A METHOD FOR THE DETERMINATION OF ^{15}N OF NUCLEOBASES IN THE MURCHISON METEORITE J. C. Stern[1,2], J. H. Doty, III[1,3] D. P. Glavin[1], J. P. Dworkin[1], [1]Goddard Center for Astrobiology, NASA Goddard Space Flight Center, Greenbelt, MD 20771, Jennifer.Stern@nasa.gov, [2]Oak Ridge Associated Universities, Oak Ridge, TN 37831 [3]Catholic University of America, Washington, DC 20064.

Introduction: Exogenous delivery of organics to the earth's surface could have been an important source of these molecules on the prebiotic earth. Nucleobases are key components in RNA and DNA and are vital to metabolism as co-enzymes. They have been detected in the Murchison meteorite and other carbonaceous chondrites [1-3], but the question of whether these compounds represent a truly exogenous source to early Earth or are present as terrestrial contamination remains unanswered. To understand the abundance and distribution of nucleobases in carbonaceous meteorites, they must be extracted from the meteorite and purified for molecular and isotopic analysis. Previous purifications of formic acid extracts of Murchison meteorite using ion-exclusion chromatography were time intensive and introduced contamination. Even after purification, identification and quantification of nucleobases was difficult due to interfering UV absorbing compounds [4] (Fig. 1).

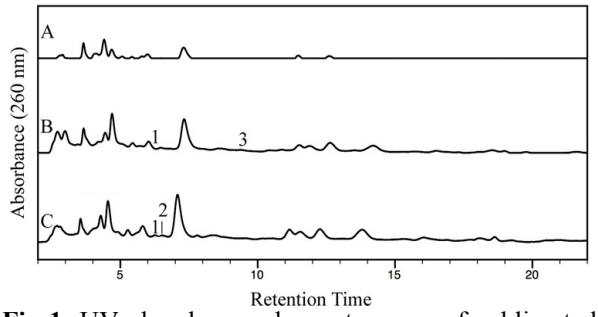

Fig 1. UV absorbance chromatograms of sublimated formic acid extracts of three CM meteorites A: ALH 83100, B: LEW 90500, and C: Murchison. The nucleobases 1: xanthine, 2: uracil, and 3: guanine were identified by their ToF mass spectra. This demonstrates that even after sample purification, there are abundant non-nucleobase chromophores present. Studying the ^{15}N abundances mitigates these interferences.

We have developed a sublimation technique that enables the separation of nucleobases from less volatile analytically interfering compounds [5-6] for analysis by Liquid Chromatography with UV Absorbance Detection and tandem time-of-flight Mass Spectrometer (LC-AD/ToF-MS). To determine the origin of nucleobases in Murchison, we have coupled our sublimation procedure with chemical derivatization and compound-specific isotope analysis (CSIA) using hybrid gas chromatography-combustion-isotope ratio mass spectrometry instrumentation with additional gas chromatography-quadrupole mass spectrometry (GC-QMS/GC-C-IRMS) capabilities for simultaneous molecular and isotopic analysis. We have chosen to measure $\delta^{15}N$ because the derivatization agent does not add any N atoms to the derivatized nucleobase and because of the absence of nitrogen in co-eluting peaks such as dicarboxylic acids, which have complicated measurements of $\delta^{13}C$ in past studies.

Here we present LC-AD/ToF-MS data showing distribution of nucleobases in the Murchison meteorite and preliminary results of sensitivity tests using GC-QMS/GC-C-IRMS for molecular and ^{15}N isotopic analysis. This data provides "proof of concept" that nucleobases can be extracted and purified from meteorite samples and analyzed for ^{15}N, thus addressing the question of the origins of nucleobases in Murchison meteorite.

Nitrogen isotopic analyses of Murchison bulk organic extracts indicate significant enrichment in ^{15}N with respect to terrestrial organic matter ($\delta^{15}N$ = +25‰ to +150‰) [7]. Labile organic matter in the Murchison meteorite (defined as organics not soluble in organic solvent but removed by aqueous alteration in the laboratory) is enriched in ^{15}N ($\delta^{15}N \leq$ +85‰) [7]. Nucleobases are extracted from Murchison via formic acid at 100°C, and would be expected to have similar enrichments in ^{15}N if they are extraterrestrial in origin.

