Antimycobacterial Activities In Vitro and In Vivo and Pharmacokinetics of Dihydromycoplaneacin A

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The in vitro activity of dihydromycoplaneacin A (DHMP A), a new cyclic peptide antibiotic, was compared with those of antimycobacterial drugs such as streptomycin, isoniazid (INH), rifampin, and ofloxacin against several clinically isolated species of mycobacteria, including Mycobacterium tuberculosis, M. intracellulare, and M. kansasii. DHMP A demonstrated stronger activities than other drugs against all species of mycobacteria tested at concentrations of <0.0125 to 25 μg/ml. A marked synergism between DHMP A and INH was demonstrated by the checkerboard technique against M. tuberculosis, M. intracellulare, and M. smegmatis, and the synergistic effect was observed by treatment of the culture of M. smegmatis with DHMP A for at least 3 h prior to treatment with INH. It was also shown that both absorption and excretion of INH in mice were faster than those of DHMP A. On the basis of these results, combination therapy with DHMP A and INH was successfully carried out in experimental tuberculosis in mice infected with M. bovis Ravenel. After a single intravenous administration of 10 mg of DHMP A per kg, its half-life in serum in mice was about 0.5 h and in dogs it was 5.5 h. A single oral administration of 50 mg of DHMP A per kg in mice gave a peak level in serum of 10 μg/ml at 2 to 4 h. A single oral administration to dogs of 12.5 mg/kg gave a peak of 5.0 μg/ml at 3 h. In these experiments, urinary recoveries within 48 h were 21.0% in mice and 25.2% in dogs. The tissue distribution level of DHMP A in mice after oral administration was in the order of liver > kidney > serum > spleen > lung. The 50% lethal doses of DHMP A for mice were more than 6,000 mg/kg orally and 1,840 mg/kg intraperitoneally.

MATERIALS AND METHODS

Antimicrobial agents tested. DHMP A was prepared by chemical reduction of MP A isolated from the culture broth of A. awajinensis in our laboratories, and its amorphous powder (purity, greater than 95%) was used throughout the experiments. Rifampin (RFP; Rifadin) and ofloxacin (OFLX) were purchased from Daiichi Pharmaceutical Co., Ltd., Japan, isoniazid (INH) and ethambutol (EB) were from Sigma Chemical Co., and streptomycin (SM) was from Meiji Seika Co., Ltd., Japan.

Susceptibility test. Mycobacterial strains used in this study were mainly clinically isolated from patients with pulmonary diseases at East Saitama Hospital, Saitama Prefecture, Japan. The susceptibility test was performed by a broth dilution technique in Dubos medium supplemented with 10% bovine serum albumin (Eiken Co., Ltd., Japan). Each mycobacterial strain grown on 1% Ogawa medium was subcultured in the Dubos medium for 1 to 2 weeks at 37°C. A 40-μl amount of 102-fold-diluted subculture with the same medium freshly prepared was inoculated into the test tubes containing serial twofold dilutions of the test drugs in 2 ml of Dubos medium and incubated at 37°C for 3 weeks. MICs were expressed as the lowest concentrations of drug at which no visible growth was observed.

Microbiological assay of DHMP A. The antibacterial activity in serum, urine, feces, and tissue homogenates was determined by the conventional paper disk-agar diffusion method on nutrient agar (Eiken Co., Ltd., Japan) with

Dihydromycoplaneacin A (DHMP A) was primarily discovered as an active metabolite in the urine of mice and dogs administered mycoplaneacin A (MP A), a major component of the novel family of antibiotics produced by a soil isolate classified as Actinoplanes awajinensis subsp. mycoplaneacinus subsp. nov. (10).

MP A possesses a cyclic structure composed of 10 amino acid residues containing three kinds of proline analogues and α-ketobutyric acid, which can be differentiated from these other minor components, MPs B, C, and D, by high-pressure liquid chromatography (6, 7).

DHMP A has α-hydroxybutyric acid as an N-acyl group, which was derived by the ketone group of the α-ketobutyric acid moiety in the parent compound, as shown in Fig. 1.

