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3 **Investigation of Pyridine Carboxylic Acids in CM2 Carbonaceous Chondrites:**  
4 **Potential Precursor Molecules for Ancient Coenzymes**

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42 **Abstract**

43  
44 The distribution and abundances of pyridine carboxylic acids (including nicotinic acid) in  
45 eight CM2 carbonaceous chondrites (ALH 85013, DOM 03183, DOM 08003, EET 96016, LAP  
46 02333, LAP 02336, LEW 85311, and WIS 91600) were investigated by liquid chromatography  
47 coupled to UV detection and high resolution Orbitrap mass spectrometry. We find that pyridine  
48 monocarboxylic acids are prevalent in CM2-type chondrites and their abundance negatively  
49 correlates with the degree of pre-terrestrial aqueous alteration that the meteorite parent body  
50 experienced. We also report the first detection of pyridine dicarboxylic acids in carbonaceous  
51 chondrites. Additionally, we carried out laboratory studies of proton-irradiated pyridine in  
52 carbon dioxide-rich ices (a 1:1 mixture) to serve as a model of the interstellar ice chemistry that  
53 may have led to the synthesis of pyridine carboxylic acids. Analysis of the irradiated ice residue  
54 shows that a comparable suite of pyridine mono- and dicarboxylic acids was produced, although  
55 aqueous alteration may still play a role in the synthesis (and ultimate yield) of these compounds  
56 in carbonaceous meteorites. Nicotinic acid is a precursor to nicotinamide adenine dinucleotide, a  
57 likely ancient molecule used in cellular metabolism in all of life, and its common occurrence in  
58 CM2 chondrites may indicate that meteorites may have been a source of molecules for the  
59 emergence of more complex coenzymes on the early Earth.

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## 67 1. INTRODUCTION

68 Carbonaceous chondrites are meteorites that contain significant amounts of organic carbon  
69 (*e.g.*, 2% by weight) with some variable amount of this organic carbon in soluble form (Sephton,  
70 2002). The organic compounds contained in these chondrites are thought to be formed either by  
71 aqueous reactions in meteorite parent bodies or via reactions occurring in the interstellar medium  
72 (on energetically-processed, icy-organic dust grains) and later incorporated into meteorite parent  
73 bodies (Cronin and Chang, 1993; Cronin *et al.*, 1993), or possibly by a combination of the two  
74 processes. The soluble organic content of carbonaceous chondrites is highly complex and is  
75 known to contain biologically relevant molecules such as amino acids (Kvenvolden *et al.*, 1970;  
76 Cronin and Moore, 1971; Cronin and Pizzarello, 1997; Meierhenrich *et al.*, 2004), nucleobases  
77 (Stoks and Schwartz, 1979, 1981; Martins *et al.*, 2008; Callahan *et al.*, 2011), and metabolic  
78 intermediates (Pizzarello *et al.*, 2001; Pizzarello *et al.*, 2004; Cooper *et al.*, 2011).

79 Numerous investigations involving the analysis of soluble organic compounds in meteorites  
80 have relied heavily on gas chromatography-mass spectrometry and, to a lesser extent, liquid  
81 chromatography-mass spectrometry. In one of our earlier studies, we had applied liquid  
82 chromatography coupled to triple quadrupole mass spectrometry to investigate nitrogen  
83 heterocyclic compounds in a variety of carbonaceous chondrites (Callahan *et al.*, 2011). Many  
84 nitrogen heterocycles are components of RNA/DNA nucleotides as well as coenzymes  
85 (cofactors), which make these compounds of significant interest for origin of life and  
86 astrobiology studies. We demonstrated that multiple reaction monitoring (MRM) mode (via  
87 triple quadrupole mass spectrometry) served as a highly sensitive means for the targeted analysis  
88 of nitrogen heterocycles. However, the low mass resolution and mass accuracy with MRM  
89 acquisitions prevented an unambiguous identification of compounds in some cases, and this led

90 us to employ high resolution/accurate mass measurements to rectify these cases. Coupled with  
91 liquid chromatography, the high mass accuracy and high mass resolution of an Orbitrap mass  
92 spectrometer eliminates most, if not all, of the matrix interferences in the extracted ion  
93 chromatograms, which allows for the unambiguous identification and quantitation of targeted  
94 compounds. Thus, we have expanded our investigation of meteoritic organic compounds by  
95 applying these state-of-the-art techniques to target additional nitrogen heterocycles such as  
96 nicotimamide, nicotinic acid, and other pyridine carboxylic acids. The delivery of these classes  
97 of compounds to the early Earth via meteorites may have been significant for the synthesis of  
98 more complex coenzymes (such as nicotinamide adenine dinucleotide, NAD) that are used  
99 throughout modern metabolism.

100 Figure 1 shows nicotinic acid (3-pyridinecarboxylic acid) and its structural isomers, picolinic  
101 acid (2-pyridinecarboxylic acid) and isonicotinic acid (4-pyridinecarboxylic acid), which have  
102 been identified in only a few carbonaceous chondrites to date, such as Murchison, a CM2  
103 chondrite, and Tagish Lake, a C2 ungrouped chondrite (Pizzarello *et al.*, 2001; Pizzarello *et al.*,  
104 2004; Pizzarello and Huang, 2005). The compound-specific isotope ratio values for nicotinic  
105 acid were  $\delta D = 129\text{‰}$  and  $\delta^{13}C = 20\text{‰}$  in the Murchison meteorite, which strongly suggests that  
106 these compounds are extraterrestrial in origin because these values are well outside of terrestrial  
107 ranges (Pizzarello *et al.*, 2004; Pizzarello and Huang, 2005). In addition, the presence of  
108 nicotinic acid, its two structural isomers, as well as several other methyl and dimethyl homologs  
109 of pyridine carboxylic acids, also points to an abiotic and extraterrestrial origin for these  
110 molecules because the observation of extensive structural diversity of organic compounds in  
111 meteorites is interpreted as indicative of an extraterrestrial origin (Sephton, 2002). The  
112 mechanism of synthesis of these pyridine carboxylic acids has yet to be determined because the

113 Murchison and Tagish Lake meteorites are carbonaceous chondrites with dissimilar organic  
114 content, yet they contain approximately the same abundances of pyridine monocarboxylic acids  
115 (Pizzarello *et al.*, 2006). Furthermore, the prevalence of nicotinic acid and its isomers in  
116 carbonaceous chondrites is unknown; therefore, we devised a method to conduct a systematic  
117 study of these compounds in several meteorites of one class (*i.e.* a systematic study of several  
118 CM2 chondrites rather than several ungrouped carbonaceous chondrites).

119 Here we report the detection and quantitation of meteoritic nicotinic acid and its structural  
120 isomers, picolinic acid and isonicotinic acid. We also report the first detection of pyridine  
121 dicarboxylic acids in meteorites. We analyzed extracts from the following eight meteorites for  
122 pyridine carboxylic acids by liquid chromatography coupled to UV detection and high resolution  
123 Orbitrap mass spectrometry (LC-UV/MS): Allan Hills (ALH) 85013, Dominion Range (DOM)  
124 03183, Dominion Range (DOM) 08003, Elephant Moraine (EET) 96016, LaPaz Ice Field (LAP)  
125 02333, LaPaz Ice Field (LAP) 02336, Lewis Cliff (LEW) 85311, and Wisconsin Range (WIS)  
126 91600. All of these meteorites are CM2 type carbonaceous chondrites, which typically contain  
127 the most diverse organic compounds (Schmitt-Kopplin *et al.*, 2010) including nitrogen  
128 heterocycles (Stoks and Schwartz, 1982; Callahan *et al.*, 2011). To our knowledge, these  
129 meteorites have never before been investigated for pyridine carboxylic acids. In addition, we  
130 carried out experiments simulating cosmic ray processing of mixed-molecular ices, which may  
131 take place in dense interstellar clouds. From these results, we discuss the potential relationship  
132 between interstellar ice chemistry and meteoritic pyridine carboxylic acids.

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## 134 **2. EXPERIMENTAL SECTION**

### 135 **2.1. Chemicals and reagents**

136 All glassware and ceramics were rinsed with ultrapure water (18.2 M $\Omega$ -cm, <5 parts-per-  
137 billion total organic carbon from a Millipore Milli-Q Integral 10 system), wrapped in aluminum  
138 foil, and heated to 500 °C overnight. Ultrapure water was used exclusively for this study.  
139 Pyridine mono- and dicarboxylic acid standards (minimum 97% purity) were purchased from  
140 Sigma-Aldrich and nicotinamide was purchased from ICN Biomedicals, Inc. Acetonitrile  
141 (99.5+%) and sulfuric acid (95-98% purity) were purchased from Sigma Aldrich, and methanol  
142 (Optima® grade) was purchased from Fisher Scientific. A standard solution of mono- and  
143 dicarboxylic acids and nicotinamide was prepared by first making individual standards in water,  
144 then mixing those standards into one solution ranging from 1-100  $\mu$ M. For liquid  
145 chromatographic separation, a 0.05% by volume sulfuric acid solution was prepared. A solution  
146 of 6 M hydrochloric acid (HCl) for the acid hydrolysis extraction was double vacuum distilled.

