A 5,6-Dihydro-isopyoverdin from *Azomonas macrocytogenes**

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From the culture medium of *Azomonas macrocytogenes* a 5,6-dihydro-isopyoverdin could be isolated which includes the same peptide chain as the accompanying isopyoverdins (azoverdins). As analogous pairs had been encountered in the pyoverdin series where a dihydro derivatives are considered to be the immediate precursors of the pyoverdins, the same biogenetic sequence can thus be assumed for the iso-series.

Introduction

In preceding publications we obtained evidence that the pyoverdin chromophore **1** is derived from the ferribactin chromophore **2** which in turn is a condensation product of D-Tyr and L-Dab (Böckmann *et al.*, 1997). **2** should also be the branching point for the formation of the recently discovered isopyoverdins **3**. If the assumption is correct that **1** and **3** are formed by analogous biogenetic steps starting from **2**, the same type of intermediates should be found and the stereochemistry of the chiral center at C-3 of **3** should be S. The correct stereochemistry was proved recently (Michalke *et al.*, 1997). We wish now to report the isolation of a 5,6-dihydro-isopyoverdin (dihydro-azoverdin) **4** from the culture medium of *Azomonas macrocytogenes* ATCC 12334.

Abbreviations: Common aminoacids, 3-letter code; Dab, 2,4-diaminobutyric acid; Hse, homoserine; (OH)Orn, N^5-hydroxy Orn; FAB-MS, fast atom bombardement mass spectrometry.

* Part LXXIV of the series "Bacterial constituents". For part LXXIII see Budzikiewicz *et al.* (1997).

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Experimental Procedures

For bacterial growth, spectroscopy, amino acid analysis, decomplexation, details of chromatography, etc. see Michalke *et al.*, 1996.

Isolation and characterization of 5,6-dihydro-azoverdin G

The culture filtrate was worked up as described earlier (Michalke *et al.*, 1996). After separation of azoverdin A by chromatography on DEAE Sephadex A-25 the fraction containing azoverdin and azoverdin G was rechromatographed on CM Sephadex C-25 with a 0.02 N pyridinium acetate buffer (pH 5.0, isocratic), detection at 340 and 405 nm. After a brown fraction (azoverdin and azoverdin G) a violet fraction containing ferri-5,6-dihydro-azoverdin G could be eluted which was rechromatographed under the same conditions. The Fe^{3+}-complex shows absorption maxima at 250, 318 and 535 nm (pH 3.0) and 250, 318 and 517 nm (pH 6.8), resp., while the free dihydro-compound shows a pH-independent absorption at 301 nm. The molecular mass of **4** was determined by FAB-MS as 1122 u, that of the Fe^{3+}-complex as 1175 u. From an aminoacid analysis follows the presence of L-Dab, L-Glu, D- and L-Hse, D- and L-(OH)Orn and D-Ser as had been observed for azoverdin G.

Transformation of dihydro-azoverdin G (**4**) *into azoverdin G.*

10 mg **4** were dissolved in 5 ml H_2O and stirred for 24 hrs with 10 mg PtO_2 . The color of the solution changed from violet to brown. The resulting azoverdin G was purified and de-complexed as described above. It was found to be identical with authentic azoverdin G by all spectroscopic evidence.

Results and Discussion

4 has the same aminoacid composition as azoverdin G and both the ^{1}H- and ^{13}C-NMR data of the amino acid portion and of the Glu side chain correspond to those of the latter. Hence the structure of these parts of **4** and of azoverdin G are identical. However, the molecular mass of **4** is 2

