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73. Yukio Kameda, Etsuko Toyoura, Yukio Kimura, and Katsuhiko Matsui : Studies on Acylase Activity and Microörganisms. Ⅷ.* Enzymatic Hydrolysis of 6-N-Benzoyl-L-lysine.

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In connection with naturally occurring ε-peptide derivatives of lysine, it was reported that the ε-amino group of lysine is attached to the carboxyl group of aspartic acid in the antibiotic, bacitracin A,[1,2] and that biocytin (6-N-biotinyl-L-lysine) was isolated from yeast.[3] On the other hand, biotinidase which acts on biocytin to yield biotin and L-lysine was isolated from the hog liver.[4]

In earlier papers we reported that a strain of *Pseudomonas* sp., KT 83, contained D-acylase and L-acylase.[5,6] In a further study of the enzyme of KT 83, we found that KT 83 hydrolyzed 6-N-benzoyl-L-lysine. L-Lysine, 6-N-benzoyl-D-lysine, and benzoic acid were obtained by incubating 6-N-benzoyl-DL-lysine with bacterial mass, acetone-dried powder, cell-free extract, or cell-free acetone powder of KT 83.*** This enzyme may be called 6-lysine-acylase.

A study of the separation of D-acylase, L-acylase, and ε-lysine-acylase from KT 83 is now under way. In the meantime a report on the ε-lysine-acylase was published by Greenstein, *et al.*[7] They observed that an enzyme system in rat kidney hydrolyzes 6-N-acetyl-L-lysine. The past discovery of ε-lysine-acylase in soil bacteria, which also effects enzymatic resolution of 6-N-benzoyl-lysine, is herein reported.

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Experimental

Growth Conditions and Preparation of KT 83 Cell-free Extract—KT 83 (*Pseudomonas* sp.) was grown in ten 1-L. Erlenmeyer flasks each containing 200 cc. of bouillon medium (pH 7.2). Growth was allowed to proceed at 25° for 3 days, and the cells were then harvested by centrifugation and washed with distilled water. The yield of the cells in wet weight was approximately 25~30 g. per 2 L. of the medium. The cells, after having been ground with alumina,[8] were extracted with 40 cc. of distilled water. The alumina and unruptured cells were removed by centrifugation for 20 mins. at 3,000*g* and then cell walls were removed by centrifugation for 30 mins. at 25,000*g*. Approximately 40 cc. of a supernatant solution was obtained.

KT 83 Cell-free Acetone Powder—40 cc. of KT 83 cell-free extract was adjusted to pH 4.5 with AcOH and centrifuged for 20 mins. at 3,000*g*. The supernatant solution was discarded, the pellets were poured into 100 cc. of acetone cooled to −5° and stirred for 5 mins. The solid was collected by suction and washed with cold acetone and ether, transferred onto a filter paper, and worked gently with a spatula until the solvent has evaporated, leaving a dry powder. The yield of the cell-free acetone powder was approximately 1.1 g.

Asymmetric Hydrolysis of 6-N-Benzoyl-DL-lysine—(i) 2.5 g. of 6-N-benzoyl-DL-lysine was dissolved in 400 cc. of water and the solution was neutralized to about pH 7.2 by the addition of 10% NaOH. To this aqueous solution, 3.2 g. (in wet weight) of KT 83 bacterial mass was added to insure complete

* Part Ⅶ : Yakugaku Zasshi, **78**(1958), in press.

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hydrolysis of the L-form of the 6-N-benzoyl-lysine and the mixture was incubated at 37°, with a few drops of toluene, for 3 days. The digest was then adjusted to pH 4.5 with AcOH. The filtrate through charcoal was evaporated *in vacuo* to a very small volume. The resulting precipitate, which is almost pure 6-N-benzoyl-D-lysine, was filtered by suction and washed with ether. Recrystallization from water gave 0.78 g. (62.4%) of 6-N-benzoyl-D-lysine as colorless leaves, m.p. 270~272°(decomp.); $[\alpha]_D^{18}$ −19.5° (c=2, 5*N* HCl). *Anal.* Calcd. for $C_{13}H_{18}O_3N_2$: C, 62.38; H, 7.25; N, 11.19. Found: C, 62.51; H, 7.32; N, 11.25.

