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Rearrangement reactions in the electrospray ionization mass spectra of pyoverdins[+]

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Abstract

The electrospray ionization mass spectra of pyoverdins, chromopeptidic siderophores of the bacterial family *Pseudomonas*, especially when analyzed by collision activation in an ion trap, show rearrangement reactions which can lead to erroneous structure proposals. Several of these processes will be described. (Int J Mass Spectrom 210/211 (2001) 603–612) © 2001 Elsevier Science B.V.

Keywords: Pyoverdins; *Pseudomonas*; Electrospray ionization; Rearrangement reaction

1. Introduction

Members of the fluorescent group of the bacterial family Pseudomonadaceae are characterized by the production of iron chelating metabolites, so-called pyoverdins. Their characteristic feature is the chromophore (1S)-5-amino-2,3-dihydro-8,9-dihydroxy-1*H*-pyrimido[1,2a]chinolin-1-carboxylic acid (**1**) (Fig. 1). The chromophore is bound by its carboxyl group amidically to the N-terminus of a peptide chain comprising of 6–12 amino acids, *L* as well as *D*, and partially modified, and by its amino group to a small dicarboxylic acid or its monoamide ("side chain"). Frequently a part of the peptide chain forms a cyclopeptidic subunit [1]. Most structural information regarding the peptide part of the pyoverdins can be obtained by collision activation in an ion trap of $[M + H]^+$ and of $[M + 2H]^{2+}$ ions formed by electrospray ionization (ESI) [2]. With the exception of the rare cases where Arg is part of the peptide chain the first proton is located essentially in the chromophore system. Fragment formation from $[M + H]^+$ proceeds therefore overwhelmingly by charge remote processes [3] and mainly B ions from the linear part of the peptide chain are formed, while fragmentation of cyclic units is not observed. In $[M + 2H]^{2+}$ the second proton according to the mobile proton scheme [4,5] can reside at the various peptide bonds and facilitate cleavage reactions there. Fragments of cyclic subunits are observed as well as secondary processes as the loss of small neutrals (H_2O, NH_3, CO, CH_2CO, and CO_2). The interpretation of the mass spectra thus obtained is complicated by the prescence of skeletal rearrangements, which feign incorrect

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[+] Part C of the series "Bacterial Constituents." For part IC see R. Sultana, S.D. Siddiqui, K. Taraz, H. Budzikiewicz, J.-M. Meyer, Tetrahedron 57 (2001) 1009.

Dedicated to Professor Dr. N. N. M. Nibbering on the occasion of his 60th birthday.

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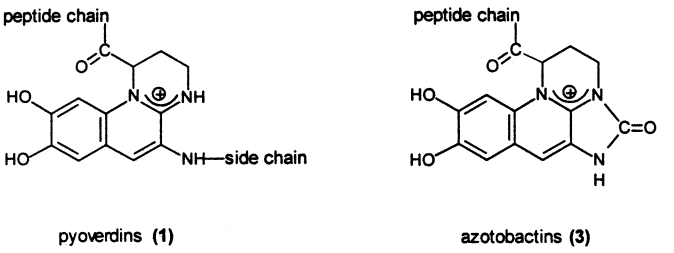


Fig. 1. General structure of pyoverdins (**1**) and azotobactins (**3**).

substitution patterns and amino acid sequences. This phenomenon will be discussed.

For the nomenclature of peptide fragments the convention proposed by Roepstorff and Fohlman [6] will be used: N-terminal fragments $H_2N–CHR^+$, $H_2N–CHR–CO^+$ and $H_2N–CHR–CO–NH^+$ are designated by the letters A, B, and C, a subscript indicates the number of amino acid residues in the fragment, and hyphens the number of additional hydrogen atoms (e.g. $H_2N–CHR–CO–NH_3^+$ would be a C_1'' ion). This formal designation is used irrespective of the actual structure of the ions (rearranged structures or location of the additional hydrogens).

