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Mono-*N*-acyl-2,6-diaminopimelic acid derivatives: Analysis by electromigration and spectroscopic methods and examination of enzyme inhibitory activity

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ABSTRACT

Thirteen mono-*N*-acyl derivatives of 2,6-diaminopimelic acid (DAP)—new potential inhibitors of the dapE-encoded *N*-succinyl-L,L-diaminopimelic acid desuccinylase (DapE; EC 3.5.1.18)—were analyzed and characterized by infrared (IR) and nuclear magnetic resonance (NMR) spectroscopies and two capillary electromigration methods: capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC). Structural features of DAP derivatives were characterized by IR and NMR spectroscopies, whereas CZE and MEKC were applied to evaluate their purity and to investigate their electromigration properties. Effective electrophoretic mobilities of these compounds were determined by CZE in acidic and alkaline background electrolytes (BGEs) and by MEKC in acidic and alkaline BGEs containing a pseudostationary phase of anionic detergent sodium dodecyl sulfate (SDS) or cationic detergent cetyltrimethylammonium bromide (CTAB). The best separation of DAP derivatives, including diastereomers of some of them, was achieved by MEKC in an acidic BGE (500 mM acetic acid [pH 2.54] and 60 mM SDS). All DAP derivatives were examined for their ability to inhibit catalytic activity of DapE from *Haemophilus influenzae* (*Hi*DapE) and ArgE from *Escherichia coli* (*Ec*ArgE). None of these DAP derivatives worked as an effective inhibitor of *Hi*DapE, but one derivative—*N*-fumaryl, Me-ester-DAP—was found to be a moderate inhibitor of *Ec*ArgE, thereby providing a promising lead structure for further studies on ArgE inhibitors.

The World Health Organization reported that bacterial infections represent a significant and permanently growing medical problem around the world due to the increasing number of disease-causing microbes that have become resistant to currently available antibiotics [1–3]. In fact, several pathogenic bacteria, some of which were thought to have been eradicated, have made a significant resurgence due to bacterial resistance to antibiotics [4,5]. For example, resistance to tetracyclines increased from 0% in 1948 to 98% in 1998 [4,5], and tuberculosis is currently one of the leading causes of death in adults by an infectious disease worldwide, which is significant given that death rates due to tuberculosis had declined to near imperceptible levels in industrial nations [6,7]. According to the Centers for Disease Control and Prevention (USA), several bacterial strains currently exhibit multidrug resistance, with more than 60% of hospital-acquired infections in the United States alone caused by the so-called ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* sp.). These findings have stimulated an intensive search for new potent antimicrobial agents that are active against drug-resistant bacterial strains [5,8].

Inhibitors of cell wall biosynthesis—vancomycin and β-lactams, to name a couple—have proven to be very potent antibiotics, evidence that interfering with cell wall synthesis has deleterious effects on bacterial cell survival. Enzymes that are targeted by these antibiotics tend to be present in all bacteria and are highly similar in structure and function. Unfortunately, only two new antibacterial drugs have emerged since 1962. Because every

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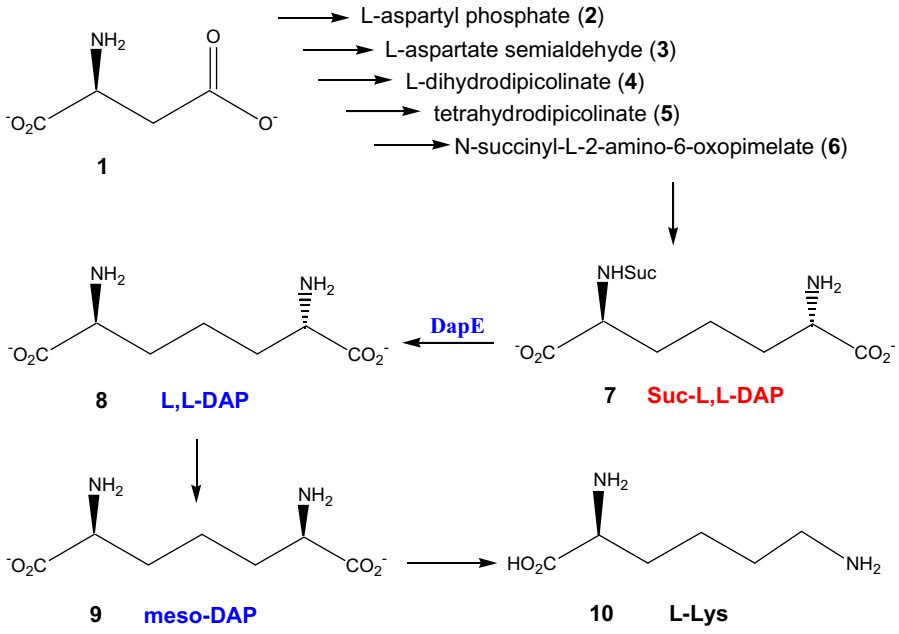

Fig.1. Biosynthetic reactions leading from aspartate to lysine: L-aspartate (**1**); *N*-succinyl-L,L-diaminopimelate (**7**); L,L-diaminopimelate (**8**); D,L-diaminopimelate (**9**); L-lysine (**10**).

antibiotic has a finite lifetime given that resistance will ultimately occur, particularly if the same enzymes are repeatedly targeted, development of new classes of inhibitors that target previously untargeted cellular enzymes is essential to retain control of infectious disease [5,8]. As such, antimicrobial peptides have been introduced as an innate defense system and shown to provide protection against a wide variety of microorganisms [9–12]. Another approach targets microbial enzymes, especially those catalyzing metabolic processes exclusive to bacteria, as potential targets for potent and selective antibiotics [8].

