Analogs of Diaminopimelic Acid as Inhibitors of meso-Diaminopimelate Dehydrogenase and LL-Diaminopimelate Epimerase*

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Analogs 1–8 of diaminopimelic acid (DAP) were synthesized and tested for inhibition of purified meso-DAP D-dehydrogenase from Bacillus sphaericus and of LL-DAP epimerase from Escherichia coli. The dehydrogenase was assayed by monitoring NADPH formation spectrophotometrically at 340 nm. N-Hydroxy DAP 4, stereoisomers of the dehydrogenase with relative rates exceeding those of the meso isomers of the thin analogs 1ab, 2ab, and 3ab. DAP epimerase was assayed by coupling the epimerization of LL-DAP to DL-DAP (Ki = 0.26 mM) with the dehydrogenase-catalyzed oxidation of DL-DAP by NADP. Lanthionine isomers 1ab and 1c were stronger inhibitors of the epimerase (Ki = 0.18 mM, K′ = 0.67 mM, and K = 0.42 mM, respectively) than the corresponding meso-sulfoxide 2ab or meso-sulfone 3ab. Other isomers of 2 and 3, as well as compounds 7 and 8, showed no epimerase inhibition. N-Hydroxy DAP 4 was the most potent competitive inhibitor (Ki = 0.0056 mM) of the epimerase, whereas N-amino DAP 5 is weaker (Ki = 2.9 mM) and 4-methylene DAP 6 is a noncompetitive inhibitor (K′ = 0.95 mM). Although none of the analogs tested showed time-dependent inactivation of either enzyme, compounds 4, 5, 6, and 7 display substantial antibacterial activities. Possible mechanisms of epimerase inhibition and significance of the DAP pathway as a target for antibiotics are discussed.

Attention has recently focused on the metabolism of meso-diaminopimelic acid (meso-DAP)† as a target for development of new antibiotics (1–5). This compound occurs in the cell wall peptidoglycans of virtually all Gram-negative and some Gram-positive bacteria (6, 7). It is also the immediate biosynthetic precursor of L-lysine in bacteria, green algae, some lower fungi, and all higher plants, but is absent in mammals. Although none of the isomers of the thio analogs 1ab, 2ab, and 3ab. DAP epimerase was assayed by coupling the epimerization of LL-DAP to DL-DAP (Ki = 0.26 mM) with the dehydrogenase-catalyzed oxidation of DL-DAP by NADP. Lanthionine isomers 1ab and 1c were stronger inhibitors of the epimerase (Ki = 0.18 mM, K′ = 0.67 mM, and K = 0.42 mM, respectively) than the corresponding meso-sulfoxide 2ab or meso-sulfone 3ab. Other isomers of 2 and 3, as well as compounds 7 and 8, showed no epimerase inhibition. N-Hydroxy DAP 4 was the most potent competitive inhibitor (Ki = 0.0056 mM) of the epimerase, whereas N-amino DAP 5 is weaker (Ki = 2.9 mM) and 4-methylene DAP 6 is a noncompetitive inhibitor (K′ = 0.95 mM). Although none of the analogs tested showed time-dependent inactivation of either enzyme, compounds 4, 5, 6, and 7 display substantial antibacterial activities. Possible mechanisms of epimerase inhibition and significance of the DAP pathway as a target for antibiotics are discussed.

Several variations of meso-DAP biosynthesis exist in different bacterial strains (9). Most bacteria (and higher plants) convert LL-DAP to the meso (DL) form with LL-diaminopimelate epimerase (EC 5.1.1.7) (10–12), but some bacteria, such as Bacillus sphaericus, bypass the LL form by means of meso (DL)-diaminopimelate D-dehydrogenase (EC 1.4.1.16) (Fig. 1) (9, 13–15). The meso (DL) isomer is then decarboxylated as the D (R) center with inversion of configuration by meso-diaminopimelate decarboxylase (EC 4.1.1.20) to form L-lysine (16, 17).

