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187. Yukio Kameda, Katsuhiko Matsui, Yukio Kimura, Etsuko Toyoura, and Masako Kimura : Studies on Acylase Activity and Microorganisms. XIX*1. Optical Resolution of Threonine, Lysine, Tryptophan, and Methionine by Acylase of Soil Bacteria.*2

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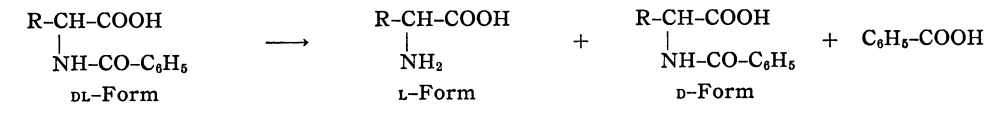
In the earlier papers,[1,2] it was reported that a strain of soil bacteria, KT 84 (*Pseudomonas* sp.), asymmetrically hydrolyzed benzoyl, dichloroacetyl, chloroacetyl or acetyl derivatives of the following 24 amino acids to produce the corresponding L-amino acids and acyl-D-amino acids in a good yield. They are alanine, 2-aminobutyric acid, valine, leucine, phenylalanine, tyrosine, *p*-methoxyphenylalanine, 3,4-methylenedioxyphenylalanine, *p*-nitrophenylalanine, serine, threonine, allothreonine, aspartic and glutamic acids, methionine, cystine, lysine, ornithine, 2,4-diaminobutyric acid, phenylglycine, *threo*- and *erythro*-3-phenyl serine, and *threo*- and *erythoro*-3-(*p*-nitrophenyl)serine. Two strains of soil bacteria, KT 104 (*Pseudomonas* sp.) and KT 85 (*Pseudomonas* sp.), had the ability to hydrolyze asymmetrically acetyl derivatives of DL-tryptophan[3] and DL-alloisoleucine,[4] respectively.

In the present work, the authors could isolate from various field areas fifteen strains (KT 202, KT 205, KT 207, etc.) of soil bacteria capable of hydrolyzing asymmetrically N-benzoyl-DL-threonine, 2,6-di-N-benzoyl-DL-lysine, N-acetyl-DL-tryptophan or N-acetyl-DL-methionine by using a synthetic medium containing a benzoyl derivative of threonine, cystine, lysine, anthranilic acid or 6-amino hexanoic acid as the sole source of carbon. All strains seemed to be rod-shaped and exhibited an acylase activity as shown in Table I. Morphological studies will be reported elsewhere.

It may be seen from Table I that KT 205, KT 218, KT 219, etc., can hydrolyze the L-form, but not the D-form of benzoyl derivatives of threonine and lysine. At the outset the ability of KT 218 to resolve N-benzoyl-DL-threonine (I) was tested in the following manner : To 0.05*M* N-benzoyl-DL-threonine solution (pH 7.2), KT 218 bacterial mass was added and the mixture was incubated at 37° with a few drops of toluene. After 2 days, L-threonine (II), $[\alpha]_D^{13}$ −28.0°, N-benzoyl-D-threonine (III), $[\alpha]_D^{15}$ −25.6°, and benzoic acid were obtained in a good yield. In the same way, it was confirmed that KT 229 and KT 83 also hydrolyzed N-benzoyl-DL-threonine (I) to yield L-threonine (II), N-benzoyl-D-threonine (III), and benzoic acid



R=CH_3-CH(OH)- (I) R=CH_3-CH(OH)- (II) R=CH_3-CH(OH)- (III)
R=$C_6H_5CONH(CH_2)_4$- (IV) R=$C_6H_5CONH(CH_2)_4$- (V) R=$C_6H_5CONH(CH_2)_4$- (VI)

*1 Part XVIII : This Bulletin, **10**, 831 (1962).
*2 Reported at the Kanto branch Meeting of the Pharmaceutical Society of Japan, Tokyo, Sep. 7, 1959.
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1) Y. Kameda, *et al.* : Nature **169**, 1016 (1952); *Ibid.*, **170**, 888 (1952).
2) *Idem* : Yakugaku Zasshi, **78**, 748, 754, 759, 763, 765, 767, 769 (1958).
3) Y. Kameda, E. Toyoura, Y. Kimura, B. Okino : This Bulletin, **6** 395 (1958). In this paper, 1-N-acetyltryptophan is misprint for N-acetyltryptophan.
4) Y. Kameda, E. Toyoura, K. Matsui : *Ibid.*, **6**, 441 (1958).

