Electronic Supplementary Information

Metabolic Precursors in Astrophysical Ice Analogs: Implications for Meteorites and Comets

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Experimental Methods

Chemicals and reagents. All glassware and ceramics were rinsed with ultrapure water (18.2 MΩ·cm, < 5 parts-per-billion total organic carbon from a Millipore Milli-Q Integral 10 system), wrapped in aluminum foil, and heated to 500 °C overnight. All standards were purchased from Sigma-Aldrich. Sulfuric acid (95-98% purity) and formic acid (>95% purity) were purchased from Sigma-Aldrich, acetonitrile (Optima® grade, 99.5+%), and methanol (Optima® grade) were purchased from Fisher Scientific. A standard solution of pyridine mono- and dicarboxylic acids was prepared by first making individual standards in water (with the exception of 3,5-pyridine dicarboxylic acid, which was prepared in 0.1 M ammonium hydroxide), then mixing those standards into one solution ranging in concentration from 1 to 100 µM. A standard solution of hydroxypyridines was prepared by dissolving individual compounds in ultrapure water, then mixing those standards into one solution ranging in concentration from 0.1 to 100 µM. For liquid chromatographic separation, a 0.05% (by volume) sulfuric acid solution was prepared.

Astrophysical ice analog experiments and *in situ* FTIR measurements. Ice films were produced by leaking gases from separate manifolds into the stainless steel high vacuum chamber (P ~ 10^{-7} torr) through tubes facing a polished aluminum substrate.[1-2] The temperature of the substrate was maintained between 20 and 300 K by a closed-cycle helium cryostat (ARS model DE-204) and a resistive heater. Samples were grown at 50 K for 4 to 28 minutes, where the total time depended on the ratio of gases deposited. Final sample thicknesses were ~2 µm as measured by 650 nm laser interferometry. Ice samples were then cooled to 20 K and exposed to a beam of 0.9 MeV protons from a Van de Graaff accelerator with a current of 150 nA to a total fluence of 1×10^{15} p+ cm^{-2}. The ice thicknesses were kept well below the stopping range for 0.9 MeV protons, ensuring that the entire bulk of the sample was processed. In irradiations of the three-component ices, the average total energy absorbed was ~19 eV $molecule^{-1}$, determined using a stopping power for pyridine + CO_2 + H_2O mixtures of 2.48×10^8 eV cm^2 g^{-1} $p+^{-1}$. The average energy absorbed in the irradiations of the pyridine + CO_2 mixtures was ~25 eV $molecule^{-1}$, using a stopping power of 2.46×10^8 eV cm^2 g^{-1} $p+^{-1}$. All stopping powers were calculated using the SRIM software package[3] assuming a density for the mixtures of ~1 g cm^{-3}. FTIR spectra of the ice films were recorded *in situ* at several temperatures with a Thermo Nicolet Nexus 670 spectrometer as 100-scan accumulations at 1 cm^{-1} resolution over the range of 5000 to 650 cm^{-1}. After irradiation, the ice was allowed to warm up to room temperature overnight under vacuum to remove any volatiles, and the spectrum of the non-volatile residue was recorded. The vacuum system was then opened and the residue-coated substrate removed. The color of the irradiated residue at room temperature ranged from faint to deep yellow, a noticeable change from the initially colorless ice sample. The residue was removed by repeatedly pipetting solvent (1:1 water:methanol) onto the substrate, and the extract was transferred into an HPLC vial. The extract was analyzed immediately by DART-MS (described below) and then stored at 187 K for later analysis by LC-UV/MS.

External mass calibration. External calibration was performed before the analyses of all samples and standards using a mixture of caffeine, MRFA (L-methionyl-arginyl-phenylalanyl-alanine acetate hydrate) peptide, and Ultramark 1621 (a mixture of fluorinated phosphazines) in an acetonitrile-methanol-water solution containing 1% acetic acid, which resulted in a typical mass accuracy of < 3 parts-per-million (ppm).

***Ex situ* sample analysis by DART-MS**. A Thermo Scientific LTQ Orbitrap XL hybrid ion trap-orbitrap mass spectrometer equipped with an IonSense DART ion source was used for the rapid determination of chemical composition in ice residues and controls. The ceramic cone on the DART source was baked at 550 °C prior to analyses. The DART parameters were: He gas (99.999% purity), 350 °C run temperature, and positive ion mode. Mass spectrometer parameters were: positive ion mode, capillary temperature of 200 °C, capillary voltage of 25 V, tube lens at 120 V, mass range m/z set to 50-500, mass resolution at 30,000 (full-width-half-maximum at m/z 400), maximum ion injection time equal to 250 ms, and acquisition time per sample of 0.55 min. A Quickstrip card was used for analyses with a 5 μL sample spot. Elemental compositions were calculated from the $[M + H]^+$ protonated molecule with introduced limits of carbon (0–30), hydrogen (0–60), nitrogen (0–10), and oxygen (0–15) and a mass tolerance of 5 ppm.

Comparison of sampling methods using DART-MS platform. In the beginning of the investigation, we analyzed the residue by two different DART techniques: (1) directly off of the substrate using the 3+D scanner accessory and (2) using Quickstrip sample cards and liquid extraction. For the latter, solvent (1:1 water:methanol) was repeatedly pipetted onto the substrate and then transferred into an HPLC vial; 5 μL of this extract was spotted onto a Quickstrip sample card for analysis. We found that extracting the residue and sampling on the Quickstrip card recovered more complex organic compounds, as observed in the mass spectrum; therefore, this method was used throughout the rest of the investigation.

