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Mechanisms of Inhibitory Action of Racemomycin-D on Plant Growth[1)]

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The mechanisms of phytogrowth-inhibitory action of racemomycin-D were investigated by means of histopathological studies of the tissues of the root and stem of *Raphanus sativus* L. var. *raphanistroides* MAKINO after treatment with the antibiotic. Severe delayed damage was observed at 36 h after treatment with racemomycin-D, the parenchymatous cells of the treated groups contained many disintegrated fragments of cytoplasm, and the cell membrane had become much thinner. At 48 h after treatment with racemomycin-D, the amount of chlorophyll in leaves of *R. sativus* of the treated groups was greatly decreased as compared with the control group, and remained at a low level thereafter.

Keywords——racemomycin-D; streptothricin antibiotic; phytogrowth-inhibitory action; cytoplasm; histopathological examination; cell membrane; chlorophyll amount; parenchymatous cell

It has already been reported that streptothricin antibiotics, a group of basic water-soluble antibiotics, show antimicrobial, insecticidal,[2,3)] ichthyotoxic,[2)] and phytogrowth-inhibitory activities.[2)] As regards the insecticidal activity, a histopathological investigation[4)] and analysis of the distribution[5)] into tissues of 5th instar larvae of the silkworm, *Bombyx mori*, showed that racemomycin-D, one of the streptothricin antibiotics, causes severe delayed damage to the Malpighian tubules.

However, no detailed work has yet been done on the mechanisms of phytogrowth-inhibitory action. Therefore, in this work, we extensively investigated the phytogrowth-inhibitory action of racemomycin-D[6)] (Fig. 1), and examined the changes in the amount of chlorophyll in the leaves in the histopathological character of the tissues of the root and stem in order to clarify the mechanism.

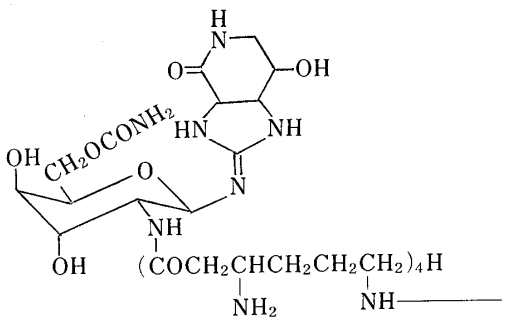

Fig. 1. Chemical Structure of Racemomycin-D

Materials and Methods

Chemicals——Racemomycin-D,[6)] one of the streptothricin antibiotics produced by *Streptomyces lavendulae* OP-2,[7)] was used. 2,4-Dichlorophenoxyacetic acid sodium salt (Tokyo Kasei Industry Co., Ltd.) was employed as a

standard.

Plants——Fresh seeds (all purchased on the market in Osaka) used in the experiment were as follows; *Raphanus sativus* L. var. *raphanistroides* MAKINO (Cruciferae), *Allium fistulosum* L. (Liliaceae), *Cucumis sativus* L. (Cucurbitaceae), *Aquilegia vulgaris* L. (Ranunculaceae), *Digitalis purpurea* L. (Scrophulariaceae), *Lactuca sativa* L. (Compositae), *Chrysanthemum coronarium* L. (Compositae), *Salvia officinalis* L. (Labiatae), *Mimosa pudica* L. (Leguminosae), *Pharbitis nil* CHOISY (Convolvulaceae), *Gypsophila elegans* BIEB (Caryophyllaceae) and *Sesamum indicum* L. (Pedaliaceae).

Germination and Cultivation——The seeds were sterilized with 1% NaClO solution and put on sterilized cotton in a tall Petri dish (9 cm in diameter and 6 cm in height). The 2 ml of distilled water containing recemomycin-D was added to seeds of the treated groups, 2 ml of distilled water was added to seeds to the control group, and 2 ml of distilled water containing 2,4-dichlorophenoxyacetic acid sodium salt was added to seeds of the standard group. Each group was germinated and cultivated at 25 °C under 600 Lux.

Concentration of Treatment Solution——For the phytogrowth-inhibitory activity test, the concentrations of racemomycin-D and 2,4-dichlorophenoxyacetic acid sodium salt were both 1000 ppm. Distilled water was used in the control group. To determine the effects of racemomycin-D on the growth of *R. sativus*, treatment concentrations of 100, 500 and 1000 ppm were used.

Phytogrowth-Inhibitory Activity Test——According to the method described by Inamori *et al.*,[2] the ratio of the average length of each treated group to that of the control group (taken as 1.00) was calculated at 7 d after the start of treatment. In order to examine the inhibitory effect of racemomycin-D on the growth of *R. sativus*, the length of the treated group was compared with that of the control group at intervals of 24 h.

