

Carbonaceous meteorites contain a wide range of extraterrestrial nucleobases

Michael P. Callahan^{a,1}, Karen E. Smith^b, H. James Cleaves II^c, Josef Ruzicka^d, Jennifer C. Stern^a, Daniel P. Glavin^a, Christopher H. House^b, and Jason P. Dworkin^a

^aNational Aeronautics and Space Administration Goddard Space Flight Center and The Goddard Center for Astrobiology, Greenbelt, MD 20771; ^bDepartment of Geosciences and Penn State Astrobiology Research Center, Pennsylvania State University, 220 Deike Building, University Park, PA 16802; ^cGeophysical Laboratory, Carnegie Institution of Washington, Washington, DC 20015; and ^dScientific Instruments Division, Thermo Fisher Scientific, Somerset, NJ 08873

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All terrestrial organisms depend on nucleic acids (RNA and DNA), which use pyrimidine and purine nucleobases to encode genetic information. Carbon-rich meteorites may have been important sources of organic compounds required for the emergence of life on the early Earth; however, the origin and formation of nucleobases in meteorites has been debated for over 50 y. So far, the few nucleobases reported in meteorites are biologically common and lacked the structural diversity typical of other indigenous meteoritic organics. Here, we investigated the abundance and distribution of nucleobases and nucleobase analogs in formic acid extracts of 12 different meteorites by liquid chromatography–mass spectrometry. The Murchison and Lonewolf Nunataks 94102 meteorites contained a diverse suite of nucleobases, which included three unusual and terrestrially rare nucleobase analogs: purine, 2,6-diaminopurine, and 6,8-diaminopurine. In a parallel experiment, we found an identical suite of nucleobases and nucleobase analogs generated in reactions of ammonium cyanide. Additionally, these nucleobase analogs were not detected above our parts-per-billion detection limits in any of the procedural blanks, control samples, a terrestrial soil sample, and an Antarctic ice sample. Our results demonstrate that the purines detected in meteorites are consistent with products of ammonium cyanide chemistry, which provides a plausible mechanism for their synthesis in the asteroid parent bodies, and strongly supports an extraterrestrial origin. The discovery of new nucleobase analogs in meteorites also expands the prebiotic molecular inventory available for constructing the first genetic molecules.

Meteorites provide a record of the chemical processes that occurred in the solar system before life began on Earth. Carbonaceous chondrites are a rare class of meteorite and are composed of various groups (e.g., CI group, CM group, and CR group) according to their composition and petrography. They are known to contain a diverse suite of organic compounds including many that are essential in contemporary biology (1, 2). Amino acids, which are the monomers of proteins, have been extensively studied in meteorites. An extraterrestrial origin for most of the amino acids detected in carbonaceous chondrites has been firmly established based on three factors: the detection of racemic amino acid mixtures (i.e., equal mixtures of D and L amino acids), wide structural diversity (including the presence of many nonprotein amino acids that are rare or nonexistent in the biosphere), and nonterrestrial values for compound-specific deuterium, carbon, and nitrogen isotope measurements (3–13). In contrast to amino acids, nucleobases in meteorites have been far less studied.

Nucleobases are substituted one-ring (pyrimidine) or two-ring (purine) nitrogen heterocyclic compounds that serve as the structural basis of information storage in RNA and DNA and, for a variety of reasons, are believed to have been essential for the origin and early evolution of life (14). The analysis of nucleobases in meteorites has been ongoing since the early 1960s (2). Determining the origin of nucleobases in meteorites has been challenging due to their low abundances relative to many other organics,

meteorite heterogeneity, experimental artifacts, and terrestrial contamination. To date, all of the purines (adenine, guanine, hypoxanthine, and xanthine) and the one pyrimidine (uracil) reported in meteorites (15–18) are biologically common and could be explained as the result of terrestrial contamination. Martins et al. performed compound-specific stable carbon isotope measurements for uracil and xanthine in the Murchison meteorite (19) and interpreted the isotopic signatures for these nucleobases as nonterrestrial. However, other meteoritic coeluting molecules (e.g., carboxylic acids known to be extraterrestrial) could have contributed to the $\delta^{13}\text{C}$ values for these nucleobases. Furthermore, there have been no observations of stochastic molecular diversity of purines and pyrimidines in meteorites, which has been a criterion for establishing extraterrestrial origin of other organic compound classes. Thus, an extraterrestrial origin for nucleobases detected in carbonaceous chondrites has never been established unequivocally, nor has the detection of nucleobases been demonstrated in more than a handful of meteorites.

