

## In Vitro and In Vivo Antibacterial Activities of Carumonam (AMA-1080), a New *N*-Sulfonated Monocyclic $\beta$ -Lactam Antibiotic

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The in vitro and in vivo antibacterial activities of carumonam (AMA-1080), a synthetic sulfazecin derivative, were compared with those of aztreonam, cefoperazone, ceftazidime, and cefsulodin. Carumonam was highly active in vitro against members of the family *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae* and weakly active against *Streptococcus pneumoniae*, but it was not active against *Staphylococcus aureus*. The MICs of carumonam for 90% of 1,156 clinical *Enterobacteriaceae* isolates were between 0.013 and 25  $\mu\text{g/ml}$ , which were the lowest MICs of the antibiotics tested. The MIC of carumonam for 90% of *Klebsiella oxytoca* was 0.2  $\mu\text{g/ml}$ , whereas that of aztreonam was 50  $\mu\text{g/ml}$ . The superiority of carumonam to aztreonam and the reference cephalosporins was also demonstrated by their activities against *Klebsiella pneumoniae* and *Enterobacter cloacae*. The MIC of carumonam for 90% of *P. aeruginosa* was 12.5  $\mu\text{g/ml}$ , which was comparable to the MICs of aztreonam and ceftazidime. Carumonam showed a high affinity for the penicillin-binding protein 3 of gram-negative bacteria, but not for the penicillin-binding proteins of *S. aureus* and *Bacteroides fragilis*. Carumonam was resistant to hydrolysis by 12 plasmid-mediated  $\beta$ -lactamases and 7 chromosomal  $\beta$ -lactamases. It was more stable than aztreonam to hydrolysis by the  $\beta$ -lactamase of *K. oxytoca*; this stability is related to the superiority of the in vitro and in vivo activities of carumonam to those of aztreonam against this species. In general, the protective activities (50% effective dose) of carumonam and reference antibiotics in mice with experimental intraperitoneal infections correlated with the in vitro activities (MIC); carumonam showed excellent protective activity against most aerobic gram-negative bacteria.

Sulfazecin is a unique *N*-sulfonated monocyclic  $\beta$ -lactam antibiotic discovered in the culture broth of *Pseudomonas acidophila* sp. nov. (3). The *N*-sulfo-2-azetidione nucleus of the sulfazecin molecule was found to be the fundamental structure accounting for the low antibacterial activity of sulfazecin. However, modifications at positions 3 and 4 of this ring altered the antibacterial properties of sulfazecin and sometimes resulted in potent antibacterial compounds with good  $\beta$ -lactamase stability (M. Ochiai, Abstr. 4th Symp. Med. Chem. Tokyo, p. 9, 1982). Of the sulfazecin-related compounds chemically synthesized, carumonam (AMA-1080, Ro 17-2301), (3*S*,4*S*)-3-[2-(2-aminothiazol-4-yl)-(Z)-2-carboxymethoxyiminoacetamido]-4-carbamoyloxy-methyl-2-azetidione-1-sulfonic acid (5) (Fig. 1), was selected for further development, and its in vitro and in vivo activities, affinity for penicillin-binding proteins (PBPs), and interaction with  $\beta$ -lactamases were compared with those of another sulfazecin-type antibiotic, aztreonam (13), and third-generation cephalosporins, cefoperazone (7) and ceftazidime (10). Cefsulodin (14) was also used for evaluating antipseudomonal activity. This work has been presented previously (M. Kondo, S. Kishimoto, M. Ochiai, K. Okonogi, and A. Imada, Proc. 13th Int. Congr. Chemother., p. 56/1-56/5, 1983; K. Okonogi and M. Kuno, Program Abstr. 23rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 578, 1983).

### MATERIALS AND METHODS

**Antibiotics.** Carumonam (dipolar ionic crystals, 976  $\mu\text{g/mg}$ ) and ceftazidime (dipolar ionic crystalline powder, 783  $\mu\text{g/mg}$ ) were prepared here, and aztreonam (dipolar ionic powder, 915  $\mu\text{g/mg}$ ) was prepared at Hoffmann-La Roche Inc., Nut-

ley, N.J. Cefsulodin (960  $\mu\text{g/mg}$ ; Takeda Chemical Industries, Ltd.), cefoperazone (947  $\mu\text{g/mg}$ ; Toyama Chemical Co., Ltd.), and benzylpenicillin (1,622 U/mg; Meiji Seika Kaisha, Ltd.) were obtained from commercial sources.

**Organisms.** Most of the clinical isolates (kindly supplied by several clinical laboratories) and laboratory strains were maintained on Mueller-Hinton agar (MHA; BBL Microbiology Systems), *Streptococcus* spp. were maintained on MHA supplemented with 10% bovine blood, and *Haemophilus influenzae* was maintained on brain heart infusion agar (Difco Laboratories) supplemented with 5% Fildes enrichment (Difco). Anaerobic bacteria were maintained on GAM semiliquid medium (Nissui). Strains of *Escherichia coli* and *Klebsiella pneumoniae* not inhibited by cefazolin at a concentration of 50  $\mu\text{g/ml}$ , strains of *Serratia marcescens* not inhibited by gentamicin at 25  $\mu\text{g/ml}$ , and strains of *Pseudomonas aeruginosa* not inhibited by sulbenicillin at 400  $\mu\text{g/ml}$  by the agar dilution method with an inoculum of  $10^5$  CFU were defined as resistant. R plasmids and their host strains, *E. coli* J53-2 and *P. aeruginosa* PU21, were generous gifts from G. A. Jacoby (4).