Quantitative Analysis of Chlorophyll in the Leaves of *Raphanus sativus* L. var. *raphanistroides* MAKINO——The A.O.A.C. method[8] was used to determine the amounts of chlorophyll in the groups treated with racemomycin-D and 2,4-dichlorophenoxyacetic acid sodium salt as well as in the control group at intervals of 24 h.

Histopathological Examination of Roots and Stems of *Raphanus sativus* L. var *raphanistroides* MAKINO——The roots and stems of each treated group were fixed with Navashin's fluid (A, 10 ml of 1% chromic acid solution and 1 ml of glacial acetic acid solution; B, 4 ml of formalin), and according to the literature,[9] they were dehydrated and embedded in paraffin. Then, they were cut into slices of 8 μm thickness, which were stained with hematoxylin–eosin solution and observed through an optical microscope at a magnification of 200.

Results

Inhibitory Effects of Racemomycin-D on Plant Growth

As shown in Table I, inhibitory effects of racemomycin-D on plant growth were detected in all of the plants examined. Racemomycin-D showed relatively strong inhibitory action

TABLE I. Inhibitory Effect of Racemomycin-D on Plant Growth

Plant	Growth (ratio)[a]	
	Racemomycin-D	2,4-D[b]
Allium fistulosum L.	0.095	0.099
Cucumis sativus L.	0.134	0.136
Aquilegia vulgaris L.	0.667	0
Digitalis purpurea L.	0.200	0
Lactuca sativa L.	0.085	0
Chrysanthemum coronarium L.	0.113	0.071
Salvia officinalis L.	0.429	0
Mimosa pudica L.	0.298	0.125
Pharbitis nil CHOISY	0.287	0.124
Gypsophila elegans BIEB	0.034	0
Sesamum indicum L.	0.067	0
Raphanus sativus L. var. *raphanistroides* MAKINO	0.220	0.170

a) Growth in control experiments after 7 d was taken as 1.00. *b*) 2,4-Dichlorophenoxyacetic acid sodium salt. Temperature, 25 °C; concentration, 1000 ppm; experimental size, 10 grains/group, 2 groups; illumination, 600 Lux.

against *Lactuca sativa* L., *Gypsophila elegans* BIEB, *Sesamum indicum* L. and *Allium fistulosum* L. Racemomycin-D had growth-inhibitory action equal to that of 2,4-dichlorophenoxyacetic acid sodium salt on *Allium fistulosum* L., *Cucumis sativus* L., and *Chrysanthemum coronarium* L.

Toxicity of Racemomycin-D to *Raphanus sativus* L. var. *raphanistroides* MAKINO

Changes of External Form——Observation of the Growth Process: After the strong phytogrowth-inhibitory action of racemomycin-D had been confirmed, we investigated how the growth process of *R. sativus* was changed by treatment with racemomycin-D. As shown in Fig. 2, until 3 d after germination, no difference of growth could be detected between the group treated with the low concentration and the control group. Thereafter, an inhibitory effect on growth was recognized in every treated group, and increased with the passage of time.

Figure 3 shows the difference of growth between the treated groups and the control group at 7 d after germination. It was found that all the treated groups showed strong growth inhibition compared with the control group.

Changes of Amount of Chlorophyll in Leaves: Direct observation revealed that the color of the surface of leaves was changed to yellow in every treated group. The leaves of the group treated with 1000 ppm of racemomycin-D were investigated to determine the amount of chlorophyll at various times after germination. As shown in Table II, the amount was markedly decreased as compared with the control group.

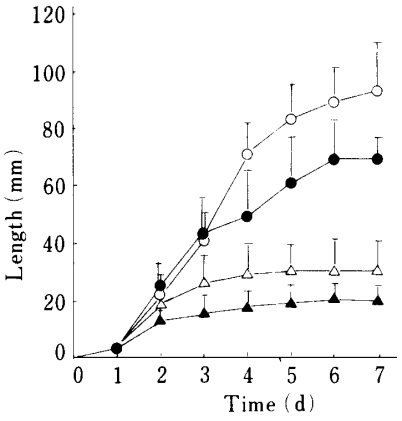

Fig. 2. Inhibitory Effect of Racemomycin-D on the Growth of *Raphanus sativus* L. var. *raphanistroides* MAKINO

—▲— 1000 ppm, —△— 500 ppm, —●— 100 ppm, --○— control. Each value represents the mean ±S.D. ($n=10$). Experimental size, 10 grains/group, 2 groups.

