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Antibacterial Activity of Ceftriaxone (Ro 13-9904), a β-Lactamase-Stable Cephalosporin

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The in vitro activity of ceftriaxone (Ro 13-9904), a parenteral cephalosporin, was compared with that of other β-lactam antibiotics. The compound was less active against *Staphylococcus aureus* and *Staphylococcus epidermidis* than was cephalothin or cefamandole, but it was comparable to cefoxitin, cefotaxime, and moxalactam in inhibiting most isolates of *S. aureus* at 3.1 μg/ml. Ro 13-9904 inhibited *Streptococcus pyogenes* and *Streptococcus pneumoniae* at concentrations below 0.25 μg/ml, but *Streptococcus faecalis* required concentrations above 25 μg/ml. *Neisseria gonorrhoeae* and *Haemophilus influenzae* were inhibited at concentrations similar to those of cefotaxime, less than 0.1 μg/ml. Ro 13-9904 was as active as cefotaxime and moxalactam against most *Enterobacteriaceae* and was the most active agent tested against *Proteus*, inhibiting all strains tested at 0.006 μg/ml. Ro 13-9904 was slightly less active than moxalactam or cefoxitin against *Bacteroides fragilis*, requiring more than 100 μg/ml to inhibit 90% of isolates, and it was less active than cefoperazone against *Pseudomonas aeruginosa*. Presence of serum, alteration of pH, and use of various media did not change the inhibitory levels. Bactericidal concentrations were similar to inhibitory levels. Ro 13-9904 was stable to most plasmid-mediated β-lactamases, but was hydrolyzed by some *Enterobacter*, *Proteus*, and *Bacteroides* β-lactamases of chromosomal origin.

An increasing number of β-lactamase-stable cephalosporins have been synthesized within the past few years. However, each has had certain gaps in its overall spectrum of activity, and multiresistant *Enterobacteriaceae* and nonfermenting aerobic gram-negative bacilli continue to cause serious infections in hospitalized patients. The oxime cephalosporins (5, 7), as well as moxalactam (6), have been shown to be active both in vitro and in clinical trials. Thus any new agents which are developed must be evaluated in comparison with these agents.

For these reasons we evaluated ceftriaxone (Ro 13-9904), (*Z*)-(6R,7R)-7-[2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetamido]-3-[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-as-triazin-3-yl)thiol methyl-8 oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylic acid disodium salt (Fig. 1), a new cephalosporin, to determine its in vitro activity in comparison with the most recently developed agents.

MATERIALS AND METHODS

Samples of Ro 13-9904 were a gift of Hoffmann-La Roche, Inc. Cephalothin and cefamandole were donated by Lilly Research Laboratories; cefoxitin was donated by Merck Sharp & Dohme; cefotaxime was donated by Hoechst-Roussel Pharmaceuticals Inc.; cefoperazone was a gift of Pfizer Inc.; carbenicillin came from Beecham Laboratories; and ceftizoxime was a gift of Fujisawa Pharmaceutical Inc.

Fresh dilutions of the compounds were prepared daily in either sterile medium or distilled water. Bacterial isolates were obtained from patients hospitalized at the Columbia-Presbyterian Medical Center, New York City. In some experiments isolates tested were known to be multiply resistant to antibiotics or to contain β-lactamases or both. Some isolates have been stored frozen for a number of years.

Susceptibility tests. Antimicrobial activity was measured by an agar dilution method with Mueller-Hinton (MH) agar unless specified otherwise. A final inoculum of 10^5 colony-forming units (CFU), prepared by dilution of a fresh overnight broth culture, was applied to agar by a replicating spot device. Broth dilutions were performed with 10^5 CFU in tubes of 1-

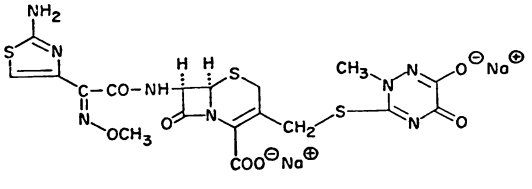

FIG. 1. *Ro 13-9904 structure: (Z)-(6R,7R)-7-[2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetamido]-3-[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-as-triazin-3-yl)thiol methyl-8 oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylic acid disodium salt.*

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TABLE 1. *Comparative activity of Ro 13-9904 and other β-lactam compounds*

