Published in final edited form as:

Biochemistry. 2017 March 14; 56(10): 1498–1503. doi:10.1021/acs.biochem.7b00055.

Three Pyrimidine Decarboxylations in the Absence of a Catalyst

Charles A. Lewis Jr.[†], Lin Shen[‡], Weitao Yang[‡], and Richard Wolfenden^{*,†}

[†]Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, North Carolina 27599-7260, United States

[‡]Department of Chemistry and Department of Physics, Duke University, Durham, North Carolina 27708-0346, United States

Abstract

The epigenetic modification of DNA by 5-methylation of cytosine residues can be reversed by the action of the TET family of dioxygenases that oxidize the methyl group to produce 5-carboxycytosine (5caC), which can be converted to cytosine in a final decarboxylation step. Likewise, 5-carboxyuracil (5caU) is decarboxylated to uracil in the last step in pyrimidine salvage. In view of the extreme difficulty of decarboxylating derivatives of orotic acid (6caU), it seemed desirable to establish the rates of decarboxylation of 5caC and 5caU in the absence of a catalyst. Arrhenius analysis of experiments performed at elevated temperatures indicates that 5caU decomposes with a rate constant of $1.1 \times 10^{-9} \text{ s}^{-1}$ ($H^\ddagger = 25 \text{ kcal/mol}$) in a neutral solution at 25 °C. The decomposition of 5caC is somewhat slower ($k_{25} = 5.0 \times 10^{-11} \text{ s}^{-1}$; $H^\ddagger = 27 \text{ kcal/mol}$) and leads to the initial accumulation of cytosine as an intermediate, followed by the relatively rapid deamination of cytosine ($k_{25} = 1.9 \times 10^{-10} \text{ s}^{-1}$; $H^\ddagger = 23.4 \text{ kcal/mol}$). Both 5caC and 5caU are decarboxylated many orders of magnitude more rapidly than 6caU is ($k_{25} = 1.3 \times 10^{-17} \text{ s}^{-1}$). *Ab initio* simulations indicate that in all three cases, the favored route of spontaneous decarboxylation in water involves direct elimination of CO₂ with the assistance of an explicit water molecule.

Graphical Abstract

*Corresponding Author: water@med.unc.edu. Phone: (919) 966-1203. Fax: (919) 966-2852.

ORCID

Richard Wolfenden: 0000-0002-3745-9099

The authors declare no competing financial interest.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.7b00055. Computational analysis of 5caC decomposition, schemes for mechanisms of decarboxylation, a table of CHELPG charges, and the optimized Cartesian coordinates (PDF)

In the absence of enzymes, some biological decarboxylation reactions are very slow indeed. The uncatalyzed decarboxylation of 6-carboxyUMP (OMP), the terminal step in pyrimidine biosynthesis (Figure 1A), proceeds spontaneously with a half-life of more than 10 million years in a neutral solution,¹ while the decarboxylation of amino acids and uroporphyrinogen takes even longer.^{2,3} It is evident that the enzymes that catalyze those reactions, with turnover numbers of $<1 \text{ s}^{-1}$, are extremely powerful catalysts and generate correspondingly high affinities for the altered substrate in the transition state.

Pyrimidine decarboxylation reactions also constitute the final step in two salvage pathways that lead to the removal of 5-methyl groups from the pyrimidine nucleus. During pyrimidine salvage in microorganisms, thymidine (5meU) is oxidized to 5-carboxyuracil (isoorotate, 5caU), which is then decarboxylated to uracil as shown in Figure 1B. During reversal of the epigenetic modification of DNA, 5-methylcytosine undergoes three successive oxidation reactions by the “TET” dioxygenase to yield 5-carboxycytosine (5caC), which is then decarboxylated to cytosine as shown in Figure 1C.⁴ In both these pathways, the final step involves removal of a carboxyl group from the pyrimidine nucleus.

