APPLIED MICROBIOLOGY, June 1969, p. 881-887

Vol. 17, No. 6
Printed in U.S.A.

Antipseudomonal Activity of α-Sulfoaminopenicillins

K. E. PRICE, D. R. CHISHOLM, F. LEITNER, M. MISIEK, AND A. GOUREVITCH
Research Division, Bristol Laboratories, Division of Bristol-Myers Company, Syracuse, New York 13201

Received for publication 7 April 1969

A series of penicillins characterized by the presence of a sulfoamino or a modified sulfoamino group in the side chain was subjected to in vitro antimicrobial screening tests. Although the most potent members of the series were less active than benzylpenicillin against gram-positive bacteria and comparably active against most gram-negative bacteria, they were, on the average, 8 to 16 times more effective against strains of *Pseudomonas aeruginosa*. In other comparative laboratory tests against *P. aeruginosa*, these compounds were about as active as carbenicillin and four to eight times more active than ampicillin. An examination of structure-activity relationships indicated that maximal potency was obtained with penicillins having an α-(aromatic or heteroaromatic)-α-sulfoaminoacetamido side chain. The compound with an α-phenyl group was comparable in activity to those having an α-(2- or 3-thienyl) group, whereas any modification in position or structure of the α-sulfoamino group reduced activity. Results of studies with a cell-free *P. aeruginosa* β-lactamase suggest that the marked inhibitory effects of α-sulfoamino penicillins for *P. aeruginosa* can be attributed, at least in part, to their high degree of resistance to this enzyme. Some derivatives, however, had weak antipseudomonal activity, despite possessing a high degree of β-lactamase resistance.

Pseudomonas aeruginosa infections of man, whether systemic or localized, have been found to be extremely refractory to chemotherapy (6, 12). Polymyxins (both B and E) and the aminoglycosidic antibiotic, gentamicin, have been successfully used for treatment of some patients, but have limited usefulness because their ranges between effective and toxic doses are very narrow (5, 9, 10, 11). Penicillins, which produce virtually no toxic effects in man, have uniformly been found to be devoid of significant inhibitory effects against this organism. Recently, however, a new semisynthetic penicillin, carbenicillin (disodium α-carboxybenzylpenicillin), was found quite active against *P. aeruginosa* strains in vitro (1, 2, 12). Furthermore, clinical efficacy has been shown for this compound in patients with *Pseudomonas*-infected burns as well as respiratory, urinary tract, and generalized *Pseudomonas* infections (3, 8, 14). The present report describes a new class of antipseudomonal penicillins whose most potent members may be sufficiently active to warrant clinical evaluation.

MATERIALS AND METHODS

All penicillins used in this study were synthesized by members of the Organic Chemistry Research Department of Bristol Laboratories (D. N. McGregor, U.S. Patent 3,381,001). Those having an α-aromatic or α-heteroaromatic group and a substituted α-amino group were prepared directly from the corresponding α-amino-penicillin. In all cases except one, the compounds had the same configuration as that of ampicillin, which was derived from R-phenylglycine [nomenclature of Cahn (4)]. The other penicillins were prepared by acylation of 6-aminopenicillanic acid with racemic mixtures of the appropriate amino acid chlorides. The purity of each of the above-mentioned penicillins was estimated to be at least 85%.

Minimal inhibitory concentration (MIC) values were determined by agar-dilution tests. In this procedure, antibiotics were incorporated into Nutrient Agar (Difco) or Trypticase Soy Agar (BBL) with 2% defibrinated sheep blood. After plates solidified, the surface was inoculated with undiluted to 100-fold-diluted overnight broth cultures by means of the Steers multiple inoculator apparatus (13). The end point, which was determined after overnight incubation at 37 C, was the lowest concentration that suppressed visible growth.

