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Comparison of In Vitro Activity of Sch 21420, a Gentamicin B Derivative, with Those of Amikacin, Gentamicin, Netilmicin, Sisomicin, and Tobramycin

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Sch 21420 is a new aminoglycoside synthesized from gentamicin B. Susceptibility tests with Sch 21420, amikacin, gentamicin, netilmicin, sisomicin, and tobramycin were performed on a variety of bacterial species including 44 with known mechanisms of resistance to aminoglycosides. Sch 21420 and amikacin had similar effects on all except *Haemophilus influenzae* and *Neisseria* species, which were more susceptible to amikacin. Except with some strains of *Serratia marcescens*, the drugs used were bactericidal. Sch 21420 and amikacin were more stable than the other four aminoglycosides in the presence of the inactivating enzymes produced by some strains. Strains which were very resistant to Sch 21420 and amikacin either were permeability mutants or produced AAC (6')-I inactivating enzyme. The effect of cations on the susceptibilities of these strains to Sch 21420 and amikacin was seen mostly with *Pseudomonas aeruginosa* and to Sch 21420 with *Acinetobacter*. Cations did not affect the susceptibilities of other *Pseudomonas* species, *Enterobacteriaceae*, *Staphylococcus aureus*, or *Streptococcus faecalis* to Sch 21420 or amikacin.

Sch 21420 is an aminoglycoside which was synthesized by a method similar to that used in the synthesis of amikacin, except that gentamicin B was used in place of kanamycin (12). The activity of this compound was found to be similar to that of amikacin against the *Enterobacteriaceae* and *Pseudomonas aeruginosa* (7, 9, 11, 13, 15, 17).

In this study, we compared the inhibitory and bactericidal activities of Sch 21420 to those of amikacin, gentamicin, netilmicin, sisomicin, and tobramycin against a wide variety of gram-positive and gram-negative organisms. The effect of differences in inoculum size and cation concentration on the activity of these six aminoglycosides was assessed. The activities of these drugs on organisms with various mechanisms of resistance (inactivating enzymes and permeability mutants) were also compared.

MATERIALS AND METHODS

Antibiotics. Antibiotic powders suitable for susceptibility tests were supplied as follows: Sch 21420, gentamicin, netilmicin, and sisomicin were from Schering Corp., Bloomfield, N.J.; amikacin was from Bristol Laboratories, Syracuse, N.Y.; and tobramycin was from Eli Lilly & Co., Indianapolis, Ind.

Organisms. A total of 507 bacterial isolates were collected in the following amounts from the various laboratories participating in this study: 182 strains of *Enterobacteriaceae*, 168 strains of gram-negative bacilli other than *Enterobacteriaceae* (including 100 strains of *P. aeruginosa*), 108 strains of staphylococci and streptococci, and 49 strains of *Neisseria* species. The various genera and species represented are shown in Tables 1 and 2. In addition, 44 strains with known aminoglycoside resistance mechanisms were also tested.

Most of the isolates were tested in duplicate by two of the collaborating laboratories (Center for Disease Control and the Sacramento Medical Center) in a manner previously reported (1, 8). A third laboratory, the Kaiser Foundation, also tested a more limited number to study the effect of inoculum size on minimum inhibitory concentrations (MICs). Very similar MICs were obtained at the participating institutions.

Antibiotic susceptibility tests. MICs were determined by the broth microdilution method with test trays prepared commercially (Micro Media Systems, San Jose, Calif.). Mueller-Hinton broth was dispensed into a single lot of trays and distributed to the participating laboratories. These trays were stored at −60°C until inoculated. The trays were thawed at room temperature (approximately 20 to 30 min) and then were inoculated with disposable inoculators delivering 5 μl to each well.

