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N-Hydroxy Amides. IV.[1] Synthesis and Properties of a Trihydroxamic Acid Anilide as a Model for Ferrioxamines

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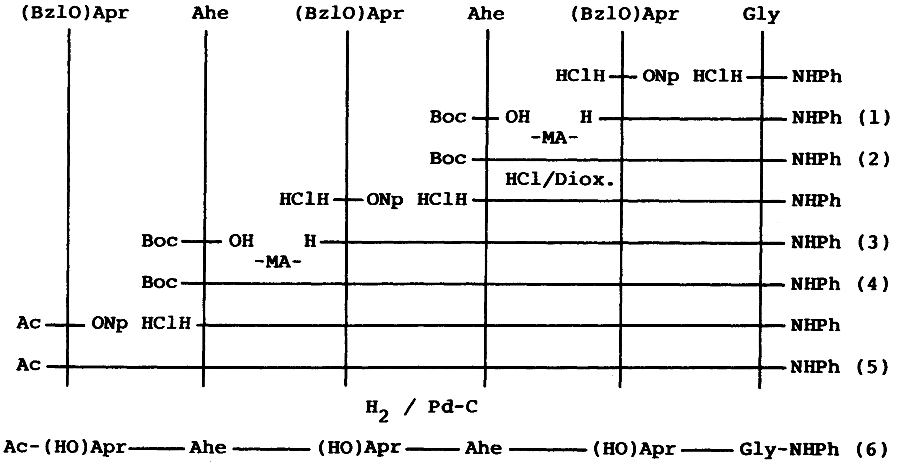
A trihydroxamic acid having a 6-aminohexanoyl-3-(hydroxyamino)propanoyl sequence has been synthesized in a stepwise manner, starting with glycinanilide. The trihydroxamic acid anilide bears a good structural resemblance with *N*-benzoyl-deferriferrioxamine B (Benzoyl-DFB). A plot of the absorbance vs. the molar ratio of iron(III) to the hydroxamate group indicates the formation of a 1 : 1 intramolecular iron(III) complex for the trihydroxamic acid anilide and Benzoyl-DFB. Cyclic voltammetry also shows that the iron complexes of both compounds have similar electrochemical properties. A solid iron(III) complex was obtained when the trihydroxamic acid anilide was mixed with tris(2,4-pentanedionato)iron(III) in methanol.

There is a growing interest in natural and synthetic iron sequestering agents.[2–6] Ferrioxamines, an important class of microbial iron-transporting compounds (siderophores), are stable octahedral iron(III) complexes of linear and cyclic trihydroxamic acids (deferriferrioxamines). They show unique biological activities, such as antibiotics and growth factors.[2,4,7–9] A characteristic structural feature for these trihydroxamic acids is a repeating unit comprising 5-hydroxyamino-1-pentanamine and succinic acid.[2,8] Deferriferrioxamine B (DFB) is used as a drug for the treatment of an iron-overload disease in humans.[4,5,8,10] In this respect, it is of interest for designing and synthesizing trihydroxamic acids of a similar nature but of different molecular compositions as a model and for comparing the important properties, such as iron(III) complex formation and the electrochemical behavior between these synthetic and natural compounds.

A previous paper reported on a synthesis of an *N*-hydroxy hexapeptide anilide containing three hydroxamic acid units in the chain.[11] The hexapeptide anilide showed a rather weak iron(III) bond, owing to the close spacing between hydroxamate groups. In an attempt to provide compounds that firmly hold iron(III), the synthesis of a linear oligoamide having a 6-aminohexanoyl-3-(hydroxylamino)propanoyl sequence has been made. This paper describes the results together with an examination of iron(III) binding properties.