Resistance of the penicillins to *P. aeruginosa* β-lactamase was determined by incubating the test compound with a cell-free extract of the organism and assaying the residual bioactivity at various time periods. The crude β-lactamase was prepared as follows. *P. aeruginosa* A9843 was grown overnight at 37 C in Heart Infusion Broth (Difco) with BL-P 1462 (α-sulfoaminobenzylpenicillin) added at 50 μg/ml as an enzyme inducer. Cells were harvested, washed twice in physiological saline, resuspended in saline at 100th the original volume, and then disrupted by subjecting 15-ml samples to a Raytheon sonic oscillator (Raytheon Mfg. Co., Waltham, Mass.) for 10 to 12 min.

After centrifugation at 30,000 to 35,000 × *g* at 4 C for 5 to 10 min, the supernatant fluids were pooled and stored at −20 C. β-Lactamase resistance determinations were carried out as follows. A 6-ml amount of a solution (2 mg/ml) of the test penicillin in 0.001 M phosphate buffer (*p*H 7.0) was added to 6 ml of the appropriately diluted crude enzyme preparation. The reaction mixture was incubated at 37 C for various time periods. At each sampling time, 1 ml of the mixture was removed and added to 4 ml of acetone to precipitate the enzyme and to prevent further hydrolysis of the compound. After centrifugation, the supernatant fluid was diluted as required, in 0.001 M phosphate buffer, (*p*H 7.0), and assayed by a standard procedure (12) on Seed Agar (BBL) plates that had been inoculated with *Sarcina lutea* ATCC 9341. Ampicillin was used as the standard. The β-lactamase resistance of the new penicillins was expressed in terms of a "resistance coefficient" (RC): $RC = t_{cmpd}/t_{amp}$; where t_{cmpd} and t_{amp} are the times required (estimated by graphic means) to hydrolyze 1 μmole of test compound and 1 μmole of ampicillin per ml, respectively.

RESULTS AND DISCUSSION

In routine antimicrobial screening tests, it was noted that a new penicillin, BL-P 1462 (α-sulfoaminobenzylpenicillin), was unusually active against *P. aeruginosa*. A comparison of the antibacterial spectrum of this compound with that of penicillin G (benzylpenicillin) is presented in Table 1.

With the exception of penicillinase-producing (penase+) staphylococci, penicillin G is 10 to 20 times more active than BL-P 1462 against gram-positive bacteria. The superior activity of the latter compound against penase + staphylococci can undoubtedly be attributed to the fact that its degree of resistance to staphylococcal penicillinase is at least three times greater than that of penicillin G (K. E. Price, *unpublished data*).

In the case of gram-negative bacilli, however, enterobacteria strains were equally susceptible to the two compounds, whereas the inhibitory activity of BL-P 1462 against *P. aeruginosa* strains was up to 17 times greater than that of penicillin G.

Since the α-sulfoamino group of BL-P 1462 appeared to be responsible for its effect against *P. aeruginosa*, additional penicillins having this group in their side chain were synthesized and tested. Table 2 shows the MIC values of various compounds for *P. aeruginosa* A9843 and *Staphylococcus aureus* A9537 as well as the ratio of the two MIC values. Also shown for some of the penicillins is their degree of resistance, relative to ampicillin, to hydrolysis by a crude β-lactamase from *P. aeruginosa* A9843.

BL-P 1462, the first compound in Table 2, had an MIC for *P. aeruginosa* of 32 and thus, was approximately 25 times less active against this organism than the *S. aureus* strain. Its resistance to the β-lactamase of *P. aeruginosa* A9843 was 10-fold greater than that of ampicillin. Similar MIC values were found for the second compound, which differs from BL-P 1462 only in that one of the oxygens in the 3-carboxyl group has been replaced by sulfur (see R_2 in Table 2). Compound 3,

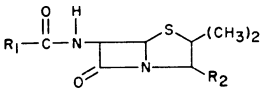
TABLE 1. *Antimicrobial spectrum of penicillin G and BL-P 1462 (α-sulfoaminobenzylpenicillin)*

