Analogs of Diaminopimelic Acid as Inhibitors of meso-Diaminopimelate Decarboxylase from Bacillus sphaericus and Wheat Germ*

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Analogs (1→6) of diaminopimelic acid have been synthesized and tested for inhibition of meso-diaminopimelate decarboxylases from Bacillus sphaericusIFO 3525 and from wheat germ (Triticum vulgarris). Difluoromethyl diaminopimelate 1 does not irreversibly inactivate or strongly competitively inhibit either enzyme. Lanthionine sulfonates (2ab, 2c, and 2d) are good competitive inhibitors (about 50% inhibition at 1 mM) of both decarboxylases. The meso and L-isomers of lanthionine sulfone (3ab and 3c) and lanthionine (6ab and 6c) are weaker competitive inhibitors (about 50% inhibition at 10–20 mM). The corresponding D-isomers (3d and 6d) are less effective. The N-modified analogs are the most potent competitive inhibitors. The inhibition constant (Kᵢ) values for B. sphaericus and wheat germ decarboxylases with N-hydroxydiaminopimelate 4 (mixture of isomers) are 0.91 and 0.71 mM, respectively; for the N-aminodiaminopimelate 5 (mixture of isomers) the Kᵢ values are 0.10 and 0.084 mM, respectively. These N-modified analogs do not effectively inhibit L-lysine decarboxylase. None of the compounds showed any time-dependent inactivation of the decarboxylases, in contrast to behavior of other pyridoxal phosphate-dependent enzymes with analogous substrate derivatives. Possible mechanisms of inhibition are discussed. In preliminary tests for antibiotic activity 4 and 5 both gave 75% growth inhibition of Bacillus megaterium at 20 μg/ml in defined media. Other analogs (1→3) showed essentially no antibacterial activity.

However, mammals lack this metabolic pathway and require L-lysine as an essential dietary constituent (9). Mammals also rapidly excrete administered diaminopimelate and small peptides containing it (10, 11), some of which act as promoters of slow-wave sleep (11, 12) or as immunoadjuvants (13). Since L-lysine is itself involved in peptidoglycan cross-linking in many Gram-positive bacteria (14) and is universally necessary for protein synthesis, analogs of meso-diaminopimelic acid which inhibit diaminopimelate decarboxylase could prove lethal to bacteria but should show little mammalian toxicity.

Meso-diaminopimelate decarboxylase from both prokaryotic and eukaryotic sources is pyridoxal phosphate dependent and catalyzes the decarboxylation of (2R,6S)-diaminopimelic acid (a meso compound) exclusively at the D-center with an unusual inversion of configuration to give L-lysine (15, 16) (Fig. 1). The enzyme is highly specific for the meso-isomer, and the D- and L-isomers of diaminopimelate are neither substrates nor effective inhibitors (17). Inhibition of the decarboxylase by L-lysine is well documented (17–22) and may assist regulation of lysine biosynthesis (21). A variety of structurally related compounds, including lanthionine (17, 18, 20), α-aminopimelic acid (17, 20), cystine (17, 19, 20), and norleucine (22) are only weak inhibitors.

A number of potentially more effective inhibitors, 1–5, can be envisioned (Fig. 2) based on substrate structure and consideration of the extensively studied mechanism of pyridoxal phosphate-dependent decarboxylases (23–25). The α-difluoromethyl analog 1 appeared especially promising because many α-difluoromethyl amino acids which have been examined are potent irreversible inhibitors of the corresponding amino acid decarboxylases (26–31). Although lanthionine 6 is only a weak competitive inhibitor of diaminopimelate decarboxylase (17, 18, 20), the sulfone 2 and sulfone 3 could be more likely to undergo pyridoxal phosphate-catalyzed β-elimination which may lead to enzyme inactivation (26, 27, 32–34). The N-hydroxy analog 4 chosen because N-hydroxyglutamic acid has been reported to be an irreversible inhibitor of various pyridoxal phosphate-dependent enzymes which metabolize glutamate, including glutamate decarboxylase (35). Finally, the N-amino (hydrazino) derivative 5 was expected to be a potent competitive inhibitor in analogy to the behavior of other α-hydrazino acids with enzymes using pyr-

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1. Reaction catalyzed by meso-diaminopimelate decarboxylase.