We analyzed the formic acid extracts of Orgueil (CI1), Meteorite Hills (MET) 01070 (CM1), Scott Glacier (SCO) 06043 (CM1), Allan Hills (ALH) 83100 (CM1/2), Lewis Cliff (LEW) 90500 (CM2), Lonewolf Nunataks (LON) 94102 (CM2), Murchison (CM2), Grosvenor Mountains (GRO) 95577 (CR1), Elephant Moraine (EET) 92042 (CR2), Graves Nunataks (GRA) 95229 (CR2), Queen Alexandra Range (QUE) 99177 (CR3), and the Almahata Sitta meteorite fragment #4 (ureilite) by liquid chromatography–mass spectrometry using both triple quadrupole detection and high resolution, accurate mass orbitrap detection. Despite the great potential of high resolution mass spectrometry to investigate highly complex samples, these techniques have rarely been applied to the study of carbonaceous chondrites (1). To our knowledge, with the exception of Murchison and Orgueil, these meteorites have not been examined for nucleobases and nucleobase analogs.

Results and Discussion

We investigated the abundance and distribution of nucleobases and nucleobase analogs in 11 different carbonaceous chondrites from three different groups (CI, CM, and CR) representing the entire range of aqueous alteration (types 1, 2, and 3) and one ureilite meteorite. Eleven out of the 12 meteorites studied contained at least the nucleobase adenine. The three CM2 carbonac-

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¹To whom correspondence should be addressed. E-mail: michael.p.callahan@nasa.gov.

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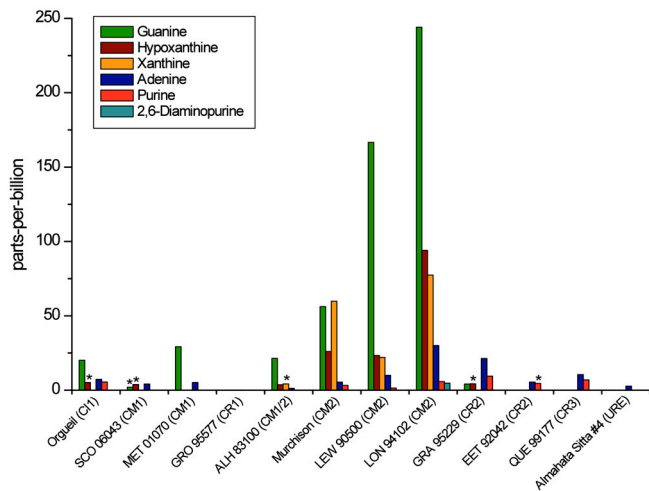


Fig. 1. Distribution of guanine, hypoxanthine, xanthine, adenine, purine, and 2,6-diaminopurine in 11 carbonaceous chondrites and one ureilite. The three CM2 carbonaceous chondrites in this study (Murchison, LEW 90500, and LON 94102) contained significantly higher (approximately 4x to 12x) abundances of purine nucleobases as well as greater structural diversity. The * represents a tentative assignment. The meteorites are roughly ordered by increasing aqueous alteration (*Right to Left*) as determined using mineralogical and isotopic evidence (38–41). The relative degree of aqueous alteration among carbonaceous chondrites within the same group and of the same petrologic type is less certain, although some ordering can be made.

