(70 eV) m/z (relative intensity) 356 [M^+, (17)], 341 (13), 299 (20), 267 (13), 239 (25), 213 (17), 185 (32), 182 (82), 157 (16), 153 (33), 141 (16), 129 (25), 119 (31), 115 (31), 105 (17), 97 (26), 91 (35), 83 (54), 81 (16), 77 (25), 69 (33), 65 (14), 57 (100).

Tridachiapyrone-F (10): 1.2 mg, white powder; UV (MeOH) λ_{max} 255, 225 nm (ϵ 5800, 7400); IR ($CHCl_3$) 2960, 2850, 1725, 1690, 1665, 1595,1450, 1390, 1370, 1325, 1220, 1050, 990, 920 cm^{-1}; ^1H NMR data, Table III; low-resolution mass spectrum (70 eV) m/z (relative intensity) 372 [M^+, (35)], 357 (8), 315 (14), 297 (12), 283 (13), 269 (15), 255 (23), 241 (22), 227 (29), 220 (12), 201 (36), 189 (28), 182 (32), 173 (31), 171 (19), 161 (24), 159 (23) 155 (20), 153 (16), 134 (56), 133 (36), 128 (31), 115 (45), 105 (44), 91 (100), 77 (41), 57 (78).

Tridachiapyrone-B (11): 1.5 mg, colorless oil; UV (MeOH) λ_{max} 248 nm (ϵ 11 900); IR ($CHCl_3$) 3020, 2995, 2925, 2880, 1710, 1660, 1650, 1635, 1600, 1590, 1450, 1400, 1370, 1310, 1250, 1160, 1025, 975, 800 cm^{-1}; ^1H and ^{13}C NMR data, Tables II and IV; low-resolution mass spectrum (70 eV) m/z (relative intensity) 412 [M^+, (6)], 356 (20), 355 (4), 327 (12), 253 (12), 241 (15), 214 (13), 213 (43), 183 (17), 182 (91), 155 (10), 153 (10), 142 (15), 128 (13), 115 (16), 105 (15), 91 (33), 83 (87), 57 (100).

Isotridachiapyrone-B (12): 1.8 mg, colorless oil; UV, IR, and low-resolution mass spectrum same as for **11**; ^1H and ^{13}C NMR, Tables II and IV.

Acknowledgment. This research was supported by Grant NA80AA-D00089 from the Office of Sea Grant, NOAA, U.S. Department of Commerce. High-resolution mass spectra were provided by Dr. C. Costello at the Massachusetts Institute of Technology mass spectral laboratory supported by a grant (principal investigator Professor K. Biemann) from the Biotechnology Research Branch, Division of Research Resources. We gratefully acknowledge support from the National Science Foundation (Grant CHE-8113507) for assistance in the purchase of a high-field NMR spectrometer. We gratefully acknowledge the use of the facilities of the Discovery Bay Marine Laboratory of the University of the West Indies, Mona, Kingston, Jamaica, W.I., during our collection trip.

Registry No. **3**, 70117-21-4; **4**, 79495-60-6; **5**, 99265-40-4; **6**, 99148-24-0; **7**, 99165-16-9; **8**, 99148-21-7; **9**, 99148-22-8; **10**, 99148-23-9; **11**, 99148-25-1; **12**, 99212-17-6.

Structure of Des(diserylglycyl)ferrirhodin, DDF, a Novel Siderophore from *Aspergillus ochraceous*

Mahbubul A. F. Jalal, Jeffrey L. Galles, and Dick van der Helm*

Department of Chemistry, University of Oklahoma, Norman, Oklahoma 73019

Received April 17, 1985

Des(diserylglycyl)ferrirhodin, DDF, a novel ferric siderophore isolated from *Aspergillus ochraceous*, was identified as N^2-[N^2-[N^5-hydroxy-N^5-(*cis*-5-hydroxy-3-methyl-1-oxo-2-pentenyl)-L-ornithyl]-N^5-hydroxy-N^5-(*cis*-5-hydroxy-3-methyl-1-oxo-2-pentenyl)-L-ornithyl]-N^5-hydroxy-N^5-(*cis*-5-hydroxy-3-methyl-1-oxo-2-pentenyl)-L-ornithine. Evidence for the structure of the siderophore was obtained from ^1H and ^{13}C NMR of its deferri and gallium(III) complex forms, from synthesis of its *N*-acetyl and methyl ester derivatives, and from degradation studies. This is the first fungal siderophore with a linear tripeptide backbone.

