Synthesis of Parabactin Analogues and Formation of Transition Metal Complexes of Parabactin and Related Compounds

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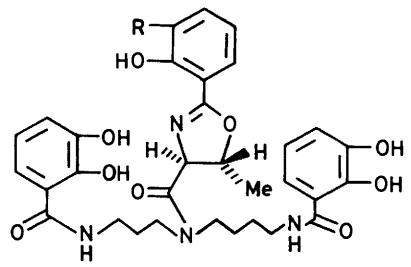
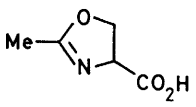
Transition metal complexes of parabactin and its related synthetic spermidine catecholamides which lack the central *o*-hydroxyphenyloxazoline were studied by the potentiometric titration method, e.s.r. spectroscopy, and fast atom bombardment mass spectrometry (FAB-MS). Direct mass spectral sampling of the parabactin–Fe^{III} complex in glycerol solution was first achieved by FAB-MS spectrometry, confirming that the composition of the parabactin–Fe^{III} complex is $[Fe(parabactin)]^{2-}$ $2Na^+$ and that the iron(III) ion binds with two catechols and an *o*-hydroxyphenyl-Δ^2-oxazoline moiety in NaOH solution. The e.s.r. *g* value (4.5) of the parabactin–Fe^{III} complex differed from those (4.3) of the 3:1 catechol–Fe^{III} complex and other parabactin-related catecholamide–Fe^{III} complexes. These results suggest that the structure of the parabactin–Fe^{III} complex differs considerably from the regular octahedral structure of the 3:1 catechol–Fe^{III} complex and may have a structure similar to the ferrimycobactin P–Fe^{III} complex, in which the iron(III) ion lies exposed in a splayed **V**-shaped cleft and binds with five oxygens and one nitrogen in a very strained octahedron.

Naturally occurring[1] and synthetic[2] iron (Fe^{2+} and Fe^{3+})-chelating compounds are of great current interest to organic chemists and biochemists because of their unique structures and biochemical functions, *i.e.*, transport of Fe^{3+} ion into micro-organisms[1a,c–e] and plants[1h,i,3] and cleavage of the double helical DNA.[4] Some of these compounds have also been shown to be potent anticancer drugs or iron-sequestering agents[1c,5] in the treatment of acute or chronic iron poisoning. Recently, several microbial iron-transporting compounds ('siderophore') have been found,[6] characterized by *X*-ray analysis[7] and synthesized.[8] Among these siderophores, parabactin (**1a**), a spermidine-containing catechol-type compound, was first isolated from an iron-depressed culture of *Paracoccus denitrificans* by Tait in 1975.[6a] The structure and absolute configuration of parabactin (**1a**) and agrobactin (**1b**) were confirmed by Neilands[6c] and Helm.[7d] Total synthesis of parabactin was achieved by Bergeron and Kline[9] and by some of the authors,[10] independently.

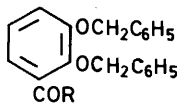
The structure of the parabactin–Fe^{III} complex needs to be further examined because recognition of siderophore iron chelation by the micro-organism's outer membrane receptors may depend on the configuration of the metal complex.[11,12] Its structure was first characterized by Neilands and his co-workers by the titration method and c.d. spectra.[13] Bergeron and Kline studied the structure of the parabactingallium(III) complex in solution by 300 MHz ^1H n.m.r. and concluded that it existed as a mixture of the two diastereoisomeric *cis*-co-ordination isomers (Λ*cis*-3C,4C and Λ*cis*-4C,3C)[14]. However, no detailed study has been reported on the direct characterization of the composition and the structure of the parabactin–Fe^{III} complex. Moreover, the role of the *o*-hydroxyphenyl-Δ^2-oxazoline moiety is still obscure. Therefore, it is necessary to confirm this by a comparative method utilising parabactin analogues lacking the central *o*-hydroxyphenyl-Δ^2-oxazoline moiety.



(1) **a** ; R = H
b ; R = OH



(8)

