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Surprisingly Low Aqueous Acidity at the α-Positions of Pyridiniums and Pyrimidinium: The Role of Solvation

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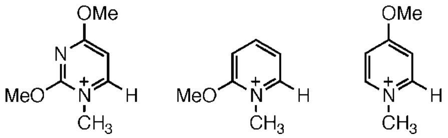
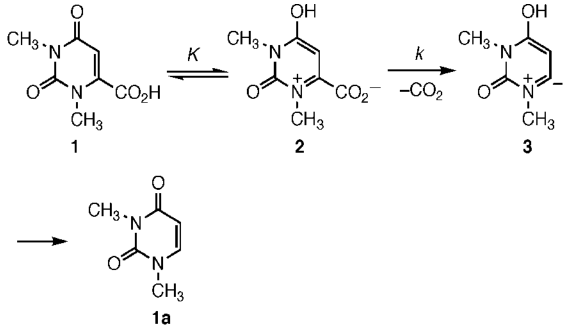
ABSTRACT



The pK_a's of the 6-CH groups of *N*-methyl-2-methoxypyridinium, *N*-methyl-4-methoxypyridinium, and 1-methyl-2,4-dimethoxypyrimidinium ions in aqueous solution were determined to be about 33. The pK_a values were nearly identical to each other and surprisingly similar to those reported for the neutral pyridones and uracil. Further determination of the enthalpies and entropies of the H−D exchange reactions revealed the role of solvation.

The decarboxylation of 1,3-dimethylorotic acid (**1**) and its analogues has been utilized as a model for the enzymatic decarboxylation catalyzed by orotidine-5′-monophosphate decarboxylase (ODCase).[1–14] Studies on the stability of the intermediates have provided support for the mechanism shown in Scheme 1, in which equilibrium formation of zwitterion **2** is followed by the loss of CO_2 and formation of ylide **3**.[6,7,13,14] Recent enzymatic studies have indicated the involvement of a carbanionic intermediate in the reaction.[15–17]

Scheme 1. Proposed Mechanism of the Decarboxylation of 1,3-Dimethylorotic Acid



A mechanistic study of the model reactions was facilitated by the systematic decarboxylation of acids **1**, **4**, and **5** to uracil **1a** and pyridones **4a** and **5a**, respectively (Figure 1). Despite their structural similarity, acid **5** decarboxylates 3 orders of magnitude faster than either acid **1** or **4**.[1,7] Studies

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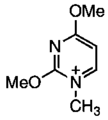
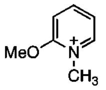
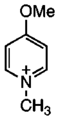
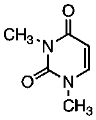
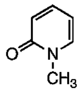
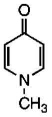
Table 1. Kinetic and Thermodynamic Parameters for H−D Exchange Reactions at the α-Position of Pyrimidinium, Pyridinium, Uracil, and Pyridone Compounds

Substrate	 **1c**	 **4c**	 **5c**	 **1a**	 **4a**	 **5a**
proton affinity[a] (kcal/mol)	273[b]	276[b]	275[b]	368[b,d] 369.9[c,d]	376[b,d] 377.0[c,d]	376[b,d] 377.0[c,d]
k_{OH} (M^{-1} s^{-1})[e]	4.0×10^{-9}	1.7×10^{-9}	1.7×10^{-8}	~3×10^{-6}[f]	3.3×10^{-8}[f]	8.3×10^{-8}[f]
pK_a	33 ± 2	34 ± 2	33 ± 2	~30[f]	32 ± 2[f]	32 ± 2[f]
$\Delta G^\ddagger$ (kcal/mol)	23.9	24.4	23.0	—	22.6[g]	22.1[g]
$\Delta H^\ddagger$ (kcal/mol)	30.0	31.4	28.1	—	21.2[g]	20.2[g]
$\Delta S^\ddagger$ (cal/mol/K)	20.7	23.7	17.2	—	–4.6[g]	–6.2[g]