ous chondrites (Murchison, LEW 90500, and LON 94102) particularly stood out because they had the most diverse and abundant set of purines measured (Fig. 1 and *SI Text*). Total purine abundances were 4 to 12 times higher in CM2 carbonaceous chondrites compared to the other meteorites examined. As the degree of aqueous alteration increases in CM carbonaceous chondrites, the overall abundance and diversity of nucleobases decreases as seen in ALH 83100 (CM1/2), SCO 06043 (CM1), and MET 01070 (CM1). Meteorites of other groups (CI and CR) with varying degrees of aqueous alteration (types 1, 2, and 3) also had comparatively less total purine abundance and structural diversity compared to CM2 carbonaceous chondrites. Furthermore, there is an apparent correlation between purine diversity and the diversity of other small organic species, such as amino acids (5), with the maximum diversity found in CM2 carbonaceous chondrites. Interestingly, this correlation does not hold for the abundances of such compounds; for example, CR2 and CR3 meteorites are rich in amino acids and small amines (5, 20, 21) but poor in purines. Based on our measurements, the CM2 carbonaceous chondrites appear to have provided a more favorable environment for the formation and/or preservation of purines compared to other types of carbonaceous chondrites.

Establishing an unambiguous extraterrestrial origin for any biological nucleobase in carbonaceous chondrites is challenging. Unlike meteoritic amino acids, nucleobases are achiral so enantiomeric ratios (i.e., D/L ratios) cannot be used to help distinguish between abiotic and biotic origins. With sample-limited meteorites, compound-specific stable isotope analysis may be impractical or impossible due to the large amount of meteorite material required. Additionally, other meteoritic organics may prevent an unambiguous stable isotope ratio measurement despite extensive sample cleanup and chromatography (19). Indigenous organic compounds in meteorites are usually present in structurally homologous series (2); therefore, finding nucleobase analogs not typically found in terrestrial biochemistry would strongly support an extraterrestrial origin for these canonical nucleobases because they are often produced concurrently in abiotic syntheses.

Purine, 2,6-diaminopurine, and 6,8-diaminopurine were unambiguously identified in LON 94102 (Fig. 2) and a larger extract of Murchison by their chromatographic retention time, accurate mass spectrum (including accurate mass measurements on multiple fragmentation products), and coinjection with standards (resulting in the detection of a single peak) (22). Additionally, two different formic acid extracts of LON 94102 were analyzed on three different liquid chromatography–mass spectrometry instruments (one triple quadrupole and two Orbitraps) in two separate laboratories [National Aeronautics and Space Administration (NASA) Goddard Space Flight Center and Thermo Scientific], which all produced similar results. Purine and 6,8-diaminopurine were identified (by their chromatographic retention time and an accurate mass measurement for the parent mass) in several other meteorites as well (Fig. 1 and *SI Text*). This demonstrates that both purine and 6,8-diaminopurine are widely distributed in carbonaceous chondrites, particularly in CM2 and CR2 meteorites, and provides additional support that purines found in these meteorites are indigenous and not terrestrial contaminants. Aside from one report of 2,6-diaminopurine occurring in cyanophage S-2L (23), these three purines are rare or absent in terrestrial biology. Studies of 8-aminoadenosine, as a potential cancer therapeutic, have shown that this compound is known to inhibit transcription by multiple mechanisms (24) so that the presence of 6,8-diaminopurine (8-aminoadenine) in meteorites is highly unlikely to be the result of terrestrial biological contamination.

All of the purines observed in Murchison and LON 94102 (i.e., adenine, guanine, hypoxanthine, xanthine, purine, 2,6-diaminopurine, and 6,8-diaminopurine) were also generated from aqueous reactions of NH_4CN (see *SI Text*). Adenine (normalized to 1) was the most abundant nucleobase in the formic acid extracted NH_4CN samples followed by purine (0.79), hypoxanthine (0.23), 6,8-diaminopurine (0.07, assuming the same response factor as 2,6-diaminopurine), 2,6-diaminopurine (0.05), guanine (0.02), and xanthine (0.01). Although the relative abundances of these purines are different than those detected in carbonaceous chondrites, this may be attributable to the extensive aqueous and energetic processing the asteroid parent bodies have undergone during their approximately 4.5 billion-year history (25) compared to the NH_4CN reactions. The presence of hydrogen cyanide and ammonia as synthetic precursor molecules has been deduced in hydrated carbonaceous chondrites based on the presence of α -amino acids, α -hydroxy acids, and iminodicarboxylic acids reported in the Murchison meteorite, which supports a Strecker-type synthesis requiring hydrogen cyanide and ammonia (13, 26–29). Additionally, abundant ammonia has been detected in the CR2 carbonaceous chondrite GRA 95229 after hydrothermal treatment (30), which was one of the meteorites in our study.

