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# Moxalactam (6059-S), a Novel 1-Oxa-β-Lactam with an Expanded Antibacterial Spectrum: Laboratory Evaluation

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Moxalactam (6059-S) {7β-[2-carboxy-2-(4-hydroxyphenyl)acetamido]-7α-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]-methyl]-1-oxa-1-dethia-3-cephem-4-carboxylic acid disodium salt} is a new semisynthetic 1-oxa-β-lactam derivative for parenteral use. It was highly active against a broad range of gram-negative microorganisms, including those resistant to other cephalosporins. Moreover, it had widely expanded antibacterial spectra which included *Haemophilus influenzae*, indole-positive *Proteus*, *Enterobacter*, *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Bacteroides fragilis*. When a large number of clinical isolates of the above-named bacilli were tested by the agar dilution method, using an inoculum size of one loopful of $10^6$ or $10^8$ organisms or both per ml, the 70% minimal inhibitory concentrations at the lower inoculum were 0.2, 0.2, 0.4, 0.8, 25, and 0.8 μg/ml, respectively. Its activity appeared to be independent of inoculum size and addition of serum. In these organisms, morphological response of the exposed cells revealed that the bacteriolytic effect of 6059-S was initiated by a concentration equivalent to the minimal inhibitory concentration. 6059-S was markedly bactericidal to both β-lactamase-producing and -nonproducing strains of *Escherichia coli*; this was well reflected by its extraordinary stability to microbial β-lactamase degradation. Administered subcutaneously in mice, 6059-S attained plasma levels and a half-life similar to those of cefazolin and exhibited potent protective efficacy against systemic infections; it also proved to be significantly more effective than either sulbenicillin or piperacillin against *Pseudomonas aeruginosa* and than either cefazolin or cefmetazole against a variety of other gram-negative bacteria.

Moxalactam (6059-S) {7β-[2-carboxy-2-(4-hydroxyphenyl)acetamido]-7α-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]-methyl]-1-oxa-1-dethia-3-cephem-4-carboxylic acid disodium salt} is a new semisynthetic 1-oxa-β-lactam antibiotic developed by Shionogi Research Laboratory (6); its chemical structure is given in Fig. 1.

A number of penicillins and cephalosporins have long maintained clinically important status for treatment of a variety of bacterial infections. Some of the *Enterobacteriaceae*, however, were still out of range of their antibacterial spectra, mainly because of either intrinsic resistance or β-lactamase production in the organisms. Thus, it became urgent to develop antibiotics possessing therapeutic effectiveness toward such resistant bacteria (13). Research performed in this laboratory demonstrated that replacement of a sulfur atom by an oxygen atom at the 1-position of the cephem nucleus enhanced antibacterial activity to a great extent (6). This finding stimulated efforts to modify the 1-oxacephem to confer β-lactamase stability and to extend the gram-negative spectrum; 6059-S was selected as an eligible antibiotic in this respect (T. Yoshida, Philos. Trans. R. Soc. London, in press). The aim of this paper was to evaluate the antibacterial activity of 6059-S both in vitro and in vivo against aerobic and anaerobic microorganisms, using selected penicillins, cephalosporins, and cephamycin derivatives for comparison.

This report was presented in part at the 18th Interscience Conference on Antimicrobial Agents and Chemotherapy (T. Yoshida, M. Narisada, S. Matsuura, W. Nagata, and S. Kuwahara, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 18th, Atlanta, Ga., abstr. no. 151, 1978).

## MATERIALS AND METHODS

**Organisms.** Cultures other than the strains specified below and anaerobic bacteria were laboratory strains maintained in our laboratory and recent clinical isolates obtained from domestic hospitals, which were identified in our laboratory. The cultures of *Escherichia coli* ML1410 RGN238 and *E. coli* ML1410 RGN823 were kindly supplied by T. Sawai (Chiba University, Chiba, Japan) and those of *Enterobacter*

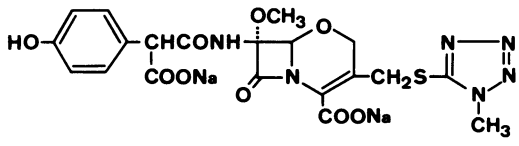

FIG. 1. *Chemical structure of 6059-S.*

*cloacae* 214 and *E. cloacae* 53 were from R. H. Richmond (University of Bristol, Bristol, England).

These cultures were transferred and incubated overnight at 37°C on slants of heart infusion (HI) agar (Eiken), except *Haemophilus influenzae* and streptococci, which were grown on brain HI agar chocolatized after the addition of 5% defibrinized horse blood and HI agar supplemented with 5% defibrinized rabbit blood, respectively.

The obligate anaerobic bacterial strains were kindly supplied by K. Ueno (Gifu University, Gifu, Japan) and maintained by transfer in GAM broth supplemented with 1.5% agar. GAM broth medium, commercially available from Nissui Co., Tokyo, Japan, contained: peptone, 10 g; soybean peptone, 3 g; proteose peptone W, 10 g; digested serum powder, 13.5 g; yeast extract, 5 g; beef extract, 2.2 g; liver extract, 1.2 g; glucose, 3 g; $K_2HPO_4$, 2.5 g; soluble starch, 5 g; NaCl, 3 g; L-cysteine hydrochloride, 0.3 g; sodium thioglycolate, 0.3 g; agar, 1.5 g; and deionized water, 1,000 ml. The final reaction of the medium reached pH 7.3 after autoclaving for 15 min. The inoculated cultures were incubated in an anaerobic chamber equilibrated with oxygen-free vapor ($N_2$, 80%; $H_2$, 10%; $CO_2$, 10%) for 24 h at 37°C and were stored at room temperature for 1 month. Storage for a longer period was possible in a 10% skim milk suspension at −78°C.

**Antibiotics.** 6059-S was prepared by Shionogi Research Laboratory. Cefamandole, cefmetazole (CS-1170), and piperacillin (T-1220) were the products of Eli Lilly & Co., Sankyo Co., and Toyama Chemical Co., respectively. The other antibiotics were purchased from commercial sources.

**Animals.** ICR strain female mice aged 5 weeks and weighing 20 to 24 g were used for both pharmacological studies and protection tests.

**Minimal inhibitory concentrations (MICs).** An antibacterial susceptibility test was performed by the agar dilution method, using a multiloop inoculating device which delivered about 0.5-μl samples of bacterial suspensions to a plating agar containing twofold dilutions of antibiotics. The inocula used were one loopful of a bacterial suspensions of $10^6$ or $10^8$ or both colony-forming units (CFU)/ml prepared from overnight cultures. The tests were usually performed on GAM agar for obligate anaerobes and on HI agar for the other organisms. *Proteus* strains were plated on medium containing 3% agar to minimize swarming. Horse serum (5%) was added to HI agar when strains of streptococci were tested. Fildes enrichment (3%; Difco) was used to supplement HI agar when *Haemophilus* strains were tested.

The MIC was the lowest concentration at which no visible colonies appeared. All cultures were incubated aerobically at 37°C for 18 to 20 h except those of obligate anaerobes, which were incubated in an anaerobic chamber as mentioned above.

