Synthesis of the Stereoisomers of β-Hydroxyhistidine and their Analytical Identification in Hydrolysates of Bacterial Peptides

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β-Hydroxyhistidine, Stereospecific Synthesis

The synthesis of the four stereoisomers of β-hydroxyhistidine and their analytical identification is reported.

Introduction

Ser (= β-hydroxy Ala) belongs to the proteinogenic amino acids while other β-hydroxy amino acids prevail in bacterial peptidic metabolites, often acting as Fe³⁺ chelating ligands. Thus, β-hydroxy Asp is frequently encountered in the Pseudomonas siderophores (pyoverdins) [1], β-Hydroxy Leu and 3-hydroxy-3-methyl Pro were recently found in a depsipeptide from Streptomyces sp. [2]. L-Erythro-β-Hydroxy His is a characteristic component of bleomycins from Streptomyces verticillus [3]. L-Threo-β-hydroxy His was found in exochelin MN from Mycobacterium neoaurum [4] and in pyoverdin PF244 from Pseudomonas fluorescens [5]. In our studies of Pseudomonas siderophores we recently encountered twice amino acids that from spectral evidence could have been β-hydroxy His [6]. Therefore, we needed authentic comparison material of all four stereoisomers. In the literature syntheses are described for the S-erythro isomer [7] and one preparation without experimental details for the R-threo isomer in about 90% purity [7]. So we decided to synthesize all four stereoisomers 1–4 for the formation of derivatives which allow a direct comparison with the material obtained from the degradation of the siderophores.

Abbreviations: Common amino acids, 3-letter code; TMS, trimethylsilyl; Chromatography: GC, gas chromatography; RP-HPLC, reversed phase high performance liquid chromatography; TLC, thin layer chromatography; Mass spectrometry: CI, chemical ionisation; EI, electron ionisation; FAB, fast atom bombardment; PI, positive ion; GC-MS, GC coupled with a mass spectrometer; NMR: DSS, 2,2-dimethyl-2-silapentane-5-sulfonate; TMS, tetramethylsilane.

<ktms> = <3(DSS) for 1H and <3(TMS) = <3(DSS) - 1.61 ppm for 13C.

Materials and Methods

Instruments and materials

NMR: Bruker (Karlsruhe) AM 300 (1H 300, 13C 75.5 MHz), chemical shifts relative to TMS with internal standard DSS using the relations δ(TMS) = δ(DSS) for 1H and δ(TMS) = δ(DSS) - 1.61 ppm for 13C.

Mass spectrometry: FAB-MS Finnigan MAT (Bremen) HSQ 30 with a FAB gun from Ion Tech (Teddington, GB), gas Xe. GC-MS Finnigan (San Jose, CA, USA) Incos 500 with Varian (Walnut Creek, CA, USA) 3400 gas chromatograph, capillary column SE-54 (Chromatographie Service, Langerwehe).

X-ray analysis: Enraf-Nonius-CAD4 Diffractometer (Nonius, Delft, NL).


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HPLC: Knauer (Berlin) HPLC pump 64; columns: Knauer Kromasil C₄ (5 μm, 250x4 mm), Macherey-Nagel (Düren) ET 200/8/4 Nucleodex β-OH (5 μm, 200x4 mm).

Column Chromatography: Merck (Darmstadt) silicagel 60 (70–230 mesh); Whatman (Whatman Balston, Tewksbury, MS, USA) cellulose powder CF 11; Serva (Heidelberg) Dowex 50 WX 8 (H⁺-form, 20–50 mesh); Serva XAD-4 (0.3–1 mm, pores 85–90 Å; Pharmacia (Freiburg) SP Sephadex C-25; Waters (Milford, MS, USA) Sep-Pak RP₁₈-cartridge.

TLC: Macherey-Nagel Polygram cellulose plates CEL 300 UV₂₅₄ (0.1 mm); Polygram silicagel plates SIL G/UV₂₅₄ (0.25 mm).

Reagents

All chemicals were of p.a. quality. Solvents were distilled prior to use and -when necessary- freed from water. For HPLC deionized and twice distilled water purified on XAD-4 resin was used.

