ChemComm

COMMUNICATION

Cite this: *Chem. Commun.*, 2015, **51**, 11787

Received 20th April 2015,
Accepted 8th June 2015

DOI: 10.1039/c5cc03272e

www.rsc.org/chemcomm

Metabolic precursors in astrophysical ice analogs: implications for meteorites and comets†

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We report the synthesis of complex organic compounds including nicotinic and quinolinic acid, two members involved in the nicotinamide adenine dinucleotide (NAD) biosynthetic pathway, in irradiated astrophysical ice analogs. If delivered to Earth by meteorites and comets, these compounds may have contributed to the origin and early evolution of life.

In dense molecular clouds, small (μm-sized) dust grains are coated with water, carbon dioxide, carbon monoxide, and other low molecular weight compounds, which form an icy mantle.[1] Energetic processing of icy grains by cosmic rays and UV photons leads to more complex molecules, as demonstrated by numerous laboratory studies simulating these environments, a few of which are cited here.[2–7] Furthermore, modeling has shown that grains would have experienced significant heating due to migration through different parts of the solar nebula, and this would also facilitate the production of more complex organic compounds.[8] The processed ice materials could then serve as ingredients in the formation of planetary systems including primitive meteoritic parent bodies. Meteorites, particularly carbonaceous chondrites, are highly complex[9] and a significant portion of meteoritic organic compounds (*e.g.*, aromatics, carboxylic acids, and amino acids) may be relics of processed interstellar or nebular ices because their high D/H ratios suggest a low temperature origin.[10–13]

In our previous study, we measured the distribution and abundances of pyridine carboxylic acids (including nicotinic acid) in eight CM2 carbonaceous chondrites by liquid chromatography-mass spectrometry.[14] Our results showed that pyridine carboxylic acids were common in CM2 chondrites, decreasing in abundance as the amount of aqueous alteration of the meteorite parent body increased. This finding suggested that aqueous phase reactions in the meteorite parent body may destroy these compounds, but their mechanism of synthesis has yet to be determined. However, the structural diversity of meteoritic pyridine monocarboxylic acids and dicarboxylic acids is suggestive of radical chemistry that may have taken place within interstellar ices.

It should be noted that pyridine has not yet been identified in the interstellar medium (in the gas or ice phases).[15] On the other hand, benzene has been identified in the circumstellar medium of the protoplanetary nebula CRL618,[16] and it was recently shown that pyridine can be produced from UV photolysis of benzene in NH_3 + H_2O ice mixtures.[17] This suggests that pyridine could be formed from benzene embedded within interstellar ices near a strong UV source such as a young star. Additionally, comets may contain pyridine, which would be another indication that pyridine is present in icy materials.[18]

In this study, we performed experiments in which pyridine + CO_2 and pyridine + CO_2 + H_2O ice samples at 20 K were irradiated with 0.9 MeV protons from a Van de Graaff accelerator. *In situ* FTIR spectroscopy was used to monitor the spectroscopic changes after each radiation dose and to identify abundant products. Additional details of the experimental apparatus and methods can be found in the ESI.†

Results of the irradiation of the three-component mixture $H_2O:CO_2:\text{pyridine} = 100:20:1$ are described here since they are representative of those from the other experiments. Fig. 1 contains the IR spectra of the $H_2O:CO_2:\text{pyridine}$ ice sample, before and after irradiation to a dose of 1×10^{15} p+ cm^{-2}, and of the room temperature residue after heating. The broad features at approximately 3300 and 1700 cm^{-1} are due to H_2O, the sharp bands at approximately 2340 and 2270 cm^{-1} are due to CO_2 and its isotopologue $^{13}CO_2$, and the small, sharp bands between 1600 and 1000 cm^{-1} are due to the bending and wagging modes of pyridine. After irradiation (Fig. 1, middle trace) the pyridine bands were smaller and a new band, assigned to CO, appeared at 2140 cm^{-1}. After warm-up to room temperature, the distinct bands gave way to broader bands that are difficult to interpret. The color of the irradiated residue at

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† Electronic supplementary information (ESI) available: Experimental methods and figure. See DOI: 10.1039/c5cc03272e

