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Pattern of OH Radical Addition to Uracil and Methyl- and Carboxyl-Substituted Uracils. Electron Transfer of OH Adducts with *N,N,N′,N′*-Tetramethyl-*p*-phenylenediamine and Tetranitromethane

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Abstract: With the use of pulse radiolysis with optical detection, the isomer distribution of the radicals formed by addition of OH to the C(5)–C(6) double bonds of uracil, thymine, 3- and 6-methyluracil, orotic acid, and isoorotic acid was determined by utilizing differences in the redox properties of the radical isomers. The radicals formed by addition of OH to C(6) (~20% in the case of uracil) oxidize *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD) with rate constants (1–2) × 10^9 M^{-1} s^{-1} to yield TMPD$^{+}\cdot$; those produced by addition of OH to C(5) (~65% in the case of thymine) reduce tetranitromethane with rate constants ~10^9 M^{-1} s^{-1} to yield $C(NO_2)_3^-$. With the exception of that from orotic acid, the radicals formed by addition of OH at C(5) undergo a base-catalyzed dehydration reaction to yield radicals which are able to oxidize TMPD to yield TMPD$^{+}\cdot$. In the case of uracil it is shown that the dehydrated OH adduct, which is identical with the one-electron oxidation product produced from the reaction of uracil with $SO_4^{-}\cdot$, has a pK_a of 9.7. In the presence of oxygen at pH ~8 the C(5) OH adducts are converted into peroxyl radicals which oxidize TMPD with rate constants ~1 × 10^8 M^{-1} s^{-1}. In the case of thymine the peroxyl radical derived from the radical formed by H abstraction from the methyl group contributes (~10% of OH) to the production of TMPD$^{+}\cdot$.

Introduction

The reactions of pyrimidines like, e.g., uracil with the radicals produced in the radiolysis of water, i.e., e_{aq}^-, H, and OH, have been intensively investigated.[2-16] These reactions are generally accepted to involve addition to the heterocyclic system. In the case of H and OH, the C(5)–C(6) double bond has been identified as the site of addition. On the basis of ESR[4-9] and product analysis[3] studies the OH radical seems to add preferentially to C(5). However, the ESR data[4-7] refer to the steady-state concentrations of the radicals, and these are not necessarily identical with the concentrations of the initially produced radicals. Analogously, with the spin trapping technique,[8,9] the apparent isomer distribution may differ from the true one as a result of incomplete scavenging of individual isomers. The precise pattern of OH addition at C(5)/C(6) is therefore still unresolved.

The radicals formed by reaction of OH with pyrimidines tend to undergo pH-dependent changes.[6,10] In basic solution, the changes follow first-order kinetics.[12-14] On the basis of ESR results, it was suggested[10] that the radicals formed by addition of OH at C(6) undergo base-catalyzed ring opening. However, this interpretation has recently been criticized.[15,16] The structure of the ring-opened radical was questioned on the basis of MO calculations.[15] More importantly, it was found[16] that in the case of uracil the radical formed by reaction with $SO_4^{-}\cdot$ is identical with that produced by OH in basic solution. The authors concluded that a base-catalyzed dehydration reaction takes place. An analogous dehydration reaction has recently been proposed[17] in the case of the OH adduct of 4-pyridone which may be considered a model compound for uracil. It was further shown[17] that the dehydrated OH adduct of 4-pyridone has oxidizing properties (with respect to ascorbate). Oxidizing properties were also found for certain aliphatic radicals characterized by delocalizability of the unpaired spin to oxygen.[18] In the present paper it is shown that the isomeric radicals formed by reaction of OH with uracil and some of its methyl- or carboxyl-substituted derivatives have individually different redox properties and may thereby be distinguished from one another. The redox properties change as a result of dehydration reactions and reactions with molecular oxygen.

Experimental Section

The substrates were obtained from Merck, Fluka, and Sigma and were used as received. The solutions typically contained 2 mM uracil or its derivatives in triply distilled water, and they were saturated with N_2O (O_2 content ≤ 4 ppm) in order to convert e_{aq}^- into OH. In cases where the production of oxygenated radicals was required, the solutions were saturated with a 4:1 mixture of N_2O and O_2. Under these conditions, 97% of e_{aq}^- reacts with N_2O to yield OH. The pH was adjusted using NaOH.

The 3-MeV van de Graaff accelerator and the optical detection system have been described.[19] The solutions were irradiated at room temperature (20 ± 2 °C) with electron pulses of 0.4–1 μs duration using doses that produced 2–4 μM radicals. Dosimetry was performed with N_2O-saturated 10 mM KCNS solutions taking $\epsilon((SCN)_2^{-}\cdot)_{480\ nm} = 7600\ M^{-1}\ cm^{-1}$ and $G(OH) = 6.0$.