Of the enzymes that catalyze these reactions, OMP decarboxylase (reaction 1A) has been examined most extensively (refs 5–7 and references cited therein). Isoorotase (5caU decarboxylase), which catalyzes reaction 1B, has been isolated only recently and shown to contain a Zn^{2+} atom coordinated by one Asp and three His residues.⁷ No enzyme that catalyzes the decarboxylation of 5caC (caCDCase, reaction 1C) has yet been isolated; however, Schiesser et al.^{8,9} have reported the slow decarboxylation of 5-caC in suspensions of stem cells; and Xu et al.⁷ have detected 5caC-decarboxylating activity in a recombinant isoorotase. More recently, Liutkevicius et al. have reported that DNA C5-methyltransferase, the principal agent of epigenetic modification of DNA, also exhibits weak decarboxylase activity when SAM and SAH are omitted from reaction mixtures.¹⁰ To establish the kinetic barriers faced by decarboxylases acting on 5caC and 5caU and compare those barriers with the barrier faced by OMP decarboxylase, it would be of interest to know the rates of these reactions in the absence of a catalyst.

The calculated CHELPG charges indicate that C6 is positively charged while C5 is negatively charged in the transition states for decarboxylation of 5caU and 5caC (Table S1),

suggesting two factors that might contribute to a difference in reactivity among the three bases. First, the interaction between C5 and a leaving -COO^- group would be expected to be repulsive while that between C6 and a leaving -COO^- group would be expected to be attractive, resulting in a slower rate of dissociation from the C6 position than from the C5 position. Moreover C5 is more basic than C6, so that it should be easier for the α -carbon atom of 5caU and 5caC than for that of 6caU to attract a proton from solvent water.

In this work, we used Arrhenius analysis to determine the kinetics and activation parameters for the decomposition of 5caC and 5caU in a neutral solution. We then compared those experimental values with those predicted using *ab initio* QM methods combined with a continuum solvent model to determine the probable mechanisms of these nonenzymatic reactions.

MATERIALS AND METHODS

5-Carboxyuracil (5caU), cytosine, *N*-acetylcysteine, *N*-acetylcysteamine, and β -mercaptoethanol were purchased from Aldrich Corp. 5-Carboxycytosine (5caC) was purchased from Princeton BioMolecular Research (Princeton, NJ).

Reaction mixtures containing the pyrimidine substrate (~ 0.025 M) in potassium phosphate buffer (0.1 M, pH 7.0) were introduced into quartz tubes, flushed with argon, sealed under vacuum, and incubated in Thermolyne 47900 ovens for enough time to achieve between 10 and 90% reaction at each temperature. After cooling, samples (0.05 mL) were prepared for ^1H nuclear magnetic resonance (NMR) spectroscopy by being mixed with 0.45 mL 99.9% D_2O , containing pyrazine (0.01 M) that had been added as a chemical shift (δ 8.60) and integration standard. Spectra were acquired at 25 °C with a Bruker Avance III HD 500 MHz NMR spectrometer equipped with a cryoprobe, using a water suppression pulse sequence (eight transients with a 60 s pulse delay). These conditions yielded integrated intensities with an estimated error of $<5\%$. NMR signals from each species were well-separated and allowed accurate quantitation of the reactant and product of each reaction. In each case, substrate decomposition followed simple first-order kinetics to at least 90% completion under the conditions examined. The resulting rate constants, obtained during experiments performed at 10 or more temperatures, yielded linear Arrhenius plots with least-squares regression coefficients of $r^2 \geq 0.95$.

