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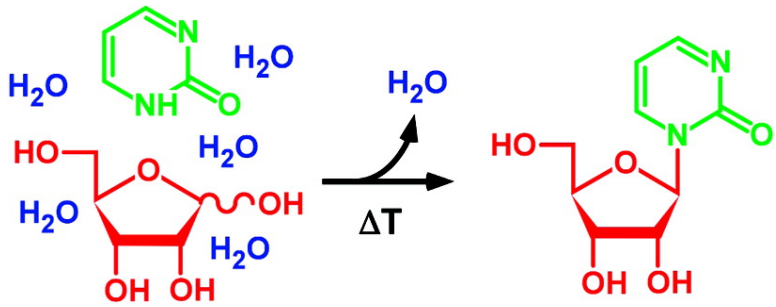

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Formation of a β-Pyrimidine Nucleoside by a Free Pyrimidine Base and Ribose in a Plausible Prebiotic Reaction

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Glycosylation of pyrimidine bases to give nucleosides under plausible prebiotic conditions has not been achieved, and this represents a serious challenge to the "RNA world" and to many of its proposed precursors.[1] Three decades ago, Orgel and co-workers demonstrated that adenine and hypoxanthine form glycosidic bonds with D-ribose when dried and heated together, although adenine gives mainly the product of attack at the NH_2 group.[2] As much as 20% of the nucleoside was reported from hypoxanthine (BRSM).[2] Unfortunately, neither cytosine nor uracil gives rise to nucleosides under these conditions, nor does guanine, a fact attributed to its low solubility.[1] Some researchers have taken to exploring completely different pathways to prebiotic pyrimidine nucleoside formation, including the construction of the cytosine base on a sugar phosphate.[3] However, suggestions that the original bases may have differed from the canonical AUCG leave open the possibility that some of these bases might form glycosides under prebiotic conditions.[4] Indeed, urazole, a five-membered ring derived from hydrazine, readily forms glycosides.[3c] In search of alternative pyrimidine bases, we have explored the condensation of ribose with 2-pyrimidinone (Figure 1) by both experiment and calculation.

Drying and heating of 2-pyrimidinone with ribose using the reaction conditions described by Orgel[2] produces zebularine in 12% yield. Zebularine formation was confirmed by mass spectrometry (m/z = 227 Da), HPLC mobility (Figure 2, peak 2), UV spectrophotometry, and NMR analysis of HPLC-purified reaction products (Supporting Information (SI)). To the best of our knowledge, this work represents the first successful synthesis of a pyrimidine nucleoside from a free base and an unactivated sugar in a plausible prebiotic reaction. Additional products were also observed, including two assigned as the β-pyranosyl (coelutes with zebularine) and α-furanosyl (peak 1) nucleosides (Figure 2, SI). The α-pyranosyl anomer may be present in low yield, but was not detected. Thus, the total yield of pyrimidine nucleosides formed in the reaction is even greater than that reported here for zebularine. As seen in Figure 2, the relative ratios of the products differ dependent upon the reaction conditions, yet typically favor β-anomers, as previously observed for ribosides of urazole.[3c]

Nucleoside formation by purines and ribose under drying conditions is an acid-catalyzed reaction that can also be promoted by divalent metal ion salts at neutral pH.[2] In the absence of Mg^{2+}, 2-pyrimidinone yields zebularine when dried and heated with ribose from a slightly acidic solution of pH 2.1 (Figure 2). The actual pH at reaction is not known, but is likely to be less than 1 because of the drying conditions. Buffering the sample pH to 6.3 reduces

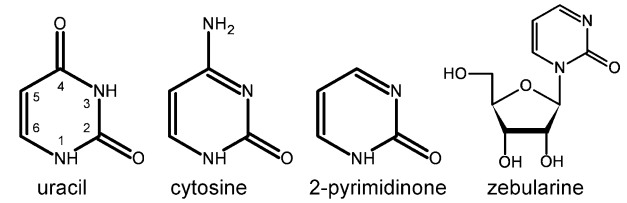

Figure 1. Chemical structures of pyrimidine bases and zebularine, the β-furanosyl ribonucleoside of 2-pyrimidinone.

