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Physiological Activity of Streptothricin Antibiotics[1)]

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The physiological activities of streptothricin antibiotics were investigated, taking racemomycin-D as a representative of the group. It was found that racemomycin-D has insecticidal activity, fish-toxicity and inhibitory activity on plant growth.

Firstly, racemomycin-D showed a weak but broad-spectrum insecticidal activity against the adults of *Blattella germanica, Musca domestica, Nilaparvata lugens, Laodelphax striatella*, and *Plutella xylostella*. A rather strong insecticidal effect on *Culex pipiens molestus* larvae was found, with mortalities of 90% and 15% at 10 ppm and 2 ppm, respectively. Though racemomycin-D showed no insecticidal effect against the larvae of *Spodoptera litura*, it showed inhibitory activity on feeding at a concentration of 300 ppm.

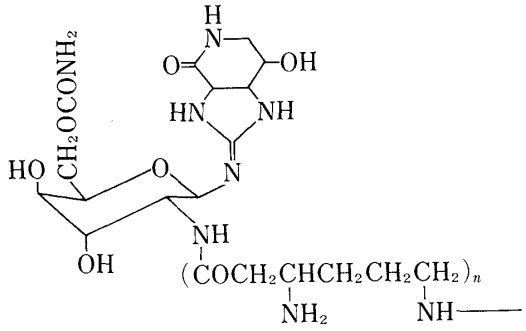
Secondly, racemomycin-D was toxic to all the fish species examined, *i.e., Misgurnus anguillicaudatus, Carassius auratus*, and *Oriziae latipes*. Among these, racemomycin-D showed particularly strong toxicity against *C. auratus* with a TLm of 3.7 ppm (after 48 hr).

Thirdly, racemomycin-D showed strong inhibitory activity on the growth of *Brassica campestris L. subsp. Napus Hook fil et Anders* var. *nippo-okifera, Arctium Lappa* L., *Petrocelium sativum*, and *Raphanus sativus* L. var. *acanthiformis* at a concentration of 500 ppm (with an inhibitory ratio of 0.1, relative to 1.0 for the control group).

Keywords——racemomycin-D; delayed toxicity; bait method; injection method; topical application method; insecticidal activity; fish-toxicity; inhibitory effect on plant growth

A streptothricin antibiotic, racemomycin-D (Fig. 1), belongs to the group of basic, water-soluble antibiotics. It is different from the other antibiotics in this group in that it has not only antibacterial activity, but also antiviral[3)] and antifungal activities. Though this streptothricin antibiotic has a broad spectrum of physiological activity, as stated above, it has not been brought into medical use because of its strong delayed toxicity.

To investigate the range of biological activities of this antibiotic, the authors studied its insecticidal activity, fish toxicity, and inhibitory activity on plant growth. The results obtained are reported in this paper.



$n=4$: racemomycin-D

Fig. 1. Chemical Structures of Streptothricin Antibiotics

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3) H. Taniyama, Y. Sawada, and T. Kitagawa, *Chem. Pharm. Bull.*, **19**, 1627 (1971).

Materials and Methods

Chemicals——Racemomycin-D[4] is one of the streptothricin antibiotics, produced by *Streptomyces lavendulae* OP-2.[5] In the experiments on insecticidal activity and fish-toxicity, streptomycin sulfate (Banyu Pharmaceutical Co., Ltd.), which belongs to the same group of basic water soluble antibiotics and has a D-glucosamine moiety in the molecule,[6] was used for comparison. In the experiment on insecticidal activity, parathion (Wako Junyaku Co., Ltd.), acephate (Chevron Chemical Co., Ltd.), fenitrothion (Sumitomo Chemical Co., Ltd.) and nereistoxin hydrogen oxalate (Takeda Chemical Ind. Ltd.) were used as standards. In the experiment on fish-toxicity, rotenone (Nakarai Chemical Co., Ltd.) was used as a standard. In the examination of the plant growth inhibition, 2,4-dichlorophenoxy-acetic acid sodium salt (Tokyo Kasei Industry Co., Ltd.) was used as a standard.