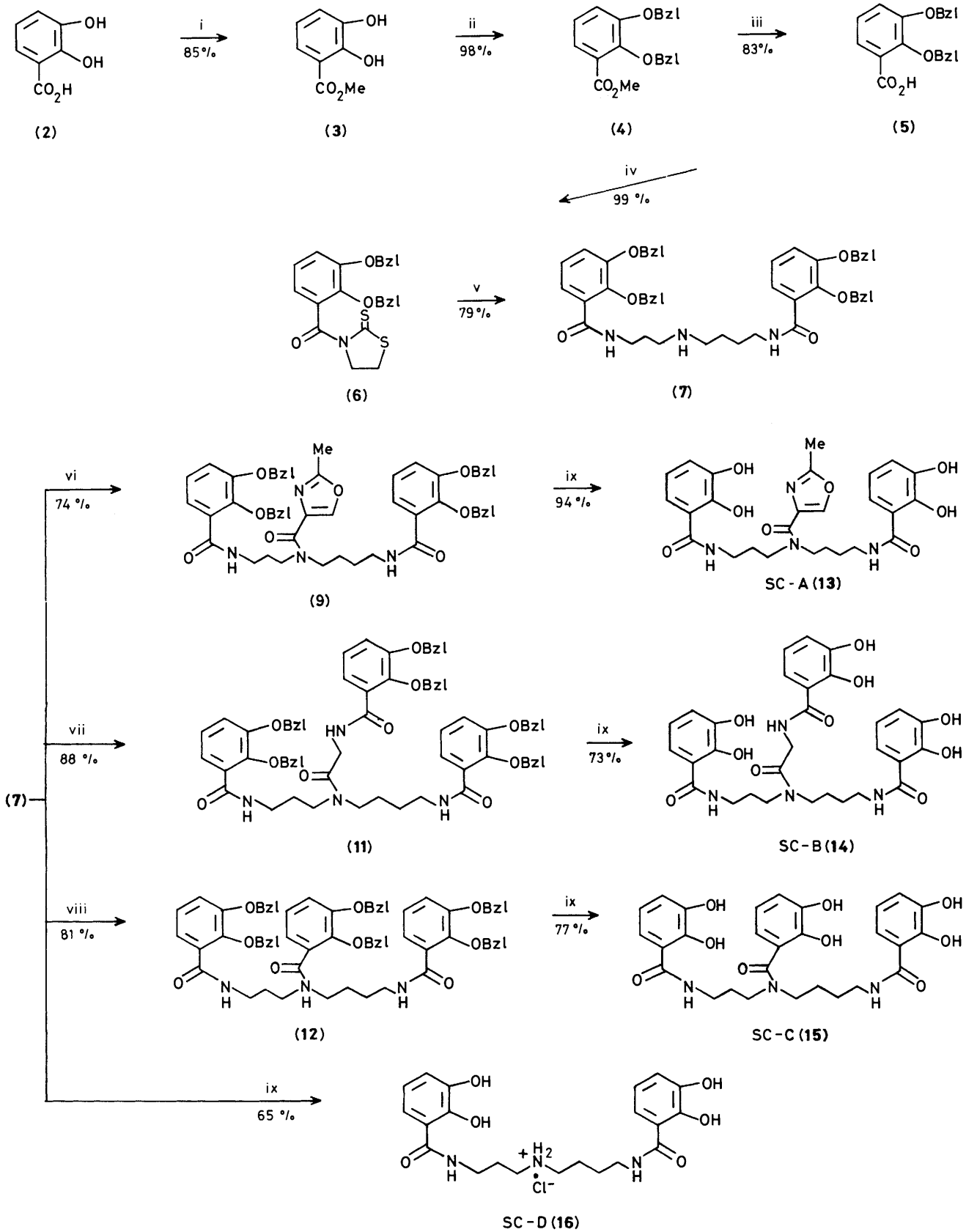


(10) R = $NHCH_2CO_2H$
(17) R = Cl

Thus, we synthesized transition metal (Fe^{3+}, Cu^{2+}, VO^{2+}) complexes with parabactin and investigated them by fast atom bombardment mass spectrometry (FAB-MS) and e.s.r. spectrometry, in order to clarify the structure of the parabactin–Fe^{III} complex and the role of the central *o*-hydroxyphenyl-Δ^2-oxazoline moiety.

Results and Discussion

Synthesis of Parabactin-related Compounds.—Molecular designs of parabactin-related spermidine-catecholamides (SC-A)



Scheme. *Reagents:* i, concentrated H_2SO_4, MeOH, reflux; ii, $PhCH_2Br$, K_2CO_3. DMF; iii, KOH, aq. MeOH; iv, TT, DCC, DMAP, CH_2Cl_2; v, spermidine, CH_2Cl_2; vi, (**8**), DPPA, DMAP, Et_3N; vii, (**10**), DPPA, DMAP, Et_3N; viii, (**17**), Et_3N, THF; ix, 10% Pd–C, 9% HCOOH, MeOH