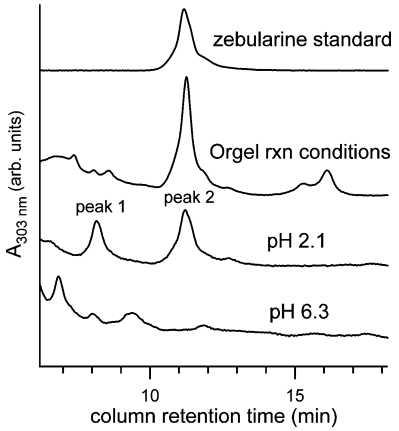

Figure 2. HPLC chromatographs of an authentic zebularine standard with products of the drying-heating reaction of 2-pyrimidinone with ribose. The Orgel reaction conditions were fifteen-fold excess of ribose, 25 mM $MgCl_2$, 50 mM $MgSO_4$, pH 6.2, dried and heated at 100 °C for 2.25 h. For the two other chromatographs shown, twofold excess of ribose, dried and heated at 75 °C for 2 h. All reactions were initially 5 mM in 2-pyrimidinone. The α-furanosyl nucleoside elutes as peak 1, the two β-anomers coelute as peak 2. The shoulder peak observed in the zebularine standard is an artifact of the HPLC gradient. See SI for additional details.

zebularine production to an unobservable level, although other reaction products are still observed (Figure 2). Thus, our results indicate that the zebularine formation is similar to the purine glycosylation reaction in that it is both acid-catalyzed and is promoted by Mg^{2+} near neutral pH.

It is of interest to compare the nucleoside yields from 2-pyrimidinone, adenine, and hypoxanthine. The relative yields of β-furanosyl nucleosides produced in three different reaction conditions were determined by HPLC analysis. The results presented in Table 1 demonstrate that 2-pyrimidinone, surprisingly, forms β-nucleosides in greater yields than both adenine and hypoxanthine. Uracil did not form nucleosides in detectable yields under any of the conditions tested. In addition, we conducted nucleoside degradation studies using the same reaction conditions that were used for the nucleoside syntheses. Transition state theory predicts

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Table 1. Comparison of β-Furanosyl Ribonucleoside Yields for Three Bases under Selected Reaction Conditions[a]

reaction	zebularine	adenosine	inosine
Orgel reaction conditions	12%	<1%	3%
pH 2.1	2%	1%	1%
pH 6.3	<1%	<1%	<1%

[a] Reaction conditions are described in Figure 2. Yields are absolute and neglect side products, which we estimate at <20%. The maximum variability in the measured yields was 45%. Previously reported nucleoside yields were calculated BRSM.[2] See SI for additional details.

Table 2. The B3LYP/6-31G(d) Predicted Activation Energies (in kcal/mol) for Glycosidic Bond Formation between Ribose and 2-Pyrimidinone or Uracil[a]

reactants	activation energy
ribose, pyrimidinone	52.7
ribose, uracil	50.8
ribose, protonated-pyrimidinone	34.3
ribose, protonated-pyrimidinone, Mg^{2+}	**20.2**
ribose, uracil, Mg^{2+}	39.9

[a] See SI for pathway structures and associated energies, and for calculation methodology.

that, under the same reaction conditions, the most easily formed nucleosides will also be those for which the glycosidic bond is most easily broken, and we observe a positive correlation between the propensity for a particular nucleoside to degrade and its formation yield (SI).

We have used high-level calculations to model the zebularine formation reaction to understand why 2-pyrimidinone readily forms a glycosidic bond with ribose. Protonated 2-pyrimidinone has a pK_a of 2.24.[5] Thus, most of the base will be protonated under the acidic reaction condition during the actual reaction, although this does not mean that the base must be the proton donor in an acid-catalyzed reaction. In the presence of Mg^{2+} at pH 6.3, less than 0.1% of the base will be protonated.

We have modeled glycosylation involving a closed sugar in analogy with other glycosylation and deglycosylation reactions,[6] although an alternative route may also exist with the open-chain sugar.[7] The stationary structures obtained at the B3LYP/6-31G(d) level reveal that protonated 2-pyrimidinone forms a complex with ribose that includes a strong H-bond between the N1 proton of 2-pyrimidinone and the O1′ hydroxyl group of ribose. (Direct proton transfer from the solvent to the ribose was not studied.) This proton is also transferred to the O1′ hydroxyl group during cleavage of the C1′−O_{OH} bond.

The reaction mechanism for zebularine formation in the presence of Mg^{2+} was also investigated at the B3LYP/6-31G(d) level. An initial complex was formed between ribose and protonated 2-pyrimidinone with the assistance of the Mg^{2+} cation. In a transition-state structure the breaking of the C1′−O_{OH} bond is facilitated by the Mg^{2+} cation and a bond is formed between the leaving HO^- group and the Mg^{2+} cation. The activation energy for this reaction step is calculated to be 20.2 kcal/mol (see Table 2).

