RAPID CONVENTIONAL AND MICROWAVE-ASSISTED DECARBOXYLATION OF L-HISTIDINE AND OTHER AMINO ACIDS VIA ORGANOCATALYSIS WITH R-CARVONE UNDER SUPERHEATED CONDITIONS

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GRAPHICAL ABSTRACT

Abstract This article reports a new methodology taking advantage of superheated chemistry via either microwave or conventional heating for the facile decarboxylation of alpha amino acids using the recoverable organocatalyst, R-carvone. The decarboxylation of amino acids is an important synthetic route to biologically active amines, and traditional methods of amino acid decarboxylation are time consuming (taking up to several days in the case of L-histidine), are narrow in scope, and make use of toxic catalysts. Decarboxylations of amino acids including L-histidine occur in just minutes while replacing toxic catalysts with green catalyst, spearmint oil. Yields are comparable to or exceed previous methods and purification of product ammonium chloride salts is aided by an isomerization reaction of residual catalyst to phenolic carvacrol. The method has been shown to be effective for the decarboxylations of a range of natural, synthetic, and protected amino acids.

Keywords Amino acids; decarboxylation; green chemistry; microwave chemistry; organocatalysis

INTRODUCTION

Biogenic amines are ubiquitous in the body, regulating a variety of functions ranging from neurotransmission to cellular signaling to various physiological
responses.[1–4] Given the physiological sensitivity to biogenic amines, the ability to synthesize these compounds or unnatural derivatives for pharmaceutical purposes is attractive. Of the biogenic amines, histamine is one of the most widely dispersed in the body and is known for its importance in the regulation of many bodily processes.[5–10]

Many biogenic amines including histamine are derived from their respective \( \alpha \)-amino acids via a variety of decarboxylase enzymes.[6,11–17] For example, study of \( L \)-histidine decarboxylase knockout (HDC KO) mice has given much insight into the effects of histamine in the body.[5,18] These mice lack the ability to produce histamine because their HDC encoding gene has been “knocked out” or removed during the mouse cloning process. Studies show that these HDC KO mice are deficient in natural killer T-cell production[19] and suffer from decreased wound healing rates.[20] It follows that current pharmaceutical uses of histamine are primarily related to the activation of dendritic cells via the \( H_4 \) receptor and subsequent immune response,[21,22] making it clear that an efficient production of high-purity histamine is immediately important.

Enzymatic decarboxylation of amino acids occurs in many organisms and has long been reported as a synthetic option for the decarboxylation of the amino acid histidine.[23,24] Many amino acids have also been shown to undergo decarboxylation upon reflux in a high boiling solvent, such as cyclohexanol, in the presence of a ketone[25–27] catalyst with cyclohex-2-en-1-one[28] or acetophenone derivatives[29] being the most recently reported catalysts. For syntheses of some of the more biologically relevant amines, previously reported procedures are slow at best and are complicated by difficult purification protocols required to remove by-products and high boiling solvents. In recent years two processes for the removal of product free amines by distillation from a high boiling solvent[30,31] have been reported. These methods assist with the problem of solvent removal for lower boiling amines; however, the reaction times were still quite long and the successful reports of difficult decarboxylations such as histidine are unsubstantiated. Most especially for free amines that exhibit high boiling points, an alternative method of isolation is needed to prevent thermal degradation.

RESULTS AND DISCUSSION

Solvent Optimization

To address the problem of long reaction times in the decarboxylations of many amino acids such as histidine, it was envisioned that chemistry at temperatures above the reflux temperature of cyclohexanol (~160 °C) may provide a solution. However, as previously noted, a significant amount of effort must be devoted to the removal of high-boiling solvents at the expense of yield and efficiency. One advantage to using cyclohexanol or polyethylene glycol reported by all previous researchers was the solubility of the amine product and insolubility of amino acids, allowing visual determination of reaction completion.

Rather than employing an even higher boiling solvent system, which would involve the same difficulties as previous methods, the possibility of a pressurized reaction system using a solvent with a lower normal boiling point was investigated.
Both microwave-promoted and hot oil bath systems were employed using a sealed 15-bar maximum pressure reaction vessel. While many solvents satisfy the criterion of greater volatility, the search was narrowed to a series of short-chain alcohol solvents to promote microwave absorption. Among the short-chain alcohol solvents, n-butanol, n-pentanol, and isopropanol proved to absorb microwaves insufficiently while ethanol, methanol, and water dissolve the reactant amino acid at the optimum reaction temperatures of $>160^\circ$C, hindering visual determination of reaction completion and failing to facilitate decarboxylation of histidine. n-Propanol was determined to be the optimum solvent for visual inspection of reaction completion that could reach the maximum safe temperature and be easily removed after reaction completion. This solvent achieves a maximum temperature in the instrument (1200 W) of 190°C (calibrated $\pm 2^\circ$C) with a vapor pressure of 15 bar as determined by the Clausius-Claperyon equation. It should also be noted that reactions performed neat resulted in poor to no yield and aprotic solvents failed to promote decarboxylation even upon heating to 190°C in an oil bath.