Siderophores are compounds produced by microorganisms under an iron deficient condition to chelate and transport extracellular iron. *Aspergillus ochraceous* produces more than a dozen siderophores most of which belong to the ferrichrome family (asperchromes).[1-3] One of these compounds, named des(diserylglylcyl)ferrirhodin (DDF) (previously termed compound I) (1), was isolated and shown[1] to possess siderophore activity in tests carried out with *Arthrobacter flavescens* Jg-9. In recent studies, it is demonstrated that it can transport ^{59}Fe(III) to the producing organism as efficiently as ferrirubin, the major siderophore of the fungus. In this report we describe the structure determination of this siderophore, on the basis of various evidences including ^1H and ^{13}C NMR of its deferri and Ga(III) complex forms.

Structure Determination

Des(diserylglycyl)ferrirhodin, DDF (1), which is ninhydrin positive and cationic at low pH, is isolated from iron-starved cultures of *A. ochraceous* by a series of chromatographic procedures described earlier.[1,2] It crystallizes in thin red fibers or needles from a number of solvent systems including ethanol–ethyl acetate and dimethylformamide–acetronitrile, but the single crystals are not large enough for X-ray diffraction studies. On the basis of microanalysis, DDF and its deferri derivative are found to have the molecular formula $C_{33}H_{53}N_6O_{13}Fe$ and $C_{33}H_{56}N_6O_{13}$, respectively. A comparison of these elemental compositons shows that the Fe(III) to ligand ratio in DDF is 1:1. The visible absorption maximum of an aqueous solution of DDF at neutral pH is at 437 nm, which is typical of a ferric hydroxamate complex. The insensitivity of the absorption maximum to pH changes in the range of 7.0 to 2.0 also indicates that the ratio of Fe(III) to ligand is 1:1 and that DDF is a trihydroxamate compound.[4,5]

Quantitative reductive hydrolysis of 1 mol of DDF (1) with HI[6,7] produces 3 mol of L-ornithine. The absolute configuration of ornithine was confirmed by polarimetry. ^1H NMR data of deferri-DDF (**2**) (Table I) show the signal for three hydroxamic acid protons, which disappears in the spectra of Ga-deferri-DDF (**3**). The hydroxamic acid functions in the fungal siderophores are formed by N^5-

(1) Jalal, M. A. F; Mocharla, R.; Barnes, C. L.; Hossain, M. B.; Powell, D. R.; Eng-Wilmot, D. L.; Grayson, S. L.; Benson, B. A.; van der Helm, D. *J. Bacteriol.* **1984**, *158*, 683.

(2) Jalal, M. A. F.; Mocharla, R.; van der Helm, D. *J. Chromatogr.* **1984**, *301*, 247.

(3) Jalal, M. A. F.; Mocharla, R.; Barnes, C. L.; Hossain, M. B.; Powell, D. R.; Benson, B. A.; van der Helm, D. In "Peptides: Structure and Function"; Hruby, V. J., Rich, H., Eds.; Pierce Chemical Company: 1983; p 503.

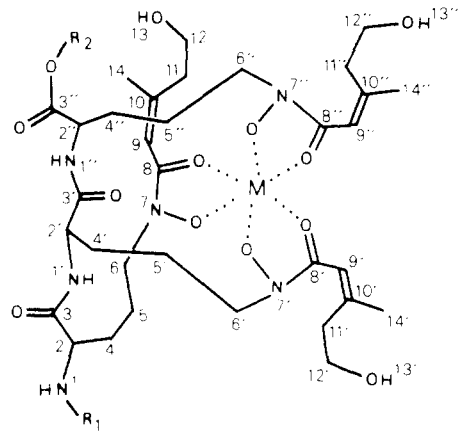
(4) Sayer, J. M.; Emery, T. F. *Biochemistry* **1968**, *7*, 184.

(5) Schwarzenbach, G.; Schwarzenbach, K. *Helv. Chim. Acta* **1963**, *46*, 1390.

(6) Emery, T.; Neilands, J. B. *J. Am. Chem. Soc.* **1961**, *83*, 1626.

