Pure & Appl. Chem., Vol. 66, Nos 10/11, pp. 2207–2210, 1994.
Printed in Great Britain.

The biosynthesis of pyoverdins

Herbert Budzikiewicz
Institut für Organische Chemie der Universität
Greinstr. 4, D-50939 Köln, Germany

Abstract.
Observations are reported wich shed some light on the biosynthesis of the siderophores of the genus *Pseudomonas* named pyoverdins.

INTRODUCTION.

Members of the so-called fluorescent group of the genus *Pseudomonas* when grown in an iron deficient medium produce complexing compounds (so-called pyoverdins) which were noted first by *Gessard* in 1882 [2]. It took, however, almost ninety years until the structure of the first member of this class was elucidated [3]. In the meantime about 20 complete or fairly complete structures of pyoverdins are known [4]. They all comprise three distinct moieties, *viz.*

(a) the dihydroxyquinoline chromophore **1** which is responsible for the fluorescence giving the bacterial class its name;

(b) a peptide chain bound to the carboxyl group of **1** usually (but not necessarily) via its N-terminus and consisting of 6 to 12 amino acids (D as well as L and comprising also unusual - as e. g. Dab or Hse - and derivatized - as N^6-acyl-N^6-hydroxy-Orn - ones); and

(c) a dicarboxylic acid (amide) bound to the amino group of **1**.

Usually several pyoverdins differing only in the nature of these dicarboxylic acids co-occur.

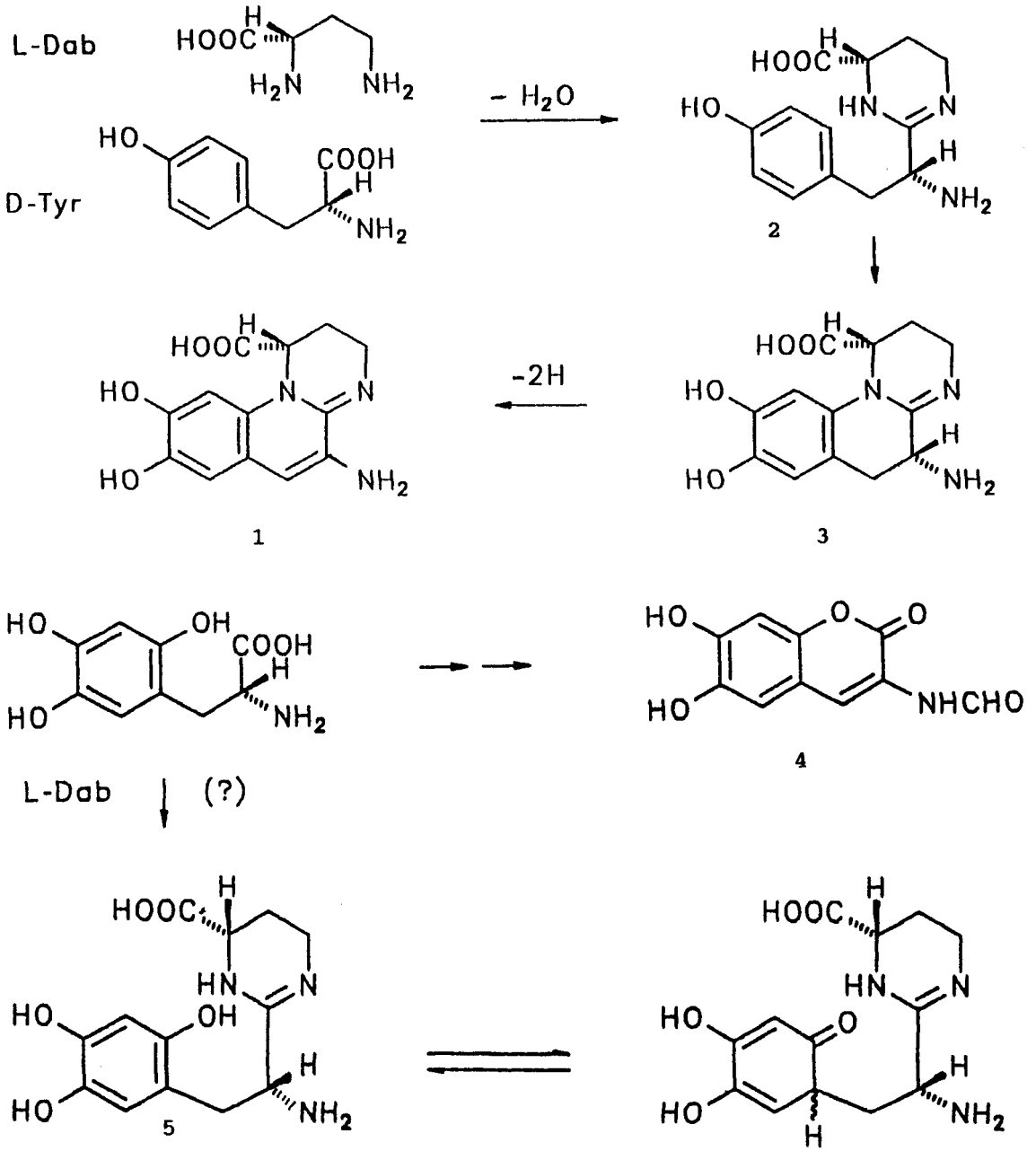
The iron (III) chelating system consists of the catechol grouping of **1** and of two hydroxamic acid units or one hydroxamic and one α-hydroxycarboxylic acid system located in the peptide chain, which has a twofold function, *viz.* to provide the complexing centers in the correct spacial position and to allow the recognition and docking of the proper pyoverdin on the bacterial cell surface. The peptide chains differ for the various *Pseudomonas* species or even subspecies.

