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142. Etsuko Toyoura : Studies on Acylase Activity and Microörganisms. XIII.*2 Optical Resolution of *p*-Methoxyphenylalanine and 3,4-Methylenedioxyphenylalanine by Metabolism of Soil Bacteria on Benzoyl Derivatives of DL-Amino Acids.

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p-Methoxyphenylalanine has not been isolated from proteins, but it was obtained as a hydrolysis product of an antibiotic, puromycin.[1] 3,4-Methylenedioxyphenylalanine has not occurred in nature yet.

In the earlier papers[2~4] it was reported that two strains (KT-231 and KT-230) of soil bacteria metabolized benzoyl derivatives of ε-N-benzoyl-DL-lysine, DL-methionine, DL-phenylalanine, DL-leucine, DL-glutamic, and DL-aspartic acids to produce L-amino acids and the corresponding benzoyl-D-amino acids.

The present work is a direct continuation of that previously reported and describes the optical resolution of *p*-methoxyphenylalanine (II) and 3,4-methylenedioxyphenylalanine (V). KT-231 metabolized benzoyl derivatives of *p*-methoxy-DL-phenylalanine and 3,4-methylenedioxy-DL-phenylalanine to yield *p*-methoxy-L-phenylalanine (III), $[\alpha]_D^{10}$ −8°, benzoyl-*p*-methoxy-D-phenylalanine (IV), m.p. 135°, $[\alpha]_D^{10}$ −7.3°, 3,4-methylenedioxy-L-phenylalanine (VI), $[\alpha]_D^{12}$ −15.0°, and benzoyl-3,4-methylenedioxy-D-phenylalanine (VII), m.p. 126°, $[\alpha]_D^{15}$ +11.0°.

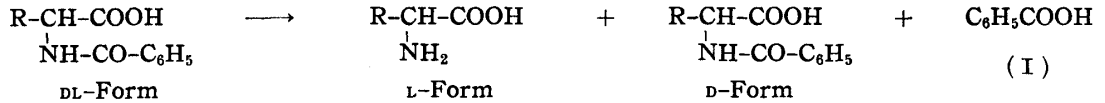
The metabolic activity of KT-231 and KT-230 is shown in Table I.

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

	KT-230	KT-231
Benzoic Acid (I)	+	+
Benzoyl-*p*-methoxy-DL-phenylalanine (II)	+	+
Benzoyl-*p*-methoxy-DL-phenylalanine (II) (without NH_4Cl)	−	−
Benzoyl-*p*-methoxy-D-phenylalanine (IV)	−	−
p-Methoxy-DL-phenylalanine	−	−
Benzoyl-3,4-methylenedioxy-DL-phenylalanine (V)	+	+
Benzoyl-3,4-methylenedioxy-DL-phenylalanine (V) (without NH_4Cl)	−	−
Benzoyl-3,4-methylenedioxy-D-phenylalanine (VII)	−	−
3,4-methylenedioxy-DL-phenylalanine	−	−

\+ 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.



R=*p*-CH_3O-C_6H_4-CH_2-(II) R=*p*-CH_3O-C_6H_4-CH_2-(III) R=*p*-CH_3O-C_6H_4-CH_2-(IV)
R=3,4-CH_2O_2-C_6H_3-CH_2-(V) R=3,4-CH_2O_2-C_6H_3-CH_2-(VI) R=3,4-CH_2O_2-C_6H_3-CH_2-(VII)

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*2 This constitutes a part of a series entitled "Studies on Acylase Activity and Microörganisms" by Y. Kameda. Part XII : This Bulletin, **7**, 785(1959).

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Experimental

Synthesis of Benzoyl-*p*-methoxy-DL-phenylalanine (II) and Benzoyl-3,4-methylenedioxy-DL-phenylalanine (V)—Benzoyl-*p*-methoxy-DL-phenylalanine, m.p. 173~174°, and benzoyl-3,4-methylenedioxyphenyl-DL-alanine, m.p. 179~181°, were prepared according to the azlactone method.

Resolution of Benzoyl-*p*-methoxy-DL-phenylalanine (II) by the Metabolism of KT-231—KT-231 was inoculated into 50 cc. of the culture medium[4] mentioned above containing 1.5 g. of benzoyl-*p*-methoxy-DL-phenylalanine and incubated at 25° for 10 days. The culture medium was heated at 80° for several min. and centrifuged to remove the cellular material. The supernatant solution 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 hr. of standing at ca. 5°, the precipitate was filtered by suction, washed with EtOH, and recrystallized from H_2O. 0.3 g.(62%) of *p*-methoxy-L-phenylalanine (III) was obtained as colorless plates, $[\alpha]_D^{10}$ −8.0°(c=2, 5*N* HCl). *Anal.* Calcd. for $C_{10}H_{13}O_3N$: C, 61.52; H, 6.71. Found: C, 61.56; H, 6.75.

The EtOH solution combined with washings from the separation of *p*-methoxy-L-phenylalanine was evaporated *in vacuo* to dryness, the residue was taken up in the minimum amount of cold water, 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 acetone-benzene to 0.35 g.(46.7%) of benzoyl-*p*-methoxy-D-phenylalanine (IV) as colorless needles, m.p. 135°; $[\alpha]_D^{10}$ −7.3°(c=2, *N* NaOH). *Anal.* Calcd. for $C_{17}H_{17}O_4N$: C, 68.21; H, 5.73. Found : C, 68.15; H, 5.83.

Resolution of Benzoyl-3,4-methylenedioxy-DL-phenylalanine (V) by Metabolism of KT-231—KT-231 was inoculated into 50 cc. of the culture mediun[4] mentioned above containing 1.5 g. of benzoyl-3,4-methylenedioxy-DL-phenylalanine and incubated at 25° for 18 days. The culture medium was treated according to the resolution procedure of benzoyl-*p*-methoxyphenylalanine and afforded 0.3 g. (61.3%) of 3,4-methylenedioxy-L-phenylalanine (VI) as colorless plates, $[\alpha]_D^{10}$ −15.0°(c=2, 5*N* HCl) (*Anal.* Calcd. for $C_{10}H_{11}O_4N$: C, 57.41; H, 5.30. Found : C, 57.49; H, 5.32) and 0.4 g.(53.4%) of benzoyl-3,4-methylenedioxy-D-phenylalanine (VII) as colorless prism, m.p. 126°, $[\alpha]_D^{10}$ +11.0°(c=2, *N* NaOH) (*Anal.* Calcd. for $C_{17}H_{15}O_5N$: C, 65.18; H, 4.82. Found : C, 65.08; H, 4.87).

Summary

The metabolic activities of KT-231 and KT-230 were tested on *p*-methoxyphenylalanine and 3,4-methylenedioxyphenylalanine and their benzoyl derivatives (Table I).

It was demonstrated that one strain (KT-231) of soil bacteria metabolized benzoyl derivatives of *p*-methoxy-DL-phenylalanine and 3,4-methylenedioxy-DL-phenylalanine to produce L-amino acids and the corresponding benzoyl-D-amino acids.

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