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Base-Promoted Reaction of 5-Hydroxyuracil Derivatives with Peroxyl Radicals

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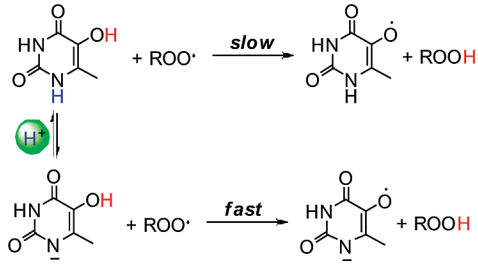
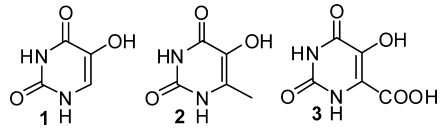
ABSTRACT



Addition of millimolar amounts of a weak base (pyridines) dramatically accelerates the reaction with peroxyl radicals of two biologically relevant uracil derivatives, 5-hydroxyuracil (HU) and 5-hydroxy-6-methyluracil (HMU). This is due to the formation of small amounts of the deprotonated form (pK_a = 8.1−8.5 in water), which reacts with peroxyl radicals much faster than the parent undissociated form, via formal H-atom transfer from the OH in the 5 position.

5-Hydroxyuracil, 5-HU (**1**, isobarbituric acid), is an effective antitoxoplasmosis agent,[1] and it has been found to effectively scavenge hydroxyl radicals produced by γ-radiolysis to form iso-dialuric acid.[2] The 5-HU residue is also one of the most relevant DNA lesions occurring by oxidative degradation of cytosine.[3]



Structurally related 5-hydroxy-6-methyluracil (HMU, **2**) is a known drug with immunotropic activity,[4] and there is evidence in the literature for its potential antioxidant activity.[5] Indeed its rate constant for reaction with ethylbenzene peroxyl radicals was reported as $k = 2.6 \times 10^4$ M^{-1} s^{-1}.[5] A recent investigation has shown that during the thermal decomposition of 2,2′-azo-isobutyronitrile (AIBN) in 2-propanol at 75 °C (in air) HMU prevented the radical-mediated oxidation of the solvent (to form acetone) as efficiently as the well-known antioxidant quercetin, by reacting with

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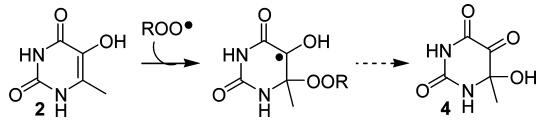
initiator-derived peroxyl radicals to yield product **4**.[6] However, the mechanism for such peroxyl-radical-quenching is far from being understood. It was suggested to occur by radical addition to the ring in the 6-position followed by disproportionation, by analogy with the known reactivity of uracil derivatives with hydroxyl radicals (Scheme 1).[6]

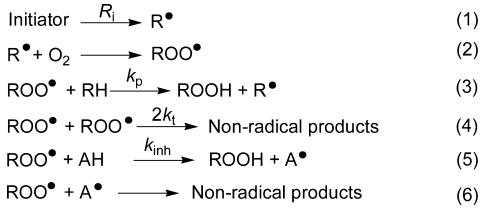
Scheme 1. Proposed 6-Addition of Peroxyl Radicals to HMU, Followed by Radical Disproportionation[6]



Given the relevance of 5-hydroxyluracil derivatives in chemical biology and in medicinal chemistry, we set up to investigate the kinetics and mechanism for their reaction with peroxyl radicals. 5-HU (**1**), HMU (**2**), and their carboxylic derivative (**3**) were prepared according to the literature.[7,8]

The reactivity with peroxyl radicals was investigated using the robust and well-established method of inhibited autoxidations, using the (AIBN) thermally initiated autoxidation of cumene (Scheme 2) in acetonitrile (50% v/v) at 30 °C, as the model system.[9,10,11] The reaction was followed by monitoring the oxygen consumption with an automatic recording gas-absorption apparatus.[10]

Scheme 2. Thermal-Initiated Autoxidation of Cumene (RH)



As can be seen in Figure 1, compound **2** gave a neat inhibition of cumene autoxidation until complete consumption (after ca. 2500 s); **1** gave a less pronounced retarding of oxygen consumption, while compound **3** had no inhibiting activity. The slope of the oxygen consumption trace during the inhibited period afforded k_{inh} values, while its length allowed the determination of the stoichiometric coefficient n, i.e., the number of peroxyl radicals trapped by one molecule of inhibitor, as listed in Table 1 (see Supporting Information for details on calculations).