At all three laboratories, an actively growing broth culture was diluted to match the turbidity of a 0.5 McFarland standard. The suspension was then diluted 1:50 in sterile water containing 0.02% Tween 80 and dispensed as described previously. The final inoculum

TABLE 1. *Ranges and modes of MICs of six aminoglycosides for 325 strains of Enterobacteriaceae and nonfermentative gram-negative bacilli*

Organism (no.)	Range of MIC (μg/ml)[a]											
	Sch 21420		Amikacin		Gentamicin		Netilmicin		Sisomicin		Tobramycin	
Pseudomonas aeruginosa (100)	0.5->64	(16)	0.5->64	(8)	0.5->64	(8)	0.25->64	(16)	0.25->64	(2)	0.25->64	(2)
Pseudomonas spp. (20)[b]	0.5->64	(1, >64)	0.25->64	(1, >64)	0.25->64	(>64)	0.25->64	(0.5, >64)	≤0.125->64	(0.5)	0.25->64	(0.25, 0.5, 64)
Acinetobacter calcoaceticus (15)	2-64	(2)	2-32	(2)	1->64	(1)	1->64	(1, 2)	0.5->64	(0.5)	0.5->64	(1)
Aeromonas hydrophila (8)	0.5-2	(1)	0.5-2	(2)	0.5-2	(1)	0.25-4	(1)	0.25-2	(0.5)	0.25-4	(0.5)
Escherichia coli (24)	0.5-4	(2)	1-4	(4)	0.5-2	(1)	0.5-2	(1)	0.5-2	(0.5)	0.5-2	(1)
Klebsiella pneumoniae (25)	0.5-4	(1)	1-8	(2)	0.25-16	(1)	0.25-4	(1)	0.25-4	(0.5)	0.25-16	(0.5)
Enterobacter spp. (25)[c]	0.5-4	(1)	0.5-4	(2)	0.25-4	(1)	0.25-2	(1)	≤0.125-1	(0.5)	0.25-1	(1)
Serratia marcescens (28)	1->64	(2)	2->64	(4)	1-32	(1)	1-16	(2)	0.5-16	(1)	1->64	(2, 4)
Proteus mirabilis (25)	1-64	(4)	0.5-16	(4)	0.5-4	(1)	0.5-8	(2)	0.5-4	(0.5)	0.5-4	(1)
Proteus spp. (indole positive) (30)[d]	0.5-16	(2)	0.5-16	(2)	0.5-16	(1)	0.25-32	(1)	0.5-8	(0.5)	0.25-16	(1)
Providencia stuartii (25)	0.5->64	(4, 8)	0.5->64	(4)	0.5->64	(16)	0.25->64	(16)	0.25-64	(8)	0.5->64	(8)

[a] Mode or modes are shown within parentheses.
[b] *Pseudomonas* species include *P. cepacia* (three), *P. maltophilia* (two), *P. acidovorans* (two), *P. fluorescens* (four), *P. putida* (three), *P. stutzeri* (six).
[c] Includes *E. cloacae* (ten), *E. aerogenes* (ten), and *E. agglomerans* (five).
[d] Includes "new" proposed taxonomic groups *Morganella morganii* (ten), *Proteus vulgaris* (ten) and *Providencia rettgeri* (ten).

TABLE 2. *Ranges and modes of MICs of Sch 21420 and five other aminoglycosides for four gram-positive species, H. influenzae, and two Neisseria species (182 strains tested)*

Organism (no.)	Range of MIC (μg/ml)[a]											
	Sch 21420		Amikacin		Gentamicin		Netilmicin		Sisomicin		Tobramycin	
Staphylococcus aureus												
Methicillin susceptible (50)[b]	0.5–4	(2)	0.5–8	(2)	≤0.125–32	(0.5)	≤0.125–2	(0.5)	≤0.125–32	(0.25)	≤0.125–32	(0.25)
Methicillin resistant (10)	2–8	(2)	1–8	(1)	≤0.125–1	(0.5)	≤0.125–2	(0.25)	≤0.125–0.5	(0.25)	0.25–16	(0.25)
Streptococcus faecalis (10)	>64	(>64)	>64	(>64)	8–64	(32)	8–32	(16)	8–64	(16)	64–>64	(64, >64)
Streptococcus pneumoniae (19)	4–64	(64)	4–64	(32)	2–32	(32)	2–32	(4)	2–32	(32)	2–32	(32)
Streptococcus pyogenes (19)	64–>64	(64)	2–8	(4)	2–8	(4)	4–8	(4)	2–8	(4)	2–8	(4)
Haemophilus influenzae (25)[c]	8–64	(32)	1–8	(4)	1–8	(4)	0.25–8	(8)	0.25–8	(8)	0.25–8	(8)
Neisseria gonorrhoeae (25)[c]	32–64	(32)	0.5–8	(2)	0.5–8	(4)	0.5–8	(1)	0.25–4	(2)	0.5–4	(1)
Neisseria meningitidis (24)	32–64	(32)	2–32	(8)	1–8	(4)	1–8	(8)	1–8	(4)	1–8	(2)

