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International Journal of Mass Spectrometry 195/196 (2000) 251–258

International Journal of Mass Spectrometry

The gas phase acid/base properties of 1,3,-dimethyluracil, 1-methyl-2-pyridone, and 1-methyl-4-pyridone: relevance to the mechanism of orotidine-5′-monophosphate decarboxylase

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Received 3 June 1999; accepted 2 September 1999

Abstract

A combination of experimental and theoretical approaches have been used to probe the gas phase acidity and basicity of 1,3-dimethyluracil, 1-methyl-2-pyridone, and 1-methyl-4-pyridone. For the acidity measurements, bracketing experiments were completed in an electrospray/quadrupole ion trap mass spectrometer. The conjugate bases of the title species were formed by collision activated decarboxylation of appropriate carboxylate precursors. The data indicates only a small variation in the acidities (ΔH_{acid} = 369.9–377.0 kcal/mol) with the uracil derivative being ~7 kcal/mol more acidic than both the pyridones. To determine the basicities of the title compounds, Cooks' kinetic method was used and a much larger variation (19 kcal/mol) was observed in the proton affinities of the neutral species: 1,3-dimethyluracil, 213.7 ± 3.0 kcal/mol; 1-methyl-2-pyridone, 222.3 ± 2.9 kcal/mol; and 1-methyl-4-pyridone, 233.1 ± 3.0 kcal/mol. ΔH_{acid} and proton affinity values were also computed at the MP2/6-31+G(*d*,*p*)//HF/6-31+G(*d*), and B3LYP/6-31+G(*d*,*p*)//HF/6-31+G(*d*) levels. There is very good agreement between the experimental values and those from both levels of theory. (Int J Mass Spectrom 195/196 (2000) 251–258) © 2000 Elsevier Science B.V.

Keywords: Acidity; Proton affinity; Uracil; Pyridone

1. Introduction

Mass spectrometry has proven to be an outstanding tool for deriving thermochemical information and with the advent of electrospray ionization techniques [1–4], it has been possible to study a wide range of biomolecules that would not be volatile enough for conventional approaches. In this paper, we describe gas phase studies of model systems related to orotidine-5′-monophosphate (OMP) decarboxylase. In biological systems, OMP decarboxylase catalyzes the decarboxylation of OMP to uridine-5′-monophosphate (UMP), the final step in pyrimidine nucleotide biosynthesis [5]. OMP decarboxylase is a remarkably proficient enzyme and accelerates the reaction by a factor of 1.4×10^{17} [6]. Several mechanisms have been put forth to explain the enzymatic rate enhancements [7–9]. The most widely accepted mechanisms involve protonation (at either of the carbonyl oxygens) followed by expulsion of CO_2 to give zwitterionic intermediates (carbene resonance forms are also possible) [7,9]. The two mechanisms are illustrated in

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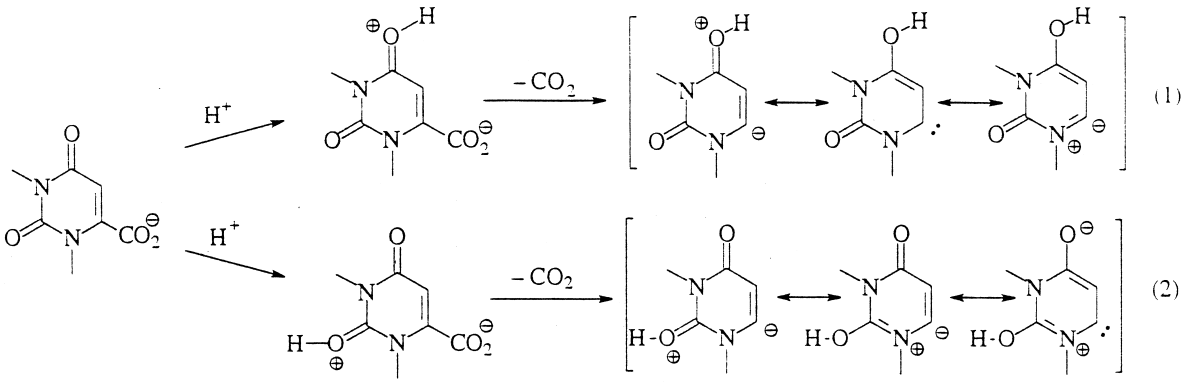
Dedicated to the memory of Professor Robert R. Squires, a truly exceptional scientist, colleague, and friend.

