EXOGENOUS AND ENDOGENOUS SOURCES OF ORGANIC COMPOUNDS
ON THE EARLY EARTH: INVESTIGATING CARBONACEOUS METEORITES
AND PLASIBLY PREBIOTIC COMPLEX MIXTURES
BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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By
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ABSTRACT

The early Earth atmosphere was thought to have been composed mostly of nitrogen and carbon dioxide with low concentrations of reduced gases (H$_2$, CH$_4$, etc.). Laboratory experiments simulating endogenous chemistry of early Earth (i.e. spark discharge with neutral gas mixtures) produce low abundances of biologically relevant molecules, which may have been a problem for the origin(s) of life. Thus, an exogenous source (e.g., meteorites, comets, and interplanetary dust particles) that delivered organic molecules may have been important in facilitating prebiotic reactions on the early Earth.

In Chapter 2, we investigated cyanide in a variety of meteorites by developing an analytical protocol that consisted of extracting a meteorite via acid-digestion and distillation using a gas-permeable membrane apparatus followed by chemical derivatization and analysis by liquid chromatography coupled to fluorescence detection and time-of-flight mass spectrometry. Cyanide is an important precursor for the synthesis of both amino acids and nucleobases. In Chapter 3, we investigated pyridine carboxylic acids in eight different CM2-type carbonaceous chondrites by liquid chromatography coupled to UV detection and high resolution Orbitrap mass spectrometry. Of particular interest was nicotinic acid (pyridine-3-carboxylic acid), which is a precursor to the coenzyme nicotinamide adenine dinucleotide (NAD), a ubiquitous molecule found in all living cells. Additionally, the synthesis of meteoritic pyridine carboxylic acids has yet to be determined. Therefore, we carried out laboratory studies of proton-irradiated pyridine in carbon dioxide-rich low-temperature ices to serve as a model of the interstellar ice chemistry that may have led to the synthesis of pyridine carboxylic acids.
Examining the synthesis and inventory of small, biologically relevant molecules in carbonaceous meteorites is useful for understanding their availability on the early Earth; however, biopolymers (e.g., nucleic acids and proteins) are extremely large and complex in structure and such molecules are not found in meteorites. In Chapter 4, we investigated the reactivity of various nitrogen heterocycles with plausibly prebiotic complex mixtures produced from spark discharge experiments in order to understand their ability to form more complex (substituted or polymeric) structures. We also investigated nitrogen heterocyclic polymers produced from 5-hydroxymethyluracil and 5-hydroxymethylcytosine by liquid chromatography-high resolution Orbitrap mass spectrometry and theoretical calculations.
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For

M and D

Dad and Su

Adam

My Grandparents, Francis and Elizabeth
An honest man, armed with all the knowledge available to us now, could only state that in some sense, the origin of life appears at the moment to be almost a miracle, so many are the conditions which would have had to have been satisfied to get it going. But this should not be taken to imply that there are good reasons to believe that it could not have started on the earth by a perfectly reasonable sequence of fairly ordinary chemical reactions. The plain fact is that the time available was too long, the many microenvironments on the earth's surface too diverse, the various chemical possibilities too numerous and our own knowledge and imagination too feeble to allow us to be able to unravel exactly how it might or might not have happened such a long time ago, especially as we have no experimental evidence from that era to check our ideas against.

-- Francis Crick, 1981

I don’t believe in the no-win scenario.

-- James T. Kirk, 2285
Chapter 1: Introduction

Early Earth and Prebiotic Chemistry. Earth formed ~4.6 billion years ago through the accretion of gas and dust swirling around the newly formed sun. Earth accreted in the habitable zone, which allowed for suitable conditions for the eventual formation of life. The transition from the prebiotic chemical reactions on early Earth to the complex biochemical reactions driven by life is currently unknown in large part because the conditions of Earth during its first 500 million years are unknown. In 1952, Stanley Miller, then a graduate student under Harold Urey, modeled the endogenous chemistry of early Earth by employing a spark discharge apparatus to simulate Earth’s early ocean-atmosphere system and the chemical reactions that occurred as an electrical discharge (simulating lightning) entered this system. These early simulations not only produced complex mixtures of abiotic organic compounds but they also produced α-amino acids, the monomers of proteins. Miller’s original reducing atmosphere used in the spark discharge experiments has since been criticized for not representing the current view of the early Earth atmosphere, which might have been dominated by nitrogen and carbon dioxide, with only relatively small concentrations of reduced gases (H₂, CH₄, etc.). Nevertheless, it has been demonstrated that an electric discharge through a neutral atmosphere (CO₂ and N₂) can also produce organic compounds, albeit in much lower abundances.

Spark discharge experiments with neutral atmospheres have been known to produce small amounts (<0.3%) of hydrogen cyanide (HCN). HCN is an important precursor molecule in prebiotic chemistry, including the synthesis of α-amino acids in spark discharge experiments (via a variation of the Strecker-cyanohydrin reaction). If spark
discharge experiments with neutral atmospheres serve as good models of prebiotic chemistry on the early Earth, then biologically relevant molecules (such as \( \alpha \)-amino acids) may have been produced in low abundances, which may have been a problem for the origin(s) of life. Alternatively, HCN can also polymerize spontaneously in basic solution, the important end product being nucleobases (the informational molecules found in the monomers of DNA and RNA); although, high HCN concentrations are required for polymerization or a concentration mechanism (like eutectic freezing) must be invoked.\(^{9-11}\)

**Meteorites.** An exogenous source of organic molecules may also have been important in facilitating prebiotic reactions on the early Earth. These extraterrestrial sources could have come from meteorites (asteroidal or planetary), comets, and interplanetary dust particles. Among these sources, meteorites are the most accessible of extraterrestrial materials that can be studied in the laboratory. Carbonaceous chondrites are one class of meteorite and represent some of the most ancient material in the Solar System. The total organic carbon content of carbonaceous meteorites is approximately 2\% by weight and 1-30\% of this organic carbon is in a soluble form, which is extremely complex and diverse.\(^{12}\) Some of these organic compounds could be synthesized (or transformed) during a period of preterrestrial aqueous alteration on the meteorite parent body (i.e. the asteroid).\(^{13}\)

**Extraterrestrial Cyanide.** Biologically important monomers, such as nucleobases and \( \alpha \)-amino acids have been identified in the soluble organic portion of carbonaceous chondrites\(^{14-18}\) and HCN is the presumed precursor molecule in the synthesis of these classes of compounds. Chapter 2 details our search for cyanide in meteorites (assuming
that some leftover cyanide remained, which was locked up in a polymeric or other form). In the middle of our investigation, Pizzarello reported the first measurement of cyanide (measured as HCN) in a meteorite.\textsuperscript{19} Pizzarello determined that the acid-treated hot water extract of the Murchison meteorite released \( \sim 400 \) nmol CN/g meteorite. This result was somewhat surprising. The fact that Pizzarello was able to extract cyanide with hot water (100 °C for 24 hours) revealed two important things. First, cyanide is accessible to aqueous fluids, \textit{i.e.} cyanide is not sequestered in an acid-digestible mineral phase. Second, cyanide should hydrolyze extensively under Pizzarello’s initial extraction conditions;\textsuperscript{11,19} thus, Pizzarello’s detection of HCN is peculiar. For these reasons, we have analyzed many different meteorites, including different groups, and expanded our study to further investigate potential sources of cyanide. Finally, if meteorites contained large abundances of cyanide, then meteorites during the Late Heavy Bombardment may have delivered essential cyanide compounds that would be available for prebiotic chemistry in addition to influencing planetary atmospheres and atmospheric chemistry. This work has been submitted to \textit{Proceedings of the National Academy of Sciences of the United States of America} and is currently undergoing changes for resubmission. The authors for the Chapter 2 manuscript are: Karen E. Smith, Michael P. Callahan, Jason P. Dworkin, and Christopher H. House.

\textbf{Metabolic Precursors in Carbonaceous Chondrites.} There are numerous studies investigating meteoritic \( \alpha \)-amino acids and nucleobases (see refs. 20-22 for review), which may be relevant to the modern genetic system. In contrast, few studies have investigated meteorites for molecules that appear in modern metabolic processes.\textsuperscript{23-26} Coenzymes are organic molecules that are essential for numerous enzyme-catalyzed
reactions in cellular metabolism. Many coenzymes have close structural similarities to nucleotides, which, in part, leads to the idea that coenzymes are also ancient and may have been an evolutionary predecessor of modern nucleic acids. Nicotinamide adenine dinucleotide (NAD) is a ubiquitous coenzyme that is involved in redox reactions in all cells. NAD contains two different nitrogen heterocycles, adenine, which has been measured in carbonaceous chondrites and nicotinamide. Nicotinamide has never been found in carbonaceous chondrites to date; however, other small molecules involved in metabolism such as pyruvic acid, oxaloacetic acid, citric acid, isocitric acid, and α-ketoglutaric acid (all members of the citric acid cycle) have been identified, which leads to the tantalizing suggestion that meteorites may have supplied the building blocks pertaining to metabolic processes. Chapter 3 discusses our search for nicotinamide and nicotinic acid, a precursor to nicotinamide, and other substituted pyridines in several CM2-type carbonaceous chondrites.

Nicotinic acid and its structural isomers have been previously measured in only two meteorites, Murchison (CM2) and Tagish Lake (C2 ungrouped), and the synthesis of these pyridine carboxylic acids has yet to be determined. The overall organic content of these two meteorites is dissimilar (which suggests multiple synthetic pathways on the meteorite parent body), yet, surprisingly, these two meteorites contain approximately the same abundances of pyridine monocarboxylic acids (which points to a single synthetic pathway). In addition to meteorite analyses, Chapter 3 also describes our laboratory studies of proton-irradiated pyridine in carbon dioxide-rich ices to serve as a model of interstellar ice chemistry, which may provide a plausible alternative explanation for the observations of pyridine carboxylic acids in dissimilar meteorites. This work is currently
under review at *Geochimica et Cosmochimica Acta*. The authors for the Chapter 3 manuscript are: Karen E. Smith, Michael P. Callahan, Perry A. Gerakines, Jason P. Dworkin, and Christopher H. House.

**The Reactivity of Nitrogen Heterocycles Under Prebiotic Conditions.** Examining the synthesis and inventory of small, biologically relevant molecules in carbonaceous meteorites is useful for understanding their availability on the early Earth; however, biomolecules, such as nucleic acids and proteins, are extremely large and complex in structure (and such molecules have not yet been found in meteorites). To elaborate further, a DNA/RNA monomer, or nucleotide, contains a nucleobase attached to a sugar-phosphate group via a N-glycosidic bond. While these nucleotide monomers are important for biology, life (as we know it) requires the polymerization of these monomers in order to form a fully functional DNA molecule, which consists of a double-stranded polymer of nucleotides strung together in a twisted helix. Despite the relative ease with which nucleobases, especially purines, have been synthesized under early Earth conditions,\(^9,10,28\) the synthesis of nucleotides has been notoriously difficult under plausibly prebiotic conditions.\(^{29,30}\) A possible first step in building a prebiotic nucleotide is to first understand the reactivity of nitrogen heterocycles under early Earth conditions. Chapter 4 details our investigation of the reactivity of select nitrogen heterocycles under the simulated complexity of early Earth using mixtures produced from a spark discharge apparatus. We also characterized products formed from the polymerization of hydroxymethylated pyrimidines. Products from these reactions may have been similar to those that led to the first biomolecules.
**Instrumentation.** This dissertation research relies heavily on liquid chromatography and mass spectrometry for sample analysis and a brief explanation of these instruments is given here (see also each chapter for more instrument and method details). We used high performance liquid chromatography (HPLC) and ultrahigh performance liquid chromatography (UHPLC) coupled to UV or fluorescence detection and mass spectrometry (*i.e.* triple quadrupole, time-of-flight, and linear ion trap-Orbitrap mass analyzers) to analyze meteorite and spark discharge reaction samples. These instruments are located in the Astrobiology Analytical Laboratory at NASA Goddard Space Flight Center. HPLC, which uses a chromatographic column and liquid mobile phase, was employed for this research to separate the components of these complex samples. On occasion, we used UHPLC, which is a variation of HPLC that uses <2 μm particle size columns with high pressure (15,000 psi) instrumentation that increases chromatographic resolution (the distance between two peak midpoints) and sensitivity. Once separated, the individual components could then be detected by UV, fluorescence, and/or mass spectrometry. We used UV or fluorescence detection because many of the compounds in our analyses were either UV active (chromophores) or fluorescent (fluorophores). Some added benefits of fluorescence detection are: the specificity of the excitation and emission characteristics and the increased sensitivity compared to UV detection. Additionally, a fluorescent tag can be attached to analytes of interest *via* chemical derivatization (as in the research described in Chapter 2). Finally, mass spectrometry is an analytical technique that measures the mass-to-charge ration of an ion. We used triple quadrupole and time-of-flight mass spectrometry for the detection, quantitation, and identification of compounds of interest. These types of mass spectrometers have been used extensively
and many reviews are available for in-depth descriptions (e.g., see refs. 31, 32). We also used Orbitrap mass spectrometry, which is particularly novel because this technique has not been used (to our knowledge) to analyze spark discharge reaction samples.

The Orbtirap is a relatively new type of high-resolution mass analyzer (which was first introduced in 2000)\textsuperscript{33} and consists of a spindle-like central electrode and a barrel-like outer electrode (altogether, about the size of a US quarter). The Orbitrap uses electrostatic fields to produce ion movement both in a circular path around the central electrode and an axial oscillation along the central electrode; the latter is used to calculate the mass-to-charge ratio of the ion. Two split halves of the outer electrode detect the image (transient) current produced by the axial oscillations of the ions, which is fast Fourier transformed to obtain the frequencies of these axial oscillations, and these frequencies are converted to the mass-to-charge ratios. The high-resolution achieved by the Orbitrap is due to its use of axial oscillation frequency, which is independent of the velocity or initial position of the ions. Mass resolution can be increased by increasing the detection time or the electrostatic field strength. In this dissertation, the Orbitrap analyzer played a prominent role in the targeted screening and quantitation of pyridine carboxylic acids in complex meteorite samples (Chapter 3) and identifying oligomers of hydroxymethyluracil and hydroxymethylcytosine using a combination of high-resolution accurate mass measurements and product ion spectra (Chapter 4).

REFERENCES

from Meteorites


Chapter 2: Extraterrestrial Cyanide in Carbonaceous Meteorites

Meteorite and other exogenous contributions become very important only if the earth had a neutral atmosphere.

-- Stanley Miller, 2003

ABSTRACT

Carbonaceous chondrites are meteorites that are known to contain a complex diversity of organic compounds including those relevant to modern biology. Cyanide is thought to be a crucial precursor molecule in the abiotic synthesis of meteoritic amino acids and nucleobases. In this chapter, we developed a method for the analysis of cyanide in acid-digested distillates of various meteorites by chemical derivatization and liquid chromatography (LC) with fluorescence detection and mass spectrometry. We find that cyanide abundance (~50 to 2500 nmol CN/g meteorite) in CM chondrites correlates with the degree of aqueous alteration that the meteorite parent body experienced, which suggests an extraterrestrial origin for cyanide. Potential sources for cyanide in CM chondrites are hydrogen cyanide polymer and metal-cyanide complexes. We identified ferricyanide in the Lewis Cliff 85311 meteorite using LC-UV detection and this compound is an unlikely terrestrial contaminant for an Antarctic meteorite. The formation of stable metal-cyanide complexes provides a plausible explanation for the survival of cyanide, which indicates that cyanide may not have been completely used up by chemical reactions occurring on the parent asteroid during the aqueous alteration phase. The exogenous delivery of cyanide to early Earth may have influenced prebiotic chemistry and the formation of numerous biologically relevant molecules.
INTRODUCTION

Meteorites are fragments of asteroids that have been colliding with Earth’s surface since its accretion ~4.6 billion years ago. Carbonaceous chondrites are a rare class of meteorite that constitute ~4% of all meteorite finds and contain some of the most ancient material in the Solar System. The total organic carbon content of carbonaceous meteorites is approximately 2% by weight, and 1-30% of this organic carbon is in a soluble form, which is extremely complex and diverse. The molecular complexity is best illustrated in studies using Fourier transform ion cyclotron resonance mass spectrometry analyzing solvent extracts of the Murchison and Maribo meteorites and the detection of tens of thousands of different molecular compositions. Organic analytes are typically found at the parts-per-million (ppm, or µg compound/g meteorite) level or lower and require both very sensitive and selective analytical methods for identification.