Organisms——Insects: Adults of *Blattella germanica* L., *Musca domestica* Macquart, *Nilaparvata lugens* Stal, and *Laodelphax striatella* Fallen, and larvae of *Spodoptera litura* Fabricius, *Plutella xylostella* Curtis, and *Culex pipiens molestus* Forskal were used. Fishes: *Misgurnus anguillicaudatus* Cantor, *Carassius auratus* Linnaeus, and *Oriziae latipes* Temminck and Schlegel were used. Plants: Seeds of *Brassica campestris* L. *subsp. Napus Hook fil et Anders* var. *nippo-okifera* Makino, *Arctium Lappa* L., *Petrocelium sativum* Nyman and *Raphanus sativus* L. var. *acanthiformis* Makino were used.

Insecticidal Activity Tests——1) Bait Method: The test solution was prepared by dissolving the chemicals in water, and was absorbed into a cotton pad in a small glass dish. The prepared bait was placed in a cage with the insects, *B. germanica* or *M. domestica*. The insect mortalities were counted 72 and 24 hr after the insect release, respectively.

2) Topical Application: Test solutions were applied onto the ventral surface of the abdomen of *B. germanica* or *M. domestica* at a volume of 2 μl/insect. The mortalities were counted 72 and 48 hr after the application, respectively.

3) Injection: Test solutions prepared with water were injected into the abdomens of *B. germanica* or *M. domestica* at a volume of 2 μl/insect. The mortality count was made 72 and 48 hr after the injection, respectively. The insects were anaestisized during the application and injection.

4) Leaf and Stem Dipping: For *S. litura* and *P. xylostella* larvae, toxicity was examined by releasing the larvae onto leaves treated with the chemicals. Leaves of soybean and cabbage plants, respectively, were dipped for 5 sec into aqueous solutions of test chemicals (containing 0.033% of a spreader, Dine,® Takeda Chemical industries, Ltd.), then dried in air, and fed to the larvae in Petri dishes.

For *L. striatella* and *N. lugens*, the toxicity of the chemicals applied to the stems of rice plants was examined. The stems were dipped for 5 sec into the test solutions, then dried in air, and placed in test tubes into which the insects were released. The insect mortalities were assessed 48 hr after the insect release.

5) Immersion Test: *C. pipiens molestus* larvae were released into aqueous solutions of the chemicals, and left for 24 hr.

Fish-Toxicity Test——A method described by Sugawara and Koyama[7] was employed for the fish-toxicity test. The TLm (median tolerance limit) was calculated as a function of time according to the Doudoroff method.[8]

Examination of the Inhibition of Plant Growth——Sterilized cotton was laid in Petri dishes with a diameter of 9 cm. Ten ml of aqueous solutions of racemomycin-D or 2,4-dichlorophenoxy-acetic acid sodium salt at various concentrations, or water (control), was added. Ten seeds of each plant were put on the cotton and left for one week. The average growth length of each plant was calculated, and the degree of growth inhibition was expressed as a ratio, relative to the average of the control.[9]

Temperature——Every experiment was carried out at 25—27°.

Results

1. Insecticidal Effect of Racemomycin-D

Bait and feeding experiments: The insecticidal effect of racemomycin-D on various insects was assessed by means of the bait test and feeding tests with treated leaves and stems. As shown in Table I, the results indicate that racemomycin-D has a broad insecticidal spectrum.

4) Y. Inamori, S. Sunagawa, M. Tsuruga, Y. Sawada, and H. Taniyama, *J. Ferment. Technol.*, **54**, 795 (1976).
5) Y. Inamori, S. Sunagawa, Y. Sawada, and H. Taniyama, *Hakkokogaku Kaishi*, **54**, 795 (1976).
6) The structure-activity relationships will be reported elsewhere.
7) H. Sugawara and K. Koyama, "Nōyaku no Seibutsu Kenteiho" Nankodo Co., Ltd., Tokyo, 1971, p. 242.
8) P. Doudoroff, *Sewage and Industrial Wastes*, **23**, 1830 (1951).
9) T. Kurihara, Y. Sakamoto, S. Ohara, and Y. Inamori, *Yakugaku Zasshi*, **98**, 802 (1978).