**Determination of MIC by the microbroth dilution method.**

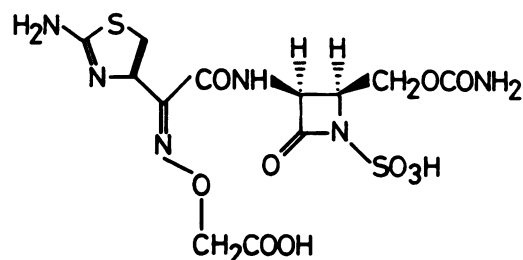


FIG. 1. Structure of carumonam (AMA-1080, Ro 17-2301).

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TABLE 1. Comparative activities of carumonam and reference antibiotics against clinical isolates

Organism (no. of strains)	Antibiotic	MIC ( $\mu\text{g/ml}$ ) <sup>a</sup>		
		Range	50%	90%
<i>Staphylococcus aureus</i> (50)	Carumonam	>100	>100	>100
	Aztreonam	>100	>100	>100
	Cefoperazone	0.78–6.25	1.56	3.13
	Ceftazidime	6.25–50	12.5	12.5
<i>Streptococcus pneumoniae</i> <sup>b</sup> (34)	Carumonam	3.13–25	12.5	25
	Aztreonam	50–>100	>100	>100
	Cefoperazone	0.05–0.2	0.1	0.2
	Ceftazidime	0.1–0.39	0.2	0.39
<i>Escherichia coli</i> (cefazolin sensitive) (103)	Carumonam	0.025–0.78	0.1	0.2
	Aztreonam	0.013–0.78	0.1	0.2
	Cefoperazone	0.006–12.5	0.39	3.13
	Ceftazidime	0.05–0.78	0.2	0.39
<i>Escherichia coli</i> (cefazolin resistant) (67)	Carumonam	0.05–12.5	0.1	3.13
	Aztreonam	0.1–50	0.2	6.25
	Cefoperazone	0.1–100	6.25	25
	Ceftazidime	0.2–50	0.39	3.13
<i>Citrobacter freundii</i> (104)	Carumonam	0.025–50	0.2	25
	Aztreonam	0.013–100	0.2	50
	Cefoperazone	0.025–>100	0.39	50
	Ceftazidime	0.05–>100	0.78	100
<i>Klebsiella pneumoniae</i> (cefazolin sensitive) (90)	Carumonam	0.013–0.2	0.1	0.2
	Aztreonam	0.013–0.39	0.1	0.2
	Cefoperazone	0.1–25	0.2	3.13
	Ceftazidime	0.05–1.56	0.39	0.78
<i>Klebsiella pneumoniae</i> (cefazolin resistant) (44)	Carumonam	0.05–6.25	0.1	0.39
	Aztreonam	0.05–>100	0.39	6.25
	Cefoperazone	0.1–>100	12.5	100
	Ceftazidime	0.2–25	0.78	6.25
<i>Klebsiella oxytoca</i> (42)	Carumonam	0.025–25	0.05	0.2
	Aztreonam	0.025–>100	1.56	50
	Cefoperazone	0.2–>100	12.5	>100
	Ceftazidime	0.1–100	0.2	0.78
<i>Enterobacter cloacae</i> (97)	Carumonam	0.025–100	0.1	6.25
	Aztreonam	0.025–100	0.2	25
	Cefoperazone	0.025–>100	0.39	25
	Ceftazidime	0.1–>100	0.39	25
<i>Serratia marcescens</i> (gentamicin sensitive) (102)	Carumonam	0.025–12.5	0.1	0.2
	Aztreonam	0.025–25	0.2	0.39
	Cefoperazone	0.1–>100	0.78	12.5
	Ceftazidime	0.05–50	0.39	0.78
<i>Serratia marcescens</i> (gentamicin resistant) (89)	Carumonam	0.05–12.5	0.39	3.13
	Aztreonam	0.05–25	0.78	6.25
	Cefoperazone	0.1–>100	50	>100
	Ceftazidime	0.1–50	0.78	3.13
<i>Proteus vulgaris</i> (93)	Carumonam	0.003–0.1	0.025	0.05
	Aztreonam	0.006–0.2	0.013	0.025
	Cefoperazone	0.2–25	1.56	3.13
	Ceftazidime	0.025–1.56	0.1	0.2
<i>Proteus mirabilis</i> (94)	Carumonam	0.006–0.025	0.013	0.025
	Aztreonam	0.006–0.025	0.013	0.013
	Cefoperazone	0.39–6.25	0.78	1.56
	Ceftazidime	0.025–0.2	0.1	0.1