[a] Mode or modes are shown within parentheses.
[b] Includes 25 penicillinase-producing strains.
[c] Includes 10 beta-lactamase-producing isolates.

was approximately 10^5 colony-forming units (CFU) per ml. For testing the fastidious streptococci, including *Streptococcus pyogenes* and *Streptococcus pneumoniae*, the inoculum was standardized in Mueller-Hinton broth containing 5% lysed rabbit blood, and 0.1 ml of this adjusted cell suspension was added to each microdilution well, giving a final concentration of 10^5 CFU/ml. In tests with *Haemophilus influenzae*, enough Filde reagent was added to the inoculum to yield a final concentration of 1%.

The MIC was recorded as the lowest concentration totally inhibiting bacterial growth (clear well) after approximately 18 h of incubation at 35°C in a forced-air incubator.

Minimum lethal concentrations (MLCs) were determined for 56 strains from nine genera by subculturing 5-μl portions from each microdilution well on Trypticase (BBL Microbiology Systems, Cockeysville, Md.) soy agar with 5% sheep blood. The subculture was made with a multiple inoculum replicator onto a plate (150 by 100 mm). After 48 h of incubation, the endpoints were read as the lowest concentration yielding no more than 0.1% survivors (99.9% killed).

The effect of varying the inoculum concentrations on MIC and MLC endpoints was studied with 56 rapidly growing faculative anaerobes. Trays were inoculated to achieve final concentrations of 10^3, 10^5, and 10^7 CFU/ml. MICs and MLCs were interpreted as described above. Comparisons of MICs and MLCs were made with results obtained with an inoculum density of 10^5 CFU/ml.

Neisseria gonorrhoeae and *Neisseria meningitidis* were tested by the agar dilution method. Proteose peptone agar with 1% hemoglobin and 1% Kellogg supplement was prepared by incorporating appropriate antibiotic concentrations. The inoculum was made by suspending colonies in Mueller-Hinton broth diluted to a concentration of 10^6 CFU/ml. Plates were then inoculated by a Steers replicator (16). The MICs were determined after 24 h of incubation in 5% CO_2 at 35°C.

RESULTS

The ranges and modes of the MICs of Sch 21420 and five other aminoglycosides for 325 enteric and nonfermentative gram-negative bacilli are shown in Table 1. Based on MICs, the most active aminoglycoside was sisomicin, followed in decreasing order by tobramycin, gentamicin, netilmicin, Sch 21420, and amikacin. However, most of these organisms were susceptible to all the drugs.

Some strains of the *Pseudomonas* species and nearly all strains of *Providencia stuartii* were very resistant to the six drugs as indicated by MICs of >64 μg/ml. Some strains of *Serratia marcescens* were very resistant to Sch 21420, amikacin, and tobramycin at the >64-μg/ml level, as were some strains of *Acinetobacter calcoaceticus* subsp. *anitratus* to gentamicin, netilmicin, sisomicin, and tobramycin. Most of these species, however, had a modal MIC within a susceptible or moderately susceptible range or

bimodal MICs with susceptible and very resistant groups, as seen with most of the results with *Pseudomonas* species other than *P. aeruginosa*.

Similar results for *H. influenzae*, *Neisseria* species, staphylococci, and streptococci are shown in Table 2. Both the methicillin-susceptible and methicillin-resistant staphylococci were susceptible to Sch 21420, amikacin, and netilmicin. Most strains were also susceptible to the other three aminoglycosides, although some strains were resistant. Of the streptococci tested, the *Streptococcus faecalis* isolates were resistant to all six drugs but most resistant to Sch 21420, amikacin, and tobramycin. The susceptibility of the *S. pneumoniae* and *S. pyogenes* strains varied, but Sch 21420 appeared to be the least effective drug. Sch 21420 was also the least active drug on strains of *H. influenzae* and *Neisseria* species. The modal MICs of the other five drugs for *H. influenzae* were 4 or 8 μg/ml but in some cases were lower for the *Neisseria* species (1 and 2 μg/ml).

