Monatshefte für Chemie 131, 645–653 (2000)

Monatshefte für Chemie
Chemical Monthly

Microbial and Enzymatic Synthesis of Optically Pure *D*- and *L*-3-Trimethylsilyl-alanine by Deracemization of *D*,*L*-5-Trimethylsilyl-methyl-hydantion

Markus Pietzsch[1,*], **Thomas Waniek**[1], **Richard J. Smith**[2], **Svetoslav Bratovanov**[2], **Stefan Bienz**[2], and **Christoph Syldatk**[1]

[1] Institute of Biochemical Engineering, University of Stuttgart, D-70569 Stuttgart, Germany
[2] Institute of Organic Chemistry, University of Zurich, CH-8057 Zurich, Switzerland

Summary. The sterospecificities of hydantoinases and N-carbamoyl amino acid amidohydrolases (N-carbamoylases) from different microbial sources were investigated for the stereoselective syntheses of the unnatural silicon-containing amino acids *D*- and *L*-3-trimethylsilyl-alanine (**3**) from the respective racemic hydantoin *D*,*L*-**1**. In a preparative biotransformation, whole resting cells of *Agrobacterium sp*. IP I 671, immobilized in a Ca-alginate matrix, were used for the synthesis of amino acid *D*-**3** in 88% yield and 95% enantiomeric excess. Since the purified *D*-N-carbamoylase from *Agrobacterium sp*. IP I 671 was shown to be 100% *D*-selective, the enantiomeric purity of 95% of *D*-**3** arising from the transformation with the immobilized cells must be explained by the participation of a further, *L*-selective N-carbamoylase or, which is more likely, by racemization of the final hydrolysis product by the action of an amino acid racemase. Isolated hydantoinases from *Bacillus thermoglucosidasius*, *Thermus sp*., *Arthrobacter aurescens* DSM 3745, and *Arthrobacter crystallopoietes* DSM 20117 turned out to be stereospecific for the conversion of the *D*-form of hydantoin *D*, *L*-**1**. The enantiomerically pure *L*-form of **3** was also prepared. It was synthesized from racemic N-carbamoyl amino acid *D*,*L*-**2** by enantiomer-specific hydrolysis of the *L*-form in presence of *L*-N-carbamoylase from *Arthrobacter aurescens* DSM 3747.

Keywords. *D*-3-Trimethylsilyl-alanine; Hydantoinase; N-Carbamoyl amino acid amidohydrolase; N-Carbamoylase; Biotransformation.

Introduction

Optically active silicon-containing amino acids represent unnatural compounds with interesting pharmaceutical behavior. When, e.g., in a biological active renin inhibitor *L*-phenylalanine was replaced by *L*-3-trimethylsilyl-alanine (*L*-**3**), in contrast to the parent compound the silicon-containing analog was largely resistant towards proteolytic digestion, whereas the inhibition constant was affected only marginally [1].

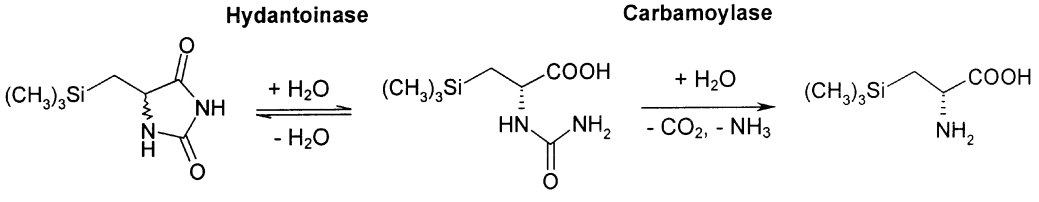
* Corresponding author

Several approaches towards optically active silicon-containing amino acids have been published. The *Schöllkopf*, *Seebach*, or *Myers* methods of asymmetric α-alkylation of glycine enolate equivalents [1–3] or the *Evans* procedure of asymmetric α-bromination of carboxylic acid derivatives followed by substitution of the bromine atom [4] gave access – at least principally – to either antipode of compound **3** in high enantiomeric excesses. The overall yields attained with these syntheses, however, were rather low, and chiral auxiliaries had to be used in stoichiometric amounts.