N_2O-saturated 10 mM KCNS solutions at pH 7.5 containing 0.2 mM *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD) were used to determine the extinction coefficient of TMPD$^{+}\cdot$. In this system $(CNS)_2^{-}\cdot$ reacts with TMPD to produce TMPD$^{+}\cdot$ ($k = 3.1 \times 10^9\ M^{-1}\ s^{-1}$). Based on $\epsilon((SCN)_2^{-}\cdot)_{480\ nm} = 7600\ M^{-1}\ cm^{-1}$, $\epsilon(TMPD^{+}\cdot)_{565\ nm}$ was found to be 12 500 M^{-1} cm^{-1}, which is equal to the value determined[20] on pro-

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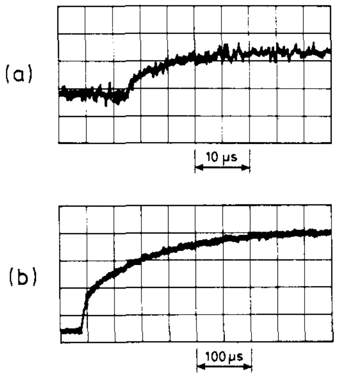

Figure 1. Buildup of $TMPD^{+}\cdot$ measured at 565 nm in N_2O-saturated solutions of 2 mM uracil and 0.1 mM TMPD: (a) pH 8.0, 330 rd; (b) pH 9.7, 340 rd.

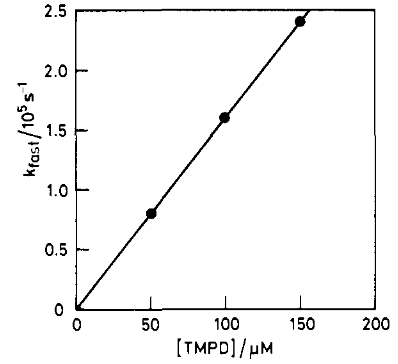

Figure 2. Dependence of the rate of formation of $TMPD^{+}\cdot_{initial}$ on TMPD concentration: [uracil] = 2.0 mM, pH 8.0.

duction of $TMPD^{+}\cdot$ by oxidation with I_2.

The yields of nitroform anion, $C(NO_2)_3^-$ ($\equiv NF^-$), were determined with reference to a N_2O-saturated aqueous solution containing 0.1 M dioxane and 1 mM tetranitromethane (TNM) for which $G(NF^-) = G(OH) + G(H)$[21] = 6.6. On the basis of KCNS dosimetry, $\epsilon(NF^-)_{350\ nm} = 13250\ M^{-1}\ cm^{-1}$, which is lower than the value[22,23] (15000 $M^{-1}\ cm^{-1}$) previously reported.[24]

The concentration of tetranitromethane was measured after converting it at pH ~12 into NF^-.

Results and Discussion

1. Use of *N,N,N',N'*-Tetramethyl-*p*-phenylenediamine (TMPD) as an Electron Donor. a. N_2O-Saturated Solutions. Figure 1 shows the buildup of the optical density at 565 nm, where $TMPD^{+}\cdot$ has an absorption maximum, after pulse irradiating a N_2O-saturated aqueous solution containing 2 mM uracil and 0.1 mM TMPD. At pH 8 a plateau value is reached within 15–20 μs after the pulse (Figure 1a), followed by a decay with essentially second-order kinetics ($2k \sim 4 \times 10^9\ M^{-1}\ s^{-1}$). At pH 9.7, however, there are two components in the buildup kinetics (Figure 1b). After the initial growth of absorption, which is the same as that at pH 8, there is a further increase, which reaches a maximum over ~500 μs, and there is no subsequent decay within several milliseconds. In both stages of the reaction the absorption spectra observed are those of $TMPD^{+}\cdot$.

As shown in Figure 2, the exponential rate of formation of the initial component, as measured at pH 8, depends linearly on the concentration of TMPD. From the slope of the straight line, the second-order rate constant for formation of $TMPD^{+}\cdot$ in this system is $1.7 \times 10^9\ M^{-1}\ s^{-1}$ (Table I).

Table I. *G* Values and Rates of Formation of the Initial Component of $TMPD^{+}\cdot$ (U-6-OH)[a]

pyrimidine	*G*(U-6-OH)[b]	*k*(U-6-OH + TMPD)/$M^{-1}\ s^{-1}$
uracil	1.1	$(1.7 \pm 0.1) \times 10^9$
thymine (5-methyluracil)	1.7	$(1.3 \pm 0.1) \times 10^9$
6-methyluracil	0.6	$(1.1 \pm 0.2) \times 10^9$
3-methyluracil	0.8	$(2.3 \pm 0.3) \times 10^9$
isoorotic acid (5-carboxyuracil)	1.9	$(1.8 \pm 0.2) \times 10^9$
orotic acid (6-carboxyuracil)	0.5	$\sim 10^9$

[a] Measured at 565 nm in N_2O-saturated aqueous solutions at pH ~8: [pyrimidine] = 2.0×10^{-3} M, [TMPD] = $0.5 - 1.5 \times 10^{-4}$ M. Average dose used = 350 rd/pulse. [b] These values include $TMPD^{+}\cdot$ formed by direct reaction of OH with TMPD (see text).

