ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1994, p. 1118–1122
0066-4804/94/$04.00+0

Vol. 38, No. 5

In Vitro and In Vivo Antibacterial Activities of KRM-1648 and KRM-1657, New Rifamycin Derivatives

KENJI FUJII,* AKIYOSHI TSUJI, SHUICHI MIYAZAKI, KEIZO YAMAGUCHI, AND SACHIKO GOTO

Department of Microbiology, Toho University School of Medicine, 21-16 Omori-nishi 5-chome, Ota-ku, Tokyo 143, Japan

Received 19 November 1993/Returned for modification 3 January 1994/Accepted 28 February 1994

The in vitro and in vivo antibacterial activities of the new rifamycin derivatives KRM-1648 and KRM-1657 were compared with those of rifampin. Rifabutin, ciprofloxacin, and clarithromycin were also tested for reference. The respective MICs of KRM-1648 and KRM-1657 for 90% of the strains tested (MIC_{90}s) were 0.016 and 0.0078 μg/ml, respectively, for methicillin-susceptible *Staphylococcus aureus*, 0.016 and 0.0039 μg/ml, respectively, for methicillin-resistant *S. aureus*, and 0.0625 and 0.016 μg/ml, respectively, for methicillin- and quinolone-resistant *S. aureus*. These MIC_{90}s of KRM-1657 were equal to or 2- to 64-fold lower than those of rifampin. KRM-1648 and KRM-1657 with MIC_{90}s of between 0.002 and 0.078 μg/ml were 2- to 128-fold more active than rifampin against *Staphylococcus epidermidis* and *Streptococcus* species, including *Streptococcus pneumoniae* and *Streptococcus pyogenes*. The MIC_{90}s of KRM-1657 for *Haemophilus influenzae* and *Neisseria gonorrhoeae* were 0.25 and 0.1 μg/ml, respectively; KRM-1657 was almost as active as rifampin and was 8- to 16-fold more active than KRM-1648 against these strains. The frequency of occurrence of spontaneous mutations to resistance to KRM-1648 and KRM-1657 was equal to that to rifampin. Against systemic infection with *S. aureus* in mice, the efficacies of KRM-1648 and KRM-1657 were comparable to that of rifampin.

The recently synthesized rifamycin derivatives 3′-hydroxy-5′-(4-isobutyl-1-piperazinyl)benzoxazinorifamycin (KRM-1648) and 3′-hydroxy-5′-(4-propyl-1-piperazinyl)benzoxazinorifamycin (KRM-1657) (Fig. 1) exhibit potent activity against a variety of mycobacteria including *Mycobacterium tuberculosis*, *Mycobacterium avium*, and *Mycobacterium intracellulare* (1, 3, 6, 7, 9, 10). These compounds have also been shown to be active against *Staphylococcus aureus* and *Bacillus subtilis* but have been shown to be inactive against *Escherichia coli* and *Klebsiella pneumoniae* (10). In the study described here, we conducted further testing to determine the in vitro activities of KRM compounds against gram-positive and gram-negative bacteria with the aim of elucidating their antibacterial properties. The frequency of occurrence of spontaneous mutants of *S. aureus* resistant to these compounds and the therapeutic efficacies of these compounds against *S. aureus* infections in mice were also determined.

MATERIALS AND METHODS

Antimicrobial agents. KRM-1648, KRM-1657, and rifabutin were obtained from Kaneka Corp. (Takasago, Japan). Other antimicrobial agents were obtained from the indicated sources: rifampin, Chong Kun Dang Corp. (Seoul, Korea); ciprofloxacin, Bayer Yakuhin Co., Ltd. (Tokyo, Japan); and clarithromycin, Taisho Pharmaceutical Co., Ltd. (Tokyo, Japan).

Test strains. The bacterial strains used in the study were control reference strains and recent clinical isolates collected between 1989 and 1992 from hospitals in several parts of Japan. All organisms were stocked frozen at the Department of Microbiology, Toho University School of Medicine, Tokyo, Japan.