The filtrate from 6-N-benzoyl-D-lysine was acidified to pH 1.0 with HCl and extracted with ether. The aqueous layer was evaporated *in vacuo*, the residue was taken up with EtOH, and the EtOH solution was neutralized with pyridine. The resulting precipitate was collected by suction and recrystallized from water and EtOH to 0.55 g. (60.2%) of L-lysine monohydrochloride as colorless crystals, m.p. 256°(decomp.); $[\alpha]_D^{17}$ +21° (c=2, 5*N* HCl). *Anal.* Calcd. for $C_6H_{15}O_2N_2Cl$: C, 39.40; H, 8.21; N, 15.32. Found: C, 39.21; H, 8.34; N, 15.45.

From the ether layer, 0.47 g. (77.0%) of benzoic acid was obtained as colorless plates, m.p. 118~120°.

(ii) To 200 cc. of a 0.025*M* 6-N-benzoyl-DL-lysine solution, 0.4 g. of KT 83 acetone-dried powder was added and the mixture was incubated at 37° for 2 days. 0.33 g. (72.2%) of L-lysine monohydrochloride, 0.36 g. (57.6%) of 6-N-benzoyl-D-lysine, and 0.24 g. (78.7%) of benzoic acid were obtained.

(iii) To 400 cc. of a 0.025*M* 6-N-benzoyl-DL-lysine solution, 4 cc. of KT 83 cell-free extract was added and the mixture was incubated at 37° for 3 days. 0.55 g. (60.2%) of L-lysine monohydrochloride, 0.93 g. (74.0%) of 6-N-benzoyl-D-lysine, and 0.45 g. (73.7%) of benzoic acid were obtained.

(iv) To 100 cc. of a 0.025*M* 6-N-benzoyl-DL-lysine solution, 0.1 g. of KT 83 cell-free acetone powder was added and the mixture was incubated at 37° for 3 days. 0.14 g. (61.3%) of L-lysine monohydrochloride, 0.22 g. (70.4%) of 6-N-benzoyl-D-lysine, and 0.11 g. (72.1%) of benzoic acid were obtained.

Summary

It was found that a strain of *Pseudomonas* sp., KT 83, contains an enzyme which hydrolyzes 6-N-benzoyl-L-lysine. By incubating 6-N-benzoyl-DL-lysine with bacterial mass, acetone-dried powder, cell-free extract, or cell-free acetone powder of KT 83, L-lysine, 6-N-benzoyl-D-lysine, and benzoic acid were obtained in a good yield.

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74. Yukio Kameda, Etsuko Toyoura, Yukio Kimura, and Buhei Okino: Studies on Acylase Activity and Microörganisms. IX.* Enzymatic Resolution of Tryptophan by a Strain of Soil Bacteria.**

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In the earlier papers[1,2] it was reported that KT 84 (*Pseudomonas* sp.) asymmetrically hydrolyzed the benzoyl, dichloroacetyl, chloroacetyl, or acetyl derivatives of the following 24 amino acids to give the corresponding L-amino acids and acylated D-amino acids in a good yield: Alanine, aminobutyric acid, valine, leucine, phenylalanine, tyrosine, *p*-methoxyphenylalanine, 3,4-methylenedioxyphenylalanine, *p*-nitrophenylalanine, serine, threonine, allothreonine, aspartic acid, glutamic acid, methionine, cystine, lysine, ornithine, 2,4-diaminobutyric acid, *threo*- and *erythro*-*β*-phenylserines, *threo*- and *erythro*-*β*-*p*-nitrophenylserines, and phenylglycine.

In an experiment of hydrolysis of acetyltryptophan by KT 84, it was found that the hydrolysis was too slow for practical application in the resolution procedure.

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