This family of antibiotics has strong activity against mycobacteria, including strains of Mycobacterium tuberculosis, M. intracellulare, and some other species of atypical mycobacteria (3, 9).

The present paper deals with the antimycobacterial activities of DHMP A in vitro and evaluation of the activity in vivo against experimental tuberculosis in mice, its pharmacokinetics in mice and dogs, and acute toxicities in mice and rats.

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Micrococcus luteus PC11001 as the test organism. A stock solution of DHMP A was prepared at a concentration of 100 μg/ml in 1/15 M phosphate buffer (pH 7.0) containing 10% methanol, from which three drug concentrations, 12.5, 6.25, and 3.13 μg/ml, were prepared with either serum from an experimental animal (serum samples) or 1/15 M phosphate buffer, pH 7.0 (for urine, tissue homogenates, and feces), as a diluent.

The lowest limit of detection of this method was 0.2 μg/ml, and its precision was ±5% for drug concentrations ranging from 1 to 100 μg/ml.

Blood samples were drawn from the carotid artery of mice and from the antecubital vein of dogs. They were allowed to clot, and the sera were used for bioassay. Fecal samples extracted with 50% aqueous methanol were centrifuged. The supernatant was used for bioassay.

Detection of DHMP A or its active metabolite in biological fluids. The ethyl acetate extracts from serum, urine, feces, and tissue homogenates were concentrated and applied on a thin-layer chromatography plate (Art 5715, F254 silica gel plate; Merck Co., Ltd., Federal Republic of Germany) and developed with a solvent system of CHCl₃-methanol (10:1) for bioautography of the active principle. The Rₜ of DHMP A in this system was 0.4.

Distribution of DHMP A and INH in organs of biological fluids in mice. Distribution of DHMP A in mice was determined as follows. Mice received an oral dose of 100 mg of DHMP A per kg and were sacrificed at 1, 2, 4, 6, 8, or 24 h after administration. The lungs, livers, kidneys, and spleens from five mice in each group were pooled after blotting each organ with filter paper to remove residual blood and homogenized in an eightfold volume of 0.9% saline. DHMP A in the supernatant was determined by the microbiological assay described above. Distribution of INH in mice was also determined as follows. Five mice in each group received a subcutaneous dose of 25 mg of INH per kg and were sacrificed at 0.5, 1, 2, or 4 h after administration. Tissue homogenates were prepared by the method described above. INH in biological fluids or tissue homogenates was determined fluorometrically by the method described by Scott and Wright (8).

Combination effects of DHMP A and other antitubercular drugs. Combination effects of DHMP A and other antitubercular drugs, such as RFP, INH, SM, and EB, were examined by the checkerboard titration method. An overnight culture of M. smegmatis ATCC 607 in nutrient broth supplemented with 2% glycerol or a 2-week culture of M. tuberculosis and M. intracellulare on 1% Ogawa medium at 37°C was suspended in fresh Dubos medium, followed by application of 20 μl of inoculum (about 5 × 10⁵ CFU) into 2 ml of Dubos medium containing DHMP A and other antitubercular drugs in a serial twofold dilution ranging from 0 to 100 μg/ml. MICs in every combination were determined after 1 or 2 weeks of incubation at 37°C. The fractional inhibitory concentration (FIC) for each component was calculated based on the formula of Elion et al. (2) as follows: FIC = (MIC of antitubercular drug in combination/MIC of antitubercular drug alone) + (MIC of DHMP A in combination/MIC of DHMP A alone). Then, a minimal FIC of the respective FICs was defined as an FIC index. Synergism was defined by an FIC index of <0.5, and antagonism was defined by an FIC index of >4.