![Diagram of reaction catalyzed by meso-diaminopimelate decarboxylase.](https://example.com/diagram.png)
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Portions of this work (including “Experimental Procedures”) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 86M-307, cite the authors, and include a check or money order for $4.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverley Press.

EXPERIMENTAL PROCEDURES

RESULTS

Synthesis of Diaminopimelate Analogos—The difluoromethyl diaminopimelate 1 was prepared as a mixture of all possible isomers (a-d, Fig. 2) by the route illustrated in Scheme 1. The known (37) di-tert-butyl diaminopimelate 7 reacted with benzaldehyde to give the corresponding diimine 8, the lithiated anion of which was treated with chlorodifluormethane by the procedure of Bey et al. (38). Deprotection under acidic conditions gave compound 1 in 15% overall yield as a mixture of stereoisomers which could not be separated using various chromatographic techniques.

The pure meso-, LL-, and DD-isomers of lanthionine (6ab, 6c, and 6d, respectively) were prepared by condensation of D- or L-cysteine with the appropriate D- or L-isomer of β-chloroalanine (39). Each lanthionine isomer was then individually oxidized with hydrogen peroxide to the corresponding sulfoxide 2ab, 2c, or 2d by a modified literature procedure (40). Since no epimerization occurs at C-2 or C-6, the sulfoxides are stereocchemically pure except for 2ab which may be an unresolved mixture of two stereoisomers at the sulfur atom. The isomerically pure meso-, LL-, and DD-lanthionine sulfoxides (3ab, 3c, and 3d, respectively) were obtained by performic acid oxidation of the corresponding lanthionines 6ab, 6c, and 6d.

Synthesis of N-hydroxydiaminopimelate 4 was accomplished as shown in Scheme 2. The monocarboxbenzoxo derivative 9 of di-tert-butyl diaminopimelate 7 was oxidized by the method of Polanski and Chimiak (41) to give 10. Deprotection of 10 with hydrogen bromide in acetic acid produces the desired N-hydroxydiaminopimelate 4 as a mixture of all possible stereoisomers (a-d).

The N-aminodiaminopimelate (hydrazone analog) 5 was prepared as illustrated in Scheme 3. Racemic α-aminopimelic acid was N-protected by the method of Sheehan and Guziec (42) to give compound 11. This was converted to its bis-acid chloride and treated with the lithium derivative of the ROxazolidone 12 described by Evans et al. (43). An easily separable mixture of 13 and its diastereomer having opposite configuration at the α-carbon is produced. Reaction of the lithiated anion of 13 with dibenzyl azodicarboxylate afforded 14 with good stereoselectivity, 2 but epimerization occurred during basic cleavage of the chiral oxazolidone auxiliary to give 15. Hydrogenolysis of the protecting groups produced the N-aminodiaminopimelate 5 as an optically inactive mixture of stereoisomers.

Interaction of Substrate Analogos with meso-Diaminopimelate Decarboxylase—The diaminopimelate decarboxylases from B. sphaericus (17) and wheat germ (T. vulgaris) (18) were tested for inhibition of release of 14CO2 from [1,7-14C]diaminopimelate (16) by substrate analogs 1-5 (Fig. 2). Although the pure meso-, LL-, and DD-isomers (a, c, and d, respectively) of the sulfur-containing amino acids 2, 3, and 6 were examined individually, the other analogs, 1, 4, and 5, were each used as statistical mixtures of all possible stereoisomers a-d. The isomeric forms of the latter compounds could not be separated by a variety of chromatographic methods. However, the preference of these decarboxylases for the meso-isomer and their absolute specificity for decarboxylation of a (R) center (17) suggested that 1a, 4a, and 5a would be the most potent inhibitors. The results are shown in Table I.