Two classes of compounds of biological importance, α-amino acids and nucleobases, have been identified in the soluble organic portion of carbonaceous chondrites. α-Amino acids are the monomers of protein and are produced abiotically on the asteroid likely via the Strecker-cyanohydrin synthesis involving aqueous aldehyde, hydrogen cyanide (HCN), and ammonia (NH₃). Nucleobases are nitrogen heterocyclic compounds that serve as the structural basis of information storage in RNA and DNA and are produced abiotically on the asteroid likely via aqueous reactions of ammonium cyanide (NH₄CN). Despite the implication of cyanide in these syntheses, it is surprising that a comprehensive investigation of cyanide in meteorites has not yet been carried out. Identifying and quantitatively measuring cyanide in carbonaceous chondrites will help link an essential precursor molecule to the known occurrence of meteoritic α-
amino acids and nucleobases and lead to a better understanding of meteorite parent body chemistry. Furthermore, cyanide is widely believed to have an important role in the origin of life on Earth; however, a major challenge for terrestrial prebiotic chemistry is the availability of cyanide. The exogenous delivery of cyanide via meteorites during the Late Heavy Bombardment could have supplemented endogenous cyanide production on early Earth and influenced prebiotic chemistry and the formation of numerous biologically relevant molecules.

There is a wide array of current analytical techniques for the identification of cyanide, some of which include: spectrophotometry/colorimetry, capillary electrophoresis with optical detection, electrochemical methods, mass spectrometry, and gas chromatography. Due to the complexity of meteorites, chromatographic techniques are usually necessary for small molecule analysis. Only one previous analytical study for the detection of cyanide in any meteorite has been reported. This study involved extracting the meteorite in hot water, acidifying the extract with phosphoric acid, and then sampling the headspace via a syringe for analysis by gas chromatography-mass spectrometry. Very few details regarding the analytical method were described in this study; for instance, recovery, quantitation, accuracy, limit of detection, and linearity were not stated. More importantly, only one meteorite, Murchison, was analyzed and the extraterrestrial or terrestrial origin of the cyanide extracted from this sample is unclear.

In this chapter, we present the detection and quantitation of cyanide in meteorites using a novel method that combines the modern analytical techniques of liquid chromatography coupled to fluorescence detection and time-of-flight mass spectrometry (LC-FD/ToF-MS) with the traditional sample preparation techniques of acid-digestion
and distillation via a gas-permeable membrane apparatus. We analyzed the following meteorites for cyanide: Allan Hills (ALH) 83100, Allan Hills (ALH) 84001, Graves Nunataks (GRA) 06100, Lewis Cliff (LEW) 85311, Lewis Cliff (LEW) 90500, Lonewolf Nunataks (LON) 94102, Murchison, Roberts Massif (RBT) 04133. All of these meteorites are carbonaceous chondrites with the exception of ALH 84001, which is a martian meteorite (see Table A-1 for petrographic classifications of each meteorite). To our knowledge, these meteorites, with the exception of Murchison, have never before been investigated for cyanide.

**EXPERIMENTAL SECTION**

**Materials.** Potassium cyanide (KCN) and methanol (Optima, 99.9%) were purchased from Fisher Scientific. Glycine, 2,3-naphthalenedicarboxaldehyde (NDA), sodium borate, sodium hydroxide (NaOH), sulfuric acid (95-98%), 3-cyanopyridine, methanol, sodium ferrocyanide [Na₄Fe(CN)₆], potassium ferricyanide [K₃Fe(CN)₆], and ammonium acetate were purchased from Sigma Aldrich. Acetonitrile (HPLC grade, 99.9%) was purchased from Acros. Ultrapure water (18.2 MΩ·cm, <5 parts-per-billion [ppb] total organic carbon) was used from a Millipore Direct Q3 UV system. Sodium borate, KCN, and serpentine were heated to 500 °C overnight to remove possible organic contaminants. KCN standards ranging in concentration from 0.01 µM to 2 µM were dissolved in 0.08 M NaOH.

**Sample Preparation, Acid-digestion, and Distillation.** All glassware and ceramics were rinsed with ultrapure water, wrapped in foil, and heated to 500 °C overnight in order to remove organic compounds. All meteorites were pulverized to a fine powder using a
mortar and pestle under a positive pressure High-Efficiency Particulate Air (HEPA) filtered laminar flow hood (Labconco). We acid-digested and distilled the following amounts of each meteorite: 204.3 mg ALH 83100, 203.6 mg ALH 84100, 206.7 mg GRA 06100, 202.8 mg LEW 85311, 209.3 mg LEW 90500, 201.1 mg LON 94102, 204.4 mg Murchison, and 207.7 mg RBT 04133. Control samples were run in parallel with the meteorites, which included reagent blanks, a serpentine blank (a hydrated magnesium silicate), and KCN standards (see Appendix A for more on method blanks). The powdered meteorite sample was placed into a MicroDIST (Lachat Instruments) sample tube of the distillation apparatus (see Figure 2-1) along with 5.8 mL ultrapure water. 750 µL of 9 M sulfuric acid (H₂SO₄) was added to the sample tube as a releasing agent for cyanide and 1.5 mL of 0.08 M NaOH was added to the top of the tube as a trapping solution for cyanide. The sample tube was separated from the NaOH trapping solution by a gas-permeable membrane within the tube. The distillation tube was sealed with a press (Lachat Instruments) and placed into a hot oil bath with a temperature range of 134-142 °C for 30 minutes. The final distillate volume in the top tube after distillation was approximately 2-3 mL. We tested two distillation apparatuses, a micro and a macro distillation apparatus and we chose the micro distillation apparatus (MicroDIST) because the final distillate volume was small enough to concentrate the sample 10 times more than the macro distillation apparatus (this was important considering the relatively low abundance of cyanide in meteorites). Additionally, the micro distillation apparatus is disposable, which reduces sample cross-contamination, is much simpler in design, and reduces material consumption (which decreases solvent volume and reduces cost).
Figure 2-1. Schematic (and accompanying photos) for the MicroDIST distillation apparatus used to acid-digest and distill cyanide from the meteorite and control samples.
**Derivatization.** Figure 2-2 shows the derivatization scheme for cyanide using NDA and glycine.\textsuperscript{16,17} The meteorite and control sample distillates were processed using the following optimized conditions: derivatization for 15 minutes at room temperature using a mixture of 50 µL 0.1 M glycine, 50 µL 10 mM sodium borate buffer (pH 9.1), 50 µL meteorite or control sample, and 50 µL 1 mM NDA (modified from ref 16, 17). Glycine and sodium borate buffer solutions were dissolved in ultrapure water, KCN standards were dissolved in 0.08 M NaOH, and NDA was dissolved in methanol. Glycine, KCN, and NDA standard solutions were made fresh daily from the powder.

**Cyanide Analysis.** Cyanide analysis was performed using liquid chromatography with fluorescence detection and time-of-flight mass spectrometry. A Waters LCT Premier time-of-flight mass spectrometer (ToF-MS) was equipped with an electrospray ionization source (ESI), which was operated in positive ion mode. ToF-MS settings were as follows: V mode (mass resolution ~ 5,000), ESI capillary voltage +3.5 kV, cone voltage 30 V, desolvation temperature 350 °C, and source temperature 120 °C. The N\textsubscript{2} cone and desolvation gases were flowed at 50 L/hr and 650 L/hr, respectively. The mass range was set to 50-500 m/z. Chromatographic separation was achieved using a Waters Acquity ultra performance liquid chromatograph (LC) with a prefilter and guard column followed by an Acquity BEH C18 2.1 × 50 mm column (1.7 µm particle size) and an Acquity BEH Phenyl 2.1 × 150 mm column (1.7 µm particle size) with a 25 µL injection volume. Samples were eluted using an isocratic flow of 35% 50 mM ammonium formate buffer with 8% methanol at pH 8 and 65% methanol at a flow rate of 150 µL/min and column temperature of 30 °C. The fluorescence excitation wavelength for the NDA-
Figure 2-2. Reaction scheme for the derivatization of cyanide via NDA and glycine.$^{16,17}$
cyanide derivative was 252 nm and the emission was monitored at 483 nm using a Waters Acquity fluorescence detector (FD).

**Extraction and analysis of LEW 85311 for Metal-Cyanide Complexes.** A powdered sample of LEW 85311 was extracted under the following procedure: 500 µL of 0.08 M NaOH was added to 266.8 mg of LEW 85311 in a 2.5-mL glass ampoule, flame-sealed, and put into an oven at 80 °C for 24 hours. A 0.05 mM [Fe(CN)$_6$]$^{3+}$ standard, a 0.05 mM [Fe(CN)$_6$]$^{4-}$ standard, and a 0.08 M NaOH method blank were run in parallel with the meteorite extract. A spike experiment was also performed, which consisted of 100 µL 0.5 mM [Fe(CN)$_6$]$^{3+}$ standard added to 100 µL LEW 85311 extract. The standards, blank, spiked meteorite extract, and meteorite extract were purified using Waters Oasis WCX solid phase extraction (SPE) cartridges. The samples were loaded onto the SPE cartridges and the iron-cyanide complexes were eluted with 0.08 M NaOH. Samples were then analyzed using liquid chromatography with UV detection. Chromatographic separation was achieved using a Waters 2695 high performance liquid chromatograph (HPLC) with a SIELC Primesep B2 2.1 × 150 mm reverse-phase/anion-exchange column with a 5 µm particle size and a 10 µL injection volume. Mobile phase (A) consisted of 100 mM ammonium acetate (pH 4), mobile phase (B) was 100 mM ammonium acetate (pH 9), mobile phase (C) was acetonitrile, and mobile phase (D) was ultrapure water. Samples were eluted at 300 µL/min using the following gradient: 30% (C) and 50% (D), which were held isocratic for the entire run, 0-5 min. 20% (A), 5-15 min. 20-0% (A), 15-20 min. 0-20% (A) with a column temperature of 40 °C. The UV detection was monitored at 230-400 nm using a Waters 2996 photodiode array detector.
RESULTS AND DISCUSSION

Measurement of Cyanide in Standard Solutions. We performed a seven-point linearity study using non-distilled solutions of KCN with a concentration range of 0.01 \( \mu \text{M} \) to 2 \( \mu \text{M} \) (or 60 fmol to 12 pmol on column). The curve was highly linear for fluorescence \( (R^2 = 0.9997) \) and for single ion mass detection at the theoretical protonated mass of 251.08 ± 0.03 Da \( (R^2 = 0.9989) \). The limit of detection was 0.1 nmol CN/g meteorite (60 fmol on column) with a signal-to-noise-ratio of 3:1. The 0.5 \( \mu \text{M} \) KCN standard was processed through the entire method from acid-digestion and distillation to derivatization and analyzed using LC-FD/ToF-MS in order to determine recovery. The average recovery for cyanide was 99% based on eight measurements with mass and fluorescence detection. We determined that the NDA-cyanide derivative showed <1% degradation after 2.25 hours and <15% degradation after 17.5 hours at room temperature. Thus, all meteorite samples were derivatized for 15 minutes and analyzed immediately.

Measurement of Cyanide in CM Carbonaceous Chondrites. CM chondrites are rich in organic compounds, which include the likely products of cyanide chemistry (e.g. \( \alpha \)-amino acids and nucleobases). We applied our method to five CM carbonaceous chondrites. Figure 2-3 shows the fluorescence and mass chromatograms of the NDA-cyanide derivative of cyanide extracted from LEW 90500. These data are representative of our results from the other four CM carbonaceous chondrites. Cyanide identification in the meteorite was made by comparison of the fluorescence and single ion mass peak \( (m/z 251.08 \pm 0.03, \text{which corresponds to the protonated molecular ion}) \) retention times to standards. Cyanide concentration was calculated by comparing the fluorescence and mass peak areas of the sample to our standard concentration curve. Additionally,
Figure 2-3. Fluorescence ($\lambda_{em} = 483$ nm) and mass chromatograms ($m/z$ 251.08 with a ±0.03 window, which corresponds to the protonated molecular ion) of the NDA-cyanide derivative from the control blank (bottom traces), KCN standard (middle traces) and the LEW 90500 meteorite (top traces). Identification was made by matching the retention time of the standard to that of the meteorite using both fluorescence and mass detection.
meteorite cyanide abundances were not corrected for recovery based on nearly 100% recovery of the standards, which indicated that there was no loss of cyanide during the acid-digestion and distillation processes. Figure 2-4 shows that all five of the CM carbonaceous chondrites in this study contain cyanide (see Table A-1 for abundances). LEW 85311 is the most abundant in cyanide (2472.1 ± 37.6 nmol CN/g meteorite), while ALH 83100 has the least amount of cyanide (49.8 ± 0.4 nmol CN/g meteorite). The cyanide abundance for Murchison in our study (94.8 ± 0.68 nmol CN/g meteorite) was lower than the abundance (~400 nmol CN/g meteorite) reported in a study of Murchison by Pizzarello. The difference in cyanide abundance in Murchison between the two studies may be due to heterogeneity in Murchison and/or differences in the analytical methods.

There is strong evidence that carbonaceous chondrites have been altered by the action of liquid water, which was derived from the melting of ice inside the asteroid by radioactive heating from short-lived radionuclides (e.g., 26Al). The degree of aqueous alteration in our five CM chondrites was estimated using previously published data of bulk hydrogen abundance, isotopic composition, and the C/H ratio (which also correlates with petrologic indicators of the extent of aqueous alteration). Figure 2-4 shows the trend of decreasing cyanide abundance with increasing aqueous alteration of the meteorite. The correlation between cyanide abundances and the aqueous alteration of the meteorites (a characteristic directly related to the asteroid) suggests that cyanide was indigenous to the meteorite parent body. In addition, lower abundances of meteoritic purines (guanine, hypoxanthine, xanthine, and adenine) in the same CM chondrites (as reported in Callahan et al. and Appendix A) also correlate with the aqueous alteration of...
Figure 2-4. Abundances of cyanide and total purines (guanine, hypoxanthine, xanthine, and adenine) in five CM carbonaceous chondrites determined by mass and fluorescence detection. Carbonaceous chondrites are ordered from right to left in order of increasing aqueous alteration. The error was calculated as the standard error of the mean from four measurements. Total purine data was taken from Callahan et al., with the exception of LEW 85311 (see Appendix A for the extraction of purines from LEW 85311).
the meteorites. The detection of cyanide in CM chondrites indicates that cyanide was possibly trapped in some stable form and not completely used up by chemical reactions that occurred during the aqueous alteration phase on the parent asteroid. The concentration of cyanide in meteorites is significantly higher (~500x) than the concentration of total purines, which demonstrates that the precursor was likely more readily available on early Earth compared to complex organic compounds.

Measurement of Cyanide in CR and Martian Meteorites. We applied our method to two CR2 carbonaceous chondrites, RBT 04133 and GRA 06100 and one martian meteorite, ALH 84001. CR carbonaceous chondrites are metal-rich meteorites, some of which are known to contain high abundances of amino acids (~200 ppm)\textsuperscript{20} and \(\text{NH}_3\) (~300 ppm)\textsuperscript{21} and low abundances of nucleobases (~40 ppb).\textsuperscript{5} Cyanide was not detected above our detection limit (0.1 nmol CN/g meteorite) in RBT 04133 and GRA 06100. It is possible that interferences, which hindered the acid-digestion and distillation and/or derivatization processes, contributed to the non-detection; therefore, we also performed a spike experiment. We spiked 202.3 mg of RBT 04133 with 500 \(\mu\)L of 2 \(\mu\)M KCN, and processed this sample using the entire meteorite work-up procedure. This experiment resulted in a 27% recovery of the spike using the mass and fluorescence data. This result demonstrated that if RBT 04133 had contained cyanide at a similar level to the CM carbonaceous chondrite with the lowest abundance of cyanide (ALH 83100 contained 49.8 ± 0.4 nmol CN/g meteorite), the cyanide concentration would have been above our limit of detection, despite possible interferences and low spike recovery.