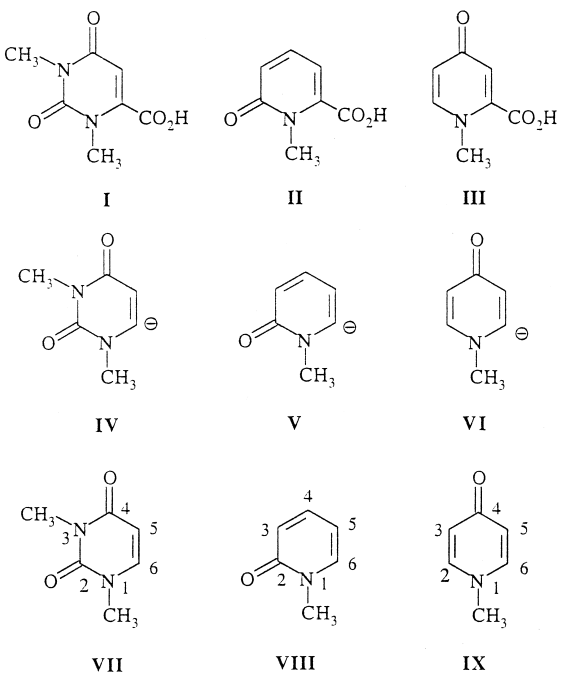
PII S1387-3806(99)00191-8



Scheme 1. Model mechanisms.

Scheme 1 using 1,3-dimethylorotic acid (**I**) as a substrate analog.

Recently, one of us has been involved in a series of nonenzymatic model studies employing simple analogs, **I–III** (Scheme 2) [10,11]. Analogs **II** and **III** are particularly interesting because they lack one of the carbonyls and therefore are limited to only one of the protonation pathways shown in Scheme 1; however, it is possible that the thermal decarboxylations occur directly from the carboxylate without protonation to give a zwitterion. As a part of the model study, it became evident that it would be very useful to have thermochemical data on the acid/base behavior of key species along the proposed reaction paths. Specifically, the proton affinities (PAs) of **IV–IX** (Scheme 2) could provide important insights into the kinetic data obtained experimentally in the thermal decarboxylations. In addition to the relevance to the mechanism of OMP decarboxylase, the acid/base properties of these species are of fundamental interest. First, there is very little data in the literature on the carbon acidity of polyfunctional heterocycles. These species can be moderately acidic and therefore their conjugate bases are viable mechanistic intermediates. Second, although the proton affinities of related species have been studied experimentally and computationally [12–14], a consistent data set is not available for analyzing the effect of the ring structure (2-pyridone versus 4-pyridone versus uracil) on the gas phase basicity. Of the species in this study, the proton affinity of **VIII** has been reported previously [12] and there are some condensed phase data (pK_as) available for **VII–IX** and their nonmethylated parent compounds [15–17].



Scheme 2. Model species.

In the present work, the proton affinities were determined by three approaches. For neutral compounds, Cooks' kinetic method was used [18,19]. In this approach, a proton bound complex of the species of interest and a base of known proton affinity is formed. Collision activated dissociation (CAD) of the complex leads to two protonated products and the

ratio of these products is a measure of the relative proton affinities of the two compounds.

$$B_1M^+B_2 \begin{array}{l} \nearrow^{k_1} \\ \searrow_{k_2} \end{array} \begin{array}{l} B_1H^+ + B_2 \quad (3) \\ B_2H^+ + B_1 \quad (4) \end{array}$$

