ASTROBIOLOGY
Volume 13, Number 10, 2013

DOI: 10.1089/ast.2013.1044

Thymine and Other Prebiotic Molecules Produced from the Ultraviolet Photo-Irradiation of Pyrimidine in Simple Astrophysical Ice Analogs

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Abstract

The informational subunits of RNA or DNA consist of substituted *N*-heterocyclic compounds that fall into two groups: those based on purine ($C_5H_4N_4$) (adenine and guanine) and those based on pyrimidine ($C_4H_4N_2$) (uracil, cytosine, and thymine). Although not yet detected in the interstellar medium, *N*-heterocycles, including the nucleobase uracil, have been reported in carbonaceous chondrites. Recent laboratory experiments and *ab initio* calculations have shown that the irradiation of pyrimidine in ices containing H_2O, NH_3, or both leads to the abiotic production of substituted pyrimidines, including the nucleobases uracil and cytosine. In this work, we studied the methylation and oxidation of pyrimidine in CH_3OH:pyrimidine, H_2O:CH_3OH:pyrimidine, CH_4:pyrimidine, and H_2O:CH_4:pyrimidine ices irradiated with UV photons under astrophysically relevant conditions. The nucleobase thymine was detected in the residues from some of the mixtures. Our results suggest that the abundance of abiotic thymine produced by ice photolysis and delivered to the early Earth may have been significantly lower than that of uracil. Insofar as the delivery of extraterrestrial molecules was important for early biological chemistry on early Earth, these results suggest that there was more uracil than thymine available for emergent life, a scenario consistent with the RNA world hypothesis. Key Words: Pyrimidine—Nucleobases—Interstellar ices—Cometary ices—Molecular processes—Prebiotic chemistry. Astrobiology 13, 948–962.

1. Introduction

Ribonucleic (RNA) and deoxyribonucleic acids (DNA) are two of the most fundamental macromolecules that constitute the building blocks of life. Both are polymers with backbones comprised of alternating phosphate and sugar units with attached nucleobases that serve as informational subunits. In RNA, the nucleobases include adenine, cytosine, guanine, and uracil, while DNA utilizes the same nucleobases with the exception that thymine (a methylated form of uracil) is substituted for uracil. Chemically, nucleobases are *N*-heterocyclic compounds that fall into one of two groups: those based on purine ($C_5H_4N_4$), which include adenine and guanine, and those based on pyrimidine ($C_4H_4N_2$), which include uracil, cytosine, and thymine.

In part because of their biological significance, scientists have sought to confirm the presence of small *N*-heterocycles, including pyrimidine, purine, and their derivatives, in the gas phase of the interstellar medium. To date, none of them have been detected (Simon and Simon, 1973; Kuan *et al.,* 2003, 2004; Charnley *et al.,* 2005; Brünken *et al.,* 2006). Nonetheless, polycyclic aromatic hydrocarbons are known to be ubiquitous in galactic and extragalactic interstellar/circumstellar environments, and polycyclic aromatic nitrogen heterocycles are expected to be present as well (Allamandola *et al.,* 1989; Puget and Léger, 1989; Roelfsema *et al.,* 1996; Galliano *et al.,* 2008). It is therefore reasonable to assume that *N*-heterocycles, including pyrimidine-based species, may be present in space where they can condense on the surface of cold, icy grains, such as those found in dense molecular clouds (Sandford *et al.,* 2004; Bernstein *et al.,* 2005).

While yet to be detected in space, purine- and pyrimidine-based compounds *have* been detected in carbonaceous chondrites, including Orgueil, Murchison, Murray, and Lonewolf Nunataks 94102 (Hayatsu, 1964; Folsome *et al.,* 1971, 1973; Hayatsu *et al.,* 1975; van der Velden and Schwartz, 1977; Stoks and Schwartz, 1979, 1981; Callahan *et al.,* 2011). The extraterrestrial origin of the nucleobases detected in the Murchison meteorite was supported by isotopic analysis (Martins *et al.,* 2008), though the presence of

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coeluting compounds may contribute to the measured isotopic enrichments. Their detection in meteorites lends further credence to their extraterrestrial formation via at least one astrophysical, non-biological process. This paper will focus exclusively on the abiotic *extraterrestrial* formation of thymine in ices containing astrophysically relevant components. For an introduction to potential *terrestrial* formation processes, please refer to papers by Schwartz and Chittenden (1977), as well as Choughuley *et al.* (1977).

Recent laboratory experiments have shown that UV photo-irradiation of pyrimidine in pure H_2O and mixed H_2O:NH_3 ices leads to the formation of many pyrimidine derivatives, including uracil, its precursor 4(3*H*)-pyrimidone, and cytosine (Nuevo *et al.*, 2009, 2012). Uracil and 4(3*H*)-pyrimidone have been reported in the Murchison, Murray, and Orgueil carbonaceous chondrites (Folsome *et al.*, 1971, 1973; Lawless *et al.*, 1972; Stoks and Schwartz, 1979), while the detection of cytosine has yet to be confirmed in meteorite samples.

Recent *ab initio* and density functional theory quantum chemical computations indicate that 4(3*H*)-pyrimidone and uracil are the most stable singly and doubly oxidized pyrimidine derivatives formed from the photo-irradiation of pyrimidine in pure H_2O ice (Bera *et al.*, 2010). More importantly, a second conclusion from that study was that the presence of H_2O as an ice matrix is essential for the formation of these oxidized compounds because it participates in the proton abstraction from the intermediate compounds to the final, stable products. This process has also been experimentally shown to assist with the addition of amino groups (Nuevo *et al.*, 2012).

Both CH_3OH and CH_4 are important components of interstellar ices (Allamandola *et al.*, 1992; Gibb *et al.*, 2004; Dartois, 2005) and may react with pyrimidine upon exposure to UV radiation to form nucleobases, including thymine, although in the case of CH_4 an additional oxygen source would be required. To study the possibility of methyl photoaddition to pyrimidine, we performed UV photolysis experiments, under astrophysical conditions, of several ices consisting of pyrimidine mixed with CH_3OH, H_2O+CH_3OH, CH_4, and H_2O+CH_4 in different relative proportions. The organic residues formed in these experiments were analyzed with gas and liquid chromatography techniques to search for the presence of pyrimidine derivatives, including the nucleobases uracil, cytosine, and thymine, as well as other compounds of prebiotic interest such as urea, glycerol, small carboxylic acids, and small amino acids. A series of quantum chemical computations was performed to better understand some of the experimental results. The computational results will be discussed extensively in an upcoming paper, but some key points will be included here where relevant.

The use of simple ice mixtures is an important tool for understanding the underlying photochemistry in astrophysically relevant ices. Starting with simple mixtures of compounds known to be present in interstellar ices allows us to better understand the conditions and reactants needed for the formation of a particular product and is necessary to understand the results of experiments on more complex and realistic ice mixtures. As such, these experiments are not intended to precisely model the overall chemistry in astrophysically relevant ices but are instead designed to probe a specific subset of reactions that may be relevant to the formation of thymine.

2. Experimental Methods

2.1. UV photo-irradiation of ices at low temperature

Sample preparation was carried out inside a vacuum cryogenic chamber evacuated by a diffusion pump (Edwards BRV 25) to a pressure of a few 10^{-8} mbar. We carried out a series of experiments in which CH_3OH:pyrimidine, H_2O:CH_3OH:pyrimidine, CH_4:pyrimidine, and H_2O:CH_4:pyrimidine gas mixtures were separately deposited onto an aluminum (Al) foil that had been pre-baked at 500°C and attached to a cold finger cooled to 15–25 K by a closed-cycle helium cryocooler (APD Cryogenics) (Allamandola *et al.*, 1988; Bernstein *et al.*, 1995). Gas mixtures were prepared in a glass line evacuated by a diffusion pump (Edwards BRV 10, background pressure $\sim 5\times10^{-6}$ mbar). H_2O (liquid, purified to 18.2 MΩ cm by a Millipore Direct-Q UV 3 device, and freeze-pump-thawed five times to remove excess dissolved gases), CH_3OH (liquid, Aldrich HPLC grade 99.9% purity, freeze-pump-thawed five times), CH_4 (gas, Matheson Tri-Gas, Research purity), and pyrimidine (liquid, Aldrich, 99% purity, freeze-pump-thawed five times) were mixed in two 1.9 L glass bulbs. The ratios between the components were determined by their partial pressures in the bulb with an accuracy of 0.05 mbar. In this work, we prepared CH_3OH:pyrimidine mixtures with relative proportions of 5:1, 10:1, and 20:1; H_2O:CH_3OH:pyrimidine mixtures with relative proportions of 20:2:1, 20:5:1, and 20:10:1; CH_4:pyrimidine mixtures with relative proportions of 18:1, 30:1, and 45:1; and finally H_2O:CH_4:pyrimidine mixtures with relative proportions of 20:5:1, 20:10:1, and 3:30:1.

In a typical experiment, a total of 32–45 mbar of gas mixture (2.7–3.7 mmol) was deposited and condensed onto the cooled Al foil. During deposition, the growing ice layer was simultaneously photo-irradiated with a microwave-powered H_2 discharge UV lamp (Opthos). This lamp emits photons mainly at 121.6 nm (Lyman-α) and a continuum centered around 160 nm, with an estimated total flux of $\sim 2\times10^{15}$ photons cm^{-2} s^{-1} (Warnek, 1962). This lamp simulates the UV radiation field observed in many astrophysical environments. The temperature of deposition was typically 14–28 K, while the deposition time varied from 20–28 h for regular UV dose experiments to 68–77 h for high UV dose experiments. The total mass of the deposited ice film typically ranged from 50 to 80 mg. Many ions and radicals are formed in the ice upon irradiation at low temperature, and if adjacent to each other in the ice matrix, they can react immediately to form more complex products. However, the majority of these ions and radicals remains trapped by the ice matrix and only reacts when the ices are slowly warmed, allowing unreacted ions and radicals to migrate and react as the ice matrix rearranges and ultimately sublimes. This warm-up occurred under static vacuum to 220 K, after which the chamber was open to air and the Al foil with the samples was removed for further analysis by high-performance liquid chromatography (HPLC) and gas chromatography coupled with mass spectrometry (GC-MS).

