[Chem. Pharm. Bull. 36(4)1577—1580(1988)]

Antimicrobial Activity on Plant-Pathogenic Microorganisms and Phytogrowth-Inhibitory Activity of Streptothricin Antibiotics, Racemomycin-A and -C

YOSHIHIKO INAMORI,* HIROKO TOMINAGA, MAKOTO OKUNO, HIROSHI SATO and HIROSHI TSUJIBO

Osaka University of Pharmaceutical Sciences, Kawai, Matsubara-shi, Osaka 580, Japan

(Received September 30, 1987)

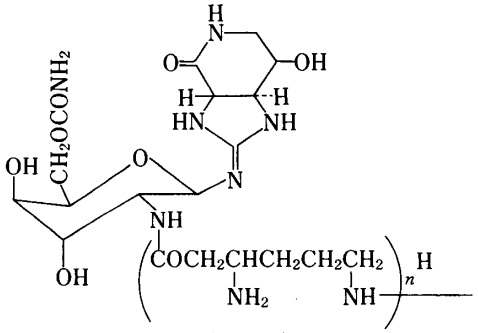
Streptothricin antibiotics, racemomycin-A and -C (RM-A and -C), which contain one and two β-lysine moieties in the molecule, were found to show antimicrobial activity on plant-pathogenic microorganisms and phytogrowth-inhibitory activity.

RM-A and -C showed antimicrobial activities against all the plant-pathogenic microorganisms tested. In particular, RM-C showed strong antifungal activity on *Fusarium oxysporum* f. sp. *lycopersici* IFO-6531 (minimal inhibitory concentration (MIC): 3.0 μg/ml). Both antibiotics strongly inhibited the growth of the root of *Brassica rapa* L. even at the low concentration of 50 ppm. These activities of RM-C were slightly stronger than those of RM-A. This would indicated that the β-lysine moiety in the molecule plays some role in the activity of streptothricin antibiotics.

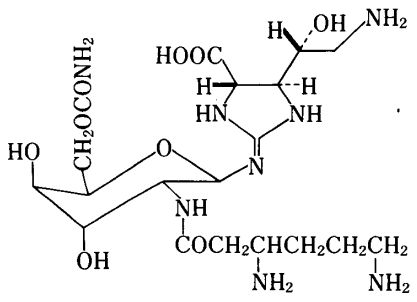
On the other hand, racemomycinic-A-acid (RM-A-acid), produced by opening the lactam ring of the streptolidine moiety of RM-A, did not show any of the above-mentioned activities. This would suggest a close relationship between the streptolidine moiety in the molecule of streptothricin antibiotics and the activities.

Keywords——racemomycin-A; racemomycin-C; racemomycinic-A-acid; streptothricin antibiotic; β-lysine moiety; streptolidine moiety; antimicrobial activity; phytogrowth-inhibitory activity; plant-pathogenic microorganism

Recemomycin-A—-E (RM-A—E) are streptothricin antibiotics which contain one to five β-lysine moieties in the molecule. These antibiotics, in spite of their broad and potent antimicrobial activity, have not been brought into medical use because of their severe delayed toxicities. However, these antibiotics have also been reported to have antiviral activity.[1] Therefore, they are of great interest, if their delayed toxicities can be overcome. Among them, RM-D (Chart 1) containing four β-lysine moieties in the molecule has been reported to show



racemomycin-A (RM-A) $n=1$
racemomycin-C (RM-C) $n=2$
racemomycin-D (RM-D) $n=4$



racemomycinic-A-acid (RM-A-acid)

Chart 1

antimicrobial,[2] insecticidal,[3-5] phytogrowth-inhibitory[3,6] and ichthyotoxic[3] activities by the authors. We also reported that RM-A (Chart 1), containing one β-lysine moiety in the molecule, has strong insecticidal activity.[7,8] However, no work has been done on the antimicrobial activity on plant-pathogenic microorganisms or on the phytogrowth-inhibitory activity of RM-A and -C (Chart 1).

In this work, the antimicrobial activities on plant-pathogenic microorganisms and the phytogrowth-inhibitory activities of RM-A, -C and racemomycinic-A-acid (RM-A-acid, Chart 1), produced by opening the lactam ring of the streptolidine moiety of RM-A, were examined. The results are presented here.

Materials and Methods

Chemicals——RM-A, RM-C and RM-A-acid were used. RM-A and -C are streptothricin antibiotics isolated from the culture broth of *Streptomyces lavendulae* OP-2[9] according to the method of Inamori *et al.*[2] RM-A-acid was prepared from RM-A according to the method of Taniyama *et al.*[10] Sodium 2,4-dichlorophenoxyacetate was used as a standard for the phytogrowth-inhibitory activity test.