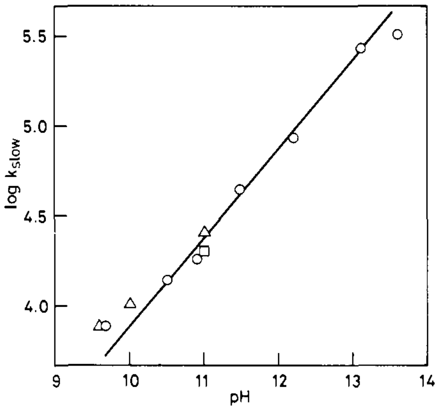

Figure 3. Effect of pH on the rate of formation of the slow component: [uracil] = 2.0 mM, average dose 350 rd/pulse. [TMPD]: □, 50 μM; ○, 100 μM; △, 150 μM.

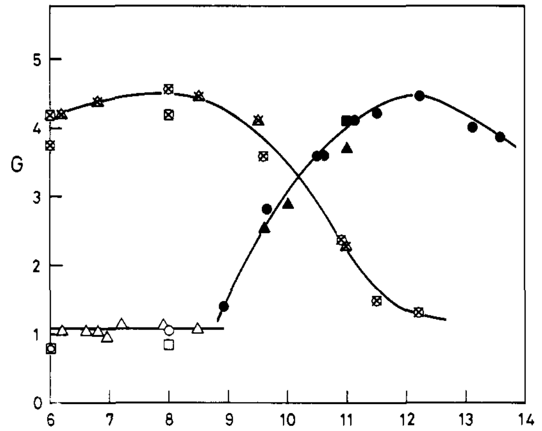

Figure 4. Dependence of $G(TMPD^{+}\cdot)$ on pH: [uracil] = 2.0 mM. [TMPD]: □, 50 μM; ○, 100 μM; △, 150 μM. The dose was 350 rd/pulse: open symbols, N_2O-saturated, ~20 μs after the pulse; filled symbols, N_2O-saturated, after reaching $TMPD^{+}\cdot$ plateau; crossed open symbols, solutions saturated with N_2O–O_2 4:1 after reaching plateau.

In contrast to the initial component, the rate of the (exponential) growth of the second component is *in*dependent of the TMPD concentration but dependent on pH. As shown in Figure 3 the rate increases with pH up to pH 13.5 where there is possibly the onset of a plateau corresponding to $\sim 3 \times 10^5\ s^{-1}$. Figure 4 shows the pH dependence of the initial and total yields of $TMPD^{+}\cdot$. At pH ≳10 the initial component cannot be distinguished from the subsequent one since the contribution of the pH-dependent component to the total $TMPD^{+}\cdot$ yield and its rate of formation increase with increasing pH. The yield of the initial component, $G(TMPD^{+}\cdot)_{initial}$, was independent of radiation dose up to ~1 krd/pulse (rd = rad), as measured in a solution containing 2 mM uracil and 0.1 mM TMPD at pH 8. Between pH 6 and 8.5, $G(TMPD^{+}\cdot)_{initial}$ is 1.1 and independent of [TMPD] in the range

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[24] There is a presently unresolved ~10% inconsistency between the KSCN and TNM[23] dosimetry systems. All yields given in the text are based on KSCN dosimetry. $\epsilon(NF^-)_{350\ nm}$ is therefore taken to be 13 250 $M^{-1}\ cm^{-1}$.

Table II. Total Yield of TMPD$^{+}\cdot$ and Rates of Formation of the Slow Component of TMPD$^{+}\cdot$ at Different pH Values[a]

	$G(\mathrm{TMPD}^{+}\cdot)_{\mathrm{total}}$	$(\mathrm{pH})_{\mathrm{threshold}}$[b]	$(\mathrm{pH})_{\max}$[b]	$k_{\mathrm{slow}}/10^5\ \mathrm{s}^{-1}$				
				pH 9	pH 10	pH 11	pH 12	pH 13
uracil	4.5	8.3	12.2		0.10	0.24	0.76	2.4
thymine	4.1	7.0	10.5	0.35	0.8	1.2	1.6	
6-methyluracil	3.7	7.2	11.4	0.25	0.9	2.0	2.2	
3-methyluracil	4.1	7.5	10.0	0.04	0.45	0.9		
isoorotic acid	4.7	~11	>13					0.6 (pH 13.3)
orotic acid		~13	>13					

[a] Measured at 565 nm in N_2O-saturated aqueous solutions: [pyrimidine] = 2.0×10^{-3} M, [TMPD] = $0.5 - 1.5 \times 10^{-4}$ M. Average dose used 350 rd/pulse. [b] See text for explanation of the symbols.

