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# Asymmetric amination of 4-methoxyphenylacetone and its related compounds with microorganisms

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**Summary.** Asymmetric amination of 4-methoxyphenylacetone and its related compounds by microorganisms was investigated. Among 630 type culture strains, 4-methoxyphenylacetone-aminating ability was found in *Brevibacterium, Chromobacterium, Flavobacterium, Mycobacterium, Pseudomonas,* and *Sarcina* spp. 4-Methoxyamphetamine produced by these microorganisms was the (*S*)-(+)-enantiomer. *B. linens* IFO 12141 was selected as the best strain. The optimum pH of amination was about 7.0, and L-alanine was the most effective amino donor for the amination. By using this strain, 37.6 m*M* (*S*)-(+)-4-methoxyamphetamine was formed with a 94% conversion yield from 4-methoxyphenylacetone. As for substrate specificity, *B. linens* IFO 12141 catalysed amination of 3,4-dimethoxyphenylacetone and 4-(4-methoxyphenyl)-2-butanone, and formed the corresponding optically active amines.

## Introduction

4-Methoxyamphetamine (PMA) and its related compounds are known as the sympathomimetic amines (Shulgin et al. 1969), and many studies on acute toxicity, behavioural effects and central serotonergic effects have been reported (Smythies et al. 1970; Menon et al. 1976; Davis et al. 1978; Harris et al. 1978). Moreover, PMA is a useful raw material for the synthesis of carbostyril derivatives, a potent bronchodilative agent.

Optically active PMA is chemically synthesized from 4-methoxyphenylacetone and (+)- or (−)-α-methylbenzylamine by the method of Nichols et al. (1973), but there is no report of enzymatic synthesis of PMA. To provide a new enzymatic method for the synthesis of optically active PMA, we screened microorganisms which catalyse asymmetric amination of 4-methoxyphenylacetone. In this paper, we report the results of screening and investigations of asymmetric amination of 4-methoxyphenylacetone and its related compounds.

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## Materials and methods

*Microorganisms.* Microorganisms from stock cultures maintained in our laboratory were used for the screening test, and *Brevibacterium linens* IFO 12141, selected as the best strain, was used for further studies.

*Screening.* The compositions of the media used for the screening test were as follows: for bacteria, 1% peptone, 1% meat extract, and 0.5% NaCl, pH 7.0; for *Mycobacterium*, 0.2% glycerol, 1% peptone, 0.5% yeast extract, 0.5% malt extract, 0.5% casamino acids, 0.2% meat extract, 0.1% $MgSO_4 \cdot 7H_2O$, and 0.005% Tween 80, pH 7.2; for yeast, 1% glucose, 0.5% peptone, 0.3% yeast extract, and 0.3% malt extract, pH 6.2; for actinomycetes, 0.4% glucose, 1% malt extract, and 0.4% yeast extract, pH 7.3.

All media were distributed in 3-ml amounts to test tubes, and sterilized. After inoculation with test microorganisms from slant cultures, shaking incubation was carried out at 30° C for 24 h (bacteria and yeast) or 48 h (*Mycobacterium* and actinomycetes) with a reciprocal shaker operated at 250 strokes/min with a 7-cm amplitude. The cells were collected by centrifugation and washed once with 3 ml saline. One millilitre of substrate solution containing 20 m*M* 4-methoxyphenylacetone, 100 m*M* $NH_4Cl$, 100 m*M* sodium L-glutamate, 100 m*M* L-alanine, 1% methyl sulfoxide, and 50 m*M* potassium phosphate buffer, pH 7.0, was added, and incubated at 30° C for 48 h. After removal of the cells by centrifugation, the supernatant was adjusted to pH 10.0 with 10 *N* NaOH and extracted with ethyl acetate. The organic layer was used for determination of PMA formation.