Results and Discussion

Synthesis. A trihydroxamic acid carrying an anilide terminal was synthesized by the reactions shown in Scheme 1. The anilide derivative was chosen in order to obtain a series of compounds as crystals. Thus, glycinanilide was coupled with *p*-nitrophenyl 3-(benzyloxyamino)propanoate H–(BzlO)Apr–ONp[12] to give *N*-[3-(benzyloxyamino)propanoyl]glycinanilide (**1**). The terminal *N*-benzyloxyamino group of **1** was acylated with 6-(Boc-amino)hexanoic acid (Boc–Ahe–OH) by a mixed anhydride procedure to give compound (**2**). The low nucleophilicity of the benzyloxyamino group allowed us to use a *p*-nitrophenyl ester without *N*-protection;[11] yet, the same benzyloxyamino group had a sufficient activity to attack a mixed anhydride. A repeated-acylation technique, needed to couple *N*-benzyloxy α-amino acid derivatives,[11] was unnecessary for the benzyloxy-β-alanyl unit. The ease of acylation is ascribed to less steric constraint around the nitrogen atom, relative to



Scheme 1.

$$CH_3\text{-}\underline{CON(OH)}\text{-}(CH_2)_2CONH(CH_2)_5\text{-}\underline{CON(OH)}\text{-}(CH_2)_2CONH(CH_2)_5\text{-}\underline{CON(OH)}\text{-}(CH_2)_2CONHCH_2CONH\text{-}Ph$$

Compound 6

$$CH_3\text{-}\underline{CON(OH)}\text{-}(CH_2)_5NHCO(CH_2)_2\text{-}\underline{CON(OH)}\text{-}(CH_2)_5NHCO(CH_2)_2\text{-}\underline{CON(OH)}\text{-}(CH_2)_5NHCO\text{-}Ph$$

Benzoyl-DFB

that of *N*-benzyloxy *α*-amino acid derivatives.[11]

A reaction cycle involving the acylation of the benzyloxyamino group with a mixed anhydride of Boc-Ahe-OH and a subsequent deprotection of the Boc-group, followed by another acylation with H-(BzlO)Apr-ONp, gave compounds **1**, **2**, **3**, **4**, and **5** in good yields. The cycle was terminated at the hexamer stage by use of Ac-(BzlO)Apr-ONp. Column chromatography at each stage provided a pure product. The desired trihydroxamic acid anilide (**6**) was obtained by catalytic hydrogenation[1,13] with Pd-C, followed by column chromatography. IR absorptions for the carbonyl groups were 1670 (sh) (-CONH-) and 1630 cm^{-1} -CON(OH)-, the latter of which is ascribed to the cis configuration.[14] ^{1}H NMR peaks were observed in the expected regions (Experimental). The compound was soluble in aqueous DMF but insoluble in water and methanol.

As a reference, compound Benzoyl-DFB was prepared according to a method described in the literature.[15] Molecular models reveal that there is a good structural analogy between compound **6** and Benzoyl-DFB; both compounds have a 9-atom spacing between the hydroxamic acid groups[8] and the same 7 atoms exist in the sequence between the phenyl group and the nearest hydroxamic acid moiety. These two compounds show similar solubilities.

Iron(III) Binding. DFB itself has a great affinity for iron(III), forming a 1:1 intramolecular iron(III) complex by the three hydroxamate groups.[8,15] Benzoyl-DFB was reported to form a stable iron(III) complex.[15] The formation constant of a similar complex of Acetyl-DFB[16] is as high as 10^{30}.

The complex-forming tendency of compound **6** was examined in a 50% aqueous DMF solution[17,18] using DFB as a reference compound. Plots of absorbance vs. the molar ratio of iron(III) to the hydroxamic acid groups are shown in Fig. 1. Both compound **6** and Benzoyl-DFB show a sharp inflection at a ratio of 1/3 as DFB does,[17,18] indicating the formation of the expected complex. The complex formation is also supported by its λ_{max} 435 nm and ε 2700, which are close to those of iron(III)-DFB.[16] From these results, the 9-atom spacing seems to be satisfactory for the formation of a stable complex, regardless of the position of the amide groups. In an aqueous DMF solution, a slight increase in the absorbance and a bathochromic shift of λ_{max} were observed in the iron(III) excess region (Fig. 1). Although the reason is still obscure, one explanation is that the spectral changes are due to a weak interaction between free, or free and complexed iron(III) and solvent amide groups.[19]