TABLE I. Acylase Activity of Soil Bacteria

Substrates / Strains	N-Bezoyl-threonine		2,6-Di-N-benzoyl-lysine		6-N-Benzoyl lysine		N-Acetyl-tryptophan		N-Acetyl-methionine		Carbon source of isolation-medium	source of soil Sample
	DL	D	DL	D	DL	D	DL	D	DL	D		
KT 202	0	0	0	0	0	0	0	0	6.2	0	N-Benzoyl-DL-threonine	(A)
KT 205	3.1	0	5.2	0	0.5	0	4.2	2.1	7.8	0.5	〃	(B)
KT 207	0	0	0.9	0	0	0	4.8	0	5.7	0	〃	(B)
KT 213	0	0	0.3	0	0	0	5.9	0	3.5	0.1	N-Benzoyl-anthranilic acid	(A)
KT 217	0.6	0	0	0	0	0	2.3	0	2.6	0	〃	(B)
KT 218	18.3	0	23.7	0	0	0	2.1	0	4.2	0	Di-N-benzoyl-L-cystine	(A)
KT 219	4.2	0	5.6	0	1.0	0	5.8	1.7	11.0	1.3	〃	(B)
KT 220	0	0	0	0	0	0	2.5	0	2.7	0	〃	(C)
KT 221	3.9	0	6.7	0	0.7	0	4.9	0.9	7.4	0.6	〃	(A)
KT 222	8.4	0	15.2	0	0	0	14.3	4.8	13.7	3.7	〃	(B)
KT 223	4.5	0	3.7	0	1.6	0	2.9	0.9	7.4	1.0	N-Benzoyl-6-amino-hexanoic acid	(B)
KT 224	0.8	0	0.4	0	0	0	0.3	0	15.4	0	〃	(B)
KT 229	7.3	0	17.1	0	0	0	20.0	5.2	75.0	15.2	〃	(B)
KT 237	0	0	1.5	0	0	0	0	0	1.4	0	2,6-Di-N-benzoyl-DL-lysine	(D)
KT 241	0.6	0	0	0	0	0	6.7	0	2.9	0	N-Benzoyl-anthranilic acid	(D)
KT 83	6.8	0	9.5	0	5.1	0	14.7	3.2	15.6	2.2	N-Benzoyl-6-amino-hexanoic acid	Yakugaku Zasshi **72**, 791 (1952).
KT 104	1.4	0	2.3	0	0	0	10.5	0	8.0	0	N-Benzoyl-anthranilic acid	This Bulletin **6**, 395 (1958).

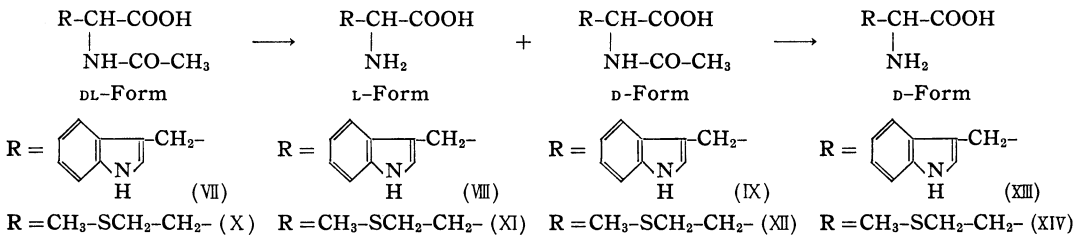
Expressed as micromoles hydrolyzed per hr. per 10 mg. acetone powder. The digest consisted of 10 mg. acetone powder, 2 cc. of H_2O, and 2 cc. of 0.05*M* neutralized substrates (pH 7.8) at 37°. Free amino acid was determined by Grassmann and Heyde's method.[8]

(A) : The school garden of Faculty of Pharmacy, Kanazawa University.
(B) : The herb garden of Faculty of Pharmacy, Kanazawa University.
(C) : A bamboo thicket near Faculty of Pharmacy, Kanazawa University.
(D) : A refuse heap of Rokujo village in Takamatsu City.