Inert techniques

Reaction flasks were evacuated (600 Pa) and heated for removal of adsorbed water, then flushed with N₂ (purity grade 2.0), passed over Cu-cat., P₂O₅ and KOH, and through conc. H₂SO₄. Syringes were dried at 110 °C for 24 hrs; during the addition of reagents and during the entire reaction the whole system was flushed by N₂ purified as described above.

Syntheses

(2S,5S,6S)-1-Benzoyl-2-t-butyl-5-[hydroxy(1’-triphenylmethyl-4’-imidazolyl)methyl]-3-methylimidazolidin-4-one (7) and (2R,5S,6S)-5-[benzoyl(1’triphenylmethyl-4’-imidazolyl)methyl]-2-t-butyl-3-methylimidazolidin-4-one (8)

Under inert conditions (v. supra) in a 250 ml three-neck flask 0.388 ml (2.75 mmol) diisopropylamine in 50 ml dry tetrahydrofuran (THF) were cooled to –78 °C. 1.72 ml (2.75 mmol) of n-butyl lithium as a 1.6 M solution in hexane was added drop by drop. The colorless solution was stirred for 20 min., then 0.65 g (25 mmol) (2S)-1-benzoyl-2-t-butyl-3-methylimidazolidin-4-one (6) in 20 ml dry THF was added in small portions. The orange-coloured solution was cooled to –100 °C. After 20 min stirring, 1.69 g (5 mmol) 1-triphenyl-4-imidazole aldehyde (5) [8] in 10 ml THF was added drop by drop and the mixture was stirred for 30 min at –100 °C (liquid N₂/diethyl ether) and subsequently for 30 min at room temperature. With the help of a syringe the mixture was transferred into a saturated aqueous NH₄Cl solution. The aqueous phase was separated and extracted 3 times with portions (20 ml each) of diethyl ether. The combined organic phases were dried over MgSO₄ and freed of the solvents i. v. By chromatography on silica gel (diethyl ether:hexane:acetone 50:26:6 v/v/v) unreacted starting materials were removed. The reaction products 7 and 8 were eluted with acetone. The partially crystalline mixture (yield 1.17 g) contained 7 and 8 in a ratio of 3:1 (by NMR).

(2S,5S,6S)-1-Benzoyl-2-t-butyl-5-[hydroxy(1’-triphenylmethyl-4’-imidazolyl)methyl]-3-methylimidazolidin-4-one (7)

M.p.: (oil);

H NMR (CDCl₃): 7.50–7.02 (m, 20 arom. H; H-C(2)); 6.40 (s, H-C(5)); 5.63 (d, J=11.2, OH); 5.31 (s, H-C(2)); 4.61 (d, J=5.5, H-C(5)); 4.12 (dd J=11.2; 5.5, H-C(6)); 2.99 (s, CH₃N); 1.01 (s, f-butyl).

¹³C NMR (CDCl₃): 173.5; 170.8; 142.5; 139.2; 139.1; 138.1; 136.4; 132.1; 129.1; 128.4; 128.3; 121.9; 80.8; 75.5; 67.7; 61.0; 41.1; 33.0; 26.5. EIL-MS: 541; 243 (100); 165; 105; 91; 77. PI-FAB-MS (thioglycerol): 599 [M+H]⁺; 243 (100).

(2R,5S,6S)-5-[benzoyl(1’triphenylmethyl-4’-imidazolylmethyl]-2-t-butyl-3-methylimidazolidin-4-one (8)

M.p.: 86 °C;

H NMR (CDCl₃): 7.98–7.05 (m, 20 arom. H; H-C(2)); 6.97 (d, J=1.5, H-C(5)); 6.28 (d, J=2.4, H-C(6)); 4.38 (d, J=2.4, H-C(5)); 2.86 (s, CH₃N); 1.01 (s, f-butyl).

¹³C NMR (CDCl₃): 173.0; 165.5; 142.4; 139.1; 137.7; 133.1; 130.5; 130.0; 129.9; 128.5; 128.4; 128.3; 121.7; 84.2; 75.8; 70.4; 62.7; 37.3; 31.1; 25.4.

