UDC 547.466.6 : 576.851.132.095.3 : 577.152

141. Etsuko Toyoura : Studies on Acylase Activity and Microörganisms. XII.*2 Optical Resolution of Glutamic and Aspartic Acids by Metabolism of Soil Bacteria on Benzoyl Derivatives of DL-Amino Acids.

(*Laboratory of Antibiotics, Faculty of Pharmacy, Kanazawa University**1)

In previous papers,[1,2] it was shown that four strains (KT-230, KT-231, KT-232, and KT-233) of soil bacteria could metabolize dibenzoyl-DL-lysine to produce ε-N-benzoyl-L-lysine and dibenzoyl-D-lysine, that benzoyl-DL-methionine, benzoyl-DL-phenylalanine, and benzoyl-DL-leucine could be resolved by the metabolism of KT-230 and KT-231, but not by KT-232 or KT-233, and that N-benzoyl-DL-threonine could be resolved by KT-233 but not by KT-230, KT-231, or KT-232. This paper describes the optical resolution of glutamic and aspartic acids.

At the outset, the metabolic activities of KT-230, KT-231, KT-232, and KT-233 were tested on glutamic and aspartic acids, and their benzoyl derivatives. The result is shown in Table I. The constituents of the culture medium used in the experiments were as follows: 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; organic substance to be tested, 0.2 g; distilled water, 100 cc.; pH 7.4~7.6 (adjusted with 10% NaOH).

From Table I, it is to be understood that KT-230 and KT-231 can utilize benzoic acid, produced from benzoyl-L-glutamic and benzoyl-L-aspartic acids by the action of their acylase, as the sole source of carbon, and that benzoyl-DL-glutamic and benzoyl-DL-aspartic acids can be resolved by the metabolism of KT-230 and KT-231, but not by KT-232 or KT-233.

Table I. Metabolic Activity of Soil Bacteria

	KT-230	KT-231	KT-232	KT-233
Benzoic acid (I)	+	+	+	+
Benzoyl-DL-glutamic acid (II)	+	+	+	+
Benzoyl-DL-glutamic acid (II) (without NH_4Cl)	−	−	+	+
Benzoyl-D-glutamic acid (VI)	−	−	−	−
DL-Glutamic acid	−	−	+	+
Benzoyl-DL-aspartic acid (V)	+	+	+	+
Benzoyl-DL-aspartic acid (V) (without NH_4Cl)	−	−	+	+
Benzoyl-D-aspartic acid (VII)	−	−	−	−
DL-Aspartic acid	−	−	+	+

\+ Within 4 days at 25°, there were luxuriant growths of bacteria on a culture medium with the particular organic compound as the source of carbon. This cultivation experiment was repeated three times in succession.
− Almost no visible growth of bacteria observed at 25° in 4 days.

The ability of KT-231 to resolve benzoyl-DL-glutamic acid was tested as follows: KT-231 was grown at 25° in the culture medium mentioned above containing benzoyl-DL-glutamic acid (II). After 7 days, L-glutamic acid (III), $[\alpha]_D^{15}$ +30.8°, and benzoyl-D-glutamic acid (IV), m.p. 134~135°, $[\alpha]_D^{15}$ +14.5°, were obtained in a good yield.

In the same way, it was confirmed that KT-231 metabolized benzoyl-DL-aspartic acid (V) to yield L-aspartic acid (VI), $[\alpha]_D^{15}$ +24.5°, and benzoyl-D-aspartic acid (VII), m.p. 177°, $[\alpha]_D^{15}$ −37.4°.

*1 Tsuchitoribanaga-machi, Kanazawa (豊浦悦子).

*2 This constitutes a part of a series entitled "Studies on Acylase Activity and Microörganisms" by Y. Kameda. Part XI : This Bulletin, **7**, 702(1959).

1) Y. Kameda, E. Toyoura, K. Matsui, Y. Kimura, S. Kitagawa : Nature, **182**, 453(1958).
2) Y. Kameda, E. Toyoura, K. Matsui : This Bulletin, **7**, 702(1959).

$$\underset{\text{DL-Form}}{\text{R-CH(NH-CO-C}_6\text{H}_5\text{)-COOH}} \longrightarrow \underset{\text{L-Form}}{\text{R-CH(NH}_2\text{)-COOH}} + \underset{\text{D-Form}}{\text{R-CH(NH-CO-C}_6\text{H}_5\text{)-COOH}} + \text{C}_6\text{H}_5\text{COOH} \quad (\text{I})$$

R=HOOC-CH_2-CH_2-(II) R=HOOC-CH_2-CH_2-(III) R=HOOC-CH_2-CH_2-(IV)
R=HOOC-CH_2-(V) R=HOOC-CH_2-(VI) R=HOOC-CH_2-(VII)

Grateful acknowledgement is offered to Professor Y. Kameda for his interest and encouragement throughout this work. Thanks are also due to Takeda Pharmaceutical Industries, Ltd. for financial help and to Mr. Y. Itatani for microanalyses.