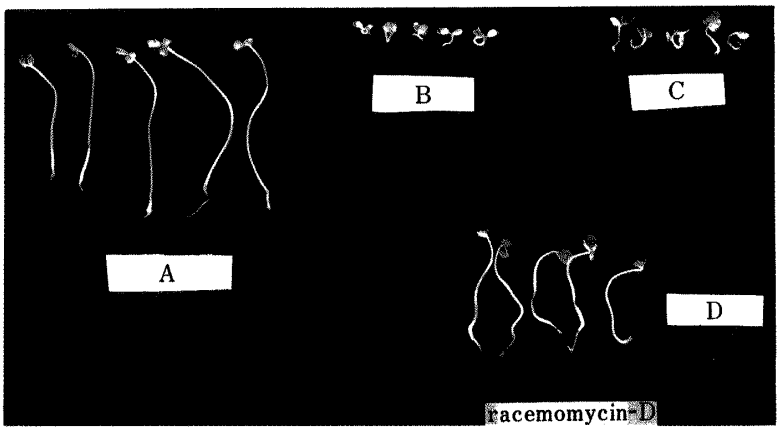

Fig. 3. Inhibitory Effects of Racemomycin-D on the Growth of *Raphanus sativus* L. var. *raphanistroides* MAKINO at 7 d after Treatment

A, control; B, 1000 ppm; C, 500 ppm; D, 100 ppm.

TABLE II. Changes of Chlorophyll Content of *Raphanus sativus* L. var. *raphanistroides* MAKINO after Treatment with Racemomycin-D

Time (h)	Chemical	Total chlorophyll (%)[b]	Chlorophyll a (%)	Chlorophyll b (%)
48	Racemomycin-D	0.006	0.004	0.002
	2,4-D[a]	0.011	0.008	0.003
	Control	0.013	0.011	0.002
72	Racemomycin-D	0.010	0.007	0.003
	2,4-D	0.010	0.008	0.002
	Control	0.108	0.096	0.012
96	Racemomycin-D	0.014	0.009	0.005
	2,4-D	0.014	0.009	0.005
	Control	0.155	0.098	0.057
120	Racemomycin-D	0.013	0.011	0.002
	2,4-D	0.014	0.012	0.002
	Control	0.134	0.084	0.051

a) 2,4-Dichlorophenoxyacetic acid sodium salt. *b*) % (wet wt.). Analytical method, A.O.A.C. method; concentration, 1000 ppm; temperature, 25 °C; illumination, 600 Lux.

