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Structure of ferrichrome-type siderophores with dissimilar N^{δ}-acyl groups: asperchrome B_1, B_2, B_3, D_1, D_2 and D_3

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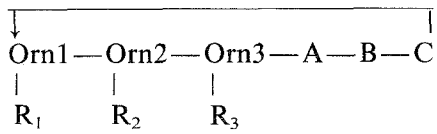
Summary. Asperchromes are a series of iron-chelating compounds which contain a cyclic hexapeptide backbone as in ferrichrome siderophores and differ from the latter in having heterogenous acyl groups in the ornithine side chains. The molecular structures of the asperchrome B and D series have been determined by ^{1}H- and ^{13}C-NMR spectroscopy; single-crystal X-ray diffraction was used to determine the detailed structural features of asperchrome B_1 and asperchrome D_1. Asperchrome B_1 crystallizes in the triclinic space group P1 with $a=1.3143(5)$ nm, $b=1.2200(5)$ nm, $c=0.8949(3)$ nm, $\alpha=105.17(4)°$, $\beta=94.03(3)°$, $\gamma=109.65(3)°$, $V=1.2843$ nm^3, $Z=1$, $\rho_x=1.446$ g cm^{-3}. Final $R=0.054$ for 4625 reflections measured at 138 K using MoK$\bar{\alpha}$. Asperchrome D_1 crystallizes in the monoclinic space group $P2_1$ with $a=1.2248(11)$ nm, $b=1.3795(9)$ nm, $c=1.3644(6)$ nm, $\beta=93.24(6)°$, $V=2.3016$ nm^3, $Z=2$, $\rho_x=1.418$ g cm^{-3}. Final $R=0.110$ for 3180 reflections measured at 138 K using MoK$\bar{\alpha}$ radiation. The conformation of the molecular backbone and iron coordination geometry in both asperchrome B_1 and D_1 compare well with those observed in other known ferrichrome siderophores. The differences in the acyl groups are illustrated and the structural results are correlated with their iron transport properties.

Key words: Siderophore — Asperchrome — Structure determination

Introduction

The ferrichrome-type siderophores are a group of iron-chelating cyclic hexapeptides which are produced by a number of fungal species in response to low iron availability. They all have the general formula:



where A and B are glycine, serine or alanine, C is always a glycine and Orn1, Orn2, Orn3 are modified ornithine residues with R_1, R_2 and R_3 acyl groups derived from one or more of a number of carboxylic acids. In recent years, the discovery and detailed structures of a number of these siderophores, such as ferrichrome (van der Helm et al. 1980), ferrichrome A (Zalkin et al. 1966; van der Helm et al. 1981), ferricrocin (Barnes et al. 1984), ferrichrysin (Norrestam et al. 1975) and ferrirubin (Barnes et al. 1985) have been reported. The results of structural investigations on these compounds show that the hexapeptide ring has the general features of the Schwyzer model (Schwyzer and Ludescher 1969) of an antiparallel β-pleated structure and that the chelation of the metal ion causes the three ornithyl side chains to fold over the hexapeptide ring into a compact globular conformation. A common feature of all these siderophores is the homogeneity of their *N*-acyl residues ($R_1=R_2=R_3$).

We recently isolated a series of ferrichrome-type siderophores from the fungus *Aspergillus ochraceous* which are structurally characterized by having heterogenous *N*-acyl groups. These compounds were given the trivial names of asperchromes B_1, B_2, B_3, C, D_1, D_2, D_3 and E; a preliminary report on their isolation and chemical structure has been published (Jalal et al. 1984a). These siderophores contain a common Orn1-Orn2-Orn3-Ser4-Ser5-Gly6 cyclic hexapeptide back-

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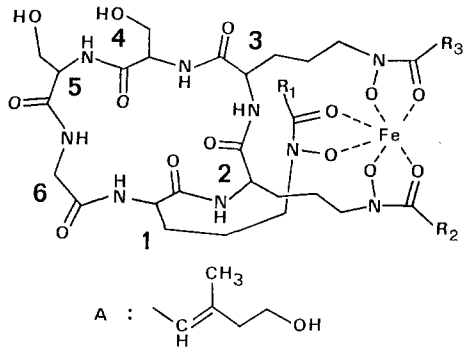

1 Ferrirubin $R_1 = R_2 = R_3 = A$

2 Asperchrome B_1 $R_1 = CH_3$, $R_2 = R_3 = A$

3 Asperchrome B_2 $R_1 = A$, $R_2 = CH_3$*, $R_3 = A$*

4 Asperchrome B_3 $R_1 = A$, $R_2 = A$*, $R_3 = CH_3$*

5 Asperchrome D_1 $R_1 = A$, $R_2 = R_3 = CH_3$

6 Asperchrome D_2 $R_1 = CH_3$, $R_2 = A$*, $R_3 = CH_3$*

7 Asperchrome D_3 $R_1 = CH_3$, $R_2 = CH_3$*, $R_3 = A$*

8 Ferrichrysin $R_1 = R_2 = R_3 = CH_3$

Fig. 1. Schematics of ferrirubin, asperchromes B_1–B_3, D_1–D_3, and ferrichrysin (* positions not confirmed)

bone with three dissimilar N^{δ}-acyl groups. The structure of these asperchromes have been determined by ^{1}H- and ^{13}C-NMR spectroscopy and by single-crystal X-ray diffraction. We report here the structure determination of the asperchrome B and D series (2–7, Fig. 1) with detailed crystallographic results of asperchrome B_1 and D_1 and their comparison with two closely related siderophores, ferrirubin (1) and ferrichrysin (8). The structural results of the other members of the asperchrome group will be reported in a separate publication.

Materials and methods

Production and purification of siderophores. Aspergillus ochraceous (isolated from air, deposited as ATCC 58722) was grown in liquid culture in iron-deficient medium. Conditions of growth and the procedures for the extraction and isolation of the siderophores have been published earlier (Jalal et al. 1984a). Individual compounds were purified by reversed-phase chromatography as described before (Jalal et al. 1984b).

Table 1. Crystal data and intensity data collection parameters for asperchrome B_1 and asperchrome D_1

Data	Parameter	Asperchrome B_1 ($C_{37}H_{58}N_9O_{16}Fe$)	Asperchrome D_1 ($C_{33}H_{52}N_9O_{15}Fe$)
Crystal data	Crystal system	Triclinic	Monoclinic
	Space group	P1	$P2_1$
	Cell parameters		
	a	1.3143 (5) nm	1.2248 (11) nm
	b	1.2200 (5) nm	1.3795 (9) nm
	c	0.8949 (3) nm	1.3644 (6) nm
	α	105.17 (4)°	
	β	94.03 (3)°	93.24 (6)°
	γ	109.65 (3)°	
	V	1.2843 nm^3	2.3016 nm^3
	Z	1	2
	ρ_x	1.446 g cm^{-3}	1.418 g cm^{-3}
	μ(MoK$\bar{\alpha}$)	3.4 cm^{-1}	3.6 cm^{-1}
	Asymmetric unit	$C_{37}H_{58}N_9O_{16}Fe + 10H_2O$	$C_{33}H_{52}N_9O_{15}Fe + 2EtOH + H_2O$
Intensity data	Radiation	MoK$\bar{\alpha}$ (graphite monochromator)	MoK$\bar{\alpha}$ (graphite monochromator)
	$2\theta_{max}$	53°	44°
	Scan width	$(1.0 + 0.20 \tan\theta)$°	$(0.90 + 0.20 \tan\theta)$°
	Horizontal aperture	$(4.5 + 0.86 \tan\theta)$ mm	$(4.50 + 0.86 \tan\theta)$ mm
	t_{max}	120 s	90 s
	Monitors	Three reflections measured every 2 h	Three reflections measured every 2 h
	Max. variation in monitor intensity	3%	4%
	Total measurements	5318	4892
	No. of observed reflections ($I \geq 2\sigma(I)$)	4625	3180
	T	138 ± 2 K	138 ± 2 K