We have previously synthesized a series of DAP analogs 1–5 (Fig. 2) which proved to be competitive inhibitors of both the bacterial (B. sphaericus) and plant (wheat) meso-DAP decarboxylases (3). The strongest of these inhibitors, N-hydroxydiaminopimelate 4 and N-aminodiaminopimelate 5, display good antibiotic activity against certain bacteria. However, Girodeau and co-workers (4) found that although the olefinic analog 6 is a weak inhibitor of the bacterial (Escherichia coli) decarboxylase, it possesses potent antimicrobial properties. This result together with the fact that various stereoisomers of 6 have similar antibacterial potency suggested that LL-DAP epimerase could be the true target enzyme (4). To assess this proposal, in the present work we examine the interaction of diaminopimelate analogs 1–8 with LL-DAP epimerase from E. coli (10) and with meso-DAP D-dehydrogenase from B. sphaericus (14, 18). The L-glutamic acid γ-hydrazone 7 and its methylated derivative 8 were chosen as potential transition state analogs for these enzymes because they possess a planar amide nitrogen at the position corresponding to the α-carbon of diaminopimelate.

**EXPERIMENTAL PROCEDURES**

**Materials**—All chemicals were of reagent grade. Diaminopimelic acid (DAP) (mixture of stereoisomers), NADP, hog kidney leucine aminopeptidase (200 units/mg), lyophilized E. coli (strain W, ATCC 9637), and reactive blue 2-agarose were purchased from Sigma. [G-3H]Dihydrochloride (1 Ci/mmol) was purchased from Amer sham Corp. as a mixture of stereoisomers in statistical (25% SS) proportion. B. sphaericus (IFO 3325) was obtained from the Institute of Fermentation, Osaka. DEAE-cellulose DE52 was purchased from Whatman; Sephadex G-200 was obtained from Pharmacia LKB Biotechnology Inc.; hydroxyapatite (Bio-Gel HTP) was acquired from Bio-Rad. Spectraor 4 dialysis tubing (M, cutoff 12,000–14,000) was purchased from Fisher. Protein was measured relative to bovine serum albumin using a Bio-Rad protein kit (19).

The following substrate analogs were synthesized as previously described (3): lanthionine 1 (pure meso, LL, and DD-isomers); lanthionine sulfoxide 2 (pure meso, LL, and DD-isomers); lanthionine sulfone 3 (pure meso, LL, and DD-isomers); N-hydroxydiaminoimelate 4 (as a mixture of stereoisomers); and N-aminodiaminopimelate 5 (as a mixture of stereoisomers). L-Glutamic acid γ-hydrazone 7 is commercially available from Sigma. A sample of 1-(5-L-
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The final stages of the diaminopimelate pathway to L-lysine in bacteria.

The racemic (DD) bis-N-benzyloxycarbonyl isomer was further purified by recrystallization (m.p. 123–125 °C) from acetoneitrile (22) before hydrogenolytic deprotection. The pure meso-diaminopimelic acid showed no measurable optical rotation and had the following properties: IR (KBr disc) 3300–2400 (m, br), 2100 (w, br), 1631 (s), 1599 (vs), 1510 (m), 1503 (m), 1413 (m), 1397 (m), 1363 cm−1; 1H NMR (300 MHz, D2O) 7.56-7.31 (m, 2H, H-6, H-5), 5.29 (t, 2H, H-2'), 4.76 (t, 2H, H-3'), 2.16 (s, 3H, CH3), 1.65 (s, 3H, CH3).

The racemic (DD + LL) bis-N-benzyloxycarbonyl-diaminopimelic acid mixture was converted to the corresponding bis-amide, hydrogenolyzed, and resolved enzymatically according to the procedure of Wade et al. (21) with the aid of leucine aminopeptidase and resolved enzymatically according to the procedure of Girodeau et al. (4). General instrumentation and procedures have been previously described (3, 16).

Resolution of Diaminopimelate Isomers—The meso and DD or LL diastereomers of diaminopimelic acid were separated by selective crystallization of their bis-N-benzyloxycarbonyl derivatives as outlined by Wade et al. (21). The meso-bis-N-benzyloxycarbonyl isomer was obtained by recrystallization (m.p. 194–195 °C) directly from acetoneitrile (22).

The following properties: IR (KBr disc) 3420 (m, br), 3300–2400 (br, s), 2100 (w, br), 1631 (s), 1599 (vs), 1510 (m), 1503 (m), 1413 (m), 1397 (m), 1363 (m) cm−1; 1H NMR (300 MHz, D2O) 7.56-7.31 (m, 2H, H-6, H-5), 5.29 (t, 2H, H-2'), 4.76 (t, 2H, H-3'), 2.16 (s, 3H, CH3), 1.65 (s, 3H, CH3).

After drying 1 week in vacuo at 64 °C, POSFAB-MS (glycerol/formic acid) 191 (MH+), 381 (M+H+), 571 (M+H+).