DISCUSSION.

The peculiar structure of the pyoverdins prompted investigations regarding their biogenesis - especially that of the chromphore **1** - which will be summarized below.

(a) The chromophore. The key to the understanding of the formation of **1** was the discovery that two other pigments can be isolated by careful work-up which have the same peptide chain as the co-occurring pyoverdins, *viz.* 5,6-dihydropyoverdins and ferribactins [5, 6]. The ferribactin chromophore (**2**) is formally a condensation product of D-Tyr and L-Dab [7, 8]. It comprises all the structural elements necessary for the formation of the 5,6-dihydro-chromophore **3** by ring closure and introduction of the second hydroxyl group. Regarding the ring closure it is of interest that a genetically modified non-fluorescent mutant of *Ps. aeruginosa* produces **4** [9]. This suggests that **2** is transformed into **5** which cyclizes by attack of the NH-group of the tetrahydropyrimidine ring at the tautomeric keto form of **5** with subsequent elimination of H_2O. Phe-hydroxylase activity has been shown for *Ps. aeruginosa* and *Ps. putida* [10, 11]. **3** (which is found preferentially in cultures with a high cell density where the local O_2 supply is reduced) can then be dehydrogenated to **1**. Further evidence for the intermediacy of **2** in the biosynthetic pathway is the isolation of an isopyoverdin with the chromophore **6** from a *Ps. putida* strain which is formed by cyclisation

via the "wrong" pyrimidine N of **2** [12].