X-ray data collection for asperchrome B_1 and D_1. Thick, platelike crystals of asperchrome B_1 were obtained from slow evaporation of an aqueous solution. The crystals were unstable at room temperature but proved to be stable at low temperature. Asperchrome D_1 crystallized as block crystals from ethanol. The crystal showed a large mosaic spread ($\approx 1.8°$). All X-ray measurements on both the compounds were carried out on an Enraf-Nonius CAD-4 automatic diffractometer fitted with a liquid N_2 low-temperature device. The crystal data and relevant data-collection parameters for the two compounds are given in Table 1. Cell parameters in each case were obtained by least-squares fit to $\pm 2\theta$ values of 48 reflections measured at 138 K using MoKα_1 radiation. Intensities were collected by employing a $\theta-2\theta$ scan technique with variable scan width. For each compound, data were corrected for Lorentz and polarization factors, but no absorption correction was applied.

Structure determination and refinement. Both the structures were determined by the heavy atom technique and successive difference Fourier syntheses. The structures were refined by using a blocked full-matrix least-squares routine with the SHELX76 program system (Sheldrick 1976). In asperchrome B_1, both the acyl groups are partially disordered; 47 hydrogen atoms (out of 58) were located from a difference Fourier map and these were included into least-squares refinement. The disordered atoms were given partial occupancy and were refined. The refinement converged to a final $R=0.054$, $R_w=0.053$ for 4625 observed reflections. Due to poor crystal quality and limited data ($2\theta_{max}=44°$), the refinement of asperchrome D_1 structure did not converge easily. The structure was refined by blocked full-matrix least-squares with anisotropic thermal parameters for the non-hydrogen atoms; 40 hydrogen atoms were placed at their calculated positions and their contributions were included into the structure factor calculations. The final R for 3180 observed reflections is 0.110.

NMR spectroscopy. Deferri compounds were prepared from their respective ferric complexes by the 8-hydroxyquinoline method (Jalal et al. 1985). ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75.4 MHz) spectra were determined on these deferri compounds at room temperature with a Varian XL300 spectrophotometer. Assignment of NMR signals was carried out by decoupling, deuterium exchange studies and chemical shift correlations to the published results obtained on compounds having similar structural units.

Results and discussion

X-ray structure of asperchrome B_1 and D_1

The final atomic parameters for asperchrome B_1 (2) and D_1 (5) are listed in Table 2. The atom

Table 2. Fractional atomic coordinates and equivalent isotropic temperature factors for non-hydrogen atoms. Upper values belong to asperchrome B_1 and lower values to asperchrome D_1

Atom	$10^4 \times x$	$10^4 \times y$	$10^4 \times z$	U_{eq}[a]
Fe	995.9 (0)	995.6 (0)	1005.2 (0)	0.0117 (3)
	187 (2)	4002 (4)	794 (2)	0.0209 (7)
O(1ε)	1670 (3)	2802 (3)	1739 (5)	0.017 (1)
	1571 (8)	3395 (8)	1335 (7)	0.018 (2)
O(1ζ)	2248 (3)	1252 (4)	2628 (5)	0.020 (2)
	1116 (9)	3999 (10)	− 377 (7)	0.032 (4)
O(2ε)	1839 (3)	1045 (3)	− 782 (5)	0.014 (1)
	766 (9)	5198 (8)	1477 (7)	0.027 (4)
O(2ζ)	675 (3)	− 802 (3)	− 38 (5)	0.016 (1)
	− 928 (10)	5016 (10)	282 (9)	0.027 (3)
O(3ε)	− 301 (3)	1072 (4)	− 126 (5)	0.018 (2)
	− 635 (8)	3542 (8)	1906 (7)	0.018 (3)
O(3ζ)	− 105 (3)	576 (4)	2434 (5)	0.018 (1)
	− 635 (10)	2861 (9)	194 (8)	0.022 (4)
N(1)	4354 (4)	5551 (4)	−1294 (6)	0.018 (2)
	4940 (12)	5562 (10)	3155 (9)	0.019 (5)
C(1α)	3834 (4)	4256 (5)	−1460 (7)	0.017 (2)
	3838 (14)	5755 (13)	2693 (12)	0.027 (4)
C(1)	2716 (4)	3789 (5)	−2494 (7)	0.015 (2)
	3009 (14)	5725 (12)	3505 (14)	0.033 (6)
O(1)	2527 (3)	4350 (4)	−3360 (5)	0.023 (2)
	3327 (11)	5986 (9)	4341 (8)	0.035 (5)
C(1β)	3845 (4)	4019 (5)	146 (7)	0.015 (1)
	3576 (14)	5045 (11)	1789 (10)	0.023 (5)
C(1γ)	3364 (5)	4776 (5)	1338 (7)	0.019 (1)
	3710 (13)	3986 (16)	2053 (10)	0.028 (5)
C(1δ)	3342 (5)	4458 (5)	2874 (7)	0.024 (1)
	3472 (17)	3307 (14)	1162 (12)	0.045 (7)
N(1ε)	2679 (4)	3182 (4)	2635 (6)	0.019 (1)
	2396 (11)	3545 (10)	718 (9)	0.023 (4)

Table 2. (continued)

