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Short communication

Artifacts related to *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide derivatization of citrulline revealed by gas chromatography–mass spectrometry using both electron and chemical ionization

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ABSTRACT

Derivatization with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) was used for gas chromatography–mass spectrometry (GC–MS) analysis of citrulline and ornithine. Aqueous 50 μl aliquots at 1 and 10 mM concentrations were dried and derivatized separately, and 70 eV electron ionization or CH_4 positive chemical ionization were used. Ornithine produced a single GC peak. Physiological citrulline concentrations produced GC artifact peaks for the ornithine derivative, and a compound consistent with elimination of a water molecule from the tri-*tert*-butyldimethylsilyl (TBDMS) citrulline derivative. A third GC peak obtained using 10 mM citrulline concentrations gave a mass spectrum consistent with a mixture of true tri- and tetra-TBDMS citrulline. Analyses of ^{13}C-ureido-labeled citrulline confirmed the presence of the true TBDMS citrulline derivatives produced from 10 mM samples and provided evidence that the TBDMS ornithine artifact results from loss of TBDMS isocyanate from tetra-TBDMS citrulline. Linear-programmed temperature GC retention index data relative to *n*-alkanes are reported for observed GC peaks.

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1. Introduction

Numerous single and multi-step derivatization methods exist for gas chromatography (GC) analysis of amino acids. Ideally, derivatization for GC will produce a single and unique stable derivative of the target analyte with acceptable volatility and polarity. As an example where this does not occur, Corso et al. [1] derivatized arginine with *N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) and produced the derivative for ornithine, showing this reagent to have limited usefulness for both of these amino acids.

Serum values of the amino acid L-citrulline (Fig. 1A) are diagnostic for various pathologic states [2–6]. We desired a derivatization method for GC analysis of citrulline to quantify serum levels in rats that normally range from 70 to 80 μM [7,8]. However, after receiving irradiation doses that cause gastrointestinal (GI) injury [5,6] rat serum citrulline concentrations fall to ≤10 μM [9].

A recent paper discussed MTBSTFA derivatization of citrulline and gas chromatography–mass spectrometry (GC–MS) with 70 eV electron ionization (EI) [10]. The addition of *tert*-butyldimethylsilyl (TBDMS) groups to the citrulline molecule at three locations (tri-TBDMS citrulline, Fig. 1B) was described, and the reported EI mass spectrum included prominent current for *m/z* 286 and 474 ions. No evidence was observed for $M^{+\bullet}$ (expected at *m/z* 517). Assignment of the GC peak to the tri-TBDMS citrulline derivative was consistent with earlier work [11] where tabular information was provided for several *m/z* values from analysis of the reported citrulline derivative, including *m/z* 474, and a nearly-absent signal at *m/z* 460 which would correspond to $[M-57]^+$ for tri-TBDMS citrulline.

When the TBDMS group is present an $[M-57]^+$ mass spectral peak is expected from EI-induced neutral loss of a *tert*-butyl fragment, and both research groups explained the lack of *m/z* 460 ion current with analysis of citrulline as a result of molecular rearrangement in the mass spectrometer ion source. However, the *m/z* values attributed to tri-TBDMS citrulline are also consistent with the ornithine derivative, including [10,11] *m/z* 474 ($[M]^{+\bullet}$), and [10] *m/z* 417 ($[M-57]^+$). Thus, in both papers the spectral data attributed to the citrulline derivative could result from degradation to the ornithine derivative.

Based on previous work [10], we attempted MTBSTFA derivatization for quantitative GC–MS analysis of citrulline at physiological concentrations as low as 10 μM. An L-citrulline standard was used that had been subjected to ^{1}H and ^{13}C NMR analyses to verify that citrulline and not ornithine was the derivatization target. A

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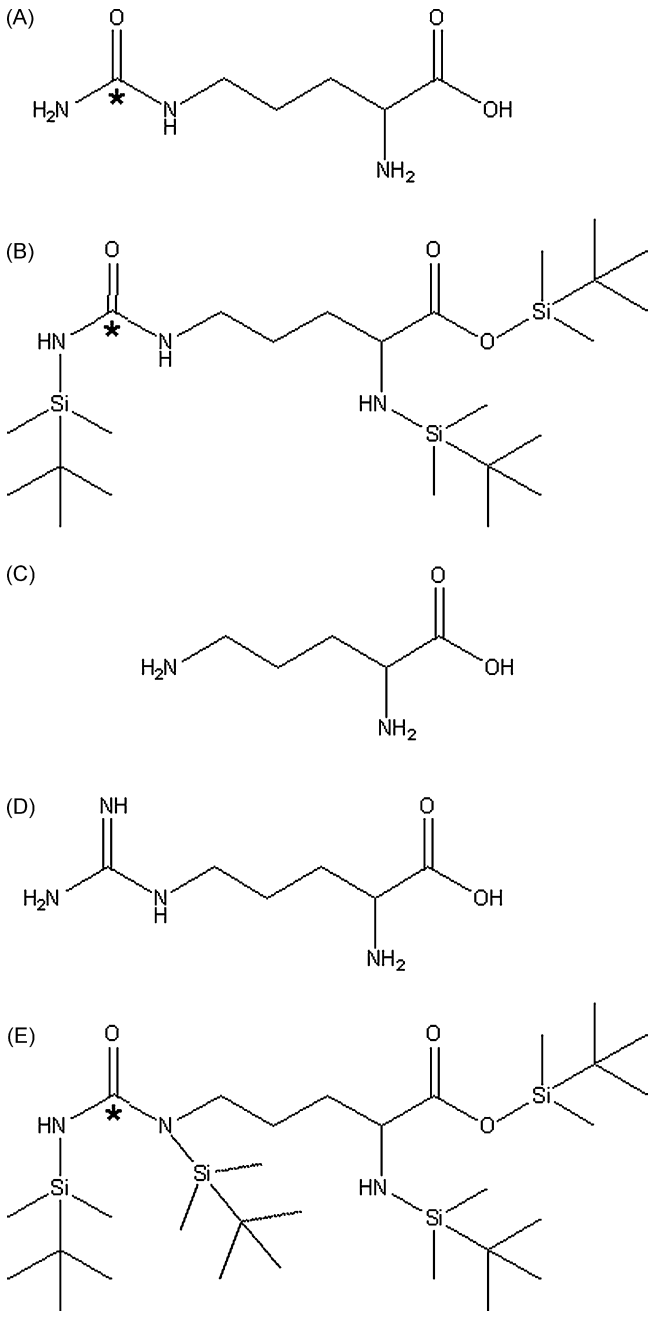