Hydrolysis of the purified bis-amide of DD-diaminopimelic acid and recrystallization from H2O/MeOH gave the DD-isomer, which upon incubation with meso-DAP d-dehydrogenase displayed an initial reaction rate 0.16% of that obtained with meso-DAP using the standard assay. The IR, 1H NMR, and positive ion fast atom bombardment mass spectrum characteristics were identical to those of the LL-isomer: [α]D −45.7 (c 0.2, 1 N HCl) (lit. (22) [α]D −45.5° (c 1, 1 N HCl)).

C6H8N2O4
Calculated: C 44.20 H 7.42 N 14.53
Found: C 43.80 H 7.32 N 14.53

after drying in vacuo at 64 °C for 7 days.

Purification of LL-Diaminopimelate Epimerase—The LL-DAP epimerase was isolated from E. coli ATCC 9637 by modification of the method of Wiseman and Nichols (10). All enzyme purification steps were performed at 4 °C. Lyophilized E. coli cells (100 g, Sigma) were suspended in 1.1 liter of buffer A (50 mM potassium phosphate, 1 mM EDTA, 10 mM diithiostreitol, pH 7.0) and after 14 h at 4 °C were lysed in three batches by sonication. The cell pellet from sonication was suspended in 1-cm light path. Enzyme activity was monitored spectrophotometrically at 340 nm with a Beckman DU-8 spectrophotometer. The Km for meso-DAP under these conditions is 1.1 mM. A unit of activity is 1 μmol/min of NADPH formed under the conditions specified.

Unless otherwise specified, all reactions involving LL-DAP epimer-
ase were performed at 25 °C, pH 7.8, in 0.1 M Tris-HCl, 1 mM EDTA, and 1 mM diithiothreitol in a cuvette of a 1-cm light path. LL-DAP epimerase activity was conveniently assayed at 340 nm using an HP-8451 diode array UV spectrophotometer by coupling the conversion of LL-DAP to meso-DAP with the NADPH-dependent meso-DAP dehydrogenase. The enzyme was assayed at 25 °C following the method of Wiseman and Nichols (10). The assay solution contained 0.4 mM LL-DAP, 0.3 mM NADP, and 0.13 unit of meso-DAP dehydrogenase in the standard pH 7.8 Tris buffer. The Km for LL-DAP is 0.36 mM. A unit of epimerase activity is 1 μmol/min NADPH formed under these conditions. The assay is linear over time for a change of NADPH unit.

For the coupled enzyme assay, the meso-DAP d-dehydrogenase activity was assayed at 25 °C in 0.1 M Tris-HCl, 1 mM EDTA, 1 mM diithiothreitol, 0.25 mM NADP, 0.33 mM meso-DAP, pH 7.8, by following the increase in absorbance at 340 nm. The Km for meso-DAP under these conditions is 0.11 mM (10). The apparent equilibrium constant for the oxidation of meso-DAP resin approximately 1 at pH 7.8 (10). A unit of activity is 1 μmol/min NADPH formed under the conditions specified.

All the analogs 1–8 were tested as substrates of meso-DAP d-dehydrogenase by replacing the meso-DAP in the standard assay procedure. Inhibition studies of both meso-DAP d-dehydrogenase and LL-DAP epimerase were performed following the corresponding enzyme assay procedure as described above, except that various concentrations of substrate analog were included in the mixture. Assays for time-dependent inhibition were accomplished by incubating the analog with the appropriate enzyme at 25 °C and withdrawing aliquots at time intervals. No time-dependent dehydrogenase inactivation was observed for any of these compounds. The conditions for incubations containing between 5 and 10 mM 10 analog for up to 60 min.

The inhibitory effect of N-hydroxydiaminopimelate 4 on LL-DAP epimerase activity was assayed by the epimerase-catalyzed release of H2 from water from [G-3H]DAP after a 40-min incubation at 25 °C as previously described by Wiseman and Nichols (10). Typically, 10 μl of a reaction mixture containing 0.0004 unit of LL-DAP epimerase in 0.1 M Tris-HCl (pH 7.8), 1 mM EDTA, 1 mM diithiothreitol, and 1.0 μCi of (D5, L plus meso)-[G-3H]DAP (24) was added to a column (0.4 ml) of Bio-Rad AG 50W-X2 ion exchange resin (H+ form). The column was washed three times with 500 μl of water, and the eluates were combined and counted for radioactivity.

