BioMetals 1993, **6**, 93–100

Ornibactins—a new family of siderophores from *Pseudomonas*

Holger Stephan, Stefan Freund, Werner Beck, Günther Jung, Jean-Marie Meyer* & Günther Winkelmann†

*Institut für Organische Chemie and †Institut für Biologie, Mikrobiologie/Biotechnologie, Universität Tübingen, Tübingen, Germany and *Institut de Biologie, Institut Le Bel, Strasbourg, France*

Received 23 November 1992; accepted for publication 4 December 1992

Novel linear hydroxamate/hydroxycarboxylate siderophores from strains of *Pseudomonas cepacia* were isolated and named ornibactins. The ornibactins represent modified tetrapeptide siderophores, possessing the sequence L-Orn^1(N^δ-OH, N^δ-acyl)-D-*threo*-Asp(β-OH)-L-Ser-L-Orn^4(N^δ-OH, N^δ-formyl)-1,4-diaminobutane. The N^δ-acyl groups of Orn^1(N^δ-OH, N^δ-acyl) may vary and represent the three acids 3-hydroxybutanoic acid, 3-hydroxyhexanoic acid and 3-hydroxyoctanoic acid, leading to a mixture of three different ornibactins, designated according to their acyl chain length as ornibactin-C4, ornibactin-C6 and ornibactin-C8. Each of the siderophores is accompanied by a small amount of a more hydrophilic component with a 16 a.m.u. higher mass. The structure elucidation was based on results from gas chromatography amino acid analysis, electrospray mass spectrometry, and one- and two-dimensional nuclear magnetic resonance techniques.

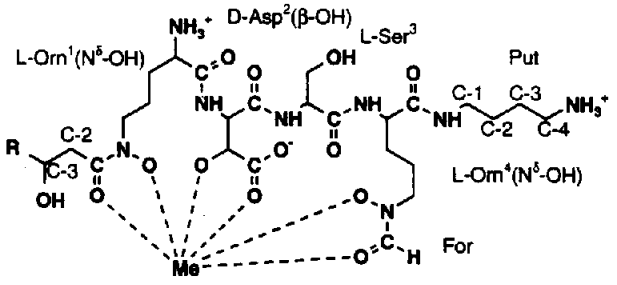
Keywords: iron, ornibactin, *Pseudomonas*, pyoverdines, siderophores

Introduction

Siderophores are iron chelating compounds which are excreted under iron limitation in virtually all microorganisms, and which function in the solubilization, transport and storage of iron. This topic has been reviewed in a recent comprehensive book on microbial iron chelates (Winkelmann 1991). The iron binding ligands involved may represent catecholates, hydroxamates or polycarboxylates. The fluorescent *Pseudomonades* like *P. fluorescens* and *P. aeruginosa* have been shown to produce different siderophores, among which the pseudobactins (Teintze *et al.* 1981, Yang & Leong 1984, Buyer *et al.* 1986), the pyoverdins (Briskot *et al.* 1989, Abdallah 1991) and pyochelin (Cox *et al.* 1981) predominate. Furthermore, ferribactins (Maurer *et al.* 1968) have been shown to represent the biogenetic precursors of pyoverdins (Taraz *et al.* 1991, Linget *et al.* 1992). All pyoverdins and pseudobactins, collectively called pyoverdin-type siderophores, share a common chromophore providing a 1,2-dihydroxy bidentate as one of the three bidental ligands. In addition, either two N^δ-hydroxyornithines or one N^δ-hydroxyornithine plus one β-hydroxyaspartic acid are involved in iron binding. The peptide backbone may be linear, as in pseudobactin (Teintze *et al.* 1981), or cyclic, as in some pyoverdins (Briscot *et al.* 1989). After complexing with Fe(III), red-brown octahedral complexes are formed showing characteristic charge-transfer bands at about 470–550 nm and formation constants of approximately 10^{25} at pH 7. While the quinoline residue is a characteristic feature of the pyoverdin-type siderophores, the ferribactins contain a precursor-like chromophore consisting of a condensation product of phenylalanine and diaminobutyric acid.

We have recently reported on the structural elucidation of siderophores from a non-fluorescent *Pseudomonas* strain, named ornibactins, which resemble the pyoverdins in their peptide structure, but lack a chromophore (Stephan *et al.* 1992). While in our previous paper emphasis was laid on the structure elucidation of the most lipophilic component—F (Figure 1), and its gallium complex, this paper will focus on the microheterogeneity of the ornibactin family.

Address for correspondence: G. Winkelmann, Institut für Biologie, Mikrobiologie/Biotechnologie, Universität Tübingen, Tübingen, Germany.



