Biological Activities of Racemomycin-B, β-Lysine Rich Streptothricin Antibiotic, the Main Component of *Streptomyces lavendulae* OP-2

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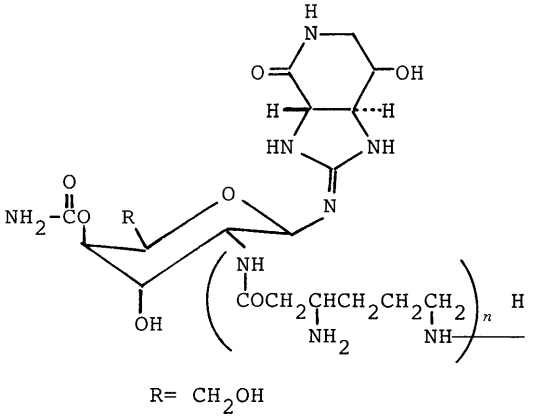
Racemomycin-B (RM-B), the main component of *Streptomyces lavendulae* OP-2 which is the basis of 50% of the antibiotics produced, is a streptothricin antibiotic which contains three β-lysine moieties in the molecule. RM-B had antimicrobial activity against plant-pathogenic microorganisms and growth-inhibitory activity against the root of *Brassica rapa* L. at the concentration of 50 ppm. It strongly inhibited the growth of *Pseudomonas syringae* pv. *tabaci* IFO-3508 (minimum inhibitory concentration (MIC): 0.4 μg/ml), and also showed antifungal activity against six kinds of *Fusarium oxysporum* species (MIC: 0.1—2.0 μg/ml). The antimicrobial activity of RM-B was much stronger than those of RM-A and -C which contain, respectively, one and two β-lysine moieties in their molecules. The above activities of RM-A, -C and -B were thus in the order of -B>-C>-A: namely, the biological activity of racemomycin compounds tended to be stronger with increase in the number of β-lysine moieties in the molecule.

Keywords racemomycin-B; racemomycin-C; racemomycin-A; β-lysine moiety; *Streptomyces lavendulae* OP-2; antimicrobial activity; plant-pathogenic microorganism; *Fusarium oxysporum*; phytogrowth-inhibitory activity

Racemomycins-A, -C, -B and -D (RM-A, -C, -B and -D, Chart 1) contain one, two, three and four β-lysine moieties in their respective molecules and belong to the group of streptothricin antibiotics. In spite of their potent antibacterial activities,[1,2] streptothricin antibiotics have not been introduced for medical use because of their severe delayed nephrotoxicity.[3-5] These antibiotics were, however, reported to show an insecticidal effect[6-10] as well as antibacterial activity. RM-A,[11] -C[11] and -D[6,12] were also found to have phytogrowth-inhibiting activity. Recently, we reported that RM-A and -C showed antimicrobial activity against plant-pathogenic microorganisms.[11] No work has yet been done to our knowledge, on the antimicrobial activity against these microorganisms or on the phytogrowth-inhibitory activity of RM-B. Strain OP-2,[13] thought to be *Streptomyces lavendulae*, was isolated from soil by the authors and was found to produce a streptothricin mixture,[2] the main components of which were identified as the antibiotics RM-B and -D.

In this paper, the antimicrobial activity of RM-B, the main component of *S. lavendulae* OP-2,[13] on plant-pathogenic microorganisms including various species of *Fusarium oxysporum* is described and compared with those of RM-A and -C. Attention is also focused on the inhibitory effect of RM-B against *Brassica rapa* L. root growth.



R= CH_2OH

racemomycin-B (RM-B) $n=3$
racemomycin-C (RM-C) $n=2$
racemomycin-A (RM-A) $n=1$
racemomycin-D (RM-D) $n=4$

Chart 1

Materials and Methods

Chemicals The antibiotics, RM-B, -A and -C are streptothricin groups isolated from the culture broth of *Streptomyces lavendulae* OP-2[13] according to the method of Inamori *et al.*[2] Sodium 2,4-dichlorophenoxyacetate was used as a standard for the phytogrowth-inhibitory activity test.

Organisms Plant-pathogenic microorganisms: The plant-pathogenic bacteria used were as follows: *Agrobacterium tumefaciens* IFO-3058, *Pseudomonas syringae* pv. *tabaci* IFO-3508, *P. syringae* pv. *phaseolicola* IFO-12656 and *P. stutzeri* IFO-12510. Plant-pathogenic fungi used were *Botryotinia fuckeliana* IFO-9760, *Ceratocystis fimbriata* IFO-4870, *Fusarium oxysporum* f. sp. *lycopersici* IFO-6531, *F. oxysporum* f. sp. *cucumerium* IFO-6384, *F. oxysporum* f. sp. *niveum* IFO-4471, *F. oxysporum* f. sp. *melonis* IFO-6385, *F. oxysporum* f. sp. *raphani* IFO-9972 and *F. oxysporum* f. sp. *conglutians* IFO-6383. The plant used was the seed of *Brassica rapa* L.

