Experimental

Material——Amides and Na-PAS : Same as described in the previous paper.[1] NaCl, Na_2SO_4, CH_3COONa, Na-citrate; JIS. guaranteed reagent.

Apparatus and Procedure——Spectrophotometry : Same as described in the previous paper.[4] Colorimetry : Same as described in the previous paper.[1]

Summary

Dissociation constants of the complex between pyrazine- or pyridine-monocarboxamide and Na-PAS in aqueous solution (K) were obtained at various temperatures (T) by the absorption spectra measurment, then heats of complex formation ΔHc were evaluated from the correlation between log K and 1/T. Thus obtained ΔHc value by the spectral method exceedingly differs from that[1] thermal method considering the experimental error, so the discussion was made on this reason.

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4) Part 1 : H. Negoro *et al.* : This Bulletin, **7**, 91 (1959).

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131. Yukio Kameda, Katsuhiko Matsui, Yukio Kimura, Etsuko Toyoura, and Eiji Takeuchi : Studies on Acylase Activity and Microörganisms. XVII.*1 Optical Resolution of Tryptophan, 2-Aminohexanedioic Acid, and 2-Aminoöctanoic Acid by Metabolism of Soil Bacteria on Benzoyl Derivatives of DL-Amino Acids.

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In previous papers[1~5] it was reported that lysine, methionine, phenylalanine, leucine, threonine, glutamic and aspartic acids, 3-(*p*-methoxyphenyl)alanine, and 3-(3,4-methylenedioxyphenyl)alanine could be assymmetrically resolved by the metabolism of soil bacteria such as KT-230 and KT-231 on benzoyl derivatives of DL-amino acids. The present work is a direct continuation and extension of the same work previously reported.

Tryptophan is one of essential amino acids, but 2-aminohexanedioic acid and 2-aminooctanoic acid have not been isolated from protein hydrolysates. D-2-Aminohexanedioic acid was obtained as a hydrolysis product of an antibiotic, cephalosporin N.[6]

*1 This work was presented at the Kantō Local Meeting of the Pharmaceutical Society of Japan, October 3, 1959. Part XVI : Ann. Rept. Fac. Pharm. Kanazawa Univ., **9**, 20 (1959).

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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).
3) E. Toyoura : *Ibid.*, **7**, 785 (1959).
4) *Idem* : *Ibid.*, **7**, 787 (1959).
5) *Idem* : *Ibid.*, **7**, 789 (1959).
6) G.G.F. Newton, E.P. Abraham : Biochem. J., **58**, 103 (1954).

Already it was shown that N-benzoyl-DL-tryptophan (II) could be metabolized by one strain (KT-305), but not by six strains (KT-230, KT-231, KT-232, KT-233, KT-301, and KT-307) of soil bacteria.[7] In this work two new strains (KT-303 and KT-304) capable of metabolizing N-benzoyl-DL-tryptophan were isolated from soil by using a synthetic culture medium containing N-benzoyl-DL-tryptophan as the sole source of carbon. The metabolic activites of KT-303, KT-304, and KT-305 were tested on tryptophan and its benzoyl derivatives (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).

Table I. Metabolic Activity of Soil Bacteria KT-303, KT-304, and KT-305

	KT-303	KT-304	KT-305
Benzoic acid (I)	+	+	+
N-Benzoyl-DL-tryptophan (II)	+	+	+
N-Benzoyl-DL-tryptophan (II) (without NH_4Cl)	−	+	−
N-Benzoyl-D-tryptophan (IV)	−	−	−
DL-Tryptophan	−	+	−

+ : There were luxuriant growths of bacteria within 4 days at 25° on a culture medium with the particular organic compound as the source of carbon. This cultivation experiment was repeated 3 times in succession.
− : Almost no visible growth of bacteria observed at 25° in 4 days.

From Table I, it is to be understood that KT-303 and KT-305 can produce L-tryptophan (III) and N-benzoyl-D-tryptophan (IV) from N-benzoyl-DL-tryptophan (II) by utilising benzoic acid (I), which was formed from N-benzoyl-L-tryptophan by the action of of their acylases, as the sole source of carbon and ammonia as the sole source of nitrogen.