Strain (no.)	Drug	MIC range (μg/ml)	MIC (μg/ml) for % of strains:	
			50%	90%
Staphylococcus aureus (20)	Ro 13-9904	1.6->50	3.1	3.1
	Cephalothin	0.05-25	0.2	1.6
	Cefamandole	0.2-25	0.4	1.6
	Cefoxitin	0.8->50	3.1	6.3
	Cefotaxime	1.6-25	1.6	3.1
	Moxalactam	3.1-25	6.2	12.5
Staphylococcus epidermidis (12)	Ro 13-9904	0.8->50	6.3	50
	Cephalothin	0.2-25	0.4	25
	Cefamandole	0.2-25	0.8	25
	Cefoxitin	0.8-50	6.3	50
	Cefotaxime	0.2->50	1.6	50
	Moxalactam	0.8->50	12.5	50
Streptococcus pyogenes (10)	Ro 13-9904	0.012-0.25	0.012	0.215
	Cephalothin	0.05-0.2	0.05	0.1
	Cefamandole	0.05-0.1	0.05	0.1
	Cefotaxime	0.01-0.2	0.05	0.1
	Penicillin	0.001-0.05	0.005	0.05
Streptococcus agalactiae (15)	Ro 13-9904	0.012-0.1	0.025	0.1
	Cephalothin	0.1-0.8	0.1	0.4
	Cefamandole	0.1-0.8	0.1	0.4
	Cefotaxime	0.05-0.2	0.1	0.1
	Penicillin	0.05-0.2	0.1	0.2
Viridans streptococci (10)	Ro 13-9904	1.1-1.6	0.4	0.8
	Cephalothin	0.2-0.8	0.2	0.4
	Cefamandole	0.1-0.4	0.1	0.4
	Cefotaxime	0.05-0.8	0.1	0.4
	Penicillin	0.01-0.4	0.1	0.2
Streptococcus pneumoniae (10)	Ro 13-9904	0.012-0.1	0.025	0.1
	Cephalothin	0.05-0.8	0.2	0.8
	Cefamandole	0.1-0.4	0.1	0.2
	Cefoxitin	0.8-3.1	1.6	3.1
	Cefotaxime	0.1-0.2	0.04	0.4
	Moxalactam	0.4-12.5	3.1	12.5
Streptococcus bovis (10)	Ro 13-9904	0.8-12.5	1.6	3.1
	Cephalothin	0.2-0.8	0.4	0.8
	Cefotaxime	0.1-1.6	0.2	0.8
	Moxalactam	0.8-6.3	1.6	3.1
	Ampicillin	0.05-0.2	0.1	0.2
Streptococcus faecalis (30)	Ro 13-9904	12.5->100	25	>100
	Cephalothin	12.5->100	25	>100
	Cefoxitin	>100	>100	>100
	Cefotaxime	6.3->100	25	50
	Moxalactam	6.3->100	50	>100
	Ampicillin	0.8-6.3	3.1	3.1
Haemophilus influenzae (15)	Ro 13-9904	0.002-0.1	0.01	0.1
	Cephalothin	1.6-12.5	3.1	12.5
	Cefamandole	0.1-1.0	0.4	0.8
	Cefotaxime	0.05-0.1	0.02	0.1
	Moxalactam	0.01-0.1	0.01	0.1
	Ampicillin	0.2->100	0.8	25

TABLE 1—*Continued*

Strain (no.)	Drug	MIC range (μg/ml)	MIC (μg/ml) for % of strains: 50%	90%
Neisseria gonorrhoeae (15)	Ro 13-9904	0.01–0.025	0.01	0.025
	Cephalothin	0.4–3.1	0.8	3.1
	Cefamandole	0.05–3.1	0.4	3.1
	Cefoxitin	0.4–3.1	0.8	1.6
	Cefotaxime	0.005–0.05	0.005	0.05
	Penicillin	0.1–>100	0.6	>100
Neisseria meningitidis (10)	Ro 13-9904	0.012–0.025	0.012	0.025
	Cephalothin	0.2–0.8	0.4	0.8
	Cefotaxime	0.012–0.025	0.012	0.025
	Moxalactam	0.025–0.05	0.025	0.05
	Penicillin	0.05–0.1	0.05	0.1
Listeria monocytogenes (11)	Ro 13-9904	0.8–25	12.5	25
	Cephalothin	0.8–25	1.6	25
	Cefamandole	0.4–25	1.6	25
	Cefoxitin	0.8–100	25	100
	Cefotaxime	0.8–100	25	100
	Ceftizoxime	0.4–12.5	6.3	12.5
Clostridium sp. (10)	Ro 13-9904	0.25–25	1.6	25

ml volume. Plates or tubes were incubated at 35°C for 18 h. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic that inhibited development of visible growth on agar or in broth. The minimal bactericidal concentration (MBC) was determined by plating 0.01-ml amounts from clear broth tubes onto blood agar plates. The MBC was the concentration at which there were fewer than five colonies after 24 h of incubation at 35°C. Susceptibility of streptococci was determined by using MH agar supplemented with 5% sheep blood. Susceptibility of *Neisseria* species and *Haemophilus* species was determined on chocolate MH agar with assays run in the presence of CO_2. Tube dilutions for these species were performed by using Levinthal broth. Anaerobic susceptibility was determined by using MH agar supplemented with sheep blood and vitamin K. Incubation of anaerobic cultures was for 48 h in GasPak jars (BBL Microbiology Systems).

Killing curves were performed in MH broth (BBL Microbiology Systems), using a fresh dilution of organisms from an overnight incubation. Samples were taken at selected intervals, immediately diluted in broth, and plated at several dilutions on MH agar. After overnight incubation the CFU were counted.

Synergy studies were performed on agar using serial twofold dilutions of both agents as previously published (1). A fourfold reduction of MIC of both agents was taken as synergy. Partial synergy was defined as a fourfold reduction in the MIC of one agent and only a twofold or no reduction in the MIC of the other agent.