That is, the 72hour mortalities of the adults of *B. germanica* were 80% and 20% at concentrations of 1000 ppm and 500 ppm, respectively. Mortalities of the adults of *M. domestica* at 500 ppm and 100 ppm were 100% and 40%, respectively. It was found that the mortalities of the adults of *N. lugens* and *L. striatella* and the larvae of *P. xylostella* were 10, 15, 25%, respectively, at 300 ppm. Toxicity to the mosquito larvae: Table II shows that the mortalities of the larvae of *C. pipiens molestus* were 90% and 15% at 10 ppm and 2 ppm, respectively. Even at low concentrations, this chemical has a rather strong insecticidal effect. However, streptomycin sulfate did not show an insecticidal effect against *B. germanica* or *M. domestica* even at concentrations of 1000 ppm and 500 ppm. Injection method: As shown in Table III, the mortalities of the adult of *B. germanica* due to the injection were 40% at 90 μg/g, and 10% at 30 μg/g.

Table I. Insecticidal Activity of Racemomycin-D Applied in Bait and the Plant

Insect	Method of treatment	Concentration of chemical (ppm)	% mortality		
			Racemomycin-D	Streptomycin	Parathion
Blattella[a] *germanica* (adult)	Bait		0[e]	0[e]	0[e]
		100	0	0	100
		500	20	0	100
		1000	80	0	100
			Racemomycin-D	Streptomycin	Parathion
Musca[b] *domestica* (adult)	Bait		0[e]	0[e]	0[e]
		10	0	0	100
		100	40	0	100
		500	100	0	100
			Racemomycin-D	Fenitrothion	
Laodelphax[c] *striatella* (adult)	Stem Dip		0[e]	0[e]	
		33	0	100	
		100	10	100	
		300	15	100	
			Racemomycin-D	Fenitrothion	
Nilaparvata[c] *lugens* (adult)	Stem Dip		0[e]	0[e]	
		33	0	0	
		100	10	30	
		300	25	85	
			Racemomycin-D	Acephate	
Plutella[d] *xylostella* (larvae)	Leaf Dip		0[e]	0[e]	
		33	0	30	
		100	5	90	
		300	10	100	

Observation time: *a*) 72 hr, *b*) 24 hr, *c*) 48 hr, *d*) 72 hr. Temperature: 26° *e*) Mortality in the control group
Experimental size: 10 insects/group, 2 groups.

Table II. Insecticidal Activity of Racemomycin-D on Larvae of *Culex pipiens molestus*

Chemical	% mortality (ppm)		
	10	2	0.4
Racemomycin-D	90	15	0
Nereistoxin Hydrogen Oxalate	100	100	95
Control	0	0	0

Observation time: 24 hr.
Temperature: 26°.
Experimental size: 10 insects/group, 2 groups.

Table III. Insecticidal Activity of Injected Racemomycin–D

Insect	Method of treatment	Concentration of chemical (μg/g)	% mortality		
			Racemomycin–D	Streptomycin	Parathion
Adults of *Blattella*[a] *germanica*	Injection		0[c]	0[c]	0[c]
		15	0	0	100
		30	10	0	100
		90	40	0	100
		150	70	0	100
Adults of *Musca domestica*[b]	Injection		Racemomycin–D	Streptomycin	Parathion
			0[c]	0[c]	0[c]
		1.5	0	0	100
		3	20	0	100
		6	30	0	100
		11	50	0	100
		17	90	0	100

Observation time: *a*) 72 hr, *b*) 48 hr.
Temperature: 26°.
Experimental size: 10 insects/group, 2 groups.
c) Mortality in the control group.