Ornibactin-C4 R = $-CH_3$

Ornibactin-C6 R = $-CH_2-CH_2-CH_3$

Ornibactin-C8 R = $-CH_2-CH_2-CH_2-CH_2-CH_3$

Figure 1. Structures of ornibactins (Me = Fe or Ga) components including numbering of putrescine (Put) and the acid residues bound to the N^{δ} of N^{δ}-hydroxy-ornithine. According to the chain length of the acid bound to Orn¹(N^{δ}-OH) the ornibactins were named ornibactin-C4, ornibactin-C6 and ornibactin-C8.

Materials and methods

Isolation and purification

Culture supernatants (5 l) of *Pseudomonas* strain TVV69 grown for 40 h in succinate medium containing 5 mM ornithine were supplemented with iron citrate (5 g) and stirred for 1 h. The solution was concentrated to 400 ml under vacuum and saturated with NaCl. The siderophore containing residue was extracted by chloroform:phenol (1:1, 200 ml), and resolubilized in water by adding diethylether (400 ml) and water (50 ml) to the mixture. The aqueous phase was washed three times with ether and evaporated to dryness under vacuum. The residue was extracted with methanol (5 × 10 ml). Further purification was carried out by gel chromatography on Sephadex LH-20 (45 × 2.5 cm) with methanol as eluent. Amber colored fractions were pooled, evaporated to dryness and redissolved in water. Gel chromatography on Biogel P2 (90 × 2.5 cm) with water as eluent yielded an amber colored fraction which was evaporated to dryness under vacuum. A total of 300 mg of siderophore mixture containing components A–F was obtained.

The components were separated by preparative high performance liquid chromatography (HPLC): Nucleosil C-18 (250 × 20 mm, 10 μm) (Grom, Herrenberg, Germany); programmable HPLC pump model 590; injection valve; Lambda-Max model 481 LC spectrophotometer; data module model 740 (all Waters, Eschborn, Germany); model 202 fraction collector (Abimed, Langenfeld, Germany); isocratic elements: component F, acetonitrile: water (17:83, 0.1% trifluoroacetic acid); component D, acetonitrile:water (7:93, 0.1% trifluoroacetic acid); component B, acetonitrile:water (2:98, 0.1% trifluoroacetic acid); flow rate: 25 ml min^{-1}.

HPLC

For analytical HPLC a System Gold equipment (Beckman Instruments, San Ramon, USA) was used; Autosampler 507; Programmable Solvent Module 126; Diode Array Detector Module 168; Nucleosil C-18 columns (5 μm; 250 × 4.6 mm) (Grom); data processing with System Gold software 6.01. As solvent system we used bidistilled water containing 0.12% trifluoroacetic acid (solvent A) and acetonitrile (Merck, Darmstadt, Germany) containing 0.08% trifluoroacetic acid (solvent B). Ornibactins were eluted by a solvent gradient from 6 to 40% B by volume in 20 min at room temperature using a flow rate of 1.3 ml min^{-1}.

Electrospray mass spectrometry

Electrospray mass spectra and tandem mass spectra were recorded on a triple-quadrupole mass spectrometer API III equipped with a pneumatically assisted electrospray (ion spray) ion source (Sciex, Thornhill, Canada). The spectra were acquired in positive mode. Solutions of ornibactins in water (1 mg ml^{-1}) were introduced into the ion source at a flow rate of 5 μm min^{-1} using a medical syringe infusion pump (model 22, Harvard Apparatus, USA).

Tandem mass spectra (MS/MS) were obtained by collision induced dissociation (CID) of the molecular ions with argon as collision gas (collision energy about 50 eV).

For LC-MS measurements, an HPLC system consisting of a solvent delivery system 140 A (Applied Biosystem Weiterstadt, Germany), an injection valve (Latek Eppelheim, Germany) and a Nucleosil C-18 column (100 × 2 mm, 5 μm) (Grom) was connected to the electrospray interface. A gradient of acetonitrile in water (10–80% within 20 min) containing 0.1% trifluoroacetic acid was used at a flow rate of 200 μl min^{-1}.

Amino acid analysis

Ornibactins (100 nmol) were hydrolyzed with 6N HCl (Suprapur, Merck, Darmstadt) or concentrated HI (p.a., Merck, Darmstadt) for 21 h at 110 °C. After removing the acid by a stream of nitrogen at 110 °C the *n*-propyl esters of the amino acids were formed by reaction with 3N HCl in propanol at 110 °C. Excess reagents were removed by a stream of nitrogen at 110 °C. Treatment of the residues with a mixture of trifluoroacetic acid anhydride and trifluoroacetic acid ethyl ester yielded the *N*-trifluoroacetyl-amino acid-*n*-propyl esters. After evaporation to dryness by a stream of nitrogen at room temperature, the residues were redissolved in toluene:acetone (3:1) and analyzed with a Sichromat 1 gas chromatograph (Siemens, München, Germany) equipped with a thermoionic detector, an Autosampler A100, an Autoderivat 100 (CAT, Tübingen, Germany) and a Spectra Physics Integrator. Glass capillary columns coated with Chirasil-Val (Frank *et al.* 1977) were used as the stationary chiral phase.