TABLE 1—Continued

Organism (no. of strains)	Antibiotic	MIC ( $\mu\text{g/ml}$ ) <sup>a</sup>		
		Range	50%	90%
<i>Proteus morgani</i> (102)	Carumonam	0.025–3.13	0.05	0.78
	Aztreonam	0.006–3.13	0.025	0.39
	Cefoperazone	0.1–50	0.78	12.5
	Ceftazidime	0.05–12.5	0.1	3.13
<i>Proteus rettgeri</i> (97)	Carumonam	0.003–0.05	0.013	0.013
	Aztreonam	0.003–0.2	0.013	0.025
	Cefoperazone	0.2–50	1.56	6.25
	Ceftazidime	0.025–1.56	0.1	0.39
<i>Proteus inconstans</i> (32)	Carumonam	0.013–0.05	0.025	0.025
	Aztreonam	0.006–0.05	0.013	0.025
	Cefoperazone	0.2–50	1.56	25
	Ceftazidime	0.05–1.56	0.2	0.78
<i>Pseudomonas aeruginosa</i> (sulbenicillin sensitive) (83)	Carumonam	0.2–25	3.13	12.5
	Aztreonam	0.39–50	6.25	12.5
	Cefoperazone	0.78–>100	3.13	25
	Ceftazidime	0.2–25	1.56	6.25
	Cefsulodin	0.2–25	3.13	6.25
<i>Pseudomonas aeruginosa</i> (sulbenicillin resistant) (69)	Carumonam	0.78–100	3.13	12.5
	Aztreonam	0.78–>100	6.25	25
	Cefoperazone	1.56–>100	50	100
	Ceftazidime	0.78–12.5	3.13	6.25
	Cefsulodin	3.13–>100	25	100
<i>Haemophilus influenzae</i> <sup>b</sup> (75)	Carumonam	0.1–0.39	0.1	0.2
	Aztreonam	0.025–0.2	0.1	0.1
	Cefoperazone	0.006–0.1	0.025	0.1
	Ceftazidime	0.05–0.2	0.1	0.2

<sup>a</sup> Determined by the microbroth dilution method with Mueller-Hinton broth (inoculum, ca.  $10^4$  CFU/ml). MICs are given for 50 and 90% of strains as indicated.

<sup>b</sup> Test medium was supplemented with 10% horse serum for *S. pneumoniae* and 5% Fildes enrichment for *H. influenzae*.

Portions (0.1 ml) of Mueller-Hinton broth (BBL) containing twofold serial dilutions of each antibiotic were dispensed into 96-well (8 by 12) microtiter plates by using a 96-channel dispenser (Dynatech Laboratories, Inc.). Bacterial suspensions ( $1.5 \mu\text{l}$ ) containing about  $10^3$  CFU were delivered to the wells with an automatic inoculator (Dynatech). The plates were incubated overnight at  $37^\circ\text{C}$ , and the optical density of each well was measured with a Titertek Multiskan (Flow Laboratories, Inc.) as described by Genta et al. (1). Growth was defined as positive when the optical density was 0.050 or more and negative when less than 0.050. The MIC was defined as the lowest concentration of antibiotic causing negative bacterial growth.

**Determination of MIC by the agar dilution method.** Approximately  $5 \mu\text{l}$  of a bacterial suspension containing  $5 \times 10^3$  or  $5 \times 10^5$  CFU was inoculated into MHA containing twofold serial dilutions of each antibiotic. The activity against anaerobic bacteria was examined on GAM agar (Nissui) by using an inoculum of  $10^5$  CFU prepared from a GAM broth (Nissui) culture incubated for 48 h. The plates were incubated at  $37^\circ\text{C}$  for 18 h, and the MIC was taken as the lowest concentration of the drug that inhibited the visible bacterial growth.

**Assay of PBPs.** Logarithmically grown cells in Trypticase soy broth (BBL) with shaking (aerobes) or in GAM broth (Nissui) without shaking (anaerobes) were harvested by centrifugation and disintegrated by grinding with alumina for *Staphylococcus aureus* or by sonication for the other organ-

TABLE 2. Affinities of carumonam and aztreonam for PBPs

Orga- nism	PBP No.	I <sub>50</sub> <sup>a</sup> (μg/ml)		Orga- nism	PBP No.	I <sub>50</sub> (μg/ml)		Orga- nism	PBP No.	I <sub>50</sub> (μg/ml)	
		CAM <sup>b</sup>	AZT			CAM	AZT			CAM	AZT
<i>S. aureus</i> FDA 209P	1	294	124	<i>K. pneumoniae</i> No. 13	1	>40	>40	<i>P. vulgaris</i> IFO 3988	1a	2.51	0.99
	2	291	125		2	>40	>40		1b	>40	>40
3	>400	275	3		0.059	0.095	2		>40	>40	
*	>400	282	4		>40	>40	3		0.045	0.067	
4	>400	>400	5/6		>40	>40	4		>40	>40	
MIC <sup>c</sup>		>100	>100		MIC	0.05	0.05		5/6	>40	>40
								MIC	0.025	0.013	
<i>E. coli</i> NIHJ JC-2	1a	2.6	0.33	<i>E. cloacae</i> IFO 12937	1a	12.1	0.77	<i>P. aeruginosa</i> P9	1a	10.4	2.76
	1b	>40	>40		1b	>40	21.1		1b	4.42	4.58
2	>40	>40	2		>40	>40	2		>40	>40	
3	0.06	0.02	3		0.045	0.05	3		0.031	0.010	
4	>40	>40	4		>40	>40	4		>40	>40	
5/6	>40	>40	5/6		>40	>40	5/6		>40	>40	
MIC		0.05	0.1	MIC	0.05	0.1	MIC	0.78	1.56		
<i>C. freundii</i> IFO 12681	1	>40	9.66	<i>S. marcescens</i> IFO 12648	1a	19.6	5.13	<i>B. fragilis</i> ATCC 25285	1	90.1	16.0
	2	>40	>40		1b	10.3	12.5		1'	66.4	12.0
3	0.24	0.13	1c		12.4	17.6	2		16.4	6.26	
4	>40	>40	2		>40	>40	3		>400	>400	
5/6	>40	>40	3		0.069	0.034	4		158	>400	
MIC		0.05	0.05		4	>40	>40		MIC	12.5	>100
				5/6	>40	>40					
				MIC	0.05	0.1					

<sup>a</sup> I<sub>50</sub>, Concentration required to inhibit [<sup>14</sup>C] benzylpenicillin binding by 50%.

