

Enigmatic Isovaline: Investigating the Stability, Racemization, and Formation of a Non-Biological Meteoritic Amino Acid

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Abstract. Among the Murchison meteoritic amino acids, isovaline stands out as being both non-biological (non-protein) and having a relatively high abundance. While approximately equal amounts of D- and L-isovaline have been reported in Murchison and other CM meteorites, the molecule's structure appears to prohibit its racemization in aqueous solutions. We recently have investigated the low-temperature solid-phase chemistry of both isovaline and valine with an eye toward each molecule's formation, stability, and possible interconversions of D and L enantiomers. Ion-irradiated isovaline- and valine-containing ices were examined by IR spectroscopy and highly-sensitive liquid chromatography/time-of-flight mass spectral methods to assess both amino acid destruction and racemization. Samples were studied in the presence and in the absence of water-ice, and the destruction of both isovaline and valine was measured as a function of radiation dose. In addition, we have undertaken experiments to synthesize isovaline, valine, and their amino acid isomers by solid-phase radiation-chemical pathways other than the oft-invoked Strecker process.

1. Introduction

Meteorites provide the only direct evidence for extraterrestrial amino acids. To date, about 70 amino acids have been reported in Murchison and other objects, with the majority of the acids being non-biological (Pizzarello et al. 2008). Although many of these amino acids are racemic (i.e., equal abundance of D and L structures), reports of enantiomeric excesses are in the literature, with some values being as large as 10% (Cronin & Pizzarello 1997).

Carbonaceous chondrites, such as Murchison, are particularly attractive to astrobiologists as these meteorites harbor a rich variety of organic molecules and are among the most primitive material in the solar system. The amino-acid inventories of CM and CI carbonaceous chondrites have been examined, and distinct differences have been found between them (Ehrenfreund et al. 2001). CM types Murchison and Murray are enriched in, among other amino acids, isovaline and α -aminoisobutyric acid compared to CI types, such as Orgeuil and Ivuna.

Our own studies of Murchison samples have concentrated on isovaline and its isomers, such as valine, all having the formula $C_5H_{11}NO_2$. Figure 1 shows the result of a liquid chromatography/time-of-flight mass spectral measurement

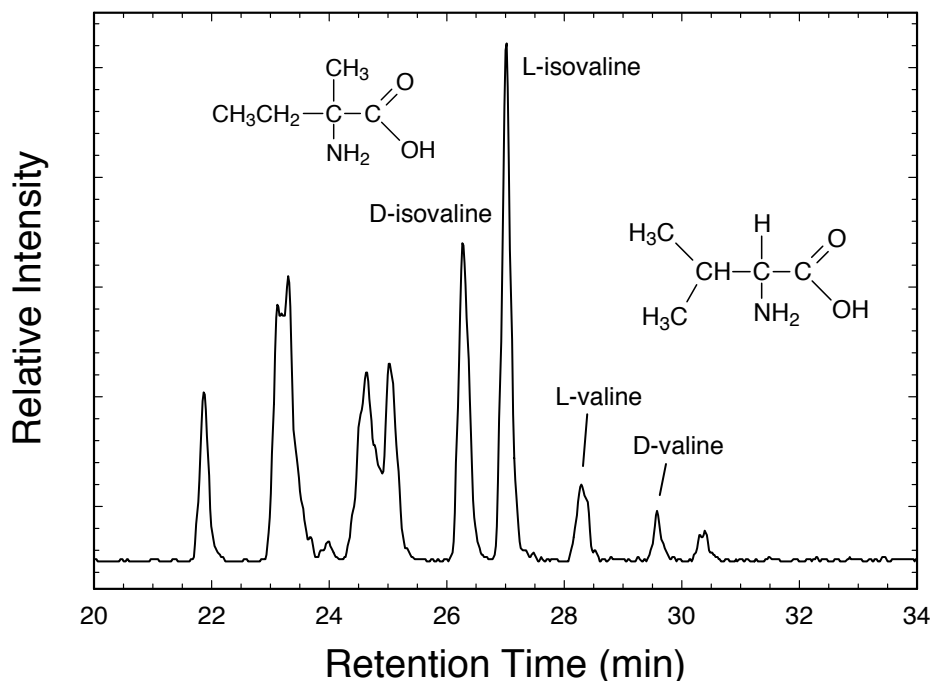


Figure 1. A liquid chromatography/time-of-flight mass spectral measurement of C₅ amino acids from a Murchison sample. Structures of isovaline and valine are shown in the left and right parts of the figure, respectively. About half of the unlabeled peaks also have been identified, but are not covered in this paper.