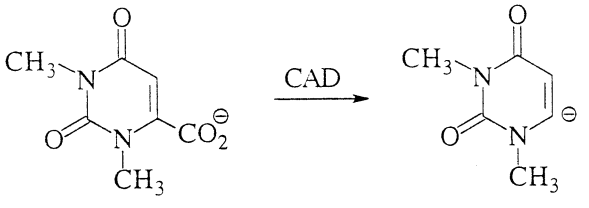
$$B_2H^+ + B_1 \overset{K}{\rightleftharpoons} B_1H^+ + B_2 \quad (5)$$

$$K \approx k_1/k_2 \quad (6)$$

$$PA_1 - PA_2 \approx RT_{eff} \ln K \quad (7)$$

The assumptions of the method are built into Eqs. (6) and (7). Eq. (6) requires that the dissociation does not have an appreciable reverse activation barrier and that the transition state for dissociation occurs late enough to truly represent the stability of the products. Both are generally true for proton bound systems. The use of Eq. (7) requires that the entropy effects in Eq. (5) be negligible. It has been assumed that this is true when the two bases have similar structures. The other required term is T_{eff}, which can be approximated experimentally (see below).

Anions **IV**–**VI** are not amenable to this approach because they are reasonably basic and would not be readily formed under electrospray conditions. Moreover, it is not clear that ionization would lead directly to deprotonation at the site of interest (several C–H bonds are available). About a decade ago, Graul and Squires [20–22] presented a series of studies on the formation of carbanions via collision-activated decarboxylation reactions and exploited this technique to obtain thermodynamic data on some carbanions. In the present systems, decarboxylation of the carboxylates from **I**–**III** should provide access to carbanions



IV (8)

IV–**VI** [e.g. Eq. (8)]. This method of generating the carbanions is also conceptually attractive because it parallels the enzymatic pathway (OMP decarboxylase). Once the carbanions are formed, their proton affinities (i.e. ΔH_{acid} of **VII**–**IX**) can be probed via the bracketing method. In this approach, the anions are allowed to react with a series of neutral acids of varying strength. The proton affinity is bracketed between the ΔH_{acid} of the weakest acid that gives a proton transfer and the strongest acid that does not. Although less precise than Cooks' method, it gives similar results and is bound by a related set of assumptions.

To support all of the experimental work, ab initio calculations have been used to determine proton affinities for each of the systems. In most cases, calculations were completed at the MP2/6-31+G(*d*,*p*)//HF/6-31+G(*d*) and B3LYP/6-31+G(*d*,*p*)//HF/6-31+G(*d*) levels; however, calculations at the MP2/6-31+G(*d*,*p*)//MP2/6-31+G(*d*) level were used on some small, model systems. Unless noted otherwise, the MP2 values will be used in the text.

2. Experimental

2.1. General

All experiments were completed in a modified Finnigan-LCQ quadrupole ion trap mass spectrometer. Ions were generated by electrospray from 10^{-4}–10^{-5} M solutions of the precursors in methanol using a flow rate of 3–5 μL/min. Typically, an electrospray needle voltage of ~4000 V was used. With the exception of **I**–**III** and **IX**, all reagents were obtained from commercial sources and used without further purification. Acid **I** was prepared through the methylation of orotic acid as reported by Curran and Augier [23]. The same method was used to prepare **II** from 2-pyridone-6-carboxylic acid. Acid **III** was prepared from 4-pyrone-2-carboxylic acid as reported by Beak and Siegel [7]. Pyridone **IX** was synthesized by the reaction of methyl iodide and 4-methoxypyridine using a method described by Beak and Bonham [24]. The identity and purity of the synthesized compounds

was verified by their ^{1}H NMR spectrum, mass spectrum, and melting point.

