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Hydrolysis of Cyclic Ureas under Microwave Irradiation: Synthesis and Characterization of 7,8-Diaminopelargonic Acid

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Abstract: A simple and efficient method for the synthesis of 7,8-diaminopelargonic acid, a key intermediate in the biotin biosynthesis pathway, is reported. The d-desthiobiotin powder was dissolved in concentrated hydrochloric acid, and the solution was exposed to microwave radiation of 2.45 GHz for varying lengths of time ranging from 60 s to 2 min. The product thus obtained was characterized by spectroscopic techniques and confirmed through bioassay. Further, the protocol was extended to the synthesis of several diamines from their corresponding cyclic ureas. The results show that the method is generally applicable and not only accelerates the hydrolysis reaction but also offers excellent yields.

Keywords: acid hydrolysis, cyclic urea, d-desthiobiotin, 7,8-diaminopelargonic acid, microwave irradiation

INTRODUCTION

Biotin (vitamin H) plays an important role in a number of carboxylation and transcarboxylation reactions, which are essential for the growth of plants, microorganisms, and animals. The biosynthesis of biotin, however, occurs only in plants and microorganisms, in four enzyme-catalyzed reactions

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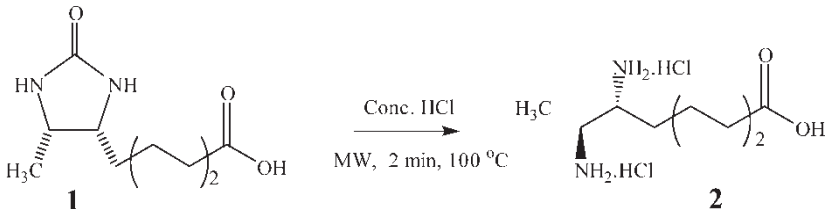
following the initial synthesis of pimeloyl-CoA. 7-Keto-8-aminopelargonic acid (KAPA), 7,8-diaminopelargonic acid (DAPA), and desthiobiotin are the intermediates in the biotin biosynthesis pathway.[1] DAPA is produced by the DAPA synthase catalyzed transfer of an amino group from *S*-adenosyl methionine (SAM) to KAPA. It is taken up by dethiobiotin synthetase, the next enzyme in the pathway, which converts it to desthiobiotin by the addition of CO_2 in an ATP-dependent reaction.[2] In the literature, very few methods are available for the synthesis of DAPA. Nudelman et al. described detailed synthesis of KAPA, racemic DAPA methyl ester, and their analogues starting from L-alanine as a chiral substrate.[3] However, we were unable to reproduce their results for DAPA synthesis. du Vigneaud et al. have reported synthesis of DAPA by hydrolyzing the cyclic urea moiety of desthiobiotin ester with minimum characterization data.[4] There are a few other methods available for the hydrolysis of cyclic ureas, but these are either specific or the conditions employed are very harsh requiring long times (2.5–19.5 h) and resulting in poor yields.[5,6] The importance of DAPA and the lack of a generally applicable method for the hydrolysis of cyclic ureas prompted us to explore an efficient and diastereospecific synthesis.

Microwave irradiation can dramatically increase reaction rates and thereby decrease the reaction time. Moreover, it has also been an attractive tool for enhancing the selectivity.[7] Additionally, modern microwave instruments allow precise control of temperature and pressure in sealed reaction tubes. Therefore, over the past two decades, organic chemists have explored its utility for various types of organic reactions. Here, we report the direct hydrolysis of cyclic urea to its corresponding diamine hydrochloride salt.

RESULTS AND DISCUSSION

Our preliminary approach involved hydrolysis of d-desthiobiotin using concentrated HCl under conditions developed by du Vigneaud et al. The high temperature (200°C) employed, along with the long duration (2.5 h) of conventional heating, results in partial charring of the product. This is however not surprising, as the melting point of DAPA is 180°C. In view of the need for improvements and our prior results in the synthesis of amino acid esters, β-amino acids, isocyanates, and antimicrobial 2-hydroxy diaryl ethers[8] employing microwave irradiation for similar thermal reactions, we decided to reinvestigate this reaction. Initial efforts focused on optimizing microwave conditions for the formation of 7,8-diaminopelargonic acid based on prior investigations of conventional thermal conditions. The above reaction was successfully carried out using microwave irradiation. Thus the method became very reproducible and gave the desired product in excellent



Scheme 1. Synthesis of 7,8-diamino pelargonic acid by microwave irradiation.

purity and good yield (98%). For the synthesis of 7,8-diaminopelargonic acid, d-desthiobiotin (50 mg) and concentrated HCl (5 mL) were placed sequentially under stirring in a sealed tube (CEM designed 20-mL pressure-rated reaction vial), and the reaction mixture was exposed to microwave irradiation for 60 s to 2 min at 100°C (Scheme 1). To confirm the purity of **2**, it was made to react with phthalic dicarboxaldehyde and β-mercaptoethanol in sodium carbonate solution at pH 10 and stored at rt for 1 h. The complex mixture thus obtained was injected onto an RP-HPLC with C18 column and run isocratically in 43% acetonitrile and 1% acetic acid with a Waters HPLC system at a flow rate of 0.5 mL/min. The DAPA derivative, which absorbs at 420 nm eluted at 17.06 min as a single peak. Further, DAPA was characterized by NMR and mass.