In our simulations, the three bases, each accompanied by one explicit water molecule, were described at the *ab initio* QM level. General solvent effects were considered implicitly with the polarizable continuum model (PCM).^{11,12} Geometries were optimized using the DFT method with the B3LYP hybrid functional^{13,14} and the 6-31+G(d) basis set in the presence of PCM. The identity of stationary points was confirmed by frequency analyses at the same level, yielding the thermal corrections to Gibbs free energies. Calculations of single-point energy and atomic CHELPG charges¹⁵ were performed at the MP2/6-311++G(d,p) level with PCM on the optimized geometries. Free energy differences between the protonated and unprotonated states of the three bases at pH 7.0 were estimated on the basis of the experimental values of $\text{p}K_a$.¹⁶ All simulations were implemented using GAUSSIAN 03.¹⁷

RESULTS

Kinetics of Decomposition of 5caU, Cytosine, and 5caC

Decarboxylation of 5caU—At pH 7, 5caU (H_6 , δ 7.98) underwent complete conversion to uracil (H_6 , δ ppm; H_5 , δ = 5.75 ppm, J = 7.75 Hz) following first-order kinetics, without observable byproducts, over the temperature range of 70–180 °C. Arrhenius analysis (blue line, Figure 2) yielded thermodynamic activation parameters of $H^\ddagger$ = 25.3 kcal/mol and $G^\ddagger$ = 29.6 kcal/mol, with an extrapolated rate constant of $1.1 \times 10^{-9} \text{ s}^{-1}$ ($t_{1/2}$ = 20 years) at 25 °C.

Deamination of Cytosine—Cytosine (H_6 , δ 7.44; H_5 , δ 5.92, J = 7.25 Hz) was converted to uracil following first-order kinetics over the temperature range of 90–180 °C. Arrhenius analysis (red line, Figure 2) yielded an extrapolated rate constant of $1.9 \times 10^{-10} \text{ s}^{-1}$ at 25 °C, with a $H^\ddagger$ of 23.4 kcal/mol. Similar values have been reported for this reaction at pH 6.8 (k_{25} = $2.7 \times 10^{-10} \text{ s}^{-1}$; $H^\ddagger$ = 22.1 kcal/mol).¹⁸

Decarboxylation of 5caC—Figure 3 shows the progress of the decomposition of 5caC in a neutral solution at 150 °C. After 5 h, roughly equal amounts of 5caC, cytosine, and uracil were present and accounted for 99% of the reaction mixture. Trace amounts (<0.7%) of 5caU (red) appeared during the first 3 h of reaction and disappeared later.

5caC (H_6 , δ 8.10) decomposition was studied across the temperature range of 90–150 °C and produced cytosine (H_6 , δ 7.44; H_5 , δ 5.92, J = 7.25 Hz) and uracil (H_6 , δ 7.4; H_5 , δ 5.75, J = 7.75 Hz) in similar amounts, following a first-order kinetics. Arrhenius analysis of 5caC decomposition (blue line, Figure 4) yielded an extrapolated rate constant at 25 °C of $5.0 \times 10^{-11} \text{ s}^{-1}$, with a $H^\ddagger$ of 27.1 kcal/mol. The calculated rates of appearance of cytosine (k_{25} = $3.22 \times 10^{-11} \text{ s}^{-1}$) and uracil (k_{25} = $3.72 \times 10^{-11} \text{ s}^{-1}$) were nearly identical with similar thermodynamic parameters.

These results imply that the decomposition of 5caC (k_{25} = $5 \times 10^{-11} \text{ s}^{-1}$ at 150 °C) to uracil proceeds mainly by decarboxylation to cytosine followed by the somewhat more rapid deamination of cytosine (k_{25} = $1.9 \times 10^{-10} \text{ s}^{-1}$) to yield uracil (Figure 5). As expected for that reaction sequence, the initial appearance of cytosine was faster than the initial appearance of uracil. If uracil had instead been formed mainly by initial deamination of 5caC to 5caU, then 5caU would have been expected to appear transiently at concentrations consistent with its known rate of decomposition to uracil (k = $1.1 \times 10^{-9} \text{ s}^{-1}$) (Figure 2). In fact, only traces of 5caU (< 0.7%) were observed, much less than would have been predicted (~5%) if 5caC decomposition proceeded mainly by deamination followed by decarboxylation (Figures S1–S3).