A typical photon flux for diffuse interstellar environments is estimated to be 8×10^{7} photons cm^{-2} s^{-1} for photons with energies higher than 6 eV (Mathis *et al.*, 1983), while fluxes

are expected to be 3–5 orders of magnitude lower in more opaque dense interstellar clouds (Prasad and Tarafdar, 1983; Shen *et al.*, 2004). Our 20–28 h experiments therefore correspond to a UV photo-irradiation of ices for about 10^4 years in diffuse media, about 10^7 to 10^9 years in dense media, and about 3 times longer than these numbers for the higher 68–77 h irradiation experiments.

2.2. HPLC and GC-MS analysis of residues at room temperature

Following extraction with 500 μL of H_2O, 5 μL of each sample was injected into a Hewlett Packard/Agilent 1100 Series high-performance liquid chromatograph (HPLC) equipped with a Phenomenex Luna 5μm Phenyl-Hexyl column (size: 250×4.60 mm, inner diameter: 5 μm). Compounds separated by the column were detected by a diode-array UV detector in five different wavelengths at 220, 245, 256, 280, and 300 nm. The method used for HPLC analysis (solvent used, preparation of the pH 5 formate buffer, solvent gradients) is given by Nuevo *et al.* (2009). Peaks in the HPLC sample chromatograms were identified by comparison of both their retention times and UV spectra with standards dissolved to 10^{-3} *M* in H_2O and injected with the same protocol.

For GC-MS analysis, 100 μL of each sample were transferred to pre-baked (500°C) vials and dried under vacuum in a desiccator for 2 h. Then, 50 μL of a 3:1:1 mixture of *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA) with 1% of *tert*-butyldimethylchlorosilane (*t*BDMCS) (Restek), dimethylformamide (Pierce, silylation grade solvent), and pyrene (Sigma-Aldrich, analytical standard, 100 ng μL^{-1} in cyclohexane) were added to the dried residues. The vials were then heated to 100°C for 1 h to convert hydrogen-containing moieties such as OH and NH_2 into their *tert*-butyldimethylsilyl (*t*BDMS) derivatives (MacKenzie *et al.*, 1987; Casal *et al.*, 2004; Schummer *et al.*, 2009). It should be noted that blanks were prepared simultaneously to, and under the same conditions as, the samples throughout each stage of the drying, derivatization, and subsequent GC-MS analysis protocols. These blanks revealed no cross contamination.

Separation was carried out with a Thermo Trace gas chromatograph coupled to a DSQ II mass spectrometer with a splitless injection, a Restek Rxi-5ms column (length: 30 m, inner diameter: 0.25 mm, film thickness: 0.50 μm), an injector temperature of 250°C, and a helium (carrier gas; Airgas, ultrapure) flow of 1.3 mL min^{-1}. The method (temperature gradient) used is described in detail by Nuevo *et al.* (2009). Masses were recorded between 50 and 550 amu (atomic mass units), and data analysis was performed with Xcalibur software (Thermo Finnigan). Peaks in the GC-MS sample chromatograms were identified by comparison of both their retention times and mass spectra with the same standards as used for the HPLC analysis, derivatized according to the same protocol as the samples.

The standards searched for in this study are the same as those used for our earlier studies of H_2O:pyrimidine, NH_3:pyrimidine, and H_2O:NH_3:pyrimidine mixtures (Nuevo *et al.*, 2009, 2012), plus a number of additional compounds, as follows:

(1) Methylpyrimidine derivatives: 2-methylpyrimidine (Aldrich, 97% purity), 4-methylpyrimidine (Aldrich, 97% purity), 5-methylpyrimidine (Aldrich, 96% purity), and 4,6-dimethylpyrimidine (Aldrich, ≥93% purity).
(2) Oxidized methylpyrimidine derivatives: 2-hydroxy-4-methylpyrimidine (hydrochloride, Aldrich, 97% purity), 2-hydroxy-4,6-dimethylpyrimidine (hydrochloride, Aldrich, 98% purity), 4-hydroxy-2,6-dimethylpyrimidine (Aldrich, ≥99% purity), 1-methyluracil (Aldrich, 99% purity), thymine (Sigma, ≥99% purity), 6-methyluracil (Aldrich, 97% purity), 4,6-dihydroxy-2-methylpyrimidine (Aldrich, 97% purity), 4,6-dihydroxy-5-methylpyrimidine (Aldrich, 97% purity), and 2,4-dihydroxy-5,6-dimethylpyrimidine (Aldrich, 97% purity).
(3) 4-Pyrimidinemethanol (Aldrich, rare chemical library).
(4) Derivatives of uracil: 5-formyluracil (Aldrich, 98% purity) and 5-(hydroxymethyl)uracil (Aldrich, 97% purity).
(5) Glycolic acid (Sigma-Aldrich, 99% purity), oxalic acid (Sigma-Aldrich, ≥99.0% purity), L-(+)-lactic acid (Sigma Aldrich, ≥98% purity), 3-hydroxypropanoic

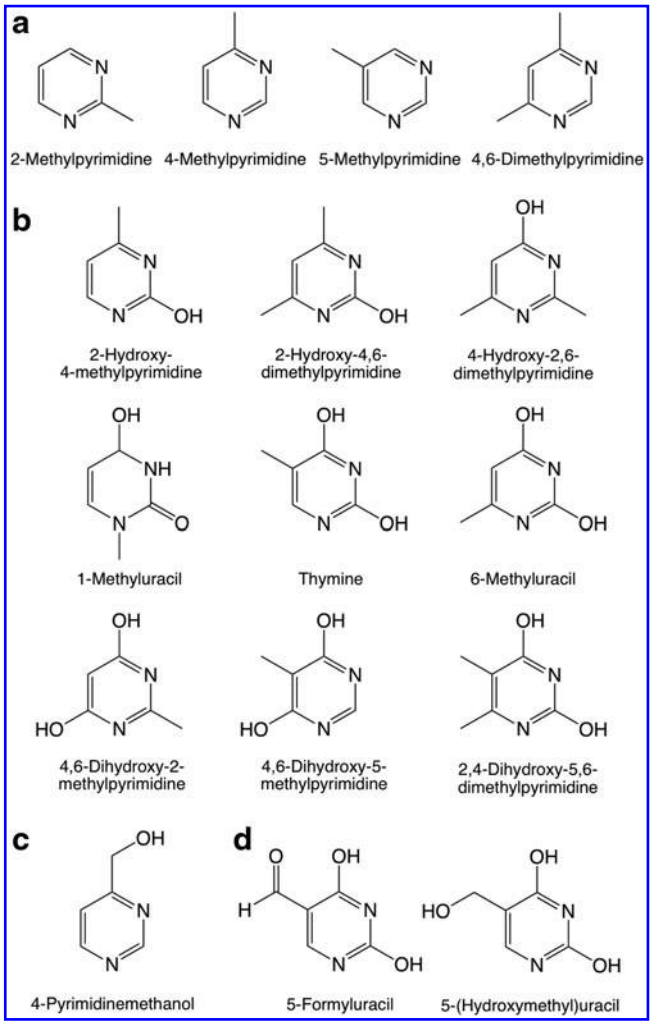

FIG. 1. Molecular structures of the additional standards searched for in this study: (**a**) methylpyrimidine derivatives, (**b**) oxidized methylpyrimidine derivatives including the nucleobase thymine, (**c**) 4-pyrimidinemethanol, and (**d**) derivatives of uracil. The structures of all other pyrimidine compounds searched for are given in Nuevo *et al.* (2009, 2012).

acid (Tokyo Chemical Industry Co., *ca.* 30% in H_2O, *ca.* 3.6 mol L^{-1}), glycerol (Sigma-Aldrich, ≥99% purity), and DL-glyceric acid (Tokyo Chemical Industry Co., 20% in H_2O, *ca.* 2 mol L^{-1}).

(6) Amino acids: *β*-alanine (Aldrich, 99% purity), sarcosine (Aldrich, 98% purity), *N*-ethylglycine (Aldrich, 98% purity), and DL-2-aminobutyric acid (Aldrich, 99% purity).

The molecular structures of these additional compounds are given in Figs. 1 (pyrimidine-based compounds) and 2 (non-pyrimidine-based compounds). The molecular structures of the other compounds searched for in our samples can be found in the references Nuevo *et al.* (2009, 2012). The chemical formulas, molecular masses, HPLC and GC-MS retention times, and mass of the most intense peaks for all searched compounds are given in Table 1.

2.3. A note about quantum chemical calculations

We performed *ab initio* second-order Møller-Plesset (MP2) perturbation theory and Z-averaged perturbation theory (ZAPT2), as well as B3LYP density functional theory quantum chemical computations along with correlation consistent polarized valence triple zeta (cc-pVTZ) basis set to understand the formation and destruction of specific species observed in the experiments described in this work. We explored multiple methylation and oxidation scenarios, and mechanistic pathways to understand these experimental observations. Detailed computational results will be reported in a separate upcoming paper (Bera *et al.*, unpublished results). However, we will mention some theoretical results in this manuscript in the appropriate context. Quantum chemical results we briefly discuss here have been obtained by widely used methods and have also been successfully used in the past to investigate the formation of uracil in irradiated H_2O:pyrimidine ices (Bera *et al.*, 2010).