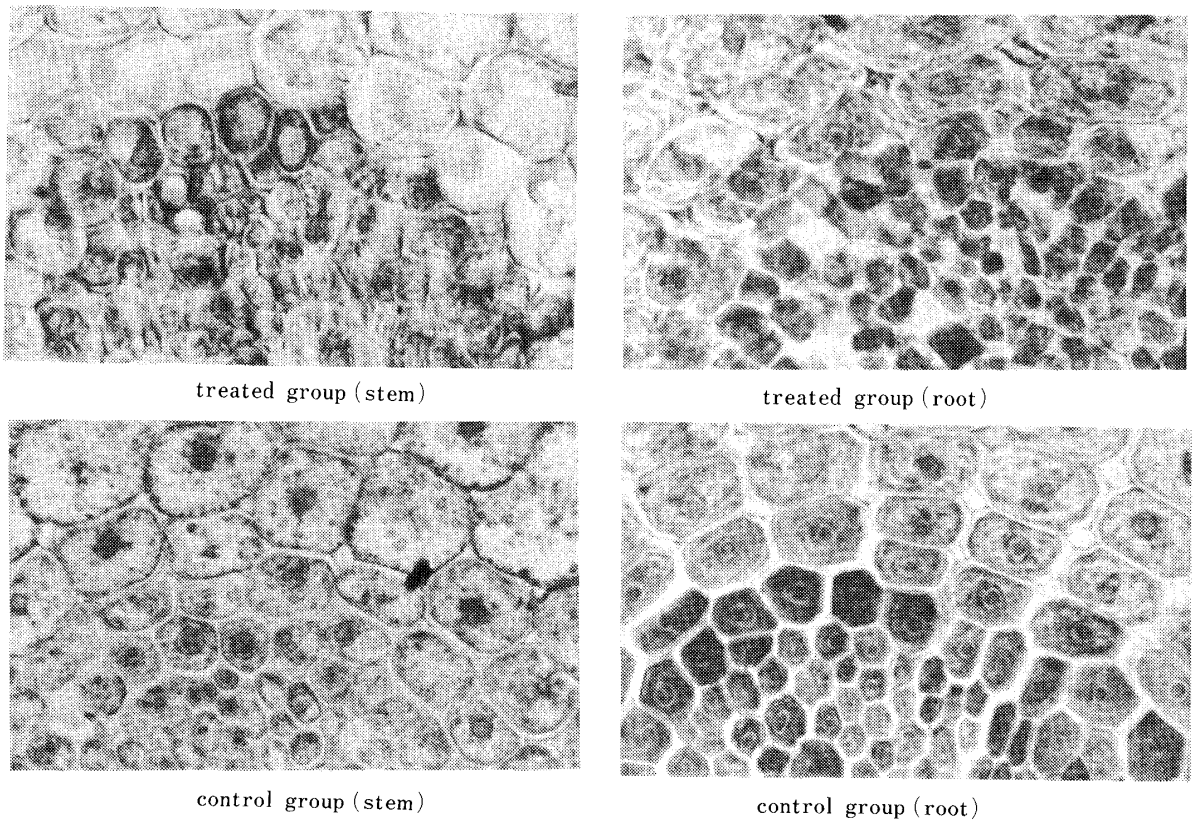

Fig. 4. Photographs of Tissues of the Root and Stem of *Raphanus sativus* L. var. *raphanistroides* MAKINO at 24 h after Treatment with Racemomycin-D Hematoxylin–Eosin Staining (×200)

Changes of Internal Form——The tissues of root and stem of *R. sativus* treated with 1000 ppm of racemomycin-D were stained with hematoxylin–eosin solution at 24 and 36 h after germination and studied. As shown in Fig. 4 at about 24 h the tissues of the treated root

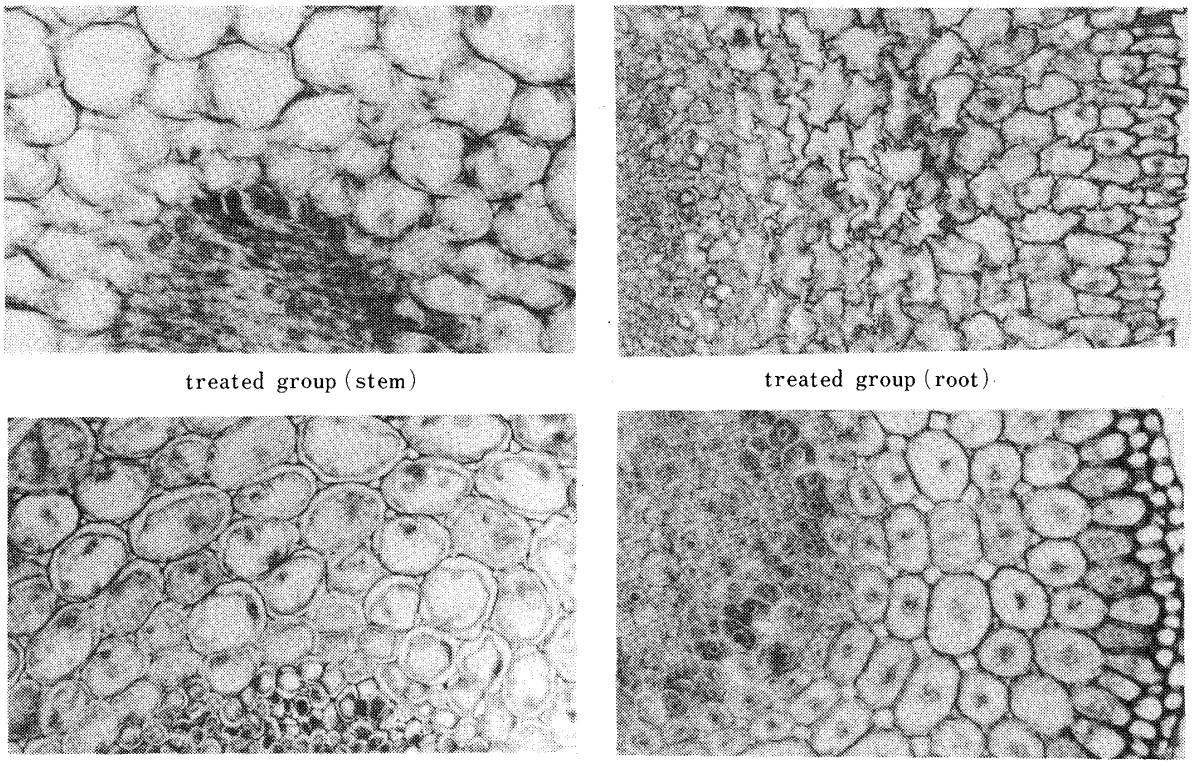

Fig. 5. Photographs of Tissues of the Root and Stem of *Raphanus sativus* L. var. *raphanistroides* MAKINO at 36 h after Treatment with Racemomycin-D Hematoxylin–Eosin Staining (×200)

and stem began to show the following changes compared with the control group: 1) the arrangement of the central cylinder tissues became irregular and 2) parenchymatous cells of the cortex were deformed with an irregular cell arrangement and a thinner cell membrane.