β-Lactamase activity of all isolates was determined with the Glaxo chromogenic cephalosporin (9). Enzymes were classified on the basis of hydrolysis of penicillins and cephalosporins (4). Organisms induced to make β-lactamase with cephalothin (25 μg/ml) were *Enterobacter cloacae*, *Citrobacter freundii*, *Morganella morganii*, and *Bacteroides fragilis*. Enzymes were osmotic shockates as described (4), except for the *Escherichia coli*, *M. morganii*, *Pseudomonas aeruginosa*, and *Bacillus cereus* enzymes, which were purified according to published methods (4).

RESULTS

The comparative in vitro activities of Ro 13-9904 and other agents are shown in Table 1. Ro 13-9904 was less active than cephalothin or cefamandole against *Staphylococcus aureus* and *Staphylococcus epidermidis*, but was similar in activity to cefoxitin, cefotaxime, and moxalactam. It failed to inhibit *S. aureus* or *S. epidermidis* isolates resistant to methicillin, as did all of the other β-lactam agents. Ro 13-9904 was more active than cephalothin or cefamandole against *Streptococcus pyogenes* and *Streptococcus agalactiae* and was similar to cefotaxime in activity against these species. Ro 13-9904 was markedly more active than cefoxitin or moxalactam against *Streptococcus pneumoniae* and had activity similar to cefotaxime against this species. Its activity against *Streptococcus bovis* was less than those of ampicillin, cephalothin, and cefotaxime, but similar to that of moxalactam. Like other cephalosporins, it failed to inhibit *Streptococcus faecalis* and *Streptococcus faecium* (not shown; MIC, >50 μg/ml). Ro 13-9904 displayed excellent activity against *Haemophilus influenzae*, including β-lactamase-produc-

TABLE 2. *Comparative activity of Ro 13-9904 and β-lactam and aminoglycoside compounds*

Strain (no.)	Drug	MIC range (μg/ml)	MIC (μg/ml) for % of strains:	
			50%	90%
Escherichia coli (47)	Ro 13-9904	0.0125–25	0.025	0.1
	Cephalothin	1.6–>100	6.3	50
	Cefamandole	0.1–>100	0.8	50
	Cefoxitin	0.8–>100	3.1	12.5
	Cefotaxime	0.0125–25	0.025	0.05
	Moxalactam	0.025–25	0.05	0.1
Klebsiella pneumoniae (32)	Ro 13-9904	0.0125–0.4	0.05	0.1
	Cephalothin	0.4–>100	12.5	>100
	Cefamandole	0.2–>100	6.3	>100
	Cefoxitin	0.8–>100	6.3	50
	Cefotaxime	0.012–1.6	0.1	0.4
	Moxalactam	0.025–1.6	0.1	0.4
	Piperacillin	3.1–>400	100	>400
Enterobacter cloacae (57)	Ro 13-9904	0.05–100	0.2	25
	Cefamandole	0.4–>100	1.6	25
	Cefotaxime	0.012–12.5	0.1	0.4
	Moxalactam	0.012–12.5	0.1	0.4
	Ceftizoxime	0.012–12.5	0.1	0.4
	Carbenicillin	3.1–>100	12.5	>100
Enterobacter aerogenes (33)	Ro 13-9904	0.006–3.1	0.05	0.2
	Cefamandole	0.2–50	0.8	3.1
	Cefotaxime	0.006–6.3	0.05	0.2
	Moxalactam	0.006–3.1	0.05	0.2
	Ceftizoxime	0.006–6.3	0.05	0.2
	Carbenicillin	0.8–>100	6.2	>100
Enterobacter, other (21)	Ro 13-9904	0.025–12.5	0.2	1.6
	Cefamandole	0.2–>100	6.3	25
	Cefotaxime	0.05–12.5	0.4	1.6
Citrobacter freundii (21)	Ro 13-9904	0.012–1.6	0.1	0.2
	Cephalothin	0.8–>100	50	>100
	Cefamandole	0.4–50	1.6	25
	Cefoxitin	1.6–>100	3.1	50
	Cefotaxime	0.025–1.6	0.1	0.4
	Moxalactam	0.025–1.6	0.1	0.4
Citrobacter diversus (29)	Ro 13-9904	0.012–0.2	0.025	0.1
	Cephalothin	0.8–25	1.6	12.5
	Cefamandole	0.1–3.1	0.2	1.6
	Cefoxitin	0.4–12.5	1.6	3.1
	Cefotaxime	0.012–0.2	0.05	0.1
	Moxalactam	0.012–0.2	0.1	0.2
Serratia marcescens (31)	Ro 13-9904	0.2–>100	25	50
	Cefamandole	>100	>100	>100
	Cefoxitin	12.5–>100	100	>100
	Cefotaxime	0.2–>100	25	50
	Ceftizoxime	0.2–>100	25	50
	Moxalactam	0.05–50	12.5	50
	Gentamicin	1.6–>25	25	25
Proteus mirabilis (32)	Ro 13-9904	<0.006–0.01	0.006	0.006
	Cephalothin	0.8–3.1	1.6	3.1
	Cefamandole	0.2–1.6	0.04	0.4
	Cefoxitin	0.4–1.6	0.8	0.8
	Cefotaxime	0.006–0.12	0.006	0.12
	Cefoperazone	0.01–3.1	0.1	1.6