[a] The values refer to the protonation of the corresponding carbanions at carbon-6. [b] Values calculated at the B3LYP/6-31+G(d,p) level. [c] Experimental values. [d] From ref.[7] [e] Extrapolated to 25 °C by Arrenhius equation. [f] From ref.[13] [g] Calculated from data in ref.[13]

on the gas-phase and solution-phase stability of the corresponding carbanions **1b**, **4b**, and **5b** have established a lack of correlation between the rate of decarboxylation and the stability of resulting carbanions.[6,7,13,14] We have demonstrated that there are large differences in the equilibrium constants or reaction enthalpies for the first step in the proposed mechanism (Scheme 1), which can explain the differences in the rate constants.[7]

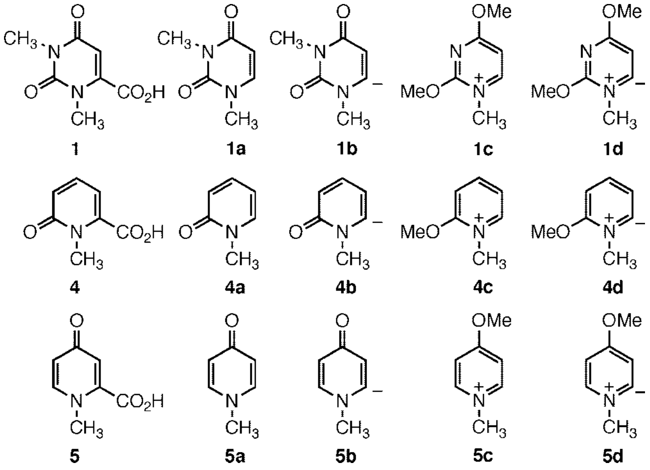


Figure 1. Substrates, products, intermediates, and their analogues in the decarboxylation reactions.

One of the assumptions involved with the proposed mechanism is that ylide **3** (Scheme 1) and its counterparts derived from acids **4** and **5** share similar stability.[7] In this report, we have investigated the stability of the methylated analogues (**1d**, **4d**, and **5d**) in the gas phase and aqueous solution.

The gas-phase proton affinity of ylides **1d**, **4d**, and **5d** was calculated and tabulated in Table 1. Unfortunately, the charge neutrality of these species makes it impossible to experimentally measure their gas-phase proton affinities with mass spectrometry. The results for the three ylides are nearly the same, and they are approximately 100 kcal/mol more stable than carbanions **1b**, **4b**, and **5b** with respect to protonation. As expected, the proximity of the positively charged nitrogen has a tremendous stabilizing effect on the carbanions. The similar values of their proton affinity suggest that the assumption in proposing the mechanism shown in Scheme 1 is valid (i.e., the decarboxylation products have equal stability).

The stability of ylides **1d**, **4d**, and **5d** in water was measured by determining the pK_a of the 6-CH group of pyrimidinium **1c** and pyridiniums **4c** and **5c**. The aqueous pK_a was determined through the measurement of the rate of hydrogen−deuterium exchange on the target carbons using NMR spectroscopy, utilizing the method developed by Richard and co-workers.[18,19] The same method was employed in measuring the pK_a of uracil **1a** and pyridones **4a** and **5a**.[8,13]

The hydrogen−deuterium exchange reaction for pyridinium **4c** in NaOCH$_3$/CH$_3$OD has been reported (and confirmed in our laboratory) to be fairly facile with first-order kinetics on the concentration of substrate and base.[20] It has also been reported that hydrogen−deuterium exchange

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(20) Beak, P.; Monroe, E. M. *J. Org. Chem.* **1969**, *34*, 589–596. The difference between the rate constants in water and methanol is quite large. We do not have a good explanation for this. We are currently investigating the effects of electron-donating and withdrawing groups on the acidity of α-CH groups of pyridinium compounds and the solvent effects of these substituted pyridinium compounds. These studies are beyond the scope of this investigation.

occur rapidly in aqueous solution for acetyl methyl groups when there is a nearby (though not adjacent) positive charge.[21] We expect the reactions in aqueous solution to be fast as well, but to our great surprise, the reactions were found to be very slow and numerous tries with buffer solutions of various pD failed to show any hydrogen−deuterium exchange. The kinetic experiments were thus carried out in NaOD/D_2O solution as reported for pyridones **4a** and **5a**.[13] The rate of hydrogen−deuterium exchange on carbon-6 of pyrimidinium **1c** and pyridiniums **4c** and **5c**follows first-order kinetics on the concentration of substrate (as shown in Figure 2) as well as NaOD. The specific rate constant at

