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**Research Paper**

# Amino Acids from Ion-Irradiated Nitrile-Containing Ices

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## Abstract

Solid $CH_3CN$ and solid $H_2O + CH_3CN$ 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, $NH_3$, acetaldehyde (formed by reaction with background and atmospheric $H_2O$), 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 $^{13}C$-labeled $CH_3CN$ 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

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organic molecules for a combined infrared (IR) and chromatographic study of radiation-chemical reactions.

Acetonitrile, $CH_3CN$, 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 (Coustenis *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 some observed and some yet-unobserved interstellar molecules. For comets, cosmic radiation can drive solid-phase chemistry by acting on nuclear ices, which then sublime as a comet approaches the Sun and release radiation products. In the case of Titan, this satellite's surface is thought to receive nitriles from the atmosphere and have regions of $H_2O$ ice (Griffith *et al.*, 2003). Condensed-phase nitrile chemistry can be initiated on Titan, regardless of the presence or absence of $H_2O$ ice, by radiation originating within the saturnian magnetosphere (Sagan and Thompson, 1984). In all these environments, ionizing radiation drives chemical change, which causes nitriles and other molecules to form new species.

Our goal was to examine the solid-phase radiation chemistry of acetonitrile, a well-documented interstellar, cometary, and Titan molecule. This molecule was part of a recent IR study of ion-irradiated nitriles in various chemical environments (Hudson and Moore, 2004). One aspect of that work was the prediction of keteneimine, $H_2C{=}C{=}NH$, in the ISM, a prediction that has since been confirmed (Lovas *et al.*, 2006). This gives confidence in the power of the laboratory approach.

In this study, we have extended our earlier work using experimental techniques of far greater sensitivity in a search for one class of biomolecules—amino acids. Our intent was to determine some of the molecules to which the presence of $CH_3CN$-containing ices can lead in a radiation environment, but which are, at present, hidden from astronomical observation.

Finally, we show in this paper that nitrile-containing ices exposed to ionizing radiation produce residual materials that, after acid hydrolysis, yield a rich suite of amino acids. Our results can be considered complementary to the detection of amino acids in UV-photolyzed ices by others (Bernstein *et al.*, 2002; Muñoz Caro *et al.*, 2002; Nuevo *et al.*, 2006).

## 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 ($\sim$2 $\mu$m) of icy material, typically at 10–25 K under dynamic vacuum ($\sim 10^{-7}$ torr). A polished metal disk, either gold or aluminum, served as the substrate (area $\approx 5$ 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 $CH_3CN$ and 274 MeV cm$^2$ g$^{-1}$ for $H_2O$ (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 $\sim$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*-phthaldialdehyde/*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 $\mu$l of water. Of this solution, 10 $\mu$l 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 $\mu$m 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; $CH_3CN$ (Sigma-Aldrich); $CH_3{}^{13}CN$, $^{13}CH_3CN$, and $^{13}CH_3{}^{13}CN$ (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 $^{13}C$-labeled $CH_3CN$ 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 $H_2O$ from experiments with pure acetonitrile, several sources of water were difficult to control. First, $H_2O$ molecules, which adhere to the walls of any vacuum system, may have been cryopumped onto pure $CH_3CN$ ices, though no such water contamination was noticed in our blanks. Second, chemical reagents were stated by the supplier as having $H_2O$ levels at the 0.001% level or lower. Third, each irradiated sample, after warming under vacuum, was briefly exposed to atmospheric $H_2O$ (5–10 min) while being rinsed from the aluminum substrate. Finally, the $CH_3CN$ 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 $CH_3CN$ and compare our new work with previous results. 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.