**Ninety-nine percent killing concentration.** A twofold dilution series of antibiotics was prepared in HI broth, using a microtiter plate (96 wells; Cooke Engineering Co.). Bacterial suspensions at the exponential growth phase were prepared by subculturing diluted overnight cultures for 2 h. Equal volumes (50 μl) of the suspension were inoculated into each well. Final populations of inoculum were adjusted to approximately $10^6$ CFU/ml. After appropriate intervals (1, 2, 3, 4, 5, and 24 h), samples of the cultures were taken and transplanted to the surface of nutrient agar by a stamping device stainless-steel rods (96 channels) calibrated to deliver 0.5 μl. The subculture plates were incubated further for 24 h.

The 99% killing concentration was defined as the lowest concentration of antibiotic permitting growth on subculture of less than five colonies. Thus, the killing rate at the minimal bactericidal concentration was at least 99%. The original plates of broth culture were incubated for 24 h, and the MICs were determined. All incubations were performed at 37°C.

**Bactericidal action.** The overnight broth cultures were diluted in Trypto-soy broth (Eiken) and incubated at 37°C. When the cultures reached the exponential growth phase, approximately $10^6$ CFU/ml, they were divided into 4.5-ml samples, and doubling dilutions (0.5 ml) of antibiotics were added to give the desired final concentrations. Incubation was continued at 37°C without shaking. Samples of cultures with or without antibiotics were removed at 0, 1, 2, 4, 6, and 24 h and subjected to 10-fold dilutions in Trypto-soy broth. After subculture in Trypto-soy agar overnight at 37°C, viability was determined by counting visual colonies.

**Morphological response.** The overnight culture was diluted 100-fold in Trypto-soy broth and incubated for 1 to 2 h at 37°C. One loopful of the bacterial suspension ($10^7$ CFU/ml), was inoculated on HI agar (Eiken) containing doubling dilutions of antibiotics. otics.

After exposure of the culture for various intervals at 37°C, microscopic observations were made by phase-contrast microscopy, and morphological changes in exposed cells were recorded.

**β-Lactamase preparations.** The medium used for β-lactamase preparations was nutrient phosphate broth (Nissui) adjusted to pH 7.2. Bacteria that produced constitutive β-lactamase were grown without shaking overnight at 37°C and harvested by centrifugation at 3,000 × *g* for 15 min at 4°C. Cells were washed with potassium phosphate buffer (0.1 M, pH 7.0) and suspended in the same buffer for sonication. Overnight cultures of inducible β-lactamase producers were diluted 10-fold with the fresh medium and incubated at 37°C for 2 h with shaking. Penicillin G was added as inducer to give a final concentration of 1 mg/ml for *Pseudomonas aeruginosa* and 100 μg/ml for the other bacteria. Incubations were continued for an additional 2 h. Bacteria were harvested and washed cells were prepared as described above. Washed organisms were sonically disrupted for 2 min in the cold with a Sonicator-150 (Ohtake). Cell debris was removed by centrifuging at 33,000 × *g* for 30 min at 4°C, and supernatant was filtered through a membrane (0.22 μm; Millipore Corp.) and stored at −78°C until used.

Some of the β-lactamase preparations were purified from the cell-free extracts by single-column chromatography. A carboxymethyl-Sephadex column was used for β-lactamases from *E. coli* 6, *E. cloacae* 214, *Proteus vulgaris* 31, and *E. cloacae* 53, and a diethylaminoethyl-Sephadex column was used for those from *E. coli* W3110 RTEM and *Klebsiella* sp. 363. Fractions containing enzyme activity were pooled, dialyzed against the same buffer at 4°C for 16 h, and stored at −78°C until used.

**Assay of β-lactamase. (i) Bioassay.** Substrates were incubated with a twofold dilution of enzyme on a microtiter plate (Cooke Engineering Co.) at 37°C for 2 h, and residual antibiotic activity was determined by the agar well assay using *E. coli* B. The initial substrate concentration was 250 μg/ml. Hydrolytic activity of β-lactamase was determined as previously described (8). The hydrolysis rate of cephaloridine was set at a value of 100.

**(ii) Spectrophotometric assay.** The procedure used for spectrophotometric assay was as detailed by O'Callaghan et al. (8) and Jansson (3) with a slight modification. The ultraviolet absorption spectra of 6059-S and the alkaline-hydrolyzed product in 0.1 M phosphate buffer (pH 7.0) are shown in Fig. 2. At a concentration of $10^{-4}$ M (56.4 μg/ml), the optical density at 270 nm decreased from 1.23 to 0.26, a change of 0.97 ($\Delta\epsilon$ = 9,700). When 6059-S was treated with an aqueous solution of sodium hydroxide (four equivalents) at 25°C, the decrease in absorbance at 270 nm followed first-order kinetics and the hydrolysis was completed in 30 min. High-pressure liquid chromatography of the resulting solution demonstrated that *N*-methyltetrazol-5-yl-thiol and a highly hydrophilic product were formed. The infrared spectrum of the products obtained by lyophilization did not show the β-lactam carbonyl bond. The nuclear magnetic resonance spectrum of the products formed in $D_2O$-NaOH supported the structure of a highly hydrophilic product assigned as a cephalosporanic acid type (personal communications). Consequently, the decrease with time in absorbance at 270 nm of 6059-S treated with the intact enzyme, as corrected for that with the denatured enzyme, correlated with the rate of β-lactam hydrolysis of 6059-S.

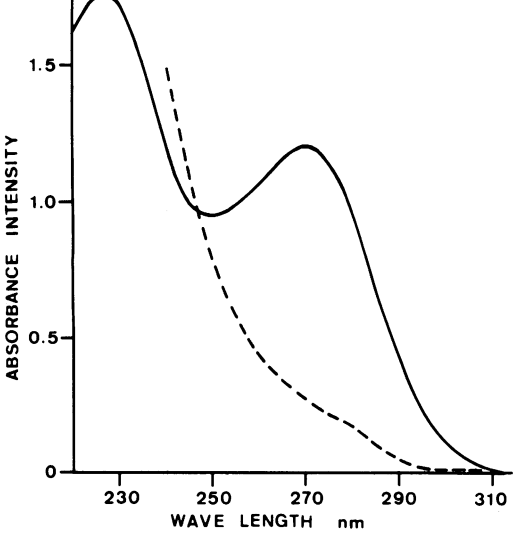


FIG. 2. *Ultraviolet absorption spectra of 6059-S solution ($10^{-4}$ M) in 0.1 M phosphate buffer (pH 7.0), before (—) and after (---) hydrolysis, by NaOH (4 × $10^{-4}$ M) at 25°C for 30 min.*

The partially purified enzyme preparations were diluted with potassium phosphate buffer (0.1 M, pH 7.0) containing 0.001% gelatin. Enzyme solution (0.2-ml aliquot) was added to 2 ml of the buffer containing substrate at 30°C, and the reaction mixture was transferred to a cuvette in a spectrophotometer (Hitachi model 200-20) with circulation of water at 30°C. The decrease in optical density was recorded with a reference of substrate solution lacking enzyme. The kinetic parameters $V_{max}$ and $K_m$ were calculated from double-reciprocal plots of $V_{max}$ against substrate concentrations, and $K_i$ was determined from plots of apparent $K_m$ against concentrations of an inhibitor.