The biological activity of DAPA synthesized by the present protocol was tested by a bioassay using the *E. coli* bioA109 (MEC1) strain (*E. coli* Genetic Stock Center, Yale University) originally described by Eisenberg and Stoner[9] and recently reported by us.[10] Since the biotin biosynthesis pathway in *E. coli* bioA109 (MEC1) strain is blocked at the level of the synthesis of DAPA, its growth in minimal M9 medium is dependent on the exogenous provision of DAPA. The synthesized DAPA supported the growth of the bacterial cells as evident by the observation of a zone of growth around the paper discs loaded with the compound. The semilogarithmic (concentration) plot of the diameters of the growth zone against the concentration of DAPA used was linear (Fig. 1). These results confirm that the synthesized DAPA is biologically active.

Encouraging results in the synthesis of DAPA under microwave conditions prompted us to evaluate whether the procedure can be extended to prepare simple diamines. A general method was desired, although primarily we were interested in making **2** from **1** for further use as a substrate for biotin biosynthesis pathway enzymes. Surprisingly, by following the above protocol, it was possible to hydrolyze other cyclic ureas to their corresponding diamines (Scheme 2) in very short periods of time. The desired products were obtained in pure form and in high yields by evaporating the obtained reaction mixture *in vacuo*; the residue was crystallized using ethanol/ether (Table 1).

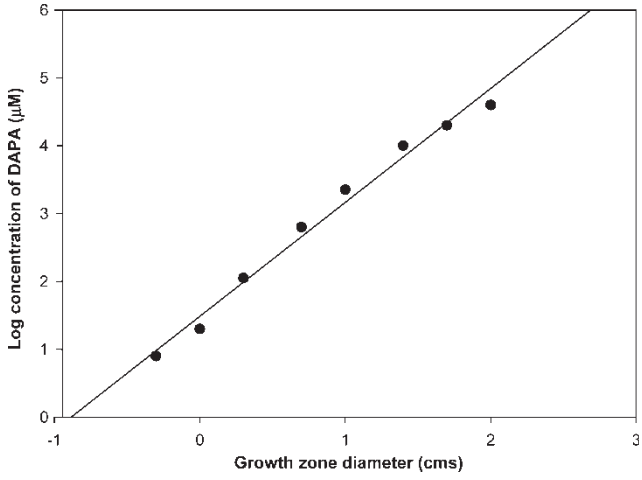
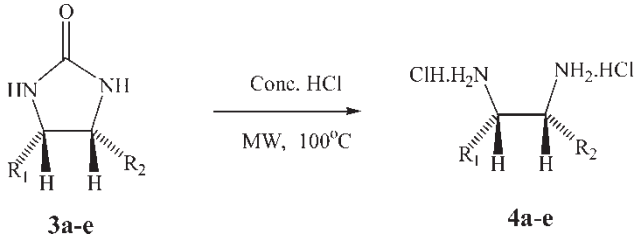


Figure 1. Growth response of *E. coli bioA109* to DAPA. Ten-μL aliquots of known concentrations of DAPA were loaded on paper discs over the agar plate, and the diameter of the zone of growth were manually determined after 15 h of incubation at 37°C.

EXPERIMENTAL

Hydrolysis of Desthiobiotin

The d-desthiobiotin (50 mg, 0.23 mmol) powder was dissolved in concentrated HCl (5 mL), and the solution was stirred in a sealed tube (CEM designed 20-mL pressure-rated reaction vial) and then exposed to microwave irradiation for 60 s to 2 min. The resulting mixture was evaporated to dryness and then thrice concentrated with 5 mL of water to remove excess HCl. The residue was dissolved in minimum ethanol and recrystallized by adding dry ether. Yield 96%; mp, 180–181°C; $[\alpha]_D^{25} = +4.12$ (c =1 in MeOH).



3a-e **4a-e**

Scheme 2. Synthesis of diamines by microwave irradiation.

Table 1. Examples for hydrolysis of cyclic urea using microwave conditions

			Conditions		Time		Yield (%)	
Entry **4**	R_1	R_2	Lit.	Exptl.	Lit.	Exptl.	Lit.	Exptl.
a	C_6H_5	C_6H_5	47% aq. HBr[5c] in reflux AcOH	Conc. HCl 100°C	24 h	3 min	98	94
b	CH_3	H		—		2 min	—	98
c	$CH_2C_6H_5$	CO_2H	2M Hcl[59] reflux	—	2.5 h 34 h drying	3 min	100	96
d	H	H	—	—	—	2 min	—	94
e	R_1-R_2=CH_2-CH=CH-CH_2	—	$Ba(OH)_2$[5d] H_2SO_4 NH_3-$CHCl_3$ 140°C		19.5 h	5 min	72	93

Hydrolysis of Cyclic Ureas

The cyclic urea (1 mmol) was dissolved in conc. HCl (6 mL), and the solution was sequentially stirred in a sealed tube (CEM designed 20-mL pressure-rated reaction vial) and then exposed to microwave irradiation for 60 s to 2 min. The resulting mixture was evaporated to dryness and then thrice concentrated with 5 mL of water to remove excess HCl. The products were dissolved in minimum ethanol and recrystallized by dry ether.

CONCLUSION

In summary, we have demonstrated the direct hydrolysis of d-desthiobiotin to 7,8-diaminopelargonic acid in concentrated HCl under microwave conditions. The synthesized DAPA was characterized both chemically and biologically. Further, the conditions reported here are also generally applicable to the hydrolysis of other cyclic ureas. Thus, the presently developed method is very useful because the reaction proceeds smoothly. Moreover, it has a high selectivity, faster rate, and offers good yields.

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