

Studies on Lysergic Acid Diethylamide and Related Compounds. VII.¹⁾
Microbial Transformation of Lysergic Acid
Diethylamide and Related Compounds

HISASHI ISHII,^{2a,d)} MAKOTO HAYASHI,^{2b)} TETSUKICHI NIWAGUCHI,
and YUJI NAKAHARA^{2c)}

Faculty of Pharmaceutical Sciences, Chiba University,^{2a,d)} Research
Institute for Chemobiodynamics, Chiba University,^{2b)} and
National Research Institute of Police Science^{2c)}

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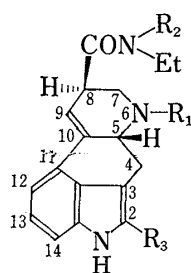
Lysergic acid diethylamide (LSD) was biotransformed by various species of microorganisms to give four metabolites; lysergic acid ethylamide (LAE), and norlysergic acid diethylamide (norLSD) are already known, but lysergic acid ethyl-2-hydroxyethylamide (LEO) and lysergic acid ethylvinylamide (LEV) have not been reported previously.

Characteristic features of *Streptomyces roseochromogenes* and *S. lavendulae* were noted in biotransformation experiments with LSD and isoLSD. The latter has an enzyme system for the demethylation of LSD and isoLSD, but the former does not.

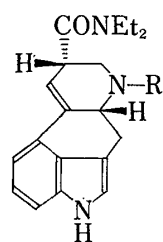
Using the technique of reincubation of metabolites, the metabolic pathway from LSD to LEV or LEO in *S. roseochromogenes* was studied, and the pathway of biotransformation from LEV to LEO was established.

Keywords—lysergic acid diethylamide; microbial transformation; lysergic acid ethylvinylamide; lysergic acid ethyl-2-hydroxyethylamide; *Streptomyces roseochromogenes*; *Streptomyces lavendulae*; N-de-ethylation

The strong hallucinogenic activity of lysergic acid diethylamide (LSD) (1) has aroused interest in its metabolic pathway. In 1957, Axelrod *et al.*³⁾ showed that LSD (1) was transformed into 2-hydroxylysergic acid diethylamide (2-OH-LSD) (2) by enzyme systems of mammalian liver microsomes supplemented with NADPH and oxygen. In 1959, Boyd⁴⁾



- 1: R₁=CH₃, R₂=Et, R₃=H (LSD)
2: R₁=CH₃, R₂=Et, R₃=OH (2-OH-LSD)
4: R₁=H, R₂=Et, R₃=H (norLSD)
5: R₁=CH₃, R₂=H, R₃=H (LAE)
6: R₁=CH₃, R₂=CH₂CH₂OH, R₃=H (LEO)
7: R₁=CH₃, R₂=CH=CH₂, R₃=H (LEV)



- 3: R₁=CH₃ (isoLSD)
8: R₁=H (iso-norLSD)

Chart 1. Structures of Substrates and Metabolites

- 1) Part VI: Y. Nakahara, T. Niwaguchi, and H. Ishii, *Tetrahedron*, **33**, 1591 (1977). Part of this study was presented at the 19th Symposium on the Chemistry of Natural Products, Hiroshima, Japan, October 1975.
2) Location: a) 1-33, Yayoi-cho, Chiba, 260, Japan; b) 1-8-1, Inohana, Chiba, 280, Japan; c) Sanban-cho, Chiyoda-ku, Tokyo, 102, Japan; d) To whom correspondence should be addressed.
3) J. Axelrod, R.O. Brady, B. Witkop, and E.V. Evarts, *Nature* (London), **178**, 143 (1956); *idem*, *Ann. N. Y. Acad. Sci.*, **66**, 435 (1957).

showed that administration of LSD (1) to rats resulted in the biliary excretion of two Ehrlich-positive metabolites. In 1962, Slaytor *et al.*⁵⁾ suggested that these metabolites could be β -glucuronides of 12-hydroxylated derivatives of LSD (1) and isolysergic acid diethylamide (isoLSD) (3), an epimer of LSD (1) at C₈. On the other hand, Szara⁶⁾ opposed this view and claimed that hydroxylation takes place at the C₁₃ position of the lysergic acid skeleton.