***Ex situ* sample analysis by LC-UV/MS**. Sample extracts and controls were also analyzed using a Thermo Scientific Accela high performance liquid chromatograph coupled to a Thermo Scientific Accela photodiode array detector (PDA) and a Thermo Scientific LTQ Orbitrap XL hybrid ion trap-orbitrap mass spectrometer equipped with an electrospray ionization (ESI) source and operated in positive ion mode. Parameters for ESI are listed in the sentences that follow. Relative flow rates for the nitrogen gas for desolvation of the electrospray were set to 45 for the sheath gas, 15 for the auxiliary gas, and 15 for the sweep gas (all in arbitrary units). The ion transfer capillary voltage and temperature were 8 V and 275 °C, respectively. The tube lens was set to 50 V. Full-scan mass spectra were acquired over a range of *m/z* 50 to 500. The mass resolution was set to 30,000 (full-width-half-maximum at m/z 400) in order to maintain a sufficient number of data points across chromatographic peaks. MS/MS data were collected in the ion trap (CID energy 28, isolation width 2 Da). Separation of pyridine carboxylic acids was accomplished by injecting 10 μL of sample solution onto two SIELC Primesep 100, 150 mm ×

2.1 mm columns (5 μm particle size) with a Primesep 100, 10 mm × 2.1 mm guard column (5 μm particle size). Samples were eluted at 200 μL min^{-1} under isocratic conditions with a mobile phase of 0.05% sulfuric acid. Separation of hydroxypyridines was accomplished by injecting 10 μL of sample solution onto a Phenomenex Kinetex PFP, 150 mm × 4.6 mm column (2.6 μm particle size) with an HPLC KrudKatcher Ultra Column In-Line Filter. Mobile phase (A) consisted of 0.1% formic acid and mobile phase (B) was 100% acetonitrile. Samples were eluted at 300 μL min^{-1} using the following gradient: 0-7 min 100-95% A, 7-15 min 95% A, 15-25 min 100% A.

Compounds were identified by comparing mass and UV chromatographic retention time, accurate mass measurements, and product ion spectra to reference standards. Quantitation of substituted pyridines was achieved using a standard concentration curve. The error in these measurements was calculated as the standard error of the mean from 2 measurements.

Hydroxypyridines. In H_2O + CO_2 + pyridine ices, hydroxypyridines were more abundant than pyridine carboxylic acids (see Table S1) and may be particularly favorable to synthesize. For example, hydroxypyridines were more abundant in the residue of an irradiated ice mixture where H_2O:pyridine = 10:1 compared to a lower abundance of pyridine carboxylic acids where CO_2:pyridine = 10:1 (and even 1:1).

	Exp. 4 ***1:2:10 P:CO_2:H_2O, non-irradiated***	***Exp. 5*** ***1:2:10 P:CO_2:H_2O***	***Exp. 6*** ***1:20:100 P:CO_2:H_2O***
2-hydroxypyridine	3 ± 3	25861 ± 404	1547 ± 66
3-hydroxypyridine	<1	15527 ± 133	1888 ± 19
4-hydroxypyridine	46 ± 8	11061 ± 220	1173 ± 103

Table S1 Abundances (in pmol) of hydroxypyridines in three different ice residues. The estimated limit of quantitation was 1 pmol. Error was calculated as standard error from two measurements.

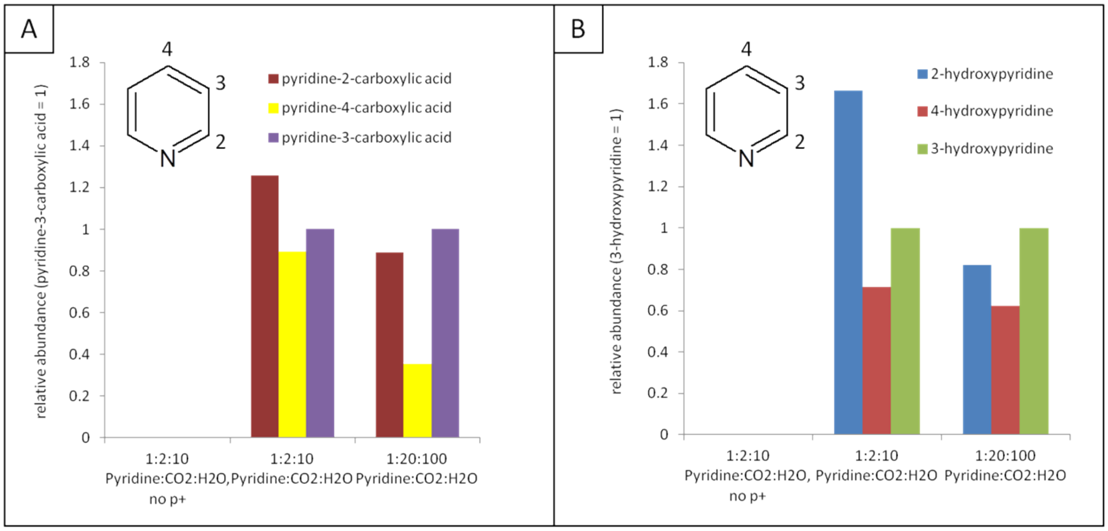

Fig. S1 (A) Relative abundances of pyridinemonocarboxylic acids in the pyridine-CO_2-H_2O ice residues. (B) Relative abundances of hydroxypyridines in the pyridine-CO_2-H_2O ice residues. Astrophysical ice analogs with higher water content begin to favor substitution at the 3-position (over the 2-position) on the pyridine ring for the pyridinecarboxylic acid and hydroxypyridine classes.

References

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3. J. F. Ziegler, M. D. Ziegler, and J. P. Biersack, *Nucl. Instrum. Methods Phys. Res. B Beam Interact. Mater. Atoms*, 2010, **268**, 1818-1823.