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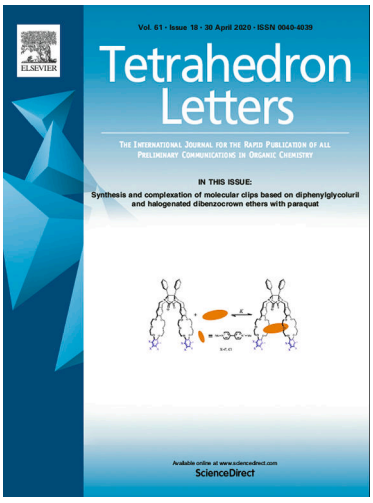

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Graphical Abstract

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Decarboxylation of orotic acid analogues: comparison of solution and gas-phase reactivity

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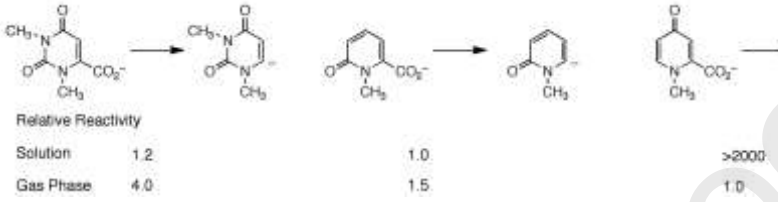

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Decarboxylation of orotic acid analogues: comparison of solution and gas-phase reactivity

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ABSTRACT

The decarboxylation of orotic acid and analogues have been investigated as a model for enzymatic decarboxylation catalyzed by orotidine-5'-monophosphate decarboxylase (ODCase). The rate of decarboxylation of 1-methyl-4-pyridone-2-carboxylic acid in solution has been reported to be three orders of magnitude greater than those of 1,3-dimethylorotic acid and 1-methyl-2-pyridone-6-carboxylic acid in solution. Here, the gas-phase decarboxylation of the three corresponding carboxylates were investigated. The carboxylate of 1,3-dimethylorotic acid decarboxylates at a faster rate and thus the relative rates of decarboxylation are different from those observed in solution. The relative rates of decarboxylation correlate well with the stability of the corresponding carbanions and the calculated activation energies for gas-phase decarboxylation. Therefore, the reactions in the gas phase seem to go through the direct decarboxylation mechanism whereas the reactions in solution likely go through zwitterionic intermediates as previously proposed.

1. Introduction

The decarboxylation of 1,3-dimethylorotic acid (**1**) and its analogues (**3** and **5**) as shown in Figure 1 has been studied as a model for the enzymatic decarboxylation catalyzed by orotidine-5'-monophosphate decarboxylase (ODCase).[1-15] 1,3-Dimethyluracil (**2**), 1-methyl-2-pyridone (**4**), and 1-methyl-4-pyridone (**6**) are formed as the sole product in respective reactions from the decarboxylation of acids **1**, **3**, and **5** at elevated temperatures.[1,8] Previous mechanistic studies have involved the investigation of the nature and stability of the putative carbanion intermediates (**7**-**9**) as well as the effects of factors such as solvent and hydrogen bonding.[1-15]

While acids **1** and **3** decarboxylate at approximately the same rate in solution, acid **5** reacts three orders of magnitude faster than either **1** or **3** (Table 1).[1,8] The large difference in the rate of decarboxylation of these acids in solution has provided a unique opportunity to investigate the factors important for the rate of the reactions. It has been demonstrated that the stability of carbanions (**7**-**9**) formed from the decarboxylation of these three acids does not play an important role in determining the rate of decarboxylation in solution.[7,8,15] In these reports, carbanions **8** and **9** are found to be equally stable while carbanion **7** was more stable by about 7 kcal/mol in the gas phase as summarized in Table 1.[7,8] The experimentally measured proton affinities of the carbanions agree with the calculated values very well.[7,8]

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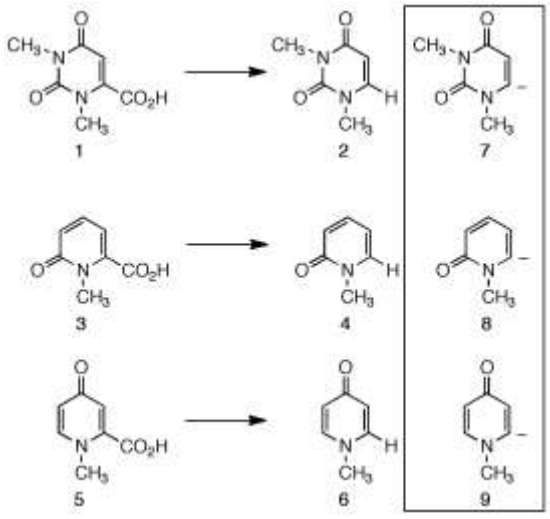

Figure 1. Decarboxylation of orotic acid analogues (**1**, **3**, and **5**) and their corresponding carbanion intermediates (**7**, **8**, and **9**)