It is also possible that RBT 04133 and GRA 06100 may not be representative of typical CR2 meteorites. For instance, it has been reported that the properties of RBT
04133 suggest that it might be a mildly thermally altered CV3 chondrite.\textsuperscript{22,23} CV chondrites are thermally altered meteorites with a much lower organic compound content.\textsuperscript{24-26} Additionally, Abreu \textit{et al.}\textsuperscript{27,28} suggested that the CR parent body of the meteorite GRA 06100 may have had significant thermal alteration in addition to aqueous alteration. Therefore, it is reasonable that thermal degradation of cyanide on the meteorite parent body was the cause for the lack of cyanide detection in these two meteorites.

Martian meteorites are igneous rocks that have been ejected from the surface of Mars by impacts. ALH 84001, a martian orthopyroxenite, is one of the most well known meteorites because it was reported to contain carbonate deposits, hydrocarbons, and structures resembling fossil bacteria;\textsuperscript{29} all of which could be considered signs of martian life; however, the evidence for martian life in ALH 84001 has been hotly debated.\textsuperscript{30-33} In the search for signatures of martian life or martian prebiotic chemistry, cyanide would be an excellent target because it plays a critical role in the abiotic synthesis of amino acids and nucleobases. Cyanide was not detected above our detection limit (0.1 nmol CN/g meteorite) in ALH 84001.

\textbf{Potential Sources for Cyanide in the Meteorites.} Cyanide can be found in nature, but most terrestrial sources come from industry and are mainly in the forms of free cyanide (HCN, CN\textsuperscript{-}), organocyanides (\textit{e.g.} CH\textsubscript{3}CN), and metal-cyanide complexes (\textit{e.g.} [Ni(CN)\textsubscript{4}]\textsuperscript{2-}).\textsuperscript{34} Our method of acid-digestion followed by distillation has been extensively used in environmental chemistry to liberate the free cyanide anion from metal-cyanide complexes (organocyanides do not typically contribute to the total cyanide content).\textsuperscript{34,35} In meteorites, cyanide may also be present in polymeric form. This notion
is supported by the identification of products of HCN polymer in other extraterrestrial environments such as dust particles from the Comet 81P/Wild 2.\textsuperscript{36} To investigate whether or not HCN polymer could release cyanide by our extraction method, we performed our acid-digestion, distillation, derivatization, and analysis on hydrogen cyanide polymer that we synthesized in the laboratory (see Appendix A). The HCN polymer was analyzed for free cyanide anion before acid-digestion and distillation. The total cyanide concentration in the HCN polymer solution increased nearly 100-fold after distillation (from 0.8 nmol CN before to 79 nmol CN after distillation) indicating that cyanide can be released from HCN polymer by this method and that HCN polymer may be a source for the detected cyanide in CM chondrites.

The insoluble organic matter (IOM) is another potential source of cyanide in carbonaceous chondrites because it is known to contain nitriles;\textsuperscript{37} however, the cyanide detected in our experiments is unlikely to come from IOM. The latter statement is supported by the fact that a similar concentration of (released) cyanide was detected in hot water extracts of the Murchison meteorite,\textsuperscript{15} and IOM would not be a contributing factor in the water-soluble portion.

Carbonaceous chondrites are known to contain Fe- and Mg-bearing minerals;\textsuperscript{38} therefore, it is possible that metal cations were available to form complexes with free cyanide on the meteorite parent body. Thus, we conducted a preliminary investigation that targeted \([\text{Fe(CN)}_6]^{4-}\) and \([\text{Fe(CN)}_6]^{3-}\) in LEW 85311, the CM2 chondrite that contained the most cyanide. Figure 2-5 shows the UV chromatograms recorded at 302 nm for the 0.08 M NaOH blank (red dash trace), LEW 85311 meteorite (bold blue trace), and LEW 85311 meteorite spiked with \([\text{Fe(CN)}_6]^{3-}\) (black trace). The UV
Figure 2-5. The UV chromatograms recorded at 302 nm for the 0.08 M NaOH blank (red dash trace), the LEW 85311 meteorite (bold blue trace), and the LEW 85311 meteorite spiked with 0.25 mM [Fe(CN)₆]³⁻ (black trace). The chromatogram for LEW 85311 (bold blue trace) was scaled by 0.5 to account for dilution that occurred in the 1:1 [Fe(CN)₆]³⁻ spiked meteorite sample. The retention time of [Fe(CN)₆]³⁻ is 3.5 min.
chromatograms for both meteorite extracts contained several broad peaks and a strong UV-absorbing background. For LEW 85311 spiked with $\text{[Fe(CN)₆]}^{3-}$, the UV peak at 3.5 minutes was noticeably larger in the spiked sample compared with the non-spiked meteorite sample and matched the chromatographic retention time of the $\text{[Fe(CN)₆]}^{3-}$ standard. In addition, the predicted peak height for the combined $\text{[Fe(CN)₆]}^{3-}$ standard and the non-spiked meteorite sample matched that of the spiked meteorite sample. Based on our results, we assign $\text{[Fe(CN)₆]}^{3-}$ as a candidate for releasing cyanide from meteorites (by our extraction techniques); however, a second characterization technique will be needed for unambiguous identification. Metal-cyanide complexes on Earth are typically from anthropogenic sources (such as industrial sites). Thus, $\text{[Fe(CN)₆]}^{3-}$ detected in LEW 85311 is most likely extraterrestrial because this meteorite was recovered in Antarctica and not likely exposed to industrial cyanide contaminants.

The formation of stable metal-cyanide complexes provides a plausible explanation for the survival of cyanide despite the aqueous alteration processes that occurred on the parent asteroid. In particular, iron cyanide complexes are strong complexes (dissociate at pH < 2), which means they would likely be stable under neutral to alkaline conditions during the aqueous alteration phase. The exogenous delivery of abundant cyanide (which could be released as HCN upon UV irradiation of the metal-cyanide complex) from CM chondrites, along with abundant NH₃ from CR chondrites, may have played a significant role in the prebiotic chemistry leading to the origin of life on Earth (and potentially elsewhere).

**Implications for cyanide in meteorites.** The high abundance of cyanide in CM carbonaceous chondrites indicates that meteoritic cyanide might have been available on
early Earth shortly after accretion because of the heavy bombardment of the Earth’s surface by asteroids. The most primitive (i.e. less aqueously altered) CM2 chondrites may have been particularly important because they contained the highest abundance of cyanide. This extraterrestrial delivery of cyanide may be significant for the origin of life on Earth (and elsewhere). On the early Earth, a weakly reducing or neutral atmosphere was likely present\textsuperscript{40}, which is known to significantly decrease the abundance of synthesized organic molecules in laboratory simulations\textsuperscript{41}. Thus, exogenous cyanide delivery \textit{via} meteorites may have supplemented potentially lower cyanide abundance produced on the early Earth\textsuperscript{42,43} and facilitated the production of critical molecules necessary for life (as we know it). In our future work, we will measure the carbon and nitrogen isotope ratios of cyanide, analyze additional CR chondrites (those without thermal alteration), and investigate meteorites for the presence of additional metal-cyanide complexes to better understand the preservation of cyanide in meteorites.

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ASSOCIATED CONTENT


REFERENCES


Chapter 3: Investigation of Pyridine Carboxylic Acids in CM2 Carbonaceous Chondrites: Potential Precursor Molecules for Ancient Metabolic Pathways

ABSTRACT

The distribution and abundances of pyridine carboxylic acids (including nicotinic acid) and nicotinamide in eight CM2 carbonaceous chondrites (ALH 85013, DOM 03183, DOM 08003, EET 96016, LAP 02333, LAP 02336, LEW 85311, and WIS 91600) were investigated by liquid chromatography coupled to UV detection and high resolution Orbitrap mass spectrometry. Nicotinamide was not identified in any of the 24 different meteorite extracts we analyzed. We find that pyridine carboxylic acids are prevalent in CM2-type chondrites and these compounds correlate with the degree of preterrestrial aqueous alteration that the meteorite parent body experienced. Additionally, we carried out laboratory studies of proton-irradiated pyridine in carbon dioxide-rich ices to serve as a model of the interstellar ice chemistry that may have led to the synthesis of pyridine carboxylic acids. Analysis of the irradiated ice residue shows that a comparable suite of pyridine mono- and dicarboxylic acids was produced, although aqueous alteration may still play a role in the synthesis (and ultimate yield) of these compounds. Nicotinic acid is a precursor to nicotinamide adenine dinucleotide, a ubiquitous and likely ancient molecule used in cellular metabolism in all of life, and its common occurrence in CM2 chondrites may indicate that meteorites may have been an additional source of molecules for the emergence of metabolic pathways on the early Earth.
INTRODUCTION

Nicotinamide adenine dinucleotide (NAD) is one of the most ancient molecules in modern cells and was likely present very early in the evolution of life. There are two ways in which modern cells can synthesize NAD: the \textit{de novo} pathway or the Preiss-Handler salvage pathway. Both pathways involve formation of a nicotinic acid mononucleotide (NaMN) intermediate, which gets converted to NAD through the use of enzymes; however, only the salvage pathway uses existing material (\textit{i.e.} nicotinic acid) as a substrate. The first organisms on early Earth were thought to be anaerobic, so its possible that the first pathway to NAD was either an anaerobic \textit{de novo} pathway or a salvage pathway in which an abiotic synthesis of nicotinic acid would be required. Abiotic nicotinic acid may have been synthesized on early Earth (likely in a nonenzymatic manner) and/or delivered \textit{via} extraterrestrial materials. Understanding the source of the metabolic precursors of NAD could help elucidate the origin of an important and ancient metabolic pathway.

There have been laboratory studies to investigate the endogenous formation of pyridine carboxylic acids under plausibly prebiotic conditions of early Earth. Friedmann \textit{et al.}\cite{friedmann2015} demonstrated that the pyridine ring could be synthesized in the form of nicotinonitrile (3-cyanopyridine) by sending an electrical discharge through a mixture of ethylene and ammonia. Nicotinonitrile is an important molecule because it can be converted to nicotinamide (Figure 3-1), a principal molecule in NAD, and nicotinic acid by hydrolysis of the cyano group (Figure B-1). In addition, Cleaves and Miller\cite{cleaves2005} demonstrated a possible nonenzymatic early Earth synthesis of nicotinic acid and its metabolic precursor, quinolinic acid (a pyridine dicarboxylic acid), by reacting
Figure 3-1. Structures of the three pyridine monocarboxylic acid isomers and nicotinamide.
dihydroxyacetone phosphate with aspartic acid.

Another possible source of pyridine carboxylic acids could have come from the exogenous delivery to early Earth by meteorites shortly after Earth’s accretion. Carbonaceous chondrites are meteorites that contain approximately 2% carbon by weight, and approximately 1-30% of this carbon is in a soluble form.\textsuperscript{9} The organic compounds contained in these chondrites are likely formed either by aqueous reactions on the meteorite parent body or in the interstellar medium (on energetically-processed, icy-organic dust grains) and later incorporated into the meteorite parent body.\textsuperscript{10,11} The soluble organic content of carbonaceous chondrites is highly complex and is known to contain biologically relevant molecules such as the building blocks of proteins (\textit{i.e.} amino acids),\textsuperscript{12-15} nucleic acids and coenzymes (\textit{e.g.}, nucleobases),\textsuperscript{16-19} and those pertaining to metabolic processes.\textsuperscript{20-22}

Figure 3-1 shows nicotinic acid (3-pyridinecarboxylic acid) and its structural isomers, picolinic acid (2-pyridinecarboxylic acid) and isonicotinic acid (4-pyridinecarboxylic acid), which have been identified in only a few carbonaceous chondrites to date, such as Murchison, a CM2 chondrite, and Tagish Lake, a C2 ungrouped chondrite.\textsuperscript{20,21,23} The compound-specific isotope ratio values for nicotinic acid were $\delta D = 129\%$ and $\delta^{13}C = 20\%$ in the Murchison meteorite, which strongly suggests that these compounds are extraterrestrial in origin.\textsuperscript{21,23} In addition, the presence of nicotinic acid, its two structural isomers, as well as several other methyl and dimethyl homologs of pyridine carboxylic acids, also points to an abiotic and extraterrestrial origin for these molecules because the observation of extensive structural diversity of organic compounds in meteorites usually is indicative of an extraterrestrial origin.\textsuperscript{9} The synthesis of these pyridine carboxylic
acids has yet to be determined because the Murchison and Tagish Lake meteorites are carbonaceous chondrites with dissimilar organic content, yet they contain approximately the same abundances of pyridine monocarboxylic acids. The prevalence of nicotinic acid and its isomers in carbonaceous chondrites is unknown; therefore, we devised a method to detect these compounds in several meteorites of one class.

In this chapter, we report the detection and quantitation of meteoritic nicotinic acid and its structural isomers, picolinic acid and isonicotinic acid. We analyzed extracts from the following eight meteorites for pyridine carboxylic acids and nicotinamide by liquid chromatography coupled to UV detection and high resolution Orbitrap mass spectrometry (LC-UV/MS): Allan Hills (ALH) 85013, Dominion Range (DOM) 03183, Dominion Range (DOM) 08003, Elephant Moraine (EET) 96016, LaPaz Ice Field (LAP) 02333, LaPaz Ice Field (LAP) 02336, Lewis Cliff (LEW) 85311, and Wisconsin Range (WIS) 91600. All of these meteorites are CM2 type carbonaceous chondrites, which typically contain the most diverse organic compounds including nitrogen heterocycles. To our knowledge, these meteorites have never before been investigated for pyridine carboxylic acids or nicotinamide. In addition, we carried out experiments simulating cosmic ray processing of mixed-molecular ices, which may take place in dense interstellar clouds. From these results, we propose a formation mechanism for these meteoritic compounds.

**EXPERIMENTAL SECTION**

**Chemicals and reagents.** All glassware and ceramics were rinsed with ultrapure water (18.2 MΩ·cm, <5 parts-per-billion total organic carbon from a Millipore Milli-Q
Integral 10 system), wrapped in aluminum foil, and heated to 500 °C overnight. Ultrapure water was used exclusively for this study. Pyridine mono- and dicarboxylic acid standards (minimum 97% purity) were purchased from Sigma-Aldrich and nicotinamide was purchased from ICN Biomedicals, Inc. Acetonitrile (99.5+%) and sulfuric acid (95-98% purity) were purchased from Sigma Aldrich, and methanol (Optima® grade) was purchased from Fisher Scientific. A standard solution of mono- and dicarboxylic acids and nicotinamide was prepared by first making individual standards in water, then mixing those standards into one solution ranging from 1-100 µM. For liquid chromatographic separation, a 0.05% by volume sulfuric acid solution was prepared. A solution of 6 M hydrochloric acid (HCl) for the acid hydrolysis extraction was double vacuum distilled.