(7) Chinard, F. P. *J. Biol. Chem.* **1952**, *199*, 91.

0022-3263/85/1950-5642$01.50/0



1, $R_1 = R_2 = H$; M = Fe(III)
2, $R_1 = R_2 = H$; (M absent, 3 NOH present)
3, $R_1 = R_2 = H$; M = Ga(III)
4, $R_1 = H$; $R_2 = CH_3$; M = Fe(III)
5, $R_1 = H$; $R_2 = CH_3$; M = Ga(III)
6, $R_1 = CH_3CO$; $R_2 = CH_3$; M = Fe(III)
7, $R_1 = CH_3CO$; $R_2 = CH_3$; (M absent, 3 N-OH present)
8, $R_1 = CH_3CO$; $R_2 = H$; M = Fe(III)
9, $R_1 = CH_3CO$; $R_2 = H$; (M absent, 3 NOH present)

hydroxylation and N^5-acylation of the ornithine residues.

NMR data (Tables I and II) show that the three ornithyl N^5-acyl groups are all identical and made of 5-hydroxy-3-methyl-*cis*-2-pentenoyl residues. The cis configuration was verified by the NOE experiment. When the CH_3 (H-14,14′,14″) proton frequency was irradiated, a negative NOE was observed in the CH (H-9,9′,9″) signal of deferri-DDF. No NOE was observed in an equivalent experiment done on deferriferrirubin,[8,9] which contains the same N^5-acyl groups but with the trans configuration.

Mild hydrolysis of DDF in methanolic HCl breaks the hydroxamic bond and releases the ornithyl N^5-acylating acid. Its proton NMR in CD_3OD shows it to be 5,6-dihydro-4-methyl-2*H*-pyran-2-one, which is the lactone formed from *cis*-5-hydroxy-3-methyl-2-pentenoic acid. Further evidence for the cis configuration of the *N*-acyl moieties comes from the GC–MS data of deferri-DDF (**2**). This compound undergoes thermal decomposition in the gas chromatograph producing a major volatile species, whose mass spectrum shows three conspicuous ion peaks. The ion at *m/e* 112 (50) is the molecular ion for the above lactone. An ion at *m/e* 113 (27) is its hydrogenated form. The ring system represented by the molecular ion of the lactone undergoes a facile retro-Diels–Alder fragmentation and produces an ion at *m/e* 82, which is incidentally the base peak of the spectrum. Origin of this lactone and its characteristic mass fragmentation may be expected only from the cis form of the N-acyl moieties in **2**.

The connectivity of the three N^5-hydroxy-N^5-(*cis*-5-hydroxy-3-methyl-1-oxo-2-pentenyl)-L-ornithine units in DDF is established by the NMR data and verified by forming its various derivatives. Three distinct multiplets for the three α-CH protons (H-2,2′,2″) and two well-separated doublets for the two NH protons (H-1′,1″) (Table I) in deferri-DDF indicate that the three ornithyl residues are joined together in a linear tripeptide. Due to the difference in the polarity of the two ends of the tripeptide backbone, the individual α-CH protons (and also the NH protons) experience a different deshielding environment and produce separate signals at different chemical shift values. Further evidence of this arrangement is supplied by the three ornithyl C=O (C-3,3′,3″) signals in deferri-DDF (**2**). The pattern of these signals are similar in all aspects to the one reported by Llinas and co-workers[10] for L-ornithyl-L-ornithyl-L-ornithine, with D_2O as the solvent.

The presence of a free carboxyl group in DDF is confirmed by forming its methyl ester derivative **4** with etherial diazomethane at room temperature. NMR spectra obtained on Ga-deferri-DDF methyl ester **5** in CD_3OD shows the OCH_3 proton signal at δ 3.67 and its carbon signal at δ 52.8. Treatment of **4** in methanolic solution with equimolar quantity of acetic anhydride produces its N-acetyl derivative **6**. Iron is removed from this compound with use of EDTA to obtain its NMR spectrum. In CD_3OD, the spectrum of deferri-DDF *N*-acetyl methyl ester (**7**) shows two methyl peaks at δ 1.98 ($NHCOCH_3$) and δ 3.71 ($COOCH_3$) in addition to the resonances present in deferri-DDF (**2**). The three α-CH proton resonances in **7** are closer together (δ 4.32, 4.38, and 4.42) compared to the same resonances in deferri-DDF (**2**) (δ 3.99, 4.23, and 4.47). Moreover, the *N*-acetyl methyl ester **7** shows three NH proton signals (δ 7.62, 7.86, and 7.95) when the spectrum is obtained in $CDCl_3$.