The ability of KT 218, KT 229, and KT 83 to resolve 2,6-di-N-benzoyl-DL-lysine (IV) was then tested. KT 218 and KT 229 hydrolyzed asymmetrically 2,6-di-N-benzoyl-DL-lysine (IV) to produce 6-N-benzoyl-L-lysine (V), $[\alpha]_D^{15}$ +19.5°, 2,6-di-N-benzoyl-D-lysine (VI), $[\alpha]_D^{15}$ +7.5°, and benzoic acid but KT 83 produced L-lysine besides the above three products. Apparently, L-lysine was produced from 6-N-benzoyl-L-lysine by 6-lysine acylase[5,6] of KT 83.

The data in Table I show that KT 229, KT 83, KT 222, etc., are capable of hydrolyzing both L- and D-forms of N-acetyl derivatives of tryptophan and methionine, while KT 218, KT 241, KT 224, KT 104, etc., hydrolyze only the L-form of acetyl amino acids. It was demonstrated that KT 241 hydrolyzed asymmetrically N-acetyl-DL-tryptophan (VII) to produce L-tryptophan (VIII), $[\alpha]_D^{15}$ −32.0°, and N-acetyl-D-tryptophan (IX), $[\alpha]_D^{15}$ −28.0°, m.p. 184~185°, and that KT 224 hydrolyzed asymmetrically N-acetyl-DL-methionine (X) to produce L-methionine (XI), $[\alpha]_D^{15}$ +22.5°, and N-acetyl-D-methionine (XII), $[\alpha]_D^{15}$ +23.0°, m.p. 102~104°. It is of interest to note that KT 224 hydrolyzed N-acetyl-L-methionine more easily than N-benzoyl-L-methionine, while KT 84[7] hydrolyzed the latter more easily.

KT 229 could hydrolyze N-acetyl-D-tryptophan (IX) and N-acetyl-D-methionine (XII) to D-tryptophan (XIII), $[\alpha]_D^{15}$ +32.0°, and D-methionine (XIV), $[\alpha]_D^{15}$ −22.5°, respectively.



The data from the experiments carried out under comparable conditions using acetone powder and cell free extract of KT 218, KT 224, and KT 241, are given in Table II.

Table II. Acylase Activity of Soil Bacteria KT 218, KT 224 and KT 241

Substrates \ Strains	KT 218		KT 224		KT 241	
	Acetone powder	Cell free ext.	Acetone powder	Cell free ext.	Acetone powder	Cell free ext.
N-Benzoyl-L-methionine	2.16	29.3	0.05	0.33	0.04	1.25
N-Acetyl-L-methionine	0.42	2.67	1.54	16.0	0.29	2.63
N-Acetyl-L-tryptophan	0.21	1.70	0.03	0.12	0.67	3.25

Expressed as micromoles hydrolyzed per hr. per mg. protein or acetone powder. Protein was determined by the phenol method of Lowry, *et al.*[11] The digests consisted of 0.5 cc. of enzyme solution, 1.5 cc. of distilled water, and 2 cc. of 0.1*M* neutralized substrates (pH 7.8) at 37°. Free amino acid was determined by Grassmann and Heyde's method.[8]

Particular attention should be directed here to the following points: 1) N-Acetyl-L-methionine is more easily hydrolyzed than N-acetyl-L-tryptophan by KT 224 or KT 218, while the latter is more easily hydrolyzed by KT 241. 2) N-Acetyl-derivative of L-methionine is more easily hydrolyzed than N-benzoyl derivative by KT 224 or KT 241, while the latter was more easily hydrolyzed by KT 218.

5) Y. Kameda, E. Toyoura, Y. Kimura, K. Matsui: This Bulletin, **6**, 394 (1958).
6) S. Wada: J. Biochem. (Tokyo), **46**, 445, 1541 (1959).
7) Y. Kameda, E. Toyoura, Y. Kimura, Y. Kanaya, J. Ishikawa, K. Yoshimura: Yakugaku Zasshi, **78**, 767 (1958).
8) W. Grassmann, W. Heyde: Z. physiol. Chem., **183**, 32 (1929).