EI-MS: 541; 243 (100); 165; 105; 91; 77. PI-FAB-MS (thioglycerol): 599 [M+H]⁺; 243 (100).

S‒Threeo-β-hydroxyhistidine-monohydrochloride-monohydrate (4)

S‒Threeo-β-hydroxyhistidine-monohydrochloride-monohydrate (4)

In a 50 ml flask the mixture of 0.78 g (1.3 mmol) 7 and 8 was heated with 20 ml 6 N HCl under stirring for 24 h at 100 °C. The aqueous phase was extracted 4 times with portions of diethyl ether (15 ml each) and then brought to dryness i. v. The obtained product was recrystallized twice from acetone/water. Yield 0.19 mg colourless needles.

M.p.: 190–200 °C (decomposition);

rot.: [α]⁴⁻³⁶⁵ = −24 ± 2° (c=1mol/l, in H₂O).
1H NMR (D2O, pH 4.3): 8.72 (d, J=1.3, H-C(2)); 7.51 (dd, J=0.9;1.3, H-C(5)); 5.42 (dd, J=5.1; 0.9, H-C(3)); 4.03 (d, J=5.1, H-C(2)).

13C NMR (D2O, pH 4.3): 171.9; 135.6; 133.5; 117.9; 65.0; 60.0.

EI-MS: 171 (M+); 109; 97 (100); 81; 69; 54; 44.


**R-Threo-β-hydroxyhistidine-mono-hydrochloride-mono-hydrate (3)**

The same procedure as described above was used starting from (2R)-1-benzoyl-2-tert-butyl-3-methylimidazolidin-4-one.

M.p.: 190–200 °C (decomposition);
rot.: [a]_rt365 = +22 ± 2° (c=1mol/l, in H2O).

1H NMR (D2O, pH 4.3): 8.72 (d, J=1.3, H-C(2)); 7.51 (dd, J=0.9;1.3, H-C(5)); 5.42 (dd, J=5.1;0.9, H-C(3)); 4.03 (d, J=5.1, H-C(2)).

13C NMR (D2O, pH 4.3): 171.9; 135.6; 133.5; 117.9; 65.0; 60.0.

EI-MS: 171 (M+); 109; 97 (100); 81; 69; 54; 44.


**N/O-Pentafluoropropionyl-O-isopropylester of β-hydroxy His.**

1.5 mg (0.067 mmol) β-hydroxy His in 1 ml isopropanol/acetylchloride (5:1 (v:v)) was heated to 110 °C for 1 h. After cooling the reaction mixture to room temp, the solvent was removed i. v. The reaction product was solved in dichloromethane, treated with ultrasonic for 1 min and heated with 0.3 ml pentafluoropropionic anhydride to 150 °C for 5 min. After cooling to room temp, the reaction mixture was brought to dryness i. v. The N,O-acyl derivative was dissolved in 100 μl dichloromethane and used for GC-MS experiments immediately.

**Silylation of β-hydroxy His with N-methyl-N-trimethylsilyltrifluoroacetamide**

1.5 mg (0.067 mmol) β-hydroxy His was heated for 2 h in 300 μl N-methyl-N-trimethylsilyltrifluoroacetamide to 130 °C. The reaction mixture was cooled to room temp. and used for GC-MS experiment immediately.

**Results and Discussion**

**Syntheses**

**R, S-Erythro-β-hydroxyhistidine (1) + (2)**

As several syntheses of R, S-erythro-β-hydroxy His are known (v. supra) the erythro racemate was synthesized for further separation and derivatisation studies according to literature [9].

**R- and S-Threo-β-Hydroxyhistidine (3) + (4)**

The enantioselective synthesis of R- and S-threo-β-hydroxy His was effected by a modification of the procedure introduced by Seebach [10] (Scheme 2). 4-Formyl-1-triphenylmethyl-imidazole aldehyde (5) [11] (the introduction of the triphenylmethyl group renders the aldehyde soluble in THF) is allowed to react with (2S)-6 or (2R)-1-benzoyl-2-tert-butyl-3-methylimidazolidin-4-one. The 2-tert butyl group shields one side of the molecule and the chirality at C-2 thus determines the course of the reaction. The mixture of 7 and 8 can be used for the further steps of the synthesis without separation. 3 was obtained with 92% ee, 4 with 94% ee. The absolute stereochemistry of 3 and 4 was confirmed by X-ray analysis of single crystals of the monohydrates-mono-hydrochlorides.