## 147 **2.2. Meteorites**

148 The following Antarctic CM2 carbonaceous chondrites analyzed in this study were interior  
149 fragments selected by the meteorite sample curator at the NASA Johnson Space Center: ALH  
150 85013 (parent 23, specific 65, mass 1.134 g), DOM 03183 (parent 15, specific 25, mass 1.196 g),  
151 DOM 08003 (parent 9, specific 14, mass 1.126 g), EET 96016 (parent 0, specific 16, mass 1.116  
152 g), LAP 02333 (parent 16, specific 24, mass 1.061 g), LAP 02336 (parent 3, specific 11, mass  
153 1.078 g), LEW 85311 (parent 24, specific 74, mass 1.138 g), and WIS 91600 (parent 59, specific  
154 61, mass 1.069 g). Individual meteorite chips, free of fusion crust, were pulverized into a fine  
155 powder using a ceramic mortar and pestle under a positive pressure High-Efficiency Particulate  
156 Air (HEPA) filtered Class 100 laminar flow hood (Labconco). The control blank for this study  
157 was crushed serpentine (a hydrated magnesium silicate mineral) heated to 500 °C for 3 hours,  
158 which was processed using identical protocols.

## 159 **2.3. Extraction and Sample Work-up**

### 160 **2.3.1. Hot water and acid-hydrolyzed hot water extracts**

161 For the extraction of pyridine carboxylic acids, samples of ALH 85013 (105.9 mg), DOM  
162 03183 (108.7 mg), DOM 08003 (98.1 mg), EET 96016 (110.0 mg), LAP 02333 (96.8 mg), LAP  
163 02336 (99.3 mg), LEW 85311 (100.2 mg), and WIS 91600 (100.0 mg) were transferred to  
164 individual glass ampoules and flame-sealed with 1 mL water each and heated to 100 °C for 24  
165 hours. The hot water extracts were then centrifuged and the supernatant was split into two 500  
166 µL portions. One 500 µL portion of the supernatant was transferred to a 10 mm glass test tube,  
167 dried under vacuum and placed into a larger 20 mm test tube. The test tube was flame-sealed  
168 and the hot water extracted dried residue was acid-hydrolyzed using 6 M HCl vapor at 150 °C  
169 for 3 hours in order to liberate any pyridine carboxylic acids that might have been in an acid-  
170 labile “bound” form, which is typical for some meteoritic organic compounds (Cronin and  
171 Moore, 1971). The acid-hydrolyzed hot water extracts were dried under vacuum then re-  
172 dissolved in 1 mL of water. The hot water and acid-hydrolyzed hot water meteorite extracts  
173 (unknown pH, but the latter was likely acidic) were desalted using conditioned cation exchange  
174 columns (Biorad Poly-Prep® AG® 50W-X8 resin, 100-200 mesh, hydrogen form). Meteorite  
175 extracts were loaded onto the columns, rinsed with water, and the pyridine carboxylic acids were  
176 eluted with 2 M NH<sub>4</sub>OH. Only the (basic) elute fraction was kept and analyzed. The desalted  
177 extracts were then dried down under vacuum and re-dissolved with 100 µL water. All meteorite  
178 extracts were stored in a -86 °C freezer until analysis.

### 179 **2.3.2. Formic acid extracts**

180 Samples of ALH85013 (206.9 mg), DOM03183 (204.8 mg), DOM08003 (204.6 mg),  
181 EET96016 (214.5 mg), LAP02333 (215.7 mg), LAP02336 (211.1 mg), LEW85311 (206.6 mg),

182 and WIS91600 (211.9 mg) were transferred to individual glass ampoules and flame-sealed with 2  
183 mL formic acid and heated to 100 °C for 24 hours. The ampoules were frozen in liquid nitrogen  
184 before opening (to minimize the risk of explosion), centrifuged, and the supernatant transferred  
185 to a test tube. The meteorite powder was rinsed with 1 mL formic acid; the supernatants were  
186 combined and then dried under vacuum. After the residue was dry, it was dissolved in 2 mL 0.1  
187 M ammonium hydroxide. Meteorite extracts were loaded onto a conditioned Waters solid phase  
188 extraction (SPE) cartridge (Oasis Max 6 cc, 150 mg sorbent) and rinsed with 6 mL 5 % ammonia  
189 in water followed by 6 mL methanol. Pyridine monocarboxylic acids were then eluted using 6  
190 mL 5% formic acid in methanol. The purified extracts were dried under vacuum and dissolved  
191 in 400  $\mu$ L 0.1 M ammonium hydroxide. All meteorite extracts were stored in a -86 °C freezer  
192 until analysis.

#### 193 **2.4. Irradiated pyridine + CO<sub>2</sub> ice experiments**

194 Proton irradiation experiments were performed in the Cosmic Ice Laboratory at the NASA  
195 Goddard Space Flight Center. The system consisted of a stainless steel high vacuum chamber (P  
196  $\sim 10^{-7}$  torr) containing a polished aluminum substrate attached to the cold finger of a closed-  
197 cycle helium cryostat (ARS model DE-204). This rotatable substrate can face a deposition tube,  
198 a beam of 0.8-MeV protons from a Van de Graaff accelerator, or an FTIR spectrometer (Hudson  
199 and Moore, 2003; Hudson and Moore, 2004). Gases (CO<sub>2</sub> and pyridine vapor) were mixed in a  
200 separate gas manifold, and ice films were produced by leaking them into the vacuum chamber in  
201 front of the cold substrate over the course of  $\sim$ 15 minutes. Final sample thicknesses were  $\sim$ 1  $\mu$ m,  
202 as measured by laser interferometry. This thickness is well below the stopping range for 0.8-  
203 MeV protons and ensures that the entire ice sample is processed. Contaminants (mainly H<sub>2</sub>O)  
204 made up no more than 1% of the final ice, based on our estimates of the background gases



205 present and the rate at which the sample was grown. After deposition, the pyridine + CO<sub>2</sub> (1:1)  
206 ice was exposed to a 0.8-MeV proton beam with a current of 150 nA until a fluence of  $1.2 \times 10^{15}$   
207 protons·cm<sup>-2</sup> was achieved. The average energy dose (energy absorbed) was 36 eV per initial  
208 molecule deposited (plausible for dense interstellar clouds), which was determined using a  
209 proton stopping power for the pyridine + CO<sub>2</sub> mixture of  $2.73 \times 10^8$  eV cm<sup>2</sup>·g<sup>-1</sup>·proton<sup>-1</sup>. This  
210 value was calculated using the SRIM software package (Ziegler *et al.*, 2010), assuming a density  
211 for the pyridine + CO<sub>2</sub> mixture of 1 g·cm<sup>-3</sup>.

212 To aid in the interpretation of the IR spectra of the irradiated ice, we also measured the IR  
213 spectra of the possible irradiation products nicotinic acid, isonicotinic acid, and picolinic acid at  
214 ~20 K. These reference spectra may also be useful for interpretation of observational data.  
215 Since these three substances are supplied as powders, a sublimation oven inside the vacuum  
216 chamber (described by Gerakines *et al.*, 2012) was used in each case to vaporize the chosen  
217 compound and produce an ~1 μm thick film on the cold substrate whose IR spectrum could then  
218 be measured.

219 After irradiation, the ice was allowed to warm up slowly to room temperature overnight,  
220 allowing volatile products to be pumped away. The remaining non-volatile (refractory) material  
221 was removed by repeatedly pipeting 250 μL water on the aluminum substrate until the yellow-  
222 colored residue was observed to be completely removed. The refractory solution was analyzed  
223 immediately by LC-UV/MS.

## 224 **2.5. Sample Analysis by LC-UV/MS**

225 All samples and controls were analyzed using a Thermo Scientific Accela high performance  
226 liquid chromatograph coupled to a Thermo Scientific Accela photodiode array detector (PDA)  
227 and a Thermo Scientific LTQ Orbitrap XL hybrid mass spectrometer. Separation of pyridine

228 carboxylic acids was accomplished by injecting 5  $\mu$ L sample solution onto an SIELC Primesep  
229 100, 150  $\times$  2.1 mm column (5  $\mu$ m particle size) with a Primesep 100, 10  $\times$  2.1 mm guard column  
230 (5  $\mu$ m particle size). Mobile phase (A) consisted of water with 0.05% sulfuric acid and mobile  
231 phase (B) was 100% acetonitrile. Samples were eluted at 200  $\mu$ L/min using the following  
232 gradient: 0-3 min. 100% A, 3-10 min. 100-95% A, 10-20 min. 95% A, and 20-30 min. 100% A.  
233 The UV spectrum (200-400 nm) was collected using the Accela PDA. UV ( $\lambda$  = 260 nm)  
234 retention time was used as additional confirmation for identification and not for quantitation due  
235 to high UV-absorbing background and complexity.

236 The Thermo Scientific LTQ Orbitrap XL hybrid mass spectrometer was equipped with an  
237 electrospray ionization (ESI) source and operated in positive ion mode. Parameters for ESI were  
238 as follows: the nitrogen gas for desolvation of the electrospray was set to 35 for the sheath gas,  
239 15 for the auxiliary gas, and 1 for the sweep gas, all in arbitrary units. The ion transfer capillary  
240 voltage and temperature were 21 V and 275  $^{\circ}$ C, respectively. The tube lens was set to 55 V.  
241 Full scan mass spectra were taken over a range of  $m/z$  50 to 500. The mass resolution was set to  
242 30,000 (at full-width-half-maximum for  $m/z$  400) in order to maintain an appropriate number of  
243 data points across chromatographic peaks. MS/MS data was collected in the Orbitrap mass  
244 analyzer (HCD energy 60% and 100%, isolation width 1 Da, and resolution 7,500). External  
245 calibration was performed using a mixture of caffeine, MRFA (L-methionyl-arginyl-  
246 phenylalanyl-alanine acetate hydrate) peptide, and Ultramark 1621 (a mixture of fluorinated  
247 phosphazines) in an acetonitrile-methanol-water solution containing 1% acetic acid, which  
248 resulted in a typical mass accuracy of <2 parts-per-million (ppm).