Organisms——Plant-pathogenic Microorganisms: Plant-pathogenic bacteria used were as follows: *Corynebacterium michiganense* IFO-12471, *Pseudomonas stutzei* IFO-12510, *Pseudomonas syringae pv. tabaci* IFO-3508, *Pseudomonas syringae pv. phaseolicola* IFO-12656 and *Agrobacterium tumefaciens* IFO-3058. Plant-pathogenic fungi used were as follows: *Aureobasidium pullulans* IFO-4464, *Botryotinia fuckeliana* IFO-9760, *Ceratocystis fimbriata* IFO-4864, *Rhizoctonia solani* IFO-30464 and *Fusarium oxysporum* f. sp. *lycopersici* IFO-6531. The plants used were *Brassica rapa* L. and *Raphanus sativus* L. var. *raphanistroides* MAKINO.

Biological Activity Tests——Antimicrobial Activity Tests: Antibacterial testing was carried out by the agar dilution method. The test bacterium was applied to heart infusion agar (Eiken Chemical Co., Ltd.) containing various concentrations of RM-A, RM-C and RM-A-acid. The plates were incubated at 27 °C for 48 h and the growth was observed with the naked eye. Antifungal testing was carried out by the agar dilution method. The media used were as follows: potato sucrose agar in all cases except for *Fusarium oxysporum* f. sp. *lycopersici* IFO-6531 (potato dextrose agar: Eiken Chemical Co., Ltd.). The test fungi were applied to these media containing various concentrations of RM-A, RM-C and RM-A-acid. The plates were incubated at 27 °C for 5 d and the growth was observed with the naked eye.

Phytogrowth-Inhibitory Activity Test: The phytogrowth-inhibitory activity was tested according to the method of Hirai *et al.*[11] Namely, aliquots (1 ml) of water solutions of RM-A, RM-C, RM-A-acid and sodium 2,4-dichlorophenoxyacetate were each diluted to the concentration of 50 ppm in 100 ml of sterilized agar (0.8%, Difco Laboratories). The agar containing RM-A, RM-C, RM-A-acid, or sodium 2,4-dichlorophenoxyacetate or water alone (control) was poured into a 500 ml sterilized beaker covered with aluminium foil. Then, 20 seeds of each plant sterilized with 70% EtOH and 1% NaClO were put on the agar and left for 7 d at a light intensity of 600 lux. The length of the root of each plant was measured and averaged. The phytogrowth-inhibitory activity was expressed as the ratio of the length of root to that of the control (1.00).

Results

Antibacterial Activities of RM-A, RM-C and RM-A-Acid on Plant-Pathogenic Bacteria

The antibacterial activities of RM-A, RM-C and RM-A-acid on plant-pathogenic bacteria were investigated by the agar dilution method and compared. As shown in Table I, RM-A and RM-C showed rather strong antibacterial activities against all plant-pathogenic bacteria tested. The antibacterial spectrum of RM-C was similar to that of RM-A, but the antibacterial activity of the former was stronger than that of the latter.

In contrast, RM-A-acid in which the lactam ring of the streptolidine moiety of RM-A is opened, showed complete loss of antibacterial activity on plant-pathogenic bacteria.

Antifungal Activities of RM-A, RM-C and RM-A-Acid on Plant-Pathogenic Fungi

The antifungal activities of RM-A, RM-C and RM-A-acid on plant-pathogenic fungi were examined by the agar dilution method and compared. As shown in Table II, RM-A and RM-C had rather strong antifungal activities on plant-pathogenic fungi. In particular, RM-C inhibited the growth of *Ceratocystis fimbriata* IFO-4864. The inhibitory activity of RM-C was

Table I. Antibacterial Activities of RM-A, RM-C and RM-A-Acid on Plant-Pathogenic Bacteria

Bacteria	MIC (μg/ml)		
	RM-A	RM-C	RM-A-acid
Agrobacterium tumefaciens IFO-3058	200	140	>500
Corynebacterium michiganense IFO-12471	10	10	>500
Pseudomonas stutzeri IFO-12510	10	10	>500
Pseudomonas syringae pv. phaseolicola IFO-12656	50	10	>500
Pseudomonas syringae pv. tabaci IFO-3508	45	15	>500

Culture conditions: 27 °C, 48 h. Medium: Heart infusion agar. Method: Agar dilution method.

Table II. Antifungal Activities of RM-A, RM-C and RM-A-Acid on Plant-Pathogenic Fungi

Fungi	MIC (μg/ml)		
	RM-A	RM-C	RM-A-acid
Aureobasidium pullulans IFO-4464	25	10	>600
Botryotinia fuckeliana IFO-9760	60	40	>600
Ceratocystis fimbriata IFO-4864	15	5	>600
Rhizoctonia solani IFO-30464	15	15	>600
Fusarium oxysporum f. sp. *lycopersici* IFO-6531	20	3	>600

Culture conditions: 27 °C, 5 d. Media: Potato sucrose agar (*Fusarium oxysporum* f. sp. *lycopersici* IFO-6531, potato dextrose agar). Method: Agar dilution method.

Table III. Inhibitory Activities of RM-A, RM-C and RM-A-Acid on Plant Growth

Plant	Growth (ratio)[a]			
	RM-A	RM-C	RM-A-acid	2,4-D[b]
Brassica rapa L.	0.30	0.20	1.10	0.06
Raphanus sativus L. *var. raphanistroides* Makino	0.65	0.27	1.01	0.10

a) Growth in control experiments after 7 d was taken as 1.00. Concentration: 50 ppm. Quantity of light: 600 lux. Experimental size: 20 seeds/group, 2 groups. *b*) Sodium 2,4-dichlorophenoxyacetate.

slightly stronger than that of RM-A.