None of the analogs exhibited any irreversible inhibition of either decarboxylase. Surprisingly, the difluoromethyl diaminopimelate 1 was not even a good competitive inhibitor. Lanthionines 6 and the sulfoxides 3 (Fig. 2) are also weak competitive inhibitors, with the meso (ab) and LL (c) isomers being generally stronger than DD (d) isomers. The lanthionine sulfoxides 2 show much stronger inhibition. Interestingly, in the case of the B. sphaericus enzyme, the LL-lanthionine sulfoxide 2c appears to be slightly more inhibitory than the meso compound 2ab. Since the N-hydroxy and N-aminodiaminopimelates 4 and 5 are the most potent inhibitors, the inhibition constants (Ki) were determined for these two analogs by standard methods (44, 45). The N-hydroxy compound 4 has Ki values of 0.71 and 0.91 mM for meso-diaminopimelinate decarboxylase from wheat germ (Km = 0.16 mM) (18) and from B. sphaericus (Km = 1.7 mM) (17), respectively. 2 The corresponding Ki values for the N-α-methoxamino analog 5 are 0.084 and 0.10 mM. Since enzyme assays were done in the presence of 50 μM pyridoxal phosphate which can be expected (35) to slowly react with 4 and 5, the assays for Kd determinations contained a large excess of these analogs (0.2-0.9 mM) to minimize interference from inhibitor depletion.

The N-hydroxy and N-α-methoxamino analogs 4 and 5 were also examined for specificity by testing them as inhibitors of L-lysine decarboxylase from B. cedaeae. Neither analog gave any time-dependent inhibition of this en-
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**Scheme 1**

**Scheme 2**

**Scheme 3**

**Table 1**

| Analog  | B. subtilis enzyme | T. vulgaris enzyme |
|---------|--------------------|--------------------|
|         | Concentration of analog (mM) | Activity of inhibitor (%) | Concentration of analog (mM) | Activity of inhibitor (%) |
| j       | 10                 | 64                 | 7                   | 88                   |
| 2ab     | 0.90               | 61                 | 0.90                | 46                   |
| 2c      | 0.90               | 56                 | 0.90                | 49                   |
| 2d      | 0.90               | 56                 | 0.90                | 57                   |
| 3a      | 10                 | 60                 | 10                  | 57                   |
| 3c      | 10                 | 71                 | 11                  | 64                   |
| 3d      | 10                 | 91                 | 10                  | 86                   |
| 4a      | 0.40               | 67                 | 0.70                | 55                   |
| 4b      | 0.40               | 7                  | 0.20                | 41                   |
| 6a      | 10                 | 52                 | 14                  | 55                   |
| 6c      | 10                 | 74                 | 16                  | 49                   |
| 6d      | 10                 | 100                | 14                  | 81                   |

* Mixture of stereoisomers.

zyme. The N-hydroxy compound 4 showed weak competitive inhibition (85% activity of control with 7 mM of this analog); the N-amino derivative 5 tested at 0.2 mM was noninhibitory.

Antibacterial Activity—Preliminary in vitro tests for antibiotic properties of diaminopimelate analogs 1→6 were done in both complex and defined media. Since most complex media derived from plant sources may be expected to contain l-lysine and some diaminopimelate, defined media lacking these amino acids was used to ensure de novo synthesis of bacterial l-lysine. The difluoromethyl analog 1 caused no inhibition of growth when tested at 400 µg/ml against Escherichia coli, Bacillus subtilis, or Bacillus cereus in defined (46, 47) liquid media or at 200 µg on discs against Arthrobacter simplex, Micrococcus roseus, or Micrococcus lysodeikticus growing on defined media on plates. None of the isomers of lantionine sulfoxide 2 or lantionine sulfone 3 caused any significant inhibition of growth when tested at 300 µg on discs against A. simplex, M. roseus, or M. lysodeikticus or at 350 µg against Streptomyces antibioticus, E. coli, B. subtilis, B. cereus, and Bacillus megaterium on defined media on plates. Very slight inhibition of B. subtilis by LL-lantionine sulfone 3c could be seen at higher concentrations (≥350 µg).

The N-hydroxy analog 4 and the hydrazino analog 5 also showed no growth inhibition at concentrations of up to 100 µg/ml against B. subtilis, B. cereus, B. megaterium, E. coli, Staphylococcus aureus, Streptococcus faecalis, Streptomyces antibioticus, and Propionibacterium thermii grown in complex media. However, in defined media (46, 47), at a concentration of 20 µg/ml, both 4 and 5 inhibited the growth of B. megaterium by 75%. At 500 µg/ml, B. subtilis growth was inhibited almost completely by both 4 and 5. E. coli and Pseudomonas aeruginosa were not affected.