While the value of k_{inh} measured for HMU (1.5×10^4 M^{-1} s^{-1}) was in good agreement with previous literature (vide supra), the much lower reactivity recorded for the unhindered 5-HU was quite surprising in light of the mechanism depicted in Scheme 1.

Also, the magnitude of k_{inh} was quite unusual for the addition of peroxyl radicals to a conjugated C=C double bond. For comparison, addition of ROO• to styrene has $k =$ 41 M^{-1} s^{-1},[12] while addition to [60]-fullerene, with 30 conjugated double bonds, has $k = 313$ M^{-1} s^{-1} ($k \sim 10$ M^{-1} s^{-1} after normalization on the number of double bonds).[11] Indeed, the measured rates would be surprisingly fast for such a kind of reaction, suggesting a different mechanism for the antioxidant behavior of HMU.

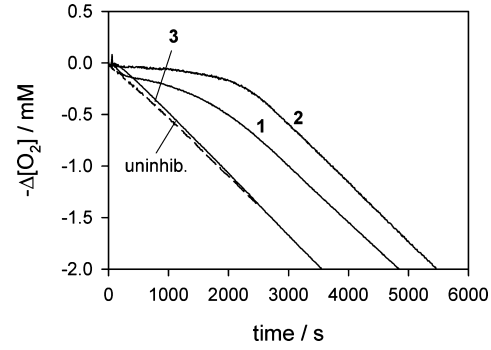


Figure 1. Oxygen consumption recorded during the autoxidation of cumene (3.5 M) in MeCN, initiated by AIBN (0.05 M) at 303 K without inhibitors (dashed line) and in the presence of **1**, **2**, or **3** in 6.3×10^{-6} M concentration.

When compounds **1**, **2**, and **3** were tested as inhibitors of the autoxidation of more oxidizable styrene, only **2** gave a significant retarding of oxygen consumption, and k_{inh} was in agreement with that measured with cumene.

Table 1. Reactivity with Peroxyl Radicals at 303 K in MeCN

compound	k_{inh}/M^{-1} s^{-1}	n	substrate
1	$(6.7 \pm 0.8) \times 10^3$	1.9 ± 0.2	cumene
2	$(1.5 \pm 0.2) \times 10^4$	2.1 ± 0.2	cumene or styrene
3	$<10^3$	n.d.	cumene

Interestingly, upon addition of millimolar amounts of pyridine to the autoxidating mixture, the antioxidant behavior

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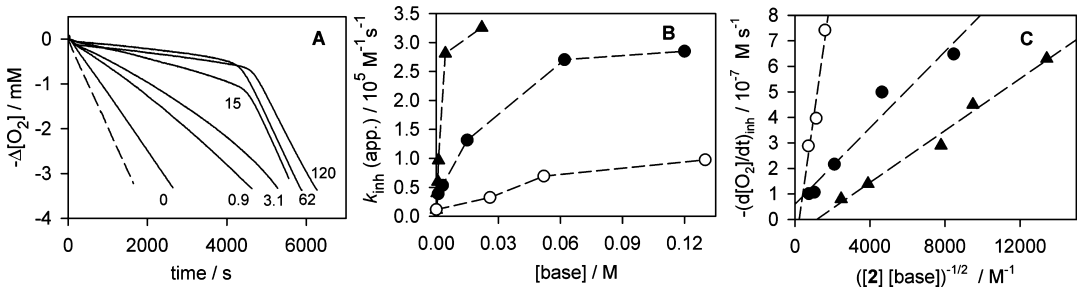

Figure 2. (A) Oxygen consumption during the autoxidation of styrene (4.3 M) in acetonitrile (AIBN = 0.05 M) at 30 °C in the absence (dashed line) and in the presence of **2** (1.5×10^{-5} M) at various [pyridine/mM]. (B) Effect of the addition of 3-Br-pyridine (○), pyridine (●), and 4-NMe_2-pyridine (▲) on the apparent inhibition constant and (C) on the rate of oxygen consumption during the inhibited period.

improved dramatically (Figure 2A). Pyridine itself did not affect the rate of styrene autoxidation.