**Meteorites.** The following Antarctic CM2 carbonaceous chondrites analyzed in this study were interior fragments selected by the meteorite sample curator at the NASA Johnson Space Center (JSC): ALH 85013 (specific 65, parent 23, mass 1.134 g), DOM 03183 (specific 25, parent 15, mass 1.196 g), DOM 08003 (specific 14, parent 9, mass 1.126 g), EET 96016 (specific 16, parent 0, mass 1.116 g), LAP 02333 (specific 24, parent 16, mass 1.061 g), LAP 02336 (specific 11, parent 3, mass 1.078 g), LEW 85311 (specific 74, parent 24, mass 1.138 g), and WIS 91600 (specific 61, parent 59, mass 1.069 g). Individual meteorite chips, free of fusion crust, were pulverized into a fine powder using a ceramic mortar and pestle under a positive pressure High-Efficiency Particulate Air (HEPA) filtered Class 100 laminar flow hood (Labconco). The control blank for this study was crushed serpentine (a hydrated magnesium silicate mineral) heated to 500 °C for 3 hours, which was processed using identical protocols.
**Hot water and acid-hydrolyzed hot water extractions.** For the extraction of pyridine carboxylic acids, samples of ALH 85013 (105.9 mg), DOM 03183 (108.7 mg), DOM 08003 (98.1 mg), EET 96016 (110.0 mg), LAP 02333 (96.8 mg), LAP 02336 (99.3 mg), LEW 85311 (100.2 mg), and WIS 91600 (100.0 mg) were transferred to individual glass ampoules and flame-sealed with 1 mL water each and heated to 100 °C for 24 hours. The hot water extracts were then centrifuged and the supernatant was split into two 500 µL portions. One 500 µL portion of the supernatant was transferred to a 10 mm glass test tube, dried under vacuum and placed into a larger 20 mm test tube. The test tube was flame-sealed and the hot water extracted dried residue was acid-hydrolyzed using 6 M HCl vapor at 150 °C for 3 hours in order to liberate any pyridine carboxylic acids that might have been in an acid-labile “bound” form, which is typical for some meteoritic organic compounds. The acid-hydrolyzed hot water extracts were dried under vacuum then re-dissolved in 1 mL of water. The hot water and acid-hydrolyzed hot water meteorite extracts were desalted using conditioned cation exchange columns (Biorad Poly-Prep® AG® 50W-X8 resin, 100-200 mesh, hydrogen form). Meteorite extracts were loaded onto the columns, rinsed with water, and the pyridine carboxylic acids were eluted with 2 M NH₄OH. The desalted extract was then dried down under vacuum and re-dissolved with 100 µL water. Nicotinamide was also a target compound in these extracts. All meteorite extracts were stored in a -86 °C freezer until analysis.

**Formic acid extractions.** Samples of ALH85013 (206.9 mg), DOM03183 (204.8 mg), DOM08003 (204.6 mg), EET96016 (214.5 mg), LAP02333 (215.7 mg), LAP02336 (211.1 mg), LEW85311 (206.6 mg), and WIS91600 (211.9 mg) were transferred to glass ampoules and flame-sealed with 2 mL formic acid and heated to 100 °C for 24 hours.
The ampoules were frozen in liquid nitrogen before opening, centrifuged, and the supernatant transferred to a test tube. The meteorite powder was rinsed with 1 mL formic acid; the supernatants were combined and then dried under vacuum. After the residue was dry, it was dissolved in 2 mL 0.1 M ammonium hydroxide. Meteorite extracts were loaded onto a conditioned Waters solid phase extraction (SPE) cartridge (Oasis Max 6 cc, 150 mg sorbent) and rinsed with 6 mL 5% ammonia in water followed by 6 mL methanol. Pyridine monocarboxylic acids were then eluted using 6 mL 5% formic acid in methanol. The purified extracts were dried under vacuum and dissolved in 400 µL 0.1 M ammonium hydroxide. Nicotinamide was also a target compound in these extracts. All meteorite extracts were stored in a -86 °C freezer until analysis.

**Irradiated pyridine + CO2 ice experiments.** Proton irradiation experiments were performed in the Cosmic Ice Laboratory at the NASA Goddard Space Flight Center. The system consisted of a stainless steel high vacuum chamber (P ~ 10\(^{-7}\) torr) containing a polished aluminum substrate attached to the cold finger of a closed-cycle helium cryostat (ARS model DE-204). This rotatable substrate can face a deposition tube, a beam of 0.8-MeV protons from a Van de Graaff accelerator, or an FTIR spectrometer.\(^{27,28}\) Gases (CO\(_2\) and pyridine vapor) were mixed in a separate gas manifold, and ice films were produced by leaking them into the vacuum chamber in front of the cold substrate over the course of ~15 minutes. Final sample thicknesses were ~1 µm, as measured by laser interferometry. This thickness is well below the stopping range for 0.8-MeV protons and ensures that the entire ice sample is processed. Contaminants (mainly H\(_2\)O) made up no more than 1% of the final ice, based on our estimates of the background gases present and the rate at which the sample was grown. After deposition, the pyridine + CO\(_2\) (1:1)
ice was exposed to a 0.8-MeV proton beam with a current of 150 nA until a fluence of $1.2 \times 10^{15}$ protons·cm$^{-2}$ was achieved. The average energy dose (energy absorbed) was 36 eV per initial molecule deposited, which was determined using a proton stopping power for the pyridine + CO$_2$ mixture of $2.73 \times 10^8$ eV cm$^2$·g$^{-1}$·proton$^{-1}$. This value was calculated using the SRIM software package,$^{29}$ assuming a density for the pyridine + CO$_2$ mixture of 1 g·cm$^{-3}$.

To aid in the interpretation of the IR spectra of the irradiated ice, we also measured the IR spectra of the possible irradiation products nicotinic acid, isonicotinic acid, and picolinic acid at ~20 K. Since these three substances are supplied as powders, a sublimation oven inside the vacuum chamber (described by Gerakines et al.$^{30}$) was used in each case to vaporize the chosen compound and produce an ~1 µm thick film on the cold substrate whose IR spectrum could then be measured.

After irradiation, the ice was allowed to warm up slowly to room temperature overnight, allowing volatile products to be pumped away. The remaining non-volatile (refractory) material was removed by repeatedly pipeting 250 µL water on the aluminum substrate until the yellow-colored residue was observed to be completely removed. The refractory solution was analyzed immediately by LC-UV/MS.

**Sample Analysis by LC-UV/MS.** All samples and controls were analyzed using a Thermo Scientific Accela high performance liquid chromatograph coupled to a Thermo Scientific Accela photodiode array detector (PDA) and a Thermo Scientific LTQ Orbitrap XL hybrid mass spectrometer. Separation of pyridine carboxylic acids was accomplished by injecting 5 µL sample solution onto a SIELC Primesep 100, 150 × 2.1 mm column (5 µm particle size) with a Primesep 100, 10 × 2.1 mm guard column (5 µm
particle size). Mobile phase (A) consisted of water with 0.05% sulfuric acid and mobile phase (B) was 100% acetonitrile. Samples were eluted at 200 µL/min using the following gradient: 0-3 min. 100% A, 3-10 min. 100-95% A, 10-20 min. 95% A, and 20-30 min. 100% A. The full UV spectrum (200-400 nm) was collected using the Accela PDA. UV (λ = 260 nm) retention time was used as additional confirmation for identification and not for quantitation due to high UV-absorbing background and complexity.

The Thermo Scientific LTQ Orbitrap XL hybrid mass spectrometer was equipped with an electrospray ionization (ESI) source and operated in positive ion mode. Parameters for ESI were as follows: the nitrogen gas for desolvation of the electrospray was set to 35 for the sheath gas, 15 for the auxiliary gas, and 1 for the sweep gas, all in arbitrary units. The ion transfer capillary voltage and temperature were 21 V and 275 °C, respectively. The tube lens was set to 55 V. Full scan mass spectra were taken over a range of m/z 50 to 500. The mass resolution was set to 30,000 (at full-width-half-maximum for m/z 400) in order to maintain an appropriate number of data points across chromatographic peaks. MS/MS data was collected in the Orbitrap mass analyzer (HCD energy 60% and 100% and isolation width of 1 Da). External calibration was performed using a mixture of caffeine, MRFA (L-methionyl-arginyl-phenylalanyl-alanine acetate hydrate) peptide, and Ultramark 1621 in an acetonitrile-methanol-water solution containing 1% acetic acid, which resulted in a typical mass accuracy of <2 parts-per-million (ppm).

Abundances were calculated only for pyridine monocarboxylic acids. Very low (or zero) recoveries of pyridine dicarboxylic acids (due to the desalting and SPE procedures
employed here) prevented accurate measurements of abundances and were not reported here. In addition, 2,6-pyridinedicarboxylic acid exhibited poor ESI efficiency, which resulted in poor sensitivity of this compound.

RESULTS AND DISCUSSION

Measurements of pyridine carboxylic acids and nicotinamide in standard solutions and meteorite extracts. We performed a seven-point linearity study using standard solutions of the three pyridine monocarboxylic acid isomers and nicotinamide using a concentration range of 1 µM to 100 µM (or 5 pmol to 500 pmol on column). The calibration curves were highly linear (R² > 0.99) for nicotinic acid, isonicotinic acid, and picolinic acid using the integrated peak area of the mass chromatogram at the theoretical protonated mass of 124.0393 ± 0.0006 Da (5 ppm mass window), and enabled accurate quantitation of these compounds in a sample. The calibration curve for nicotinamide was also highly linear (R² > 0.99) using the integrated peak area of the mass chromatogram at the theoretical protonated mass of 123.0558 ± 0.0006 Da (5 ppm mass window). Our estimated limit of detection for each pyridine carboxylic acid isomer and nicotinamide is ~1 part-per-billion (ppb or ng compound/g meteorite).

Figure 3-2 shows a typical mass chromatogram of the serpentine procedural blank (bottom trace), the standard mix of all three pyridine monocarboxylic acid isomers (middle trace), and the LAP 02336 meteorite (top trace). These data are representative of our results from the other CM2 carbonaceous chondrites. Nicotinic acid, isonicotinic acid, and picolinic acid in the meteorite were identified based on chromatographic retention time, accurate mass measurements, and product ion spectra. The high mass
Figure 3-2. Mass chromatograms (m/z 124.0393 with a 5 ppm window corresponding to the protonated molecular ion) of picolinic acid (5.4 min.), isonicotinic acid (9.0 min.), and nicotinic acid (11.6 min.) of the LAP 02336 carbonaceous chondrite formic acid extract (top trace) and reference standards (middle trace). Identification was made by characteristic retention time (determined from reference standards) and accurate mass measurements. Pyridine monocarboxylic acids were not detected (<1 ppb) in the serpentine control blank.
accuracy and high mass resolution of the Orbitrap mass spectrometer eliminates most, if not all, of the matrix interferences in the mass chromatograms, which allows for the unambiguous identification and quantitation of targeted compounds. We did not detect nicotinic acid, isonicotinic acid, or picolinic acid in our procedure blank (above our detection limits), which suggests that our laboratory analytical protocols did not introduce additional pyridine carboxylic acids. The abundances of nicotinic acid and its isomers were adjusted based on recoveries of standards processed using our desalting or SPE procedure (see Table B-3 for recoveries).

**Abundances of pyridine carboxylic acids (and nicotinamide) in meteorite extracts.** We analyzed two different hot water extracts, non-acid-hydrolyzed and acid-hydrolyzed, of eight CM2 carbonaceous chondrites in order to determine the abundances of nicotinic acid and its two isomers, picolinic acid and isonicotinic acid. Seven of the eight CM2 chondrites in this study, with the exception of WIS 91600, contained all three pyridine monocarboxylic acid isomers in both extracts. The abundances of pyridine monocarboxylic acid isomers in the hot water extract were approximately equivalent. This result is similar to what was observed in earlier studies of hot water extracts of two carbonaceous chondrites (a CM2 chondrite and a C2 ungrouped chondrite). WIS 91600 was the only meteorite that did not contain any pyridine monocarboxylic acids above our detection limits (<1 ppb), which was likely due to thermal alteration on the parent body that led to the decomposition of organic compounds. Of the meteorites that contained pyridine monocarboxylic acids in both extracts, DOM 03183 contained the least amount of total pyridine monocarboxylic acids (61 ppb), also likely due to thermal alteration of the meteorite parent body. Figure 3-3a shows the abundances of pyridine
Figure 3-3. The abundances and distribution of pyridine monocarboxylic acids in the (a) hot water, (b) acid-hydrolyzed, and (c) formic acid extracts determined by mass detection. Carbonaceous chondrites are ordered from right to left in order of increasing aqueous alteration based on the method by Alexander et al.\textsuperscript{32} The error was calculated as the standard error of the mean from three measurements.
monocarboxylic acids in the hot water extracts (see also table B-4 for abundances), which range from approximately 61 ppb to 253 ppb total pyridine monocarboxylic acids (excluding WIS 91600). Figure 3-3b shows the abundances of pyridine monocarboxylic acids in the acid-hydrolyzed hot water extracts (see also Table B-5 for abundances), which are noticeably higher than the values for the hot water extracts ranging from 128 ppb to 497 ppb total pyridine monocarboxylic acids (excluding WIS 91600). The difference in the hot water vs. the acid-hydrolyzed hot water extracts is the release of approximately 23-166% more pyridine monocarboxylic acids likely from an acid-labile bound form (e.g. precursor molecules, complex bonded species). We investigated 3-cyanopyridine, which can be hydrolyzed to nicotinic acid, as a potential precursor molecule (see Appendix B).

We analyzed formic acid extracts of eight CM2 carbonaceous chondrites in order to obtain the abundances of nicotinic acid, isonicotinic acid, and picolinic acid. All of the eight CM2 chondrites contained all three structural isomers of pyridine carboxylic acids in the formic acid extract. Figure 3-3c shows the abundances of pyridine monocarboxylic acids (see also Table B-6 for abundances), which are significantly more abundant in the formic acid extract (ranging from 163 ppb to 1377 ppb total pyridine monocarboxylic acids) compared to both of the hot water extracts. The elevated abundances may be due to higher extraction efficiency with formic acid, which has been known to be effective for liberating nitrogen heterocycles from meteorites.33,17,19 In addition, picolinic acid abundance seems to increase significantly, especially in DOM 08003, although the exact reason for this is unknown.
All CM2 carbonaceous chondrites have experienced some amount of preterrestrial aqueous alteration. Aqueous alteration is a secondary process that occurs for a finite period of time on the meteorite parent body in which ice in the asteroid core melts via heat produced through decay of various radionuclides, such as $^{26}$Al. This period of liquid water on the asteroid alters the chemistry of the mineral matrix and may affect the synthesis or destruction of organic compounds. In Figure 3-3a-c, for all extracts, the meteorites are arranged from right to left in order of increasing aqueous alteration based on the method by Alexander et al. using previously reported bulk hydrogen abundance, isotopic composition, and C/H ratio information (which also correlates with petrologic indicators for the degree of aqueous alteration). In the acid-hydrolyzed hot water and formic acid extracts, there is a noticeable decrease in abundance of pyridine monocarboxylic acids with increasing aqueous alteration. This trend suggests that aqueous alteration may have had an overall destructive effect on these compounds. LEW 85311, the least aqueously altered (most primitive) meteorite of this study, contains the highest abundance of total pyridine monocarboxylic acids (>1.3 ppm).

Finally, we unambiguously identified a few pyridine dicarboxylic acids (3,4-pyridinedicarboxylic acid, 2,5-pyridinedicarboxylic acid, and 3,5-pyridinedicarboxylic acid) in our meteorite extracts, and these organic compounds appear to be common products as well. To our knowledge, this is the first report of pyridine dicarboxylic acids in meteorites. One tantalizing observation is that we did not detect nicotinamide in any of the meteorite extracts, which suggests that CM2 carbonaceous chondrites might not have been a significant source of this compound for early Earth (see Appendix B for more details). In addition, we were unable to detect 2,3-pyridinedicarboxylic acid
(quinolinic acid) in our meteorite extracts. The lack of detection for quinolinic acid may have been attributed to very poor recoveries during the desalting procedure (≈7%). On the other hand, other pyridine dicarboxylic acids were detected despite similar low recoveries.