**Microbiological assay of antibiotics.** Concentrations of antibiotics were determined by the band culture assay method, using as test organisms *E. coli* 7437 for 6059-S and *Bacillus subtilis* ATCC 6633 for cefazolin. The band culture method was first described by Okubo and Fujimoto (10) and modified in this laboratory. Agar bands containing test organisms ($10^5$ CFU/ml) were each prepared by pouring 3 ml of melted agar into 20 gutters (5 by 5 by 150 mm) arranged in a glass plate (7 by 150 by 200 mm). Two wells were bored in each solidified agar band by removing agar plugs of 5-mm length. A 50-μl aliquot of the sample or the standard solutions was added to the well. Two wells in the same agar band were used for each solution. The standard solutions, diluted at 2-log intervals, were used in six agar bands, and the rest of the bands were used for the sample solutions. The agar plates were incubated overnight at 37°C, covered by a glass plate. The lengths of inhibitory zones appearing on the agar bands were measured to the nearest 0.1 mm. The amounts of antibiotics in the test samples were calculated form the regression curves (length versus logarithm of concentration) obtained with the standard solutions.

**Determination of plasma levels.** Mice were given 0.2 ml of antibiotic solution in physiological saline at appropriate concentrations by subcutaneous injection. Twenty mice were used for each dose, and four mice were sacrificed at each interval. Blood samples were taken by cardiac puncture by using a heparinized syringe at 0.25, 0.5, 1, 1.5, and 2 h after injection. Blood plasmas were separated by centrifugation, and the antibiotic activity was determined by the band culture method.

**Urinary and biliary recovery.** A principle of the procedure was described previously (18) and modified in this laboratory. Mice were fed a liquid diet (containing 4% glucose, 0.5% Casamino Acids, and trace vitamin mixture) for 20 h before antibiotic administration. Ten animals were used for each dosage. After administration, each mouse was placed on a stainless-steel screen set in a 200-ml flask containing 20 ml of phosphate buffer (0.1 M, pH 7.0). At 2 h after admin-

istration, the mouse was clamped at the orifice of the urethra and sacrificed. An incision was made in the urinary bladder, and the bladder was washed with the buffer containing urine excreted over 2 h. The resulting extracts were used for the determination of urinary recovery. The liver and gall bladder were removed quickly and put on a piece of Dry Ice. The frozen gall bladder was removed from the liver and placed in 10 ml of ice-cold buffer solution together with whole intestine (from the duodenum to the end of the ileum), which was cut longitudinally. The resulting mixtures were centrifuged at 9,000 × *g* for 10 min after intermittent shaking in the cold. The supernatant was used for bioassay to determine biliary recovery.

**Protection test.** Acute lethal infections in mice were produced by intraperitoneal challenge of the organism suspended in 5% hog gastric mucin (American Laboratories, Inc.). The test organisms were grown on HI agar overnight at 37°C. The bacterial cells on the surface of the slant were suspended in HI broth (Eiken) supplemented with 10% bovine serum to a density of approximately $10^9$ CFU/ml. For *P. aeruginosa*, 10% skim milk was used as the supplement. These suspensions were stored at −78°C until used. After determination of the 50% lethal dose (the number of organisms required to kill 50% of the mice within 3 days), 20 to 1,000 50% lethal doses were used for the challenge. The challenged mice were treated by subcutaneous administration at 1 and 5 h postinfection. Eight mice were used for each dose level. The survival rate of the treated mice was determined at 7 days postinfection, and the single dosage required to protect 50% of the treated mice (50% effective dose) was calculated by probit analysis.

## RESULTS

**Antibacterial activity in vitro.** The MICs of 6059-S against 1,082 strains of clinical isolates of various bacterial species were measured by the agar dilution method, using two different inoculum sizes. When 94 strains of *Staphylococcus aureus* were tested, the MICs were distributed in concentrations of 6.25 and 12.5 μg/ml. There was no difference in susceptibility between penicillin-susceptible strains and penicillinase-producing strains. Both *Streptococcus pyogenes* (18 strains) and *Streptococcus pneumoniae* (46 strains) were more susceptible to 6059-S than *S. aureus*. More than 90% of the strains of streptococci tested were inhibited by 1.56 and 3.13 μg/ml, respectively.

The distributions of gram-negative bacteria susceptible to 6059-S were compared with those of arbitrarily selected β-lactam antibiotics such as cefazolin, cefmetazole, cefamandole, cefoxitin, ampicillin, sulbenicillin, and piperacillin (see Table 1).

Most strains of *H. influenzae*, *E. coli*, *Klebsiella* sp. and *Proteus* sp., either indole positive or negative, *Enterobacter* sp., and *Citrobacter freundii* were inhibited at concentrations of 0.39 μg or less of 6059-S per ml. However, a few strains were susceptible at less than this concentration to the cephalosporins used for comparison, which mostly required at least 10-fold-higher concentrations than 6059-S for the same effect.

Of the 23 strains of *H. influenzae* tested, 6 were resistant to ampicillin but showed rather low MICs of 6059-S; hence, it was evident that ampicillin resistance did not affect susceptibility to 6059-S. The 210 strains of indole-positive *Proteus* consisted of the following four species: 63 *P. vulgaris*, 75 *P. morganii*, 32 *P. rettgeri*, and 40 *P. inconstans*. The activities of 6059-S were almost equal in the four groups, with MICs ranging from 0.05 to 0.39 μg/ml. Cefmetazole was more active than cefazolin, but the distribution of its MICs fluctuated over a wide range of concentrations.

Of the 85 strains of *Enterobacter* sp., which included 27 strains of *E. aerogenes* and 58 strains of *E. cloacae*, 14 strains tended to be less susceptible to 6059-S. These strains were highly resistant to cefamandole.

*Serratia marcescens* was very susceptible to 6059-S; MICs were below 0.78 μg/ml for 76% of the strains tested, whereas the lowest MIC of cefmetazole was 6.25 μg/ml.

Against *P. aeruginosa*, 6059-S was slightly more active than sulbenicillin but four times less active than piperacillin. The median MICs were 25, 50, and 6.25 μg/ml, respectively. Of the 112 strains tested, 11 strains were not inhibited by 400 μg of either sulbenicillin or piperacillin per ml with the heavy inoculum, whereas they were found to be susceptible to 200 μg or less of 6059-S per ml.

6059-S was also active against obligate anaerobic bacteria (Table 2). Most of strains were inhibited by concentrations ranging from 0.2 to 6.25 μg/ml. Some of the gram-negative anaerobes, such as *Bacteroides fragilis* subsp. *fragilis*, were more susceptible to 6059-S than to cephamycin-type compounds. Of the 56 strains of clinical isolates tested, 39 were inhibited by 6059-S at a concentration below 0.78 μg/ml and were eight times more susceptible than either cefoxitin or cefmetazole, although the remaining strains showed MICs similar for these three antibiotics (Table 1).

