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# 132. Yukio Kameda and Tomoo Ōmori : Studies on Acylase Activity and Microörganisms. XVIII.[*1] A Strain of Soil Bacteria capable of resolving Lysine by its Metabolism on $N^2$-Benzoyl or $N^2$-Phenacyl Derivative of $N^6$-Benzoyl-DL-lysine.

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In the earlier papers[1~4] it was reported that five strains (KT-230, KT-231, KT-232, KT-233, KT-301) of soil bacteria metabolized di-$N^2$,$N^6$-benzoyl-DL-lysine (I) to produce $N^6$-benzoyl-L-lysine (III) and di-$N^2$,$N^6$-benzoyl-D-lysine (IV), but not metabolized $N^2$-phenacyl-$N^6$-benzoyl-DL-lysine (II), and on the other hand, that one strain (KT-311) of soil bacteria metabolized (II) to produce (III) and $N^2$-phenacyl-$N^6$-benzoyl-D-lysine (V), but not metabolized (I). Incidentally, the metabolic activities of KT-230, KT-231, KT-232, KT-233, KT-301, and KT-311 are shown in Table I.

Table I. Metabolic Activity of Soil Bacteria, KT-230, KT-231, KT-232, KT-233, KT-301, KT-311, and KT-313

| | KT-230, KT-231 KT-232, KT-233 | KT-301 | KT-311 | KT-313 |
|---|---|---|---|---|
| Benzoic acid (VI) | + | + | + | + |
| Di-$N^2$,$N^6$-benzoyl-DL-lysine (I) | + | + | − | + |
| Di-$N^2$,$N^6$-benzoyl-DL-lysine (I) (without $NH_4Cl$) | − | − | − | − |
| Di-$N^2$,$N^6$-benzoyl-D-lysine (IV) | − | − | − | − |
| $N^6$-Benzoyl-DL-lysine | − | − | − | − |
| Phenylacetic acid (VII) | − | + | + | + |
| $N^2$-Phenacyl-$N^6$-benzoyl-DL-lysine (II) | − | − | + | + |
| $N^2$-Phenacyl-$N^6$-benzoyl-DL-lysine (II) (without $NH_4Cl$) | − | − | − | − |
| $N^2$-Phenacyl-$N^6$-benzoyl-D-lysine (V) | − | − | − | − |

+ : Within 4 days at 25° a luxuriant growth of bacteria was obtained 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 was observed at 25° in 4 days. The constituents of the culture medium used in the experiments are as follows : organic substance to be tested, 0.2 g.; $NH_4Cl$, 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; distilled water, 100 cc.; pH 7.4~7.6 (adjusted with 10% NaOH).

In the present work a new strain of soil bacteria designated KT-313 was isolated, which metabolized di-$N^2$,$N^6$-benzoyl-DL-lysine (I) and $N^2$-phenacyl-$N^6$-benzoyl-DL-lysine (II) to produce $N^6$-benzoyl-L-lysine (III), di-$N^2$,$N^6$-benzoyl-D-lysine (IV) and $N^2$-phenacyl-$N^6$-benzoyl-D-lysine (V), respectively. The metabolic activity of KT-313 is shown in Table I.

From Table I, it is to be understood that KT-313 can produce $N^6$-benzoyl-L-lysine (III) and di-$N^2$,$N^6$-benzoyl-D-lysine (IV) or $N^2$-phenacyl-$N^6$-benzoyl-D-lysine (V) from di-$N^2$,$N^6$-benzoyl-DL-lysine (I) or $N^2$-phenacyl-$N^6$-benzoyl-DL-lysine (II) by utilizing benzoic acid or phenylacetic acid, which was formed from di-$N^2$,$N^6$-benzoyl-L-lysine (IIIB) or $N^2$-phenacyl-$N^6$-benzoyl-L-lysine (IIIP) by the action of their acylase, as the sole

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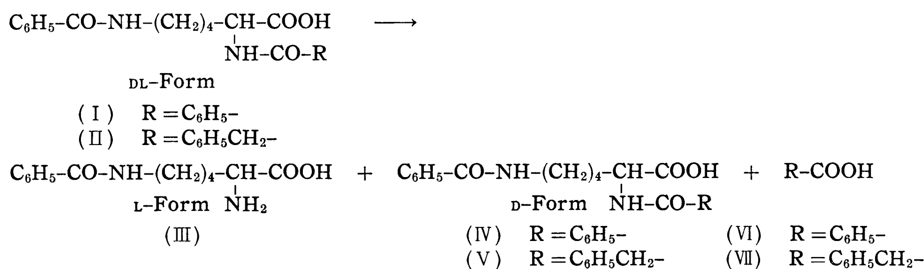
[*2] Tsuchitoribanaga-machi, Kanazawa (亀田幸雄, 大森知雄).