of a Murchison extract, looking only at isovaline, valine, and their isomers. The enhancement of isovaline over valine is obvious as are substantial apparent excesses of L-isovaline and L-valine over their D counterparts.

We recently have performed experiments on isovaline and valine to investigate their relative thermal and radiolytic stabilities in order to possibly explain their different CM and CI meteoritic abundances. We also have examined possible interconversions between these molecules, as well as racemization of each. Finally, we have undertaken experiments designed to synthesize isovaline and valine from known and suspected meteoritic compounds.

2. Experimental

Our experiments involved mid-infrared spectroscopy coupled with liquid chromatography and time-of-flight mass spectral (LC/ToF-MS) analyses. Relevant details for both techniques are in earlier papers from our laboratories (Hudson et al. 2005; Glavin et al. 2006). In brief, experiments began with the condensation of an ice sample onto a pre-cooled aluminum mirror in a vacuum chamber interfaced to the beamline of a van de Graaff accelerator. Mid-IR spectra of the resulting ices were recorded in situ in the 10 - 300 K range. Samples were irradiated to doses of 1 - 10 eV molecule⁻¹ and then brought slowly up to

room temperature overnight. Residual materials were removed by washing the metal substrate with water, and then analyzed by LC/ToF-MS without acid hydrolysis. Analytical and procedural blanks were performed at each step of our work. Reagents used were of the highest purity available from Sigma-Aldrich and Cambridge Isotopes.

3. Results

3.1. Thermal Studies

Our initial experiments concerned the thermal stability of isovaline and valine. In separate experiments, each amino acid (AA) was sublimed under vacuum from a small pyrex tube onto the 10 K aluminum mirror. Reflecting a mid-IR beam off the AA-coated metal surface produced an IR spectrum that could be followed as the sample was warmed. Variations in IR bands were seen near 100 K as the amino acid converted from a non-ionic to a zwitterionic form, the main change being loss of a C=O stretching feature near 1717 cm^{-1} . Further warming gave no additional changes and each amino acid was stable under vacuum up to the highest temperature examined. In short, there was no difference in thermal behavior between valine and isovaline at 10 - 300 K. Thus, temperature effects alone cannot explain the enhancement of one of these molecules over the other in carbonaceous chondrites.

3.2. Radiation Stabilities

Our second set of experiments involved the same preparation just described, but each amino acid sample was irradiated at 10 K under vacuum with a 0.8 MeV proton beam, to simulate exposure to cosmic radiation. For both valine and isovaline, a dose of about $1.4\text{ eV molecule}^{-1}$ sufficed to destroy half the sample molecules; separate experiments with AA + H₂O-ice mixtures gave essentially the same result. When the temperature was raised to 80 K, the dose to destroy half the AA molecules dropped slightly, to about $1.0\text{ eV molecule}^{-1}$. Our conclusion is that there is insufficient difference in the radiation stabilities of valine and isovaline to explain how one of these molecules might become enhanced over the other in an extraterrestrial radiation environment.

3.3. Amino Acid Reactions

Warming irradiated samples to room temperature, while under vacuum, left a residual material that could be washed from the aluminum substrate with water. These residues were analyzed with LC/ToF-MS for both isomerization and racemization of starting materials. We found no interconversion of isovaline and valine, excluding yet another possible means for an enhancement of one isomer over the other. However, we did find that in all cases racemization took place. A 10 K irradiation to a dose of $10\text{ eV molecule}^{-1}$ converted pure L-valine into a 1:2 D:L valine mixture (i.e., D-to-L ratio of 0.5). A D-isovaline sample behaved almost exactly the same in an identical experiment. The radiation dose for Murchison fragments has been estimated as only about 530 Mrad, or about $1\text{ eV molecule}^{-1}$ (Bonner et al. 1979). This means that the racemization we

observed is probably much greater than background radiation will produce in the meteoritic case.