**Catalyst Optimization**

Another factor affecting the reaction rate and overall ease of purification of the product mixture is the identity and load of the catalyst. Previously 1% v/v of cyclohex-2-en-1-one has been reported by Hashimoto and coworkers for a wide range of amino acid decarboxylations.\cite{28} Significant amounts of impurities were observed in the resulting reaction mixture in attempts to reproduce these experiments. Alternatively, it was reported that when acetophenone and derivatives were used at 20 mol% for decarboxylation of histidine, modest success was observed after $>54$ h. In catalyst optimization experiments, cyclohex-2-en-1-one proved to provide a greater catalytic effect at 20 mol% than acetophenone. It was envisioned that the “enone” R-carvone, the natural product spearmint oil, may retain the catalytic advantage over acetophenone while providing an alternative method of removal of the catalyst based on the known isomerization reaction to phenolic carvacrol\cite{32} (Scheme 1). R-Carvone provides the added advantage of replacing cyclohex-2-en-1-one, a relatively expensive catalyst having acute human toxicity.\cite{33} It was observed during preliminary experiments that the rate of reaction significantly increased at greater catalyst load than the 1 mol% load previously reported by Hashimoto. As a general rule, the catalytic effect becomes appreciable at about 0.1 mol equivalents for both cyclohex-2-en-1-one and R-carvone and peaks at about 2 mol equivalents for the amino acid decarboxylation in this study. The reaction times in minutes of a series of microwave-assisted decarboxylations of phenylalanine in n-propanol at 190°C with varying load of R-carvone are given in Fig. 1.

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**Scheme 1.** Isomerization of R-carvone to phenolic carvacrol.
Decarboxylations of a series of amino acids were performed at the 2 equivalent catalyst load, comparing the catalytic abilities of cyclohex-2-en-1-one, acetophenone, both carvone enantiomers, and the simple ketone acetone under solvent-optimized conditions. Five min were allowed for the microwave to achieve maximum reaction temperature of 195 °C. Additional reflux time at the maximum temperature was allowed if necessary. Reaction completion was determined by the transformation of amino acid slurry to clear solution as shown in Fig. 2. A summary of catalyst optimization experiments may be viewed in Table 1.

In these reactions R-carvone showed reactivity similar to that of previous catalysts. Decarboxylations with S-carvone as catalyst were also effective, though decarboxylation of L-histamine required 50 min reflux as opposed to 25 min. Given these results, decarboxylations of D-amino acids with R-carvone as catalyst may also be performed as desired. With catalytic effect confirmed, it remained to be seen whether the decarboxylations occur cleanly with minimal by-products. Isolation of products using the other catalysts under the higher temperature conditions and catalyst load proved impossible due to decomposition of products and/or catalysts.

**Catalyst Removal and Purification**

Given that decarboxylations of amino acids with ketone catalysts is thought to occur through an imine intermediate\[^{26,27}\] and the observed rise in impurities as a result of increasing the catalyst load following the Hashimoto procedure, it became

![Figure 1](image1.png)

*Figure 1. Decarboxylation times of phenylalanine with variable molar ratio of R-carvone.*

![Figure 2](image2.png)

*Figure 2. Generic decarboxylation for determination of catalytic effect.*
apparent that the fate of the decarboxylated imine should be investigated. Gas chromatography–mass spectrometry (GC-MS) analysis of the product mixture after the decarboxylation of phenylalanine at the 2 mol equivalent catalyst load shows all decarboxylated imine, no free amine, and minimal volatile impurities. The reaction mixture was then diluted with aqueous hydrochloric acid, excess R-carvone was removed via ether wash, and then the free-based product was partitioned to the organic phase via aqueous sodium hydroxide wash. A significant degree of hydrolysis was expected, however, in GC-MS analysis of the organic extract the imine of the decarboxylated product is quite stable and persists as shown by the chromatogram and mass spectrum in Fig. 3.

