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Beauveria bassiana ATCC 7159 Contains an *L*-Specific α-Amino Acid Benzamidase

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Summary. Biotransformation of a series of racemic N-benzoyl α-amino acids by the fungus *Beauveria bassiana* ATCC 7159 results in isolation of the corresponding *D*-amino acid benzamides in high enantiomeric purity and yield.

Keywords. Amino acid; *Beauveria bassiana*; Biocatalysis; Biotransformation, Kinetic resolution

Introduction

Enzymatic methods for the kinetic resolution of racemic α-amino acids are well established [1]. Methods have been developed that involve the stereoselective hydrolysis of carboxylic acid ester [2] or amino amide [3] derivatives; either *D*- or *L*-specific hydrolysis can occur, and the product may be isolated as the free amino acid or as the unhydrolyzed substrate [4]. These transformations are summarized in Fig. 1.

Enantioselective hydrolyses involving amide derivatives of the amino group are most frequently reported using an amino acylase enzyme preparation [5] or a whole cell biocatalyst containing such an enzyme [6] for hydrolysis of the acetamide group. Reports of the kinetic resolution of other amino amide derivatives of α-amino acids by aminoacylase enzymes include *inter alia* examples of hydrolyses of N-chloroacetyl [7], N-trifluoroacetyl [7], N-methoxyacetyl [7], N-benzyl [8], and N-benzyloxycarbonyl [8] derivatives.

The enzymatic hydrolysis of N-benzoyl (benzamide) derivatives of α-amino acids has been only rarely reported, in spite of an early account of the use of takadiastase for the enantioselective hydrolysis of such compounds [9]. A "*D*-deacylase powder" from an unidentified pseudomonad has been reported to hydrolyze the *D* enantiomer of N-benzoylphenylalanine in an enantioselective manner [10], and *L*-N-benzoylglutamic acid is converted to the free amino acid by an enzyme described as urethane hydrolase IV from an unidentified microbial source [11], but the hydrolysis of N-benzoyl derivatives has not been systematically

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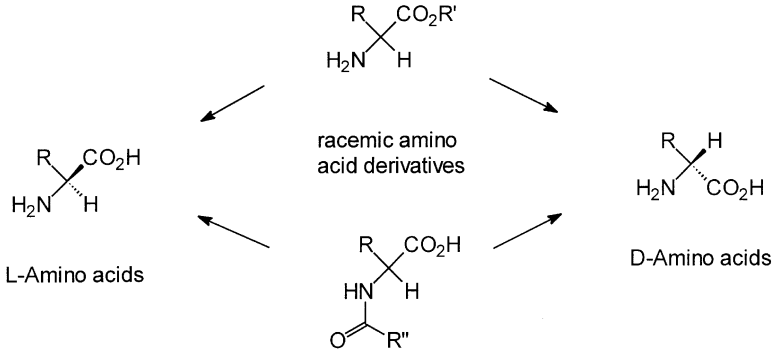


Fig. 1. Kinetic resolution of amino acid derivatives

investigated as a means for kinetic resolution of the naturally occurring α-amino acids.

The fungus *Beauveria bassiana* ATCC 7159 is one of the most frequently used whole cell biocatalysts, being exceeded in application only by baker's yeast, *Pseudomonas putida*, and *Aspergillus niger* [12]. It catalyzes a wide variety of reactions which have recently been reviewed [13], including the hydrolysis of amides. In this report we describe the use of *B. bassiana* for the *L*-specific enantioselective hydrolysis of a series of N-benzoyl α-amino acids.

Results and Discussion

Biotransformation of the N-benzoyl derivatives of racemic α-amino acids by *Beauveria bassiana* ATCC 7159 results in the enantioselective hydrolysis of the natural *L*-isomer, leaving the residual *D*-N-benzoyl amino acid in high enantiomeric excess. These results are summarized in Fig. 2 and Table 1.