3.4. Amino Acid Syntheses

The experiments just described show that meteoritic abundance differences between isovaline and valine cannot be easily explained by an instability of either molecule relative to the other, or by a radiation-induced equilibrium that favors either isomer. Since an explanation is not possible on the basis of molecular destruction, we have begun to study formation paths for these two amino acids. We also are interested in simply being able to make isovaline. Although the oft-invoked Strecker reaction might lead to this compound in mixed molecular ices, a close inspection of the literature shows that laboratory detections of isovaline are based on the acid hydrolyses of reaction products (Nuevo et al. 2007). In other words, what has been shown is that amino-acid precursors, sometime called bound amino acids, can be formed and then, after strong-acid hydrolysis, isovaline is detected. What we seek is something more like the meteoritic case, the formation and direct detection of isovaline itself.

We knew from experience that molecules will fragment on irradiation and that the individual pieces will add to unsaturated molecules. For example, irradiation of H_2O produces H and OH radicals which add to acetylene to make vinyl alcohol (Hudson & Moore 2003), and irradiation of CH_3OH forms H and CH_2OH radicals which add to CO to make glycolaldehyde (Hudson et al. 2005). We thus reasoned that C_4 -saturated alkylamines ($\text{C}_4\text{H}_{11}\text{N}$) would under fragmentation to form H and $\text{C}_4\text{H}_{10}\text{N}$ radicals which would add to CO_2 to make C_5 amino acids. Figure 2 summarizes the expected reaction paths. Supporting this strategy was the known presence of primary amines in both Murchison (Jungclaus et al. 1976) and Stardust (Sandford et al. 2006) samples.

On irradiation at 10 K of mixtures of $^{13}\text{CO}_2$ and either isobutylamine or *sec*-butylamine, an IR band immediately formed at 1717 cm^{-1} , the same feature mentioned earlier for valine and isovaline. This band persisted up to room

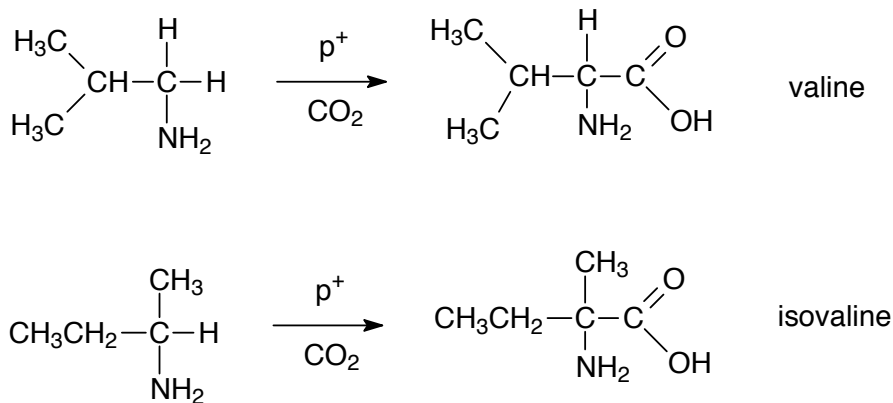


Figure 2. Reactions showing the formation of C_5 amino acids from proton-irradiated CO_2 and C_4 amines. The top reaction begins with isobutylamine, and the bottom reaction begins with *sec*-butylamine.