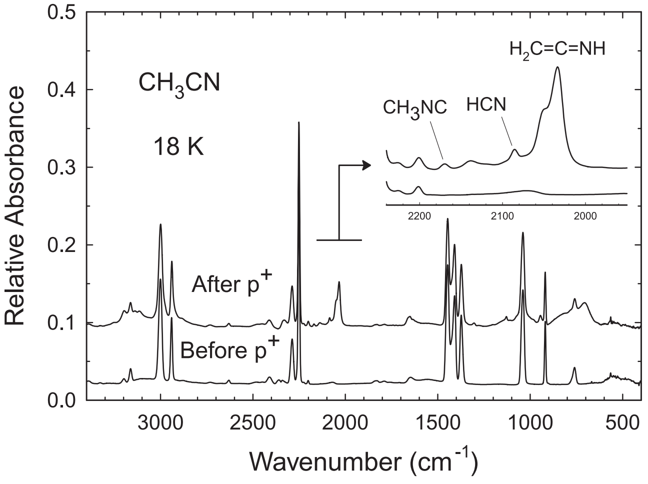


**FIG. 1.** IR spectra of an amorphous $CH_3CN$ ice before and after 0.8 MeV proton irradiation to a dose of about 1 eV $molecule^{-1}$.

TABLE 1. POSITIONS ($CM^{-1}$) OF SELECTED PRODUCTS IN IRRADIATED ICE

| | *Product Molecule and Position ($cm^{-1}$)* | | | |
|---|---|---|---|---|
| *Ice irradiated** | *$CH_3NC$ $\nu_2$* | *HCN $\nu_1$* | *$H_2CCNH$ $\nu_3$* | *$CH_4$ $\nu_4$* |
| $CH_3CN$ | 2170 | 2086 | 2033 | 1303 |
| $^{13}CH_3CN$ | 2169 | 2086 | 2025 | 1296 |
| $CH_3{}^{13}CN$ | 2133 | 2054 | 1983 | 1303 |
| $^{13}CH_3{}^{13}CN$ | 2134 | 2054 | 1974 | 1295 |

*The positions of the CN-stretching vibration for the four isotopic variants of $CH_3CN$ were as follows, all in $cm^{-1}$: 2252 ($CH_3CN$), 2247 ($^{13}CH_3CN$), 2202 ($CH_3{}^{13}CN$), and 2200 ($^{13}CH_3{}^{13}CN$).

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

Figure 1 is an IR spectrum of amorphous $CH_3CN$ 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 $CH_3{}^{13}CN$, $^{13}CH_3CN$, and $^{13}CH_3{}^{13}CN$, these bands were found to have the positions given in Table 1. The isotopic shifts agree with those seen by others for $CH_3NC$ and $H_2CCNH$ (*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 $^{13}C$ shift of 32 $cm^{-1}$. This feature can now be assigned to HCN, since the $^{13}C$-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 $^{13}C$ isotopic shifts agree with those for $CH_4$ (Jacox, 1979b).

Returning to Fig. 1, growth in the 3200 and 700 $cm^{-1}$ regions that was observed after irradiation of $CH_3CN$ is most reasonably explained by the formation of amines. However, many molecules with an $-NH_2$ 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, $NCCH_2CH_2CN$; but the spectral signals are too weak for a firm identification.

Similar spectral changes were seen in irradiated $H_2O$ + $CH_3CN$ ice mixtures, initially having 20:1 and 10:1 ratios, but to a smaller degree due to dilution.

### *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-

TABLE 2. PRODUCTS IDENTIFIED IN UNHYDROLYZED RESIDUES FROM IRRADIATED $^{13}CH_3{}^{13}CN$ ICE

| *Molecule* | *Formula* | *Method of detection* |
|---|---|---|
| Hydrogen cyanide | $HCN$ | GC-MS |
| Acetaldehyde | $CH_3C(O)H$ | GC-MS |
| Succinonitrile | $NCCH_2CH_2CN$ | GC-MS |
| 2-Aminosuccinonitrile | $NCCH(NH_2)CH_2CN$ | GC-MS |
| Hydroxyacetonitrile | $HOCH_2CN$ | GC-MS |
| Pentanedinitrile | $NC(CH_2)_3CN$ | GC-MS |
| 3-Aminocrotonitrile | $(CH_3)C(NH_2)CHCN$ | GC-MS |
| Acetamide | $CH_3C(O)NH_2$ | GC-MS |
| Ammonia | $NH_3$ | GC-MS and LC* |
| Methylamine | $CH_3NH_2$ | GC-MS and LC |
| Ethylamine | $CH_3CH_2NH_2$ | GC-MS and LC |
| *n*-Propylamine | $CH_3CH_2CH_2NH_2$ | GC-MS and LC |
| *i*-Propylamine | $(CH_3)_2CHNH_2$ | GC-MS and LC |
| Ethanolamine | $HOCH_2CH_2NH_2$ | LC |

*LC methods detected only primary amines.