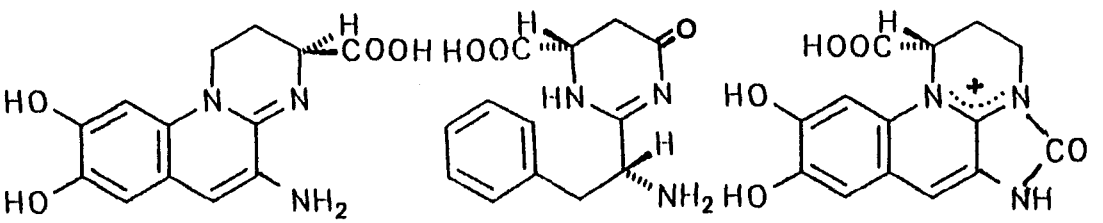


It could be shown that ^{14}C-Tyr is incorporated into **1** for *Ps. aeruginosa* while *Ps. putida* apparently uses Phe (and not Tyr or DOPA) introducing the second hydroxyl at a later stage [10]. As stated above condensation of D-Tyr with L-Dab would lead directly to **2**. Such condensation products of Dab with other amino acids (*i. a.*, Gln and Ser) have been found in various pyoverdin peptide chains [13, 14]. A research group from Minsk [15] demonstrated, however, that for non-fluorescent mutants of *Ps. putida* whose pyrimidine biosynthesis was blocked at different stages the pyoverdin production was restored by auxotrophic dihydroorotate. This would mean that not Dab but rather Asp is the second precursor of **1** and **7** rather than **2** would be the intermediate. In addition to the moot point whether the Dab-condensation products in the peptide chains are also dihydroorotic acid derivatives conclusions based

on auxotrophism in mutants blocked in the genetic realm have to be treated with caution. Studies with labelled alledged precursors of **2** are on the way. That **2** is actually the precursor of **1** for *Ps. chlororaphis* is very likely from the following experiment: The strain ATCC 9446 when grown with $(^{15}NH_4)_2SO_4$ as the only nitrogen source produces both a pyoverdin and a ferribactin labelled in all positions with ^{15}N. Feeding experiments with the labelled ferribactin yielded a pigment which in its FAB mass spectrum showed the mass of the $[M + H]^+$ ion of the completely labelled pyoverdin [24].

(b) The peptide chain. Rather little is known in this area. There is evidence that the synthesis is effected by non-ribosomal polyfunctional enzyme systems [16, 17] in analogy to that of peptide antibiotics [18]. These enzymes apparently have also epimerase activity to provide the D-amino acids encountered in the pyoverdins. In agreement with the assumption of (at least partial) extracellular processes is the observation [19] of a change in membrane proteins and an increase of broth proteins for various *Ps. spp.* when grown under iron deficiency. Formation of the hydroxamic acids from Orn (N-hydroxylation and -acylation) seems to be an early step in the peptide chain synthesis [20].

(c) Dicarboxylic acids. All dicarboxylic acid (amides) encountered sofar belong to the citric acid cycle. The starting point is Glu in agreement with the observation that ferribactins were found with Glu only. Glu and α-ketoglutaric acid form an equilibrium during the growth of the bacteria the rate of interconversion changing constantly. Succinamide can be found from the start of the pyoverdin production in agreement with the observation that dihydropyoverdins with a Glu and a succinamide side chain are found [21].



6 **7** **8**

(d) Combination of the three parts. *Meyer* pronounced the opinion [17] that the first step in the biosynthesis of pyoverdins is the formation of an amino acid chain comprising the elements of **2** and Glu (see preceding section). *Maksimova* [15] owing to her conclusion that the formation of **2** is connected with the pyrimidine bases synthesis postulates an independent building of the three elements. Irrespective of the fact whether her ideas are correct there is circumstantial evidence that at least the formation of **2** and of the peptide chain are indepedent processes as, *i. a.*, the formation of **4** by a mutant strain not able to attach the peptide chain [9]. In addition, for all pyoverdins whose structure has been elucidated sofar the amino acids of the peptide chain are linked by peptidic bonds, i. e., via their α-amino groups [4] (the only exception mentioned in the literature [22] - the ε-amino group of Lys - is doubtful [4]) while the peptide chain itself can be attached to the carboxyl group of **1** by an amide as well as an ester bond. Glu is bound to the amino group of **1** always by its 5-carboxyl group.

CLASSIFICATION OF *Pseudomonas*.

The classification system of the genus *Pseudomonas* is under discussion [23]. In this context the unique structures of the pyoverdins gain in importance: The different peptide chains of the various species and subspecies are a safe means for an identification as sofar

only one exception of this rule has been found: *Ps. fluorescens* ATCC 13525 and *Ps. chlororaphis* ATCC 9446 produce identical pyoverdins and pseudobactins [24]; the identifications of these two strains should, therefore, be rechecked). The fact that *Azomonas macrocytogenes* ATCC 12334 produces a pyoverdin [25] demonstrates its close relationship to the fluorescent group of *Pseudomonas*. A recent discovery in this laboratory [24] allows the same conclusion for *Azotobacter vinelandii*: The chromophore of its siderophores (azotobactins) differs from **1** by a CO-bridge between two N-atoms (**8**) [26, 27]. We could now show that *Ps. chlororaphis* produces a pyoverdin, a ferribactin and an azotobactin having the same peptide chain.

