

PC-904, a New Semisynthetic Penicillin

GERALD P. BODEY,* SUZANNE WEAVER, AND THERESA PAN

Department of Developmental Therapeutics, The University of Texas System Cancer Center M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Received for publication 31 May 1977

Sodium 6{D(-)- α (4-hydroxyl-1,5 naphthyridine-3-carboxamido)phenylacetamido} penicillanate (PC-904) is a new semisynthetic penicillin with broad-spectrum activity against gram-positive cocci and gram-negative bacilli. At a concentration of 1.56 $\mu\text{g/ml}$, it inhibited 100% of isolates of *Proteus mirabilis*, 89% of *Pseudomonas aeruginosa*, 67% of *Escherichia coli*, and 45% of *Enterobacter* spp. At a concentration of 12.5 $\mu\text{g/ml}$, it inhibited 75% of *Klebsiella* spp. and 67% of *Serratia marcescens*. PC-904 failed to inhibit the growth of gram-negative bacilli when large inocula were used. Some differences were noted when organisms were tested in different media or at different hydrogen ion concentrations. It is more active than mezlocillin, azlocillin, ticarcillin, carbenicillin, and amoxicillin against *E. coli*, *Klebsiella* spp., and *P. aeruginosa*.

Modifications of the penicillin molecule have led to important advances in antibiotic therapy. Ampicillin broadened the spectrum of penicillins to include some gram-negative bacilli, including *Escherichia coli* and *Proteus mirabilis*. The synthesis of carbenicillin resulted in a penicillin with antipseudomonal activity (1). Since then a variety of new semisynthetic penicillins has been investigated. Sodium 6 {D(-)- α (4-hydroxyl-1,5 naphthyridine-3-carboxamido)phenylacetamido} penicillanate (PC-904) is a new penicillin whose chemical structure is shown in Fig. 1. This report presents results of in vitro studies of PC-904 which indicate that it may be a potentially useful new antibiotic.

MATERIALS AND METHODS

Susceptibility tests were conducted on 496 clinical isolates of gram-negative bacilli and 113 clinical isolates of gram-positive cocci, using the dilution technique with an automatic microtiter system (Canalco, Autotiter instruction manual). All gram-negative bacilli and *Staphylococcus aureus* isolates to be tested were inoculated into Mueller-Hinton broth (Difco) and incubated at 37°C for 18 h. *Streptococcus pyogenes* and *S. pneumoniae* were incubated in tryptose phosphate broth. For gram-negative bacilli and *S. aureus*, a 0.05-ml sample of a 10^{-3} dilution of this broth culture (approximately 10^5 colony-forming units [CFU/ml]) was used as the inoculum. For other gram-positive cocci, a 0.05-ml sample of a 10^{-2} dilution of this broth culture (approximately 10^6 CFU/ml) was used as the inoculum for susceptibility testing.

All gram-negative bacilli used in this study were cultured from blood obtained from patients between 1971 and 1976. The patients were hospitalized at The M. D. Anderson Hospital and Tumor Institute

and had underlying malignant diseases. A total of 100 isolates each of *Pseudomonas aeruginosa*, *Klebsiella* spp., *Enterobacter* spp., and *E. coli*, 60 isolates of *Proteus* spp., and 36 isolates of *Serratia* spp. were used. All gram-positive cocci used in this study were cultured from specimens obtained from hospitalized patients, most of whom did not have cancer. A total of 36 isolates of *S. pyogenes*, 14 isolates of *S. pneumoniae*, and 48 isolates of *S. aureus* were used. The susceptibility of isolates of *S. aureus* to penicillin G was determined by the broth dilution method. Isolates inhibited by less than 0.10 $\mu\text{g/ml}$ were selected as penicillin G susceptible, and those isolates requiring more than 25 $\mu\text{g/ml}$ for inhibition of growth were selected as penicillin G resistant.

