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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 authors[2] 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).

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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 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 flasks and incubated at 37° for 7 days: NH_4Cl 0.1 g, K_2HPO_4 0.1 g, $MgSO_4 \cdot 7H_2O$ 0.05 g, 1% $CaCl_2 \cdot 6H_2O$ 2 drops, 1% $FeCl_3 \cdot 6H_2O$ 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–H_2O to give colorless plates of L-methionine, mp 275—278° (dec.), yield 0.62 g (83%) *Anal.* Calcd. for $C_5H_{11}NO_2S$: C, 40.26; H, 7.43; N, 9.39. Found: C, 40.08; H, 7.65; N, 9.52. $[\alpha]_D^{20}$ +22.4° (c=2, 5 N HCl). The compound was characterized as its benzoate, mp 95—97°. *Anal.* Calcd. for $C_{12}H_{15}NO_3S$: C, 56.91; H, 5.97; N, 5.53. Found: C, 57.16; H, 6.08; N, 5.23. $[\alpha]_D^{20}$ −19.3° (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 H_2O. 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 H_2O to give white needles of N-phenylacetyl-D-methionine, mp 109—110°, yield 0.85 g (63%). *Anal.* Calcd. for $C_{13}H_{17}NO_3S$: C, 58.43; H, 6.36; N, 5.24. Found: C, 58.37; H, 6.40; N, 5.15. $[\alpha]_D^{20}$ +13.0° (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 previously[4] and gave colorless leaves of ε-N-benzoyl-L-lysine, mp 270—272° (dec.), yield 0.41 g (65.5%). *Anal.* Calcd. for $C_{13}H_{18}N_2O_3$: C, 62.38; H, 7.25; N, 11.19. Found: C, 62.46; H, 7.01, N, 10.81. $[\alpha]_D^{20}$ +18.8° (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 $C_{21}H_{24}N_2O_4$: C, 68.46; H, 6.57; N, 7.60. Found: C, 67.59; H, 6.71; N, 7.53. $[\alpha]_D^{20}$ −3.5° (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).

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