

ISOLATION OF PURINES AND PYRIMIDINES FROM THE MURCHISON METEORITE USING SUBLIMATION. D. P. Glavin¹, and J. L. Bada², ¹NASA Goddard Space Flight Center, Code 915, Greenbelt, MD 20771. ²Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093.

Introduction: The origin of life on Earth, and possibly on other planets such as Mars, would have required the presence of liquid water and a continuous supply of prebiotic organic compounds. The exogenous delivery of organic matter by asteroids, comets, and carbonaceous meteorites could have contributed to the early Earth's prebiotic inventory by seeding the planet with biologically important organic compounds [1]. A wide variety of prebiotic organic compounds have previously been detected in the Murchison CM type carbonaceous chondrite including amino acids, purines and pyrimidines [2]. These compounds dominate terrestrial biochemistry and are integral components of proteins, DNA and RNA.

Several purines including adenine, guanine, hypoxanthine, and xanthine, as well as the pyrimidine uracil, have previously been detected in water or formic acid extracts of Murchison using ion-exclusion chromatography and ultraviolet spectroscopy [3,4]. However, even after purification of these extracts, the accurate identification and quantification of nucleobases is difficult due to interfering UV absorbing compounds [3]. In order to reduce these effects, we have developed an extraction technique using sublimation [5] to isolate purines and pyrimidines from other non-volatile organic compounds in Murchison acid extracts.

Sample Preparation and Sublimation

Experiments: A powdered sample of the Murchison meteorite (104 mg) was sealed in a clean test tube with 1 mL of 95% formic acid (Sigma-Aldrich) and incubated in a heating block set at 100°C for 24 h. As a control, 100 mg of crushed serpentine that had been heated in air at 500°C for 3 h was processed similarly. Half of the formic acid extract was dried under vacuum, re-dissolved in double-distilled (dd) 0.01 M HCl and analyzed for purines and pyrimidines via HPLC separation with detection by UV absorption ($\lambda = 260$ nm). The remaining formic acid extracts were then sealed separately under 0.5 Torr air in a quartz glass sublimation apparatus and heated in a tube furnace set at 450°C for 5 min. A cold finger, attached to the sublimation tube was kept in liquid nitrogen throughout the entire experiment. After sublimation was complete, the apparatus was removed from the furnace and opened to atmospheric pressure. The residue on the end of the coldfinger was rinsed with 0.01 M HCl, and the resulting solution was analyzed by HPLC. In addition, a Murchison meteorite sample (105 mg) that had not been extracted

in formic acid, was heated directly inside the sublimation apparatus.

HPLC Results and Discussion: Prior to sublimation heating, the Murchison formic acid extract eluted as several small HPLC peaks with retention times similar to adenine, guanine, hypoxanthine, and xanthine, and possibly uracil (Fig. 1a). A large unidentified peak in the chromatogram with a retention time of ~ 5 min and showing significant tailing, made it difficult to accurately quantify these nucleobases, especially uracil, in the Murchison formic acid extract. However, this large non-volatile organic component was removed after sublimation of the Murchison formic acid extract at 450°C and peaks corresponding to adenine, hypoxanthine, xanthine and uracil were readily identified (Fig. 1c). We did not detect any guanine after sublimation at this temperature, and although there are no apparent structural reasons for the low sublimation recoveries of guanine relative to other purines such as adenine, this finding is consistent with earlier reports [5,6].

We were unable to identify any purines or pyrimidines on the cold finger after heating the Murchison meteorite sample directly at 450°C (Fig. 1b). This result is surprising since all of these nucleobases, with the exception of guanine, have previously been sublimed from a pure standard mixture at the same temperature with recoveries ranging from 50 to 85% [5]. The presence of a kerogen-like organic polymer in Murchison may inhibit the sublimation of these nucleobases directly from the meteorite [7]. Two small, unidentified peaks (labeled 'X' in Fig. 1b) were detected after direct sublimation of Murchison, however these peaks did not have retention times similar to any of the nucleobases tested. Because peaks with a similar retention time were not detected in a serpentine blank that was sublimed at the same temperature (Fig. 1d), these peaks are likely thermal degradation products.