TABLE 2— *Continued*

Strain (no.)	Drug	MIC range (μg/ml)	MIC (μg/ml) for % of strains: 50%	90%
Proteus, indole positive (29)	Ro 13-9904	0.006–25	0.025	3.1
	Cefamandole	0.4–>100	12.5	>100
	Cefoxitin	1.6–>100	12.5	>100
	Cefotaxime	0.006–12.5	0.1	3.1
	Cefoperazone	0.4–50	1.6	12.5
	Moxalactam	0.1–12.5	0.2	3.1
Providencia (32)	Ro 13-9904	<0.003–0.2	0.02	0.1
	Cefamandole	0.2–>50	0.8	6.3
	Cefoxitin	0.8–12.5	0.8	1.6
	Cefotaxime	0.025–0.4	0.05	0.2
	Ceftizoxime	0.001–0.025	0.006	0.025
	Cefoperazone	0.2–50	12.5	50
	Moxalactam	0.06–0.1	0.1	0.1
Salmonella (18)	Ro 13-9904	<0.025–100	0.05	0.05
	Cephalothin	3.1–100	3.1	50
	Cefamandole	0.2–100	0.4	25
	Cefoxitin	1.6–6.3	1.6	6.3
	Cefotaxime	0.05–0.1	0.05	0.1
	Cefoperazone	0.4–100	0.8	25
	Ampicillin	1.6–>100	6.3	>100
Shigella (17)	Ro 13-9904	<0.006–0.2	0.01	0.025
	Cephalothin	25–>100	>100	>100
	Cefamandole	0.2–12.5	3.1	6.3
	Cefoxitin	0.4–12.5	1.6	1.6
	Cefotaxime	0.006–0.8	0.025	0.05
	Ampicillin	6.3–>100	>100	>100
Acinetobacter (23)	Ro 13-9904	0.006–>100	50	>100
	Cefamandole	1.6–>100	>100	>100
	Cefoxitin	1.6–>100	>100	>100
	Cefotaxime	0.05–>100	50	>100
	Cefoperazone	0.8–>100	100	>100
	Moxalactam	1.6–>100	25	>100
Pseudomonas aeruginosa (82)	Ro 13-9904	0.8–>100	50	>100
	Cefotaxime	0.4–>100	25	>100
	Ceftizoxime	0.8–>100	50	>100
	Moxalactam	0.8–>100	12.5	>100
	Cefoperazone	1.6–>100	3.1	25
	Carbenicillin	12.5–>100	100	>100
	Piperacillin	1.6–100	3.1	25
	Gentamicin	0.2–25	0.8	25
Pseudomonas, other (*P. maltophilia, P. cepacia, P. stutzeri, P. diminuta*) (17)	Ro 13-9904	>50	>50	>50
Bacteroides (12)	Ro 13-9904	1.6–>100	6.3	50
	Cefamandole	12.5–>100	50	>100
	Cefoxitin	3.1–12.5	6.3	12.5
	Cefotaxime	0.8–>100	12.5	>100
	Moxalactam	0.8–50	1.6	6.3
	Carbenicillin	12.5–>100	50	>100
	Penicillin G	6.3–>100	12.5	>100
Bacteroides fragilis subsp. *fragilis* (16)	Ro 13-9904	0.8–>100	25	>100
	Cefamandole	12.5–>100	100	>100
	Cefoxitin	3.1–>100	12.5	>100
	Cefotaxime	1.6–>100	50	>100
	Moxalactam	3.1–>100	12.5	>100
	Carbenicillin	12.5–>100	50	>100

ing, ampicillin-resistant isolates, being as active as cefotaxime and moxalactam. The compound had excellent activity against both *Neisseria gonorrhoeae*, including β-lactamase-producing isolates, and *Neisseria meningitidis*: it was as active as cefotaxime and considerably more active than cephalothin. Like a number of the new cephalosporins (6, 8), Ro 13-9904 had relatively poor activity against *Listeria* and against various clostridial species, including *Clostridium perfringens*.