However, RM-A-acid did not show any antifungal activity on any of the plant-pathogenic fungi tested even at the high concentration of 600 μg/ml.

Phytogrowth-Inhibitory Activities of RM-A, RM-C and RM-A-Acid

The inhibitory activities of RM-A, RM-C and RM-A-acid on plantgrowth were tested and compared. The results are summarized in Table III. RM-A and RM-C inhibited the growth of the root of two kinds of plants. In particular, both antibiotics exhibited strong phytogrowth-inhibitory activities on *Brassica rapa* L. even at the low concentration of 50 ppm. The inhibitory activity of RM-C was stronger than that of RM-A. On the other hand, RM-A-acid showed no phytogrowth-inhibitory activity.

Discussion

It was found that RM-A and -C, containing one and two β-lysine moieties in the molecule, showed rather strong antimicrobial activities on plant-pathogenic microorganisms, as well as phytogrowth-inhibitory activities.

Antimicrobial Activity on Plant-Pathogenic Microorganisms

RM-A and -C had rather strong antimicrobial activities against all of the plant-pathogenic microorganisms tested (Tables I and II). The inhibitory activity of these antibiotics

on plant-pathogenic microorganisms is reported for the first time in this paper. Although the toxicity (LD_{50}: 300 mg/kg, i.v. in mice)[12] of RM-A was lower than that of streptomycin (LD_{50}: 200 mg/kg, i.v. in mice), it should be emphasized that, unlike streptomycin, RM-A showed rather strong antifungal activity on *Ceratocystis fimbriata* IFO-4864. A pot test of RM-A on *Ceratocystis fimbriata* is in progress.

Phytogrowth-Inhibitory Activity

RM-A and -C inhibited the growth of the root of two kinds of plants even at the low concentration of 50 ppm (Table III). Among streptothricin antibiotics, RM-D has already been reported to show strong inhibitory activity on plant growth by the authors.[3,6] These findings indicate that the phytogrowth-inhibitory activity may be a common biological activity of streptothricin antibiotics.

The above-mentioned activities of RM-C were stronger than those of RM-A. It was also reported that the insecticidal activity tended to be stronger with increase in the number of β-lysine moieties in the molecule; these may play some role in the activity of the antibiotics, but mechanisms involved are still not clear. It was found that RM-A-acid completely lacked the above-mentioned activities, indicating that the lactam ring of the streptolidine moiety is essential. Namely, the streptolidine moiety in the molecule is considered to play an important role in the activity of streptothricin antibiotics. Taniyama *et al.*[10] have already reported that RM-A-acid showed no antibacterial activity. It was also reported that RM-A-acid showed no insecticidal activity.[7] The relationship between chemical structure and phytogrowth-inhibitory activity of streptothricin antibiotics is reported for the first time in this paper. The results strongly suggest a close relationship between the lactam ring of the streptolidine moiety of streptothricin antibiotic and the activity.

Acknowledgement The authors thank Mr. T. Kobayashi, Osaka University of Pharmaceutical Sciences, for his support during this project.

References and Notes

1) H. Taniyama, Y. Sawada and T. Kitagawa, *Chem. Pharm. Bull.*, **19**, 1627 (1971).
2) Y. Inamori, S. Sunagawa, M. Tsuruga, Y. Sawada and H. Taniyama, *J. Ferment. Technol.*, **56**, 15 (1978).
3) 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).
4) M. Kubo, Y. Kato, K. Morisaka, K. Nomoto and Y. Inamori, *Chem. Pharm. Bull.*, **31**, 325 (1983).
5) Y. Kato, M. Kubo, K. Morisaka, Y. Waku, K. Hayashiya and Y. Inamori, *Chem. Pharm. Bull.*, **31**, 305 (1983).
6) M. Kubo, Y. Kato, N. Ōta, T. Takemoto, K. Nomoto, H. Tsujibo and Y. Inamori, *Chem. Pharm. Bull.*, **33**, 2910 (1985).
7) 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).
8) Y. Inamori, M. Kubo and H. Tsujibo, *Chem. Pharm. Bull.*, **35**, 1509 (1987).
9) Y. Inamori, S. Sunagawa, Y. Sawada and H. Taniyama, *Hakko-Kogaku Kaishi*, **54**, 795 (1976).
10) H. Taniyama, Y. Sawada and T. Kitagawa, *J. Antibiot.*, **24**, 662 (1971).
11) A. Hirai, H. Uchiyama and M. Sugiura, "Shokubutsu Saibo Ikushu Nyumon, Seibutsukagaku Jikkenho," Vol. 16, Gakkai Syuppan Center Co., Ltd., Tokyo, 1983, p. 19.
12) H. Taniyama, Y. Sawada and T. Kitagawa, *J. Antibiot.*, **24**, 662 (1971).