

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.);  $[\alpha]_D^{18} -19.5^\circ$  (c=2, 5N 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.);  $[\alpha]_D^{18} +21^\circ$  (c=2, 5N 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 0.025M 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 0.025M 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 0.025M 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 Microorganisms. IX.\*

Enzymatic Resolution of Tryptophan by a Strain of Soil Bacteria.\*\*

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In the earlier papers<sup>1,2)</sup> 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*- $\beta$ -phenylserines, *threo*- and *erythro*- $\beta$ -*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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1) Y. Kameda, E. Toyoura, H. Yamazoe, Y. Kimura, Y. Yasuda : *Nature*, **170**, 888(1952).

2) Y. Kameda, *et al.* : *Yakugaku Zasshi*, **78**(1958), in press.

This paper describes the fact that KT 104, a strain of *Pseudomonas* sp. isolated from the soil in this laboratory, had the ability to hydrolyze 1-N-acetyl-DL-tryptophan asymmetrically. L-Tryptophan and 1-N-acetyl-D-tryptophan were obtained by incubating 1-N-acetyl-DL-tryptophan with the bacterial mass, acetone-dried powder, or cell-free extract of KT 104.

The authors wish to express their grateful appreciation to Dr. K. Ogata of Institute for Fermentation for his help in determining the genus of KT 104, and to Mr. Y. Itatani of Kanazawa University for his microanalyses.

### Experimental

**Isolation and Characterization of KT 104**—The composition of the culture medium for isolation of KT 104 was as follows: N-Benzoylanthranilic acid, 0.2 g.; NH<sub>4</sub>Cl, 0.1 g.; K<sub>2</sub>HPO<sub>4</sub>, 0.1 g.; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.05 g.; 1% soln. of CaCl<sub>2</sub>·6H<sub>2</sub>O, 2 drops; 1% soln. of FeCl<sub>3</sub>·6H<sub>2</sub>O, 1 drop; distilled water, 100 cc.; pH 7.0~7.2 (adjusted with 10% NaOH). 0.2 g. of a soil sample, taken from a bamboo thicket at Hiraguri village in Kanazawa, was inoculated into 10 cc. of the above culture medium and incubated at 25° for 3~6 days. If luxuriant growth of bacteria occurred, a loop of the culture fluid was transferred to a new culture medium of the same composition. Such transplantation was repeated at least three times. The bacterial suspension of the last generation was then planted in an agar medium, containing of 1.5% agar in the above culture medium. Culture experiments were carried out in order to determine whether the microbe isolated from the agar plate could grow in the above medium. KT 104 can be cultivated successively in the above medium containing N-benzoylanthranilic acid as the sole source of carbon, and ammonia as the sole source of nitrogen.

KT 104 belongs to the *Pseudomonas* group and has the following characters: Aerobic, short rod-shaped, motile, polar flagella; gram-negative; yields water-soluble yellowish green pigment that diffuses through the medium; optimal temperature at around 28°. KT 104 can be cultivated in a synthetic medium containing benzoic, *p*-hydroxybenzoic, salicylic, anisic, or phenylacetic acid as the sole source of carbon, and ammonia as the sole source of nitrogen, at 25°, for at least 3 generations (cf. the above culture medium).

**Growth Conditions and Preparation of Acetone Powder of KT 104**—KT 104 (*Pseudomonas* sp.) was grown in five 1-L. Erlenmeyer flasks each containing 200 cc. of bouillon (pH 7.2). Growth was allowed to proceed at 25° for 3 days, the cells were harvested by centrifugation, and washed with distilled water. The yield of cells, in wet weight, was approximately 8 g. The cells thus obtained were placed in 300 cc. of acetone cooled to -5° and stirred vigorously for 5 mins. The solid obtained was collected by suction and washed first with cold acetone and then with cold ether. The filter cake of cells was transferred onto a sheet of filter paper and worked gently with a spatula until the solvent evaporated, leaving a dry powder which weighed approximately 0.8 g.

**Preparation of Cell-free Extract of KT 104**—KT 104 cells were grown in 1 L. of bouillon as mentioned above. About 8 g. (in wet weight) of the bacterial mass, after having been ground with alumina for 20~30 mins., was extracted with 40 cc. of 0.02M phosphate buffer (pH 7.8) or with distilled water. The alumina and unruptured cells were removed by centrifugation for 20 mins. at 3,000g and then cell walls were removed by centrifugation for 30 mins. at 20,000g. Approximately 40 cc. of a straw-colored supernatant solution was obtained.

**Preparation of Acetyl-DL-tryptophan**—It was prepared from 4-acetamido-4,4-diethoxycarbonylbutyraldehyde phenylhydrazone according to the procedure of Warner and Moe<sup>3)</sup> as colorless plates, m.p. 201~203°(decomp.).