Experimental

Preparation of Substrates——N-Benzoyl-DL-threonine, 2,6-di-N-benzoyl-DL-lysine, 6-N-benzoyl-DL-lysine, N-acetyl-DL-tryptophan, N-acetyl-DL-methionine, di-N-benzoyl-L-cystine were prepared according to the previously described procedure.[2] N-Benzoyl-anthranilic[9] and N-benzoyl-6-aminohexanoic[10] acids were prepared by the benzoylation of anthranilic acid and 6-aminohexanoic acid respectively. N-Benzoyl-anthranilic acid, m.p. 178~179°; N-Benzoyl-6-aminohexanoic acid, m.p. 79~80°.

Isolation of Soil Bacteria——The culture medium used for isolation of soil bacteria was composed of carbon source (N-benzoyl-DL-threonine, N-benzoyl-anthranilic acid, di-N-benzoyl-L-cystine, N-benzoyl-6-aminohexanoic acid or 2,6-di-N-benzoyl-DL-lysine), 0.2 g.; NH_4Cl, 0.1 g.; K_2HPO_4, 0.1 g.; $MgSO_4 \cdot 7H_2O$, 0.05 g.; 1% soln. of $CaCl_2 \cdot 6H_2O$, 2 drops; 1% soln. of $FeCl_3 \cdot 6H_2O$, 1 drop; dist. H_2O, 100 cc.; pH 7.0~7.2 (adjusted with 10% NaOH). 0.2 g. of soil sample 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 bouillon agar.

Preparation of the Acetone Powder of Soil Bacteria——Soil bacteria were grown in 200 cc. of bouillon (pH 7.2) at 25° for 3~5 days and the cells were then harvested by centrifugation and washed with dist. H_2O. The cells were poured into 100 cc. of Me_2CO cooled to −5° and the mixture was stirred for 5 min. The solid were collected by suction, washed with cold Me_2CO and then Et_2O, and dried in a desiccator.

Preparation of Cell Free Extract of Soil Bacteria KT 218. KT 224 and KT 241——i) KT 218 was grown in ten of 1 L. Erlenmeyer flask each containing 200 cc. of bouillon medium (pH 7.2) at 25° for 5 days, and the cells were then harvested by centrifugation and washed with dist. H_2O. The yield of cells in wet weight was ca. 6 g. After having been ground with alumina, the cells were extracted with 30 cc. of dist. H_2O and the alumina and cellular debris were removed by centrifugation, yielding 28 cc. (containing ca. 200 mg. of protein) of a supernatant solution (KT 218 cell free extract). Protein was determined by the phenol method of Lowry, *et al.*[11]

ii) 47 cc. (containing ca. 500 mg. of protein) of cell free extract of KT 224 was obtained from 12 g. (in wet weight) of bacterial cells grown in 2 L. of bouillon at 25° for 3 days.

iii) 48 cc. (containing 650 mg. of protein) of cell free extract of KT 241 was obtained from 15 g. (in wet weight) of bacterial cells grown in 2 L. of bouillon at 25° for 3 days.

Asymmetric Hydrolysis of N-Benzoyl-DL-threonine (I) by the Suspension of KT 218——2.23 g. (0.01*M*) of N-benzoyl-DL-threonine (I) was suspended in 200 cc. of H_2O and brought into solution at pH 7.8 by addition of 10% NaOH. To this aqueous solution, 1 g. (in wet weight) of KT 218 bacterial mass was added and the mixture was allowed to digest at 37° with a few drops of toluene. Samples were withdrawn at intervals and determined for free amino acids according to the Grassmann and Heyde's method.[8] When no further increase in amino acids was noted (1~2 days), the digestion was continued overnight to insure complete hydrolysis of the L-form of N-benzoyl-DL-threonine. The digest was then adjusted to pH 4.5 by the addition of AcOH. After heating for several min., the coagulated protein and insoluble mass were removed by centrifugation. The supernatant which came through charcoal was concentrated *in vacuo*. The residue was treated with an excess of EtOH. After standing for several hrs. at 5°, the precipitate was filtered by suction, washed several times with hot EtOH, and crystallized from hot H_2O and EtOH to 0.38 g. (68.6%) of L-threonine (II) as colorless plates, m.p. 250°(decomp.), $[\alpha]_D^{13}$ −28.0° (c=2, H_2O). *Anal.* Calcd. for $C_4H_9O_3N$: C, 40.33; H, 7.62; N, 11.76. Found: C, 40.45; H, 7.69; N, 11.61.