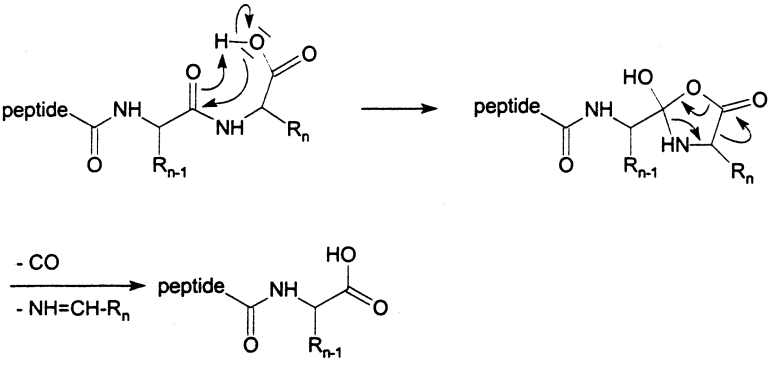
2. Experimental

2.1. Mass spectra conditions

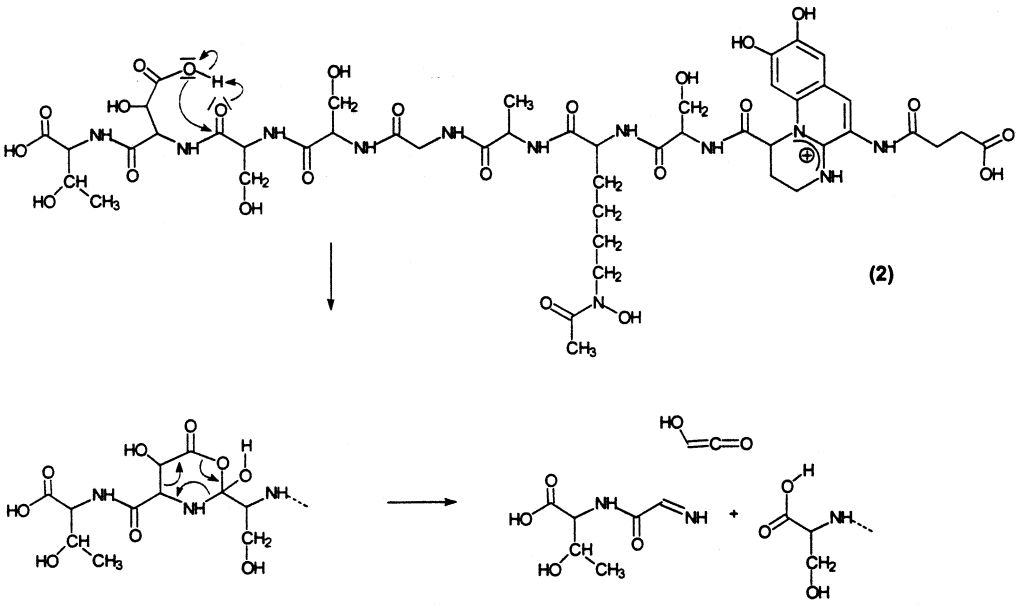
The mass spectral analyses were performed with a MAT 900 ST instrument with an EBT geometry and equipped with an ESI II ion source (Finnigan MAT, Bremen, Germany); spray voltage 3.4–3.6 kV, capillary temperature 230 °C. The samples were dissolved in a mixture consisting of water, methanol, and acetic acid 50:50:0.1 (v/v). All masses given in the text are nominal masses. Fragmentation induced by collision activation (CA) was effected in the ion trap with $\sim 2 \times 10^{-3}$ Pa He as bath gas. The compounds investigated stem from the collection of this institute. For their isolation and characterization see the respective references.

2.2. Trifluoroacetylation of pyoverdins

To 0.3 mg pyoverdin dissolved in 60 μL waterfree dimethylformamide (DMF) 20 μL each of DMF solutions containing 0.089 μL ethyl-diisopropylamine and 0.066 μL S-ethyl trifluorothioacetate, respectively, were added. After shaking for 3 h at room



Scheme 1.



Scheme 2.

temperature 10 ml of 0.05 m acetic acid were added. The solution was brought onto a Sep-Pak RP18 cartridge (Waters, Milford, MA, USA) activated and rinsed with water. Reagents were washed out with 15 mL 0.1 m acetic acid and the derivatized pyoverdin was desorbed with methanol/acetic acid 7:3 (v/v). The solution was brought to dryness i.v. (~1kPa) at ~30 °C and stored at −22 °C.

