68767-15-7; **8b** (isomer 1), 87828-34-0; **8b** (isomer 2), 87828-35-1; **8c**, 68767-17-9; **8d**, 68767-16-8; **8e**, 87762-21-8; **9** (n = 1), 936-52-7; **9** (n = 2), 670-80-4; **9** (n = 3), 7182-08-3; **10**, 68266-50-2; **11a**, 87762-22-9; **11b** (isomer 1), 87762-23-0; **11b** (isomer 2), 87762-25-2; **11c**, 68767-23-7; **11d**, 68767-22-6; **11e**, 87762-24-1; **12**, 43153-04-4; **13a**, 69956-76-9; **13b**, 69956-77-0; **13c**, 69956-78-1; **14** (n = 1), 120-92-3; **14** (n = 2), 108-94-1; **14** (n = 3), 502-42-1; **15** (isomer 1), 87828-36-2; **15** (isomer 2), 87828-37-3.

Synthesis and Antimicrobial Activity of Clindamycin Analogues: Pirlimycin,[1,2] a Potent Antibacterial Agent

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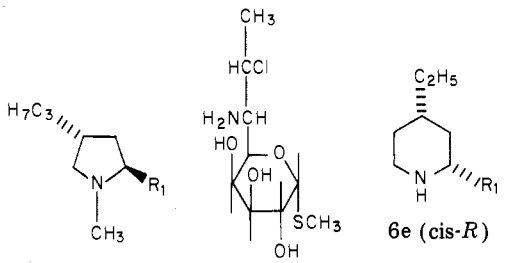
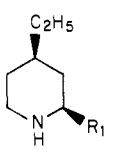
The preparation of a series of analogues of clindamycin is described in which the naturally occurring five-membered cyclic amino acid amide portion of the molecule is replaced by a four-, six-, or seven-membered cyclic amino acid amide. The most interesting compound is pirlimycin (**7e**, U-57,930E), in which the (2*S*-*trans*)-4-*n*-propylhygramide portion of clindamycin is replaced by (2*S*-*cis*)-4-ethylpipecolamide. This structural modification results in significantly favorable changes in toxicity, metabolism, and antibacterial potency. Although the in vitro antibacterial activity of clindamycin and pirlimycin are nearly identical, the latter compound is 2–20 times more active than clindamycin when administered to mice experimentally infected with strains of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Bacteroides fragilis*, and *Plasmodium berghei*. Pirlimycin is absorbed in rats and mice following both subcutaneous and oral administration. It readily penetrates *B. fragilis* induced abscesses in mice and is sequestered within these abscesses. A drug concentration of at least 60 times the required inhibitory concentration is maintained for 6 h following a single subcutaneous dose of 200 mg/kg. Urinary excretion of total bioactivity consists only of intact pirlimycin with no other antibacterially active metabolites being detected. Pirlimycin is tolerated well in rats and mice at the administered levels.

The synthesis and structure of the antibiotic clindamycin (**1**; Chart I) and the potency and spectrum of its antibacterial activity were described in earlier communications from this laboratory.[3] Further investigation into the chemistry and biology of the lincomycin/clindamycin family of antibiotics resulted in changes in the sugar-ring portion of the molecule at C-1–C-4 and in replacement of the chlorine atom at C-7 of clindamycin by a nitrile group, as well as by several different sulfur- and oxygen-containing moieties.[3,4] Alterations introduced into the amino acid amide portion of clindamycin included variations in the length of the alkyl side chain and replacement of the methyl group attached to the nitrogen atom by a longer chain alkyl group, by a 2-hydroxyethyl function, and by a hydrogen.[5] A group of compounds was also prepared in which the cyclic amino acid amide portion of clindamycin was replaced by a noncyclic amino acid amide.[6]

Chart I[a]

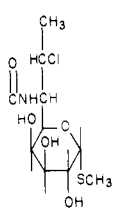


1 (clindamycin) 2 6e (cis-*R*)



7e (pirlimycin, cis-*S*)

[a] R_1 =



This paper describes a further examination of the structure–activity relationships of a series of analogues in which the size of the cyclic amino acid amide portion of the molecule was increased or decreased by one, two, or three methylene groups. In the six-membered ring series, we also investigated the effect on antibacterial activity of changes in N-substitution and in the length, branching, and position of the alkyl side chain. Pirlimycin (**7e**, U-57,930E) was the most interesting compound prepared in this study.

Chemistry. The synthesis of amides of general structure **5** was carried out as shown in Scheme I. The amino acids **3a–c**,[1] were obtained from commercial sources, while **3d–j** were prepared by a modification of the method of

(1) A preliminary announcement of a portion of this work was presented earlier: (a) Birkenmeyer, R. D. *Abstr. 20th Intersci. Conf. Antimicrob. Ag. Chemother.* **1980**, abstr 65. (b) Lewis, C.; Stern, K. F.; Zurenko, G. E. *Ibid.* **1980**, abstr 66.

(2) Pirlimycin is the generic name for the (2*S*-*cis*)-4-ethylpipecolamide of methyl (7*S*)-7-chloro-7-deoxythiolincosaminide.

(3) (a) Birkenmeyer, R. D.; Kagan, F. *J. Med. Chem.* **1970**, *13*, 616–619. (b) Magerlein, B. J. "Structure–Activity Relationships Among the Semisynthetic Antibiotics"; Perlman, D., Ed.; Academic Press: New York, 1977; pp 601–651.

(4) (a) Bannister, B.; Birkenmeyer, R. D.; Kagan, F. U.S. Patent 3 316 243, 1967. (b) Birkenmeyer, R. D. U.S. Patent 3 849 396, 1974. (c) Birkenmeyer, R. D.; Kagan, F. U.S. Patent 3 513 155, 1970. (d) Bannister, B. *J. Chem. Soc., Perkin Trans. 1* **1972**, 3025–3030. (e) *Ibid.* **1972**, 3031–3036. (f) Birkenmeyer, R. D. U.S. Patent 3 549 615, 1970. (g) Bannister, B. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1676–1682. (h) *Ibid.* **1974**, 360–369. (i) Bannister, B. U.S. Patent 3 915 954, 1975. (j) Magerlein, B. J.; Kagan, F. *J. Med. Chem.* **1969**, *12*, 974–977.

(5) (a) Magerlein, B. J.; Birkenmeyer, R. D.; Kagan, F. *J. Med. Chem.* **1967**, *10*, 355–359. (b) Birkenmeyer, R. D. U.S. Patent 3 787 390, 1974. (c) Birkenmeyer, R. D.; Dolak, L. A. *Tetrahedron Lett.* **1970**, 5049–5051.

(6) Morozowich, W. U.S. Patent 3 853 843, 1974.