Organisms used for studies of the effect of inoculum size on the activity of PC-904 were incubated in Mueller-Hinton broth for 18 h at 37°C. It was assumed that approximately 10^8 CFU/ml were present after incubation, which was subsequently confirmed by subculturing 0.1-ml samples of sheep blood agar and performing colony counts after 24 h of incubation at 37°C. Serial 10-fold dilutions of the broth culture were made, using Mueller-Hinton broth, so that 10^7 and 10^5 CFU/ml were used as inocula. An inoculum of 10^6 CFU/ml was used in all other studies of gram-negative bacilli. Studies of the effect of pH on the activity of PC-904 were conducted in Mueller-Hinton broth, and pH values were adjusted to 6.4, 7.2, and 8.2 with phosphate buffer. Studies comparing the activity of PC-904 with those of mezlocillin, azlocillin, carbenicillin, ticarcillin, and amoxicillin were conducted in Mueller-Hinton broth. Fifty isolates each of *E. coli*, *Klebsiella* spp., *P. aeruginosa*, *Enterobacter* spp., and *P. mirabilis*, 10 isolates of indole-positive *Proteus* spp., and 36 isolates of *Serratia marcescens* were used. Isolates were selected so that organisms with differing susceptibilities to PC-904 were included.

PC-904 was supplied as a powder by Sumitomo

Chemical Co., Ltd., Hyogo, Japan. Mezlocillin and azlocillin were supplied as powders by Delbay Pharmaceuticals, Inc., Bloomfield, N.J. Carbenicillin, ticarcillin, and amoxicillin were supplied by Beecham Pharmaceuticals, Bristol, Tenn. Twofold serial dilutions of the antibiotics were made with Mueller-Hinton broth, and the minimal inhibitory concentration (MIC) was determined after incuba-

tion at 37°C for 18 h. All wells containing trace growth or no discernible growth were subcultured on sheep blood agar. A calibrated pipette was used to transfer 0.01 ml of the inoculum. The minimum bactericidal concentration (MBC) was determined after incubation at 37°C for 18 h. The MBC was defined as the lowest concentration of drug that yielded less than five colonies on subculture to sheep blood agar (99% kill). Comparative studies were conducted simultaneously, and all studies were performed in triplicate.

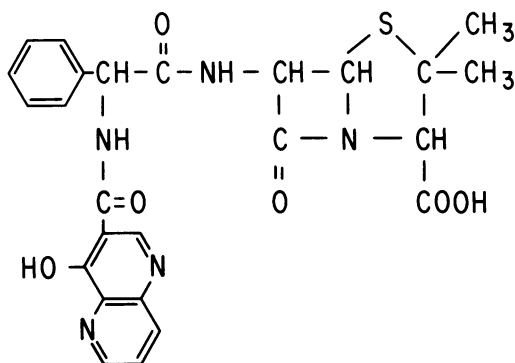


FIG. 1. Chemical structure of PC-904.

RESULTS

The *in vitro* activity of PC-904 against gram-positive cocci and gram-negative bacilli is shown in Fig. 2. *S. pyogenes* and *S. pneumoniae* were quite susceptible to this antibiotic, with 93 and 100% of isolates, respectively, inhibited by 0.012 $\mu\text{g}/\text{ml}$. Penicillin G-susceptible *S. aureus* were also susceptible, but PC-904 was not as active as penicillin G, all of these isolates were inhibited by 0.1 μg of penicillin G per ml, but less than 10% were inhibited by 0.1 μg of PC-904 per ml.