In 1996, *Yamananka et al.* reported on the first biotransformation for the preparation of a silicon-containing amino acid. With *L*-aminoacylase from porcine liver as the biocatalyst, *L*-**3** was prepared by enantiomer-specific hydrolysis of *L*-N-acetyl-3-trimethylsilyl-alanine from the racemate [5]. Since this reaction represents a kinetic resolution, a yield of 50% can be obtained at its best, and the transformation is thus not efficient. The same drawback suffers the kinetic resolution of a silicon-containing N-carbamoyl amino acid (synthesis of *D*-*p*-trimethylsilylphenyl-alanine) by the action of a *D*-N-carbamoylase from *Blastobacter sp.* A17 p-4 [6].

If applicable to silicon-containing substrates, no limitation with respect to yields would be present in the hydantoinase process that was studied and developped in our group [7]. The hydantoinase process consists of a casade of two hydrolytic enzymes *i.e.* a hydantoinase and a N-carbamoylase (see Scheme 1), in combination with chemical or enzymatic racemization of the starting hydantoin. The hydantoinase is responsible for the partial hydrolysis of a hydantoin to the respective N-carbamoyl amino acid and the N-carbamoylase for the subsequent deaminocarbonylation to the corresponding amino acid. Because 5-monosubstituted hydantoins racemize spontaneously already under slightly alkaline conditions, enzymatically accepted enantiomeric form of the hydantoin is constantly resupplied from the racemate. A theoretical yield of 100% is thus possible for the formation of enantiomerically pure amino acids departing from racemic starting materials.

We describe in the following that silicon-containing amino acids are in fact accessible by the hydantoinase process. Several biocatalysts of different micro-organisms have been studied and were found to hydrolyze enantio-selectively one component of *D*,*L*-5-trimethylsilylmethyl-hydantoin (*D*,*L*-**1**) or *D*,*L*-N-carbamoyl-3-trimethylsilyl-alanine (*D*,*L*-**2**), respectively. The biocatalysts investigated are whole resting cells of *Agrobacterium sp*. IP I 671 and its enriched N-carbamoylase [8], commercially available hydantoinase from *Bacillus thermoglucosidasius* and



1 **2** **3**

Scheme 1

Thermus sp., purified hydantoinase from *Arthrobacter aurescens* DSM 3745 [9], and recombinant *L*-N-carbamoylase from *Arthrobacter aurescens* DSM 3747 [10].

Results and Discussion

Biotransformations with whole cells of Agrobacterium sp. IP I 671

It has been shown by *Runser et al.* in 1990 that whole cells of *Agrobacterium sp*. IP I 671 catalyze the hydrolysis of *D*,*L*-5-(*p*-hydroxyphenyl)-hydantoin to form enantiomerically pure *D*-(*p*-hydroxyphenyl)-glycine in excellent yield [11]. We have tested the same system for the biotransformation of our silicon-containing test substrate, hydantoin *D*,*L*-**1**. Hence, racemic hydantoin *D*,*L*-**1** was incubated with immobilized cells of *Agrobacterium sp*. IP I 671 (25% cell wet mass (CWM) in Ca-alginate) under anaerobic conditions and delivered the *D*-form of the desired amino acid in 88% yield and 95% *ee*. The kinetics of the transformation, together with the mass balance, are graphically shown in Fig. 1a.

It is imperative for the transformation that it is carried out under anaerobic conditions. In presence of oxygen, partial decomposition of the desired amino acid is observed. Figure 1b shows that the curve for the mass balance (of substrate, intermediate, and product) declines rapidly up to a reaction time of approximately 40 h. After this time, the concentration of the amino acid as well as the mass balance remain almost constant.

The degradation of the amino acid might be explained by the action of an amino acid dehydrogenase. In *Agrobacterium sp*. IP I 671, the gene coding for an enzyme with close homologies to such amino acid dehydrogenases was found to be located on the same operon as the hydantoinase and the N-carbamoyl amino acid amidohydrolase [12]. Evidently, the enzyme responsible for the degradation reaction is deactivated after approximately 40 h, not affecting the transformation any longer.

The silicon-containing amino acid *D*-**3**, formed in a preparative biotransformation of 500 mg of hydantoin *D*,*L*-**1**, was isolated and purified by means of ion exchange chromatography. The amino acid had to be separated from a number of other compounds that were liberated from the whole cell biocatalyst. As is recognized from the reversed phase HPLC chromatograms shown in Fig. 2, several compounds that are not related to the silicon-containing substrate were found in the broth of both the biotransformation and a substrate-free incubation of the cells in the buffer.