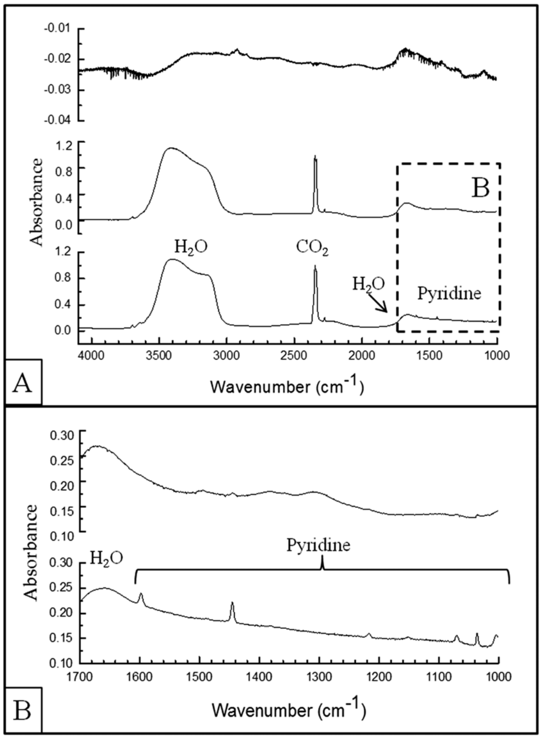

Fig. 1 (A) IR spectra of the $H_2O:CO_2$:pyridine = 100 : 20 : 1 ice mixture at 20 K before p+ irradiation (bottom trace), at 20 K after irradiation to a dose of 1×10^{15} p+ cm^{-2} (middle trace), and the refractory residue at room temperature after irradiation to a total dose of 1×10^{15} p+ cm^{-2} (top trace). (B) A close-up view of the region from 1700 to 1000 cm^{-1} showing pyridine bands before (bottom trace) and after proton irradiation (top trace).

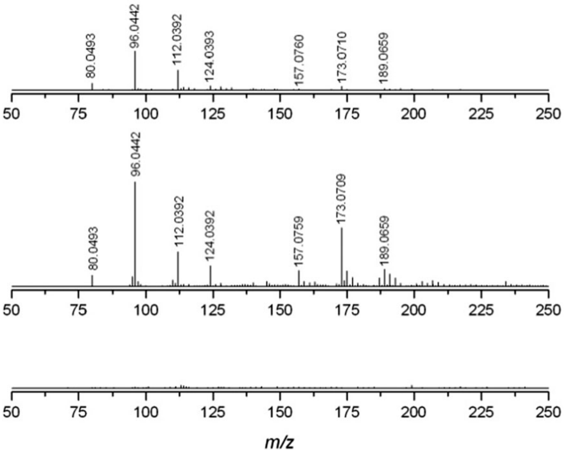

Fig. 2 DART mass spectra of the residue of a non-irradiated $H_2O:CO_2$:pyridine = 10 : 2 : 1 ice mixture (bottom trace), $H_2O:CO_2$:pyridine = 10 : 2 : 1 irradiated to a dose of 1×10^{15} p+ cm^{-2} (middle trace), and $H_2O:CO_2$:pyridine = 100 : 20 : 1 irradiated to a dose of 1×10^{15} p+ cm^{-2} (top trace).

room temperature ranged from faint to deep yellow, a noticeable change from the initially colorless ice sample.

We analyzed the refractory residues by high resolution orbitrap mass spectrometry using a direct analysis in real-time (DART) ion source and high performance liquid chromatography coupled to UV spectroscopy and high resolution orbitrap mass spectrometry (LC-UV/MS), which are more sensitive and specific techniques than FTIR. Fig. 2 shows DART mass spectra of the residues of non-irradiated and irradiated pyridine + CO_2 + H_2O mixtures. Elemental composition from accurate mass measurements (relative mass error <3 ppm) suggests that some of the new products in the residues of the irradiated ice mixtures are: pyridine monocarboxylic acids (*m*/*z* 124.0392), hydroxypyridines (*m*/*z* 96.0442), dihydroxypyridines (*m*/*z* 112.0392), bipyridine(s) (*m*/*z* 157.0759), hydroxy-substituted bipyridine(s) (*m*/*z* 173.0710), and hydroxy (mono–mono/di)-substituted bipyridine(s) (*m*/*z* 189.0659). High molecular weight species up to *m*/*z* 400 were detected in the $H_2O:CO_2$:pyridine = 10 : 2 : 1 residue, which demonstrates that significant molecular complexity can result from the irradiation of relatively simple ice mixtures. In general, products are less concentrated in the $H_2O:CO_2$:pyridine = 100 : 20 : 1 mixture and there is less molecular complexity based on the DART data; however, this may be due to abundances below detection limits rather than lack of product formation. Leftover pyridine (*m*/*z* 80.0493) was also detected in the residues of the irradiated ice samples indicating that this volatile species could be trapped in the residue that was produced (otherwise, pyridine sublimes at approximately 180 K, which is why it is absent in the non-irradiated control sample).