The synergistic effect of DHMP A and INH on A. smegmatis ATCC 607 was analyzed by incubating the culture with either of the drugs for various periods of time (DHMP A, 0, 3, 7, and 20 h; INH, 0, 1, 3, and 7 h) and removing the drug by centrifugation at 5,000 rpm for 15 min, followed by determination of the MIC for the rest of this pretreated culture (5 × 10⁴ CFU/ml) in Dubos medium at 37°C for 7 days. The drug concentration in each pretreatment was equivalent to the MIC against the test organism, 0.2 μg/ml for DHMP A and 12.5 μg/ml for INH.

Experimental animals. Strain ICR/JCL male mice, weighing 20 to 22 g; strain ddY male mice, weighing 22 to 24 g; and strain NMRI male and female mice, weighing 20 to 22 g, were used. Strain Fischer male and female rats weighing 150 to 200 g were used. Male beagle dogs weighing 10 to 15 kg were used. The dogs were fasted for 15 to 18 h prior to administration of the drug.

In vivo activity of DHMP A. (i) Efficacy of DHMP A on experimental tuberculosis in mice. Eighty male ddY mice were inoculated intravenously with 3 × 10⁶ CFU of M. tuberculosis H₃⁷Rv. They were divided into eight groups (10 mice in each). Treatment by oral administration of DHMP A, MP A, and RFP started on day 3 after challenge and continued for 21 days. All mice were sacrificed on day 31 after challenge. The lungs and spleens from five randomly selected mice in each group were separately homogenized in 1/15 M phosphate buffer (pH 7.0) in saline to make a 10% homogenate; 0.1-ml portions were poured onto 1% Ogawa medium, and viable colonies were counted after incubation for 4 weeks at 37°C.

(ii) Efficacy of combination therapy (DHMP A with INH) on a model of lethal infection with M. bovis Ravenel. Four groups of ddY male mice (10 mice in each group) were inoculated intravenously with 0.5 × 10⁷ to 2.5 × 10⁷ CFU of M. bovis Ravenel. Each group of mice received DHMP A or INH alone, a combination of both drugs, or saline as a control. DHMP A was orally administered to the mice at a dose of 4 mg in a 0.2-ml suspension in distilled water per mouse after 5 days of infection, and 4 h later, INH was given subcutaneously at a dose of 0.1 mg in 0.2 ml of saline per mouse, and treatment was continued once a day for 3 weeks. The efficacy was assessed by percent survival and by body weight gain over 40 and 100 days, respectively, after challenge.
(iii) Efficacy of combination therapy (DHMP A with INH) on experimental tuberculosis. The regimen for *M. tuberculosis* R-KM infection was similar to that described above, except that treatment started on day 12 after challenge.

Oral administration. DHMP A was dissolved in cold water at low doses between 12.5 and 200 mg/kg (DHMP A is more soluble in cold water than in hot water) or suspended in 0.5% carboxymethyl cellulose at high doses of more than 200 mg/kg. RFP was dissolved in 25% aqueous propylene glycol. The total volume of the sample solution for each dose was 0.2 ml in mice by oral gavage and 1 ml/kg of body weight in dogs by oral intubation.

Intravenous administration. The solution of DHMP A injected into mice was an emulsion of 1 ml of medium-chain-length triglyceride (Nissin Oil Co., Ltd., Japan) and 100 mg of polyoxymethylene glycol laurate ester (MYL-10) in 9 ml of 0.9% physiological saline. The vehicle for dogs was 15% ethanol in polyoxymethylene glycol 400.

Intrapertioneal administration. Intrapertioneal administration of the drug was carried out after suspension in 0.5% carboxymethyl cellulose.

Urinary and fecal excretion. After administration of the antibiotics, urine and feces samples from three animals confined to a metabolic cage were collected at appropriate intervals and pooled every 24 h.

Pharmacokinetic analysis. Pharmacokinetic analysis of DHMP A concentrations in serum after oral and intravenous administration was done by nonlinear least-squares program NONLIN 74 (5) with the following equations: area under the curve = dose/k_{el}/V and t_{1/2} = 0.693/k_{el}, where k_{el} is the first-order elimination rate constant, V is the apparent volume of distribution, and t_{1/2} is the half-life.