The kinetic constants Vmax and Ka were obtained from initial velocity data using the statistical method of Wilkinson (23). Competitive inhibition constants Ki were obtained using the relationship

\[ K_{a} = K_{a} + [I]/K_{a} \]

where Ka and Ki are apparent Ka values in the presence and absence of inhibitor (I), respectively (24). Noncompetitive inhibition constants Ki were calculated using the relationship

\[ V_{max} = V_{max}'(1 + [I]/K_{a}) \]

where Vmax and Vmax' are apparent Vmax values in the presence and absence of inhibitor (I), respectively (24).

Antibacterial Testing—All bacteria used were from the American Type Culture Collection (Rockville, MD) and identified by the following accession numbers: Arthrobacter simplex 6946, Bacillus subtilis 6051, Bacillus cereus 27348, Bacillus megaterium 15374, Escherichia coli 9637, Micrococcus roseus 196, Micrococcus luteus (glycosidicis) 4898, Pseudomonas aeruginosa 10145, Staphylococcus aureus 6533P, Streptomyces antibioticus 8663. Conditions under which these organisms were grown as well as experimental details for the growth inhibition studies have previously been reported (3).

RESULTS

Interaction of Substrate Analogs with meso-DAP d-Dehydrogenase—The diaminopimelate analogs 1–8 were first examined to determine whether they were substrates for the dehydrogenase which was isolated from B. sphaericus (18). Each pure isomer ab, c, and d of the sulfur-containing amino acids 1, 2, and 3 was tested individually whereas only the pure L (S)-isomers of 7 and 8 were employed. The other analogs 4, 5, and 6 were each used as mixtures of all possible stereoisomers a−d. The dehydrogenase assay monitors NADPH formation spectrophotometrically at 340 nm. Reaction rates (relative to meso-DAP as a standard) of the analogs which can act as substrates are shown in Table I. As previously reported (14), the enzyme exhibits strict specificity for meso-isomers and barely accommodates substitutions in the main carbon chain. Hence the c (LL) and d (DD) stereoisomers of 1–3 as well as compounds 7 and 8 could not detectably act as substrates for meso-DAP dehydrogenase. The Km for meso-lanthionine 1ab is 5.8 mM compared with 1.1 mM for meso-DAP, with a relative Vmax 1% of that observed for the natural substrate. Oxidation of the lanthionine sulfur gives compounds (2ab or 3ab) which are even poorer substrates.

Interestingly, N-hydroxydiaminopimelate 4, N-aminodiaminopimelate 5, and 4-methylene diaminopimelate 6 have relative reaction rates which exceed those of the sulfur analogs.

The reactive analogs (1ab, 2ab, 3ab, 4, 5, and 6) were tested for time-dependent inhibition of the dehydrogenase by incubation with the enzyme and removal of aliquots for standard assay at varying time intervals. No time-dependent dehydrogenase inactivation was observed for any of these compounds for incubations containing between 5 and 10 mM analog for up to 60 min.

Interaction of Substrate Analogs with LL-DAP Epimerase—All of the analogs described above were individually examined for inhibition of LL-diaminopimelate epimerase which was isolated from E. coli (10). A coupled enzyme assay (10) was used in which the conversion of LL-DAP to meso-DAP by the epimerase was measured by further transformation of this product to L-A^1-tetrahydrodipicolinate by excess meso-DAP dehydrogenase with concomitant formation of NADPH. The results are shown in Table II. None of the compounds exhibited any time-dependent irreversible inhibition since incubation of LL-DAP epimerase over 60 min with any of them at concentrations up to 4 mM caused no inactivation of the enzyme. When the sulfur-containing amino acids were tested for competitive inhibition, the LL- and DD-isomers of both lanthionine sulfides (2c and 2d) and lanthionine sulfones (3c and 3d) displayed no detectable effect on the epimerase, but their corresponding meso isomers, 2ab and 3ab, were very weak competitive inhibitors (Ki = 11 mM and Ki = 21 mM, respectively). The parent lanthionines 1 are much stronger inhibitors of LL-DAP epimerase than their oxidized derivatives, with the meso isomer 1ab being a mixed competitively

### Table I

Diaminopimelate analogs as substrates for meso-diaminopimelate d-dehydrogenase from B. sphaericus

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Concentration (mM)</th>
<th>Relative Velocity</th>
<th>Km (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>meso-DAP</td>
<td>10</td>
<td>100</td>
<td>1.1</td>
</tr>
<tr>
<td>1ab</td>
<td>10</td>
<td>1.08</td>
<td>5.8</td>
</tr>
<tr>
<td>2ab</td>
<td>14</td>
<td>0.091</td>
<td></td>
</tr>
<tr>
<td>3ab</td>
<td>10</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*See Fig. 2 for structures of analogs.
Inhibitors of Diaminopimelate Dehydrogenase and Epimerase