The enantiomeric excess (*ee*) of the products listed in Table 2 were determined by chiral stationary phase HPLC analysis with reference to authentic enantiopure standards. Yields refer to isolated, purified material and are based on the total amount of racemic substrate used. Low yields in the case of the sulfur-containing amino acids may be attributable to the formation of the corresponding sulfoxides as side products. *Beauveria bassiana* is known to convert N-protected thia-amino acids to the corresponding sulfoxides with high diastereomeric selectivity [14], but this reaction is slower than hydrolysis of the benzamide group, taking 3–5 days for completion compared with the 1–2 days required for the hydrolytic process. A time course study for the hydrolysis of *DL*-N-benzoylalanine is presented in Fig. 3, from which it is apparent that complete conversion of the *L*-enantiomer has been

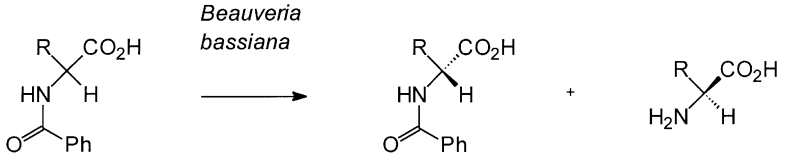
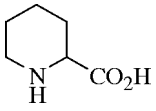
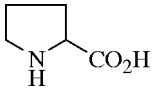
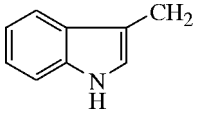
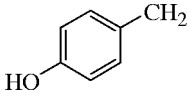


Fig. 2. Kinetic resolution of N-benzoyl amino acid derivatives by *B. bassiana* ATCC 7159

Table 1. Kinetic resolution of N-benzoyl amino acid derivatives by *B. bassiana* ATCC 7159

(±)-N-Benzoyl α-amino acid substrate		*D*-N-Benzoyl α-amino acid product	
	Substituent	Yield/%	*ee*/%
Alanine	CH_3	20	>95
Asparagine	CH_2CONH_2	28	80
Aspartic acid	CH_2CO_2H	23	>95
Cysteine	CH_2SH	17	>95
Ethionine	$CH_2CH_2SCH_2CH_3$	16	>95
Glutamic acid	$CH_2CH_2CO_2H$	22	>95
Glutamine	$CH_2CH_2CONH_2$	25	20
Leucine	$CH_2CH(CH_3)_2$	23	>95
Methionine	$CH_2CH_2SCH_3$	18	>95
Phenylalanine	$CH_2C_6H_5$	30	>95
Phenylglycine	C_6H_5	27	>95
Pipecolinic acid		20	>95
Proline		20	>95
S-Methylcysteine	CH_2SCH_3	32	>95
Tryptophan		21	>95
Tyrosine		17	20
Valine	$(CH_3)_2CH$	28	>95

[a] Refer to structures of Fig. 2; complete structures are shown for pipecolinic acid and proline only

attained after 36–48 hours incubation. Similar studies were not performed for the other substrates listed in Table 2, but on the basis of the data presented in Table 2 a 72 hour incubation period was selected as standard. The moderate *ee* observed for hydrolyses of racemic N-benzoyl asparagine, glutamine and tyrosine may be attributable to incomplete conversion of these substrates over the period of their biotransformation.

The hydrolysis of benzamides by *B. bassiana* appears to be selective for α-amino acid derivatives. Benzamides of β-amino acids such as 3-aminobutyric acid and 3-amino-3-phenylpropionic acid were not hydrolyzed by *B. bassiana*, substrates being recovered unchanged following incubation for five days. In other studies [15–19] many different amino substrates protected as their N-benzoyl derivatives have been hydroxylated by *B. bassiana* without loss of the benzoyl group; indeed, presence of the latter functionality in the substrate is often beneficial to the yield and stereoselectivity of such processes, as the benzamide unit may act as a binding group for the hydroxylase enzymes present in *B. bassiana* [18, 19]. As shown in the present

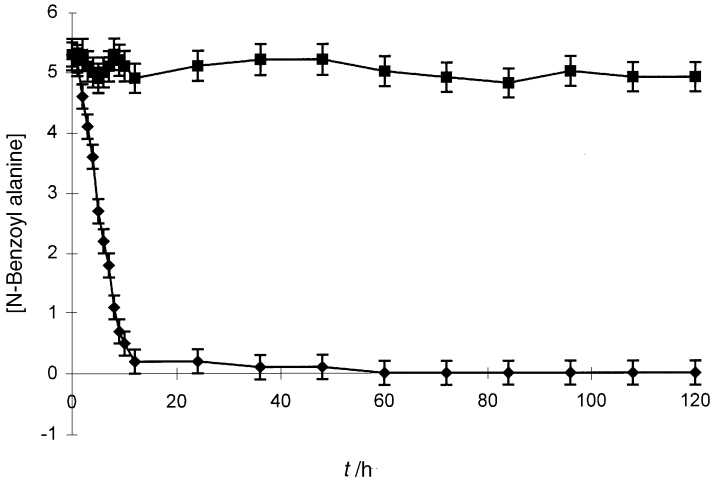


Fig. 3. Time course for the hydrolysis of *DL*-N-benzoylalanine by *B. bassiana* ◆: *L*-N-benzoyl alanine, ■: *D*-N-benzoyl alanine

study, however, the hydrolysis of racemic N-benzoyl α-amino acids by *B. bassiana* presents a simple method for the preparation of the *D*-benzoyl α-amino acids listed in Table 1 in moderate yield and, for most examples, high enantiomeric purity.