Acute toxicities. Acute toxicities (50% lethal doses [LD_{50}s]) of the drug were determined in mice and rats (20 animals in each group) by oral and intraperitoneal administration. LD_{50}s in each experiment were calculated by the method described by Litchfield and Wilcoxon (4).

RESULTS

In vitro antimycobacterial activities. Antimycobacterial activities of DHMP A in Dubos medium were the best among the antituberculous drugs tested against *M. tuberculosis*, *M. intracellulare*, *M. gordonae*, and *M. nonchromogenicum*. MICs of DHMP A against these organisms ranged between <0.0125 and 25, 0.05 and >25, <0.0125 and 0.4, and 0.4 and 25 μg/ml, respectively. The DHMP A MICs for 90% of isolates of mycobacteria were 0.05 μg/ml for *M. tuberculosis*, 0.78 μg/ml for *M. kansasii*, and 25 μg/ml for *M. intracellulare*. It was also active against *M. bovis* (Table 1).

Combination effects of DHMP A and antituberculous drugs in vitro. Combination effects of DHMP A and other antituberculous drugs, such as RFP, INH, SM, and EB, were examined by the checkerboard technique against *M. smegmatis* ATCC 607 in Dubos medium. A marked synergistic

| Organism (no. of isolates) | Drug | MIC (μg/ml)* | 50% | 90% | For individual isolatesb |
|--------------------------|------|--------------|-----|-----|-------------------------|
| *M. tuberculosis* (38)   | DHMP A | <0.0125–25 | <0.0125 | 0.05 |
|                          | SM    | 0.05–>100   | 0.39 | 50  |
|                          | INH   | 0.2–>100    | 0.39 | 25  |
|                          | RFP   | 0.25–>100   | 0.1  | >100|
|                          | OFLX  | 0.1–25      | 0.39 | 0.78|
| *M. intracellulare* (20) | DHMP A | 0.05–>25 | 1.56 | 25  |
|                          | SM    | 0.78–>100   | 25   | >100|
|                          | INH   | 25–>100     | 50   | >100|
|                          | RFP   | 0.05–>100   | 6.25 | 50  |
|                          | OFLX  | 0.78–25     | 3.13 | 12.5|
| *M. kansasii* (20)       | DHMP A | 0.2–25    | 0.39 | 0.78|
|                          | SM    | 3.13–>100   | 12.5 | 50  |
|                          | INH   | 3.13–>100   | >100 | >100|
|                          | RFP   | 0.39–0.78   | 0.39 | 0.39|
|                          | OFLX  | 0.78–12.5   | 1.56 | 3.13|
| *M. bovis* (5)           | DHMP A | 0.025–0.2 | 0.025 | 0.05, 0.1, 0.2 |
|                          | SM    | <0.05–0.78  | <0.05, 0.2, 0.39 | 0.78 |
|                          | INH   | <0.02–0.39  | <0.02, 0.39 |
|                          | RFP   | <0.025–0.05 | <0.025, 0.05 |
|                          | OFLX  | 0.39–0.78   | 0.39, 0.78 |
| *M. fortuitum* (4)       | DHMP A | >25        | >25  |
|                          | SM    | >100        | >100 |
|                          | INH   | >100        | >100 |
|                          | RFP   | 50          | 50   |
|                          | OFLX  | 0.78–3.13   | 0.78, 1.56, 3.13 |
| *M. gordonae* (3)        | DHMP A | <0.0125–0.39 | <0.0125, 0.025, 0.39 |
|                          | SM    | 0.78–12.5   | 0.78, 1.56, 12.5 |
|                          | INH   | 100–>100    | 100, >100 |
|                          | RFP   | 0.05–1.56   | 0.05, 0.2, 1.56 |
|                          | OFLX  | 0.78–3.13   | 0.78, 3.13 |
| *M. nonchromogenicum* (3)| DHMP A | 0.39–25    | 0.39, 25 |
|                          | SM    | 100–>100    | 100, >100 |
|                          | RFP   | 25–100      | 25, >100 |
|                          | OFLX  | 12.5–>25    | 12.5, >25 |

* 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.

b Inferior number indicates number of isolates for which MIC was as indicated.
ACTIVITY OF DHMP A AGAINST MYCOBACTERIA

Isoniazid (INH), Rifampicin (RFP), Streptomycin (SM), Ethambutol (EB).