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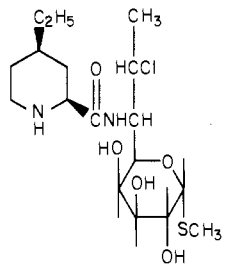


7e

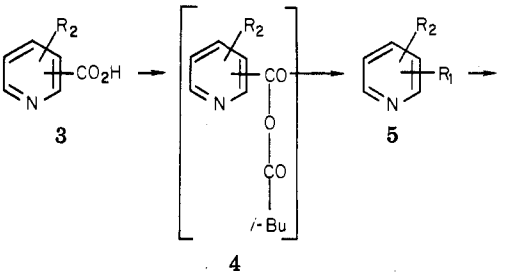
Solomon.[7] In the latter cases, the 2-bromo-4-alkylpyridine intermediates were converted to the acids via metal–halogen exchange, followed by carbonation with CO_2. Compounds **3k,m** may be prepared by known procedures.[8] The acids were reacted with isobutyl chloroformate to give the mixed anhydrides **4**, which were coupled with methyl (7*S*)-7-chloro-7-deoxythiolincosamide[9] [7-Cl-MTL (**2**), the sugar amine portion of clindamycin (Chart I)] to give the unsaturated amino acid amides **5**. Hydrogenation of **5** in the presence of PtO_2 resulted in an approximately 1:1 mixture of two isomeric products, **6** and **7**. These pairs of isomers had different mobilities upon silica gel TLC. When the reduction mixture of **6** and **7** possessed significant antibacterial activity, as determined by in vitro biological assay, the more polar isomer invariably was more potent than the less polar one. Previous work in the clindamycin analogue program has shown that replacement of the (2*S*)-4-*n*-propylhygric acid portion of the molecule with the corresponding *R* acid results in greatly diminished antibacterial activity.[10] Therefore, we tentatively assigned the cis-*S* configuration to the more active and more polar of the pairs of reduction products and the cis-*R* structure to the less active and less polar ones. X-ray crystallographic studies of the isomeric pair **6e** and **7e** (Chart I) confirmed the above conclusion.[11] Pirlimycin (**7e**) is the (2*S*-*cis*)-4-ethylpipecolamide of 7-Cl-MTL, and **6e** is its cis-*R* isomer.

Scheme II depicts the synthesis of the group of analogues containing a substituent on the nitrogen atom of the six-membered cyclic amino acid amide. Reductive alkylation of the appropriate substrate with formaldehyde gave the *N*-methyl compounds **8–12**, while treatment with ethylene oxide introduced the α-hydroxyethyl group, compounds **13–15**.

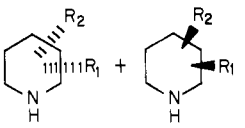
The synthesis of a four-membered cyclic amino acid amide analogue of clindamycin is described in Scheme III. After first protecting the amine function of the commercially available 2(*S*)-azetidenecarboxylic acid as its *N*-benzyloxycarbonyl (Cbz) derivative, we converted the acid to its mixed anhydride. This intermediate was coupled with **2**, and the Cbz group was removed with Pd/cyclohexene to give the desired compound **16**. The *R* and *S* seven-membered cyclic amino acid amide analogues **17** and **18** were obtained when a racemic mixture of hexahydro-1*H*-azepine-2-carboxylic acid was treated as in Scheme III.[12] When racemic quinuclidine-2-carboxylic acid was used as the starting material in the above reaction sequence, the *R* and *S* bicyclic amides **19** and **20** were obtained.[13] The Cbz protective group was unnecessary in the latter case.

(7) Solomon, W. *J. Chem. Soc.* **1946**, 934–936.
(8) (a) Jerchel, D.; Heider, J. *Justus Liebigs Ann. Chem.* **1958**, *613*, 153–170. (b) Matsumoto, I.; Tomimoto, K. *Chem. Abstr.* **1973**, *78*, 136084.
(9) (a) Magerlein, B. J.; Kagan, F. *J. Med. Chem.* **1969**, *12*, 760–784.
(10) Magerlein, B. J., personal communication.
(11) Chidester, C. G., personal communication.
(12) Nagasawa, H. T.; Elberling, J. A.; Fraser, P. S. *J. Med. Chem.* **1971**, *14*, 501–508.

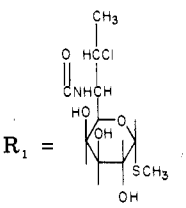
Scheme I. Synthesis of Pirlimycin and Analogues



3 4 5



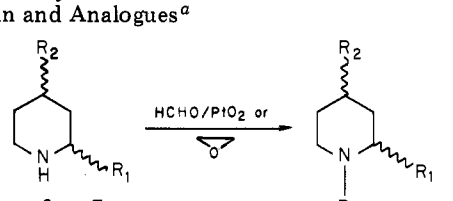
6 7



compds 3–7	position of CO_2H or R_1	R_2
a	2	H
b	3	H
c[a]	4	H
d	2	3-CH_3
e	2	4-C_2H_5
f	2	4-*n*-C_3H_7
g	2	4-*n*-C_4H_9
h	2	4-*t*-C_4H_9
i	2	4-*n*-C_6H_{13}
j[b]	2	4-*n*-C_8H_{17}
k	2	5-C_2H_5
l	2	6-CH_3
m	2	6-C_2H_5

[a] 6C = 7C. [b] Compound 6j was not isolated.

Scheme II. Synthesis of N-Substituted Pirlimycin and Analogues[a]



6 or 7 8–15

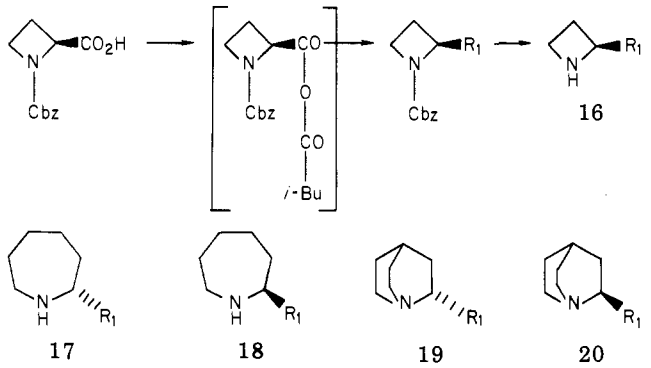
no.	R_1	R_2	R_3
8	*R*	H	CH_3
9	*S*	H	CH_3
10	*R*	C_2H_5	CH_3
11	*S*	C_2H_5	CH_3
12	*S*	*n*-C_6H_{13}	CH_3
13	*R*	C_2H_5	CH_2CH_2OH
14	*S*	C_2H_5	CH_2CH_2OH
15	*S*	*n*-C_4H_9	CH_2CH_2OH

[a] R_1 and R_2 are the same as in Scheme I.

Biology. The compounds described here were tested in vitro for their ability to inhibit the growth of a number of aerobic and anaerobic bacteria. The bioactivity of these compounds was compared with the data for a clindamycin-susceptible strain of *S. aureus*, as shown in Table I. The following conclusions were drawn from these data: (1) The pyridyl portion of the amino acid amide system, present in analogues **5a–m**, was undesirable. None of these compounds had significant antibacterial activity. (2)

(13) Renk, E.; Grob, C. A. *Helv. Chim. Acta* **1954**, *37*, 2119–2123.