**Effect of inoculum size.** When the inoculum size was increased from $10^6$ to $10^8$ CFU/ml, the activity declined only slightly against all the gram-negative bacteria tested, and no more than a twofold increase in MIC was noted in most cases (Table 1). With gram-positive strains, however, the susceptibility against 6059-S varied little with increase in inoculum size.

**Influence of medium pH and the addition of serum.** The effects of pH and the addition of human serum on the agar dilution MICs of 6059-

TABLE 1. *Distribution of susceptibilities of clinical isolates of gram-negative bacteria to 6059-S and selected β-lactam antibiotics*

| Organism (no. of strains) | Inoculum[a] | Antibiotic | MIC (μg/ml)[b] to inhibit the tested strain by: | | | |
|---|---|---|---|---|---|---|
| | | | 50% | 70% | 90% | 100% |
| *E. coli* (114) | Low | 6059-S | 0.2 | 0.2 | 0.39 | 50 |
| | High | 6059-S | 0.2 | 0.39 | 0.78 | 100 |
| | Low | Cefazolin | 3.13 | 6.25 | 12.5 | >100 |
| | Low | Cefmetazole | 1.56 | 1.56 | 3.13 | 100 |
| *Klebsiella* sp. (132) | Low | 6059-S | 0.2 | 0.2 | 0.39 | 3.13 |
| | High | 6059-S | 0.39 | 0.39 | 0.78 | 3.13 |
| | Low | Cefazolin | 3.13 | 6.25 | 25 | >100 |
| | Low | Cefmetazole | 1.56 | 1.56 | 3.13 | 25 |
| *P. mirabilis* (63) | Low | 6059-S | 0.2 | 0.2 | 0.39 | >100 |
| | High | 6059-S | 0.39 | 0.39 | 0.39 | >100 |
| | Low | Cefazolin | 12.5 | 12.5 | 12.5 | 50 |
| | Low | Cefmetazole | 3.13 | 6.25 | 6.25 | >100 |
| Indole-positive *Proteus* sp. (210) | Low | 6059-S | 0.2 | 0.2 | 0.39 | 3.13 |
| | High | 6059-S | 0.2 | 0.39 | 0.39 | 3.13 |
| | Low | Cefazolin | >100 | >100 | >100 | >100 |
| | Low | Cefmetazole | 6.25 | 6.25 | 12.5 | >100 |
| *Enterobacter* sp. (85) | Low | 6059-S | 0.2 | 0.39 | 12.5 | >100 |
| | High | 6059-S | 0.4 | 0.78 | 25 | >100 |
| | Low | Cefazolin | >100 | >100 | >100 | >100 |
| | Low | Cefamandole | 3.13 | 6.25 | >100 | >100 |
| *S. marcescens* (82) | Low | 6059-S | 0.39 | 0.78 | 25 | 100 |
| | High | 6059-S | 0.78 | 1.56 | 25 | >100 |
| | Low | Cefazolin | >100 | >100 | >100 | >100 |
| | Low | Cefmetazole | 12.5 | 25 | >100 | >100 |
| *C. freundii* (47) | Low | 6059-S | 0.10 | 0.20 | 1.56 | 6.25 |
| | High | 6059-S | 0.20 | 0.39 | 6.25 | 6.25 |
| | Low | Cefazolin | 100 | 100 | >100 | >100 |
| | Low | Cefmetazole | 25 | 50 | 100 | >100 |
| *P. aeruginosa* (112) | Low | 6059-S | 25 | 25 | 50 | 400 |
| | High | 6059-S | 50 | 100 | 200 | 400 |
| | Low | Sulbenicillin | 50 | 50 | 200 | >400 |
| | Low | Piperacillin | 6.25 | 6.25 | 100 | >400 |
| *H. influenzae* (23) | Low | 6059-S | 0.10 | 0.20 | 0.78 | 0.78 |
| | Low | Cefazolin | 25 | 25 | 50 | 50 |
| | Low | Ampicillin | 0.78 | 0.78 | 3.13 | 6.25 |
| *B. fragilis* subsp. *fragilis* (56) | Low | 6059-S | 0.78 | 0.78 | 6.25 | 12.5 |
| | High | 6059-S | 1.56 | 3.13 | 6.25 | 12.5 |
| | Low | Cefoxitin | 6.25 | 12.5 | 12.5 | 25 |
| | Low | Cefmetazole | 6.25 | 6.25 | 12.5 | 50 |

[a] Low and high inocula indicate one loopful of $10^6$ and $10^8$ CFU/ml, respectively.
[b] MIC was determined by the agar dilution method.

S were determined with seven organisms, including *S. aureus, E. coli, Klebsiella pneumoniae, P. vulgaris, E. cloacae, S. marcescens,* and *P. aeruginosa.* The activity of 6059-S was not influenced by a change of pH of 6 to 8 or by addition of human serum up to 50%.

**Bactericidal activity.** Time course experiments on the 99% killing concentration were studied with 29 clinical isolates of *E. coli* which consisted of 15 strains susceptible to ampicillin and 14 strains resistant to ampicillin (MIC, >100 μg/ml). The 99% killing concentrations of 6059-S were lowest after a 2- to 3-h incubation period and reached 0.1 to 0.39 μg/ml, which remained virtually unchanged for a 24-h period (Fig. 3). 6059-S was extremely bactericidal to both ampicillin-susceptible and -resistant *E. coli.* Moreover, no significant differences were observed between the MICs and the short-term killing concentration in either type of strains. In contrast, cefazolin showed striking differences in 99% killing concentrations with ampicillin-sus-

TABLE 2. *Antibacterial spectra of 6059-S, cefoxitin, and cefmetazole against aerobic bacteria tested by the agar dilution method*

| Organism | MIC[a] (μg/ml) | | |
|---|---|---|---|
| | 6059-S | Cefoxitin | Cefmetazole |
| *Bacteroides fragilis* subsp. *fragilis* | | | |
| W-1 | 0.78 | 6.25 | 6.25 |
| 2508 | 0.78 | 25 | 25 |
| 2552 | 0.78 | 12.5 | 6.25 |
| *B. fragilis* subsp. *thetaiotaomicron* IMA9 | 0.78 | 1.56 | 3.13 |
| *B. fragilis* subsp. *distasonis* Ju-11-1 | 1.56 | 12.5 | 25 |
| *B. fragilis* subsp. *vulgarus* HS-68 | 1.56 | 6.25 | 12.5 |
| *B. fragilis* subsp. *ovatus* 2505 | 6.25 | 25 | 25 |
| *Fusobacterium varium* B1083 | 6.25 | 3.13 | 3.13 |
| *F. necrophorum* 8C | 0.39 | 0.1 | 0.1 |
| *F. nucleatum* FN-1 | 0.78 | 0.2 | 0.1 |
| *Veillonella parvula* 10790 | 1.56 | 0.2 | 0.1 |
| *Peptococcus anaerobius* ATCC 14956 | 1.56 | 0.39 | 0.39 |
| *Peptostreptococcus anaerobius* B-38 | 6.25 | 0.78 | 1.56 |
| *Eubacterium limosum* ATCC 8486 | 6.25 | 0.78 | 0.39 |
| *Propionibacterium acnes* P-15 | 0.39 | 0.1 | 0.05 |
| *Clostridium perfringens* NH-6 | 0.1 | 0.78 | 0.1 |

[a] Test was performed with an inoculum of one loopful of $10^6$ CFU/ml and GAM agar, with ingredients as given in the text.