Preparation of test drug solution. For in vitro testing, KRM-1648, KRM-1657, rifampin, and clarithromycin were dissolved in dimethyl sulfoxide at 3.2 mg/ml. Rifabutin and ciprofloxacin were dissolved in dimethylformamide and 0.1 N NaOH, respectively, at 3.2 mg/ml. Drug solutions were diluted with medium broth for use. All drugs were stored at −20°C.

For in vivo testing, rifampin was dissolved (and the other three rifamycin derivatives were suspended) in 2.5% gum arabic solution containing 0.05% Tween 80 (polysorbate 80) at 1.6 mg/ml and was then diluted with the same vehicle to achieve the appropriate concentrations. The compounds were

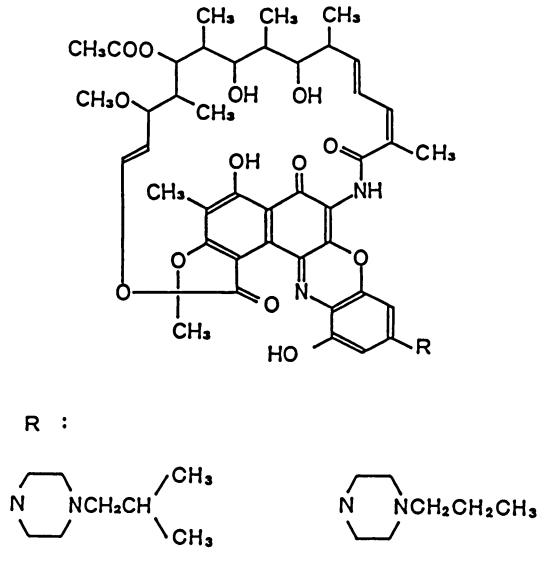

FIG. 1. Structures of KRM-1648 and KRM-1657.

* Corresponding author. Mailing address: Biochemical Research Laboratories, Kaneka Corporation, 1-8, Miyamae-machi, Takasago-cyo, Takasago-shi, Hyogo 676, Japan. Phone: 81-794-45-2426. Fax: 81-794-45-2459.

TABLE 1. MICs of KRM-1648, KRM-1657, and other antibiotics for gram-positive and gram-negative bacteria obtained by the broth microdilution method

Strain	MIC (μg/ml)[a]			
	KRM-1648	KRM-1657	RMP	RBT
Staphylococcus aureus 209-P	0.0039	0.002	0.0078	0.016
Staphylococcus aureus Smith	0.0078	0.002	0.0078	0.0313
Staphylococcus aureus Terajima	0.0039	0.0005	0.0078	0.016
Staphylococcus aureus Newman	0.0078	0.0005	0.002	0.0078
Staphylococcus aureus ATCC 29213	0.0313	0.0039	0.0078	0.0313
Staphylococcus aureus ATCC 25923	0.0313	0.0078	0.016	0.0625
Staphylococcus epidermidis ATCC 13228	0.002	0.0039	0.0078	0.0078
Micrococcus luteus ATCC 9341	0.0039	0.0039	0.0078	0.0039
Streptococcus pneumoniae type II	0.002	0.001	0.0078	0.0078
Enterococcus faecalis TMS 64	0.25	0.0313	0.5	0.125
Bacillus subtilis ATCC 6633	0.016	0.0313	0.0313	0.016
Escherichia coli NIHJJC 2	>32	>32	16	16
Klebsiella pneumoniae IFO 3512	>32	>32	16	16
Salmonella typhimurium S 60	>32	>32	16	16
Salmonella typhimurium ATCC 13311	4	0.125	4	4
Pseudomonas aeruginosa IFO 3445	>32	>32	32	16
Serratia marcescens IFO 12468	>32	>32	32	32
Acinetobactor calcoaceticus NCTC 7488	>32	16	2	4

[a] RMP, rifampin; RBT, rifabutin.