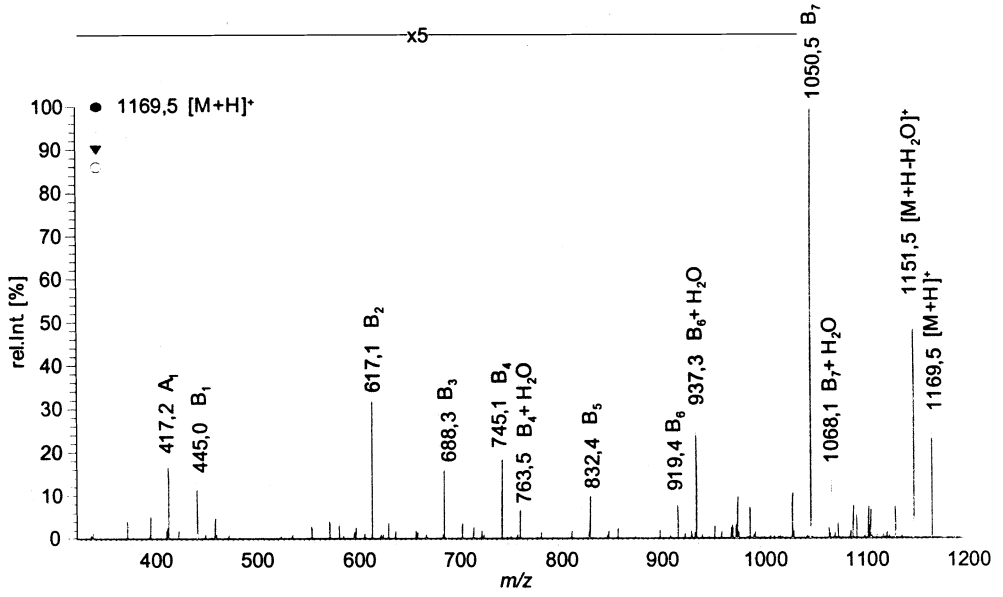
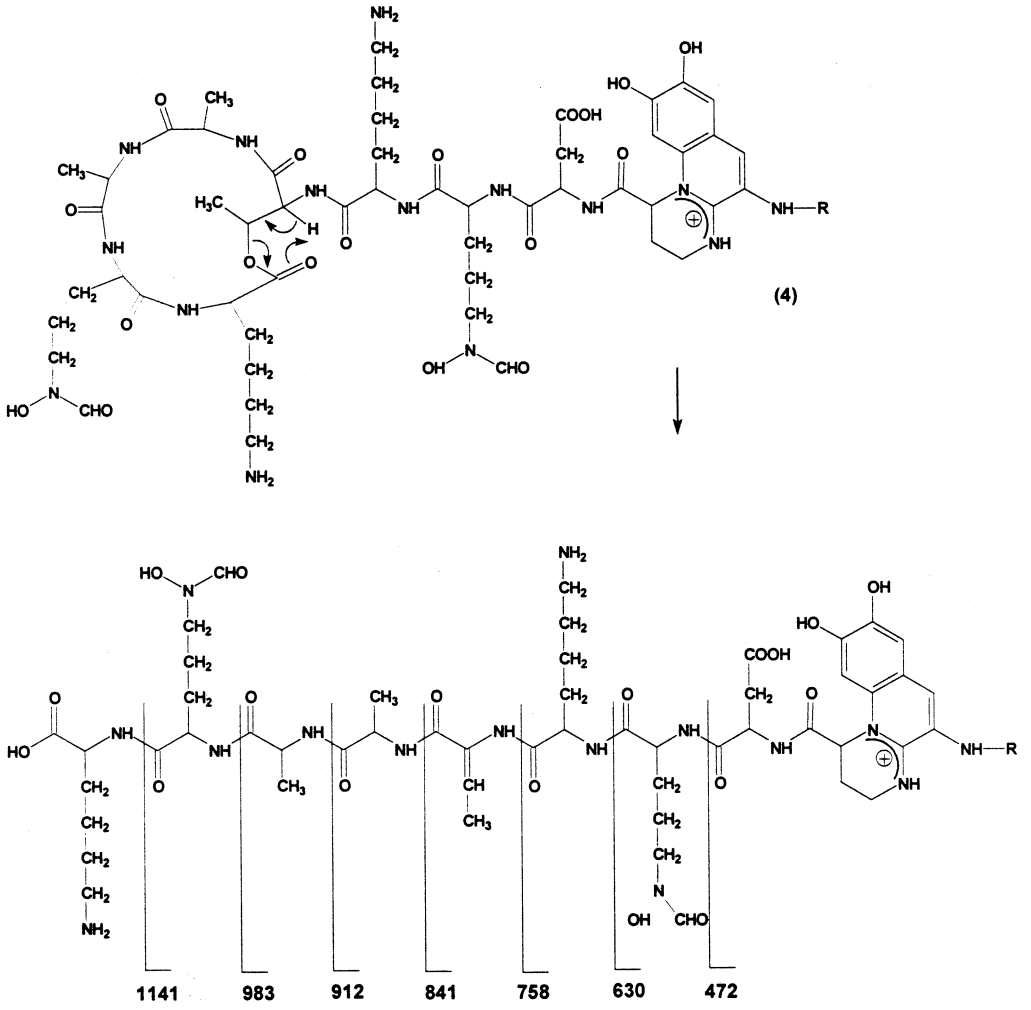


Fig. 2. ESI CA spectrum of $[M + H]^+$ of the pyoverdin from *Pseudomnas fluorescens* Pl9 (**2**).



Scheme 3.