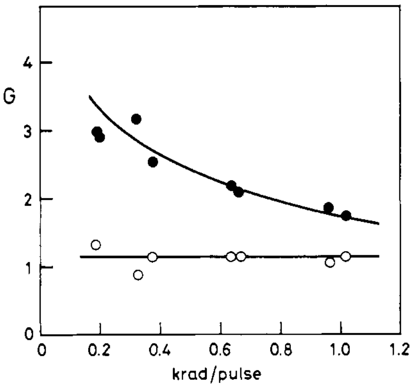

Figure 5. Dependence of $G(\mathrm{TMPD}^{+}\cdot)$ on dose/pulse at pH 9.7: [uracil] = 2.0 mM, [TMPD] = 0.1 mM; (○) initial component, (●) slow component.

0.05 and 0.15 mM. In comparison, the total yield of TMPD$^{+}\cdot$, $G(\mathrm{TMPD}^{+}\cdot)_{\mathrm{total}}$, depended on dose rate (Figure 5). The yields of TMPD$^{+}\cdot$ shown in Figure 4 were therefore determined at a constant dose rate of 350 rd/pulse. The yield of TMPD$^{+}\cdot$ is seen to increase with increasing pH to a maximum value which corresponds to $G = 4.5$ at pH ~12.5. In order to correct this value for loss due to radical–radical reactions, a dose rate variation was again performed and from these data the yield of TMPD$^{+}\cdot$ at zero dose rate was calculated[25] to correspond to $G = 5.1$, which accounts for >90% of the initially produced OH radicals.

Results analogous to those described for uracil were obtained on reaction of OH with methyl- and carboxyl-substituted uracils in the presence of TMPD. Table I contains the yields and the rate constants for formation of TMPD$^{+}\cdot$ via the initial components. The *rate constants* are seen to be relatively independent of the individual structure of the uracil derivative; however, the *yields* of initially formed TMPD$^{+}\cdot$ are sensitive to structural differences: the yields are considerably larger if the compounds are substituted at C(5) as compared to C(6). In contrast to the other compounds, with thymine and isoorotic acid, the decay of the initial component of TMPD$^{+}\cdot$ does not proceed to the zero level.

With thymine and 3- and 6-methyluracil the pH-dependent componens in the growth of TMPD$^{+}\cdot$ are considerably faster, at the same pH, than that in the case of uracil (Table II). As a consequence, the pH at which the second component begins to become noticeable ($\equiv \mathrm{pH}_{\mathrm{threshold}}$) and the pH at which the maximum total yield of TMPD$^{+}\cdot$ is reached ($\equiv \mathrm{pH}_{\max}$) are both lower than in the case of uracil. With isoorotic and orotic acid, however, the second component is either very slow (isoorotic) or not present even at pH 13 (orotic acid).

b. Solutions Saturated with N_2O/O_2, 4:1. The concentration of oxygen in such solutions is 0.28 mM; i.e., >97% of e_{aq}^- reacts with N_2O to produce OH radicals. On reacting OH with 2 mM uracil in these solutions in the presence of, e.g., 0.1 mM TMPD, TMPD$^{+}\cdot$ was found to be produced. The buildup of TMPD$^{+}\cdot$ after the pulse, as monitored at 565 nm, consisted of two components. With both components the (exponential) rate of production of TMPD$^{+}\cdot$ was linearly dependent on [TMPD]; i.e., for both components the rate-determining step involves reaction of uracil-derived radicals with TMPD. The rate constant for formation of TMPD$^{+}\cdot$ via the initial ("fast") component is similar to that found with the same system in the absence of O_2, while the rate constant for the second ("slow") component is $1.4 \times 10^8\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$, as determined between pH 6 and 11. The yield of TMPD$^{+}\cdot$, as formed via the fast process, corresponds to $G = 1.1$, which is the same as that found in the absence of O_2, and it is independent of pH (pH 6–12). The maximum total yield of TMPD$^{+}\cdot$ (formed via the fast plus slow processes at a dose rate of 350 rd/μs) corresponds to $G = 4.7$ and is observed at pH ~8. The G value obtained by extrapolating data from a dose rate variation to zero dose rate is 5.2. This value accounts for 95% of the OH radicals. At pH >8 $G(\mathrm{TMPD}^{+}\cdot)$ decreases (Figure 4) to reach the value ~1.1 at pH >12; i.e., at pH >12 TMPD$^{+}\cdot$ is produced only via the fast component.