CM2-type carbonaceous chondrites may have been an important exogenous source for organic compounds on the early Earth. The most primitive (i.e. lowest degree of aqueous alteration) CM2 chondrites may have been especially important because they contain the highest abundance of pyridine carboxylic acids. The extraterrestrial source of nicotinic acid may have implications for the origin of life on Earth especially with regard to ancient metabolic pathways. Nicotinic acid and other pyridine carboxylic acids are readily soluble in water, which suggests that these compounds could be easily removed from the meteorite and possibly participate in chemical reactions on early Earth. It is interesting to point out that both nicotinic acid and adenine have been measured in meteorites, which are molecular precursors to NAD. However, some NAD components, such as adenine, are reported in very low ppb abundances in meteorites, which makes it difficult to envision how useful concentrations built up on early Earth without complementary endogenous synthesis or the use of alternative molecules. Furthermore, the synthesis of NAD or its intermediates, such as nicotinic acid β-riboside, may be challenging under prebiotic conditions. For example, the synthesis of nicotinic acid β-riboside is difficult in the laboratory, and it is usually produced using multiple synthetic organic steps (without relevant, plausibly prebiotic conditions). Also, NAD-mediated reactions in living organisms require the β-configuration, and a stereoselective synthesis presents additional difficulties. Nevertheless, alternative routes to synthesize NAD may
be feasible. For example, nucleotides have been successfully synthesized using multi-component synergetic reactions, which bypasses trying to couple ribose to a nitrogen heterocycle in the sequential fashion traditionally employed. A similar scenario may be applicable to the synthesis of NAD, although it may or may not involve pyridine carboxylic acid.

Irradiation experiments with pyridine-CO$_2$ ices: connection with meteoritic organics? The interstellar-parent body hypothesis states that organic compounds in meteorites may have been produced via radiation chemistry (and photochemistry) that took place on the icy organic mantles of interstellar grains or via aqueous phase chemistry on the asteroid parent body. For the former scenario, atoms and molecules condense on dust grains in dense interstellar clouds (T < 30 K). Energetic processing of icy grains by cosmic rays and/or photons would lead to more complex molecules, as demonstrated by numerous laboratory studies. Furthermore, theoretical modeling of the dynamical evolution of ice grains in the solar nebula has shown that warming would also occur, which would further enable the production of complex organics. A significant portion of meteoritic organics (e.g. aromatics, carboxylic acids, amino acids) may be relics of interstellar grain processes because they are enriched in deuterium and exhibit a high degree of branching and structural diversity (indicative of reactions involving radicals and ions).

From our investigation and previous studies, substituted pyridines are structurally diverse in meteorites (with methyl, dimethyl, monocarboxylic acid, and dicarboxylic acid substitutions observed), which suggest that this compound class might be the products of interstellar grain processes. In order to test this hypothesis (which also relates to the
interstellar-parent body hypothesis), we proton-irradiated a 1:1 mixture of pyridine and CO$_2$ held at a very low temperature. This experiment is only a first approximation for the formation of interstellar pyridine carboxylic acids because pyridine has not yet been identified in interstellar ices (although, the presence of pyridine in interstellar ice might still be possible). Figure 3-4 (top three traces) shows the IR spectra of the deposited sample recorded before and after proton irradiation at ~20 K and the irradiated sample after warm-up to room temperature. There are many new IR bands as a result of irradiation. Figure 3-4 (bottom three traces) also shows the IR spectra of nicotinic acid, isonicotinic acid, and picolinic acid (deposited in separate experiments) at ~20 K recorded for reference to the irradiated sample. For the irradiated sample at ~20 K, we assign the broad IR bands centered around 1290 cm$^{-1}$ and 1720 cm$^{-1}$ to characteristic bands of nicotinic acid and isonicotinic acid, which may have formed in situ during the low temperature irradiation of the pyridine-CO$_2$ ice. These bands correspond to the in-plane deformation of C-H and the carbonyl stretch of nicotinic acid$^{48}$ and isonicotinic acid. There do not appear to be any common IR bands between the picolinic acid IR spectrum and the irradiated pyridine-CO$_2$ ice spectrum at ~20 K. After warm-up to room temperature, the IR spectrum becomes very broad and difficult to interpret with respect to the pyridine carboxylic acid isomer reference spectra.

We analyzed the refractory residue of the proton-irradiated pyridine-CO$_2$ ice by our LC-UV/MS method. A variety of pyridine monocarboxylic acids, pyridine dicarboxylic acids, and hydroxypyridines were identified, the latter may have formed from residual water in the irradiation setup. We find that the distribution of the three pyridine carboxylic acid isomers identified in the refractory residue is similar to the distribution of
Figure 3-4. *In situ* IR spectra of the deposited pyridine + CO$_2$ sample recorded before and after proton irradiation at ~20 K and the irradiated sample after warm-up to room temperature (top traces). *In situ* IR spectra of nicotinic acid, isonicotinic acid, and picolinic acid deposited (in separate experiments) at ~20 K for reference to the irradiated sample (bottom traces). Common IR bands between the proton-irradiated sample spectra and the nicotinic acid and isonicotinic acid spectra are highlighted in gray.
isomers in the least altered (most primitive) meteorite, LEW 85311 (the abundance of picolinic acid > nicotinic acid > isonicotinic acid). More aqueously altered meteorites generally have a different distribution of pyridine monocarboxylic acids compared to those measured in the irradiated ice residue. We also identified four pyridine dicarboxylic acids (3,4-, 2,3-, 2,5-, 3,5-pyridinedicarboxylic acids) in the irradiated pyridine-CO$_2$ ice, three of which (3,4-, 2,5-, 3,5-pyridinedicarboxylic acids) we also found in the majority of the CM2 carbonaceous chondrites in this study.

The similar distribution of pyridine carboxylic acid isomers in irradiated pyridine-CO$_2$ ice and the most primitive meteorite coupled with the extended structural diversity of dicarboxylic acids in both samples is suggestive of radical chemistry and a possible interstellar origin for these molecules. However, previous studies have determined that nicotinic acid in the Murchison meteorite had a $\delta^{12}D = +129$‰ and $\delta^{13}C = +20$‰, which clearly suggested an extraterrestrial origin but the magnitude of deuterium enrichment did not approach values of interstellar molecules. However, it is unknown whether significant H/D exchange occurred during the analytical (aqueous extraction) protocol utilized. Other synthetic routes may have been possible. Fischer-Tropsch type reactions have been previously proposed for the synthesis of alkyl pyridines from aldehydes and ammonia, and similar reactions could be feasible for pyridine carboxylic acids.

For possible future work, irradiation experiments could be conducted with more realistic ice compositions, which would include starting from simpler precursors and the addition of H$_2$O, which is the dominant component of interstellar ices. We could also measure compound-specific isotope ratios of pyridine carboxylic acids in LEW 85311,
which is known through this study to contain high abundances of these compounds. In addition, LEW 85311’s low degree of aqueous alteration (for a CM2 chondrite) may have better-preserved signatures of potential interstellar chemistry.

CONCLUSIONS

1. Liquid chromatography coupled to Orbitrap mass spectrometry permitted the unambiguous identification of pyridine carboxylic acids in meteorites based on chromatographic retention time and accurate mass measurements. The linear response over a wide concentration range, along with high recoveries from both desalting and solid phase extraction protocols, enabled the quantitation of pyridine monocarboxylic acids in meteorites. Low recoveries of pyridine dicarboxylic acids inhibited accurate quantitation and revised purification techniques will be needed for these molecules in future studies.

2. Pyridine carboxylic acids were identified in all eight of the CM2-type carbonaceous chondrites (formic acid extract). The highest abundance of pyridine monocarboxylic acids was found in LEW 85311, which is the most primitive (least aqueously altered) meteorite in this study. In the formic acid extract, LEW 85311 contained: 511 ppb picolinic acid, 294 ppb isonicotinic acid, and 572 ppb nicotinic acid.

3. Pyridine monocarboxylic acids inversely correlate with the degree of aqueous alteration of CM2-type meteorite parent bodies (acid-hydrolyzed hot water extract and formic acid extract). There is a decrease in abundance of pyridine carboxylic acids as aqueous alteration of the meteorite parent body increases, which suggests that aqueous phase reactions on the meteorite parent body may have had a destructive effect on these compounds.
4. The abundance of pyridine monocarboxylic acids significantly increased in the acid-hydrolyzed hot water and formic acid extracts (compared to the hot water extract). These observations suggest that some of the pyridine monocarboxylic acids existed in an acid-labile bound form.

5. The full suite of pyridine monocarboxylic acids and a variety of pyridine dicarboxylic acids were identified in the non-volatile residue of proton-irradiated pyridine + CO$_2$ ice. A similar suite of pyridine mono- and dicarboxylic acids was measured in CM2 carbonaceous chondrites (only 2,3-pyridinedicarboxylic acid was absent among the targeted compounds) and suggests a connection between interstellar chemistry and the pyridine carboxylic acids observed in meteorites. However, more realistic interstellar ice compositions (e.g. the inclusion of H$_2$O and simpler organic precursors) are needed in future studies.

6. Nicotinamide was not identified in any of the 24 different meteorite extracts we analyzed. One possible explanation for this might be that the nicotinamide (if it existed on the meteorite parent body) was hydrolyzed to form nicotinic acid during the period of aqueous alteration on the meteorite parent body.

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ASSOCIATED CONTENT

Supporting Information in Appendix B. (1) Cyanopyridines. (2) Nicotinamide. Figure B-1: Hydrolysis of 3-cyanopyridine. Table B-1: Hydrolysis of 3-cyanopyridine. Table B-1: Hydrolysis of nicotinamide. Table B-3: Recoveries for desalted and SPE standards. Table B-4: Abundances of pyridine carboxylic acids in eight CM2-type meteorites from the hot water extraction. Table B-5: Abundances of pyridine carboxylic acids in eight CM2-type meteorites from the hot water acid-hydrolyzed extraction. Table B-6: Abundances of pyridine carboxylic acids in eight CM2-type meteorites from the formic acid extraction.

REFERENCES


Chapter 4: Exploring the Fate of Nitrogen Heterocyclic Compounds in Complex Prebiotic Mixtures

The chief obstacle to understanding the origin of RNA-based life is identifying a plausible mechanism for overcoming the clutter wrought by prebiotic chemistry.

-- Gerald F. Joyce, 2002

INTRODUCTION

All of the known life on Earth uses nucleic acids (DNA and RNA) as genetic molecules. Nucleic acids are composed of nucleotides, which are repeating subunits of nucleobases (nitrogen heterocycles) held in place by a sugar phosphate backbone. The prebiotic synthesis of these genetic macromolecules has been a longstanding question in origin(s) of life research because the structure and composition of DNA is likely too complex to have formed spontaneously on the early Earth. RNA has been proposed as an evolutionary precursor to DNA (the RNA world hypothesis) because of its dual ability to catalyze reactions and store genetic information.\(^1,2\) However, RNA has a similar complex sugar-phosphate backbone to DNA, and the difficulty of synthesis and instability of D-ribose and the possible scarcity of polyphosphates on the early Earth\(^3,4\) makes it unlikely to have been produced prebiotically. The difficulty in a prebiotic synthesis of RNA has led to the idea that alternative nucleic acid structures such as peptide nucleic acids (PNA), threose nucleic acids (TNA), and glycol nucleic acids (GNA)\(^5\) may have preceded RNA.\(^8\) All of these structures are comparatively simpler than RNA because they contain the same nitrogen heterocycles, but have different backbones; however, none of these alternative nucleic acid molecules have been demonstrably produced prebiotically. Components of PNA have been synthesized under prebiotic conditions;\(^9\) however, a complete and convincing prebiotic synthesis of PNA has yet to be
demonstrated. In order to bridge the gap between prebiotic chemistry and the RNA world, a primitive nucleic acid (and their nucleotide monomers) should have a robust prebiotic synthesis.

The synthesis of nucleotides under plausibly prebiotic conditions has been notoriously difficult in the laboratory. The direct addition of nucleobases to ribose does not yield ribonucleotides in high yield for purines and does not work at all for the pyrimidines,\textsuperscript{10,11} however, an abiotic pathway to forming activated pyrimidine ribonucleotides by simultaneous formation of the nucleobase and the ribose has been shown.\textsuperscript{12} This result is intriguing as it demonstrates an alternative abiotic route to forming ribonucleotides, however, the prebiotic plausibility of this organic synthesis has been questioned.\textsuperscript{13} This synthesis requires that a number of highly concentrated and reactive reagents be reacted in a precise sequence, a scheme that might be unlikely to have occurred in natural environments on early Earth. A more plausible reaction might be the direct addition of prebiotically formed electrophiles, such as acrolein, to nucleobases.\textsuperscript{14-16} The products of these additions include N\textsuperscript{1}-substituted pyrimidines and N\textsuperscript{9}-substituted purines,\textsuperscript{14} which are the same positions of the ribose attachment to pyrimidines and purines in DNA and RNA. However, it is unclear how the acrolein-substituted nucleobases would function as nucleotide equivalents in alternative genetic systems that would be useful to a pre-RNA world scenario.

Unlike the difficulties involved with nucleotide synthesis, laboratory synthesis of nucleobases under plausible prebiotic conditions has been readily demonstrated through formamide reactions\textsuperscript{17} and hydrogen cyanide (HCN) polymerization.\textsuperscript{18,19} Additionally, endogenous early Earth synthesis of nucleobases may have also been supplemented by
exogenous delivery of nucleobases by carbonaceous chondrite meteorites.\textsuperscript{20-22} Despite the possible availability of nucleobases on early Earth, alternative nucleic acids may have consisted of alternative nucleobases that were similar to the bases found in DNA and RNA. Possible candidates for alternative nucleobases might be 5-hydroxymethyluracil (HMU) and 5-hydroxymethylcytosine (HMC), which are not part of the five canonical base set traditionally found in DNA and RNA, but are known to occur in modern biology.\textsuperscript{23-24} HMU has also been synthesized under plausible prebiotic conditions by reacting uracil with formaldehyde.\textsuperscript{25} Additionally, the hydroxymethyl site of HMU is highly reactive with various nucleophiles (e.g. ammonia, hydrogen cyanide, glycine, \textit{etc.}). For this reason, an attempt was made to incorporate HMU into a peptide backbone to produce PNA.\textsuperscript{26} Although this attempt was unsuccessful, HMU oligomeric material was identified as a byproduct of the reaction, but was never fully characterized.

The prebiotic synthesis of nucleotides (or nucleotide-like molecules) is one step toward forming more complex genetic macromolecules; however, the complexity of prebiotic chemistry on early Earth is a challenge to forming RNA-like compounds because unwanted products can also be produced. Laboratory experiments using a spark discharge apparatus and a mixture of reduced gases, which simulate an ocean-atmosphere system on early Earth, produced amino acids (the monomers of protein) and other low molecular weight compounds (supporting the “prebiotic or primordial soup” hypothesis).\textsuperscript{27,28} However, this reducing atmosphere has been criticized for not representing the current view of the early Earth atmosphere, which might have been dominated by nitrogen and carbon dioxide, with only relatively small concentrations of reduced gases (H\textsubscript{2}, CH\textsubscript{4}, \textit{etc.}).\textsuperscript{29-31} Nevertheless, it has been demonstrated that spark
discharge experiments using a neutral atmosphere (CO\textsubscript{2} and N\textsubscript{2}) still produced organic compounds, albeit, in much lower abundances.\textsuperscript{32}

In this chapter, we report on the reactivity of select nitrogen heterocycles under the simulated complexity of early Earth using mixtures produced from a spark discharge apparatus. We characterized the products from these reactions using liquid chromatography coupled to UV and/or fluorescence detection and mass spectrometry. During our investigation of nitrogen heterocycles, we determined that 5-hydroxymethyluracil can readily self-oligomerize consistent with earlier observations by Cleaves.\textsuperscript{26} We also discovered that 5-hydroxymethylcytosine can self-oligomerize independent of the organic compounds produced from the spark discharge apparatus. We characterized some of the products from the oligomerization reactions of hydroxymethylated nucleobases by liquid chromatography-high resolution Orbitrap mass spectrometry and theoretical calculations.

