Pergamon

*Bioorganic & Medicinal Chemistry*, Vol. 2, No. 6, pp. 429-432, 1994

0968-0896/94 $7.00 + .00

0968-0896(94)E0031-V

# Enzymatic Asymmetric Synthesis of α-Methyl Arylalkylamines and α-Methyl Arylalkylalcohols by Arylalkyl Acylamidases

Jun Ogawa, Sakayu Shimizu* and Hideaki Yamada†

*Department of Agricultural Chemistry, Kyoto University, Kitashirakawa-Oiwakecho, Sakyo-ku, Kyoto 606, Japan*

**Abstract**—With the novel microbial enzyme, 'arylalkyl acylamidase', optically active α-methyl arylalkylamines and α-methyl arylalkylalcohols have been obtained through enantioselective hydrolysis of their racemic amides and esters. (*S*)-Enantiomers of 1-methylbenzylamine, 1-methyl-3-phenylpropylamine and 1-methyl-3-phenylpropanol of high optical purity (> 94 % *e.e.*) were synthesized with the cells of *Nocardia erythropolis* IAM 1440 or *Cellulomonas fimi* AKU 671. (*R*)-Enantiomer of 1-methyl-3-phenylpropylamine and (*S*)-enantiomer of 1-methyl-2-phenylpropanol of high optical purity (> 95 % *e.e.*) were synthesized with the crude preparation of arylalkyl acylamidase of *Pseudomonas putida* Sc2 AKU 881.

## Introduction

Optically active amines exhibit unique physiological activities, acting as hallucinogens,[1] and have great synthetic potential as optically active nitrogen-containing compounds.[2] There have been some reports of microbial or enzymatic production of optically active amines through asymmetric acylation of amines[3] and asymmetric amination of carbonyl compounds.[4] For the synthesis of optically active amines, we investigated the asymmetric hydrolysis of racemic *N*-acyl amines with microorganisms[5] and found a novel enzyme, 'arylalkyl acylamidase', which catalyzes the hydrolysis of *N*-acyl arylalkylamines to arylalkylamines. There are some types of arylalkyl acylamidases, having different enantioselectivities towards some *N*-acyl-α-methyl arylalkylamines. One is specific for (*R*)-enantiomer of a *N*-acyl-α-methyl arylalkylamine and the other for (*S*)-enantiomer. Arylalkyl acylamidases also catalyze the hydrolysis of arylalkyl esters at considerable rates.[6] Therefore, they are promising catalysts not only for the preparation of optically active α-methyl arylalkylamines but also for that of α-methyl arylalkylalcohols. In this paper, we report the screening of microorganisms having high arylalkyl acylamidase activity, and the synthesis of optically active α-methyl arylalkylamines and α-methyl arylalkylalcohols with these potent biocatalysts.

## Results and Discussion

*Screening of microorganisms having high arylalkyl acylamidase activity*

Arylalkyl acylamidase activity was assayed among 165 bacteria and 51 actinomycetes from our stock cultures using racemic *N*-acetyl-1-methyl-3-phenylpropylamine (**1a**) as the substrate. Four bacteria, belonging to the genera *Bacillus*, *Corynebacterium*, *Cellulomonas* and *Acinetobacter*, and four actinomycetes, belonging to the genera *Nocardia* and *Rhodococcus*, showed high hydrolysis activity (> 25 % molar yield) and produced only (*S*)-1-methyl-3-phenylpropylamine (**2**), but no strain could produce (*R*)-**2**.

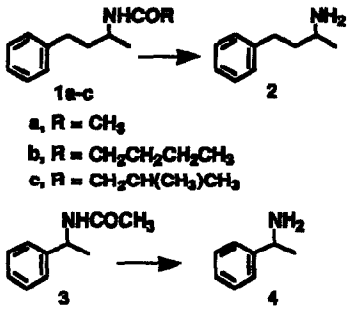
To obtain (*R*)-**2**-producing microorganisms, about 950 strains which utilized (*R*)-**1a** as a major carbon source were tested. Fifty-five strains produced (*R*)-**2** (> 10 % molar yield), and the most promising one, which produced (*R*)-**2** with an approximate 20 % molar yield, was taxonomically identified as *Pseudomonas putida* Sc2 (AKU 881).