Table III. G Values and Rates of Reaction of U-5-OH with TNM[a] and Total Yields of OH Adducts

pyrimidine	k(U-5-OH + TNM)/$10^9\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$	G(U-5-OH) (= $G(\mathrm{NF}^-)$)	([U-5-OH] + [U-6-OH][b])/[OH]
uracil	1.9	4.9	1.02
thymine	1.5	3.6	0.91
6-methyluracil	2.7	5.3	1.00
isoorotic acid	1.7	3.8	0.98
orotic acid	0.2	5.2	0.97

[a] [pyrimidine] = 5.0×10^{-4} M, [TNM] = 5×10^{-5}–5×10^{-4} M. pH ~6. Average dose used: 300 rd/pulse. [b] The values were calculated using $G(\mathrm{OH}) = 5.69$ and 5.92 for solutions containing 2 and 0.5 mM uracil, respectively (see text and ref 26).

Analogous observations were made with the other uracils. In the case of thymine the maximum yield of TMPD$^{+}\cdot$, as measured at pH ~7, was larger by ~10% than that determined at pH 10.5 in N_2O-saturated solution. This situation is different from the uracil case, where the maximum yields of TMPD$^{+}\cdot$ are the same in N_2O/O_2 and in N_2O-saturated solutions (Figure 4).

2. Use of Tetranitromethane (TNM) as an Electron Acceptor. Pulse irradiation of N_2O-saturated aqueous solutions at pH ~6 containing 0.5 mM (substituted) uracil and 0.05–0.5 mM tetranitromethane (TNM) leads to the production of the nitroform anion (NF^-). There was only one component in the formation of NF^-. Under the experimental conditions the OH radicals react exclusively with the uracil to yield OH adducts. Except for orotic acid, the rate constants for formation of NF^- by reaction of TNM with the OH adducts (Table III) are $(1-3) \times 10^9\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$. The yields of NF^- (Table III) are less than that of OH[26] and depend on the structure of the uracil: with the 5-substituted uracils (i.e., thymine and isoorotic acid), the yields are considerably smaller than with uracil itself and, especially, with the 6-substituted uracils (e.g., orotic acid). This dependence on structure is complementary to that observed with $G(\mathrm{TMPD}^{+}\cdot)_{\mathrm{initial}}$, the fast component in the formation of TMPD$^{+}\cdot$. As seen in column 4 of Table III, except for thymine, $G(\mathrm{NF}^-)$ and $G(\mathrm{TMPD}^{+}\cdot)_{\mathrm{initial}}$ add up to ~100% of $G(\mathrm{OH})$.[26]

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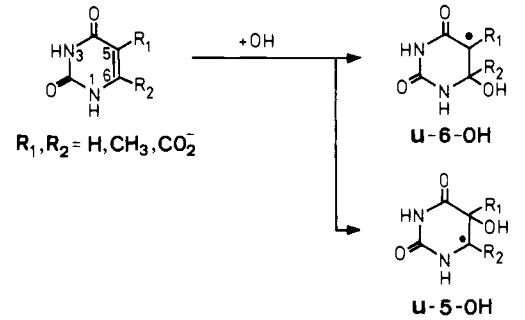
[25] Steenken, S.; O'Neill, P. *J. Phys. Chem.* **1977**, *81*, 505.

[26] For 0.5 mM uracil solutions $G(\mathrm{OH})$ is calculated as 5.92 (see section 3a).

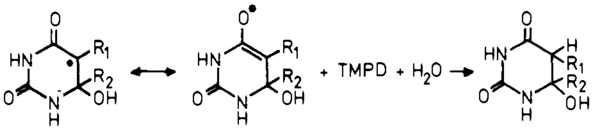
3. Reaction Mechanism. a. Nature of the Oxidizing Radicals in the Absence of Oxygen. In N_2O-saturated solutions of uracil there is a competition for e_{aq}^- between uracil and N_2O which leads to a reduction in the yield of OH. Based on $k(e_{aq}^- + N_2O) = 8.7 \times 10^9$, $k(e_{aq}^- + \text{uracil}) = 9.3 \times 10^9\ M^{-1}\ s^{-1}$,[11] and $G(e_{aq}^-) = 3.2$, for a 2 mM uracil solution at pH ~7, $G(OH) = 5.69$. The OH radical may react with uracil ($k = 6.3 \times 10^9\ M^{-1}\ s^{-1}$)[27] or with TMPD ($k \sim 1 \times 10^{10}\ M^{-1}\ s^{-1}$)[28] to yield $TMPD^{+}\cdot$.[29] Thus, for a 2 mM uracil solution containing 0.1 mM TMPD, the yield of $TMPD^{+}\cdot$ formed by direct reaction with OH, $G(TMPD^{+}\cdot)_{direct}$, is calculated to be ~0.4.