**DISCUSSION**

It is helpful to consider the mechanism of meso-diaminopimelate decarboxylase in order to understand its behavior with substrate analogs. During decarboxylation of α-amino acids by pyridoxal phosphate-dependent decarboxylases the bond between the α-carbon and the carboxyl carbon of the substrate is expected to be nearly perpendicular to the plane of the cofactor's conjugated π system (Fig. 3) (23-25). The cofactor essentially stores the electrons of the cleaved bond until protonation from solvent can occur. This reaction proceeds with retention of configuration with all pyridoxal phosphate-dependent α-decarboxylases investigated to date except for meso-diaminopimelate decarboxylase which shows inversion (15, 16). If a substrate analog is used which has a leaving group (especially fluorine) attached to a β-carbon of the α-amino acid, the intermediate incipient anion (B or B+ Fig. 3) can in theory cause elimination to generate an electron-deficient conjugated system. Stereoelectronic considerations for elimination reactions (48) require that the bond between
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Normal Mechanism

\[ \text{PLP-ENZ} \] + \[ \text{N-ENZ} \] → \[ \text{OVERALL REACTION} \]

Potential Inhibition by β-Elimination

\[ A_1 \] → \[ B_1 \] → \[ C_1 \]

Inhibition by N-Modification

\[ A_3 \] → \[ B_3 \] → \[ C_3 \]

FIG. 3. Mechanism of diaminopimelate decarboxylase and possible modes of inhibition by substrate analogs. In each case the initial enzyme-bound substrate-cofactor complex is labeled A and the intermediate decarboxylated complex before protonation is designated by B. Hollow arrows in reactive complexes D1 and D2 indicate possible sites of nucleophilic attack.

Based on such considerations and on extensive precedent with other pyridoxal phosphate-dependent α-decarboxylases (26-31), the α-difluoromethyl diaminopimelate 1 was expected to be a potent inactivator of the meso-diaminopimelate decarboxylases. However, the total lack of irreversible or even strong competitive inhibition shows that this analog cannot bind effectively to the enzyme active site. Apparently both the plant and bacterial diaminopimelate decarboxylases enforce the stringent stereochemical requirement for the DL-isomer of substrate by a “tight fit” in the region surrounding the α-carbon and do not permit replacement of the α-hydrogen by a larger group (e.g., difluoromethyl). This contrasts with the behavior of most other pyridoxal phosphate-dependent α-decarboxylases which easily accommodate an α-methyl or α-difluoromethyl group (30, 50).

Elimination of a group “X” at the β-carbon of the side chain should circumvent this difficulty and lead to enzyme...
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inhibition. The sulfoxides 2 and sulfones 3 were investigated because a number of pyridoxal phosphate-catalyzed β-eliminations of sulfur-containing groups (including thiols) are known (51, 52), and the parent meso-lanthionine 6ab is decarboxylated by diaminopimelate decarboxylase at 5% of the rate of the natural substrate (17). Although the LL- and meso-isomers of lanthionine sulfoxide 2 and sulfone 3 are competitive inhibitors (Table I), they do not show time-dependent inactivation of the decarboxylases. The failure of the desired elimination reaction may be due to poor leaving ability of the sulfur-containing group caused by lack of protonation. Alternatively, it may be due to misalignment of the bond between the β-carbon and sulfur, i.e. the distal binding site for the side chain places the sulfur in position R’ or R” instead of at X in intermediate B2 (Fig. 3). If an unfavorable conformation is responsible for failure of elimination, replacement of the β-hydrogens of meso-diaminopimelate by halogen (e.g. fluorine) may afford a potent irreversible inhibitor of the decarboxylase. The reasons for stronger inhibitory activity of the sulfoxides 2 relative to the sulfones 3 or the parent lanthionines 6 are not clear. This effect may be due to some specific secondary binding of the sulfoxide functionality because the “wrong” DD- and LL-isomers also appear to compete effectively.