The free NH_2 group of DDF (in methanolic solution) reacts with an equimolar quantity of acetic anhydride to form *N*-acetyl DDF (**8**). Deferri *N*-acetyl DDF (**9**) shows 3 NH proton signals at δ 7.37 ($J_{\alpha,NH}$ = 6.4 Hz), 8.05 ($J_{\alpha,NH}$ = 7.7 Hz) and 8.36 ($J_{\alpha,NH}$ = 7.3 Hz) and the *N*-acetyl CH_3 signal at δ 1.83 in its NMR spectrum obtained in $(CD_3)_2SO$.

Discussion

DDF contains N^5-acylated hydroxyornithine groups which are identical with those found in fusarinine B,[4] fusigen,[11] and ferrirhodin.[8] Whereas fusarinine B contains three linear hydroxamic acid functions with head to tail connectivities[12] similar to fusigen, which is the cyclized form of fusarinine B, the head to head hydoxamate connectivity pattern of DDF is identical with the one found in ferrirhodin, so that DDF can be described as N^2-[N^2-[N^5-hydroxy-N^5-(*cis*-5-hydroxy-3-methyl-1-oxo-2-pentenyl)-L-ornithyl]-N^5-hydroxy-N^5-(*cis*-5-hydroxy-3-methyl-1-oxo-2-pentenyl)-L-ornithyl]-N^5-hydroxy-N^5-(*cis*-5-hydroxy-3-methyl-1-oxo-2-pentenyl)-L-ornithine.

The absence of a cyclic hexapeptide ring (characteristic feature of ferrirhodin and other ferrichromes) affects the iron-binding property of the hydroxamic groups, as the absorption maximum of DDF shows a 28-nm bathochromic shift when the pH is lowered from 2.0 to 1.7, while most ferrichromes are unchanged by this pH change.

The N^5-acyl group in DDF and its trans isomer are common to a large number of fungal siderophores. In some compounds, the terminal hydroxyl group is further acylated. To establish the distinction between the cis and the trans isomer and also between their free and *O*-acylated forms by ^1H and ^{13}C NMR, the spectra of a number of known siderophores are obtained in $(CD_3)_2SO$, and the data on their N^5-acyl groups are presented in Table III. These data clearly show that the 5-hydroxy-3-methyl-2-pentenoyl residues in DDF are cis and that their terminal hydroxyls are free.

Table I shows that on complexation with gallium(III), the high-field NH resonance moves further upfield to δ 6.77 (J = 6.6 Hz), and the low-field NH signal shifts further downfield to δ 9.92 (J = 1.9 Hz). This large change in the

(8) Keller-Schierlein, W. *Helv. Chim. Acta* **1963**, *46*, 1920.
(9) Barnes, C. L.; Hossain, M. B.; Jalal, M. A. F; Eng-Wilmot, D. L.; Grayson, S. L.; Benson, B. A.; Agarwal, S. K.; Mocharla, R.; van der Helm, D. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1985**, *C41*, 341.
(10) Llinas, M.; Wilson, D. M.; Klein, M. P.; Neilands, J. B. *J. Mol. Biol.* **1976**, *104*, 853.
(11) Diekmann, H.; Zähner, H. *Eur. J. Biochem.* **1967**, *3*, 213.
(12) Maehr, H. *Pure Appl. Chem.* **1971**, *28*, 603.

Table I. ^1H Chemical Shifts of Deferri-DDF (2) and Ga-deferri-DDF (3) in $(CD_3)_2SO$ (300 MHz)

