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Uracil Radicals in the Gas Phase: Specific Generation and Energetics

Jill K. Wolken, Erik A. Syrstad, Shetty Vivekananda, and František Tureček*

Department of Chemistry, Bagley Hall
Box 351700, University of Washington
Seattle, Washington 98195-1700

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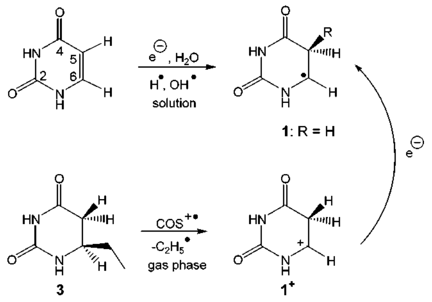
Nucleobase residues are the prime target for attack by energetic particles in the process of radiative and oxidative DNA damage.[1] There are currently two accepted mechanisms of nucleobase radical formation.[1,2] The first comprises direct addition to the nucleobase of small radicals (H, OH, etc.) that are formed by radiolysis of water or other chemical reactions (Scheme 1).[1] The other mechanism consists of electron capture followed by protonation of the transient anion radical with the solvent, resulting in net hydrogen-atom addition to the nucleobase (Scheme 2).[2] While the kinetics of radical and electron additions to nucleobases have been studied in detail in solution and frozen matrices,[3,4] relatively little is known about the intrinsic nature and reactivity of the transient nucleobase radicals that are the presumed reaction intermediates.[5]

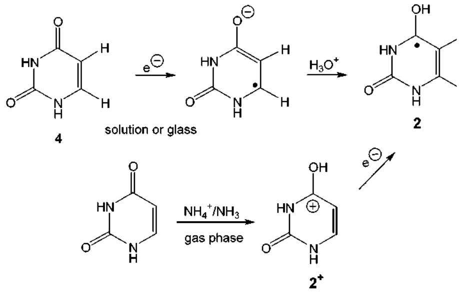
Here we report on the specific preparation in the gas phase of two isomeric radicals, 5,6-dihydropyrimidine-2,4(1*H*,3*H*)-dion-6-yl (**1**) and 4-hydroxy-3,4-dihydropyrimidine-2(1*H*)-on-4-yl (**2**), which are derived from the RNA nucleobase uracil. Although RNA is more resistant than DNA to oxidative and other radical-induced degradation,[6] this increased stability is thought to arise from the chemical properties of the ribose moiety, rather than the presence of uracil.[7] Radical **1** corresponds to an H-atom adduct to C-5 in uracil and represents the elusive transient intermediate of the direct addition mechanism. Radical **2** is an H-atom adduct to O-4 in uracil and represents a transient intermediate of the electron capture−protonation mechanism.

We have shown previously that heterocyclic radicals can be formed in two steps in the gas phase (Scheme 1).[8] In the first step, a gas-phase ion is synthesized that has the bond connectivity of the desired radical. The ion is selected by mass, accelerated to a high velocity (119 000 m s^{-1} for the uracil ions), and discharged by a glancing collision with dimethyl disulfide as a polarizable electron donor. Because of the short duration of the electron-transfer event ($<10^{-14}$ s) the nascent radical is formed with the structure and geometry of the precursor ion.[9] Stable radicals and their dissociation products are then ionized and detected as cations by neutralization−reionization mass spectrometry.[10]

Scheme 1



Scheme 2



Cation $\mathbf{1}^+$ was synthesized by dissociative ionization of 6-ethyldihydrouracil (**3**)[11] (Scheme 1). The selection of the neutral precursor and the ionization conditions were guided by high-level ab initio calculations.[12] The calculated ionization energy of **1** (6.82 eV) was lower than that of $C_2H_5^{\bullet}$(8.12 eV)[14] which indicated preferential formation of $\mathbf{1}^+ + C_2H_5$ with the charge located in the uracil fragment. The appearance energy for the formation of $\mathbf{1}^+$ was calculated as AE = 10.1 eV, and the requisite energy was provided by charge-exchange ionization with $COS^{+\bullet}$ (IE(COS) = 11.18 eV). This resulted in an abundant formation of $\mathbf{1}^+$ that was characterized by its unique collisionally activated dissociation (CAD) spectrum.[15] Cation $\mathbf{2}^+$ was synthesized by selective gas-phase protonation of uracil (Scheme 2). This synthesis was also guided by ab initio calculations that provided the topical proton affinity at O-4 as PA = 858 kJ mol^{-1}.[16,17] Hence, protonation with NH_4^+ (PA = 853 kJ mol^{-1}) occurred selectively at O-4 to give the stable ion $\mathbf{2}^+$ that was characterized by a CAD spectrum that differed substantially from that of $\mathbf{1}^+$.[15]