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 $^{13}CH_3{}^{13}CN$ and $H_2O$ + $^{13}CH_3{}^{13}CN$ 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 $^{13}CH_3{}^{13}CN$ 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 $M+H^+$ 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 $G$(Gly) ~ 0.001.

Finally, Fig. 4 is a fluorescence detection liquid chromatography (FD-LC) chromatogram of the acid-hydrolyzed, OPA/NAC-tagged material from irradiated $^{13}CH_3{}^{13}CN$ 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 $^{13}CH_3{}^{13}CN$ at ~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 irradi-

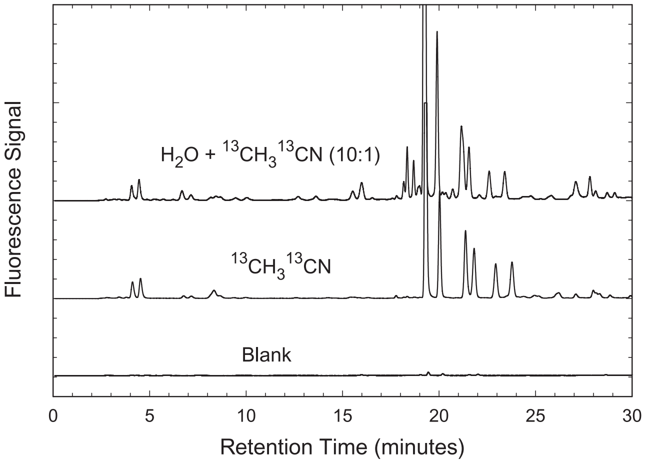
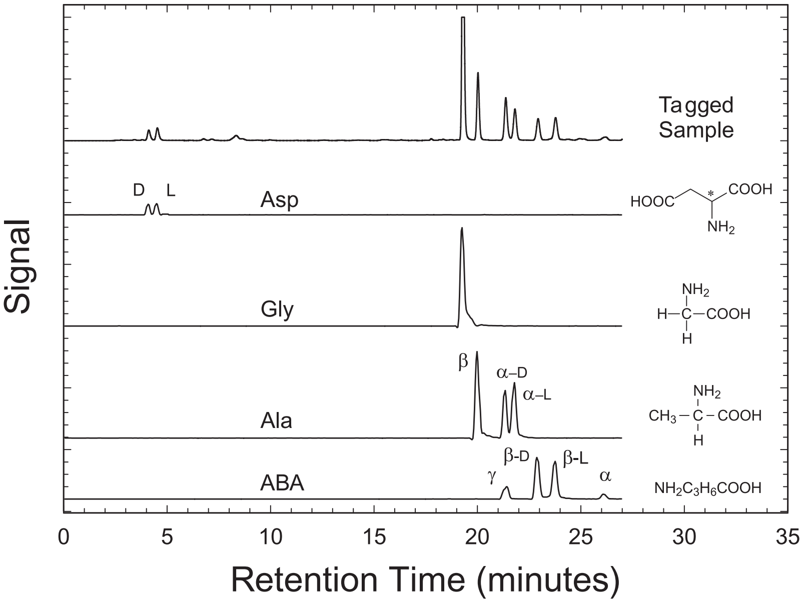


**FIG. 2.** FD-LC chromatograms of acid-hydrolyzed OPA/NAC-tagged residues from irradiated $H_2O$ + $^{13}CH_3{}^{13}CN$ and $^{13}CH_3{}^{13}CN$ samples. The vertical expansion on the upper trace is 10 times that of the middle trace.

