Research Paper

Amino Acids from Ion-Irradiated Nitrile-Containing Ices

Reggie L. Hudson,1,2 Marla H. Moore,2,3 Jason P. Dworkin,2,3 Mildred P. Martin,2,4 and Zachary D. Pozun2,5

Abstract

Solid CH3CN and solid H2O + CH3CN were ion irradiated near 10 K to initiate chemical reactions thought to occur in extraterrestrial ices. The infrared spectra of these samples after irradiation revealed the synthesis of new molecules. After the irradiated ices were warmed to remove volatiles, the resulting residual material was extracted and analyzed. Both unhydrolyzed and acid-hydrolyzed residues were examined by both liquid and gas chromatographic–mass spectral methods and found to contain a rich mixture of products. The unhydrolyzed samples showed HCN, NH3, acetaldehyde (formed by reaction with background and atmospheric H2O), alkyamines, and numerous other compounds, but no amino acids. However, reaction products in hydrolyzed residues contained a suite of amino acids that included some found in carbonaceous chondrite meteorites. Equal amounts of D- and L-enantiomers were found for each chiral amino acid detected. Extensive use was made of 13C-labeled CH3CN to confirm amino acid identifications and discriminate against possible terrestrial contaminants. The results reported here show that ices exposed to cosmic rays can yield products that, after hydrolysis, form a set of primary amino acids equal in richness to those made by other methods, such as photochemistry. Key Words: Amino acids—Ices—Prebiotic chemistry—Radiation chemistry—Infrared spectra. Astrobiology 8, 771–779.

Introduction

Laboratory work over the past 50 years has shown that the energetic processing of gas-phase and condensed-phase mixtures of simple organic and inorganic molecules yields organic products that, on further treatment, form amino acids. This work is of interest to astrobiologists as it suggests possible synthetic pathways to biomolecules starting with extraterrestrial conditions. Organics synthesized by the processing of interstellar materials could have been incorporated into cometary ice and then delivered to early Earth.

Photo- and radiation-chemical treatments of cosmic-ice analogs have consistently shown the formation of amino-acid precursors, rather than the amino acids themselves. These precursors, about which relatively little is known (Botta and Bada, 2002), hydrolyze to form amino acids (e.g., Kobayashi et al., 1995; Bernstein et al., 2002; Muñoz Caro et al., 2002; Nuevo et al., 2006), resulting in a situation reminiscent of the so-called “bound” meteoritic amino acids, which are detected only after extraction, such as with strong mineral acids. Such acid treatments, though foreign to interstellar and cometary environments, induce processes that should occur by water hydrolysis over long periods of time, the acid simply catalyzing the reactions.

Many aspects of prebiotic chemistry can be, and have been, studied in laboratories; but such work involves a large number of variables and unknowns. This has led us to seek out model systems with which to uncover reaction products, test chemical predictions, and further an understanding of reaction mechanisms in ices. This paper describes one such study. Here, we have selected a member of a single class of

1Department of Chemistry, Eckerd College, St. Petersburg, Florida.
2NASA Astrobiology Institute, Goddard Center for Astrobiology, Greenbelt, Maryland.
3NASA Goddard Space Flight Center, Greenbelt, Maryland.
4Catholic University of America, Washington, DC.
5University of Pittsburgh, Pittsburgh, Pennsylvania.
organic molecules for a combined infrared (IR) and chromatographic study of radiation-chemical reactions.

Acetonitrile, CH$_3$CN, was chosen for our work as it is the simplest organic nitrile, contains three of the four major biogenic elements, does not spontaneously polymerize, and has been the subject of earlier radiation studies with which we can compare our own results. Acetonitrile is also one of few molecules that have been detected in cometary comae (e.g., Huggins, 1882; Magee-Sauer et al., 1999; Woodney et al., 2002), Titan’s atmosphere (Courstenis et al., 1999), and the interstellar medium (ISM) (Bell et al., 1998). Concerning the latter, nitriles compose the largest class of molecules and ions reported in the ISM. Many of the approximately 140 interstellar species listed in a recent compilation (Hudson, 2006) are so complex that their source is thought to be reactions initiated by cosmic radiation incident on ice mantles of interstellar grains, as opposed to gas-phase chemistry. This suggests that nitriles as a group may be responsible both for the formation of molecules in association with meteoroids and cometary dust, and for the formation of complex species on the surfaces of grains.