Penicillin G-resistant *S. aureus* strains were

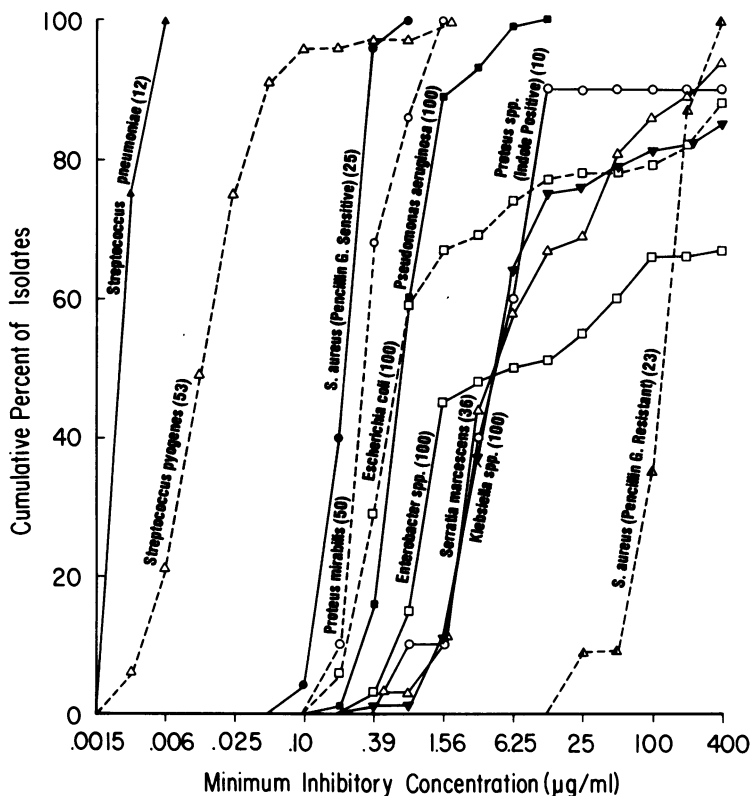


FIG. 2. *In vitro* activity of PC-904 against clinical isolates of bacteria. The numbers in parentheses indicate the number of isolates tested.

also resistant to this antibiotic. *P. mirabilis* was the most susceptible of all gram-negative bacilli; all isolates were inhibited by 1.56 μg of PC-904 per ml. The majority of isolates of *P. aeruginosa* were inhibited by 1.56 $\mu\text{g}/\text{ml}$. Although 74% of isolates of *E. coli* were inhibited by 6.25 $\mu\text{g}/\text{ml}$, most of the remaining isolates were resistant. Nine of the 10 isolates of indole-positive *Proteus* spp. were inhibited by 12.5 $\mu\text{g}/\text{ml}$, but the remaining isolate was resistant. Seventy-five percent of isolates of *Klebsiella* spp., 67% of *S. marcescens* and 61% of *Enterobacter* spp. were inhibited by 12.5 μg or less of PC-904 per ml. Generally, the MBC was the same or one dilution less than the MIC.

The effect of inoculum size on the MIC and MBC was determined for 10 isolates each of *P. aeruginosa*, *K. pneumoniae*, and *E. coli*. Concentrations of 10^5 and 10^7 CFU/ml were used for these studies. At a concentration of 10^5 CFU/ml, the MIC and MBC were the same for most isolates. Eight isolates of *K. pneumoniae* and nine isolates each of *E. coli* and *P. aeruginosa* were inhibited by 12.5 μg of PC-904 per ml. None of the isolates were inhibited by 400 μg of PC-904 per ml when an inoculum of 10^7 CFU/ml was used.

The effect of media on the activity of PC-904 against 10 isolates each of *P. aeruginosa*, *K. pneumoniae*, and *E. coli* was determined. No substantial differences were observed when nutrient broth, Mueller-Hinton broth, Trypticase soy broth (Baltimore Biological Laboratory), and brain heart infusion broth were used for testing *P. aeruginosa*. The MIC for most isolates of *E. coli* was twice as high in nutrient broth as in the other media. PC-904 was substantially less active against *K. pneumoniae* when brain heart infusion broth was used.

The effect of pH on the susceptibility of 10 isolates each of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* was determined. The studies were conducted in Mueller-Hinton broth, and pH values were adjusted to 6.4, 7.2, and 8.0 by the addition of phosphate buffer. The greatest effect was observed against isolates of *K. pneumoniae*, which were more susceptible at acid pH. A twofold increase in drug concentration was required to inhibit these organisms at a pH of 8.0. The pH had little effect on the activity of PC-904 against isolates of *P. aeruginosa* and *E. coli*.

The activity of PC-904 against 50 isolates of various gram-negative bacilli was compared with those of carbenicillin, ticarcillin, amoxicillin, mezlocillin, and azlocillin (Fig. 3-7). However, only 36 isolates of *S. marcescens* were available for testing (Fig. 5). PC-904 was the most active penicillin against *E. coli*, but was only slightly more active than mezlocillin.