249 Abundances were calculated only for pyridine monocarboxylic acids. The error for these  
250 measurements was estimated to be <10%, which was calculated as the relative standard deviation

251 from 21 measurements of each pyridine monocarboxylic acid standard (63 measurements in  
252 total) and verified by three measurements of each pyridine monocarboxylic acid (9  
253 measurements in total) in the meteorite LAP 02333. Very low (or zero) recoveries of pyridine  
254 dicarboxylic acids (due to the desalting and SPE procedures employed here) prevented accurate  
255 measurements of abundances and were not reported here. In addition, 2,6-pyridinedicarboxylic  
256 acid exhibited poor ESI efficiency, which resulted in poor sensitivity for this compound.

257

### 258 **3. RESULTS AND DISCUSSION**

#### 259 **3.1. Measurements of pyridine carboxylic acids in standard solutions and meteorite** 260 **extracts.**

261 We performed a seven-point linearity study using standard solutions of the three pyridine  
262 monocarboxylic acid isomers using a concentration range of 1  $\mu\text{M}$  to 100  $\mu\text{M}$  (or 5 pmol to 500  
263 pmol on column). The calibration curves were highly linear ( $R^2 > 0.99$ ) for nicotinic acid,  
264 isonicotinic acid, and picolinic acid using the integrated peak area of the extracted ion  
265 chromatogram at the theoretical protonated mass of  $124.0393 \pm 0.0006$  Da (5 ppm mass  
266 window), and enabled accurate quantitation of these compounds in a sample. Our estimated  
267 limit of detection for each pyridine carboxylic acid isomer is  $\sim 1$  part-per-billion (ppb or ng  
268 isomer/g meteorite).

269 Figure 2 shows typical extracted ion chromatograms of the serpentine procedural blank  
270 (bottom trace), the standard mix of all three pyridine monocarboxylic acid isomers (middle  
271 trace), and the LAP 02336 meteorite (top trace). These data are representative of our results  
272 from the other CM2 carbonaceous chondrites. Nicotinic acid, isonicotinic acid, and picolinic  
273 acid in the meteorite were unambiguously identified based on chromatographic retention time,

274 accurate mass measurements, and product ion spectra. We did not detect nicotinic acid,  
275 isonicotinic acid, or picolinic acid in our procedure blank (above our detection limits), which  
276 suggests that our laboratory analytical protocols did not introduce additional pyridine carboxylic  
277 acids. The abundances of nicotinic acid and its isomers were adjusted based on recoveries of  
278 standards processed using our desalting or SPE procedure (see Supporting Information for table).

279 Figure 3 shows typical extracted ion chromatograms of the serpentine procedural blank  
280 (bottom trace), the standard mix of pyridine dicarboxylic acid isomers (middle trace), and the  
281 LAP 02333 meteorite (top trace). Additionally, product ion spectra for these compounds are  
282 shown in Figure 4. We unambiguously identified 3,4-pyridinedicarboxylic acid, 2,5-  
283 pyridinedicarboxylic acid, and 3,5-pyridinedicarboxylic acid in multiple meteorite extracts. To  
284 our knowledge, this is the first report of pyridine dicarboxylic acids in meteorites, and these  
285 organic compounds appear to be common in CM2 chondrites as well. However, we were unable  
286 to detect 2,3-pyridinedicarboxylic acid (quinolinic acid) in our meteorite extracts. The lack of  
287 detection for quinolinic acid may have been attributed to very poor recoveries during the  
288 desalting procedure (~7%). On the other hand, other pyridine dicarboxylic acids were detected  
289 despite similar low recoveries.

290 One interesting observation is that we did not detect nicotinamide in any of the meteorite  
291 extracts, which suggests that CM2 carbonaceous chondrites might not have been a significant  
292 source of this compound for early Earth (see Supporting Information for more details).

### 293 **3.2. Abundances of pyridine carboxylic acids in meteorite extracts.**

294 We analyzed two different hot water extracts, non acid-hydrolyzed and acid-hydrolyzed, of  
295 eight CM2 carbonaceous chondrites in order to determine the abundances of nicotinic acid and  
296 its two isomers, picolinic acid and isonicotinic acid. Seven of the eight CM2 chondrites in this

297 study, with the exception of WIS 91600, contained all three pyridine monocarboxylic acid  
298 isomers in both extracts. The abundances of pyridine monocarboxylic acid isomers in the hot  
299 water extract were approximately equivalent. This result is similar to what was observed in  
300 earlier studies of hot water extracts of two carbonaceous chondrites (a CM2 chondrite and a C2  
301 ungrouped chondrite) (Pizzarello *et al.*, 2006 ). WIS 91600 was the only meteorite that did not  
302 contain any pyridine monocarboxylic acids above our detection limits (<1 ppb), which was likely  
303 due to thermal alteration on the parent body (Choe *et al.*, 2010) that led to the decomposition of  
304 organic compounds. Of the meteorites that contained pyridine monocarboxylic acids in both  
305 extracts, DOM 03183 contained the least amount of total pyridine monocarboxylic acids (61  
306 ppb), also likely due to thermal alteration of the meteorite parent body (Alexander *et al.*, 2012).  
307 Figure 5a shows the abundances of pyridine monocarboxylic acids in the hot water extracts,  
308 which range from approximately 61 ppb to 253 ppb total pyridine monocarboxylic acids  
309 (excluding WIS 91600). Figure 5b shows the abundances of pyridine monocarboxylic acids in  
310 the acid-hydrolyzed hot water extracts, which are noticeably higher than the values for the hot  
311 water extracts ranging from 128 ppb to 497 ppb total pyridine monocarboxylic acids (excluding  
312 WIS 91600). The difference in the hot water vs. the acid-hydrolyzed hot water extracts is the  
313 release of approximately 23-166% more pyridine monocarboxylic acids likely from an acid-  
314 labile bound form (*e.g.*, precursor molecules, complex bonded species). However, another  
315 possibility for the lower abundances of pyridine monocarboxylic acids in the hot water extracts  
316 compared to the hot water acid-hydrolyzed extracts might be due to the desalting step. Hot water  
317 meteorite extracts were (likely) neutral to basic pH, which might have resulted in some loss of  
318 pyridine monocarboxylic acids during the water rinse rather than the elution step. We

319 investigated both 3-cyanopyridine and nicotinamide, which can be hydrolyzed to nicotinic acid,  
320 as a potential precursor molecule (see Supporting Information).

321 We analyzed formic acid extracts of eight CM2 carbonaceous chondrites in order to obtain  
322 the abundances of nicotinic acid, isonicotinic acid, and picolinic acid. All of the eight CM2  
323 chondrites contained all three structural isomers of pyridine carboxylic acids in the formic acid  
324 extract. Figure 5c shows the abundances of pyridine monocarboxylic acids, which are  
325 significantly more abundant in the formic acid extract (ranging from 163 ppb to 1377 ppb total  
326 pyridine monocarboxylic acids) compared to both of the hot water extracts (non acid-hydrolyzed  
327 and acid-hydrolyzed). The elevated abundances may be due to higher extraction efficiency with  
328 formic acid, which has been known to be effective for liberating nitrogen heterocycles from  
329 meteorites (van der Velden and Schwartz, 1977; Stoks and Schwartz, 1981; Callahan *et al.*,  
330 2011). In addition, picolinic acid abundance seems to increase significantly, especially in DOM  
331 08003, although the exact reason for this is unknown. A summary of abundances (in numerical  
332 form) for pyridine monocarboxylic acids in hot water, hot water acid-hydrolyzed, and formic  
333 acid extracts can be found in the Supporting Information.

334 All CM2 carbonaceous chondrites have experienced some amount of preterrestrial aqueous  
335 alteration. Aqueous alteration is a secondary process that occurs for a finite period of time on the  
336 meteorite parent body in which ice in the asteroid core melts via heat produced through decay of  
337 various radionuclides, such as  $^{26}\text{Al}$  (Grimm and McSween, 1989). This period of liquid water on  
338 the asteroid alters the chemistry of the mineral matrix and may affect the synthesis or destruction  
339 of organic compounds. In Figure 5a-c, for all extracts, the meteorites are arranged from right to  
340 left in order of increasing aqueous alteration based on the method of Alexander *et al.* (2012 and  
341 2013) using previously reported bulk hydrogen abundance, isotopic composition, and C/H ratio

342 information (which also correlates with petrologic indicators for the degree of aqueous  
343 alteration) (see Supporting Information for additional details). In the acid-hydrolyzed hot water  
344 and formic acid extracts, there is a noticeable decrease in abundance of pyridine monocarboxylic  
345 acids with increasing aqueous alteration. This trend suggests that aqueous alteration may have  
346 had an overall destructive effect on these compounds, similar to previous studies involving  
347 amino acids (*e.g.*, Glavin *et al.*, 2010) and nucleobases (Callahan *et al.*, 2011). LEW 85311, the  
348 least aqueously altered (most primitive) meteorite of this study, contains the highest abundance  
349 of total pyridine monocarboxylic acids (>1.3 ppm).