**FIG. 3.** Amino acid identifications in the acid-hydrolyzed OPA/NAC-tagged residue of irradiated $^{13}CH_3{}^{13}CN$. The top trace is an FD-LC chromatogram. The other traces are ToF-MS signals, recorded in our instrument's positive electrospray mode, for these tagged amino acids (and mass ranges): Asp (399.092–399.128), Gly (339.074–339.115), Ala (354.094–354.136), and ABA (369.113–369.157). Two small features at 7 min are for L- and D-glutamic acid, in that order. The enantiomers of $\alpha$-amino-*n*-butyric acid coeluted, and $\gamma$-amino-*n*-butyric acid eluted just before D-Ala (shoulder).



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 $CH_3CN$*

Little work on the radiation products of condensed-phase $CH_3CN$ is available for comparison to our results. Bradley and Wilkinson (1967) irradiated liquid $CH_3CN$ 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 $CH_3CN$ to $^{60}Co$ $\gamma$-rays at 25°C and found $CH_4$, HCN, $(CH_2CN)_2$, 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 $CH_3CN$. In their earliest experiments, the products reported for $H_2O + CH_3CN$ solutions were $H_2$, $NH_3$, and $CH_3C(O)H$ (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 $CH_3CN$-containing liquids gave the same products we have identified in ices, namely HCN, $CH_4$, and $(CH_2CN)_2$. 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 $CH_3CN$ to both $CH_3NC$ and $H_2CCNH$. This too has been confirmed in the present work.

TABLE 3. AMINO ACIDS IDENTIFIED BY FD-LC/TOF-MS IN RESIDUES FROM IRRADIATED ICES[a,b]

| | *Abundance relative to glycine = 100* | |
|---|---|---|
| *Amino acid* | *$^{13}CH_3{}^{13}CN$ Ice* | *$H_2O$ + $^{13}CH_3{}^{13}CN$ Ice* |
| Glycine (Gly) | 100 | 100 |
| D, L-Alanine (D, L-Ala) | 80 | 14 |
| $\beta$-Alanine ($\beta$-Ala) | 34 | 34 |
| D, L-$\alpha$-Amino-*n*-butyric acid (D, L-$\alpha$-ABA) | 9 | 0 |
| D, L-$\beta$-Amino-*n*-butyric acid (D, L-$\beta$-ABA) | 26 | 9 |
| $\gamma$-Amino-*n*-butyric acid ($\gamma$-ABA) | 26 | 14 |
| D, L-Glutamic acid (D, L-Glu) | 6 | 2 |
| D, L-Aspartic acid (D, L-Asp) | 36 | 8 |

[a] The overall amino acid percent yield was ~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 ~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.

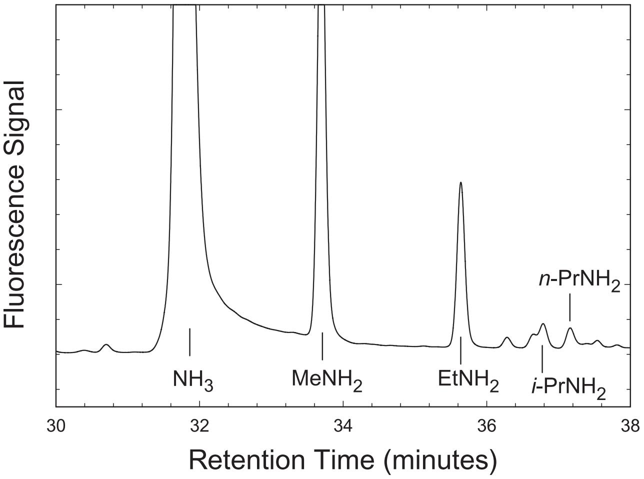


**FIG. 4.** FD-LC chromatogram showing primary amines in the acid-hydrolyzed OPA/NAC-tagged residue from irradiated $^{13}CH_3{}^{13}CN$. Identifications were confirmed by elution times and mass spectra.