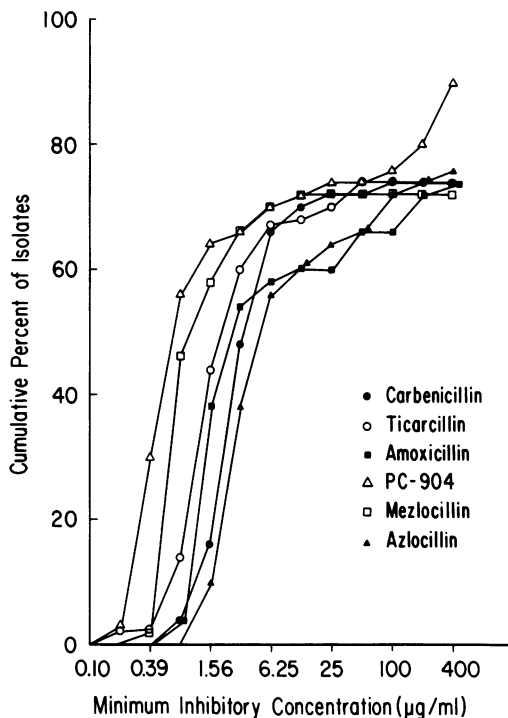


FIG. 3. Comparative activity of penicillins against 50 isolates of *E. coli*. Antibiotics were tested simultaneously in triplicate.

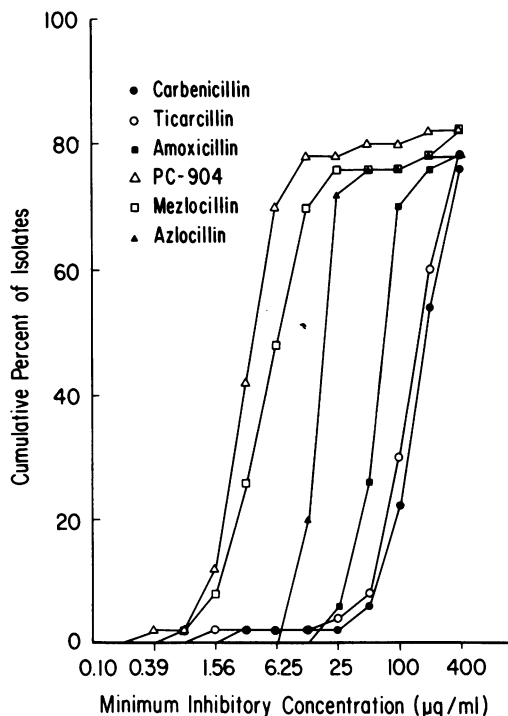


FIG. 4. Comparative activity of penicillins against 50 isolates of *Klebsiella* spp.

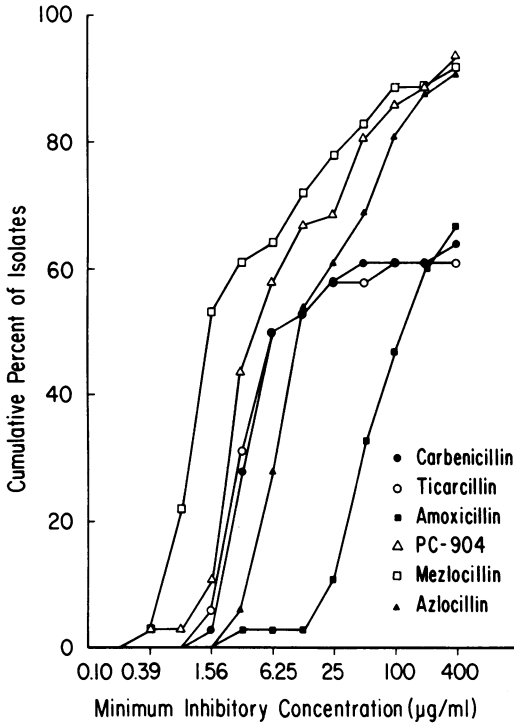


FIG. 5. Comparative activity of penicillins against 36 isolates of *S. marcescens*.