<sup>b</sup> Abbreviations: CAM, carumonam; AZT, aztreonam.

<sup>c</sup> MICs (in micrograms per milliliter) were determined by the agar dilution method on MHA with an inoculum of about 10<sup>3</sup> CFU except for the MIC determination of *B. fragilis*, which was performed in GAM broth with an inoculum of 10<sup>4</sup> CFU/ml.

isms. Competitive binding affinities of the antibiotics for PBPs were measured by the method of Nozaki et al. (9). The binding affinities of antibiotics for each PBP were expressed in terms of the concentration (in micrograms per milliliter) required to prevent [<sup>14</sup>C]benzylpenicillin binding by 50% (I<sub>50</sub>).

**Sources and assay of β-lactamases.** The purification of the β-lactamases of *Enterobacter cloacae* TN1282, *Citrobacter freundii* GN1706, *P. aeruginosa* U31, *S. marcescens* TN81, *Proteus vulgaris* GN4413, *Bacteroides fragilis* V284-3, *S. aureus* 1840, *E. coli* TN713 (TEM-1), *E. coli* TN649 (OXA-1), and *P. aeruginosa* GN3407 (PSE-1) has been described (11). The enzyme of *Klebsiella oxytoca* TN1719 was purified by the procedure used for the purification of the enzyme of *S. marcescens* TN81: carboxymethyl cellulose column chromatography and Sephadex G-100 gel filtration. Plasmid-mediated β-lactamases were extracted from *E. coli* J53-2 and *P. aeruginosa* PU21. Each of the enzyme preparations contained only one kind of β-lactamase, as indicated by iso-electric focusing (15).

The hydrolysis rates of carumonam and aztreonam were measured microbiologically and spectrophotometrically as described below, and the hydrolysis rates of the remaining antibiotics were measured microiodometrically as described previously (11).

One unit of β-lactamase was defined as the amount of enzyme that hydrolyzed 1 μmol of benzylpenicillin (penicillinase) or cephaloridine (cephalosporinase) per min at 30°C.

**(i) Microbiological method.** The reaction mixture (0.5 ml), containing 0.2 mM monocyclic β-lactam antibiotic, 50 mM phosphate buffer (pH 6.9), and an appropriate amount of the β-lactamase, was incubated at 30°C. At intervals, 25-μl portions of the reaction mixture were withdrawn and added to 475 μl of methanol to stop the reaction. The amount of residual antibiotic was determined by the paper disk method with *E. coli* LD-2 as the test organism.

**(ii) Spectrophotometric method.** The reaction mixture (2 ml), containing 0.2 mM substrate, 50 mM phosphate buffer (pH 6.9), and an appropriate amount of the β-lactamase, was incubated at 30°C in a Gilford spectrophotometer, and the

TABLE 3. Stabilities of carumonam and reference antibiotics to hydrolysis by penicillinases

Source or type of enzyme	Relative rate of hydrolysis <sup>a</sup>			
	Carumonam	Aztreonam	Cefoperazone	Ceftazidime
<i>Klebsiella oxytoca</i> TN1719 (IVb) <sup>b</sup>	<0.01	4.33	1.55	<0.01
<i>Staphylococcus aureus</i> 1840	<0.01	<0.01	0.19	<0.01
TEM-1	<0.01	<0.01	11.2	<0.01
TEM-2	<0.01	0.02	12.0	<0.01
HMS-1	0.05	0.06	11.5	<0.05
SHV-1	0.02	0.08	10.4	<0.01
OXA-1	0.08	0.30	8.10	<0.05
OXA-2	<0.02	<0.02	15.4	0.04
OXA-3	<0.02	0.04	8.16	<0.03
PSE-1	<0.01	<0.01	0.38	<0.05
PSE-2	0.63	1.24	17.6	<0.1
PSE-3	0.16	0.15	0.09	<0.3
PSE-4	0.01	<0.01	0.31	<0.01

<sup>a</sup> The activity was determined by using 0.2 mM preparations of each substrate and was expressed as the relative rate of hydrolysis, taking the hydrolysis rate for benzylpenicillin as 100.

<sup>b</sup> Classification by Richmond and Sykes (12).

decrease in optical density at 215 or 320 nm was recorded. The differences in molar extinction coefficient ( $\Delta\epsilon$ ,  $M^{-1}cm^{-1}$ ) between the antibiotics and the corresponding hydrolyzed compounds at 215 and 320 nm, respectively, were as follows: carumonam, 1,340 and 420; aztreonam, 1,320 and 490.

**Protective effect.** Organisms were cultured overnight at 37°C in brain heart infusion and suspended in 5% mucin (Difco). Four-week-old male Slc:ICR mice weighing 19 to 23 g were infected intraperitoneally with 0.5 ml of the bacterial suspension. The challenge doses contained about 30 to 100 times the number of bacteria required to kill 50% of the control mice. Five mice at each dose level were individually given 0.2 ml of an antibiotic solution subcutaneously. Mice infected with *P. aeruginosa* were given antibiotics at 0, 2, and 4 h, and mice infected with other bacteria were given antibiotics at 0 h after infection. All experiments were repeated at least five times. The 50% effective dose (in milligrams per kilogram) was calculated by the probit method (6) from the survival rate recorded on day 5 after infection.