3. Results and discussion

3.1. $[B+H_2O]^+$ ions

Ions of the formal mass $[B + H_2O]^+$ are actually $[B' + OH]^+$ ions. They are formed from $[M + H]^+$ by a CA induced charge remote process involving the transfer of an OH group from an amino acid to the preceding amide carbonyl group accompanied by the loss of the C-terminal part of the molecule. This process was described originally for an OH transfer from the C-terminal carboxyl group to the penultimate amide carbonyl (Scheme 1) and it was confirmed by ^{18}O labeling. It is especially pronounced when a basic amino acid (Arg etc.) is present in the peptide chain (localization of the ionizing H^+) [7,8]. OH transfer is possible also from the side chains (cf. [9]) of amino acids [Asp-COOH, Ser-OH, N-formyl-N-hydroxy Orn (abbreviated Fho), etc.] to the neighboring amide carbonyl with concomitant loss of the rest of the chain. An example is the formation of $[B_6 + H_2O]^+$ from **2**, (Scheme 2, Fig. 2) and of $[B_4 + H_2O]^+$ by an analogous transfer from Ser, in addition to the corresponding rearrangement from the terminal amino acid ($[B_7 + H_2O]^+$, note that this part of the spectrum is not enlarged) [10]. In pyoverdins the ionizing proton

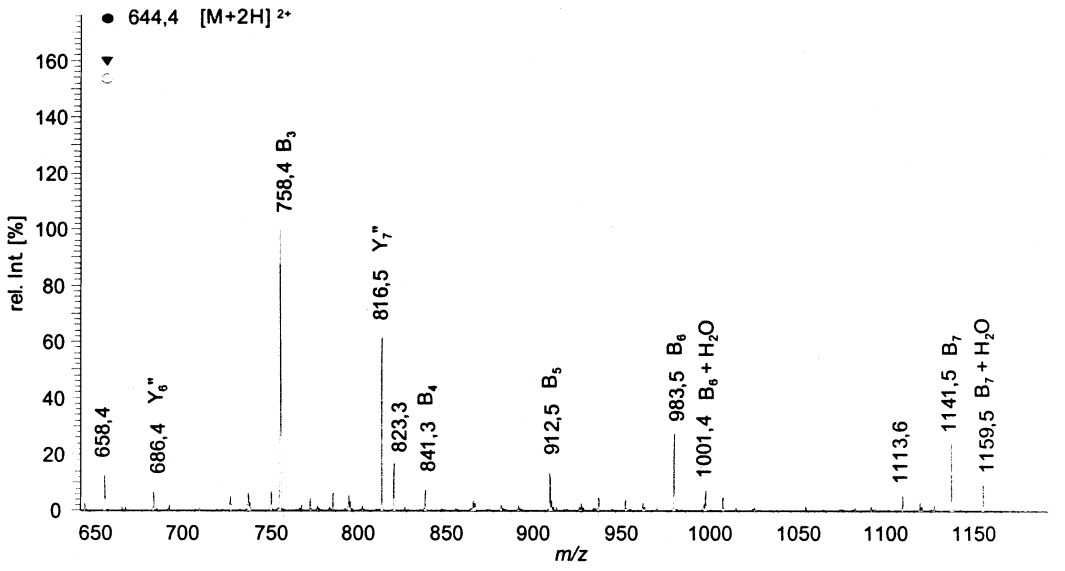


Fig. 3. Partial trap CA spectrum of pyoverdin 7SR1 (**4**).

is localized in the chromophore **1**. Azotobactins have the chromophore **3** which is per se a cation. OH transfer leads therefore to $[B + OH]^+$ ions [10]. $[B_{\omega-1} + H_2O]^+$ ions are also observed when the C-terminal free carboxyl group is formed by a McLafferty rearrangement from a cyclodepsipeptidic lactone (Scheme 3, Fig. 3): **4** yields $[B_6 + H_2O]^+$ (*m/z* 1001), transfer of OH from Fho, and $[B_7 + H_2O]^+$ (*m/z* 1151), transfer from the newly formed C-terminal Lys-COOH) [10].

The formation of $[B + H_2O]^+$ ions can also be observed starting from $[M + 2H]^{2+}$ as in the case of **5** (Fig. 4) [11]. Thus, $[B_3 + H_2O]^+$ (Scheme 4, transfer of OH from Fho to Lys and cleavage of the amide bond between the two amino acids) appears in the mass spectrum (Fig. 5, Table 1) [2]. A dissociation into two singly charged particles must take place here (location of one H^+ in the chromophore and the other one in the cyclic part; such precursors have been invoked [12] to explain fragmentation of the cyclic subunit).