The enantiomeric excess of the *D*-3-trimethylsily-alanine (*D*-**3**) obtained by the hydantoinase process with whole cells of *Agrobacterium sp*. IP I 671 was determined to be 95%. Since isolated *D*-N-carbamoylase from the same microorganism turned out to be 100% *D*-selective (see below), not delivering traces of the *L*-antipode of **3**, the lower enantiomeric purity obtained by the biotransformation in presence of the whole cells was rather surprising. It can be explained, however, with the possible participation of a further, *L*-selective N-carbamoylase – as shown to be present, *e.g*., in a *Blastobacter* strain [6] – or with the more likely possibility of racemization of *D*-3-trimethylsilyl-alanine (*D*-**3**) by the action of an amino acid racemase [13]. Altogether, several of the drawbacks commonly associated with the use of whole cell

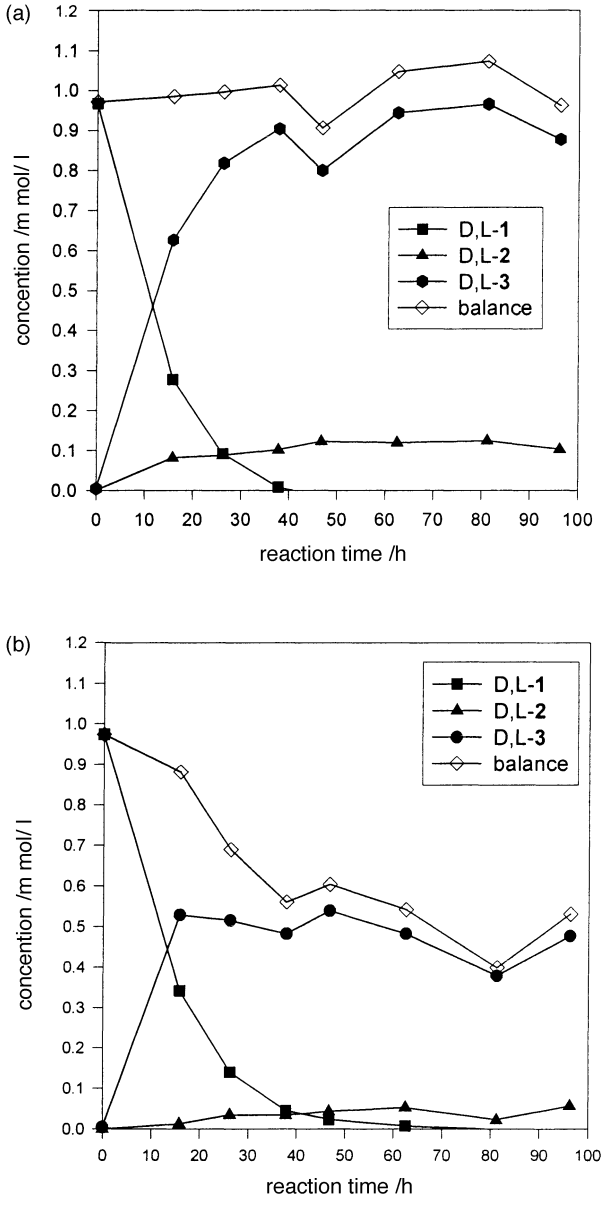


Fig. 1. Hydrolysis of *D,L*-**1** with immobilized cells of *Agrobacterium sp.* IP I 671 as the biocatalyst under (a) anaerobic and (b) aerobic conditions

biocatalysts [14] were experienced with the whole cell biotransformation of *D,L*-**1**, too. Therefore, several isolated enzymes, either in free or in immobilized form, were investigated for their ability to enantio-selectively hydrolyze racemic hydantoin *D,L*-**1** or racemic N-carbamoyl amino acid *D,L*-**2**.