**FIG. 2.** Effects of combining DHMP A with several kinds of antituberculous drugs. Test organism, M. smegmatis ATCC 607. Test medium, Dubos medium supplemented with 10% bovine serum albumin.

An antagonistic effect was observed for the combination of DHMP A with INH, and the minimal FIC index between them was 0.250 to 0.375. No antagonistic effect was observed between DHMP A and the other three drugs (Fig. 2). A similar synergistic effect of DHMP A with INH was also apparent on clinically important mycobacteria, such as M. tuberculosis H37Rv IFM 2029 and M. intracellulare IFM 2073 (Fig. 3).

The effect of pretreatment with either of the two drugs on their synergistic effect was examined. The MIC of DHMP A was not affected even when the bacterial cells were pretreated with INH at 12.5 μg/ml (the MIC) for 7 h, but that of INH was lowered from 12.5 to 3.13 μg/ml by pretreatment with 0.2 μg of DHMP A per ml (the MIC) for 3 h or more (Fig. 4).

**FIG. 3.** Synergistic effects of DHMP A with INH on several species of mycobacteria. Test medium, Dubos medium supplemented with 10% bovine serum albumin.

Comparative studies on tissue distributions of DHMP A and INH. Prior to the trial of in vivo combination therapy, tissue distributions of these two drugs were also examined. INH was rapidly absorbed, and in various tissues the peak concentrations were determined after a single oral (DHMP A) or subcutaneous (INH) dose.

**FIG. 4.** Effect of preincubation with DHMP A and INH. Test organism, M. smegmatis ATCC 607. Test medium, Dubos medium supplemented with 10% bovine serum albumin.
concentration was observed within 30 min after administration of 25 mg/kg subcutaneously. It was also rapidly eliminated from all of the tissues and became undetectable at 4 h. Absorption of DHMP A (100 mg/kg) given orally was slower than that of INH, and peak concentrations in liver and other tissues, including serum, were observed at 2 and 4 h after administration, respectively. The tissues with the highest concentrations were liver and kidney, followed by serum, spleen, and lung (Fig. 5). The active principle in all tissues tested was identified to be DHMP A itself as a sole active compound by thin-layer chromatography on silica gel. Concentrations of DHMP A in all of the tissues tested gradually decreased, but the level in serum remained at 0.5 µg/ml even after 8 h (Fig. 5). These results combined with the results of pretreatment studies shown in Fig. 4 suggested that the two drugs might not coexist in tissues if they were administered simultaneously and the in vivo efficacy of combining DHMP A with INH might be greater if DHMP A was given orally 4 h prior to subcutaneous administration of INH.

In vivo activities of DHMP A. The efficacy of DHMP A alone on experimental tuberculosis in mice infected with *M. tuberculosis* H₃₇Rv was determined by comparison of the number of residual viable units of the mycobacteria in lung and spleen with that in animals that received vehicle only, DHMP A, MP A, or RFP. A significant decrease in the number was observed in the spleens of animals that received RFP, DHMP A, and MP A but not in the lungs, and the order of effectiveness in the spleen was 0.5 mg of RFP, 4 mg of DHMP A, 4 mg of MP A, 0.25 mg of RFP, and 2 mg of DHMP A per mouse per day (Fig. 6).

First, combination therapy with DHMP A and INH was carried out in the *M. bovis* Ravenel model of lethal infection in mice. DHMP A was administered orally at a dose of 4 mg per mouse after 5 days of infection, and 4 h later, INH was
given subcutaneously at a dose of 0.1 mg per mouse; treatment continued once a day for 3 weeks. Efficacy was assessed by percent survival for 100 days after challenge. Mean survival days (± standard deviation) of four groups (not treated or treated with DHMP A alone, INH alone, or a combination of DHMP A and INH) were 20.9 ± 1.4, 52.7 ± 7.6, 39.0 ± 6.2, and 89.2 ± 5.4, respectively (Fig. 7).