The compound inhibited members of the *Enterobacteriaceae* at lower concentrations than did the older cephalosporins or even a number of the second-generation agents of this class (Table 2). Its activity against *E. coli* and *K. pneumoniae* was similar to those of cefotaxime and moxalactam. It was less active than moxalactam, cefotaxime, or ceftizoxime against *E. cloacae*, but had equal activity against *Enterobacter aerogenes* and activity equal to that of cefotaxime against other enterobacters. Both *Citrobacter freundii* and *Citrobacter diversus* were inhibited by Ro 13-9904 at concentrations similar to those of moxalactam and ceftizoxime. Although some *Serratia* species were inhibited at low concentrations (<3 μg/ml), the compound was no more active than ceftizoxime, cefotaxime, or moxalactam against resistant isolates. Ro 13-9904 was the most active agent tested against *Proteus mirabilis*. It had activity similar to those of moxalactam and cefotaxime against *Providencia*, *Salmonella*, and *Shigella* species. It was as active as cefotaxime and moxalactam against *Morganella*, *Proteus rettgeri*, and *Proteus vulgaris* and more active than cefoperazone. *Acinetobacter* strains in general were as resistant to Ro 13-9904 (MIC, >50 μg/ml) as they were to the other agents. Ro 13-9904 inhibited carbenicillin-susceptible *P. aeruginosa* at concentrations below 25 μg/ml (data not shown), but it was much less active than piperacillin or cefoperazone, which inhibited the majority of these relatively resistant isolates at concentrations of ≤25 μg/ml. Ro 13-9904 did not inhibit other *Pseudomonas* species such as *P. maltophilia* or *P. cepacia*. Although Ro 13-9904 was more active than carbenicillin and cefotaxime against *B. fragilis*, it was one dilution less active than cefoxitin or moxalactam, and concentrations of 25 μg/ml were required to inhibit 50% of the isolates.

Effect of alteration of test conditions. The effect of growth medium upon MICs was tested for *S. aureus*, *E. coli*, *Klebsiella pneumoniae*,

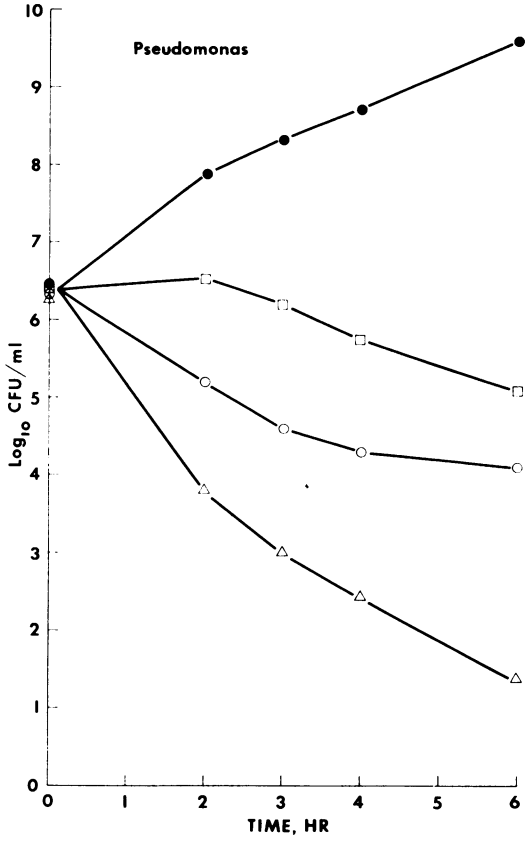

FIG. 2. *Time-kill curve against a β-lactamase-producing E. coli strain with Ro 13-9904 (▲) compared with cefotaxime (●), cefoxitin (□), and cefamandole (△) at concentrations twice the MICs, which were Ro 13-9904, 0.025 μg/ml; cefotaxime, 0.025 μg/ml; cefoxitin, 1.6 μg/ml; cefamandole, 16 μg/ml.*

TABLE 3. *Inoculum effect on Ro 13-9904 activity*

Strain	Concn (μg/ml) at inoculum of:					
	10^7		10^5		10^3	
	MIC	MBC	MIC	MBC	MIC	MBC
Pseudomonas sp. 3414	50	>100	0.4	12.5	0.2	0.4
Klebsiella sp. 4021	12.5	50	0.1	0.4	0.1	0.1
Enterobacter aerogenes 3943	50	100	0.1	0.4	0.1	0.1
E. cloacae 3834	25	25	0.1	0.2	0.05	0.05
Proteus mirabilis 2334	50	50	0.006	0.006	0.006	0.0125
P. rettgeri	25	50	0.1	0.1	0.1	0.1

TABLE 4. *Comparative activity of newer cephalosporins against cephalothin- and carbenicillin[a]-resistant, β-lactamase-containing bacteria*

Strain	MIC (μg/ml) of drug:					
	Ro 13-9904	Moxalactam	Cefotaxime	Ceftizoxime	Cefoxitin	Cefamandole
Escherichia coli	0.05	0.1	0.05	0.05	1.6	100
E. coli	0.05	0.4	0.1	0.1	12.5	3.1
Enterobacter aerogenes	0.05	0.8	0.02	1.6	>400	50
E. cloacae	25	6.2	12.5	12.5	>400	>400
E. cloacae	0.1	0.2	0.05	0.1	>400	3.1
E. hafniae	0.8	0.4	0.8	0.8	>50	>50
Salmonella typhimurium	0.02	0.1	0.05	0.1	1.6	12.5
Shigella sonnei	0.2	0.2	1.6	0.8	100	50
Proteus mirabilis	0.05	0.1	3.1	1.6	>400	>400
P. mirabilis	0.001	0.8	0.2	0.1	25	>50
P. inconstans	0.05	0.4	0.2	0.2	1.6	>50
P. vulgaris	0.05	0.8	0.1	0.1	3.1	>200
P. rettgeri	0.05	0.1	0.05	0.1	100	200
Klebsiella pneumoniae	0.1	0.8	0.05	0.1	25	50
K. pneumoniae	0.1	0.2	0.4	0.2	100	25
Serratia marcescens	25	12.5	25	12.5	>400	>400
Pseudomonas aeruginosa	50	6.2	25	25	>400	>400
P. aeruginosa	100	12.5	50	100	>400	>400
Bacteroides fragilis	25	1.6	25	25	12.5	>400
B. fragilis	50	3.2	50	25	25	>400
Citrobacter freundii	0.8	0.4	0.4	0.4	>100	>100
Staphylococcus epidermidis	>25	>25	>25	>25	>25	>25
S. aureus	>25	>25	>25	>25	>25	>25