Experiments began with the preparation of a thin film (~2 μm) of icy material, typically at 10–25 K under dynamic vacuum (~10$^{-7}$ torr). A polished metal disk, either gold or aluminum, served as the substrate (area ~ 5 cm$^2$) for ice growth. To maintain a reflective surface, the substrates were cleaned by buffing with ethanol (rather than being pyrolyzed) and then held under vacuum in the irradiation chamber at least overnight. Next, the ice film that formed on the substrate was irradiated with a 0.8 MeV H$^+$ beam from a Van de Graaff accelerator at a rate of a few hundred krad/sec. This process was repeated until the desired dose was achieved, typically 1–10 eV per molecule (a few thousand Mrad) for IR experiments and 10–20 eV per molecule for chromatographic experiments. Radiation doses were determined as described earlier and with the use of a stopping power of 285 MeV cm$^2$ g$^{-1}$ for CH$_3$CN and 274 MeV cm$^2$ g$^{-1}$ for H$_2$O (Hudson and Moore, 2004, 2006).

Transmission IR spectra of the ice film and subsequent residue were recorded after a sample was first prepared. They were also recorded periodically throughout the irradiation and during and after the post-irradiation warm-up. Two IR spectrometers were used in this work: a Bruker Vector 22 and a Mattson Polaris. A spectrum collected with either instrument consisted of a 60–200 scan accumulation at a resolution of 2 or 4 cm$^{-1}$.

After proton irradiation, the ice sample was allowed to warm under dynamic vacuum to room temperature over ~12 hours, which removed most volatiles from the metal substrate and left a residual material behind. This residue was extracted with high-performance liquid chromatography (HPLC)–grade acetonitrile, collected, and then split into three portions for analyses, as follows:

1. One portion was examined for traces of volatile material by way of gas chromatography–mass spectrometry (GC-MS). However, the focus of our work was not the analysis of volatiles by GC-MS, so only a few of the observed products were identified.

2. A second aliquot of the extract was dried and then derivatized with an o-phthalaldehyde/N-acetyl-L-cysteine (OPA/NAC) reagent, according to Glavin et al. (2006), to separate enantiomers and facilitate detection of primary amines by fluorescence HPLC with parallel positive and negative electrospray time-of-flight mass spectrometry (FD-LC/ToF-MS) with use of a Waters 2695 HPLC and LCT Premier system. For fluorescence measurements, the excitation wavelength was 340 nm, and the emission wavelength was 450 nm.

3. The third aliquot of the radiation-produced residue was dried, acid hydrolyzed by exposure to 6 M HCl vapor at 150°C for 3 hours, redried, and then dissolved in 50 μl of water. Of this solution, 10 μl was analyzed by OPA/NAC with FD-LC/ToF-MS, and the remainder was stored for future use.

Materials and Methods

The work described here involved two separate types of experiments: low-temperature ion irradiation with in situ IR spectroscopy and room-temperature chromatographic analyses. Details of both techniques are in print (Hudson and Moore, 2004; Glavin et al., 2006), so here we only give brief descriptions of each.

Experiments began with the preparation of a thin film (~2 μm) of icy material, typically at 10–25 K under dynamic vacuum (~10$^{-7}$ torr). A polished metal disk, either gold or aluminum, served as the substrate (area ~ 5 cm$^2$) for ice growth. To maintain a reflective surface, the substrates were cleaned by buffing with ethanol (rather than being pyrolyzed) and then held under vacuum in the irradiation chamber at least overnight. Next, the ice film that formed on the substrate was irradiated with a 0.8 MeV H$^+$ beam from a Van de Graaff accelerator at a rate of a few hundred krad/sec. This process was repeated until the desired dose was achieved, typically 1–10 eV per molecule (a few thousand Mrad) for IR experiments and 10–20 eV per molecule for chromatographic experiments. Radiation doses were determined as described earlier and with the use of a stopping power of 285 MeV cm$^2$ g$^{-1}$ for CH$_3$CN and 274 MeV cm$^2$ g$^{-1}$ for H$_2$O (Hudson and Moore, 2004, 2006).

Transmission IR spectra of the ice film and subsequent residue were recorded after a sample was first prepared. They were also recorded periodically throughout the irradiation and during and after the post-irradiation warm-up. Two IR spectrometers were used in this work: a Bruker Vector 22 and a Mattson Polaris. A spectrum collected with either instrument consisted of a 60–200 scan accumulation at a resolution of 2 or 4 cm$^{-1}$.

After proton irradiation, the ice sample was allowed to warm under dynamic vacuum to room temperature over ~12 hours, which removed most volatiles from the metal substrate and left a residual material behind. This residue was extracted with high-performance liquid chromatography (HPLC)–grade acetonitrile, collected, and then split into three portions for analyses, as follows:

1. One portion was examined for traces of volatile material by way of gas chromatography–mass spectrometry (GC-MS). However, the focus of our work was not the analysis of volatiles by GC-MS, so only a few of the observed products were identified.

