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Abundant extraterrestrial purine nucleobases in the Murchison meteorite: Implications for a unified mechanism for purine synthesis in carbonaceous chondrite parent bodies

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ABSTRACT

Extraterrestrial nucleobases are of key interest owing to their implications for the chemical evolution of primordial molecules in the solar system and their potential contributions to prebiotic chemistry on the early Earth. Recent advances in analytical techniques capable of detecting femto-mole-order nucleobases have allowed us to identify all five exogenous nucleobases—uracil, cytosine, thymine, adenine, and guanine—in carbonaceous chondrites and to quantify uracil obtained from the carbonaceous asteroid (162173) Ryugu. In this study, we optimized the analytical method using hot-water extraction followed by 6 M hydrochloric acid (HCl) extraction from a sample of the CM2 Murchison meteorite to reassess the abundances and distributions of extraterrestrial nucleobases. The target analyses performed using high-performance liquid chromatography paired with electrospray-ionization, high-resolution mass spectrometry revealed that purine nucleobases were substantially more enriched in the subsequent 6 M HCl extract (951 ± 104 parts per billion, ppb) than in the hot-water extract (199 ± 3 ppb). The most abundant nucleobase was guanine (649 ± 103 ppb in total). The 6 M HCl extract not only contained canonical nucleobases but also included rare nucleobase analogs from the purine family, such as 8-substituted purines. Unlike purines, we preferentially detected pyrimidine nucleobases in the hot-water extract and the acid hydrolysate (185 ± 17 ppb and 297 ± 5 ppb, respectively) rather than in the 6 M HCl extract (51 ± 4 ppb). The disparate distributions of purine and pyrimidine bases in the Murchison meteorite suggests that purines are more robustly incorporated than pyrimidines into meteoritic matrices and/or insoluble macromolecular organic matter. We propose a unified formation mechanism for purine nucleobases—which involves the synthesis of 5-aminoimidazole derivatives from hydrogen cyanide and its related molecules—that can account for the molecular distributions of the extraterrestrial purine nucleobases found in the Murchison meteorite.

1. Introduction

Nucleobases, which are nitrogen (N)-containing heterocyclic compounds, are essential components of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) that carry and transfer genetic information crucial for all life on Earth. They are subdivided into two principal categories: pyrimidine nucleobases, including uracil ($C_4H_4N_2O_2$), cytosine ($C_4H_5N_3O$), and thymine ($C_5H_6N_2O_2$), which have a single six-membered N-heterocyclic ring; and purine nucleobases adenine ($C_5H_5N_5$) and guanine ($C_5H_5N_5O$), which are characterized by a six-membered ring and a five-membered ring, for a total of two N-heterocyclic rings. Although xanthine ($C_5H_4N_4O_2$) and hypoxanthine ($C_5H_4N_4O$) are not present in nucleic acids, they are crucial intermediates in the biochemical synthesis and degradation of purine nucleotides.

The aforementioned pyrimidine and purine nucleobases have been identified successfully in carbonaceous chondrites using a combination of gas or liquid chromatography and mass spectrometry (Stoks and Schwartz, 1979, 1981; Martins et al., 2008; Oba et al., 2022). The isotopic compositions of organic compounds in carbonaceous meteorites

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are strongly indicative of their extraterrestrial origin (Pizzarello et al., 2006). However, with respect to meteoritic nucleobases, the carbon isotope ratios ($\delta^{13}C$) have only been measured once previously (for uracil and xanthine, Martins et al., 2008) and their nitrogen and hydrogen isotopic compositions ($\delta^{15}N$ and δD, respectively) remain undetermined. Consequently, the extraterrestrial origin of meteoritic nucleobases has been evaluated based on their molecular distributions (i.e., differences in relative abundances compared with those of terrestrial life) and the identification of unusual purine and pyrimidine bases (e.g., purine, 2,6-diaminopurine, 6,8-diaminopurine, isocytosine, 1-methyluracil, and 6-methyluracil), which are rare in the biosphere (Callahan et al., 2011; Oba et al., 2022). The detection of a wide variety of indigenous nucleobases in carbonaceous chondrites highlights their cosmochemical importance. These extrateresstial materials are potentially ubiquitous sources of the components of genetic material during the formation of the solar system and might play a pivotal role in the exogenous delivery to the early Earth (Chyba and Sagan, 1992).

Recent advances in analytical methods involving high-performance liquid chromatography paired with electrospray-ionization, high-resolution mass spectrometry (HPLC/ESI-HRMS) have facilitated the efficient separation of nucleobases from their structural isomers in complex mixtures, allowing for detection of nucleobases based on exact mass (sufficient for determining their chemical formulas) at levels of parts per billion (ppb) to parts per trillion (ppt) (Oba et al., 2019, 2022). Thymine and cytosine were first identified in carbonaceous meteorites using this HPLC/HRMS technique for the exact determination of “underivatized formulas” along with the detection of other nucleobases (e.g., uracil, adenine, and guanine) and various N-heterocyclic compounds, including imidazoles and nicotinic acids (Oba et al., 2022). To date, samples from the carbonaceous asteroid (162173) Ryugu have provided us with a valuable opportunity to investigate organic molecular evolution, including a wide variety of amino acids, nucleobases, carboxylic acids, amines, polyaromatic cyclic hydrocarbons (PAHs), and other prebiotic molecular signatures (Naraoka et al., 2023; Parker et al., 2023; Oba et al., 2023a, 2023b; Aponte et al., 2023; Yoshimura et al., 2023). We expect that other novel organic molecules will be identified in further surveys from the group of approximately 20,000+ molecular species within the spatial heterogeneity (Schmitt-Kopplin et al., 2023; Hashiguchi et al., 2023). Among these N-heterocyclic molecules from the Ryugu aggregate samples (A0106 & C0107), Oba and coworkers have succeeded in the qualitative identification of uracil, imidazole carboxylic acids (structural isomers of uracil), and nicotinic acid (commonly known as niacin, a B3 vitamin) (Oba et al., 2023a).

The wet-chemistry processes used for extraction and purification are as crucial as the analytical methods employed for the effective recovery of organic molecules from meteorite samples. In the past decade, the extraction and purification procedures for meteoritic amino acids have largely been standardized across different laboratories (Pizzarello et al., 2012; Chan et al., 2016; Koga and Naraoka, 2017; Glavin et al., 2020; Koga et al., 2021; Naraoka et al., 2023). These procedures involve the extraction of powdered meteorite samples with ultrapure water at 100 °C–110 °C for more than 12 h (commonly termed “hot-water extraction”) and then purifying the extracts using a cation-exchange column. To the best of our knowledge, nucleobases in meteorites have been extracted and purified using several different procedures in previous studies (e.g., Stoks and Schwartz, 1979; Shimoyama et al., 1990). Various types of purine bases were initially identified in formic acid (HCOOH) extracts that were heated at 100 °C for 24 h and purified using solid-phase extraction (Callahan et al., 2011). Subsequent work (Oba et al., 2022) demonstrated that extraction and purification procedures using water or 2% hydrochloric acid (HCl) at room temperature (~25 °C) could recover not only purine bases but also pyrimidine bases. Furthermore, uracil and N-heterocyclic compounds were detected in the acid-hydrolyzed hot-water extract (heated at 105 °C for 20 h with 6 M HCl) of the Ryugu samples (Oba et al., 2023a). However, the differences in the molecular distributions and abundances of the nucleobases extracted using neutral water or acidic solvents remain unclear.

Considering the aforementioned factors, in this study, we report an optimized analytical method and a representative application using hot-water extraction followed by 6 M HCl extraction from the CM2 Murchison meteorite to investigate the abundances and distributions of extraterrestrial nucleobases in the respective extracts, and to consider their formation mechanisms.

2. Materials and methods

2.1. Chemicals and reagents

Authentic standards for the nucleobases and other N-heterocyclic compounds were purchased from Tokyo Chemical Industry, Sigma-Aldrich, FUJIFILM Wako Pure Chemical, Combi-Blocks, Toronto Research Chemicals, and BLD Pharmatech Ltd. Sodium hydroxide pellets (NaOH, 99.99% trace-metal basis) were obtained from Sigma-Aldrich and dissolved in MilliQ-water (Millipore Milli-Q grade, 18.2 MΩ) to prepare an aqueous NaOH solution. A stock mixed nucleobase solution was prepared by combining individual nucleobase standards dissolved in the NaOH aqueous solution. Ultrapure water and 6 M HCl (Tama Chemicals Co., Ltd., Japan; Tama pure AA-10 grade), 3 M NaOH solution (Kanto Chemical Industry Co., Ltd., Japan; ultrapur^{TM} grade), methanol (FUJIFILM Wako Pure Chemical Corporation, Japan; for Tof-MS grade), and ammonia solution (FUJIFILM Wako Pure Chemical Corporation, Japan; Guaranteed Reagent grade, 28% in water) were obtained for the extraction and purification procedures. Solutions of 1 M and 0.1 M HCl, 1 M NaOH, and 10% NH_4OH were prepared and diluted from the abovementioned solvents using ultrapure water. Ultrapure water, acetonitrile (Tof-MS grade), and formic acid (LC–MS grade; >99.5% purity) were sourced from FUJIFILM Wako Pure Chemical for HPLC/ESI-HRMS analyses.

All the glassware and sea sand (FUJIFILM Wako Pure Chemical Corporation, Japan; 30–50 mesh) used for procedural blanks were rinsed with MilliQ-water, wrapped in aluminum foil, and subsequently heated at 450 °C for 5 h in air prior to use.