2.2. Bracketing experiments

The electrospray ionization source was optimized for the carboxylates derived from **I**–**III**. These ions were isolated in the ion trap and then subjected to collision activated dissociation by a resonant excitation pulse (0.5–1.5 V for ~30 ms). This process led to large signals for **IV**–**VI** and subsequently all other ions were ejected from the trap. We observed that some decarboxylation occurred during electrospray ionization, but this could be suppressed by reducing the temperature of the heated desolvation capillary. Once a stable signal for the carbanion was established, the neutral reagent was added to the helium buffer gas of the ion trap using a gas handling system that has been described previously [25,26]. In short, the neural reagent is delivered by a syringe pump (30–300 μL/h) to a fast flow of helium (0.5–2 L/min). The great majority of the gas is diverted and ~0.25 mL/min is drawn into the ion trap. Using the mixing ratio (reagent/He), the He pressure in the trap (1.75×10^{-3} Torr), and a correction for differential effusion, the partial pressure of the reagent in the trap can be determined. The neutral is added continuously throughout the experiment. To test for proton transfer, the ions (**IV**–**VI**) were allowed to react with the neutral reagent (~5×10^{-7} Torr) for 100–300 ms (time between the isolation of **IV**–**VI** and the ejection of all ions to obtain a mass spectrum). Rapid proton transfer (i.e. near the collision rate) was taken as evidence that the reaction was exothermic. In most cases, adducts between the anions and neutrals reagents were also observed.

2.3. Cooks' kinetic experiments

Solutions of the species of interest and the reference base were subjected to electrospray ionization and the mixed proton bound complexes were isolated. For CAD, the dimer ions were activated for 3–10 ms with an activation voltage of ~0.5 V. About 1000 scans were averaged for product ions with low intensities. Experimental uncertainty in the abundance ratios of the fragment ions was estimated to be ±12%. Although it is preferable to use standard bases whose structures are similar to the species of interest, it was not always possible for these systems (partly due to the lack of appropriate standards and also to spurious results with polyfunctional species). The inability to use similar standards will eliminate some of the cancellation of errors normally associated with Cooks' method and increase the uncertainties. In terms of T_{eff}, numerous studies in our lab on a range of species indicate that it is slightly above ambient temperature for CAD in the Finnigan quadrupole ion trap [27,28]. For the present study we have employed an average value (T_{eff} = 328 K) obtained from previous work in our trap. Given the general uncertainties in the reference PAs and the approximations in Eq. (7) this arbitrary choice of T_{eff} should have only a modest effect on the uncertainty of the measurements (±0.3 kcal/mol). Overall, it is expected that under these circumstances, Cooks' values have uncertainties similar to those of bracketing experiments.

2.4. Calculations

Calculations were completed with the GAUSSIAN94 [29] quantum mechanical package on an SGI Octane, an IBM 39H, or an HP 735 computer. Optimizations were completed without constraints and harmonic frequency calculations were done at the HF/6-31+G(*d*) level. All proton affinities are corrected for zero point vibrational energy (HF) scaled by 0.9135 [30]. For **IV**–**IX** and their protonation products, optimizations were completed at the HF/6-31+G(*d*) level followed by single point calculations at the MP2/6-31+G(*d*,*p*) and B3LYP/6-31+G(*d*,*p*) levels. For the nonmethylated analogs (uracil, 2-pyridone, and 4-pyridone) and their deprotonation products, optimizations were completed at the MP/6-31+G(*d*) level followed by single point calculations at the MP2/6-31+G(*d*,*p*) level. Given the modest level of theory, no thermal corrections were made and the results refer to 0 K (values at 300 K should be ~1 kcal/mol higher due to the lost translational energy of the proton).