Preparation of the gallium complexes

A solution of ornibactin (10 μmol) in water (40 ml) was added to a solution of 8-hydroxyquinoline (145 mg, 1 mmol) in methanol (25 ml) and stirred for 16 h at room temperature. The following procedures were carried out in iron-free glassware (Matzanke *et al.* 1986). The reaction mixture was extracted with dichloromethane (5 × 20 ml). To the aqueous phase a solution of gallium sulfate (15 mg, 35 μmol) in diluted sulfuric acid (about 0.1 N) was added and the mixture was allowed to react for 30 min at room temperature. Then the solution was adjusted to neutral pH and lyophilized. The crude product was purified by preparative HPLC under the same conditions as described for the Fe(III), complexes and the purity was checked by analytical HPLC and electrospray mass spectrometry.

Nuclear magnetic resonance (NMR)

High resolution NMR spectra were obtained on a BRUKER AMX 400 spectrometer (Bruker Physics, Karlsruhe, Germany) interfaced to a X32 computer and equipped with an inverse triple resonance probe. Solutions (5 mM) of the gallium complexes of components B, D and F in [D_6]dimethyl sulfoxide were used. Chemical shifts were referenced to the solvent peak ($\delta(^1H) = 2.50$ p.p.m., $\delta(^{13}C) = 39.5$ p.p.m.).

One-dimensional proton spectra were recorded with 128 scans and a data size of 16 Kbyte. After zerofilling to 32 Kbyte the free induction decay was apodized by a e^x window function.

(H,C)-Heteronuclear multiple quantum coherence [(H,C)-HMQC] spectra with a BIRD pulse to supress the signals of protons bound to ^{12}C were acquired with 256 experiments of 32 scans and a data size of 2 Kbyte in the F2 dimension. The sweep width was set to 10 p.p.m. in the F2 dimension and to 100 p.p.m. in F1 dimension. All spectra were recorded at 305 K in the phase sensitive absorption mode with quadrature detection in both dimensions, using the time-proportional phase-incrementation method (TPPI). During acquisition, proton decoupling was achieved with a GARP composite pulse sequence. Data processing consisted of zerofilling up to 2 Kbyte (F2) and 1 Kbyte in the F1 dimension. A squared sine bell weighting function shifted $\pi/3$ was used in both dimensions. A baseline correction in both dimensions as well as a skyline subtraction in the F2 dimension greatly enhanced the signal to noise ratio.

Results

Isolation of the siderophore mixture and purification of components B, D and F

The non-fluorescent *Pseudomonas* strain TVV69 was isolated from a rice rhizosphere by V. Tran Van and T. Heulin (Centre de Pédologie, Nancy, France). It was grown in succinate medium containing 5 mM L-ornithine at 30 °C for 40 h. After addition of iron citrate to the culture supernatant, the ferric ornibactins were isolated by a modification of the chloroform:phenol extraction method (Meyer & Abdallah 1978) followed by gel chromatography on Sephadex LH-20 and Biogel P2 yielded 300 mg of the mixture of components A to F from 5 l of culture supernatant. The reversed-phase HPLC chromatogram (Figure 2) indicates a mixture of three main siderophores (components B, D and F), each accompanied by a small shoulder (components A, C and E). The main components were purified by preparative HPLC, and characterized by analytical HPLC, capillary zone electrophoresis and electrospray mass spectrometry.

Electrospray mass spectrometry

The molecular masses of the ferric complexes of components B, D and F were determined by electrospray mass spectrometry. Using this technique it is possible to minimize the amount of fragmentation during the ionization process and to observe the predominant molecular-ion peak (Bruins *et al.* 1987). The obtained molecular masses were 790 a.m.u. for component F, 762 a.m.u. for component D and 734 a.m.u. for component B. Accordingly, the mass difference between the three ornibactins is 28 a.m.u. in each case.

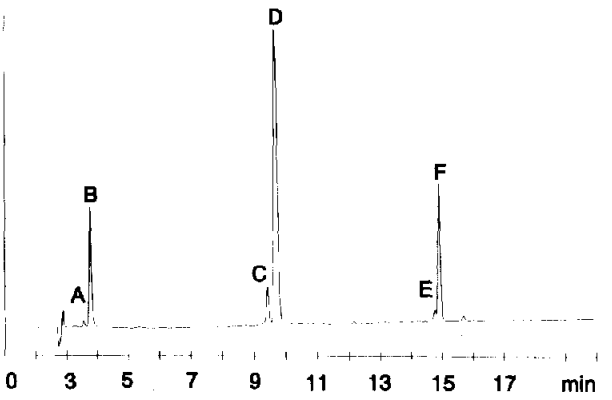


Figure 2. HPLC separation of the ornibactin mixture on a reversed-phase column (Nucleosil C-18, 5 μm, 4.6 × 250 mm) using a gradient (6–40% within 20 min) of acetonitrile (containing 0.08% trifluoroacetic acid) in water (containing 0.12% trifluoroacetic acid) at a flow rate of 1.3 ml min^{-1}. Absorption was monitored at 218 nm. Retention times of the siderophores were: 3.54 min (component A), 3.75 min (component B = ornibactin-C4), 9.41 min (component C), 9.64 min (component D = ornibactin-C6), 14.74 min (component E) and 14.88 min (component F = ornibactin-C8).