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 : Yakugaku Zasshi, **80**, 362 (1960).
4) *Idem* : This Bulletin, **7**, 789 (1959).

source of carbon and ammonia as the sole source of nitrogen. KT-313 metabolized di-$N^2$,$N^6$-benzoyl-DL-lysine (I) and $N^2$-phenacyl-$N^6$-benzoyl-DL-lysine (II) to yield the following compounds respectively: $N^6$-benzoyl-L-lysine (III), $[\alpha]_D^{20}$ +19°, di-$N^2$,$N^6$-benzoyl-D-lysine (IV), m.p. 146~147°, $[\alpha]_D^{20}$ +7.5°, and $N^2$-phenacyl-$N^6$-benzoyl-D-lysine (V), m.p. 134~135°, $[\alpha]_D^{20}$ −3.3°.




KT-313 seemingly belongs to the *Pseudomonas* group and has the following characters: aerobic; rod-shaped; gram-negative; yield water-soluble yellowish green pigment that diffuses through the medium.

It is of interest that KT-313 hydrolyzed benzoyl (IIIB) and phenacyl (IIIP) derivatives of $N^6$-benzoyl-L-lysine (III), while KT-301 hydrolyzed (IIIB), but not (IIIP), and KT-311 hydrolyzed (IIIP), but not (IIIB).

## Experimental

**Preparation of Substrates**——Di-$N^2$,$N^6$-benzoyl-DL-lysine (I) and $N^2$-phenacyl-$N^6$-benzoyl-DL-lysine (II) were prepared according to the procedure described previously.[5]

**Isolation and Characterization of Soil Bacteria KT-313**——The constituents of the culture medium for isolation of KT-313 were as follows: $N^2$-phenacyl-$N^6$-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% $CaCl_2 \cdot 6H_2O$, 2 drops; 1% $FeCl_3 \cdot 6H_2O$, 1 drop; dist. $H_2O$, 100 cc.; pH 7.4~7.6 (adjusted with 10% NaOH). A soil sample (0.2 g.) was taken from a coppice at Yudani village in Toyama Prefecture, inoculated into 10 cc. of the above culture medium, and incubated at 25° for 6 days. If luxuriant growth of bacteria occurred, a loop of the culture fluid was transfered to a new culture medium of the same composition. Such transplantation was repeated at least 3 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.

The newly isolated soil bacteria was designated KT-313 and its metabolic activity is shown in Table I. KT-313 seemingly belongs to the *Pseudomonas* group and has the following characters; aerobic, rod-shaped, gram-negative and yields water-soluble yellowish green pigment that diffuses through the medium.

**Resolution of Di-$N^2$,$N^6$-benzoyl-DL-lysine (I) by the Metabolism of KT-313**——100 cc. of the sterilized medium mentioned above containing 1.77 g. of di-$N^2$,$N^6$-benzoyl-DL-lysine in 200 cc. Erlenmeyer flask was seeded with a loop of KT-313 and incubated at 25° for 17 days. The culture thus obtained was heated at 80° for several min. and centrifuged for 10 min. at 8,000 g. to remove the insoluble mass. 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 ca. 5 cc. of $H_2O$, neutralized with conc. $NH_4OH$, and the resulting precipitate was collected by suction. Recrystallization from $H_2O$ gave 0.5 g. (80%) of $N^6$-benzoyl-L-lysine (III) as colorless leaves, m.p. 270°(decomp.); $[\alpha]_D^{20}$: +19°(c=2, 5*N* HCl). *Anal.* Calcd. for $C_{13}H_{18}O_3N_2$: C, 62.38; H, 7.25. Found: C, 62.51; H, 7.08.

