Absolute Configuration of the Isopyoverdin Chromophore*

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By ozonolytic degradation of the chromophore of isopyoverdin from *Pseudomonas putida* BTP 1 L-2,4-diaminobutyric acid was obtained. This proves that the C-3 of the chromophore is S-configurated.

Introduction

In preceding publications we presented 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. **2** (or one of its hypothetic derivatives; Böckmann *et al.*, 1997) should also be the biogenetic branching point for the formation of the recently discovered isopyoverdin (Jacques *et al.*, 1995) chromophore **3a**. In this case the configuration of the chiral center at C- 3 of **3** should be S as it should be derived from the α-C of L-Dab. Now we report degradation studies which prove that the assumption is correct.

Abbreviations. Common amino acids, 3-letter code; Dab, 2,4-diaminobutyric acid; GC/MS, gas chromatography combined with mass spectrometry; RP-HPLC, reversed phase high performance liquid chromatography.

* Part LXXI of the series "Bacterial Constituents". For part LXX see Böckmann *et al.* (1997).

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Experimental Procedures

For the culture of *Pseudomonas putida* BTP1, the isolation of isopyoverdin from the culture medium and for other experimental details not reported here see Jacques *et al.* (1995).

Hydrolysis of isopyoverdin

Ten portions of 50 mg of isopyoverdin were dissolved in 8 ml 3M HCl each. N_2 was bubbled through the solutions for 10 min which subsequently were kept at 110 °C for 5 days. The hydrolysate was brought to dryness and redissolved in H_2O. This procedure was repeated 3 times. The combined residues were dissolved in 30 ml 1M HCl, adsorbed on SepPak RP-18 (WATERS, Milford, USA) and washed with H_2O to remove the amino acids from the hydrolysate. The chromophore **3b** (the NH_2-group of **3a** is replaced by an OH-group during hydrolysis; Michels *et al.*, 1991) was desorbed with CH_3OH/H_2O 9:1 (v/v), the eluate was brought to dryness and redissolved in 40 ml 1M HCl. Purification was achieved by RP-HPLC on Nucleosil 100 C-18, 5 µm (KNAUER, Berlin) with a gradient 0.1% CF_3COOH/CH_3OH going from 10 to 50% CH_3OH within 40 min. Detection at 254 nm.

Ozonolysis

Through a suspension of 50 mg **3b** in 3 ml dry $CHCl_3$ cooled to -30 °C O_3 (Ozon Generator FISCHER, Meckenheim; 3 vol.-%, gas stream 10 l/hr; O_2 dried by passing through conc. H_2SO_4 and over silicagel) was bubbled for 1 hr (a deep blue color developped), then dry dimethylformamide was added drop by drop until **3b** was dissolved completely. The treatment with O_3 was continued for 7 hrs, afterwards the solution was allowed to warm up over 16 hrs to 5 °C. Cooling to -30 °C, treatment with O_3 for 8 hrs and warming up over 16 hrs was repeated 3 times, then the solution was brought to room temp. Material deposited at the end of the gas inlet tube was dissolved with CH_3OH. To the combined solutions which were concentrated i.v. to 4 ml, 15 ml HCOOH, 5 ml H_2O_2 (30%) and 3 drops conc. H_2SO_4 were

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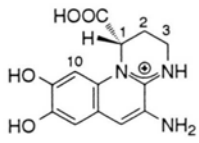

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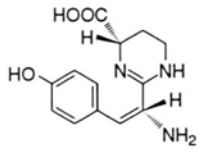

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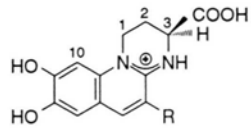

3a: R=NH_2
3b: R=OH

added. After warming to 60 °C for 30 min the mixture was again concentrated i.v. to 4 ml, and after addition of 5 ml H_2O_2 (30%) it was refluxed for 5 hrs and subsequently kept for 50 hrs at room temp. The reaction mixture was concentrated i.v., diluted with H_2O and concentrated again. This procedure was repeated 3 times. Finally the oily residue was freed from solvents at 40 °C and 0.01 torr. After transformation into the TAP derivative (Michels *et al.*, 1991) gas chromatography on a Chirasil-L-Val column (CHROMPACK, Middelburg, NL) and GC/MS on a L-Chirasil-Val column (Macherey & NAGEL, Düren) in comparison with authentic D,L-TAP-Dab demonstrated that L-Dab had been obtained.

Results and Discussion

By independent measures (X-ray, CD and chemical degradation) it had been shown (Michels *et al.*. 1991) that the chromophore **1** common to all pyoverdins is S-configurated at C-1. Comparison of the CD-spectra confirmed this observation for additional members of this class which had been investigated subsequently. When the first isopyoverdin had been found (Jacques *et al.*, 1995) it was surmised that its chromophore **3a** could have the same precursor as the pyoverdin chromophore **1**, *viz.* that of a ferribactin (**2a**) derived from D-Tyr and L-Dab, and hence the configuration of **3a** at C-3 should also be S. The degradation results reported above confirm this hypothesis and substantiate the assumption that pyoverdins and isopyoverdins belong to the same biogenetic family (Böckmann *et al.*, 1997).

Acknowledgements

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