The effect that increasing the inoculum concentration had on the MICs of the six aminoglycosides for 10 representative strains is shown in Table 3. In most cases, no increase or only one dilution increase in the MIC resulted when the inoculum was increased from 10^3 to 10^5 CFU/ml. *Proteus vulgaris*, *Staphylococcus aureus*, and *P. aeruginosa* strains were most affected, and *Escherichia coli* strains were least affected. The changes in MICs by drug were not greatly different, but amikacin and tobramycin were most affected, and gentamicin was least affected. The changes in MICs resulting from increasing the inocula from 10^5 to 10^7 CFU/ml were more marked. *P. aeruginosa* and *Proteus rettgeri* were the most affected, and *Enterobacter aerogenes* and *Klebsiella pneumoniae* were the least affected. Sch 21420 and gentamicin were the most affected aminoglycosides, and netilmicin and sisomicin showed the least change.

The MLCs and MICs of 56 selected strains and the six aminoglycosides are shown in Table 4. For 87% of the strains, the MLC and MIC were either the same (i.e., ratio = 1) or differed by one dilution (ratio = 2). For only 5% of the strains were the ratios ≥8, and all but one of these were *S. marcescens*. Most of the ratios of 4 were also obtained with *S. marcescens*.

The effects of cation supplementation of the media on the MICs obtained with Sch 21420 and amikacin are shown in Table 5. The greatest effect was noted on the Sch 21420 MICs when this drug was tested against *P. aeruginosa*. In unsupplemented media, MICs of Sch 21420 were slightly less than those of amikacin; but in cation-supplemented media, the Sch 21420 MICs were slightly higher than those for amikacin.

TABLE 3. *MICs obtained for six aminoglycosides when inoculum concentrations of representative strains of nine bacterial genera were increased*

Organism	MIC (μg/ml)																	
	Sch 21420			Amikacin			Gentamicin			Netilmicin			Sisomicin			Tobramycin		
	3[a]	5	7	3	5	7	3	5	7	3	5	7	3	5	7	3	5	7
Pseudomonas aeruginosa 12298	8	16	>64	4	8	>64	4	8	>64	4	16	64	1	4	32	0.5	2	32
Escherichia coli 12315	2	2	16	2	4	16	2	2	8	2	2	8	1	2	4	2	2	8
Enterobacter cloacae 12364	1	2	8	1	2	8	0.5	1	4	0.5	1	2	0.25	0.5	2	0.5	1	4
Enterobacter aerogenes 36914	0.5	2	8	1	4	8	0.5	1	2	0.5	1	2	0.25	0.5	1	0.5	1	2
Klebsiella pneumoniae 36943	1	2	8	1	2	8	1	1	2	1	1	2	0.5	1	2	0.5	1	2
Morganella morganii 12324	1	2	8	1	4	4	0.5	1	4	0.5	1	4	0.5	1	2	0.5	1	4
Proteus rettgeri 12322	1	2	32	0.5	1	32	2	4	64	2	8	16	2	4	16	2	8	32
Proteus vulgaris 23223	0.5	4	16	0.25	2	16	0.25	1	8	0.25	1	4	0.25	0.5	2	0.125	1	4
Serratia marcescens 36951	2	2	32	2	4	16	2	2	8	2	2	8	0.5	1	4	4	16	32
Staphylococcus aureus 18046	1	4	8	1	4	8	0.25	1	4	0.25	0.5	2	0.125	0.5	1	0.125	0.5	2

[a] Inoculum concentration in CFU per milliliter on a $\log_{10}$ scale.

