

Optical Resolution of Methionine and ϵ -N-Benzoyl-lysine by Metabolism of *Escherichia coli* on Phenylacetyl Derivatives

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Nine strains of *Escherichia coli* were tested for the metabolic activities on methionine, ϵ -N-benzoyl-lysine and their phenylacetyl derivatives, and it was observed that *Escherichia coli* IFO-3470 and IFO-3550 asymmetrically metabolized N-phenylacetyl-DL-methionine and α -N-phenylacetyl- ϵ -N-benzoyl-DL-lysine to yield L-methionine, ϵ -N-benzoyl-L-lysine and each corresponding D-form of phenylacetyl derivatives, respectively.

The facts indicate the possibility of the resolution of other amino acids involving their analogs by the metabolism of *Escherichia coli* on phenylacetyl derivatives.

Keywords—resolution of amino acids; methionine; ϵ -N-benzoyl-lysine; *Escherichia coli*; metabolism; acylase activity

The authors²⁾ reported a practical method of the optical resolution of racemic amino acids by the metabolism of the soil bacteria on N-benzoyl derivatives. For example, the strains KT-230 and KT-231 of *Pseudomonas* sp. asymmetrically metabolized N-benzoyl derivatives of the following DL-amino acids to yield L-amino acids and the corresponding N-benzoyl-D-amino acids: methionine, phenylalanine, leucine, ϵ -N-benzoyl-lysine, glutamic acid, aspartic acid, *p*-methoxy-phenylalanine, 3,4-methylenedioxy-phenylalanine, and 2-amino-hexanedioic acid. Incidentally, the strains KT-230 and KT-231 metabolized N-benzoyl derivatives of the amino acids, but not N-phenylacetyl derivatives.

This report presents the result of the optical resolution of DL-methionine and ϵ -N-benzoyl-DL-lysine by the metabolism of *Escherichia coli* on phenylacetyl derivatives.

The metabolic activities of 9 strains of *Escherichia coli* on phenylacetic acid, DL-methionine, ϵ -N-benzoyl-DL-lysine and their α -N-phenylacetyl derivatives were examined.

TABLE I. Metabolic Activity of Various Strains of *Escherichia coli*

Substrate	IFO-3470 3550	IFO-3139, 3471 3544, 3545 3551, 3552	IFO-3806
Phenylacetic acid	+ ^{a)}	+	—
Phenylacetyl-DL-Met	+	—	—
Phenylacetyl-D-Met	—	—	—
DL-Met	—	—	—
α -N-Phenylacetyl- ϵ -N-Benzoyl-DL-Lys	+	—	—
α -N-Phenylacetyl- ϵ -N-Benzoyl-D-Lys	—	—	—
ϵ -N-Benzoyl-DL-Lys	—	—	—

a) +: luxuriant growth of the bacteria was observed at 37° within 4 days.

—: almost no visible growth was observed.

1) Location: Ho-3, Kanagawa-machi, Kanazawa.

2) Y. Kameda, E. Toyoura and K. Matsui, *Chem. Pharm. Bull.* (Tokyo), 7, 702 (1959); E. Toyoura, *ibid.*, 7, 785 (1959); *idem, ibid.*, 7, 787 (1959).

From Table I, it is assumed that *Escherichia coli* IFO-3470 and IFO-3550 metabolized N-phenylacetyl-DL-methionine and α -N-phenylacetyl- ϵ -N-benzoyl-DL-lysine to yield L-methionine, ϵ -N-benzoyl-L-lysine and each corresponding D-form of phenylacetyl derivatives, respectively. As described in Experimental, *Escherichia coli* IFO-3470 or IFO-3550 was incubated at 37° for 7 days in the synthetic medium containing N-phenylacetyl-DL-methionine. In the case of both strains, L-methionine and N-phenylacetyl-D-methionine were obtained in good yield.

Therefore, it is to be understood that *Escherichia coli* IFO-3470 and IFO-3550 utilized phenylacetic acid which was liberated from N-phenylacetyl-L-methionine by the action of their acylases, to yield L-methionine and N-phenylacetyl-D-methionine.

In the same way, *Escherichia coli* IFO-3470 and IFO-3550 asymmetrically metabolized α -N-phenylacetyl- ϵ -N-benzoyl-DL-lysine to yield ϵ -N-benzoyl-L-lysine and α -N-phenylacetyl- ϵ -N-benzoyl-D-lysine.

The facts indicate the possibility of the optical resolution of other amino acids involving their analogs, if suitable strains of *Escherichia coli* could be isolated.

Experimental

Preparation of Substrates—N-Phenylacetyl-DL-methionine and α -N-phenylacetyl- ϵ -N-benzoyl-DL-lysine were prepared according to the procedure described previously.³⁾

Strains of *Escherichia coli*—The strains were originally obtained from the Institute for Fermentation, Osaka, Japan. The stock cultures were maintained on slopes of nutrient agar at 4°.