[a] Cephalothin MIC, ≥ 50 μg/ml; carbenicillin MIC, ≥ 200 μg/ml.

Serratia marcescens, *P. aeruginosa*, and *E. cloacae*. The MICs and MBCs of representative isolates (five each), tested in brain heart infusion, Trypticase soy, nutrient, and MH media, were within a single dilution of each other in all instances. Comparison of the MICs in MH broth and in MH agar revealed no differences for *E. coli*, *K. pneumoniae*, *S. marcescens*, *P. aeruginosa*, *E. cloacae*, *P. mirabilis*, and *S. aureus*.

Table 3 illustrates the effect of inoculum size on both MICs and MBCs of representative isolates, all of which produced β-lactamases. At inocula of 10^3 and 10^5 CFU, there was no significant difference between MIC and MBC except for *P. aeruginosa*, in which there was a 32-fold difference between the MIC and MBC at 10^5 CFU. An 8- to 32-fold difference between MIC and MBC was seen for 10 strains of *P. aeruginosa* at 10^5 CFU. There was a definite increase in the MICs and MBCs for all isolates, including the extremely susceptible *P. mirabilis*, when an inoculum of 10^7 CFU was used. Indeed, the MIC at 10^7 CFU of all of these organisms was 128-fold greater or more than the MIC and MBC at 10^5 CFU.

The MICs and MBCs for five isolates each of *E. coli*, *S. marcescens*, *K. pneumoniae*, *E. aerogenes*, *E. cloacae*, and *P. aeruginosa* differed by less than one tube dilution when tested in MH medium adjusted to pH 6, 7, and 8, indicating that pH had no effect on MICs or MBCs.

We determined that Ro 13-9904 was 93.6% protein bound at a concentration of 1.56 μg/ml, 94% bound at 6.25 μg/ml, and 91.9% bound at 25 μg/ml. We determined the MICs and MBCs of representative organisms in 50% human serum. There was no appreciable increase in the MIC or MBC when the assay was performed in serum for most *Enterobacteriaceae*, but the MIC of *P. aeruginosa* was eightfold greater.

Killing-curve studies were performed with Ro 13-9904 and other agents against susceptible *E. coli*, *K. pneumoniae*, and *P. mirabilis*. Figure 2 shows a comparison of the effect of Ro 13-9904, cefotaxime, and cefoxitin on a β-lactamase-producing *E. coli*. The decrease in CFU was similar for all of the agents, which were present at concentrations twice the MIC.

Table 4 shows a direct comparison of Ro 13-9904 and other new compounds against organisms resistant to cephalothin and carbenicillin. Ro 13-9904 showed activity comparable to cefotaxime, ceftizoxime, and moxalactam and was superior, in a number of instances, to cefoxitin and cefamandole. However, Ro 13-9904 did not inhibit isolates resistant to moxalactam or cefotaxime. Table 5 shows that Ro 13-9904 did inhibit all ampicillin-resistant *E. coli* strains, all

cephalothin- and gentamicin-resistant *K. pneumoniae* strains, all carbenicillin- or cefamandole-resistant *Citrobacter* strains, carbenicillin-resistant *E. cloacae* isolates, and all carbenicillin-resistant *Morganella*, *P. rettgeri*, and *Providencia* isolates. It inhibited a few *Serratia* strains that were resistant to carbenicillin and cefoxitin, but it did not inhibit *P. aeruginosa* isolates resistant to piperacillin or gentamicin.

β-Lactamase stability of Ro 13-9904. The stability of Ro 13-9904 to β-lactamases of various organisms is shown in Table 6. Ro 13-9904 was not hydrolyzed by the β-lactamase of *S. aureus* and underwent minimal hydrolysis by plasmid-mediated TEM-type β-lactamase from *E. coli* or by plasmid-mediated OXA-type β-lactamase from *Shigella*. However, β-lactamases of *Citrobacter*, *Enterobacter*, *Pseudomonas*, *P. vulgaris*, *Morganella*, and *Bacteroides* did hydrolyze Ro 13-9904, but did not hydrolyze cefoxitin or moxalactam.