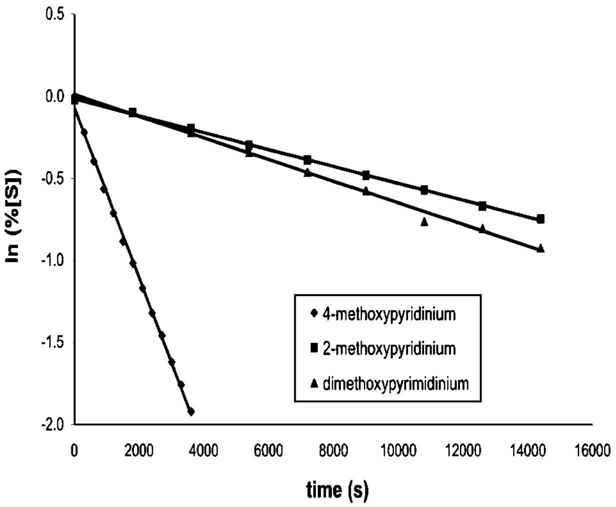

Figure 2. Rate plot for the disappearance of H-6 at 90 °C.

1 N NaOD/D_2O, k_{OD}, was determined by following the disappearance of H-6 by NMR.

The rate of hydrogen−deuterium exchange was measured over the temperatures of 60−90 °C and was extrapolated to 25 °C by Arrhenius plot as shown in Figure 3. A secondary solvent deuterium isotope effect of $k_{DO}/k_{HO} = 1.46$ was utilized as in previous studies[8,13] and the rate constants for the hydroxide-catalyzed deprotonation, k_{OH}, for pyrimidinium **1c** and pyridiniums **4c** and **5c**were calculated as listed in Table 1. The reaction between the carbanion and water was considered to be diffusion-controlled, and a value of $\sim 10^{11}$ s^{-1} was assumed for the rate constant, k_{HOH}, as in previous studies.[8,13] The pK_a values of the 6-CH groups of pyrimidinium **1c** and pyridiniums **4c** and **5c** are thus estimated using eq 1.

$$pK_a = pK_w + \log(k_{HOH}/k_{HO}) \qquad (1)$$

On the basis of the hydrogen–deuterium exchange model outlined above, the data indicate that the pK_a values of pyrimidinium **1c** and pyridiniums **4c** and **5c** (Table 1) are slightly higher than those for pyridones **4a** and **5a**. The results are surprising because the adjacent positive charge is expected to greatly stabilize the negative charge at carbon-6 and thus to significantly increase the acidity of the 6-CH groups. An “internal return” mechanism for the hydrogen−deuterium exchange reaction, in which the removed proton reattaches to the carbanion without diffusing away, is ruled out by the observation of a sizable deuterium isotope effect. In an internal return mechanism, the deuterium isotope effect is expected to be nonexistent or very small. In our experiment with the 6-deutereted substrate, the deuterium isotope effect was estimated to be 6 ± 1, within the range of primary isotope effects.

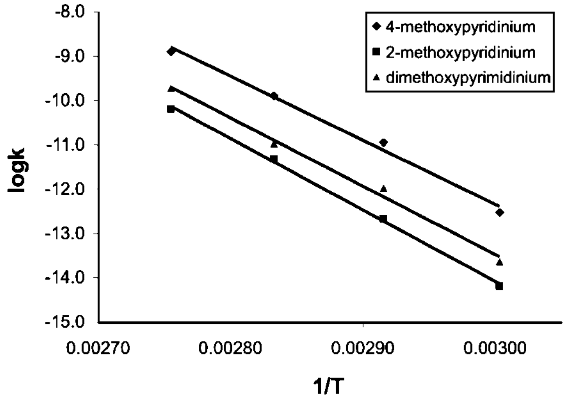

Figure 3. Arrhenius plot for the rates of deuterium exchange.