The molecular ions can be fragmented by the introduction of a collision gas to obtain additional structural information (Thomson *et al*. 1982). These daughter spectra proved to be virtually identical in all three cases, indicating that components B, D and F possess closely related structures, differing only in one part of the molecule.

Amino acid analysis

Amino acid analysis yielded identical results for all ferric ornibactin components (B, D and F). After hydrolysis with 6N HCl for 22 h at 110 °C, L-serine and L-ornithine were identified in all samples as *N*-trifluoroacetyl-amino acid-*n*-propyl esters by gas chromatography (GC) using an enantioselective Chirasil-Val column (Frank *et al*. 1977). Ornithine is formed by partial reduction of N^{δ}-hydroxy-ornithine present in the molecule which was proved by reductive hydrolysis with concentrated HI. All samples contained two additional peaks which were assigned to D-*threo*-*β*-hydroxy-aspartic acid by co-injection of a standard sample and to 1,4-diaminobutane (putrescine) by GC-MS. Therefore components B, D and F are composed of the same amino acids, including putrescine. Consequently the differences between the components have to be found in the remaining part of the molecule, the acid bound to the N^{δ} of N^{δ}-hydroxy-ornithine in position one. The mass differences of 28 a.m.u. found in the electrospray mass spectra suggest the loss of one ethylene fragment ($-C_2H_4-$) from component F to component D and of another ethylene fragment from component D to component B. This is in agreement with the observation in reversed-phase HPLC that component F is the most lipophilic component of the mixture followed by components D and B.

NMR experiments

To unravel the structure of the acid bound to the N^{δ} of N^{δ}-hydroxy-ornithine in position one NMR spectra of the gallium complexes of component B and D were acquired and compared with the results of the gallium–component F complex (Stephan *et al*. 1992).

The one-dimensional proton spectra of the gallium complex of component B (Figure 3a), component D (Figure 3b) and component F (Figure 3c) appeared to be very similar in the NH- and $C^{\alpha}H$-region of the spectra, indicating again that the peptide moieties of the molecules are identical. Distinct differences were found in the aliphatic region of the spectra.

The protons bound to the carbon atoms C-4 to C-7 in the acid residue of component F (Figure 1) showed resonances between 1.45 and 1.2 p.p.m. Due to the assumed shorter hydrocarbon chains in the acid moieties in components B and D, less signals are expected in this region. This was clearly observable in the spectra even without exact assignment of the peaks.

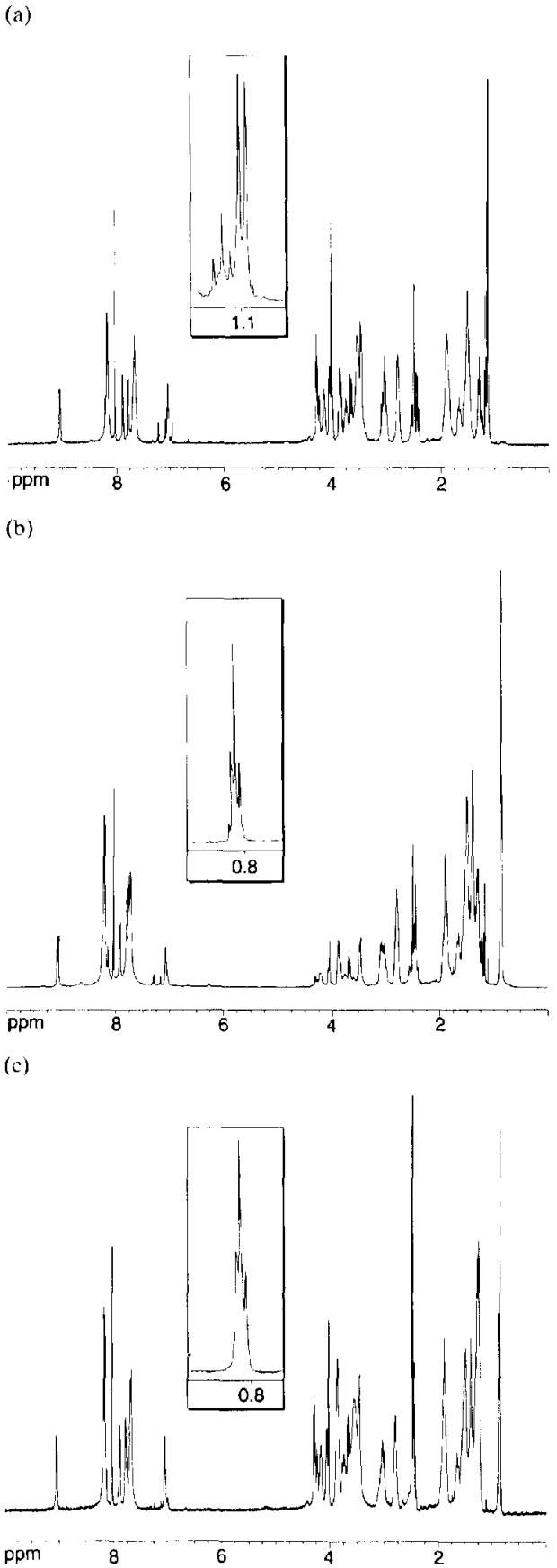
The terminal methyl group (C-8) in the acid residue of component F (Figure 1) corresponds to a proton signal at 0.86 p.p.m. with a triplet fine structure due to $^{3}J_{H,H}$- coupling with the two protons of the adjacent methylene group (Figure 3c, inset). A similar triplet at 0.87 p.p.m. was found for the terminal methyl group (C-6) of the acid moiety of component D (Figure 3b, inset). On the other hand, the terminal methyl group (C-4) in component B (Figure 3a, inset) appeared as a doublet at 1.13 p.p.m. indicating that just one proton is connected to the adjacent carbon (C-3).