Through this screening, we selected *Nocardia erythropolis* (IAM 1440) and *Cellulomonas fimi* (AKU 671) as (*S*)-enantiomer-producing biocatalysts, and *P. putida* Sc2 as an (*R*)-enantiomer-producing one.

*Synthesis of (S)-α-methyl arylalkylamines with cells containing (S)-specific arylalkyl acylamidase*

With washed cells of *N. erythropolis* or *C. fimi*, the synthesis of (*S*)-α-methyl arylalkylamines through the enantioselective hydrolysis of their amides was performed (Scheme I). With 5 % (w/v) racemic **1a** as substrate, optically active (*S*)-**2** (99 % *e.e.*) was produced in about 35 % molar yields by these microorganisms. (*S*)-**2** was also synthesized with the same catalysts from *N*-valeryl- and *N*-isovaleryl-1-methyl-3-phenylpropylamine (**1b** and **1c**) with 92 and 97 % *e.e.*, respectively. With 1 % (w/v) racemic *N*-acetylbenzylamine (**3**) as substrate, these microorganisms also produced (*S*)-1-methylbenzylamine (**4**) with 99 % *e.e.*, in about 30 % molar yields (Table 1).

†Present address: Department of Biotechnology, Kansai University, 3-3-35 Yamatecho, Suita, Osaka 564, Japan.




**Scheme I.** Synthesis of optically active α-methyl arylalkylamines.

*Synthesis of (R)-α-methyl arylalkylamines with (R)-specific arylalkyl acylamidase*

The arylalkyl acylamidase of *P. putida* Sc2 responsible for the (*R*)-specific hydrolysis of racemic **1a** was an extracellular one.[6] The enzyme was prepared from a culture supernatant of the organism and used for the synthesis of (*R*)-α-methyl arylalkylamines through the enantioselective hydrolysis of their amides (Scheme I). As shown in Table 2, with 5 % (w/v) and 15 % (w/v) racemic **1a** as substrates, (*R*)-**2** with 92 and 95 % *e.e.*, was obtained in 27 and 12 % molar yields, respectively. With 1 % (w/v) **1a** as substrate, the optical purity of the product was rather low (85 % *e.e.*, 40 % molar yield). These results suggest that a high substrate concentration is necessary to obtain (*R*)-**2** of high optical purity. The enzyme preparation used here did not show enantioselectivity towards the hydrolysis of either valeramide (**1b**) or isovaleramide (**1c**). Because the purified arylalkyl acylamidase did not hydrolyze **1b** or **1c**,[6] it is likely that these substrates were hydrolyzed by contaminating hydrolases in the enzyme preparation. Hydrolysis of **3** was not observed with either the purified arylalkyl acylamidase or the present enzyme preparation.

**Table 1.** Synthesis of (*S*)-α-methyl arylalkylamines

| | | *N. erythropolis* | | *C. fimi* | |
|---|---|---|---|---|---|
| | | Molar | % e.e. | Molar | % e.e. |
| Substrate[a] | Product | yield (%) | | yield (%) | |
| **1a**(5) | **2** | 35 | 99(*S*) | 32 | 99(*S*) |
| **1b**(5) | **2** | 46 | 92(*S*) | 45 | 92(*S*) |
| **1c**(5) | **2** | 33 | 97(*S*) | 26 | 97(*S*) |
| **3** (1) | **4** | 34 | 98(*S*) | 31 | 99(*S*) |

[a]The initial concentration of each substrate in % (w/v) is given in parenthesis.
All reactions were carried out for 24 h.

**Table 2.** Synthesis of (*R*)-α-methyl arylalkylamines.

| | | Reaction | Molar | % e.e. |
|---|---|---|---|---|
| Substrate[a] | Product | time (h) | yield (%) | |
| **1a**(1) | **2** | 3 | 40 | 85(*R*) |
| **1a**(5) | **2** | 24 | 27 | 92(*R*) |
| **1a**(15) | **2** | 96 | 12 | 95(*R*) |
| **1b**(5) | **2** | 24 | 22 | 3(*R*) |
| **1c**(5) | **2** | 24 | 21 | 0 |
| **3** (1) | **4** | 24 | tr | n.d. |

[a]The initial concentration of each substrate in % (w/v) is given in parenthesis.
tr = trace, n.d. = not determined

Table 3. Synthesis of optically active α-methyl arylalkylalcohols.