| Amino acid | Phe | His | Trp | Tyr |
|------------|-----|-----|-----|-----|
| R-Carvone  | 5   | 25  | 5   | 10  |
| S-Carvone  | 5   | 50  | 5   | 16  |

*Completed in ∼72 h in an oil bath with decomposition of product.*
It was observed that the desired imine hydrolysis occurs readily only after heating in aqueous acid at >50 °C. Even after treatment of the reaction mixture with many times the reaction volume of 2.0 M HCl, it proved difficult to adequately remove all traces of the imine at system equilibrium. However, 5 min reflux at 190 °C in 2 M HCl accomplished both hydrolysis of the imine and isomerization of R-carvone to carvacrol as shown in Fig. 4. Carvacrol is then easily removed via ether extraction. It should be noted that gentle reflux at 80 °C allows the imine to hydrolyze in equilibrium and ∼80% of R-carvone catalyst may be recovered via three sequential refluxes and extractions with ether. Alternatively, near quantitative recovery is possible via soxhlet extraction with warm toluene. If R-carvone is recovered via the three quick extractions, a final high-temperature reflux should be performed to isomerize residual R-carvone to carvacrol. All methods of amino acid decarboxylation in the literature fail to account for the quantity and reactivity of imine that may remain, perhaps necessitating the extensive further purification required by these authors to achieve pharmaceutical purity. Product amines are isolated as hydrochloride salts by rotary evaporation of water under reduced pressure and further drying overnight in a vacuum oven.

RESULTS AND SCOPE

A generic representation of the organocatalytic decarboxylations of a range of alpha amino acids is given in Scheme 2 for both microwave and conventional heating procedures. The reaction times for the decarboxylation of selected amino acids of interest are reported in Table 2 along with isolated yields of the amine hydrochloride or dihydrochloride salts for the optimized reaction conditions highlighted in Scheme 2. While the range of applicability for decarboxylations using this procedure was quite good, the procedure proved ineffective for the natural amino acids Arg, Asp, L-DOPA, Glu, Ser, Asn, Gln, Cys, and Met, given the sensitivity of specific functional groups to the reaction conditions.
The slight differences in the overall reaction times reported between microwave (MW) heating and oil bath heating are the result of the necessary differences in experimental protocols. In the microwave reactor initial heating occurs over a 5-min period and temperature is computer controlled by an infrared thermometer in a continuous feedback system to ±2 °C. Conventional heating was performed in a preheated bath with observed temperature oscillations of no more than ±5 °C. Figure 5 shows a representative 1H NMR for the product amine hydrochloride salts in D2O. The solvent peak arising from acidic proton exchange was suppressed. Note that no organic impurities are observed in 1H NMR of the hydrochloride salts using dimethylsulfoxide (DMSO-d6) as solvent.

Further decarboxylations were investigated with unnatural amino acids and protected amino acids. Results are summarized in Table 3. Note that the procedure allows for the simultaneous decarboxylation and deprotection of the protected amino acids investigated. It should be noted that decarboxylated imines are present with protecting groups in tact in GC-MS chromatograms after the initial step.

Table 2. Optimized decarboxylations of natural amino acids

| Amino acid | MW | Oil bath |
|------------|----|----------|
|            | Reaction time (min) | Yield (%) | Reaction time (min) | Yield (%) |
| Ala        | 5  | 60       | 38         | 74         |
| Gly        | 13 | 86       | 40         | 59         |
| His        | 25 | 87       | 12         | 92         |
| Ile        | 9  | 69       | 12         | 76         |
| Leu        | 5  | 72       | 5          | 69         |
| Lys        | 12 | 73       | 17         | 93         |
| Phe        | 5  | 76       | 5          | 78         |
| Pro        | 5  | 80       | 5          | 48         |
| Thr        | 5  | 59       | 12         | 41         |
| Trp        | 20 | 53       | 9          | 72         |
| Tyr        | 20 | 53       | 40         | 67         |
| Val        | 5  | 79       | 9          | 55         |

*aReaction times represent total programmed time including 5 min initial heating period.

*bRequires 80 °C hydrolysis with soxhlet extraction.