**Characterisation of the enantiomers 1 – 4**

**Chromatography**

(a) RP-HPLC. Derivatisation with dansyl chloride [12] resulted in a mixture of mono- and didansylate. On the achiral Kromasil C4 column using an acetic acid (20 mm)/methanol gradient (80 to 2% acetic acid (27 min); 2% acetic acid (8 min)) the threo monodansylates eluted before the erythro monodansylates while for the didansylates the order was reversed. On the chiral Nucleodex column using a TEA-acetic acid (pH 3.9) buffer/ methanol gradient (90 to 45% buffer (18 min); 45 to 2% buffer (9 min); 2% buffer (5 min)) the sequence for the monodansylates is S-threo, R-threo, S-erythro, R-erythro, and for the didansylates S-erythro, S-threo, R-erythro, R-threo.

(b) GC-MS. For the analysis of amino acids both with achiral and chiral columns the N/O-perfluoroacyl isopropyl esters were used [13]. Best results were obtained with pentafluoropropionic anhydride. However, not the mass spectrum of the expected N,O-acyl derivative was observed, but rather that of two decomposition products formed by the loss of pentafluoropropionic acid and of isopropanol, resp. [14].

Trimethylsilylation of β-hydroxy His gave a mixture of O,O-di-TMS and of N,N,O,O-tetra-TMS.
derivatives. The best results were obtained with N-methyl-N-trimethylsilyltrifluoroacetamide. On a SE-54 column both the di- and tetra-TMS derivatives of the erythro isomers eluted before those of the threo-isomers.

**NMR spectroscopy**

In Tables I and II the $^1$H and $^{13}$C NMR spectral data of threo- and erythro-$\beta$-hydroxy His are compiled. In the $^1$H-spectra the shift differences are about 0.1 ppm for the protons in the aliphatic chain and for the 5'-ring proton. Surprisingly the largest shift differences in the $^{13}$C-spectra cannot be found in the asymmetric carbon centers C-2 and C-3, but rather in those neighboring them, viz. C-1 and C-4'.

| Table I. $^1$H NMR data of threo- and erythro-$\beta$-hydroxy His at pH 4.3. |
|---|---|---|---|
| Atom | threo $\delta$ (ppm) | $J$ (Hz) | erythro $\delta$ (ppm) | $J$ (Hz) | $\Delta$ ppm |
| 2 | 4.03 | d, 5.1 | 4.17 | d, 3.7 | 0.14 |
| 3 | 5.42 | d.dd, 5.1, 0.9 | 5.51 | d.dd, 3.7, 0.9 | 0.09 |
| 2' | 8.72 | d, 1.3 | 8.70 | d, 1.3 | 0.02 |
| 5' | 7.51 | d.dd, 0.9, 1.3 | 7.42 | d.dd, 0.9, 1.3 | 0.09 |

| Table II. $^{13}$C NMR data of threo- and erythro-$\beta$-hydroxy His at pH 4.3. |
|---|---|---|---|
| Atom | threo $\delta$ (ppm) | erythro $\delta$ (ppm) | $\Delta$ ppm |
| 1 | 171.9 | 171.1 | 0.8 |
| 2 | 60.0 | 60.0 | 0.0 |
| 3 | 65.0 | 64.9 | 0.1 |
| 2' | 135.6 | 135.1 | 0.5 |
| 4' | 133.5 | 132.1 | 1.4 |
| 5' | 117.9 | 117.6 | 0.3 |

**Conclusions**

The four stereoisomeric $\beta$-hydroxy His ($RR$, $SS$ = threo-, $RS$, $SR$ = erythro-) can be distinguished by their chromatographic and NMR-spectroscopic properties. However, the differences are rather small and in the absence of other criteria (as by a differentiation between the D- and L- ($R$- and $S$-) amino acids by enzymatic methods [15]) reference compounds should be available.
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