Modification of the substrate nitrogen presented an attractive alternative approach to inhibition of the decarboxylase. Cooper and Griffith (35) have observed that N-hydroxyglutamate irreversibly inhibits pyridoxal phosphate-dependent glutamate decarboxylase. These workers suggest formation of a very stable nitrooxime A4 (Fig. 3) in the active site as the cause of inhibition. Although N-hydroxysuccinimide 4 is a good competitive inhibitor of diaminopimelate decarboxylase from both wheat germ and  S. cerevisiae, no irreversible inactivation was seen. The compound is specific for this enzyme and does not inhibit L-lysine decarboxylase. Although enzyme-catalyzed decarboxylation of 4 appears unlikely, the possible occurrence of this process has not been rigorously disproven. Reversibility of the inhibition may result from binding of the N-hydroxy analog in the active site by initiation of transamination with the cofactor enzyme complex without completion of nitrooxime formation. Alternatively, the nitrooxime A4 may form but may undergo rapid and reversible enzyme-catalyzed cleavage or diffusion out of the active site. In either case, the behavior of plant and bacterial diaminopimelate decarboxylases is similar and again in strong contrast to precedent with other pyridoxal phosphate-dependent enzymes.

The N-aminodiaminopimelate 5 was expected to be a very potent competitive inhibitor because hydrazino substrate analogs have inhibition constants in the range of 1–2 μM with a pyridoxal phosphate-dependent histidine decarboxylase (30) and with aspartate aminotransferase (36). Presumably a hydrazino results from interaction of such analogs with the cofactor in the active site (A4, Fig. 3). Because of the intervening nitrogen, such species are incapable of the normal reactions (e.g. decarboxylation) and are also much more stable to hydrolysis than normal substrate imines. Taking into consideration the strict stereochemical requirements of diaminopimelate decarboxylases and the fact that analog 5 is probably a statistically mixture of all stereoisomers (25% would be the correct 5a isomer), the observed inhibition constants (Ki = 100 μM for B. cerevisiae enzyme; K = 84 μM for wheat germ enzyme) indicate quite potent inhibition.

The lack of antibacterial activity of the difluoromethyl diaminopimelate 1 and the sulfur-containing analogs 2 and 3 is in accord with their inability to irreversibly inhibit meso-diaminopimelate decarboxylase. Although the N-modified analogs 4 and 5 are better competitive inhibitors of this enzyme, their antibiotic action is very limited in preliminary microbiological tests. Since  B. megaterium is not affected by 4 and 5 in complex media but is strongly inhibited in defined media lacking L-lysine, one major difficulty appears to be rapid transport of exogenous L-lysine which circumvents the metabolic block. This could potentially be avoided by co-administration of a compound which interferes with transport of this amino acid. Another problem may be ineffective transport of the N-modified analogs 4 and 5 themselves into bacterial cells; this may account for the very limited antibiotic spectrum in defined media. It may be possible to overcome this problem by incorporation of the N-hydroxy- and N-aminodiaminopimelates 4 and 5 into dipeptides. Payne and co-workers (53–56) have shown that related peptides can enter bacterial cells more effectively, will release the toxic amino acid by hydrolysis, and will greatly enhance antibacterial activity. Studies on such dipeptides and on other inhibitors of enzymes in the diaminopimelate pathway are in progress.

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Supplementary Material on Analogs of Diaminopimelic Acid as Inhibitors of meso-Diaminopimelate Decarboxylase from Brevibacterium Sp. and Weiz Germ

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Experimental Procedures

Materials

All chemicals were of reagent grade. Dimethyl acetamide (DMA) was distilled under argon atmosphere. Dispropylamine was distilled from calcium hydride under argon atmosphere. Brevibacterium sp. cultures were grown in Diaminopimelate medium, 7 g was prepared from Diaminopimelic acid (mixture of isomers) by the method of Hino et al. [27]. 2-[3H]-propionyl-1,3-aminoadipic acid (2H-PAAD) was prepared from Brevibacterium sp. cultures as described in reference 27. Pyruvylpyruvate was prepared by the method of Payne [28]. 2-[3H]-propionyl-1,3-aminoadipic acid (2H-PAAD) was prepared from Brevibacterium sp. cultures as described in reference 27.