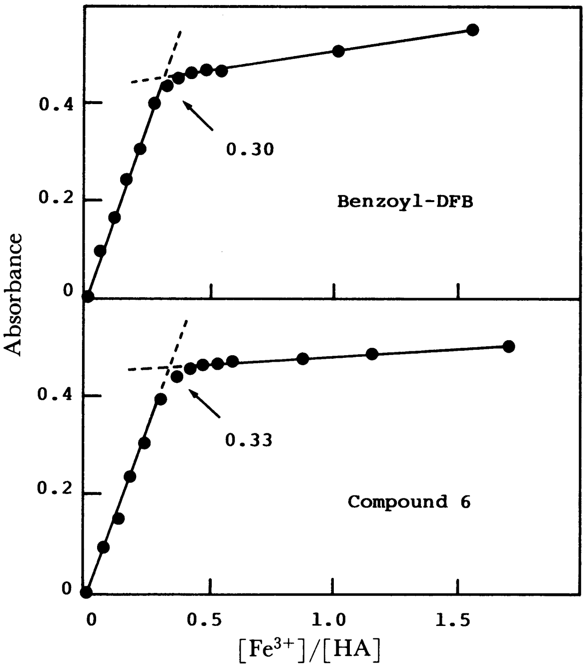


Fig. 1. Absorbance vs. the ratio of iron(III) to the hydroxamic acid (HA) units in 50% aqueous DMF solution at apparent pH=3.0 and 25 °C, ionic strength 0.1 with KNO_3. Absorbance of λ_{max} was determined for Benzoyl-DFB at 430—470 nm and for compound **6** at 423—445 nm.

An examination of CPK molecular models of the iron(III) complexes reveals that there are several molecular arrangements leading to a stable complex.[15] When compound **6** was mixed with tris(2,4-pentanedionato)iron(III) in methanol, the compound dissolved readily and an iron(III) complex was formed as a solid when the solvent was evaporated. The complex, soluble in water, showed a visible spectrum similar to that of ferrioxamine B.[16]

Cyclic Voltammetry. In order to determine the usefulness of compound **6** as a siderophore model, it was worthwhile to determine the electrochemical properties of the iron(III) complex. To this end, the cyclic voltammetry was carried out at a neutral pH in an aqueous solution. The results are summarized in Table 1 and some details are given in Table 2. It has been reported that DFB with iron(III) (ferrioxamine B) exhibits a reversible one-electron oxidation-reduction wave at pH 8 when a mercury electrode is used.[20]

Table 1. Results of Cyclic Voltammetry[a)]

Ligand	$E_{1/2}$ vs. SCE[b)] mV	ΔE mV
DFB	−698[c)]	60[c)]
	−705	65[d)]
Benzoyl-DFB	−710	35
Compound **6**	−703	35

a) Conditions: 0.05 M sodium borate-0.05 M sodium phosphate buffer, pH 8.0; ionic strength 0.5 with 1 M KCl. b) Saturated calomel electrode. c) Ref. 20. d) $i_{pc}/i_{pa}=1.00$.

Table 2. Cyclic Voltammetry Data[a)]

Ligand	Scan rate mVs^{-1}	i_{pc} A	i_{pa} A	i_{pc}/i_{pa}
Benzoyl-DFB	500	42	29	1.44
	200	21	15	1.40
	100	14	10	1.40
	50	8.5	6.0	1.40
	20	5.5	4.0	1.37
Compound **6**	500	51	38	1.34
	200	26	20	1.30
	100	17	13	1.30
	50	11	8.5	1.29
	20	6	5	1.20

a) Details of the data in Table 1.