The thermodynamics of activation of the reactions presented here are summarized in Table 1, and Figure 6 compares the rate constants of these reactions with those that have been established for other biological decarboxylation reactions. The rate constants observed at pH 7 indicate that the decarboxylation of 5caU proceeds ~20-fold more rapidly than that of 5caC, whereas the decarboxylation of orotate (6caU) proceeds more than 5 orders of magnitude more slowly.¹⁹

Nonreactivity of Thiols as Potential Catalysts of 5caC Decarboxylation

Earlier, Schiesser et al.⁹ reported that thiols catalyzed the decarboxylation of 5caC incorporated into 30-mer oligonucleotide substrates. When we examined the potential effect of added *N*-acetylcysteamine (0–1.0 M) on the rate of decomposition of 5CaC, we observed no significant enhancement (<10%) in rate even at a thiol concentration of 1 M.

Summary of Results

Rate constants and thermodynamics of activation observed for the present reactions are summarized in Table 1 and Figure 6. The decarboxylation of 5caU takes place approximately 20-fold more rapidly than that of 5caC. In contrast, the decarboxylation of orotate (6caU) proceeds many orders of magnitude more slowly, with a rate constant ($k_{25} = 1.3 \times 10^{-17} \text{ s}^{-1}$) not very different from those for the decarboxylation of glycine ($k_{25} = 9 \times 10^{-18} \text{ s}^{-1}$)² and uroporphyrinogen ($k_{25} = 2 \times 10^{-17} \text{ s}^{-1}$).⁴ Of the many reactions catalyzed by enzymes, only the hydrolysis of aliphatic phosphomonoester dianions ($k_{25} = 2 \times 10^{-20} \text{ s}^{-1}$)²⁰ appears to be slower.

DISCUSSION

Biological decarboxylation reactions are usually considered to proceed by simple elimination of CO₂. In water, the alternative possibility that the scissile carboxylate group undergoes covalent hydration, followed by elimination of bicarbonate, arises. Bicarbonate is less reactive and better than CO₂ as a solvated reaction product, reducing the likelihood of recombination. In a computational study of the decarboxylation of trichloroacetate, the free energy barrier for a mechanism involving water addition was shown to be comparable to the barrier against a mechanism involving direct decarboxylation.²¹

In this work, we examined these alternatives by comparing the $G^\ddagger$ values observed for the decarboxylation of 5caU, 5caC, and 6caU with the values obtained by *ab initio* calculations. The experimental and calculated free energy barriers for these reactions are summarized in Table 2.

For the nonenzymatic decarboxylation of 5caU (isoorotate), a mechanism involving direct elimination of CO₂ exhibited a calculated free energy of activation of 33.0 kcal/mol. In this mechanism, one water molecule close to C5 forms a complex with 5caU and stabilizes the transition state (Scheme S1). Another mechanism, involving addition of water to the carboxylate group of isoorotate, followed by elimination of CO₂ (Scheme S2), exhibited a much higher barrier, 52.8 kcal/mol. For a mechanism involving C5-protonated 5caU (Scheme S3) in which a proton is transferred from the 5-carboxylate group to the α -carbon, the calculated free energy barrier was 31.3 kcal/mol at pH 7.0, with a pK_a of 4.5. Comparison of those values with the experimental value for decarboxylation of isoorotate ($G^\ddagger = 29.6 \text{ kcal/mol}$) suggests that direct elimination of CO₂ from the unprotonated and protonated transition states makes similar contributions to the uncatalyzed reaction.

The enzyme isoorotase (5caU decarboxylase) has been isolated and shown to contain a Zn²⁺ ion, coordinated by one Asp residue and three His residues that are conserved in isoorotases from different species. On the basis of the crystal structure of the enzyme from *Cordyceps*

militaris, Ding and his associates have proposed that Asp323 acts as a nucleophile toward the scissile carbonyl group of the substrate to generate a tetrahedral intermediate in the elimination of bicarbonate, or as a general base that abstracts a proton from a water molecule that adds to the scissile carbonyl group of the substrate.⁷ Either of those processes would involve covalent hydration, in contrast to the mechanism favored for the nonenzymatic reaction by the findings presented here. Comparison of the rate constant of the uncatalyzed reaction with the value of k_{cat} reported for isoorotase⁷ indicates that this enzyme produces a relatively modest rate enhancement ($k_{\text{cat}}/k_{\text{non}}$) in the neighborhood of 10⁸-fold.