Loss of the C-terminal amino acid with backtrans-

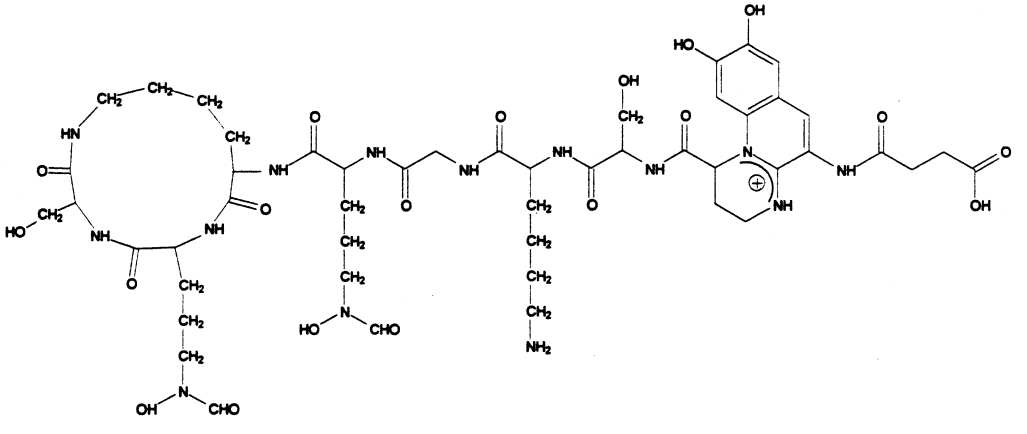
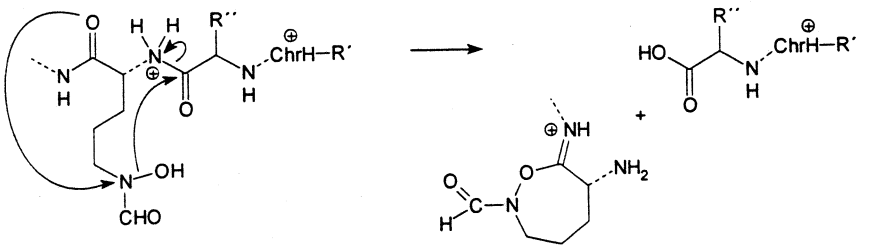


Fig. 4. Pyoverdin **5** from *Pseudomonas fluorescens* 13525 and *Pseudomonas chlororaphis* 9446.



Scheme 4. R′=acyl side chain and R″=CH_2-CH_2-CH_2-CH_2-NH_2 (Lys).

fer of one oxygen from the carboxyl group has also been reported for peptide molecular ions formed by the attachment of a monovalent metal cation under fast atom bombardment conditions, $[M + Met]^+$ giving $[B_{\omega-1} + OH + Met]^+$. Two basically different mechanisms were discussed, one with the metal cation localized somewhere in the peptide chain involving an OH shift from the COOH group [13,14] in analogy to Scheme 1, and the other one having a proton attached to a basic center of the peptide, starting with an attack of the carboxylate anion at the neigboring peptide bond and transfer of O^- with concomitant migration of the metal cation [15,16], the main argument being that methyl esters do not show migration of the OCH_3 group [16]. For $[M + H]^+$ species as discussed previously conceivably zwitterionic species having two protons localized at basic centers of the peptide chain and a terminal COO^- group could be considered resulting in an O^- transfer, but this mechanism would certainly not hold for a rearrangement involving e.g. the OH group of Ser. Charge localization in the peptide chain and a subsequent low activation energy concerted process typical for charge remote reactions after low energy collision [17] seems to be the prerequisite for the formation of the rearrangement ions discussed here.

3.2. Migration of formyl and acetyl groups

Many pyoverdins have in their peptide chains N-formyl- (or N-acetyl-) N-hydroxy Orn (Fho, Aho) units. These hydroxamic acids serve as ligands for the complexation of Fe^{3+}. The CHO group of Fho can migrate to a free ϵ-NH_2 group of Lys or to a free