Under present conditions with a carbon electrode at pH8, the same compound showed a slightly different value of the peak-to-peak separation (ΔE). The data may be interpreted as indicating a similar reversible redox process since $i_{pa}/i_{pc}=1.0$ and $i_{pc}\propto v^{1/2}$.[21)] Half-wave potentials ($E_{1/2}$) obtained for iron(III) with compound **6** and Benzoyl-DFB are very close to that of DFB, as expected from the similar 9-atom spacing; however, the value of the peak-to-peak separation is different from that of DFB. Although a value 35 mV for both compound **6** and Benzoyl-DFB may correspond to a two-electron transfer process, it is improbable that the introduction of a phenyl group in the remote position can change the electron-transfer mode, that is, from a one-electron-transfer to a two-electron transfer. The observed small separation and the deviation of i_{pa}/i_{pc} values from unity for both compound **6** and Benzoyl-DFB are considered rather to be due to a weak adsorption of these ligands to the carbon electrode through phenyl groups; a case of weak adsorption of both reactant and product. A plot of i_{pc} vs. either v or $v^{1/2}$ from the data in Table 2 does not necessarily yield a straight line. A situation is known in which as adsorption occurs the peak separation narrows.[22)]

In short, compound **6** and Benzoyl-DFB behave similarly during the formation of an iron(III) complex. Also, the complexes formed show a quasi-reversible redox wave in an electrochemical determination.

Experimental

All the melting points are uncorrected. IR spectra were obtained with a JASCO model A-302 spectrophotometer and ^{1}H NMR spectra were recorded on a JEOL JNM-FX 200 spectrometer with TMS as internal standard. HPLC was carried out with a JASCO model TWINCLE apparatus by using a column packed with a Finepak SIL C_{18}. A microanalysis was performed in the Analytical Section, Institute of Physical and Chemical Research, Saitama. TLC was carried out by using Merck precoated silica gel 60F$_{254}$ plates: R_f^1, THF-hexane (5:1); R_f^2, $CHCl_3$–MeOH–C_6H_6 (6:1:3); R_f^3, $CHCl_3$–MeOH (3:1).

The iron(III) complex molar ratio was determined with a Hitachi model 320 spectrophotometer in a 50% aqueous DMF solution, as before.[17,18)] 1 M = 1 mol dm^{-3}.

H–(BzlO)Apr–Gly–NHPh (1). A solution containing *p*-nitrophenyl 3-(benzyloxyamino)propanoate hydrochloride[12)] (2.22 g, 6.3 mmol) and Et_3N (0.64 g, 6.3 mmol) in $CHCl_3$ (15 mL) was added to a solution of glycinanilide hydrochloride (1.18 g, 6.3 mmol) and Et_3N (0.64 g, 6.3 mmol) in $CHCl_3$ (25 mL); the mixture was stirred at 30 °C for 48 h. Chloroform (100 mL) was added to this and the $CHCl_3$ layer was washed with aqueous Na_2CO_3 and water, and dried (Na_2SO_4). Evaporation of the solvent gave a crude product (1.91 g, 93%). Purification by column chromatography (Wako gel C-300 with THF-hexane, 3:2 v/v) and reprecipitation from EtOAc–hexane gave a solid, 1.62 g (78%). The hydrochloride of **1** was obtained as crystals by a treatment of the solid with 3.6 M HCl in dioxane, mp 147—148 °C: R_f^1=0.54, R_f^2=0.46, R_f^3=0.90. Found: C, 59.23; H, 6.12; N, 11.55%. Calcd for $C_{18}H_{21}N_3O_3\cdot HCl$: C, 59.42; H, 6.10; N, 11.55%.