TABLE 5. *Activity of Ro 13-9904 against isolates resistant to other β-lactam antibiotics*

Strain	Resistant to[a]:	No. of strains tested/susceptible to Ro 13-9904[b]
Escherichia coli	Ampicillin	25/25
	Piperacillin	15/15
	Cefazolin	5/5
	Cefamandole	3/3
Klebsiella pneumoniae	Cephalothin	15/15
	Cefamandole	6/6
	Cefoxitin	2/2
	Gentamicin[c]	5/5
Enterobacter cloacae	Carbenicillin	10/10
	Piperacillin	6/6
	Cefamandole	6/6
Citrobacter	Carbenicillin	15/15
	Piperacillin	7/7
	Cefamandole	4/4
Proteus, Providencia, Morganella	Carbenicillin	18/18
	Piperacillin	18/15
	Cefoxitin	3/3
Serratia	Carbenicillin	25/2
	Piperacillin	25/2
	Cefoxitin	20/1
	Cefotaxime	10/0
Bacteroides	Carbenicillin	15/3
	Cefoxitin	5/0
	Cefoperazone	5/0
	Moxalactam	3/0
Pseudomonas	Carbenicillin	24[d]/8
	Piperacillin	7[d]/0
	Cefotaxime	10/2
	Gentamicin	10/0

[a] Isolates were not inhibited by <25 μg of the agent per ml.
[b] Isolates were inhibited by <12.5 μg/ml.
[c] Isolates were not inhibited by 10 μg/ml.
[d] Isolates were not inhibited by 50 μg/ml.

Ro 13-9904 did not inhibit the hydrolysis of cephaloridine by plasmid β-lactamases of *S. aureus* or *E. coli*. It did inhibit the hydrolysis of cephaloridine by some of the chromosomally mediated β-lactamases of *Morganella*, *Citrobacter*, and *Enterobacter*, but it was less effective than were cefoxitin or cefotaxime.

Synergy studies. To ascertain the effect of the combination of Ro 13-9904 and other agents, Ro 13-9904 was combined with gentamicin and with carbenicillin and tested against 32 isolates of *E. coli*, *K. pneumoniae*, *S. marcescens*, *S. aureus*, *C. freundii*, *P. aeruginosa*, *P. rettgeri*, and *E. cloacae*. Table 7 shows that complete synergy of Ro 13-9904 and gentamicin was demonstrated for only 16% of isolates and 19% of isolates when Ro 13-9904 and carbenicillin were combined. Overall, some degree of synergy was found for 59 and 44% of the isolates tested with either combination. Antagonism was not seen for the carbenicillin–Ro 13-9904 combination. Figure 3 shows the effect of Ro 13-9904 and gentamicin when tested against *P. aeruginosa*.

DISCUSSION

The last few years have seen the introduction of a large number of cephalosporin antibiotics which have increased the antibacterial spectrum of older agents to include β-lactamase-producing *Enterobacteriaceae* and *Bacteroides*. Cefotaxime (5), moxalactam (6), cefoperazone (8), and ceftizoxime (2) have been shown to inhibit organisms resistant to cefamandole, cefuroxime, and cefoxitin. Ro 13-9904 has been another addition to this group of potent β-lactam antibiotics. In general, this agent has shown activity against most members of the *Enterobacteriaceae* similar to those of cefotaxime, ceftizoxime, and moxalactam. It was more active against *P. mirabilis* than other compounds, but some indole-positive *Proteus* isolates had MICs of 25 μg/ml, as did some *E. cloacae*. In both cases this resistance could be correlated with instability to the chromosomally mediated β-lactamases of these organisms. This compound was much less active than cefoperazone or piperacillin against *P. aeruginosa* and was less active than cefoxitin or moxalactam against *B. fragilis* subsp. *fragilis*, which contains a β-lactamase-that hydrolyzes Ro 13-9904.

Although Ro 13-9904 was less active against *S. aureus* and *S. epidermidis* than older agents such as cephalothin, it was as active as cefoxitin and better than moxalactam. It was as active as

TABLE 6. *β-Lactamase stability of Ro 13-9904*

β-Lactamase source	Type of β-lactamase[a]	Relative rate of hydrolysis[b] of drug:		
		Ro 13-9904	Cephalothin	Cefotaxime
Escherichia coli[c]	Pen	0.1	19	0
Klebsiella pneumoniae	Cepha	6	69	0
Enterobacter cloacae[d]	Cepha	11	186	0
Citrobacter freundii[d]	Cepha	21	100	0
Serratia marcescens	Cepha	0	125	0
Morganella morganii[d]	Cepha	10	198	0
Proteus vulgaris[d]	Cepha	25	175	0
Shigella sonnei[c]	Pen	0.2	24	0
Pseudomonas aeruginosa[c]	Pen	0	10	0
P. aeruginosa	Cepha	36	266	0
Bacteroides fragilis[d]	Cepha	128	140	76
Staphylococcus aureus[c]	Pen	0	0	0
Bacillus cereus	Both	16	100	0

[a] Pen, Primarily penicillin substrate; Cepha, primarily cephalosporin substrate; Both, both types.
[b] Rate in relation to cephaloridine (100%), except for *S. aureus* and *B. cereus*, which are based upon hydrolysis of penicillin G (100%). Rates for cefoxitin and moxalactam were 0 for all strains shown.
[c] Plasmid mediated.
[d] Induced with cephalothin.