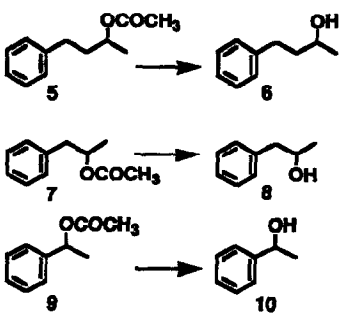
| | | *N. erythropolis* | | | *C. fimi* | | | *P. putida* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Substrate | Product | Reaction time (h) | Molar yield (%) | %e.e. | Reaction time (h) | Molar yield (%) | %e.e. | Reaction time (h) | Molar yield (%) | %e.e. |
| 5 | 6 | 3 | 20 | 82(*S*) | 2 | 26 | 94(*S*) | 44 | 8 | 22(*R*) |
| 7 | 8 | 3 | 23 | 27(*S*) | 2 | 30 | 26(*S*) | 24 | 9 | 99(*S*) |
| 9 | 10 | 3 | 5 | 27(*S*) | 6 | 15 | 57(*S*) | 120 | tr | n.d. |

All reactions were carried out with 5% (w/v) substrate.
tr = trace, n.d. = not determined

*Synthesis of optically active α-methyl arylalkylalcohols with arylalkyl acylamidase*

The arylalkyl acylamidase of *P. putida* Sc2 has been shown to catalyze the hydrolysis of several esters of arylalkylalcohols. A culture supernatant of *P. putida* Sc2, and washed cells of *N. erythropolis* and *C. fimi* were used for the synthesis of optically active α-methyl arylalkylalcohols through the enantioselective hydrolysis of their acetic acid esters (Scheme II). As shown in Table 3, optically active (*S*)-1-methyl-3-phenylpropanol (**6**), 82 % *e.e.* and 94 % *e.e.*, was obtained with the *N. erythropolis* and *C. fimi* cells, respectively (20 and 26 % molar yields, respectively) from 5 % (w/v) corresponding racemic acetic acid ester (**5**) as substrates. (*S*)-1-Methyl-2-phenylethanol (**8**), 99 % *e.e.* (9 % molar yield), was obtained with the enzyme preparation of *P. putida* Sc2 from 5 % (w/v) corresponding racemic acetic acid ester (**7**) as substrate. It was interesting that the enzyme preparation specific for (*R*)-**1a** showed the specificity for (*S*)-**7**. As for the other α-methyl arylalkylalcohols tested, the optical purities of the products were low.

The data shown here suggest that these biocatalysts are useful tools for the preparation of optically active α-methyl arylalkylamines and α-methyl arylalkylalcohols.




Scheme II. Synthesis of optically active α-methyl arylalkylalcohols.

## Experimental Section

*Microorganisms and media*

Microorganisms preserved in our laboratory (AKU culture collection) and ones isolated from soil samples were used. Medium A comprised 1.5 g (*R*)-**1a**, 1 g $KH_2PO_4$, 1 g $K_2HPO_4$, 0.3 g $MgSO_4 \cdot 7H_2O$, 0.1 g yeast extract (Oriental Yeast, Japan) and 1 g $NH_4Cl$, in 1 L of tap water, pH 7.0. Medium B comprised 1.5 g racemic **1a**, 1 g $KH_2PO_4$, 1 g $K_2HPO_4$, 0.3 g $MgSO_4 \cdot 7H_2O$, 3 g yeast extract, 3 g meat extract (Mikuni Co., Japan), 10 g glycerol and 2 g Polypepton (Daigo Nutritional, Japan), in 1 L of tap water, pH 7.0.

*Isolation of* (R)*-1a-utilizing microorganisms*

Soil samples were inoculated into 2 mL of medium A in test tubes (16 x 165 mm). After being aerobically cultured at 28 °C with reciprocal shaking (300 rpm), each soil suspension was transferred to medium A and then cultured until the growth of microorganisms was apparent. Then the microorganisms were isolated on 2 % agar plates containing medium A.