Table IV. Insecticidal Activity of Topically Applied Racemomycin–D

Insect	Method of treatment	Concentration of chemical (μg/g)	% mortality		
			Racemomycin–D	Streptomycin	Parathion
Adults of *Blattella*[a] *germanica*	Topical application		0[c]	0[c]	0[c]
		15	0	0	100
		30	10	0	100
		90	20	0	100
		150	30	0	100
		300	50	0	100
Adults of *Musca domestica*[b]	Topical application		Racemomycin–D	Streptomycin	Parathion
			0[c]	0[c]	0[c]
		15	0	0	100
		30	30	0	100
		60	80	0	100
		160	90	0	100

Observation time: *a*) 72 hr, *b*) 48 hr.
Temperature: 26°.
Experimental size: 10 insects/group, 2 groups.
c) Mortality in the control group.

Table V. Time Course of Mortality of *Blattella germanica* baited with Racemomycin–D

Dose (ppm)	Number of dead(out of 10 insects) at the indicated time (hr) after baiting			
	24	48	72	96
1000	0	1	8	10
500	0	0	2	6
100	0	0	0	1
Control	0	0	0	0

Temperature: 26°.
Experimental size: 10 insects/group, 2 groups.

In addition, it was found that the mortalities in *M. domestica* were 90% and 20% at 20 μg/g and 3 μg/g, respectively. On the other hand, streptomycin sulfate did not show an insecticidal effect even at high dosages, as indicated in Table III. Topical application method: The insecticidal effects of racemomycin-D against *B. germanica* and *M. domestica* were investigated by topical application. As shown in Table IV, the mortalities of *B. germanica* were 50% at a dosage of 300 μg/g and 10% at 30 μg/g. The mortalities of *M. domestica* were 80% and 30% at dosages of 60 and 30 μg/g, respectively. However, streptomycin sulfate was not insecticidal even at high concentrations.

As presented in Table V, racemomycin-D showed delayed toxicity against the adults of *B. germanica*. It appears that this delayed toxicity occurs in a manner similar to that reported for mammalian toxicity.[4] Effect on feeding activity: Though racemomycin-D did not show lethal activity against the larvae of *S. litura*, it was found that the chemical inhibited the larval feeding activity (see Fig. 2). That is, the number of feeding marks on leaves treated with a concentration of 300 ppm was far less than that in the control group (with water). A much stronger feeding inhibition was found at a concentration of 1000 ppm. The decrease of feeding marks in the acephate treatment group was attributable to the lethal action of the chemical.