Our DART-MS analysis indicated that the radiation-induced chemistry of pyridine is similar to that of benzene in that oligomerization and partial hydrogenation occurs.[19] We also surmise that there are likely many structural isomers for these oligomers due to the nitrogen heteroatom. These chemical transformations are still evident in the irradiated residues of pyridine + CO_2 and pyridine + CO_2 + H_2O ice mixtures, although, to a lesser extent. These results may be significant because this could potentially make astronomical observation of pyridine in interstellar ices difficult.

Structural isomers were likely produced in the residues of the irradiated ice samples, which cannot be easily distinguished and accurately quantified using DART-MS. As a result, we utilized LC-UV/MS focusing our analyses on pyridine carboxylic acids and hydroxypyridines. We optimized our previously published LC-UV/MS method[14] for pyridine carboxylic acids by adding a second HPLC mixed-mode column in sequence and modifying chromatographic conditions in order to improve the separation of pyridine dicarboxylic acid isomers and reduce overall runtime. Fig. 3 shows typical extracted ion chromatograms of a sample residue compared to reference standards. Picolinic acid, isonicotinic acid, and nicotinic acid were chromatographically resolved and clearly identified in the extracted ion chromatogram of the residue of the irradiated $H_2O:CO_2$:pyridine = 100 : 20 : 1 ice mixture. A suite of pyridine dicarboxylic acids, including quinolinic acid (2,3-pyridinedicarboxylic acid), was detected in the residues of irradiated ice mixtures; however, abundances were much lower than for the pyridine monocarboxylic acids, as indicated in Table 1. Only three of six pyridine dicarboxylic acids could be quantified, likely because sensitivity for 2,6-pyridinedicarboxylic acid was poor and 2,4- and 2,5-pyridinedicarboxylic acids were not well resolved for accurate quantitation.

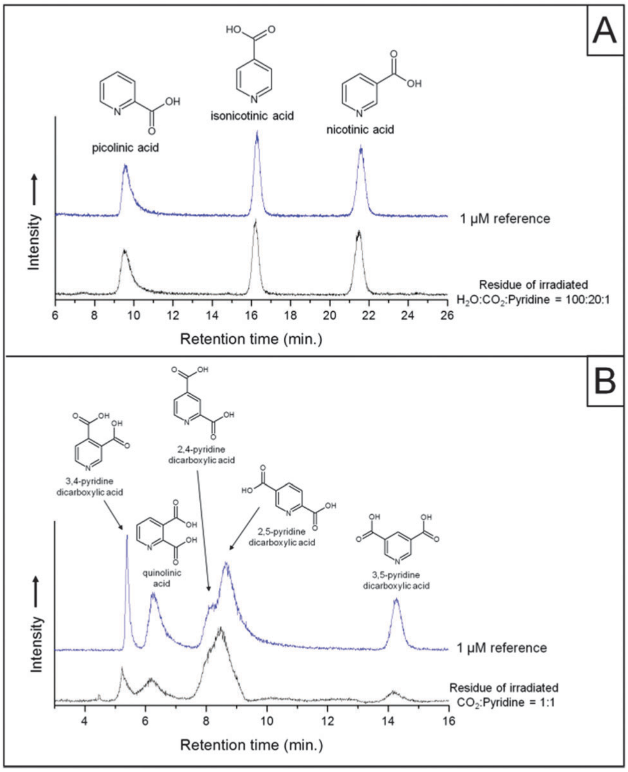

Fig. 3 (A) Extracted ion chromatograms at *m/z* 124.0393 (with a 5 ppm window) of the $H_2O:CO_2$:pyridine = 100 : 20 : 1 residue irradiated to a dose of 1×10^{15} p+ cm^{-2} (bottom trace) and reference standards (top trace). (B) Extracted ion chromatograms at *m/z* 168.0291 (with a 5 ppm window) of the CO_2 : pyridine = 1 : 1 residue irradiated to a dose of 1×10^{15} p+ cm^{-2} (bottom trace) and reference standards (top trace).