Scheme III. Synthesis of Pirlimycin Analogues with Smaller and Larger Ring Systems[a]



[a] R_1 is the same as in Scheme I.

Compounds possessing a saturated cyclic amino acid amide system may be very potent antibiotics, provided certain other criteria were met. (3) The highest degree of antibacterial activity was more likely to occur when the saturated cyclic amino acid amide system was derived from an *S* amino acid rather than from an *R* amino acid. (4) In the saturated six-membered cyclic amino acid amide system, the position of attachment of the acyl function to the piperidine ring was critical. This moiety must be substituted at C-2 of the piperidine ring for significant antibacterial activity. (5) Introduction of an additional piperidine ring substituent in the form of an alkyl group resulted in an increase or decrease of antibacterial activity. The nature of this change depended upon the position of substitution of the alkyl group, its chain length and its degree of branching. The most favorable site of attachment of the alkyl group to the piperidine ring was at C-4, with the highest antibacterial activity being obtained when this group was a straight chain of from two to eight carbon atoms in length. Introduction of the highly branched *tert*-butyl function into the C-4 position resulted in a significant decrease of activity. (6) Expansion of the piperidine cyclic amino acid amide ring system by one methylene group resulted in compound **18**, which had antibacterial activity similar to the six-membered ring analogue **7a**. (7) Contraction of the piperidine cyclic amino acid amide ring system by two methylene groups resulted in compound **16**, which had greatly diminished antibacterial activity. (8) Substitution of the piperidine nitrogen atom of the cyclic amino acid amide by a methyl or 2-hydroxyethyl group diminished antibacterial activity.

Pirlimycin (7e), which not only possessed high antibacterial activity but also demonstrated lower acute toxicity than clindamycin, was selected for more extensive testing. Tables II and III contain the results of a comparison of the in vitro antibacterial activities of pirlimycin and clindamycin when tested against an expanded group of aerobic and anaerobic bacteria. Pirlimycin demonstrated activity similar to that of clindamycin for clindamycin-susceptible bacteria, including *S. aureus* isolates that are resistant to many other antibiotics. Pirlimycin is cross-resistant with clindamycin; i.e., those isolates resistant to inhibition by clindamycin are also resistant to pirlimycin.

The significant difference in the in vivo activity of these two antibiotics is shown in Table IV. Pirlimycin, administered either orally or subcutaneously, demonstrated activity superior to that of clindamycin in the mouse protection assay in which animals were infected with *S. aureus*, *S. pyogenes*, or *S. pneumoniae*. Pirlimycin was considerably more active than clindamycin against *P. berghei*, and with a CD_{50} of 16 mg/kg was nearly as active as chloroquine (CD_{50} = 11.5 mg/kg), the drug of choice for treatment of *Plasmodium* infections in humans. Pirlimycin was also more active than clindamycin against *B. fragilis*, although the CD_{50} end point was not reached in this experiment. Both pirlimycin and clindamycin were inactive at the highest levels tested against *K. pneumoniae*, *P. vulgaris*, *E. coli*, and *P. multocida*.

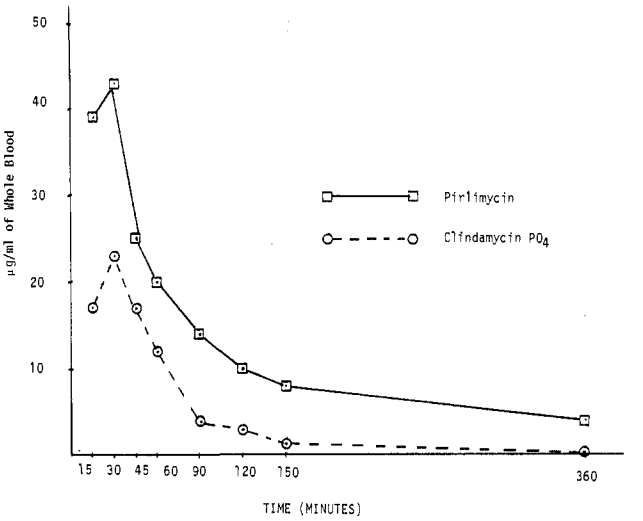


Figure 1. Concentration of clindamycin and pirlimycin bioactivity in whole blood in *B. fragilis* infected mice following a single subcutaneous dose of 200 mg/kg.

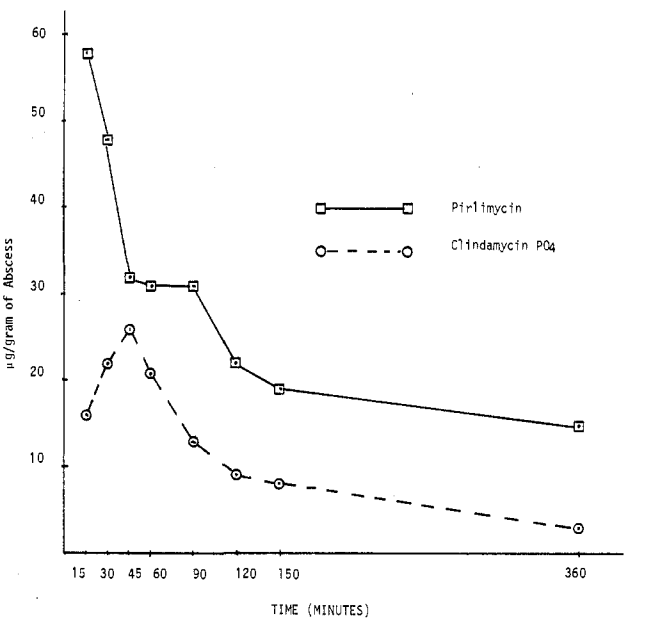


Figure 2. Concentration of clindamycin and pirlimycin bioactivity in abscess in *B. fragilis* infected mice following a single subcutaneous dose of 200 mg/kg.

Blood and abscess levels of pirlimycin and clindamycin in *B. fragilis* infected mice are shown graphically in Figures 1 and 2. Although blood levels of both compounds (Figure 1) peaked rapidly (30 min), pirlimycin maintained a higher level than clindamycin throughout the 6-h test period. Figure 2 shows that although both antibiotics readily penetrated *B. fragilis* abscesses, pirlimycin produced significantly higher and more persistent levels of drug than did clindamycin. A tabulation of the blood and abscess level data, along with the abscess level/blood level ratio, is presented in Table V. Both compounds were sequestered within the abscess; i.e., the abscess level/blood level

Table I. Biological Activity of Pirlimycin and Analogues

no.	MIC, mcg/mL	MIC, mcg/mL, against *S. aureus*	
		R compd	*S* compd
5a	62.5		
5b	>100		
5c	>1000		
5d	>100		
5e	1000		
5f	>100		
5g			
5h	>100		
5i	50		
5j	>10		
5k	62.5		
5l	>25		
5m	>25		
6a, 7a		25	3.9
6b, 7b		>80	>80
6c, 7c	>80		
6d, 7d		25	12.5
6e, 7e		250	0.1
6f, 7f		>25	0.5
6g, 7g		31.2	0.06
6h, 7h		>25	2.0
6i, 7i		0.5	0.03
7j			0.3
6k, 7k		2.5	2.0
6l, 7l		>25	>25
6m, 7m		>25	>25
8, 9		>100	3.9
10, 11		>100	0.2
12			0.3
13, 14		>160	2.5
15			0.16
16			25
17, 18		>100	1.56
19, 20		50	>100

[a] Minimum inhibitory activity of drug (in mcg/mL). [b] UC 76.

ratio was >1. Even at 6 h after treatment, a drug concentration of at least 60 times the MIC (15 μg/mL blood level vs. a MIC of 0.25 μg/mL) was maintained for pirlimycin.