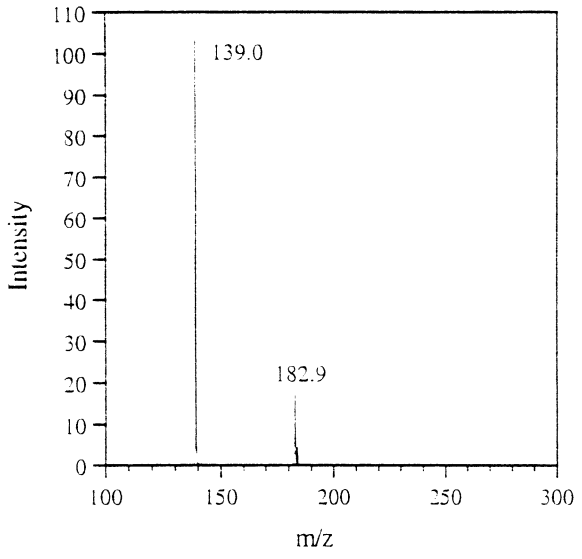


Fig. 1. CAD spectrum of the M-1 anion of **I**(*m/z* = 183). The decarboxylation product **(IV)** appears at *m/z* = 139. The enhanced ^{13}C isotope peak at *m/z* 4 is due to preferential decarboxylation of the *m/z* = 183 species.

3. Results and discussion

3.1. Proton affinities of *IV–VI* (ΔH_{acid}s of *VII–IX*)

Collision activated dissociation of carboxylate precursors proved to be an excellent way to generate carbanions **IV**–**VI**. A typical CAD spectrum from the decarboxylation of the M-1 anion of **I** is shown in Fig. 1. With moderate activation energies, carbanion **IV** is formed as the exclusive product.

The results of the bracketing studies for **IV**–**VI** are shown in Table 1 along with the corresponding computational values. **IV** readily reacts with acetone and butanone, but fails to undergo proton transfer with methacrylonitrile or propionitrile. This brackets the proton affinity at 369.9 ± 3.1 kcal/mol [31]. The MP2 computed value, 367.6 kcal/mol, is in good agreement. **V** and **VI** have similar proton affinities and the same bracket was obtained for both. Each gives a rapid proton transfer with butanol and propanol, but not with ethanol. This results in a bracketed proton affinity of 377.0 ± 2.9 kcal/mol. The MP2 calculated values for **V** and **VI** (375.5 and 375.8 kcal/mol, respectively) are very close to the experimental bracket. Finally, the B3LYP values are somewhat smaller than the MP2 values, but still in general agreement.

In doing these experiments, there was some concern about the presence of other acidic sites because bracketing experiments probe the most acidic site in a substrate, not necessarily the initial site of deprotonation (proton transfers in the collision complex can cause isomerization to the most stable anion). To investigate this potential problem, the proton affinities of all the carbanions derived from uracil, 2-pyridone, and 4-pyridone (the nonmethylated parents of **VII**–**IX**) were calculated. The results are listed in Table 2.

Table 1
Proton affinity data for anions[a]

Reference acid	ΔH_{acid}	**IV**	**V**	**VI**
Butanone	367.2 ± 2.8	yes	—	—
Acetone	369.1 ± 2.1	yes	—	—
Methacrylonitrile	370.7 ± 2.1	no	yes	yes
Propionitrile	375.0 ± 2.1	no	yes	yes
Butanol	375.4 ± 2.1	—	yes	yes
Propanol	375.7 ± 1.3	—	yes	yes
Ethanol	378.3 ± 1.0	—	slow	slow
Toluene	380.6 ± 1.0	—	no	no
Bracketed PA (300 K)		369.9 ± 3.1	377.0 ± 2.9	377.0 ± 2.9
Calculated PA (0 K)[b]		367.6 (366.0)	375.5 (374.0)	375.8 (375.0)

[a] Values in kcal/mol. Reference values from [31].
Comments refer to presence or absence of rapid proton transfer from reference acid.
[b] Calculations at the MP2/6-31+G(*d*,*p*)//HF/6-31+G(*d*) level.
Results at the B3LYP/6-31+G(*d*,*p*)//HF/6-31+G(*d*) level given parenthetically.

Table 2
Calculated proton affinities (0 K) of carbanions derived from uracil, 2-pyridone, and 4-pyridone[a]

Deprotonation site[b]	Parent		
	Uracil	2-Pyridone	4-Pyridone
6	365.2	374.3	375.9
5	377.6	384.3	393.2
4	—	389.1	—
3	—	392.9	—

[a] Calculated at the MP2/6-31+G(*d*,*p*)//MP2/6-31+G(*d*) level including scaled zero-point energy corrections.