2. A second aliquot of the extract was dried and then derivatized with an o-phthalaldehyde/N-acetyl-L-cysteine (OPA/NAC) reagent, according to Glavin et al. (2006), to separate enantiomers and facilitate detection of primary amines by fluorescence HPLC with parallel positive and negative electrospray time-of-flight mass spectrometry (FD-LC/ToF-MS) with use of a Waters 2695 HPLC and LCT Premier system. For fluorescence measurements, the excitation wavelength was 340 nm, and the emission wavelength was 450 nm.

3. The third aliquot of the radiation-produced residue was dried, acid hydrolyzed by exposure to 6 M HCl vapor at 150°C for 3 hours, redried, and then dissolved in 50 μl of water. Of this solution, 10 μl was analyzed by OPA/NAC with FD-LC/ToF-MS, and the remainder was stored for future use.

For GC-MS measurements, separation was carried out with a Thermo DSQ GC-MS with a splitless injection, an injector temperature of 200°C, and a flow of 1 ml/min He. A Restek RTX Amine column was used (30 m, 0.25 mm inner diameter, 0.5 μm film thickness) with a Restek base-deactivated guard column (5 m). The temperature was ramped from 100 to 140°C at 20°C/min, held at 140°C for 10 minutes, ramped at 1°C/minute to 145°C, held at 145°C for 5
minutes, and finally ramped at 20°C/min to 250°C, where it was held for 10 min. Masses were recorded from 50 to 650
*m/z*, and data analysis was performed with Xcalibur software (Thermo Finnigan).

The reagents (and their sources) used in our work were as follows: Millipore polished water with a resistivity greater
than 18.2 MΩ cm; CH3CN (Sigma-Aldrich); CH313CN, 13CH313CN, and 13CH313CN (Cambridge Isotopes). Analytical
standards were from Sigma-Aldrich and of the highest purity available, and they were not further purified before use. The FD-LC/ToF-MS buffers and label are described in detail in Glavin et al. (2006).

Throughout this project, extensive procedural and experimental (i.e., non-irradiation) blanks were obtained for comparison to results on irradiated ices. The use of 13C-labeled
CH3CN was important for discriminating between actual reaction products and background contamination. However, the major irradiation amino acid products were well above background contamination levels.

Though a reasonable attempt was made to exclude H2O from experiments with pure acetonitrile, several sources of water were difficult to control. First, H2O molecules, which adhere to the walls of any vacuum system, may have been cryopumped onto pure CH3CN ices, though no such water contamination was noticed in our blanks. Second, chemical reagents were stated by the supplier as having H2O levels at the 0.001% level or lower. Third, each irradiated sample, after warming under vacuum, was briefly exposed to atmospheric H2O (5–10 min) while being rinsed from the aluminum substrate. Finally, the CH3CN used to extract the samples was not degassed, and the liquid extractions were stored under air prior to analysis. However, no change in oxygen-containing, or other, products was seen with different storage times.

Results

We present our work in two parts. First, we describe the IR spectral measurements of irradiated ices that contained CH3CN and compare our new work with previous results.

![Figure 1](https://example.com/figure1.png)

**FIG. 1.** IR spectra of an amorphous CH3CN ice before and after 0.8 MeV proton irradiation to a dose of about 1 eV molecule^{-1}.

<table>
<thead>
<tr>
<th>Ice irradiated*</th>
<th>CH3NC</th>
<th>HCN</th>
<th>H2CCNH</th>
<th>CH4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH3CN</td>
<td>2170</td>
<td>2086</td>
<td>2033</td>
<td>1303</td>
</tr>
<tr>
<td>13CH3CN</td>
<td>2169</td>
<td>2086</td>
<td>2025</td>
<td>1296</td>
</tr>
<tr>
<td>CH313CN</td>
<td>2133</td>
<td>2054</td>
<td>1983</td>
<td>1303</td>
</tr>
<tr>
<td>13CH313CN</td>
<td>2134</td>
<td>2054</td>
<td>1974</td>
<td>1295</td>
</tr>
</tbody>
</table>

*The positions of the CN-stretching vibration for the four isotopic variants of CH3CN were as follows, all in cm^{-1}: 2252 (CH3CN), 2247 (13CH3CN), 2202 (CH313CN), and 2200 (13CH313CN).

We then consider some of the less-abundant, but astrobiologically important, radiation products that are difficult to impossible to detect and identify with conventional IR spectroscopy alone. These products were separated with chromatographic methods and detected with fluorescence and mass spectral measurements.