Antimicrobial Activity Test Antimicrobial testing was carried out by four means: 1) Assay method: agar dilution method, 2) incubation temperature: fungi and bacteria, 27 °C, 3) Incubation time: fungi, 5 d, bacteria, 48 h and 4) medium: fungi, potato sucrose agar (*Fusarium oxysporum* sp., potato dextrose agar), bacteria, heart infusion agar.

Growth-Inhibitory Activity Test of RM-B on the Root of *Brassica rapa* L.[14] Aliquots (1 ml) of water solutions of RM-B and sodium 2,4-dichlorophenoxyacetate were diluted to the concentration of 50 ppm in 100 ml of sterilized agar (0.8% Difco). The agar, containing RM-B 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 *Brassica rapa* 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. Phytogrowth-inhibitory activity was expressed as the ratio of root length to that of control (1.00).

Results

Antimicrobial Activity of Racemomycin-B (RM-B) on Plant-Pathogenic Microorganisms Including Various Species of *Fusarium oxysporum* RM-B showed strong antimicrobial activity against all of the plant-pathogenic microorganisms tested (Table I). In particular, this antibiotic strongly inhibited the growth of *Pseudomonas syringae* pv. *tabaci* IFO-3508; its minimum inhibitory concentration for this bacterium was 0.4 μg/ml. The antibacterial activity of RM-B was much stronger than those of RM-A[11] and -C.[11] As shown, RM-B, -C[11] and

TABLE I. Antimicrobial Activity of Racemomycin Compounds on Plant-Pathogenic Microorganisms Including Various Species of *Fusarium oxysporum*

Microorganism	Antimicrobial activity (MIC[a]: μg/ml)		
	RM-B	RM-C	RM-A
Bacteria			
Pseudomonas syringae pv. *tabaci* IFO-3508	0.4	15.0[11]	45.0[11]
Pseudomonas stutzeri IFO-12510	4.0	10.0[11]	10.0[11]
Pseudomonas syringae pv. *phaseolicola* IFO-12656	4.0	10.0[11]	50.0[11]
Agrobacterium tumefaciens IFO-3058	30.0	140.0[11]	200.0[11]
Fungi			
Botryotinia fuckeliana IFO-9760	20.0	40.0	60.0
Ceratocystis fimbriata IFO-4870	10.0	5.0	15.0
Fusarium oxysporum f. sp. *cucumerium* IFO-6384	0.1	2.5	8.0
Fusarium oxysporum f. sp. *niveum* IFO-4471	0.2	1.5	8.0
Fusarium oxysporum f. sp. *melonis* IFO-6385	0.2	2.0	8.0
Fusarium oxysporum f. sp. *raphani* IFO-9972	0.5	3.5	8.0
Fusarium oxysporum f. sp. *conglutians* IFO-6383	1.0	1.5	3.0
Fusarium oxysporum f. sp. *lycopersici* IFO-6531	2.0	3.0[11]	20.0[11]

Culture conditions: bacteria, 27 °C, 48 h; fungi, 27 °C, 5 d. Medium: bacteria, heart infusion agar, fungi; potato sucrose agar (*Fusarium oxysporum* species, potato dextrose agar). Assay method: agar dilution method. *a*) Minimum inhibitory concentration.

-A[11] had rather strong antifungal activity against *Fusarium oxysporum* f. sp. *lycopersici* IFO-6531, and this activity was further examined by the agar dilution method on five kinds of *F. oxysporum* sp. All three compounds had rather strong antifungal activity against all *F. oxysporum* sp. tested. Especially, RM-B strongly inhibited the growth of *F. oxysporum* f.sp. *cucumerium* IFO-6384 (minimum inhibitory concentration (MIC): 0.1 μg/ml). In fact, the activity of RM-B on all six kinds of this species tested was much stronger than those of RM-A and -C.

Growth-Inhibitory Activity of Racemomycin-B (RM-B) on the Root of *Brassica rapa* L. Like RM-A,[11] -C[11] and -D,[6,12] RM-B showed strong inhibition of root growth of *Brassica rapa* L. at a low concentration of 50 ppm (with an inhibitory ratio of 0.17, relative to 1.0 for the control). Nonetheless, this was weaker than that of sodium 2,4-dichlorophenoxyacetate (inhibitory ratio of 0.05, relative to 1.0 for the control, 50 ppm) used as a standard. However, RM-B inhibition on this plant at the concentration of 50 ppm was stronger than those of RM-C (inhibitory ratio of 0.20, relative to 1.0 for the control)[11] and RM-A (0.30).[11] RM-B did not inhibit the germination of the seed of *Brassica rapa* L. at this concentration.