3. Results

3.1. HPLC analysis of the residues

3.1.1. CH_3OH:pyrimidine and H_2O:CH_3OH:pyrimidine mixtures. HPLC analysis of the residues produced from UV photo-processing of ices containing only methanol and pyrimidine showed numerous peaks, of which we could only rigorously identify a few with HPLC. Although there is some variation in the chromatograms of the samples, even between residues made from ices of the same starting composition, the identifiable photo-products are consistent regardless of both the starting mixture ratios and radiation dose. The only peaks we identified (Table 2) are a large, broad peak in the 17–19 min range due to unreacted pyrimidine, as well as a much smaller peak around 13 min, seen in some but not all chromatograms, due to 4-pyrimidinemethanol. The pyrimidine peak in the chromatogram of a photo-processed sample is significantly broadened relative to its standard or an unprocessed sample, which likely indicates that pyrimidine is interacting with other dissolved compounds. A peak also appears in these samples with a retention time consistent with 4(3*H*)-pyrimidone; however, the UV spectrum is not a perfect match. The mismatch of these UV spectra may be a result of a coeluent obscuring the data since, as we know from GC-MS results presented later (Section 3.2), this compound is present.

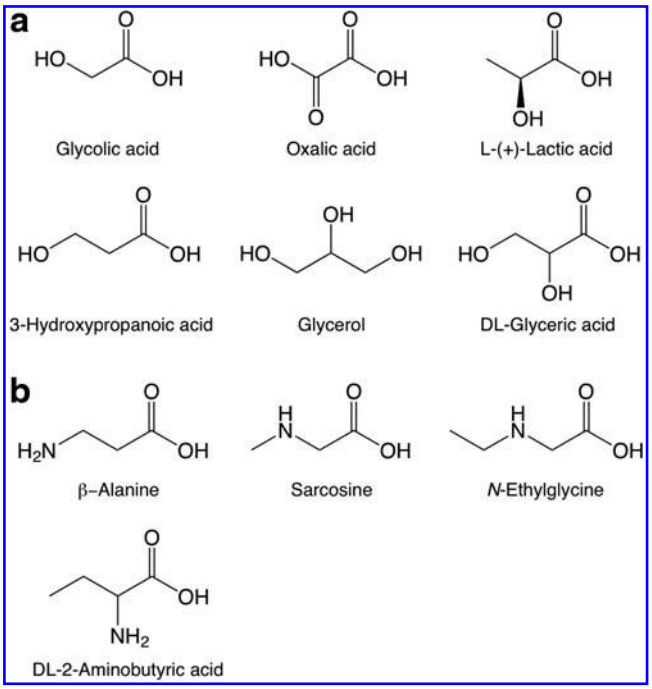

FIG. 2. Molecular structures of the additional acyclic standards searched for in this study: (**a**) glycolic acid, oxalic acid, L-(+)-lactic acid, 3-hydroxypropanoic acid, glycerol, and DL-glyceric acid; and (**b**) the amino acids β-alanine, sarcosine, *N*-ethylglycine, and DL-2-aminobutyric acid. The structures of urea and other amino acids were given by Nuevo *et al.* (2009, 2012).

Photo-processed residues from H_2O:CH_3OH:pyrimidine ices also show the production of 4-pyrimidinemethanol, as well as the production of 4(3*H*)-pyrimidone, which elutes at a retention time (R_t) near 8.71 min. Figure 3 shows the HPLC chromatogram ($\lambda = 256$ nm) of a representative H_2O:CH_3OH:pyrimidine = 20:5:1 residue and confirms the presence of 4(3*H*)-pyrimidone (peak **1**) and 4-pyrimidinemethanol (peak **2**). As with the CH_3OH:pyrimidine mixtures, the identified photo-products in H_2O:CH_3OH:pyrimidine mixtures are highly consistent between experiments, regardless of the starting mixture or radiation dose.

It should be noted that a peak is present in some of the samples from both series of experiments at the expected retention time for thymine. This does not provide definitive proof of the presence of thymine, however, because the UV spectrum of this peak differs from that of our thymine standard. There are different possible explanations for this observation. First, it is possible that thymine is not present, and some unidentified compound with some similar UV spectral features and a similar retention time is present in our samples. Alternatively, it is possible that thymine is present, but it is coeluting with one or more other compounds that change the UV absorption profile of the peak.

3.1.2. CH_4:pyrimidine and H_2O:CH_4:pyrimidine mixtures. HPLC analyses of residues produced from ices containing methane and pyrimidine also show numerous peaks, of which we have only been able to identify a few. Indeed, as

Table 1. List of All the Standards Searched for with HPLC and GC-MS Techniques

Species	*Formula*	*Molecular mass (amu)*	R_t *(HPLC) (min)*	R_t *(GC-MS) (min)*[a]	*GC-MS peak (amu)*[b]
Pyrimidine[c]	$C_4H_4N_2$	80	18.80	*n.d.*	—
1,4,5,6-Tetrahydropyrimidine	$C_4H_8N_2$	84	7.45[d]	14.02/13.89	141 (1)
2,2′-Bipyrimidine[c]	$C_8H_6N_4$	158	24.04	17.71/17.56	158 (0)
Pyrimidine *N*-oxide[c]	$C_4H_4N_2O$	96	8.65	*n.d.*	—
2-Hydroxypyrimidine	$C_4H_4N_2O$	96	7.84	11.15/11.13	153 (1)
4(3*H*)-Pyrimidone	$C_4H_4N_2O$	96	8.71	10.20/10.03	153 (1)
Uracil	$C_4H_4N_2O_2$	112	8.23	20.76/20.60	283 (2)
4,6-Dihydroxypirimidine	$C_4H_4N_2O_2$	112	6.21	21.43/21.22	283 (2)
Dihydrouracil	$C_4H_6N_2O_2$	114	8.45	24.06/23.90	285 (2)
Barbituric acid	$C_4H_4N_2O_3$	128	5.69	29.20/29.02	413 (3)
Isobarbituric acid	$C_4H_4N_2O_3$	128	7.12	29.87/29.65	413 (3)
2-Aminopyrimidine	$C_4H_5N_3$	95	20.01	12.45/12.32	152 (1)
4-Aminopyrimidine	$C_4H_5N_3$	95	7.54	15.06/14.91	152 (1)
2,4-Diaminopyrimidine	$C_4H_6N_4$	110	7.06	25.98/25.81	281 (2)
4,5-Diaminopyrimidine	$C_4H_6N_4$	110	6.93	26.97/26.86	281 (2)
2,4,6-Triaminopyrimidine	$C_4H_7N_5$	125	8.21[e]	35.87/35.52	410 (3)
2-Methylpyrimidine	$C_5H_6N_2$	94	26.49	*n.d.*	—
4-Methylpyrimidine	$C_5H_6N_2$	94	28.64	*n.d.*	—
5-Methylpyrimidine	$C_5H_6N_2$	94	31.37	*n.d.*	—
4,6-Dimethylpyrimidine	$C_6H_8N_2$	108	41.25	*n.d.*	—
4-Pyrimidinemethanol	$C_5H_6N_2O$	110	12.98	13.30/13.15	167 (1)
2-Pyrimidinecarbonitrile[c]	$C_5H_3N_3$	105	29.14	*n.d.*	—
Cytosine	$C_4H_5N_3O$	111	6.62	24.53/24.32	282 (2)
Isocytosine	$C_4H_5N_3O$	111	8.06	22.78/22.57	282 (2)
2,4-Diamino-6-hydroxypyrimidine	$C_4H_6N_4O$	126	7.52[e]	32.93/32.69	411 (3)
5-Aminouracil	$C_4H_5N_3O_2$	127	6.88	31.10/30.93	412 (3)
6-Aminouracil	$C_4H_5N_3O_2$	127	7.42	31.75/31.55	412 (3)
2-Amino-4,6-dihydroxypyrimidine	$C_4H_5N_3O_2$	127	6.25	30.66/30.47	412 (3)
2-Hydroxy-4-methylpyrimidine	$C_5H_6N_2O$	110	12.30	12.49/12.35	167 (1)
2-Hydroxy-4,6-dimethylpyrimidine	$C_6H_8N_2O$	124	22.39	13.72/13.55	181 (1)
4-Hydroxy-2,6-dimethylpyrimidine	$C_6H_8N_2O$	124	19.74	12.19/12.07	181 (1)
1-Methyluracil	$C_5H_6N_2O_2$	126	14.19	22.30/22.11	183 (1)
Thymine	$C_5H_6N_2O_2$	126	15.64	22.38/22.20	297 (2)
6-Methyluracil	$C_5H_6N_2O_2$	126	13.22	21.45/21.29	297 (2)
4,6-Dihydroxy-2-methylpyrimidine	$C_5H_6N_2O_2$	126	6.82	21.15/20.99	297 (2)
4,6-Dihydroxy-5-methylpyrimidine	$C_5H_6N_2O_2$	126	8.11	22.71/22.54	297 (2)
2,4-Dihydroxy-5,6-dimethylpyrimidine	$C_6H_8N_2O_2$	140	26.91	23.41/23.26	311 (2)
5-Formyluracil	$C_5H_4N_2O_3$	140	10.62	25.97/25.79	311 (2)
5-(Hydromethyl)uracil	$C_5H_6N_2O_3$	142	7.95	30.91/30.73	427 (3)
Orotic acid	$C_5H_4N_2O_4$	156	6.21	32.54/32.35	441 (3)
5-Nitrouracil	$C_4H_3N_3O_4$	157	13.13	27.49/27.32	328 (2)
2-Amino-5-nitropyrimidine	$C_4H_4N_4O_2$	140	35.53	21.91/21.75	197 (1)
Pyridine[c]	C_5H_5N	79	27.19	*n.d.*	—
Purine	$C_5H_4N_4$	120	15.98	20.12/19.97	178 (1)[f]
Hydantoin	$C_3H_4N_2O_2$	100	7.33	22.26/22.10	271 (2)
Urea	CH_4N_2O	60	15.53[d]	18.24/18.16	231 (2)
Glycolic acid	$C_2H_4O_3$	74	*n.d.*	14.92/14.74	247 (2)
Oxalic acid	$C_2H_2O_4$	90	4.74[d]	15.87/15.69	261 (2)