*Hydrolysis of racemic hydantoin D,L-***1** *with hydantoinases*

Racemic hydantoin *D,L*-**1** was treated with H_2O in the presence of immobilized hydantoinase from *Arthrobacter crystallopoietes* DSM 20117 (on Eupergit C,

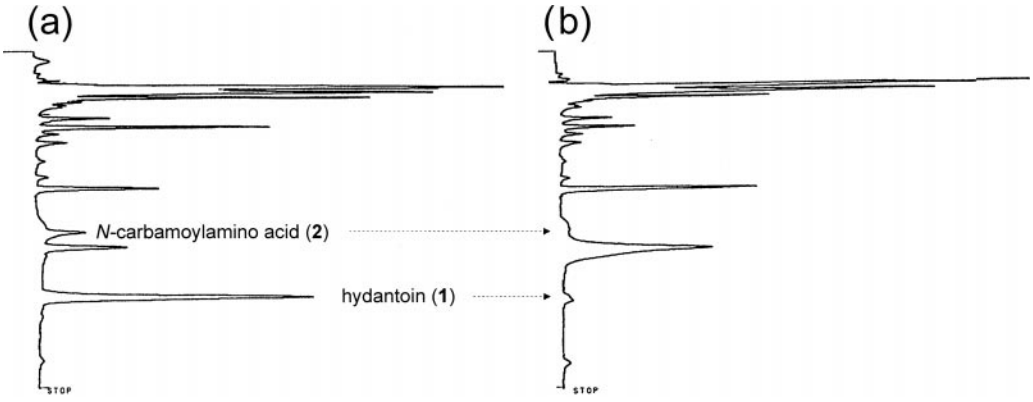


Fig. 2. (a) HPLC chromatogram of a sample taken during the biotransformation of *D*,*L*-**1** in presence of immobilized cells of *Agrobacterium sp*. IP I 671 and (b) HPLC chromatogram of a reference sample of the broth of the same cells without substrate

[15]), free hydantoinase from *Arthrobacter aurescens* DSM 3745 [9], and the commercially available hydantoinases from *Bacillus thermoglucosidasius* and *Thermus sp*. All four enzymes were found to catalyze the partial hydrolysis of *D*,*L*-**1** to the N-carbamoyl amino acid **2**. As disclosed by HPLC on a chiral medium, all enzymes were stereospecific for the *D*-enantiomer, delivering exclusively the *D*-form of compound **2**. The stereochemical results of the reactions, except for the transformation in presence of the hydantoinase from *Arthrobacter aurescens* DSM 3745, correspond to our expectations, which are based on the enzyme-catalyzed transformations of other hydantoin derivatives. The hydantoinase from *Arthrobacter aurescens* DSM 3745 represents a special case. It is known to change its enantiomeric preference with the substrate, converting, *e.g*., the hydantoins of tryptophan [9] and phenylalanine [15] with *L*-selectivity and the hydantoin of methionine with *D*-selectivity [9]. The silicon-containing hydantoin **1** is the first substrate which is converted by *Arthrobacter aurescens* DSM 3745 with complete *D*-stereospecificity.

*Hydrolysis of racemic N-carbamoyl amino acid D,L-***2** *with free and immobilized D- and L-N-carbamoylases*

Since hydantoinases catalyze the reversible hydrolysis of hydantoins to N-carbamoyl amino acids, most related biotransformations deliver mixtures of substrates and products. In the case of silicon-containing hydantoin *D*,*L*-**1**, an equilibrium mixture of *D*,*L*-**1** and *D*-**2** in a ratio of approximately 20:80 was formed (corresponds to a conversion of about 80%). To shift the equilibrium towards the side of the product, an N-carbamoylase with matching stereospecifity should be added to the system. Thus, two N-carbamoylases, catalyzing the irreversible deaminocarbonylation of N-carbamoyl amino acids to the free amino acids, were investigated for their substrate- and stereoselectivity. Enriched *D*-N-carbamoylase from *Agrobacterium sp*. IP I 671, used in free form, catalyzed the hydrolysis of the *D*-enantiomer of racemic N-carbamoyl-3-trimethylsilyl-alanine *D*,*L*-**2**. As can be seen from Fig. 3, the enzyme-