The ability of KT-303 and KT-305 to resolve N-benzoyl-DL-tryptophan was tested as follows: KT-303 or KT-305 was grown at 25° in the culture medium mentioned above containing N-benzoyl-DL-tryptophan (II). After 6 days, L-tryptophan (III), $[\alpha]_D^{20}$ −32° and N-benzoyl-D-tryptophan (IV), m.p. 98~100°, $[\alpha]_D^{20}$ +37° were obtained.

Subsequently, the metabolic activities of KT-230 and KT-231 were tested on 2-aminohexanedioic and 2-aminooctanoic acids, and their benzoyl derivatives. The results are shown in Table II.

From Table II, it is to be understood that KT-230 and KT-231 can utilize benzoic acid, produced from N-benzoyl-L-2-aminohexanedioic and N-benzoyl-L-2-aminooctanoic

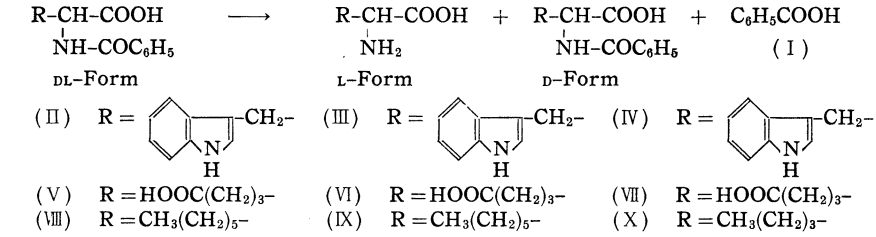
Table II. Metabolic Activity of Soil Bacteria KT-230 and KT-231

	KT-230	KT-231
Benzoic acid (I)	+	+
N-Benzoyl-DL-2-aminohexanedioic acid (V)	+	+
N-Benzoyl-DL-2-aminohexanedioic acid (V) (without NH_4Cl)	−	−
N-Benzoyl-D-2-aminohexanedioic acid (VII)	−	−
DL-2-Aminohexanedioic acid	−	−
N-Benzoyl-DL-2-aminooctanoic acid (VIII)	+	+
N-Benzoyl-DL-2-aminooctanoic acid (VIII) (without NH_4Cl)	−	−
N-Benzoyl-D-2-aminooctanoic acid (X)	−	−
DL-2-Aminooctanoic acid	−	−

+ : There were luxuriant growths of bacteria within 4 days at 25° on a culture medium with the particular organic compound as the source of carbon. This cultivation experiment was repeated 3 times in succession.
− : Almost no visible growth of bacteria observed at 25° in 4 days.

7) E. Toyoura: Yakugaku Zasshi, **80**, 362 (1960).

acids by the action of their acylases as the sole source of carbon, and that N-benzoyl-DL-2-aminohexanedioic (V) and N-benzoyl-DL-2-aminooctanoic acids (VIII) can be resolved by the metabolism of KT-230 and KT-231. KT-230 or KT-231 metabolized N-benzoyl-DL-2-aminohexanedioic and N-benzoyl-DL-2-aminooctanoic acids to yield the following compounds respectively: L-2-aminohexanedioic acid (VI), $[\alpha]_D^{20}$ +26°, N-benzoyl-D-2-aminohexanedioic acid (VII), m.p. 181~182°, $[\alpha]_D^{20}$ −25° and L-2-aminooctanoic acid (IX), $[\alpha]_D^{20}$ +24°, N-benzoyl-D-2-aminooctanoic acid (X), m.p. 72~74°, $[\alpha]_D^{20}$ −9.5°.



Experimental

Benzoyl-DL-tryptophan——To a solution of DL-tryptophan (10 g.) dissolved in *N* NaOH (120 cc.), BzCl (8.4 g.) was added with stirring at 5~10° and stirred additionally for 1 hr. at room temperature. The reaction mixture was acidified with HCl. The separated crude N-benzoyl-DL-tryptophan was collected by suction and washed several times with petr. ether to remove BzOH, and recrystallization from Me_2CO and benzene gave colorless crystals, m.p. 194. Yield, 12.5 g. (81.2%). *Anal.* Calcd. for $C_{18}H_{16}O_3N_2$: C, 70.11; H, 5.23. Found: C, 70.23; H, 5.36.