## RESULTS

**In vitro antibacterial activity.** The MICs of carumonam against 1,467 clinical isolates were determined by the microbroth dilution method in Mueller-Hinton broth and compared with the MICs of aztreonam, cefoperazone, ceftazidime, and cefsulodin (Table 1). Carumonam and aztreonam were inactive against *S. aureus*. Carumonam showed some activity against *Streptococcus pneumoniae*; it was more active than aztreonam but less active than the reference cephalosporins. The MICs of carumonam required to inhibit 90% (MIC<sub>90</sub>) of such *Enterobacteriaceae* organisms as cefazolin-sensitive *E. coli* and *K. pneumoniae*, cefazolin-resistant *K. pneumoniae*, *K. oxytoca*, gentamicin-sensitive *S. marcescens*, and indole-positive and -negative *Proteus* spp. were 0.78  $\mu\text{g/ml}$  or less. These values were usually smaller than the MIC<sub>90</sub>s of the reference antibiotics, especially cefoperazone. The activities of carumonam and aztreonam were almost comparable, but a big difference was observed in their activities against *K. oxytoca*, which produces Richmond's type IVb  $\beta$ -lactamase in large quantities (K.

Okonogi and M. Kuno, Program Abstr. 23rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 325, 1983). Carumonam was more effective than all of the reference antibiotics against cefazolin-resistant *K. pneumoniae* and more effective than ceftazidime against indole-negative and -positive *Proteus* spp. Against other *Enterobacteriaceae* organisms, the MIC<sub>90</sub>s of carumonam were 3.13  $\mu\text{g/ml}$  or more as follows: cefazolin-resistant *E. coli*, 3.13  $\mu\text{g/ml}$ ; *C. freundii*, 25  $\mu\text{g/ml}$ ; *E. cloacae*, 6.25  $\mu\text{g/ml}$ ; and gentamicin-resistant *S. marcescens*, 3.13  $\mu\text{g/ml}$ . These values were equal to or smaller than the MIC<sub>90</sub>s of aztreonam and ceftazidime. The MIC<sub>90</sub> of carumonam for 83 sulbenicillin-sensitive and 69 sulbenicillin-resistant *P. aeruginosa* strains was 12.5  $\mu\text{g/ml}$ , which was comparable to the MIC<sub>90</sub>s of aztreonam and ceftazidime. More than 90% of 75 strains of *H. influenzae* were inhibited at a carumonam concentration of 0.2  $\mu\text{g/ml}$ . This value was comparable to the MICs of aztreonam, cefoperazone, and ceftazidime. The MICs of carumonam against laboratory strains, as determined by the agar dilution method with  $5 \times 10^3$  CFU of bacteria as inocula, were as follows: *Pseudomonas cepacia* E-34, 3.13  $\mu\text{g/ml}$ ; *Pseudomonas maltophilia* IFO 14161, 3.13  $\mu\text{g/ml}$ ; *Vibrio cholerae* Inaba, 0.78  $\mu\text{g/ml}$ ; *Flavobacterium meningosepticum* IFO 12535, >100  $\mu\text{g/ml}$ ; *Achromobacter xylosoxidans* IID1344, >100  $\mu\text{g/ml}$ ; and *Acinetobacter calcoaceticus* IFO 13006, 12.5  $\mu\text{g/ml}$ .

Carumonam was active against a limited number of species of gram-positive and gram-negative anaerobes. The only species that showed MICs less than 12.5  $\mu\text{g/ml}$  with an inoculum of  $10^5$  CFU were *Peptococcus aerogenes*, *Bacteroides fragilis* subsp. *vulgatus*, *Bacteroides praecutis*, *Fusobacterium nucleatum*, and *Fusobacterium russii*; more than 20 other species of anaerobes showed MICs of 100  $\mu\text{g/ml}$  or more (data not shown).

**Affinity for PBPs.** The affinity profiles of carumonam and aztreonam for PBPs of various bacteria are summarized in Table 2. Both antibiotics showed high affinities for PBP-3 of the family *Enterobacteriaceae*. The I<sub>50</sub>s for PBP-3 were of the same order as the respective MICs. The two monocyclic  $\beta$ -lactam antibiotics showed weak affinity for part or all of the PBP-1; the I<sub>50</sub>s were far higher than those for PBP-3. PBP-2, PBP-4, and PBP-5/6 were insensitive to both antibiotics up to the highest tested concentration of 40 or 400

TABLE 4. Stabilities of carumonam and reference antibiotics to hydrolysis by cephalosporinases

Source of enzyme (type) <sup>a</sup>	Relative rate of hydrolysis <sup>b</sup>			
	Carumonam	Aztreonam	Cefoperazone	Ceftazidime
<i>Enterobacter cloacae</i> TN1282 (1a)	<0.01	<0.01	2.94	0.02
<i>Citrobacter freundii</i> GN1706 (1a)	<0.01	<0.01	1.67	<0.01
<i>Pseudomonas aeruginosa</i> U31 (1d)	<0.1	<0.1	4.30	<0.1
<i>Serratia marcescens</i> TN81 (1)	<0.01	<0.01	11.6	0.03
<i>Proteus vulgaris</i> GN4413 (1c)	<0.01	0.66	10.5	0.08
<i>Bacteroides fragilis</i> V284-3	0.07	1.01	29.1	4.62

<sup>a</sup> Classification by Richmond and Sykes (12).

<sup>b</sup> The activity was determined by using 0.2 mM preparations of each substrate and was expressed as the relative rate of hydrolysis, taking the hydrolysis rate for cephaloridine as 100.