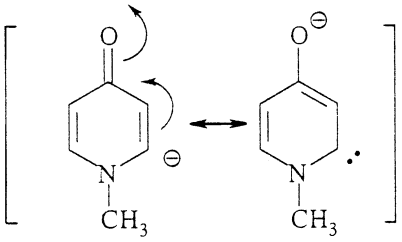
[b] See Scheme 2 for numbering protocol. In each case, position 6 results in carbanions analogous to **IV**–**VI**.

In 4-pyridone, positions 5 and 6 are equivalent to positions 3 and 2.

In every case, the most stable carbanion is derived from deprotonation at position 6, the anionic site found in **IV**–**VI**. The differences are significant and there is little doubt that the experiments are probing the proton affinities of carbanions with the negative charge at the desired site. Of course the most acidic sites in uracil, 2-pyridone and 4-pyridone are the nitrogens, but those positions are methylated in **VII**–**IX**.

Overall, the systems have relatively low proton affinities for sp^2 hybridized carbanions. For example, the anion derived from pyridine has a proton affinity of ~391 kcal/mol [31]. Apparently the ions derive substantial stabilization from the presence of the carbonyl functional groups in the ring. This is not surprising and can be explained either by an ion-dipole effect or the presence of resonance forms that allow the charge to reside on the oxygen (an example is shown below for **VI**).

Resonance forms of this type incorporate a carbene center at position 6, but Lee and Houk [9] have shown



VI

that related structures can be surprisingly stable. Our results indicate that the orientation of the carbonyl (**V** or **VI**) has little effect on its stabilizing power. It is not surprising that the addition of a second carbonyl functional group (**IV**) provides further stabilization of the carbanion. However, it is interesting to point out that **IV** is an unusually stable anion (PA is 21 kcal/mol below that of the carbanion from pyridine) and in a low dielectric medium (gas phase) it is about as basic as an enolate. This leads one to speculate that the surprisingly high efficiency of OMP decarboxylase could be partially explained by the unexpected relative stability of the decarboxylation product in a low-dielectric medium.

*3.2. Proton affinities of **VII–IX***

The results from the proton affinity studies of the neutral species are listed in Table 3. A few comparisons can be made to existing data. Cook et al. [12,32] have reported a proton affinity of 221.3 kcal/mol for **VIII** based on an equilibrium study in an ion cyclotron resonance (ICR) spectrometer. Our value, 222.3 kcal/mol, is in good agreement. Previous experimental (208.6 kcal/mol) [32] and computational (205.6 kcal/mol) [13] values are also available for uracil. Not surprisingly, we find that the dimethyl derivative (**VII**) has a somewhat higher proton affinity, 213.7 kcal/mol. Finally, Kallies and Mitzner [14] have used density functional theory to calculate a proton affinity of 229.7 kcal/mol for 4-pyridone. Again, we find that the methyl derivative, **IX**, has a slightly higher proton affinity (233.1 kcal/mol). Further support for the assigned PAs comes from our calculations, which are very close to the experimental values in each case. It is interesting to note that in the species with amide linkages (**VII** and **VII**), the calculated PA is very sensitive to the orientation of the added proton. There is approximately a 4 kcal/mol advantage to having it anti rather than syn to the amide nitrogen. A smaller effect had been noted by Chandra et al. in uracil [13].

A striking feature of the experimental data is that there is a much larger variation in the proton affinities of these species than was observed with the anions (19 kcal/mol versus 7 kcal/mol). This may be

Table 3
Proton affinity data for neutrals[a]

Structure	Reference[b]	ln (k_1/k_2)[c]	PA	PA (calc.)[d]
VII	N-Methylacetamide	−1.96	213.7 ± 3.0	211.8 (213.6)
	2-Methoxyaniline	4.16		
VIII	Pyridine	−0.15	222.3 ± 2.9	223.0 (222.5)
	s-Butylamine	−0.46		
IX	Triethylamine	1.81	233.1 ± 3.0	234.2 (233.3)
	Diethylmethylamine	−0.91		