**Infrared spectra of irradiated nitrile-containing ices**

Figure 1 is an IR spectrum of amorphous CH3CN ice, recorded at ∼18 K before and after 0.8 MeV proton irradiation. Several new post-irradiation features are seen, and some were assigned in our previous paper (Hudson and Moore, 2004). The insert of Fig. 1 shows three such assignments, at 2170, 2086, and 2033 cm^{-1}. On repeating this particular experiment with CH313CN, 13CH3CN, and 13CH313CN, these bands were found to have the positions given in Table 1. The isotopic shifts agree with those seen by others for CH3NC and H2CCNH (e.g., Jacox, 1979a). An earlier uncertainty (Hudson and Moore, 2004) with the 2086 cm^{-1} band’s assignment is now removed by the observed 13C shift of 32 cm^{-1}. This feature can now be assigned to HCN, since the 13C-isotopic shifts near 10 K for HCN and CN^{-} are 33.5 and 42.3 cm^{-1}, respectively (Mielke and Andrews, 1990; Forney et al., 1992). The final column of Table 1 contains data for an IR feature near 1300 cm^{-1}. The 13C isotopic shifts agree with those for CH4 (Jacox, 1979b).

Returning to Fig. 1, growth in the 3200 and 700 cm^{-1} regions that was observed after irradiation of CH3CN is most reasonably explained by the formation of amines. However, many molecules with an -NH2 functional group absorb in these regions, so a more specific assignment is not possible with IR spectroscopy alone. Similarly, weak features that grew in near 1003, 964, and 820 cm^{-1} are suggestive of succinonitrile, NCCH2CH2CN; but the spectral signals are too weak for a firm identification.

**Analysis of residues from irradiated nitrile-containing ices**

Volatile material in our radiation residues was analyzed with GC-MS without derivatization. Table 2 gives some identifications, based on comparisons of fragmentation patterns and elution times of authentic standards. For hydroxyace-
tonitrile, an authentic standard was unavailable, so library data were used for the identification.

To test for the presence of free amino acids in our samples, a portion of the extract from each residue was derivatized for 1 minute with OPA/NAC to produce fluorescently labeled primary amines and then subjected to FD-LC/ToF-MS analysis. Ammonia and five primary amines, listed in Table 2, were detected, though no amino acids were found. Detections were confirmed by the comparison of retention times and the exact mass spectra of OPA/NAC derivatized products in both positive and negative electrospray (Glavin et al., 2006).

To promote the hydrolysis of bound amino acids, a portion of each radiation residue was acid hydrolyzed, dried, derivatized with OPA/NAC, and subjected to FD-LC/ToF-MS analysis. These samples gave a new set of chromatographic features, which were identified as being from amino acids. Results from two such acid-hydrolyzed, tagged residues are shown in Fig. 2. It is seen that essentially the same products appeared in residues from irradiated $^{13}\text{CH}_3^{13}\text{CN}$ and $\text{H}_2\text{O} + ^{13}\text{CH}_3^{13}\text{CN}$ ices, but in a lower concentration in the latter. Figure 2 also shows a blank from unirradiated acetonitrile.

The middle chromatogram of Fig. 2, the one from the $^{13}\text{CH}_3^{13}\text{CN}$ residue, is copied at the top of Fig. 3. The four traces below it were taken by simultaneously recording the mass-spectral signal over the mass ranges indicated. These ranges represent the full width at half maximum of the M$^+\text{H}^+$ peak. Assignments to specific tagged amino acids are given in Fig. 3, along with labels for the stereochemistry. For these mass ranges, data were acquired in the instrument’s positive electrospray (ES$^+$) mode and confirmed with the negative electrospray mode (ES$^-$). Table 3 lists all our identifications, each made as in Fig. 3, along with the abundance of each amino acid. Note that only racemic mixtures of amino acids were produced in our experiments.

Our amino acid products can be quantified with three different reaction yields. The total amino acid percent yield was estimated to be on the order of 0.1% for each ice studied, based on the initial amount of acetonitrile present. Table 3 gives relative yields, compared to glycine. A conservative order-of-magnitude estimate gave a radiation chemical yield of $G(\text{Gly}) \sim 0.001$.

Finally, Fig. 4 is a fluorescence detection liquid chromatography (FD-LC) chromatogram of the acid-hydrolyzed, OPA/NAC-tagged material from irradiated $^{13}\text{CH}_3^{13}\text{CN}$ but showing features with longer retention times than those in Fig. 2 and Fig. 3. Identifications of several primary amines are given. These same amines were also seen in unhydrolyzed residues (Table 2).