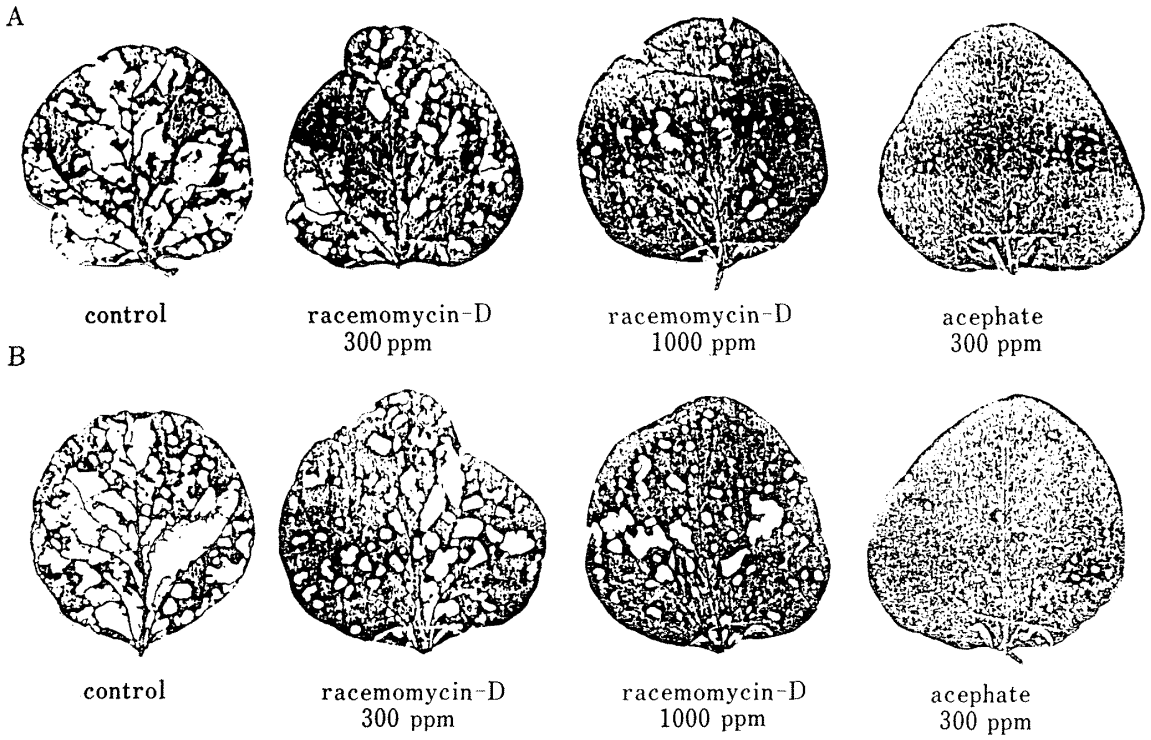

Fig. 2. Inhibitory Effect of Racemomycin-D on the Feeding of Larvae of *Spodoptera litura* (Soybean leaves)

The results of two series (A and B) of test are shown.

2. Fish-Toxicity of Racemomycin-D

The toxicity of racemomycin-D to *O. latipes*, *C. auratus* and *M. anguillicaudatus* was investigated. The TLm at 48 hours was, as shown in Table VI, 100 ppm in *M. anguillicaudatus*, 3.7 ppm in *C. auratus* and 5 ppm in *O. latipes*. Thus, racemomycin-D showed a toxic effect on fishes. On the other hand, streptomycin sulfate showed no toxic effects on these three species of fish even at a concentration of 500 ppm. However, the toxicity of racemomycin-D was less than that of rotenone.

Table VI. Ichthytoxicity of Racemomycin–D

Fish	TLm (ppm, 48 hr)		
	Racemomycin–D	Streptomycin	Rotenone
Oriziae latipes	5.0	>500	0.030
Carassius auratus	3.7	>500	0.033
Misgurnus anguillicaudatus	100.0	>500	0.037

Calculation of TLm: Doudoroff method.
Temperature: 27°.
Experimental size: 10 fishes/group, 2 groups.

Table VII. Inhibitory Effect of Racemomycin–D on Plant Growth

Plant	Growth (ratio)[a]					
	Racemomycin–D Concentration (ppm)			2,4-D[b] Concentration (ppm)		
	1000	500	100	1000	500	100
Brassica campestris subsp. Napus Hook fil et Anders var. *nippo okifera*	0.04	0.12	0.60	0.047	0.09	0.10
Raphanus sativus	0.07	0.13	0.59	0	0	0
Arctium Lappa L.	0.08	0.12	0.61	0.01	0.08	0.18
Petrocelium sativum	0.01	0.16	0.52	0.001	0.03	0

a) Growth in control experiments after 7 days was taken as 1.00.
b) 2,4-dichlorophenoxy-acetic acid sodium salt.
Temperature: 27°.
Experimental size: Ten grains/group, 2 groups.

3. Inhibitory Effect of Racemomycin-D on Plant Growth

The inhibitory effect of racemomycin-D on plant growth was investigated using the plant species described above. As shown in Table VII, the results indicate that racemomycin-D at 500 ppm had an extremely strong inhibitory effect on the growth of four species of plants (inhibitory index of approximately 0.1, relative to 1.0 for the control group). Moreover, it was shown to have an inhibitory effect on plant growth at a level as low as 100 ppm (index of approximately 0.5, relative to 1.0 for the control group).