Recently, two of us⁷⁾ (T. N. and Y. N.) reexamined the metabolites of LSD (1) in subcellular level experiments using several small animals, rats, guinea pigs and rabbits, and found that LSD (1) was subject to two types of dealkylation, at the N₆-methyl group and at the amide to give norlysergic acid diethylamide (norLSD) (4) and lysergic acid ethylamide (LAE) (5). Their structures were confirmed by comparison with synthetic specimens.^{8,9)} Later, Siddik *et al.*¹⁰⁾ reported that intraperitoneal injection of ethyl-¹⁴C-labeled LSD (1) into rats resulted in excretion of LAE (5) and norLSD (4) together with 13-hydroxy- and 14-hydroxy-lysergic acid diethylamide and their glucuronides.

To investigate the metabolic pathway of LSD (1) in mammals in detail, the structure of minor metabolites should be clarified. However, it is difficult to obtain enough of these metabolites in subcellular level experiments to establish the chemical structures, so we planned to carry out the microbial transformation of LSD (1) using several microorganisms and compare the metabolites in microorganisms with the minor ones in mammals.¹¹⁾ Two new metabolites, lysergic acid ethyl-2-hydroxyethylamide (LEO) (6) and lysergic acid ethylvinylamide (LEV) (7), were isolated together with two known ones, norLSD^{7,8)} (4) and LAE^{7,9)} (5), from the mixture of microbial metabolites. The structural establishment of these new metabolites by chemical procedures will be reported in the subsequent paper.¹²⁾ In this paper, we describe in detail experiments on the microbial transformation of LSD (1) and demonstrate the usefulness of microbial experiments for studies of metabolism even in mammals. We examined the microbial transformation of LEO (6), LEV (7), and isoLSD (3) under the same fermentation conditions, because these metabolites, LEO (6) and LEV (7), might be metabolic intermediates in the formation of LAE (5) from LSD (1) and the differences in the mode of metabolism between LSD (1) and isoLSD (3) also seemed of interest.

Materials and Methods

Materials—Preparations of the materials used as substrates or authentic samples in this study were reported in the previous paper. LEO (6) and LEV (7), obtained by biotransformation with microorganisms, were used as substrates in the reincubation experiments.

Lysergic Acid Diethylamide⁹⁾ (LSD) (1)—Colorless prisms, mp 82—84°. *Rf* values: 0.49 (solv. A); 0.27 (solv. B); 0.87 (solv. C).

Norlysergic Acid Diethylamide⁸⁾ (norLSD) (4)—Colorless needles, mp 187—188°. *Rf* values: 0.18 (solv. A); 0.05 (solv. B); 0.45 (solv. C).

Lysergic Acid Ethylamide⁹⁾ (LAE) (5)—An oily product. *Rf* values: 0.40 (solv. A); 0.21 (solv. B); 0.74 (solv. C).

Lysergic Acid Ethyl-2-hydroxyethylamide (LEO) (6)—Colorless needles, mp 108—109°. *Rf* values: 0.25 (solv. A); 0.11 (solv. B); 0.64 (solv. C). The structural establishment of this material (6) by chemical means will be described in the subsequent paper.¹²⁾

- 4) E.S. Boyd, *Arch. Int. Pharmacodyn. Ther.*, **120**, 292 (1959).
- 5) M.B. Slaytor and S.E. Wright, *J. Med. Pharm. Chem.*, **5**, 483 (1962).
- 6) S. Szara, *Life Sci.*, **2**, 662 (1963).
- 7) T. Niwaguchi, T. Inoue, and Y. Nakahara, *Biochem. Pharmacol.*, **23**, 1073 (1974).
- 8) Y. Nakahara and T. Niwaguchi, *Chem. Pharm. Bull.* (Tokyo), **19**, 2337 (1971).
- 9) Y. Nakahara and T. Niwaguchi, *Yakugaku Zasshi*, **94**, 407 (1974).
- 10) Z.H. Siddik, R.D. Barnes, L.G. Dring, R.L. Smith, and R.T. Williams, *Biochem. Soc. Trans.*, **3**, 290 (1975).
- 11) After completion of our work, Smith *et al.* reported several studies along the same line. R.V. Smith and J.P. Rosazza, *Arch. Biochem. Biophys.*, **161**, 551 (1974); *idem*, *J. Pharm. Sci.*, **64**, 1737 (1975); J.P. Rosazza, A.W. Stockliski, M.A. Gustafson, J. Adrian, and R.V. Smith, *J. Med. Chem.*, **18**, 791 (1975).
- 12) H. Ishii, T. Niwaguchi, Y. Nakahara, and M. Hayashi, *J.C.S. Perkin I*, "in press".