Other work

We also explored the role temperature might play in our results. Radiation experiments with $^{13}\text{CH}_3^{13}\text{CN}$ at $\sim 18$ K were carried out at 90 K, with virtually no differences in the amino acids eventually detected. We found, as in previous cases (Hudson and Moore, 2004), that radiation dose, as opposed to temperature, is what drives the ice chemistry.

In another experiment, we irradiated as usual and then brought the sample up to room temperature under vacuum. At that point, we recooled the residual material and irradiated.

![FIG. 2.](image_url) FD-LC chromatograms of acid-hydrolyzed OPA/NAC-tagged residues from irradiated $\text{H}_2\text{O} + ^{13}\text{CH}_3^{13}\text{CN}$ and $^{13}\text{CH}_3^{13}\text{CN}$ samples. The vertical expansion on the upper trace is 10 times that of the middle trace.

### Table 2. Products Identified in Unhydrolyzed Residues from Irradiated $^{13}\text{CH}_3^{13}\text{CN}$ Ice

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Formula</th>
<th>Method of detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen cyanide</td>
<td>HCN</td>
<td>GC-MS</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>CH$_3$(O)H</td>
<td>GC-MS</td>
</tr>
<tr>
<td>Succinonitrile</td>
<td>NCCH$_3$CH$_2$CN</td>
<td>GC-MS</td>
</tr>
<tr>
<td>2-Aminosuccinonitrile</td>
<td>NC(NH$_2$)CH$_2$CN</td>
<td>GC-MS</td>
</tr>
<tr>
<td>Hydroxycetanitrite</td>
<td>HOCH$_2$CN</td>
<td>GC-MS</td>
</tr>
<tr>
<td>Pentanedinitrile</td>
<td>NC(CH$_2$)$_2$CN</td>
<td>GC-MS</td>
</tr>
<tr>
<td>3-Aminocrotonitrile</td>
<td>(CH$_3$)C(NH$_2$)CHCN</td>
<td>GC-MS</td>
</tr>
<tr>
<td>Acetamide</td>
<td>CH$_3$(O)NH$_2$</td>
<td>GC-MS</td>
</tr>
<tr>
<td>Ammonia</td>
<td>NH$_3$</td>
<td>GC-MS and LC*</td>
</tr>
<tr>
<td>Methylamine</td>
<td>CH$_3$NH$_2$</td>
<td>GC-MS and LC</td>
</tr>
<tr>
<td>Ethylamine</td>
<td>CH$_3$CH$_2$NH$_2$</td>
<td>GC-MS and LC</td>
</tr>
<tr>
<td>n-Propylamine</td>
<td>CH$_3$CH$_2$CH$_2$NH$_2$</td>
<td>GC-MS and LC</td>
</tr>
<tr>
<td>i-Propylamine</td>
<td>(CH$_3$)$_2$CHNH$_2$</td>
<td>GC-MS and LC</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>HOCH$_2$CH$_2$NH$_2$</td>
<td>LC</td>
</tr>
</tbody>
</table>

*LC methods detected only primary amines.
ated the residue at 90 K and again found the same set of amino acids seen in Fig. 3, Table 2, and Table 3. This suggests that the amino acid precursors made in our experiments are apparently stable over a wide range of temperature and radiolysis conditions.

Discussion

Comparisons to earlier work on CH$_3$CN

Little work on the radiation products of condensed-phase CH$_3$CN is available for comparison to our results. Bradley and Wilkinson (1967) irradiated liquid CH$_3$CN with 4 MeV $e^-$, $^{60}$Co $\gamma$-rays and 250 kV X-rays and identified CH$_4$ and H$_2$ as major products, along with a water-soluble residual solid containing succinonitrile. Asycough et al. (1968) exposed liquid CH$_3$CN to $^{60}$Co $\gamma$-rays at 25°C and found CH$_4$, HCN, (CH$_2$CN)$_2$, and H$_2$ as the four most abundant products. Beginning in the early 1970s, Draganić and coworkers published a set of papers on the radiation products of aqueous solutions of various nitriles, including CH$_3$CN. In their earliest experiments, the products reported for H$_2$O + CH$_3$CN solutions were H$_2$, NH$_3$, and CH$_2$(O)H (Draganić et al., 1973). Later it was found that the dried residue from these irradiated mixtures could be hydrolyzed to give amino acids, primarily glycine (Draganić et al., 1980).