Boc–Ahe–(BzlO)Apr–Gly–NHPh (2). A mixture of Boc–Ahe-OH (0.783 g, 3.39 mmol) and Et_3N (0.342 g, 3.39 mmol) in THF (20 mL) was chilled at −12 °C, and Bu^{i}OCOCl (0.442 g, 3.25 mmol) in THF (5 mL) was added and the mixture was kept at −15 °C for 15 min. To this were slowly added HCl·H–(BzlO)Apr–Gly–NHPh (0.986 g, 2.71 mmol) and Et_3N (0.274 g, 2.71 mmol) in $CHCl_3$ (15 mL); the mixture kept for 3 h at −15 °C and for 20 h at room temperature. After evaporation of the solvent, the residue was extracted with EtOAc (100 mL), washed with 5% $NaHCO_3$, 5% citric acid, and water, and dried (Na_2SO_4). Evaporation of the solvent gave a crude product, which was purified by chromatography (Wako gel C-300 with $CHCl_3$–MeOH). Recrystallization from THF–Et_2O– petroleum ether gave 1.03 g (70%) of **2**, mp 105—106 °C: R_f^1=0.73, R_f^2=0.49, R_f^3=0.94. Found: C, 64.39; H, 7.55; N, 10.30%. Calcd for $C_{29}H_{40}N_4O_6$: C, 64.42; H, 7.46; N, 10.36%.

H–(BzlO)Apr–Ahe–(BzlO)Apr–Gly–NHPh (3). HCl·H–Ahe–(BzlO)Apr–Gly–NHPh was obtained by deprotection of **2** with 3.6 M HCl in dioxane.

A mixture of HCl·H–(BzlO)Apr–ONp (0.12 g, 0.34 mmol) and Et_3N (0.034 g, 0.34 mmol) in DMF (5 mL) was added to a solution containing HCl·H–Ahe–(BzlO)Apr–Gly–NHPh (0.161 g, 0.34 mmol) and Et_3N (0.034 g, 0.34 mmol) in DMF (10 mL); the mixture was stirred for 48 h at 30 °C, and evaporated. An extraction of the residue with EtOAc, washing (aqueous Na_2CO_3 and water), drying (Na_2SO_4), solvent evaporation, and column chromatography (Wako gel C-300 with THF–hexane) afforded 0.191 g (92%) of **3** as an oil. The oily product was converted into the hydrochloride of **3** (syrup) with 3.6 M HCl in dioxane: R_f^1=0.40, R_f^2=0.39, R_f^3=0.92.

Boc–Ahe–(BzlO)Apr–Ahe–(BzlO)Apr–Gly–NHPh (4). To a mixed anhydride solution of Boc–Ahe–OH prepared as above, was slowly added a solution of HCl·H–(BzlO)Apr–Ahe–(BzlO)Apr–Gly–NHPh (0.884 g, 1.35 mmol) and Et_3N (1.37 g, 1.35 mmol) in DMF; the mixture was kept at −16°C for 4 h, and at room temperature for 20 h. Concentration of the solution, extraction with EtOAc, washing (5% $NaHCO_3$, 5% citric acid, and H_2O), and drying (Na_2SO_4) gave a crude product (1.05 g, 94%) of **4**, which was purified by chromatography (LH-20 with MeOH) to give **4** as an oil, 0.955 g (85%): R_f^1=0.54, R_f^2=0.42, R_f^3= 0.92.

Ac–(BzlO)Apr–ONp. *O*-Benzylhydroxylamine (1.97 g, 16 mmol) in Et_2O (40 mL) was added to a solution of *p*-nitrophenyl acrylate (2.90 g, 15 mmol) in Et_2O (40 mL); the mixture stirred for 15 h at room temperature and concentrated to give an oil. The oil was acetylated with Ac_2O (10 mL) in THF (20 mL) at 70°C for 1 h and evaporated. The resulting residue was crystallized on addition of Et_2O–petroleum ether, 2.90 g (54%). Recrystallization from Et_2O– petroleum ether gave the product, 1.67 g (36%); mp 73—74°C. Found: C, 60.33; H, 5.06; N, 7.82%. Calcd for $C_{18}H_{19}N_2O_6$: C, 60.16; H, 5.33; N, 7.80%.