In the second experiment, combination therapy with DHMP A and INH was examined in experimental tuberculosis in mice infected with M. tuberculosis R-KM. The regimen was similar to that in the first experiment described above, except that treatment started on day 12 after challenge. Treatment continued for 4 weeks, but no detectable reduction in viable units of the pathogen in the lung and spleen was noted compared with those of the nontreated group (Fig. 8).

Pharmacokinetics in mice. (i) Intravenous administration. After administration of 10 mg of DHMP A per kg, its $t_{1/2b}$ in serum was 0.5 h, and urinary and fecal excretions within 48 h were 19.6 and 78.0% of the dose, respectively (Fig. 9).

(ii) Oral administration. A single oral administration (50 mg/kg) of DHMP A revealed a peak level in serum of 10.0 μg/ml at 2 to 4 h, and its urinary recovery was 21.5% (Fig. 10).

Pharmacokinetics in dogs. The $t_{1/2}$ in serum after a single intravenous DHMP A dose of 10 mg/kg was 5.5 h, and its urinary excretion within 48 h was 37% (Fig. 9). Single oral DHMP A doses of 12.5 and 25 mg/kg gave peaks of 5.0 and 9.0 μg/ml, respectively, in serum at 3 h after administration (Fig. 10). Recoveries of the antibiotic in urine within 48 h were 25.2 and 22.4%, respectively.

LD₅₀. Both mice and rats tolerated an oral DHMP A dose of 6,000 mg/kg in 0.5% carboxymethyl cellulose suspension. No toxic signs were observed in the animals, and there was a normal increase in body weight over the 14-day observa-

FIG. 8. In vivo efficacy of DHMP A alone and in combination with INH in experimental tuberculosis in mice. Treatment started on day 12 after challenge and continued for 28 days (day 0 is the first day of treatment). See the legend to Fig. 7 for details.

![Graph showing efficacy of DHMP A and INH in experimental tuberculosis in mice.](image)

FIG. 9. Levels in blood and excretion of DHMP A in mice and dogs after intravenous administration. For dogs, each value represents the mean ± the standard error ($n = 3$).

![Graph showing pharmacokinetics of DHMP A in mice and dogs after intravenous administration.](image)

FIG. 10. Levels in blood and excretion of DHMP A in mice and dogs after oral administration. For dogs, each value represents the mean ± the standard error ($n = 3$). N.T., Not tested.

| Dose (mg/kg) | Mouse | Dog |
|-------------|-------|-----|
| 50          | 21.0  | 22.4| 25.2 |
| 25          | 22.4  | N.T.|
| 12.5        | 25.2  | N.T.|

| Recovery (%) | Urine (0–48 hrs) | Feces |
|--------------|------------------|-------|
| 20           | 78               | 59    |
| 37           | 78               | 59    |
| 0.5          | 5.5              |       |
DISCUSSION

DHMP A was found to be the most active among the drugs tested against several species of clinically important mycobacteria, such as *M. tuberculosis*, *M. intracellulare*, *M. bovis*, *M. gordonae*, *M. kansasi*, and *M. nonchromogenicum*.