Lysergic Acid Ethylvinylamide (LEV) (7)—Colorless needles, mp 86–88°. *Rf* values: 0.61 (solv. A); 0.60 (solv. B); 0.92 (solv. C). The structural establishment of this material (7) by chemical means will be described in the subsequent paper.¹²⁾

Isolysergic Acid Diethylamide^{8,11)} (isoLSD) (3)—Colorless needles, mp 180–182°. *Rf* values: 0.30 (solv. A); 0.10 (solv. B); 0.45 (solv. C).

Isonorlysergic Acid Diethylamide¹³⁾ (isonorLSD) (8)—An oily product. *Rf* values: 0.45 (solv. A); 0.05 (solv. B); 0.40 (solv. C).

Analytical Procedure—Thin-layer chromatography (TLC) was carried out on commercially available Silica gel G plates, Merck No. 5721 glass plates (0.25 mm thick), using the following mixed solvent systems: i) chloroform–*n*-hexane–methanol [4: 2: 1 (v/v/v)] (solv. A); ii) acetone–chloroform [4: 1 (v/v)] (solv. B); iii) chloroform–methanol [4: 1 (v/v)] (solv. C). Spots on TLC were visualized under UV irradiation at 365 nm and by spraying with Ehrlich reagent (*p*-dimethylaminobenzaldehyde in alcoholic hydrogen chloride).

Quantitative analysis of each metabolite was performed by developing the mixture of metabolites on a TLC plate using 10–20 μ l of medium with solv. A, followed by measurement of the fluorescence intensity of each spot at λ_{ex} . 330 nm and λ_{em} . 410 nm with a Hitachi MPF-2A fluorescence spectrophotometer equipped with a J-201 integrator, a J-301 digital recorder, and a TLC accessory. The yields of unchanged LSD (1) and metabolites were taken as corresponding to the ratios of their fluorescence intensities relative to the total.

Mass spectra were obtained with JEOL JMS-01SG spectrometers.

Cultures—Ten types of bacteria and ten types of fungi shown in Table I were used. Organisms were maintained on oatmeal–soybean agar slants for bacteria and on meat extract agar slants for fungi. Microorganisms were grown in a soybean medium for bacteria [soybean meal, 30 g; soluble starch, 20 g; K_2HPO_4 , 1 g; NaCl, 1 g; city water 1 l; pH adjusted to 7.2 with 1 N NaOH] and soybean–glucose–yeast extract medium for fungi [soybean meal, 5 g; glucose, 40 g; yeast extract (Difco Lab.), 5 g; NaCl, 1 g; KH_2PO_4 , 4.1 g; K_2HPO_4 , 0.8 g; distilled water, 1 l]. The medium was sterilized in an autoclave at 121° for 15 min before use. One loopful of microorganisms was inoculated into the medium, and preincubated at 28° on a reciprocating shaker at 60 rpm for 48 hr.

The preincubated culture was inoculated into the second medium, which was incubated at 28°. After 48 hr incubation of bacteria and 72 hr incubation of fungi, the substrate was added to the medium at a concentration of 0.1 mg/ml of medium (0.01%) and cultivation was continued until the consumption of the substrate had reached a maximum [5 days for bacteria and 10 days for fungi].

Isolation and Purification of Metabolites—Three volumes of ethanol were added to the harvested culture and filtered or centrifuged. The precipitate was washed with ethanol. The washings were combined with the filtrate and evaporated to 1/5 volume *in vacuo*. The condensed solution (*ca.* pH 8) was extracted four times with ethyl acetate. The combined extract was evaporated to dryness *in vacuo*. The residue was dissolved in benzene and extracted with 1% tartaric acid. The acid layer was adjusted to *ca.* pH 8 with NaHCO_3 and extracted four times with ethyl acetate. The ethyl acetate extract was evaporated to dryness *in vacuo* to give a mixture of metabolites.

Each metabolite was isolated by preparative TLC of the mixture of metabolites on Silica gel G using solv. A, and identified by comparison with an authentic sample (mass spectrum and TLC in solv. A, B, and C).