Recently, we described a series of inhibitors for the bacterial enzyme N^{α}-acetyl-L-ornithine deacetylase (ArgE),[2] which catalyzes the conversion of N^{α}-acetyl-L-ornithine to L-ornithine in the fifth step of the biosynthetic pathway for arginine, a necessary step for bacterial growth [13]. Based on bacterial genetic information, the *meso*-diaminopimelate (mDAP)/lysine biosynthetic pathway (Fig. 1) offers several potential antibacterial targets that have yet to be explored [14–17]. One of the products of this pathway, lysine (**10**), is required in protein synthesis and is also used in the peptidoglycan layer of gram-positive bacteria cell walls. A second product, *meso*-diaminopimelic acid (mDAP) (**9**), is an essential component of the peptidoglycan cell wall in gram-negative bacteria, providing a link between polysaccharide strands. It has been shown that deletion of the gene encoding for one of the enzymes in the mDAP/lysine biosynthetic pathway, the DapE-encoded *N*-succinyl-L,L-diaminopimelic acid desuccinylase (DapE; EC 3.5.1.18) [18], is lethal to *Helicobacter pylori* and *Mycobacterium smegmatis* [19,20]. Even in the presence of lysine-supplemented media, *H. pylori* was unable to grow. Therefore, DapE is essential for cell growth and proliferation and is a part of a biosynthetic pathway that is the only source of lysine in bacteria. Because there are no similar biosynthetic pathways in mammals, inhibitors that target one or more of the enzymes in the mDAP/lysine biosynthetic pathway are hypothesized to exhibit selective toxicity only against bacteria, providing a previously nondescribed class of antimicrobial agents [14,21].

With the aim of developing new antimicrobial agents that target DapE enzymes, a methodology for the quick and efficient preparation of mono-*N*-acylated 2,6-diaminopimelic acid (DAP) derivatives was recently reported [22]. These compounds were designed as competitive inhibitors of the mono-*N*-succinyl–DAP hydrolysis reaction catalyzed by DapE, in which the structure of the N-linked succinate moiety was altered to inhibit the enzyme's ability to cleave the adjacent amide bond. The syntheses of a series of 13 DAP derivatives (Table 1) bearing different N-linked acyl side chains comprising (i) a hydrophobic aliphatic or aromatic moiety (**A1**–**A6**), (ii) an aliphatic moiety terminated by a carboxyl group (**C1**–**C4**), (iii) an aliphatic moiety terminated by an ester group (**E1**–**E3**), and their purification and characterization by high-performance liquid chromatography (HPLC), elemental analysis, and electrospray ionization–mass spectrometry (ESI–MS) were described previously [22].

We report here the analytical characterization of these DAP derivatives in order to confirm their structural properties using infrared (IR) and ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopies. We also performed qualitative and quantitative analysis of each of these compounds by two capillary electromigration methods: capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC). These two techniques are excellent tools for separation of both ionogenic and electroneutral compounds [23–25] and are frequently used for analysis and characterization of amino acids and their derivatives [26–28]. In addition, these DAP derivatives were characterized by their effective electrophoretic mobilities determined by CZE in acidic and alkaline classical or isoelectric background electrolytes (BGEs) or by MEKC in acidic and alkaline BGEs containing a micellar pseudostationary phase constituted by the anionic detergent sodium dodecyl sulfate (SDS) or the cationic detergent

[2] *Abbreviations used:* ArgE, N^{α}-acetyl-L-ornithine deacetylase; mDAP, *meso*-diaminopimelate or *meso*-diaminopimelic acid; DapE, dapE-encoded *N*-succinyl-L,L-diaminopimelic acid desuccinylase; DAP, 2,6-diaminopimelic acid; HPLC, high-performance liquid chromatography; IR, infrared; NMR, nuclear magnetic resonance; CZE, capillary zone electrophoresis; MEKC, micellar electrokinetic chromatography; BGE, background electrolyte; SDS, sodium dodecyl sulfate; CTAB, cetyltrimethylammonium bromide; Tris, tris(hydroxymethyl)aminomethane; H_3PO_4, phosphoric acid; AcOH, acetic acid; NaOH, sodium hydroxide; IDA, iminodiacetic acid; ACN, acetonitrile; MeOH, methanol; FT, Fourier transform; UV, ultraviolet; *Hi*DapE, DapE from *Haemophilus influenzae*; UV–Vis, ultraviolet–visible; *Ec*ArgE, ArgE from *Escherichia coli*; L-NAO, N^{α}-acetyl-L-ornithine; MU, mobility units.