As shown in Fig. 5, at 36 h after treatment the tissues of the root and stem showed the following changes: 1) necrosis of cells was more apparent than at 24 h, and parenchymatous cells of the stem were deformed into a wavelike form, and 2) the cell membrane of parenchymatous cells of the root and stem became thinner, and there were many disintegrated fragments of cytoplasm.

Discussion

Racemomycin-D, one of the streptothricin antibiotics, showed relatively strong growth inhibitory action against all the plants examined (Table I). The inhibitory activity was as strong as that of 2,4-dichlorophenoxyacetic acid sodium salt on some plants. The experiment of *R. sativus* showed that racemomycin-D has a delayed inhibitory activity on plant growth (Fig. 2). This result is cosistent with the fact that racemomycin-D had no effect on the germination of *R. sativus*. Thus, it was found that racemomycin-D has severe delayed toxicity against all the plants examined here, as well as mammals[10-12] and insects.[2,3]

One of the reasons for the delayed inhibitory action of racemomycin-D on plant growth was the inhibition of biosynthesis of chlorophyll (Table II), which resulted in a decrease of chlorophyll in the leaves from 48 h after treatment. The amounts of chlorophyll in the group treated with racemomycin-D were almost equal to those in the group treated with 2,4-dichlorophenoxyacetic acid (Table II). Another reason for the inhibitory action of

racemomycin-D on plant growth was severe damage to cells of the root and stem of *R. sativus*. In particular, the cell membranes of parenchymatous cells of the cortex and other cells became thinner, and many disintegrated fragments of cytoplasm were observed (Figs. 4 and 5).

We are planning to investigate the structure–activity relation for inhibition of chlorophyll biosynthesis in *R. sativus*.

References and Notes

1) This work was presented at the 103rd Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1983.
2) T. Takemoto, Y. Inamori, Y. Kato, M. Kubo, K. Morimoto, K. Morisaka, M. Sakai, Y. Sawada and H. Taniyama, *Chem. Pharm. Bull.*, **28**, 2884 (1980).
3) M. Kubo, Y. Kato, K. Morisaka, Y. Inamori, K. Nomoto, T. Takemoto, M. Sakai, Y. Sawada and H. Taniyama, *Chem. Pharm. Bull.*, **29**, 3727 (1981).
4) Y. Kato, M. Kubo, K. Morisaka, Y. Waku, K. Hayashiya and Y. Inamori, *Chem. Pharm. Bull.*, **31**, 305 (1983).
5) M. Kubo, Y. Kato, K. Morisaka, K. Nomoto and Y. Inamori, *Chem. Pharm. Bull.*, **31**, 325 (1983).
6) Y. Inamori, S. Sunagawa, M. Tsuruga, Y. Sawada and H. Taniyama, *J. Ferment. Technol.*, **56**, 15 (1978).
7) Y. Inamori, S. Sunagawa, Y. Sawada and H. Taniyama, *Hakko-Kogaku Kaishi*, **54**, 795 (1976).
8) H. Kihara, Y. Yamamoto and S. Hosono, "Shokubutsu No Senshokutaisū No Kenkyū," Yokendo Co., Ltd., Tokyo, 1931, p. 169.
9) "Official Methods of Analysis of the Association of Official Agricultural Chemists," 8th ed. The Association, Washington D.C., 1955, p. 122.
10) Y. Inamori, S. Sunagawa, M. Tsuruga, Y. Sawada, H. Taniyama, G. Saito and K. Daigo, *Chem. Pharm. Bull.*, **26**, 1147 (1978).
11) Y. Inamori, K. Morimoto, K. Morisaka, G. Saito, Y. Sawada and H. Taniyama, *Chem. Pharm. Bull.*, **27**, 230 (1979).
12) Y. Inamori, Y. Kato, K. Morimoto, K. Morisaka, G. Saito, Y. Sawada and H. Taniyama, *Chem. Pharm. Bull.*, **27**, 2570 (1979).