The nonenzymatic decarboxylation of 5caC proceeds with an experimentally observed $G^\ddagger$ value of 31.4 kcal/mol. This reaction also seems likely to proceed by direct elimination of CO₂, for which the calculated $G^\ddagger$ values for decarboxylation were 38.4 kcal/mol for the C5 unprotonated transition state (Scheme S4) and 31.9 kcal/mol for the C5-protonated form (Scheme S6) of the substrate ($pK_a = 4.5$). Another mechanism, involving addition of water to the carboxylate group of 5caC followed by elimination of CO₂ (Scheme S5), exhibited a much higher barrier (59.2 kcal/mol) and is therefore unlikely. As noted in the introduction, no enzyme that catalyzes the decarboxylation of 5caC has yet been isolated or characterized, although activity has been observed *in vitro*.

The nonenzymatic decarboxylation of derivatives of 6caU (orotic acid) proceeds with an experimental $G^\ddagger$ value of 40.4 kcal/mol, which may be compared with mechanisms that would involve direct elimination of CO₂ from the unprotonated substrate (Scheme S7) ($G^\ddagger = 37.0$ kcal/mol) or from the O4-protonated substrate (Scheme S9) ($G^\ddagger = 40.6$ kcal/mol). The uncatalyzed decarboxylation of 1-methylorotate has been shown to be insensitive to changing pH (or H₀) in the range between -1 and 8,²² but because of the rarity of the O4-protonated species ($pK_a = 2.5$) in a neutral solution, the free energy barrier for a reaction requiring the protonated species would increase from 40.6 to 46.7 kcal/mol, 6 kcal/mol higher than the barrier observed experimentally. Thus, elimination of CO₂ from 6caU at pH 7.0 appears to be dominated by reaction of the orotate monoanion as concluded in earlier work.

In summary, all three pyrimidine decarboxylations appear to proceed by similar mechanisms involving direct elimination of CO₂. As suggested in the introduction, their differences in rate can be rationalized in terms of electron density at the scissile carbon atom.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This work was supported by National Institutes of Health Grant GM-18325.

We are grateful to Gottfried Schroeder for a computational analysis of the kinetics of 5caC decomposition.

ABBREVIATIONS

5caC	5-carboxycytosine
5caU	5-carboxyuracil (isoorotic acid)
6caU	6-carboxyuracil (orotic acid)

