Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 20, pp. 2623-2628, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)10028-2

DECARBOXYLATION OF 1,3-DIMETHYLOROTIC ACID REVISITED: DETERMINING THE ROLE OF N-1

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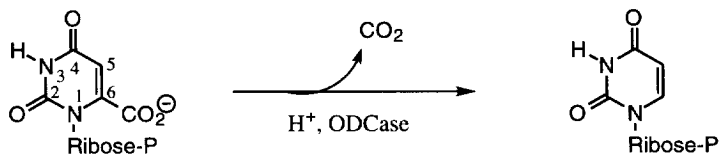
Abstract. The decarboxylation of 1,3-dimethylorotic acid was found to be only about two to three times as fast as that of citrazinic acid (1-deazaorotic acid). It is thus concluded that the positive charge development on the adjacent N-1 is not as important as previously proposed. © 1997 Elsevier Science Ltd.

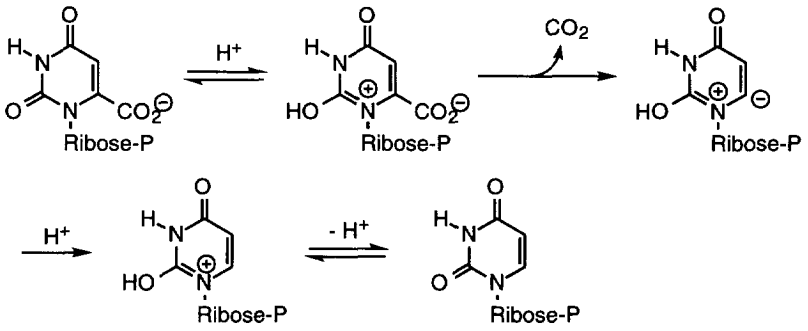
Orotidine-5'-monophosphate decarboxylase (ODCase) catalyzes the final step of de novo pyrimidine nucleotide biosynthesis, the conversion of orotidine 5'-monophosphate (OMP) to uridine 5'-monophosphate (Scheme I).[1] This unique reaction involves decarboxylation of a vinylic carboxylate, which, if direct, would yield a vinylic carbanion with no stabilization by delocalization into a π-system. Enzymes that catalyze decarboxylation of α-ketoacids, where there is a similar stabilization issue, typically form a covalent thiamine pyrophosphate adduct of the substrate to provide a conjugated electron sink for delocalization of the incipient carbanion.[2] In contrast, ODCase has no known cofactor; yet it is an extremely proficient catalyst.[3] Because of its intriguing chemistry, the catalytic mechanism of ODCase has been the target of significant research interest. In this communication, we report some interesting developments in the model studies on decarboxylation of OMP analogs.

SCHEME I



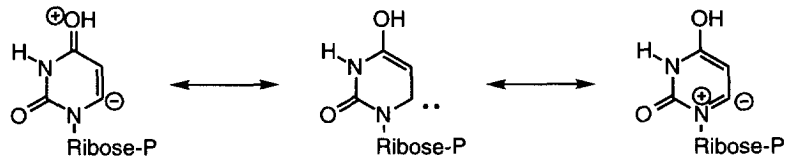
In one of the earliest studies, Beak and Siegel examined the nonenzymatic decarboxylation of 1,3-dimethylorotic acid (**1**) and several related compounds as models for the enzymatic reaction.[4] Their results led to the proposal of a "nitrogen ylide" mechanism for decarboxylation. In this proposal, initiation of the reaction involves protonation (or other electrophilic activation) of the substrate to yield a betaine structure with a positive charge localized at N-1 (Scheme II). The positive charge then facilitates subsequent decarboxylation through inductive stabilization of the adjacent, incipient, vinylic carbanion in the intermediate/transition state.

SCHEME II



An alternative covalent mechanism involving nucleophilic attack at C-5 by an active site residue and protonation at C-6 prior to decarboxylation/elimination was also proposed by analogy to the mechanism of thymidylate synthetase.[5] Although this would avoid formation of the vinylic carbanion, a number of subsequent enzymological studies strongly disfavor this mechanism. For example, substrate analog and inhibitor studies support the involvement of a C-6 based carbanion as an intermediate/transition state.[6] Additionally, kinetic isotope effect studies[7,8] are consistent with a noncovalent mechanism for the enzymatic reaction, such as that in Scheme II, and thus provide evidence against the covalent alternative. However, none of the enzymatic studies, thus far, address the most important element of the mechanism proposed by Beak and Siegel,[4] which is the role played by N-1 in the decarboxylation reaction.