The EtOH solution was combined with washing from the separation of L-threonine and evaporated *in vacuo* to dryness. The residue was taken up in the minimum amount of cold H_2O and brought to pH 1.0 by addition of HCl. The aqueous mixture was extracted with AcOEt and the extract was evaporated *in vacuo* to dryness. The residue was washed several times with petr. ether to remove BzOH and recrystallized from AcOEt and benzene to 0.85 g. (73.9%) of N-benzoyl-D-threonine (III) as colorless plates, m.p. 145~146°, $[\alpha]_D^{15}$ −25.5° (c=2, H_2O). *Anal.* Calcd. for $C_{11}H_{13}O_4N$: C, 59.18; H, 5.87; N, 6.28. Found: C, 59.25; H, 5.99; N, 6.45. BzOH (0.40 g., 66.5%) was obtained from petr. ether as colorless plates, m.p. 118~120°.

Asymmetric Hydrolysis of N-Benzoyl-DL-threonine (I) by the Suspension of KT 229——To 200 cc. of 0.05*M* N-benzoyl-DL-threonine (I) solution, 5 g. (in wet weight) of KT 229 bacterial mass was

9) A. Brückner: Ann., **205**, 130 (1880).
10) J. Eck, C. Marvel: J. Biol. Chem., **106**, 387 (1934).
11) O. H. Lowry, *et al.*: *Ibid.* **193**, 265 (1951).

added and the mixture was incubated at 37° with a few drops of toluene for 1 day. L-Threonine (II) (0.31 g., 52%), N-benzoyl-D-threonine (III) (0.58 g., 52%), and BzOH (0.43 g., 70%) were obtained.

Asymmetric Hydrolysis of N-Benzoyl-DL-threonine (I) by the Suspension of KT 83——To 200 cc. of 0.05*M* N-benzoyl-DL-threonine (I) solution, 8 g. (in wet weight) of KT 83 bacterial mass was added and the mixture was incubated at 37° with a few drops of toluene for 1 day. L-Threonine (II) (0.30 g., 50%), N-benzoyl-D-threonine (III) (0.60 g., 50%), and BzOH (0.43 g., 70.5%) were obtained.

Asymmetric Hydrolysis of 2,6-Di-N-benzoyl-DL-lysine (IV) by the Acetone Powder of KT 218——A suspension of 3.54 g. of 2,6-di-N-benzoyl-DL-lysine (IV) in 200 cc. of H_2O was brought into solution at pH 7.8 by addition of 10% NaOH. To this aqueous solution, 0.5 g. of KT 218 acetone powder was added and the mixture was allow to digested at 37° with a few drops of toluene. After 2 days, the insoluble mass was removed by centrifugation for 30 min. at 3000 g. The supernatant was adjusted to pH 4.5 with AcOH, evaporated *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 10 cc. of H_2O, neutralized with conc. NH_4OH and the resulting precipitate was collected by suction. Recrystallization from H_2O gave 1.10 g. (88.1%) of 6-N-benzoyl-L-lysine (V) as colorless leaves, m.p. 265~267°(decomp.), $[\alpha]_D^{15}$ +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, 7.46; N, 11.36.

The AcOEt layer was evaporated *in vacuo* to dryness, and the residue was washed several times with petr. ether to remove BzOH. Recrystallization from Me_2CO-benzene gave 1.48 g. (83.6%) of 2,6-di-N-benzoyl-D-lysine (VI) as colorless plates, m.p. 144~145°, $[\alpha]_D^{15}$ +7.5°(c=2, EtOH). *Anal.* Calcd. for $C_{20}H_{22}O_4N_2$: C, 67.78; H, 6.26; N, 7.91. Found: C, 67.61; H, 6.41; N, 8.02. From the petr. ether solution, 0.07 g. (11.6%) of BzOH was obtained as colorless plates, m.p. 118~120°.