^1H chemical shift, δ				
deferri-DDF (2)	Ga-DDF (3)	inventory of H	structural group	position
1.45–1.80 (m)	1.22–2.15 (m)	12	CH_2CH_2	4, 4′, 4″, 5, 5′, 5″
1.87 (s)	1.89 (s, 3 H), 1.91 (s, 6 H)	9	CH_3	14, 14′, 14″
2.64 (t, J = 6.7 Hz)	2.40–2.70 (m)	6	$=CCH_2$	11, 11′, 11″
3.40–3.62 (m)	3.35–4.00 (m)	6	CH_2N	6, 6′, 6″
3.54 (t, J = 6.7 Hz)	3.47–3.58 (m)	6	CH_2O	12, 12′, 12″
3.73 (m)	3.95 (m, 1 H)	3	CHN	2, 2′, 2″
3.93 (m, $J_{\alpha,NH}$ = 5.6 Hz)	4.15 (m, 2 H)			
4.23 (m, $J_{\alpha,NH}$ = 7.3 Hz)				
4.76 (br s)	5.00 (br s)	6	OH	13, 13′, 13″
			NH_3^+	1
6.27 (s, 2 H)	6.03 (s)	3	CH=	9, 9′, 9″
6.35 (s, 1 H)	6.06 (s), 6.08 (s)			
7.65 (d, J = 5.6 Hz)	6.77 (d, J = 6.6 Hz)	2	NH	1′, 1″
8.67 (d, J = 7.3 Hz)	9.92 (d, J = 1.9 Hz)			
10.00 (br s)	-	3	NOH	7, 7′, 7″

Table II. ^{13}C Chemical Shifts of Deferri-DDF (2) and Ga-deferri-DDF (3) in $(CD_3)_2SO$ (75.4 MHz)

^{13}C chemical shifts, δ				
deferri-DDF, (2)	Ga-DDF (3)	inventory of C	structural group	position
22.37	19.72 (1)	3	CH_2	5, 5′, 5″
22.80	21.16 (2)			
22.86				
25.18 (3)	24.56	3	CH_3	14, 14′, 14″
	25.04			
	25.51			
29.21 (1)	26.36 (1)	3	CH_2	4, 4′, 4″
29.35 (2)	27.96 (2)			
36.34 (3)	36.53 (1)	3	CH_2	11, 11′, 11″
	36.74 (2)			
46.63	48.20	3	CH_2N	6, 6′, 6″
46.89	49.08			
47.42	49.36			
52.50 (1)	51.68	3	CH	2, 2′, 2″
52.96 (2)	52.56			
	56.84			
59.58 (3)	59.32	3	CH_2OH	12, 12′, 12″
	59.61			
	59.85			
117.00	111.81	3	CH=	9, 9′, 9″
117.17	112.82			
117.54	113.37			
150.15	150.25	3	C=	10, 10′, 10″
150.61	150.70			
150.98	152.58			
166.35 (3)	158.72	3	NOHC=O	8, 8′, 8″
	158.95			
	159.22			
169.61	171.14	3	C=O	3, 3′, 3″
170.10	171.94			
173.37	173.85			

NH proton chemical shifts and in their $J_{\alpha,NH}$ coupling constants indicate that a significant difference exists in the peptide backbones of the deferri- and the metal-chelate forms of DDF. A comparison of various features in the ^1H and ^{13}C spectra of deferri-DDF (2) and Ga-deferri-DDF (3) (such as proton resonance multiplicity, chemical shift spread' and degree of nonequivalence between similar carbon atoms on different monomer units) indicates that the metal chelation causes large steric change in the DDF molecule.

The resonance of the middle ornithyl NH proton (H-1′, δ 6.77) of Ga-deferri-DDF shows less thermal shift (+1.75 × 10^{-3} ppm/°C) and a slower deuterium exchange rate compared to the other NH resonance (H-1″, δ 9.92) (−3.0 × 10^{-3} ppm/°C). Moreover, the resolution of its splitting as a doublet becomes sharper with the increase of temperature from 23 to 70 °C. Based on these observations, it is suggested that this NH(1′) proton is involved in an intramolecular hydrogen bond. The known structures of all the ferrichromes based on X-ray diffraction studies[9,13-15] show that the NH proton of the middle ornithyl residue is involved in a strong intramolecular hydrogen bond with the oxygen atom of the N–O group of the same ornithyl residue. A space-filling model of DDF shows the feasibility of an intramolecular hydrogen bond. In that respect, the Fe(III) environment of DDF is likely to resemble ferrichromes more closely than any other group of siderophores.