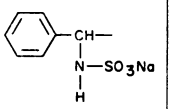
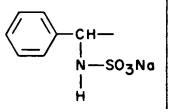
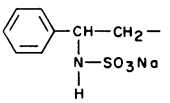
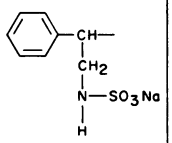
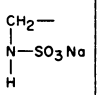
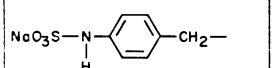
Organism	No. of strains tested	Medium	Inoculum[a]	MIC[b] (μg/ml)		
				Penicillin G (1)	BL-P 1462 (2)	Ratio of 1/2
Staphylococcus aureus (penase −)	2	NA	10^2	0.07	1.0	0.07
S. aureus (penase +)	4	NA	10^2	29.1	10.9	2.7
Streptococcus pyogenes	4	TSA	10^1	0.008	0.25	0.03
S. faecalis	4	TSA	10^1	6.3	125.0	0.05
Diplococcus pneumoniae	4	TSA	Undiluted	0.06	0.45	0.13
Listeria monocytogenes	2	TSA	10^1	1.0	8.9	0.11
Escherichia coli	3	NA	10^2	28.5	63.0	0.45
Klebsiella pneumoniae	2	NA	10^2	106.0	89.5	1.2
Proteus mirabilis	2	NA	10^2	0.9	1.1	0.82
P. morganii	2	NA	10^2	250.0	177.0	1.41
Pseudomonas aeruginosa A9843	1	NA	10^2	>500.0	63.0	>8.0
P. aeruginosa A9923	1	NA	10^2	>500.0	79.2	>6.3
P. aeruginosa A9930	1	NA	10^2	250.0	14.4	17.4

[a] Dilution of 18-hr culture.

[b] MIC is the geometric mean of the minimal inhibitory concentrations. The Steers multiple inoculator apparatus (13) was used to inoculate appropriate dilutions of the cultures onto NA (Nutrient Agar) and TSA (Trypticase Soy Agar + 2% sheep blood) plates containing various concentrations of the penicillins.

TABLE 2. *Antimicrobial activity and β-lactamase resistance of penicillins having a sulfoamino group in the side chain.*



COMPOUND NUMBER	R_1 (EPIMERIC FORM)[b]	R_2	MINIMUM INHIBITING CONCENTRATION[a] (μg/ml)			P. AERUGINOSA β-LACTAMASE RESISTANCE COEFFICIENT (RC)[c]
			P. AERUGINOSA (1)	S. AUREUS (2)	RATIO 1/2	
1	(R) 	—COONa	32	1.25	25	10
2	(R) 	—COSNa	63	1.25	50	—
3	(R,S) 	—COONa	500	2.5	200	6.7
4	(R,S) 	—COONa	>250	3.2	>78	29
5		—COONa	>2000	16.0	>125	8.8
6		—COONa	>500	.25	>2000	0.4

[a] MINIMUM INHIBITING CONCENTRATION VALUES OBTAINED BY USING STEERS MULTIPLE INOCULATOR APPARATUS (14) TO ADD 10^2 DILUTIONS OF 18-HR. CULTURES TO NUTRIENT AGAR (DIFCO) PLATES CONTAINING VARIOUS CONCENTRATIONS OF THE PENICILLINS.

[b] NOMENCLATURE FROM CAHN (4).

[c] SEE "MATERIALS AND METHODS" FOR DEFINITION OF RC. AMPICILLIN HAS BEEN ARBITRARILY ASSIGNED AN RC VALUE OF 1.0.

whose phenyl and sulfoamino groups are present as substituents on the β- rather than α-carbon, still possessed resistance to the pseudomonal penicillinase but had drastically reduced *Pseudomonas*-inhibitory activity. That this was due to a change in spectrum rather than an overall loss in potency is indicated by the marked increase in the *Pseudomonas* to *Staphylococcus* MIC ratio. The decrease in the inhibitory effect against *P. aeruginosa* can probably be attributed, at least in part, to the shift of the sulfoamino group to the β-carbon, since compound 4, the α-phenyl-β-sulfoamino analogue of BL-P 1462, was equally ineffective against this organism. As was the case with compound 3, this one was also resistant to hydrolysis by the pseudomonal β-lactamase and was fairly active against the *Staphylococcus* strain.