Pirlimycin was tolerated well in rats and mice at the administered levels, and its acute toxicity was one-half that of clindamycin. The intraperitoneal LD_{50} in mice was 600 mg/kg for pirlimycin and 300 mg/kg for clindamycin.[14]

Measurement of the total antibacterial activity present in the urine of rats treated with pirlimycin showed that it consisted only of intact drug and that no antibacterially active metabolites were present.[15] This was in contrast to the results obtained when clindamycin was assayed in a similar manner. In this case, four antibacterially active metabolites were detected.

Previous work described the introduction of a chlorine atom into the sugar amine portion of lincomycin.[3] This change resulted in a new antibiotic, clindamycin, which, when compared with its parent, demonstrated enhanced potency and a broadened spectrum of antimicrobial activity, including antiplasmodial activity. We have found that when this change in the sugar portion of the molecule was combined with several modifications in the amino acid amide moiety, a new compound was obtained having further biological advantages. This compound, pirlimycin, in addition to desirable changes in metabolism, demonstrated greatly increased in vivo potency in both the antibacterial and antiplasmodial areas, as well as a significant reduction in acute toxicity.

(14) The authors are indebted to E. R. Larsen, R. Weaver, and J. E. Gray of The Upjohn Co. for the toxicity data.

(15) The authors are indebted to T. F. Brodasky of The Upjohn Co. for TLC analysis of rat urine.

Experimental Section

Chemistry. Melting points were taken in a Thomas-Hoover Unimelt apparatus and are uncorrected. TLC was carried out on glass plates coated with silica gel G (Analtech). Silica gel, 0.05–02 mm (EM Reagents), was employed for column chromatography. ^{13}C NMR spectra were obtained for all compounds listed in Tables I–III and were consistent with those expected for the compounds being analyzed. Spectra were recorded on a Varian CFT-20 or Varian FT-80A spectrophotometer. Samples were dissolved in acetone-d_6 containing 10% Me_4Si, in methanol-d_4, or in D_2O, with Me_4Si as an external standard. Optical rotations were measured at *c* 1.0. Elemental analyses were within 0.4% of theoretical values except where noted. The elemental composition of some compounds was established by high-resolution mass spectrometry (HRMS) of the appropriate Me_3Si derivative. All HRMS determinations were within 6 millimass units of the theoretical values. Organic solutions were dried over $MgSO_4$ and evaporated in vacuo at 50 °C. The following examples represent typical preparations.

4-Ethyl-2-pyridinecarboxylic Acid (3e).[20] Anhydrous ethyl ether (1 L) was cooled to −60 °C under a nitrogen atmosphere, and 181 mL (0.29 mol) of a 1.6 M solution of *n*-butyllithium in hexane was added. A solution of 50.0 g (0.27 mol) of 2-bromo-4-ethylpyridine in 100 mL of ether was added dropwise over a 15-min period, maintaining the temperature at less than −50 °C. The mixture was warmed to 40 °C for 15 min, recooled to −60 °C, and then transferred onto a rapidly stirred slurry of dry ice in ether via a cannula. The resulting pale blue reaction mixture was stirred for 2 h, by which time it had warmed to room temperature and had turned pale yellow. Water (500 mL) was added, and the layers were separated. The ether was extracted with 100 mL of 0.2 N NaOH (aqueous layer was pH 11). The combined aqueous layers were extracted with 100 mL of ether, acidified to pH <1 with concentrated HCl, and extracted twice with 100-mL portions of ether. The aqueous phase was concentrated in vacuo to a thick paste. The residue was taken up in 400 mL of hot 2-propanol, and the insoluble salts were removed by filtration, followed by washing with 75 mL of hot 2-propanol. HCl gas was passed into the solution for 15 s, 1.5 L of ether was added, and the solution was cooled with ice. The resulting crystalline product was removed by filtration, washed with cold ether, and dried 2 h at 60 °C under reduced pressure to afford 29.6 g (0.16 mol, 59%) of 4-ethyl-2-pyridinecarboxylic acid hydrochloride as a white crystalline solid, mp 173–176 °C.

4-Ethyl-2-pyridinecarboxylic 3-Methylbutanoic Anhydride (4e). A solution of 67 g (0.357 mol) of **3e**, 71.5 g (0.714 mol) of triethylamine, and 2.5 L of acetonitrile was cooled to 10 °C, and 47.6 g (0.354 mol) of isobutyl chloroformate was added in one portion. This mixture (solution A) was stirred at 10 °C for 1 h and then used in the following preparation.

4-Ethyl-2-pyridinecarboxamide of 7-Cl-MTL (5e). A mixture of 1.5 L of acetone, 1.5 L of H_2O, and 97.7 g (0.357 mol) of **2** was warmed until all of the solid had dissolved. The mixture was cooled to 30 °C, and solution A from the preceding preparation was added in one portion. After the reaction mixture was stirred at 25 °C for 18 h, the acetone and acetonitrile were removed under vacuum. The white mushy residue was collected by filtration and dried to give 95 g of pure product. Workup of the filtrate by chromatography over silica gel with a $CHCl_3$/MeOH (6:1) solvent system gave an additional 10 g of product for an overall yield of 75%. For physicochemical data on **5e** and its analogues, see Table VI.