REFERENCES.

1. Part LXI of th series "Bacterial Constituents". For part LX see Ref. [8]
2. M. C. Gessard, *Ann. Inst. Pasteur Paris* **6**, 801 (1892).
3. M. Teintze, M. B. Hossain, C. L. Barnes, J. Leong and D. v. d. Helm, *Biochemistry* **20**, 6446 (1981).
4. H. Budzikiewicz, *FEMS Microbiol. Rev.* **104**, 209 (1993).
5. K. Taraz, D. Seinsche and H. Budzikiewicz, *Z. Naturforsch.* **46c**, 522 (1991).
6. H. Budzikiewicz, H. Schröder and K. Taraz, *Z. Naturforsch.* **47c**, 26 (1992).
7. K. Taraz, R. Tappe, H. Schröder, U. Hohlneicher, I. Gwose, H. Budzikiewicz, G. Mohn and J. F. Lefèvre, *Z. Naturforsch.* **46c**, 527 (1991).
8. G. Filsak, K. Taraz and H. Budzikiewicz, *Z. Naturforsch.* **49c**, in press (1994).
9. I. Longerich, K. Taraz, H. Budzikiewicz, L. Tsai and J.-M. Meyer, *Z. Naturforsch.* **48c**, 425 (1993).
10. N. P. Maksimova, O. V. Blashevich and Yu. K. Fomichev, *Mikrobiologiya* **61**, 818 (1992) (English tranlation: *Microbiology* **61**, 567 (1992)).
11. A. Stintzi and J.-M. Meyer, reported at the *Conference on Iron and Microbial Iron Chelates*, Brugge, Belgium (1993).
12. P. Jacques, I. Gwose, D. Seinsche, K. Taraz, H. Budzikiewicz, H. Schröder, M. Ongena and P. Thonart, *Natural Prod. Lett.* **3**, 213 (1993).
13. P. Demange, A. Bateman, Ch. Mertz, A. Dell, Y. Piemont and M. A. Abdallah, *Biochemistry* **29**, 7348 (1990).
14. S. Gipp, J. Hahn, K. Taraz and H. Budzikiewicz, *Z. Naturforsch.* **46c**, 534 (1991).
15. N. P. Maksimova, O. V. Blazhevich and Yu. K. Fomichev, *Molekularn. gen. microbiol. virusol.* No. 5, 22 (1993).
16. N. Menhart and T. Viswanatha, *Biochem. Biophys. Acta* **1038**, 47 (1990).
17. J.-M. Meyer, reported at the *Conference on Iron and Microbial Iron Chelates*, Brugge, Belgium (1993).
18. H. Kleinkauf and H. v. Döhren, *Ann. Rev. Microbiol.* **41**, 259 (1987).
19. J. M. Meyer, M. Mock and M. A. Abdallah, *FEMS Microbiol. Lett.* **5**, 395 (1979).
20. P. Visca, L. Serino and N. Orsi, *J. Bacteriol.* **174**, 5729 (1992).
21. H. Schäfer, K. Taraz and H. Budzikiewicz, *Z. Naturforsch.* **46c**, 398 (1991).
22. M. Persmark, T. Frejd and B. Mattiasson, *Biochemistry* **29**, 7348 (1990).
23. J. de Vos, reported at the *4th Int. Symp. on Pseudomonas*, Vancouver, B. C., Canada (1993).
24. U. Hohlneicher, Dissertation, Univ. zu Köln (1994).
25. S. K. Collinson, M. A. Abdallah and W. J. Page, *J. Gen. Microbiol.* **136**, 2297 (1990).
26. P. Demange, A. Bateman, A. Dell and M. A. Abdallah, *Biochemistry* **27**, 2745 (1988).
27. H. Budzikiewicz, E. M. Schaffner and K. Taraz, *Natural Prod. Lett.* **1**, 9 (1992).