350 We note that some caution should be used when interpreting organic abundances with the  
351 meteorites' inferred degree of aqueous alteration because it is well known that organic  
352 abundances can vary considerably across small spatial distances in meteorite stones (Pizzarello *et*  
353 *al.*, 2003; Herd *et al.*, 2011). Consequently, analyses of additional fragments of the same  
354 meteorite could lead to much different abundances than those given in our study (since we  
355 analyzed only one small fragment for each meteorite) and potentially alter any observed  
356 correlations. Nevertheless, intra-meteorite variation in the organic abundances does not change  
357 the primary observation of this investigation, which is nicotinic acid and other pyridine  
358 carboxylic acids appear to be fairly common, and sometimes in relatively high concentrations, in  
359 an inter-meteorite comparison using eight different meteorites.

### 360 **3.3. Irradiation experiments with pyridine-CO<sub>2</sub> ices: connection with meteoritic organics?**

361 The interstellar-parent body hypothesis holds that organic compounds in meteorites may  
362 have been produced via radiation- and photochemistry that took place on the icy organic mantles  
363 of interstellar grains or via aqueous phase chemistry in the meteorite parent bodies (Cronin and  
364 Chang, 1993; Cronin *et al.*, 1993). For the former scenario, atoms and molecules condense on

365 dust grains in dense interstellar clouds ( $T < 30$  K). Energetic processing of icy grains by cosmic  
366 rays and/or photons can lead to more complex molecules, as demonstrated by numerous  
367 laboratory studies (Bernstein *et al.*, 1999; Bernstein *et al.*, 2001; Gerakines *et al.*, 2004; Elsila *et*  
368 *al.*, 2007; Hudson *et al.*, 2008; Nuevo *et al.*, 2009; Nuevo *et al.*, 2010; Nuevo *et al.*, 2012).  
369 Furthermore, theoretical modeling of the dynamical evolution of ice grains in the solar nebula  
370 has shown that warming would also occur, which would further enable the production of  
371 complex organics (Ciesla and Sandford, 2012). A significant portion of meteoritic organics (*e.g.*,  
372 aromatics, carboxylic acids, amino acids) may be relics of interstellar grain processes because  
373 they are enriched in deuterium and exhibit a high degree of branching and structural diversity  
374 (indicative of reactions involving radicals and ions) (Sephton and Gilmour, 2000; Sandford *et al.*,  
375 2001; Pizzarello and Huang, 2005).

376 From our investigation and previous studies (Stoks and Schwartz, 1982; Pizzarello *et al.*,  
377 2001), substituted pyridines are structurally diverse in meteorites (with methyl, dimethyl,  
378 monocarboxylic acid, and dicarboxylic acid substitutions observed), which suggest that this  
379 compound class might be the product of interstellar grain processes. In order to test this  
380 hypothesis (which also relates to the interstellar-parent body hypothesis), we proton-irradiated a  
381 1:1 mixture of pyridine and CO<sub>2</sub> held at a very low temperature. This experiment is only a first  
382 approximation for the formation of interstellar pyridine carboxylic acids because pyridine has not  
383 yet been identified in interstellar ices (though the presence of pyridine in interstellar ice might  
384 still be possible). Figure 6 (top three traces) shows the IR spectra of the deposited sample  
385 recorded before and after proton irradiation at  $\sim 20$  K and the irradiated sample after warm-up to  
386 room temperature. In the non-irradiated pyridine and CO<sub>2</sub> ice sample, the most intense, sharp  
387 peaks in the 800-2000 cm<sup>-1</sup> range can all be assigned to pyridine (the majority being wagging



388 and bending modes). Figure 6 (bottom three traces) also shows the IR spectra of nicotinic acid,  
389 isonicotinic acid, and picolinic acid (deposited in separate experiments) at ~20 K recorded for  
390 reference to the irradiated sample. There are many new IR bands as a result of irradiation, which  
391 indicate new products formed. For the irradiated sample at ~20 K, we assign the broad IR bands  
392 centered around  $1290\text{ cm}^{-1}$  and  $1720\text{ cm}^{-1}$  to characteristic bands of nicotinic acid and  
393 isonicotinic acid, which may have formed *in situ* during the low temperature irradiation of the  
394 pyridine-CO<sub>2</sub> ice. These bands correspond to the in-plane deformation of C-H and the carbonyl  
395 stretch of nicotinic acid (Kumar and Yadav, 2011) and isonicotinic acid. There do not appear to  
396 be any common IR bands between the picolinic acid IR spectrum and the irradiated pyridine-CO<sub>2</sub>  
397 ice spectrum at ~20 K. Band broadening in the IR spectrum may be due to increased  
398 intermolecular interactions, which supports the notion that pyridine carboxylic acids were  
399 synthesized upon irradiation (because there would be strong hydrogen bonding interaction  
400 between carboxylic acid groups). After warm-up to room temperature, the IR spectrum becomes  
401 very broad and difficult to interpret with respect to the pyridine carboxylic acid isomer reference  
402 spectra.

403 We analyzed the refractory residue of the proton-irradiated pyridine-CO<sub>2</sub> ice by our LC-  
404 UV/MS method. A variety of pyridine monocarboxylic acids, pyridine dicarboxylic acids, and  
405 hydroxypyridines were identified, the latter may have formed from residual water in the  
406 irradiation setup. We find that the distribution of the three pyridine carboxylic acid isomers  
407 identified in the refractory residue is similar to the distribution of isomers in the least altered  
408 (most primitive) meteorite, LEW 85311 (the abundance of picolinic acid > nicotinic acid >  
409 isonicotinic acid). More aqueously altered meteorites generally have a different distribution of  
410 pyridine monocarboxylic acids compared to those measured in the irradiated ice residue. We

411 also identified four pyridine dicarboxylic acids (3,4-, 2,3-, 2,5-, 3,5-pyridinedicarboxylic acids)  
412 in the irradiated pyridine-CO<sub>2</sub> ice, three of which (3,4-, 2,5-, 3,5-pyridinedicarboxylic acids) we  
413 also found in the majority of the CM2 carbonaceous chondrites in this study.

414 The similar distribution of pyridine carboxylic acid isomers in irradiated pyridine-CO<sub>2</sub> ice  
415 and the most primitive meteorite coupled with the extended structural diversity of dicarboxylic  
416 acids in both samples is suggestive of radical chemistry and a possible interstellar origin for  
417 these molecules. However, previous studies have determined that nicotinic acid in the  
418 Murchison meteorite had a  $\delta D = +129\%$  and  $\delta^{13}C = +20\%$  (Pizzarello *et al.*, 2004; Pizzarello  
419 and Huang, 2005), which clearly suggested an extraterrestrial origin but the magnitude of  
420 deuterium enrichment did not approach values of interstellar molecules. However, this  $\delta D$  value  
421 may be a minimum value because the original H or D on the carboxylic acid group of nicotinic  
422 acid would have been removed very quickly after placing the meteoritic pyridine carboxylic acid  
423 compounds in water (for extraction purposes). Additionally, it is unknown whether significant  
424 H/D exchange would occur for the four aromatic H or D during aqueous extraction and work-up.  
425 Other synthetic routes may have been possible. Fischer-Tropsch type reactions have been  
426 previously proposed for the synthesis of alkyl pyridines from aldehydes and ammonia (Stoks and  
427 Schwartz, 1982), and similar reactions could be feasible for pyridine carboxylic acids.

428 Some open questions include: (1) do irradiation experiments with more realistic ice  
429 compositions (*e.g.*, starting from simpler precursors and the addition of H<sub>2</sub>O) still lead to the  
430 production of nicotinic acid (and other pyridine carboxylic acids) or will compounds such as  
431 hydroxypyridines dominate instead, and (2) what are the compound-specific isotope ratios of  
432 pyridine carboxylic acids in other CM2 carbonaceous chondrites (in particular LEW 85311,

433 which experienced a low degree of aqueous alteration yet contained the highest abundances of  
434 pyridine carboxylic acids)? We will address these questions in future investigations.

### 435 **3.4. Implications for prebiotic chemistry on early Earth**

436 Nicotinamide adenine dinucleotide may be one of the most ancient molecules in modern cells  
437 and was likely present very early in the evolution of life (Raffaelli, 2011). There are two ways in  
438 which modern cells can synthesize NAD: the *de novo* pathway or the Preiss-Handler salvage  
439 pathway (Preiss and Handler, 1957a, b, c, 1958a, b). Both pathways involve formation of a  
440 nicotinic acid mononucleotide (NaMN) intermediate, which gets enzymatically converted to  
441 NAD; however, only the salvage pathway uses existing nicotinic acid as a substrate. The first  
442 organisms on early Earth are widely thought to have been anaerobic, so it is possible that the first  
443 pathway to NAD was either an anaerobic *de novo* pathway or a salvage pathway in which an  
444 abiotic synthesis of nicotinic acid would be required.

445 Several laboratory studies have investigated the endogenous formation of pyridine carboxylic  
446 acids under plausibly prebiotic early Earth conditions. For example, Friedmann *et al.* (1971)  
447 demonstrated that the pyridine ring could be synthesized in the form of nicotinonitrile (3-  
448 cyanopyridine) by sending an electrical discharge through a mixture of ethylene and ammonia.  
449 Nicotinonitrile is an important molecule because it can be converted to nicotinamide (Figure 1),  
450 the redox active moiety in NAD, and nicotinic acid by hydrolysis of the cyano group. In  
451 addition, Cleaves and Miller (2001) demonstrated a possible nonenzymatic early Earth synthesis  
452 of nicotinic acid and its metabolic precursor, quinolinic acid (2,3-pyridinedicarboxylic acid), by  
453 reacting dihydroxyacetone phosphate with aspartic acid.