The mere presence of a sulfoamino group on the α-carbon may be all that is required for β-lactamase resistance, but it is certainly not sufficient to confer *Pseudomonas* growth-inhibiting properties upon the penicillin. This point is clearly illustrated by results obtained with compound 5, sulfoaminomethylpenicillin, which had no measurable antipseudomonal activity but was nevertheless quite resistant to the β-lactamase preparation.

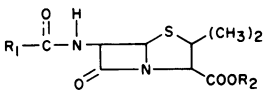
The final compound shown in Table 2, *p*-sulfoaminobenzylpenicillin, had the greatest ac-

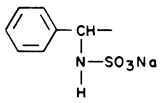
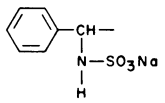
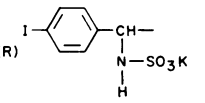
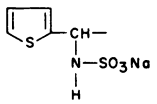
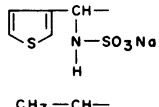
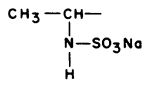
tivity against *S. aureus* but was not inhibitory for *P. aeruginosa* nor resistant to its β-lactamase. This finding suggests that the sulfoamino group may have to be on the α-carbon to confer significant antipseudomonal activity. It further indicates that there are specific structural requirements for possession of β-lactamase resistance. Evidence that β-lactamase resistance may be required for good antipseudomonal activity is given by the above example and also by results obtained with *N*-phenylsulfoaminomethylpenicillin (not shown in Table 2). This penicillin, which had an RC value of only 0.8, was found to have no antipseudomonal activity (MIC = > 500), despite the fact that its MIC for *S. aureus* was 0.32 μg/ml.

Since the results shown in Table 2 suggest that an antipseudomonal effect is obtained only when the penicillin side chain has a sulfoamino group as one of the substituents on a disubstituted α-carbon, a comparison of the activity of several α-sulfoamino derivatives which have a second substituent present on the α-carbon was made (Table 3).

The first two compounds are diastereoisomers of α-phenyl-α-sulfoaminomethylpenicillin. Configuration does appear to have an effect on antibacterial activity, since the "S" form is only about one-fourth as inhibitory for *P. aeruginosa* as the "R" form. The fact that the ratios of the *Pseudomonas* to *Staphylococcus* MIC values are the

TABLE 3. *Antimicrobial activity and β-lactamase resistance of α-substituted-α-sulfoamino penicillins.*



COMPOUND NUMBER	R_1 (EPIMERIC FORM)[b]	R_2	MINIMUM INHIBITING CONCENTRATION[a] (μg/ml) P. AERUGINOSA (1)	S. AUREUS (2)	RATIO 1/2	P. AERUGINOSA β-LACTAMASE RESISTANCE COEFFICIENT (RC)[c]
1	(R) 	—Na	32	1.25	25	10
2	(S) 	—Na	125	5.0	25	—
3	(R) 	—K	125	.32	400	—
4	(R) 	—Na	32	1.25	25	13
5	(R) 	—Na	32	1.25	25	17
6	(R) 	—Na	> 500	6.3	> 80	5.7

[a] MINIMUM INHIBITING CONCENTRATION VALUES OBTAINED BY USING STEERS MULTIPLE INOCULATOR APPARATUS (14) TO ADD 10^2 DILUTIONS OF 18-HR. CULTURES TO NUTRIENT AGAR (DIFCO) PLATES CONTAINING VARIOUS CONCENTRATIONS OF THE PENICILLINS.

[b] NOMENCLATURE FROM CAHN (4).

[c] SEE "MATERIALS AND METHODS" FOR DEFINITION OF RC. AMPICILLIN HAS BEEN ARBITRARILY ASSIGNED AN RC VALUE OF 1.0.

same for both compounds indicates that this is due to a difference in overall potency and not to a change in spectrum.