Experimental Section

Spectral Analysis. Proton NMR spectra were determined at 300 MHz and carbon-13 spectra at 75.4 MHz. Spectra are obtained in $(CD_3)_2SO$, CD_3OD, and occasionally in $CDCl_3$ and referenced to internal Me_4Si. ^1H resonances were assigned and ^1H–^{13}C connectivities were established on the basis of selective homo- and heteronuclear decoupling experiments with the double irradiation method, deuterium exchange studies, and chemical shift correlation with known siderophores based on L-ornithine. APT pulse sequence experiments were carried out with a τ delay of 6 or 8 ms to obtain CH_2, C, and C=O carbon signals up and CH_3 and CH signals down.

Production and Isolation. Growth of *A. ochraceous* and isolation of DDF has been described previously.[1,2]

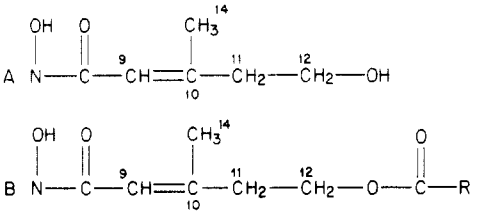
DDF (1). Red crystalline fibers were obtained from ethanol solution equilibrated with ethyl acetate. λ_{max} H_2O pH 2.0–7.0 437 nm (ε 3.403), pH 1.7 465 nm. Paper electrophoresis, 5.4 cm/h toward cathode at pH 2.0 at 1000 V field strength, neutral at pH 5.0. Solubility: water, alcohols, dimethyl formamide, dimethyl sulfoxide. R_f on silica gel layers with chloroform–methanol–water, 35:12:2 (CMW):0.10. Anal. Calcd for $C_{33}H_{53}N_6O_{13}Fe$: C, 49.69; H, 6.70; N, 10.54; O, 26.08; Fe, 7.00. Found: C, 49.47; H, 6.62; N, 10.43; O, 26.19; Fe, 6.94.

Deferri-DDF (2). DDF (50 mg) was dissolved in 5 mL of water, and 0.5 g of recrystallized 8-hydroxyquinoline was added to it. The mixture was incubated overnight at 40 °C, and then unreacted 8-hydroxyquinoline and its ferric complex were removed by chloroform extraction. If red color persisted in the siderophore solution, it was incubated with a second lot of 8-hydroxyquinoline until the solution became colorless. 8-hydroxyquinoline and its ferric complex were removed as before, and the aqueous solution was lyophilized to a white powder: ^1H and ^{13}C NMR Table I and II; R_f (CMW): 0.18, ninhydrin positive. Anal. Calcd for $C_{33}H_{56}N_6O_{13}$: C, 53.22; H, 7.53; N, 11.29. Found: C, 53.53; H, 7.20; N, 11.14.

Ga-deferri-DDF (3). To a solution of **2** (25 mg in 0.5 mL water) was added 0.5 mL of 5% Ga(III) nitrate solution (gold label, Aldrich). The solution was mixed and passed through a column

(13) van der Helm, D.; Baker, J. R.; Eng-Wilmot, D. L.; Hossain, M. B.; Loghry, R. A. *J. Am. Chem. Soc.* **1980**, *102*, 4224.
(14) Barnes, C. L.; Eng-Wilmot, D. L.; van der Helm, D. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* 1984, *C40*, 922.
(15) Norrestam, R.; Stensland, B.; Branden, C. I. *J. Mol. Biol.* **1975**, *99*, 501.

Table III. ^1H (300 MHz) and ^{13}C (75.4 MHz) Resonances of 5-Hydroxy-3-methylpent-2-enoyl Residues in Various Fungal Siderophores, Solvent $(CD_3)_2SO$



compd, deferri-form of	type of acyl group (no. of moieties)	position 9		position 10	position 11		position 12		position 14	
		^1H	^{13}C	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
ferrirubin[8,9]	A trans (3)	6.22	116.2	151.2	2.25	43.8	3.53	59.1	2.03	18.2
coprogen[17]	A trans (2)	6.22	116.2	151.0	2.25	43.8	3.53	59.1	2.03	18.2
	B[a] trans (1)	6.22	117.5	148.8	2.39	40.0[c]	4.18	62.3	2.03	18.0
asperchrome C[3]	A trans (2)	6.22	116.2	151.2	2.25	43.8	3.53	59.1	2.03	18.2
	B[b] trans (1)	6.22	117.0	149.6	2.40	40.0[c]	4.18	62.3	2.03	18.7
triacetylfusigen[11]	B[a] cis (3)	6.22	118.4	149.6	2.80	32.0	4.18	62.5	1.87	25.2
ferrirhodin[8]	A cis (3)	6.27	117.0	151.2	2.64	36.3	3.54	59.6	1.87	25.2
DDF	A cis (3)	6.27 (2)	117.0	150.2	2.64	36.3	3.54	59.6	1.87	25.2
		6.35 (1)	117.2	150.6						
			117.5	151.0						

[a] R = L-ornithyl residue. [b] R = CH_3. [c] Under solvent peak.