TABLE 7. *Synergy of Ro 13-9904 with gentamicin or carbencillin*

Strain	No. of strains tested	No. of strains showing:			
		Synergy with:		Partial synergy with:	
		Carbenicillin	Gentamicin	Carbenicillin	Gentamicin
Escherichia coli	5	1	1		
Klebsiella pneumoniae	5				4
Serratia marcescens	3		2		1
Staphylococcus aureus	3			1	
Citrobacter freundii	2			1	2
Pseudomonas aeruginosa	5	1	1	3	3
Proteus rettgeri	4	2		1	2
Enterobacter cloacae	5	2	1	2	2

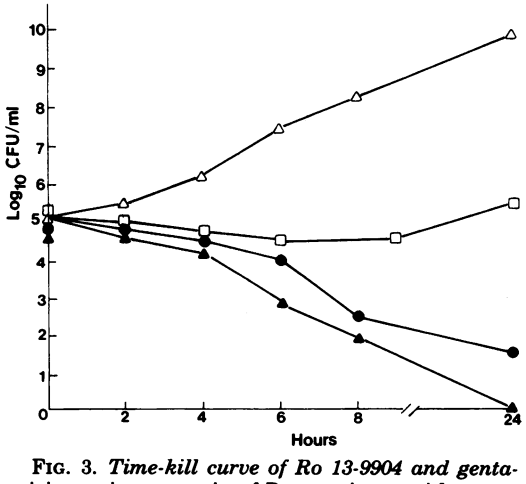

FIG. 3. *Time-kill curve of Ro 13-9904 and gentamicin against a strain of P. aeruginosa with a gentamicin MIC of 0.1 μg/ml and an Ro 13-9904 MIC of 1.6 μg/ml. Growth curve without antibiotics (●); 0.025 μg of gentamicin per ml (○); 0.4 μg of Ro 13-9904 per ml (□); gentamicin at 0.025 μg/ml and Ro 13-9904 at 0.4 μg/ml (△).*

the new agents against group A streptococci, *Haemophilus*, and *Neisseria*.

Ro 13-9904 was not as stable against β-lactamases as is cefoxitin (3) or moxalactam (6), but it resisted hydrolysis by the most common plasmid-mediated β-lactamases present in *E. coli*.

Ro 13-9904, when combined with aminoglycosides or with carbenicillin, rarely showed a synergistic action, but antagonism was not encountered as has been reported for combinations of cefoxitin, cefotaxime, and others of the newer β-lactams (5–8).

These in vitro results indicate that this agent has the antibacterial potential of the other new β-lactams. In view of its reported long half-life (J. Spicehandler, personal communication), the compound may prove to be extremely useful in the clinical setting if it possesses the low toxic potential which the older cephalosporins have had.

LITERATURE CITED

1. **Fu, K. P., and H. C. Neu.** 1978. A comparative study of the activity of cefamandole and other cephalosporins

and analysis of the β-lactamase stability and synergy of cefamandole with aminoglycosides. J. Infect. Dis. 137(Suppl.):38–48.
2. **Fu, K. P., and H. C. Neu.** 1980. Antibacterial activity of ceftizoxime, a β-lactamase-stable cephalosporin. Antimicrob. Agents Chemother. 17:583–590.
3. **Neu, H. C.** 1974. Cefoxitin, a semisynthetic cephamycin antibiotic: antibacterial spectrum and resistance to hydrolysis by gram-negative beta-lactamases. Antimicrob. Agents Chemother. 6:170–176.
4. **Neu, H. C.** 1980. Antibiotic inactivating enzymes and bacterial resistance, p. 454–473. *In* V. Lorian (ed.), Antibiotics in laboratory medicine. The Williams & Wilkins Co., Baltimore.
5. **Neu, H. C., N. Aswapokee, P. Aswapokee, and K. P. Fu.** 1979. HR 756, a new cephalosporin active against gram-positive and gram-negative aerobic and anaerobic bacteria. Antimicrob. Agents Chemother. 15:273–281.
6. **Neu, H. C., N. Aswapokee, K. P. Fu, and P. Aswapokee.** 1979. Antibacterial activity of a new 1-oxa cephalosporin compared with that of other β-lactam compounds. Antimicrob. Agents Chemother. 16:141–149.
7. **Neu, H. C., and K. P. Fu.** 1978. Cefuroxime, a beta-lactamase-resistant cephalosporin with a broad spectrum of gram-positive and -negative activity. Antimicrob. Agents Chemother. 13:657–664.
8. **Neu, H. C., K. P. Fu, N. Aswapokee, P. Aswapokee, and K. Kung.** 1979. Comparative activity and β-lactamase stability of cefoperazone, a piperazine cephalosporin. Antimicrob. Agents Chemother. 16:150–157.
9. **O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shinglar.** 1972. Novel method for detection of β-lactamases by using a chromogenic cephalosporin substrate. Antimicrob. Agents Chemother. 1:283–288.