References

1. Radzicka A, Wolfenden R. A proficient enzyme. *Science*. 1995; 267:90–93. [PubMed: 7809611]
2. Snider MJ, Wolfenden R. The rate of spontaneous decarboxylation of amino acids. *J Am Chem Soc*. 2000; 122:11507–11508.
3. Lewis CA Jr, Wolfenden R. Uroporphyrinogen decarboxylation as a benchmark for the catalytic proficiency of enzymes. *Proc Natl Acad Sci U S A*. 2008; 105:17328–17333. [PubMed: 18988736]
4. Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, He C, Zhang Y. Tet Proteins Can Convert 5-Methylcytosine to 5-Formylcytosine and 5-Carboxylcytosine. *Science*. 2011; 333:1300–1303. [PubMed: 21778364]
5. Miller BG, Wolfenden R. Catalytic proficiency: the unusual case of OMP decarboxylase. *Annu Rev Biochem*. 2002; 71:847–885. [PubMed: 12045113]
6. Hu H, Boone A, Yang W. Mechanism of OMP decarboxylation in orotidine 5-monophosphate decarboxylase. *J Am Chem Soc*. 2008; 130:14493–14503. [PubMed: 18839943]
7. Xu S, Li W, Zhu J, Wang R, Li Z, Xu GL, Ding J. Crystal structures of isoorotate decarboxylase reveal a novel catalytic mechanism of 5-carboxyuracil decarboxylation and shed light on the search for DNA decarboxylase. *Cell Res*. 2013; 23:1296–1309. [PubMed: 23917530]
8. Schiesser S, Hackner B, Pfaffeneder T, Muller M, Hagemeyer C, Truss M, Carell T. Mechanism and stem-cell activity of 5-carboxycytosine decarboxylation determined by isotope tracing. *Angew Chem, Int Ed*. 2012; 51:6516–6520.
9. Schiesser, et al. Deamination, oxidation, C-C bond cleavage reactivity of 5-hydroxymethylcytosine, 5-formylcytosine and 5-carboxycytosine. *J Am Chem Soc*. 2013; 135:14593–14599. [PubMed: 23980549]
10. Liutkevicius Z, Kriukienė E, Liutytė J, Rudytė M, Urbanavičiūtė G, Klimasauskas S. Direct decarboxylation of 5-carboxylcytosine by DNA C5-methyltransferases. *J Am Chem Soc*. 2014; 136:5884–5887. [PubMed: 24716540]
11. Miertuš S, Scrocco E, Tomasi J. (1981) Electrostatic interaction of a solute with a continuum. A direct utilization of *ab initio* molecular potentials for the prevision of solvent effects. *Chem Phys*. 1981; 55:117–129.
12. Tomasi J, Mennucci B, Cammi R. Quantum mechanical continuum solvation models. *Chem Rev*. 2005; 105:2999–3093. [PubMed: 16092826]
13. Lee C, Yang W, Parr RG. Development of the Colle-Salvetti correlation-energy formula into a functional of electron density. *Phys Rev B: Condens Matter Mater Phys*. 1988; 37:785–789.
14. Becke AD. (1993) Density functional thermochemistry. III The role of exact exchange. *J Chem Phys*. 1993; 98:5648–5652.
15. Breneman CM, Wiberg KB. Determining atomcentered monopoles from molecular electrostatic potentials. The need for high sampling density in formamide conformational analysis. *J Comput Chem*. 1990; 11:361–373.
16. Bashford D, Karplus MJ. Multiple-site titration curves of proteins: an analysis of exact and approximate methods for their calculation. *J Phys Chem*. 1991; 95:9556–9561.
17. Frisch, MJ., Trucks, GW., Schlegel, HB., Scuseria, GE., Robb, MA., Cheeseman, JR., Montgomery, JA., Jr, Vreven, T., Kudin, KN., Burant, JC., Millam, JM., Iyengar, SS., Tomasi, J., Barone, V., Mennucci, B., Cossi, M., Scalmani, G., Rega, N., Petersson, GA., Nakatsuji, H., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Klene, M., Li, X., Knox, JE., Hratchian, HP., Cross, JB., Bakken, V., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, RE., Yazyev, O., Austin, AJ., Cammi, R., Pomelli, C.,

Ochterski, JW., Ayala, PY., Morokuma, K., Voth, GA., Salvador, P., Dannenberg, JJ., Zakrzewski, VG., Dapprich, S., Daniels, AD., Strain, MC., Farkas, O., Malick, DK., Rabuck, AD., Raghavachari, K., Foresman, JB., Ortiz, JV., Cui, Q., Baboul, AG., Clifford, S., Cioslowski, J., Stefanov, BB., Liu, G., Liashenko, A., Piskorz, P., Komaromi, I., Martin, RL., Fox, DJ., Keith, T., Al-Laham, MA., Peng, CY., Nanayakkara, A., Challacombe, M., Gill, PMW., Johnson, B., Chen, W., Wong, MW., Gonzalez, C., Pople, JA. Gaussian 03, revision D.02. Gaussian, Inc; Wallingford, CT: 2004.