(*continued*)

TABLE 1. (CONTINUED)

Species	*Formula*	*Molecular mass (amu)*	R_t *(HPLC) (min)*	R_t *(GC-MS) (min)*[a]	*GC-MS peak (amu)*[b]
Lactic acid	$C_3H_6O_3$	90	*n.d.*	14.55/14.39	261 (2)
3-Hydroxypropanoic acid	$C_3H_6O_3$	90	*n.d.*	16.60/16.41	261 (2)
Glycerol	$C_3H_8O_3$	92	9.89[d]	23.15/23.03	377 (3)
Glyceric acid	$C_3H_6O_4$	106	*n.d.*	24.61/24.43	391 (3)
Glycine	$C_2H_5NO_2$	75	*n.d.*	16.17/16.00	246 (2)
DL-Alanine	$C_3H_7NO_2$	89	*n.d.*	15.70/15.53	260 (2)
β-Alanine	$C_3H_7NO_2$	89	*n.d.*	18.01/17.81	260 (2)
Sarcosine	$C_3H_7NO_2$	89	*n.d.*	17.05/16.88	260 (2)
N-Ethylglycine	$C_3H_5NO_3$	103	*n.d.*	18.25/18.08	274 (2)
N-Formylglycine	$C_3H_5NO_3$	103	*n.d.*	21.77/21.59	274 (2)
DL-2-Aminobutyric acid	$C_3H_5NO_3$	103	*n.d.*	17.32/17.17	274 (2)

Each *t*BDMS group added to a compound increases its mass by 114 amu.

Note: Retention times are reported with two decimals for the purpose of demonstrating the order of elution only. Actual retention times will vary from experiment to experiment.

[a]GC-MS chromatograms were measured with two different columns of the same model (Restek Rxi-5ms, see Section 2.2), so that retention times for compounds appear to be slightly shorter with the second column. Retention times in the text refer to the shortest column, which was used to separate most of the samples discussed here.

[b]Masses reported here (in atomic mass units) correspond to the mass of the most intense peak for each standard in the GC-MS mass spectra, that is, the total mass of the derivatized compound (M*) minus the mass of one *tert*-butyl group ($[M^*–57]^+$ fragment), except for compounds that are not derivatized (see note c). Numbers between parentheses are the number of *t*BDMS groups attached to the parent molecules.

[c]Compounds not derivatized by the MTBSTFA + 1% *t*BDMCS agent (no *t*BDMS group attached). Among those compounds, only 2,2′-bipyrimidine displays a peak in GC-MS chromatograms.

[d]The HPLC peaks of those compounds are too weak to obtain clear UV spectra.

[e]The HPLC chromatograms of those compounds display several peaks. The retention times given here are for their most intense peaks.

[f]The mass of derivatized purine is 178 amu rather than 177 amu as expected for *t*BDMS derivatives.

n.d. = Not detected.

shown on the chromatogram ($\lambda = 256$ nm) of a CH_4:pyrimidine = 45:1 residue (Fig. 4), the only identified peaks in these samples (Table 2) belong to 4(3*H*)-pyrimidone ($R_t = 8.87$ min, peak **1**), unreacted pyrimidine (18.73 min), and 4-methylpyrimidine (25.51 min, peak **4**). In this sample, 4(3*H*)-pyrimidone must be formed either from trace background contaminant H_2O present in the vacuum chamber, from oxygen liberated from the foil substrate, or from exposure of the residue to the air following extraction from the vacuum chamber. The formation of 4(3*H*)-pyrimidone under similar conditions was previously observed in residues produced from the UV irradiation of NH_3:pyrimidine ice mixtures (Nuevo *et al.*, 2012). Interestingly, 4-methylpyrimidine (peak **4**) is the only methylated derivative that could be unambiguously identified.

In two of our CH_4:pyrimidine experiments (one with a regular radiation dose and one with a high radiation dose), we also observed a weak peak whose retention time is consistent with thymine (Fig. 4, peak **3***). If it were due to thymine, this peak could be a result of reactions with trace contaminant water in our vacuum system.

The deliberate inclusion of H_2O in the original ice does not result in the formation of any additional oxidized compounds that could be identified with HPLC. One difference observed with the addition of H_2O to the starting ice mixtures was the formation of 5-methylpyrimidine in some samples, found to be, from the HPLC traces, 2–7 times less abundant than 4-methylpyrimidine in our residues. It should be noted that residues resulting from the addition of H_2O to the starting mixture lack definitive proof of the presence of thymine at any level of initial concentration or radiation. However, as with the H_2O:CH_3OH:pyrimidine experiments, a peak appears at its expected retention time, though it may be partly due to coelution with another unidentified compound. To verify the identification of thymine in HPLC chromatograms, we injected an H_2O:CH_4:pyrimidine = 20:10:1 sample and again the same sample spiked with some

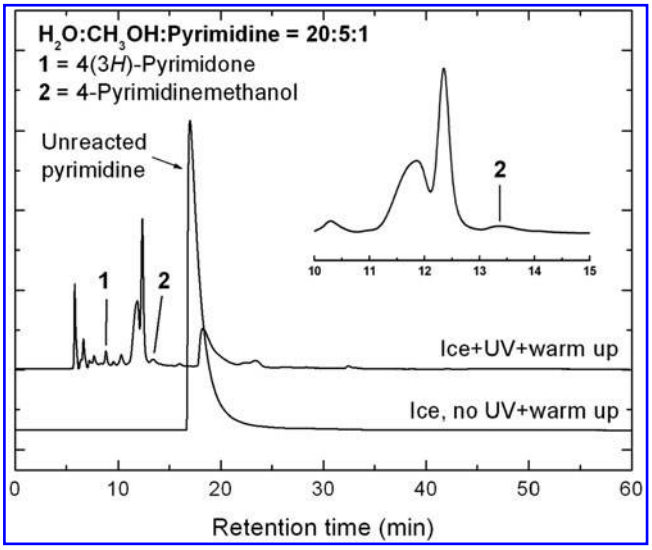

FIG. 3. HPLC chromatogram ($\lambda = 256$ nm) of an H_2O:CH_3OH:pyrimidine = 20:5:1 residue (top trace) showing the detections of 4(3*H*)-pyrimidone ($R_t = 8.81$ min, peak **1**) and 4-pyrimidinemethanol (13.30 min, peak **2**). The bottom trace corresponds to a blank experiment in which a similar mixture was not irradiated and the resulting residue analyzed following the same protocol as the sample.

Table 2. Species Searched for and Detected with HPLC (L) and GC-MS (G) in the Residues Formed from the UV Photo-Irradiation of CH_3OH:Pyrimidine, H_2O:CH_3OH:Pyrimidine, CH_4:Pyrimidine, and H_2O:CH_4:Pyrimidine Ice Mixtures

Species	*CH_3OH: Pyrimidine*	*H_2O:CH_3OH: Pyrimidine*	*CH_4: Pyrimidine*	*H_2O:CH_4: Pyrimidine*
Pyrimidine	L*	L*	L*	L*
1,4,5,6-Tetrahydropyrimidine		G[a]		G[a,b,c]
2,2′-Bipyrimidine				
Pyrimidine *N*-oxide				
2-Hydroxypyrimidine	G[a]	G		G[*,a]
4(3*H*)-Pyrimidone	L[*,a], G*	L[†], G*	(L, G)[*,e]	(L, G)*
Uracil	G*	G*	G[*,c,e]	G*
4,6-Dihydroxypirimidine	G[a]	G[*,a]	G[c,e]	G[*,a]
Dihydrouracil				
Barbituric acid				
Isobarbituric acid	G[a]	G[a]		G*
2-Aminopyrimidine	G*	G	G[*,a,c]	G[*,a]
4-Aminopyrimidine	G*	G*	G*	G*
2,4-Diaminopyrimidine				
4,5-Diaminopyrimidine				
2,4,6-Triaminopyrimidine				
2-Methylpyrimidine				
4-Methylpyrimidine			L[f]	L[f]
5-Methylpyrimidine				L[‡,f]
4,6-Dimethylpyrimidine				
4-Pyrimidinemethanol	L, G*	(L, G)*	G[*,e]	G*
2-Pyrimidinecarbonitrile				
Cytosine				G[a,c]
Isocytosine				G[a,b,c]
2,4-Diamino-6-hydroxypyrimidine				
5-Aminouracil				G[a,c]
6-Aminouracil				G[a,c]
2-Amino-4,6-dihydroxypyrimidine				
2-Hydroxy-4-methylpyrimidine		G[b]		G*
2-Hydroxy-4,6-dimethylpyrimidine				
4-Hydroxy-2,6-dimethylpyrimidine				G[a,b,c]
1-Methyluracil				
Thymine	G[b]		G[a,c,e]	L, G[‡,d]
6-Methyluracil				
4,6-Dihydroxy-2-methylpyrimidine				
4,6-Dihydroxy-5-methylpyrimidine				G[a]
2,4-Dihydroxy-5,6-dimethylpyrimidine	L[b]			
5-Formyluracil				
5-(Hydromethyl)uracil				
Orotic acid	G[b]			
5-Nitrouracil				
2-Amino-5-nitropyrimidine				
Pyridine				
Purine				
Hydantoin	G[b,c]	G[a,b,c]		
Urea	G[*,a]	G*	G[a,c,e]	G[a]

(*continued*)

TABLE 2. (CONTINUED)

Species	*CH_3OH: Pyrimidine*	*H_2O:CH_3OH: Pyrimidine*	*CH_4: Pyrimidine*	*H_2O:CH_4: Pyrimidine*
Glycolic acid	G[*,d]	G[*,d]	G[d]	G[*,d]
Oxalic acid	G[*,d]	G[*,d]	G[d]	G[*,d]
Lactic acid	G[*,a]	G[*,a]		G*
3-Hydroxypropanoic acid	G	G[a]		G[a]
Glycerol	G*	G*		G[a,c]
Glyceric acid	G*	G*	G[c,e]	G
Glycine	G[*,c]	G[*,a,c]	G[a,c,e]	G[*,c]
DL-Alanine	G[b,c]			G[b,c]
β-Alanine				
Sarcosine				
N-Ethylglycine				
N-Formylglycine		G[a]		G[a]
DL-2-Aminobutyric acid				

Blanks indicate non-detections. The identification of thymine with HPLC is supported by the analysis of samples spiked with thymine (see Section 3.1.2).