2.2. Meteorite samples and sample preparation

The extraction and purification procedures for the meteorite sample are depicted in Fig. 1. A fragment of the CM2 Murchison meteorite (~4 g), devoid of visible evidence of fusion crust, was authenticated and purchased from a meteorite trading company (an official member of the Meteoritical Society & Japan Association of Mineralogical Sciences) for nucleobase analyses. The Murchison meteorite chip was pulverized into fine powder using a pristine agate mortar and pestle, which were cleaned with ultrapure water and Tof-MS grade methanol. We did not perform any sample processing to exclude large chondrules during the pulverization of the meteorite sample. A portion of the powdered sample (1.0 g) was flame-sealed in a glass ampoule with 5 mL of ultrapure water, sonicated for 15 min in an ultrasonic bath, and heated at 110 °C for 12 h for hot-water extraction. The supernatant was separated from the meteorite residue, and half of the hot-water extract was dried and hydrolyzed in ultrapure 6 M HCl at 110 °C for 12 h. Herein, the unhydrolyzed and acid-hydrolyzed hot-water extracts are referred to as “HW (#1)” and “HW-HCl (#2)” extracts, respectively. The meteorite residue remaining after the hot-water extraction was subjected to a second extraction procedure using 3 mL of ultrapure 6 M HCl at 110 °C for 12 h in a flame-sealed glass ampoule. The HCl supernatant was separated from the meteorite residue; hereafter, this supernatant is referred to as the “HCl (#3)” extract. The recovery rates of nucleobases following both hot-water and 6 M HCl extraction were also examined (Supplementary material, Table S1).

The HW (#1), HW-HCl (#2), and HCl (#3) extracts were dried under a stream of nitrogen and redissolved in 0.1 M HCl for performing a desalting procedure using an improved method of cation-exchange

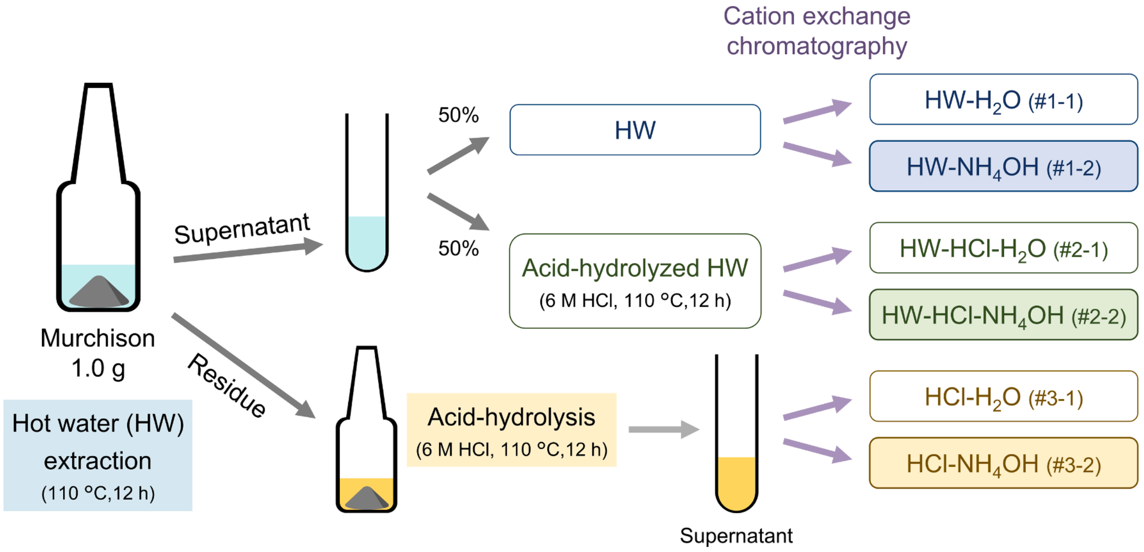

Fig. 1. Flow diagram for the extraction and purification procedures used for the Murchison-meteorite sample in the present study. Here, we note that the carbon and nitrogen isotopic compositions of individual organic molecules (e.g., nitrogen-containing amino groups, imino groups, and N-heterocycles) are consistent before and after the cation exchange chromatography (Takano et al., 2021).

chromatography reported by Takano et al. (2010). In brief, 10 mL of AG 50 W-X8 cation-exchange resin (Bio-Rad Laboratories, Inc.; analytical grade, 200–400 mesh, hydrogen form) was placed in a disposable column and rinsed with solvents in the following order: 30 mL of 1 M HCl, ultrapure water, 1 M NaOH, ultrapure water, 1 M HCl, and ultrapure water. The reconstituted HW, HW-HCl, and HCl extracts were individually loaded onto separate cation-exchange chromatography columns. The cation-exchange resins were washed with 50 mL of ultrapure water to recover acidic, neutral, and weakly basic compounds [referred to as “H_2O fraction (#x-1),” where x corresponds to the number of each extract]. Subsequently, 30 mL of a 10% NH_4OH was loaded onto the H_2O-washed cation-exchange resins to elute basic compounds, including most nucleobases [referred to as “NH_4OH fraction (#x-2)”]. The H_2O fractions were dried and redissolved in methanol to remove excess salts. The methanol-extracted H_2O and NH_4OH fractions of the HW, HW-HCl, and HCl extracts were dried and reconstituted into ultrapure water. Hence, we obtained six fractions from the Murchison meteorite [i.e., HW-H_2O (#1-1), HW-NH_4OH (#1-2), HW-HCl-H_2O (#2-1), HW-HCl-NH_4OH (#2-2), HCl-H_2O (#3-1), and HCl-NH_4OH (#3-2)] (Fig. 1). Simultaneously, we prepared procedural blanks comprising baked sea sand using the same protocol as that applied to the Murchison-meteorite samples; we analyzed the blanks to validate the background signal during each procedure.

2.3. HPLC/ESI-HRMS analyses

The aforementioned six fractions obtained from the Murchison meteorite, the procedural blanks, and authentic standards of the targeted molecules were analyzed using an online HPLC/ESI-HRMS system comprising an UltiMate 3000 and Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ mass spectrometer (Thermo Fischer Scientific Inc., Waltham, MA, USA) with a mass resolution of 140,000 at a mass-to-charge ratio m/z = 200 (e.g., Oba et al., 2022; 2023a). The HPLC instrument was outfitted with a reversed-phase separation column maintained at 40 °C. For the detection and quantification of most purine nucleobases, we employed the following isocratic HPLC eluent program with an Inert-Sustain PFP column (1.0 mm × 250 mm, particle size = 3 μm, GL Sciences Inc., Tokyo, Japan): solvent A (water) and solvent B (acetonitrile + 0.1% formic acid) = 90:10, held for 20 min with a flow rate of 0.05 mL/min. We used a different solvent A (water + 0.1% formic acid) in the ratio A:B = 100:0 or 90:10 for better separation of several purine nucleobases from their structural isomers in the PFP column. For pyrimidine nucleobase analyses, we used the following gradient HPLC eluent program with the HyperCarb™ column (2.1 mm × 150 mm, particle size = 3 μm, Thermo Fischer Scientific Inc., Waltham, MA, USA): solvent A (water + 0.1% formic acid) and solvent B (acetonitrile + 0.1% formic acid) = 100:0 at $t = 0$ min, followed by a linear gradient of A:B = 70:30 at 20 min with a flow rate of 0.2 mL/min.

We then introduced the compound solution separated using either the PFP or HyperCarb™ column into a HESI-II probe (Thermo Fischer Scientific Inc., Waltham, MA, USA) and heated it at 280 °C for desolvation. The spray voltage and capillary temperature of the ion-transfer system were 3.5 kV and 300 °C, respectively. To detect various organic molecules in the HW and HCl extracts, we recorded the mass spectra of the vaporized compounds in the positive ions over an m/z range of 60.5–400, with the mass determined to an accuracy better than 5 ppm, as defined by [(measured m/z) – (calculated m/z)]/(calculated m/z) × 10^6 (ppm). The mass accuracy was occasionally calibrated using the exact masses of protonated tyrosine (m/z = 182.0812), *tert*-butylamine (m/z = 74.0964), and a fragment ion of *tert*-butylamine (m/z = 57.0699). A positive ion with m/z = 83.0604, corresponding to an acetonitrile dimer, was used as the lock mass.

For robust identification and quantification of the nucleobases, we performed the tandem mass spectrometry (MS/MS) experiments using the same ionization conditions as those used for the full-scan analyses. We subjected the targeted positive ions isolated by the quadrupole (using an isolation window of 0.4 m/z) to high-energy collisions with N_2 gas to produce fragmented ions and monitored specific mass ranges using an Orbitrap MS with a mass resolution of 140,000 at m/z = 200. Furthermore, we identified the nucleobases and other targeted compounds in the Murchison-meteorite extracts based on their chromatographic retention times, exact masses, and mass-fragmentation patterns in the MS/MS measurements.