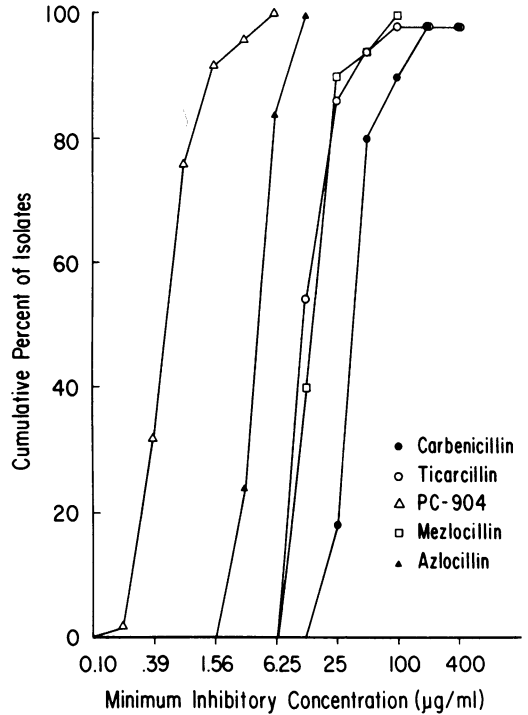


FIG. 7. Comparative activity of penicillins against 50 isolates of *P. aeruginosa*.

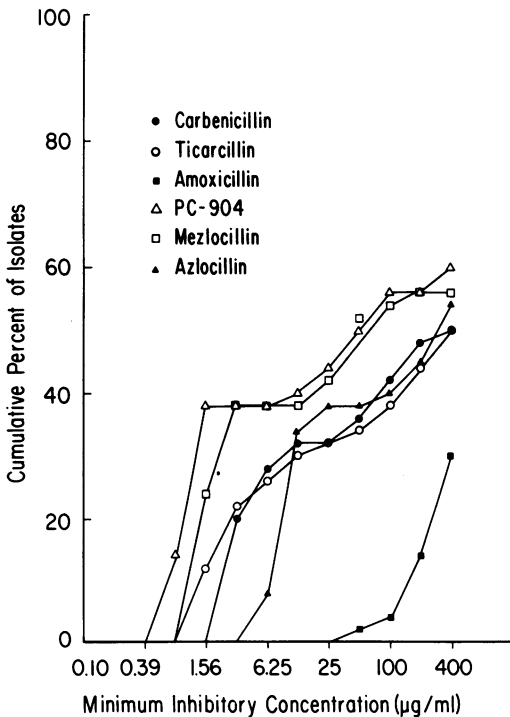


FIG. 6. Comparative activity of penicillins against 50 isolates of *Enterobacter* spp.

Azlocillin was the least active penicillin against most isolates. PC-904 was the most active penicillin against *Klebsiella* spp., but about 20% of isolates were resistant to 400 µg/ml. Mezlocillin was nearly as active as PC-904 and azlocillin had some activity, but the remaining penicillins were essentially inactive. Mezlocillin was somewhat more active than PC-904 against *S. marcescens*. About 40% of isolates were resistant to carbenicillin and ticarcillin, and amoxicillin had minimal activity against these organisms. PC-904 and mezlocillin were the most active penicillins against *Enterobacter* spp., but 40% of isolates were resistant to 400 µg/ml. Amoxicillin was inactive against most isolates. All of the penicillins were quite active against *P. mirabilis*, but azlocillin was slightly less active than the others (not shown). Two isolates were resistant to amoxicillin but were susceptible to the other penicillins. Mezlocillin was more active than PC-904 against the 10 isolates of indole-positive *Proteus* spp. (not shown). Amoxicillin was inactive and azlocillin was only marginally active. PC-904 was substantially more active than the other penicillins against *P. aeruginosa*. All of the isolates tested were inhibited by 6.25 µg/ml, whereas none of the isolates were inhibited by this concentration of ticarcillin, mezlocillin, or carbenicillin. In general, PC-904 was eight

times more active than azlocillin. Amoxicillin was inactive against *P. aeruginosa*.