Atom	$10^4 \times x$	$10^4 \times y$	$10^4 \times z$	U_{eq}[a]
C(1ζ)	2928 (5)	2349 (5)	3089 (7)	0.019 (1)
	2087 (14)	3792 (10)	− 213 (11)	0.018 (4)
C(1η)	3974 (5)	2665 (6)	4123 (8)	0.030 (1)
	2895 (15)	3768 (12)	− 960 (12)	0.034 (6)
C(1θ)	—	—	—	—
	2641 (15)	3905 (14)	−1940 (12)	0.041 (6)
C(1ν1)	—	—	—	—
	3511 (15)	3733 (13)	−2609 (12)	0.047 (5)
C(1ν2)	—	—	—	—
	1588 (13)	4285 (11)	−2416 (11)	0.035 (4)
C(1κ)	—	—	—	—
	4165 (16)	4632 (15)	−2892 (14)	0.057 (6)
O(1κ)	—	—	—	—
	4914 (13)	4890 (13)	−2042 (12)	0.106 (6)
N(2)	1974 (4)	2710 (4)	−2485 (6)	0.016 (2)
	1995 (12)	5458 (11)	3247 (9)	0.025 (5)
C(2α)	967 (4)	2173 (5)	−3626 (7)	0.015 (2)
	1155 (18)	5464 (15)	3931 (13)	0.043 (7)
C(2)	171 (4)	2826 (5)	−3221 (6)	0.014 (2)
	1052 (16)	4458 (15)	4472 (14)	0.041 (5)
O(2)	− 390 (3)	2969 (4)	−4245 (5)	0.024 (2)
	847 (14)	4395 (11)	5332 (8)	0.080 (7)
C(2β)	358 (4)	815 (5)	−3802 (6)	0.014 (1)
	5 (14)	5688 (13)	3555 (12)	0.027 (4)
C(2γ)	958 (5)	− 36 (5)	−4470 (8)	0.019 (1)
	− 101 (15)	6716 (12)	3002 (13)	0.034 (6)
C(2δ)	1848 (4)	− 102 (5)	−3409 (7)	0.016 (1)
	320 (14)	6803 (12)	2010 (11)	0.023 (6)
N(2ε)	1566 (4)	− 90 (4)	−1850 (5)	0.013 (1)
	− 19 (11)	5964 (10)	1404 (4)	0.024 (5)
C(2ζ)	928 (4)	−1026 (5)	−1422 (7)	0.017 (1)
	− 826 (17)	5797 (15)	751 (13)	0.038 (7)
C(2η)	544 (4)	−2260 (5)	−2514 (7)	0.018 (1)
	−1690 (18)	6593 (14)	561 (14)	0.039 (6)
C(2θ)	− 473 (5)	−3056 (6)	−2696 (7)	0.022 (1)
	—	—	—	—
C(2ν1)	− 767 (5)	−4325 (5)	−3801 (7)	0.022 (1)
	—	—	—	—
C(2ν2)	−1381 (6)	−2767 (6)	−1944 (9)	0.035 (1)
	—	—	—	—
C(2κ)	−1137 (5)	−4376 (6)	−5456 (8)	0.032 (1)
	—	—	—	—
O(2κ)1[b]	−1473 (5)	−5511 (6)	−6540 (8)	0.044 (2)
O(2κ)2	−2090 (18)	−4266 (20)	−5606 (27)	0.031 (5)
	—	—	—	—
N(3)	58 (4)	3154 (4)	−1694 (6)	0.019 (2)
	1139 (12)	3658 (11)	3900 (10)	0.034 (5)
C(3α)	− 806 (4)	3596 (5)	−1143 (7)	0.016 (2)
	969 (14)	2684 (14)	4267 (12)	0.023 (6)
C(3)	− 349 (4)	4945 (5)	− 162 (7)	0.018 (2)
	2046 (19)	2154 (15)	4524 (13)	0.052 (8)
O(3)	− 940 (3)	5429 (4)	514 (6)	0.029 (2)
	2005 (11)	1302 (11)	4807 (10)	0.055 (5)
C(3β)	−1494 (5)	2803 (5)	− 220 (7)	0.019 (1)
	252 (14)	2083 (13)	3494 (11)	0.023 (5)
C(3γ)	−2071 (5)	1480 (5)	−1231 (7)	0.018 (1)
	− 925 (18)	2419 (15)	3459 (13)	0.052 (8)
C(3δ)	−2251 (5)	552 (5)	− 312 (7)	0.022 (1)
	−1634 (14)	2212 (12)	2539 (12)	0.029 (6)
N(3ε)	−1215 (4)	705 (4)	581 (5)	0.015 (1)
	−1111 (11)	2661 (11)	1730 (9)	0.027 (5)

Table 2. (continued)

Atom	$10^4 \times x$	$10^4 \times y$	$10^4 \times z$	U_{eq}[a]
C(3ζ)	−1069 (4)	418 (5)	1859 (6)	0.014 (1)
	−1144 (15)	2346 (14)	780 (13)	0.024 (6)
C(3η)	−2026 (5)	− 112 (5)	2611 (7)	0.024 (1)
	−1711 (20)	1392 (17)	547 (17)	0.057 (8)
C(3θ)	−2183 (6)	−1144 (6)	2961 (9)	0.034 (2)
	—	—	—	—
C(3ν2)	−1530 (65)	−1950 (6)	2489 (9)	0.037 (2)
	—	—	—	—
C(3ν1)1[b]	−3196 (9)	−1824 (11)	3509 (15)	0.028 (2)
C(3ν1)2	−3001 (12)	−1330 (14)	4296 (20)	0.021 (3)
	—	—	—	—
C(3κ)1[b]	−2892 (9)	−1692 (10)	5189 (14)	0.035 (3)
C(3κ)2	−3825 (14)	−2621 (16)	3701 (22)	0.034 (3)
	—	—	—	—
O(3κ)1[b]	−3730 (7)	−2561 (7)	5733 (10)	0.036 (2)
O(3κ)2	−4298 (10)	−3006 (11)	4972 (14)	0.032 (3)
	—	—	—	—
N(4)	732 (4)	5541 (4)	− 94 (6)	0.017 (22)
	3030 (14)	2621 (11)	4405 (11)	0.041 (6)
C(4α)	1268 (4)	6849 (5)	728 (7)	0.016 (2)
	4021 (16)	2266 (15)	4741 (13)	0.042 (7)
C(4)	2260 (4)	7303 (5)	− 59 (7)	0.016 (2)
	4767 (14)	3138 (15)	4906 (12)	0.039 (6)
O(4)	2593 (3)	6581 (3)	− 956 (5)	0.022 (2)
	4475 (10)	3954 (12)	4643 (8)	0.044 (4)
C(4β)	1620 (5)	7051 (6)	2458 (8)	0.023 (1)
	4527 (16)	1559 (16)	4006 (14)	0.050 (6)
O(4β)	1946 (4)	8293 (4)	3311 (6)	0.035 (1)
	4846 (11)	2070 (10)	3140 (9)	0.038 (4)
N(5)	2776 (4)	8511 (4)	321 (6)	0.016 (2)
	5781 (12)	2950 (11)	5300 (9)	0.032 (5)
C(5α)	3760 (4)	9025 (5)	− 342 (7)	0.017 (2)
	6585 (15)	3666 (14)	5288 (11)	0.033 (6)
C(5)	4638 (4)	8552 (5)	120 (7)	0.016 (2)
	6657 (14)	3981 (18)	4193 (11)	0.039 (4)
O(5)	4941 (4)	8674 (4)	1472 (5)	0.029 (2)
	6458 (10)	3421 (9)	3486 (8)	0.034 (4)
C(5β)	4168 (5)	10417 (5)	313 (7)	0.023 (1)
	7730 (13)	3226 (12)	5668 (11)	0.035 (4)
O(5β)	5117 (3)	10922 (4)	− 352 (5)	0.026 (1)
	7618 (8)	3004 (8)	6705 (7)	0.031 (3)
N(6)	5052 (4)	8015 (4)	−1083 (6)	0.018 (2)
	6967 (11)	4951 (11)	4049 (10)	0.027 (3)
C(6α)	5856 (5)	7486 (5)	− 844 (8)	0.024 (2)
	6904 (16)	5374 (12)	3072 (11)	0.035 (7)
C(6)	5427 (4)	6138 (5)	− 980 (7)	0.018 (2)
	5788 (15)	5756 (13)	2682 (13)	0.028 (6)
O(6)	6087 (3)	5640 (4)	− 811 (6)	0.028 (2)
	5820 (10)	6253 (10)	1878 (9)	0.045 (5)
Solvent atoms (asperchrome B_1)				
OW(1)	510 (4)	4035 (4)	3456 (6)	0.034 (1)
OW(2)	6930 (5)	4800 (5)	1418 (8)	0.045 (1)
OW(3)	2052 (4)	6163 (5)	5602 (6)	0.034 (1)
OW(4)	4817 (4)	1478 (5)	6795 (7)	0.043 (1)
OW(5)	6350 (5)	3789 (5)	6669 (8)	0.052 (1)
OW(6)1[b]	6522 (7)	2330 (8)	2310 (10)	0.032 (2)
OW(6)2[b]	6319 (10)	2683 (11)	2058 (14)	0.040 (3)
OW(7)	4188 (4)	9407 (5)	4248 (7)	0.036 (1)

Table 2. (continued)