As described in section 1.a, of the two types of radical produced by reaction of OH with the uracils, one type oxidizes TMPD directly whereas the other develops oxidizing properties only after OH^- catalyzed conversion. The former is produced with minor yield, which contains $G(TMPD^{+}\cdot)_{direct}$. The radical is identified as that formed by addition of OH to C(6) (U-6-OH) (reaction 1A). The fact that the $G(TMPD^{+}\cdot)_{initial}$ values are independent of [TMPD] in the range 0.05–0.15 mM demonstrates that the contribution of the direct reaction OH + TMPD is within the limits of experimental error. U-6-OH is of the β-oxoalkyl type which has recently been shown[30,31] to have oxidizing properties (reaction 2).



(1A)

(1B)



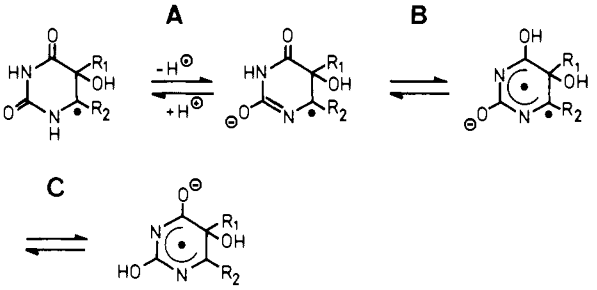
$+ \ TMPD^{\bullet +} + OH^-$ (2)

The assignment of the initial components in the buildup of $TMPD^{+}\cdot$ to the U-6-OH's is supported by the dependence on structure of G(U-6-OH). If the 6 position is substituted by a methyl or carboxyl group, G(U-6-OH) is considerably smaller than in the unsubstituted case or if substitution is at C(5). This is suggested to be due to steric hindrance of OH attack by the substituent. From this dependence on structure it can also be deduced that the radical that oxidizes TMPD is not formed by H abstraction from a NH group in which case the same radical (U· or $U^-\cdot$) would be formed as that in eq 4–6. The yield of production of such a radical would be expected to be independent of the substitution at C(5)/C(6).

From Table I it is seen that the *rate constants* for reduction of the U-6-OH's by TMPD are relatively insensitive to the type of substitution at C(5)/C(6).

Since in the absence of reactive species $TMPD^{+}\cdot$ has a lifetime exceeding the order of seconds, the fast decay (ms) of the initial components must be due to reaction with U-5-OH, the radicals formed by addition of OH to C(5) (reaction 1B). In the case of thymine and isoorotic acid the consumption of $TMPD^{+}\cdot$ does not proceed to completion. Since with these compounds the yields of U-6-OH are larger than with the other substrates and since the U-5-OH's decay also by reaction with each other, their concentration drops below the level necessary to remove $TMPD^{+}\cdot$ quantitatively.

The slow formation of $TMPD^{+}\cdot$ at pH >8 demonstrates that an oxidizing radical is produced from a nonoxidizing precursor by an OH^--catalyzed reaction. At pH ~12 and low dose rates this conversion is ≥90% complete. The oxidizing radical is suggested to be formed from U-5-OH or its anion[32] via keto–enol tautomerization (reaction 3B) followed by water or OH^- elimination (reactions 4B and 4C).



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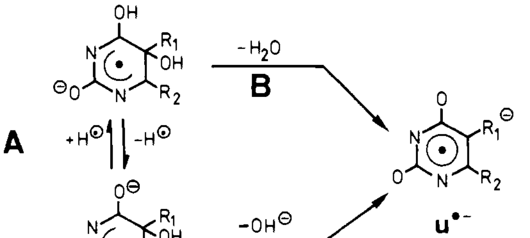
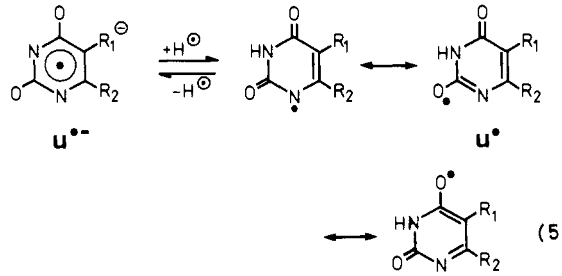
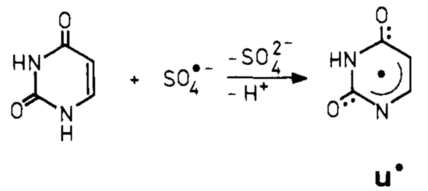


(4)



(5)

Reactions analogous to (3) have recently been demonstrated to occur with OH adducts of 2- and 4-pyridones.[17] The dehydration reaction (eq 4) is similar to that of OH adducts of phenols[33–35] and of 4-pyridone.[17] This type of reaction has been suggested[16] on the basis of ESR results involving the reaction of uracil with $SO_4^-\cdot$ (reaction 6), in which the same radical is produced as that in eq 3–5.