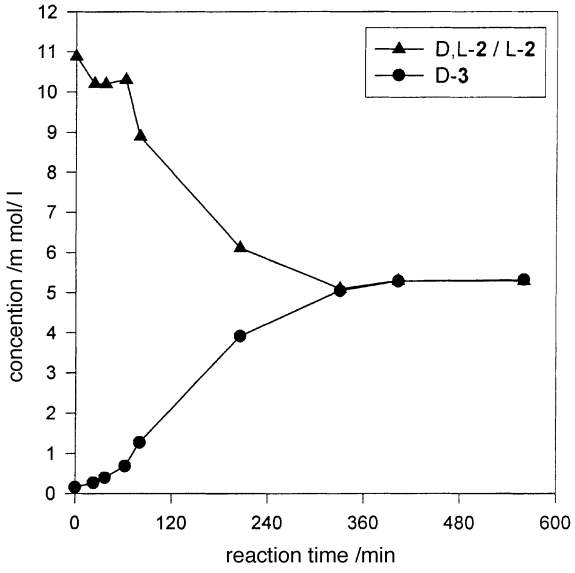


Fig. 3. Hydrolysis of *D,L*-N-carbamoyl-3-trimethylsilyl-alanine (*D,L*-**2**) with enriched *D*-N-carbamoylase from *Agrobacterium sp*. IP I 671 as the biocatalyst

catalyzed hydrolysis leads to the expected 50% conversion only, and it was shown by HPLC that exclusively *D*-3-trimethylsilyl-alanine (*D*-**3**) was formed in the process. On the other hand, the hydrolysis of *D,L*-**2** by the action of the N-carbamoylase from *Arthrobacter aurescens* DSM 3747 (immobilized enzyme), which is known for its *L*-stereospecifity, delivered exclusively the *L*-enantiomer of **3** after 50% conversion.

In summary, the hydantoinase process, an elegant method for the synthesis of enantiomerically pure unnatural amino acids, proved applicable for the production of enantiomerically pure *D*-3-trimethylsilyl-alanine (*D*-**3**), too. Four isolated hydantoinases, two N-carbamoylases, and a whole cell biocatalyst were tested as biocatalysts. They all proved to accept the silicon-containing substrates, and the biotransformations led with high stereoselectivities to the respective hydrolysis products. It is currently under investigation, whether other 5-monosubstituted silicon-containing hydantoins and N-carbamoyl amino acids are stereospecifically converted by these enzymes as well.

Experimental

General

Unless otherwise stated, all chemicals were of reagent grade and purchased from Fluka Chemie AG. Aqueous solutions were prepared with ultrapure H_2O (NANOpure II, Fa. Barnstedt, Newton, MA, USA). *D,L*-5-Trimethylsilylmethyl-imidazolidin-2,4-dione (*D,L*-5-trimethylsilymethyl-hydantoin, *D,L*-**1**), *D,L*-N-carbamoyl-3-trimethylsilyl-alanine (*D,L*-**2**), and *D,L*-**3**-trimethylsilyl-alanine (*D,L*-**3**) were synthesized as described previously [17, 18]. Since the non SI-unit U ($\mu mol \cdot min^{-1}$) is commonly used in the literature, this unit was also used in the present contribution. 1 U corresponds to 16.67 nKat ($10^{-9} mol \cdot s^{-1}$).

Microorganisms

Agrobacterium sp. IP I 671 was obtained from the Institute Pasteur (Paris, France) and cultivated according to *Meyer* and *Runser* [19]. After centrifugation, the whole cells were immobilized by entrapment in Ca-alginate as described previously for cells of *Trigonopsis variabilis* [20] with the following modifications: the cell wet mass (50 g) was suspended in 25 cm^3 of a 0.9% NaCl solution and mixed with 204 g of a solution of 4 g Na-alginate in 200 cm^3 of H_2O.

Enzymes

Enriched *D*-N-carbamoylase from *Agrobacterium sp*. IP I 671 and hydantoinases from *Bacillus thermoglucosidasius* (Hyd 1, carrier-fixed) and *Thermus sp*. (Hyd 2, suspension) were gifts from Roche Diagnostics (Penzberg, Germany). Hydantoinase from *Arthrobacter aurescens* DSM 3745 was purified as described previously [9]. Hydantoinase from *Arthrobacter crystallopoietes* DSM 20117, purified according to the literature [21], was immobilized on Eupergit C (Röhm Pharma, Germany) [15]. *L*-N-Carbamoylase from *Arthrobacter aurescens* DSM 3747 was purified from recombinant *Escherichia coli* [22] and immobilized on EAH-Sepharose [23].