We found that pyridine monocarboxylic acids were produced in all five residues that were irradiated. Picolinic acid was found in the highest abundance among the three monocarboxylic acid isomers in the residues of irradiated pyridine + CO_2 ices. Nicotinic acid was next in abundance followed by isonicotinic acid. The distribution of pyridine monocarboxylic acids noticeably changed with the addition of water. Eventually, nicotinic acid became the favored isomer in the residue of the irradiated ice with the highest water content. Interestingly, the same trend was also observed for hydroxypyridines; 2-hydroxypyridine (similar in structure to picolinic acid) was favored in the residue of the irradiated $H_2O:CO_2$:pyridine = 10 : 2 : 1 ice mixture and 3-hydroxypyridine (similar in structure to nicotinic acid) was favored in the residue of the irradiated $H_2O:CO_2$: pyridine = 100 : 20 : 1 ice mixture (see Fig. S1, ESI†). The exact mechanism for the preferred substitution at the 3-position is unknown. Regardless of the mechanism, this outcome may have important implications for the chemistry of primitive bodies as well as prebiotic chemistry; however, we note that some caution should be used when evaluating product distributions because our ice mixtures were limited to three components under select conditions rather than the full molecular inventory of interstellar/astrophysical ices (which could affect overall product distribution). Moreover, there may be the possibility of further reaction(s) of products, such as isomerization reactions of pyridine carboxylic acids. These scenarios will be the subjects of future research.

Fig. 4 shows the relative abundances (nicotinic acid = 1) of pyridine monocarboxylic acids calculated from extracted ion chromatograms in six different ice residues compared to eight CM2 carbonaceous chondrites measured from our previous study.[14] We find that the ratios of picolinic to isonicotinic to nicotinic acid in the residue of the irradiated $H_2O:CO_2$: pyridine = 100 : 20 : 1 ice mixture (0.89 : 0.35 : 1.00) resembles the least aqueously altered (most primitive) chondrites, in particular,

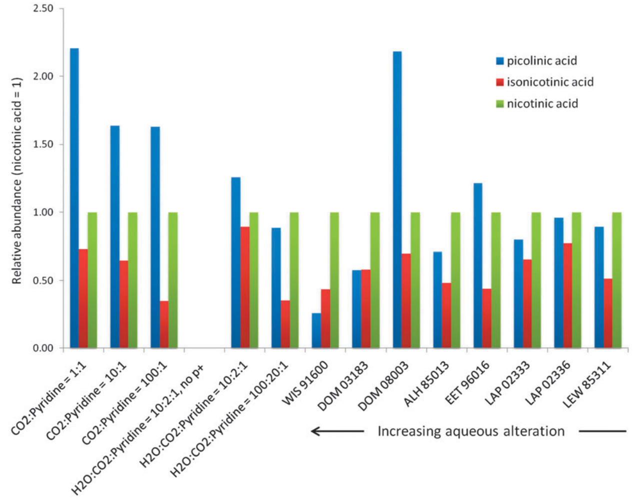

Fig. 4 Relative abundances (nicotinic acid = 1) of pyridinemonocarboxylic acids in six different ice residues compared to formic acid extracts of eight CM2 carbonaceous chondrites.

Table 1 Abundances (in pmol) of pyridine mono- and dicarboxylic acids and in six different ice residues. The estimated limit of quantitation for both pyridine mono- and dicarboxylic acids was 1 pmol. Error was calculated as standard error from two measurements. Note: the abundances for 2,4-pyridinedicarboxylic acid and 2,5-pyridinedicarboxylic acid could not be calculated due to coelution in the extracted ion chromatogram (see Fig. 3 at ~8.4 min)