3. Results

3.1. Chromatographic separations and identifications of purine nucleobases

Fig. 2 shows the baseline resolution of the extracted-ion

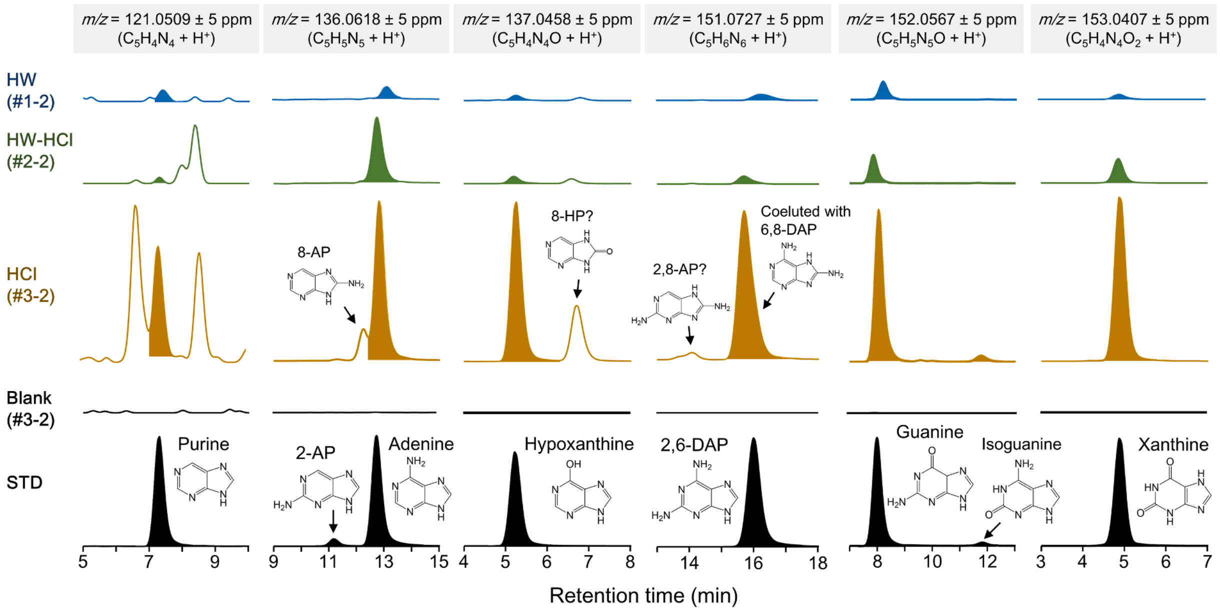

Fig. 2. Purine nucleobases detected in the HW, HW-HCl, and HCl extracts of the Murchison meteorite. Selected regions of the HPLC/HRMS extracted-ion chromatograms for the purine nucleobases in the HW (#1-2), HW-HCl (#2-2), and HCl (#3-2) extracts of the Murchison meteorite. We obtained these results via MS/MS measurements using an InertSustain PFP column and a solvent A without 0.1% formic acid. Peak identifications are as follows: 5–10 min at $m/z = 121.0509$ for purine; 9–15 min at $m/z = 136.0618$ for adenine, 2-aminopurine (2-AP), and 8-aminopurine (8-AP, tentatively identified as shown in Fig. 3); 4–8 min at $m/z = 137.0458$ for hypoxanthine and 8-hydroxypurine (8-HP, tentatively identified based on the presence of other 8-substituted purines); 13–18 min at $m/z = 151.0727$ for 2,6-diaminopurine (2,6-DAP), 6,8-diaminopurine (6,8-DAP, coeluted with 2,6-DAP as shown in Fig. 3), and 2,8-diaminopurine (2,8-DAP, tentatively identified based on its possible structure as an isomer of 2,6-DAP and 6,8-DAP); 7–13 min at $m/z = 152.0567$ for guanine and isoguanine; and 3–7 min at $m/z = 152.0567$ for xanthine. The vertical axis for each mass chromatogram from a Murchison-meteorite sample and from a procedural blank at the same m/z is normalized on the same scale to emphasize the detection of purine nucleobases abundant in the HCl-NH_4OH (#3-2) fraction.

chromatograms of the purine nucleobases obtained from the NH_4OH fractions of HW (#1-2), HW-HCl (#2-2), and HCl (#3-2) from the Murchison meteorite along with those from procedural blanks and authentic standards during the MS/MS measurements. These measurements offer substantial advantages—such as robust identifications based on the fragmentation patterns of the targeted parent ions and the selectivity of the parent ions—which we achieved via the quadrupole using the 0.4 m/z isolation window. This approach helps minimize potential matrix effects during the quantification of targeted molecules within complex organic mixtures, such as those found in meteorite extracts. Under the measurement conditions used in this study, most of the parent ions remained intact, and a linear correlation was confirmed between the peak area of the surviving parent ions and the amounts of nucleobase standards injected. The InertSustain PFP column and solvent A, which lacks 0.1% formic acid, facilitated the optimal separation between adenine and 2-aminopurine as well as between guanine and isoguanine. This is illustrated in the mass chromatograms obtained from authentic standards shown in Fig. 2.

The HPLC/HRMS analyses performed in this study yielded considerably larger chromatographic peaks for the purine nucleobases from the HCl extract (#3-2) than from the HW extracts (#1-2 and #2-2) (Fig. 2). Furthermore, various structural isomers of the targeted purine nucleobases were discernibly detected only in the HCl extract (#3-2). The identification of purine, 2-aminopurine, isoguanine, and 2,6-diaminopurine in the HCl extract demonstrates their extraterrestrial origin considering the rarity of these purine bases in biology. The mass chromatograms of the HCl extract (#3-2) at $m/z = 136.0618$ displayed the presence of another structural isomer of adenine and 2-aminopurine ($C_5H_5N_5$) (possibly 8-aminopurine, as discussed later). Chromatographic peaks corresponding to structural isomers of hypoxanthine ($C_5H_4N_4O$) or 2,6-diaminopurine ($C_5H_6N_6$) were also visible in the mass chromatograms at $m/z = 137.0458$ or 151.0727, respectively, using the aforementioned HPLC condition (potentially 8-hydroxypurine and 2,8-diaminopurine, respectively, as described later).

The HCl extract (#3-2) from the Murchison meteorite sample was analyzed using an alternate solvent A (water + 0.1% formic acid) to achieve better separation of the purine nucleobases and their structural isomers using the PFP column (Fig. 3). Fig. 3a displays the mass chromatograms of the parent ion ($m/z = 151.0727$, corresponding to $[C_5H_6N_6 + H]^+$) and the daughter ion ($m/z = 124.0618$, corresponding to $[C_4H_5N_5 + H]^+$) obtained via MS/MS measurements performed on the HCl extract and an authentic 2,6-diaminopurine standard. This fragmentation pattern can be explained by the dissociation of HCN from the parent ion on their chemical formulas. Moreover, the aforementioned daughter ion ($m/z = 124.0618$) was not detected in the 2,6-diaminopurine standard. Conversely, the structural isomer of 2,6-diaminopurine in the HCl extract (with a retention time of 14.1 min in Fig. 3a) did exhibit the daughter ion at $m/z = 124.0618$. This particular fragmentation pattern for the 2,6-diaminopurine isomer was previously reported by Callahan et al. (2011), demonstrating that the daughter ion at $m/z = 124.0618$ is unique to the parent ion 6,8-diaminopurine (depicted as $C_4H_6N_5$ in Fig. 2 of Callahan et al., 2011). Thus, although an authentic 6,8-diaminopurine standard was not available in this study, the chromatographic peak observed at 14.1 min in the HCl extract was likely derived from 6,8-diaminopurine. Consequently, the prominent chromatographic peak at $m/z = 151.0727$ observed in the HCl extract in Fig. 2 (at 15.7 min) probably resulted from the coelution of 2,6-diaminopurine and 6,8-diaminopurine. Considering that the structural isomers of purine nucleobases have functional groups at positions 2, 6, and 8, the minor chromatographic peak at $m/z = 151.0727$ eluting at 14.1 min in Fig. 2 and 11.3 min in Fig. 3 may correspond to 2,8-diaminopurine.

Similar to the separation of 2,6-diaminopurine and 6,8-diaminopurine, the HPLC conditions involving the use of solvent A with 0.1% formic acid enabled excellent separation of adenine and its structural isomers (Fig. 3b). An authentic 2-aminopurine standard exhibited the mass-fragmentation pattern from the parent ion at $m/z = 136.0618$ ($C_5H_5N_5 + H^+$) to the daughter ion at $m/z = 109.0509$ ($C_4H_4N_4 + H^+$). This daughter ion was detected in the HCl extract at the same retention