Pirlimycin (7e). A mixture of 4.05 g (0.01 mol) of **5e**, 40 mL of H_2O, 60 mL of MeOH, 1.0 mL of 37% HCl, and 8.0 g of PtO_2 was reduced on a Parr hydrogenator at 50 psi and 25 °C for 18 h. Analysis of the reaction mixture by TLC in a CHCl/MeOH (6:1) solvent system showed that all of the starting material was gone and that two more polar materials were present in a ratio of about 1:1. The reaction was filtered, and the filtrate was concentrated under vacuum to a white, crystalline mush. These crystals were collected by filtration, and the filtrate was saved. The white crystals, which were the most polar of the two products observed upon TLC of the reduction mixture, were recrystallized

Table II. Activity of Clindamycin and Pirlimycin against Aerobic Bacteria

organism	UC no.	MIC, μg/mL clindamycin	MIC, μg/mL pirlimycin
Staphylococcus aureus	6685[a]	>25	>25
Staphylococcus aureus	6686[a]	0.05	0.20
Staphylococcus aureus	6687[a]	0.025	0.20
Staphylococcus aureus	6688[a]	>25	>25
Staphylococcus aureus	6689[a]	0.05	0.78
Staphylococcus aureus	6690[a]	0.025	0.20
Staphylococcus aureus	6691[a]	0.10	0.20
Staphylococcus aureus	6692[a]	>25	>25
Staphylococcus aureus	6693[a]	0.05	0.78
Staphylococcus aureus	6694[a]	>25	>25
Staphylococcus aureus	6695[a]	0.10	0.39
Staphylococcus aureus	6696[a]	0.10	0.39
Staphylococcus aureus	6675	0.05	0.39
Staphylococcus aureus	76	0.05	0.10
Staphylococcus aureus	570	0.20	0.39
Staphylococcus aureus	571	0.20	0.78
Staphylococcus aureus	746	0.05	0.05
Staphylococcus epidermidis	719	0.10	0.20
Staphylococcus epidermidis	3389	0.10	0.20
Streptococcus faecalis	694	25	6.25
Streptococcus pyogenes	152	≤0.012	≤0.012
Streptococcus mitis	153	≤0.012	0.05
Streptococcus mitis	871	≤0.012	≤0.012
Streptococcus pneumoniae I	41	≤0.012	≤0.012
Streptococcus pneumoniae III	3213	≤0.012	≤0.012
Escherichia coli	45	>25	>25
Proteus vulgaris	93	>25	>25
Klebsiella pneumoniae	58	12.5	>25
Salmonella schottmuelleri	126	>25	>25
Pseudomonas aeruginosa	95	>25	>25
Pasteurella multocida	264	3.9	62.5

[a] Multiple antibiotic resistance.

Table III. Activity of Clindamycin and Pirlimycin against Anaerobic Bacteria

organism	UC no.	MIC, μg/mL clindamycin	MIC, μg/mL pirlimycin
Bacteroides fragilis	6513	0.06	0.12
Bacteroides fragilis	6428	0.12	0.25
Bacteroides fragilis	6864	3.9	2.0
Bacteroides fragilis	6862	7.8	15.6
Bacteroides thetaiotaomicron	6512	2.0	0.5
Bacteroides distasonis	6518	0.12	≤0.03
Bacteroides melaninogenicus	6326	0.06	0.06
Clostridium perfringens	247	0.06	0.12
Clostridium perfringens	6509	0.06	0.12
Clostridium novyi B	6329	0.06	0.12
Clostridium tertium	6508	7.8	7.8
Clostridium cadaveris	6510	≤0.03	0.06
Clostridium sordellii	6505	2.0	0.5
Clostridium tetani	6521	≤0.03	≤0.03
Clostridium botulinum A	6506	0.25	≤0.03
Clostridium bifermentans	6507	0.50	0.06
Clostridium difficile	6834	7.8	3.9
Clostridium difficile	6857	250	125
Clostridium difficile	6858	3.9	3.9
Clostridium difficile	6860	500	500
Clostridium difficile	6861	3.9	2.0
Proprionibacterium acnes	6564	0.06	0.12
Proprionibacterium acnes	6575	<0.03	0.06
Eubacterium limosum	6515	2.0	2.0
Eubacterium lentum	6522	0.50	1.0
Actinomyces naeslundii	5920	0.25	0.25
Fusobacterium nucleatum	6516	0.12	0.12
Fusobacterium nucleatum	6324	0.06	0.06
Fusobacterium varium	6052	15.6	3.9
Fusobacterium necrophorum	6568	0.06	0.06
Peptococcus asaccharolyticus	6214	0.50	0.25
Peptococcus magnus	6258	0.06	0.06
Peptoccus aerogenes	6319	≤0.03	0.06
Peptostreptococcus anaerobius	6321	0.12	0.12

Table IV. Antibacterial Activity of Clindamycin and Pirlimycin in Experimentally Infected Mice

organism	UC no.	median protective dose (CD_{50}, mg/kg) with 95% confidence interval			
		clindamycin[a]	pirlimycin[a]	clindamycin[b]	pirlimycin[b]
Staphylococcus aureus	76	5.7 (4.2-7.8)	0.4 (0.3-0.5)	12.3 (8.8-17.3)	2.9 (2.1-4.2)
Streptococcus pyogenes	152	2.3 (1.6-3.3)	0.25 (0.2-.33)	12.3 (10.2-14.8)	2.9 (2.0-4.1)
Streptococcus pneumoniae I	41	40 (30.7-52.2)	1.7 (1.2-2.3)	45.5 (31.5-60.2)	20.3 (14.7-28)
Streptococcus pneumoniae III	3213	20 (13.1-30.6)	2.7 (1.9-3.7)	37.9 (25.7-55.9)	17.7 (12.4-25.1)
Klebsiella pneumoniae	58	>320	>320	>400	>400
Proteus vulgaris	347	>160	>160	>400	>400
Escherichia coli	311	>160	>160	>400	>400
Pasteurella multocida	264	>160	>160	>400	~400
Bacteroides fragilis	6428	14.4 (10.7-19.3)	<6.25	ND[d]	ND
Plasmodium berghei[c]		53 (46-61)	16 (12-22)	ND	>50

[a] Subcutaneous administration. [b] Oral administration. [c] Subcutaneous CD_{50} for chloroquine = 11.5 (8.8-15) mg/kg. [d] Not determined.

Table V. Concentration of Clindamycin and Pirlimycin Bioactivity in Blood and Abscess in *Bacteroides fragilis*[a] Infected Mice Following a Single Subcutaneous Dose of 200 mg/kg

time of bleeding, min	clindamycin			pirlimycin		
	A[b]	B[c]	A/B[d]	A[b]	B[c]	A/B[d]
15	16	17	0.94	58	39	1.5
30	22	23	0.96	48	43	1.1
45	26	17	1.5	32	25	1.3
60	21	12	1.8	31	20	1.5
90	13	4	3.3	31	14	2.2
120	9	3	3	22	10	2.2
150	8	1.4	5.7	19	8	2.4
360	3	~0.2	15	15	4	3.7

[a] *Bacteroides fragilis* UC 6428; clindamycin MIC = 0.12 μg/mL; pirlimycin MIC = 0.25 μg/mL. [b] A = abscess (μg/g of abscess). [c] B = blood (μg/mL of whole blood). [d] A/B = ratio of abscess in blood.

from water to give a 40% yield of pirlimycin (**7e**). For physicochemical data on **7e** and its analogues, see Table VII.

(2*R*-*cis*)-4-Ethylpipecolamide of 7-Cl-MTL (6e). The filtrate saved during the preparation of **7e** was concentrated to dryness under vacuum; the residue was converted to its free base and chromatographed over silica gel with a $CHCl_3$/MeOH (6:1) solvent system. In this manner, the least polar material mentioned in the preparation of **7e** was obtained. It was converted to its HCl salt and recrystallized from acetone/water to give a 35% yield of **6e**.