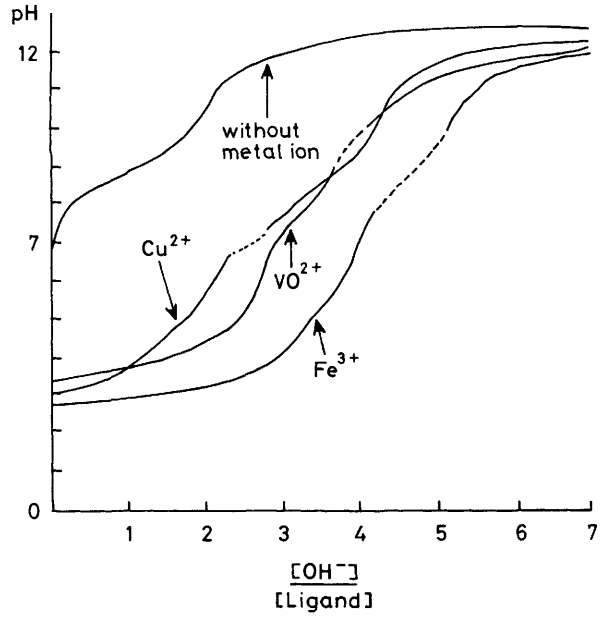


Figure 1. Titration curves of parabactin and parabactin–metal complexes

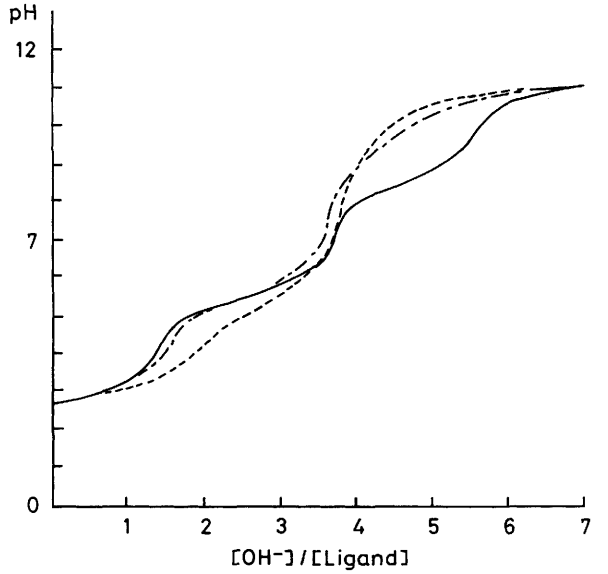


Figure 2. Titration curves of catechol–Fe^{III} complexes. ——; Fe^{III}:ligand 1:3. —•—; Fe^{III}:ligand 1:2. – – – –; Fe^{III}:ligand = 1:1

Table 1. Potentiometric titration for metal complexes of parabactin and its related compounds

Compound		Metal ion[a]	Protons released from the ligand in the metal complex
Parabactin	(**1a**)	Fe^{3+}	4—5
		Cu^{2+}	4
		VO^{2+}	3—4
SC-A	(**13**)	Fe^{3+}	4
SC-B	(**14**)	Fe^{3+}	6
SC-C	(**15**)	Fe^{3+}	5
SC-D	(**16**)	Fe^{3+}	4

[a] Metal:ligand = 1:1.

(**13**)—(SC-D) (**16**) were made as follows. Compound (SC-A) (**13**) possesses an oxazole moiety instead of an *o*-hydroxy-Δ^2-oxazoline moiety in parabactin. In compound (SC-B) (**14**), the distance between the central tertiary nitrogen atom of the spermidine moiety and the catechol group of the 2,3-dihydroxybenzoylglycinyl moiety is the same as that in parabactin. Compound (SC-C) (**15**) has a 2,3-dihydroxybenzoyl moiety which is directly connected to the central tertiary nitrogen atom of the spermidine moiety. Compound (SC-D) (**16**) lacks the central *o*-hydroxyphenyl-Δ^2-oxazoline moiety.

Thus, all spermidine-catecholamidines (**13**)—(**16**) were synthesized by the reactions depicted in the Scheme. The synthetic precursors (**9**)—(**12**) were efficiently prepared by condensation between three corresponding carboxylic acids and a key compound (**7**) which was furnished by monitored aminolysis[15] of 3-(2,3-dibenzyloxybenzoyl)-1,3-thiazolidine-2-thione (**6**) with spermidine.

Transition Metal Complexes of Parabactin and Related Compounds.—The structures of metal complexes of (**1a**) and (**13**)—(**16**) were examined by the potentiometric titration method, FAB-MS, and e.s.r. spectroscopy. The results of the potentiometric titrations are summarized in Figure 1 and Table 1. In the reaction of parabactin (**1a**) with Fe^{3+}, four protons of the phenolic hydroxy groups of the ligand were released at neutral pH (Figure 1), but five protons were presumed to be released finally, from comparison with the results for the reaction of catechols with Fe^{3+} (Figure 2). This observation indicates that iron(III) ion can chelate with two catechols and an *o*-hydroxyphenyl-Δ^2-oxazoline group as proposed by Neilands.[1a] In the reactions with Cu^{2+} and VO^{2+}, three or four protons were found to be released from the ligand of (**1a**) (Figure 1 and Table 1) indicating that these metal ions can chelate with only two catechol groups. In complex formation between spermidine-catecholamides and Fe^{3+}, all catechol protons were released except in the case of (SC-C) (**15**), in which five of the six protons were released (entries 4—7, Table 1). The cavity in (SC-C) (**15**) may be too small to form a chelation structure with Fe^{3+} using all catechol protons.

On addition of 0.25M-iron(III) chloride solution to a solution of parabactin in 0.2M-sodium phosphate buffer (pH 7.4), a purple colour developed showing an absorption maximum at 515 nm. The ε value at 515 nm increased slowly and after *ca.* 3 h reached a maximum (ε 3 375 based on iron concentration) (ε 3 525 at 513 nm in 0.2M-borate–KCl–Na_2CO_3 buffer, pH 9.6). These findings agreed well with Tait's data.[6a]

We next tried to confirm the composition of the parabactin–Fe^{III} complex ion by the FAB-MS method. FAB-MS which utilises a 'soft' ionization method seems to be efficient for the characterization of transition metal complexes in solution.[16] If the iron(III) ion chelates with five phenolate oxygens of two catechols and an *o*-hydroxyphenyl-Δ^2-oxazoline, the parabactin–Fe^{III} complex may be a doubly negatively charged complex like $[Fe(parabactin)]^{2-}$. Therefore, two sodium ions bind with the complex to neutralize the charges. The molecular weight of the parabactin–Fe^{III} complex, [Fe(parabactin)·2Na] = $C_{32}H_{31}N_4O_9FeNa_2$, is thus 717 (Figure 3). Two kinds of parabactin–Fe^{III} complex ions were detected at *m/z* 718 $(M + H)^+$ and 740 $(M + Na)^+$ as shown in Figure 4. The (SC-A)–Fe^{III} complex had mass spectral peaks at *m/z* 581, 603, and 625 which were assigned to $[Fe(SC\text{-}A) + 3H]^+$, $[Fe(SC\text{-}A) + Na + 2H]^+$, and $[Fe(SC\text{-}A) + 2Na + H]^+$, respectively.