In general, these earlier radiation experiments with CH$_3$CN-containing liquids gave the same products we have identified in ices, namely HCN, CH$_4$, and (CH$_2$CN)$_2$. The earlier report of a solid residue also is similar to what we have observed, as is the formation of amino acids after hydrolysis of the residue. Our own earlier study (Hudson and Moore, 2004) showed a radiation-induced isomerization of CH$_3$CN to both CH$_3$NC and H$_2$CCNH. This too has been confirmed in the present work.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Ice $^{13}$CH$_3^{13}$CN</th>
<th>Ice $^{13}$CH$_3^{13}$CN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine (Gly)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>D, L-Alanine (D, L-Ala)</td>
<td>80</td>
<td>14</td>
</tr>
<tr>
<td>$\beta$-Alanine ($\beta$-Ala)</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>D, L-$\alpha$-Amino-$n$-butyric acid (D, L-$\alpha$-ABA)</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>D, L-$\beta$-Amino-$n$-butyric acid (D, L-$\beta$-ABA)</td>
<td>26</td>
<td>9</td>
</tr>
<tr>
<td>$\gamma$-Amino-$n$-butyric acid ($\gamma$-ABA)</td>
<td>26</td>
<td>14</td>
</tr>
<tr>
<td>D, L-Glutamic acid (D, L-Glu)</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>D, L-Aspartic acid (D, L-Asp)</td>
<td>36</td>
<td>8</td>
</tr>
</tbody>
</table>

$^a$The overall amino acid percent yield was $\sim 0.1\%$ for each ice, based on the original amount of $^{13}$CH$_3^{13}$CN present. Relative chromatographic abundances are thought to be accurate to $\sim 20\%$ (or better) for each value given.

$^b$With the exception of D- and L-$\alpha$-ABA, which could not be separated, all chiral amino acids were determined to be racemic.
These can be explained as follows: from frozen CH₃CN, to give CH₄, have been published and Sprague, 1982). Rate constants for H-atom abstraction dant radiation products from CH₃CN are HCN and CH₄. were unknown. In previous and present work, two abundance reactions, but in other cases the reaction pathways were expected from previous work and reasonably establishment information on radiation chemical change see, for example, Swallow (1973).

Some of the radiation products seen in our experiments were expected from previous work and reasonably established reactions, but in other cases the reaction pathways were unknown. In previous and present work, two abundant radiation products from CH₃CN are HCN and CH₄. These can be explained as follows:

\[
\begin{align*}
\text{CH}_3\text{CN} & \rightarrow \text{H} + \text{CH}_3\text{CN} \\
\text{H} + \text{CH}_3\text{CN} & \rightarrow \text{HCN} + \text{CH}_3 \\
\text{CH}_3 + \text{CH}_3\text{CN} & \rightarrow \text{CH}_4 + \text{CH}_2\text{CN}
\end{align*}
\]

Supporting these reactions are low-temperature electron-spin resonance experiments, which show that the radicals H, CH₃CN, and CH₃ are present in irradiated CH₃CN (Williams and Sprague, 1982). Rate constants for H-atom abstraction from frozen CH₃CN, to give CH₄, have been published (Sprague and Williams, 1971). Succinonitrile, hinted at in our IR data and confirmed by GC-MS, probably arises from radical-radical reactions:

\[
\text{CH}_2\text{CN} + \text{CH}_3\text{CN} \rightarrow \text{NC-CH}_2\text{-CH}_2\text{-CN}
\]

A similar dimerization gives ethylene glycol (HO-CH₂-CH₂-OH) from CH₂OH radicals in irradiated CH₂OH (Hudson and Moore, 2000), and H₂C-CH₃ from CH₃ radicals in irradiated CH₄ (Moore and Hudson, 1998). The other products we identified by IR in irradiated CH₃CN, namely CH₃NC and H₂CCNH, were formed by intermolecular isomerizations (Hudson and Moore, 2004).

The behavior of the isotopic shifts in Table 1 is consistent with these reactions. The shift of the 1300 cm⁻¹ band of CH₄ was zero when the starting material was changed from CH₃CN to CH₃¹³CN but not when either ¹³CH₃CN or ¹³CH₃¹²CN was used. This showed that the CH₄ produced originated in the methyl (-CH₃) group of the original nitrile, as expected. Similarly, the carbon of the cyano (-CN) group in the starting nitrile was always found to be the carbon attached to the nitrogen in the CH₃NC, HCN, and H₂CCNH product, as expected.