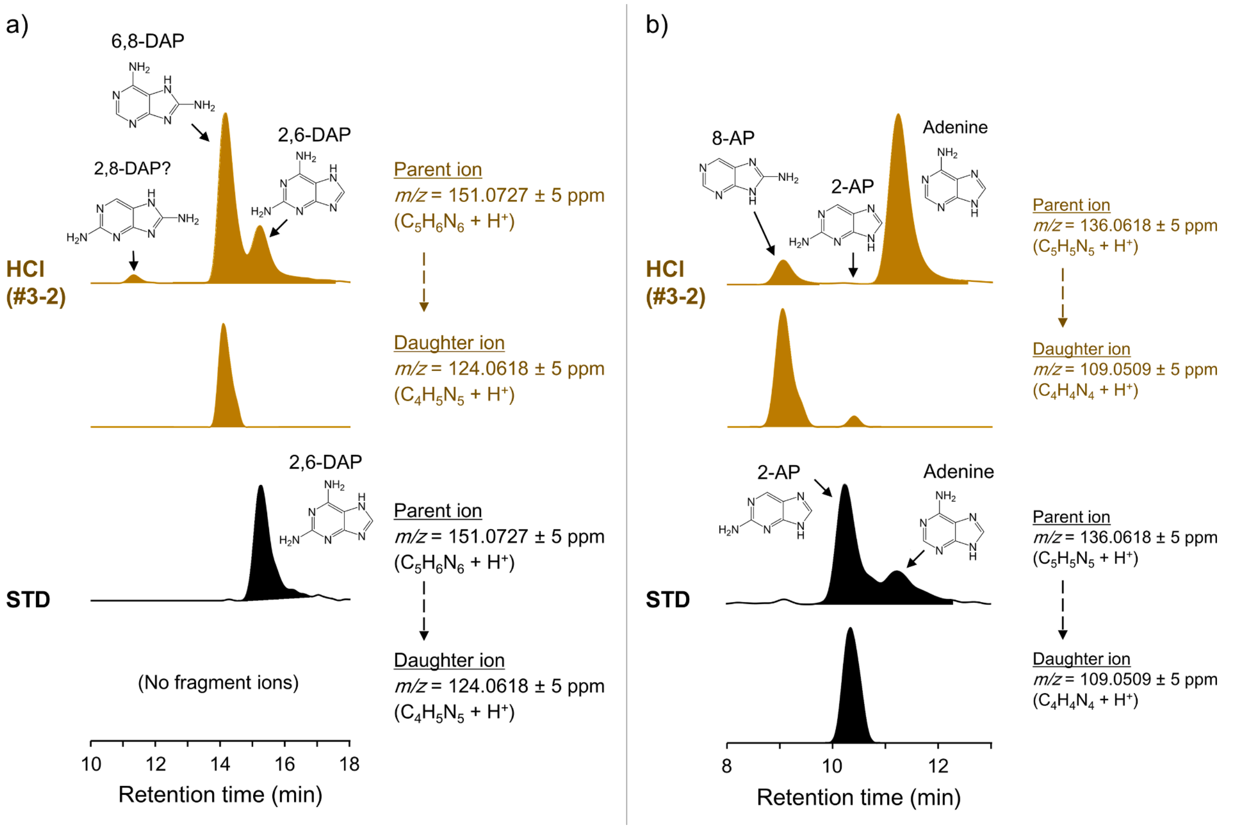

Fig. 3. Structural isomers of unusual purine nucleobases discovered in the HCl extract of the Murchison meteorite. Selected regions of the HPLC/HRMS extracted-ion chromatograms of a) $C_5H_6N_6$ purine isomers and b) $C_5H_5N_5$ purine isomers in the HCl extract (#3-2). We obtained these results from MS/MS measurements using an InertSustain PFP column and a solvent A containing 0.1% formic acid. Peak identifications are as follows: a) 10–18 min at $m/z = 151.0727$ (parent) and 124.0618 (daughter) for 2,6-diaminopurine (2,6-DAP) and its tentatively identified isomers [6,8-diaminopurine (6,8-DAP) and 2,8-diaminopurine (2,8-DAP)]; and b) 8–13 min at $m/z = 136.0618$ (parent) and 109.0509 (daughter) for adenine, 2-aminopurine (2-AP), and their tentatively identified isomer [8-aminopurine (8-AP)].

time as the standard, indicating the presence of 2-aminopurine in the Murchison sample. However, the peak area of 2-aminopurine at $m/z = 136.0618$ was significantly smaller than those of adenine and another chromatographic peak that determined for a retention time of 9.0 min. Given the positioning of the functional groups in the structural isomers of adenine and 2-aminopurine, the unidentified peak that appeared at a retention time at 9.0 min is likely to be derived from 8-aminopurine. Consequently, the order of abundance in the purine isomers ($C_5H_5N_5$) is as follows: adenine (i.e., 6-aminopurine) > 8-aminopurine ≫ 2-aminopurine. Because hypoxanthine (i.e., 6-hydroxypurine) can be produced via the hydrolysis of adenine, the relative abundances of hypoxanthine structural isomers may display trends similar to those of adenine isomers. If this hypothesis is correct, the unidentified peak at $m/z = 137.0458$ in Fig. 2 may originate from 8-hydroxypurine.

Furthermore, we were able to separate xanthine (i.e., 2,6-dihydroxypurine) and its two structural isomers (possibly 6,8-dihydroxypurine and 2,8-dihydroxypurine) from the HCl extract using the HPLC condition, with solvent A containing 0.1% formic acid (Fig. S1). The presence of xanthine isomers in the Murchison meteorite sample may be partially supported by laboratory experiments in which three purine species bearing two OH moieties were synthesized via ultraviolet photoirradiation of purine with H_2O in interstellar-ice analogs (Materese et al., 2017). While we could not identify several structural isomers of purine nucleobases, such as 6,8-diaminopurine and 8-aminopurine, due to the lack of authentic standards, these biologically-rare purine molecules were absent in the procedural blanks, indicating their extraterrestrial origin.

3.2. Purine nucleobase abundances in the HW and HCl extracts

The concentrations of purine nucleobases in the HW (#1-2), HW-HCl (#2-2), and HCl (#3-2) extracts of the Murchison meteorite sample are presented in Table 1. The total extractable abundance of purine nucleobases in the Murchison meteorite sample was determined to be 1305 ± 104 ppb (parts per billion, equivalent to $ng \cdot g^{-1}$), approximately nine times higher than values reported in previous studies (150 ppb by Callahan et al., 2011, and 152 ppb by Oba et al., 2022). The sum of the purine nucleobases in each extract varied from 90 ± 10 ppb in the HW extract (#1-2) to 1090 ± 104 ppb in the HCl extract (#3-2). The most abundant purine nucleobase in the HCl extract was guanine (542 ± 103 ppb); its abundance significantly exceeded those reported in previous studies that used different extraction procedures [for instance, water extraction at room temperature: 72 ppb (Oba et al., 2022); 95% formic-acid extraction at 100 °C: 56 ppb (Callahan et al., 2011)]. The relative abundances of the purine nucleobases in the HCl extract compared with the total extractable abundance (i.e., (HCl)/(HW-HCl + HCl)] ranged from 70% for adenine to 92% for hypoxanthine, suggesting that a majority of the purine nucleobases in the Murchison meteorite sample were strongly bound to mineral phases and/or labile parts of high-molecular-weight insoluble organic matter (IOM) and could not be recovered using hot-water extraction.

The unusual purine nucleobases were also significantly more abundant in the HCl (#3-2) extract than in the HW-HCl (#2-2) extract (139 ± 1 ppb vs. 16 ± 1 ppb, respectively; Table 1). Isoguanine, the structural isomer of guanine, was the most abundant among the unusual purine nucleobases (81 ± 1 ppb), comparable with adenine (84 ± 1 ppb). Purine ($C_5H_4N_4$), 2-aminopurine, and 2,6-diaminopurine, which were

Table 1
Concentrations of purine and pyrimidine nucleobases (ppb, ng·g^{-1}) in the HW, HW-HCl, and HCl extracts of the Murchison meteorite.[a,b]

Name	**Molecular formula**	**Hot Water (HW)**			**HCl**	**Total Extractable**	**Ratio**
		HW(#1-1, #1-2)	**HW-HCl**(#2-1, #2-2)	**Ratio** (HW)/(HW-HCl)	(#3-1, #3-2)	(HW-HCl + HCl)	(HCl)/(Total extracts)
Canonical purine bases							
Adenine	$C_5H_5N_5$	9 ± 1 (2)	37 ± 1 (2)	24%	84 ± 4 (2)	121 ± 4	70%
Hypoxanthine	$C_5H_4N_4O$	4 ± 2 (2)	11.9 ± 0.3 (2)	36%	141 ± 1 (2)	153 ± 2	92%
Guanine	$C_5H_5N_5O$	59 ± 9 (2)	107 ± 3 (2)	55%	542 ± 103 (2)	649 ± 103	83%
Xanthine	$C_5H_4N_4O_2$	9 ± 3 (2)	43 ± 1 (2)	22%	185 ± 14 (2)[c]	227 ± 14	81%
Sum		**81 ± 9**	**199 ± 3**	**41%**	**951 ± 104**	**1149 ± 104**	**83%**
Unusual purine bases							
Purine	$C_5H_4N_4$	0.11 ± 0.03 (2)	0.81 ± 0.01 (2)	13%	3.2 ± 0.1 (2)	4.0 ± 0.1	80%
2-Aminopurine	$C_5H_5N_5$	n.d.	n.d.		0.5 ± 0.1 (2)	0.5 ± 0.1	
8-Aminopurine[d]	$C_5H_5N_5$	n.d.	n.d.		31 ± 1 (2)	31 ± 1	
2,6-DAP+6,8-DAP[e]	$C_5H_6N_6$	1.7 ± 0.5 (2)	2.9 ± 0.1 (2)	60%	24 ± 1 (2)	27 ± 1	89%
2,6-Diaminopurine (2,6-DAP)	$C_5H_6N_6$	n.a.	n.a.		(7.5 ± 0.6)[f]		
6,8-Diaminopurine[d] (6,8-DAP)	$C_5H_6N_6$	n.a.	n.a.		(16.6 ± 0.3)[f]		
Isoguanine	$C_5H_5N_5O$	7 ± 1 (2)	12 ± 1 (2)	57%	81 ± 1 (2)	93 ± 2	87%
Sum		**9 ± 2**	**16 ± 1**	**56%**	**139 ± 1**	**155 ± 2**	**90%**
Sum of all purines		**90 ± 10**	**215 ± 3**	**42%**	**1090 ± 104**	**1305 ± 104**	**84%**
Pyrimidine bases							
Cytosine	$C_4H_5N_3O$	9.4 ± 0.5 (2)	19 ± 1 (2)	50%	10.6 ± 0.4 (2)	29 ± 1	36%
Uracil[g]	$C_4H_4N_2O_2$	101 ± 14 (2)	193 ± 3 (2)	52%	20 ± 3 (2)	213 ± 4	9%
Thymine[g]	$C_5H_6N_2O_2$	53 ± 10 (2)	61 ± 4 (2)	87%	14 ± 2 (2)	74 ± 5	18%
1-Methyluracil[g]	$C_5H_6N_2O_2$	4 (1)	4 (1)	94%	n.d.		0%
6-Methyluracil[g]	$C_5H_6N_2O_2$	18 (1)	20 (1)	90%	7 (1)		26%
Sum of pyrimidines		**185 ± 17**	**297 ± 5**	**62%**	**51 ± 4**	**348 ± 7**	**15%**
Sum of all nucleobases		**275 ± 20**	**512 ± 6**	**54%**	**1141 ± 104**	**1653 ± 104**	**69%**
Ratio (Purines)/(All nucleobases)		**33%**	**42%**		**96%**	**79%**	

[a] n.d. = nucleobases were not detected (below the detection limit); n.a. = not analyzed in the extracts. The reported uncertainties (δx) are based on the standard deviation (σx) of two separate measurements (n) with a standard error: $\delta x = \sigma x \times (n)^{-1/2}$.