In contrast to the parabactin–Fe^{III} complex, the relative intensities of the (SC-A)–Fe^{III} complex were very weak, and the peak due to the protonated (SC-A) (**13**), *m/z* 527, was also observed. No FAB-MS ions of the Fe^{III} complex and other

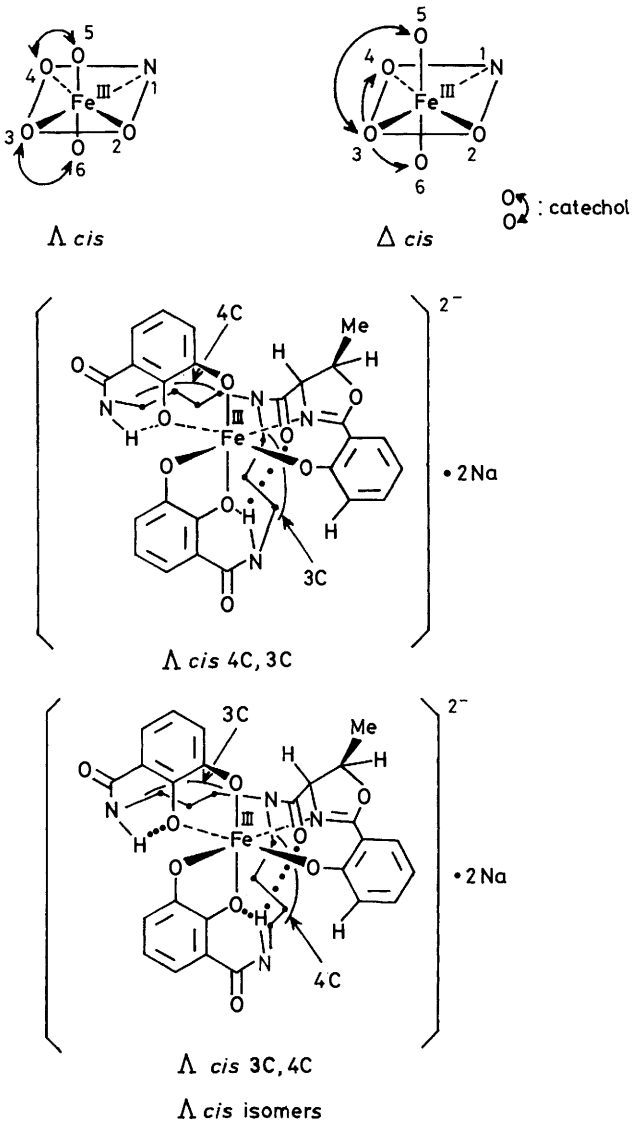


Figure 3. Possible structure of parabactin–Fe^{III} complex in solution

Table 2. E.s.r. parameters for Cu^{II} complex of parabactin and related compounds

Complex	pH	$g(g_\varepsilon)$	$g(g_x, g_y)$	$10^{-4}A/cm^{-1}$
Parabactin–Cu^{II}	6.4	2.248	2.050	193 17
Parabactin–Cu^{II}	11.8	2.249	2.050	189 16
Acetylacetone–Cu^{II}		2.285	2.060	167 17
Bleomycin–Cu^{II}		2.211	2.055	183

transition metal complexes of (SC-B) (**14**)—(SC-D) (**16**), which lack the central *o*-hydroxyphenyl-Δ^2-oxazoline moiety, could be detected. These results show that only the parabactin–Fe^{III} complex has both enough affinity for the low-volatility solvent glycerol and chemical stability in the course of argon bombardment.

Figure 5 shows the e.s.r. spectra for Cu^{2+} and Fe^{3+} complexes of parabactin at pH 6 and 77 K. The e.s.r. pattern and