**Table II**

Inhibition of LL-diaminopimelate epimerase by substrate analogs 1–8

![Image](image-url)

**See "Experimental Procedures" for assay conditions; K_m for LL-DAP was determined as 0.26 mM.**

<table>
<thead>
<tr>
<th>Substrate analog</th>
<th>Concentration</th>
<th>K_m (mM)</th>
<th>K' (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1ab</td>
<td>0.1–0.4</td>
<td>0.18</td>
<td>0.67</td>
</tr>
<tr>
<td>1c</td>
<td>0.4–4.0</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>1d</td>
<td>4.0</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>2ab</td>
<td>4.0</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>2c</td>
<td>4.0</td>
<td>NI</td>
<td></td>
</tr>
<tr>
<td>2d</td>
<td>4.0</td>
<td>NI</td>
<td></td>
</tr>
<tr>
<td>3ab</td>
<td>4.0</td>
<td>21.0</td>
<td></td>
</tr>
<tr>
<td>3c</td>
<td>4.0</td>
<td>NI</td>
<td></td>
</tr>
<tr>
<td>3d</td>
<td>4.0</td>
<td>NI</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.008–0.04</td>
<td>0.0056</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.4–3.2</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.4</td>
<td>9.95</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4.0</td>
<td>NI</td>
<td></td>
</tr>
</tbody>
</table>

6  See Fig. 2 for structures of analogs.

7  NI, no inhibition.

![Image](image-url)

**Discussion**

Meso-DAP dehydrogenase was investigated first as a possible target for diaminopimelate analogs because this enzyme also provides a convenient assay for LL-DAP epimerase. Although the dehydrogenase is more widely distributed in bacteria than originally suspected (15), it still seems likely that many species employ the epimerase pathway to arrive at meso-diaminopimelate. Careful studies by Misono and Soda (14) on the dehydrogenase from *B. sphaericus* showed that it consists of two identical subunits (each of molecular weight 40,000), donates the 4-pro-S hydrogen of its cofactor NADPH to the substrate in the reductive reaction, and is highly specific for the meso isomer of diaminopimelate. An apparently nonessential thiol group (25) and a tryptophan residue (14) are known to be in the active site. Product inhibition studies indicated that the sequence of addition of substrates in the oxidative deamination is NADP⁺ followed by meso-diaminopimelate and that the order of release of products is ammonia, then L-Δ¹-tetrahydrodipicolinate (or L-α-amino-γ-ketopimelate), and finally NADPH (14).

The behavior of meso-diaminopimelate and its thia analog, meso-lanthionine 1ab, as substrates (Table I) is in excellent agreement with previous observations (14), as is the failure of any DD- or LL-isomers to be transformed. The requirements of the dehydrogenase are obviously very strict. A comparison of the physical dimensions of lanthionine 1ab (26) and diaminopimelate indicates that the increase in bond length (1.51 Å for C–S versus 1.54 Å for C–C) is partly offset by the smaller C–S–C bond angle such that the overall length of lanthionine is only slightly greater (about 5%). A certain amount of steric bulk can be tolerated at the central atom (γ-carbon of DAP) since at least one isomer (probably meso) of the methylene analog 6 is a modest substrate. However, electronic effects may be critical. The electronegativity of sulfur lowers the pK_a values of the protonated carboxyl and amino groups of 1ab by about 0.5–0.8 units (27), and this effect should be even greater for the sulfone 3ab and sulfone 3ab which are effectively nonsubstrates. Meso-Diaminopimelate analogs which are missing a functional group at either end such as the D- or L-isomers of α-aminopimelic acid or of lysine also are not transformed (14). Surprisingly, the N-hydroxy analog 4 (statistical mixture of isomers) is an excellent substrate for the dehydrogenase. Making the reasonable assumption that the DD- and DD-isomers (4c and 4d) do not react (in analogy to diaminopimelate) and that only one of the other two isomers is converted, the correct isomer (25% of the mixture, probably 4b) is oxidized nearly as well as the natural substrate. The structures of the products are presently under investigation, but they may be derived from a nitronate intermediate, Δ¹-tetrahydrodipicolinate N-oxide (Fig. 3). Preliminary observations suggest that a product undergoes a slow dehydrogenase-catalyzed reduction by NADPH. meso-DAP
Dehydrogenase also oxidizes analogs 5 and 6 but at a much slower rate. It is again likely that only a single isomer (probably 5b and 6ab) can act as a substrate, but the possibility that other isomers are substrates (e.g., 4a and/or 5a with oxidation occurring at the N-hydroxyl or N-amino sites) cannot be rigorously excluded at present. Confirmation of this will only be possible when currently ongoing stereospecific syntheses of each isomer of 4, 5, and 6 are completed.