Atom	$10^4 \times x$	$10^4 \times y$	$10^4 \times z$	U_{eq}[a]
OW(8)1[b]	7326 (11)	2358 (12)	4755 (16)	0.064 (4)
OW(8)2[b]	7215 (8)	2013 (9)	5140 (10)	0.025 (2)
OW(9)	4162 (5)	7673 (5)	5692 (7)	0.047 (1)
OW(10)1[b]	6128 (6)	5002 (7)	4444 (11)	0.062 (2)
OW(10)2[b]	6118 (28)	5223 (32)	5588 (48)	0.075 (9)
Solvent atoms (asperchrome D_1)				
O(Et1)	6963 (9)	4711 (9)	7487 (8)	0.047 (3)
C(Et1)1	7711 (16)	5447 (15)	7961 (14)	0.060 (6)
C(Et1)2	8846 (18)	5029 (16)	7879 (15)	0.071 (6)
O(Et2)1	−4685 (10)	2338 (9)	− 454 (8)	0.055 (4)
C(Et2)1	−3776 (17)	2886 (15)	− 729 (14)	0.060 (6)
C(Et2)2	−3485 (18)	3743 (18)	31 (16)	0.086 (8)
OW(1)	4330 (14)	6439 (14)	−1331 (12)	0.119 (6)

[a] $U_{eq} = 1/3 \Sigma\Sigma U_{ij} a_i^* a_j^* (a_i \cdot a_j)$
[b] Disordered

Table 3. Peptide dimensions. Estimated SD range: distance = 0.0005–0.0008 nm for asperchrome B_1 (upper values) and 0.002–0.003 nm for asperchrome D_1 (lower values); angle = 0.2–0.4° for asperchrome B_1 and ≈1° for asperchrome D_1 (lower values)

Bond	Value (nm) for					
	$i=1$	$i=2$	$i=3$	$i=4$	$i=5$	$i=6$
N(i)-C($i\alpha$)	0.1457	0.1450	0.1474	0.1463	0.1474	0.1440
	0.148	0.143	0.145	0.136	0.140	0.145
C($i\alpha$)-C(i)	0.1520	0.1523	0.1532	0.1533	0.1530	0.1517
	0.155	0.158	0.153	0.152	0.156	0.153
C(i)-O(i)	0.1223	0.1221	0.1227	0.1245	0.1201	0.1236
	0.124	0.122	0.124	0.123	0.125	0.130
C(i)-N($i+1$)	0.1351	0.1354	0.1350	0.1335	0.1355	0.1321
	0.132	0.136	0.138	0.135	0.141	0.128
Angle	**Value (°) for**					
	$i=1$	$i=2$	$i=3$	$i=4$	$i=5$	$i=6$
N(i)-C($i\alpha$)-C(i)	107.4	112.6	112.7	105.7	109.3	116.9
	109	112	112	106	107	117
C($i\alpha$)-C(i)-O(i)	120.2	121.2	121.2	121.6	121.1	119.2
	118	123	118	121	123	114
C($i\alpha$)-C(i)-N($i+1$)	117.8	116.9	115.4	116.6	115.8	117.5
	118	116	120	116	115	119
O(i)-C(i)-N($i+1$)	121.8	121.8	123.3	121.7	123.1	123.3
	124	122	122	123	122	127
C(i)-N($i+1$)C($i+1,\alpha$)	118.2	123.7	121.4	120.1	122.4	123.0
	122	122	124	120	121	120

numbering scheme used for the crystallographic results is shown in a scheme in Fig. 2. Stereoviews of the two siderophore molecules are shown in Fig. 3a (asperchrome B_1) and Fig. 3b (asperchrome D_1).

The bond distances and angles in the hexapeptide ring for both the compounds are listed in Table 3. Conformational angles of the hexapeptide ring and selected torsion angles in the three ornithine side chains are shown in Table 4 and

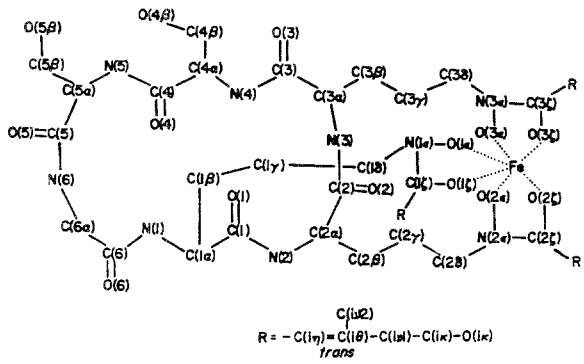

Fig. 2. Atom numbering scheme for the asperchromes and related siderophores

compared with the corresponding angles observed in two closely related siderophores: ferrirubin (1) and ferrichrysin (8). The bond lengths in the iron chelate rings for both the compounds are shown in Fig. 4 and the average values of the geometrical parameters of the iron coordination are compared with those of ferrirubin in Table 5.

In the solid state, both the siderophore molecules display some common features observed in other known ferrichrome-type structures:

1. The *Λ-cis* coordination geometry for the metal ion.
2. The hexapeptide ring conformation with only one intramolecular N–H...O hydrogen bond stabilizing a β(II) turn [N(1)...O(4) = 0.2979 nm in asperchrome B_1 and 0.3077 nm in asperchrome D_1] with Ser5 and Gly6 residues at the corners.
3. The β(I) turn with ornithine residues 2 and 3 at the corners does not result in a hydrogen bond [N(4)...O(1) = 0.4156 nm in asperchrome B_1 and = 0.4675 nm in asperchrome D_1].
4. An intramolecular hydrogen bond between the Orn2 amide group with the Orn2 oxime oxygen [N(2)...O(2ε) is 0.2810 nm in asperchrome B_1 and 0.2794 nm in asperchrome D_1] linking the peptide backbone with the iron coordination.
5. Four of the six amide groups (N1, N2, N3 and N4) are either buried inside or internally hydrogen-bonded.

The results in Tables 4 and 5 show that the presence of different acyl groups does not affect the geometry of the coordination octahedra or the hexapeptide conformation to a significant degree. A comparison of the peptide ring torsion angles shows that both ϕ_1 and ψ_1 in asperchrome B_1 differ by about 10° from those in asperchrome D_1 and that the change in these torsion angles is such that the O(1)...N(4) distance is much longer in D_1 (0.4675 nm as compared to 0.4156 nm in B_1 and 0.4170 nm in ferrirubin).

Figure 5 shows the spatial distribution of acyl groups in asperchrome D_1, asperchrome B_1 and the three independent molecules of ferrirubin (5). The terminal —CH_2—CH_2—OH group in most of the acyl groups is flexible and takes up quite random orientation (in some cases the group is disor-

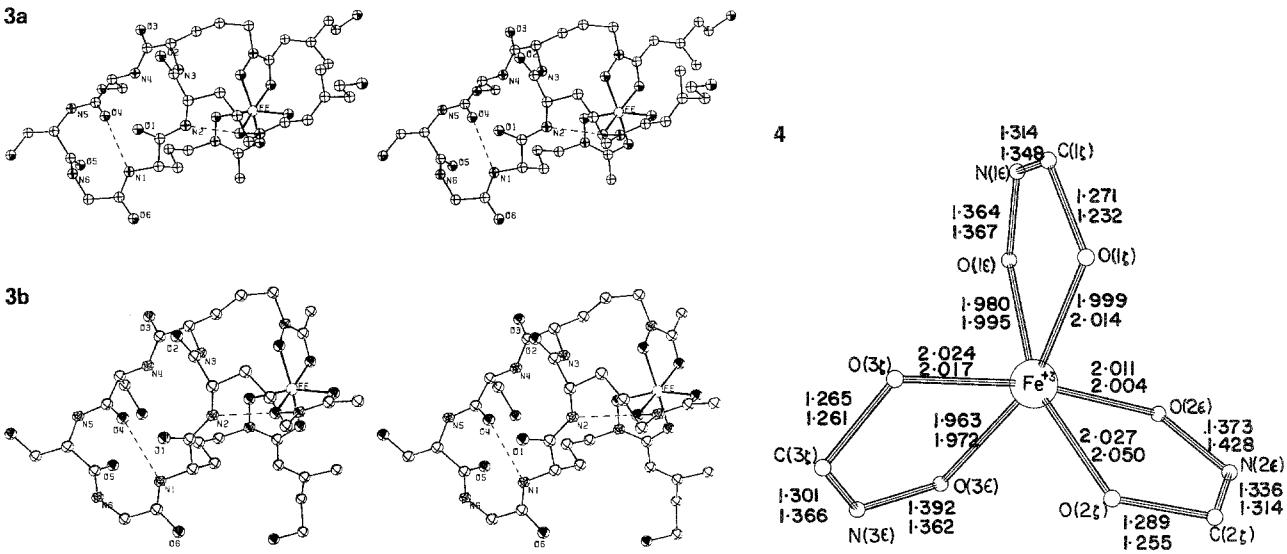

Fig. 3a, b. Stereoview of a single molecule of **(a)** asperchrome B_1 and **(b)** asperchrome D_1. *Dashed lines* indicate intramolecular hydrogen bonds