TABLE 2. MICs of KRM-1648, KRM-1657 and other antibiotics for anaerobic bacteria obtained by the broth microdilution method

Strain	MIC (μg/ml)[a]			
	KRM-1648	KRM-1657	RMP	RBT
Peptostreptococcus anaerobius ATCC 27337	0.00024	0.00012	0.0005	0.0005
Peptostreptococcus asaccharolyticus ATCC 14963	≤0.00003	≤0.00003	0.001	≤0.00003
Peptostreptococcus magnus ATCC 29328	0.125	0.125	0.25	0.0078
Eubacterium lentum GAI 7506	≤0.00003	≤0.00003	0.001	≤0.00003
Eubacterium limosum ATCC 8486	≤0.00003	≤0.00003	0.001	≤0.00003
Eubacterium aerofaciens GAI 5570	0.0625	0.002	0.125	0.0078
Clostridium histolyticum GAI 5608	0.5	0.0625	0.125	0.016
Clostridium perfringens NCTC 4946	0.0078	0.016	0.0039	0.016
Clostridium botulinum A 62	0.5	1	0.0625	0.0313
Clostridium botulinum B okra	0.0313	0.0625	0.0039	0.0078
Veillonella parvula ATCC 10790	32	>32	2	1
Bacteroides fragilis GM 7000	1	2	0.0313	0.0313
Bacteroides vulgatus ATCC 29327	0.125	0.125	0.0625	0.0078
Fusobacterium varium GAI 5560	>32	>32	>32	>32
Fusobacterium mortiferum VIP 4249	>32	>32	>32	>32
Fusobacterium nucleatum ATCC 25586	2	4	1	2

[a] RMP, rifampin; RBT, rifabutin.

then administered orally to infected mice in a volume of 0.1 ml/10 g of body weight.

Determination of MICs. The MICs were determined by the broth microdilution method. The media used for MIC determination were cation-supplemented Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) and Mueller-Hinton broth supplemented with 5% lysed horse blood–15 μg of NAD per ml–5 mg of yeast extract (Difco) per ml to support the growth of *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Haemophilus influenzae*. Gifu Anaerobic Medium broth (Nissui Pharmaceutical, Tokyo, Japan) was used for obligate anaerobes. The MICs for *Neisseria gonorrhoeae* were determined by the twofold serial agar dilution method. The media contained GC agar (Difco) supplemented with 2% defined supplemented solution (20 g of glucose, 0.5 g of glutamine, and 0.001 g of cocarboxylase in 100 ml of distilled water).

An overnight broth culture (about 10^8 CFU/ml) of a bacterial suspension, prepared by transferring bacterial colonies grown on suitable agar plates into saline, was diluted with saline, and the mixture was inoculated into drug-containing wells to a final concentration of ca. 5×10^5 CFU/ml (aerobic bacteria) or 5×10^6 CFU/ml (anaerobic bacteria). After incubation of microtiter plates at 35°C for 18 to 24 h, the MIC, defined as the lowest concentration of antimicrobial agent resulting in the complete inhibition of growth, was determined.

Determination of frequency of occurrence of spontaneous mutants. Spontaneous mutants of *S. aureus* 209-P resistant to KRM-1648, KRM-1657, and other antibiotics were isolated by plating 0.1 ml of an overnight culture that had been concentrated to about 10^7 CFU/ml as a final inoculum onto agar plates containing concentrations of each drug four times those of the MIC. After 18 h of cultivation at 35°C, the number of bacterial colonies grown on drug-supplemented agar plates was counted. The frequency of occurrence of spontaneous mutants was calculated from the number of bacterial colonies that grew on the agar plates.