TABLE 4. *MLC/MIC ratios of Sch 21420 and five other aminoglycosides for 56 strains from nine genera*

Organism (no.)	Aminoglycoside	No. of strains at MLC/MIC ratios:			
		1	2	4	≥8
P. aeruginosa (6)	Sch 21420	3	2	1	0
	Amikacin	1	3	2	0
	Gentamicin	4	1	1	0
	Netilmicin	3	3	0	0
	Sisomicin	5	1	0	0
	Tobramycin	4	2	0	0
S. aureus (5)	Sch 21420	3	2	0	0
	Amikacin	4	1	0	0
	Gentamicin	5	0	0	0
	Netilmicin	3	1	1	0
	Sisomicin	3	2	0	0
	Tobramycin	4	1	0	0
S. marcescens (9)	Sch 21420	4	2	3	0
	Amikacin	2	3	3	1
	Gentamicin	0	6	2	1
	Netilmicin	0	1	1	7
	Sisomicin	1	1	2	5
	Tobramycin	4	2	2	1
Other *Enterobacteriaceae* (36)[a]	Sch 21420	26	6	4	0
	Amikacin	23	11	2	0
	Gentamicin	24	10	1	1
	Netilmicin	31	4	1	0
	Sisomicin	30	5	1	0
	Tobramycin	27	9	0	0

[a] Enteric species include *E. coli* (nine), *K. pneumoniae* (ten), *Morganella morganii* (two), *P. rettgeri* (four), *P. vulgaris* (three), *E. cloacae* (five), and *E. aerogenes* (three). The ratios were randomly distributed among these strains.

The second largest cation effects were found with Sch 21420 and *A. calcoaceticus* subsp. *anitratus*. The differences in the amikacin MICs for these strains with and without cation supplementation were negligible. The differences in the MICs of both drugs were minor in the test with other *Pseudomonas* species, *Enterobacteriaceae*, *S. aureus*, and *S. faecalis*.

The effect of cation supplementation on the MICs of amikacin and Sch 21420 for strains with known resistance mechanisms was also examined. These data are shown in Table 6. The major differences in the MICs in the two media were species specific rather than enzyme specific. For example, the differences between the MICs of strains of *K. pneumoniae* and *S. marcescens* with AAC(3)-II enzyme obtained in the two media were negligible for Sch 21420 and amikacin, but *P. aeruginosa* strains with this enzyme had markedly different MICs in the two media.

The geometric mean MICs of Sch 21420 and amikacin for the strains with known mechanisms of resistance were very similar as shown in Table 6. For two strains of *P. aeruginosa* with AAC(3)-II inactivating enzyme, the Sch 21420 mean MIC was twice the amikacin mean MIC (20 versus 10 μg/ml). However, with three strains of *S. aureus*, *Streptococcus liquefaciens*, and *Moraxella* species with AAC (6′) enzyme, the amikacin mean MIC was approximately twice the Sch 21420 mean MIC (11.3 versus 28 μg/ml). Sch 21420 and amikacin were very active on the organisms with APH(3′)-I, APH(2″), ANT(2″), AAC(3)-II (some strains), AAC(3)-III, and AAC(2′) enzymes, marginally active on organisms with

TABLE 5. *Effect of cation supplementation on the MICs of Sch 21420 and amikacin for P. aeruginosa (n = 100) and A. calcoaceticus subsp. anitratus (n = 15)*[a]

MIC (μg/ml)	No. of *P. aeruginosa* strains				No. of *A. calcoaceticus* subsp. *anitratus* strains			
	Sch 21420		Amikacin		Sch 21420		Amikacin	
	Cat−[b]	Cat+[b]	Cat−	Cat+	Cat−	Cat+	Cat−	Cat+
≤0.12	0	0	0	0	0	0	0	0
0.25	1	0	0	0	0	0	0	0
0.5	6	1	3	1	0	0	0	0
1	22	1	8	1	7[c]	0	1	0
2	44[c, d]	0	49[c, d]	1	6[d]	0	9[c, d]	10[c, d]
4	15	4	27	18	0	10[c, d]	3	2
8	7	30	8	47	0	3	1	1
16	2	35[c, d]	3	17	2	0	1	0
32	2	20	1	11	0	0	0	2
64	0	6	0	3	0	0	0	0
>64	1	3	1	1	0	2	0	0

[a] Similar studies with 182 strains of *Enterobacteriaceae*, 10 strains of *S. faecalis*, 60 strains of *S. aureus*, and 20 strains of *Pseudomonas* species (other than *P. aeruginosa*) showed that cations had little effect on the MICs of these two aminoglycosides.

[b] Cat−, Mueller-Hinton broth without supplementation; Cat+, Mueller-Hinton broth supplemented to contain 50 mg of calcium and 25 mg of magnesium per liter.