Fig. 4. Bond distances in the iron coordination. Values are given in Å (1 Å ≡ 0.1 nm) asperchrome B_1 *(upper)* and asperchrome D_1 *(lower)*. Estimated standard deviation range: 0.003–0.007 Å for B_1, 0.010–0.019 Å for D_1

Table 4. Comparison of the average geometrical parameters of the iron coordinaton in asperchrome B_1, asperchrome D_1 and ferrirubin[a]. Number in *parentheses* is: $\sigma=[\Sigma(\Delta)^2/N(N-1)]^{1/2}$

Parameter	Value in		
	asperchrome B_1	asperchrome D_1	ferrirubin
Fe-O(ε) (nm)	0.1985 (14)	0.1990 (10)	0.1994 (10)
Fe-O(ζ) (nm)	0.2017 (9)	0.2027 (12)	0.2029 (5)
N-O(ε) (nm)	0.1376 (8)	0.1386 (20)	0.1384 (7)
C-O(ζ) (nm)	0.1275 (7)	0.1249 (9)	0.1288 (12)
N-C (nm)	0.1317 (10)	0.1343 (15)	0.1322 (9)
O(ε)...Fe...O(ζ) (°)	78.8 (4)	78.3 (4)	78.5 (4)
O(ε)...O(ζ) (nm)	0.2540 (3)	0.2552 (20)	0.2546 (13)
Ligand bite[b]	1.27	1.26	1.27
Twist angle[c]	42.7 (17)	42.2 (21)	43.6 (12)

[a] Mean of three independent molecules (Barnes et al. 1985)
[b] Ratio of O(ε)...O(ζ) distances to Fe-O distances
[c] Angle of rotation of O(1ε)-O(2ε)-O(3ε) face of coordination octahedron with respect to O(1ζ)-O(2ζ)-O(3ζ) face

Table 5. Selected torsion angles. Estimated SD range: $<1°$ for asperchrome B_1 (B), 1–3° for asperchrome D_1 (C)

Angle	Value (°) for					
		Orn1 ($i=1$)	Orn2 ($i=2$)	Orn3 ($i=3$)	Ser ($i=4$)	Ser ($i=5$)	Gly ($i=6$)
C($i-1$)-N(i)-C($i\alpha$)-C(i)							
	A	−152	− 71	−107	−166	− 54	84
	B	−155	− 74	−112	−154	− 59	92
	C	−164	− 92	−101	−154	− 55	83
	D	−155	− 78	−124	−166	− 58	90
N(i)-C($i\alpha$)-C(i)-N($i+1$)							
	A	−168	− 42	6	177	130	3
	B	−163	− 42	7	169	127	− 1
	C	−151	− 41	1	176	150	− 10
	D	−163	− 29	19	177	137	− 7
C($i\alpha$)-C(i)-N($i+1$)-C($i\alpha+1$)							
	A	−177	−166	177	−177	176	179
	B	−171	−169	177	178	−178	180
	C	−176	−175	170	167	−171	−168
	D	−176	−170	170	170	179	−178
N(i)-C($i\alpha$)-C($i\beta$)-C($i\gamma$)							
	A	60	− 64	− 70			
	B	53	− 65	− 61			
	C	54	− 57	− 73			
	D[a]	—	—	—			
C($i\alpha$)-C($i\beta$)-C($i\gamma$)-C($i\delta$)							
	A	177	75	160			
	B	177	80	150			
	C	−179	74	158			
	D[a]	—	—	—			
C($i\beta$)-C($i\gamma$)-C($i\delta$)-N($i\varepsilon$)							
	A	− 56	48	− 60			
	B	− 60	40	− 55			
	C	− 53	45	− 58			
	D[a]	—	—	—			

A = average of three independent molecules of ferrirubin (Barnes et al. 1985); B = asperchrome B_1; C = asperchrome D_1; D = ferrichrysin (Norrestam et al. 1975)
[a] Data not available

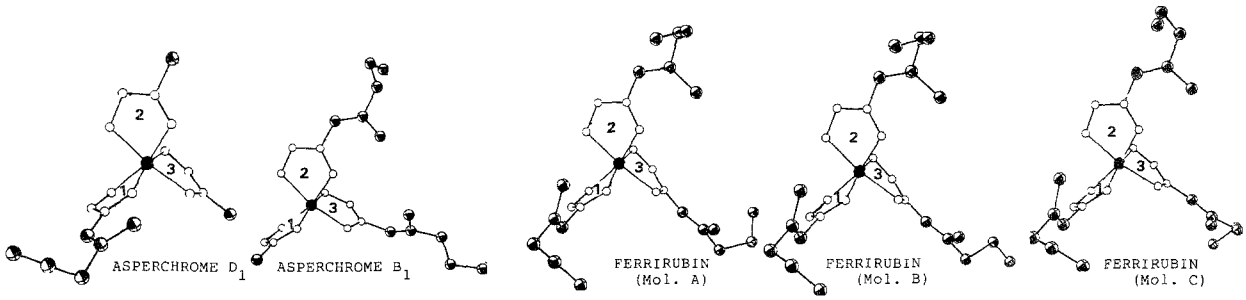

Fig. 5. Spatial distribution of acyl groups in asperchrome B_1 and D_1, and in ferrirubin. Mol. A and Mol. B found in triclinic form and Mol. C in monoclinic form of ferrirubin (Barnes et al. 1985)

Table 6. Acyl group conformation

Molecule	Torsion angle (°) $O(i\zeta)$-$C(i\zeta)$-$C(i\eta)=C(i\theta)$		
	$i=1$	$i=2$	$i=3$
Asperchrome D_1	−5.0	...	...
Asperchrome B_1	...	41.3	−50.9
Ferrirubin 1, mol. A[a]	44.4	13.8	−27.4
Ferrirubin 2, mol. B	43.0	12.0	−21.7
Ferrirubin 3, mol. C	45.1	26.0	− 7.5

[a] Barnes et al. 1985

dered). Table 6 shows the torsion angles $O(i\zeta)$-$C(i\zeta)$-$C(i\eta)=C(i\theta)$, for each of the molecules in Fig. 5. Although there are significant out-of-plane deviations, the double bond lies, in general, close to the plane of the hydroxamate group. The deviation is least in asperchrome D_1 and the largest in asperchrome B_1. The out-of-plane rotation of the double bond in Orn3 is opposite to that for Orn1 and Orn2.

Asperchrome B_1 interacts extensively with the solvent molecules (16 hydrogens bonds) and forms only two weak intermolecular hydrogen bonds, $O(3)\ldots O(2\kappa)$ $[x, y+1, z+1]=0.3172$ nm and $N(5)\ldots O(2\zeta)$ $[x, y+1, z]=0.3163$ nm. Asperchrome D_1 forms two hydrogen bonds with a water molecule and two with ethanol molecules and two fairly strong intermolecular hydrogen bonds, $N(5)\ldots O(1)$ $[1-x, -\frac{1}{2}+y, 1-z]=0.2952$ nm, $N(6)\ldots O(3)$ $[1-x, \frac{1}{2}+y, 1-z]=0.2696$ nm.