In the presence of growing concentrations of pyridine, the apparent rate constant for peroxyl radical trapping by **2** increased proportionally and exceded 10^5 M^{-1} s^{-1} at pyridine concentrations higher than 10 mM, thereby matching the reactivity of the reference antioxidant α-tocopherol (k_{inh} = 6.8×10^5 M^{-1} s^{-1} in MeCN[9b]).[13]

Qualitatively similar results were obtained with **1**, which remained consistently less reactive than **2** under identical conditions, but not with the carboxylic derivative **3**, at any tested concentration of pyridine (see Supporting Information).

The observed dependence of the reactivity on the concentration of the weak base pyridine (pK_a = 5.3) is reminiscent of the SPLET mechanism, originally proposed to explain the high reactivity of phenols with DPPH radicals in protic solvents or in the presence of a base.[14,15] According to this mechanism, reaction occurs though a sequence of deprotonation of the inhibitor AH (aided by the base, B) followed by a very fast electron transfer to the radical species, as described by eqs 7−9.

$$AH + B \overset{K_{diss}}{\rightleftharpoons} A^- + BH^+ \qquad (7)$$

$$A^- + ROO^\bullet \xrightarrow{k_8} A^\bullet + ROO^- \qquad (8)$$

$$ROO^- + BH^+ \xrightarrow{fast} ROOH + B \qquad (9)$$

Indeed, as shown in Figure 2B, when we replaced pyridine with the weaker base 3-bromopyridine (pK_a = 2.8) or with the stronger 4-dimethylamino-pyridine (pK_a = 9.7), qualitativly similar results were obtained; however, the apparent reactivity of **2** (and **1**) became, respectively, lower and higher than that recorded upon addition of pyridine. Pre-equilibration of the inhibitor with a base to yield the actual reactive species implies that the rate of oxygen consumption during the inhibited period is described by eq 10, hence it should be reversely proportional to the square root of the concentration of both the inhibitor and the base, as shown in Figure 2C (see also Supporting Information).

$$-\frac{d[O_2]}{dt} = \frac{k_p[RH]R_i}{2k_8\sqrt{k_{diss}[B][AH]}} \qquad (10)$$

5-HU (**1**) is a weak acid with $pK_a(1)$ = 8.11 and $pK_a(2)$ = 11.48;[16] therefore, partial deprotonation under our reaction condition to form the electron-rich monoanion should be a feasible process and could justify its enhanced reactivity in the presence of a base. However, this information is not available for compounds **2** and **3** to aid the rationalization of their higher and much lower reactivities, respectively. Furthermore, there is uncertainty concerning the preferred site of deprotonation for 5-HU derivatives. We determined acidity in water by UV spectroscopy, analyzing spectral changes in the pH range 1.8−14,[17] while to gather the latter information we performed DFT calculations at the B3LYP/6-311+G(d,p) level, using a polarizable continuum model,[18] to locate the most stable neutral tautomers and mono- and dianions of compounds **1**, **2**, and **3**. To lend support to our identification of the most stable structures, we also calculated their low energy electronic transitions[18] and matched them with experimental UV−vis spectra (Figure 3).

Results are summarized in Scheme 3 (see Supporting Information).

For HMU (**2**), three distinct dissociations were observed in water, which we attributed, in the order, to the ring N−H in the 1 position, the O−H substituent in 5, and the ring N−H in 3. While the first dissociation has $pK_a(1)$ just slightly higher than the literature value for $pK_a(1)$ of 5-HU, as expected from the small ED contribution of the 6-methyl

(13) In the presence of pyridine, the reactivity of α-tocopherol is instead decreased by H-bonding to pyridine itself.