[b] Sample extracts were analyzed by HPLC/ESI-HRMS as shown in Figs. 2–4.

[c] The concentration of xanthine was calculated by subtracting the co-eluting peak areas derived from its structural isomers (Fig. S1).

[d] These compounds were tentatively identified based on the results of MS/MS experiments (Fig. 3).

[e] 2,6-DAP and 6,8-DAP were not separated on the HPLC/HRMS chromatograms using the solvent A (H_2O + 0.1% formic acid).

[f] Assuming that 6,8-DAP has the same ionization efficiency as 2,6-DAP, the concentrations of 2,6-DAP and 6,8-DAP were determined by the HPLC/HRMS analyses using solvent A without 0.1% formic acid (Fig. 3).

[g] These pyrimidine nucleobases were detected only in the H_2O fractions (i.e., #1-1, #2-1, and #3-1).

identified in previous studies (Callahan et al., 2011; Oba et al., 2022), were also more abundant in the HCl extract. The presence of these unusual purine nucleobases in the HCl extract from the Murchison-meteorite sample indicates their extraterrestrial origin. Therefore, the purine nucleobases adenine, hypoxanthine, guanine, and xanthine, found abundantly in the HCl extract (Table 1), can be considered indigenous to the Murchison-meteorite sample.

The HW-HCl extract (#2-2) contained approximately twice as many purine nucleobases as the HW extract (#1-2) (Table 1), suggesting that the HW extract contained a similar amount of bound-form purine nucleobases as the free-form. Notably, the average relative abundance of canonical purine nucleobases in the HW extract compared with the HW-HCl extract [i.e., (HW)/(HW-HCl)] was close to the average relative abundance of unusual purine nucleobases (41% and 56%, respectively). Thus, most of the bound-form canonical purine nucleobases along with the bound-form noncanonical purine nucleobases are indigenous to the Murchison-meteorite sample. It is worth noting that the bound-form nucleobases remained intact without undergoing hydrolysis under the aqueous conditions on the Murchison meteorite's parent body. The condition of parent body is considered more alkaline, of longer duration, and at lower temperature (Brearley, 2006) than those of the hot-water extraction procedure we employed in this study. Identification of bound-form purine nucleobases in the HW extract will be the focus of future investigations.

3.3. *Pyrimidine nucleobases in the HW and HCl extracts*

We investigated the molecular distributions of pyrimidine nucleobases and other nitrogen-containing compounds in the Murchison-meteorite extracts using a Hypercarb column. Fig. 4 displays the extracted-ion chromatograms of the pyrimidine nucleobases obtained from the HW, HW-HCl, and HCl extracts of the Murchison meteorite, a procedural blank, and authentic standards during the MS/MS measurements. Cation-exchange chromatography, which was performed prior to the HPLC/HRMS analyses, completely fractionated uracil and thymine into the H_2O fractions (#1-1, #2-1, and #3-1) and cytosine into the NH_4OH fractions (#1-2, #2-2, and #3-2). Although most of the uracil structural isomers ($C_4H_4N_2O_2$) eluted into the NH_4OH fractions (Fig. S2), trace amounts of imidazole-2-carboxylic acid (0.3 ppb) and imidazole-4-carboxylic acid (0.3 ppb) were detected in the HW (#1-1) and HCl (#3-1) extracts, respectively. The mass chromatogram at m/z = 127.0502 in Fig. 4 reveals the presence of thymine and its structural isomers (1-methyluracil and 6-methyluracil), indicating that the HW and HCl extracts of the Murchison-meteorite sample contained not only canonical pyrimidine nucleobases but also rare pyrimidine nucleobases, as observed in a previous study (Oba et al., 2022).

Unlike purine nucleobases, abundant pyrimidine nucleobases were detected in the HW and HW-HCl extracts (Fig. 4 and Table 1). The distinct distributions of canonical purine and pyrimidine nucleobases are shown in Fig. 5. The relative abundances of the canonical pyrimidine nucleobases in the HCl extract compared with the total extractable abundance varied from 9% for uracil to 36% for cytosine, indicating that

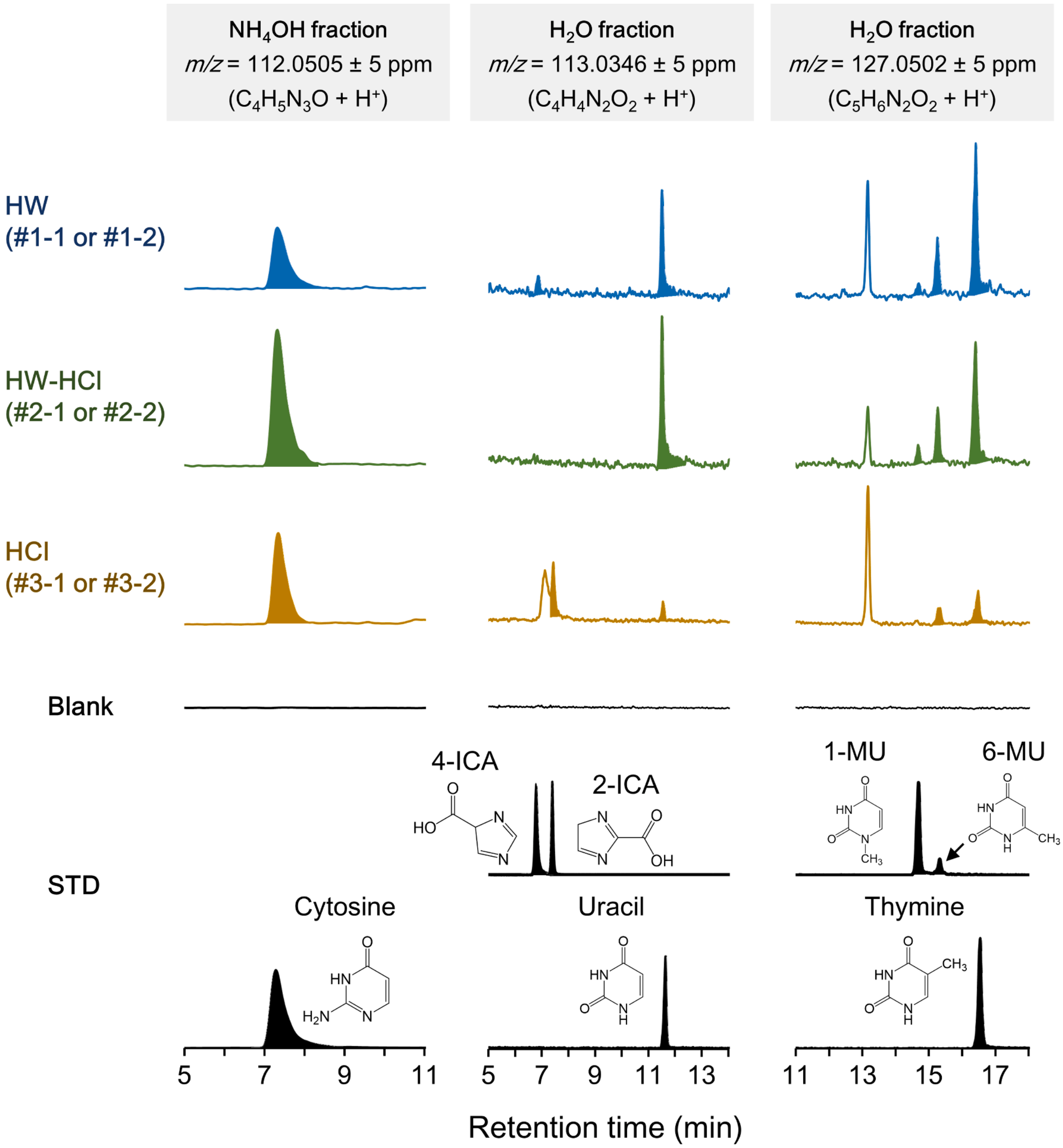

Fig. 4. Pyrimidine nucleobases detected in the HW, HW-HCl, and HCl extracts of the Murchison meteorite. Selected regions of the HPLC/HRMS extracted-ion chromatograms of the pyrimidine nucleobases in the HW, HW-HCl, and HCl extracts of the Murchison meteorite. We obtained these results from MS/MS measurements using a Hypercarb column. We detected uracil and thymine in the H_2O fractions (#1-1, #2-1, and #3-1) and cytosine in the NH_4OH fractions (#1-2, #2-2, and #3-2). Peak identifications are as follows: 5–11 min at $m/z = 112.0505$ for cytosine; 5–14 min at $m/z = 113.0346$ for uracil, imidazole-2-carboxylic acid (2-ICA), and imidazole-4-carboxylic acid (4-ICA); and 11–18 min at $m/z = 127.0502$ for thymine, 1-methylureacil (1-MU), and 6-methylureacil (6-MU). The vertical axis for each mass chromatogram at the same m/z is normalized on the same scale to emphasize the detection of the pyrimidine nucleobases that are abundant in the HW and HW-HCl extracts.

most pyrimidine nucleobases in the Murchison-meteorite sample were not as strongly bound to the mineral phases and/or IOM as were the purines. The relative abundances of the purine nucleobases in the HCl extract compared with the total extractable abundance were at least 70%. The total extractable abundance of pyrimidine nucleobases in the Murchison-meteorite sample was 348 ± 7 ppb, representing 21% of the total nucleobase abundance. Consequently, the Murchison-meteorite sample examined in this study contained a significantly higher abundance of purines (comprising 79% of the total nucleobase abundance) than pyrimidines (Table 1).