	Exp. 1 CO_2 : P = 1 : 1	Exp. 2 CO_2 : P = 10 : 1	Exp. 3 CO_2 : P = 100 : 1	Exp. 4 $H_2O:CO_2$: P = 10 : 2 : 1, non-irradiated	Exp. 5 $H_2O:CO_2$: P = 10 : 2 : 1	Exp. 6 H_2O: CO_2 : P = 100 : 20 : 1
Picolinic acid	17 516 ± 567	4318 ± 7	713 ± 10	<1	4707 ± 243	129 ± 5
Nicotinic acid	7937 ± 254	2693 ± 64	450 ± 19	<1	3739 ± 313	145 ± 15
Isonicotinic acid	5867 ± 258	1746 ± 48	162 ± 12	<1	3341 ± 212	51 ± 13
3,4-Pyridinedicarboxylic acid	148 ± 25	<1	<1	<1	19 ± 2	<1
Quinolinic acid (2,3-pda)	127 ± 5	<1	<1	<1	15 ± 0	<1
3,5-Pyridinedicarboxylic acid	79 ± 14	<1	<1	<1	43 ± 3	<1

LEW 85311 (0.89 : 0.51 : 1.00). Hydroxypyridines, which were produced in high abundances in residues of irradiated ices (see ESI†), were also identified in multiple chondrites. This may indicate that meteoritic pyridine-related compounds were originally produced in polar H_2O-rich ices that were eventually incorporated into meteorite parent bodies. There are also exceptions to this observation. In Fig. 4, the ratios of picolinic to isonicotinic to nicotinic acid for the chondrite DOM 08003 (2.18 : 0.70 : 1.00) match very well to the residue of the irradiated CO_2 : pyridine = 1 : 1 ice mixture (2.21 : 0.73 : 1.00), indicating that these isomers may have been produced in icy grains that developed a nonpolar CO_2-rich mantle. The distribution of pyridine carboxylic acids in WIS 91600 and DOM 03183 do not resemble any of our ice residues; however, these two particular meteorites show signs of additional thermal alteration, which may have influenced the distribution of organic compounds.[20,21] Furthermore, the actual abundance of pyridine carboxylic acids is quite low in WIS 91600 and DOM 03183 compared to the other meteorites analyzed, which also points to thermal alteration on the parent body altering the organic composition.

Comets are primitive bodies in the solar system that may have remained relatively unchanged since their formation. The most abundant volatile species in comets are H_2O and CO_2, the latter as high as $\sim$30% (relative to water) based on a recent survey of 18 comets using the Japanese infrared satellite AKARI.[22] Pyridine was suggested to be present in the dust of comet 1P/Halley as measured by the PUMA mass spectrometer on board Vega 1.[18] Thus, the results from our studies of pyridine in CO_2-rich water ices may also suggest that cometary nuclei could contain a rich array of hydroxypyridines and pyridine carboxylic acids. Our observation that nicotinic acid is preferentially synthesized over its other two structural isomers in ice residues where the water content was the highest may be of significance to cometary chemistry. Furthermore, the presence of nicotinic acid in comets would increase the overall amount of this compound delivered o early Earth by supplementing meteoritic delivery.[14] Current missions such as Rosetta will advance our understanding of cometary composition including complex organic compounds, which may validate these experimental models.[23]

Nicotinamide adenine dinucleotide (NAD) is a coenzyme ubiquitous in modern cells, and thought to be ancient in origin since many coenzymes share a ribonucleotidyl group despite having dissimilar roles.[24] NAD is synthesized through two metabolic pathways: (1) the *de novo* pathway where the first step involves the synthesis of quinolinic acid from amino acids or (2) the Preiss–Handler salvage pathway in which components such as nicotinic acid are recycled back to NAD.[25,26] Our laboratory experiments have demonstrated that nicotinic acid and quinolinic acid, two members involved in the initial stages of the NAD biosynthetic pathway, can be synthesized in interstellar/cometary ice analogs. It is conceivable that astrophysical ice chemistry may have ramifications for the origin and early evolution of life on Earth by providing these types of organic compounds (*via* comets and meteorites) that would eventually be incorporated into ancient pyridine coenzymes and metabolic processes. An exogenous source might have been essential to establish these pathways before enzymes were available to catalyze their synthesis.

The authors thank T. Ward, E. Gerashchenko, and S. Brown for operation of the proton accelerator, M. Loeffler for assistance with some experimental measurements and helpful discussions, R. Hudson for helpful discussions, and three anonymous reviewers. This work was supported by a NASA Postdoctoral Program Fellowship administered by Oak Ridge Associated Universities through a contract with NASA, the NASA Astrobiology Institute *via* the Goddard Center for Astrobiology, and the NASA Cosmochemistry Program.

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