[c] Modal MIC.

[d] Median MIC.

TABLE 6. *MICs of Sch 21420 and five aminoglycosides determined for 44 organisms having known resistance mechanisms*

Organism (no.)	Inactivating enzyme[a]	Geometric mean MIC (μg/ml) in Mueller-Hinton broth							
		Cation supplemented[b]						Nonsupplemented	
		Sch 21420	Amikacin	Gentamicin	Netilmicin	Sisomicin	Tobramycin	Sch 21420	Amikacin
Escherichia coli (1)	APH(3′)-I	2	4	2	1	2	2	4	8
Staphylococcus aureus (5)	APH(3′)-IV	15.2	10.4	1.5	1.4	0.75	2	13.2	23.2
S. aureus (1)	APH(2″)	2	8	>64	4	64	64	2	4
E. coli (1), *Streptococcus liquefaciens* (1)	ANT(2″)	1.5	2	48	1.5	34	>64	1.5	3
Pseudomonas aeruginosa (2)	ANT(2″)	8	8	>64	16	64	64	2	2
S. aureus (2)	ANT(4′)	6	2	≤0.12	0.25	0.25	8	4	2
P. aeruginosa (2)	AAC(3)-I	16	16	>64	24	64	4	2	2
P. aeruginosa (3)	AAC(3)-Ia	18.7	17.3	>64	>64	>64	7.3	1.7	1.7
P. aeruginosa (2)	AAC(3)-II	20	10	>64	20	>64	>64	2	3
Klebsiella pneumoniae (1), *Serratia marcescens* (1)	AAC(3)-II	1.5	2	>64	40	16	20	1.5	3
E. coli (1)	AAC(3)-III	0.5	0.5	64	8	16	8	0.25	0.5
Providencia stuartii (1)	AAC(2′)	4	4	>64	16	64	64	4	4
S. aureus (1), *S. liquefaciens* (1), *Moraxella* spp. (1)	AAC(6′)	11.3	28	>64	48.7	>64	>64	8.7	28
P. aeruginosa (1)	AAC(6′)-I	>64	>64	16	>64	64	>64	>64	32
P. aeruginosa (5)	AAC(6′)-II	32.8	26.4	>64	>64	>64	64	3.2	3.6
P. aeruginosa (8)	Perm[c]	>64	>64	>64	>64	62	30.5	26.7	33
Pseudomonas maltophilia (2)	Perm	>64	>64	>64	48	24	64	24	>64
E. coli (2)	Perm	64	64	16	12	8	24	48	>64

[a] See references 5 and 6 for classification of enzymes.
[b] Mg^{2+} and Ca^{2+}.
[c] Perm, Permeability mutant strains.

APH(3')-IV, AAC(3)-Ia, AAC(3)-II (some strains), and AAC(6') enzymes, and generally inactive on organisms with AAC(6')-I, AAC(6')-II enzymes, and the permeability mutants. With most of these strains, Sch 21420 and amikacin were more active than the other four aminoglycosides, the exception being five *S. aureus* strains with APH(3')-IV enzymes and some of the strains with permeability mutations. The least active aminoglycoside with these strains was gentamicin, which was very active only with an *E. coli* strain with APH(3')-I enzyme (all six aminoglycosides were very active against this strain) and five strains of *S. aureus* producing an APH(3')-IV enzyme.

DISCUSSION

Sch 21420 was synthesized from gentamicin B by procedures similar to those used in producing amikacin from kanamycin A (12). Thus, it was deemed probable that the biological activities of the two compounds would be similar. These data, as well as those of others (7, 9, 11, 13, 15, 17), generally support this supposition. Although each group of investigators has found some differences in MICs for various species, these differences were generally quite small. It can be concluded that the differences between the MICs of these two aminoglycosides are seldom more than one dilution, and thus, they are of essentially equal potency against the organisms isolated from clinical infections. Our data indicate that the exceptions may be *S. pyogenes*, *H. influenzae*, and *Neisseria* species, organisms rarely treated by aminoglycoside antimicrobial agents.