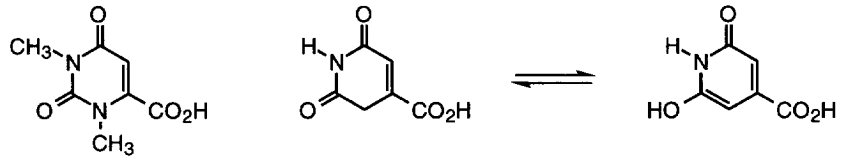
SCHEME III



Very recently, Lee and Houk[9] proposed a third mechanism for the enzymatic decarboxylation based on the results of quantum mechanical calculations of the energies of the ground state and potential intermediates in the decarboxylation of anionic orotate. While their results showed that protonation at O-2 to yield the zwitterionic ylide proposed by Beak and Siegel would substantially accelerate the rate of decarboxylation, they found that

protonation at O-4 gave a further stabilized intermediate that has both a zwitterionic and a neutral carbene resonance structure as shown in Scheme III. The results suggest that the greatest rate enhancement will occur with protonation of O-4 (which is more basic than O-2) to give the neutral carbene in a medium of low dielectric constant. In contrast to the proposal by Beak and Siegel,[4] this proposal predicts that development of positive charge at position 1 in OMP would not be important in the enzymatic reaction.

Prior to the appearance of Lee and Houk's proposal, we had designed a few experiments to investigate the role of N-1, or more specifically, the role of positive charge development at position 1, in the enzymatic decarboxylation. For the primary enzymatic experiment, we are synthesizing a 1-deaza analog of OMP, which cannot form a positive charge adjacent to the incipient carbanion. As a preliminary control we have evaluated the extent of rate enhancement provided by N-1 in the nonenzymatic decarboxylation, by comparing the rate of decarboxylation of the model compounds 1,3-dimethylorotic acid (**1**) and citrazinic acid (**2**).



1 **2**

1,3-Dimethylorotic acid (**1**), which models OMP, was extensively studied by Beak and Siegel.[4] Their data strongly suggest that in the neutral polar solvent sulfolane ($\varepsilon = 43.3$), acid **1** forms a zwitterionic betaine with a positively charged nitrogen at N-1 (similar to the structure in Scheme II), which facilitates decarboxylation to the ylide through dipole stabilization of the carbanion intermediate. Citrazinic acid (**2**) is a 1-deaza analog of OMP whose analogous tautomeric structure (shown above) does not contain a positive charge at position 1 (using OMP numbering). According to the proposed mechanism, the lack of positive charge at position 1 should result in a greatly reduced rate of decarboxylation for acid **2** compared to acid **1**. For comparison, decarboxylation of N-methyl-4-pyridinecarboxylic acid occurs ~1600-fold more slowly than the 2-carboxylic acid analog.[10] Thus, the decarboxylation of acids **1** and **2** was investigated to probe the role of N-1 in the nonenzymatic decarboxylation, and to provide insight into the enzymatic decarboxylation of OMP. The rates of decarboxylation were determined manometrically by following the evolution of carbon dioxide using conditions and methods similar to those of Beak and Siegel.[4,11]

As summarized in Table 1, the rates were measured in both neutral and basic solvents. The results suggest that one mechanism may play a more important role than the other depending on the reaction conditions. In the neutral, high dielectric solvent sulfolane ($\varepsilon = 43.3$), the 1-deaza analog (**2**) required an elevated temperature to obtain a measurable rate,[12] indicating that it decarboxylates much more slowly than the OMP analog (**1**). This result is consistent with the "ylide" mechanism prevailing under these conditions, since the absence of the positive charge adjacent to the carbanion in analog **2** should slow the reaction. However, in the less polar, but basic solvent isoquinoline ($\varepsilon = 10.7$), the rates differ by only 2- to 3-fold rather than the expected two to three

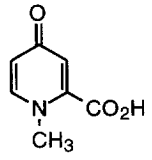
orders of magnitude, suggesting a less important role for positive charge development adjacent to the carbanion under these conditions. Although the reactants under these conditions are presumed to be carboxylate anions rather than zwitterions, hydrogen bonding or proton transfer to O-4 by protonated solvent, concerted with decarboxylation, would yield neutral carbene intermediates analogous to that proposed by Lee and Houk.[9] Thus, these results are consistent with the calculations of Lee and Houk, which showed that as the dielectric constant of the solvent is decreased, formation of a neutral carbene intermediate should be highly favored over a charged or zwitterionic intermediate.[9]