EXPERIMENTAL AND THEORETICAL METHODS

**Chemicals and Reagents.** Ultrapure water (18.2 M\textsubscript{Ω}·cm, <5 parts-per-billion [ppb] total organic carbon) was used exclusively for this study from a Millipore Direct Q3 UV system. All glassware was rinsed with water, wrapped in foil, and heated to 500 °C overnight in order to remove organic compounds. All chemicals were purchased from Sigma Aldrich with the exceptions of HMU and sodium bicarbonate, which were purchased from Alfa Aesar, and HMC, which was purchased from MP Biomedicals. Methanol (Optima\textsuperscript{®} grade) was purchased from Fisher Scientific. For liquid chromatographic separation, a solution of 10 mM ammonium formate with 2% methanol
(pH ~3.8) was prepared. For the spark discharge experiments, a buffer of 36 mM sodium bicarbonate (pH 8.5) was prepared.

**Spark Discharge Experiments.** Complex organic mixtures were produced using a spark discharge apparatus. The spark apparatus\textsuperscript{33,34} consisted of a BD-50E Tesla coil (Electrotechnics), a custom-made 1000-mL round bottom flask, two tungsten electrodes, and a gas inlet (see Figure 4-1). The spark apparatus contained 15 mL of sodium bicarbonate buffer at pH 8.5 under an atmosphere of nitrogen (40%), carbon dioxide (10%), methane (25%), and hydrogen (25%). The gases were sparked for 48 hours and the sodium bicarbonate solution was maintained at a temperature of approximately 5 °C using an ice bath. After completion of the spark discharge experiment, the solution was removed from the flask without exposure to air by handling in a nitrogen glove box.

For the survey of heterocycle reactivity, nitrogen heterocycle solutions (10\textsuperscript{-2} M – 10\textsuperscript{-6} M) were reacted *ex situ* with 1 mL aliquots of the spark product at 100 °C for 24 hours. For the focused study, isoxanthopterin was reacted *ex situ* with 1 mL aliquots of the spark product at -20 °C to 100 °C for 1 day up to a few months. All samples were filtered using an Acrodisc syringe filter and analyzed by liquid chromatography coupled to UV (and/or fluorescence) detection and mass spectrometry. Standards and control samples containing only spark organics were processed in parallel.

**Analysis of Products from the Survey of Nitrogen Heterocycles.** The survey of nitrogen heterocycle reactivity was performed using a Waters Alliance 2695 high performance liquid chromatograph coupled to a Waters 2996 photodiode array detector
Figure 4-1. Spark discharge apparatus including BD-50E Tesla coil, gas inlet, and two tungsten electrodes.
(PDA), a Waters 2475 multiwavelength fluorescence detector, and a Waters Quattro Micro API triple quadrupole mass spectrometer (QqQ-MS). The QqQ-MS was equipped with an electrospray ionization source (ESI), which was operated in positive ion mode. QqQ-MS settings were as follows: ESI capillary voltage +3.8 kV, cone voltage 35 V, desolvation temperature 350 °C, and source 120 °C. The N₂ cone and desolvation gases were flowed at 50 L/hr and 650 L/hr, respectively. The mass range was set to 50-500 m/z. Chromatographic separation was achieved using a Phenomenex guard column followed by a Phenomenex Luna phenyl-hexyl 4.6 × 250 mm column (5 µm particle size) with a 10-20 µL injection volume. Mobile phase (A) consisted of 100% methanol and mobile phase (B) was 10 mM ammonium formate buffer with 2% methanol (pH ~3.8). Samples were eluted at 0.5 mL/min using the following gradient: 0-20 min. 0-20% A, 20-22 min. 20-100% A, 22-32 min. 100% A, 32-34 min. 100-0% A, and 34-44 min. 0% A with a column temperature of 30 °C. The UV spectrum from 200-400 nm was collected using the Waters PDA. The fluorescence excitation/emission wavelengths varied depending on the heterocycle.

**Accurate Mass Measurements by Time-of-Flight Mass Spectrometry.** Accurate mass measurements were made by infusing solutions directly into a Waters LCT Premier time-of-flight mass spectrometer (ToF-MS) operated in V mode (mass resolution ~ 5,000). ESI and ToF-MS settings were similar to the settings used for the QqQ-MS. Cesium ions (m/z = 132.9054) supplied from a cesium iodide solution spiked into the sample solution were used as a lockmass for internal mass calibration and resulted in a typical relative mass error of <10 ppm. Accurate mass, along with the utilization of the natural isotope pattern, enabled the assignment of elemental formulae for small
molecules. We used these methods to identify products from the reactions between isoxanthopterin and the spark discharge mixture.

**Reactions with 5-hydroxymethyluracil and 5-hydroxymethylcytosine.** We produced nitrogen heterocycle oligomers by heating 0.01 M 5-hydroxymethyluracil (HMU) in water at 100 °C for 24 hours in flame-sealed glass ampoules. The same conditions were used for 5-hydroxymethylcytosine (HMC) as well as equimolar mixtures of HMU and HMC in order to produce mixed oligomers. We analyzed these samples by liquid chromatography coupled to UV detection and high resolution Orbitrap mass spectrometry.

**Investigation of HMU and HMC.** HMU and HMC oligomers were analyzed using a Thermo Scientific Accela high performance liquid chromatograph coupled to a Thermo Scientific Accela PDA and a Thermo Scientific Linear Ion Trap-Orbitrap XL hybrid mass spectrometer. Separation of oligomeric material was accomplished using a Phenomenex Luna phenyl-hexyl 4.6 × 250 mm column (5 µm particle size) and a 10 µL sample injection volume. Mobile phase (A) consisted of 10 mM ammonium formate with 2% methanol (pH ~3.9) and mobile phase (B) was 100% methanol. Samples were eluted at 300 µL/min using the following gradient: 0-40 min. 100-60% A, 40-60 min. 60-15% A, 60-100 min. 15% A, and 100-115 min. 100% A with a column temperature of 40 °C. The UV spectrum (200-400 nm) was collected using the Accela PDA.

The Thermo Scientific LTQ Orbitrap XL hybrid mass spectrometer was equipped with an ESI source and operated in positive ion mode. Parameters for ESI were as follows: the nitrogen gas for desolvation of the electrospray was set to 60 for the sheath gas, 20 for the auxiliary gas, and 2 for the sweep gas (all in arbitrary units). The ion
transfer capillary voltage and temperature were 15 V and 250 °C, respectively. The tube lens was set to 135 V in order to bias the detection of high molecular weight compounds. Full scan mass spectra were taken over a range of m/z 100 to 2000. The mass resolution was set to 30,000 (at full-width-half-maximum for m/z 400) in order to maintain an appropriate number of data points across chromatographic peaks. Product ion spectra (MS/MS) were collected in the Orbitrap mass analyzer (HCD energy 40% and isolation width of 0.8 Da). External calibration was performed using a mixture of caffeine, MRFA (L-methionyl-arginyl-phenylalanyl-alanine acetate hydrate) peptide, and Ultramark 1621 in an acetonitrile-methanol-water solution containing 1% acetic acid, which resulted in a mass accuracy of <2 parts-per-million (ppm).

**Theoretical Calculations.** We performed density functional theory (DFT) calculations using the Gaussian 09 package\textsuperscript{35} with the B3LYP density functional method\textsuperscript{36,37} and the 6-31G** basis set\textsuperscript{38} in order to determine the optimized geometry of the HMU dimer. The sums of the electronic and thermal free energies were calculated for each reactant (HMU and uracil) and three possible products (two HMU dimers and water).

**RESULTS AND DISCUSSION**

**Products from Reactions Between Spark Mixtures and Nitrogen Heterocycles.** Very few plausible prebiotic syntheses of nucleotides have been shown in the laboratory;\textsuperscript{10} therefore, in an attempt to demonstrate the first steps toward producing a nucleotide alternative, we surveyed the reactivity of several nitrogen heterocycles in a complex mixture similar to the presumed conditions of early Earth.\textsuperscript{39} For nitrogen
heterocycles, we selected substituted purines, pyrimidines, and pterins, which served as analogs for the canonical DNA/RNA nucleobases based on their structural similarity and their ability to base pair (Figure 4-2). For example, isoxanthopterin was used as a guanine analog (see Figure 4-2). All of the nitrogen heterocycles that we used in this study are UV active (chromophores), which facilitated their detection by UV spectroscopy in addition to mass spectrometry after liquid chromatographic (LC) separation. Additionally, some of these heterocycles, namely the pterins,\textsuperscript{40} are highly fluorescent (fluorophores). The use of fluorescent nitrogen heterocycles allowed for fluorescence detection with picomole sensitivity as well as reduced chromatographic interference (only molecules containing the added heterocycle should have both the appropriate excitation and emission characteristics). We identified products formed between the spark mixture and the selected nitrogen heterocycle using the following criteria: (1) the disappearance/reduction of the nitrogen heterocycle UV (or fluorescence) peak and (2) the appearance of new peaks in the UV (or fluorescence) chromatogram, which were not found in the spark mixture control blank. We classified our results in a general way by designating the nitrogen heterocycle as either “reactive” or “unreactive.”

Figure 4-3 shows the UV chromatograms of the nitrogen heterocycles in our study (with the exception of isoxanthopterin and HMU, which are shown later in this chapter) that were reacted with the spark discharge mixture for 24 hours at 100 °C. UV chromatograms of the spark mixture control blank are also shown at the UV wavelength specific to each nitrogen heterocycle. The pterins in our study (isoxanthopterin, 6-methylpterin, and xanthopterin) were all reactive with the spark discharge mixture. The
Figure 4-2. Canonical nucleobases (top left) and their analog counterparts (top right) are shown with base pairing sites indicated by dashed lines. We studied the reactivity of ten nucleobase analogs using complex mixtures provided by a spark discharge apparatus.
Figure 4-3. UV chromatograms of spark mixture (left column) and select nitrogen heterocycles reacted with the spark mixture at 100 °C for 24 hours (right column) are shown. The UV wavelength is specific for each nitrogen heterocycle and is shown in the left column. Areas shaded in blue indicate the formation of new nitrogen heterocycle adducts. The spark mixture at 295 nm appears to contain intense peaks, but this is due to low intensity UV peaks for 2-aminopyrimidine upon comparison. Isoxanthopterin and 5-hydroxymethyluracil were also studied and were both reactive (see Figure 4-4 and Figure 4-6).
6-methylpterin and xanthopterin both formed two major products (adducts) with the spark mixture (highlighted in blue in Figure 4-3). The two adducts formed between xanthopterin and the spark mixture have nominal masses of m/z 182 and 196. The two 6-methylpterin adducts have nominal masses of m/z 194 and 221. Isoxanthopterin formed one major product, which will be discussed further in the Isoxanthopterin Expanded Study section of this chapter. One of the pyrimidines, HMU, was also very reactive. The mass and UV chromatograms for HMU contained several new peaks and were very complex (this is in contrast to the results for isoxanthopterin), which will be discussed further in the Products from Reactions between HMU and the Spark Mixture section. The remaining six nitrogen heterocycles were found to be relatively “unreactive,” although there may have been minor products that formed. The UV data are more difficult to interpret than fluorescence data (which was only available for a few fluorescent heterocycles) because several molecules in the spark mixture control blank absorb UV light, which makes the UV chromatograms more complex and minor products harder to spot. All of the reactions between nitrogen heterocycles and spark mixtures were carried out under limited reaction conditions (i.e. one spark mixture, one temperature, and reacted for only 24 hours), which might also explain the lack of reactivity for some of the nitrogen heterocycles. Thus, it is conceivable that an unreactive heterocycle may become reactive under a different set of reaction conditions.

**Isoxanthopterin Expanded Study.** We carried out an expanded investigation of isoxanthopterin because it proved to be a reactive molecule in our survey of nitrogen heterocycles. We reacted isoxanthopterin with the spark mixture at three different temperatures and analyzed the products using liquid chromatography with fluorescence
detection and mass spectrometry. Figure 4-4 shows the fluorescence chromatograms of isoxanthopterin (retention time 14.6 min.) reacted with the spark mixture at -20 °C, 25 °C, and 100 °C. For the -20 °C reaction, which was allowed to react over the course of a few months, two peaks were observed in the fluorescence chromatogram: the unreacted isoxanthopterin peak and an unknown peak (retention time 23.8 min.). For the 25 °C reaction (also reacted over a few months), most (>95%) of the initial isoxanthopterin was converted to the same unknown compound at 23.8 min. Finally, for the 100 °C reaction (reacted for 24 hours), the unknown compound at 23.8 min. was again the major reaction product, although several less intense peaks were also observed in the fluorescence chromatogram. One of these minor products may be amidine-substituted isoxanthopterin (22.7 min.) based on accurate mass measurements, which would then suggest the presence of HCN and NH₃ in the spark mixture. We identified the major product at 23.8 min. as an adduct between cyanide and isoxanthopterin (cyano-isoxanthopterin) by fluorescence characteristics, accurate mass measurements (m/z 205.0467) to assign elemental composition (Figure 4-5), and product ion spectra. The cyano-isoxanthopterin product was also confirmed by a separate reaction between isoxanthopterin and sodium cyanide (at 100 °C for 24 hours) and had an identical retention time, m/z, and product ion spectrum. These results were surprising because the reaction led to the formation of a single dominant product despite the complexity of the spark discharge mixture. In addition, the reaction occurred over a wide range of temperatures, which suggests that this reaction may be robust and potentially better suited for further prebiotic chemistry. However, the location of the cyanide addition to the isoxanthopterin remains unknown,
Figure 4-4. Fluorescence chromatograms of isoxanthopterin (excitation $\lambda = 350$ nm, emission $\lambda = 430$ nm) standard (D) and isoxanthopterin standard reacted with the spark mixture at various temperatures and durations: (C) -20 °C for a few months, (B) 25 °C for a few months, and (A) 100 °C for 24 hours. All chromatograms contain the newly formed cyano-isoxanthopterin with increasing peak intensity as the temperature increases. Note: some of the fluorescence peaks are off scale.
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<th>Mass Error (ppm)</th>
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<td>205.0501</td>
<td>16.6</td>
<td>C₁₁H₉O₄</td>
</tr>
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**Figure 4-5.** ToF-MS accurate mass measurements for isoxanthopterin reference standard (180.0512 Da) and a major product (205.0467 Da) of the reactions between isoxanthopterin and spark mixture. In combination with fluorescence data, we can deduce the molecular formula of the m/z 205.0467 compound by starting with isoxanthopterin as the base molecule. Formula assignments are shaded in gray. Mass error = [(theoretical mass – experimental mass)/theoretical mass]×10⁶.
which ultimately makes it difficult to evaluate the functionality of this product as a nucleotide alternative (e.g. base-pairing capabilities may be inhibited). In future work, the cyano-isoxanthopterin could be isolated for NMR studies in order to determine the structure of the adduct. If cyanide added to a position on the ring that would not inhibit base-pairing capabilities, then the reactivity of the cyano-isoxanthopterin could be further explored as a possible nucleotide analog, perhaps with an existing polymeric structure.

**Products from Reactions between HMU and the Spark Mixture.** HMU was also found to be very reactive with the spark mixture. We reacted HMU with the spark mixture at 100 °C for 24 hours and analyzed the products using liquid chromatography with UV detection and mass spectrometry. Figure 4-6 shows the UV chromatogram of the HMU (retention time 13.0 min.) reacted with the spark mixture, which contains numerous new peaks. We identified the compound at 19.6 min. as an adduct between cyanide and HMU (i.e. uracil-5-acetonitrile) by UV data, molecular formulae from accurate mass measurements (m/z 152.0453), and product ion spectra. This product has been previously reported from the reaction of hydrogen cyanide and HMU at 100 °C for 25 hours. Also, we tentatively identified a variety of functional groups that attached to HMU based on UV data and molecular formulae from accurate mass measurements. Some of the possible functional groups we identified were: amine, alkyl amine, amide, amidine, nitrile, carboxylic acid, and imidazole groups (labeled as “modified HMU” in Figure 4-6). These results are in contrast to isoxanthopterin, which reacted with organic compounds from the spark mixture to form primarily one product (i.e. the cyano adduct), while HMU reacted with spark organic compounds to form an assortment of products. These results are intriguing because they demonstrate the variable reactivity between...
Figure 4-6. UV chromatogram (260 nm) showing the variety of compounds produced from reactions between the spark discharge mixture and 5-hydroxymethyluracil.
nitrogen heterocycles and a complex prebiotic mixture. It is possible that a molecule like HMU, which can react with a complex prebiotic mixture to form a variety of products with different functional groups, is more useful in a pre-RNA world scenario where specific genetic machinery has not yet been developed.