Radicals **1** and **2** were generated from the corresponding cations by collisional electron transfer with CH_3SSCH_3 and characterized by neutralization−reionization (NR) mass spectra (Figure 1). These differed substantially from the CAD spectra of the cations,[15] so that the dissociations observed on NR can be assigned to radicals **1** and **2**. Both **1** and **2** showed survivor ions (*m/z* 113) which indicated that fractions of the corresponding uracil radicals were stable on the 5.1 μs time scale of the measurements. However, the dissociation products and reaction mechanisms differed for **1** and **2**.

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* Correspondence author. Telephone: (206) 685-2041. Fax: (206) 685-3478. E-mail: turecek@chem.washington.edu.

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[12] Optimized geometries and harmonic frequencies were calculated with B3LYP/6-31+G(d,p). Single-point energies were calculated at the levels shown in Table 1. B3-PMP2 refers to averaged B3LYP and PMP2 energies (ref 13). Effective QCISD(T)/6-311+G(3df,2p) energies were obtained as described in ref 13.

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[15] CAD spectra were obtained at 10 keV with air as collision gas. The major fragment ions (*m/z*, relative intensity) were as follows. $\mathbf{1}^+$: 70 (65.6), 43 (5.7), 42 (5.0), 28 (3.7). $\mathbf{2}^+$: 112 (12.9), 70 (14.5), 69 (6.1), 68 (6.5), 57 (3.3), 44 (7.2), 43 (3.5), 42 (5.4), 28 (5.4).

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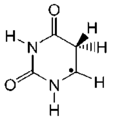
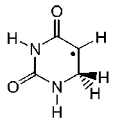
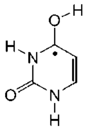
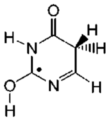
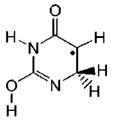
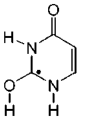
Table 1. Relative Energies of Uracil Radicals

	 **1**	 **6**	 **2**	 **7**	 **8**	 **5**
method	relative energy^{a,b}					
B3LYP/6-31+G(d,p)	0	8	41	41	64	107
B3LYP/6-311+G(3df,2p)	0	9	34	42	65	105
PMP2/6-311+G(3df,2p)	0	12	37	43	65	110
B3−PMP2/6-311+G(3df,2p)^c	0	11	35	42	65	107
QCISD(T)/6-311+G(3df,2p)^d	0	13	38	42	62	104

^{a} At 0 K in units of kJ mol^{-1}. ^{b} From single-point energies on B3LYP/6-31+G(d,p) optimized geometries and ZPVE corrections. ^{c} From averaged B3LYP and PMP2 single-point energies. ^{d} From effective QCISD(T) energies (ref 13).

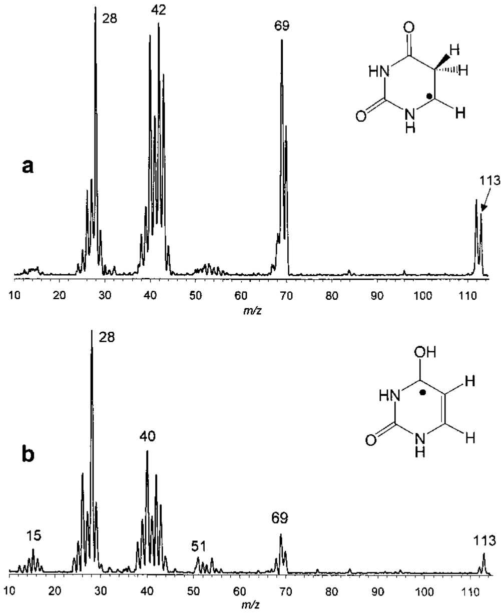


Figure 1. Neutralization (CH_3SSCH_3, 70% transmittance)/reionization (O_2, 70% transmittance) mass spectra of (a) **1**$^+$ and (b) **2**$^+$ obtained at 8200 eV ion kinetic energy.