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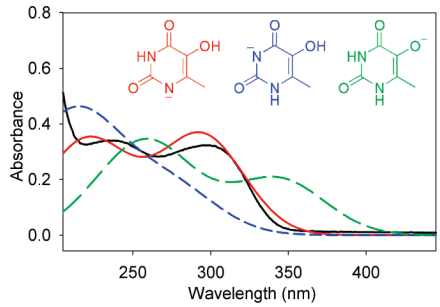
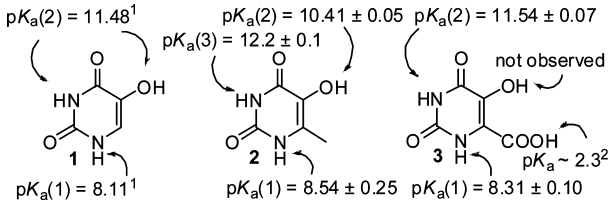


Figure 3. UV spectrum of **2** (7.2×10^{-5} M in water) at pH 9.3 (black line) and TD-DFT-calculated spectra for the three most stable monoanions.

substituent, we suggest that the value of pK_a(2) for 5-HU reported by Albert and Phillips back in 1956[16] is actually the average of those due to dissociation of the O−H in 5 and the ring N−H in 3.

Scheme 3. pK_a Values for Compounds **1**, **2**, and **3** in Water at 25 °C



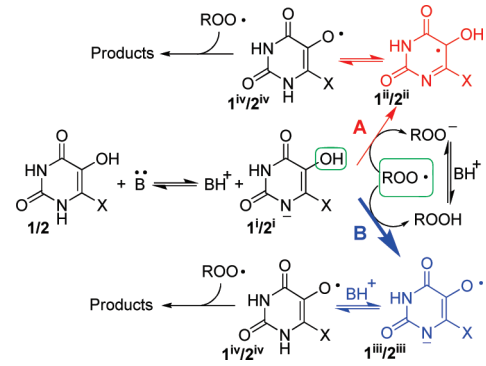
[1] Values from ref 16. [2] Deprotonation resulted in small spectral changes, preventing accurate measurements.

Clearly, pK_a values alone do not promptly justify the higher reactivity of **2** compared to **1**. Particularly, they do not justify the absence of reactivity of compound **3**. The dissociation of the carboxylic group does not significantly alter the acidity of the ring N−H in the 1 position, which is expected to be the key step for the SPLET mechanism. Interestingly, it impairs the subsequent deprotonation of the −OH in 5, showing the occurrence of a strong intramolecular hydrogen bond between the carboxylate group and the −OH in 5. Although the deprotonation of the −OH group is not expected to contribute to the SPLET reaction with peroxyl radicals in the presence of pyridine, the lack of reactivity of compound **3** indicates that the −OH in the 5 position has a key role in the quenching of peroxyl radicals.

We propose the base-promoted reaction of 5-hydroxyuracil derivatives with peroxyl radicals to occur mainly by another mechanism, triggered by pre-equilibration of the compound with the base, as depicted in Scheme 4.

While path A describes the SPLET mechanism, path B suggests the reaction to occur by formal hydrogen atom

Scheme 4. Mechanism of the Base-Promoted Reaction of **1** (X = H) and **2** (X = Me) with Peroxyl Radicals



transfer (HAT or PCET) from the monoanion, which is structurally related to ascorbate, whose reactivity in acetonitrile has recently been attributed to a HAT (PCET) mechanism.[19] Radicals $\mathbf{1}^{iv}$ and $\mathbf{1}^{iii}$ were actually identified by ESR spectroscopy from one-electron oxidation of **1** at pH 4 and 5.5.[20] The lack of reactivity of compound **3** is clearly due to intramolecular H-bonding, which would impair hydrogen-atom transfer from the hydroxylic group, while it is unclear to which extent H-bonding would disfavor ET to the peroxyl radical.

The above considerations suggest that path B is the mechanism for reaction of 5-HU derivatives with peroxyl radicals, although contribution from the SPLET mechanism cannot be completely ruled out and, most likely, a combination of the two meachanisms is responsible for the observed reactivity. Therefore, k_8 in eq 10 should be considered as arising from both processes ($k_{ET/HAT}$).

In summary, we demonstrated that the reactivity of 5-hydroxyuracil derivatives with peroxyl radicals depends on their protonation state, paralleling the behavior of vitamin C. Since in aqueous environments a relevant fraction of deprotonated forms would be available at pH 7.4, this chemistry has relevant biological implications.

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

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