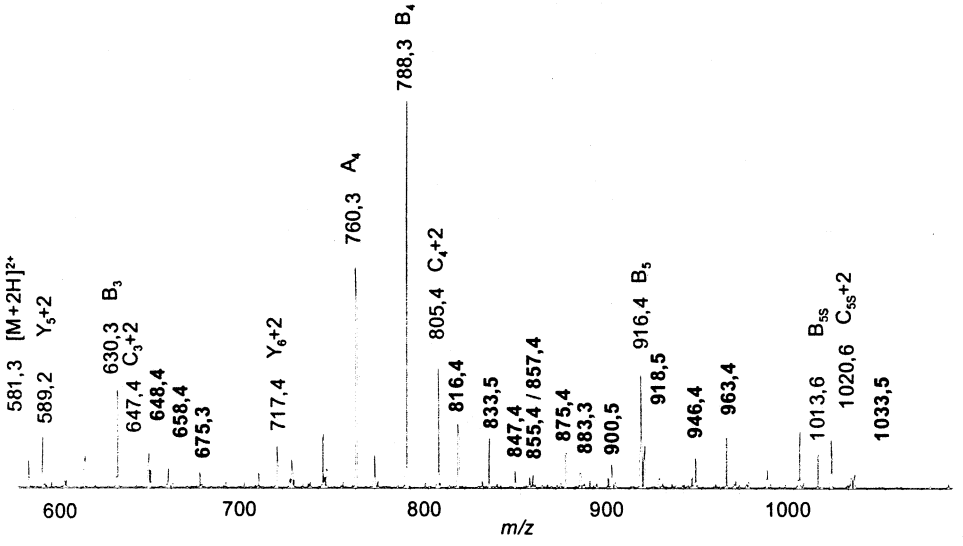


Fig. 5. Enlarged parts of the trap CA-MS^2 spectrum of $[M + 2H]^{2+}$ (*m/z* 581.3) of pyoverdin **5** (rearranged ions are marked in **bold**).

Table 1
Rearranged ions observed in the trap-CA MS^2 spectrum of $[M + 2H]^{2+}$ of **5**

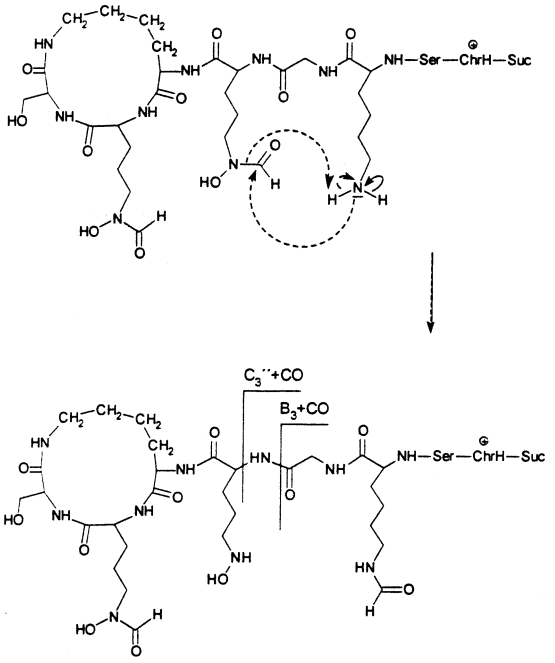
m/z		*m/z*		*m/z*	
648,4	$B_3 + H_2O$	847,4	A_4 + Ser	900,5	A_4 + Fho−H_2O
658,4	B_3 + CO	855,4	A_4 + Fho−NH_3−H_2O−CO	918,5	A_4 + Fho
675,3	C_3'' + CO	857,4	B_4 + Ser−H_2O	946,4	B_4 + Fho
816,4	B_4 + CO	875,4	B_4 + Ser	963,4	C_4'' + Fho
833,5	C_4'' + CO	883,3	A_4 + Fho−NH_3−H_2O	1033,5	B_4 + Fho + Ser

δ-NH_2 group of Orn with concomitant back transfer of one H. This results in the formation of ϵ-formyl Lys/δ-formyl Orn. All B and C″ ions containing this newly formed species are shifted by 28 u (+CO) (Scheme 5). This explains, for example the formation of $[B_3/C''_3 + CO]^+$ (*m/z* 658 and 675 in Fig. 5). An analogous migration of the formyl group from Fho unit in the cycle leads to $[B_4/C''_4 + CO]^+$ (*m/z* 816 and 833 in Fig. 5). As previously noted, from the doubly charged $[M + 2H]^{2+}$ two singly charged fragments are formed. Occasionally the rearrangement ions can have an appreciable abundance. One should also expect the formation of [A + CO] ions, but they are isobaric with normal B ions and hence do not appear as additional ions in the spectrum. That free amino groups act as the receptors of the migrating formyl groups becomes evident from the observation that the rearrangement ions are not observed when the Lys/Orn-ω-NH_2 group is trifluoroacetylated.

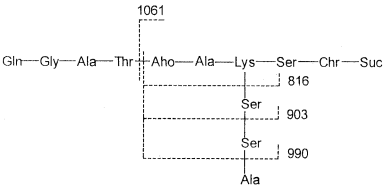
An analogous migration of acetyl groups from Aho to Lys has also been observed (+42 u). Not every pyoverdin containing Fho/Aho in addition to Lys or Orn yields these rearrangement ions. The structural requirements (distance between the donor and the acceptor unit, etc.) are a moot point.