TABLE 5. Antibacterial activities of carumonam and reference antibiotics against  $\beta$ -lactamase-producing bacteria<sup>a</sup>

Organism	Type of $\beta$ -lactamase <sup>b</sup>	MIC ( $\mu$ g/ml) of:			
		Carumonam	Aztreonam	Cefoperazone	Ceftazidime
<i>Citrobacter freundii</i> GN1706	Ia	3.13	6.25	25	50
<i>Escherichia coli</i> GN206	Ib	6.25	3.13	0.39	1.56
<i>Proteus vulgaris</i> GN4413	Ic	0.1	0.1	1.56	0.1
<i>Pseudomonas aeruginosa</i> U31	Id	12.5	6.25	50	12.5
<i>Escherichia coli</i> T7	III (TEM)	0.2	0.39	>100	0.78
<i>Escherichia coli</i> TN713	III (TEM)	0.05	0.05	12.5	0.2
<i>Klebsiella oxytoca</i> TN1711	IVb	0.1	25	>100	0.39
<i>Escherichia coli</i> TN649	Va (OXA)	0.1	0.1	0.39	0.39
<i>Pseudomonas aeruginosa</i> GN3407	Vc (PSE)	6.25	6.25	50	6.25
<i>Escherichia coli</i> J53-2 (R plasmids)					
R <sup>-</sup>		0.025	0.025	0.025	0.1
R1	TEM-1	0.05	0.05	0.39	0.2
RP4	TEM-2	0.05	0.05	>100	0.2
R997	HMS-1	0.05	0.05	>100	0.2
R1010	SHV-1	0.05	0.05	12.5	0.39
RGN238	OXA-1	0.1	0.05	0.2	0.2
R46	OXA-2	0.05	0.05	0.2	0.39
R57b	OXA-3	0.1	0.1	0.78	0.2
<i>Pseudomonas aeruginosa</i> PU21 (R plasmids)					
R <sup>-</sup>		3.13	6.25	6.25	1.56
R2	TEM-1	6.25	12.5	>100	3.13
RP1	TEM-2	6.25	12.5	>100	1.56
pMG90	OXA-1	12.5	12.5	25	3.13
RIP64	OXA-3	6.25	6.25	100	25
RPL11	PSE-1	12.5	12.5	100	3.13
R151	PSE-2	6.25	12.5	>100	3.13
Rms149	PSE-3	6.25	6.25	25	3.13
pMG19	PSE-4	6.25	6.25	100	3.13

<sup>a</sup> Determined by the agar dilution method by using MHA with an inoculum of about  $10^5$  CFU.

<sup>b</sup> The types of  $\beta$ -lactamases of clinical isolates (the first nine organisms) were indicated by the classification by Richmond and Sykes (12) and those of the R plasmids, which were introduced into *E. coli* J53-2 and *P. aeruginosa* PU21, were indicated by the classification of Matthew (8).

$\mu$ g/ml. Carumonam and aztreonam also showed high affinities for PBP-3 of *P. aeruginosa* P9. The  $I_{50}$ s, however, were far smaller than the respective MICs. PBP-1 bound both antibiotics to some extent, but less efficiently than PBP-3. The PBPs of *S. aureus* FDA 209P and *B. fragilis* ATCC 25285 showed little affinity to carumonam and aztreonam.

**Interaction with  $\beta$ -lactamases and activity against  $\beta$ -lactamase-producing bacteria.** The stabilities of carumonam and the reference antibiotics to hydrolysis by penicillinases (Table 3) and cephalosporinases (Table 4) were determined.

The rates of hydrolysis of carumonam and aztreonam were determined spectrophotometrically, microbiologically, or by both methods. The two methods gave almost the same results; for example, the rates of hydrolysis (micromoles per minute per milliliter of enzyme preparation) of carumonam and aztreonam by 3,150 U of the *P. vulgaris* GN4413  $\beta$ -lactamases per ml were, respectively, 0.134 and 16.9 in the spectrophotometric assay (320 nm) and 0.103 and 20.8 in the microbiological assay. The penicillinase of *K. oxytoca* TN1719 is a chromosomal enzyme, whereas the other penicillinases are plasmid mediated. The cephalosporinases are chromosomal enzymes.

Carumonam was stable to  $\beta$ -lactamases, although it was hydrolyzed slightly by several plasmid-mediated  $\beta$ -lactamases and the enzyme of *B. fragilis* V284-3. Aztreonam was less stable than carumonam to several  $\beta$ -lactamases, such as those of *K. oxytoca*, *P. vulgaris*, and *B. fragilis*.

Carumonam was more stable to cephalosporinases, but was slightly less stable than ceftazidime to some penicillinases.

Table 5 shows the antibacterial activities of carumonam and reference antibiotics against  $\beta$ -lactamase-producing bacteria. The superiority of carumonam to aztreonam was seen in its activity against type IVb  $\beta$ -lactamase-producing *K. oxytoca*, and its superiority to cefoperazone was seen in its activity against many  $\beta$ -lactamase-producing bacteria. Against strains of *E. coli* and *P. aeruginosa* harboring  $\beta$ -lactamase plasmids, carumonam, like aztreonam and ceftazidime, showed an activity similar to that observed against the plasmid-free host bacteria. The activity of cefoperazone against many bacteria was impaired by the presence of the  $\beta$ -lactamase plasmids. These results are consistent with the stability of the antibiotics to  $\beta$ -lactamases, as shown in Tables 3 and 4. Since there was a large difference in the activities of carumonam and aztreonam against *K. oxytoca* TN1711, we compared their activities against an additional 26 strains of clinically isolated *K. oxytoca* possessing various levels of  $\beta$ -lactamase activity. Carumonam showed potent activity against all of these strains, whereas aztreonam showed weak activity against strains with high levels of  $\beta$ -lactamases; the two antibiotics showed comparable activities when the activity of the penicillinase was below 0.1 U/mg (dry weight), but aztreonam was 16 to 500 times less active than carumonam when the penicillinase level was greater than 1 U/mg (dry weight) of cells.