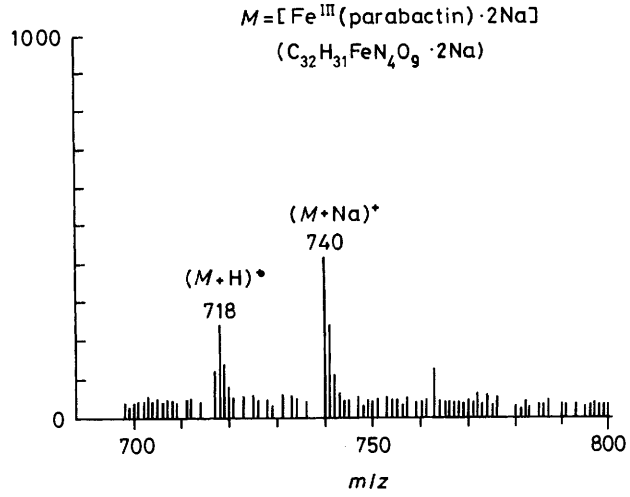


Figure 4. FAB mass spectrum for parabactin–Fe^{III} complex

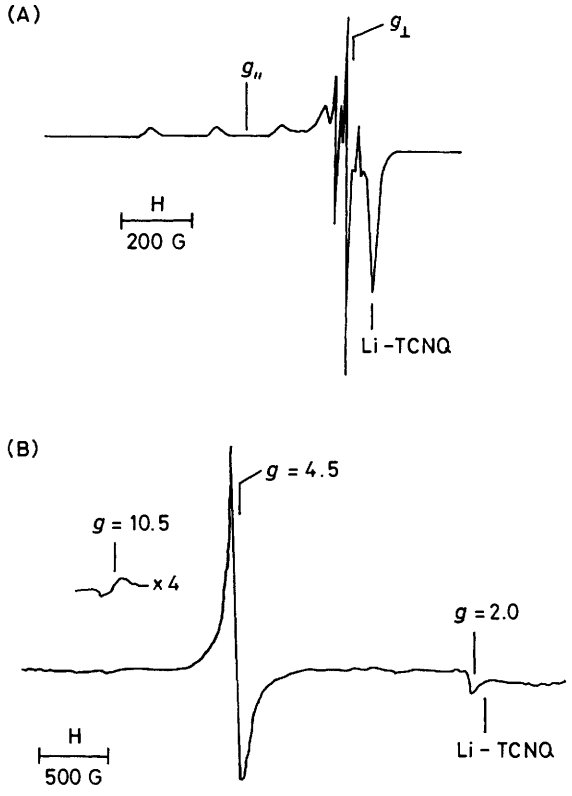


Figure 5. E.s.r. spectra for (A) Cu^{II} and (B) Fe^{III} complex of parabactin in 50% ethanol solution at pH 6 and 77 K

its parameters of the 1:1 parabactin–Cu^{II} complex are very similar to those of the 2:1 acetylactone–Cu^{II} complex (Table 2) giving closely spaced lines due to two copper isotopes of ^{63}Cu (natural abundance 69.1%) and ^{65}Cu (30.9%),[17] where the ligand nitrogen hyperfine structure was not observed. The co-ordination geometry for the Cu^{II} complex is thus suggested to have tetragonal symmetry with the Cu^{2+} ion chelating with two catechols. The results agree with the titration data (Table 1).

The purple 1:1 parabactin–Fe^{III} complex revealed a rather isotropic *g*-anisotropy (*g* 4.5), which has been documented for the electronic transition in the middle of Kramer's doublet defined as the high-spin iron(III) ligand field with a rhombic co-ordination geometry, that is, distorted octahedral geo-

Table 3. E.s.r. parameters for Fe^{III} complexes of parabactin and related compounds

Complex[a]	pH	g values at 77 K		
Parabactin–Fe^{III}	6.7	10.5	4.5	−2
	11.7	10.4	4.5	−2
Catechol–Fe^{III}[b]	2.7—11.7		4.3	2.3, 2.0
(SC-A)–Fe^{III}	7.4	9.6	4.3	−2
(SC-B)–Fe^{III}	7.1	9.6	4.3	−2
(SC-C)–Fe^{III}	7.0	8.4	4.3	−2
(SC-D)–Fe^{III}	7.0	9.6	4.3	−2

[a] Fe^{III} : ligand 1:1. [b] Fe^{III}: ligand 1:3.

metry.[18,19] In fact, the *g* value of the parabactin–Fe^{III} complex (4.5) differs from those (4.3) of the 3:1 catechol–Fe^{III} complex and other spermidine catecholamides–Fe^{III} complexes which lack the *o*-hydroxyphenyl-Δ^2-oxazoline moiety (Table 3). Recently such a rather isotropic *g* anisotropy (*g* 4.4—4.5) and some distorted octahedral geometry were observed in the case of the mugineic acid–Fe^{III} complex.[20] Thus, we realised that the structure of the parabactin–Fe^{III} complex differs considerably from the regular octahedral structure of the 3:1 catechol–Fe^{III} complex and may have a structure similar to ferrimycobactin P[7a] having a central *o*-hydroxyphenyl-Δ^2-oxazoline–Fe^{III} complex in which the iron(III) ion lies exposed in a splayed **V**-shaped cleft and binds with five oxygens and one nitrogen in a very distorted octahedron as confirmed by *X*-ray analysis.

Based on the potentiometric titration data and the present e.s.r. parameters, the parabactin–Fe^{III} complex was confirmed to have a distorted octahedral structure having two strong catecholate–Fe^{III} co-ordination sets as well as relatively weak oxazoline nitrogen–Fe^{III} and *o*-phenolate–Fe^{III} co-ordination sets.

The differences of the FAB-MS and of *g* values in the e.s.r. spectra between the parabactin–Fe^{III} complex and the related spermidine-catecholamide–Fe^{III} complexes show that the *o*-hydroxyphenyl-Δ^2-oxazoline moiety may play an important role in determining the structure and configuration of the parabactin–Fe^{III} complex and in controlling the stability of the complex and the ease of release of the iron atom.

Experimental

General Methods.—M.p.s were determined with a Yanagimoto microapparatus. I.r. spectra were recorded using KBr plates on a JASCO A-202 spectrometer, E.i. (electron impact) and fast atom bombardment mass spectra were recorded on a JEOL JMS-DX300 mass spectrometer. ^1H N.m.r. spectra were recorded on a JEOL JNM-FX100 spectrometer in $CDCl_3$ with $SiMe_4$ as internal standard unless otherwise stated. Extracts were dried over Na_2SO_4. Merck silica gel 60H was used for flash column chromatography. Sephadex LH20 (Pharmacia Fine Chemicals) was used for column chromatography.