Table 1. First-Order Rate Constants for Decarboxylation in Neutral and Basic Solvents at 206 °C

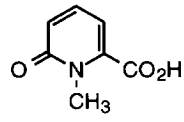
	Rate Constant, s^{-1}	
Substrate	Neutral (Sulfolane)	Ionized (Isoquinoline)
1	7.5 (±1.3) x 10^{-4}	1.6 (±0.4) x 10^{-3}
2	3.1 (±0.3) x 10^{-4} [a]	6.2 (±1.7) x 10^{-4}

[a]This rate constant was determined at 230 °C.[12]

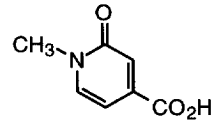
In light of our new results, it is of interest to compare the rate of decarboxylation of **2** measured here in isoquinoline at 206 °C with the rates obtained by Beak and Siegel[4] for compounds **3–5** under the same conditions. They found that compound **4** decarboxylates at about the same rate as **1** and **2**, while **5** does not decarboxylate, even at 300 °C, and **3** decarboxylates about 10^3-fold faster than compounds **1**, **2**, and **4**.



3



4



5

The facile decarboxylation of compounds **1**, **3**, and **4**, which can form N-1 cations, and the absence of decarboxylation in compound **5**, which cannot, was proposed as evidence for the role of dipole stabilization by the positive charge at N-1.[4] However, further comparison of the structures suggests that the adjacent positive charge is not the only feature contributing to the differences in observed rates. For example, both compounds **3** and **4** can form adjacent positive charges, but **3** reacts 10^3-fold faster. One explanation for this may be the significantly higher basicity of the oxygen in a 4-pyridone (**3**) vs. a 2-pyridone (**4**).[13] It has been reported that the pK_a of the protonated 4-pyridone is about 2-3 units higher than that of 2-pyridone.[14] The enhanced basicity of the oxygen in **3** may lead to an increase in the extent of hydrogen bonding or proton transfer to oxygen during decarboxylation, which would generate a neutral carbene intermediate (as in Scheme III) with an expected lower

energy than an anioic intermediate, as suggested by the work of Lee and Houk.[9] The lack of decarboxylation in **5** may then reflect both the lower basicity of oxygen in 2-pyridone, and hence, less hydrogen bonding or proton transfer in the transition state, coupled with the lack of an adjacent positive charge. The enhanced rate for compound **2** relative to **5** may be due to the enhanced basicity of O-4 (OMP numbering).[15] This explanation of the differences in rate constants can be better seen by comparing the stability of possible carbanion and carbene resonance structures of the intermediates resulting from the decarboxylation of acids **2-5,** as shown in Figure 1. Among these structures, **3'a** and **4'a** are inductively stabilized carbanions (i.e., ylides), while **2'b** and **3'b** are stabilized carbene structures due to the relatively high basicity of O-4. Thus, acid **5** does not decarboxylate since intermediate **5'** is stabilized neither as a carbanion nor as a carbene; acid **3** decarboxylates 10^3-fold faster since intermediate **3'** can be stabilized in both the carbanion or carbene resonance form; acids **2** and **4** decarboxylate at intermediate rates, since **2'** and **4'** can be stabilized as a carbene or as a carbanion, respectively.