Asymmetric Hydrolysis of 2,6-Di-N-benzoyl-DL-lysine (IV) by the Acetone Powder of KT 229——To 200 cc. of 0.05*M* 2,6-di-N-benzoyl-DL-lysine (IV) solution, 0.5 g. of KT 229 acetone powder was added and the mixture was incubated at 37° under toluene for 2 days. 6-N-Benzoyl-L-lysine (V) (1.20 g., 96%), 2,6-di-N-benzoyl-D-lysine (VI) (1.42 g., 80.5%), and BzOH (0.12 g., 20%) were obtained.

Asymmetric Hydrolysis of 2,6-Di-N-benzoyl-DL-lysine (IV) by the Acetone Powder of KT 83——To 200 cc. of 0.05*M* 2,6-di-N-benzoyl-DL-lysine solution (IV), 0.4 g. of KT 83 acetone powder was added and the mixture was incubated at 37° under toluene for 2 days. The digest was treated as mentioned above. From the AcOEt layer, 1.24 g. (70.6%) of 2,6-di-N-benzoyl-D-lysine (VI) and 0.95 g. of BzOH were obtained. The aqueous layer was adjusted to pH 5.5 with conc. NH_4OH and evaporated *in vacuo* to a small volume. The resulting precipitate was collected by suction and recrystallized from H_2O to give 0.15 g. (12%) of 6-N-benzoyl-L-lysine (V). Then, the filtrate from (V) was acidified with HCl to pH 1.0 and evaporated *in vacuo* to dryness. The residue was taken up in EtOH and the solution was neutralized with pyridine. The resulting precipitate was filtered by suction and recrystallized from H_2O and EtOH. L-Lysine monohydrochloride (0.50 g., 55%) was obtained as colorless crystals, m.p. 256°(decomp.), $[\alpha]_D^{15}$ +21.0°(c=2, 6*N* HCl). *Anal.* Calcd. for $C_6H_{15}O_2N_2Cl$: C, 39.40; H, 8.21; N, 15.32. Found: C, 39.31; H, 8.45; N, 15.49.

Asymmetric Hydrolysis of N-Acetyl-DL-tryptophan (VII) by the Acetone Powder of KT 241——To 500 cc. of 0.05*M* N-acetyl-DL-tryptophan (VII) solution, 0.8 g. of KT 241 acetone powder was added and the mixture was incubated at 37° under toluene for 3 days. The digest was treated in the same manner as reported previously[3] to yield 1.8 g. of L-tryptophan (VIII), m.p. 275~277°(decomp.), $[\alpha]_D^{15}$ −32.0°(c=1, H_2O) (*Anal.* Calcd. for $C_{11}H_{12}O_2N_2$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.65; H, 5.82; N, 13.93.) and 2.6 g. (84.5%) of N-acetyl-D-tryptophan (IX), m.p. 184~185°, $[\alpha]_D^{15}$ −28.0°(c=2, EtOH) (*Anal.* Calcd. for $C_{13}H_{14}O_3N_2$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.56; H, 5.86; N, 11.46.).

Enzymatic Hydrolysis of N-Acetyl-D-tryptophan (IX) by the Acetone Powder of KT 229——A suspension of 1.23 g. of N-acetyl-D-tryptophan (IX) in 200 cc. of H_2O was brought into solution at pH 7.8 by addition of 10% NaOH. To this aqueous solution, 0.4 g. of KT 229 acetone powder was added to insure complete hydrolysis of N-acetyl-D-tryptophan and the mixture was incubated at 37° under toluene for 2 days. The digest was then adjusted to pH 4.5 with AcOH. The filtrate which came through charcoal was evaporated *in vacuo* until crystallization began. EtOH was then added and the whole was allowed to stand for 12 hr. in a refrigerator. The precipitated D-tryptophan (XIII) was filtered by suction. Recrystallization from 70% EtOH yielded 0.61 g. (60%) of D-tryptophan as colorless scaly crystals, $[\alpha]_D^{15}$ +32.0°(c=1, H_2O). *Anal.* Calcd. for $C_{11}H_{12}O_2N_2$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.51; H, 6.10; N, 13.81.