These data on MICs also indicate that strains which are basically aminoglycoside susceptible, i.e., those which do not have inactivating enzymes or are not permeability mutants, may be slightly more susceptible to gentamicin, netilmicin, sisomicin, and tobramycin than to Sch 21420 or amikacin (sisomicin is the most active of these antibiotics). However, for most strains which produce inactivating enzymes, Sch 21420 and amikacin are markedly more active than the other four aminoglycosides.

The remarkable similarity between the results obtained with Sch 21420 and amikacin also is shown in the studies with strains having known resistance patterns. In early studies on the effect of aminoglycoside-inactivating enzymes on amikacin, it was thought that only AAC(6') enzymes were active (14). Since then, there have been reports that APH(2"), APH(3'), and ANT(4') enzymes may have activity against amikacin (2, 3, 5, 6, 10). However, amikacin resistance due to these enzymes may not always be demonstrable by in vitro susceptibility tests (2, 5, 10). It has been reported that (i) the effect of AAC(6') enzyme on amikacin is variable (5); (ii) that the APH(3')-IV enzyme from *S. aureus* will inactivate amikacin (an effect which is not apparent in susceptibility test) (2); and (iii) that *S. aureus* strains with ANT(4') enzyme were only slightly resistant to amikacin, even though amikacin is susceptible to the enzyme (10).

Our data (Table 6) generally support these conclusions. The strains with AAC(6') enzymes had amikacin and Sch 21420 MICs ranging from very susceptible to marginally resistant to very resistant. MICs were higher with *S. aureus* strains that produced APH(3')-IV enzyme than with strains that produced APH(3')-I or APH(2") enzymes but still were within the susceptible or moderately susceptible range as reported by Courvalin and Davies (2). The *S. aureus* strains that produced ANT(4') enzyme were quite susceptible to amikacin and Sch 21420 as indicated by the MICs as reported by Le Goffic et al. (10). In the instance of some strains where amikacin and Sch 21420 MICs were elevated to marginally susceptible or marginally resistant levels, we do not know whether they were indicative of a low level of enzymatic activity, a reduction in the capacity of the drug to enter the cells, or a limited innate resistance to these drugs.

Sch 21420 and amikacin were inactive on the 12 permeability mutants, as were the other four aminoglycosides in most cases. The exceptions were the marginal susceptibilities of two strains of *E. coli* to sisomicin and netilmicin. In fact, these two strains were more susceptible to all the drugs than were the other 10 permeability mutants.

The data show that Sch 21420 and the other five aminoglycosides tested in this study were bactericidal for most of the strains tested. The lethal activity on *S. marcescens* was decreased for all six aminoglycosides but especially for netilmicin and sisomicin. Of the six drugs tested, Sch 21420, amikacin, and tobramycin were most bactericidal.

The effect of cations on the activity of Sch 21420 and amikacin is species specific and does not appear to be correlated with any resistance mechanism. The effect is mainly limited to *P. aeruginosa* (Tables 5 and 6) and is apparent with both drugs. To a lesser degree, it was also noted with *A. calcoaceticus* subsp. *anitratus*, but a significant effect was seen only with Sch 21420. The effect of cations on *P. aeruginosa* is well known (4), but data on the effect on the *Pseudomonas* species and nonfermentative organisms is limited. These data show that cations have little effect on other *Pseudomonas* species (including *P. cepacia*, *P. maltophilia*, *P. acido-*

vorans, *P. fluorescens*, *P. putida*, and *P. stutzeri*). However, the results obtained with the *A. calcoaceticus* subsp. *anitratus* suggest that other nonfermentative species should be studied to determine whether cations affect their susceptibility to some aminoglycosides. Cations appear to have no significant effect on the susceptibilities of *Enterobacteriaceae*, *S. aureus*, and *S. faecalis* to aminoglycosides.

In conclusion, Sch 21420 is a new aminoglycoside which has activity very similar to that of amikacin. It has a wide antimicrobial spectrum against both gram-negative and gram-positive bacteria. It is bactericidal for most of these organisms, but with *S. marcescens*, the lethal activity is diminished.