N-Benzoyl-DL-2-aminohexanedioic Acid——To a solution of DL-2-aminohexanedioic acid (3 g.) dissolved in *N* NaOH (70 cc.) BzCl (3.2 g.) was added with stirring at 5~10° and stirred additionally for 1 hr. at room temperature. The reaction mixture was acidified with HCl. The separated crude N-benzoyl-DL-2-aminohexanedioic acid was collected by suction and washed several times with petr. ether to remove BzOH, and recrystallization from H_2O gave colorless plates, m.p. 184°. Yield, 3.6 g. (73%). *Anal.* Calcd. for $C_{13}H_{15}O_5N$: C, 58.86; H, 5.70. Found: C, 58.61; H, 5.73.

N-Benzoyl-DL-2-aminooctanoic Acid——N-Benzoyl-DL-2-aminooctanoic acid, m.p. 127°, was prepared according to the procedure described previously.[8]

Isolation and Characterization of KT-303 and KT-304——The constituents of the culture medium for isolation of KT-303 and KT-304 were as follows: N-Benzoyl-DL-tryptophan, 0.2 g.; NH_4Cl, 0.1 g.; K_2HPO_4, 0.1g., $MgSO_4 \cdot 7H_2O$, 0.05 g.; 1% soln. of $CaCl_2 \cdot 6H_2O$, 2 drops; 1% soln. of $FeCl_3 \cdot 6H_2O$, 1 drop; distilled H_2O, 100 cc.; pH 7.4~7.6 (adjusted with 10% NaOH). 0.5 g. of soil sample, taken from Ishiura-shrine in Kanazawa city (KT-303) or Nurse Hostel of Kanazawa University (KT-304) was inoculated into 10 cc. of the above culture medium and incubated at 25° for 4~7 days. If luxuriant growth of bacteriaoccurred a loop of the culture fluid was transferred to a new culture medium of the same constituent. Such transplantation was repeated at least two times. The bacterial suspension of the last generation was then planted in bouillon agar. Culture experiments were carried out in order to determine whether the microbe isolated from the agar plate could grow in the above medium. KT-303 is rod-shaped and KT-304 is seemingly *Coccobacillus*. The metabolic activity of KT-303 and KT-304 is shown in Table I.

Resolution of N-Benzoyl-DL-tryptophan (II) by the Metabolism of KT-303 or KT-305——i) KT-303 was inoculated into 100 cc. of the culture medium mentioned above containing 1.54 g. of N-benzoyl-DL-tryptophan and incubated at 25° for 6 days. The culture medium was adjusted to pH 4.5 with AcOH, evaporated *in vacuo* to small volume, acidified with HCl to pH 1.0, and extracted with Et_2O. The aqueous layer was neutralized with conc. NH_4OH and evaporated *in vacuo* to dryness. The residue was dissolved in 10 cc. of H_2O, passed through a column of Amberlite IR-120 and the column was washed with distilled H_2O until Cl^- ion was no longer found in the effluent. Then the adsorbed amino acid was eluted with 0.3~0.5*N* NH_4OH and the eluate was evaporated *in vacuo* to dryness. Recrystallization from 65% EtOH gave 0.24 g. (47.2%) of L-tryptophan (III) as colorless leaves, m.p. 280°(decomp.), $[\alpha]_D^{20}$: −32°(c=0.5, H_2O) *Anal.* Calcd. for $C_{11}H_{12}O_2N_2$: C, 64.69; H, 5.92. Found: C, 64.81; H, 5.80. The Et_2O layer was evaporated to dryness. The residue washed several times with petr. ether to remove BzOH, and recrystallized from H_2O to 0.35 g. (45.6%) of N-benzoyl-D-

8) Y. Kimura: This Bulletin, in press.

tryptophan (IV), as pinkish platelets, m.p. 98~100°, $[\alpha]_D^{20}$: +37°(c=2, Me_2CO). *Anal.* Calcd. for $C_{18}H_{16}O_3N_2$: C, 70.11; H, 5.23. Found: C, 70.23; H, 5.45.

ii) KT-305 was grown at 25° for 6 days in 100 cc. of the above medium containing 1.54 g. of N-benzoyl-DL-tryptophan (II). L-Tryptophan (III) 0.22 g. (43.1%) and 0.40 g. (52.0%) of N-benzoyl-D-tryptophan were obtained.