(H,C)-HMQC spectra (Müller 1979) were used for the structure elucidation of the acid moiety in component F. Proton signals overlapping in the one-dimensional spectrum were clearly resolved in the carbon dimension making it possible to unravel the exact number of methylene groups in the aliphatic region. Four methylene groups were easy to distinguish, three occurring at an identical proton shift [$\delta(^{1}H) = 1.27$ p.p.m.] corresponding to the methylene groups C-5 to C-7 and one at $\delta(^{1}H) =$ 1.40 p.p.m. (C-4). The terminal methyl group (C-8) [$\delta(^{1}H) = 0.86$ p.p.m.] was found in an isolated region of the spectrum (Figure 4c).

The HMQC of component D (Figure 4b) revealed only three peaks which were assigned to the acid moiety. The signal of the terminal methyl group (C-6) appears at 0.87 p.p.m., one signal at 1.34 p.p.m. corresponding to the methylene protons at C-5 and one signal at 1.39 p.p.m. resembling the methylene protons at C-4. All signals are shifted down-field in the carbon dimension compared with component F, due to the shorter chain length. Component B exhibits only a cross peak for the methyl protons at C-4 [$\delta(^{1}H) = 1.13$ p.p.m.]. The proton and carbon signals of the other residues were found at almost identical positions in the spectra.

Structures of components B and D (ornibactin-C4 and ornibactin-C6)

Summarizing the results of the amino acid analyses, electrospray mass spectrometry and NMR spectro-



scopy, components B, D and F proved to form a family of closely related siderophores being identical in the peptide backbone (L-Orn[1](N^{δ}-OH, N^{δ}-acyl-)-D-*threo*-Asp(β-OH)-L-Ser-L-Orn[4](N^{δ}-OH, N^{δ}-formyl)-1,4-diaminobutane) but with variations in the chain length of the acid bound to the ornithine in position one. As the most lipophilic siderophore of the family, component F utilizes 3-hydroxyoctanoic acid to form one of the hydroxamate groups neccesary for iron chelating, we named this compound ornibactin-C8, while components D and B, containing the shorter acids 3-hydroxyhexanoic acid and 3-hydroxybutanoic acid, respectively, were named ornibactin-C6 and ornibactin-C4 (Figure 1).

Although the peptide sequence was not determined in the case of ornibactin-C4 and -C6, the fragmentation patterns in the tandem electrospray mass spectra and the proton and carbon chemical shift positions strongly suggest an identical sequence as reported for ornibactin-C8 (component F).

Ornibactin A, C and E

Each of the siderophores described so far is accompanied by a small amount of a slightly more hydrophilic component, denoted components A, C and E, respectively (Figure 2). Their isolation is difficult due to their low abundance and their very similar retention times in HPLC.

To obtain information about the nature of these compounds, the ornibactin mixture was subjected to LC-MS experiments. A microbore HPLC system was connected online to the electrospray source of the mass spectrometer serving as detector (Gläßgen *et al.* 1992). Components A/B, C/D and E/F could not be separated during the HPLC run judging from the total ion current (Figure 5). The extracted mass spectra of the first peak corresponding to components A and B showed two signals, one at 734 a.m.u. (the mass of ornibactin-C4) and an additional one at 750 a.m.u. Pairs of signals with identical mass difference of 16 a.m.u. could also be found in the mass spectra of the other two HPLC peaks (components C/D and E/F). If these additional mass signals would occur mainly in the front flanks of the HPLC

Figure 3. ^{1}H-NMR spectra (400 MHz) of the gallium complexes of component B (ornibactin-C4) (**a**), component D (ornibactin-C6) (**b**) and component F (ornibactin-C8) (**c**) in [D_6]DMSO at 305 K referenced to the solvent peak ($\delta(^1H)$ = 2.50 p.p.m.). Extented plots of the signals of the terminal methyl groups of the acid moieties are shown in the insets.