The sum of pyrimidine nucleobases in each extract ranged from 51 ± 4 ppb in the HCl extract to 297 ± 5 ppb in the HW-HCl extract. The abundances of cytosine and uracil but not that of thymine in the HW extract increased approximately two-fold after acid hydrolysis, indicating the presence of bound-form cytosine and uracil but not that of thymine. The bound-form pyrimidine nucleobases may be indigenous to the Murchison meteorite given that bound-form canonical purine nucleobases are of extraterrestrial origin, as discussed earlier. The relative abundance of thymine in the HW extract compared with that in the HW-HCl extract (87%, Table 1) was similar to those of its structural isomers that do not occur in nucleic acids (94% for 1-methyluracil and 90% for 6-methyluracil), indicating that these $C_5H_6N_2O_2$ isomers are of extraterrestrial origin.

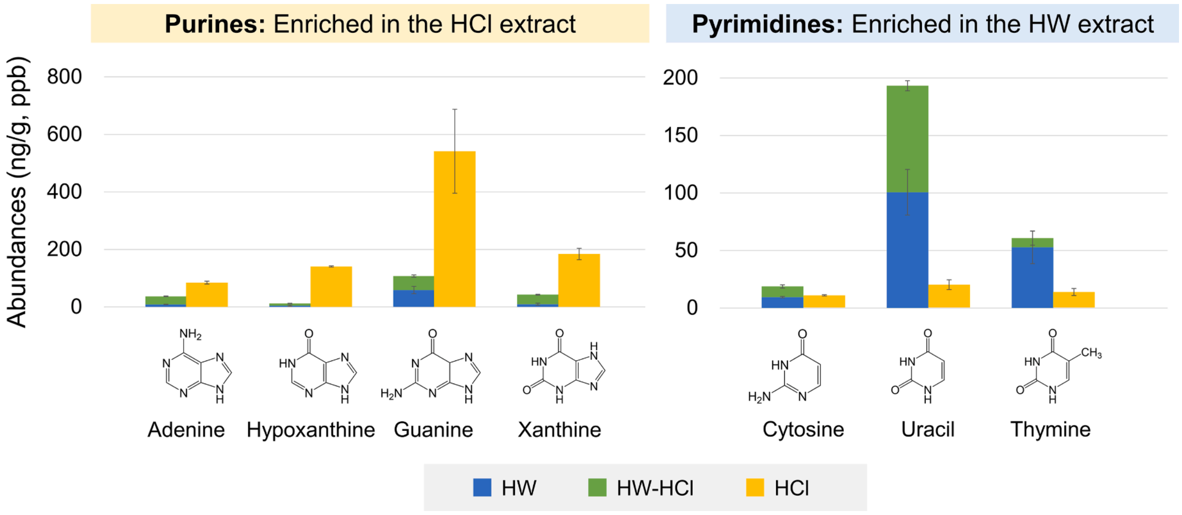

Fig. 5. Comparison of the abundances of canonical purine and pyrimidine nucleobases detected in the HW, HW-HCl, and HCl extracts of the Murchison meteorite.

3.4. Other N-containing compounds in the HW and HCl extracts

Furthermore, we examined the molecular distributions of N-containing compounds, including glycine, imidazole, imidazole carboxylic acids, and pyridine carboxylic acids, for comparison with those of nucleobases (Fig. S2). We quantified these N-containing compounds in the NH_4OH fractions of the HW and HCl extracts of the Murchison-meteorite sample (Table 2). Glycine, the simplest amino acid, was detected at an abundance approximately twice that of the total of purine and pyrimidine nucleobases (3.90 ppm versus 1.65 ± 0.10 ppm, Tables 1 and 2). The relative abundance of glycine in the HCl extract compared with the total extractable abundance was 48%, which is intermediate between the corresponding relative abundances of purines and pyrimidines (84% and 15%, respectively, see Table 1). The Murchison sample contained glycine at a concentration of 2033 ppb in the HW-HCl extract (#2-2) and 1858 ppb in the HCl extract (#3-2). The glycine concentration in the HW-HCl extract was calculated to be 27 nmol/g, which is consistent with the value reported by Glavin et al. (2021) (32 ± 3 nmol/g from a 1.0 g Murchison-meteorite extract). Thus, although the HPLC conditions employed in this study did not allow for the separation and quantification of other amino acids (e.g., the C3 amino acids D- and L-alanine, β-alanine, and sarcosine), the Murchison-meteorite sample analyzed in this study is expected to exhibit an amino-acid distribution similar to those reported in previous studies. Moreover, Koga and Naraoka (2017) observed a similar total extractable abundance of glycine (3566 ± 165 ppb) from a different Murchison-meteorite sample despite slight differences in the relative abundances of glycine in the HCl extracts (~61%) compared with the current study (48%). This observation suggests that performing 6 M HCl extraction after hot-water extraction effectively recovered organic molecules present in the meteorite matrices and/or labile parts of IOM, thus mitigating the heterogeneity in the values of detected-molecule abundances reported in different studies on the identical meteorite sample.

We identified imidazole ($C_3H_4N_2$), one of the simplest five-membered-ring N-heterocyclics, in the HCl extract (#3-2); among the Murchison-meteorite extracts, it had a notably high concentration of 832 ppb (Table 2 and Fig. S2). This value is close to the total abundance of purine nucleobases in the same extract: 951 ± 104 ppb (Table 1). The imidazole carboxylic acids, which are structural isomers of uracil ($C_4H_4N_2O_2$), also exhibited higher concentrations in the HCl extract than in the HW extracts (Table 2). Interestingly, the HCl extract contained imidazole-4-carboxylic acid at a concentration (291 ppb) that was approximately 21 times larger than that of imidazole-2-carboxylic acid (14 ppb) (Table 2 and Fig. S2). Compared with the total extractable abundances, the relative abundances of five-membered-ring N-heterocycles in the HCl extract ranged from 61% for imidazole-2-

Table 2
Concentrations of N-containing compounds other than nucleobases (ppb, ng·g^{-1}) in the HW, HW-HCl, and HCl extracts of the Murchison meteorite.[a,b]

Name	Molecular Formula	Hot Water (HW)			HCl	Total Extractable	Ratio
		HW(#1-2)	HW-HCl(#2-2)	Ratio(HW/(HW-HCl)	(#3-2)	(HW-HCl + HCl)	(HCl)/(Total extracts)
Amino acid							
Glycine	$C_2H_5NO_2$	916 (1)	2033 (1)	45%	1858 (1)	3892	48%
Five-membered N-heterocycles							
Imidazole	$C_3H_4N_2$	117 (1)	260 (1)	45%	832 (1)	1092	76%
Imidazole-2-carboxylic acid	$C_4H_4N_2O_2$	8 (1)	9 (1)	92%	14 (1)	23	61%
Imidazole-4-carboxylic acid	$C_4H_4N_2O_2$	44 (1)	61 (1)	72%	291 (1)	352	83%
Six-membered N-heterocycles							
Pyridine-2-carboxylic acid (Picolinic acid)	$C_6H_5NO_2$	n.d.	n.d.		detected[c]		
Pyridine-3-carboxylic acid (Nicotinic acid)	$C_6H_5NO_2$	228 (1)	239 (1)	95%	65 (1)	304	21%
Pyridine-4-carboxylic acid (Isonicotinic acid)	$C_6H_5NO_2$	105 (1)	118 (1)	89%	28 (1)	146	19%

[a] n.d. = N-containing compounds were not detected (below the detection limit).
[b] Sample extracts were analyzed by HPLC/ESI-HRMS as shown in Fig. S2.
[c] Picolinic acid was tentatively detected in the HCl extract.

carboxylic acid to 83% for imidazole-4-carboxylic acid. Conversely, six-membered-ring N-heterocyclics exhibited significantly lower relative abundances in the HCl extract, ranging from 19% for pyridine-4-carboxylic acid (isonicotinic acid) to 21% for pyridine-3-carboxylic acid (nicotinic acid). Considering that imidazole and its carboxylic-acid derivatives are polar compounds with high water solubility, the difference in the distributions of imidazoles and pyridines in the HW and HCl extracts cannot be simply explained based on their water solubility. This suggests that imidazoles and purine nucleobases were entrapped together within the host-mineral matrices and/or labile parts of IOM of the Murchison-meteorite sample. Therefore, the N-heterocyclic compounds exhibited inverse distributions between the HW and HCl extracts depending on their ring structures (Tables 1 and 2).

3.5. *Hydrolysis resistance of purine and pyrimidine nucleobases*

We investigated the potential degradation of nucleobases via hydrolysis during hot-water and 6 M HCl extraction. A detailed description of the experimental procedure is provided in the Supplementary text. Table S1 displays the recoveries of nucleobases following heat treatment in H_2O or 6 M HCl. Under hot-water extraction conditions, cytosine exhibited a 90% recovery rate, with uracil detected as the hydrolysis product, constituting 7% of the starting nucleobase concentration (by mol%). Most of the cytosine remained undecomposed during 6 M HCl extraction, exhibiting a higher recovery rate of 97%, with uracil production decreasing to 1%. Of all the nucleobases tested, hypoxanthine exhibited the highest susceptibility to hydrolysis, retaining 67% and 13% after heating in H_2O and 6 M HCl, respectively. Adenine and guanine were minimally decomposed under hot-water extraction conditions, yielding 99% of adenine and 100% of guanine, respectively, while 45% of adenine and 33% of guanine decomposed under 6 M HCl extraction conditions, producing 9% of hypoxanthine and 25% of xanthine, respectively. Conversely, uracil, thymine, and xanthine were negligibly decomposed under hot-water and 6 M HCl extraction conditions, with yields of 96%–99%.