Sample Preparations.—Parabactin (**1**) was synthesized as described previously.[10] Spermidine-cathecholamides were prepared as follows.

Methyl 2,3-*dihydroxybenzoate* (**3**). A few drops of concentrated H_2SO_4 were added to a solution of 2,3-dihydroxybenzoic acid (**2**) (6.16 g, 40 mmol) in MeOH (160 ml). After being refluxed for 20 h, the mixture was evaporated under reduced pressure to give an oil, which was dissolved in benzene (200 ml). The mixture was washed with brine, dried, and evaporated under reduced pressure to give an oil, which was purified by flash column chromatography ($CHCl_3$) to afford compound (**3**) (6.6 g, 85%) as prisms, m.p. 79.5—80.5 °C (from $CHCl_3$–hexane); $\nu_{max.}$ 3 460 and 1 665 cm^{-1}; δ 3.93 (3 H, s), 5.73 (1 H, br s), 6.60—7.44 (3 H, m), and 10.84 (1 H, br s) (Found: C, 56.8; H, 4.7%; M^+, 168. $C_8H_8O_4$ requires C, 57.15; H, 4.8%; *M*, 168).

Methyl 2,3-*dibenzyloxybenzoate* (**4**). Anhydrous K_2CO_3 (8.2 g, 60 mmol) and benzyl bromide (7.8 ml, 66 mmol) were added to a solution of methyl 2,3-dihydroxybenzoate (**3**) (5 g, 30 mmol) in DMF (100 ml) under N_2 with stirring. After being stirred at room temperature overnight, the mixture was poured into cold water and extracted with a large amount of EtOAc. The extract was washed with brine, dried, and evaporated under reduced pressure to give an oil, which was purified by flash chromatography on a short silica gel column with $CHCl_3$ to afford compound (**4**) (10.2 g, 98%) as needles, m.p. 63.5—64 °C (from EtOAc–hexane); $\nu_{max.}$ 1 728 cm^{-1}; δ 3.82 (3 H, s), 5.08 and 5.12 (each 2 H, s), and 6.80—7.60 (13 H, m) (Found: C, 75.9; H, 5.65%; M^+, 348. $C_{22}H_{20}O_4$ requires C, 75.85; H, 5.8%; *M*, 348).

2,3-*Dibenzyloxybenzoic acid* (**5**). An excess of 40% KOH in water was added to a solution of methyl 2,3-dibenzyloxybenzoate (**4**) (8 g, 23 mmol) in THF (20 ml). After being refluxed for 2 h, the mixture was acidified by aqueous 10% HCl and extracted with EtOAc. The extract was treated as usual to give compound (**5**) (6.3 g, 83% yield) as needles, m.p. 123—124 °C (from EtOAc–hexane); $\nu_{max.}$ 3 450 and 1 685 cm^{-1}; δ 1.60 (1 H, br s), 5.20 and 5.26 (each 2 H, s), and 7.04—7.84 (13 H, m) (Found: C, 75.4; H, 5.3%; M^+, 334. $C_{21}H_{18}O_4$ requires C, 75.45; H, 5.45%; *M*, 334).

3-(2,3-*Dibenzyloxybenzoyl*)-1,3-*thiazolidine*-2-*thione* (**6**). 1,3-Thiazolidine-2-thione (1.96 g, 16.5 mmol) and 2,3-dibenzyloxybenzoic acid (**5**) (5 g, 15 mmol) were dissolved in $CHCl_3$. To the solution was added dicyclohexylcarbodi-imide (3.4 g, 16.5 mmol) and 4-dimethylaminopyridine (DMAP) (50 mg) with stirring under ice-cooling. The mixture was stirred at room temperature under N_2 overnight, the precipitate was filtered off, and the filtrate was evaporated under reduced pressure to give an oil, which was chromatographed on a silica gel column with $CHCl_3$ to afford amide (**6**) as yellow prisms, m.p. 106—107 °C (from $CHCl_3$–Et_2O); $\nu_{max.}$ 1 670 cm^{-1}; δ 2.80 (2 H, t, *J* 8 Hz), 4.28 (2 H, t, *J* 8 Hz), 5.07 and 5.10 (each 2 H, s), and 6.80—7.80 (13 H, m) (Found: C, 66.2; H, 4.85, N, 3.35%; M^+, 435. $C_{24}H_{21}NO_3S_2$ requires C, 66.2; H, 4.85; N, 3.2%; *M*, 435).

N^1,N^{10}-*Bis*-(2,3-*dibenzyloxybenzoyl*)*spermidine* (**7**). A solution of spermidine (550 mg, 3.9 mmol) in CH_2Cl_2 (20 ml) was added to a yellow solution of 3-(2,3-dibenzyloxybenzoyl)-1,3-thiazolidine-2-thione (**6**) (3 g, 6.9 mmol) in CH_2Cl_2 (80 ml). After being stirred at room temperature under N_2 for 3 h, the mixture was washed with 2% NaOH solution to remove 1,3-thiazolidine-2-thione. The usual work-up gave an oil, which was chromatographed by the flash technique on a silica gel column (10% MeOH in $CHCl_3$) to afford diamide (**7**) (1.91 g, 79%) as a pale yellow oil; $\nu_{max.}$ 3 370 and 1 638 cm^{-1}; δ 1.08—1.84 (7 H, m), 2.20—2.60 (4 H, m), 3.04—3.52 (4 H, m), 5.05 and 5.14 (each 4 H, s), and 6.80—8.20 (28 H, m) (Found: C, 75.4; H, 6.6; N, 5.3%; M^+, 777. $C_{49}H_{51}N_3O_6$ requires C, 75.65; H, 6.6; N, 5.4%; *M*, 777).