Discussion

So far, streptothricin antibiotics have been reported to have antibacterial, antifungal, and antiviral action[3] and lethal effects on common tapeworms[10] in cats, dogs and sheep. In addition, we report in this paper that a streptothricin antibiotic is toxic to insects and fish as well as inhibitory with respect to plant growth.

First of all, racemomycin-D showed a weak but broad insecticidal spectrum against six insect species examined (Tables I—IV). A rather strong insecticidal activity was demonstrated in *C. pipiens molestus*, with a mortality of 90% at a concentration of 10 ppm (Table II). Streptothricin showed a delayed toxicity in *B. germanica* (Table V). Similar delayed toxicity

10) W.E. Brown, J. Szanto, E. Meyers, T. Kawamura, and K. Arima, *J. Antibiot.*, **30**, 886 (1977).

has been observed in mammals,[4] but the mechanism has not yet been investigated in detail. The authors have concluded that the action in mammals involves a strong renal toxicity[11-13] based on experiments with mice and rats. We hypothesized that the delayed toxicity might be due to a strongly toxic "opened lactam" metabolite. However, it is not clear whether its insecticidal activity is due to the induction of disorders in the excretory system of the insects, because the insect excretory organ (Malpighian vessel) is very different physiologically and biochemically from the kidney of mammals.

The insecticidal substances produced by *Streptomyces* species include flavensomycin,[14] C-1051,[15] destomycin A,B,[16] netropsin,[17] piericidin A,B,[18] antimycin,[19] and macrotetrolides (tetranactin,[20] nonactin,[21] *etc.*). However, no antibiotic has so far been found with such a strong insecticidal effect on mosquito larvae as the streptothricin antibiotics. In this respect, the insecticidal activity of streptothricin antibiotic is of considerable interest.

It was found that racemomycin-D, at a dosage of 300 ppm, has an inhibitory effect on the feeding activity of *S. litura* larvae. Many substances have been found in plants with feeding deterrent activity, but an antibiotic with this kind of activity has never previously been reported.

Secondly, recemomycin-D was found to be toxic to the three species of fish examined (Table VI). The only ichthyotoxic substance previously known to be produced by microorganisms is antimycin.[22] In contract, streptomycin sulfate, which belongs to the same group of basic water-soluble antibiotics, showed neither insecticidal activity nor toxicity to fishes, even at high concentrations. The difference of activities between these two substances does not simply correspond to the difference in the mammalian toxicity (racemomycin-D: LD_{50}; 9.33 mg/kg *i.v.* in mice, and streptomycin: LD_{50}; 200 mg/kg *i.v.* in mice), but must be due to instrinsic physiological activity of the streptothricin antibiotic, because racemomycin-A,[23] which exhibits low mammalian toxicity (LD_{50}; 300 mg/kg *i.v.* in mice), still showed insecticidal activity and delayed toxicity against the adult of *B. germanica*.

Thirdly, the streptothricin antibiotic had inhibitory activity on the growth of the four plant species examined, though the acitvity at low concentrations was slightly less than that of 2,4-dichlorophenoxy-acetic acid sodium salt. However, at a concentration of 500 ppm the activity was extremely strong. Even at 100 ppm the streptothricin was shown to have an inhibitory effect on plant growth (Table VII). Many fungal substances which inhibit plant growth have been reported. However, the only plant growth inhibitors previously reported to be produced by *Streptomyces* species are cycloheximide[24] and antimycin.[25]

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12) Y. Inamori, K. Morimoto, K. Morisaka, G. Saito, Y. Sawada, H. Taniyama, and H. Matsuda, *Chem. Pharm. Bull.*, **27**, 230 (1979).
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As stated above, this is the first report of the insecticidal activity, ichthyotoxicity and plant growth inhibitory activity of streptothricin antibiotics. Among the compounds previously isolated from *Streptomyces* species, only antimycin shows broad-spectrum physiological activity. Thus, the physiological activities of streptothricin antibiotics are of considerable interest, and we are now investigating the mechanisms of these actions.