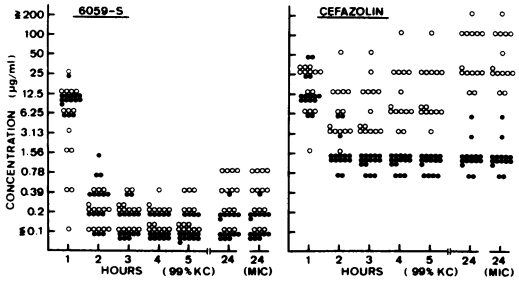


FIG. 3. *Time course of 99% killing concentration (99%KC) of 6059-S (left) and cefazolin (right) against 29 strains of E. coli clinically isolated. The test was performed in HI broth with a mean inoculum of 1.2 × $10^6$ CFU/ml. Each point represents the minimum concentration required to kill one strain, using a killing rate of at least 99%. Symbols: ●, ampicillin-susceptible strains; ○, ampicillin-resistant strains.*

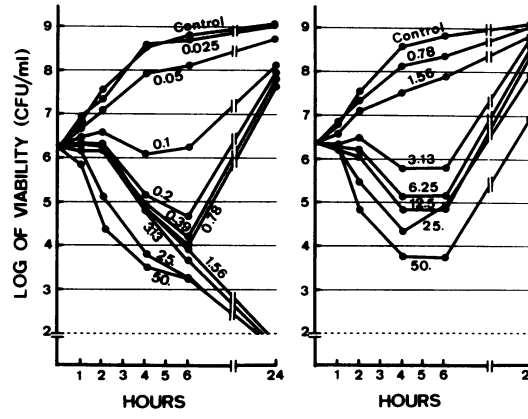


FIG. 4. *Bactericidal activity of 6059-S (left) and cefmetazole (right) against S. marcescens ATCC 13880 tested in HI broth. Numbers indicate concentrations in micrograms per milliliter.*

ceptible and -resistant *E. coli*, and the higher concentrations were always needed to kill ampicillin-resistant strains (Fig. 3, right).

The bactericidal effects against *S. marcescens* ATCC 13880 and *P. aeruginosa* PS-24 were examined by determining the viability of bacterial cells exposed to 6059-S, and the activity of 6059-S was compared with those of cefmetazole and sulbenicillin, respectively. 6059-S reduced the growth rate of *S. marcescens* at 0.1 μg/ml and decreased viable cells at a concentration of 0.2 μg/ml or more after 4 h of incubation (Fig. 4). At concentrations up to 0.78 μg/ml, a decrease in viability was followed by regrowth, but a concentration of 1.56 μg/ml, four times the MIC, caused a marked increase in killing after 24 h of incubation. In the case of cefmetazole, a concentration of at least 6.25 μg/ml, one-half the MIC, was necessary for decreasing viability after 4 h of incubation. At 24 h of incubation, however, extensive growth of this organism occurred at all concentrations up to 50 μg/ml. Thus, the killing effects against *S. marcescens* were very different for both antibiotics; 6059-S exerted a greater killing effect at low concentrations.

Against *P. aeruginosa* PS-24, bactericidal activities of 6059-S and sulbenicillin were found to

be almost equal (Fig. 5). Both antibiotics had the same agar dilution MIC, 100 μg/ml, with the heavy inoculum. Although regrowth was observed at concentrations up to 50 μg/ml, the killing rate increased progressively at concentrations greater than the MIC.

**Morphological response of organisms.** Microscopic observation of various gram-negative organisms exposed to twofold dilutions of 6059-S revealed that morphological changes occurred sequentially from filaments to bulge forms to lysis as the concentrations were increased. Minimum concentrations of 6059-S forming filaments and producing lysis were compared with those of selected antibiotics in Table 3. After 2 h of exposure of *E. coli* NIHJ JC-2 to 6059-S, filamentous forms were observed at 0.025 μg/ml and lysis of cells was seen at 0.1 μg/ml, a value identical to the MIC; these concentrations were eight times lower than those of cefazolin.

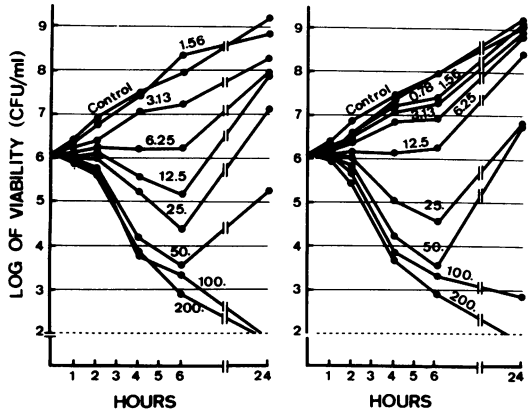


FIG. 5. *Bactericidal activity of 6059-S (left) and sulbenicillin (right) against P. aeruginosa Ps-24 tested in HI broth. Numbers indicate concentrations in micrograms per milliliter.*

In *K. pneumoniae* SRL-1, 6059-S and cefazolin produced lysis at the same concentration (3.13 μg/ml) after 2 h of exposure. However, 6059-S enhanced the lytic effect after a prolonged incubation and induced lysis of this organism at 0.1 μg/ml, which was also equal to the MIC, whereas a lytic effect by cefazolin was unchanged for 5 h of incubation. *P. vulgaris* CN-329 showed a response similar to that of *K. pneumoniae* exposed to 6059-S, and a lytic effect was completed at 5 h of exposure.

Against *P. aeruginosa* PS-24, 6059-S produced filamentous forms at 0.39 μg/ml, but carbenicillin required eight times that concentration to induce a similar response. Both antibiotics induced lysis at a similar concentration, although the MIC of carbenicillin was found to be four times that of 6059-S.

**β-Lactamase stability.** Gram-negative bacteria that were highly resistant to cephalosporins or penicillins, or both, were selected as sources of β-lactamases. Among β-lactamases produced by these organisms were included species-specific (Richmond types I and IV) and R-plasmid-mediated β-lactamases containing both TEM (Richmond type III) and OXA (Richmond type V) types (14, 15).

The results shown in Table 4 clearly demonstrated that 6059-S was extremely stable to all β-lactamases tested, and no hydrolysis was observed by the bioassay method. On the other hand, cefoxitin was hydrolyzed to some extent by β-lactamases from *E. coli* 6 and *E. cloacae* 92.