In vivo activity. The test organisms used to generate systemic infections in mice were cultured on Mueller-Hinton agar at 35°C for 18 h and were then suspended in 5% gastric mucin (Difco). Male Crj-ICR mice (weight, 18 to 22 g; Charles River, Atsugi, Japan) in groups of six mice each were inoculated intraperitoneally with 0.5 ml of a bacterial suspension containing 1.5×10^6 CFU (*S. aureus* Smith) or 2.5×10^7 CFU (methicillin-resistant *S. aureus*). One hour after inoculation, mice received a single dose of KRM-1648, KRM-1657, rifampin, or rifabutin ranging from 0.016 to 16 mg/kg of body weight orally by using an intragastric tube. The total number of mice that survived at each dose level was recorded on day 5 after inoculation, and the 50% effective dose (ED_{50}) was

TABLE 3. Antibacterial activities of KRM-1648, KRM-1657, and other antibiotics against 180 clinically isolated strains

Organism (no. of strains)	Drug[a]	MIC (μg/ml)		
		Range	50%	90%
Methicillin-susceptible *Staphylococcus aureus* (20)[b]	KRM-1648	0.0039–0.016	0.0078	0.016
	KRM-1657	0.002–0.0078	0.0039	0.0078
	RMP	0.002–0.016	0.0039	0.0078
	RBT	0.002–0.0625	0.016	0.0313
	CPFX	0.125–32	0.25	1
	CAM	0.0625–>32	0.0625	16
Methicillin-resistant *Staphylococcus aureus*[b,c] (20)	KRM-1648	0.002–0.016	0.0078	0.016
	KRM-1657	0.0005–0.0078	0.0039	0.0039
	RMP	0.0039–0.016	0.0078	0.0078
	RBT	0.0313–0.0625	0.0313	0.0625
	CPFX	0.0078–8	0.5	2
	CAM	0.125–>32	>32	>32
Methicillin- and quinolone-resistant *Staphylococcus aureus*[b,d] (16)	KRM-1648	0.001–0.125	0.0078	0.0625
	KRM-1657	≤0.00024–0.016	0.0039	0.016
	RMP	0.0039–1	0.0078	1
	RBT	0.016–2	0.0313	2
	CPFX	16–>32	>32	>32
	CAM	32–>32	>32	>32
Staphylococcus epidermidis (20)[b]	KRM-1648	0.0039–0.016	0.0078	0.0078
	KRM-1657	0.002–0.0078	0.0078	0.0078
	RMP	0.002–0.0625	0.0078	0.016
	RBT	0.016–0.0625	0.0313	0.0625
	CPFX	0.0625–8	0.125	0.5
	CAM	0.016–0.0625	0.0313	0.0625
Streptococcus pneumoniae (20)[b]	KRM-1648	0.00024–0.0625	0.001	0.002
	KRM-1657	0.00006–0.0313	0.0005	0.002
	RMP	0.016–2	0.016	0.25
	RBT	0.0005–0.25	0.0039	0.0313
	CPFX	0.25–2	1	2
	CAM	0.0078–>32	0.0313	>32
Streptococcus pyogenes (20)[b]	KRM-1648	0.0005–0.0078	0.002	0.0078
	KRM-1657	0.0005–0.0078	0.001	0.0039
	RMP	0.0078–0.0625	0.0313	0.0625
	RBT	0.002–0.0313	0.016	0.016
	CPFX	0.125–1	0.5	0.5
	CAM	0.002–>32	0.0313	1
Haemophilus influenzae (20)[b]	KRM-1648	0.5–32	2	4
	KRM-1657	0.125–1	0.125	0.25
	RMP	0.0625–1	0.25	0.25
	RBT	0.125–2	0.5	0.5
	CPFX	0.0039–0.0313	0.0078	0.0078
	CAM	0.0625–8	2	4
Neisseria gonorrhoeae, non-penicillinase producing (31)[e]	KRM-1648	0.1–0.78	0.39	0.78
	KRM-1657	≤0.006–0.1	0.05	0.1
	RMP	≤0.006–0.39	≤0.006	0.2
	RBT	0.2–1.56	0.78	0.78
	CPFX	≤0.006–0.1	≤0.006	0.05
	CAM	0.05–3.13	1.56	3.13
Neisseria gonorrhoeae, penicillinase producing (13)[e]	KRM-1648	0.39–1.56	0.78	0.78
	KRM-1657	0.012–0.78	0.05	0.1
	RMP	≤0.006–0.78	0.025	0.2
	RBT	0.39–3.13	0.78	1.56
	CPFX	≤0.006–0.39	0.025	0.39
	CAM	0.05–3.13	1.56	1.56