A two-step mechanism has been proposed to explain the large difference in rate constants observed in solution and is shown in Figure 2.[1,8,15] In this mechanism, an equilibrium leading to the formation of a zwitterionic structure such as **10** is followed by the loss of CO_2 and formation of the carbanion **11**.[1,8,15] When the zwitterionic structure **10** is much less stable than acid **1**, i.e. equilibrium constant $K << 1$, the observed rate constant is simply the product of the K and the rate constant k for the second step. The reaction rates are largely dependent on the value of K since

the rate constants *k* for the loss of CO_2 from the zwitterionic structures derived from all three acids are similar. It has been well documented by the differences in molecular properties (such as polarity, basicity and proton affinity of the ring carbonyl groups) that the corresponding zwitterionic resonance structure contributes much more to the overall structure of 4-pyridone than to those of 2-pyridone and uracil from their corresponding zwitterionic resonance structures.[16,17] Theoretical calculations have provided support for the proposed mechanism by showing that the zwitterionic structure derived from acid **5** is indeed much more stable than those from acids **1** and **3**.[8,15]

Table 1. Solution-phase rate constants for the decarboxylation of orotic acid analogues (**1**, **3**, and **5**) and gas-phase proton affinities of corresponding carbanions (**7**-**9**) as reported in ref. 8.

Substrate	Rate Constant in sulfolane (s-1)	Rate Constant in isoquinoline (s-1)	Measured Proton Affinity of Carbanions (kcal/mol)	Calculated Proton Affinity of Carbanions (kcal/mol)
1	7.5×10^{-4}	1.6×10^{-3}	369.9	367.6
3	1.2×10^{-3}	1.3×10^{-3}	377.0	375.5
5	0.32	3.2	377.0	375.8

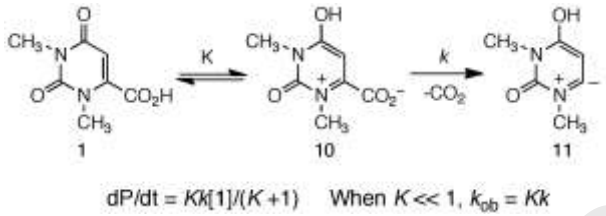

Figure 2. Proposed mechanism for the decarboxylation of orotic acid analogues and the resulting rate equation

The proposed mechanism has thus nicely explained the results from the investigations of the decarboxylation reactions of orotic acid analogues in the solution, especially under neutral conditions where the acid remains mostly protonated. However, the mechanism of the reactions in the gas phase has never been investigated. In this Letter, these gas phase reactions were probed using mass spectrometry and the relative rates of decarboxylation are found to be largely dependent on the stability of the carbanion intermediates (**7**-**9**). Furthermore, the relative rates of decarboxylation in the gas-phase correlate well with the gas-phase activation energies for decarboxylation calculated using density function theory (DFT).

2. Results and Discussio

We would like to investigate the kinetics of the decarboxylation of the orotic acid analogues in the gas phase to compare with those in solution. The methanolic solutions of the acids were introduced to the mass spectrometer via electrospray. Deprotonation of the acids occurred during the electrospray process and the resulting carboxylates **12**-**14** were isolated and subjected to collision induced dissociation (CID) to give the carbanions **7**-**9** as gas-phase products (Figure 3). The percent conversion from carboxylate to carbanion was measured as a function of applied collision energy for each of the analogs to determine the relative propensity of each carboxylate to react. Since mass spectrometry operates on ions, the rates of decarboxylation are measured from the negatively charged carboxylates thus most closely parallel the solution-phase reactions under basic conditions (Column 3, Table 1).

The relative energy required for gas-phase decarboxylation of the three carboxylates **12**, **13** and **14** can be estimated by measuring the minimum collision energy required to activate a given fragmentation pathway during an MS/MS experiment.[18]

Figure 4 shows the graph of product ion yield from the carboxylates **12**-**14** plotted against the collision energy applied during CID. For all three carboxylates, the onset of fragmentation occur at a collision energy of about 14-15%.

Since the onset collision energy values are close, the relative propensity for decarboxylation is more readily seen by comparing the extent of fragmentation near the onset collision energy as seen in Figure 5. Qualitatively, it is clear that carboxylate **12** produces more carbanion product than carboxylate **13** and carboxylate **14** produces the least amount of carbanion product. The percent of product formation at 15% collision energy was approximately 19%, 7%, 5% for the decarboxylation of carboxylates **12**, **13** and **14**., respectively. Figure 5 also demonstrates that all three carboxylates cleanly decarboxylate at relatively low collision energies without any competing fragmentation pathways.