Scheme 2. Summary of (a) microwave and (b) conventional heating one-pot decarboxylation procedures.
Decarboxylation of L-histidine and other L-amino acids has been accomplished via organocatalysis with R-carvone and subsequent one-pot hydrolysis under solvent superheated conditions using both conventional heating and microwave irradiation. Decarboxylation is more rapid than previous methods as the vessels are heated to 190 °C over 5 min. R-Carvone catalyst may be recovered via extraction with diethyl ether or via soxhlet extraction with toluene if the hydrolysis is conducted at 80 °C; however, to obtain the greatest purity it is necessary to conduct a high-temperature hydrolysis to isomerize residual R-carvone to carvacrol. Isolated yields of amine hydrochloride salts are comparable or improved over previous methods ranging from 60 to 90% with purity of hydrochloride salts estimated to be >99% by $^1$H NMR.

### Table 3. MW decarboxylations of selected unnatural amino acids

| Amino acid         | Reaction time (min)$^a$ | Yield (%) |
|--------------------|-------------------------|-----------|
| 4-Amino-Phe        | 8                       | 99        |
| 4-Bromo-Phe        | 5                       | 47        |
| 4-Methyl-Phe       | 5                       | 99        |
| 4-Nitro-Phe        | 5                       | 74        |
| 3,5-Dibromo-Tyr    | 5                       | 41        |
| 3-Iodo-Tyr         | 5                       | 77        |
| Cycloleucine       | 16                      | 40        |
| N-Trityl-His$^{[a]}$ | 5                      | 86        |
| N-Formyl-Trp$^{[b]}$ | 5                      | 74        |
| O-t-Butyl-Tyr$^{[c]}$ | 5                      | 81        |
| O-Acetyl-Tyr$^{[c]}$ | 5                      | 76        |
| O-2,6-Dichlorobenzyl-Tyr$^{[c]}$ | 5 | 64 |

$^a$Isolated product is histamine dihydrochloride.

$^b$Isolated product is tryptamine hydrochloride.

$^c$Isolated product is tyramine hydrochloride.

### SUMMARY

Decarboxylation of L-histidine and other L-amino acids has been accomplished via organocatalysis with R-carvone and subsequent one-pot hydrolysis under solvent superheated conditions using both conventional heating and microwave irradiation. Decarboxylation is more rapid than previous methods as the vessels are heated to 190 °C over 5 min. R-Carvone catalyst may be recovered via extraction with diethyl ether or via soxhlet extraction with toluene if the hydrolysis is conducted at 80 °C; however, to obtain the greatest purity it is necessary to conduct a high-temperature hydrolysis to isomerize residual R-carvone to carvacrol. Isolated yields of amine hydrochloride salts are comparable or improved over previous methods ranging from 60 to 90% with purity of hydrochloride salts estimated to be >99% by $^1$H NMR.
This process provides a more versatile and efficient option in the synthesis of biologically active amines from amino acids given the demonstrated versatility with not only natural amino acids but protected and synthetic derivatives as well.

**EXPERIMENTAL**

The 5-mmol scale microwave experiments were performed in Milestone 25-mL, 15-bar glass pressure reactors inside the Milestone StartSYNTH™ microwave reactor with external infrared (IR) temperature control. Conventional heating experiments were performed in silicone oil in identical reaction vessels. Solvents and reagents were purchased from Sigma-Aldrich or Chem-Impex International and used without additional purification. FT NMR experiments were recorded at 400 MHz in D$_2$O solvent.

**General Decarboxylation Procedure**

A magnetic stir bar, 3 mL of n-PrOH, 10 mmol of R-carvone, and 5 mmol of amino acid were charged to a pressure vessel. The vessel was heated from room temperature to 190 °C over 5 min with stirring. If necessary the reaction vessel was maintained at 190 °C for an additional time until the slurry became clear. The vessel was allowed to cool to below the solvent boiling point, carefully vented to release evolved CO$_2$, and analyzed via GC-MS to verify the presence of decarboxylated imine. Ten mL of 2 M HCl was then added in a one-pot fashion, and, in all cases but threonine, the reaction vessel was heated to 190 °C over 5 min with stirring and then allowed to cool. In the case of the temperature-sensitive threonine, soxhlet extraction with toluene was performed at 80 °C to remove R-carvone from the reaction mixture. The aqueous reaction mixture was washed three times with 25 mL of ether, and then water solvent was distilled off from the hydrochloride salt. The hydrochloride salt was transferred to a vacuum oven and dried overnight at 150 °C and 10 Torr. The hydrochloride salt was then weighed, melting point was obtained, and the salt was analyzed via NMR spectroscopy. Additional spectra and experimental data may be viewed in supplementary information.

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**SUPPLEMENTAL MATERIAL**

Supplemental experimental data for this article can be accessed on the publisher’s website.

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