(6)

In order to confirm the identity of the species produced in reactions 3–5 with that in reaction 6, the optical absorption spectra

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(27) Farhataziz; Ross, A. B. *Nat. Stand. Ref. Data Ser., Nat. Bur. Stand.* **1977**, *59*, 66.

(28) Steenken, S., unpublished data.

(29) Rao, P. S.; Hayon, E. *J. Phys. Chem.* **1975**, *79*, 1063.

(30) Steenken, S. *J. Phys. Chem.* **1979**, *83*, 595.

(31) Steenken, S.; Neta, P. *J. Phys. Chem.* **1979**, *83*, 1134.

(32) The pK_a of "the" OH adduct of uracil has been determined as 9.5 (ref 13, 14). Since U-5-OH is the major radical formed, the pK_a of 9.5 is assigned to U-5-OH.

(33) Land, E. J.; Ebert, M. *Trans. Faraday Soc.* **1967**, *63*, 1181.

(34) Adams, G. E.; Michael, B. D. *Trans. Faraday Soc.* **1967**, *63*, 1171.

(35) O'Neill, P.; Steenken, S. *Ber. Bunsenges. Phys. Chem.* **1977**, *81*, 550.

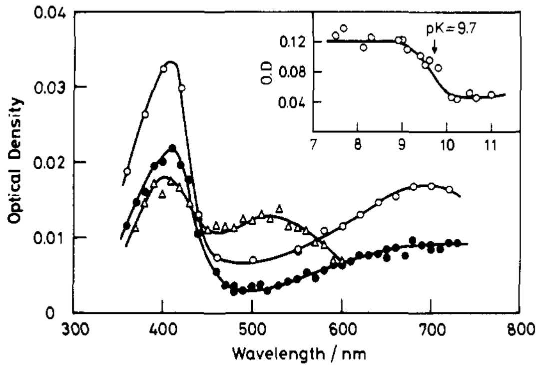
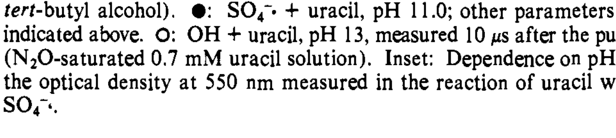


Figure 6. Absorption spectra of radicals produced by the reactions of uracil with OH and $SO_4^{-}\cdot$. Δ: $SO_4^{-}\cdot$ + uracil, pH 8.1, measured ≤2 μs after the pulse (Ar-saturated 0.7 mM uracil, 60 mM $K_2S_2O_8$, 0.06 M *tert*-butyl alcohol). ●: $SO_4^{-}\cdot$ + uracil, pH 11.0; other parameters as indicated above. O: OH + uracil, pH 13, measured 10 μs after the pulse (N_2O-saturated 0.7 mM uracil solution). Inset: Dependence on pH of the optical density at 550 nm measured in the reaction of uracil with $SO_4^{-}\cdot$.

of the radicals were measured in the pH range 7.5–11 using the same concentrations of reagent as in ref 16. Figure 6 shows that the radical produced in reaction 6 has two different spectra in different pH ranges and that the pK_a (reverse eq 5) is 9.7. The spectrum measured using $SO_4^{-}\cdot$ at pH 8.1 is in good agreement with that previously reported;[16] that obtained at pH 11 is qualitatively similar to that[11,12,14] determined on reaction of OH at pH 13. This spectrum was recorded 10 μs after the pulse, i.e., after completion of reactions 3 and 4 (see Table II). The last two spectra can be made to quantitatively coincide if it is assumed that in the $SO_4^{-}\cdot$ system $G(U^{-}\cdot) = G(SO_4^{-}\cdot) = 3.3$ and that in N_2O saturated solutions $G(U^{-}\cdot) = G(\text{U-5-OH}) = 4.9$. The former value (3.3) is from the equation given by Balkas et al.,[36] taking into account that $SO_4^{-}\cdot$ can react with OH^- ($k = 6.5 \times 10^7$ M^{-1} s^{-1}), while the latter value (4.9) is from the experiments with TNM (see section 2 and Table III). The optical experiments are thus in support of the ESR data,[16] confirming the identity of the radical formed in reactions 3–5 with that in reaction 6.