DISCUSSION

PC-904 is an interesting new semisynthetic penicillin with broad-spectrum activity against gram-positive cocci and gram-negative bacilli. A concentration of 1.56 μg or less of PC-904 per ml inhibited most isolates of *P. mirabilis*, *P. aeruginosa*, and *E. coli*. This penicillin also is active against many isolates of *Enterobacter* spp., *Klebsiella* spp., and *S. marcescens*. Mezlocillin is another new semisynthetic penicillin which has elicited considerable interest because of its broad-spectrum activity in vitro against gram-negative bacilli (2). Our studies indicate that PC-904 is slightly more active than mezlocillin against most isolates of *Enterobacteriaceae*. A major advantage of PC-904 over mezlocillin is its substantially greater activity against *P. aeruginosa*. Indeed, PC-904 was considerably more active against these organisms than the other antipseudomonal penicillins, carbenicillin, ticarcillin, and azlocillin. Although we did not test them simultaneously, PC-904 appears to be more active against *P. aeruginosa* than BL-P1654 and pirbenicillin (3, 4).

In general, our results are similar to those reported by Noguchi et al. (6). These investigators found that PC-904 was more active than carbenicillin against isolates of *E. coli*, *Klebsiella* spp., and *P. aeruginosa*. They did not observe any significant decrease in activity when large inocula were used, whereas we found that PC-904 was inactive against large inocula. This difference in results may be due to different techniques used in the two studies. In the past, we have found the effect of inoculum size to differ depending upon the penicillin. Large inocula of gram-negative bacilli were not inhibited by azlocillin. Whereas large inocula of *P. aeruginosa* were generally not inhibited by mezlocillin and pirbenicillin, large inocula of *E. coli* were inhibited, but the MIC was considerably higher. Other investigators

have reported this adverse effect of large inocula with other semisynthetic penicillins (2, 5, 7). These results suggest that a population of bacteria contains cells with varying susceptibility to these penicillins. A large inoculum has a greater likelihood of including cells that are inherently resistant to the antibiotic. The potential clinical importance of this observation is unclear.

PC-904 is potentially an important new penicillin. Its in vitro activity against *P. aeruginosa* is similar to that of aminoglycoside antibiotics, and it has a broader spectrum of activity against gram-negative bacilli than most other penicillins. It has been found to be effective for the treatment of experimental animal infections (6). This antibiotic deserves further investigation to ascertain its pharmacological and toxicological properties.

ACKNOWLEDGMENT

This work was supported by Public Health Service grant CA 05831 from the National Cancer Institute.

LITERATURE CITED

1. Acred, P., D. M. Brown, E. T. Knudsen, G. N. Rolinson, and R. Sutherland. 1967. New semisynthetic penicillin active against *Pseudomonas pyocyanea*. *Nature* (London) New Biol. 215:25-30.
2. Bodey, G. P., and T. Pan. 1977. Mezlocillin: in vitro studies of a new broad-spectrum penicillin. *Antimicrob. Agents Chemother.* 11:74-79.
3. Bodey, G. P., V. Rodriguez, and S. Weaver. 1976. Pirbenicillin: a new semisynthetic penicillin with broad-spectrum activity. *Antimicrob. Agents Chemother.* 9:668-674.
4. Bodey, G. P., and D. Stewart. 1971. In vitro studies of semisynthetic α -(substituted-ureido) penicillins. *Appl. Microbiol.* 21:710-717.
5. Neu, H. C., and E. B. Winshell. 1971. In vitro studies of semisynthetic penicillin, 6-[D(-)- α -carboxy-3-thienylacetamido] penicillanic acid (BRL 2288), active against *Pseudomonas*, p. 385-389. *Antimicrob. Agents Chemother.* 1970.
6. Noguchi, H., E. A. Tobiki, T. Nakagome, and T. Komatsu. 1976. PC-904, a novel broad-spectrum semisynthetic penicillin with marked antipseudomonal activity: microbiological evaluation. *Antimicrob. Agents Chemother.* 9:262-273.
7. Standiford, H. C., A. C. Kind, and W. M. Kirby. 1969. Laboratory and clinical studies of carbenicillin against gram-negative bacilli, p. 286-291. *Antimicrob. Agents Chemother.* 1968.