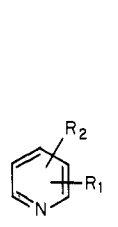
***N*-Methylpirlimycin (12).** A mixture of 5.0 g (0.012 mol) of pirlimycin (**7e**), 5 mL of 37% formaldehyde, 150 mL of EtOH, and 5 g of PtO_2 was reduced on a Parr hydrogenator at 50 psi and 25 °C for 18 h. The reaction was filtered, the filtrate was evaporated to dryness under vacuum, and the residue was chromatographed over silica gel with a $CHCl_3$MeOH (6:1) solvent system. A 39% yield of product was obtained as a white solid.

***N*-(2-Hydroxyethyl)pirlimycin (14).** A solution of 5.0 g (0.012 mol) of pirlimycin (**7e**) and 200 mL of MeOH was cooled to 0 °C, and 40 mL of ethylene oxide was added. After standing for 18 h at 0 °C, the reaction was evaporated to dryness under vacuum, and the residue was purified by chromatography over silica gel with a $CHCl_3$/MeOH (6:1) solvent system. A 70% yield of product was obtained as a white solid.

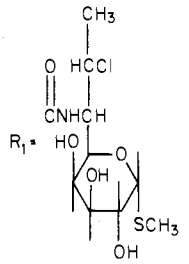
2(*S*)-Azetidenecarboxamide of 7-Cl-MTL (16). A solution of 1.0 g (2 mmol) of the *N*-Cbz precursor of **16**, 1.0 g of palladium black, 10 mL of cyclohexene, and 30 mL of 95% EtOH was stirred and heated at reflux for 30 min. The reaction was filtered, and the filtrate was evaporated to dryness under vacuum. The residue was dissolved in H_2O and washed with $CHCl_3$, and the aqueous phase was lyophilized to give a 65% yield of **16**.

Biology. In Vitro Antimicrobial Activity. Aerobic Bacteria. The minimum inhibitory concentration (MIC) for aerobic bacteria was determined by a microplate broth dilution technique. Serial twofold dilutions of the antibiotics were prepared in 50 mL

Table VI. Physical Data for Compound 5



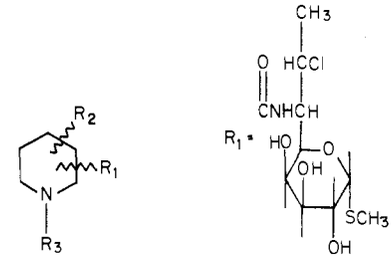
5



no.	position of R_1		mp, °C	$[\alpha]^{25}_D$,[a] deg	formula	anal.
5a	2	H	96-100	+286	$C_{15}H_{21}ClN_2O_5S$	C, H, Cl, N, S
5b	3	H		+224[b]	$C_{15}H_{21}ClN_2O_5S$	C, H, Cl, N, S
5c	4	H	189-190	+216[c]	$C_{15}H_{21}ClN_2O_5S$	C, H, Cl, N, S
5d	2	3-CH_3	179-181	+250[b]	$C_{16}H_{23}ClN_2O_5S$	C, H, Cl, N, S
5e	2	4-C_2H_5	92-95	+293	$C_{17}H_{25}ClN_2O_5S$	C, H, Cl, N, S
5f	2	4-*n*-C_3H_7	123-128	+285[d]	$C_{18}H_{27}ClN_2O_5S$	C, H, Cl, N, S
5g	2	4-*n*-C_4H_9	120-123	+279	$C_{19}H_{29}ClN_2O_5S$	C, H, Cl, N, S
5h	2	4-*t*-C_4H_9	95-98	+261	$C_{19}H_{29}ClN_2O_5S$	HRMS
5i	2	4-*n*-C_6H_{13}		+247[d]	$C_{21}H_{33}ClN_2O_5S$	HRMS
5j	2	4-*n*-C_8H_{17}		+208[b]	$C_{23}H_{37}ClN_2O_5S$	C, H, Cl, N, S
5k	2	5-C_2H_5		+267	$C_{17}H_{25}ClN_2O_5S$	C, H, N, S; Cl[e]
5l	2	6-CH_3	177-179	+232[b]	$C_{16}H_{23}ClN_2O_5S$	C, H, Cl, N, S
5m	2	6-C_2H_5	175-176	+213[b]	$C_{17}H_{25}ClN_2O_5S$	C, H, Cl, N, S

[a] Optical rotations were run in $CHCl_3$ unless otherwise noted. [b] MeOH. [c] EtOH. [d] Acetone. [e] Cl: calcd, 8.76; found, 8.35.

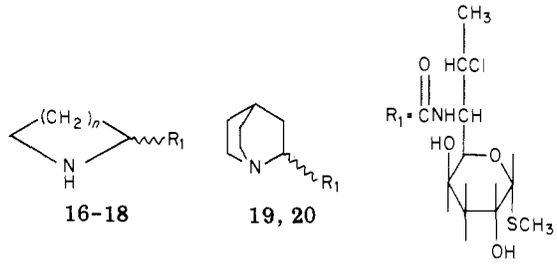
Table VII. Physical Data for Compounds 6-15