*Detected in all samples.

†Detected only in samples produced from the irradiation of ices with the regular UV dose.

‡Detected only in samples produced from the irradiation of ices with a high UV dose.

[a]Compounds often eluting at the same retention time as one or more other unidentified species (coeluents).

[b]Tentative detection in one or two samples.

[c]Detected at trace levels.

[d]Compounds also present in blank samples but with smaller abundances than in the residues (see Section 3.2.1).

[e]Compounds formed from the reaction of pyrimidine with residual H_2O in the vacuum chamber (see Sections 3.1 and 3.2 and Nuevo *et al.,* 2012).

[f]The HPLC detection of 4- and 5-methylpyrimidine in CH_4:pyrimidine and H_2O:CH_4:pyrimidine samples is sometimes ambiguous because the peak may also be assigned to other isomers.

thymine added from the prepared standard. The HPLC chromatogram of the spiked sample shows a significant increase in intensity for the peak assigned to thymine, supporting our identification. However, the UV spectrum of this spiked peak also indicates that thymine may be coeluting with another unidentified compound.

3.1.3. Summary of HPLC results. The HPLC analysis of residues produced from the UV photolysis of CH_3OH:pyrimidine, H_2O:CH_3OH:pyrimidine, CH_4:pyrimidine, and H_2O:CH_4:pyrimidine ice mixtures provided limited information about their chemical composition. Of the many peaks seen on the chromatograms, only a few could be conclusively identified in each sample. Of particular note is the lack of any methylpyrimidines in the residues produced from CH_3OH-containing ices and the lack of 5-methylpyrimidine in the residues produced from CH_4:pyrimidine ices. The former result seems to suggest that UV photo-induced methylation of pyrimidine in CH_3OH-containing ices is not an efficient process, while the latter result suggests that methylation of position 4 may be favored, at least in the absence of H_2O. It should be noted that, if we consider methylation of pyrimidine from a strictly statistical point of view, position 4 is expected to be favored, since two possible addition sites on the pyrimidine ring (positions 4 and 6) can yield 4-methylpyrimidine, while 2- and 5-methylpyrimidine can only be formed by addition at one location (positions 2 and 5, respectively). This could partially explain the differences in relative abundances of 4- and 5-methylpyrimidine observed in our H_2O:CH_4:pyrimidine samples.

A peak is present in the chromatograms of many of our samples at the expected retention time for thymine; however, the UV spectrum of this peak indicates that thymine is probably coeluting with an unidentified compound. Because of this uncertainty, HPLC analyses are insufficient for making a definitive identification of thymine, though our results are not inconsistent with its presence. To make a more definitive statement about thymine formation, GC-MS analysis is essential.

Estimating yields of the various product molecules applicable to astrophysical environments is beyond the scope of this work, since these are simple model ices. However, yields can be reported in a general sense if we ratio the peak area for any given photo-product in the HPLC chromatograms with that of unreacted pyrimidine and take into account the relative intensities of their UV spectra at a given wavelength. Such calculated yields represent upper limits on conversion efficiencies since they assume that the amount of initial pyrimidine consumed is very small compared with the initial quantity of pyrimidine in the ice mixtures. Typical conversion efficiencies for these compounds calculated in this way lie between 0.01% and 2%, a number consistent with previously obtained conversion efficiencies for UV photolysis products in astrophysically relevant ices (Allamandola *et al.,* 1988; Bernstein *et al.,* 1995).

3.2. GC-MS analysis of the residues

Gas chromatography coupled with mass spectrometry provides an independent, complementary method for identifying compounds in our samples. The difference in separation techniques provides us with an alternative method to isolate compounds that may coelute in the liquid phase. Additionally, GC-MS is useful for identifying compounds that

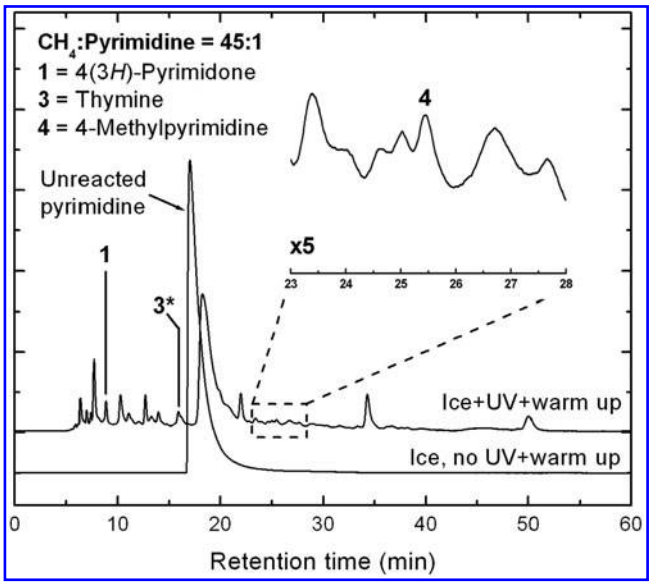

FIG. 4. HPLC chromatogram ($\lambda = 256$ nm) of a CH_4:pyrimidine = 45:1 residue (top trace) showing the detection of 4(3*H*)-pyrimidone ($R_t = 8.87$ min, peak **1**), 4-methylpyrimidine (25.51 min, peak **4**), and comparison with the corresponding no-UV control (bottom trace). The peak denoted **3*** is consistent with the presence of thymine (see Section 3.1.2).

have nondescript UV spectra within the range of our HPLC detector. GC-MS identifications of compounds were performed by comparing single-ion chromatograms (SICs) of samples with standards for the same mass corresponding to the most intense fragment of their *t*BDMS derivatives (M*–57 amu), which in most cases is due to the derivatized compound (M*) that has lost one *tert*-butyl (–$C(CH_3)_3$) group (57 amu) (Table 1; Casal *et al.,* 2004; Schummer *et al.,* 2009). The main drawback of this protocol for our application is that many underivatized compounds, including simple methylpyrimidines lacking –OH or –NH_2 moieties, cannot be detected.

In addition to our sample chromatograms, GC-MS figures in this paper may show plots of procedural blanks that correspond to non-irradiated samples produced from the same mixtures as the given residues, the injection of the derivatizing agent (MTBSTFA) alone (Section 2.2), or a chromatographic standard. Chromatograms of no-UV controls show very few peaks other than those seen for the derivatizing agent itself, which are due to known fragments and by-products of MTBSTFA and *t*BDMCS (Nuevo *et al.,* 2009, 2012; Schummer *et al.,* 2009).

In the following sections, we discuss compounds that were reliably observed in most or all of our residues. Note that additional compounds identified in only a few of our samples are mentioned, but since they are not reliably reproduced in measurable quantities they will not be discussed in detail here.

3.2.1. CH_3OH:pyrimidine mixtures. The same samples produced from the UV irradiation of CH_3OH:pyrimidine ices with different relative proportions ranging from 5:1 to 20:1 and analyzed with HPLC (Section 3.1.1) were analyzed with GC-MS. The GC-MS total-ion chromatogram (TIC) of a typical residue produced from the UV irradiation of a CH_3OH:pyrimidine = 20:1 ice mixture is shown in Fig. 5 (top trace). The products identified did not vary much as a result of the initial proportions of the starting materials, although their relative abundances did. A series of molecules, including 4(3*H*)-pyrimidone ($R_t = 10.12$ min, $m/z = 153$ amu), 2-aminopyrimidine (12.34 min, 152 amu), 4-pyrimidinemethanol (13.24 min, 167 amu), 4-aminopyrimidine (14.99 min, 152 amu), uracil (20.71 min, 283 amu), and 4,6-dihydroxypyrimidine (21.38 min, 283 amu), were identified in this residue, as well as in all other CH_3OH:pyrimidine residues we examined (Table 2). All these molecules, with the exception of 4-pyrimidinemethanol, have been previously observed in samples obtained from H_2O:pyrimidine and H_2O:NH_3:pyrimidine ices (Nuevo *et al.,* 2009, 2012).

In several samples, there is a tentative identification of a small quantity of thymine (297 amu, standard eluting at 22.20 min, see Table 1); however, this result was not consistently reproduced.

A peak at ~12.70 min with a main mass at 158 amu was observed in every sample and likely corresponds to one of the bipyrimidine isomers, as suggested by previous studies (Nuevo *et al.,* 2009, 2012; Bera *et al.,* 2010). However, a precise identification of this peak cannot be made at this time because the only available standard, 2,2′-bipyrimidine, does not provide a match.

There are a number of other peaks observed in each sample for which we can form strong hypotheses about their identity, but we cannot confirm them because of the lack of corresponding standards. Some examples of these are peaks in the 152 amu SICs that likely correspond to the addition of an NH_2 functional group to pyrimidine, with the exclusion of 2- or 4-aminopyrimidine (for which we have the standards). Using the same reasoning, we can also deduce that the peak at 17.63 min ($m/z = 153$ amu) is likely 5-hydroxypyrimidine, because it does not match the standards for 2-hydroxypyrimidine and 4(3*H*)-pyrimidone, though it shares the same parent mass, and because pyrimidine *N*-oxide is not derivatized (Table 1). Additional peaks at 181, 183, 283, 285, 297, 311, 413, and 427 amu are present in all or most samples and are consistent with increasingly functionalized pyrimidines after addition of combinations of CH_3, OH, NH_2, CH_2OH, OCH_3, and/or COOH groups.