N-[3-(2,3-*Dibenzyloxybenzoylamino*)*propyl*]-N-[4-(2,3-*dibenzyloxybenzoylamino*)*butyl*]-2-*methyloxazole*-4-*carboxamide* (**9**). A solution of diphenylphosphoryl azide (605 mg, 2.2 mmol) in DMF (5 ml) was added to a solution of the carboxylic acid (**8**) (254 mg, 2 mmol) and compound (**7**) (1.7 g, 2 mmol) in DMF (10 ml) under ice-cooling with stirring. After addition of Et_3N (0.3 ml) and DMAP (25 mg) under the same conditions, the mixture was stirred at room temperature under N_2 for 3 days. The usual work-up gave an oil. Purification by flash column chromatography (20% acetone in $CHCl_3$) gave compound (**9**) (1.31 g, 74%) as an oil; $\nu_{max.}$ 3 400, 1 645, and 1 535 cm^{-1}; δ 1.08—1.84 (6 H, m), 2.20 and 2.36 (3 H, each s), 5.08 and 5.14 (each 4 H, s), 6.80—7.84 (26 H, m), and 7.96 (3 H, m) [Found: C, 73.15; H,

6.05; N, 6.25%; $(M + H)^+$, 887. $C_{54}H_{54}N_4O_8$ requires C, 73.1; H, 6.15; N, 6.3%; *M*, 886].

N-(2,3-*Dibenzyloxybenzoyl*)*glycine* (**10**). A solution of glycine (825 mg, 11 mmol) and Et_3N (1 52 ml, 11 mmol) in water (50 ml) was added to a solution of 3-(2,3-dibenzyloxybenzoyl)-1,3-thiazolidine-2-thione (**6**) (4.35 mg, 10 mmol) in THF (50 ml). After being stirred at room temperature for 24 h, the mixture was acidified with cold aqueous 10% HCl and extracted with EtOAc. The extract was treated as usual to give an oil, which was chromatographed on Sephadex LH 20 with MeOH to afford compound (**10**) (2.33 g, 60%) as needles, m.p. 119—120 °C (from EtOAc–hexane); $\nu_{max.}$ 3 350, 1 740, and 1 630 cm^{-1}; δ 4.08 (2 H, d), 5.14 (4 H, s), 6.80—7.88 (13 H, m), 8.62 (1 H, br t, *J* 6 Hz), and 8.82 (1 H, br s) (Found: C, 70.4; H, 5.3; N, 3.65%; M^+, 391. $C_{23}H_{21}NO_5$ requires C, 70.55; H, 5.4; N, 3.6%; *M*, 391).

N-[3-(2,3-*Dibenzyloxybenzoylamino*)*propyl*]-N-[4-(2,3-*dibenzyloxybenzoylamino*)*butyl*]-N-2,3-*dibenzyloxybenzoylglycylamide* (**11**). A solution of diphenylphosphoryl azide (605 mg, 2.2 mmol) in DMF (5 ml) was added to a solution of the carboxylic acid (**10**) (782 mg, 2 mmol) and the compound (**7**) (1.7 g, 2.2 mmol) in DMF (10 ml) under ice-cooling with stirring. After addition of Et_3N (0.3 ml) and DMAP (25 mg) under the same conditions, the mixture was stirred at room temperature under N_2 for 2 days and treated as usual to give the amide (**11**) (2.02 g, 88%) as an oil; $\nu_{max.}$ 3 375 and 1 640 cm^{-1}; δ 1.00—1.80 (6 H, m), 2.80—3.40 (8 H, m), 4.06 (2 H, t, *J* 4 Hz), 4.80—5.28 (12 H, m), 6.80—7.88 (39 H, m), 8.06 (2 H, m), and 8.76 (1 H, t, *J* 4 Hz) [Found: C, 75.25; H, 6.05; N, 4.9%; $(M + H)^+$, 1 151. $C_{72}H_{70}N_4O_{10}$ requires C, 75.1; H, 6.15; N, 4.85%; *M*, 1 150].

N-[3-(2,3-*Dibenzyloxybenzoylamino*)*propyl*]-N-[4-(2,3-*dibenzyloxybenzoylamino*)*butyl*]-2,3-*dibenzyloxybenzoylamide* (**12**). Thionyl chloride (0.22 ml, 3 mmol) and a drop of DMF were added to the suspension of the carboxylic acid (**5**) (735 mg, 2.2 mmol) in dry benzene (20 ml). The mixture was refluxed for 4 h and the solvent and the excess of thionyl chloride were evaporated under reduced pressure to give an oil, which was dissolved in THF (20 ml). To the THF solution under ice-cooling was added a solution of the diamide (**7**) (1.55 g, 2 mmol) in THF (30 ml), and Et_3N (0.3 ml). The mixture was stirred at room temperature under N_2 overnight. The solvent was evaporated under reduced pressure to give an oil. Purification of the residue by flash column chromatography (5% MeOH in $CHCl_3$) gave the compound (**12**) (1.77 g, 81%) as an oil; $\nu_{max.}$ 3 400, 1 645, and 1 540 cm^{-1}; δ 0.80—1.80 (6 H, m), 2.60—3.80 (8 H, m), 4.80—5.28 (12 H, m), and 6.60—8.12 (41 H, m) [Found: C, 76.45; H, 6.05; N, 3.75%; $(M + H)^+$, 1 094. $C_{70}H_{67}N_3O_9$ requires C, 76.85; H, 6.15; N, 3.85%; *M*, 1 093].