no.	R_1[a]	R_2	R_3	mp, °C	$[\alpha]^{25}_D$,[b] deg	formula	anal.
6a	2(*R*)	H	H	220-230	+206	$C_{15}H_{27}ClN_2O_5S\cdot HCl$	C, H, Cl, N, S
6b	3(*R*)	H	H			$C_{15}H_{27}ClN_2O_5S\cdot HCl$	HRMS
6c[c]	4	H	H	250[e]		$C_{15}H_{27}ClN_2O_5S\cdot HCl$	C, H, Cl, N, S[d]
6d	2(*R*)	3-CH_3	H		+208	$C_{16}H_{29}ClN_2O_5S\cdot HCl$	HRMS
6e	2(*R*)	4-C_2H_5	H	205-210	+206	$C_{17}H_{31}ClN_2O_5S\cdot HCl$	C, H, Cl, N, S
6f	2(*R*)	4-*n*-C_3H_7	H	182-185	+172	$C_{18}H_{33}ClN_2O_5S\cdot HCl$	HRMS
6g	2(*R*)	4-*n*-C_4H_9	H	180-183	+165[f]	$C_{19}H_{35}ClN_2O_5S\cdot HCl$	HRMS
6h	2(*R*)	4-*t*-C_4H_9	H		+190	$C_{19}H_{35}ClN_2O_5S\cdot HCl$	C, H, Cl, N, S
6i	2(*R*)	4-*n*-C_6H_{13}	H	160-165	+179	$C_{21}H_{39}ClN_2O_5S\cdot HCl$	H, N, S, Cl; C[g]
6k	2(*R*)	5-C_2H_5	H	157-160	+213	$C_{17}H_{31}ClN_2O_5S\cdot HCl$	HRMS
6l	2(*R*)	6-CH_3	H	214-217	+208	$C_{16}H_{29}ClN_2O_5S\cdot HCl$	HRMS
6m	2(*R*)	6-C_2H_5	H	222[e]	+187	$C_{17}H_{31}ClN_2O_5S\cdot HCl$	HRMS
7a	2(*S*)	H	H	180-183	+231[h]	$C_{15}H_{27}ClN_2O_5S$	C, H, Cl, N, S
7b	3(*S*)	H	H			$C_{15}H_{27}ClN_2O_5S\cdot HCl$	HRMS
7c[c]	4	H	H	250[e]		$C_{15}H_{27}ClN_2O_5S\cdot HCl$	C, H, Cl, N, S[d]
7d	2(*S*)	3-CH_3	H		+203	$C_{16}H_{29}ClN_2O_5S\cdot HCl$	HRMS
7e	2(*S*)	4-C_2H_5	H	210-212	+181[f]	$C_{17}H_{31}ClN_2O_5S\cdot HCl$	C, H, Cl, N, S
7f	2(*S*)	4-*n*-C_3H_7	H	217-220	+157	$C_{18}H_{33}ClN_2O_5S\cdot HCl$	H, N, S, Cl; C[i]
7g	2(*S*)	4-*n*-C_4H_9	H	224-226	+178	$C_{19}H_{35}ClN_2O_5S\cdot HCl$	C, H, Cl, N, S
7h	2(*S*)	4-*t*-C_4H_9	H	257-259	+165[f]	$C_{19}H_{35}ClN_2O_5S\cdot HCl$	C, H, Cl, N, S
7i	2(*S*)	4-*n*-C_6H_{13}	H	213-215	+158	$C_{21}H_{39}ClN_2O_5S\cdot HCl$	C, H, Cl, N, S
7j	2(*S*)	4-*n*-C_8H_{17}	H	183-186	+146	$C_{23}H_{43}ClN_2O_5S\cdot HCl$	HRMS
7k	2(*S*)	5-C_2H_5	H	203-206	+197	$C_{17}H_{31}ClN_2O_5S\cdot HCl$	HRMS
7l	2(*S*)	6-CH_3	H	212-216		$C_{16}H_{29}ClN_2O_5S\cdot HCl$	HRMS
7m	2(*S*)	6-C_2H_5	H	180[e]	+202	$C_{17}H_{31}ClN_2O_5S\cdot HCl$	HRMS
8	2(*R*)	H	CH_3	245-247	+219[f]	$C_{16}H_{29}ClN_2O_5S\cdot HCl$	C, H, Cl, N, S
9	2(*S*)	H	CH_3	229-234	+172[f]	$C_{16}H_{29}ClN_2O_5S\cdot HCl$	C, H, Cl, N, S
10	2(*R*)	4-C_2H_5	CH_3	165-168	+217	$C_{18}H_{33}ClN_2O_5S\cdot HCl$	C, H, Cl, N, S
11	2(*S*)	4-C_2H_5	CH_3	182-184	+158	$C_{18}H_{33}ClN_2O_5S\cdot HCl$	C, H, Cl, N, S
12	2(*S*)	4-*n*-C_6H_{13}	CH_3	170-173	+126	$C_{22}H_{41}ClN_2O_5S\cdot HCl$	HRMS
13	2(*R*)	4-C_2H_5	CH_2CH_2OH	190-192	+243	$C_{19}H_{35}ClN_2O_6S$	C, H, Cl, N, S
14	2(*S*)	4-C_2H_5	CH_2CH_2OH	218-220	+151	$C_{19}H_{35}ClN_2O_6S\cdot HCl$	HRMS
15	2(*S*)	4-*n*-C_4H_9	CH_2CH_2OH	210-212	+145	$C_{21}H_{39}ClN_2O_6S\cdot HCl$	C, H, Cl, N, S

[a] Notation describes the position of substitution of the amino acid amide in the ring and its configuration. [b] Optical Rotations were run in MeOH unless otherwise noted. [c] 6c = 7c. [d] S: calcd, 7.65; found, 7.23. [e] Decomposition. [f] H_2O. [g] C: calcd, 50.09; found, 49.26. [h] EtOH. [i] C: calcd, 46.85; found, 46.25.

Table VIII. Physical Data for Compounds 16-20



16-18 19, 20

no.	position of R_1	*n*	formula	anal.
16	2(*S*)	1	$C_{13}H_{23}ClN_2O_5S$	HRMS
17	2(*R*)	4	$C_{16}H_{29}ClN_2O_5S$	HRMS
18	2(*S*)	4	$C_{16}H_{29}ClN_2O_5S$	HRMS
19	2(*R*)		$C_{17}H_{29}ClN_2O_5S$	HRMS
20	2(*S*)		$C_{17}H_{29}ClN_2O_5S$	HRMS

of brain/heart infusion broth (Difco) in the wells of a microplate. Each well was then inoculated with 50 μL of a standardized cell suspension to yield a final concentration of 10^5 viable cells per milliliter of drug-supplemented medium. The microplates were incubated at 37 °C for 20 h, and the MIC was read as the lowest concentration of drug that inhibited visible growth of the organism.

In Vitro Antimicrobial Activity. Anaerobic Bacteria. The MIC for anaerobic bacteria was determined by an agar dilution method. Serial twofold dilutions of antibiotic were prepared in 1.0-mL aliquots of Schaedler broth (BBL), 9.0 mL of molten (47 °C) Wilkens–Chalgren agar medium was added, and the drug-supplemented agar was poured into sterile petri dishes. After the agar solidified, the plates were prereduced for 3 h in a Coy anaerobic chamber (atmosphere of 85% nitrogen, 10% hydrogen, 5% carbon dioxide). Test strains of bacteria were grown on Schaedler agar for 48 h prior to antibiotic assay. Colonies were harvested with a sterile swab, and a cell suspension was prepared to equal the turbidity of a 0.5 McFarland standard. A 0.001-mL drop of the cell suspension was applied to the surface of prereduced, drug-supplemented agar with a Steers replicator. The entire procedure was carried out inside the anaerobic chamber. The plates were incubated anaerobically at 37 °C for 48 h, and the MIC was read as the least amount of drug that inhibited growth. A very faint film of growth, or less than three colonies, was considered negative.

In Vivo Antimicrobial Activity. Efficacy Studies in Mice. The procedures for testing compounds in infected mice were described previously.[16] Male white CF-1 mice, weighing 18–20

(16) Lewis, C.; Wilkins, J. R.; Schwartz, D. F.; Nikitas, C. T. *Antibiot. Annu.* **1955–1956**, 897–902.

g, were selected at random from animal pools for use in the protection tests. Techniques for establishing mouse infections and maintenance of cultures will not be further described here.