Analytical methods

RP-HPLC: The concentrations of **1** and **2** were determined with reversed phase HPLC on a Gromsil ODS 1 PE column (5 µm, 250×4,6 mm, Grom, Herrenberg, Germany; eluent: 7 cm^3 of aq. H_3PO_4 (85%) in 2000 cm^3 of H_2O and 500 cm^3 of MeOH; flow rate: 1.0 $cm^3\ min^{-1}$) according to Ref. [9] with UV-detection at 210 nm. Retention times: **1**: 35.2 min; **2**: 28.0 min.

Quantification of **3**: Concentrations of **3** were measured photometrically after reaction of the amino acid with ninhydrin [24]. A 100 mm^3 sample (concentration of amino acid between 0.1 and 1 m*M*) was mixed with 100 mm^3 of acetate buffer (21.33 g of AcONa and 6.9 cm^3 of AcOH in 100 cm^3 of H_2O) and kept at 60°C for 5 min. To this mixture, 100 mm^3 of a ninhydrin solution (174 mg of ninhydrin and 174 mg of hydrindantin×2 H_2O in 15 cm^3 of 2-methoxyethanol) were added, and the sample was kept at 60° for 20 min. Afer cooling to 23°C it was diluted with *i*-PrOH/H_2O (1:1, 1000 mm^3), and the extinction was measured at a $\lambda = 570$ nm with a spectrophotometer (Ultrospec III, Pharmacia, Freiburg, Germany). Calibration was done with *D,L*-**3** in concentrations between 0.1 and 1 m*M*.

HPLC on a chiral phase: The separation of the enantiomers of *D,L*-**1** and *D,L*-**2** was accomplished by HPLC on an ET 200/4 Nucleodex β-PM column (Macherey-Nagel, Düren, Germany; eluent: 7 cm^3 H_3PO_4 (85%) in 2000 cm^3 H_2O and 500 cm^3 MeOH, *pH* adjusted to 3.7 with conc. aq. NaOH solution; flow rate: 0.2 $cm^3 \cdot min^{-1}$) coupled to a Spectra System (Thermo Separation Products, Egelsbach, Germany) with UV detection at 210 nm. Retention times: (*L*)-**1**: 77.0 min, (*D*)-**1**: 92.8 min, $\alpha = 1.21$, (*L*)-**2**: 28.2 min, (*D*)-**2**: 25.6 min, $\alpha = 1.10$.

*Bioconversion of D,L-**1** with immobilized whole cells of Agrobacterium sp. IP I 671*

Analytical scale: Under a blanket of N_2, racemic hydantoin *D,L*-**1** (9.3 mg, 50 µmol) was suspended in 50 cm^3 of a 0.1 *M* Tris/HCl-buffer containing 1% of $CaCl_2$ (*pH* 8.5), and immobilized cells of *Agrobacterium sp*. IP I 671 (5 g) were added at 37°C (a reference reaction was carried out without the N_2 atmosphere). The mixture was kept at 37°C, and samples were taken at intervals of 10–20 h and analyzed to determine the concentrations of **1**, **2**, and **3** as described above.

Preparative scale: Since large amounts of Tris/HCl buffer and $CaCl_2$ are difficult to remove from the reaction products the preparative scale bioconversion was carried out in pure H_2O. Under a blanket of N_2, racemic hydantoin *D,L*-**1** (460 mg, 2.5 mmol) was suspended in H_2O (200 cm^3). Immobilized cells (30 g) were added, the mixture was kept at 37°C, and samples were drawn and

analyzed in regular intervals to follow the reaction. After complete consumption of *D,L*-**1** (26 h), the immobilizate was filtered off, the filtrate passed through a membrane filter (0.45 µm)., and concentrated to 35 cm^3 by rotary evaporation. A portion of the concentrate (25 cm^3) was applied with a flow rate of 0.25 $cm^3 \cdot min^{-1}$ to a chromatography column (XK16/20, packed with 20 g of Dowex 50 WX8, 400 mesh, H^+) connected to an FPLC-system equipped with a UV spectrometer and a conductivity cell (Pharmacia, Freiburg, Germany). The column was washed with H_2O until absorption and conductivity remained constant before the amino acid was eluted by washing with 0.5 *M* NH_4OH at a flow rate of 10 $cm^3 \cdot min^{-1}$. Samples containing amino acid were detected by reaction with ninhydrin on a TLC plate, collected, concentrated at a temperature of < 50°C, and dried at 10^{-3} torr to yield 242 mg (1.5 mmol, 88%) of *D*-**3** (95% *ee*, see below) which was virtually pure (^{1}H NMR).