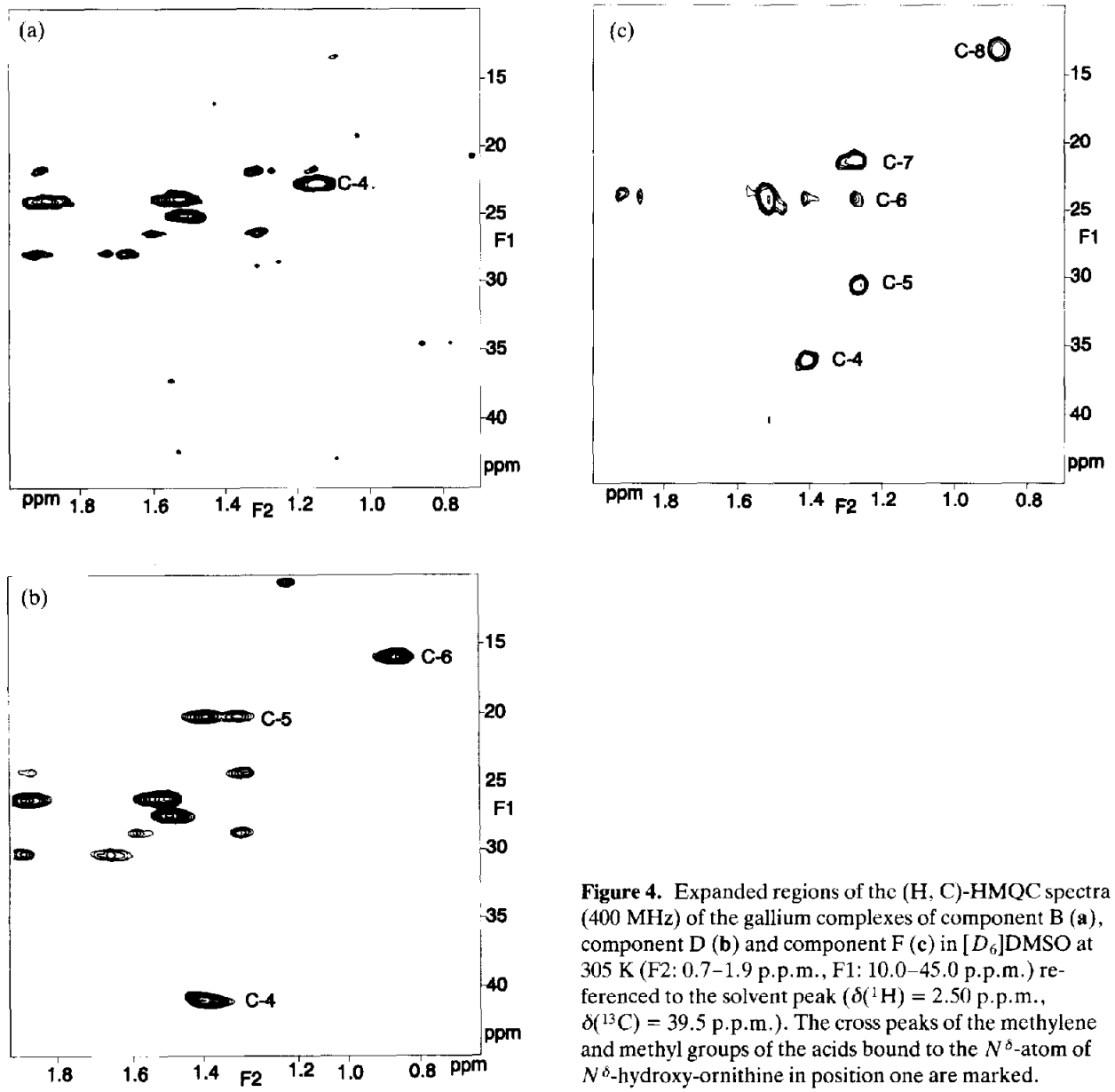


Figure 4. Expanded regions of the (H, C)-HMQC spectra (400 MHz) of the gallium complexes of component B (**a**), component D (**b**) and component F (**c**) in [D_6]DMSO at 305 K (F2: 0.7–1.9 p.p.m., F1: 10.0–45.0 p.p.m.) referenced to the solvent peak ($\delta(^1H) = 2.50$ p.p.m., $\delta(^{13}C) = 39.5$ p.p.m.). The cross peaks of the methylene and methyl groups of the acids bound to the N^{δ}-atom of N^{δ}-hydroxy-ornithine in position one are marked.