3.3. Migration of amino acid and peptide units

The ω-amino groups of Lys and Orn can also be acceptors for amino acid and even oligopeptide residues stemming from the C-terminal part of B ions. Similar rearrangement processes have been described for peptides [3,18], processes which do not occur if the amino group is acetylated. The authors assume bond formation between the terminal CO group of the B ion with the amino group yielding a cyclopeptidic structure which, by opening of another peptide bond yields fragments where parts of the peptide chain are bound to Lys or Orn, respectively. These fragments are especially annoying, since they suggest wrong sequences. Ions where one or more amino acid resi-



Scheme 5. CO transfer from Fho to Lys in the Ca spectrum of pyoverdin **4** (see Fig. 5).



Scheme 6. Structure of the rearranged ion B_{11}^{2+} of the pyoverdin **6** (see Fig. 8).

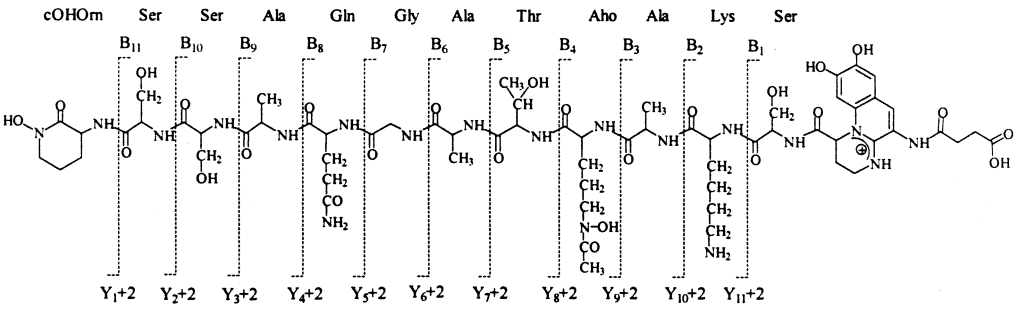


Fig. 6. Pyoverdin **6** from *Pseudomonas fluorescens* 4975.

dues had migrated to Lys or Orn can be observed in the ion trap CA spectra of pyoverdins. Also here they are suppressed when the Lys/Orn-amino group is trifluoroacetylated.

In Fig. 6 ions can be seen whose masses (e.g. *m/z* 1061, 990, 903) cannot be reconciled with the established sequence of amino acids of **6** (Fig. 7) [19]. These ions corresponding to a consecutive loss of Ala, Ser, Ser recur in the CA spectrum of B_{11}^{2+} (Fig. 8). The mass *m/z* 1061 corresponds to a B_4 species where the tripeptidic unit Ser-Ser-Ala had migrated to Lys (attack of the C-terminal CO^+ of B_{11} at the Lys ϵ-NH_2 with concomitant loss of Thr-Ala-Gly-Gln. The rearrangement results in a turning around of the tripeptide unit (Scheme 6). Subsequent loss of Ala, Ser, and Ser yields the three fragments mentioned giving finally B_4. CA of B_{10}^{2+} shows an analogous behavior. After transfer of Ser-Ala an ionic species is formed (*m/z* 974) which loses Ala and then Ser. In B_9^{2+} only Ala can be transferred (*m/z* 987) and subsequently be lost to give B_4. Although in the CA spectrum of $[M + 2H]^{2+}$ the whole series of (singly charged) B ions can be seen (Fig. 6), from B_{11}^{2+} only the losses of the three rearranged amino acid residues are observed, but no alternative loss of Aho and Ala and no further B fragmentation.