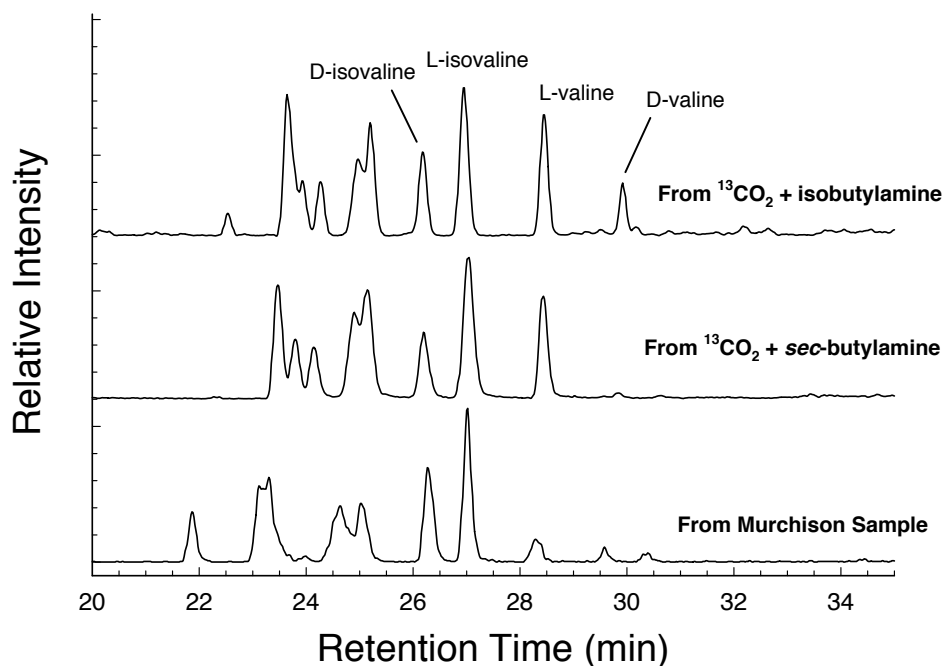


Figure 3. C_5 amino acids made in irradiated $^{13}CO_2$ + amine ices compared to C_5 amino acids in a Murchison sample (bottom trace).

temperature and could be seen regardless of the irradiation temperature. Our conclusion is that amino acids were formed in these low-temperature experiments, but that IR spectroscopy alone cannot identify the specific amino acids produced.

Irradiated $^{13}CO_2$ + amine ices were warmed under vacuum to room temperature, and the residues washed from the cold finger and analyzed by LC/ToF-MS. Figure 3 shows the results of two ice experiments and our Murchison analysis. Comparisons with blanks and standard amino acid samples left no doubt that valine and isovaline were formed in our experiments. The use of $^{13}CO_2$ also was important for confirming that the results were from the irradiation of the samples, and not atmospheric or other contamination.

4. Discussion

Our results show that isovaline's enhancement over valine in Murchison is not due to either a difference in thermal or radiation stabilities between the two molecules or a radiation-induced isomerization that favors one molecule over the other. As for these amino acids' formation, our work (Figure 3) shows unequivocal evidence, for the first time, of isovaline and valine syntheses by energetic processing of a cosmic-ice analog. Future work will examine the relative formation rates of these amino acids.

Our results also speak to the question of enantiomeric excesses of meteoritic amino acids. The amines we used were either achiral (isobutylamine) or a

racemic mixture (1:1 D:L *sec*-butylamine). In both cases, Figure 3 shows that a substantial excess of L-isovaline and L-valine, over their D-counterparts, appears to have been produced by our reactions. An alternative and more-likely interpretation of Figure 3 is that another C₅ amino acid isomer is co-eluting with L-isovaline and L-valine, artificially boosting each molecule's LC peak. There are twelve different acyclic C₅H₁₁NO₂ amino acid isomers (twenty-three counting enantiomers), so co-elution is a distinct possibility that must be checked molecule by molecule. We plan to do this in the future, and to study the C₂, C₃, and C₄ radiation products for comparison to meteoritic results.

Finally, the bottom trace of Figure 3 shows a substantial L-isomer excess from a Murchison sample. This could be interpreted as a preference for nature to favor L amino acids, as is the case in terrestrial biology. However, such an interpretation must be viewed with caution in light of our success in making large apparent enantiomeric excesses from optically-inactive starting materials.

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