A marked synergistic effect was observed in the combination of DHMP A with INH, and the minimal FIC index was 0.250 to 0.375 against *M. tuberculosis* as well as *M. intracellulare*. When bacterial cells were exposed to either DHMP A or INH for various periods of time and then to the other drugs, a synergistic effect was recognized only when the cells were treated with DHMP A 3 h before INH. Comparative studies on tissue distributions of DHMP A and INH in mice revealed that both absorption and excretion of INH were faster than those of DHMP A. Therefore, mice infected with *M. bovis* Ravenel 5 days before were administered DHMP A orally and then INH 4-h-delayed spiking once daily, and therapy was continued for 3 weeks. The mean survival of the group treated with DHMP A and INH was 89 days, compared with 21, 39, and 53 days for the nontreated, INH alone, and DHMP A alone groups, respectively. Thus, the combination effect of these two drugs was clearly demonstrated in a lethal infection of experimental tuberculosis in mice. But it was not so effective in the second experiment of experimental tuberculosis, in which treatment was started on day 12 after challenge. After oral and intravenous administration of DHMP A in mice and dogs, the recovery in urine suggested that absorption of the antibiotic after oral administration might be more than 80% of the given dose and that it was excreted mainly through the biliary tract. Although a higher dose of DHMP A than of RFP was necessary to protect mice from experimental tuberculosis, its lower toxicity than that of RFP reported in the literature (1) and broader antimycobacterial spectrum, as well as its higher level of oral and intravenous administration in dogs, suggested the possible clinical usefulness of DHMP A alone as well as in combination therapy with INH not only in the treatment of tuberculosis but also in the treatment of diseases caused by atypical mycobacteria.

LITERATURE CITED

1. Avery, G. S. (ed.). 1971. Rifampicin: a review. Drugs 1:355–398.
2. Elton, G. B., S. Swiger, and G. H. Witchings. 1954. Antagonists of nucleic acid derivatives. J. Biol. Chem. 208:477–488.
3. Haneishi, T., M. Nakajima, A. Shiraishi, T. Katayama, A. Torikata, Y. Kawahara, K. Kurihara, and M. Arai. 1985. A new antimycobacterial antibiotic, dihydromycoplanecin A. I. In vitro activities and pharmacokinetics in mice and dogs, p. 2544–2545. In J. Ishigami (ed.), Recent advances in chemotherapy (Proceedings of the 14th International Congress of Chemotherapy, Kyoto, 1985). University of Tokyo Press, Tokyo.
4. Litchfield, J. T., Jr., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96:99–113.
5. Metzler, C. M., G. L. Elfring, and A. J. McEween. 1974. A package of computer programs for pharmacokinetic modeling. Biometrics 30:562–576.
6. Nakajima, M., A. Torikata, Y. Ichikawa, T. Katayama, A. Shiraishi, T. Haneishi, and M. Arai. 1983. Mycoplanecins, novel antimycobacterial antibiotics from *Actinoplanes awajinensis* subsp. *mycolanicus* subsp. nov. II. Isolation, physicochemical characterization and biological activities of mycoplanecin A. J. Antibiot. 36:961–966.
7. Nakajima, M., A. Torikata, H. Tamaoki, T. Haneishi, M. Arai, T. Kinoshita, and H. Kuwano. 1983. Mycoplanecins, novel antimycobacterial antibiotics from *Actinoplanes awajinensis* subsp. *mycolanicus* subsp. nov. III. Structural determination of mycoplanecin A. J. Antibiot. 36:967–975.
8. Scott, E. M., and R. C. Wright. 1967. Fluorometric determination of isonicotinic acid hydrazide in serum. J. Lab. Clin. Med. 70:355–360.
9. Takahashi, H., E. Kondo, Y. Koseki, T. Haneishi, M. Arai, and T. Tokunaga. 1985. A new antimycobacterial antibiotic, dihydromycoplanecin A in combination with isoniazid on the experimental mycobacterioses in mice, p. 2546–2547. In J. Ishigami (ed.), Recent advances in chemotherapy (Proceedings of the 14th International Congress of Chemotherapy, Kyoto, 1985). University of Tokyo Press, Tokyo.
10. Torikata, A., R. Enokita, T. Okazaki, M. Nakajima, S. Iwado, T. Haneishi, and M. Arai. 1983. Mycoplanecins, novel antimycobacterial antibiotics from *Actinoplanes awajinensis* subsp. *mycolanicus* subsp. nov. I. Taxonomy of producing organism and fermentation. J. Antibiot. 36:957–960.