**Hydroxymethylated Nucleobase Oligomerization.** During our investigation of the reaction between nitrogen heterocycles and spark discharge organics, we observed that HMU readily self-oligomerizes into a variety of different structures, consistent with past observations by Cleaves. To further investigate HMU oligomerization, we reacted 0.1 M HMU with 0.1 M uracil at 100 °C for 24 hours because HMU and uracil may have both been present on early Earth (since HMU is easily synthesized from uracil and formaldehyde). After removing the sample solution from the oven, a white precipitate eventually formed. We isolated some of the solid material, redissolved it in dimethyl sulfoxide, and then diluted this solution with water containing 0.1% acetic acid in a 1:1 ratio. This solution was directly infused into the Orbitrap mass spectrometer with methanol. Figure 4-7 shows the electrospray mass spectrum with several distinct mass envelopes. The mass peaks highlighted in red are spaced by 124 Da, indicating the addition of a uracil molecule to the hydroxymethyl site followed by the loss of water, and accurate mass measurements supported the notion that distinct oligomers were produced. These results are not entirely surprising because the hydroxymethyl group of HMU is known to be highly reactive based on previous studies. Two types of oligomers were observed: (1) uracil-CH$_2$-uracil and (2) uracil-CH$_2$-HMU, the latter structure facilitated continued oligomerization. In the ESI mass spectrum, these structures were observed as sodium or potassium adducts. We also note that the peak intensity decreases as
Figure 4-7. Positive ion electrospray mass spectrum of 5-hydroxymethyluracil oligomers measured on an LTQ-Orbitrap XL mass spectrometer. The inset zooms in on higher m/z oligomers with lower peak intensity (the hexamer and heptamer). Peaks highlighted in red are sodium adducts of uracil-CH$_2$-uracil oligomers labeled with their corresponding length.
molecular size increases and that the longest oligomer is seven nitrogen heterocycles long.

Product ion spectra were acquired for additional confirmation of oligomeric structure. For example, Figure 4-8 shows the product ion spectrum of an HMU trimer. The middle box of Figure 4-8 shows the isolated protonated trimer (m/z 361.0891) at 0% collision energy. Only the protonated trimer is observed. As the collision energy is increased (15% collision energy setting), daughter ions start to appear as a result of fragmentation of the protonated trimer parent molecule (bottom box of Figure 4-8). Accurate mass measurements were acquired for parent and daughter ions, which led to an unambiguous assignment of molecular formulae. Two characteristic daughter ions at m/z 249.0617 and 125.0343 were observed in the product ion spectrum, which indicates cleavages between the methylene group and the heterocycle (*i.e.* U-CH$_2$-U-CH$_2^+$ and U-CH$_2^+$, respectively, top box of Figure 4-8). From these measurements, it is quite clear that oligomers were synthesized at the reactive hydroxymethyl site, in which a nitrogen heterocycle is added and a water molecule is lost. These additions may continue until either uracil is added or the 5-hydromethyluracil is converted to uracil – both situations “terminate” the reaction.

In order to further elucidate the structure of the HMU oligomers, we investigated two likely sites of addition of uracil to HMU (forming the HMU dimer) using DFT calculations. We calculated energies for the reactions of two possible HMU dimers (see Dimers A and B in Figure 4-9) at the B3LYP/6-31G** level in order to find the optimized geometry of the HMU dimer. These calculations were done in the gas phase at 25 °C and 125 °C. The calculated free energies of the reaction for HMU Dimer A were -6 kJ/mol at 25 °C and -4 kJ/mol at 125 °C. The energies for HMU Dimer B were the
Figure 4-8. Product ion spectra of a 5-hydroxymethyluracil trimer. Parent trimer ion (m/z 361.0891) at 0% collision energy is shown (middle box) as well as the parent trimer and characteristic daughter ions (m/z 249.0618 and m/z 125.0346) at 15% collision energy (bottom box). Suggested structures for the parent and daughter ions are also shown (top box). Accurate mass measurements and molecular formulae are shown for all ions.
Figure 4-9. Optimized structures of two possible 5-hydroxymethyluracil dimers, Dimer A (top box) and Dimer B (bottom box). Dimer A consists of a uracil-CH$_2$-uracil (N$_3$ substitution) and Dimer B consists of a uracil-CH$_2$-uracil (N$_1$ substitution). DFT calculations were performed at the B3LYP/6-31G** level.
lowest of the two dimers at -23 kJ/mol at 25 °C and -20 kJ/mol at 125 °C. The energy calculations of the HMU dimer support the notion that the oligomerization linkage is between the C⁵-CH₂ position of HMU and the N¹ deprotonated position of a second HMU or uracil molecule followed by the loss of water (Dimer B in Figure 4-9), which was also implied by Cleaves²⁶ using UV spectroscopy of bulk oligomeric HMU. However, both dimers may have been synthesized. Multiple peaks were observed in the LC-MS chromatograms, which suggest that a number of structural isomers were produced for dimers and higher order oligomers. Interestingly, the HMU oligomers are so robust that they formed in the presence of a complex distribution of spark organics, where abundant competing nucleophiles could inhibit oligomerization.

We observed a similar phenomenon of oligomerization with 5-hydroxymethylcytosine. We measured cytosine oligomers (i.e. cytosine with a bridging methylene group) of different sizes (up to the tetramer) in the LC-MS chromatogram. Cytosine oligomers were determined by accurate mass measurements, and these measurements were further supported by observation of their sodium and potassium adducts. For the protonated cytosine dimer (cytosine-CH₂-cytosine), two major peaks in ~3:1 ratio were observed, which may correspond to the N¹ and N³ substituted molecules.

Intriguingly, in 5-hydroxymethylcytosine solutions, we also measured dimers and trimers that incorporated both cytosine and uracil into the structures, which suggested that deamination of the 5-hydroxymethylcytosine to 5-hydroxymethyluracil occurred readily under our reaction conditions (100 °C for 24 hours), leading to the potential of having “informational-like” oligomers (because they have sequence information now). Figure 4-10 (bottom box) is the accurate mass chromatogram showing three peaks, which
Figure 4-10. LC-Orbitrap accurate mass chromatogram (bottom box) of three isomeric dimers produced from reactions containing 5-hydroxymethylcytosine. Product ion spectra are shown (middle box) of characteristic peaks of the three isomeric ions eluting at 15.4, 17.35, and 19.1 min. as well as suggested isomeric structures from left to right (top box): C-CH2-U (N1 substitution), C-CH2-U (N3 substitution), and U-CH2-C (N1 substitution).
correspond to isomeric dimer structures (m/z 236.0778). Possible structures of the dimers are shown (top box) as evidenced by the characteristic product ion spectrum peak (middle box). The fragmentation suggests that both sequences were synthesized (U-C and C-U). For the trimer corresponding to the uracil-CH₂-cytosine-CH₂-cytosine (or cytosine-CH₂-cytosine-CH₂-uracil), we observed 10 peaks in the LC-MS chromatogram (m/z 397.0770, [C₁₄H₁₄N₈O₄+K]⁺). An even greater diversity of mixed trimers (e.g. uracil-CH₂-cytosine-CH₂-uracil, m/z 397.0531, [C₁₄H₁₂N₇O₅+K]⁺ and cytosine-CH₂-uracil-CH₂-cytosine, m/z 396.0691, [C₁₄H₁₃N₈O₄+K]⁺) was measured in reactions of equimolar 5-hydroxymethyluracil and 5-hydroxymethylcytosine. These results have revealed that oligomers of hydroxymethylated nucleobases can readily form in solution. These nucleobase oligomers may be part of a realistic inventory of potentially functional compounds for pre-RNA world models and serve as the first step to understanding chemical evolution from the pre-RNA world to the RNA world.

Oligomerization of hydroxymethylated nucleobases might be useful in the development of simple informational polymers, especially if more than one type of nucleobase can be incorporated into the same polymer. However, the spacing between HMU nucleobases is the length of one carbon-carbon bond plus one carbon-nitrogen bond (< 3 Angstroms in total) which is very short; therefore, it might be difficult for these oligomers to adopt a structure similar to DNA or RNA that would support base-pairing (e.g., even the separation of stacked nucleobases in DNA is greater at 3.4 Angstroms). Interestingly, the HMU heptamer might be long enough to support RNA-like translational abilities. Studies have shown that a ribozyme merely five nucleotides long can perform an aminoaeryl group transfer, which activates amino acids and helps match them to the
appropriate codon for protein synthesis.\textsuperscript{41} Potential future directions might include: examining the chemical stability of the hydroxymethylated nucleobase oligomers in solution, experiments to extend the length of the hydroxymethylated nucleobase oligomers, and/or determining the melting profiles of isolated hydroxymethylated nucleobase oligomers using temperature-controlled UV spectroscopy in order to determine, if any, secondary structures that these oligomers are capable of forming.

**SUMMARY**

We investigated the reactivity of select nitrogen heterocycles under the simulated complexity of early Earth using a spark discharge apparatus. Under a narrow range of reaction conditions, we found that four out of ten heterocycles surveyed were reactive (\textit{i.e.} formed adducts with the spark mixture). Products from the reactions between nitrogen heterocycles and spark discharge mixtures were determined by UV or fluorescence characteristics, product ion spectra, and accurate mass measurements from time-of-flight mass spectrometry. Three of the reactive heterocycles were pterins and one was a hydroxymethyl-pyrimidine. It is unclear why the pterins readily formed adducts with the spark mixture, but this can be explored further in the future. In order to pinpoint the site of reactivity, one possibility would be to react the spark discharge mixture with other substituted pterins, which would block certain sites in a systematic fashion, and see if the pterins were still reactive. The 5-hydroxymethyluracil was known previously to be highly reactive;\textsuperscript{25} therefore, it was not surprising that this molecule was also very reactive with the spark mixture. While the detection of substituted nitrogen heterocycles in complex mixtures is intriguing, it is unclear whether the nitrogen
heterocycle adducts can function as nucleotide alternatives. In the case of the pterin molecules, it is unknown whether base-pairing capabilities were inhibited with the addition of spark organic compounds and for all nitrogen heterocycles adducts, only small functional groups were identified (e.g., nitrile groups, methyl groups, etc.), which is very different from the ribose-phosphate addition to nucleobases in nucleotides.

During our survey of nitrogen heterocycle reactivity, we observed the oligomerization of hydroxymethylated nucleobases. These oligomers contained repeated units of uracil-CH$_2$ terminated either by uracil or HMU (termination in HMU facilitated continued oligomerization), which were characterized by LC retention time, accurate mass measurements from high resolution Orbitrap mass spectrometry, product ion spectra, and theoretical calculations. The longest oligomer observed was an HMU heptamer (seven nitrogen heterocycles long). The oligomers were robust and formed even in the reactions with spark discharge mixtures, where abundant competing nucleophiles could have inhibited oligomerization. Mixed oligomers were also produced from solutions containing HMC only and mixtures of HMU and HMC, which make sequence information possible much like DNA and RNA. However, the structure of the oligomers may not be comparable to DNA and RNA because the nucleobases are close together and twisted so that base-pairing sites are facing in opposite directions, which is not ideal for templating. Interestingly, the heptamer (longest oligomer identified in solution) may be long enough to support RNA-like translational abilities; however, the structure of the oligomers would most likely prove to be prohibitive of such capabilities.

REFERENCES


APPENDIX A: Supporting Information for Chapter 2

(1) Safety Considerations. Cyanide is highly toxic and should be handled with care. Avoid ingestion and contact with skin. If cyanide solutions are acidified, highly toxic HCN gas may be released. Handling of cyanide should be performed by trained personnel in a well-ventilated fume hood. See ref (1) for complete safety procedures.

(2) Method Blanks. Two method blanks were used in this study: a procedural blank and a serpentine blank. The procedural blank consisted of 500 µL 0.08 M NaOH added to 5.5 mL ultrapure water plus 750 µL 9 M sulfuric acid, which was distilled and derivatized using the same procedure as the meteorites. The procedural blank fluorescence and mass peak areas were subtracted from all standards and meteorite samples. The serpentine blank consisted of 203.6 mg serpentine (free of organic carbon) added to 5.8 mL ultrapure water plus 750 µL 9 M sulfuric acid, which was distilled and derivatized using the same procedure as the meteorites. The serpentine blank is different from the procedural blank because it contains the meteorite mineral matrix, which rules out the possibility of making cyanide through mineral interactions during the acid-digestion and distillation procedure. The procedural blank and serpentine blank showed identical results.

(3) Preparation, Acid-digestion, and Distillation of HCN Polymer. Hydrogen cyanide polymer was made under the following procedure. First, HCN gas was produced by heating KCN and stearic acid under vacuum, then the gas was collected in a test tube cooled by a liquid nitrogen bath and double distilled under vacuum. HCN gas was then expanded into an evacuated glass bulb of known volume and frozen into a known mass of degassed (frozen-pumped-thawed) ultrapure water to a final concentration of 2 M. NH$_3$
gas was also frozen into ultrapure water in the same manner to produce a 2 M solution. Once these solutions were thawed, they were combined to give a final solution of 1 M NH$_4$CN. The final NH$_4$CN solution was refluxed for 2 days during which an insoluble black solid formed. The solution was stored in a 50-mL centrifuge tube at room temperature with minimal headspace (air) and allowed to react (polymerize) for 2 years. The final color of the solution was dark brown with black solids.

Two aliquots of HCN polymer (~2 g each of combined liquid and black solid) were pipetted into tared MicroDIST distillation sample tubes (Lachat Instruments) and centrifuged under vacuum in a Centrivap (Labconco) overnight to a final dry weight of 20 mg each. 1 mL of 0.08 M NaOH was added to each sample tube with dry polymer, touch-mixed using a Vortex-Genie 2 (Scientific Industries), then centrifuged for 5 minutes. 500 $\mu$L of the supernatant was transferred to a separate vial in order to measure the free cyanide anion content of the polymer solution. 5.5 mL ultrapure water was added to the remaining 500 $\mu$L of polymer solution in the distillation sample tube. 750 $\mu$L 9 M sulfuric acid was added to the polymer solution as a cyanide releasing agent and the full distillation tube was assembled. The HCN polymer sample was distilled for 30 minutes then derivatized with the NDA procedure the same as the meteorites and control samples.

(4) Study of Organocyanides: Acetonitrile and 3-cyanopyridine. Acetonitrile was distilled under the following conditions: 500 $\mu$L of acetonitrile was added to 5.5 mL ultrapure water plus 750 $\mu$L 9 M sulfuric acid, which was acid-digested, distilled, and derivatized using the same procedure as the meteorites. In addition, non-distilled acetonitrile was analyzed for free cyanide anion in order to determine the free cyanide
released during acid-digestion and distillation. Non-distilled acetonitrile was prepared as follows: 500 µL of acetonitrile was added to 2 mL 0.08 M NaOH and derivatized using the same procedure as the meteorites. 3-cyanopyridine was acid-digested and distilled under the following conditions: 500 µL of 10 µM 3-cyanopyridine was added to 5.5 mL ultrapure water plus 750 µL 9 M sulfuric acid, which was acid-digested, distilled, and derivatized using the same procedure as the meteorites. In addition, non-distilled 3-cyanopyridine was analyzed for free cyanide anion in order to determine the free cyanide released after acid-digestion and distillation. Non-distilled 3-cyanopyridine was prepared as follows: 500 µL of 10 µM 3-cyanopyridine was added to 2 mL 0.08 M NaOH and derivatized using the same procedure as the meteorites.