**Asymmetric Hydrolysis of 1-N-Acetyl-DL-tryptophan**—i) 6.15 g. of 1-N-acetyl-DL-tryptophan was suspended in 500 cc. of water and brought into solution at pH 7.8 by addition of 10% NaOH. To this aqueous solution, 8 g. (wet weight) of the cells of KT 104 was added and allowed to digest at 37° with a few drops of toluene. Samples were withdrawn at intervals and determined for free amino acid according to the Grassmann and Heyde's method. When no further increase in amino acid was noted (1~2 days), the digestion was continued overnight to insure complete hydrolysis of the L-form of acetyl-DL-tryptophan. The digest was then adjusted to pH 4.5 by the addition of AcOH. After heating for several mins., the coagulated protein and insoluble mass were removed by centrifugation. The supernatant which came through charcoal was concentrated *in vacuo* until crystallization began. EtOH was then added and the whole was allowed to stand for 12 hrs. in a refrigerator. The partially precipitated L-tryptophan was filtered by suction. By concentration of the filtrate and addition of EtOH, some additional precipitate was obtained. Recrystallization of the combined precipitate from 70% EtOH yielded 1.6 g. (62.6%) of L-tryptophan as colorless scaly crystals,  $[\alpha]_D^{25} -32^\circ(c=1, H_2O)$ . Anal. Calcd. for C<sub>11</sub>H<sub>12</sub>O<sub>2</sub>N<sub>2</sub>: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.60; H, 5.71; N, 13.95.

3) D. T. Warner, O. A. Moe: J. Am. Chem. Soc., **70**, 2765(1948).

The filtrate from L-tryptophan was concentrated to dryness and the residue was dissolved in 8 cc. of water. The solution was acidified with HCl and the resulting precipitate was collected by suction. Recrystallization from 50% MeOH gave 2.6 g. (84.5%) of 1-N-acetyl-D-tryptophan, m.p. 184~185°,  $[\alpha]_D^{15} -28^\circ$  (c=2, EtOH). *Anal.* Calcd. for  $C_{13}H_{14}O_3N_2$ : C, 63.40; H, 5.73; N, 11.38. Found: C, 63.62; H, 5.86; N, 11.46.

(ii) To 300 cc. of 0.05M 1-N-acetyl-DL-tryptophan solution, 0.8 g. of KT 104 acetone-dried powder was added and the mixture was incubated at 37° for 2~3 days. 1.0 g. (65%) of L-tryptophan and 1.5 g. (81%) of 1-N-acetyl-D-tryptophan were obtained.

(iii) To 500 cc. of 0.05M 1-N-acetyl-DL-tryptophan solution, 40 cc. of KT 104 cell-free extract was added and the mixture was incubated at 37° for 1~2 days. 1.8 g. (70.5%) of L-tryptophan and 2.6 g. (84.5%) of 1-N-acetyl-D-tryptophan were obtained.

### Summary

It was found that a strain of *Pseudomonas* sp., KT 104, isolated from the soil of a bamboo thicket in Kanazawa, has the ability to hydrolyze asymmetrically 1-N-acetyl-DL-tryptophan to give L-tryptophan and 1-N-acetyl-D-tryptophan. The strain may be used in the form of bacterial mass, acetone-dried powder, or cell-free extract.

KT 104 can be cultivated in a synthetic medium containing N-benzoylanthranilic, benzoic, *p*-hydroxybenzoic, salicylic, anisic, or phenylacetic acid as the sole source of carbon, and ammonia as the sole source of nitrogen, at 25°, for at least 3 generations.

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### 75. Michio Takido: Studies on the Constituents of the Seeds of *Cassia obtusifolia* L. I. The Structure of Obtusifolin.

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The seeds of *Cassia obtusifolia* L. (Leguminosae) are used as purgative and tonic, and have been employed as a yellow dyestuff in this country.

Some chemical studies have been made on the constituents of this seed. Elborne<sup>1)</sup> and Shimoyama<sup>2)</sup> isolated emodin, and recently Kariyone and Tsukita<sup>3)</sup> by paper chromatographical study reported the existence of chrysophanol or physcion, chrysophanol anthrone, emodin, aloe-emodin, and rhein.

In the present work, chrysophanol (IV), physcion (VII), and a new anthraquinone derivative, to which the name obtusifolin is proposed, were isolated from the seeds by column chromatography.

Obtusifolin, yellow needles, m.p. 237~238°, whose analyses agreed with  $C_{16}H_{12}O_5$  involving one methoxyl group, is insoluble in water and 5%  $NaHCO_3$ , but soluble in 5%  $Na_2CO_3$  to give an orange solution.

It gives a purple-blue color in conc.  $H_2SO_4$ , brown with  $FeCl_3$ , and orange in alcoholic  $Mg(OAc)_2$  solution. The infrared spectrum of obtusifolin indicated the presence of a free hydroxyl group and both chelated and non-chelated carbonyl. It was therefore suggested that obtusifolin is an anthraquinone derivative with at least one free hydroxyl group in the  $\beta$ -position.

Obtusifolin gives diacetate, light yellow needles, m.p. 187~188°; dimethyl ether,

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1) Elborne: *Pharm. J., Trans.*, **3**, 242(1889).

2) J. Shimoyama: *Apotheker Ztg.*, **11**, 537(1896).

3) T. Kariyone, K. Tsukita: *Yakugaku Zasshi*, **74**, 223, 225(1954).