The amines suggested by our IR spectra (Fig. 1), and confirmed by the FD-LC/ToF-MS results of Fig. 4, probably have their source in H-atom addition reactions and subsequent fragmentations and recombinations. In earlier work, we found that CO, a triply bonded molecule, was reduced to CH₃OH by proton irradiation of an H₂O + CO ice mixture (Hudson and Moore, 1999) and that C₂H₂, with a CC triple bond, was reduced to C₂H₆ (Moore and Hudson, 1998). This led us to suspect that solid CH₃CN, with its CN triple bond, could produce an amine by radiolysis, as observed. The overall reaction sequences for these reductions are as follows:

\[
\begin{align*}
\text{C}=\text{O} & \rightarrow \text{H}_2\text{C}=\text{O} \rightarrow \text{H}_3\text{C}-\text{OH} \\
\text{HC}=\text{CH} & \rightarrow \text{H}_2\text{C}==\text{CH}_2 \rightarrow \text{H}_2\text{C}=\text{CH}_3 \\
\text{CH}_3\text{C}=\text{N} & \rightarrow \text{CH}_3\text{C}-\text{NH} \rightarrow \text{CH}_3\text{CH}_2\text{-NH}_2
\end{align*}
\]

We did not unequivocally detect CH₃-CH-NH, as strong bands of other molecules obscure most of its IR features (Gerakines et al., 2004), but amine-like bands were found near 3200 and 700 cm⁻¹.

More difficult to rationalize, but of primary interest for this paper, are the many products identified in residues from irradiated CH₃CN. The IR-observed ketenimine isomer, after partial reduction, is probably the source of NH₃ and CH₃C(O)H (acetaldehyde), both detected chromatographically. Their presence is understandable since imines can hydrolyze to ammonia and aldehydes (Draganić et al., 1973). The reaction sequence is shown below:

\[
\text{H}_2\text{C}==\text{C}==\text{N}-\text{H} \rightarrow \text{H}_3\text{C}-\text{CH}==\text{N}-\text{H} \quad \text{(reduction)}
\]

\[
\text{H}_2\text{O} + \text{H}_2\text{C}==\text{C}==\text{N}-\text{H} \rightarrow \text{NH}_3 + \text{CH}_3\text{C(O)H} \quad \text{(hydrolysis)}
\]

The radiation-induced hydrolysis of CH₃CN itself can account for the acetamide, CH₃C(O)NH₂, that was observed as follows:

\[
\begin{align*}
\text{CH}_3\text{CN} + \text{H}_2\text{O} & \rightarrow \text{CH}_3\text{C(O)H}==\text{NH} \quad \text{(H, OH addition)} \\
\text{CH}_3\text{C(O)H}==\text{NH} & \rightarrow \text{CH}_3\text{C(O)H}==\text{NH}_2 \quad \text{(tautomerism)}
\end{align*}
\]

In general, reaction sequences involving combinations of radical addition (e.g., H, OH, CN), tautomerism, and hydrolysis can explain many of the other products we observed.

Amino acids in ices

This paper presents evidence that cosmic radiation acting on organic ices produces molecular precursors that, after hydrolysis, yield a set of amino acids (Table 3) comparable to those found in photochemical studies. In a few cases, the reaction pathways seem clear, such as the conversion by acid...
hydrolysis of 2-aminosuccinonitrile (Table 2) into aspartic acid:

\[
\text{NC-CH(NH}_2\text{-CH}_2\text{-CN} \rightarrow \text{HOOC-CH(NH}_2\text{-CH}_2\text{-COOH}}
\]

However, attempts to write such specific synthetic paths for most other amino acids would be highly speculative as the relevant reactions have not yet been rigorously tested.

The formation of \(\text{\alpha-}\)amino acids, in an astrobiological context, is often discussed with reference to the Strecker synthesis, which involves the reaction of a mixture of \(\text{H}_2\text{O}, \text{HCN}, \text{NH}_3\), and an aldehyde (Strecker, 1850). Specifically, the overall sequence from acetaldehyde to alanine is as follows:

![Strecker synthesis diagram]

In our experiments, all the Strecker reactants were present in the residues from irradiated \(\text{CH}_3\text{CN}\), so it might be expected that \(\text{\alpha-}\)amino acids were present as well. However, in no case were any amino acids detected without acid hydrolysis, which leaves questions about their meteoritic formation unanswered. See Elsila et al. (2007) for one of the few studies of amino acid reactions in ices, including considerations of both Strecker and non-Strecker pathways.