We investigated acetonitrile and 3-cyanopyridine, which served as model organocyanide compounds in meteorites, in order to see if these types of compounds could release cyanide using our experimental method. There are few reports of organocyanides in meteorites; however, benzonitrile, dicyanobenzene, and dicyanotoluene were identified in the Tagish Lake meteorite (C2 ungrouped carbonaceous chondrite). The 3-cyanopyridine did not release any cyanide after distillation (cyanide concentration was below limit of detection before and after distillation). Based on our results for 3-cyanopyridine, we hypothesize that cyano-substituted aromatic compounds (potentially in CM chondrites) are unlikely to release cyanide using the acid-digestion and distillation procedure. Acetonitrile (methyl cyanide) is the simplest organic nitrile and has been observed in the interstellar medium, in cometary comae, and in the Murchison meteorite (as common pyrolysis products). Therefore, acetonitrile can be considered a potential organonitrile in meteorites. The total
cyanide concentration in the acetonitrile increased 11-fold upon distillation (from 0.1 nmol CN before to 1.1 nmol CN after distillation), which was less conversion than the HCN polymer. Nonetheless, acetonitrile should also be considered a potential source for the detected cyanide in CM chondrites, and future work will target acetonitrile and other simple alkyl nitriles.

(5) Extraction of LEW 85311 for Purines. 206.6 mg of powdered LEW 85311 was extracted in a flame-sealed glass ampoule with 95% formic acid at 100 °C for 24 hours. The extract was purified using a Waters Oasis Max SPE cartridge. Purines (guanine, hypoxanthine, xanthine, and adenine) were eluted using 5% formic acid in methanol. The extract was analyzed using a Thermo Scientific Accela LC coupled to a Thermo Scientific LTQ Orbitrap XL hybrid mass spectrometer with mass resolution of approximately 60,000 and mass accuracy <<5 parts-per-million. For more experimental detail see Callahan et al.\textsuperscript{11}

Total purines were defined as the sum of guanine, hypoxanthine, xanthine, and adenine abundances, which were the most abundant purines measured in carbonaceous chondrites in the Callahan et al. study.\textsuperscript{11} Low abundant purines such as purine and 2,6-diaminopurine were not included because these compounds were not detected in all of the CM chondrites studied here (the highest sum of purine and 2,6-diaminopurine was 0.08 nmol purines/g meteorite detected in LON 94102). Additionally, 6,8-diaminopurine was not included because the abundance was not determined in the Callahan et al. study\textsuperscript{11} due to a lack of pure reference standard. The purine abundances for LEW 85311 were: 0.06 nmol guanine/g meteorite, 0.95 nmol hypoxanthine/g meteorite, 4.07 nmol xanthine/g
meteorite, 0.08 nmol adenine/g meteorite (with an estimated error of ~5% based on standard injections).

**APPENDIX A REFERENCES**


### Table A-1

The average abundance of cyanide in different meteorites determined by mass and fluorescence detection. The error was calculated as the standard error of the mean from four measurements.

<table>
<thead>
<tr>
<th>Meteorite</th>
<th>Type</th>
<th>Average Cyanide Abundance nmol CN/g meteorite</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALH 83100</td>
<td>CM1/2</td>
<td>49.8 ± 0.4</td>
</tr>
<tr>
<td>LEW 90500</td>
<td>CM2</td>
<td>148.0 ± 5.7</td>
</tr>
<tr>
<td>Murchison</td>
<td>CM2</td>
<td>94.8 ± 0.68</td>
</tr>
<tr>
<td>LON 94102</td>
<td>CM2</td>
<td>421.2 ± 25.7</td>
</tr>
<tr>
<td>LEW 85311</td>
<td>CM2</td>
<td>2472.1 ± 37.6</td>
</tr>
<tr>
<td>RBT 04133</td>
<td>CR2 (CV3?)</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>GRA 06100</td>
<td>CR2</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>ALH 84001</td>
<td>orthopyroxenite</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>(martian)</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX B: Supporting Information for Chapter 3

(1) Cyanopyridines. We investigated the possible conversion of a potential precursor for nicotinic acid that could result from our three extraction procedures. We tested 3-cyanopyridine, which has a nitrile group that can be converted to a carboxylic acid group (resulting in nicotinic acid) by hydrolysis (see Figure B-1). Additionally, 3-cyanopyridine is a product from spark discharge experiments of ethylene and ammonia.\(^1\) Ethylene has been reported in the interstellar medium\(^2\) and ammonia has been directly measured in meteorites.\(^3\) Our results show that there is minimal nicotinic acid converted from the 3-cyanopyridine standard during the hot water and formic acid extractions (Table B-1). This may hold true for the meteorite as well; however, the meteorite is much more complex than standards so we cannot state this conclusively. More nicotinic acid is formed from the 3-cyanopyridine standard during the acid-hydrolyzed hot water extraction but the recovery is still low (31%). If we extrapolate these results to our meteorite extracts, 3-cyanopyridine (and nicotinamide) could be measured in both the hot water and formic acid extracts. However, we did not detect these molecules in any of our meteorite extracts. One possible reason for this result is that these potential precursor molecules have already been converted to carboxylic acids during the lengthy aqueous alteration phase on the asteroid. This hypothesis may be supported by past analyses of meteorite organics that show a large abundance and diversity of carboxylic acids but very little in terms of molecules containing nitriles.\(^4\) On the other hand, if this were true, the more aqueously altered meteorites would have more pyridine monocarboxylic acids if there were abundant cyanopyridines to start with (yet the opposite trend was observed).
(2) Nicotinamide. We investigated the possible hydrolysis of nicotinamide to nicotinic acid that could have occurred during our three meteorite extraction procedures using reference standards. Our results show that there is little to moderate conversion of nicotinamide to nicotinic acid in the hot water (3%) and formic acid (29%) extractions (Table B-2). In contrast, all of the nicotinamide in the acid-hydrolyzed hot water extraction was converted to nicotinic acid. These results suggest that the absence of nicotinamide in the eight meteorites studied was not due to our meteorite extraction procedures; however, this conclusion includes the assumption that the meteorite matrix does not play a significant role during the extraction process. Thus, the possibility of detecting nicotinamide would have been in the hot water extraction or the formic acid extraction (to within 1 ppb). Regardless of the analytical protocols employed, any nicotinamide that may have been originally present may have already been converted to nicotinic acid during the lengthy aqueous alteration phase on the asteroid.

SUPPORTING INFORMATION REFERENCES

Figure B-1. The hydrolysis of 3-cyanopyridine to nicotinic acid via nicotinamide.
<table>
<thead>
<tr>
<th>Extraction</th>
<th>3-cyanopyridine % remaining after extraction procedure</th>
<th>Nicotinamide % converted from 3-cyanopyridine</th>
<th>Nicotinic Acid % converted from 3-cyanopyridine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot Water (water, 100 °C, 24 hrs)</td>
<td>53</td>
<td>43</td>
<td>1</td>
</tr>
<tr>
<td>Acid-hydrolyzed Hot Water (6 M HCl, 150 °C, 3 hrs)</td>
<td>0</td>
<td>0</td>
<td>31</td>
</tr>
<tr>
<td>Formic Acid (formic acid, 100 °C, 24 hrs)</td>
<td>80</td>
<td>13</td>
<td>3</td>
</tr>
</tbody>
</table>

Table B-1. Hydrolysis of 3-cyanopyridine reference standard to nicotinic acid using the three meteorite extraction procedures (hot water, acid-hydrolyzed hot water, and formic acid extractions). There is some conversion of 3-cyanopyridine to nicotinamide and minimal conversion of nicotinamide to nicotinic acid in the hot water and formic acid extractions.
Table B-2. Hydrolysis of nicotinamide reference standard to nicotinic acid using the three meteorite extraction procedures (hot water, acid-hydrolyzed hot water, and formic acid extractions). There is some conversion of nicotinamide to nicotinic acid in the hot water and formic acid extractions. All of the nicotinamide was converted to nicotinic acid in the acid-hydrolyzed extract.

<table>
<thead>
<tr>
<th>Extraction</th>
<th>Nicotinamide % converted to nicotinic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hot Water</strong></td>
<td></td>
</tr>
<tr>
<td>(water, 100 °C, 24 hrs)</td>
<td>3</td>
</tr>
<tr>
<td><strong>Acid-hydrolyzed Hot Water</strong></td>
<td></td>
</tr>
<tr>
<td>(6 M HCl, 150 °C, 3 hrs)</td>
<td>&gt;100</td>
</tr>
<tr>
<td><strong>Formic Acid</strong></td>
<td></td>
</tr>
<tr>
<td>(formic acid, 100 °C, 24 hrs)</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>% Recovery, Desalting</td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Nicotinic Acid</td>
<td>94 ± 4</td>
</tr>
<tr>
<td>Isonicotinic Acid</td>
<td>97 ± 5</td>
</tr>
<tr>
<td>Picolinic Acid</td>
<td>86 ± 3</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>64 ± 2</td>
</tr>
<tr>
<td>2,3-pyridinedicarboxylic acid</td>
<td>6.8 ± 0.8</td>
</tr>
<tr>
<td>2,5-pyridinedicarboxylic acid</td>
<td>7.7 ± 0.8</td>
</tr>
<tr>
<td>3,4-pyridinedicarboxylic acid</td>
<td>7.2 ± 0.8</td>
</tr>
<tr>
<td>3,5-pyridinedicarboxylic acid</td>
<td>20 ± 2</td>
</tr>
</tbody>
</table>

Table B-3. The average percent recoveries for the desalted and solid phase extracted (SPE) standards. Abundances of pyridine carboxylic acids from all of the carbonaceous condrites in this study were adjusted based on these recoveries. The error was calculated as the standard error of the mean from three measurements. Note: We did not have a reference standard for 2,4-pyridinedicarboxylic acid available in lab. However, based on peak shape comparison of meteorite mass chromatograms and remaining reference standards, 2,4-pyridinedicarboxylic acid was not likely present (or had poor ESI efficiency).
<table>
<thead>
<tr>
<th>Meteorite</th>
<th>Type</th>
<th>Picolinic Acid (ppb)</th>
<th>Isonicotinic Acid (ppb)</th>
<th>Nicotinic Acid (ppb)</th>
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</thead>
<tbody>
<tr>
<td>WIS 91600</td>
<td>CM2</td>
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<td>&lt;1</td>
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<td>DOM 03183</td>
<td>CM2</td>
<td>5.1</td>
<td>29.6</td>
<td>26.5</td>
</tr>
<tr>
<td>DOM 08003</td>
<td>CM2</td>
<td>42.9</td>
<td>38.0</td>
<td>58.7</td>
</tr>
<tr>
<td>ALH 85013</td>
<td>CM2</td>
<td>30.0</td>
<td>51.3</td>
<td>58.5</td>
</tr>
<tr>
<td>EET 96016</td>
<td>CM2</td>
<td>59.9</td>
<td>44.2</td>
<td>97.4</td>
</tr>
<tr>
<td>LAP 02333</td>
<td>CM2</td>
<td>55.6</td>
<td>64.6</td>
<td>74.5</td>
</tr>
<tr>
<td>LAP 02336</td>
<td>CM2</td>
<td>44.7</td>
<td>64.3</td>
<td>70.7</td>
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<tr>
<td>LEW 85311</td>
<td>CM2</td>
<td>105.9</td>
<td>63.5</td>
<td>84.2</td>
</tr>
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</table>

**Table B-4.** The abundances of pyridine carboxylic acids in eight CM2-type meteorites from the hot water extraction as determined by mass detection. The error for these measurements was 10%, which was calculated as the relative standard deviation from 21 measurements of each pyridine carboxylic acid standard (63 measurements in total) and verified by three measurements of each pyridine carboxylic acid (9 measurements in total) in the meteorite, LAP 02333.
<table>
<thead>
<tr>
<th>Meteorite</th>
<th>Type</th>
<th>Picolinic Acid</th>
<th>Isonicotinic Acid</th>
<th>Nicotinic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>WIS 91600</td>
<td>CM2</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>DOM 03183</td>
<td>CM2</td>
<td>9.9</td>
<td>49.2</td>
<td>69.4</td>
</tr>
<tr>
<td>DOM 08003</td>
<td>CM2</td>
<td>65.7</td>
<td>59.6</td>
<td>96.5</td>
</tr>
<tr>
<td>ALH 85013</td>
<td>CM2</td>
<td>36.9</td>
<td>62.8</td>
<td>85.1</td>
</tr>
<tr>
<td>EET 96016</td>
<td>CM2</td>
<td>100.5</td>
<td>59.8</td>
<td>122.2</td>
</tr>
<tr>
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<td>CM2</td>
<td>138.0</td>
<td>133.2</td>
<td>157.7</td>
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<tr>
<td>LAP 02336</td>
<td>CM2</td>
<td>113.7</td>
<td>142.3</td>
<td>188.4</td>
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<tr>
<td>LEW 85311</td>
<td>CM2</td>
<td>217.5</td>
<td>98.2</td>
<td>182.4</td>
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</table>

**Table B-5.** The abundances of pyridine carboxylic acids in eight CM2-type meteorites from the hot water acid-hydrolyzed extraction as determined by mass detection. The error for these measurements was 10%, which was calculated as the relative standard deviation from 21 measurements of each pyridine carboxylic acid standard (63 measurements in total) and verified by three measurements of each pyridine carboxylic acid (9 measurements in total) in the meteorite, LAP 02333.
Table B-6. The abundances of pyridine carboxylic acids in eight CM2-type meteorites from the formic acid extraction as determined by mass detection. The error for these measurements was 10%, which was calculated as the relative standard deviation from 21 measurements of each pyridine carboxylic acid standard (63 measurements in total) and verified by three measurements of each pyridine carboxylic acid (9 measurements in total) in the meteorite, LAP 02333.

<table>
<thead>
<tr>
<th>Meteorite</th>
<th>Type</th>
<th>Picolinic Acid</th>
<th>Isonicotinic Acid</th>
<th>Nicotinic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>WIS 91600</td>
<td>CM2</td>
<td>25.1</td>
<td>42.0</td>
<td>96.3</td>
</tr>
<tr>
<td>DOM 03183</td>
<td>CM2</td>
<td>70.2</td>
<td>70.8</td>
<td>121.9</td>
</tr>
<tr>
<td>DOM 08003</td>
<td>CM2</td>
<td>482.2</td>
<td>153.7</td>
<td>221.0</td>
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<tr>
<td>ALH 85013</td>
<td>CM2</td>
<td>98.8</td>
<td>67.4</td>
<td>139.6</td>
</tr>
<tr>
<td>EET 96016</td>
<td>CM2</td>
<td>322.0</td>
<td>116.7</td>
<td>265.1</td>
</tr>
<tr>
<td>LAP 02333</td>
<td>CM2</td>
<td>197.1</td>
<td>161.5</td>
<td>246.8</td>
</tr>
<tr>
<td>LAP 02336</td>
<td>CM2</td>
<td>318.4</td>
<td>256.9</td>
<td>332.1</td>
</tr>
<tr>
<td>LEW 85311</td>
<td>CM2</td>
<td>510.7</td>
<td>294.1</td>
<td>571.8</td>
</tr>
</tbody>
</table>
Vita

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Education

Ph.D. Geosciences and Biogeochemistry  
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Publications

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(2013) Investigation of pyridine carboxylic acids in CM2 carbonaceous chondrites:  
Potential precursor molecules for ancient metabolic pathways. Submitted to  
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Extraterrestrial cyanide in carbonaceous meteorites. Submitted to PNAS.

P. E. McGovern, B. P. Luley, N. Rovira, A. Mirzoian, M. P. Callahan, K. E. Smith,  
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C. H. House, and J. P. Dworkin (2011) Carbonaceous meteorites contain a wide  
range of extraterrestrial nucleobases. PNAS, 108, 13995-13998.

Awards

• NASA Earth and Space Sciences Fellowship (NESSF) (2012-2013)  
• Pennsylvania Space Grant Travel Award (2011)  
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  Meeting, San Diego, 2012  
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• Geosciences Representative, EMS Graduate Student Council (2008-2009)