Ac–(BzlO)Apr–[Ahe–(BzlO)Apr]$_2$–Gly–NHPh (5). HCl·H-Ahe-(BzlO)Apr-Ahe-(BzlO)Apr-Gly-NHPh (**4′**) was obtained by deprotection of **4** with 3.6 M HCl in dioxane.

Ac–(BzlO)Apr–ONp (0.455 g, 1.27 mmol) was added to a solution of the hydrochloride (0.813 g, 1.06 mmol) and Et_3N (0.107 g, 1.06 mmol) in DMF (20 mL). The solution was stirred for 48 h at 30°C, and the solvent was evaporated to give a residue. Extraction of the residue with EtOAc, washing (aqueous Na_2CO_3 and water), drying (Na_2SO_4), and evaporation of the solvent gave a crude product of **5**. Column chromatography (Wako gel C-300 with THF–hexane) afforded 0.855 g (85%) of **5**, mp 99—101°C; R_f^1= 0.23, R_f^2=0.35, R_f^3=0.94. Found: C, 65.49; H, 7.30; N, 10.00%. Calcd for $C_{52}H_{67}N_7O_{10}$: C, 65.73; H, 7.11; N, 10.32%.

Ac–(HO)Apr–[Ahe–(HO)Apr]$_2$–Gly–NHPh (6). A mixture containing the hexaamide (**5**) (0.60 g, 0.63 mmol) and 10% Pd-C (0.12 g) in MeOH (30 mL) was subjected to hydrogenation with H_2 at 35°C for 40 h. DMF (30 mL) was added; the hot suspension was filtered. Concentration of the filtrate and column chromatography (Toyo pearl HW-40 with DMF) afforded 0.35 g (82%) of **6**, mp 175—176°C (decomp); R_f^2=0.03, R_f^3=0.44; IR, $\nu_{C=O}$ 1670 (sh) and 1630 cm^{-1}; ^1H NMR (DMSO-d_6) δ=1.35 (12H, m, $-CH_2-CH_2-CH_2-$), 2.00 (3H, s, CH_3-), 2.30 (8H, m, $N-CH_2-$ and $-CH_2-CO$ in -Ahe-), 3.00 (6H, m, $-CH_2-CO$ in -Apr-), 3.70 (6H, m, $N-CH_2-$ in -Apr-), 3.85 (2H, d, $N-CH_2-CO$), 7.30 (5H, m, $-C_6H_5$), 7.78 (3H, -NH-CO), 8.20 (1H, -NH-Ph), and 9.6—9.8 (3H, N-OH). Found: C, 54.62; H, 7.42; N, 14.31%. Calcd for $C_{31}H_{49}N_7O_{10}$: C, 54.77; H, 7.27; N, 14.43%.

Iron(III) Complex of 6. Tris(2,4-pentanedionato)iron(III) (13 mg) was added to a methanolic suspension (5 mL) of **6** (26 mg). The suspension became a clear solution. The mixture was left at room temperature for 1 d, and concentrated to give a crude product, 25 mg (64%). Reprecipitation from MeOH to ether and drying under vaccum (60 °C, 1 d) gave a sample; λ_{max} 420 nm, ε=2590 (in water). Found: C, 50.38; H, 6.63; N, 11.25; Fe, 7.37%. Calcd for $C_{31}H_{46}N_7O_{10}\cdot Fe\cdot CH_3OH$: C, 50.26; H, 6.59; N, 12.82; Fe, 7.30%.

Cyclic Voltammetry. To a DMF solution containing a trihydroxamic acid ligand was added 0.90 equivalent of an aqueous $Fe(NO_3)_3$ solution. The solvent was then evaporated. To this was added a phosphate buffer solution (pH 8.2 and ionic strength 0.50 with KCl). This was filtered and adjusted to pH 8.00 with 1 M HCl.

Cyclic voltammetry was carried out for solutions, thus obtained, by generating triangular waves at an ambient temperature. A carbon electrode was used for measurements with a saturated calomel electrode as a reference and a platinum wire as an auxiliary electrode. Current-voltage curves were recorded on an X–Y recorder.

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