Resolution of N-Benzoyl-DL-2-aminohexanedioic Acid (V) by the Metabolism of KT-231——KT-231 was inoculated into 200 cc. of the culture medium mentioned above containing 2.65 g. of N-benzoyl-DL-2-aminohexanedioic acid (V) and incubated at 25° for 11 days. The culture medium was adjusted to pH 4.5 with AcOH, concentrated *in vacuo* to a small volume, acidified with HCl to pH 1.0 and extracted with AcOEt. The aqueous layer was evaporated *in vacuo* to dryness. The residue was dissolved in 4 cc. of H_2O, neutralized with aniline, and added with 30 cc. of EtOH. The resulting precipitate was collected by suction. Recrystallization from H_2O-EtOH gave 0.62 g. (77%) of L-2-aminohexanedioic acid (VI) as colorless plates, m.p. 207°, $[\alpha]_D^{20}$: +26°(c=2, 5*N* HCl). *Anal.* Calcd. for $C_6H_{11}O_4N$: C, 44.71; H, 6.88; N, 8.69. Found: C, 44.36; H, 6.90; N, 8.65.

The AcOEt layer was evaporated *in vacuo* to dryness. The residue was washed several times with petr. ether to remove BzOH, and recrystallized from Me_2CO and benzene to 0.77 g. (58%) of N-bonzoyl-D-2-aminohexanedioic acid (VII) as colorless crystals, m.p. 181~182°, $[\alpha]_D^{20}$: −25°(c=1, 2*N* NaOH). *Anal.* Calcd. for $C_{13}H_{15}O_5N$: C, 58.86; H, 5.70; N, 5.28. Found: C, 58.81; H, 5.78; N, 5.22. From the petr. ether, 0.12 g. (20%) of BzOH, m.p. 118~120°, was obtained.

Resolution of N-Benzoyl-DL-2-aminooctanoic Acid (VIII) by the Metabolism of KT-230——KT-230 was inoculated into 200 cc. of the culture medium mentioned above containing 2.63 g. of N-benzoyl-DL-2-aminooctanoic acid (VIII) and incubated at 25° for 10 days. The culture medium was adjusted to pH 4.5 with AcOH, evaporated *in vacuo* to dryness, and the residue was treated with an excess of EtOH. After a few hour of standing at ca. 5°, the precipitate was collected by suction, washed with EtOH, and recrystallized from 50% EtOH, to 0.58 g. (73%) of L-2-aminooctanoic acid (IX) as colorless leaves, m.p. 305~307°(decomp.), $[\alpha]_D^{20}$: +24°(c=1, 5*N* HCl). *Anal.* Calcd. for $C_8H_{17}O_2N$: C, 60.34; H, 10.76. Found: C, 60.51; H, 10.62.

The EtOH solution with combined washings from the separation of L-2-aminooctanoic acid was evaporated *in vacuo* to dryness. The residue was taken up in the minimum amount of cold H_2O, brought to pH 1.0 by addition of HCl, and extracted with AcOEt. The extract was evaporated *in vacuo* to dryness, the residue was washed several times with petr. ether to remove BzOH, and recrystallized from Me_2CO and benzene to 0.94 g. (71%) of N-benzoyl-D-2-aminooctanoic acid as colorless plates, m.p. 72~74°, $[\alpha]_D^{20}$: −9.5°(c=2, Me_2CO). *Anal.* Calcd. for $C_{15}H_{21}O_3N$: C, 68.41; H, 8.04. Found: C, 68.52; H, 8.21.

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Summary

The metabolic activities of 3 strains (KT-303, KT-304, and KT-305) of soil bacteria were tested on tryptophan and its benzoyl derivatives (Table I), and it was found that KT-303 and KT-305 metabolized benzoyl-DL-tryptophan to produce L-tryptophan and benzoyl-D-tryptophan respectively.

Two strains (KT-230 and KT-231) of soil bacteria had the activity to metabolize benzoyl derivatives of 2-aminohexanedioic acid and 2-aminooctanoic acids to yield L-2-aminohexanedioic acid, N-benzoyl-D-2-aminohexanedioic acid, L-2-aminooctanoic acid, and N-benzoyl-D-2-aminooctanoic acid respectively.

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