Introduction of an iodo radical into the *para*-position of the phenyl ring of R-(α-phenyl-α-sulfoaminomethyl)penicillin (compound 3) resulted in decreased antipseudomonal but increased antistaphylococcal activity. The degree of resistance of the penicillin to *P. aeruginosa* β-lactamase was not determined.

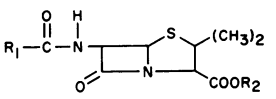
The next two compounds (4 and 5) are α-sulfoamino-α-(2- or 3-thienyl) methylpenicillins. Their antibacterial spectra and degree of resistance to *Pseudomonas* β-lactamase are similar to those of compound 1 (BL-P 1462). The potency of the compounds also appears comparable to that of BL-P 1462.

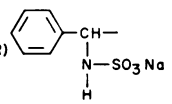
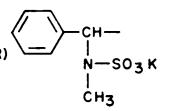
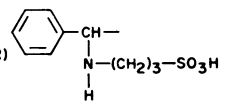
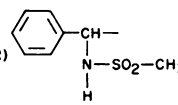
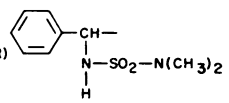
The final compound, α-methyl-α-sulfoaminomethylpenicillin, had very poor antipseudomonal activity. Thus, cyclic substituents in α-substituted α-sulfoaminomethylpenicillins appear to confer greater *Pseudomonas*-inhibitory effects than short-chain aliphatic groups. Cyclic substituents are not required, however, for preservation of the antistaphylococcal activity of the compound or resistance to the β-lactamase preparation.

Since an α-phenyl substituent appears to be associated with good anti-*Pseudomonas* activity, a series of derivatives containing this group (benzylpenicillins) were substituted at the α-carbon with modified sulfoamino groups in an effort to further increase activity against this organism (Table 4).

Compound 2, where an *N*-methyl group has been introduced into the α-sulfoamino moiety, has essentially the same spectrum (*Pseudomonas*

TABLE 4. *Antimicrobial activity and β-lactamase resistance of α-substituted benzylpenicillins.*



COMPOUND NUMBER	R_1 (EPIMERIC FORM)[b]	R_2	MINIMUM INHIBITING CONCENTRATION[a] (μg/ml)			P. AERUGINOSA β-LACTAMASE RESISTANCE COEFFICIENT (RC)[c]
			P. AERUGINOSA (1)	S. AUREUS (2)	RATIO 1/2	
1	(R) 	—Na	32	1.25	25	10
2	(R) 	—K	125	2.5	50	>50
3	(R) 	—H	>500	.32	>1560	—
4	(R) 	—K	>500	.63	>800	—
5	(R) 	—K	125	.63	200	14.3

[a] MINIMUM INHIBITING CONCENTRATION VALUES OBTAINED BY USING STEERS MULTIPLE INOCULATOR APPARATUS (14) TO ADD 10^2 DILUTIONS OF 18-HR. CULTURES TO NUTRIENT AGAR (DIFCO) PLATES CONTAINING VARIOUS CONCENTRATIONS OF THE PENICILLINS.

[b] NOMENCLATURE FROM CAHN (4).

[c] SEE "MATERIALS AND METHODS" FOR DEFINITION OF RC. AMPICILLIN HAS BEEN ARBITRARILY ASSIGNED AN RC VALUE OF 1.0.

to *Staphylococcus* MIC ratio) as compound 1 (BL-P 1462). There is an indication, however, that relative to BL-P 1462, the antibacterial potency of the penicillin is somewhat less, although its resistance to the β-lactamase preparation is significantly greater.

Incorporation of a propyl group into the α-substituent so as to give α-(3-sulfopropylamino)-benzylpenicillin results in a compound with little antipseudomonal, but high antistaphylococcal, activity. The resistance of the compound to β-lactamase was not determined.