454 CM2-type carbonaceous chondrites may have been an important exogenous source for  
455 organic compounds on the early Earth. The measurement of nicotinic acid in many different

456 CM2 carbonaceous chondrites suggests that this compound (and more generally speaking, this  
457 class of compounds) was common in these types of meteorites. Nicotinic acid and other pyridine  
458 carboxylic acids are readily soluble in water, which suggests that these compounds could be  
459 easily removed from the meteorite and possibly participate in chemical reactions on early Earth.  
460 It is interesting to point out that nicotinic acid and adenine, both of which are molecular  
461 precursors to NAD, have been measured in meteorites. However, adenine was reported in very  
462 low ppb abundances in meteorites (Callahan *et al.*, 2011), which makes it difficult to envision  
463 how useful concentrations built up on early Earth without complementary endogenous synthesis  
464 or the use of alternative molecules.

465 Finally, the synthesis of NAD or its intermediates, such as nicotinic acid  $\beta$ -riboside, may be  
466 challenging under prebiotic conditions. For example, the synthesis of nicotinic acid  $\beta$ -riboside is  
467 difficult in the laboratory, and it is usually produced using multiple synthetic organic steps  
468 (without relevant, plausibly prebiotic conditions) (Franchetti *et al.*, 2004). Also, NAD-mediated  
469 reactions in living organisms require the  $\beta$ -configuration, and a stereoselective synthesis presents  
470 additional difficulties. Nevertheless, alternative routes to synthesize NAD may be feasible. For  
471 example, nucleotides have been successfully synthesized using multi-component synergetic  
472 reactions, which bypasses trying to couple ribose to a nitrogen heterocycle in the sequential  
473 fashion traditionally employed (Powner *et al.*, 2009). A similar scenario may be applicable to  
474 the synthesis of NAD, although it may not involve pyridine carboxylic acid.

475

#### 476 **4. CONCLUSIONS**

477 1. Liquid chromatography coupled to Orbitrap mass spectrometry permitted the unambiguous  
478 identification of pyridine carboxylic acids in meteorites based on chromatographic retention time

479 and accurate mass measurements. The linear response over a wide concentration range, along  
480 with high recoveries from both desalting and solid phase extraction protocols, enabled the  
481 quantitation of pyridine monocarboxylic acids in meteorites. Low recoveries of pyridine  
482 dicarboxylic acids inhibited accurate quantitation and revised purification techniques will be  
483 needed for these molecules in future studies; however, to our knowledge, this is the first report of  
484 pyridine dicarboxylic acids in carbonaceous meteorites.

485 2. Pyridine carboxylic acids were identified in all eight of the CM2-type carbonaceous  
486 chondrites (formic acid extract). The highest abundance of pyridine monocarboxylic acids was  
487 found in formic acid extract of LEW 85311, which is the most primitive (least aqueously altered)  
488 meteorite in this study.

489 3. Pyridine monocarboxylic acids negatively correlate with the degree of aqueous alteration of  
490 CM2-type meteorite parent bodies (acid-hydrolyzed hot water extract and formic acid extract).  
491 There is a decrease in abundance of pyridine carboxylic acids as aqueous alteration of the  
492 meteorite parent body increases, which suggests that aqueous phase reactions in the meteorite  
493 parent body may have destroyed some of these compounds.

494 4. The abundance of pyridine monocarboxylic acids significantly increased in the acid-  
495 hydrolyzed hot water and formic acid extracts (compared to the hot water extract). These  
496 observations suggest that some of the pyridine monocarboxylic acids existed in an acid-labile  
497 bound form.

498 5. The full suite of pyridine monocarboxylic acids and a variety of pyridine dicarboxylic acids  
499 were identified in the non-volatile residue of proton-irradiated pyridine + CO<sub>2</sub> ice. A similar  
500 suite of pyridine mono- and dicarboxylic acids was measured in CM2 carbonaceous chondrites  
501 (only 2,3-pyridinedicarboxylic acid was absent among the targeted compounds) and suggests a

502 connection between interstellar chemistry and the pyridine carboxylic acids observed in  
503 meteorites. However, more realistic interstellar ice compositions (*e.g.*, the inclusion of H<sub>2</sub>O and  
504 simpler organic precursors) are needed in future studies.

505 6. Nicotinamide was not identified in any of the 24 different meteorite extracts we analyzed.  
506 One possible explanation for this might be that the nicotinamide (if it existed on the meteorite  
507 parent body) was hydrolyzed to form nicotinic acid during the period of aqueous alteration on  
508 the meteorite parent body.