**In vivo antibacterial activity.** The protective effect of

TABLE 6. Protective effects of carumonam and reference antibiotics against experimental intraperitoneal infection in mice

Organism (challenge dose, CFU per animal)	Antibiotic	ED <sub>50</sub> (mg/kg) <sup>a</sup>	MIC <sup>b</sup> (μg/ml)
<i>Escherichia coli</i> O111 (10 <sup>6</sup> )	Carumonam	0.021 (0.017–0.024)	0.013
	Aztreonam	0.020 (0.016–0.023)	0.013
	Cefoperazone	0.019 (0.015–0.023)	0.013
	Ceftazidime	0.038 (0.030–0.047)	0.05
<i>Escherichia coli</i> T7 (10 <sup>4</sup> )	Carumonam	0.144 (0.125–0.166)	0.1
	Aztreonam	0.352 (0.297–0.417)	0.2
	Cefoperazone	66.7 (55.0–81.4)	25
	Ceftazidime	0.358 (0.296–0.430)	0.39
<i>Citrobacter freundii</i> TN518 (10 <sup>6</sup> )	Carumonam	0.058 (0.050–0.066)	0.1
	Aztreonam	0.165 (0.136–0.202)	0.1
	Cefoperazone	0.339 (0.282–0.409)	0.39
	Ceftazidime	0.218 (0.181–0.261)	0.39
<i>Klebsiella pneumoniae</i> DT (10 <sup>3</sup> )	Carumonam	0.056 (0.045–0.067)	0.025
	Aztreonam	0.047 (0.037–0.057)	0.013
	Cefoperazone	0.797 (0.570–1.108)	0.025
	Ceftazidime	0.037 (0.029–0.046)	0.05
<i>Klebsiella oxytoca</i> TN1711 (10 <sup>6</sup> )	Carumonam	0.067 (0.056–0.080)	0.025
	Aztreonam	10.7 (8.84–12.9)	3.13
	Cefoperazone	313 (251–403)	50
	Ceftazidime	0.136 (0.109–0.168)	0.1
<i>Enterobacter cloacae</i> TN618 (10 <sup>5</sup> )	Carumonam	0.053 (0.047–0.061)	0.1
	Aztreonam	0.059 (0.051–0.069)	0.1
	Cefoperazone	0.093 (0.075–0.118)	0.1
	Ceftazidime	0.109 (0.093–0.128)	0.2
<i>Serratia marcescens</i> TN66 (10 <sup>3</sup> )	Carumonam	0.110 (0.091–0.134)	0.05
	Aztreonam	0.112 (0.091–0.136)	0.05
	Cefoperazone	2.35 (1.86–2.95)	0.78
	Ceftazidime	0.166 (0.141–0.198)	0.2
<i>Proteus vulgaris</i> GN4712 (10 <sup>3</sup> )	Carumonam	0.282 (0.215–0.377)	0.013
	Aztreonam	0.099 (0.077–0.130)	0.006
	Cefoperazone	9.16 (7.05–13.0)	0.78
	Ceftazidime	0.281 (0.185–0.391)	0.05
<i>Proteus mirabilis</i> IFO 3849 (10 <sup>6</sup> )	Carumonam	0.132 (0.106–0.163)	0.025
	Aztreonam	0.073 (0.058–0.090)	0.013
	Cefoperazone	2.42 (1.78–3.38)	1.56
	Ceftazidime	0.111 (0.082–0.143)	0.2
<i>Pseudomonas aeruginosa</i> U31 (10 <sup>6</sup> )	Carumonam	15.8 (13.8–18.1)	6.25
	Aztreonam	21.8 (18.4–25.9)	6.25
	Cefoperazone	137 (114–166)	25
	Ceftazidime	9.79 (8.25–11.6)	6.25
	Cefsulodin	5.03 (4.25–5.95)	0.78
<i>Pseudomonas aeruginosa</i> P9 (10 <sup>3</sup> )	Carumonam	1.96 (1.66–2.34)	0.78
	Aztreonam	5.67 (4.69–6.91)	1.56
	Cefoperazone	10.8 (9.09–12.8)	3.13
	Ceftazidime	1.41 (1.16–1.71)	0.78
	Cefsulodin	0.855 (0.717–1.020)	0.78

carumonam in mice infected intraperitoneally with several strains of gram-negative bacteria was compared with the protective effects of aztreonam, cefoperazone, ceftazidime, and cefsulodin (Table 6). In mice infected with two strains of *E. coli*, the protective effect of carumonam was similar to or slightly superior to that of the other antibiotics. Carumonam was much more potent than aztreonam against infection with *K. oxytoca* TN1711. Carumonam was more active than

TABLE 6—Continued

Organism (challenge dose, CFU per animal)	Antibiotic	ED <sub>50</sub> (mg/kg) <sup>a</sup>	MIC <sup>b</sup> (μg/ml)
<i>Pseudomonas aeruginosa</i> GN3407 (10 <sup>5</sup> )	Carumonam	6.80 (5.90–7.84)	3.13
	Aztreonam	18.3 (14.8–22.8)	6.25
	Cefoperazone	439 (355–542)	50
	Ceftazidime	3.71 (3.11–4.46)	3.13
	Cefsulodin	39.2 (32.7–47.5)	25

<sup>a</sup> Antibiotics were administered subcutaneously immediately after infection by *Enterobacteriaceae* organisms and 0, 2, and 4 h after infection by *P. aeruginosa*. The 50% effective doses (ED<sub>50</sub>s) were determined as described in the text. The 95% confidence limits are given in parentheses.