Although the last two derivatives both have potent antistaphylococcal activity, only compound 5, which is α-(*N*,*N*-dimethylsulfamido)-benzylpenicillin, is inhibitory for *P. aeruginosa*. This compound was less active than BL-P 1462 (compound 1) but comparably resistant to the pseudomonal β-lactamase. The β-lactamase resistance of α-(methanesulfonamido)benzylpenicillin (compound 4) was not determined.

On the basis of the data shown in Tables 1 to 4, it appears that maximal antipseudomonal activity is obtained with penicillins having an α-(unsubstituted aromatic or heteroaromatic)-α-sulfoaminoacetamido side chain. The compound with the α-phenyl group is comparable in activity to those with an α-(2- or 3-thienyl) radical, whereas any modification in position or structure of the α-sulfoamino group appears to be detrimental to activity. The structural requirements for resistance to *Pseudomonas* β-lactamase seem much less rigid, although the mere presence of a sulfoamino group in the side chain is not sufficient, as attested to by the relatively high susceptibility of *p*-sulfoaminobenzylpenicillin to enzymatic hydrolysis. Finally, antistaphylococcal activity was noticeably poorer for those penicillins lacking an aromatic group in the side chain.

If one assumes a relationship between antipseudomonal activity and β-lactamase resistance, the above findings give rise to two important questions. First, what is responsible for the variation in antipseudomonal activity possessed by this large group of closely related, enzyme-resistant and antimicrobially potent compounds and, second, how can simple α-substituents protect the β-lactam ring from enzymatic hydrolysis? Regarding the first question, it seems probable that variations in activity can be attributed to the relative ability of the compounds to reach the site of action or their degree of affinity for the enzymes involved in cross-linking cell wall peptidoglycan strands, or both. As far as the second question is concerned, it is possible that the negative charge on the α- or β-carbon substituent prevents enzy-

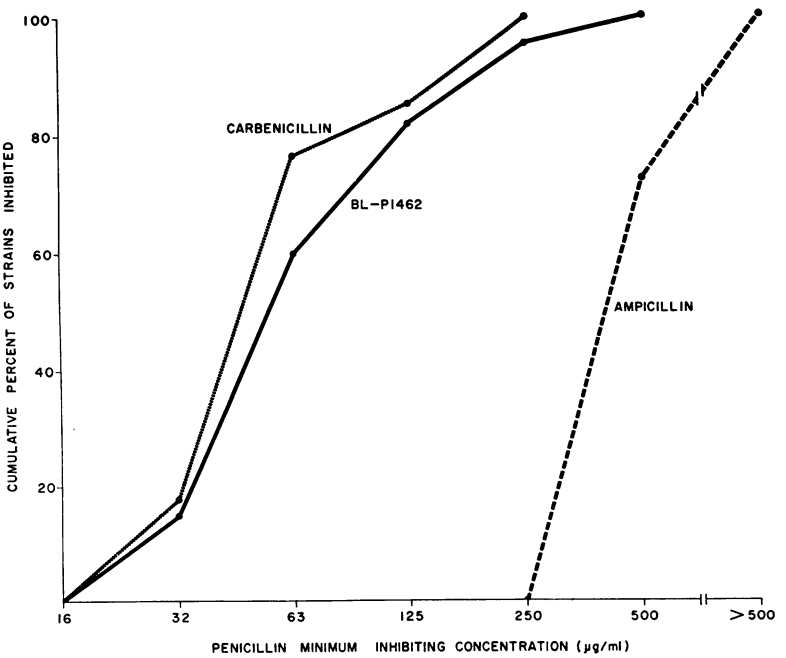


FIG. 1. *MIC values of the three penicillins for P. aeruginosa (22 strains) were determined by an agar-dilution method. A 10^2 dilution of each 18-hr culture was added by means of the Steers multiple inoculator apparatus (13) to Nutrient Agar (Difco) plates containing various concentrations of the antibiotics.*

matic hydrolysis through electrostatic repulsion or, alternately, the substituent may hinder enzyme attachment in essentially the same way that the bulky methoxy groups of methicillin hinder attachment to staphylococcal enzymes. Such blocking could occur with these α-substituents because, although small, they are quite polar and thus are surrounded by a relatively stable and quite large hydration sphere. Carbenicillin, which is extremely resistant to the penicillinase of *P. aeruginosa* strain A9843 (K. E. Price, *unpublished data*), also has a highly polar group (−COONa) on the α-carbon.