General information

Infrared (IR) spectra were recorded on a Nicolet T-99 FT-IR instrument. Mass spectra (MS) were obtained on Kratos A.E. K-50-50 high vacuum double focusing magnetic sector mass spectrometer. Proton nuclear magnetic resonance (NMR) spectra were recorded on Varian XL-90 (50 MHz) and XEI-700 (200 MHz) instruments in the specified deuterated solvent with tetramethylsilane (TMS) or deuterated sodium J-trimethylsilylJ-propionate (TSP) as internal standards. 19F and 13C NMR spectra were obtained on a Bruker WH-90 (21 MHz) NMR spectrometer. The 1H NMR spectra were recorded on Bruker WM-80 (80 MHz) and WH-200 (200 MHz) instruments in the specified deuterated solvent with tetramethylsilane (TMS) or deuterated sodium J-trimethylsilylJ-propionate (TSP) as internal standards. 19F and 13C NMR spectra were obtained on a Bruker WH-90 (21 MHz) NMR spectrometer. The 1H NMR spectra were recorded on Bruker WM-80 (80 MHz) and WH-200 (200 MHz) instruments in the specified deuterated solvent with tetramethylsilane (TMS) or deuterated sodium J-trimethylsilylJ-propionate (TSP) as internal standards.
Inhibitors of meso-Diaminopimelate Decarboxylase

(2.5 g) was dissolved in glacial acetic acid (10 ml) and added to cooled (4°C) glacial acetic acid (10 ml) into which hydrogen bromide gas had been passed for 5-10 min. The mixture was immediately added to ice water (50 ml), then during 10-15 min (50 ml) was stirred vigorously at room temperature for 3 h. The phases were separated and the aqueous phase was washed with dichloromethane (2 x 50 ml). Concentration of the organic phase to dryness was carried out under an orange fume. This was applied to a column of ion exchange resin (AG 50W-X4, 50-100 mesh, H+ form, 100 ml bed volume) and eluted with aqueous 1 M ammonium buffer (pH 3.3) (60) and detected with ninhydrin spray. Concentration in vacuo left 30 mg (1%) of a white solid. 235°C (decomp.); 195 (Hg); 220 (Br); 254, 555 (M+NH4); 551 (M+H); 577 (M+Na); 592 (M+K); 615 (M+Rb); 641 (M+Cs); 297 (M+Na+2); 82.2 (H2); 6.26 (H2O).

Compounds 9 and 10 were isolated by the procedure of Pollak and Chinias (41). The product was purified by flash chromatography (64) (25% ether/dichloromethane: 60% acetic acid/hexane) to give 45% of 5a as a colorless oil. 1H NMR (CDCl3, 300 MHz): 5.16 (s, 1 H, H-11); 4.73 (t, J = 2.3 Hz, 2 H, H-12); 3.73 (s, 3 H, 3 COO-); 3.57 (s, 3 H, 3 COO-); 3.35 (s, 3 H, 3 COO-); 3.21 (s, 2 H, H-13); 2.06 (s, 3 H, 3 COO-); 1.24 (s, 6 H, 6 CH3); 1.14 (s, 3 H, 3 COO-). Anal. Calc. for C24H29NO12: C, 58.96; H, 4.83; N, 5.86. Found: C, 58.84; H, 4.90; N, 5.70.

To a cooled (4°C) solution of 10 (0.34 g, 1.2 mmol) in glacial acetic acid (5 ml) was added a saturated solution of hydrogen bromide in acetic acid (1 ml). The mixture was allowed to warm to room temperature and, after 30 min, water (5 ml) was added. The mixture was concentrated to about one half and the residue was dissolved in acetic acid (1 ml). The solution was cooled to 4°C and hydrogen bromide was bubbled through for 3-10 min. After 20 min further at 4°C, water (10 ml) was added and the mixture was applied to an ion exchange column (AG 50W-X4, 50-100 mesh, H+ form, 100 ml bed volume) and eluted with 2% aqueous ammonia to give 17% (4%) of an apparently chromatographically pure 5a.

1M-HCl (pH 1.2) and cooling to 0°C. Piperonal (15%) was added and the reaction mixture was concentrated to dryness. To the residue was added a tetrachlorocarbon (500 ml) and the residue was dissolved in acetic acid (1 ml). The solution was cooled to 4°C and hydrogen bromide was bubbled through for 3-10 min. After 20 min further at 4°C, water (10 ml) was added and the mixture was applied to an ion exchange column (AG 50W-X4, 50-100 mesh, H+ form, 100 ml bed volume) and eluted with 2% aqueous ammonia to give 17% (4%) of an apparently chromatographically pure 5a.