Since most bacteria appear to employ L-DAP epimerase to make meso-diaminopimelate, this enzyme initially appeared to be a very attractive target for antibiotic development. The epimerase was detected over 30 years ago in E. coli (28) but was only recently purified and fully characterized (10). It exists as an active monomer of molecular weight 34,000, requires no cofactors and probably no metal, and appears to use an active site thiol group as one of two bases involved in the deprotonation-protonation process of diaminopimelate epimerization (10). The mechanism resembles that of proline racemase (29). Hence some anionic character might be expected to develop at the α-carbon, and the carbon framework at that site should be planar in the transition state.

This suggests that elimination of leaving groups on the β-carbon or on the adjacent nitrogen may be possible (Fig. 4). A diaminopimelate analog having an L-configuration at the distal binding site and a planar center at the position corresponding to the α-carbon should be a transition state mimic. In principle, such a compound could also be administered as well as be generated in situ by an elimination process.

None of the analogs 1–8 is able to irreversibly inactivate the epimerase. The meso-lanthionine 1ab and its Ll-isomer 1c are good mixed-competitive and competitive inhibitors, respectively, but the Dl-isomer 1d is much less effective (Table II), in accord with the stereochemical requirements of the enzyme for its natural substrates. Oxidation of the sulfur to a sulfoxide or sulfone drastically lowers the affinity for the epimerase’s active site, just as with the dehydrogenase. Whether this is primarily due to electronic effects (greater electronegativity) or to changes in overall geometry is not certain, but it is interesting to note that sulfoxide 2ab is a far better competitive inhibitor of the highly specific meso-diaminopimelate decarboxylase than either its reduced or oxidized analogs 1ab or 3ab, respectively (3).

Compounds 4–7 all have antibiotic activity against certain bacteria (3, 4, 30–32) with 6 and 7 strongly affecting E. coli, but they vary enormously in their ability to inhibit the Ll-DAP epimerase from this organism. The reason for the extremely potent inhibition by the N-hydroxy analog 4 is unknown, but it may be that elimination of water from the 4a or 4c isomer occurs to generate an α-imine bound to the enzyme as a planar transition state analog (Fig. 4). This could potentially be attacked by the active site thiol at the α-carbon in a reversible fashion with eventual release from the enzyme. Interestingly, the N-amino analog 5 is 3 orders of magnitude less effective as a competitive inhibitor than 4. This may reflect the inability of the terminal hydrazine amino group to leave through elimination, or it may be due to a change in site of protonation of the hydrazino moiety relative to the N-hydroxy group.

The antibiotic effects of 7, which does not inhibit the epimerase, can be ascribed to interference with glutamine metabolism (30–32). However, the relatively modest noncompetitive inhibition of the epimerase by strongly antibiotic 4-methylene diaminopimelate 6 indicates that this enzyme is not the site of action for this olefinic analog as previously suggested (4). It is interesting to note that bicyclomycin, an antibiotic which impairs cleavage of diaminopimelyl peptide bonds in peptidoglycan (5, 33), also bears a methylene group on the diaminopimelate chain.

Several factors suggest that Ll-DAP epimerase may also not be the enzyme responsible for the antimicrobial activity of the N-hydroxy analog of 4. One is that the N-amino derivative 5 is an equally effective antibiotic but is a much poorer epimerase inhibitor. A more relevant and direct observation was made by Stragier and co-workers (34). They produced insertion mutants of the dap F gene in E. coli, thereby incapacitating the epimerase. Nevertheless, these mutants still grow despite a large accumulation of Ll-DAP, possibly because other amino acid epimerases slowly produce meso-DAP for peptidoglycan synthesis. Although the N-hydroxy analog 4 is an effective antibiotic against B. megaterium, its failure to destroy E. coli may be due to problems of transport into the cell. This difficulty could potentially be overcome by incorporation of 4 into a dipeptide which can readily enter the cell and release the inhibitor by hydrolysis (35–38). Studies on such dipeptides as well as on formation of pure isomers of 4 and their effects on earlier enzymes in the diaminopimelate pathway are in progress.

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REFERENCES
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