Purine, 2,6-diaminopurine, and 6,8-diaminopurine were not detected (above our parts-per-billion detection limits) in the procedural blanks, nucleobase procedural samples, serpentine control samples, Murchison soil sample (see *SI Text*), or Antarctic ice sample (see *SI Text*), which strongly suggests that these compounds are indigenous to the meteorites. Because adenine, guanine, hypoxanthine, and xanthine were observed in both the soil and Antarctic ice samples (though at different ratios and lower abundances than observed in meteorites; see *SI Text*), it could still be argued that these nucleobases are the result of terrestrial contamination. On the other hand, these same nucleobases are also synthesized concurrently with purine, 2,6-diaminopurine, and 6,8-diaminopurine in reactions of NH_4CN . Furthermore, the distributions of purines measured in the nine Antarctic meteorites appear to correlate with meteorite petrology and the extent of parent body alteration rather than with the content of the terrestrial environments from which they were recovered, arguing against terrestrial contamination from the ice. Based on the elevated abundances of these compounds in the meteorites compared with terrestrial sources, we propose that the adenine,

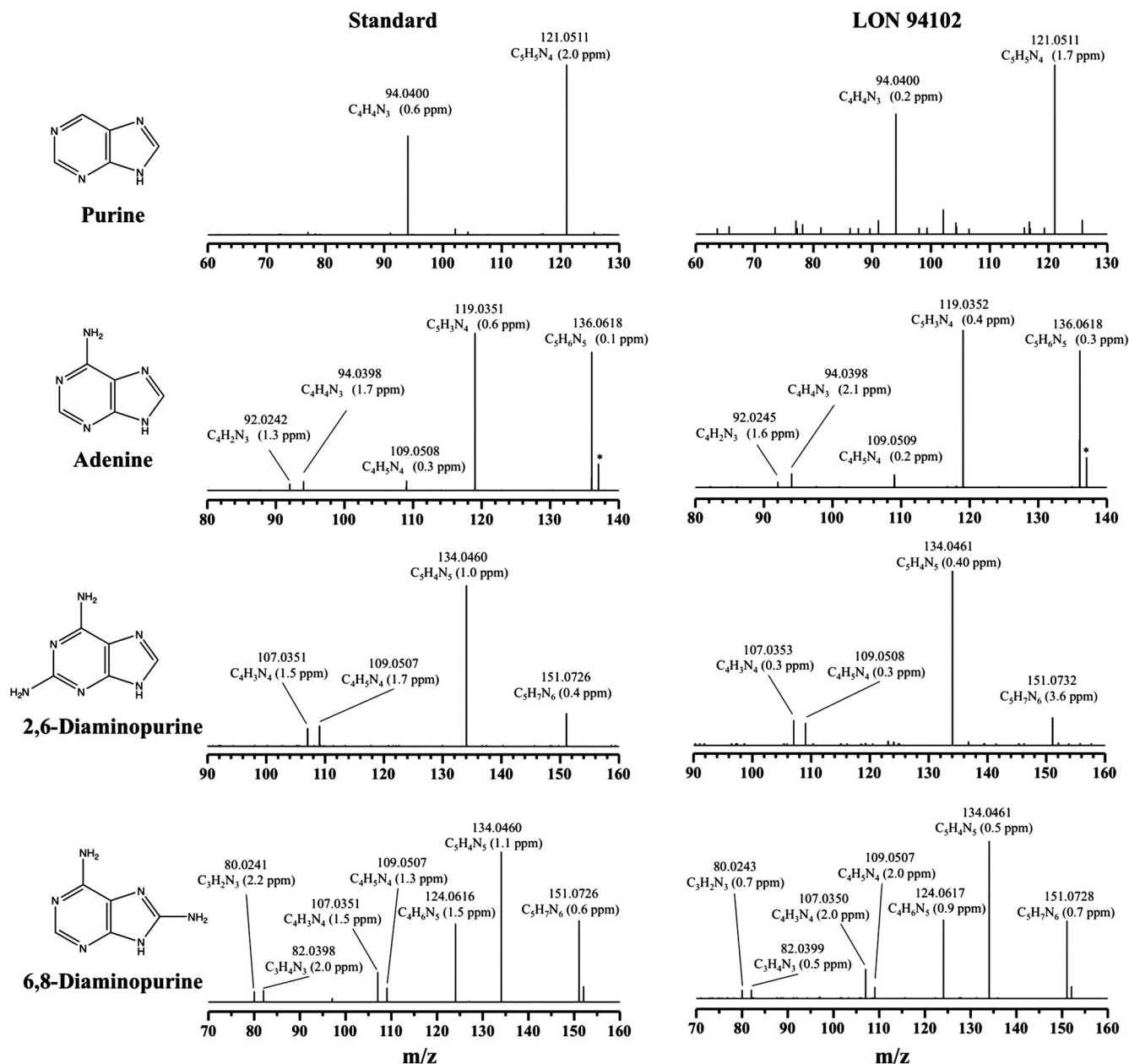


Fig. 2. Mass-selected fragmentation spectra of reference standards (left spectra) and compounds found in the meteorite LON 94102 (right spectra) measured on an LTQ Orbitrap XL hybrid mass spectrometer using an HCD (higher energy collision dissociation) setting of 90 to 100%. Purine, adenine, 2,6-diaminopurine, and 6,8-diaminopurine were identified using accurate mass measurements on the parent mass and multiple fragment masses and chromatographic retention time. Mass accuracy of less than 5 ppm allows for the unambiguous assignment of elemental formulae. The * represents inferences in the fragmentation spectra that are present in both the meteorite and reference standard spectra.

guanine, hypoxanthine, and xanthine observed in CM2 meteorites are largely extraterrestrial, but could potentially also contain traces of terrestrial contamination.

The presence of extraterrestrial purines in meteorites has far-reaching implications. The first cellular systems on the early Earth were presumably assembled from three components: nucleic acids, proteins, and cell membranes (31). Potential molecular subunits for constructing all of these macromolecular species (e.g., amino acids, amphiphilic compounds, and from this study—a variety of purine nucleobases) have been identified in meteorites and appear to be indigenous. Thus, meteorites may have served as a molecular kit providing essential ingredients for the origin of life on Earth and possibly elsewhere.