<sup>b</sup> MIC was determined by the agar dilution method on MHA by using an inoculum of about 10<sup>3</sup> CFU.

aztreonam in mice infected with *C. freundii* TN518, but it was slightly less active than aztreonam in mice infected with *Proteus* spp. In mice infected with three strains of *P. aeruginosa*, carumonam had an excellent protective effect; carumonam was significantly more effective than cefoperazone, slightly more effective than aztreonam, and slightly less effective than ceftazidime. Carumonam was slightly less effective than cefsulodin against two of three *P. aeruginosa* strains, but it was significantly more effective against strain GN3407, which produced PSE-1 β-lactamase (see Table 5).

## DISCUSSION

Carumonam, a new synthetic *N*-sulfonated monocyclic β-lactam antibiotic, exerts a potent antibacterial activity against a wide range of aerobic gram-negative bacteria including members of the family *Enterobacteriaceae*, *P. aeruginosa*, and *H. influenzae*, but it is not active or only weakly active against gram-positive and anaerobic bacteria. Carumonam is as active as aztreonam and ceftazidime against most gram-negative bacteria, but it is superior to the reference antibiotics used against certain bacterial species. It was more potent than aztreonam against ceftazolin-resistant *K. pneumoniae*, *K. oxytoca*, and *E. cloacae* and more potent than ceftazidime against *K. pneumoniae*, *E. cloacae*, and *Proteus* spp. Carumonam was more potent than cefoperazone against most gram-negative bacteria.

The excellent activity of carumonam against gram-negative bacteria is related to its high affinity for their PBP-3. For the *Enterobacteriaceae* organisms, the I<sub>50</sub>s for carumonam were almost equivalent to the respective MICs. This was not true for *P. aeruginosa*, for which the MIC was much higher than the I<sub>50</sub> for PBP-3. This suggests that carumonam penetrates easily through the outer membrane of *Enterobacteriaceae* organisms but that it is restricted by the outer membrane in *P. aeruginosa*. The PBPs of *S. aureus* showed little affinity to carumonam. This fact might be related to the inactivity of carumonam against this organism. The affinity profile for PBPs of aztreonam was similar to that of carumonam, although aztreonam tended to show a higher affinity for PBP-1 in some species. Our results demonstrating the affinity of aztreonam for the PBPs of various bacteria are consistent with those of Georgopapadakou et al. (2). These investigators also suggested that aztreonam is inactive against *S. aureus* because it lacks affinity for the PBPs of this organism. Carumonam showed low affinities for the PBPs of this organism. *B. fragilis* ATCC 25285; its affinity levels were comparable to those of aztreonam. Georgopapadakou et al. (2) concluded that aztreonam is inactive against *B. fragilis* because it lacks affinity for the PBPs of this organism. However, this

may not be the only reason for this inactivity since carumonam, which has a similar affinity for the PBPs of the same organism, showed stronger activity than aztreonam. Other factors, such as hydrolysis by  $\beta$ -lactamases and the permeability barrier, should be taken into account. We demonstrated here that carumonam is more than 10 times more resistant than aztreonam to hydrolysis by the  $\beta$ -lactamase of *B. fragilis* (see Table 4). This difference might be reflected in the difference in the antibacterial activities of the two structurally related monocyclic  $\beta$ -lactam antibiotics.

Another factor that contributes to the excellent activity of carumonam against gram-negative bacteria is its resistance to  $\beta$ -lactamases; carumonam was more than 100 times more stable than benzylpenicillin to penicillinases and more than 1,000 times more stable than cephaloridine to cephalosporinases. Reflecting its good stability to  $\beta$ -lactamases, carumonam showed good antibacterial activity against  $\beta$ -lactamase-producing bacteria. Aztreonam showed similar stability to carumonam, except that the former was more readily hydrolyzed than the latter by the penicillinase of *K. oxytoca*; *K. oxytoca* strains with high  $\beta$ -lactamase activity were resistant to aztreonam and susceptible to carumonam. Cefoperazone, which is less stable than carumonam to  $\beta$ -lactamases, was less active than carumonam against many gram-negative bacteria.

Carumonam showed potent protective activity in mice intraperitoneally infected with gram-negative bacteria. The protective effect of carumonam and the reference antibiotics reflected their in vitro antibacterial activity. Carumonam was more potent than aztreonam in protecting against *K. oxytoca* and strains of *P. aeruginosa* and more potent than cefoperazone against many strains of the family *Enterobacteriaceae* and *P. aeruginosa*. The protective activities of carumonam and ceftazidime did not differ very much; the differences between the 50% effective doses were within fourfold.

The present results with carumonam are encouraging. This new sulfazecin-type antibiotic, like aztreonam and ceftazidime, has an expanded spectrum against gram-negative bacteria including members of the family *Enterobacteriaceae*, *Pseudomonas* spp., and *H. influenzae*. Moreover, carumonam is active against bacteria that are less susceptible to the other two antibiotics, making it a strong candidate for clinical evaluation.

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