### *Reactions in irradiated $CH_3CN$-containing ices*

The high-energy ions incident on our ices are responsible for initiating chemical reactions, but only indirectly for the final products. Each 0.8 MeV $H^+$ passing through a sample traces out a path containing thousands of secondary electrons that cause molecular ionizations and excitations that ultimately result in bond breakage to give free radicals and other unstable intermediates. The reactions of these intermediates form the molecular products we detect. For additional 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 $CH_3CN$ are HCN and $CH_4$. These can be explained as follows:

$$CH_3CN \rightarrow H + CH_2CN$$

$$H + CH_3CN \rightarrow HCN + CH_3$$

$$CH_3 + CH_3CN \rightarrow CH_4 + CH_2CN$$

Supporting these reactions are low-temperature electron-spin resonance experiments, which show that the radicals H, $CH_2CN$, and $CH_3$ are present in irradiated $CH_3CN$ (Williams and Sprague, 1982). Rate constants for H-atom abstraction from frozen $CH_3CN$, to give $CH_4$, 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:

$$CH_2CN + CH_2CN \rightarrow NC\text{-}CH_2\text{-}CH_2\text{-}CN$$

A similar dimerization gives ethylene glycol ($HO\text{-}CH_2\text{-}CH_2\text{-}OH$) from $CH_2OH$ radicals in irradiated $CH_3OH$ (Hudson and Moore, 2000), and $H_3C\text{-}CH_3$ from $CH_3$ radicals in irradiated $CH_4$ (Moore and Hudson, 1998). The other products we identified by IR in irradiated $CH_3CN$, namely $CH_3NC$ and $H_2CCNH$, 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 $CH_4$ was zero when the starting material was changed from $CH_3CN$ to $CH_3{}^{13}CN$ but not when either $^{13}CH_3CN$ or $^{13}CH_3{}^{13}CN$ was used. This showed that the $CH_4$ produced originated in the methyl ($-CH_3$) 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 $CH_3NC$, HCN, and $H_2CCNH$ 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 $CH_3OH$ by proton irradiation of an $H_2O$ + CO ice mixture (Hudson and Moore, 1999) and that $C_2H_2$, with a CC triple bond, was reduced to $C_2H_6$ (Moore and Hudson, 1998). This led us to suspect that solid $CH_3CN$, with its CN triple bond, could produce an amine by radiolysis, as observed. The overall reaction sequences for these reductions are as follows:

$$C{\equiv}O \rightarrow H_2C{=}O \rightarrow H_3C\text{-}OH$$

$$HC{\equiv}CH \rightarrow H_2C{=}CH_2 \rightarrow H_3C\text{-}CH_3$$

$$CH_3\text{-}C{\equiv}N \rightarrow CH_3\text{-}CH{=}NH \rightarrow CH_3\text{-}CH_2\text{-}NH_2$$

We did not unequivocally detect $CH_3\text{-}CH{=}NH$, 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 $CH_3CN$. The IR-observed ketenimine isomer, after partial reduction, is probably the source of $NH_3$ and $CH_3C(O)H$ (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:

$$H_2C{=}C{=}N\text{-}H \rightarrow H_3C\text{-}CH{=}N\text{-}H \text{ (reduction)}$$

$$H_2O + H_3C\text{-}CH{=}N\text{-}H \rightarrow NH_3 + CH_3C(O)H \text{ (hydrolysis)}$$

The radiation-induced hydrolysis of $CH_3CN$ itself can account for the acetamide, $CH_3C(O)NH_2$, that was observed as follows:

$$CH_3CN + H_2O \rightarrow CH_3C(OH){=}NH \text{ (H, OH addition)}$$

$$CH_3C(OH){=}NH \rightarrow CH_3C(O)\text{-}NH_2 \text{ (tautomerism)}$$

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.