Results and Discussion

Various kinds of microorganisms are available for the microbial transformation of LSD (1). We obtained four metabolites, norLSD (4), LAE (5), LEO (6), and LEV (7). The experimental results can be summarized as follows. i) *Streptomyces roseochromogenes* metabolized LSD (1) to the greatest extent (75%) of all the microorganisms used in this work, giving LAE (5), LEO (6) and LEV (7) without norLSD (4). ii) In contrast, *S. lavendulae* forms norLSD (4) practically as a sole metabolite. iii) These observations indicate that *S. roseochromogenes* and *S. lavendulae* have quite different enzyme systems for the metabolism of LSD (1). The former has a degradative enzyme system acting on an ethyl group of the N,N-diethylamide function but the latter has an N-demethylation system acting on the N₆-methyl group. iv) *S. massasporeus*, *S. platensis*, *S. rimosus*, *S. fulvissimus*, and *Cunninghamella echinulata* produced all four metabolites from LSD (1), suggesting that these microorganisms have the enzyme systems existing in both *S. roseochromogenes* and *S. lavendulae*. v) Two fungi, *C. echinulata* and *Gliocladium deliquenscens*, produce LEV (7), a

13) T. Niwaguchi, Y. Nakahara, and H. Ishii, *Yakugaku Zasshi*, **96**, 673 (1976).

TABLE I. Percentage of Unchanged LSD and Metabolites produced by Bacteria and Fungi

Strain (number ^{a)})	Unchanged LSD (1) (%)	Metabolites (%)			
		norLSD (4)	LAE (5)	LEO (6)	LEV (7)
Bacteria					
<i>Streptomyces</i>					
<i>S. massasporeus</i> (ISP 5035)	55	13	10	15	0.5
<i>S. platensis</i> (ISP 5041)	80	2	6	9	0.5
<i>S. rimosus</i> (ISP 5060)	65	0.5	15	10	1.5
<i>S. roseus</i> (ISP 5076)	97	—	—	—	—
<i>S. violaceus</i> (ISP 5082)	98	—	—	—	—
<i>S. lavendulae</i> (IFM 1031)	60	35	1	1	—
<i>S. roseochromogenes</i> (IFM 1081)	25	—	37	20	2
<i>S. fulvissimus</i> (ISP 5593)	60	0.5	20	5	0.5
<i>S. aureofaciens</i> (IFO 12843)	90	—	7	—	—
<i>Mycobacterium</i>					
<i>M. smegmatis</i> (IFO 13167)	88	0.5	8	—	—
Fungi					
<i>Cunninghamella</i>					
<i>C. flakesleecana</i> (IFO 4443)	75	—	8	9	—
<i>C. echinulata</i> (IAM 8209)	64	6	13	6	5
<i>Rhizopus</i>					
<i>R. stolonifer</i> (IFO 6300)	88	4	5	—	—
<i>Claviceps</i>					
<i>C. purpurea</i> (IFO 5782)	99	—	—	—	—
<i>Corticium</i>					
<i>C. sasakii</i> (IFO 6258)	98	—	—	—	—
<i>Trameetes</i>					
<i>T. sanguinea</i> (IFO 6490)	90	—	6	—	—
<i>Aspergillus</i>					
<i>A. niger</i> (IAM 3003)	99	—	—	—	—
<i>A. ochraceus</i> (IFO 4069)	94	—	4	—	—
<i>Penicillium</i>					
<i>P. chrysogenum</i> (IFO 9252)	75	13	5	—	—
<i>Gliocladium</i>					
<i>G. deliquescens</i> (IFO 9016)	65	—	18	8	5

a) ISP=International Cooperative Project for the Description and Deposition of Type Cultures of *Streptomyces*. IFM=Research Institute for Chemobiodynamics, Chiba University, Chiba, Japan.
IFO=Institute for Fermentation, Osaka, Japan.
IAM=Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan.

new metabolite. vi) The most widely distributed metabolite in the microbial transformation of LSD (1) is LAE (5); *S. roseochromogenes* gave the best yield.

The formation of norLSD (4) from LSD (1) could be explained by N-demethylation, which is fairly common in the metabolism of foreign compounds. However, it should be noted here that norLSD (4) and LAE (5) were common metabolites in the biotransformation of LSD (1) in mammals⁷⁾ and in microorganisms. Moreover, one of us (T. N.)¹⁴⁾ recently established one of the minor metabolites as LEV (7) using the sample obtained in this experiment. This shows that microbial transformation experiments can be useful¹¹⁾ for the structural establishment of minor mammalian metabolites.