Six β-lactamases were partially purified, and kinetic parameters, $V_{max}$, $K_m$, and $K_i$, were measured by using the spectrophotometric assay at 30°C. No hydrolysis of 6059-S was observed with

TABLE 3. *Morphological changes of gram-negative organisms exposed to 6059-S and selected antibiotics for 2 and 5 h*[a]

| Organism | Antibiotic | Minimum concn (μg/ml) | | | MIC (μg/ml; 20 h) |
|---|---|---|---|---|---|
| | | Filament (2 h) | Lysis | | |
| | | | 2 h | 5 h | |
| *E. coli* NIHJ JC-2 | 6059-S | 0.025 | 0.1 | 0.1 | 0.1 |
| | Cefazolin | 0.2 | 0.8 | 0.8 | 1.6 |
| *K. pneumoniae* SRL-1 | 6059-S | 0.05 | 3.1 | 0.1 | 0.2 |
| | Cefazolin | 0.8 | 3.1 | 1.6 | 3.1 |
| *P. vulgaris* CN-329 | 6059-S | 0.05 | 1.6 | 0.2 | 0.2 |
| | Cefmetazole | 0.8 | 1.6 | 1.6 | 3.1 |
| *P. aeruginosa* PS-24 | 6059-S | 0.4 | 100 | 12.5 | 50 |
| | Carbenicillin | 3.1 | 100 | 25 | 200 |

[a] Test was performed on HI agar inoculated with one loopful of $10^7$ CFU/ml. Emergence of morphological response was observed under a phase-contrast microscope after incubation at 37°C for 2 h and 5 h. At 20 h, the MIC was determined.

TABLE 4. *β-Lactamase stability and agar dilution MICs*

| Organism | Class of β-lactamase[a] | Relative hydrolysis rate[b] | | | | MIC (μg/ml)[c] | | |
|---|---|---|---|---|---|---|---|---|
| | | 6059-S | CFX | CEZ | ABPC | 6059-S | CFX | CEZ |
| *E. coli* 6 | I | <1 | 6 | 81 | 5 | 0.78 | 50 | 25 |
| *P. vulgaris* 31 | I | <1 | <2 | 120 | 53 | 0.20 | 3.13 | 800 |
| *P. morganii* 8 | I | <1 | <4 | 170 | 10 | 0.20 | 12.5 | 400 |
| *P. rettgeri* 5 | I | <1 | <1 | 81 | 0.2 | 0.10 | 100 | 1,600 |
| *P. inconstans* 31 | I | <1 | <3 | 100 | <1 | 0.10 | 3.13 | 25 |
| *E. aerogenes* 10 | I | <1 | <2 | 62 | 1 | 6.25 | 200 | 1,600 |
| *E. cloacae* 92 | I | <1 | 2 | 62 | 3 | 12.5 | 800 | 1,600 |
| *E. cloacae* 214 | I | <1 | <1 | 110 | <3 | 25 | 800 | >3,200 |
| *S. marcescens* HIG | I | <1 | <4 | 210 | <2 | 0.20 | 6.25 | 800 |
| *C. freundii* 27 | I | <1 | <3 | 120 | <3 | 0.20 | 400 | 400 |
| *P. aeruginosa* 30 | I | <1 | <1 | 120 | 8 | 50 | 3,200 | >3,200 |
| *B. fragilis* R-1-23 | I | <1 | <1 | 45 | 6 | 0.78 | 6.25 | 25 |
| *E. coli* W3110 RTEM | III | <1 | <3 | 66 | 200 | 0.78 | 6.25 | 6.25 |
| *E. coli* ML1410 RGN823 | III | <1 | <1 | 38 | 150 | 0.20 | 3.13 | 25 |
| *Klebsiella* sp. 363 | IV | <1 | <1 | 120 | 370 | 0.10 | 3.13 | 1,600 |
| *E. cloacae* 53 | IV | <1 | <3 | 81 | 250 | 0.39 | 12.5 | 1,600 |
| *E. coli* ML1410 RGN238 | V | <1 | | 53 | 700 | 0.20 | 6.25 | 3.13 |

[a] Class of β-lactamase is according to Richmond's classification (15).

[b] Relative hydrolysis rates were determined by microbiological assay. The hydrolysis rate of cephaloridine was set at a value of 100. CEZ, Cefazolin; CFX, cefoxitin; ABPC, ampicillin.

[c] MICs were determined by the agar dilution method. Inoculum size was one loopful of $10^6$ CFU/ml.

TABLE 5. *Kinetic parameters of various types of β-lactamase*[a]

| Source of β-lactamase | Class | Relative $V_{max}$[b] | | | $K_i$ (μM) of 6059-S | $K_m$ (μM) | | |
|---|---|---|---|---|---|---|---|---|
| | | 6059-S | CEZ | ABPC | | CER | CEZ | ABPC |
| *E. coli* 6 | Ib | <0.04 | 130 | 1.7 | 0.36 | 750 | 1,400 | 8.2 |
| *E. cloacae* 214 | Ia | <0.04 | 170 | <0.1 | 0.062 | 670 | 2,100 | —[c] |
| *P. vulgaris* 31 | Ic | <0.05 | 440 | 11 | 39 | 170 | 240 | 7.1 |
| *E. coli* W3110 RTEM | IIIa | <0.05 | 14 | 160 | 810 | 790 | 500 | 72 |
| *Klebsiella* sp. 363 | IV | <0.03 | 60 | 180 | >2,000 | 180 | 29 | 130 |
| *E. cloacae* 53 | IVa | <0.04 | 12 | 94 | 150 | 190 | 10 | 48 |

[a] Determined by the spectrophotometric assay at 30°C. Enzymes were partially purified. CEZ, Cefazolin; ABPC, ampicillin; CER, cephaloridine.

[b] $V_{max}$ is relative to an arbitrary value of 100 for cephaloridine.

[c] —, Not determined.

this method (Table 5). 6059-S strongly inhibited the enzymes from *E. coli* 6 and *E. cloacae* 214 and showed low $K_i$'s, indicating that 6059-S bound to these enzymes with high affinity but was not hydrolyzed by them.

The MICs of 6059-S were compared with those of cefoxitin and cefazolin (Table 4). Several organisms were resistant to cefoxitin, and almost all of them were highly resistant to cefazolin. However, all the organisms tested were extremely susceptible to 6059-S, although to a lesser extent against *Enterobacter* sp.

**Antibacterial activity in vivo.** The therapeutic efficacy of 6059-S in experimental infection of mice was compared with those of cefazolin and cefmetazole, and the results are shown in Table 6. 6059-S showed excellent in vivo activity against gram-negative organisms, which included highly resistant organisms such as indole-positive *Proteus*, *Enterobacter* sp., and *S. marcescens*. Doses of <1.4 mg of 6059-S per kg were enough to protect mice against these organisms. There was virtually no difference in effectiveness between organisms that were cefazolin susceptible and those that were cefazolin resistant. In the case of cefazolin, however, 30- to 250-fold-higher doses were required for the same effect against cefazolin-susceptible strains, and 120- to 5,000-fold-higher doses were required against cefazolin-resistant strains. The protective effects of cefmetazole were more pronounced than those of cefazolin. The 50% effective doses of cefmetazole, however, were as high as 1.2 to 135 mg/kg, 8- to 1,300-fold higher than those of 6059-S.