Radical **1** underwent highly specific (>95%) loss of a hydrogen from C-5 to form the most stable tautomer of uracil (**4**).[16] The mechanism of the hydrogen loss was established by specific deuterium labeling of the H-5, H-6, and H-1/H-3 positions in the precursor ion **1**$^+$.[18] Loss of H-5 amounted to ~45% of total dissociations of **1**. The remaining ~55% dissociations were due to ring cleavages forming HNCO (detected as $HNCO^{+\bullet}$ at *m/z* 43) and C_3H_4NO (*m/z* 70, Figure 1a). The latter ring fragment underwent subsequent dissociations to form mainly C_2H_4N (*m/z* 42), CO, and HCNH (*m/z* 28) that were abundant in the NR spectrum of **1**.[19] There are three possible modes of ring cleavage in **1** producing HNCO that were not distinguished by experiment. However, the ring cleavage resulting from dissociation of the C-4−C-5 and C-2−N-3 bonds was calculated to be energetically most favorable.

Table 2. Kinetics of H-Atom Additions to Uracil

	position			
	C-5	C-6	O-2	O-4
$-\Delta H_{add}{}^a$	114	101	9	75
$E_{TS}{}^b$	19.0	27.1	63.5	45.4
$E_{Arrh}{}^c$	19.8	27.7	64.1	46.2
log A^d	12.98	12.96	12.98	13.08
log $k_{298}{}^e$	9.52	8.11	1.74	4.98
% at 298 K^f	96	4	0	0

^{a} 0 K reaction enthalpies for H-atom additions to uracil in kJ mol^{-1} from effective QCISD(T)/6-311+G(3df,2p) energy calculations and B3LYP/6-31+G(d,p) ZPVE corrections. ^{b} Transition-state energies for H-atom additions. ^{c} Arrhenius activation energies. ^{d} Arrhenius preexponential factors. ^{e} Calculated rate constants at 298 K in mol^{-1} cm^3 s^{-1}. ^{f} Fractions of H-atom additions at the indicated positions in uracil.

Radical **2** also showed two types of dissociations. Loss of H involved the O-4 hydrogen atom to form **4**, as established by deuterium labeling. The by-far predominating dissociations (~85%) were ring cleavages that produced small fragments, mainly HNCO, C_2H_2N, and CO (Figure 1b). The NR spectrum thus clearly indicated that the heterocyclic ring in **2** was destabilized by H-atom addition to O-4 and prone to facile cleavage.

The energetics of several uracil radicals was elucidated by ab initio calculations. Table 1 shows the relative energies of H-atom adducts to O-2 (**5**), O-4 (**2**), C-5(**1**), C-6 (**6**), and tautomers **7** and **8** that were obtained at several levels of theory. Radical **1** was the most stable H-atom adduct to uracil that was followed closely by **6**. Adducts to the carbonyl oxygens in uracil (**2** and **5**) were substantially less stable than **1**.

Do the uracil radical relative energies correlate with the site reactivities toward H-atom addition? To answer this question we calculated the relevant transition-state (TS) energies for H-atom additions to uracil in positions C-5, O-4, O-2, and C-6, forming **1**, **2**, **5**, and **6**, respectively. The TS energies were then used to calculate bimolecular rate constants at 298 K (k_{298})[20] and the corresponding Arrhenius parameters (Table 2). The k_{298} values showed unambiguously that C-5 was the most reactive position in uracil that accounted for >96% H-atom additions. Addition to C-6 competed poorly at 298 K, and the carbonyl oxygens O-2 and O-4 were virtually unreactive.

In summary, two pivotal intermediates of H-atom addition to uracil were generated specifically in the gas-phase. Radical **1** is the most stable isomer out of the (uracil + H) adducts and is predicted to account for 96% of H-atom additions at 298 K.

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[17] The other topical proton affinities in uracil are 826, 784, 731, and 738 kJ mol^{-1} for protonation at O-2, C-5, N-1, and N-3, respectively (ref 16a).

[18] Details of labeling experiments and calculations will be reported in full papers, see: Wolken, J. K.; Tureček, F. *J. Phys. Chem. A*, submitted for publication, April 2001, and Syrstad, E. A.; Vivekananda, S. Tureček, F. *J. Phys. Chem. A*, submitted for publication, April 2001.

[19] The reference $C_3H_4NO^{+\bullet}$ ion was prepared by dissociative ionization of **3** and its NR mass spectrum showed prominent fragments of C_2H_4N (*m/z* 42), CO, and HCNH (*m/z* 28).

[20] From standard transition-state theory formulas (see: Levine, I. N. *Physical Chemistry*, 3rd ed.; McGraw-Hill: New York, 1988), without considering H-atom tunneling.