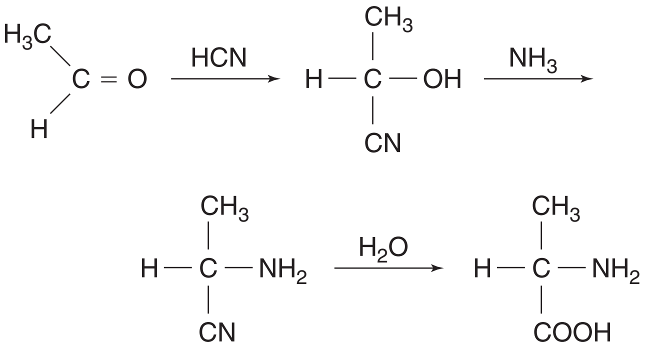
### *Amino acids in ices*

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:

$$\mathrm{NC\text{-}CH(NH_2)\text{-}CH_2\text{-}CN} \rightarrow \mathrm{HOOC\text{-}CH(NH_2)\text{-}CH_2\text{-}COOH}$$

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 $\alpha$-amino acids, in an astrobiological context, is often discussed with reference to the Strecker synthesis, which involves the reaction of a mixture of $H_2O$, HCN, $NH_3$, and an aldehyde (Strecker, 1850). Specifically, the overall sequence from acetaldehyde to alanine is as follows:



In our experiments, all the Strecker reactants were present in the residues from irradiated $CH_3CN$, so it might be expected that $\alpha$-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 $H_2O + CH_3OH + NH_3 + HCN$ mixture and reported glycine, alanine, and serine. Muñoz Caro *et al.* (2002) photolyzed a $H_2O + CH_3OH + NH_3 + CO + CO_2$ ice and found 16 amino acids. Nuevo *et al.* (2006) used a $H_2O + CH_3OH + NH_3$ 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 $CH_3CN$ and $H_2O$, 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 $\alpha$-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 4. AMINO ACIDS IDENTIFIED IN ICE-PROCESSING EXPERIMENTS

| *Amino acids detected* | *Bernstein* et al. *(2002)*<br>$H_2O$<br>$CH_3OH$<br>$NH_3$<br>HCN<br>Far-UV photolysis | *Muñoz Caro* et al. *(2002)*<br>$H_2O$<br>$CH_3OH$<br>$NH_3$<br>CO<br>$CO_2$<br>Far-UV photolysis | *Nuevo* et al. *(2006)*<br>$H_2O$<br>$CH_3OH$<br>$NH_3$<br>Far-UV photolysis | *This work (2008)*<br>$CH_3CN$<br>and<br>$H_2O$ $CH_3CN$<br>0.8-MeV $p^+$ radiolysis | *Kobayashi* et al. *(1995)*<br>$H_2O$<br>$CH_4$<br>$NH_3$<br>3-MeV $p^+$ radiolysis |
|---|---|---|---|---|---|
| Gly | x | x | x | x | x |
| $\alpha$-Ala | x | x | x | x | x |
| $\beta$-Ala | x[a] | x | x | x | x |
| Ser[b] | x | x | | | |
| Asp | | x | x | x | |
| $\alpha$-ABA | | x | x | x | x |
| $\beta$-ABA | | | | x | x |
| $\gamma$-ABA | | | | x | x |
| Glu | | | | x | |
| Val[b] | | x | | | |
| Di- and secondary amino acids | | x[c] | x[c] | | |

[a] Elsila *et al.* (2007).

[b] Ser, serine; Val, valine.

[c] Detection of 1,2,3-triaminopropane also reported.

own experiments, radiolysis produced $CH_4$ and $NH_3$ (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 $CH_4 + NH_3$ and $CH_4 + H_2O$ combinations in the last column will give HCN and $CH_3OH$ 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 icy environments that contain $CH_3CN$. 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 $H_2O$ ice (Griffith *et al*., 2003), any surface $CH_3CN$ 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 bioorganics can be synthesized by reactions in complex ice mixtures, even simple $H_2O + CH_3CN$ ices can also generate many amino acids.

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## 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*-phthaldialdehyde/*N*-acetyl-L-cysteine; ToF-MS, time-of-flight mass spectrometry.

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