Fresh stock solutions of the antibiotics were prepared at the beginning of each experiment and diluted so that the desired dose was contained in 0.2 mL when administered subcutaneously or in 0.5 mL when administered by oral intubation. The drug was administered immediately after the infecting dose and daily for 4 consecutive days thereafter. The test animals were observed for 3 additional days. Evaluation of antibiotic activity was based on the median protective dose (CD_{50}) as determined in groups of 10 animals challenged with 100 × LD_{50} of the microorganism. Calculation of the CD_{50} was by the method of Spearman and Karber.[17]

The procedures for testing antibiotics in *Plasmodium berghei* infected mice were different from those employed for the rest of the bacteria and have also been described previously.[18] The *Bacteroides fragilis* abscess model is similar to that described by Walker, Nitzan, and Wilkins.[19] The infection is produced by the subcutaneous injection of a *B. fragilis* culture grown in a semisolid medium, which results in the development of palpable subcutaneous abscesses. A human clinical isolate of *B. fragilis* was grown anaerobically on Schaedler Agar at 37 °C for 72 h. Six isolated colonies were harvested with a sterile loop and a cell suspension made in 2.0 mL of prereduced BHI broth. A 0.5-mL aliquot of this suspension was used to inoculate serum vials of prereduced BHI semisolid medium (BHI broth plus 0.25% agar). The vials were incubated anaerobically at 37 °C for 20 h. After incubation, the number of viable cells was determined by plate count, and 0.5 mL of culture was used to inoculate mice subcutaneously under the loose skin of the groin on the left side of the mouse. Untreated animals develop palpable subcutaneous abscesses in 4-7 days. Drug solutions were prepared in distilled water and administered in a 0.2-mL subcutaneous dose. Infected animals received one dose 4 h postinfection and two doses daily for 2 days on the side opposite the infected site. Mice were examined 7 days after infection for the presence or absence of abscesses, evident by palpation. The median protective dose (CD_{50}), i.e., the amount of drug required to protect 50% of the animals from abscess formation, was calculated from the 7-day data.

Pharmacology. Blood and abscess levels of pirlimycin and clindamycin in *B. fragilis* infected mice were determined by microbiological assay. Mice were infected with *B. fragilis* as previously described. Seven days postinfection, groups of five mice each were dosed with 200 mg/kg of pirlimycin or clindamycin and sacrified at various time intervals following dosage. Blood samples were collected by cardiac puncture, and abscesses were excised. These materials were assayed for antibiotic activity by standard microbiological methods.

Excretion of bioactivity via the urine of rats given a single subcutaneous or oral dose of pirlimycin or clindamycin was determined by standard microbiological methods. Animals were housed in individual metabolism cages for collection of urine.

Registry No. 1, 18323-44-9; 2, 22965-79-3; 3a, 98-98-6; 3b, 59-67-6; 3c, 55-22-1; 3d, 4021-07-2; 3e·HCl, 79415-18-2; 3f, 87999-87-9; 3g, 83282-39-7; 3h, 42205-74-3; 3i, 8799-88-0; 3j, 87999-89-1; 3k, 770-08-1; 3l, 934-60-1; 3m, 4080-48-2; 4e, 88015-20-7; 5a, 78788-61-1; 5b, 78788-62-2; 5c, 78788-63-3; 5d, 87999-90-4; 5e, 78788-60-0; 5f, 87999-91-5; 5g, 78788-74-6; 5h, 87999-92-6; 5i, 87999-93-7; 5j, 87999-94-8; 5k, 78788-66-6; 5l, 87999-95-9; 5m, 87999-96-0; 6a, 88154-62-5; 6b, 87999-97-1; 6c, 87999-98-2; 6d, 87999-99-3; 6e·HCl, 80081-63-6; 6f, 88000-00-4; 6g, 79464-95-2; 6h, 88000-01-5; 6i, 88000-02-6; 6k, 88080-01-7; 6l, 88000-03-7; 6m, 88000-04-8; 7a, 78822-42-1; 7b, 88000-05-9; 7d, 88080-02-8; 7e·HCl, 78822-40-9; 7f, 88080-03-9; 7g, 78788-73-5; 7h, 88080-04-0; 7i, 88080-05-1; 7j, 88000-06-0; 7k, 88080-06-2; 7l, 88080-07-3; 7m, 88080-08-4; 8, 88080-09-5; 9, 88080-10-8; 10, 88000-07-1; 11, 88080-11-9; 12·HCl, 88000-08-2; 13, 88000-09-3; 14·HCl, 88080-12-0; 7e, 79548-73-5; 15, 88000-10-6; 16, 88000-11-7; 16 (*N*-Cbz derivative), 88015-21-8; 17, 88000-12-8; 18, 88015-22-9; 19, 88000-13-9; 20, 88000-14-0; 2-bromo-4-ethylpyridine, 54453-91-7.

(17) Spearman, C.; Karber, G. "Statistical Methods in Biological Assays", 2nd ed.; Hafner: New York, 1964; pp 523-530.
(18) Lewis, C. *J. Parasitol.* **1968**, 169-170.
(19) Walker, C. B.; Nitzan, D.; Wilkins, T. D. *Antimicrob. Agents Chemother.* **1977**, 435-440.
(20) We thank R. C. Thomas for the preparation of this compound.

Notes

Studies on 1,2,3-Triazoles. 10.[1] Synthesis and Antiallergic Properties of 9-Oxo-1*H*,9*H*-benzothiopyrano[2,3-*d*]-1,2,3-triazoles and Their *S*-Oxides

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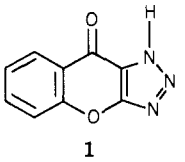
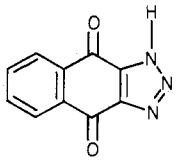
Selected derivatives of 9-oxo-1*H*,9*H*-benzothiopyrano[2,3-*d*]-1,2,3-triazole, a new heterocyclic ring system, and their *S*-oxides have been prepared and evaluated for antiallergic activity in the rat passive cutaneous anaphylaxis screen. Several of the compounds show intravenous potencies similar to or greater than that of disodium cromoglycate, the most potent being 6,7-dimethyl-9-oxo-1*H*,9*H*-benzothiopyrano[2,3-*d*]-1,2,3-triazole and its 4,4-dioxide.

Derivatives of benzopyrano[2,3-*d*]-1,2,3-triazole (**1**) and



1



2

the related naphtho[2,3-*d*]-1,2,3-triazole (**2**) have recently been shown to be potent inhibitors of the IgE-mediated passive cutaneous anaphylaxis (PCA) reaction in the rat,[1,2] and one compound, BRL 22321A, the sodium salt of the 6,7-dimethyl derivative of **2**, has undergone extensive pharmacological evaluation.[3] Of particular interest was

(1) Part 9. Buckle, D. R.; Smith, H.; Spicer, B. A.; Tedder, J. M. *J. Med. Chem.* **1983**, *26*, 714.
(2) Buckle, D. R.; Outred, D. J.; Rockell, C. J. M.; Smith, H.; Spicer, B. A. *J. Med. Chem.* **1983**, *26*, 251.

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