509

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524

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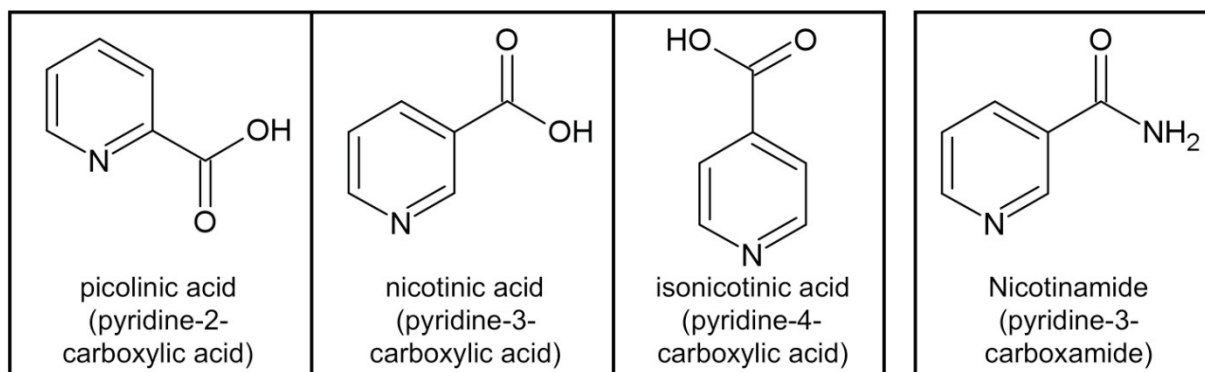
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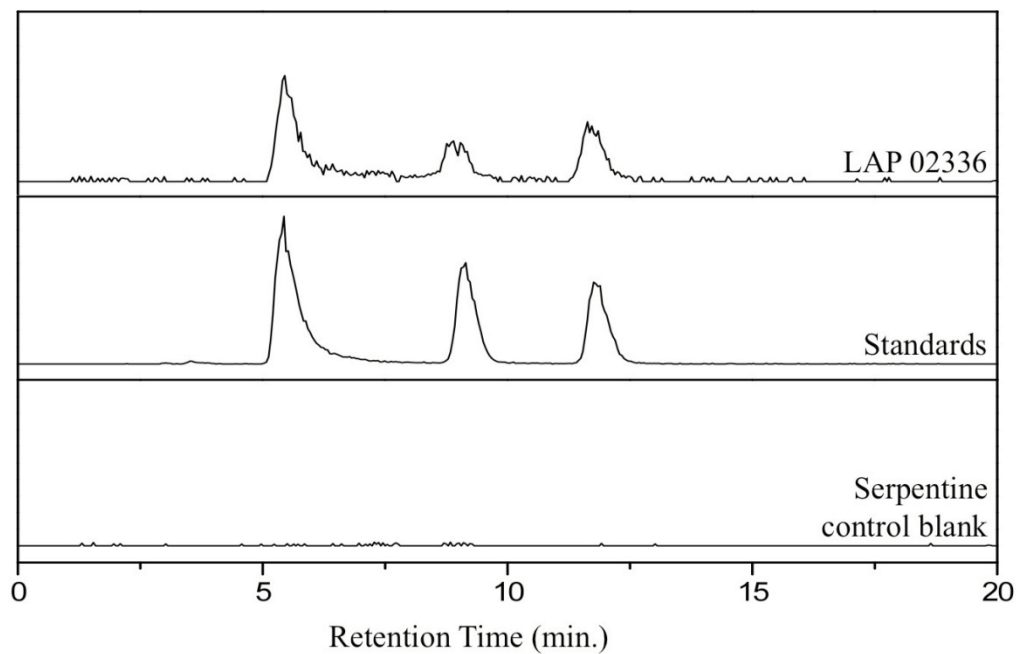
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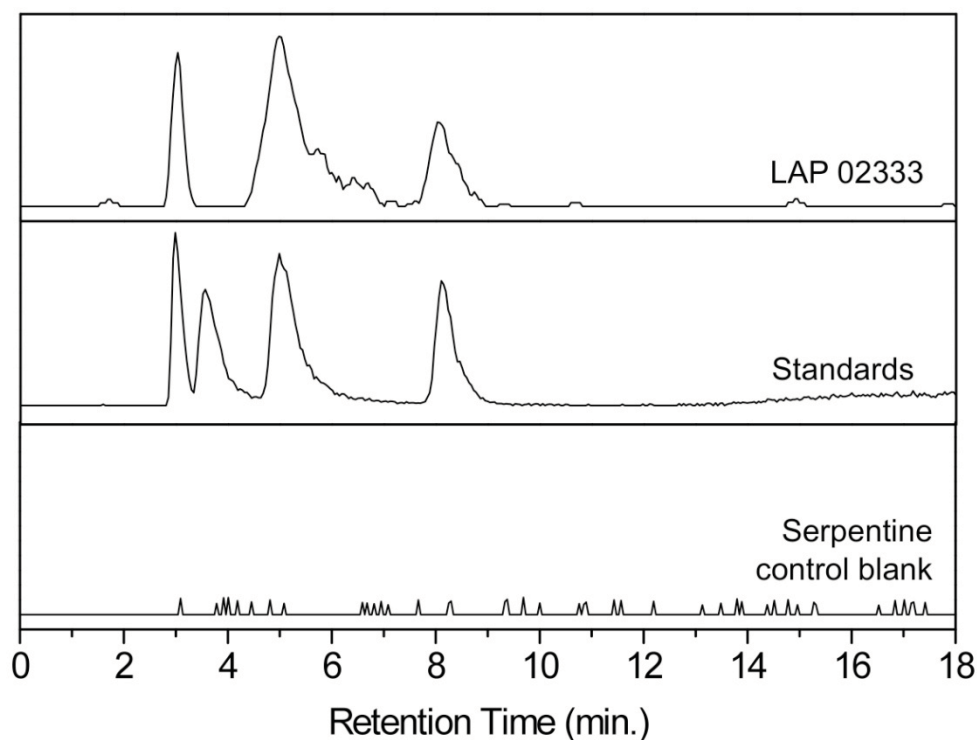
662  
663 **Figure 1.** Structures of the three pyridine monocarboxylic acid isomers and nicotinamide.  
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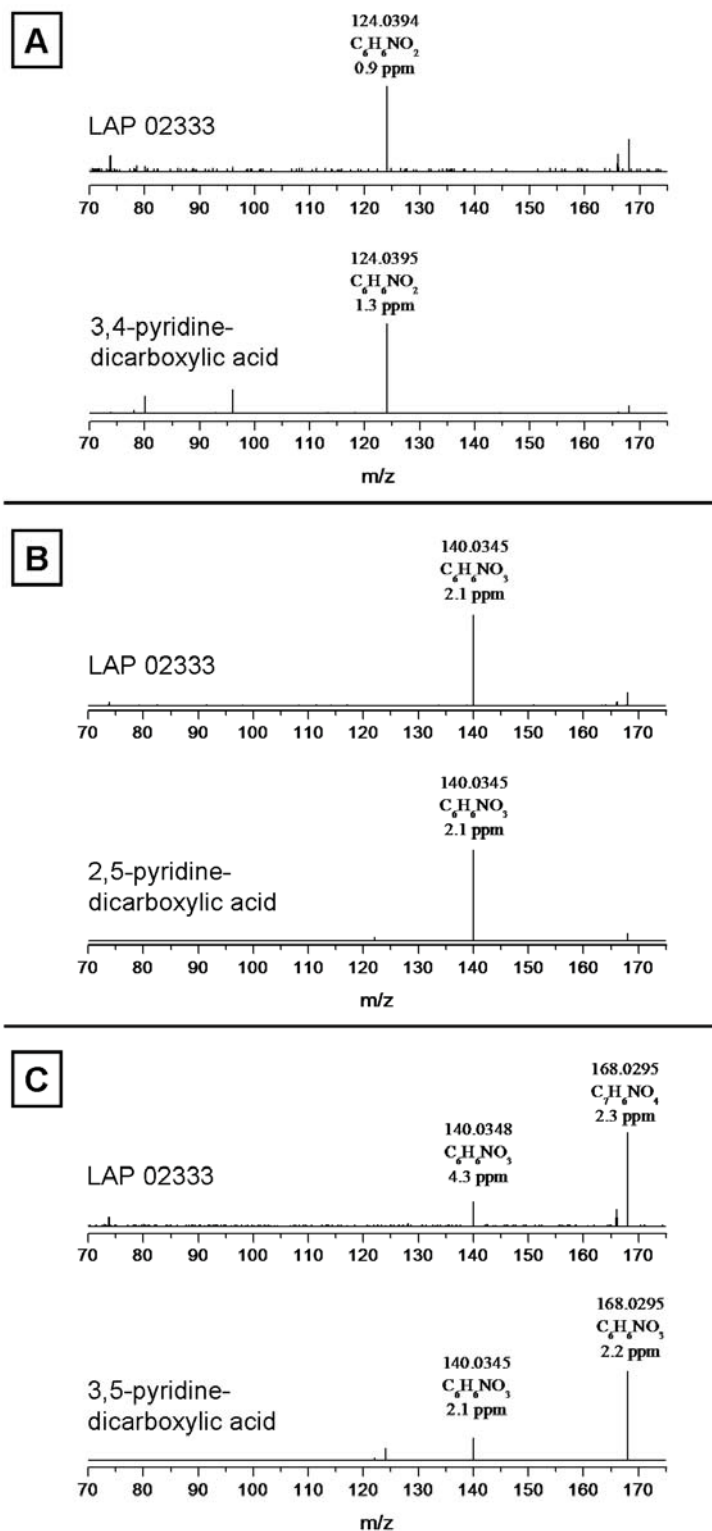


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686 **Figure 2.** Extracted ion chromatograms ( $m/z$  124.0393 with a 5 ppm window corresponding to  
687 the protonated molecular ion) of picolinic acid (5.4 min.), isonicotinic acid (9.0 min.), and  
688 nicotinic acid (11.6 min.) of the LAP 02336 carbonaceous chondrite formic acid extract (top  
689 trace) and reference standards (middle trace). Pyridine monocarboxylic acids were not detected  
690 in the serpentine control blank (bottom trace).

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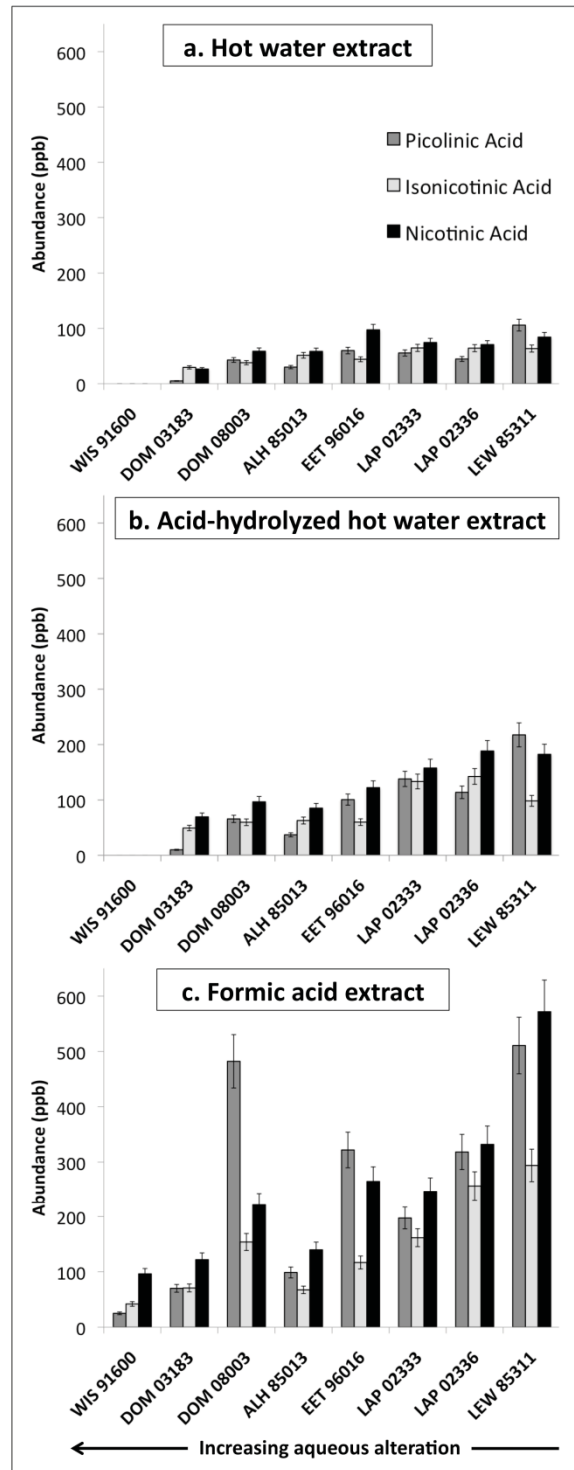