Discussion

Since *Streptomyces lavendulae* OP-2[13] produced RM-B with three β-lysine moieties in its molecule as a main component (50% of producing antibiotics),[2] this strain seemed the most appropriable for the investigation of RM-B biological activity. The antimicrobial activity on plant-pathogenic microorganisms and phytogrowth-inhibitory activity were therefore examined and RM-B was found to demonstrate both activities.

Antimicrobial Activity on Plant-Pathogenic Microorganisms The antimicrobial activity of RM-B on plant-pathogenic microorganisms was characterized by the following three points: 1) It was stronger than those of RM-A and -C, 2) RM-B most strongly inhibited the growth of *P. syringae* pv. *tabaci* IFO-3508 (MIC: 0.4 μg/ml, see Table I) among the bacteria tested and 3) it showed broad and potent antifungal activity on all *F. oxysporum* species tested (MIC: 0.1—1.0 μg/ml, Table I). *F. oxysporum* sp. are well known to be not only plant-pathogenic fungi but also the organisms causing keratomycosis, and no low toxicity antifungal substance on these fungi has yet been discovered. In this respect, the strong antifungal activity of RM-B on this species is of great interest, if its delayed toxicity can be overcome. Efforts to reduce the toxicity of racemomycin compounds are now under way.

Phytogrowth-Inhibitory Activity Like RM-A,[11] -C[11] and -D,[6,12] RM-B also showed strong growth inhibition of the root of *Brassica rapa* L. Thus all four racemomycin compounds have this activity.

In both activities examined, RM-B was found to be strongest followed by RM-C and then by RM-A; the biological activity of racemomycin compounds therefore tended to be stronger with increase in the number of β-lysine moieties in the molecule. This was also true of insecticidal activity as reported by the authors,[7] thus confirming the importance of the β-lysine moiety in the molecule in these compounds. The amount of RM-D on hand was, unfortunately, too small to permit examination of biological activity. Research on the basis of the relationship between the number of β-lysine moieties and the biological activity of racemomycin compounds is continuing.

References

1) H. Taniyama, F. Miyoshi and K. Kageyama, *Yakugaku Zasshi*, **82** (1962).
2) Y. Inamori, S. Sunagawa, M. Tsuruga, Y. Sawada and H. Taniyama, *J. Ferment. Technol.*, **56**, 15 (1978).
3) Y. Inamori, S. Sunagawa, M. Tsuruga, Y. Sawada, H. Taniyama, G. Saito and K. Daigo, *Chem. Pharm. Bull.*, **26**, 1147 (1978).
4) Y. Inamori, K. Morimoto, K. Morisaka, G. Saito, Y. Sawada, H. Taniyama and H. Matsuda, *Chem. Pharm. Bull.*, **27**, 230 (1979).
5) Y. Inamori, Y. Kato, K. Morimoto, K. Morisaka, G. Saito, Y. Sawada and H. Taniyama, *Chem. Pharm. Bull.*, **27**, 2570 (1979).
6) 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).
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) M. Kubo, Y. Kato, K. Morisaka, K. Nomoto and Y. Inamori, *Chem. Pharm. Bull.*, **31**, 325 (1983).

9) Y. Kato, M. Kubo, K. Morisaka, Y. Waku, K. Hayashiya and Y. Inamori, *Chem. Pharm. Bull.*, **31**, 305 (1983).
10) Y. Inamori, M. Kubo and H. Tsujibo, *Chem. Pharm. Bull.*, **35**, 1509 (1987).
11) Y. Inamori, H. Tominaga, M. Okuno, H. Sato and H. Tsujibo, *Chem. Pharm. Bull.*, **36**, 1577 (1988).
12) M. Kubo, Y. Kato, N. Ōta, T. Takemoto, K. Nomoto, H. Tsujibo and Y. Inamori, *Chem. Pharm. Bull.*, **33**, 2910 (1985).
13) Y. Inamori, S. Sunagawa, Y. Sawada and H. Taniyama, *Hakko-Kogaku Kaishi*, **54**, 795 (1976).
14) A. Hirai, H. Uchiyama and M. Sugiura, "Seibutsu Kenteiho," Nankodo Co., Ltd., Tokyo, 1971. p. 242.