*Culture conditions. Brevibacterium linens* IFO 12141 was cultured in the following medium; 0.5% glucose, 1% peptone, 1.25% yeast extract, 1% meat extract, and 0.5% NaCl, pH 7.0. A 100-ml portion of the medium in a 500-ml flask was autoclaved at 120° C for 10 min, inoculated with one loopful of slant culture, and incubated at 30° C for 24 h on a reciprocal shaker operated at 140 strokes/min with a 7-cm amplitude. The cells harvested by centrifugation from culture broth were used as intact cells.

*Asymmetric amination of 4-methoxyphenylacetone and related compounds.* The standard conditions for asymmetric amination of 4-methoxyphenylacetone and related compounds were as follows; a reaction mixture containing 30 m*M* 4-methoxyphenylacetone or related compounds, 500 m*M* L-alanine, 15% methyl sulfoxide,

100 m*M* potassium phosphate buffer (pH 7.0), and intact cells harvested from 100 ml culture broth (final volume 25 ml), was incubated at 30° C for 72 h.

*Isolation and purification of PMA and related amines.* After the reaction, the cells were removed by centrifugation, and the supernatant adjusted to pH 10.0 with 10 *N* NaOH and extracted with ethyl acetate. The organic layer was washed with a small amount of saturated aq. NaCl solution, and dried over anhydrous $Na_2SO_4$. The organic layer was concentrated under reduced pressure and a small amount of 2-propanol containing 10% hydrochloric acid was added to the concentrated solution, and then stored at room temperature. The precipitate of amine hydrochloride was collected by filtration, and dried under reduced pressure. The absolute configurations of the products were determined by comparison of the values of specific rotation with those given in the literature (Nichols et al. 1973).

*Analytical methods.* Identification and determination of PMA and other amines were performed by thin-layer chromatography using the ascending technique on a Merck $60F_{254}$ plate (solvent; chloroform-methanol-acetic acid, 85:15:3, by vol.). The chromatograms were sprayed with ninhydrin solution and heated on a Thermolyne Type 1900 hot plate (Dubuque, Ia, USA). The coloured spots were compared with authentic amines. PMA, 4-methoxyphenylacetone, related amines and ketones were also determined using a Shimadzu (Kyoto, Japan) LC-3A high-performance liquid chromatograph (column, Nucleocil 5C18, Macherey-Nagel, FRG; carrier, 30% methanol containing 0.5% acetic acid; detection, UV 273 nm). Optical rotations were determined by a Perkin-Elmer (Norwalk, Conn, USA) 243 polarimeter at 20° C, enantiomeric purity being calculated from the optical rotation value.

*Chemicals.* 3-Methoxyphenylacetone, 4′-methoxypropiophenone, 4′-chloropropiophenone were purchased from Aldrich Chemical Co. (Milwaukee, Wisc, USA), and other ketones were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). Authentic PMA and 3,4-dimethoxyamphetamine were prepared by methods described in the literature (Nichols et al. 1973). Authentic 3-(4-methoxyphenyl)-1-methylpropylamine was prepared by the method described by Sterling Drug Inc (1983). L-Alanine of analytical standard grade produced by Tanabe Seiyaku Co. (Osaka, Japan) was used. The other chemicals were analytical grade.

## Results and discussion

### *Screening of microorganisms*

The ability to form PMA from 4-methoxyphenylacetone was screened for 630 strains including 333 strains of bacteria, 226 strains of yeasts, 71 strains of actinomycetes. As shown in Table 1, six species of bacteria belonging to the genera *Brevibacterium, Chromobacterium, Flavobacterium, Mycobacterium, Pseudomonas,* and *Sarcina* were found to form PMA from 4-methoxyphenylacetone. The product was isolated as PMA hydrochloride and identified by comparing its infrared and nuclear magnetic resonance spectra and melting point with those of an authentic specimen. The specific rotation of PMA hydrochloride isolated from each reaction mixture is also listed in Table 1. From the results, PMA produced by these microorganisms was the (*S*)-(+)-enantiomer, and the enantiomeric purity was very high. Among these microorganisms, *B. linens* IFO 12141 was selected and used for further experiments.