peaks they could be assigned to the corresponding low abundance components in the siderophore mixture. Therefore six HPLC chromatograms were extracted out of the data set showing the occurrence of ions with a mass of 750, 734, 778, 762, 806 and 790 a.m.u., respectively (Figure 5). This kind of data processing allows us to resolve HPLC peaks not completely separated during the run. The peak measured at 750 a.m.u. in fact possessed a lower intensity and had a slightly shorter retention time (2.08 min) compared with the one at 734 a.m.u. (2.17 min). Hence the first peak was assigned to correspond to component A. The same properties were found in the other HPLC peaks, proving a molecular weight of 750 a.m.u. for component A, 778 a.m.u. for component C and 806 a.m.u. for component E.

From the identical mass differences of 16 a.m.u. between the main components and the minor fractions, the similar retention times of both in HPLC runs and their identical UV/V is spectra it can be assumed that the components A, C and E are derivatives of the ornibactins-C4, -C6 and -C8 (components B, D and F), respectively, rather than possessing a completely independent structure. The additional mass of 16 a.m.u. could originate from an

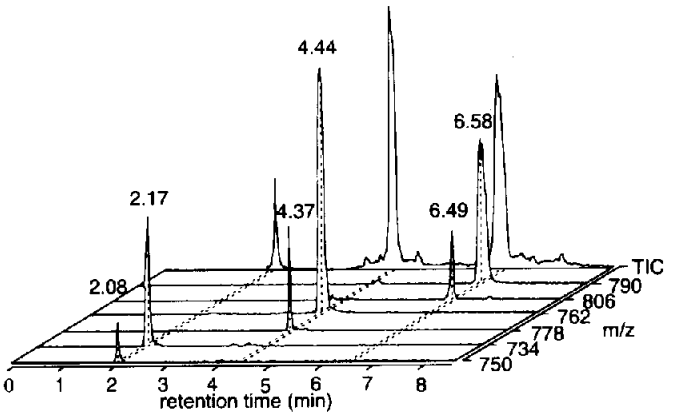


Figure 5. HPLC-MS separation of the ornibactin mixture on a reversed-phase column (Nucleosil C18, 5 μm, 100 × 2 mm) using a gradient (10–80% within 20 min) of acetonitrile (containing 0.1% trifluoroacetic acid) in water (containing 0.1% trifluoroacetic acid) at a flow rate of 200 μl min^{-1}. Total ion current (TIC) and mass extracted chromatograms for ions of 750, 734, 778, 762, 806 and 790 a.m.u. with corresponding retention times are shown.

additional hydroxy group or, less likely, from an additional amino function in the molecule. This modification present in components A, C and E might be due to an hydroxylation either at the aliphatic chains by a non-specific hydroxylation enzyme or at the amide functions by an hypothetic enzyme responsible for the formation of hydroxamate groups.

Discussion

The ornibactins-C4, -C6 and -C8 represent a new family of siderophores possessing the same peptide sequence but showing microheterogeneity in the acyl residues bound to ornithine in position one. All of these siderophores are accompanied by small amounts of compounds (components A, C and E) which are probably byproducts of the biosynthesis with similar structure. The ornibactins represent the first family of peptide siderophores isolated from *P. cepacia* and related non-fluorescent species, although the non-peptidic cepabactins have been described earlier (Meyer *et al.* 1989). According to their non-fluorescent property the isolated ornibactins also lack the commonly found chromophore containing the catecholate binding unit for ferric ions. Therefore, instead of the missing catecholate bidentate, a second hydroxamate is present which has led to the name ornibactin. The presence of two outer hydroxamate and one central hydroxycarboxylate residues may render the ornibactins very alike to the shizokinen, arthrobactin and aerobactin family with regard to iron binding properties and water solubility. As shown in the present investigation, the chain length of the acid moiety of the ornibactins varies from four to eight carbon atoms.

As shown by Oyaizu & Komagata (1983), 3-hydroxy fatty acids are wide-spread in *Pseudomonas* species and are found only in Gram-negative bacteria and not in Gram-positive bacteria. Long-chain 3-hydroxy fatty acids are generally linked by ester or amide bonds to lipid A and are commonly liberated by methanolysis and detected by GC. Because of the evaporation methods used, only non-volatile, long-chain fatty acids (much longer than eight carbon atoms) are detected. As shown in the present investigation, short-chain 3-hydroxy fatty acids also occur in *Pseudomonas* species and are inserted in that part of the peptide siderophore where the quinoline residue is located in the case of the pyoverdins.

Therefore, it will be of interest to investigate whether or not the acyl chain influences iron transport mediated by the ornibactins. Although earlier work of Hohnadel & Meyer (1988) has shown that the specificity of iron transport in the pyoverdin family is mainly based on the diversity of the peptide backbone, the use of ornibactins in the study of receptor specificity will help to elucidate the molecular requirements of the siderophore–receptor interactions in the producing strain and related *Pseudomonas* strains.