Experimental

Materials and methods

Melting points were determined on a Kofler hot stage and are uncorrected. The ^{1}H NMR spectra were recorded on a Bruker Avance series 300 spectrometer in CD_3OD solution using residual CH_3OH as the internal standard; chemical shifts are reported in ppm (δ), and the multiplicities are quoted as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). The ^{13}C NMR spectra were recorded at 75 MHz on the same spectrometer in CD_3OD solution. Mass spectra were obtained in the +FAB mode using a Kratos 1S spectrometer. IR spectra were obtained using a Mattson Research Series FTIR spectrometer. Optical rotations were recorded at ambient temperature in methanol as solvent using a Rudolph Autopol 3 polarimeter. Enantiomeric excess (*ee*) was determined using an ASTEC Chirobiotic T^{TM} column 250×4.6 mm with acetonitrile/methanol/acetic acid/triethylamine 455/545/2/2 solvent at a flow rate of 1 cm^3/min with UV detection at 254 nm. TLC was performed on Merck silica gel F_{254} plates (0.2 mm), and column chromatography used Merck silica gel 9385 (230–400 mesh). *Beauveria bassiana* ATCC 7159 was maintained on *Sabouraud* dextrose agar slopes, grown at 26°C and stored at 4°C.

Preparation of substrates

With the exception of the N-benzoyl tyrosine, all substrates were prepared in 85–95% yield by the following general procedure: the racemic amino acid (1 g) was dissolved in sodium hydroxide solution (20 cm^3, 2 *M*), and the solution cooled to 0°C. Benzoyl chloride (1.1 mole equivalents) was added in ten portions over a period of 1 h while the solution was stirred vigorously. The *pH* was checked periodically and adjusted if necessary to >12 by the addition of 2 *M* NaOH. When the addition was complete, the solution was stirred at room temperature for 2 h, then re-cooled to 0°C and adjusted to $pH = 2$ by the addition of 5% HCl. The precipitated product was separated by

filtration, dried, and crystallized from 95% ethanol. Products from proline and pipecolinic acid did not precipitate in crystalline form and were isolated by extraction with CH_2Cl_2 and evaporation of the extract to give a solid residue which was then crystallized. All products gave satisfactory spectroscopic data and melting points in agreement with those reported [20].

N-Benzoyltyrosine was prepared by a modification of the above procedure in which methanol (10 cm^3) was added to the reaction mixture following the 2 h stirring period at room temperature. The mixture was then stirred at room temperature overnight and subsequent steps performed as described above.

Biotransformations with B. bassiana

A growth medium (1 dm^3) composed of glucose (10 g) and corn steep liquor (20 g) in distilled water, adjusted to *pH* 4.85 with 1 *M* NaOH, was distributed in 5 1 dm^3 *Erlenmeyer* flasks which were stoppered with foam plugs and sterilized by autoclaving at 121°C for 20 min. The flasks were allowed to cool and then inoculated under sterile conditions with *B. bassiana* taken from a 3-day old agar slope. The flasks were allowed to stand overnight at 27°C and then placed on a rotary shaker (1″orbit) at 180 rpm, 27°C. After three days, a solution of the appropriate substrate (0.5 g) in 95% ethanol (10 cm^3) was added, and growth was allowed to continue for a further 3 days. The fungal mass was removed by filtration, and the filtrate was adjusted to *pH* 3 and continuously extracted with CH_2Cl_2 for 4 days. The extract was then evaporated to give a residue that was examined by TLC and chiral stationary phase HPLC and subjected to column chromatography using ethyl acetate/methanol solvent mixtures containing 2% acetic acid. The solvent composition was changed from 0% methanol to 40% methanol in 10% steps, and elution with the final mixture was continued until all product was removed from the column. All products had physical constants (melting points and optical rotation data) as reported [20] and exhibited spectroscopic data consistent with their structures.

Acknowledgements

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