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 712 **Figure 3.** Extracted ion chromatograms ( $m/z$  168.0291 with a 5 ppm window corresponding to  
 713 the protonated molecular ion) of 3,4-pyridinedicarboxylic acid (3.0 min.), 2,5-  
 714 pyridinedicarboxylic acid (5.0 min.), and 3,5-pyridinedicarboxylic acid (8.1 min.) of the LAP  
 715 02333 carbonaceous chondrite acid-hydrolyzed hot water extract (top trace) and reference  
 716 standards (middle trace). Note that 2,3-pyridinedicarboxylic acid (3.6 min.) was not detected in  
 717 LAP 02333 and other meteorites. Pyridine dicarboxylic acids were not detected in the serpentine  
 718 control blank (bottom trace).  
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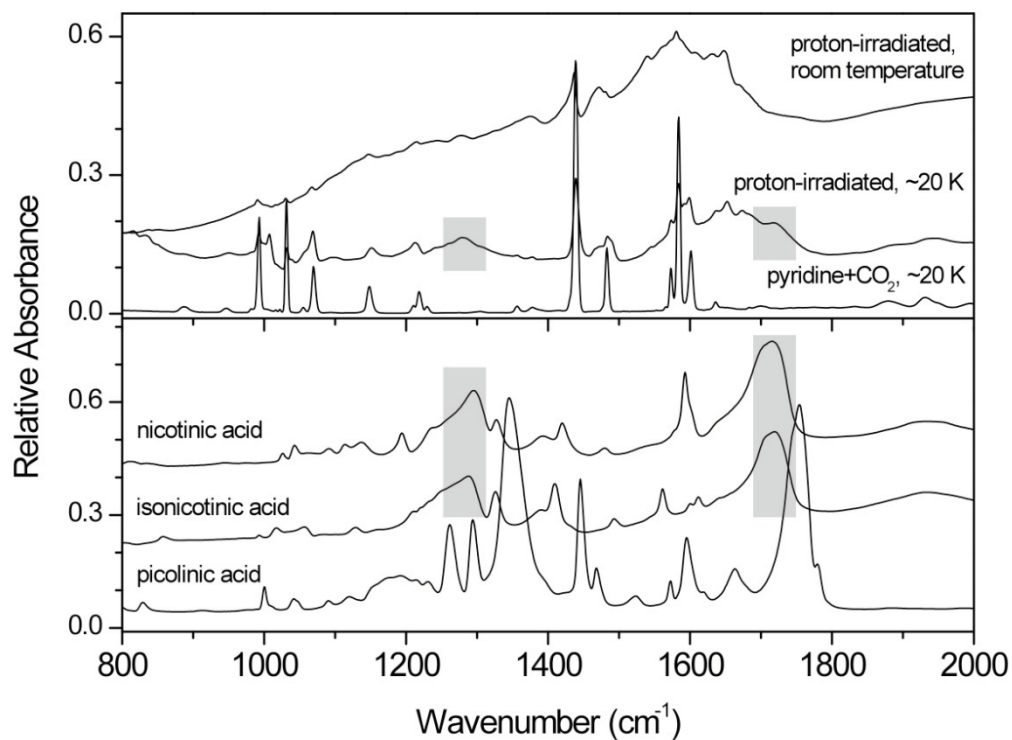


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**Figure 4.** Product ion spectra (HCD 60%, resolution 7,500) of reference standards and compounds found in LAP 02333. Three compounds were identified: 3,4-pyridinedicarboxylic acid (A), 2,5-pyridinedicarboxylic acid (B), and 3,5-pyridinedicarboxylic acid (C).



**Figure 5.** The abundances and distribution of pyridine monocarboxylic acids in the (a) hot water, (b) acid-hydrolyzed hot water, and (c) formic acid extracts determined by mass detection. Carbonaceous chondrites are ordered from right to left in order of increasing aqueous alteration based on the method by Alexander *et al.* (2012).



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727 **Figure 6.** *In situ* IR spectra of the deposited pyridine + CO<sub>2</sub> sample recorded before and after  
 728 proton irradiation at ~20 K and the irradiated sample after warm-up to room temperature (top  
 729 traces). *In situ* IR spectra of nicotinic acid, isonicotinic acid, and picolinic acid deposited (in  
 730 separate experiments) at ~20 K for reference to the irradiated sample (bottom traces). Common  
 731 IR bands between the proton-irradiated sample spectra and the nicotinic acid and isonicotinic  
 732 acid spectra are highlighted in gray.

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742 **Investigation of Pyridine Carboxylic Acids in CM2 Carbonaceous Chondrites:**  
743 **Potential Precursor Molecules for Ancient Coenzymes**

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753 **SUPPORTING INFORMATION**

754 **(1) Cyanopyridines.** We investigated the possible conversion of a potential precursor for  
755 nicotinic acid that could result from our three extraction procedures. We tested 3-cyanopyridine,  
756 which has a nitrile group that can be converted to a carboxylic acid group (resulting in nicotinic  
757 acid) by hydrolysis (see Figure S-1). Additionally, 3-cyanopyridine is a product from energetic  
758 processing of ethylene and ammonia (Friedmann *et al.*, 1971); therefore, 3-cyanopyridine as a  
759 precursor to nicotinic acid in meteorites is plausible. Our results show that there is minimal  
760 nicotinic acid converted from the 3-cyanopyridine standard during the hot water and formic acid  
761 extractions (Table S-1). This may hold true for the meteorite as well; however, the meteorite is  
762 much more complex than standards so we cannot state this conclusively. More nicotinic acid is  
763 formed from the 3-cyanopyridine standard during the acid-hydrolyzed hot water extraction, but  
764 the recovery is still low (31%). If we extrapolate these results to our meteorite extracts, 3-  
765 cyanopyridine (and nicotinamide) could be measured in both the hot water and formic acid  
766 extracts. However, we did not detect 3-cyanopyridine in any of our meteorite extracts. One  
767 possible reason for this result is that these potential precursor molecules have already been  
768 converted to carboxylic acids during the lengthy aqueous alteration phase on the asteroid. This  
769 hypothesis may be supported by past analyses of meteorite organics that show a large abundance



770 and diversity of carboxylic acids but very little in terms of molecules containing nitriles  
771 (Sephton, 2002). On the other hand, if this were true, the more aqueously altered meteorites  
772 would have more pyridine monocarboxylic acids if there were abundant cyanopyridines to start  
773 with, yet the opposite trend was observed (but this assumes that the carboxylic acids are the end  
774 product of hydrolysis).

775 **(2) Nicotinamide.** We investigated the possible hydrolysis of nicotinamide to nicotinic acid  
776 that could have occurred during our three meteorite extraction procedures using reference  
777 standards. There is little to moderate conversion of nicotinamide to nicotinic acid in the hot  
778 water (3%) and formic acid (29%) extracts (Table S-2). In contrast, all of the nicotinamide in the  
779 acid-hydrolyzed hot water extraction was converted to nicotinic acid. Thus, the possibility of  
780 detecting nicotinamide would be in the hot water extraction or the formic acid extraction (to  
781 within 1 ppb). We did not detect nicotinamide in any of our meteorite extracts. Regardless of  
782 the analytical protocols employed, any nicotinamide that may have been originally present may  
783 have already been converted to nicotinic acid during the lengthy aqueous alteration phase on the  
784 asteroid.

785 **(3) Estimating degree of aqueous alteration in CM2 carbonaceous chondrites.** We  
786 plotted  $\delta D$  vs. bulk C/H and  $\delta D$  vs. bulk H for each meteorite using values previously  
787 determined in Alexander *et al.* (2012 and 2013), and is shown in Figure S-2. These plots form  
788 well-defined trends and appear to reflect varying degrees of aqueous alteration. Heated-CM2s  
789 (*e.g.*, DOM 03183 and WIS 91600) were not plotted because these types of CMs were previously  
790 known to plot well away from these trends (Alexander *et al.*, 2013). In the study by Alexander  
791 *et al.* (2013), significant differences were found in the bulk compositions for multiple samples  
792 from the same meteorites in some cases, which subsequently influenced how they were classified

793 (and hence, their inferred degree of aqueous alteration). Thus, the determination of aqueous  
794 alteration for meteorites examined in our study were meant to serve as estimates only since we  
795 analyzed different stones of the same meteorites listed in Alexander *et al.* (2012 and 2013).

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820 **Figure S-1.** The hydrolysis of 3-cyanopyridine to nicotinic acid via nicotinamide.