### *Asymmetric amination of 4-methoxyphenylacetone and its related compounds by B. linens IFO 12141*

Some details of asymmetric amination of 4-methoxyphenylacetone by *B. linens* IFO 12141 were studied. Table 2 shows the effect of amino donor on the reaction. Among inorganic ammonium salts and L-amino acids tested, L-alanine was found to be the most effective amino donor for the reaction. Figure 1 shows the effect of pH on the reaction. The optimum pH for asymmetric amination was about pH 7.0. On the basis of these results, subsequent experiments were carried out using L-alanine as the amino donor at pH 7.0.

Table 3 shows the results of investigating substrate specificity. *B. linens* IFO 12141 also catalysed amination of acetophenone, 4′-methoxyacetophenone, methoxyphenylacetones, and 4-(4-methoxyphenyl)-2-butanone, and formed corresponding amines. On the other hand, no amine was formed from propiophenone, benzylethylketone, and benzyl-*n*-propylketone derivatives. From these results, it was found that the position of the carbonyl group had an effect on substrate specificity, and the methylketone moiety was essential for amination.

Table 4 shows the results of asymmetric amination of 4-methoxyphenylacetone, 3,4-dimethoxyphenylacetone, and 4-(4-methoxyphenyl)-2-butanone. By using *B. linens* IFO 12141, 37.6 m*M* (*S*)-(+)-methoxyamphetamine was formed with a 94% conversion yield from 40 m*M* 4-methoxyphenylacetone. 3,4-Dimethoxyphenylacetone and 4-(4-methoxyphenyl)-2-butanone were also aminated to 3,4-dimethoxyamphetamine and 3-(4-me-

**Table 1.** 4-Methoxyamphetamine (PMA)-forming microorganisms

| Microorganism | PMA formed (m*M*) | $[\alpha]_D$ (c = 1, water)[a] |
|---|---|---|
| *Brevibacterium linens* IFO 12141 | 6.1 | +24.4 |
| *Chromobacterium iodinum* IFO 3558 | 4.4 | +19.7 |
| *Flavobacterium rigense* FERM-P 3556 | 3.1 | +23.8 |
| *Mycobacterium smegmatis* ATCC 607 | 2.6 | +22.2 |
| *Pseudomonas riboflavina* IAM 1093 | 4.0 | +20.6 |
| *Sarcina albida* IAM 1012 | 0.9 | +21.5 |

[a] The specific rotation of optically pure (*S*)-(+)-4-methoxyamphetamine hydrochloride is +22.4° in the literature (Nichols et al. 1973)

**Table 2.** Effect of amino donor on asymmetric amination of 4-methoxyphenylacetone

| Amino donor | PMA formed (m*M*) |
|---|---|
| None | 17.4 |
| $NH_4Cl$ | 20.7 |
| $(NH_4)_2SO_4$ | 19.0 |
| $(NH_4)_2HPO_4$ | 20.8 |
| $CH_3COONH_4$ | 19.2 |
| $HCOONH_4$ | 19.1 |
| L-Alanine | 28.2 |
| Sodium L-glutamate | 20.4 |

The reaction mixture containing 30 m*M* 4-methoxyphenylacetone, 500 m*M* of each amino donor, 15% methyl sulfoxide, 100 m*M* potassium phosphate buffer, and intact cells, pH 7.0, was incubated at 30° C for 72 h