LITERATURE CITED

1. **Barry, A. L., C. Thornsberry, R. N. Jones, P. C. Fuchs, T. L. Gavan, and E. H. Gerlach.** 1977. Cefuroxime, an in vitro comparison to six other cephalosporins. Proc. R. Soc. Med. **70**(Suppl. 9):63–70.
2. **Courvalin, P., and J. Davies.** 1977. Plasmid-mediated aminoglycoside phosphotransferase of broad substrate range that phosphorylates amikacin. Antimicrob. Agents Chemother. **11**:619–624.
3. **D'Amato, R. F., C. Thornsberry, C. N. Baker, and L. A. Kirven.** 1975. Effect of calcium and magnesium ions on the susceptibility of *Pseudomonas* species to tetracycline, gentamicin, polymyxin B, and carbenicillin. Antimicrob. Agents Chemother. **7**:596–600.
4. **Davies, J., and P. Courvalin.** 1977. Mechanisms of resistance to aminoglycosides. Am. J. Med. **62**:868–872.
5. **Drasar, F. A.** 1978. Detection of aminoglycoside degrading enzymes, p. 70–75. *In* D. S. Reeves, I. Phillips, J. D. Williams, and R. Wise (ed.), Laboratory methods for antimicrobial chemotherapy. Churchill Livingstone, New York.
6. **Jackson, G. G.** 1976. Aminoglycosides. A historical overview. Am. J. Med. (Suppl.) 6–13.
7. **Jones, R. N., A. L. Barry, P. C. Fuchs, T. L. Gavan, E. H. Gerlach, H. Sommers, and C. Thornsberry.** 1978. 1-N-(S-3-amino-2-hydroxypropionyl) gentamicin B (Sch 21420): a collaborative in vitro susceptibility comparison with amikacin and gentamicin against 12,984 clinical bacterial isolates. Curr. Microbiol. **1**:359–364.
8. **Jones, R. N., C. Thornsberry, A. L. Barry, P. C. Fuchs, T. L. Gavan, and E. H. Gerlach.** 1977. BL-S786, a new parenteral cephalosporin. II. In vitro antimicrobial activity comparison with six related cephalosporins. J. Antibiot. **30**:583–592.
9. **Kabins, S. A., and C. Nathan.** 1978. In vitro activity of Sch 21420, derivative of gentamicin B, compared to that of amikacin. Antimicrob. Agents Chemother. **14**:786–787.
10. **Le Goffic, F., A. Martel, M. L. Capmau, B. Baca, P. Goebel, H. Chardon, C. J. Soussy, J. Duval, and D. H. Bouanchaud.** 1976. New plasmid-mediated nucleotidylation of aminoglycoside antibiotics in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **10**:258–264.
11. **Miller, G. H., P. J. S. Chiu, and J. A. Waitz.** 1978. Biological activity of Sch 21420, the 1-N-S-α-hydroxy-β-aminopropionyl derivative of gentamicin B. J. Antibiot. **31**:688–696.
12. **Nagabhushan, T. L., A. B. Cooper, H. Tsai, P. J. L. Daniels, and G. H. Miller.** 1978. The syntheses and biological properties of 1-N-(S-4-amino-2-hydroxybutyryl)-gentamicin B and 1-N-(S-3-amino-2-hydroxypropionyl)-gentamicin B. J. Antibiot. **31**:681–687.
13. **Neu, H. C., and K. P. Fu.** 1978. 1-N-HAPA gentamicin B, a new aminoglycoside active against gentamicin resistant isolates—activity compared to other aminoglycosides. J. Antibiot. **31**:385–393.
14. **Price, K. E., M. D. DeFuria, and T. A. Pursiano.** 1976. Amikacin, an aminoglycoside with marked activity against antibiotic-resistant clinical isolates. J. Infect. Dis. **134**(Suppl.):249–261.
15. **Sanders, C. C., W. E. Sanders, Jr., and R. V. Goering.** 1978. In vitro studies with Sch 21420 and Sch 22591: activity in comparison with six other aminoglycosides and synergy with penicillin against enterococci. Antimicrob. Agents Chemother. **14**:178–184.
16. **Steers, E., E. Foltz, B. S. Graves, and J. Riden.** 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemother. (Washington, D.C.) **9**:307–311.
17. **Yu, P. K. W., and J. A. Washington II.** 1978. In vitro evaluation of a semisynthetic derivative of gentamicin B (Sch 21420). Antimicrob. Agents Chemother. **13**:891–892.