[a] RMP, rifampin; RBT, rifabutin; CPFX, ciprofloxacin; CAM, clarithromycin.
[b] The organisms were tested by the broth dilution method.
[c] MIC of oxacillin was above 4 μg/ml.
[d] MIC of ciprofloxacin was above 16 μg/ml.
[e] The organisms were tested by the agar dilution method.

TABLE 4. Frequency of occurrence of spontaneous mutants of *S. aureus* 209-P resistant to KRM-1648, KRM-1657, and other antibiotics

Drug	Frequency (selective concn [μg/ml][a])
KRM-1648	1.1×10^{-6} (0.016)
KRM-1657	1.6×10^{-6} (0.0078)
Rifampin	3.2×10^{-6} (0.0313)
Rifabutin	1.2×10^{-6} (0.0625)
Ciprofloxacin	1.0×10^{-7} (1)
Clarithromycin	$<5.0 \times 10^{-8}$ (0.25)

[a] Four times the corresponding MIC.

calculated by the method of van der Waerden (8). All untreated mice died within 1 day after inoculation.

RESULTS

Antibacterial activity. The results of susceptibility testing of standard strains of bacteria are given in Tables 1 and 2. KRM-1648 and KRM-1657 exhibited strong activity against gram-positive bacteria but were inactive against most of the gram-negative bacteria tested. The spectra of antibacterial activities of the KRM compounds were similar to those of rifampin and rifabutin.

The activities of KRM compounds against 180 clinical isolates of bacteria are given in Table 3. KRM-1657 and KRM-1648 exhibited potent activity against both methicillin-susceptible *S. aureus* (MICs for 90% of strains tested [MIC_{90}s], 0.0078 and 0.016 μg/ml, respectively) and methicillin-resistant *S. aureus* (MIC_{90}s, 0.0039 and 0.016 μg/ml, respectively). They had activities comparable to that of rifampin (MIC_{90}s, 0.0078 and 0.0078 μg/ml, respectively) and were more active than the other drugs tested. Against methicillin- and quinolone-resistant *S. aureus*, KRM-1657 was the most active compound (MIC_{90}, 0.016 μg/ml) among the drugs tested. Against *Staphylococcus epidermidis*, *S. pneumoniae*, and *S. pyogenes*, KRM-1657 and KRM-1648 exhibited potent activity, with MIC_{90}s ranging from 0.002 to 0.0078 μg/ml; their activities against these strains were much greater than those of the reference drugs tested. Against *H. influenzae*, KRM-1657 exhibited good activity (MIC_{90}, 0.25 μg/ml), comparable to those of rifampin and rifabutin, and was more active than KRM-1648 and clarithromycin but was less active than ciprofloxacin. Against non-penicillinase-producing- and penicillinase-producing strains of *N. gonorrhoeae*, KRM-1657 also exhibited good activity (MIC_{90}, 0.1 μg/ml), as did rifampin; its activity against these strains was greater than those of the other drugs tested.