It is of particular interest that LEO (6) and LEV (7) as well as LAE (5) were formed in our microbial experiments. Since N-deethylation is a well-known mode of biotransformation of foreign compounds having a diethylamide group,¹⁵⁾ LAE (5) is not surprising as a metabo-

14) T. Niwaguchi and T. Inoue, unpublished results.

15) B.K. Koe and R. Pinson Jr., *J. Med. Chem.*, **7**, 635 (1964); E.H. Wiseman, E.S. Schreiber, and R. Pinson Jr., *Biochem. Pharmacol.*, **13**, 1421 (1964); E.G.C. Clarke, "Isolation and Identification of Drugs," The Pharmaceutical Press, London, 1969, p. 443.

lite of LSD (1) with *S. roseochromogenes*. However, the formation of a vinylamide [LEV (7)] or a β -hydroxyethylamide group [LEO (6)] from a diethylamide function [LSD (1)] is quite new as far as we know.

It is natural to expect that LEV (7) might be a precursor of LEO (6) in the metabolism of LSD (1) because of the co-existence of both compounds in the mixture of metabolites. Thus LEV (7) was treated again with *S. roseochromogenes* under the same fermentation conditions, giving LEO (6) and LAE (5) in 20% and 50% yields, respectively. Since the hydration of LEV (7) to LEO (6) is of anti-Markovnikov type, which would require an enzymatic system, we may safely deduce that LEO (6) is a metabolite of LEV (7). It

TABLE II. Metabolism of LEV (7) and LEO (6) by *S. roseochromogenes*

Substrate	Unchanged substrate (%)	Metabolites (%)	
		LAE (5)	LEO (6)
LEV (7)	20	50	20
LEO (6)	100	—	—

should be added here that this finding simply means the possibility of the presence of a sequential process [LSD (1)→LEV (7)→LEO (6)], allows us to extinguish the direct step to LEO (6) from LSD (1) in *S. roseochromogenes*, because there are several reports on ω -oxidation of an alkyl side chain in the metabolism of foreign compounds.¹⁶⁾

In the field of studies on the biotransformation of foreign compounds, there are many reports on N-dealkylation of amine derivatives. A mechanism in which oxygenation at an α -carbon atom relative to nitrogen initiates N-dealkylation of the amine has been accepted in general. If this mechanism is also correct for the biogenetic N-dealkylation of an amide group, the fact that LAE (5) was isolated from the mixture of metabolites of LSD (1) suggests the presence of an intermediate (9) which was hydroxylated at an α -carbon atom of an ethylamide group of LSD (1). This intermediate (9) (Chart 2) should also be a common

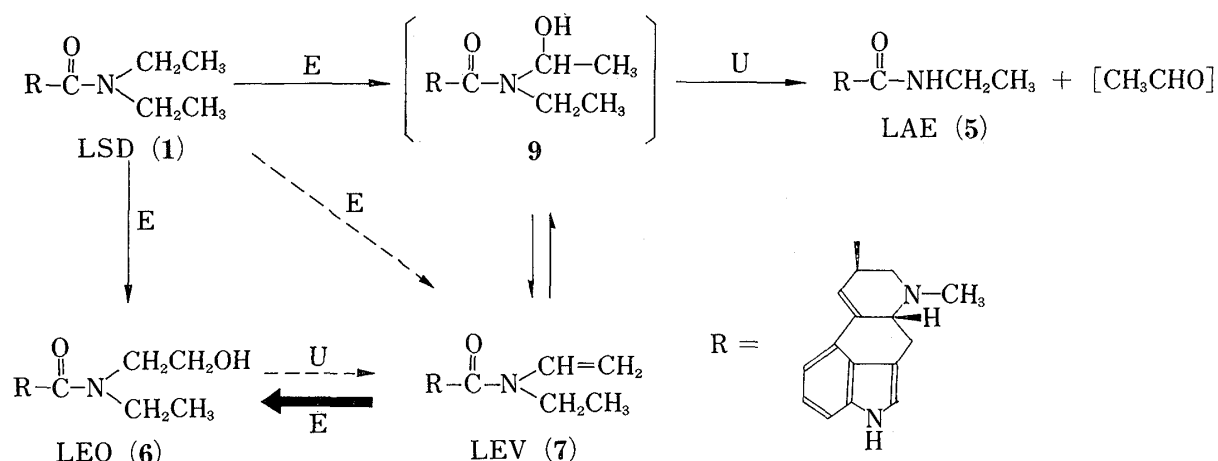


Chart 2. Proposed Reaction Sequence for the Metabolism of LSD (1) to LAE (5) and LEO (6) by Several Bacteria and Fungi