The results of the aforementioned hydrolysis experiment suggest that some of the nucleobases in the Murchison-meteorite sample may have been degraded during hot-water and 6 M HCl extraction employed in this study. However, the HPLC/HRMS analyses revealed that the Murchison-meteorite sample yielded a substantially greater abundance of purine nucleobases in the 6 M HCl extract than in the HW extract (as shown in Table 1). This result implies that the initial concentrations of purine nucleobases in the Murchison-meteorite residue following hot-water extraction may have been higher than those measured via the HPLC/HRMS analyses. An alternative explanation is that the presence of phyllosilicate minerals and inorganic species in the Murchison meteorite may have served as a protective shield for purine nucleobases against hydrolysis during 6 M HCl extraction. In contrast to purine bases, cytosine demonstrated greater vulnerability to hydrolysis under hot-water extraction conditions. These results emphasize the necessity for optimizing the extraction procedure based on the specific nucleobases being targeted in carbonaceous meteorites. Furthermore, since the targeted pyrimidine nucleobases — cytosine, uracil, and thymine — were not significantly decomposed in the heated 6 M HCl solution, their scarcity in the HCl extract of the Murchison meteorite cannot be ascribed to decomposition during the acid extraction procedure we employed in this study.

4. Discussion

4.1. *Formation mechanism of abundant purine nucleobases in the Murchison meteorite*

The HPLC/ESI-HRMS analyses performed in this study confirm that abundant purine nucleobases can be effectively extracted from the Murchison meteorite using 6 M HCl after hot-water extraction (Table 1). Notably, the concentrations of guanine, xanthine, and hypoxanthine exceeded those of other purine nucleobases in the 6 M HCl extract. To provide a comprehensive explanation of the molecular distribution within the Murchison meteorite, we have compiled the mechanisms of purine nucleobase formation proposed in prior research (Shaw, 1950; Oró and Kimball, 1961, 1962; Ferris and Orgel, 1966a, b; Sanchez et al., 1968; Hudson et al., 2012) in Fig. 6. Purine nucleobases can be chemically synthesized from HCN, NH_3, and HCN-related compounds, including cyanogen, formamide, formamidine, urea, and guanidine (formation mechanisms for these molecules are illustrated in Fig. S3). The most important step during purine synthesis is the generation of 5-amino-4-substituted imidazoles, such as 5-aminoimidazole-4-carboxamide (AICA), 5-aminoimidazole-4-carbonitrile (AICN), 5-aminoimidazole-4-carboxamidine (AICAI), and 5-aminoimidazole.

As shown in Fig. 6, guanine, xanthine, and hypoxanthine can be synthesized from AICA in the presence of reactants such as HCN, formamidine, guanidine, cyanogen, cyanate, and urea (Shaw, 1950; Sanchez et al., 1968). Considering that prior HPLC/ESI-HRMS analyses (Oba et al., 2022) detected AICA in a Murchison-meteorite sample, AICA may have been the most abundant among the 5-amino-4-substituted imidazoles produced during the formation of extraterrestrial purine nucleobases in the parent body of the Murchison meteorite. Moreover, notably, AICA can be synthesized via the hydrolysis of AICN and AICAI. The abundances of adenine, isoguanine, and 2,6-diaminopurine—which can be synthesized from AICN and/or AICAI (Shaw, 1950; Oró and Kimball, 1961, 1962; Ferris and Orgel, 1966b; Sanchez et al., 1968)—are lower than those of guanine, xanthine, and hypoxanthine (Table 1). This suggests that the hydrolysis rates of AICN and AICAI may surpass the production rates of less-abundant purine nucleobases from the corresponding imidazole derivatives in the Murchison meteorite's parent body.

The formation pathways summarized in Fig. 6 also include the synthesis of purine ($C_5H_4N_4$) via the reaction of HCN with formamide (Hudson et al., 2012). Interestingly, this formation pathway generates 2-aminoacetonitrile as an intermediate molecule, which produces the amino acid glycine after hydrolysis (Aponte et al., 2017). The 2-aminoacetonitrile reacts with formamide to produce 5-aminoimidazole, which further reacts with another formamide molecule to produce purine (Hudson et al., 2012). To the best of our knowledge, although the formation pathway of 2-aminopurine has not been demonstrated in previous laboratory experiments, the formation of 2-aminopurine from 5-aminoimidazole and formamidine can be posited based on their structural similarities to purine (Fig. 6). Given that purine and 2-aminopurine were the least-detected among the purine nucleobases in the Murchison meteorite sample (Table 1), we consider the formation pathways involving 5-aminopurine to be less active than those involving AICA, AICN, and AICAI.

The formation mechanisms depicted in Fig. 6 can also account for the synthesis of 8-aminopurine, 6,8-diaminopurine, and 2,8-diaminopurine, all of which were tentatively identified in the Murchison-meteorite sample (Fig. 3). During the formation of the imidazole ring, aminomalononitrile (an HCN trimer), diaminomaleonitrile (an HCN tetramer), and 2-aminoacetanitrile react with formamidine to produce AICN, AICAI, and 5-aminoimidazole, respectively. We hypothesize that if these precursor molecules react with guanidine—a compound that can also be readily produced from HCN and formamidine (Fig. S3)—the resulting imidazole derivatives would possess an amino group at the 2-position of the imidazole ring. For instance, a reaction between the HCN tetramer and guanidine can yield 2,5-diaminoimidazole-4-carboxamidine. Subsequent reactions of this imidazole derivative with formamidine, HCN, or formamide can afford 6,8-diaminopurine instead of adenine. The synthesis pathways of 8-aminopurine and 2,8-diaminopurine can also be accounted for with the proposed formation mechanisms. These purines can be formed via the reaction of 2,5-diaminoimidazole with formamide or formamidine, respectively, as illustrated in Fig. 6. These reactions demonstrate the versatility of the proposed formation mechanism,

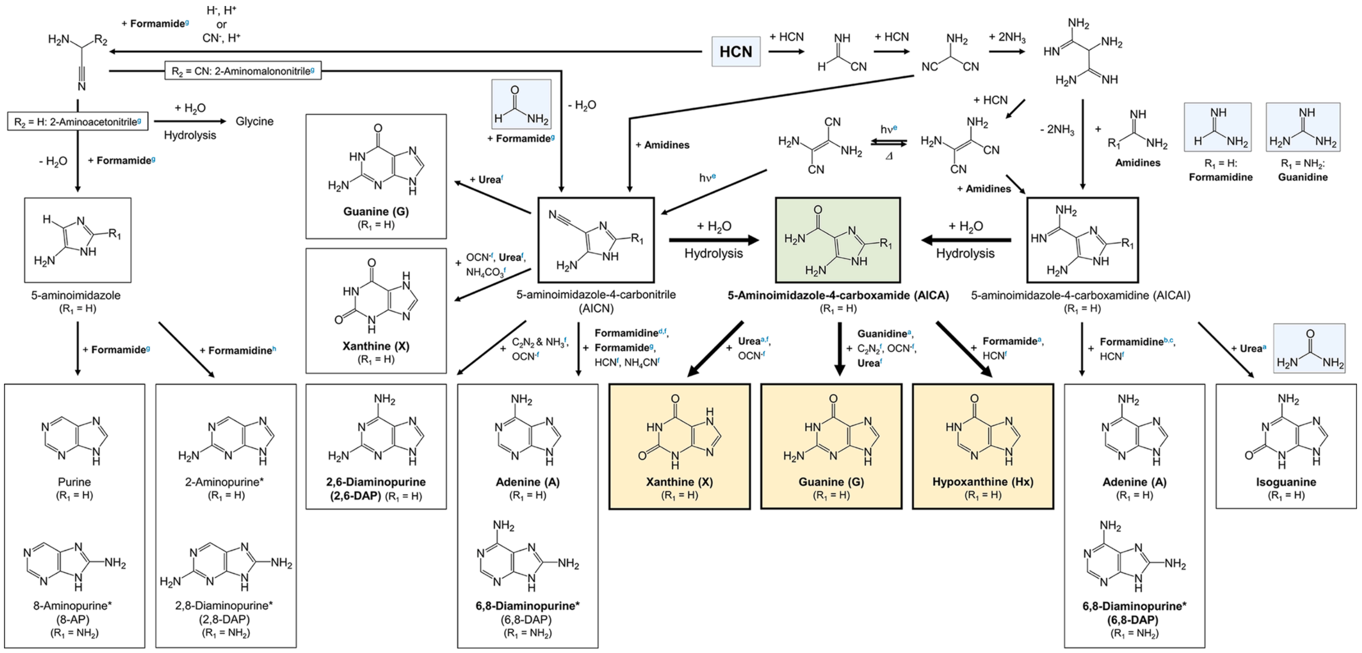

Fig. 6. Summary of the proposed formation pathways of purine nucleobases from HCN and its related molecules via the formation of 5-aminoimidazole derivatives. *The purine nucleobases tentatively identified in this study. [a]Shaw (1950). [b]Oró and Kimball (1961). [c]Oró and Kimball (1962). [d]Ferris and Orgel (1966b). [e]Ferris and Orgel (1966a). [f]Sanchez et al. (1968). [g]Hudson et al. (2012). [h]These reaction pathways are hypothesized based on the structural similarities in this class of compounds. The starting molecule, hydrogen cyanide (HCN), is highlighted in blue, along with its derivatives formamidine, guanidine, formamide, and urea. The critical precursor compound, 5-aminoimidazole-4-carboxamide (AICA), is highlighted in green. Finally, the resulting purines—specifically guanine, xanthine, and hypoxanthine, which were abundantly discovered in the Murchison meteorite (Table 1)—are highlighted in yellow. This color-coding scheme provides enhanced visibility and clearly distinguishes between the initial materials, the precursor, and the end products in the proposed purine-nucleobase formation pathway.

which accounts for various purine nucleobases detected in the Murchison meteorite.