Frequency of occurrence of spontaneous mutants. The frequency of occurrence of spontaneous mutants of *S. aureus* 209-P resistant to the test drugs is given in Table 4. The concentration used for selection was four times the corresponding MIC, and colonies that grew on agar plates were considered resistant to the respective drugs; MICs were in some cases more than 16-fold higher (for KRM compounds, rifampin, and rifabutin) and in other cases were 8-fold higher (for ciprofloxacin) than those before selection (data not shown). The frequency of occurrence of spontaneous mutants of *S. aureus* 209-P resistant to the KRM compounds (1.1×10^{-6} for KRM-1648; 1.6×10^{-6} for KRM-1657) was almost the same as those to rifampin (3.2×10^{-6}) and rifabutin (1.2×10^{-6}), but greater than those to ciprofloxacin (1.0×10^{-7}) and clarithromycin ($<5.0 \times 10^{-8}$).

In vivo efficacy in mice. The in vivo efficacies of the KRM compounds were compared with those of rifampin and rifabutin against septicemia caused by *S. aureus* infection in mice. The results are summarized in Table 5. Against infection with the *S. aureus* Smith strain, KRM-1648 and KRM-1657 exhibited equally strong protective effects, with ED_{50}s of 0.063 and 0.063 mg/kg, respectively; their efficacies were comparable to that of rifampin (ED_{50}, 0.099 mg/kg) and greater than that of rifabutin (ED_{50}, 0.63 mg/kg). Against infection with methicillin-resistant *S. aureus* 235, the ED_{50}s of KRM-1648, KRM-1657, rifampin, and rifabutin were 0.500, 0.397, 0.315, and 1.260 mg/kg, respectively.

DISCUSSION

The new rifamycin derivatives KRM-1648 and KRM-1657 have been shown to exhibit strong in vitro and in vivo antimycobacterial activities, particularly against *M. tuberculosis* and members of the *M. avium* complex (1, 3, 6, 7, 9, 10).

The findings of the present study demonstrated that KRM-1648 and KRM-1657 have antibacterial activity against gram-positive bacteria, but they have poor activity against most of the gram-negative bacteria tested. The spectra of antibacterial activities of both of these KRM compounds were almost the same as those of rifampin and rifabutin.

Of the four rifamycin derivatives tested, KRM-1657 was the most active against most bacteria; this was followed by rifampin, KRM-1648, and rifabutin. Although the activity of KRM-1657 against methicillin-susceptible and -resistant *S. aureus* was only comparable to or slightly greater than those of rifampin and KRM-1648 against methicillin- and quinolone-resistant *S. aureus*, it was 4- and 64-fold more active than KRM-1648 and rifampin, respectively. KRM-1648 and KRM-

TABLE 5. Comparison of activities of KRM-1648, KRM-1657, and other antibiotics against systemic infections in mice

Organism	Inoculum (CFU/mouse)[a]	Drug[b]	MIC (μg/ml)	ED_{50} (mg/kg) [95% confidence interval])
S. aureus Smith	1.5×10^6	KRM-1648	0.0078	0.063 (0.046–0.085)
		KRM-1657	0.002	0.063 (0.046–0.085)
		Rifampin	0.0078	0.099 (0.079–0.125)
		Rifabutin	0.0313	0.630 (0.500–0.794)
S. aureus 235, methicillin resistant	2.5×10^7	KRM-1648	0.0039	0.500 (0.500–0.500)
		KRM-1657	0.002	0.397 (0.315–0.500)
		Rifampin	0.0039	0.315 (0.235–0.422)
		Rifabutin	0.016	1.260 (0.941–1.688)

[a] With 5% mucin.
[b] One hour after inoculation, mice were treated with single doses of drugs by using an intragastric tube (six mice per dose).

1657 also exhibited high degrees of activity against *S. pneumoniae*, *S. pyogenes*, *H. influenzae*, and *N. gonorrhoeae.*

Against systemic infections with methicillin-susceptible and -resistant *S. aureus* in mice, KRM-1648 and KRM-1657 each had as strong a protective effect as rifampin, and their good efficacies in vivo were consistent with their high degrees of in vitro activity against *S. aureus*. The findings of pharmacokinetic studies have indicated that the concentrations of KRM compounds in plasma are lower than that of rifampin but that their concentrations in certain organs (lung and spleen) are comparable to or greater than those of rifampin following oral administration to mice at 20 mg/kg (7, 10). Moreover, KRM-1648 and KRM-1657 exhibit fewer toxic effects than rifampin in mice when administered orally once a day for 5 days at a dose of 600 mg/kg (4).