Acknowledgments

We thank the Deutsche Forschungsgemeinschaft for financial support. The technical assistance of G. Nicholson and A. Cansier is gratefully acknowledged.

References

Abdallah MA. 1991 Pyoverdins and pseudobactins. In: Winkelmann G, ed. *Handbook of Microbial Iron Chelates*. Boca Raton, FL: CRC Press.

Briskot G, Taraz K, Budzikiewicz. 1989 Pyoverdin-type siderophores from *Pseudomonas aeruginosa*. *Liebigs Ann Chem*, **1989**, 375–384.

Bruins AP, Covey TR, Henion JD. 1987 Ion spray interface for combined liqud chromatography/atmospheric pressure ionisation mass spectroscopy. *Anal Chem* **59**, 2642–2646.

Buyer JS, Wright JM, Leong J. Structure of pseudobactin A 214, a siderophore from bean-deleterious *Pseudomonas*. *Biochemistry* **25**, 5492–5499.

Cox CD, Rinehart KL, Moore ML, Cook JC. 1981 Pyochelin: novel structure of an iron-chelating growth promotor for *Pseudomonas aeruginosa*. *Proc Natl Acad Sci USA* **78**, 4256–4260.

Frank H, Nicholson GJ, Bayer E. 1977 Rapid gas chromatographic separation of amino acid enantiomers with a novel chiral stationary phase. *J Chromatogr Sci* **14**, 174–176.

Gläßgen WE, Seitz HU, Metzger J. 1992 High-performance liquid chromatography/electrospray mass spectrometry and tandem mass spectrometry of anthocyanins from plant tissues and cell cultures of *Daucus Carota* L. *Biol Mass Spectrom* **21**, 271–277.

Hohnadel D, Meyer J-M. 1988 Specificity of pyoverdine-mediated iron uptake among fluorescent *Pseudomonas* strains. *J Bacteriol* **170**, 4865–4873.

Linget C, Stylianou DG, Dell A, Wolff RE, Piemont Y, Abdallah M. 1992 Bacterial siderophores: The structure of a desferribactin produced by a *Pseudomonas fluorescens* ATCC 13525. *Tetrahedron Lett* **33**, 3851–3854.

Matzanke BF, Ecker DJ, Yang TS, Huynh BH, Müller G, Raymond KN. 1986 *Escherischia coli* iron enterobactin uptake monitored by Mössbauer spectroscopy. *J Bacteriol* **167**, 674–680.

Maurer B, Müller A, Keller-Schierlein W, Zähner H. 1968 Ferribactin, ein Siderophor aus *Pseudomonas fluorescens* Migula. *Arch Microbiol* **60**, 326–339.

Meyer JM, Abdallah MA. 1978 The fluorescent pigment of *Pseudomonas fluorescens:* biosynthesis, purification and physiochemical properties. *J Gen Microbiol* **107**, 319–328.

Meyer JM, Hohnadel D, Hallé F. 1989 Cepabactin from *Pseudomonas cepacia*, a new type of siderophore. *J Gen Microbiol* **135**, 1479–1487.

Müller L. 1979 Sensitivity enhanced detection of weak nuclei using heteronuclear multiple quantum coherence. *J Am Chem Soc* **101**, 4481–4484.

Oyaizu H, Komagata K. 1982 Grouping of *Pseudomonas* species on the basis of cellular fatty acid composition and the quinone system with special reference to the existence of 3-hydroxy fatty acids. *J Gen Appl Microbiol* **29**, 17–40.

Stephan H, Freund S, Meyer JM, Winkelmann G, Jung G. 1993 Structure elucidation of the gallium–ornibactin complex by 2D-NMR spectroscopy. *Liebigs Ann Chem* 43–48.

Taraz K, Tappe R, Schröder H, Hohleicher U, Gwose I, Budzikiewicz H, *et al*. 1991 Ferribactins—the biogenetic precursors of pyoverdins. *Z Naturforsch* **46c**, 527–533.

Thomson BA, Iribarne JV, Dziedzic PJ. 1982 Liquid ion evaporation/mass spectroscopy/mass spectroscopy for the detection of polar and labile molecules. *Anal Chem* **54**, 2219–2244.

Teintze M, Hossain MB, Barnes CL, Leong J, van der Helm D. 1981 Structure of ferric pseudobactin, a siderophore from a plant growth promoting *Pseudomonas*. *Biochem* **20**, 6446–6457.

Winkelmann G, ed. 1991 *Handbook of Microbial Iron Chelates*. Boca Raton, FL: CRC Press.

Yang CC, Leong. 1984 Structure of pseudobactin 7SR1, a siderophore from plant-deleterious *Pseudomonas*. *Biochemistry* **23**, 3534–3540.