At the end of the paper, Supplement Tables 1 and 2 give a complete listing of the bond distances (in Å) and bond angles (in °), respectively, for asperchromes B_1 and D_1.

NMR spectra of asperchrome B_1, D_1 and their isomers

The structure determination of asperchrome B_1 and D_1 by X-ray diffraction facilitated the identification of the other isomers by NMR spectroscopy. Both asperchrome B_1 and D_1 have two additional isomers, depending on the position of the odd *N*-acyl group in the three ornithine units. Reversed-phase chromatography yielded separation of these isomers as asperchrome B_2 and B_3 (isomers of asperchrome B_1) and asperchrome D_2 and D_3 (isomers of asperchrome D_1). However, asperchrome D_2 and D_3 could not be separated completely and their NMR spectra were obtained on a mixture of both compounds.

Tables 7 and 8 present the ^1H- and ^{13}C-NMR chemical shifts of the deferri form of these asperchromes (2–7) and ferrirubin (1). Chemical shifts of asperchromes B_1, B_2 and B_3 are similar and thus they are treated as a single group in the tables. Asperchrome D_1, D_2 and D_3 are also grouped together for the same reason. It is obvious from the tables that, except for the *N*-acyl groups, ^1H and ^{13}C chemical shifts of asperchrome B_1, B_2, B_3, D_1, D_2 and D_3 and ferrirubin are very similar. Only slight differences exist in ^1H signals of some NH groups in the peptide ring. The reduction in the number of N^δ-anhydromevalonoyl groups from three (in ferrirubin) to two (in asperchrome B_1–B_3) and finally to one (in asperchrome D_1–D_3) is reflected in the decrease in intensity of the ^1H- and ^{13}C-NMR signals arising from these groups. The presence of one N^δ-acetyl group in asperchrome B_1–B_3 and two of these groups in asperchromes D_1–D_3 is shown by the appearance of a new methyl signal (at $\delta=1.97$ and 20.3 ppm for ^1H and ^{13}C respectively) with their relative intensities corresponding to their number.

It has been possible to distinguish between asperchrome B_2 and B_3 (nor D_2 and D_3) from the NMR results obtained in this study. The signals arising form different N^δ-acyl groups superimpose on each other and the isomers cannot be assigned a specific structure (e.g. whether asperchrome B_2 contains its single N^δ-acetyl group in or-

Table 7. ^{1}H-NMR chemical shifts (δ) of deferriasperchromes (B_1–B_3 and D_1–D_3) compared to those of deferriferrirubin. Solvent is $(CD_3)_2SO$

Residue	Proton group	δ (ppm) for deferri form of		
		ferrirubin	asperchrome B_1–B_3	asperchrome D_1–D_3
Glycyl	CH_2	3.60–3.90 (2H)	3.60–3.90 (2H)	3.60–3.90 (2H)
	NH	8.53 (1H)	8.45–8.53 (1H)	8.49 (1H)
Seryl	CH	3.9–4.15 (2H)	3.9–4.15 (2H)	3.9–4.15 (2H)
	CH_2	3.50–3.70 (4H)	3.50–3.70 (4H)	3.50–3.70 (4H)
	OH	4.96, 5.12 (2H)	4.96, 5.12 (2H)	4.96, 5.12 (2H)
	NH	8.02, 8.08 (2H)	7.96–8.02 (2H)	7.97 (2H)
Ornithyl	CH	3.90–4.15, 4.20 (3H)	3.90–4.15, 4.20 (3H)	3.90–4.15, 4.20 (3H)
	$CH_2(\beta)$	1.60–1.90 (6H)	1.60–1.90 (6H)	1.60–1.90 (6H)
	$CH_2(\gamma)$	1.45–1.75 (6H)	1.45–1.75 (6H)	1.45–1.75 (6H)
	$CH_2(\delta)$	3.45–3.65 (6H)	3.45–3.65 (6H)	3.45–3.65 (6H)
	NH	7.50, 7.98, 8.34 (3H)	7.50, 7.98, 8.32 (3H)	7.50, 7.98, 8.28 (3H)
	NOH	9.75 (3H)	9.75 (3H)	9.75 (3H)
N-Acyl	CH	6.22 (3H)	6.22 (2H)	6.22 (1H)
	CH_2—	2.25 (6H)	2.24 (4H)	2.24 (2H)
	CH_2O—	3.53 (6H)	3.53 (4H)	3.53 (2H)
	OH	4.62 (3H)	4.56 (2H)	4.56 (1H)
	=C—CH_3(*iv*2)	2.03 (9H)	2.03 (6H)	2.03 (3H)
	$CH_3(i\eta)$	—	1.97 (3H)	1.97 (6H)

Table 8. ^{13}C-NMR chemical shifts (δ) of deferriasperchromes (B_1–B_3 and D_1–D_3) compared to those of deferriferrirubin. Solvent is $(CD_3)_2SO$

Residue	Carbon group	δ (ppm) for deferri form of		
		ferrirubin	asperchrome B_1–B_3	asperchrome D_1–D_3
Glycyl	CH_2	43.0 (1C)	43.2 (1C)	43.1 (1C)
	>C=O	169.2 (1C)	169.2 (1C)	168.6 (1C)
Seryl	CH	55.6,56.2 (2C)	55.6, 56.3 (2C)	55.5, 56.1 (2C)
	CH_2	60.8 (2C)	60.8 (2C)	60.6 (2C)
	>C=O	170.4, 170.9 (2C)	170.6, 170.0 (2C)	169.9,170.3 (2C)
Ornithyl	CH	52.8, 53.4, 54.0 (3C)	52.8, 53.4, 54.4 (3C)	52.6, 53.3, 54.0 (3C)
	$CH_2(\beta)$	28.0, 28.3, 28.4 (3C)	28.0, 28.3, 28.4 (3C)	27.9, 28.2, 28.3 (3C)
	$CH_2(\gamma)$	23.0, 23.4, 23.6 (3C)	23.0, 23.3, 23.4 (3C)	22.8, 23.3, 23.5 (3C)
	$CH_2(\delta)$	46.6 (3C)	46.6 (3C)	46.5 (3C)
	>C=O	172.0 (2C), 172.4 (1C)	172.2 (2C), 172.4 (1C)	171.4 (2C), 171.8 (1C)
N-Acyl	>C=O	167.2(3C)	167.3 (3C)	166.5 (3C)
	CH	116.2 (3C)	116.6 (2C)	116.1 (1C)
	C	151.2 (3C)	151.8 (2C)	151.1 (1C)
	CCH_2	43.8 (3C)	43.8 (2C)	43.7 (1C)
	CH_2OH	59.1 (3C)	59.3 (2C)	59.1 (1C)
	=C—CH_3(*iv*2)	18.2 (3C)	18.2 (2C)	18.2 (1C)
	$CH_3(i\eta)$	—	20.3 (1C)	20.3 (2C)

nithine 2 or 3). Asperchrome B_1 and D_1, whose structures have been unequivocally determined by X-ray diffraction, are, however, structurally very different from their isomers. In both asperchrome B_1 and D_1, the odd N^{δ}-acyl group is on ornithine 1, the side chain which is perpendicular (axial) to the plane of the hexapeptide ring. The side chains of ornithine 2 and 3, on the other hand, are more or less continuous (equitorial) with the plane of the hexapeptide ring.