E=enzymatic process, U=undefined process, either enzymatic or spontaneous.
 \leftarrow =confirmed process, \dashleftarrow =assumed process, \dashrightarrow =unextinguished process.

16) A.M. Guarino, W.D. Conway, and H.M. Fales, *European J. Pharmacol.*, **8**, 244 (1969); J.E. Bakke, J.D. Robbins, and V.J. Feil, *J. Agric. Food Chem.*, **19**, 462 (1971); M. Keise and W. Lenk, *Biochem. Pharmacol.*, **22**, 2575 (1973); D.J. Tocco, A.E.W. Duncan, F.A. Deluna, H.B. Hucker, V.F. Gruber, and W.A. Vandenhuevel, *Drug Metabolism and Disposition*, **3**, 361 (1975).

precursor for LEV (7), because loss of a proton of the hydroxy group of the intermediate (9) yields the former (5), while loss of a β -proton of 1-hydroxyethylamide gives the latter (7). These considerations led us to propose the metabolic pathway of LSD (1) in *S. roseochromogenes* shown in Chart 2.

At this point, we should take account of the possibility of transformation from LEO (6) to LEV (7) by dehydration. We attempted to examine the reincubation of LEO (6) under the same conditions, but the starting material was recovered intact. Since it is possible that LEO (6) might not be permeable into the cells due to their hydrophilic character, the significance of this result in relation to the possible role of LEO (6) in the metabolic pathway from LSD (1) to LEV (7) is uncertain. Furthermore, direct formation of LEV (7) from LSD (1) by dehydrogenase could not be rigidly excluded because the presence of an enzymatic system attacking an α -hydrogen atom adjacent to a heteroatom has been reported.¹⁷⁾

We also examined the biotransformation of isoLSD (3), an epimer of LSD (1) at C₈. It was metabolized by *S. lavendulae* to give isonorLSD (8), but not by *S. roseochromogenes*, which left the starting material unchanged. These findings confirm our assumption that the

TABLE III. Metabolism of isoLSD (3) by *S. roseochromogenes* and *S. lavendulae*

Strain	Unchanged isoLSD (3) (%)	Metabolite: iso-norLSD (8) (%)
<i>S. roseochromogenes</i>	100	—
<i>S. lavendulae</i>	70	13

enzyme systems of these microorganisms for the metabolism of foreign compounds are strikingly different. In the subcellular level experiments using several small animals, LSD (1) was biotransformed into LAE (5), LEV (7), and norLSD (4) as mentioned above. On the other hand, isoLSD (3) was subject to dealkylation at the N₆-methyl group to give isonorLSD (8) but not at the amide group.¹⁸⁾ Taking into consideration of the results obtained in microbial experiments, we could conclude that mammalian tissues have two enzyme systems corresponding to the modes of dealkylation, respectively. In other words, experiments with *S. roseochromogenes* and *S. lavendulae* are suitable for studies of mammalian metabolic pathways in dealkylation of an N,N-dialkylamide group and of an N₆-alkyl group, respectively, as models.

Finally, many researchers have reported oxygenation at C₂, C₁₂, C₁₃, or C₁₄ in the indole nucleus of LSD (1) in various experiments on the metabolism of LSD (1). Although it is well known that several aromatic compounds are oxygenated at the aromatic ring by various species of fungi, we could not find such a derivative of LSD (1) in a mixture of fungal metabolites of LSD (1).

Acknowledgement We thank Prof. T. Arai, Chiba University, for providing many strains of *Streptomyces* used in this work.

17) R.W. Chadwick, L.T. Chuang, and K. Williams, *Pesticide Biochem. Physiol.*, **5**, 575 (1975).

18) T. Niwaguchi, T. Inoue, and T. Sakai, *Biochem. Pharmacol.*, **23**, 3063 (1974).