*Screening method*

Each strain was grown in 1 mL of medium B in a test tube (15 x 105 mm) at 28 °C for 2–5 days with reciprocal shaking (300 strokes/min), and then the culture broth was analyzed for **1a** and **2** by TLC as described previously.[5] The microorganisms that produced **2** in the culture broth were selected and grown in medium B (1 mL) at 28 °C with shaking. On the third day, 1 % (w/v) racemic **1a** and 1 % (w/v) Tween 80 were added, and then the incubation was continued for a further 2 days. Reactions were stopped by adding 1 mL of ethanol. After centrifugation, the supernatant was analyzed for **2** and the optical purity by HPLC, as described below.

*Synthesis of* (S)*-α-methyl arylalkylamines*

*N. erythropolis* IAM 1440 and *C. fimi* AKU 671 were cultured in medium B for 2 days at 28 °C. The cells were harvested by centrifugation and washed with 0.85 % (w/v) NaCl, and then used for the reaction. Reactions were carried out at 30 °C with shaking. The reaction mixture comprised a substrate, 100 mM potassium phosphate (pH 7.0), 10 % (v/v) ethanol and 0.5 g (wet weight) of washed cells, in a total volume of 25 mL.

The molar yields were determined by HPLC on an ODS column [Cosmosil $5C_{18}$, 4.6 x 100 mm, Nacalai Tesque, Japan; mobile phase, $CH_3OH/H_2O$ = 45/50 (v/v), pH 2.5; flow rate, 1.0 mL/min; detection, UV 254 nm]. The retention times were 2.4 and 1.7 min for **2** and **4**, respectively. The optical purities of **2** and **4** were determined by HPLC on an ODS column [Cosmosil $5C_{18}$, 4.6 x 250 mm; mobile phase, $CH_3OH/H_2O$ = 50/50 (v/v), pH 2.5; flow rate, 1.0 mL/min; detection, UV 254 nm], after the formation of diastereomeric derivatives with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate. The retention times were 59.8 and 64.1 min for (*R*)-**2** and (*S*)-**2**, and 24.5 and 27.2 min for (*R*)-**4** and (*S*)-**4**, respectively.

*Synthesis of* (R)-*α-methyl arylalkylamines*

The crude enzyme of *P. putida* Sc2 AKU 881 was prepared as described previously.[6] The enzyme preparation obtained on solid ammonium fractionation (12 mg protein/mg) was used as the enzyme. Reactions were carried out at 30 °C with shaking. The reaction mixture comprised a substrate, 100 mM potassium phosphate (pH 7.0), 5 % (w/v) Tween 80 and 6.25 mL of the crude enzyme, in a total volume of 25 mL. Other conditions were the same as given above.

*Synthesis of optically active α-methyl arylalkylalcohols*

All reactions were carried out at 30 °C with shaking. The reaction mixture comprised a substrate, 200 mM potassium phosphate (pH 7.0) and 7.5 mL of the crude enzyme from *P. putida* Sc2, or 0.75 g (wet weight) of cells of *N. erythropolis* IAM 1440 or *C. fimi* AKU 671, in a total volume of 25 mL. The molar yields were determined by HPLC on an ODS column [Cosmosil $5C_{18}$, 4.6 x 100 mm; mobile phase, $CH_3OH/H_2O$ = 50/50 (v/v), pH 2.5; flow rate, 1.0 mL/min; detection, UV 254 nm]. The retention times were 3.3, 4.8 and 8.2 min for **6**, **8** and **10**, respectively. Determination of the optical purities of **6**, **8** and **10** was carried out after the isolation of **6**, **8** and **10** from the reaction mixtures with a SEP-PAK Silica cartridge (Waters Associates, U.S.A.), and successive derivatization with benzoylchloride for **6** or acetylchloride for **8**. Measurement of the optical purities of the isolated **10**, and derivatized **6** and **8** was carried out by HPLC on a Chiralcel OB column [Daicel Chemical Industries, Ltd, Japan; mobile phase, hexane/2-propanol = 90/10 (v/v); flow rate, 0.5 mL/min; detection, UV 254 nm]. The retention times were 14.2 and 18.7 min for (*S*)-**10** and (*R*)-**10**, 11.3 and 13.4 min for acetic acid esters of (*R*)-**8** and (*S*)-**8**, and 12.0 and 14.0 min for benzoic acid esters of (*R*)-**6** and (*S*)-**6**, respectively.

(*Received* 26 *October* 1993; *accepted* 20 *January* 1994)

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