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 0.7 g. (79%) of di-$N^2$,$N^6$-benzoyl-D-lysine (IV), as colorless leaves, m.p. 146~147°; $[\alpha]_D^{16}$: +7.5°(c=2, EtOH). *Anal.* Calcd. for $C_{20}H_{22}O_4N_2$: C, 67.78; H, 6.26. Found: C, 67.90; H, 6.11. From the petr. ether, BzOH was scarcely obtained.

5) Y. Kameda, E. Toyoura, Y. Kimura, K. Matsui, H. Saito: Yakugaku Zasshi, 78, 759 (1958).

**Resolution of $N^2$-Phenacyl-$N^6$-benzoyl-DL-lysine (II) by the Metabolism of KT-313**——KT-313 was inoculated into 100 cc. of the culture medium mentioned above containing 1.84 g. of $N^2$-phenacyl-$N^6$-benzoyl-DL-lysine and incubated at 25° for 5 days. The culture medium was treated according to the resolution procedure of di-$N^2$,$N^6$-benzoyl-DL-lysine and afforded 0.33 g. (52.8%) of $N^6$-benzoyl-L-lysine (III) as colorless leaves, m.p. 270° (decomp.); $[\alpha]_D^{20}$ : +19° (c=2, 5N HCl) (*Anal.* Calcd. for $C_{13}H_{18}O_3N_2$ : C, 62.38; H, 7.25. Found : C, 62.53; H, 6.44.), 0.85 g. (93%) of $N^2$-phenacyl-$N^6$-benzoyl-D-lysine (V) as colorless leaves, m.p. 134~135°; $[\alpha]_D^{8}$ : −3.3° (c=3, EtOH) (*Anal.* Calcd. for $C_{21}H_{24}O_4N_2$: C, 63.73; H, 7.55. Found : C, 63.93; H, 7.78), and 0.04 g. (10%) of phenylacetic acid, m.p. 74~76°. (III) was characterized as its phenylacetate (III P), m.p. 134~135°; $[\alpha]_D^{8}$ : +3.3 (c=3, EtOH), obtained in 67.9% yield after recrystallization from $Me_2CO$ and benzene. The mixture of (V) and (III P) melted at ca. 149°. Incidentally, $N^2$-Phenacyl-$N^6$-benzoyl-DL-lysine melts at 150~151°.

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## Summary

A certain strain (KT-313) isolated from soil had the metabolic activity shown in Table I and could metabolize di-$N^2$,$N^6$-benzoyl-DL-lysine (I) and $N^2$-phenacyl-$N^6$-benzoyl-DL-lysine (II) to produce $N^6$-benzoyl-L-lysine (III), di-$N^2$,$N^6$-benzoyl-D-lysine (IV) and $N^2$-phenacyl-$N^6$-benzoyl-D-lysine (V), respectively. KT-313 seemingly belongs to the *Pseudomonas* group. It is of interest that KT-313 hydrolyzed benzoyl (IIIB) and phenacyl (IIIP) derivatives of $N^6$-benzoyl-L-lysine (III), while KT-301 hydrolyzed (IIIB), but not (IIIP), and KT-311 hydrolyzed (IIIP), but not (IIIB).

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## 133. Tchan Gi Bak*[1] and Shigeaki Kuwano : Reversible and Irreversible Inhibitions of Glutamic and Arginine Decarboxylase Activities of *Escherichia coli* by Gallic Acid and *d*-Catechin.

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Kimura, *et al.* have found that tannins and related compounds inhibit the decomposition of amino acids to form diamines [1], hydrogen sulfide[2] and indole[3] by pyridoxal phosphate-dependent enzymes of *Escherichia coli.* It has also been shown that gallic acid and *d*-catechin, characteristic components of pyrogallol- and catechol-tannins respectively, inhibit the enzyme reactions through somewhat different mechanisms.

The present paper deals with the kinetic studies on the inhibition of two pyridoxal phosphate-requiring enzymes of *E. coli,* i.e. glutamic and arginine decarboxylases, by gallic acid and *d*-catechin. It was especially aimed to elucidate in details the differences in inhibition mechanisms due to gallic acid and *d*-catechin. Efforts were also made to show that these inhibitions are caused by both reversible and irreversible

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1) K. Kimura, S. Kuwano, H. Hikino : Yakugaku Zasshi, **78**, 236 (1958).
2) K. Kimura, K. Yamauchi, S. Kuwano : This Bulletin, **7**, 426 (1959).
3) *Idem* : *Ibid.*, **7**, 531 (1959).