Structure activity relationship

All asperchromes are ferrichrome-type siderophores with a *Λ-cis* iron coordination geometry. They use the ferrichrome receptor system in the membrane during iron transport in *Neurospora crassa* (Huschka et al. 1986). With ^{55}Fe-labelled siderophores which contain a common cyclo(-Ser-Ser-Gly-Orn-Orn-Orn) backbone, it has been shown that the presence of an increased number of N^{δ}-anhydromevalonoyl groups decreases the transport efficiency of the siderophores in *N. crassa* (Huschka et al. 1986). The transport rate in this fungus shows the order: ferrichrysin > asperchrome D_1 > asperchrome B_1 > ferrirubin. Ferrirhodin contains three N^{δ}-*cis*-anhydromevalonoyl groups instead of the *trans*-acyl groups found in its isomer, ferrirubin. A preferred conformation of these *cis*-acyl groups makes the iron atom less accessible in ferrirhodin than in ferrirubin (van der Helm et al. 1987). However, transport studies have revealed that ferrirhodin is taken up as much as ferrichrysin, whereas ferrirubin is the least active siderophore in this fungus (Huschka et al. 1986).

Both asperchrome B_1 and D_1 strongly inhibit uptake of coprogen by *N. crassa* (Huschka et al. 1986). The presence of at least one N^{δ}-anhydromevalonoyl group in the molecule is thought to be necessary for coprogen transport-inhibition activity. The same study has also shown that the transport activities of the three isomers of asperchrome B are not significantly different from one another.

These observations clearly demonstrate the general role of the functional groups of the iron coordination in transport and uptake, but the results do not show a great specificity for the iron environment of the compounds and the spatial details of the acyl groups seem relatively unimportant. There is, however, a clear indication for the function of the *cis*- and *trans*-isomers of the acyl groups, as is indicated by the distinct differences for ferrirubin and ferrirhodin in both uptake and inhibition. Further uptake studies with compounds in the ferrichrome series in a variety of microorganism systems may possibly establish a more precise function of the acyl groups in the process of iron transport. However, it has to be realized that one cannot expect a common process for iron uptake in all fungi while in each case the transport may be expected to be a multistep process, possibly involving a number of structural variables in the siderophores.

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SUPPLEMENTARY TABLE 1

Bond Distances.

	Asperchrome B_1	Asperchrome D_1
Fe-O(1ε)	1.980(3)	1.995(11)
Fe-O(1ζ)	1.999(4)	2.014(19)
Fe-O(2ε)	2.011(3)	2.004(12)
Fe-O(2ζ)	2.027(3)	2.050(13)
Fe-O(3ε)	1.963(3)	1.972(10)
Fe-O(3ζ)	2.024(3)	2.017(13)
O(1ε)-N(1ε)	1.364(6)	1.367(16)
O(1ζ)-C(1ζ)	1.271(6)	1.232(20)
O(2ε)-N(2ε)	1.373(4)	1.428(17)
O(2ζ)-C(2ζ)	1.289(5)	1.255(23)
O(3ε)-N(3ε)	1.392(5)	1.362(18)
O(3ζ)-C(3ζ)	1.265(6)	1.261(21)
N(1)-C(1α)	1.457(5)	1.48(2)
N(1)-C(6)	1.321(7)	1.28(2)
C(1α)-C(1)	1.520(7)	1.55(2)
C(1α)-C(1β)	1.539(6)	1.59(2)
C(1)-O(1)	1.223(5)	1.24(2)
C(1)-N(2)	1.351(6)	1.32(2)
C(1β)-C(1γ)	1.537(6)	1.51(3)
C(1γ)-C(1δ)	1.522(6)	1.55(2)
C(1δ)-N(1ε)	1.455(6)	1.46(2)
N(1ε)-C(1ζ)	1.314(5)	1.35(2)
C(1ζ)-C(iη)	1.474(8)	1.46(2)
C(1η)-C(1θ)	-	1.37(2)
C(1θ)-C(1ν2)	-	1.50(2)
C(1θ)-C(1ν1)	-	1.46(2)
C(1ν1)-C(1κ)	-	1.54(3)
C(1κ)-O(1κ)	-	1.48(2)
N(2)-C(2α)	1.450(7)	1.43(2)
C(2α)-C(2)	1.523(6)	1.58(3)
C(2α)-C(2β)	1.538(6)	1.50(3)
C(2)-O(2)	1.221(5)	1.22(2)
C(2)-N(3)	1.354(5)	1.36(2)
C(2β)-C(2γ)	1.533(6)	1.61(2)
C(2γ)-C(2δ)	1.491(7)	1.48(2)
C(2δ)-N(2ε)	1.466(5)	1.47(2)
N(2ε)-C(2ζ)	1.366(6)	1.31(2)
C(2ζ)-C(2η)	1.458(6)	1.54(3)
C(2η)-C(2θ)	1.330(8)	-
C(2θ)-C(2ν2)	1.508(8)	-
C(2θ)-C(2ν1)	1.506(6)	-
C(2ν1)-C(2κ)	1.505(7)	-
[a]C(2κ)-O(2κ)	1.371(7) 1.305(21)	- -
N(3)-C(3α)	1.474(6)	1.45(2)
C(3α)-C(3)	1.532(6)	1.53(3)
C(3α)-C(3β)	1.540(6)	1.57(2)
C(3)-O(3)	1.227(6)	1.24(3)
C(3)-N(4)	1.350(7)	1.38(3)
C(3β)-C(3γ)	1.521(6)	1.51(3)
C(3γ)-C(3δ)	1.532(6)	1.51(2)
C(3δ)-N(3ε)	1.458(7)	1.45(2)
N(3ε)-C(3ζ)	1.301(5)	1.37(2)
C(3ζ)-C(3η)	1.501(7)	1.51(3)
C(3η)-C(3θ)	1.328(6)	-
C(3θ)-C(3ν2)	1.512(8)	-
[a]C(3θ)-C(3ν1)	1.505(12) 1.671(14)	- -
[a]C(3ν1)-C(3κ)	1.479(13) 1.507(19)	- -
[a]C(3κ)-O(3κ)	1.462(12) 1.443(17)	- -
N(4)-C(4α)	1.463(5)	1.36(3)
C(4α)-C(4)	1.533(7)	1.52(3)
C(4α)-C(4β)	1.514(6)	1.55(3)
C(4)-O(4)	1.245(5)	1.23(3)
C(4)-N(5)	1.335(5)	1.35(2)
C(4β)-O(4β)	1.405(6)	1.45(2)
N(5)-C(5α)	1.474(6)	1.40(2)
C(5α)-C(5)	1.530(6)	1.56(2)
C(5α)-C(5β)	1.529(6)	1.59(2)
C(5)-O(5)	1.201(5)	1.25(2)
C(5)-N(6)	1.355(5)	1.41(3)
C(5β)-O(5β)	1.438(7)	1.46(2)
N(6)-C(6α)	1.440(6)	1.45(2)
C(6α)-C(6)	1.517(6)	1.53(3)
C(6)-O(6)	1.236(6)	1.30(2)

[a]Disordered Atoms

SUPPLEMENTARY TABLE 2

Bond Angles (°)

	Asperchrome B_1	Asperchrome D_1
O(1ε)-Fe-O(1ζ)	79.4(1)	77.6(4)
O(1ε)-Fe-O(2ε)	87.3(1)	84.4(4)
O(1ε)-Fe-O(2ζ)	164.1(1)	161.6(5)
O(1ε)-Fe-O(3ε)	90.3(1)	92.3(4)
O(1ε)-Fe-O(3ζ)	104.6(1)	102.5(5)
O(1ζ)-Fe-O(2ε)	96.4(1)	99.8(5)
O(1ζ)-Fe-O(2ζ)	95.9(1)	97.2(5)
O(1ζ)-Fe-O(3ε)	164.5(1)	161.0(6)
O(1ζ)-Fe-O(3ζ)	92.3(1)	88.1(5)
O(2ε)-Fe-O(2ζ)	78.1(1)	79.0(5)
O(2ε)-Fe-O(3ε)	94.7(1)	95.2(4)
O(2ε)-Fe-O(3ζ)	166.4(1)	170.5(5)