Furthermore, a product of HCN polymerization from 1 M NH_4CN (which underwent reaction at room temperature for approximately 6 months) exhibited a more prominent chromatographic peak of 6,8-diaminopurine compared with that of 2,6-diaminopurine, as indicated in the supporting information of Callahan et al. (2011). In this instance, the molecular distribution of diaminopurine isomers is consistent with that observed in case of the Murchison meteorite analyzed in this study (as shown in Fig. 3), suggesting that the purine nucleobases present in the Murchison meteorite were likely synthesized via HCN polymerization, following the formation pathways depicted in Fig. 6. Although pyrimidine (cytosine, uracil, and thymine) and purine nucleobases (adenine, xanthine, and hypoxanthine) can be synthesized under interstellar-ice conditions (Oba et al., 2019), HCN-polymerization-type reactions, which have been demonstrated by previous studies (Shaw, 1950; Oró and Kimball, 1961, 1962; Ferris and Orgel, 1966a, b; Sanchez et al., 1968), can potentially generate various purine nucleobases more efficiently when they occur in the presence of liquid water within the parent bodies of carbonaceous chondrites. Therefore, the proposed formation mechanisms for purine nucleobases offer a comprehensive explanation for the presence of all purine nucleobases identified in the Murchison meteorite.

4.2. *Potential correlation between nucleobase formation mechanisms and the spatial distributions of N-heterocyclics within the Murchison meteorite*

The proposed formation mechanisms for extraterrestrial purine nucleobases (Figs. 6 and S3) may be linked to the observed distribution of imidazole molecules within the Murchison meteorite. Both purine nucleobases and imidazole molecules were found abundantly in the HCl extract from this meteorite (Table 2). In particular, we detected imidazole-4-carboxylic acid in significantly greater amounts than its structural isomer, imidazole-2-carboxylic acid, indicating the preferential production of imidazole molecules with a functional group at the 4-position (equivalent to the 5-position) of the imidazole ring. This observation aligns with the structure of 5-substituted imidazole molecules, which are crucial intermediates in the formation of purine nucleobases, as illustrated in Fig. 6. When AICA loses its amino group at the 4-position of the imidazole ring, imidazole-4-carboxylic acid can be readily generated through the hydrolysis of the amide group at the 5-position. The high concentrations of purine nucleobases and imidazole-4-carboxylic acid in the HCl extract suggest the concurrent syntheses of these molecules.

The origin of the purines and imidazoles released during the 6 M HCl extraction of the Murchison-meteorite sample remains uncertain. The most plausible source is the mineral matrices, which are primarily composed of phyllosilicates. The total extractable abundance of purine nucleobases in the Murchison-meteorite sample (using HW and 6 M HCl extraction) was approximately nine times higher than that reported in a previous study using formic-acid extraction (Callahan et al., 2011), as shown in Table 1. Since formic acid is known to react readily with carbonate (e.g., Baltrusaitis et al., 2006), the results suggest that carbonate is not the major host-mineral matrices trapping purines and imidazoles. Furthermore, associations of organic matter with phyllosilicate minerals have been observed in carbonaceous chondrites (Yesiltas and Kebukawa, 2016; Yesiltas et al., 2017) and asteroid Ryugu (Ito et al., 2022; Viennet et al., 2023; Hashiguchi et al., 2023). Given this background, it is plausible that purine nucleobases may be trapped in meteorite matrices comprising phyllosilicates, which are more resistant to dissolution by organic acids and efficiently liberated via 6 M HCl extraction owing to the dissolution of host-mineral matrices. Alternatively, if the disparity in matrix-mineral dissolution between HCl and formic acids is irrelevant to the molecular distribution of purine nucleobases, the Murchison-meteorite sample analyzed in this study may have contained matrix regions with high concentrations of purine nucleobases owing to heterogeneity in the spatial distributions of organic molecules in meteorites (Naraoka and Hashiguchi, 2018; Hashiguchi and Naraoka, 2019; Naraoka et al., 2019).

Another potential source of abundant purines and imidazoles is IOM. As discussed in a previous study (Sephton et al., 2003), some free organic matter, which is extractable using common organic solvents, may be genetically related to a part of IOM. The observed molecular distribution may also be explained if acid-labile parts of IOM contain abundant purines and imidazoles and release these molecules during 6 M HCl extraction. An additional extraction experiment using hydrofluoric acid on the Murchison meteorite would be useful to distinguish the source of the molecular classes by dissolving host-mineral matrices without strong alteration of the acid-labile parts of IOM. Therefore, based on current knowledge, we consider purine nucleobases and imidazoles in the 6 M HCl extract to have been synthesized concurrently during the accretion of the planetesimals that formed the parent body of the Murchison meteorite and subsequently coincorporated within the host-mineral matrices and/or IOM. Alternatively, the precursor materials for purines and imidazoles might be incorporated into the host-mineral matrices. Within this environment, the precursors might have undergone reactions to produce purines and imidazoles, potentially catalyzed by the minerals themselves (Bennett et al., 2013).

In contrast to the five-membered N-heterocyclics and purine nucleobases, the six-membered N-heterocycles, such as pyrimidine nucleobases and pyridine carboxylic acids, exhibited a different distribution pattern. They were more abundant in the HW extracts than in the HCl extract (Tables 1 and 2). This molecular distribution might suggest that both free- and bound-forms of six-membered N-heterocycles dissolve readily in hot water. Conversely, the abundant purines and five-membered N-heterocycles in the HCl extract (Tables 1 and 2) might indicate that, after the HW extraction, most of these molecules were still retained in the meteorite matrices and/or a labile part of IOM. They were primarily retrievable through the subsequent HCl extraction process. Further exploration of these phenomena will shed more light on the complex processes governing the synthesis of meteoritic organic molecules, including nucleobases, and the subsequent evolution of these molecules within the mineral matrices and/or IOM of meteorite parent bodies.

4.3. *Prospects for the isotopic analyses of extraterrestrial nucleobases*

A crucial discovery in this study is the notably high concentration of purine nucleobases in the HCl extract of the Murchison-meteorite sample (Table 1). In particular, the total extractable abundance of guanine (649 ± 103 ppb) is approximately an order of magnitude larger than the values reported in previous studies, ranging from 56 (Callahan et al., 2011) to 72 (Oba et al., 2022) ppb. Given that compound-specific isotopic analysis usually demands at least sub-ppm concentrations of organic molecules in meteorite samples, the 6 M HCl extraction procedure used in our study offers considerable advantages for measuring the isotopic compositions of purine nucleobases. The $\delta^{13}C$ values of nucleobases in the Murchison meteorite have only been reported once before, specifically for uracil and xanthine (Martins et al., 2008). Although the $\delta^{15}N$ values of nucleosides have been measured in a biological sample once previously (Strable et al., 2011), to the best of our knowledge, no studies have been conducted on the nitrogen-isotope ratios of meteoritic nucleobases to date. Therefore, the optimized extraction procedures from this study are invaluable for future investigations to determine the carbon and nitrogen isotopic compositions of nucleobases in extraterrestrial samples.

5. Conclusions

In this study, we employed HPLC/ESI-HRMS to investigate the molecular distributions of nucleobases and several nitrogen-containing compounds in HW and 6 M HCl extracts of the Murchison meteorite. Notably, we found purine nucleobases to be substantially more enriched in the 6 M HCl extracts than in the HW extracts, with guanine being the most abundant purine nucleobase. Moreover, we found purines with an amino group at the 8-position (i.e., 8-aminopurine and 6,8-diaminopurine) to be more abundant than those with an amino group at the 2-position (i.e., 2-aminopurine and 2,6-diaminopurine). Conversely, pyrimidine nucleobases were more enriched in the HW extracts than in the 6 M HCl extracts. The total extractable abundance of pyrimidine nucleobases in the Murchison meteorite sample was approximately four times lower than that of purine nucleobases. Acid hydrolysis of the HW extract approximately doubled the concentrations of purine and pyrimidine nucleobases, excluding thymine, suggesting that comparable amounts of bound-form purines and pyrimidines coexist with free-form purines and pyrimidines.

The HPLC/ESI-HRMS analyses revealed different molecular distributions of the nitrogen heterocycles between the HW extract and 6 M HCl extract, depending on whether their ring structures were five-membered, six-membered, or purine-derived fused-ring structures. Hot-water extraction recovered approximately 85% of the total pyrimidine nucleobases and approximately 80% of total pyridine carboxylic acids. The subsequent 6 M HCl extraction yielded approximately 84% of the total purine nucleobase and approximately 84% of the total imidazole molecules. These distinct distributions of 1) pyrimidines + pyridines and 2) purines + imidazoles are unlikely to be explained solely by differences in their water solubility. We detected the amino acid glycine at a similar concentration in both the HW and HCl extracts, demonstrating an intermediate pattern between purines and pyrimidines.

The co-occurrence of purines and imidazoles in the HCl extract is consistent with the proposed formation mechanism of purine nucleobases via the synthesis of imidazole derivatives. This mechanism, based on the synthesis of AICA, can account for the enrichment of guanine, xanthine, and hypoxanthine in the Murchison meteorite. The same mechanism can also explain the synthesis of 8-substituted purines, all of which were tentatively identified in the Murchison-meteorite sample. The significantly greater abundance of imidazole-4-carboxylic acid in the 6 M HCl extract than that of its structural isomer provides further corroborates the unified mechanism of purine synthesis via imidazole derivatives. Although these purine nucleobases and imidazoles (imidazole and imidazole-4-carboxylic acid) were released upon the dissolution of host-mineral matrices and/or the alteration of labile parts of IOM during 6 M HCl extraction, further investigations using different extraction procedures are essential to accurately identify their sources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

Data are available through Mendeley Data at https://doi.org/10.17632/m6w642z6bb.1.

Appendix A. Supplementary material

The supplementary material is comprised of a document that provides a detailed description of the nucleobase hydrolysis experiments and data that support the primary results reported here in the main text. Supplementary material to this article can be found online at https://doi.org/10.1016/j.gca.2023.10.024.

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