Analytical Techniques: Work presented here is based on the analysis of the nucleobase standards cytosine, uracil, xanthine, guanine, hypoxanthine, thymine, and adenine. Identification and quantification of nucleobases was carried out using a Waters Alliance 2695 HPLC separations module, a Waters 2695 UV absorbance PDA, a Waters LCT Premier Time-of-Flight Mass Spectrometer. A sample consisting of a standard mix from stock solutions was dried down and sealed in a clean test tube with 95% formic acid, then incubated at 100°C for 24 hours. The supernatant was then dried down in a sublimation apparatus and sealed at 50 millitorr. The custom-made apparatus consists of a quartz sample tube and a borosilicate cold finger; this design has been successfully used to sublime purines and pyrimidines [5]. The quartz sample tube is then heated to 550°C for 5 minutes, and the cold finger is cooled with liquid nitrogen. The apparatus is allowed to return to room temperature before being rinsed with 0.1 M NH_4OH. A small fraction of the NH_4OH extract was then injected onto the LC-AD/-ToF-MS.

CSIA of nucleobases was carried out using a Thermo MAT 253 IRMS and Thermo DSQ II quadropole MS. The sample was derivatized for GC-QMS/GC-C-IRMS analysis with 3:1 N-methyl-N-[tert-butyldimethylsilyl] trifluoroacetimide : dimethyl formamide (MTBSTFA : DMF) for 90 minutes at 90°C.

Samples were injected into a Thermo Trace GC Ultra with a Rxi – 5ms column (30 m, 0.25 mm ID, 0.5 μm film thickness). At the terminal end of the column, a small split (~ 10%) of the sample was sent to the quadrupole MS for compound identification, and the rest was combusted via a Thermo GC-C III interface, where CO_2 was cryogenically trapped, and N_2 sent to the IRMS for isotopic analysis.

Results and Discussion: Experiments have shown that sublimation has a higher recovery rate than previously used methods of purification [6]. Recovery rates and the sensitivity of each instrument are given in Table 1. Despite the fact that our recovery rates are less than 100%, we find that no isotopic fractionation occurs during the sublimation process in experiments using adenine as a standard. The ToF-MS provides exact mass, allowing for the formula of unknown compounds to be determined. Compounds are additionally identified by comparing the exact mass and retention time of an unknown to a known standard that is run under the same conditions.

Table 1. Sublimation recovery rates and instrument sensitivities (mol/L).

Compound	Sublimation	ToF-MS	IRMS
Cytosine	93%	1.5×10^{-12}	5.4×10^{-3}
Uracil	80%	5.0×10^{-11}	3.1×10^{-3}
Xanthine	85%	3.0×10^{-12}	3.8×10^{-3}
Guanine	86%	1.5×10^{-11}	8.8×10^{-3}
Hypoxanthine	86%	1.0×10^{-12}	3.9×10^{-3}
Thymine	96%	3.0×10^{-12}	3.7×10^{-3}
Adenine	96%	3.0×10^{-12}	4.4×10^{-3}

Table 2. Recovery of Nucleobases from Murchison Meteorite Formic Acid Extracts [3-4].

Nucleobase	Amount (ppb)
Adenine	267
Cytosine	<30,000
Thymine	<3
Guanine	234
Uracil	63
Hypoxanthine	215
Xanthine	530

Preliminary data from ^{15}N isotopic analysis of nucleobase standards (Fig. 2) indicate that reasonable precision (± 2‰) can be achieved with ~5 nmol of nucleobase on column. Because bulk labile organics in Murchison have been shown to have significant enrichment in ^{15}N with respect to terrestrial organics, this is an acceptable standard deviation in determining whether these compounds are extraterrestial in origin.

Based on previous studies of nucleobase abundance in Murchison meteorite from previous sublimation studies (Table 2) and IRMS sensitivity data (Table 1), we will need 15 grams of Murchison to get 3-4 replicate measurements.

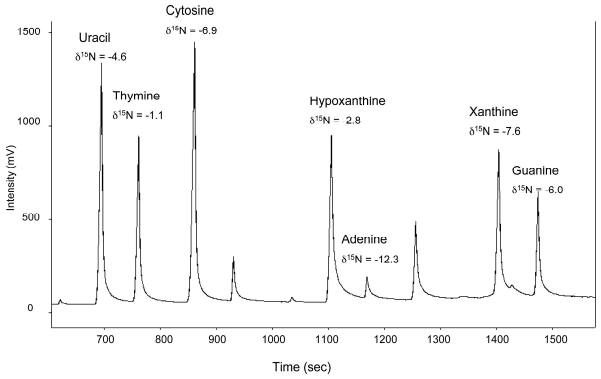

Fig. 2. GC-C-IRMS chromatogram of an injection of ~5 nmol of nucleobases with $\delta^{15}N$ values. Standard deviations are all less than 2‰.

Conclusions: In order to definitively determine the origin of the nucleobases found in meteorites, compound-specific isotopic information is needed. The coupling of LC-ToF analysis for identification and quantification of these compounds with ^{15}N analysis by GC-C-IRMS is a powerful tool that will shed light on the origins of nucleobases in meteorites and extraterrestrial samples.

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