The frequencies of occurrence of spontaneous mutants of *S. aureus* 209-P resistant to KRM-1648 and KRM-1657 were similar to those for rifampin and rifabutin and greater than those for ciprofloxacin and clarithromycin. This suggests that combination therapy is preferable when these compounds are used for the treatment of infection. *S. aureus* has been shown to easily develop resistance to rifampin (2); correspondingly, rifampin has been used in combination with other antibiotics in the treatment of bacterial infections (5). In conclusion, KRM-1648 and KRM-1657 are new rifamycin derivatives with potent antibacterial activities and therefore merit further study.

REFERENCES

1. **Emori, M., H. Saito, K. Sato, H. Tomioka, T. Setogawa, and T. Hidaka.** 1993. Therapeutic efficacy of the benzoxazinorifamycin KRM-1648 against experimental *Mycobacterium avium* infection induced in rabbits. Antimicrob. Agents Chemother. **37:**722–728.
2. **Eng, R. H. K., S. M. Smith, F. J. Buccini, and C. E. Cherubin.** 1985. Differences in ability of cell-wall antibiotics to suppress emergence of rifampicin resistance in *Staphylococcus aureus*. J. Antimicrob. Chemother. **15:**201–207.
3. **Kuze, K., T. Yamamoto, R. Amitani, and K. Suzuki.** 1991. In vivo activities of new rifamycin derivatives against mycobacteria. Kekkaku **66:**7–12. (In Japanese.)
4. **Saito, H., H. Tomioka, K. Sato, M. Emori, T. Yamane, K. Yamashita, K. Hosoe, and T. Hidaka.** 1991. In vitro antimicrobial activities of newly synthesized benzoxazinorifamycins. Antimicrob. Agents Chemother. **35:**542–547.
5. **Sande, M. A., and W. M. Sheld.** 1980. Combination antibiotic therapy of bacterial endocarditis. Ann. Intern. Med. **92:**390–395.
6. **Tomioka, H., H. Saito, K. Fujii, K. Sato, and T. Hidaka.** 1993. In vitro antimicrobial activity of benzoxazinorifamycin, KRM-1648, against *Mycobacterium avium* complex, determined by the radiometric method. Antimicrob. Agents Chemother. **37:**67–70.
7. **Tomioka, H., H. Saito, K. Sato, T. Yamane, K. Yamashita, K. Hosoe, K. Fujii, and T. Hidaka.** 1992. Chemotherapeutic efficacy of a newly synthesized benzoxazinorifamycin, KRM-1648, against *Mycobacterium avium* complex infection induced in mice. Antimicrob. Agents Chemother. **36:**387–393.
8. **van der Waerden, B. L.** 1940. Wirksamkeits- und Konzentraionsberstimmung durch Tierversuche. Arch. Exp. Pathol. Pharmakol. **195:**389–412.
9. **Yamamoto, T., R. Amitani, F. Kuze, and K. Suzuki.** 1990. In vitro activities of new rifamycin derivatives against *Mycobacterium tuberculosis* and *M. avium* complex. Kekkaku **65:**805–810. (In Japanese.)
10. **Yamane, T., T. Hashizume, K. Yamashita, E. Konishi, K. Hosoe, T. Hidaka, K. Watanabe, H. Kawaharada, T. Yakamoto, and F. Kuze.** 1993. Synthesis and biological activity of 3′-hydroxy-5′-aminobenzoxazinorifamycin derivatives. Chem. Pharm. Bull. **41:** 148–155.