In addition to the substituted pyrimidines observed in these samples, several other compounds were detected, including some compounds whose production must involve the rupture of the pyrimidine ring. One of these compounds is hydantoin, an oxidized carbon and nitrogen heterocyclic molecule with a 5-member ring that was previously found in residues produced from H_2O:CH_3OH:NH_3 ice mixtures and that may be involved in the prebiotic synthesis of oligopeptides (de Marcellus *et al.,* 2011). Additionally, acyclic molecules, including glycine (standard eluting at 16.00 min, $m/z = 246$ amu), urea (18.16 min, 231 amu), glycerol (23.03 min, 377 amu), and traces of alanine (15.53 min, 260 amu), were observed (Table 2). Glycolic acid (14.74 min, 247 amu) and oxalic acid (15.69 min, 261 amu) were also identified in each sample, although it should be noted that a fraction of each of these compounds comes from the derivatization procedure, as they both appear in blank samples—including those that have only had the derivatization agent added to an empty vial—at a predictable background level. However, our samples show peaks for glycolic and oxalic acids that are 3–10 times stronger than these

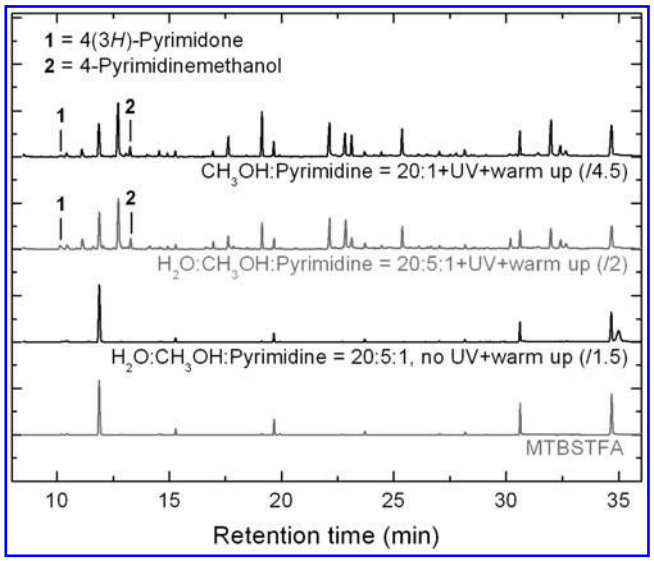

FIG. 5. Total-ion chromatograms for typical residues produced from CH_3OH:pyrimidine = 20:1 (top trace) and H_2O:CH_3OH:pyrimidine = 20:5:1 mixtures (top middle trace), as well as from a no-UV control (top bottom trace) and a procedural blank prepared with only the derivatization agent (MTBSTFA, bottom trace).

background levels. Finally, all sample chromatograms also show the presence of lactic (2-hydroxypropanoic) acid (14.39 min, 261 amu) as well as glyceric (2,3-dihydroxypropanoic) acid (24.43 min, 391 amu), and some of them show the presence of small quantities of 3-hydroxypropanoic acid (16.41 min, 261 amu), which are probably mainly formed from photo-processed CH_3OH alone. The observed urea and glycine may form from photo-dissociated pyrimidine fragments. Both these compounds were also previously found in residues produced from the irradiation of H_2O:NH_3:pyrimidine ice mixtures (Nuevo *et al.,* 2012).

Experiments in which ice mixtures were irradiated with a higher UV dose added several new compounds to the list of identified species, including 2-hydroxy-4-methylpyrimidine (12.35 min, 167 amu) and isobarbituric acid (29.65 min, 413 amu). Additionally, there are more unidentified peaks that are consistent with additions of other combinations of CH_3, OH, NH_2, CH_2OH, OCH_3, and/or COOH groups to pyrimidine that were not observed in low radiation dose experiments.

3.2.2. H_2O:CH_3OH:pyrimidine mixtures. This series of experiments tested the effect of introducing H_2O to the CH_3OH:pyrimidine mixtures, with initial H_2O:CH_3OH:pyrimidine compositions ranging from 20:2:1 to 20:10:1. The top middle trace of Fig. 5 shows the GC-MS chromatogram of a typical H_2O:CH_3OH:pyrimidine = 20:5:1 residue. As with CH_3OH:pyrimidine residues, the products identified varied little with the proportions of the initial ice components and include 4(3*H*)-pyrimidone, 2- and 4-aminopyrimidine, 4-pyrimidinemethanol, uracil, 4,6-dihydroxypyrimidine, and isobarbituric acid (Table 2). Among these compounds, the only photo-product not previously observed in experiments utilizing a normal radiation dose in CH_3OH:pyrimidine mixtures was isobarbituric acid. Also similar to CH_3OH:pyrimidine residues, unidentified peaks include a bipyrimidine isomer other than 2,2′-bipyrimidine (~12.80 min, 158 amu) and a peak at 17.59 min (153 amu) likely due to 5-hydroxypyrimidine. Finally, peaks consistent with increasingly substituted functionalized pyrimidines with CH_3, OH, CH_2OH, OCH_3, and/or COOH groups are also observed (see Section 3.2.1).

Finally, hydantoin and all the collection of acyclic compounds identified in CH_3OH:pyrimidine residues (glycine, urea, glycolic acid, oxalic acid, lactic acid, 3-hydroxypropanoic acid, glycerol, and glyceric acid) were observed in each of these samples (Table 2), with the addition of *N*-formylglycine, a non-biological amino acid that was previously detected in H_2O:pyrimidine and H_2O:NH_3:pyrimidine samples (Nuevo *et al.,* 2012). The only additional identifiable compound found in high UV dose experiments was oxalic acid. Additionally, there were more unidentified peaks whose mass spectra are consistent with combinations of CH_3, OH, NH_2, CH_2OH, OCH_3, and/or COOH groups and that were not observed in low radiation dose experiments.

3.2.3. CH_4:pyrimidine mixtures. In this series of experiments, the initial compositions ranged from 18:1 to 45:1, and no source of oxygen was intentionally introduced to the system. Since methylated pyrimidines with no other functional groups cannot be derivatized by our procedure, many of the possible products that can be formed from CH_4 and pyrimidine cannot be observed with GC-MS. The only molecule positively identified with GC-MS analysis in any of these samples that could have originated from the intended starting materials was 4-aminopyrimidine (14.91 min, 152 amu). We also observed a peak belonging to an unidentified bipyrimidine isomer in each sample (~12.70 min, 158 amu). In addition to these photo-products, we observed a number of oxidized pyrimidines, the most abundant being 4(3*H*)-pyrimidone, that could not have formed from the starting materials alone. As previously stated, we hypothesize that oxidized products formed either by reacting with trace background H_2O (which represents the primary contaminant in our vacuum chamber), from the release of O from the Al foil substrate, or by exposure of reactive species to air after the sample is extracted from the vacuum system but prior to analysis. The presence of oxidized pyrimidines in samples lacking an oxygen source has been previously observed in both the HPLC and GC-MS chromatograms of NH_3:pyrimidine samples (Nuevo *et al.,* 2012).

The high UV dose experiments revealed similar results, with the additional identification of 2-aminopyrimidine (12.32 min, 152 amu) as well as unidentified peaks likely belonging to isomers of diaminopyrimidines other than 2,4- and 4,5-diaminopyrimidine (281 amu), for which we have standards. Additionally, other unidentified compounds with masses consistent with oxidized pyrimidines were detected.

3.2.4. H_2O:CH_4:pyrimidine mixtures. In this series of experiments, residues were produced from H_2O:CH_4:pyrimidine ice mixtures (initial concentrations of 20:10:1, 20:5:1, and 3:30:1), and the following molecules were identified in each sample: 4(3*H*)-pyrimidone, 2-hydroxypyrimidine, 2- and 4-aminopyrimidine, 2-hydroxy-4-methylpyrimidine, 4-pyrimidinemethanol, uracil, 4,6-dihydroxypyrimidine, cytosine (24.28 min, 282 amu), and isobarbituric acid. The unidentified isomer of bipyrimidine eluting around 12.70 min is also present, as well as peaks for masses of 181, 183, 283, 285,