A notable exception was that 6059-S was less effective than cefazolin in protecting the mice challenged with *S. aureus*.

Against pseudomonal infection, 6059-S was clearly superior to sulbenicillin and piperacillin (Table 7), although the activity of these antibiotics was rather low in comparison with the results obtained in the above-mentioned organisms. It was noted that 6059-S showed two to three times the therapeutic activity of piperacillin, although the majority of pseudomonal strains were four times more susceptible in vitro to piperacillin than to 6059-S.

**Pharmacological properties.** The plasma levels obtained after subcutaneous injection of 6059-S in mice are shown in Table 8. When 6059-S was administered at 2.5 to 40 mg/kg, the plasma levels were proportional to the doses and reached a peak at 15 min after injection, declining thereafter at a similar rate with these doses.

At a dose of 20 mg of 6059-S per kg, the plasma levels were almost identical to those of cefazolin. The half-life of 6059-S was 25.0 min, equivalent to that of cefazolin (23.1 min). The extent to which 6059-S and cefazolin were excreted into the urine and bile of mice is shown in Table 9. Similar total recovery with both antibiotics was observed during the first 2-h period; however, a significant amount of 6059-S was excreted into the bile (11.4 ± 4.2%), i.e., approximately four times that of cefazolin. After

TABLE 6. *Protective effect on experimental infections in mice*

| Organism | Challenge (CFU/mouse) | Challenge $LD_{50}$[a] | $ED_{50}$ (mg/kg per injection)[b] | | | MIC (μg/ml)[c] | | |
|---|---|---|---|---|---|---|---|---|
| | | | 6059-S | CEZ | CMZ | 6059-S | CEZ | CMZ |
| CEZ susceptible | | | | | | | | |
| *S. aureus* Smith | $9 \times 10^5$ | 80 | 10.6 | 0.12 | 2.9 | 6.25 | 0.2 | 0.78 |
| *S. aureus* C-14 | $2 \times 10^6$ | 930 | 10.2 | 0.58 | 2.2 | 6.25 | 0.2 | 1.56 |
| *E. coli* EC-14 | $3 \times 10^4$ | 20 | 0.05 | 2.4 | 1.2 | 0.1 | 0.78 | 0.78 |
| *E. coli* 377 | $4 \times 10^6$ | 90 | 0.13 | 14.8 | 8.4 | 0.1 | 6.25 | 3.13 |
| *K. pneumoniae* SRL-1 | $3 \times 10^2$ | 30 | 0.84 | 29.8 | 23.1 | 0.1 | 0.78 | 0.78 |
| *P. mirabilis* PR-4 | $4 \times 10^6$ | 120 | 0.25 | 8.0 | 11.1 | 0.1 | 3.13 | 1.56 |
| *H. influenzae* 88562 | $1 \times 10^6$ | 110 | 0.03 | 8.3 | 6.5 | 0.02 | 1.56 | 0.78 |
| *E. cloacae* CL-113 | $1 \times 10^6$ | 120 | 0.09 | 12.5 | 5.5 | 0.1 | 6.25 | 6.25 |
| CEF resistant | | | | | | | | |
| *E. coli* 73 | $4 \times 10^6$ | 90 | 0.25 | 38.3 | 5.6 | 0.2 | 25 | 1.56 |
| *K. pneumoniae* 363 | $2 \times 10^7$ | 70 | 0.37 | >500 | 7.7 | 0.05 | >100 | 0.39 |
| *K. pneumoniae* KL-184 | $3 \times 10^6$ | 120 | 0.27 | >500 | 5.3 | 0.2 | >100 | 0.78 |
| *P. vulgaris* CN-329 | $7 \times 10^5$ | 110 | 0.03 | 56.4 | 16.3 | 0.2 | >100 | 1.56 |
| *P. morganii* MOR-50 | $3 \times 10^6$ | 110 | 1.4 | 178 | 10.6 | 0.2 | >100 | 3.13 |
| *E. aerogenes* TB-510 | $1 \times 10^5$ | 150 | 0.14 | 77.1 | 135 | 0.39 | 50 | >100 |
| *E. cloacae* CL-25 | $6 \times 10^5$ | 100 | 0.10 | >500 | 133 | 0.39 | >100 | >100 |
| *E. cloacae* CL-47 | $4 \times 10^6$ | 130 | 0.17 | 363 | 90.7 | 0.1 | >100 | >100 |
| *S. marcescens* ATCC 13880 | $3 \times 10^4$ | 90 | 0.99 | >500 | 60.1 | 0.39 | >100 | 12.5 |

[a] $LD_{50}$, 50% lethal dose.

[b] Antibiotics were administered subcutaneously 1 and 5 h after intraperitoneal infection. $ED_{50}$, 50% effective dose; CEZ, cefazolin; CMZ, cefmetazole (CS-1170).

[c] MICs were determined by the agar dilution test, using an inoculum of one loopful of $10^6$ CFU/ml.

TABLE 7. *Protective effect on experimental infections of P. aeruginosa in mice*

| Strain | Challenge (CFU/mouse) | $LD_{50}$[a] | $ED_{50}$ (mg/kg per injection)[b] | | | MIC (μg/ml)[c] | | |
|---|---|---|---|---|---|---|---|---|
| | | | 6059-S | SBPC | PIPC | 6059-S | SBPC | PIPC |
| PS-24 | $3 \times 10^3$ | 200 | 11.6 | 35.7 | 28.3 | 25 | 50 | 6.25 |
| E-2 | $7 \times 10^3$ | 100 | 14.7 | 99.2 | 37.2 | 50 | 100 | 12.5 |
| NC-5 | $3 \times 10^3$ | 100 | 27.1 | 239 | 60.9 | 50 | 100 | 12.5 |
| X-239 | $7 \times 10^5$ | 60 | 18.5 | 58.6 | 29.8 | 25 | 50 | 6.25 |
| PA-18 | $3 \times 10^6$ | 30 | 24.8 | 169 | 134 | 25 | >400 | 400 |
| PA-116 | $4 \times 10^6$ | 30 | 64.4 | 185 | 143 | 12.5 | 25 | 6.25 |
| PI-67 | $2 \times 10^6$ | 20 | 25.0 | 77 | 46.8 | 6.25 | 12.5 | 3.13 |

[a] $LD_{50}$, 50% lethal dose.

[b] Antibiotics were administered subcutaneously 1 and 5 h after intraperitoneal infection. $ED_{50}$, 50% effective dose; SBPC, sulbenicillin; PIPC, piperacillin (T-1220).

[c] MICs were determined by the Agar dilution test, using an inoculum of one loopful of $10^6$ CFU/ml.