Bernstein et al. (2002), Muñoz Caro et al. (2002), Nuevo et al. (2006), and Elsila et al. (2007) generated recent laboratory results on amino acid synthesis in ices. In all cases, a frozen mixture (~10 K) was photolyzed with vacuum-UV light, and volatiles were removed by warming. The residual material then was acid hydrolyzed and chromatographically analyzed, and amino acids were found. Bernstein et al. (2002) studied a 4-component \(\text{H}_2\text{O} + \text{CH}_3\text{OH} + \text{NH}_3 + \text{HCN}\) mixture and reported glycine, alanine, and serine. Muñoz Caro et al. (2002) photolyzed a \(\text{H}_2\text{O} + \text{CH}_3\text{OH} + \text{NH}_3 + \text{CO} + \text{CO}_2\) ice and found 16 amino acids. Nuevo et al. (2006) used a \(\text{H}_2\text{O} + \text{CH}_3\text{OH} + \text{NH}_3\) mixture and found 5 primary biological amino acids among the products, after acid treatment. Our work has followed a similar sequence of ice processing, sample warming, and analysis of a hydrolyzed residue. An important difference is that our reactants have been confined to \(\text{CH}_3\text{CN}\) and \(\text{H}_2\text{O}\), and still amino acids have been detected. In fact, our ices are among the simplest yet reported to make amino acids.

Table 4 shows a comparison of our radiation results with photochemical products and with the older analyses of 3-MeV \(p^+\) irradiated ices by Kobayashi et al. (1995). Such comparisons allow for searches for particular markers of energetic processing. The first few lines of the table show that some of the same amino acids are found in both types of experiments. One difference is that serine has not yet been found in the radiation work. Additional differences are obvious in the middle and bottom of the table, and all cases show the difficulty of producing isovaline and \(\text{\alpha-}\)aminoisobutyric acid, both found in meteorites (Ehrenfreund et al., 2001).

One conclusion suggested by Table 4 is that energetic processing of almost any organic ice that contains C, H, N, and O probably results in formation of amino acid precursors, which can be hydrolyzed to give the acids themselves. In our

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>(\alpha)-Ala</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>(\beta)-Ala</td>
<td>x(^a)</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Ser(^b)</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Asp</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>(\alpha)-ABA</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>(\beta)-ABA</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>(\gamma)-ABA</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Glu</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Val(^b)</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Di- and secondary amino acids</td>
<td>x(^c)</td>
<td>x(^c)</td>
<td>x(^c)</td>
<td>x(^c)</td>
<td>x(^c)</td>
</tr>
</tbody>
</table>

\(^a\)Elsila et al. (2007).
\(^b\)Ser, serine; Val, valine.
\(^c\)Detection of 1,2,3-triaminopropane also reported.
own experiments, radiolysis produced CH₄ and NH₃ (both detected), which were used as starting materials in the ices of Kobayashi et al. (1995), so general agreement in the last 2 columns of Table 4 is expected. In fact, similar considerations should apply for comparisons among all the experiments in the table. For example, the CH₄ + NH₃ and CH₄ + H₂O combinations in the last column will give HCN and CH₃OH products on irradiation, which are reagents listed in the first column; so, again, similar reaction products are expected. Additional work is needed to determine whether the differences in products of Table 4 are from anything more than differences in the analytical techniques employed. Finally, it is probably worth emphasizing again that each amino acid in Table 4 was only detected after an acid treatment of a residual material from an ice-processing experiment. For processed ices to contribute amino acids to early Earth, for example, the ices and their residual materials would need to have undergone either hydrolysis or some sort of parent-body aqueous alteration.

Summary

Our results show that ionizing radiation produces amino acid precursors in ices that contain CH₃CN. By extension, other nitriles in ices should also give rise to amino acids. Among the extraterrestrial environments where this might occur are cometary nuclei and the ices of solar system moons. For example, since Titan is now known to possess H₂O ice (Griffith et al., 2003), any surface CH₃CN will give rise to complex nitriles and amino acid precursors, driven by the magnetospheric radiation of Saturn. Cometary collisions with Earth’s moon, Mars, and other worlds should have delivered amino acids, or their precursors, to those bodies. Moreover, given the degree to which comets delivered material to early Earth, the types of amino acids derived from processed laboratory ices should have been available when life originated. Finally, our work shows that, while bioorganic can be synthesized by reactions in complex ice mixtures, even simple H₂O + CH₃CN ices can also generate many amino acids.

Acknowledgments

NASA funding through the Goddard Center for Astrobiology and Planetary Atmospheres is acknowledged. R.L.H. also acknowledges the support of NASA for grants NNG05GJ46G and NNG05GJ58A.

Abbreviations

FD-LC, fluorescence detection liquid chromatography; GC-MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; IR, infrared; ISM, interstellar medium; LC, liquid chromatography; OPA/NAC, o-phthalaldehyde/N-acetyl-L-cysteine; ToF-MS, time-of-flight mass spectrometry.

References


Address reprint requests to:
Reggie L. Hudson
Department of Chemistry
Eckerd College
4200 54th Avenue South
St. Petersburg, FL 33711

E-mail: hudsonrl@eckerd.edu