(2 × 30 cm) of Sephadex G15. The fractions containing Ga-deferri-DDF were revealed by TLC on silica gel with CMW (R_f 0.10) followed by spraying with 2% $FeCl_3$ in 0.1 N HCl. The pooled fractions were concentrated, purified twice by gel-filtration and finally lyophilized to a white powder: ^1H and ^{13}C NMR; Tables I and II.

DDF Methyl Ester (4) and Ga-deferri-DDF Methyl Ester (5). Diazomethane was prepared from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide according to the standard method.[16] DDF (1) (25 mg) or Ga-deferri-DDF (3) was dissolved in 25 mL of methanol to which was added a freshly prepared etherial solution of excess diazomethane. The reaction mixture was left at room temperature for 1 h and then evaporated under reduced pressure. 4 and 5 were further purified by having been passed through a silica gel column (1.5 × 25 cm) (pretreated with 8-hydroxyquinoline to remove traces of iron in the case of 5 with CMW as the eluting solvent). On silica gel layers, both 4 and 5 have the same R_f value (0.34). Ga-deferri-DDF methyl ester (5), white powder: ^1H NMR (CD_3OD) δ 1.20–2.30 (m, 4, 4′, 4″, 5, 5′, 5″), 1.96 (s, 3 H), 1.98 (s, 6 H), (14, 14′, 14″), 2.56–2.90 (m, 11, 11′, 11″), 3.50–3.85 (m, 6, 6′, 6″, 12, 12′, 12″), 3.67 (s, OCH_3), 3.87–4.73 (m, 2, 2′, 2″), 6.04, 6.06, 6.14 (s, 9, 9′, 9″); ^{13}C NMR (CD_3OD), δ 21.7 (1C) and 23.0, (2C) (5, 5′, 5″), 25.4, 25.6, 26.0 (14, 14′, 14″), 28.6 (2C) and 29.8 (1C) (4, 4′, 4″), 38.0, 38.3, and 38.4 (11, 11′, 11″), 52.8 (OCH_3), 54.8, 55.3, 59.2 (2, 2′, 2″), 61.6, 61.8, 62.0 (12, 12′, 12″), 113.6, 114.4, 114.9 (9, 9′, 9″), 153.2, 153.4, 154.8 (10, 10′, 10″), 161.5, 161.6, 161.8 (8, 8′, 8″), 173.7 (2C), 176.4 (1C) (3, 3′, 3″).

***N*-Acetyl DDF (8) and Deferri *N*-Acetyl DDF (9).** To a solution of DDF (16 mg in 10 mL methanol) was added 2 μL of acetic anhydride. The reaction mixture was left at room temperature for 1 h, and then the solvents were removed under vacuum. *N*-Acetyl DDF (8) formed was further purified by chromatography on a silica gel column (1.5 × 25 cm) by using CMW (R_f on silica gel layers, 0.23) as the eluting solvent. Fe(III) was removed from 8 by the 8-hydroxyquinoline method described earlier. Deferri *N*-acetyl DDF (9), white powder: ^1H NMR ($(CD_3)_2SO$ δ 1.25–1.8 (m, 4, 4′, 4″, 5, 5′, 5″), 1.83 (s, $NCOCH_3$), 1.85 (s, 14, 14′, 14″), 2.62 (m, 11, 11′, 11″), 3.40–3.62 (m, 6, 6′, 6″), 3.52 (t, 12, 12′, 12″, J = 6.77 Hz), 3.82, 4.09, 4.25 (3m, 2, 2′, 2″), 4.80 (s, 13, 13′, 13″), 6.24 (2 H), 6.40 (1 H) (s, 9, 9′, 9″), 7.37 (d, 1 NH, $J_{\alpha,NH}$ = 6.4 Hz), 8.05 (d, 1 NH, $J_{\alpha,NH}$ = 7.7 Hz), 8.36 (d, 1 NH, $J_{\alpha,NH}$ = 7.3 Hz), (1, 1′, 1″), 9.86 (2 H), 10.05 (1 H), (s, 7, 7′, 7″).