General Method of Debenzylation of the Diamides (**7**), (**9**), (**11**), *and* (**12**).—The diamide (1—1.4 mmol) was dissolved in 90% HCO_2H–MeOH (1 : 9) (100—160 ml). To the solution, a catalytic amount of 10% Pd–C was added and stirred under N_2. The solid Pd–C was filtered off. The filtrate was evaporated under reduced pressure to give an oil. Crystallization from 5% HCl as the HCl salt or purification of the residue by flash column chromatography gave the spermidine-catecholamides (**13**)—(**16**).

Spermidine-catecholamide (**13**).—Purification by flash column chromatography gave compound (**13**) as a amorphous substance (94%); $\nu_{max.}$ 3 360, 1 635, and 1 545 cm^{-1}; δ(CD_3OD) 1.40—2.12 (6 H, m), 2.24 and 2.42 (3 H, each s), 3.00—4.00 (8 H, m), 6.52—7.36 (6 H, m), and 8.16 (1 H, s) [Found: C, 59.1; H, 5.9; N, 10.3%; $(M + H)^+$, 527. $C_{26}H_{30}N_4O_8$ requires C, 59.3; H, 5.75; N, 10.65%; *M*, 526].

Spermidine-catecholamide (**14**).—Purification by flash column chromatography gave compound (**14**) as a amorphous substance (73%); $\nu_{max.}$ 3 370, 1 635, and 1 545 cm^{-1}; δ(CD_3OD) 1.40—2.20 (6 H, m), 2.80—3.80 (8 H, m), 4.30 (2 H, s), and 6.40—7.60 (9 H, m) [Found: C, 58.8; H, 5.75; N, 9.0%; $(M + H)^+$, 611. $C_{30}H_{34}N_4O_{10}$ requires C, 59.0; H, 5.6; N, 9.2%; *M*, 610].

Spermidine-catecholamide (**15**).—Purification by flash column chromatography gave compound (**15**) as a amorphous substance (77%); $\nu_{max.}$ 3 400, 1 635, and 1 545 cm^{-1}; δ(CD_3OD) 1.20—2.20 (6 H, m), 1.80—2.88 (8 H, m), and 6.40—7.40 (9 H, m) [Found: C, 60.6; H, 5.65; N, 7.4%; $(M + H)^+$, 554. $C_{28}H_{31}N_3O_9$ requires C, 60.75; H, 5.65; N, 7.6%; *M*, 553].

Spermidine-catecholamide (**16**).—The usual work-up gave the compound (**16**) as a pale grey amorphous hydrochloride (65%); $\nu_{max.}$ 3 420, 3 200, 1 640, and 1 540 cm^{-1}; δ(CF_3CO_2H, with CH_2Cl_2 as internal standard) 1.60—2.28 (6 H, m), 3.00—4.12 (8 H, m), and 6.68—7.96 (8 H, m) [Found: C, 55.1; H, 6.2; N, 9.1%; $(M - HCl)^+$, 417. $C_{21}H_{28}ClN_3O_6$ requires C, 55.55; H, 6.2; N, 9.25%; *M*, 454].

FAB-MS Measurements.—The parabactin–Fe^{III} complex was prepared as follows. To a solution of parabactin (1.4 ng) in EtOH (0.2 ml), 5mM-$FeCl_3$ (0.22 ml) was added, and then 0.1M-NaOH (0.22 ml) was slowly added with stirring. After being stirred at room temperature for 30 min, the purple solution (*ca.* 0.1 ml) was directly combined with glycerol on an electrode. Mass spectra were obtained after the usual work-up. Other spermidine-cathecolamide–Fe^{III} complexes were similarly treated.

Electronic Spectra.—A solution of parabactin (0.366 mg) in EtOH (0.25 ml) was added to 0.2M-sodium phosphate buffer (pH 7.4, 2 ml), and then 2.5mM-$FeCl_3$ (2 ml) was added to the mixture. Electronic spectra were measured at 10 min intervals with a Shimadzu UV260 spectrometer under air at room temperature.

Potentiometric Titration.—The potentiometry was carried out as described previously.[21] The 1:1 metal–ligand solution (2.0 mM) in 50% EtOH solvent (μ 0.1, KNO_3) was titrated with carbonate-free 0.1M-KOH at 25 °C. The solution was stirred by a magnetic stirrer and CO_2-free nitrogen was passed slowly into the solution during the titration. The solution of $FeCl_3$, $CuCl_2$, and $VOSO_4$ were standardised complexometrically with EDTA.

E.s.r. Spectra.—E.s.r. spectra were measured by a JEOL FE2XG X-band spectrometer with 100 KHz magnetic field modulation at liquid nitrogen temperature (77 K). The magnetic field was calibrated by the splitting of Me^{II} in MgO (Δ_{3-4} 8.69 mT) and *g* values were standardized using Li-TCNQ (*g* 2.00252) as a reference.

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