To obtain a sample for analysis, 28 mg (0.17 mmol) of *D*-**3** were dissolved in conc. aq. HCl, and the solvent was removed by evaporation. A solution of 0.16 cm^3 (0.51 mmol) of trimethylsilyl-diethylamine in 3 cm^3 of *THF* was added, and the mixture was stirred for 1 h. The precipitate was filtered off and washed with Et_2O (8 cm^3), the combined organic phases were concentrated, and the residue was dried at 10^{-3} torr. Hydrolysis was effected by the addition of acetone (1 cm^3) and H_2O (4 cm^3) and stirring for 30 min. The solvent was evaporated, the solid residue washed with acetone (2 cm^3), MeOH (1 cm^3), and Et_2O (4 cm^3), and dried at 10^{-3} torr to give 12.3 mg (45%) of *D*-**3** (95% *ee*).

$[\alpha]_D^{25} = -26.4$ ($c = 0.22$, 2 *M* aq. HCl) literature values for *L*-**3**: +31.0 ($c = 0.51$, 4 M aq. HCl) [2], +33.3 ($c = 0.526$, 4 *M* aq. HCl) [3], +21.4 ($c = 3.2$, aq. HCl) [4].

To determine the enantiomeric purity of the sample, *D*-**3** was transformed into *D*-**1** by reaction with KOCN and HCl [25] and analyzed by HPLC on a chiral phase. The analytical data of *D*-**3** (^{1}H and ^{13}C NMR spectroscopy elementary analysis, MS) were identical with those obtained for the racemic amino acid from which *D,L*-**1** was originally synthesized.

*Biotransformations of D,L-**1** with free and immobilized hydantoinases*

Hydantoinase from *Arthrobacter aurescens* DSM 3745 (31 mU; activity for the conversion of the standard *D,L*-5-benzylhydantoin) was added to 1 cm^3 of a 1 m*M* solution of *D,L*-**1** in 0.1 *M* Tris/HCl-buffer (*pH* = 8.5) at 37°C, and the mixture was kept at this temperature for the transformation. At a conversion of 8%, attained after several hours, 100 mm^3 of the mixture were diluted with 900 mm^3 of HPLC eluent, and, after centrifugation, the concentrations of substrate and product enantiomers were analyzed by chiral HPLC. Analogously, 0.5 cm^3 of a 1 m*M* soln. of *D,L*-**1** in 0.1 *M* Tris/HCl-buffer (*pH* 8.5) were treated with the hydantoinase from *Thermus sp.* (10 mm^3, 120 U; activity for the conversion of *D,L*-5-phenylhydantoin), the hydantoinase from *Bacillus thermoglucosidasius* (30 mg, 6.8 U; activity for the conversion of *D,L*-5-(*p*-hydroxyphenyl-hydantion), and the hydantoinase from *Arthrobacter crystallopoietes* DSM 20117 (70 mg, 0.1 U; activity for the conversion of *D,L*-5-(*p*-hydroxyphenyl)-hydantoin), respectively. At 3%, 16%, and 6% conversion, attained after several hours of reaction at 37°C, 100 mm^3 of the mixtures were diluted with 900 mm^3 of HPLC eluent and analyzed as described above by chiral HPLC.

*Biotransformations of D,L-**2** with free N-carbamoylases*

Racemic N-carbamoyl amino acid *D,L*-**2** (20 mg, 0.1 mmol) was dissolved in 10 cm^3 of 0.1 *M* Tris/HCl-buffer (*pH* 8.5), lyophilized *D*-N-carbamoylase from *Agrobacterium sp.* IP I 671 (4 mg, 0.8 U; activity for the conversion of *D,L*-N-carbamoylphenylalanine) was added, and the mixture was kept at 37°C. At different time intervals (see Fig. 3) samples were taken and analyzed by RP-HPLC and photometrically after reaction with ninhydrin. Chiral HPLC of the samples revealed that only *D*-**2** was hydrolyzed. Analogously, *D,L*-**2** (1000 mm^3 of a 3 mM soln. in 0.1 *M* Tris/HCl-buffer (*pH* 8.5)) was treated with immobilized *L*-N-carbamoylase from *Arthrobacter aurescens* DSM 3747 (59.3 mg)

at 37°C. After 2 h, a sample was taken and centrifuged, and the supernatant was analyzed by chiral HPLC to reveal that solely *L*-2 was hydrolyzed.