The identification of purine, 2,6-diaminopurine, and 6,8-diaminopurine in the meteorites Murchison and LON 94102

also expands the inventory of nucleobases that could have been available during the origins of life. The stability of 2,6-diaminopurine is similar to that of adenine, guanine, and xanthine and the accumulation of these compounds on the early Earth may have been possible (32). Furthermore, 2,6-diaminopurine can base pair with uracil (or thymine), and the additional amino group permits the formation of three hydrogen bonds (33). Because meteorites may have provided a significant source of prebiotic organic material including purines, it is plausible that alternative nucleobases such as 2,6-diaminopurine, 6,8-diaminopurine, xanthine, and hypoxanthine were available for constructing the first genetic molecules. It has been proposed that an “expanded genetic alphabet” was present, and perhaps required, in the RNA World (34); conversely an all-purine primitive RNA has also been proposed (35).

Materials and Methods

We employed a targeted approach for analysis that focused on the five canonical RNA/DNA nucleobases (adenine, guanine, cytosine, thymine, and uracil) as well as 17 nucleobase analogs (see *SI Text*), which have been synthesized under plausible prebiotic conditions in the laboratory (with the exception of 3,7-dimethylxanthine; theobromine) (36, 37). Meteorite analysis was carried out using either a Waters 2695 high performance liquid chromatograph (LC) coupled to a Waters 2996 photodiode array detector and Waters Quattro Micro API triple quadrupole mass spectrometer operating in multiple reaction monitoring (MRM) mode or a Thermo Scientific Accela LC coupled to a LTQ Orbitrap XL hybrid mass spectrometer. Typically, initial screening and quantitation of compounds was performed by the LC-triple quadrupole mass spectrometer in MRM mode while unambiguous structural confirmation was obtained using the LC-Orbitrap mass spectrometer, which permits high mass resolution (approximately 60,000 for our target masses) and excellent mass accuracy ($\ll 5$ ppm). Experimental details are provided in *SI Text*.

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