The gas phase proton affinities of ylides **1d**, **4d**, and **5d** are much lower than the carbanions **1b**, **4b**, and **5b**, as expected. However, the pK_a values in aqueous solution are very similar The discrepancy in the results can be attributed to solvation effect. The ease of deprotonation of cationic acids to form zwitterions in the gas phase compared to solution has been discussed previously.[18,22,23] As shown in Scheme 2, the solvation of substrates and products is similar for deprotonation of a neutral acid by an anionic base while the solvation of the zwitterionic product is much weaker than the cationic acid and anionic base. Betaine provides a good example of this effect. It is over 100 kcal/mol more acidic than acetic acid in the gas phase, but only about three pK_a units more acidic in water.[22,23] However, it is surprising that solvation completely eliminates the enhanced acidity of the cationic species in the present systems.

To further investigate the effect of solvation on the hydrogen−deuterium exchange reaction, the activation free energy, enthalpy, and entropy of the reactions were determined, and results are shown in Table 1. The results show that the similar values of the activation free energy ($\Delta G^{\ddagger}$) of the hydrogen−deuterium exchange reaction of the positively charged substrates (**1c**, **4c**, and **5c**) and neutral substrates (**4a** and **5b**) are coincidental. The pyridinium and pyrimidinium ions have much higher values of activation enthalpy ($\Delta H^{\ddagger}$) and activation entropy ($\Delta S^{\ddagger}$) than the neutral

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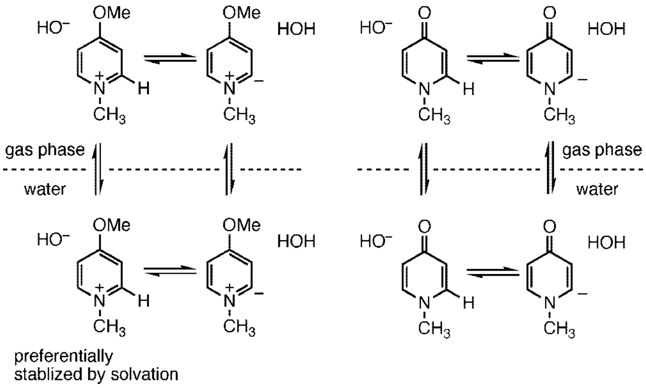
Scheme 2. Deprotonation of Cationic and Neutral Acids by an Anionic Base in the Gas-Phase and Water



pyridones. The entropy values are consistent with those reported for the reaction between oppositely charged ions in water.[24,25]

The larger values for pyridinium and pyrimidinium ions indicate the role of solvation. The transition states of the hydrogen–deuterium exchange reactions resemble the carbanions. In the cases of cationic substrates, the transition states resemble the ylides **1d**, **4d**, and **5d**, which are neutral and much less heavily solvated than the substrates. Thus, strongly bound and well-oriented solvent molecules will be released in the transition state, explaining the higher $\Delta S^{\ddagger}$ values. The loss of solvation (and thus stabilization) in the transition from the substrate to the transition state also explains the higher $\Delta H^{\ddagger}$ values in the cationic species (entropy-enthalpy compensation), but complete balancing is not generally expected, which in this case, leads to the cationic and neutral species having similar estimated pK_a's.

Despite the difference in values regarding the stability of ylides **1d**, **4d**, and **5d** in the gas phase and aqueous solution, the three ylides do share similar stability in both phases, as proposed in the mechanistic hypothesis shown in Scheme 1.[7] The results reported here have provided further support for the proposed mechanism for the decarboxylation of model reactions. It should be pointed out that the solvation effect for the decarboxylation step in Scheme 1 should not be as strong due to the charge neutrality of both the zwitterionic carboxylate and carbanion. On the other hand, the stabilization effect of a less polar environment is obvious from the comparison of results in the gas phase and aqueous solution. The effect of the solvent, and thus the environment, can be further seen in the large difference in the specific rates of hydrogen–deuterium exchange for pyridinium **5c** in aqueous solution and methanol solution.[20] These results indicate the potential impact of the nonpolar environment at the active site of ODCase on the stabilization of the ylide intermediate.[18,26–28] However, whether the enzymatic reaction follows the same mechanism remains to be seen.

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Supporting Information Available: Experimental procedures and spectra data. This material is available free of charge via the Internet at http://pubs.acs.org.

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