Resolution of N-Phenylacetyl-DL-Methionine by the Metabolism of *Escherichia coli* IFO-3470 and IFO-3550—a) *Escherichia coli* IFO-3470 was inoculated in 200 ml of the following medium containing 2.68 g of N-phenylacetyl-DL-methionine in a 1000-ml Erlenmeyer flask and incubated at 37° for 7 days: NH_4Cl 0.1 g, K_2HPO_4 0.1 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.05 g, 1% $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ 2 drops, 1% $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 1 drop, distilled water 100 ml, pH 7.4–7.6. The culture was harvested by centrifugation at $10000 \times g$ for 10 min. The supernatant was adjusted to pH 4.5 with AcOH, evaporated *in vacuo* to dryness. The residue was washed several times with hot EtOH (100 ml) and recrystallized from EtOH-H₂O to give colorless plates of L-methionine, mp 275–278° (dec.), yield 0.62 g (83%). *Anal.* Calcd. for $\text{C}_5\text{H}_{11}\text{NO}_2\text{S}$: C, 40.26; H, 7.43; N, 9.39. Found: C, 40.08; H, 7.65; N, 9.52. $[\alpha]_D^{20} + 22.4^\circ$ ($c=2$, 5 N HCl). The compound was characterized as its benzoate, mp 95–97°. *Anal.* Calcd. for $\text{C}_{12}\text{H}_{15}\text{NO}_3\text{S}$: C, 56.91; H, 5.97; N, 5.53. Found: C, 57.16; H, 6.08; N, 5.23. $[\alpha]_D^{20} - 19.3^\circ$ ($c=2$, EtOH). EtOH washing (100 ml) of the residue was evaporated *in vacuo* to dryness, the resulting residue was dissolved in a small amount of H₂O. The solution was acidified with 1 N HCl to pH 1.0 and extracted with three 30 ml portions of EtOAc. After removal of EtOAc *in vacuo*, the residue was washed several times with petr. ether to remove phenylacetic acid and recrystallized from H₂O to give white needles of N-phenylacetyl-D-methionine, mp 109–110°, yield 0.85 g (63%). *Anal.* Calcd. for $\text{C}_{13}\text{H}_{17}\text{NO}_2\text{S}$: C, 58.43; H, 6.36; N, 5.24. Found: C, 58.37; H, 6.40; N, 5.15. $[\alpha]_D^{20} + 13.0^\circ$ ($c=2$, EtOH).

b) *Escherichia coli* IFO-3550 was incubated at 37° for 5 days in 100 ml of the medium containing 1.34 g of N-phenylacetyl-DL-methionine in a 500-ml Erlenmeyer flask. Treatment of the cultured broth by the same procedure described above gave L-methionine, yield 0.21 g (56.3%) and N-phenylacetyl-D-methionine, yield 0.39 g (58.2%).

Resolution of α -N-Phenylacetyl- ϵ -N-Benzoyl-DL-Lysine by the Metabolism of *Escherichia coli* IFO-3470 and IFO-3550—a) *Escherichia coli* IFO-3470 was incubated at 37° for 5 days in 100 ml of the medium mentioned above containing 1.84 g of α -N-phenylacetyl- ϵ -N-benzoyl-DL-lysine. The cultured broth was treated exactly according to the procedure described previously⁴⁾ and gave colorless leaves of ϵ -N-benzoyl-L-lysine, mp 270–272° (dec.), yield 0.41 g (65.5%). *Anal.* Calcd. for $\text{C}_{13}\text{H}_{18}\text{N}_2\text{O}_3$: C, 62.38; H, 7.25; N, 11.19. Found: C, 62.46; H, 7.01; N, 10.81. $[\alpha]_D^{20} + 18.8^\circ$ ($c=2$, 5 N HCl) and colorless leaves of α -N-phenylacetyl- ϵ -N-benzoyl-D-lysine, mp 134–135°, yield 0.78 g (84.8%). *Anal.* Calcd. for $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_4$: C, 68.46; H, 6.57; N, 7.60. Found: C, 67.59; H, 6.71; N, 7.53. $[\alpha]_D^{20} - 3.5^\circ$ ($c=2$, EtOH).

b) *Escherichia coli* IFO-3550 was incubated at 37° for 5 days in the medium containing 1.84 g of α -N-phenylacetyl- ϵ -N-benzoyl-DL-lysine. The cultured broth was treated by the same procedure, and ϵ -N-benzoyl-L-lysine, yield 0.30 g (48.0%) and α -N-phenylacetyl- ϵ -N-benzoyl-D-lysine, yield 0.63 g (68.5%) were obtained.

3) Y. Kameda, E. Toyoura, Y. Kimura, K. Matsui and H. Saito, *Yakugaku Zasshi*, **78**, 759 (1958).

4) Y. Kameda and T. Omori, *Chem. Pharm. Bull.* (Tokyo), **10**, 831 (1962).