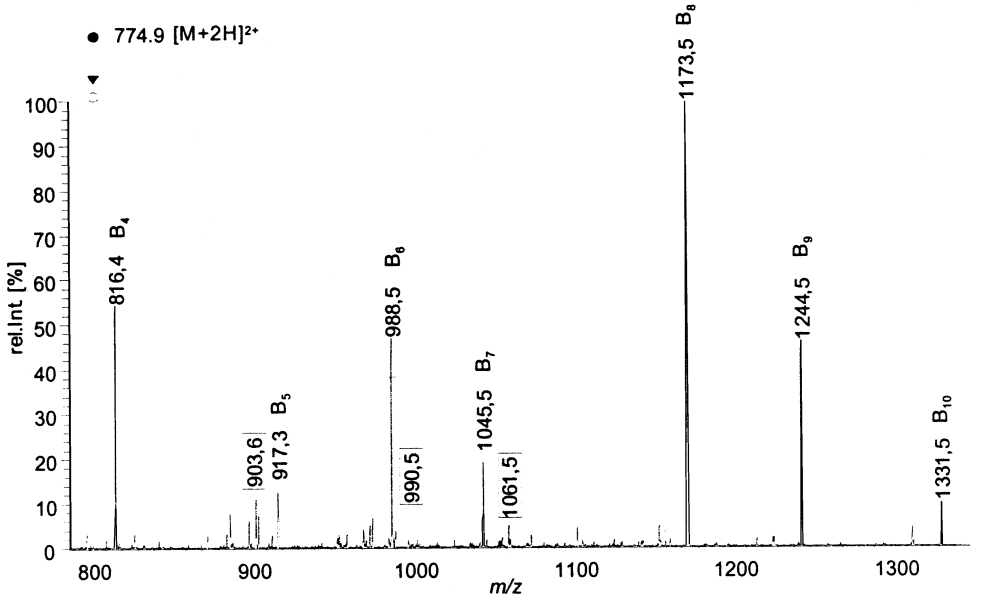


Fig. 7. Partial trap CA spectrum of $[M + 2H]^{2+}$ of pyoverdin **6**.

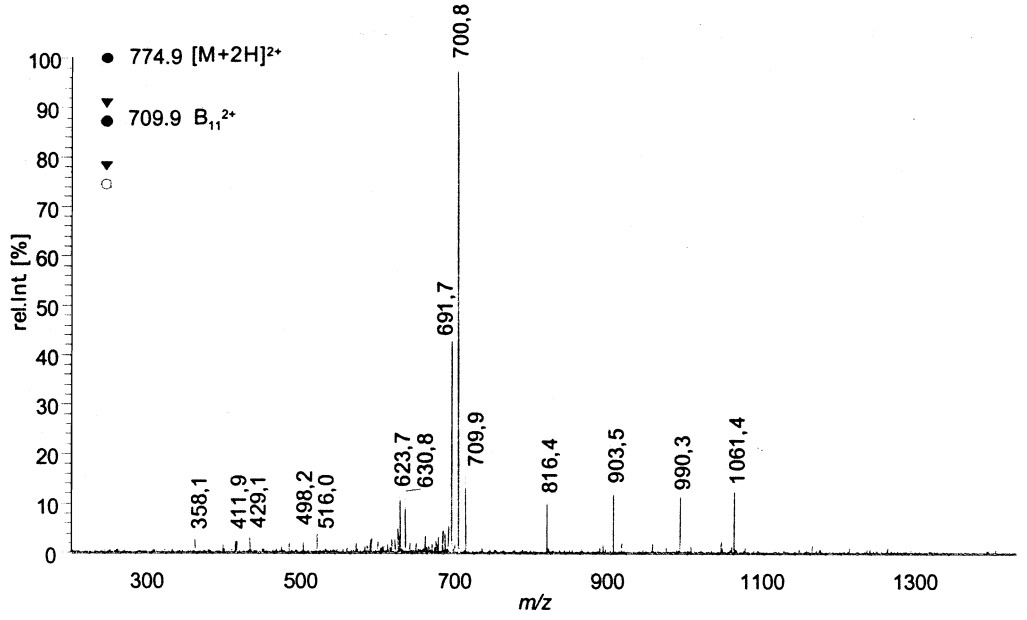


Fig. 8. Trap CA MS^3 spectrum of the ion B_{11}^{2+} from pyoverdin **6**.

These rearrangement ions are not observed in the CA spectra of $[M + H]^+$ or of the singly charged B ions. This suggests that only the high-energy B ions formed by a cleavage of a protonated amide bond (and not those formed by a charge remote process) undergo rearrangement and subsequent fragmentation. This is in agreement with the transfer of parts of the cycle (e.g. Fho and Fho + Ser, *m/z* 946 and 1033 in the spectrum of **5**, Fig. 5) to B_4. Cleavages in the cyclic part of a pyoverdin occur only when a second H^+ is located in this part of the molecule. Again, nothing can be said about the structural requirements of these rearrangement reactions.

4. Conclusions

Pyoverdins when subjected to mass spectrometric analysis after ionization by protonization show rearrangement reactions which have been observed for simple peptides, but also unexpected processes due to the presence of modified amino acids. They are preferentially found in doubly charged species where one proton can induce fragmentation in the peptide chain whereas the other one is localized at a center of high basicity (here generally the chromophore). Processes of this type become especially evident after collision activation of ions selected in an ion trap, and then can exceed by orders of magnitude fragments formed by the straightforward fragmentations of the original amino acid backbone. The peptide chain of pyoverdins contains amino acids modified to hydroxamic acids (Fho, Aho, etc.) and cyclic substructures. Especially rearrangement processes involving these structural realms could easily lead to erroneous structural conclusions.

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