O(2ζ)-Fe-O(3ε)	97.0(1)	97.1(5)
O(2ζ)-Fe-O(3ζ)	90.7(1)	95.0(5)
O(3ε)-Fe-O(3ζ)	79.0(1)	78.3(4)
N(1ε)-O(1ε)-Fe	110.6(2)	110.5(8)
Fe-O(1ζ)-C(1ζ)	112.5(3)	116.0(8)
Fe-O(2ε)-N(2ε)	112.1(2)	110.9(8)
Fe-O(2ζ)-C(2ζ)	113.2(2)	112(1)
Fe-O(3ε)-N(3ε)	111.6(2)	112.6(8)
Fe-O(3ζ)-C(3ζ)	113.5(3)	116(1)
N(1)-C(1α)-C(1)	107.4(3)	108(1)
N(1)-C(1α)-C(1β)	111.6(3)	111(1)
C(1)-C(1α)-C(1β)	116.2(4)	115(1)
C(1α)-C(1)-O(1)	120.2(4)	118(2)
C(1α)-C(1)-N(2)	117.8(3)	118(1)
O(1)-C(1)-N(2)	121.8(5)	124(2)
C(1α)-C(1β)-C(1γ)	114.7(3)	113(1)
C(1β)-C(1γ)-C(1δ)	112.4(3)	113(1)
C(1γ)-C(1δ)-N(1ε)	112.0(4)	108(1)
C(1δ)-N(1ε)-O(1ε)	113.7(3)	113(1)
C(1δ)-N(1ε)-C(1ζ)	129.8(5)	130(1)
O(1ε)-N(1ε)-C(1ζ)	116.4(4)	116(1)
N(1ε)-C(1ζ)-O(1ζ)	119.0(5)	117(1)
N(1ε)-C(1ζ)-C(1η)	121.3(4)	119(1)
O(1ζ)-C(1ζ)-C(1η)	119.7(4)	124(1)
C(1ζ)-C(1η)-C(1θ)	-	124(2)
C(1η)-C(1θ)-C(1ν1)	-	117(2)
C(1η)-C(1θ)-C(1ν2)	-	128(2)
C(1ν1)-C(1θ)-C(1ν2)	-	115(1)
C(1θ)-C(1ν1)-C(1κ)	-	116(2)
C(1ν1)-C(1κ)-O(1κ)	-	108(1)
C(1)-N(2)-C(2α)	118.2(3)	122(1)
N(2)-C(2α)-C(2)	112.6(3)	112(2)
N(2)-C(2α)-C(2β)	112.4(3)	118(1)
C(2)-C(2α)-C(2β)	107.6(4)	104(2)
C(2α)-C(2)-O(2)	121.2(4)	123(2)
C(2α)-C(2)-N(3)	116.9(4)	116(1)
O(2)-C(2)-N(3)	121.8(4)	121(2)
C(2α)-C(2β)-C(2γ)	115.5(4)	113(1)
C(2β)-C(2γ)-C(2δ)	119.1(4)	119(1)
C(2γ)-C(2δ)-N(2ε)	111.8(4)	110(1)
C(2δ)-N(2ε)-O(2ε)	115.7(3)	112(1)
C(2δ)-N(2ε)-C(2ζ)	127.7(3)	134(1)
O(2ε)-N(2ε)-C(2ζ)	115.7(3)	113(1)
N(2ε)-C(2ζ)-O(2ζ)	118.2(3)	123(2)
N(2ε)-C(2ζ)-C(2η)	119.7(4)	118(2)
O(2ζ)-C(2ζ)-C(2η)	122.1(4)	119(2)
C(2ζ)-C(2η)-C(2θ)	122.6(4)	-
C(2η)-C(2θ)-C(2ν1)	118.2(4)	-
C(2η)-C(2θ)-C(2ν2)	124.9(4)	-
C(2ν1)-C(2θ)-C(2ν2)	116.8(5)	-
C(2θ)-C(2ν1)-C(2κ)	110.6(3)	-
C(2ν1)-C(2κ)-O(2κ)	114.9(4)[a]	-
	111.2(8)	-
C(2)-N(3)-C(3α)	123.7(4)	122(1)
N(3)-C(3α)-C(3)	112.7(4)	112(1)
N(3)-C(3α)-C(3β)	111.0(4)	110(1)
C(3)-C(3α)-C(3β)	110.5(3)	110(1)
C(3α)-C(3)-O(3)	121.2(5)	118(2)
C(3α)-C(3)-N(4)	115.4(4)	120(2)
O(3)-C(3)-N(4)	123.4(4)	122(2)
C(3α)-C(3β)-C(3γ)	111.8(3)	111(1)
C(3β)-C(3γ)-C(3δ)	114.1(3)	118(1)
C(3γ)-C(3δ)-N(3ε)	110.7(4)	107(1)
C(3δ)-N(3ε)-O(3ε)	115.2(3)	117(1)
C(3δ)-N(3ε)-C(3ζ)	127.6(4)	127(1)
O(3ε)-N(3ε)-C(3ζ)	116.8(4)	116(1)
N(3ε)-C(3ζ)-O(3ζ)	118.3(4)	116(2)
N(3ε)-C(3ζ)-C(3η)	120.7(4)	118(2)
O(3ζ)-C(3ζ)-C(3η)	121.0(4)	126(2)
C(3ζ)-C(3η)-C(3θ)	120.8(5)	-
[a]C(3η)-C(3θ)-C(3ν1)	124.0(6) 112.9(5)	- -
C(3η)-C(3θ)-C(3ν2)	124.6(5)	-
[a]C(3ν1)-C(3θ)-C(3ν2)	109.8(5) 121.4(5)	- -
[a]C(3θ)-C(3ν1)-C(3κ)	108.8(8) 107.2(9)	- -
[a]C(3ν1)-C(3κ)-O(3κ)	112.3(8) 110.8(10)	- -
C(3)-N(4)-C(4α)	121.4(4)	124(2)
N(4)-C(4α)-C(4)	105.7(3)	106(2)
N(4)-C(4α)-C(4β)	110.0(3)	113(1)
C(4)-C(4α)-C(4β)	111.3(4)	110(2)
C(4α)-C(4β)-O(4β)	110.6(3)	111(2)
C(4α)-C(4)-O(4)	121.6(4)	121(2)
C(4α)-C(4)-N(5)	116.6(3)	116(2)
O(4)-C(4)-N(5)	121.7(4)	123(2)
C(4)-N(5)-C(5α)	120.1(3)	120(2)
N(5)-C(5α)-C(5)	109.3(3)	107(1)
N(5)-C(5α)-C(5β)	107.9(3)	110(1)
C(5)-C(5α)-C(5β)	110.9(4)	109(1)
C(5α)-C(5β)-O(5β)	108.1(3)	105(1)
C(5α)-C(5)-O(5)	121.1(4)	123(2)
C(5α)-C(5)-N(6)	115.8(4)	115(2)
O(5)-C(5)-N(6)	123.1(4)	122(1)
C(5)-N(6)-C(6α)	122.4(4)	121(1)
N(6)-C(6α)-C(6)	116.9(4)	117(1)
C(6α)-C(6)-O(6)	119.2(5)	114(1)
C(6α)-C(6)-N(1)	117.5(4)	119(1)
O(6)-C(6)-N(1)	123.3(4)	127(2)

[a]Disordered Atoms