TABLE 8. *Mouse plasma concentrations of 6059-S and cefazolin after a single subcutaneous injection*

| Antibiotic | Dose (mg/kg) | Plasma concn (μg/ml ± SD)[a] at: | | | | |
|---|---|---|---|---|---|---|
| | | 15 min | 30 min | 60 min | 90 min | 120 min |
| 6059-S | 40 | 90.9 ± 2.6 | 57.0 ± 6.7 | 25.6 ± 3.9 | 13.5 ± 2.3 | 4.4 ± 1.2 |
| 6059-S | 20 | 38.8 ± 5.2 | 27.9 ± 2.8 | 12.9 ± 2.8 | 5.6 ± 2.5 | 2.1 ± 0.9 |
| 6059-S | 10 | 22.4 ± 2.8 | 14.3 ± 1.0 | 6.0 ± 1.3 | 3.0 ± 1.4 | 0.9 ± 0.2 |
| 6059-S | 5 | 12.6 ± 1.3 | 7.0 ± 1.0 | 3.1 ± 1.0 | 0.9 ± 0.1 | <0.6 |
| 6059-S | 2.5 | 5.1 ± 0.4 | 3.1 ± 0.5 | 1.2 ± 0.5 | <0.6 | <0.6 |
| Cefazolin | 20 | 40.6 ± 2.2 | 35.2 ± 6.6 | 10.5 ± 2.5 | NT[b] | 2.0 ± 0.5 |

[a] Average from four mice at each interval. SD, standard deviation.
[b] NT, Not tested.

TABLE 9. *Urinary and biliary recovery of 6059-S and cefazolin after a single subcutaneous dose of 20 mg/kg*

| Antibiotic | % Recovery (±SD)[a] in 2 h | | |
|---|---|---|---|
| | Urine | Bile | Total |
| 6059-S | 71.8 ± 7.4 | 11.4 ± 4.2 | 83.3 ± 7.0 |
| Cefazolin | 82.9 ± 9.7 | 3.0 ± 2.4 | 86.0 ± 8.7 |

[a] Average from 10 mice. SD, Standard deviation.

oral administration of 40 mg/kg in mice, 6059-S was found to be poorly absorbed: the urinary recovery did not exceed 2% of the administered dose, and 61% of the dose was recovered from intestinal contents during the first 2-h period.

## DISCUSSION

6059-S possesses a unique chemical structure, namely, the 1-oxa-β-lactam nucleus substituted by 7α-methoxy and phenylmalonyl groups (Fig. 1), which plays an important role in broadening the antibacterial spectrum and protecting the molecule from β-lactamase attack. These structural requirements have been discussed elsewhere (Yoshida, in press). Several characteristic features of 6059-S were demonstrated in the present study: (i) antibacterial activity favoring gram-negative rather than gram-positive bacteria; (ii) high susceptibility of organisms almost independent of their β-lactamase production; (iii) extremely extended antibacterial spectrum; (iv) bactericidal effect closely correlated to MIC; (v) high and sustained plasma levels after parenteral dosing; and (vi) potent in vivo efficacy.

The superior activity of 6059-S against a wide range of *Enterobacteriaceae* isolates was fully demonstrated (Table 1). Cephamycin group antibiotics such as cefoxitin and cefmetazole were reported to extend their spectrum over indole-positive *Proteus* species and *Serratia* (17), whereas 6059-S exhibited more than 10-fold-greater activity in vitro. Moreover, 6059-S was more bactericidal than cefmetazole, and regrowth was not observed with the former at a concentration as low as 1.56 μg/ml but was observed with the latter at all concentrations up to 50 μg/ml (Fig. 4). This effect explains a greater protective activity of 6059-S than expected from the in vitro activity (Table 6). The activity of 6059-S was also prominent against *Enterobacter* strains as compared with that of cefamandole, which has been known to have excellent activity against members of this genus (4).

Recently, several "α-ureido"-type antipseudomonal penicillins such as piperacillin (T-1220) have been introduced and generally surpass carbenicillin in their activity in vitro (16). Against *P. aeruginosa*, 6059-S was about four times less active in vitro but consistently more active in vivo than piperacillin (Table 7).

6059-S was found to be resistant to β-lactamases, including R-plasmid-mediated and cephalosporinase types (Tables 4 and 5). Cephalosporins with β-lactamase resistance, such as cefoxitin, cefuroxime, and HR-756, have been reported (2, 9, 11). However, cefoxitin was hydrolyzed to some extent by β-lactamases from *E. coli* 6 and *E. cloacae* 92 (Table 4), which corresponded to similar findings reported by other investigators (7, 11). Cefuroxime was also hydrolyzed by some of the type I, IV, and V β-lactamases, as previously reported (9). Wise suggested the β-lactamase susceptibility of HR-756 from observations of the pronounced inoculum effect on the MICs against *B. fragilis* (19). These findings suggested that 6059-S is more stable to any type of β-lactamase produced by the representative gram-negative bacteria than are these other antibiotics. The extreme resistance of 6059-S to β-lactamase degradation might contribute in part to its potent activity against bacteria highly resistant to other cephalosporins and cephamycin group antibiotics. *Enterobacteriaceae* organisms have been known to produce two different types of β-lactamases simultaneously (12). 6059-S might be useful against frequent emergence of such resistant organisms, as its resistance to β-lactamase degradation was found to be independent of the enzyme type.

When 6059-S was administered subcutaneously in mice, the plasma concentration ($C$ [mi-

crograms per milliliter]) of 6059-S after 15 min could be simulated by using the following empirical formula: $C = 1.8 \times 2(1 - t/23.2) \times D$, where $D$ is dosage in milligrams per kilogram and $t$ is time in minutes after subcutaneous dosing. This equation makes it possible to estimate practically unmeasurable concentrations in plasma after the administration of doses even lower than 2.5 μg/ml. It was apparent from the results in Table 6 that the plasma concentrations of 6059-S usually reached levels beyond the MICs against individual bacteria infected, when administered in doses equivalent to the 50% effective doses. Consequently, the in vivo efficacy of 6059-S, as well as the bacteriolytic action, must be dependent on its MIC.

The time course of the plasma level of 6059-S was found to be similar to that of cefazolin (Table 8), which was in agreement with the values reported by Fare et al. (1). The similarity of 6059-S to cefazolin in plasma level and the half-life was also noted by Matsuura et al. (S. Matsuura, T. Yoshida, K. Sugeno, Y. Harada, M. Harada, and S. Kuwahara, 18th ICAAC abstr. no. 152) in a variety of experimental animals. Moreover, the ultimate half-life in plasma in normal volunteers was about 2.5 h (J. Kurihara, K. Matsumoto, Y. Uzuka, H. Shishido, T. Nagatake, H. Yamada, T. Yoshida, T. Oguma, Y. Kimura, and Y. Tochino Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 19th, Boston, Mass., abstr. no. 171, 1979), which was considerably longer than those reported for other injectable cephalosporins (5).

In addition to a favorable pharmacokinetic profile in humans, the broad antibacterial spectrum and high activities of 6059-S over both aerobes and anaerobes lent support to a potent therapeutic efficacy at lower doses on clinically important bacteria regardless of the increasing incidence of resistance caused by β-lactamases. Clinical trials currently underway may prove the relevance of this.

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