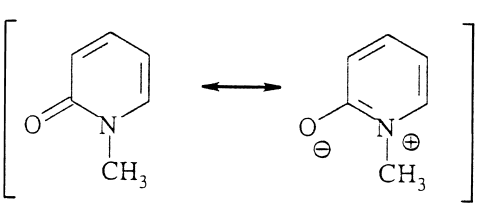
[a] Values in kcal/mol.
[b] PAs of reference bases [32]: N-methylacetamide = 212.4; 2-methoxyaniline = 216.3; pyridine = 222.0; *s*-butylamine = 222.2; triethylamine = 234.7; diethylmethylamine = 232.1; uncertainties in these values are generally ±2 kcal/mol.
[c] See Eq. (3) with the reference as B_1, T_{eff} = 328 K is used to calculate PA.
[d] Calculated at MP2/6-31+G(*d*,*p*)//HF/6-31+G(*p*) level (0 K).
Results at the B3LYP/6-31+G(*d*,*p*)//HF/6-31+G(*d*) level given parenthetically.

explained by differences in the site of protonation. In the anions (**IV**–**VI**), a localized, sp^2 carbanion is protonated in each case and interactions with the rest of the structure are either through space or via indirect conjugation (polarization of the π-system orthogonal to the carbon lone pair). Protonation of **VII**–**IX** occurs on carbonyls of fundamentally different functional groups (imide, amide, and unsaturated ketone, respectively) so a larger variation is not surprising. Of the two isomeric species, **IX** is much more basic than **VIII**. This may be explained in part by examining the aromatic resonance forms of the neutral species.

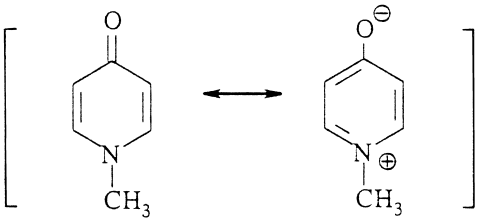
The aromatic resonance forms for both compounds involve a charge separation, but it is much shorter in **VII**. This will provide electrostatic stabilization to the oxyanion and make **VIII** more stable and less basic than **IX** (protonation removes the formal charge separation and eliminates this effect in both cases). Support for this argument comes from the fact that **VIII** is calculated to be 11 kcal/mol more stable than **IX**. **VII** is the least basic of the series because the imide functional group reduces electron density on the carbonyl oxygens. A similar order of basicities exists in aqueous solution for the nonmethylated, parent compounds (uracil, 2-pyridone, and 4-pyridone). Their conjugate acids have pK_a values of 0.6, 0.7 (1.25 has also been reported), and 3.27, respectively [15–17]. Although the order is the same, the variation in basicity is much smaller. These species are very weak bases in solution so extensive solvation (hydrogen bonding) accompanies protonation resulting in a leveling effect that makes the basicity less sensitive to structural differences in the substrate.

4. Conclusions

The computational and experimental results indicate that there is a rather small variation (7 kcal/mol)



VIII



IX

in the ΔH_{acid} values of 1,3-dimethyluracil, 1-methyl-2-pyridone, and 1-methyl-4-pyridone. These compounds are moderate gas phase acids and fall in the range of simple alcohols and ketones. In contrast, there is a very large variation (19 kcal/mol) in the proton affinities of these species. The results suggest that the compounds could have very different reactivity patterns if protonation is a prerequisite to decarboxylation. A detailed discussion of the mechanistic ramifications of these results as well as a reanalysis of kinetic data for the thermal decarboxylation of the carboxylic acid derivatives of the title compounds will be presented in the near future.

Acknowledgements

This investigation was supported by a Research Infrastructure in Minority Institutions award from the National Center for Research Resources with funding from the Office of Research on Minority Health National Institutes of Health no. 5 P20 RR11805. FC thanks the U.S. Department of Education for funding via the GAANN program (P200A70233).

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