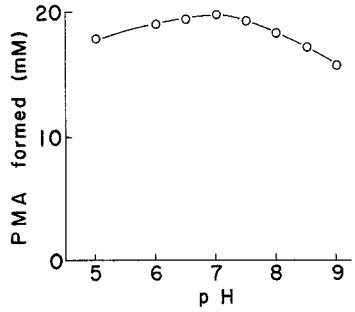
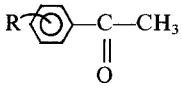
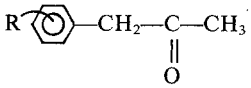
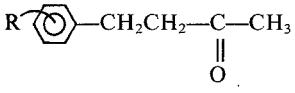
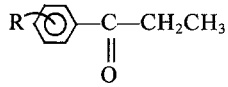
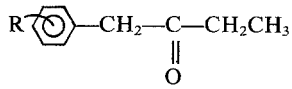
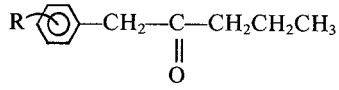



**Fig. 1.** Effect of pH on 4-methoxyamphetamine (PMA) formation. The reaction mixture containing 20 m*M* 4-methoxyphenylacetone, 500 m*M* L-alanine, 15% methyl sulfoxide, 100 m*M* buffer, and intact cells was incubated at 30° C for 72 h. Buffer solutions used were as follows: pH 5.0, $CH_3COOH$—$CH_3COONa$; pH 6.0–7.5, $KH_2PO_4$—$K_2HPO_4$; pH 8.0–9.0, TRIS(hydroxymethyl)aminomethane-HCl

**Table 3.** Substrate specificity

| Substrate | R | Amine formation |
|---|---|---|
|  | H | + |
| | 4′-$CH_3O$ | + |
| | 4′-$CH_3$ | − |
| | 4′-Cl | − |
| | 4′-OH | − |
| | 2′-OH | − |
|  | 4-$CH_3O$ | + + + |
| | 3-$CH_3O$ | + + |
| | 2-$CH_3O$ | + + |
| | 3,4-$CH_3O$ | + + + |
|  | H | − |
| | 4-$CH_3O$ | + + |
| | 4-OH | − |

| Substrate | R | Amine formation |
|---|---|---|
|  | H | − |
| | 4′-$CH_3O$ | − |
| | 4′-$CH_3$ | − |
| | 4′-Cl | − |
|  | H | − |
| | 4-$CH_3O$ | − |
| | 4-$CH_3$ | − |
| | 4-Cl | − |
|  | H | − |

Amine formation was detected by thin-layer chromatography as follows: −, no amination; +, weak amination; + +, good amination; + + +, very good amination. R, side groups

**Table 4.** Asymmetric amination of 4-methoxyphenylacetone and its related compounds

| Substrate | Conc (m*M*) | Product (m*M*) | Conversion yield (%) | $[\alpha]_D$ (c=1, $H_2O$) | % e.e. |
|---|---|---|---|---|---|
| 4-Methoxyphenylacetone | 40 | 37.6 | 94.0 | +24.4[a] | >99 |
| 3,4-Dimethoxyphenylacetone | 40 | 34.4 | 86.0 | +23.5[a] | >99 |
| 4-(4-Methoxyphenyl)-2-butanone | 40 | 14.3 | 35.8 | − 5.9[b] | 98.3 |

[a] The specific rotation of optically pure (*S*)-(+)-4-methoxyamphetamine hydrochloride and (*S*)-(+)-3,4-dimethoxyamphetamine hydrochloride are +22.4° and +23.1° in the literature (Nichols et al. 1973)

[b] The specific rotation of (−)-3-(4-methoxyphenyl)-1-methylpropylamine hydrochloride described in Sterling Drug Inc (1983) is −6.0°

e.e., enantiomeric excess

thoxyphenyl)-1-methylpropylamine in a molar yield 86.0% and 35.8%, respectively. Each product was isolated from the reaction mixture as the hydrochloride salt and the specific rotation was measured. As shown in Table 4, all products were optically active and the enantiomeric purities were very high.

The present results provide useful information for the application of microorganisms to the asymmetric synthesis of 4-methoxyamphetamine and its related amines. The mechanism of the reaction and the characteristics of the enzyme-catalysed asymmetric amination are being investigated further.

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