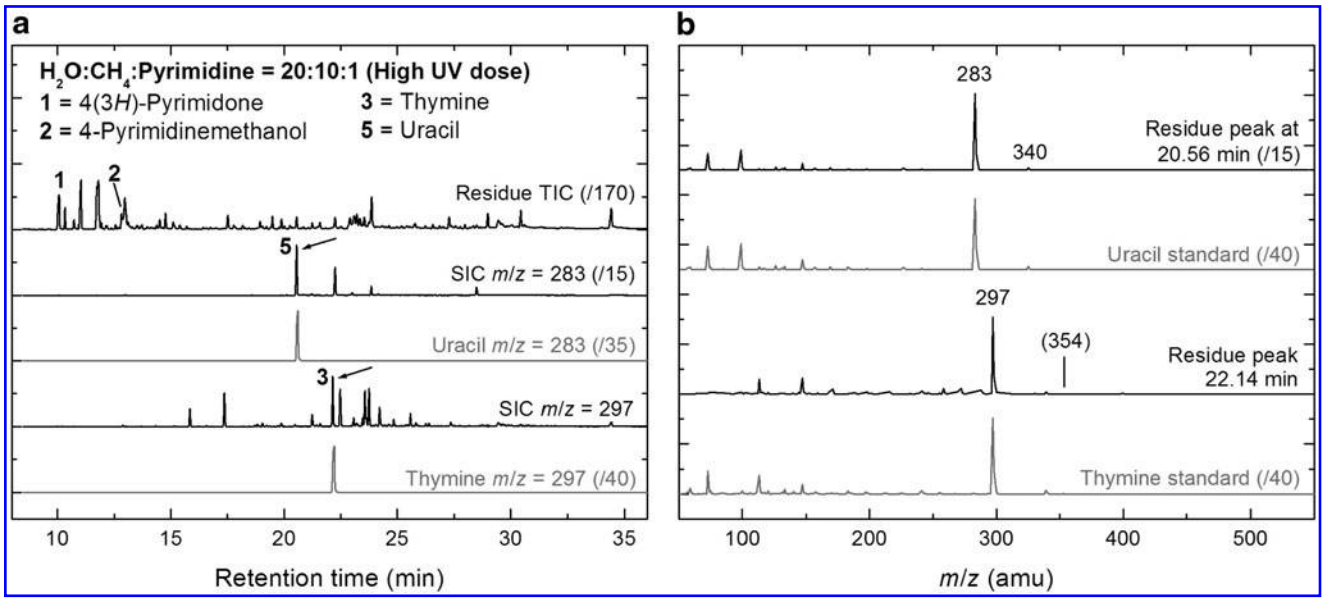

FIG. 6. (**a**) Sample TIC of a residue from an H_2O:CH_4:pyrimidine sample exposed to a high UV dose (top trace). The top middle and bottom middle traces show sample SICs of masses corresponding to uracil (283 amu, peak **5**) and thymine (297 amu, peak **3**), respectively. The middle and bottom traces show SICs of the uracil and thymine standards for their same respective masses. (**b**) Comparison between the mass spectra of the sample peak eluting at 20.56 min (top trace) with that of the uracil (top middle trace), and comparison between the mass spectra of the sample peak at 22.14 min (bottom middle trace) with that of the thymine standard (bottom trace). Both comparisons indicate positive identifications of these nucleobases.

311, 413, 427, and 441 amu, also found in CH_3OH:pyrimidine and H_2O:CH_3OH:pyrimidine samples, that are consistent with substituted functionalized pyrimidines (Sections 3.2.1 and 3.2.2). In addition, the acyclic compounds identified in the previous residues produced from H_2O-contaning starting ices (glycine, *N*-formylglycine, alanine, urea, glycolic acid, oxalic acid, lactic acid, 3-hydroxypropanoic acid, glycerol, and glyceric acid) are also present in these samples.

For all the mixtures in this series, a weak peak consistent with thymine is present at retention times around 22.13 min. However, with the exception of one residue in which thymine could be clearly identified, background noise usually made a conclusive identification difficult. In contrast, some of the residues produced from the high UV dose experiments unambiguously show the production of thymine (Fig. 6).

3.2.5. H_2O:5-methylpyrimidine mixtures. Among the multiple pathways leading to the formation of thymine from the addition of OH and CH_3 groups to pyrimidine, preliminary results from our quantum chemical computations suggest that, although not the preferred overall reaction route, once pyrimidine is methylated, oxidation should proceed in a relatively efficient manner. This led us to perform two experiments involving the photolysis of H_2O:5-methylpyrimidine ice mixtures with relative proportions 140:1 and 280:1 (mainly constrained by the very low vapor pressure of 5-methylpyrimidine). The goal of these experiments was to provide a better understanding of both the efficiency of the oxidation of 5-methylpyrimidine and its photo-stability to UV radiation. Results from these experiments show that the irradiation of H_2O:5-methylpyrimidine ices leads to the production of thymine in addition to photo-products which were not detected in our CH_3OH- and CH_4-containing experiments, such as 5-(hydroxymethyl)uracil ($R_t = 30.64$ min, $m/z = 427$ amu) and orotic acid (32.26 min, 441 amu). However, these experiments also showed the presence of oxidized pyrimidines lacking any methyl group, such as 4(3*H*)-pyrimidone. Results of these H_2O:5-methylpyrimidine experiments therefore suggest that a limiting factor for thymine production, and presumably for the production of other methylated pyrimidines, is that CH_3 groups are readily cleaved from the pyrimidine ring and may be replaced by competing moieties from the surrounding ice, in particular oxygen atoms.

3.2.6. Summary of GC-MS results. The GC-MS analysis of residues produced from the UV photolysis of CH_3OH:pyrimidine, H_2O:CH_3OH:pyrimidine, CH_4:pyrimidine, and H_2O:CH_4:pyrimidine ice mixtures provided significantly more information than their corresponding HPLC data. GC-MS chromatograms reveal an array of substituted pyrimidines that remained remarkably similar regardless of relative starting concentrations, radiation dose, and composition. Thymine was found in small quantities only in samples produced from CH_3OH:pyrimidine (tentatively) and H_2O:CH_4:pyrimidine starting mixtures. UV photolysis of H_2O:5-methylpyrimidine ices resulted in the formation of thymine and other previously unobserved pyrimidine variants, including 5-(hydroxymethyl)-uracil and orotic acid. Thus, these experiments demonstrated that photolytic cleavage of the methyl group from the pyrimidine ring is possible in these ices and demonstrated the effect of competition with OH moieties.

4. Discussion

4.1. Pyrimidine methylation in CH_3OH- versus CH_4-containing ices

Water (H_2O) has been shown experimentally to be readily photo-dissociated into OH radicals that can efficiently be

added to the pyrimidine ring (Nuevo *et al.*, 2009, 2012). When CH_3OH replaces H_2O in the starting ice mixtures, it is expected to similarly release OH radicals after photo-dissociation that can oxidize pyrimidines, in addition to CH_3, CH_2OH, and OCH_3 radicals. Öberg *et al.* (2009) reported photo-dissociation branching ratios of about 1:1:5 for CH_3/OH, OCH_3, and CH_2OH radicals from CH_3OH. The higher abundance of CH_2OH radicals is supported by the presence of 4-pyrimidinemethanol in all our CH_3OH:pyrimidine and H_2O:CH_3OH:pyrimidine residues, which is likely formed from the addition of a CH_2OH moiety to the pyrimidine ring. Interestingly, although 4-pyrimidinemethanol was identified in all HPLC and GC-MS chromatograms (Table 2), there is no evidence for the presence of other pyrimidinemethanol isomers, suggesting that addition in position 4 is highly favored. Such a selective stereochemistry was previously observed for the addition of OH and NH_2 groups to pyrimidine, which favored the formation of 4(3*H*)-pyrimidone and 4-aminopyrimidine, respectively (Nuevo *et al.*, 2009, 2012; Bera *et al.*, 2010). Similarly, when not unfavorably competing with its oxidation, the methylation of pyrimidine seems to favor the formation of 4-methylpyrimidine over that of the two other isomers (Section 3.1.3).

The detection of oxidized pyrimidines, including 4(3*H*)-pyrimidone, uracil, 4,6-dihydroxypyrimidine, as well as other unidentified oxidized variants (Table 2) in CH_3OH:pyrimidine residues confirms the oxidizing power of CH_3OH. However, we note that the addition of H_2O to CH_3OH:pyrimidine mixtures resulted in the production of highly oxidized compounds, such as isobarbituric acid (Section 3.2.2), that were not previously detected in the CH_3OH-only experiments. The strong similarities between the photo-products formed in the presence or absence of H_2O—in fact, even the similarities between these residues and those from previous H_2O:pyrimidine experiments (Nuevo *et al.*, 2009)—indicate that the photo-oxidation of pyrimidine is a robust process. In other words, when UV-labile oxygen is available in the residue, it is readily added to pyrimidine to produce a fairly consistent set of products. This is further demonstrated by the fact that changing the relative concentration of the starting ice components does not significantly alter the composition of the resulting residues.

In contrast, no methylpyrimidines were positively identified from the photolysis of CH_3OH-containing ices, which suggests that the production of these molecules under our experimental conditions may not be efficient, even when ices are subjected to a higher dose of UV photons (Section 3.1.3). It should be noted that similar results were observed by Elsila *et al.* (2006), in which irradiation of methanol and quinoline yielded minute quantities of methylquinolines that were not identifiable with HPLC. We know from its photo-dissociation branching ratios that methanol may not be an efficient source of CH_3 groups (Öberg *et al.*, 2009). However, the dearth of methylpyrimidines produced in the CH_3OH:pyrimidine may also be due to any or all of the following: (i) the CH_3 addition process itself is inefficient, (ii) CH_3 addition unfavorably competes with OH addition, (iii) methylpyrimidines are rapidly oxidized, or (iv) methylpyrimidines easily undergo further substitutions that remove or replace the methyl groups.

Experiments in which the source for methyl groups was methane instead of methanol (CH_4:pyrimidine and H_2O:CH_4:pyrimidine mixtures) provide some insights into these possibilities. First, the CH_4:pyrimidine experiments demonstrated that CH_3 groups are readily added to pyrimidine, with a regioselective preference for an addition to position 4 of the ring (Section 3.1.3). Though quantum chemical computations indicate that methyl addition is less efficient than oxidation, experimental results show that oxidation does not completely prevent methylation. In fact, the addition of H_2O to the starting ices seems either to enable or increase the yield of 5-methylpyrimidine over mixtures with no H_2O. The presence of methylpyrimidines in H_2O-containing ices also suggests that methylpyrimidines are not absent because of their rapid oxidation. Although the presence of methylpyrimidines in our methane experiments suggests that they can survive under our experimental conditions, the experiments that started with methylpyrimidines showed that radiation processing in ice certainly can result in the subsequent loss of the methyl group or its replacement with other competing moieties.

Finally, we note that the presence of H_2O can actually enhance methylation of pyrimidine. Residues from our H_2O:CH_4:pyrimidine ices showed the presence of both 4- and 5-methylpyrimidine, while our CH_4:pyrimidine ices only produced detectable 4-methylpyrimidine. This effect is similar to that previously observed for the addition of NH_2 groups to pyrimidine in H_2O:NH_3:pyrimidine ice mixtures (Nuevo *et al.*, 2012). MP2/ZAPT2 and B3LYP (with cc-pVTZ basis set) quantum chemical computations indicate that this is because H_2O plays the role of a proton acceptor, which helps stabilize intermediate species that lead to the formation of the final products (Bera *et al.*, 2010, and unpublished results). While quantum chemical computations demonstrate the important role H_2O can play in the methylation process, they also indicate that oxidation is modestly more efficient than methyl addition.