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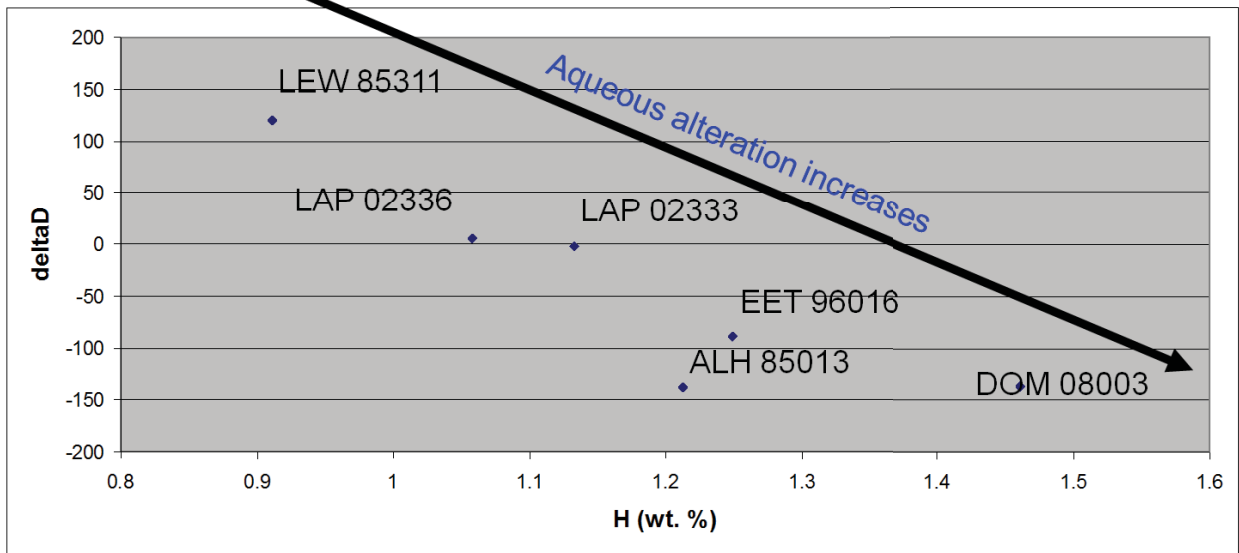
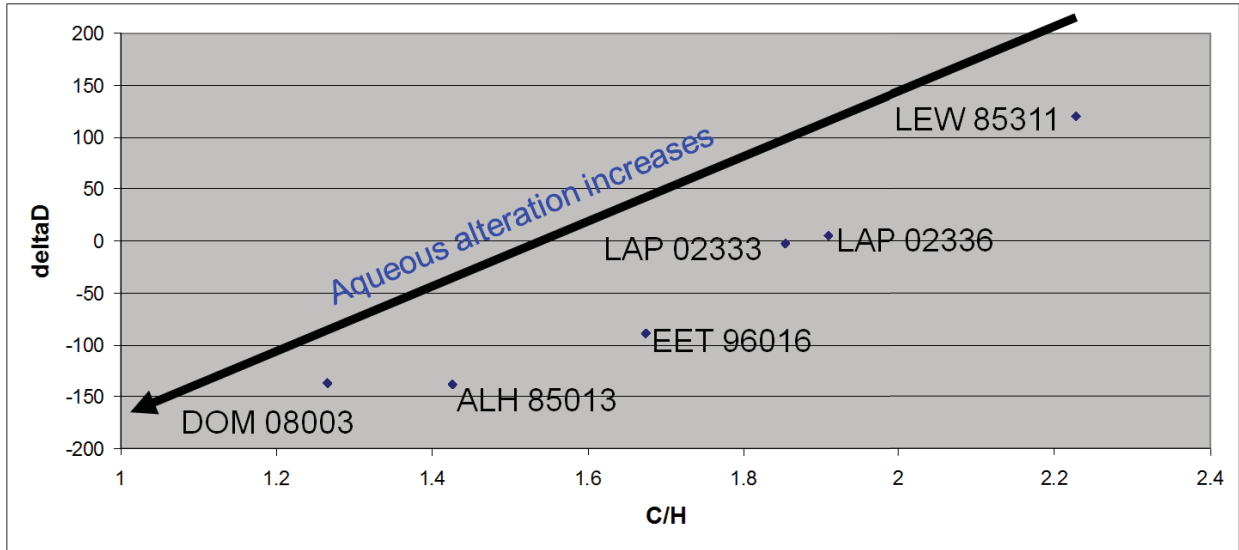
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849 **Figure S-2.** Plots of  $\delta D$  vs. bulk C/H (top plot) and  $\delta D$  vs. bulk H (bottom plot) of carbonaceous  
 850 chondrites to estimate degree of aqueous alteration. Data taken from Alexander *et al.* (2012 and  
 851 2013).

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<b>Extraction</b>	<b>3-cyanopyridine % remaining</b> after extraction procedure	<b>Nicotinamide % converted</b> from 3-cyanopyridine	<b>Nicotinic Acid % converted</b> from 3-cyanopyridine
<b>Hot Water</b> (water, 100 °C, 24 hrs)	53	43	1
<b>Acid-hydrolyzed Hot Water</b> (6 M HCl, 150 °C, 3 hrs)	0	0	31
<b>Formic Acid</b> (formic acid, 100 °C, 24 hrs)	80	13	3

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859 **Table S-1.** Hydrolysis of 3-cyanopyridine reference standard to nicotinic acid using the three  
860 meteorite extraction procedures (hot water, acid-hydrolyzed hot water, and formic acid  
861 extractions).

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<b>Extraction</b>	<b>Nicotinamide <u>% converted to</u> nicotinic acid</b>
<b>Hot Water</b> (water, 100 °C, 24 hrs)	3
<b>Acid-hydrolyzed Hot Water</b> (6 M HCl, 150 °C, 3 hrs)	>100
<b>Formic Acid</b> (formic acid, 100 °C, 24 hrs)	29

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878 **Table S-2.** Hydrolysis of nicotinamide reference standard to nicotinic acid using the three  
879 meteorite extraction procedures (hot water, acid-hydrolyzed hot water, and formic acid  
880 extractions). There is some conversion of nicotinamide to nicotinic acid in the hot water and  
881 formic acid extractions. All of the nicotinamide was converted to nicotinic acid in the acid-  
882 hydrolyzed extract.

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	<i>% Recovery, Desalting</i>	<i>% Recovery, SPE</i>
Nicotinic Acid	94 ± 4	75 ± 1
Isonicotinic Acid	97 ± 5	75 ± 2
Picolinic Acid	86 ± 3	76 ± 3
Nicotinamide	64 ± 2	--
2,3-pyridinedicarboxylic acid	6.8 ± 0.8	--
2,5-pyridinedicarboxylic acid	7.7 ± 0.8	--
3,4-pyridinedicarboxylic acid	7.2 ± 0.8	--
3,5-pyridinediacrboxylic acid	20 ± 2	--

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898 **Table S-3.** The average percent recoveries for the desalted and solid phase extracted (SPE)  
899 standards. Abundances of pyridine carboxylic acids from all of the carbonaceous condrites in  
900 this study were adjusted based on these recoveries. Dashes indicate that these compounds were  
901 not recovered after SPE. The error was calculated as the standard error of the mean from three  
902 measurements. *Note:* We did not have a reference standard for 2,4-pyridinedicarboxylic acid  
903 available in lab. However, based on peak shape comparison of meteorite extracted ion  
904 chromatograms and remaining reference standards, 2,4-pyridinedicarboxylic acid was not likely  
905 present (or had poor ESI efficiency).

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Meteorite	Type	Picolinic Acid (ppb)	Isonicotinic Acid (ppb)	Nicotinic Acid (ppb)
WIS 91600	CM2	<1	<1	<1
DOM 03183	CM2	5.1 ± 0.5	29.6 ± 3	26.5 ± 3
DOM 08003	CM2	42.9 ± 4	38.0 ± 4	58.7 ± 6
ALH 85013	CM2	30.0 ± 3	51.3 ± 5	58.5 ± 6
EET 96016	CM2	59.9 ± 6	44.2 ± 4	97.4 ± 10
LAP 02333	CM2	55.6 ± 6	64.6 ± 6	74.5 ± 7
LAP 02336	CM2	44.7 ± 4	64.3 ± 6	70.7 ± 7
LEW 85311	CM2	105.9 ± 11	63.5 ± 6	84.2 ± 8

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934 **Table S-4.** The abundances of pyridine carboxylic acids in eight CM2-type meteorites from the  
935 hot water extraction as determined by mass detection. The error for these measurements was  
936 calculated as the relative standard deviation from 21 measurements of each pyridine carboxylic  
937 acid standard (63 measurements in total) and verified by three measurements of each pyridine  
938 carboxylic acid (9 measurements in total) in the meteorite, LAP 02333.

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Meteorite	Type	Picolinic Acid	Isonicotinic Acid	Nicotinic Acid
WIS 91600	CM2	<1	<1	<1
DOM 03183	CM2	9.9 ± 1	49.2 ± 5	69.4 ± 7
DOM 08003	CM2	65.7 ± 7	59.6 ± 6	96.5 ± 10
ALH 85013	CM2	36.9 ± 4	62.8 ± 6	85.1 ± 8
EET 96016	CM2	100.5 ± 10	59.8 ± 6	122.2 ± 12
LAP 02333	CM2	138.0 ± 14	133.2 ± 13	157.7 ± 16
LAP 02336	CM2	113.7 ± 11	142.3 ± 14	188.4 ± 19
LEW 85311	CM2	217.5 ± 22	98.2 ± 10	182.4 ± 18

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962 **Table S-5.** The abundances of pyridine carboxylic acids in eight CM2-type meteorites from the  
963 hot water acid-hydrolyzed extraction as determined by mass detection. The error for these  
964 measurements was calculated as the relative standard deviation from 21 measurements of each  
965 pyridine carboxylic acid standard (63 measurements in total) and verified by three measurements  
966 of each pyridine carboxylic acid (9 measurements in total) in the meteorite, LAP 02333.

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Meteorite	Type	Picolinic Acid	Isonicotinic Acid	Nicotinic Acid
WIS 91600	CM2	25.1 ± 2	42.0 ± 42	96.3 ± 10
DOM 03183	CM2	70.2 ± 7	70.8 ± 7	121.9 ± 12
DOM 08003	CM2	482.2 ± 48	153.7 ± 15	221.0 ± 22
ALH 85013	CM2	98.8 ± 10	67.4 ± 7	139.6 ± 14
EET 96016	CM2	322.0 ± 32	116.7 ± 12	265.1 ± 26
LAP 02333	CM2	197.1 ± 20	161.5 ± 16	246.8 ± 25
LAP 02336	CM2	318.4 ± 32	256.9 ± 26	332.1 ± 33
LEW 85311	CM2	510.7 ± 51	294.1 ± 29	571.8 ± 57

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989 **Table S-6.** The abundances of pyridine carboxylic acids in eight CM2-type meteorites from the  
990 formic acid extraction as determined by mass detection. The error for these measurements was  
991 calculated as the relative standard deviation from 21 measurements of each pyridine carboxylic  
992 acid standard (63 measurements in total) and verified by three measurements of each pyridine  
993 carboxylic acid (9 measurements in total) in the meteorite, LAP 02333.

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