Acknowledgements

We thank Dr. *W. Tischer* and Dr. *W. Bacher* (Roche Diagnostics, Penzberg, Germany) for the provision of the immobilized hydantoinases from *Bacillus thermoglucosidasius* and *Thermus sp*. and of the enriched *D*-N-carbamoylase from *Agrobacterium sp*. IP I 671.

References

[1] Weidmann B (1992) Chimia **46**: 312
[2] Fitzi R, Seebach D (1988) Tetrahedron **44**: 5277
[3] Myers Ag, Gleason JL, Yoon T, Kung DW (1997) J Am Chem Soc **119**: 656
[4] Walkup RD, Cole DC, Whittlesey BR (1995) J Org Chem **60**: 2630
[5] Yamanaka H, Fukui T, Kawamoto T, Tanaka A (1996) Appl Microbiol Biotechnol **45**: 51
[6] Tsuji Y, Yamanaka H, Fukui T, Kawamoto T, Tanaka A (1997) Appl Microbiol Biotechnol **47**: 114
[7] Pietzsch M, Syldatk C (1995) Hydrolysis and formation of hydantoins. In: Drauz K, Waldmann H (eds) Enzyme Catalysis in Organic Synthesis. VCH-Verlag, Weinheim, p 409
[8] Runser SM, Meyer PC (1993) Eur J Biochem **213**: 1315
[9] May O, Siemann M, Pietzsch M, Kiess M, Mattes R, Syldatk C (1988) J Biotechnol **61**: 1
[10] Wilms B, Wiese A, Syldatk C, Mattes R, Altenbuchner J, Pietzsch M (1999) J Biotechnol **68**: 101
[11] Runser S, Chinski N, Ohleyer E (1990) Appl Microbiol Biotechnol **33**: 382
[12] Hils M (1998) Mutanten der D-Carbamoylase zur Bildung aktiven Enzyms bei Expression des Gens in *Escherichia coli* und Analyse eines Genclusters für die Enzyme des Hydantoin-Abbaus aus *Agrobacterium sp*. IP I-671. PhD Thesis, Institute of Industrial Genetics, Stuttgart, Germany
[13] Möller A, Syldatk C, Schulze M, Wagner F (1988) Enzyme Microb Technol **10**: 618
[14] Faber K (1997), Biotransformations in Organic Chemistry – A Textbook. Springer, Berlin
[15] Pietzsch M, Schwämmle A, Syldatk C (in prep.)
[16] Waniek T (1999) Untersuchungen zur Substratspezifität und Enantioselektivität mikrobieller Hydantoinasen. PhD Thesis, Institute of Biochemical Engineering, Stuttgart, Germany
[17] Porter TH, Shive W (1968) J Med Chem **11**: 402
[18] Smith RJ, Bratovanov S, Bienz S (1997) Tetrahedron **53**: 13695
[19] Meyer P, Runser S (1993) FEMS Microbiol Lett **109**: 67
[20] Huber P, Bratovanov S, Bienz S, Syldatk C, Pietzsch M (1996) Tetrahedron Asymm **7**: 69
[21] Siemann M, Alvarado-Marín Á, Pietzsch M, Syldatk C (1998) J Molec Catal B, Enzymatic **6**: 387
[22] Pietzsch M, Wiese A, Ragnitz K, Wilms B, Altenbuchner J, Mattes R, Syldatk C (1999) J Chromatogr B **737**: 179
[23] Ragnitz K, Syldatk C, Pietzsch M (in prep.)
[24] Fahmy RA, Niederwieser A, Pataki G, Brenner M (1961) Helv Chim Acta **44**: 2022
[25] Dakin HD (1919) Biochem J **13**: 398

Received December 20, 1999. Accepted January 7, 2000