Fig. 1. (A) citrulline; (B) tri-TBDMS citrulline; (C) ornithine; (D) arginine; and (E) tetra-TBDMS citrulline. *For citrulline and TBDMS citrulline derivatives, denotes the position for ^{13}C label when ^{13}C-ureido citrulline was the derivatization target.

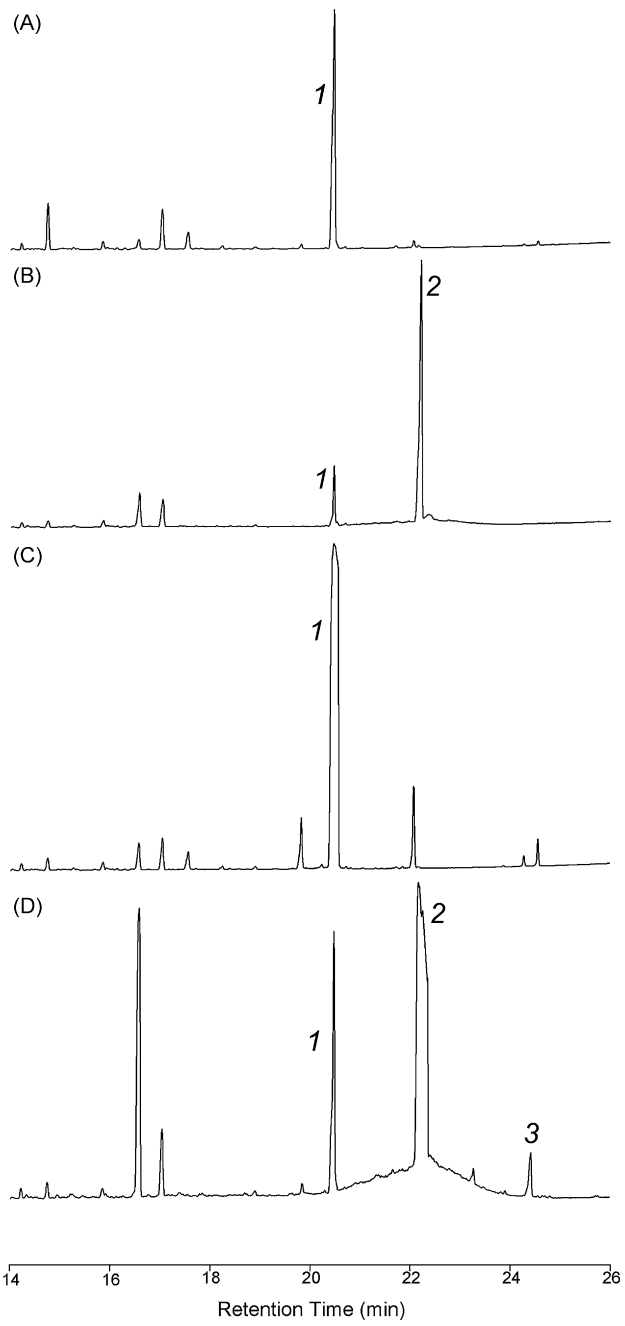

Fig. 2. GC–MS chromatograms, GC peak identities per Table 1, EI analyses following MTBSTFA derivatization: (A) 1 mM ornithine; (B) 1 mM citrulline; (C) 10 mM ornithine (the peak near 22 min has apparent $[M]^{+\bullet}$ of *m/z* 502 by EI analysis, also consistent with positive CI data, not to be confused with peak 2 observed in traces (B) and (D); (D) 10 mM citrulline, with mass spectra from elevated baseline between peaks 1 and 3 closely matching peak 1, although ion current is also present suggesting the presence of TBDMS-isocyanate.

quadrupole mass filter detector was used with 70 eV EI, and a GC peak was seen with mass-spectral characteristics similar to those reported earlier for the citrulline derivative [10,11]. However, the mass spectrum resulting from these initial efforts was also nearly identical to that reported by Corso et al. [1] for the ornithine derivative. Additional GC peaks were observed, and poor precision was seen for the GC peak in question.

To explain these initial observations, citrulline, ^{13}C-ureido citrulline, and ornithine were derivatized using MTBSTFA. Arginine samples were also derivatized to reproduce earlier results [1] where this produced the ornithine derivative. Analyses were completed by GC–MS with 70 eV EI, and also with positive chemical ionization (CI) using CH_4 reagent to verify the molecular masses of the respective derivatives and related compounds. For comparison, ornithine and arginine structures are provided in Fig. 1C and D respectively.

2. Materials and methods

2.1. Chemicals

Standards were purchased for L-citrulline (98%, Sigma, St. Louis, MO), L-ornithine hydrochloride (99%, Sigma), L-arginine (99%, Sigma), and ^{13}C-ureido citrulline (>95%, >98% ^{13}C enrichment, ProSpect Pharma, Columbia, MD). The derivatization reagent was