Experimental

Synthesis of Benzoyl-DL-glutamic (II) and Benzoyl-DL-aspartic Acids (V)—Benzoyl-DL-glutamic and benzoyl-DL-aspartic acids were prepared according to the method of E. Fischer.[3] Benzoyl-DL-glutamic acid, m.p. 155~156°. Benzoyl-DL-aspartic acid, m.p. 162°.

Resolution of Benzoyl-DL-glutamic Acid (II) by the Metabolism of KT-231—KT-231 was inoculated into 200 cc. of the culture medium mentioned above containing 2.51 g. of benzoyl-DL-glutamic acid and incubated at 25° for 7 days. The culture was centrifuged to remove the cellular material. The supernatant solution was concentrated *in vacuo* to 1/10 the original volume. The concentrate was acidified with dil. HCl and extracted several times with AcOEt. The aqueous layer was evaporated *in vacuo* to dryness and the residue was extracted with EtOH containing HCl gas to remove insoluble salts. HCl-EtOH solution was then evaporated *in vacuo* and the residue was refluxed with 10% HCl for 2 hr. The HCl solution was evaporated *in vacuo* to dryness, the residue was dissolved in a small volume of H_2O, and the solution was neutralized with conc. NH_4OH. The resulting precipitate was collected by suction and recrystallized from H_2O to 0.47 g.(64%) of L-glutamic acid (III) as colorless plates, m.p. 195~196°, $[\alpha]_D^{15}$ +30.8°(c=2, 2*N* HCl). *Anal.* Calcd. for $C_5H_9O_4N$: C, 40.81; H, 6.17; N, 9.52. Found: C, 40.77; H, 6.26; N, 9.66.

The AcOEt layer was evaporated to dryness *in vacuo* and the residual oil crystallized on standing in a refrigerator. The crystallized residue was washed several times with boiling petr. ether to remove BzOH and finally crystallized from Me_2CO-benzene to 1.0 g.(80%) of benzoyl-D-glutamic acid (IV) as colorless needles, m.p. 134~135°, $[\alpha]_D^{15}$ +14.5°(c=4, H_2O). *Anal.* Calcd. for $C_{12}H_{13}O_5N$: C, 57.37; H, 5.22; N, 5.58. Found: C, 57.41; H, 5.31; N, 5.68.

From the petr. ether solution, 0.11 g.(18%) of BzOH (I) was obtained as colorless plates, m.p. 118~119°.

Resolution of Benzoyl-DL-aspartic Acid (V) by the Metabolism of KT-231—KT-231 was inoculated into 200 cc. of the culture medium mentioned above containing 2.38 g. of benzoyl-DL-aspartic acid and incubated at 25° for 8 days. The culture medium was centrifuged to remove the cellular material, concentrated *in vacuo* to 1/10 the original volume. The concentrate was acidified with dil. HCl and extracted several times with AcOEt. The aqueous layer was concentrated *in vacuo* to a small volume, neutralized with conc. NH_4OH, and stood in a refrigerator. The resulting precipitate was collected by suction and recrystallized from H_2O to 0.47 g.(71%) of L-aspartic acid (VI) as colorless plates, $[\alpha]_D^{15}$ +24.5°(c=2, 5*N* HCl). *Anal.* Calcd. for $C_4H_7O_4N$: C, 36.09; H, 5.30; N, 10.52. Found: C, 36.11; H, 5.46; N, 10.78.

The AcOEt layer was evaporated to dryness *in vacuo*, the crystallized residue was washed several times with boiling petr. ether to remove BzOH, and finally recrystallized from H_2O to 0.7 g.(58%) of benzoyl-D-aspartic acid (VII) as colorless needles, m.p. 177°; $[\alpha]_D^{15}$ −37.4°(c=2, 2*N* KOH). *Anal.* Calcd for $C_{11}H_{11}O_5N$: C, 55.69; H, 4.67; N, 5.91. Found: C, 55.60; H, 4.78; N, 5.81.

From the petr. ether solution, 0.11 g.(18%) of BzOH (I) was obtained as colorless plates, m.p. 118~120°.

Summary

The metabolic activities of 4 strains (KT-230, KT-231, KT-232, and KT-233) of soil bacteria were tested on glutamic and aspartic acids and their benzoyl derivatives, and it was found that benzoyl-DL-glutamic and benzoyl-DL-aspartic acids are resolved by the metabolism of KT-230 and KT-231, but not by KT-232 and KT-233. It was also demonstrated that KT-231 metabolized benzoyl-DL-glutamic and benzoyl-DL-aspartic acids to yield L-amino acids and the corresponding benzoyl-D-amino acids.

(Received March 30, 1959)

3) Y. Kameda, *et al.* : Yakugaku Zasshi, **78**, 765(1958).