BL-P 1462, one of the most active members of this group of penicillins, was evaluated against a number of *P. aeruginosa* strains in comparative in vitro tests with ampicillin and carbenicillin (Fig. 1).

Ampicillin failed to suppress growth of any strain at 250 μg/ml, whereas BL-P 1462 and carbenicillin each inhibited more than 50% of the strains at a concentration of 63 μg/ml and at least 80% at 125 μg/ml. On the basis of these results, there appears to be little difference in the antipseudomonal activities of BL-P 1462 and carbenicillin.

In view of this finding and because BL-P 1462 has proven to be efficacious in experimental *P. aeruginosa* infections, whether systemic in the mouse or localized in the urinary tract of rats (K. E. Price, *unpublished data*), it will be subjected to thorough pharmacological and toxicological examination to determine its suitability for clinical investigation.

ACKNOWLEDGMENTS

We acknowledge the capable technical assistance of Theresa Pursiano, Thomas Hallett, James Quigley, and Thomas Ingram.

LITERATURE CITED

1. Acred, P., D. M. Brown, E. T. Knudsen, G. N. Rolinson, and R. Sutherland. 1967. New semi-synthetic penicillin active against *Pseudomonas pyocyanea*. Nature. 215:25–30.
2. Bodey, G. P., and L. M. Terrell. 1968. In vitro activity of carbenicillin against gram-negative bacilli. J. Bacteriol. 95:1587–1590.
3. Brumfitt, W., A. Percival, and D. A. Leigh. 1967. Clinical and laboratory studies with carbenicillin. A new penicllin active against *Pseudomonas pyocyanea*. Lancet 1:1289–1293.
4. Cahn, R. S. 1964. An introduction to the sequence role—a system for the specification of absolute configuration. J. Chem. Educ. 41:116–125.
5. Darrell, J. H., and P. M. Waterworth. 1967. Dosage of gentamicin for Pseudomonas infections. Brit. Med. J. 2:535–537.
6. Feingold, D. S., and F. Oski. 1965. *Pseudomonas* infection. Treatment with immune human plasma. Arch. Intern. Med. 116:326–328.
7. Grove, D. C., and W. A. Randall. 1955. Assay methods of antibiotics. A laboratory manual. Medical Encyclopedia, Inc. New York.
8. Jones, R. J., and E. J. Lowbury. 1967. Prophylaxis and therapy for *Pseudomonas aeruginosa* infection with carbenicillin and with gentamicin. Brit. Med. J. 3:79–82.
9. Lowbury, E. J. L., and D. M. Jackson. 1968. Local chemoprophylaxis for burns with gentamicin and other agents. Lancet 1:654–657.
10. Nishiura, T., Y. Kawada, M. Tahara, H. Mizutani, and R. Miyamura. 1967. Oral administration of sodium colistin methanesulfonate on urinary tract infections. J. Antibiot. 20:377–379.
11. Olesen, S., and P. O. Madsen. 1967. Intravenous administration of sodium colistimethate in urinary tract infections. Curr. Therap. Res. 9:283–287.
12. Smith, C. B., and M. Finland. 1968. Carbenicillin: activity in vitro and absorption and excretion in normal young men. Appl. Microbiol. 16:1753–1760.
13. Steers, E., E. L. Foltz, and B. S. Graves. 1959. Inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemother. 9:307–311.
14. van Rooyen, C. E., J. F. Ross, G. W. Bethune, and A. C MacDonald. 1967. Bacteriological observations on carbenicillin in the control of *Pseudomonas aeruginosa* infection in burns. Can. Med. Ass. J. 97:1227–1229.