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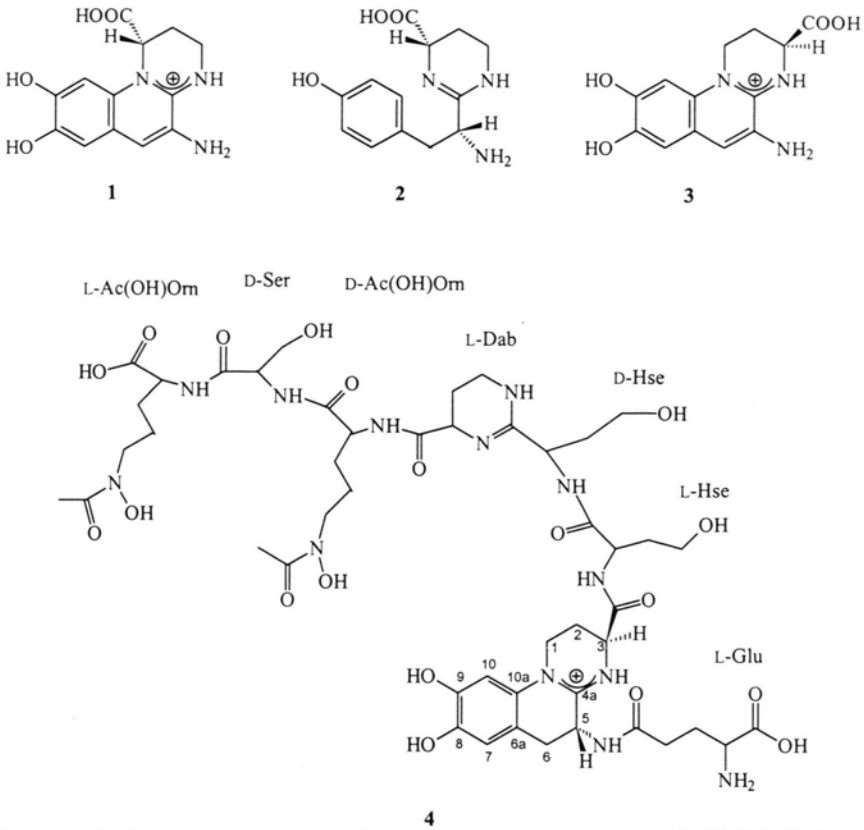

units higher and the UV/Vis data correspond to those of 5,6-dihydro-pyoverdins. Accordingly, the ^{1}H- and ^{13}C-data (Tables I and II) of the positions 4a to 10a of the chromophore match those of the 5,6-dihydro-pyoverdin Pp2 (Gwose *et al.*, 1992) while those of the positions C- 1 to C-3 agree with those of azoverdin G (Michalke *et al.*, 1996) in accordance with the position of the carboxyl group at C-3 rather than at C-1. From these data it follows that **4** has the structure of a 5,6-dihydro-iso-pyoverdin. This could be confirmed by the oxidative transformation of **4** into azoverdin G.

Table I. ^{1}H NMR data of the chromophores of 5,6-dihydro-azoverdin G (**4**), (H_2O, pH 3.0) azoverdin G (H_2O, pH 4.3) and 5,6-dihydro-pyoverdin Pp2 (D_2O, pH 3.0).

	5,6-dihydro-azoverdin G (**4**)	azoverdin G	5,6-dihydro-pyoverdin Pp2
Chr-1	3.71/4.01	3.86/4.40	5.28
Chr-2	2.48	2.58	2.50/2.57
Chr-3	4.59	4.60	3.23/3.68
Chr-5	5.10	–	5.50
Chr-6	3.03	7.81	3.03/3.06
Chr-7	6.80	6.88	6.81
Chr-10	6.85	6.94	6.85

Table II. ^{13}C NMR data of the chromophores of 5,6-dihydro-azoverdin G (**4**), (H_2O, pH 3.0) azoverdin G (H_2O, pH 4.3) and 5,6-dihydro-pyoverdin Pp2 (D_2O, pH 3.0).

	5,6-dihydro-azoverdin G (**4**)	azoverdin G	5,6-dihydro-pyoverdin Pp2
CO	172.0	173.3	171.2
Chr-1	43.2	43.9	56.6
Chr-2	23.5	22.9	23.4
Chr-3	51.9	51.2	37.3
Chr-4a	160.4	149.2	161.1
Chr-5	48.5	117.1	48.3
Chr-6	29.0	139.6	29.1
Chr-6a	117.1	115.3	117.4
Chr-7	117.3	113.0	117.6
Chr-8	143.8	146.3	143.4
Chr-9	145.2	152.6	145.1
Chr-10	106.2	101.7	105.7
Chr-10a	129.9	133.9	129.2

5,6-Dihydro-pyoverdins were found to co-occur with the corresponding pyoverdins in the fermentation broth especially when a high cell density and consequently a certain lack of oxygen prevails. They are the immediate biogenetic precursors of the pyoverdins. On the currently accepted biogenetic scheme (Böckmann *et al.*, 1997) the branching point for the formation of isopyoverdins are the ferribactin intermediates **2** preceding the ring closure. The discovery of the first 5,6-dihydro-isopyoverdin shows that the subsequent biogenetic steps are the same in both series.

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