Comparison of results obtained from all calculations performed provides the following conclusions: (1) Acid catalyzes glycosidic bond formation by facilitating cleavage of the C1′−O_{OH} bond, and the acidic proton can be delivered by the protonated base; (2) Mg^{2+} facilitates the reaction between ribose and protonated 2-pyrimidinone by both lowering the energy of the transition states and by holding ribose and the protonated base in close proximity and in a relative orientation that is compatible with the transition states and the nucleoside product.

It is instructive to consider that, at neutral pH, the bases that form glycosides under the present conditions, namely adenine, hypoxanthine, and pyrimidinone, all have a reacting system that consists of an amidine moiety (the imidazole portion of the purine) or a vinylogous amidine moiety. This feature allows a ring nitrogen atom that carries an in-plane electron pair to act as a nucleophile while the second nitrogen atom, which carries an in-plane hydrogen atom, can simultaneously or subsequently lose this hydrogen as a proton. Cytosine, in its canonical keto-amino tautomer, also has such a structure, but the lone pair is on a nitrogen atom that is sterically hindered, being flanked by an NH_2 and a carbonyl group, apparently preventing reaction.

The pK_a of N1−H of uracil is about 9.5, so that there is less than 1% of the anion at neutral pH. However, Kimura et al. have shown that placement of the uracil base near a cationic species can selectively reduce the pK_a of N1−H by more than 2 pH units.[8] Thus, coordination of uracil within a supramolecular assembly, such as in a coaxial stack with cationic intercalators,[4b] might sufficiently lower the N1−H pK_a to allow glycosylation.

If 2-pyrimidinone nucleosides existed in the first RNA-like polymers, they could have been replaced by a post glycosylation base modification or by evolution. Conversion to uridine could have occurred by the addition of water across the N3−C4 bond,[9] followed by hydride transfer of the C-4 hydrogen to an acceptor (e.g., glyoxylate). The latter process would have been driven by the superior stability of uridine against glycosidic bond hydrolysis (SI) and greater functionality of the uracil substituents for base pairing.[10] In any case, we suggest that the studies presented here indicate what might have been the essential features of pyrimidine (or pyrimidine-like) bases in early life.

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Supporting Information Available: Sample preparation and data analysis details, NMR spectra, computational details, including energy state diagrams. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Zubay, G.; Mui, T. *Origins Life Evol. Biosphere* **2001**, *31*, 87−102.

(2) Fuller, W. D.; Sanchez, R. A.; Orgel, L. E. *J. Mol. Evol.* **1972**, *1*, 249−257.

(3) (a) Ingar, A.-A.; Luke, R. W. A.; Hayter, B. R.; Sutherland, J. D. *Chem. Biol. Chem.* **2003**, *4*, 504−507.

(4) (a) Benner, S.; Burgstaller, P.; Battersby, T.; Jurczyk, S. In *The RNA World*; Gesteland, R. F., Atkins, J. F., Eds.; CSHL Press: Cold Spring Harbor, New York, 1999; pp 163−181. (b) Hud, N. V.; Anet, F. A. L. *J. Theor. Biol.* **2000**, *205*, 543−562. (c) Kolb, V. M.; Dworkin, J. P.; Miller, S. L. *J. Mol. Evol.* **1994**, *38*, 549−557. (d) Mittapalli, G. K.; Osornio, Y. M.; Guerrero, M. A.; Reddy, K. R.; Krishnamurthy, R.; Eschenmoser, A. *Angew. Chem., Int. Ed.* **2007**, *46*, 2478−2484. (e) Mittapalli, G. K.; Reddy, K. R.; Xiong, H.; Munoz, O.; Han, B.; De Riccardis, F.; Krishnamurthy, R.; Eschenmoser, A. *Angew. Chem., Int. Ed.* **2007**, *46*, 2470−2477.

(5) Brown, D. J. *Nature* **1950**, *165*, 1010.

(6) (a) Horenstein, N. A. *Adv. Phys. Org. Chem.* **2006**, *41*, 275−314. (b) Berti, P. J.; McCann, J. A. B. *Chem. Rev.* **2006**, *106*, 506−555.

(7) Dworkin, J. P.; Miller, S. L. *Carbohydr. Res.* **2000**, *39*, 359−365.

(8) Kimura, E.; Kitamura, H.; Koike, T.; Shiro, M. *J. Am. Chem. Soc.* **1997**, *119*, 10909−10919.

(9) Frick, L.; Yang, C.; Marquez, V. E.; Wolfenden, R. *Biochemistry* **1989**, *28*, 9423−9430.

(10) Gildea, B.; McLaughlin, L. W. *Nucleic Acids Res.* **1989**, *17*, 2261−2281.

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