4.2. Formation of thymine

We estimated the production yield of thymine in the residue formed from the irradiation of an H_2O:CH_4:pyrimidine = 20:10:1 ice mixture with a high UV dose (Fig. 6) to be about 2×10^{-5}, which means that one molecule of thymine is formed for every 5×10^{4} molecules of pyrimidine irradiated. This is an order of magnitude smaller than for uracil from H_2O:pyrimidine experiments and for cytosine from H_2O:NH_3:pyrimidine experiments irradiated with a regular photon dose (Nuevo *et al.*, 2012). To explain such a low efficiency for the formation of thymine in our experiments, one needs to understand the processes that take place during each step of its formation from pyrimidine, which requires a number of ingredients and experimental conditions as follows:

(1) A CH_3 source is needed. CH_3OH and CH_4 are both seen in interstellar ices (Allamandola *et al.*, 1992; Gibb *et al.*, 2004; Dartois, 2005) and are valid sources of CH_3 radicals. CH_3OH is typically much more abundant than CH_4 in interstellar ices, but the results of our experiments suggest that CH_4 is a more efficient source of CH_3 radicals than CH_3OH.
(2) The formation of thymine requires the presence of an oxygen source, but not so much as to dominate the chemistry and inhibit the methylation of pyrimidine. The results of our experiments indicate that both H_2O and CH_3OH can serve as efficient sources of oxygen.

(3) Theoretical considerations suggest that a proton acceptor in the ice matrix, such as H_2O, is helpful as we have seen in previous experiments and with quantum chemical computations (Bera *et al.,* 2010, and unpublished results). As with uracil, the relative abundance of H_2O should be high enough to hydrate the intermediate compounds and extract the excess proton to stabilize the formation of the final products (Bera *et al.,* 2010) but not so high that the oxidation of pyrimidine becomes so dominant that it inhibits the addition of CH_3 groups. The issue of competition was shown in the experiments involving H_2O:5-methylpyrimidine discussed in Section 3.2.5.

(4) Thymine requires a high abundance of UV photons to be formed. Indeed, its formation requires the addition of three groups to the pyrimidine ring and therefore requires a higher number of photons than uracil and cytosine, which are formed from the addition of two groups each (Nuevo *et al.,* 2009, 2012). On the other hand, a high number of UV photons can also be a hindrance, because while they are essential for the formation of thymine, photons can also destroy or further modify previously formed thymine, particularly when further photolysis enables competition with other moieties like oxygen.

Our experimental results do not show any clearly preferred pathway for the formation of thymine. They do, however, suggest that the addition of methyl groups may play a role in limiting the overall efficiency of the process in ices resembling those observed in an astrophysical environment. To better understand the mechanisms and chemical pathways of thymine formation, quantum computations with the use of *ab initio* MP2 and ZPAT2 methods as well as B3LYP density functional methods were performed and will be discussed in a forthcoming separate article. Finally, although we do not explicitly study the photo-induced destruction of thymine, there is clearly a balance struck between the formation and degradation processes. This balance will determine the overall abundance of thymine present in the final residues, and it is expected that similar processes would occur in astrophysical environments.

4.3. Comparison with meteorites and astrobiological implications

Several compounds identified in our samples have been detected in carbonaceous chondrites such as the Murchison meteorite, in particular, oxidized pyrimidines that include 4(3*H*)-pyrimidone (sometimes referred to as 4-hydroxypyrimidine) and uracil, as well as CH_2OH-containing pyrimidines that include 4-pyrimidinemethanol (sometimes referred to as 4-hydroxymethylpyrimidine) (Folsome *et al.,* 1971). In the same study, two isomers of 4-hydroxy-X-methylpyrimidine (where X = 2 and/or 6) were also reported, indicating the presence of pyrimidines substituted by both one OH group and one CH_3 group, though they are different from those detected in some of our H_2O:CH_4:pyrimidine samples (Table 2). Although their presence in our samples cannot be ruled out from our GC-MS data (Section 3.2), these meteoritic compounds could not be rigorously searched for because standards are not commercially available.

Uracil is the only pyrimidine-based nucleobase that has thus far been reported in meteorites (Stoks and Schwartz, 1979). In that work, the presence of thymine could not be confirmed, but upper limits placed on its concentration in the Allende, Orgueil, Murchison, and Murray meteorites were an order of magnitude lower than those for uracil. As previously stated, the extraterrestrial origin of uracil in Murchison was supported by $\delta^{13}C$ measurements by Martins *et al.* (2008), though it has been debated whether this value could be at least partly due to coeluting compounds (Callahan *et al.,* 2011).

The absence of cytosine in meteorites may be explained by its conversion into uracil via hydrolysis (Shapiro, 1999; Nelson *et al.,* 2001), which is one of the steps of the protocol applied for the analysis of meteoritic samples. Thus, the presence of cytosine in meteorites cannot be ruled out. However, the non-detection of thymine cannot be explained by a similar conversion issue, which suggests that there could be another reason for its absence. To date, there has not been a significant discussion explaining the non-detection of thymine in meteorites, and more generally, the presence of nucleobases in meteorites is not fully understood.

Assuming that photo-processes on icy grains play an important role in the production of nucleobases that are present in meteorites, our experimental results suggest a few possible explanations for the non-detection of thymine. First, methyl addition is an essential step for the formation of thymine, but this reaction appears mostly inefficient in H_2O- and CH_3OH-rich ices. Although methylation readily occurs in CH_4-rich ices, observational data suggest that CH_4 is typically an order of magnitude less abundant than CH_3OH in interstellar ices (Gibb *et al.,* 2004, and references therein). Second, the formation of thymine requires at least three substitutions, which can only efficiently take place in environments providing higher UV radiation fields, as may have been the case in the outer regions of the solar nebula (Ciesla and Sandford, 2012). Additionally, since the formation of thymine requires more UV photons than that of cytosine or uracil, it (or its possible precursors) may be more susceptible to photolytic destruction during or after its formation. Collectively, these results suggest that extraterrestrial thymine, abiotically produced via UV ice photolysis, may not have been delivered to primitive Earth by asteroids or comets in comparable abundances to other molecules of prebiotic interest produced in the same manner, including uracil and cytosine.

Interestingly, thymine is only found in DNA and not in RNA. The RNA world hypothesis proposes that an early period in the emergence of life existed, during which primitive biological reactions were dominated solely by RNA, which has no need of thymine (Gilbert, 1986; Joyce, 1989, 2002). If the extraterrestrial delivery of abiotically produced nucleobases was an important factor in the beginning of life on Earth (Oró, 1961; Chyba and Sagan, 1992), then our results, which suggest that thymine would be produced in lower abundances than uracil and cytosine, as well as the apparent paucity of thymine in meteorites, are not inconsistent with such models or the suggestion that the use of thymine—and consequently DNA—appeared at a later terrestrially driven stage in the complexity of biology.

5. Conclusions

Experiments involving the UV photolysis of mixed molecular ices containing CH_4 and pyrimidine in the presence or absence of H_2O led to the formation of small quantities of

methylated pyrimidine derivatives. In contrast, similar experiments in which CH_4 was substituted with CH_3OH did not yield measurable quantities of methylpyrimidines but may have produced small quantities of thymine in the presence of excess H_2O. Finally, irradiation experiments of 5-methylpyrimidine in H_2O ice showed that the methyl group can be cleaved from the pyrimidine ring by UV photolysis.

Forming thymine from pyrimidine requires three substitutions, two oxidations, and one methylation, and thus more photochemical steps than for forming the nucleobases uracil and cytosine. In interstellar ices, H_2O is by far the most abundant component in astrophysical ices, with CH_3OH trailing by an order of magnitude and CH_4 falling roughly another order of magnitude lower than that of CH_3OH. Since our experiments have shown that CH_3OH is not as effective a reagent for the methylation of pyrimidine as CH_4, under interstellar/protosolar conditions oxidation of pyrimidine may likely dominate its methylation. In conjunction with the higher photon dose requirement, this suggests that the formation of thymine under astrophysical conditions may not be an efficient process compared to the formation of uracil and cytosine.

In a scenario in which molecules important for life such as nucleobases were formed via astrophysical ice photoprocesses and delivered to the primitive Earth via comets and meteorites, our experimental results suggest that the abundance of uracil delivered may have been significantly higher than that of thymine. This is consistent with the detection of uracil and the non-detection of thymine in meteorites. Further, if the delivery of extraterrestrial nucleobases to Earth played a role in the origin of life, uracil—one of the subunits of RNA—may have played a more important role, while thymine—one of the building blocks of DNA—may have only been involved in prebiotic/biotic chemistry at a later stage in Earth's history.

Acknowledgments

This work was supported by NASA grants from the NASA Astrobiology Institute and Origins of Solar Systems programs. C.K.M., M.N., and S.A.S. would like to acknowledge R.L. Walker (NASA Ames) for technical support. P.P.B. and T.J.L. would like to acknowledge financial support from NASA to investigate the formation and evolution of carbon-based material in the Universe. We would also like to thank two anonymous reviewers of this manuscript for useful comments and suggestions.

Author Disclosure Statement

No competing financial interests exist.

Abbreviations

cc-pVTZ, correlation consistent polarized valence triple zeta; GC-MS, gas chromatography coupled with mass spectrometry; HPLC, high-performance liquid chromatography, high-performance liquid chromatograph; MP2, second-order Møller-Plesset perturbation theory; MTBSTFA, *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide; SIC, single-ion chromatogram; *t*BDMCS, *tert*-butyldimethylchlorosilane; *t*BDMS, *tert*-butyldimethylsilyl; TIC, total-ion chromatogram; ZAPT2, second-order Z-averaged perturbation theory.

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Submitted 14 June 2013
Accepted 14 September 2013