Asymmetric Hydrolysis of N-Acetyl-DL-methionine (X) by the Acetone Powder of KT 224——To 400 cc. of 0.05*M* N-acetyl-DL-methionine (X) solution, 0.4 g. of KT 224 acetone powder was added and the mixture was incubated at 37° under toluene for 2 days. The digest was treated by the previously reported procedure[7] to yield 1.19 g. (80%) of L-methionine (XI) as colorless scaly crystals, m.p. 276~278°, $[\alpha]_D^{15}$ +22.5°(c=2, 5*N* HCl) (*Anal.* Calcd. for $C_5H_{11}O_2NS$: C, 40.26; H, 7.43; N, 9.39. Found: C, 40.15; H, 7.50; N, 9.46) and 1.4 g. (73%) of N-acetyl-D-methionine (XII) as colorless plates,

m.p. 103~105°, $[\alpha]_D^{15}$ +23.0° (c=2, H_2O) (*Anal.* Calcd. for $C_7H_{13}O_3NS$: C, 43.97; H, 6.85; N, 7.33. Found : C, 44.05; H, 6.77; N, 7.46.).

Enzymatic Hydrolysis of N-Acetyl-D-methionine (XII) by the Acetone Powder of KT 229——A suspension of 1.9 g. of N-acetyl-D-methionine (XII) in 400 cc. of H_2O was brought into solution at pH 7.8 by addition of 10% NaOH. To this aqueous solution, 0.4 g. of KT 229 acetone powder was added to insure complete hydrolysis of N-acetyl-D-methionine and the mixture was incubated at 37° under toluene for 1 day. The digest was then adjusted to pH 4.5 with AcOH. The filtrate which came through charcoal was evaporated *in vacuo* until crystallization began. EtOH was then added and the whole was allowed to stand for 12 hr. in a refrigerator. The precipitated D-methionine was filtered by suction. Recrystallization from H_2O and EtOH yielded 1.1 g. (74%) of D-methionine (XIV) as colorless scaly crystals, m.p. 276~278°(decomp.), $[\alpha]_D^{15}$ −22.3° (c=2, 5*N* HCl). *Anal.* Calcd. for $C_5H_{11}O_2NS$: C, 40.26; H, 7.43; N, 9.39. Found : C, 40.37; H, 7.56; N 9.41.

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Summary

Fifteen strains (KT 202, KT 205, KT 207, etc.) of soil bacteria capable of asymmetric hydrolysis of N-benzoyl-DL-threonine, 2,6-di-N-benzoyl-DL-lysine, N-acetyl-DL-lysine, N-acetyl-DL-tryptophan or N-acetyl-DL-methionine were isolated by using a synthetic medium containing N-acyl-derivatives of amino acids as the sole source of carbon (Table I).

N-Benzoyl-DL-threonine was asymmetrically hydrolyzed to L-threonine, N-benzoyl-D-threonine and benzoic acid by the acylase of KT 218, KT 229, and KT 83, while 2,6-di-N-benzoyl-DL-lysine, to 6-N-benzoyl-L-lysine, 2,6-di-N-benzoyl-D-lysine and benzoic acid by KT 218 and KT 229.

KT 241 and KT 224 hydrolyzed asymmetrically N-acetyl-DL-tryptophan and N-acetyl-DL-methionine to L-tryptophan and N-acetyl-D-tryptophan, and L-methionine and N-acetyl-D-methionine, respectively.

It was observed that N-acetyl-D-tryptophan and N-acetyl-D-methionine were hydrolyzed by several strains (KT 229, KT 83, KT 222, etc.) of soil bacteria.

It seems of interest to note that N-acetyl-L-methionine was more easily hydrolyzed by KT 224 or KT 218 than N-acetyl-L-tryptophan, while the latter was more easily hydrolyzed by KT 241, and that the N-acetyl derivative of L-methionine was more easily hydrolyzed by KT 224 or KT 241 than the N-benzoyl derivative, while the latter was more easily hydrolyzed by KT 218.

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