18. Schroeder GK, Wolfenden R. Rates of spontaneous disintegration of DNA and the rate enhancements produced by DNA glycosylases and deaminases. *Biochemistry*. 2007; 46:13638–13647. [PubMed: 17973496]
19. Lewis CA Jr, Wolfenden R. Uroporphyrinogen decarboxylation as a benchmark for the catalytic proficiency of enzymes. *Proc Natl Acad Sci U S A*. 2008; 105:17328–17333. [PubMed: 18988736]
20. Lad C, Williams NH, Wolfenden R. The rate of hydrolysis of phosphomonoester dianions and the catalytic proficiencies of protein and inositol phosphatases. *Proc Natl Acad Sci U S A*. 2003; 100:5607–5610. [PubMed: 12721374]
21. Howe RW, Kluger R. Decarboxylation without CO₂: Why bicarbonate forms directly as trichloroacetate is converted to chloroform. *J Org Chem*. 2014; 79:10972–10980. [PubMed: 25340631]
22. Lewis CA Jr, Wolfenden R. Orotic acid decarboxylation in water and nonpolar solvents, and a potential role for desolvation in the action of OMP decarboxylase. *Biochemistry*. 2009; 48:8738–8745. [PubMed: 19678695]

Figure 1.
Pyrimidine decarboxylation reactions.

Figure 2. Arrhenius plots for the decarboxylation of 5caU (blue) and the deamination of cytosine (red) over the temperature range of 70–180 °C in 0.1 M potassium phosphate buffer (0.1 M) at 25 °C.

Figure 3.

Time course of decomposition of 5caC (blue), showing the appearance and disappearance of cytosine (green), the appearance of uracil (purple), and the transient appearance of traces of 5caU (red) at pH 7 and 150 °C. The left panel shows a detail of the first 12 h of the reaction.

Figure 4. Decomposition of 5caC. Arrhenius plot for the decomposition of 5caC (blue diamonds) in potassium phosphate buffer (0.1 M, pH 7), over the temperature range of 90–150 °C. Cytosine (green triangles) and uracil (red squares) appeared at rates that were nearly equivalent.

Figure 5.
Alternative pathways for the conversion of 5caC to uracil.

Figure 6.
Biological decarboxylation reactions in the absence of a catalyst (25 °C).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1
First-Order Rate Constants and Thermodynamics of Activation in 0.1 M Potassium Phosphate Buffer (pH 7.0)

	$t_{1/2}$ (25 °C)	rate constant k_{25} °C (s ⁻¹)	$H^\ddagger$ (kcal/mol)	$G^\ddagger$ (kcal/mol)	$T^\ddagger$ (kcal/mol)	$S^\ddagger$ (kcal/mol)
5caU decarboxylation	29 y	1.08×10^{-9}	25.3	29.6		-4.3
cytosine deamination	170 y	1.88×10^{-10}	23.4	30.7		-7.4
5caC decomposition	630 y	4.98×10^{-11}	27.1	31.4		-4.35
6caU decarboxylation	2.4 by	1.3×10^{-17}	42.8	40.4		+2.5

Table 2

Free Energy Barriers for Decarboxylation in a Neutral Solution at 25 °C (kilocalories per mole)

	exp	unprotonated		protonated
		water complex ^a	water addition	water complex
5-carboxyuracil	29.6	33.0	52.8	31.3 (27.9) ^b
5-carboxycytosine	31.4	38.4	59.2	31.9 (28.5)
6-carboxyuracil	40.4	37.0	54.8	46.7 (40.6)

^a“Water complex” denotes the reaction path with direct elimination of CO₂.^bThe numbers in parentheses are the calculated reaction free energy barriers starting from the reactant in the protonated form. Free energy differences between protonated and unprotonated states are calculated as

$$\Delta G_{U \rightarrow P} = \ln(10) k_B T (\text{pH} - \text{p}K_a)$$

and added to the numbers in parentheses, yielding the free energy barriers expected for reaction of the protonated species at pH 7.0.