It is important to emphasize that the purines identified in formic acid extracts of Murchison were not detected in water extracts [4]. This suggests that the purines are either bound to other organics, or were produced (e.g. oligomerization of HCN) during acid extraction. Although a previous study has shown that the synthesis of adenine from HCN in acid is highly temperature dependent and inefficient at 100°C [8], we cannot rule out the possibility that some purines may have been synthesized during formic acid

extraction of Murchison. We found that in previous formic acid extraction and sublimation experiments using pure nucleobase mixtures, thermal deamination of the nucleobases did not occur [5]. Therefore, the production of hypoxanthine and xanthine by thermal deamination of adenine and guanine during the extraction procedure is very unlikely.

Conclusions: We have identified the purines adenine, hypoxanthine, and xanthine, as well as the pyrimidine uracil after sublimation of the formic acid extract of the Murchison meteorite using HPLC and UV absorption detection. The concentrations of these nucleobases in our extracts ranged from 145 to 356 ppb and are similar to those originally reported by Schwartz and coworkers (see Table 1). These preliminary findings need to be confirmed by gas chromatography mass spectrometry.

The origin of the purines and pyrimidines in Murchison has not yet been clearly established. However, van der Velden and Schwartz note that the large amounts of xanthine and the apparent lack of the pyrimidines cytosine and thymine in Murchison, does not correlate with the distribution of nucleobases found in geological environments on Earth [9]. Moreover, the abundance of extraterrestrial amino acids and the low level of terrestrial amino acid contaminants found in Murchison [10], support the idea that the purines and pyrimidines are indigenous to the meteorite. In the future, nitrogen isotopic measurements of the nucleobases in Murchison should be carried out in order to firmly establish the origin of these compounds.

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Table 1. Recovery of Nucleobases from Murchison Meteorite Formic Acid Extracts (in ppb).

Nucleobase	This Study*	Schwartz [3,4]
Adenine	204	267
Cytosine	< 11	< 30,000
Thymine	< 255	< 3
Guanine	< 16	234
Uracil	145	63
Hypoxanthine	232	215
Xanthine	356	530

* sublimed at 450°C for 5 min

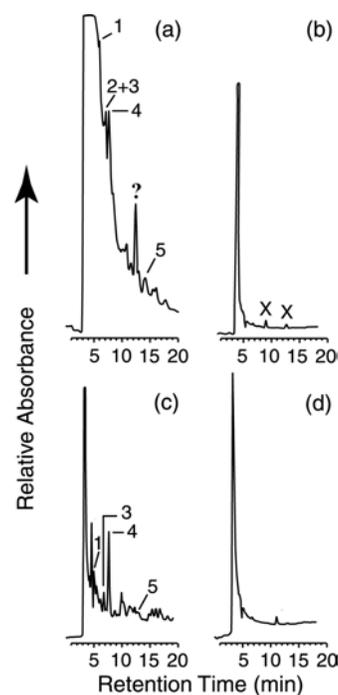


Fig. 1. The 0- to 20-min region (no peaks were observed outside of this time period) of the HPLC chromatograms. Peaks were identified by comparison of the retention times with those of a standard run in parallel, as follows: 1, uracil; 2, guanine; 3, hypoxanthine; 4, xanthine; 5, adenine; and X, unknown. (a): UV absorbance ($\lambda=260$ nm) of the formic acid extract from the Murchison meteorite sample, (b): sublimed unextracted meteorite heated at 450°C for 5 min, (c): the cold finger residue after heating a formic acid extract of the meteorite at 450°C for 5 min, and (d): a serpentine blank carried through the same processing procedures as the Murchison sample. The conditions for purine and pyrimidine separations for the mobile phase at 25°C were as follows: 100 % 0.1 M potassium phosphate buffer (pH = 4.9) and flow rate 1 ml/min on a Phenomenex Jupiter C-18 RP column (4.6 x 250 mm).