist-free, and this is the isomer ferrioxamine D_1 assumes in the crystal structure. One can, therefore, conclude that the most probable isomer of **4** is Δ-C-trans,trans and that of ferrioxamine B and D_1 is Δ(or Λ)-N-cis,cis. Following the same line of argument, it can be proposed that the second most probable isomer for **4** would be Δ-N-cis,cis, because this isomer requires a single twist in the chain end containing only a methyl group. Similarly, for ferrioxamine B (or D_1), the second most probable isomer is Δ(or Λ)-N-cis,trans. This may explain why only two isomers could be identified in the case of Cr^{3+} and also of Ga^{3+} complexes of ferrioxamine B.[44-46]

Structure-activity relationships have been investigated for coprogens in *Neurospora crassa*. The number of *trans*-anhydromevalonoyl groups in these siderophores have been found to be an important determinant in the iron-uptake process. As these groups are replaced gradually by acetyl groups, the uptake activity reduces proportionally (i.e., iron-uptake rate is in the order, coprogen > neocoprogen I > neocoprogen II).[12]

The molecular structures of the ligands in the coprogen family are very different from the ones observed in the ferrichrome family. Furthermore, coprogens assume predominantly a Δ-trans coordination geometry, while all the members of the ferrichrome family show a Λ-cis geometry. Yet the two groups of siderophores reveal competition with each other during iron transport process in *N. crassa*. A shared transport system with separate receptor proteins has been proposed for coprogen and ferrichromes.[48] Neither the uptake of coprogen nor its competition with ferrichromes has been observed in *Penicillium parvum* indicating that this fungus lacks the coprogen receptor system and does not recognize this siderophore. Recent studies have revealed that the immediate surrounding of the iron atom in ferrichromes is involved in the process of coprogen uptake inhibition.[12] The members of the ferrichrome family that have *trans*-anhydromevalonic acid groups as their *N*-acyl moiety exert maximum inhibition on coprogen uptake in *N. crassa*.

Another shared uptake system has been recognized in *Escherichia coli*, in which a common outer membrane receptor, fhuE, is used by coprogen, ferric rhodotorulate, and ferrioxamines (B and D_1).[14,15,49] Ferrioxamine D_1 exists as a mixture of Λ-cis and Δ-cis isomers in the crystal structure,[34] while all coprogens exist predominantly as the Δ-trans optical isomer. It has been mentioned earlier that the molecules of **4** and ferrioxamine D_1 (Δ-form), in their respective crystal structures, superpose quite well (Figure 4). The orientation of the hydroxamate groups (cis or trans) seems to have little influence on the activity of these siderophores in this system. An analogous observation has been made in the case of retroferrichrome, in which the orientation of all three hydroxamate groups of ferrichrome is reversed (i.e, the C=O groups takes the position of N-O groups and vice versa). Such a reversal of the hydroxamate groups was found to have little effect on the iron transport activity.[50] Chirality of the iron atom (Λ or Δ), on the other hand, seems to be far more important in relation to uptake activity.[51]

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Supplementary Material Available: A packing diagram, atomic parameters, and bond distances for neocoprogen I (8 pages). Ordering information is given on any current masthead page.

(47) The nomenclature for the iron coordination of the diastereoisomers of coprogen is based on the rules outlined by Leong and Raymond.[44] (i) Viewed down the C_3 axis, the chelate rings 1, 2, and 3 are arranged clockwise for Λ isomers and counterclockwise for Δ isomers. (ii) If ring 1 has the carbon atom of the hydroxamate group below the nitrogen, it is denoted "C". If the reverse is true it is called "N". (iii) For rings 2 and 3, each is called "cis" or "trans", depending upon whether it has the same or opposite relative orientation with respect to the C_3 axis as does ring 1. (iv) The ring nearest to the free amino terminus (in ferrioxamine B) is designated as ring 1. Because in the coprogens there is no unique N-terminus (as in ferrioxamine B or D_1), we propose that the chelate rings are designated such that the diketopiperazine ring be placed between rings 1 and 2. The resulting isomer designations (Figure 5) are applicable to all members of the coprogen family and constitute a correction of the nomenclature used in a recent review.[2]

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Kinetics of Cyclopropyl Radical Reactions. 3. Study of Some 1-Substituted Cyclopropyl Radicals by EPR Spectroscopy. The Inversion Barrier for 1-Methylcyclopropyl[1]

S. Deycard,[2] L. Hughes, J. Lusztyk, and K. U. Ingold*

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Abstract: The 1-methyl-, 1-ethoxy-, and 1-chlorocyclopropyl radicals have been observed by low-temperature EPR spectroscopy in "frozen" configurations in which the ring hydrogens that are syn and anti to the unpaired electron's orbital have different hyperfine splittings. The $a^H(syn)/a^H(anti)$ ratios are 1.5 (CH_3), 1.8 (EtO), and 1.9 (Cl), all considerably lower than the ratio of ca. 3.3 found by Kawamura et al.[25] for methyl-substituted 1-fluorocyclopropyl radicals. The out-of-plane angles of the 1-substituent have been calculated from measured a^{13C_α} values to be 22.5° (cyclopropyl), 22.9° (CH_3), 29.1° (EtO), and 5.8° (Me_3Si). These angles are considerably smaller than those that have been calculated for some of these radicals by ab initio and other methods. Variable-temperature EPR spectroscopy on 1-methylcyclopropyl yields the following Arrhenius equation for its inversion: $\log (k_{inv}/s^{-1}) = (13.1 \pm 0.3) - (3.1 \pm 0.2)/2.3RT$ kcal/mol. For 1-ethoxycyclopropyl the rate constant for rotation about the C–OEt bond can be represented by $\log (k_{rot}/s^{-1}) = (12.5 \pm 0.2) - (5.8 \pm 0.2)/2.3RT$. The barrier to inversion of this radical is ≥9 kcal/mol. The 1-chlorocyclopropyl radical could only be observed at very low temperatures.

In Part 1[3] we reported the first absolute rate constants for some reactions of the cyclopropyl radical in solution.[4] In Part 2[5] we described how a combination of absolute rate data, stereospecific deuterium labeling of appropriate radical precursors, and chemical

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