A solution of 5a (0.34 g, 1.2 mmol) in glacial acetic acid (5 ml) was added and the mixture was concentrated to dryness. To the residue was added a tetrachlorocarbon (500 ml) and the residue was dissolved in acetic acid (1 ml). The solution was cooled to 4°C and hydrogen bromide was bubbled through for 3-10 min. After 20 min further at 4°C, water (10 ml) was added and the mixture was applied to an ion exchange column (AG 50W-X4, 50-100 mesh, H+ form, 100 ml bed volume) and eluted with 2% aqueous ammonia to give 17% (4%) of an apparently chromatographically pure 5a.
Inhibitors of meso-Diaminopimelate Decarboxylase

Lysine decarboxylase from Bacillus subtilis (Type 1) was obtained from Sigma Chemical Co. and assayed by measuring $^{14}$C$_2$O$_2$ evolution from L-[1-$^{14}$C]lysine following the method of Monod and Fisher (68). Diaminopimelate decarboxylase was isolated from Saccharomyces cerevisiae (87, 222) by the method of Asad-Sg (171) and from wheat germ (Triticum vulgare) by modification of the method of Menzels and Creveling (181). After ammonium sulfate fractionation as previously described (18), the enzyme was further purified by column chromatography on Bio-Gel P-2 (18). The enzyme was eluted from the column with 0.1 M NaCl in buffer A (0.05 M Tris-HCl pH 7.0, 0.05 M NaCl, 0.1% thimerosal, and 10 mg of pyridoxal phosphate per ml). The peak was collected and concentrated by ultrafiltration, dialyzed against 0.1 M NaCl, and applied to a hydroxylapatite column (Bio-Gel HTP). The enzyme was eluted from the column with 20 mM buffer A. Lysine decarboxylase was eluted with 20 mM buffer A containing 0.1 M NaCl. The enzyme was eluted with the same buffer containing 0.15 M NaCl, concentrated by ultrafiltration, dialyzed against buffer A, and applied to a hydroxylapatite column (Bio-Gel HTP). Diaminopimelate decarboxylase was eluted with 20 mM buffer A and had a specific activity of 0.076 units/mg. Enzyme activity was assayed by measuring the release of $^{14}$CO$_2$ from L-[1-$^{14}$C]lysine as previously described (67). Assays of inhibition were accomplished in the same way, except that amine decarboxylase was included in the assay. Assays for time-dependent inhibition were performed by incubating the assay mixture with enzyme at 30°C and withdrawing aliquots of the mixture at 0, 15, and 30 minutes incubation time for assay by the above procedure. Control experiments were performed simultaneously.

Rates of $^{14}$CO$_2$ production were analyzed by the statistical method of Wilkinson (44). Competitive inhibition constants $K_i$ for analogues 4 and 5 were obtained from experiments in which $^{14}$CO$_2$ was measured as a function of diaminopimelate concentration using the relationship $K_i = K_i [I] + K_i [I]$. Where $K_i$ and $K_i$ are apparent $K_i$ values in the presence and absence of inhibitor $I$, respectively (45).

Antimicrobial Testing

All bacterial cultures were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and identified by the following succession numbers: Actinomyces naeslundii 6545, Neisseria subflava 6501, N. cerevisiae 27740, P. aeruginosa 13724, Pseudomonas aeruginosa 103, E. coli 222, P. aeruginosa 6309, Aerobacter aerogenes 9484, Staphylococcus aureus 6360, Micrococcus luteus 6360. All organisms were grown aerobically with shaking.

Inhibition of growth in liquid culture was performed in roller tubes containing 10 ml of medium, sterilized by autoclaving for 30 min at 130°C, and inoculated with 1% of an overnight culture. Analogues were added as filter-sterilized (Millipore 0.45 μm) aqueous solutions. Growth was observed for 18 h or until tubes with no growth showed adequate turbidity (0.5, 600 μm × 0.1). Experimental values were recorded relative to these controls. All tests were performed in duplicate.

For disc inhibition tests, plates containing media solidified with 1.5% agar were inoculated with 0.2 ml of an overnight culture, spread over the surface and allowed to dry. Seven filter discs (Whatman No. 1, 3 mm) were impregnated with up to 600 μg of analog and placed on the agar surface. The plates were incubated for 24 h. Inhibition was scored as the diameter of the clear zone.