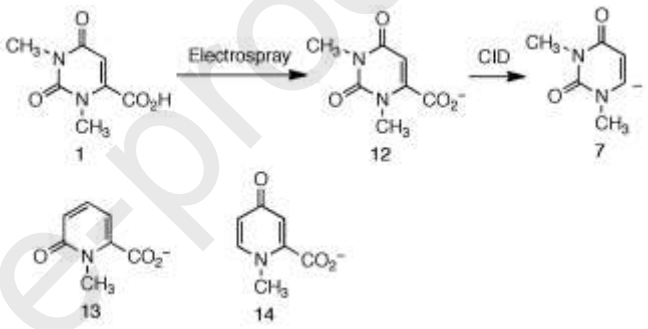

Figure 3. Electrospray ionization of orotic acid analogues to carboxylates and subsequent decarboxylation to carbanions

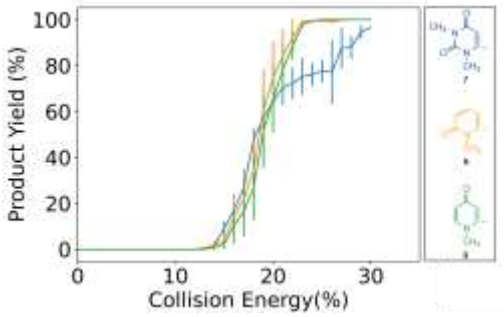

Figure 4. Product yields of product carbanions **7**-**9** as a function of collision energy applied to reactant carboxylates **12**-**14**

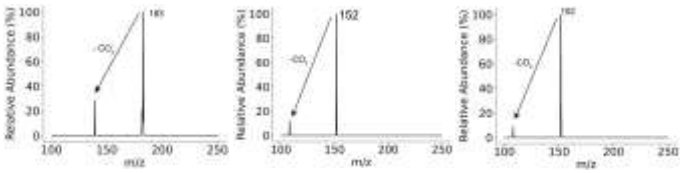

Figure 5. MS/MS spectra of the carboxylates **12** (*m/z* 183), **13** (*m/z* 152) and **14** (*m/z* 152) respectively at 15% collision energy. The decarboxylated product ion peak appears 44 *m/z* units lower than the reactant peak in each spectrum.

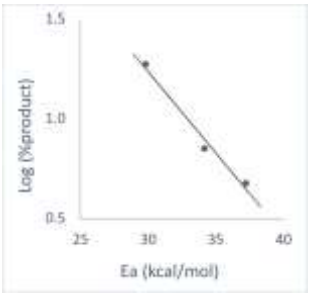

Figure 6. Plot of the logarithm of the percentage of the carbanion product formed at 15% collision energy against the calculated activation energy ($R^2 = 0.98$)

The decarboxylation reactivity of the carboxylates derived from orotic acid and its analogues in the gas phase is in complete contrast to what was previously observed in solution. In the gas phase, carboxylate **12** decarboxylates faster than carboxylates **13** and **14**; whereas in solution, carboxylate **14** decarboxylates three orders of magnitude faster than carboxylates **12** and **13**.

The order of the gas-phase reactivity for the decarboxylation of carboxylates **12-14** seems to correlate with the gas-phase stability of carbanions **7-9** (Table 1). In order to fully explain the reactivity order in the gas phase, DFT analysis on the activation energy of the reactions was carried out. DFT calculations reveal that all three analogs undergo a barrier-less loss of CO_2 which was confirmed by linear transit calculations.[10,19,20] Therefore, the differences in energy between the reactants (the carboxylates) and products (the carbanions) represent the energy of activation for the decarboxylation reactions. The calculated activation energies for the decarboxylation of the carboxylates **12-14** were found to be 29.9, 34.2, and 37.3 kcal/mol, respectively. When the logarithm of initial rates of the reactions represented by the percentages of carbanion products formed at 15% collision energy (19%, 7%, and 5%, respectively) were plotted against the activation energy (E_a), a linear relationship was obtained with R^2 value of 0.98 as shown in Figure 6. Therefore, the different reaction propensities for the reactions in the gas phase can be fully explained by the different activation energies and the reactions go through the direct decarboxylation mechanism in contrast to the zwitterionic mechanism proposed to occur in solution. The stability of the carbanion products plays a very important role in determining the rates of the decarboxylation in the gas phase because the activation energies of the reactions are largely determined by the stability of the carbanions.

3. Conclusions

In summary, the reactivity of the carboxylates **12-14** (derived from orotic acid analogues **1**, **3**, and **5**, respectively) in the gas phase are very different from previously observed solution-phase de faster than acids **1** and **3** in solution; whereas in the gas phase, carboxylate **12** (derived from acid **1)** decarboxylates faster than carboxylates **13** and **14** (derived from acids **3** and **5**, respectively). Different mechanisms are thus operating in the gas phase and in solution. In solution, the stability of the carbanion products (**7-9**) does not play an important role in determining the rates of the reactions; whereas in the gas phase, the stability of the carbanion products plays a very important role by its influence on the activation energies of the reactions. The gas-phase reactivity of the carboxylates correlates very well with the calculated activation energies and the reactions go through the direct decarboxylation mechanism in contrast to the zwitterionic mechanism in solution.

Acknowledgments

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Highlights

- Decarboxylation of orotic acid analogues are studied in the gas phase.
- Reactions in the gas phase and solution operate by different mechanism.
- Calculated activation energies correlate with the reaction rate in the gas phase.
- The stability of the carbanions plays different role in the gas phase and solution.