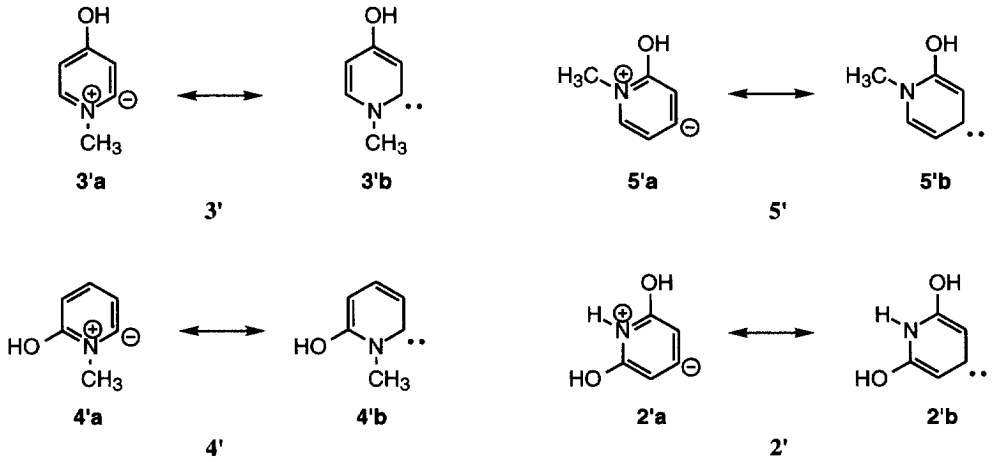


Figure 1. Intermediates from the Decarboxylation of Model Compounds

The most important conclusion from the results and reanalysis presented here is that both formation of an adjacent positive charge and protonation of (or hydrogen bonding to) the enone oxygen can contribute to the stabilization of the vinylic carbanion upon decarboxylation. The extent to which each contributes depends on the structure and may indeed vary further as a function of the dielectric constant of the medium, as predicted by the work of Lee and Houk.[9] Which of these factors will be more important in the enzymatic decarboxylation of OMP remains to be seen. The synthesis of the corresponding 1-deaza substrate analog is currently underway.

Acknowledgment. This work was supported by San Francisco State University and by a grant from National Center for Research Resources (Research Infrastructure in Minority Institutions (P20 RR11805) with funding from the Office of Research on Minority Health), National Institutes of Health. We are indebted to Drs. Scott Gronert and James Keeffe (San Francisco State University) for helpful discussions concerning the kinetic measurement. We also thank Drs. Jeehiun K. Lee and K. N. Houk (University of California at Los Angles) for making the results of the computational studies available to us prior to publication.

References and Notes

1. Lieberman, I.; Kornberg, A.; Simms, E.S. *J. Biol. Chem.* **1955**, *215*, 403.
2. Bruice, T. C.; Benkovic, S. *Bioorganic Mechanisms*; Benjamin: New York, 1966; Vol 2.
3. Radzicka, A.; Wolfenden, R. *Science* **1995**, *267*, 90.
4. Beak, P.; Siegel, B. *J. Am. Chem. Soc.* **1976**, *98*, 3601.
5. Silverman, R. B.; Groziak, M. P. *J. Am. Chem. Soc.* **1982**, *104*, 6434.
6. Levine, H. L.; Brody, R. S.; Westheimer, F. H. *Biochemistry* **1980**, *19*, 4993.
7. Acheson, S. A.; Bell, J. B.; Jones, M. E.; Wolfenden, R. *Biochemistry* **1990**, *29*, 3198.
8. Smiley, J. A.; Paneth, P.; O'Leary, M. H.; Bell, J. B.; Jones, M. E. *Biochemistry* **1991**, *30*, 6216.
9. Lee, J. K.; Houk, K. N. *Science* **1997**, *276*, 942.
10. Haake, P.; Mantecon, J. *J. Am. Chem. Soc.* **1964**, *86*, 5230.
11. The rate constants for the decarboxylation of 1,3-dimethylorotic acid (**1**) obtained by us are very similar to those obtained by Beak and Siegel.[4] The amount of carbon dioxide produced from the decarboxylation reaction was quantitated by the amount of barium carbonate collected upon reaction with a saturated barium hydroxide solution.[4]
12. Our studies on decarboxylation of related compounds show that the rate constant generally increases by 2- to 3-fold upon raising the reaction temperature from 206 °C to 230 °C. Based on these observations, we estimate a rate constant of 1 x 10^{-4} s^{-1} for citrazinic acid (**2**) in sulfolane at 206 °C.
13. Tieckelmann, H. In *The Chemistry of Heterocyclic Compounds, Pyridine and Its Derivatives, Supplement Part Three*; Abramovitch, R. A. Ed.; Wiley: New York, 1974; Chapter XII.
14. The pK_a's of protonated 2-pyridone and 4-pyridone have been reported to be 0.75–1.2 and 3.2, respectively, see: (a) Dean, J. A. In *Lange's Handbook of Chemistry*; McGraw-Hill: San Francisco, 1992; pp 8.19–8.71. (b) Jencks, W. P.; Reglenstein, J. In *Handbook of Biochemistry and Molecular Biology*; Fasman, G. Ed.; CRC: Cleveland, 1976; p 338.
15. Citrazinic acid (**2**) is more readily protonated than 2-pyridone. For discussions on basicity of related compounds such as glutaconimide, see 13.

(*Received in USA* 6 *August* 1997; *accepted* 11 *September* 1997)