DDF *N*-Acetyl Methyl Ester (6) and Deferri-DDF *N*-Acetyl Methyl Ester (7). To a solution of (4) (20 mg in 10 mL of methanol) was added 2.5 μL of acetic anhydride, and the reaction mixture was left at room temperature for 1 h. The solvent was removed under vacuum, and 6 was further purified by chromatography on a silica gel column (1.5 × 25 cm) in CMW (R_f on silica gel layer, 0.82). 6 could not be deferriated with the 8-hydroxyquinoline method, because the deferri compound (7) is soluble in chloroform which is used to extract 8-hydroxyquinoline and its ferric complex. An alternate procedure using Na–EDTA was utilized for this purpose. 6 was dissolved in water containing excess Na–EDTA and left at room temperature. When the solution was completely decolorized, 7 was extracted into phenol–chloroform 1:1. Six volumes of diethyl ether were added to the phenol–chloroform extract, and 7 was extracted back into water followed by washing with sufficient ether. The aqueous solution was then lyophilized yielding a white powder. ^1H NMR (CD_3OD) (with a drop of C_6H_6) δ 1.60–1.90 (m, 4, 4′, 4″, 5, 5′, 5″), 1.92 (s, 14, 14′, 14″), 1.98 (s, *N*-acetyl CH_3), 2.72 (m, 11, 11′, 11″), 3.60–3.75 (m, 6, 6′, 6″), 3.69 (s, OCH_3), 3.71 (m, 12, 12′, 12″), 4.34–4.43 (m, 2, 2′, 2″), 6.37 (s, 9, 9′, 9″); ^{13}C (CD_3OD) δ 22.5 (*N*-acetyl CH_3), 24.3, 24.4 (5, 5′, 5″), 25.1 (14, 14′, 14″), 29.5, 30.1 (4, 4′, 4″), 37.6 (11, 11′, 11″), 52.8 (OCH_3), 53.6, 54.3, 54.7 (2, 2′, 2″), 61.4 (12, 12′, 12″), 119.0 (9, 9′, 9″), 152.3 (10, 10′, 10″), 169.5 (8, 8′, 8″), 173.6, 173.8, 174.0, 174.3 (3, 3′, 3″ and N-acetyl C=O).

Nonreductive Hydrolysis of DDF. To a solution of DDF in methanol (20 mg/5 mL) was passed dry HCl gas, just enough to decolorize the solution. The solution was then evaporated to dryness, the residue was dissolved in 10 mL of water, and the lactone was extracted in ether. The ether extract was dried over $MgSO_4$ and evaporated to yield 5,6-dihydro-4-methyl-2*H*-pyran-2-one. ^1H NMR (CD_3OD) δ 2.03 (3 H, CH_3, 4) 2.45 (2 H, CH_2, 5) 4.40, (2 H, CH_2, 6), 5.78 (1 H, CH, 3).

Reductive Hydrolysis of DDF. L-Ornithine produced[6] was quantitatively measured by the spectroscopic method of Chinard[7] (2.81, 2.96, 2.89 mol/mol of 1), and its absolute configuration was determined by polarimetry.

Acknowledgment. This work was supported by a Grant from the National Institute of General Medical Sciences (GM-21822). We thank Drs. D. Lacefield and P. Roberts of Civil Aeromedical Institute, FAA, Oklahoma, for providing us the facilities for polarimetry.

Registry No. 1, 99376-94-0; 2, 99376-95-1; 3, 99376-96-2; 4, 99376-97-3; 5, 99376-98-4; 6, 99376-99-5; 7, 99377-00-1; 8, 99397-61-2; 9, 99377-01-2.

(16) Darbre, A. In "Handbook of Derivatives for Chromatography"; Blau, K., King, G. S., Eds.; Heyden and Sons Inc.: Philadelphia 1978; p 92.

(17) Keller-Schierlein, W.; Diekmann, H. *Helv. Chim. Acta* **1970**, *53*, 2035.