As shown by the formation of $TMPD^{+}\cdot$, the radical from reactions 3–5 or 6 oxidizes TMPD (reaction 7); the rate constant



(7)

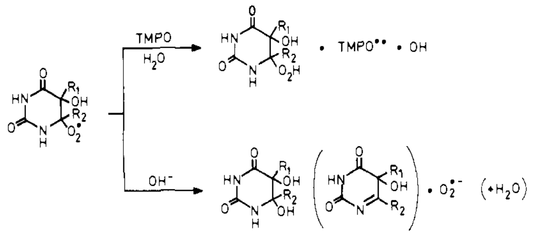
u•⁻ u⁻

for this reaction $k(\text{U}\cdot \text{ or } \text{U}^{-}\cdot + \text{TMPD}) \geq 2 \times 10^9$ M^{-1} s^{-1}. This follows from the observation that at pH ~12 the rate of formation of $TMPD^{+}\cdot$ was still independent of [TMPD] in the range 0.05–0.15 mM.

The rates of dehydration, k_{deh}, of the uracil OH adducts (Table II) increase with increasing $[OH^-]$. Unlike the case of the OH adduct of 4-pyridone,[17] where the dependence of k_{deh} on pH followed a titration curve, i.e., where k_{deh} became independent of pH at pH > pK_a (OH adduct), for uracil (Figure 3) the dependence may be roughly represented by the empirical relation $k_{deh} = (7.6 \times 10^5)[OH^-]^{1/2}$ s^{-1}. This dependence is suggested to reflect approximately equal contributions of reactions 3B and 4C to the kinetics of formation of $U^{-}\cdot$ plus the involvement of the protonation equilibrium 4A. The rate of reaction 4C is probably considerably faster than that of reaction 4B, because of the additional electron density of the doubly ionized OH adduct in reaction 4C.

From Table II it is seen that in the case of substitution of the uracil system with (electron-donating) methyl groups the rates of dehydration increase as compared to uracil. As a result, the limiting pH at which the dehydration reaction becomes visible, $pH_{threshold}$, and the lowest pH at which the maximum yield of $TMPD^{+}\cdot$ is observed, pH_{max}, are both lower with the methylated uracils than with uracil. An analogous enhancing effect on dehydration rates due to substitution by methyl has been observed with 1,2-dihydroxyalkyl radicals.[18] On the other hand, substitution by (electron-withdrawing) carboxyl groups leads to a drastic decrease in the rates of dehydration such that this reaction becomes noticeable only at very high pH. This result is analogous with those involving OH adducts of 4-pyridones[17] and of phenols,[37] where substitution by carboxyl also results in a reduction in the rate of dehydration.

b. Nature of the Radicals in Solutions Containing Oxygen. In solutions saturated with N_2O/O_2 4:1 and containing 2 mM uracil and, e.g., 0.1 mM TMPD, the production of $TMPD^{+}\cdot$ occurs in two kinetically distinguishable steps. On the basis of the rate constant and yield of $TMPD^{+}\cdot$ formation via the fast process, this involves the same radical as that observed in the absence of oxygen, i.e., the radical U-6-OH (reaction 2). The fact that after completion of the slow reaction the total yield of TMPD at pH ~8 (Figure 4) approximates that of OH indicates that U-5-OH is converted into an oxidizing radical by reaction with oxygen. Oxygen normally reacts with organic radicals by addition to yield peroxyl radicals with diffusion-controlled rates.[38] Since the rate constant for formation of $TMPD^{+}\cdot$ by the peroxy radicals (reaction 8A) is only 1.4×10^8 M^{-1} s^{-1}, at dose rates of 300 rd/μs there



(8A)

(8B)

is some 15% loss of initially produced radicals by radical–radical reactions. However, the value obtained for zero dose rate (G = 5.2) accounts for 95% of the initially produced radicals.

The decrease with pH of the yield of $TMPD^{+}\cdot$ is explained by the base-catalyzed decomposition of oxygenated U-5-OH to yield a radical product ($O_2^{-}\cdot$) that is unable to oxidize TMPD. This is shown in reaction 8B.[39] $O_2^{-}\cdot$ is, in fact, unreactive toward TMPD. The rate constant for oxidation of TMPD by $O_2^{-}\cdot$ was determined (using a solution containing 0.3 mM oxygen, 0.1 M formate, and 1 mM TMPD) to be $<10^6$ M^{-1} s^{-1}.

Unimolecular elimination of $O_2^{-}\cdot$ from α-hydroxyperoxyl radicals has previously been observed;[40,41] it is of importance if the carbon carrying $-O_2\cdot$ is substituted by an electron-donating group.[41] In the case of U-5-OH, C(6) is substituted by NH.

As a model for reaction 8A, $HOC(CH_3)_2CH_2O_2\cdot$ was produced by reacting OH with *tert*-butyl alcohol in the presence of 0.3 mM O_2 and 0.05–0.2 mM TMPD. The formation of $TMPD^{+}\cdot$ was observed with $k(HOC(CH_3)_2CH_2O_2\cdot + \text{TMPD}) = 5.5 \times 10^7$ M^{-1} s^{-1}.[42] A reaction of this type, i.e., one that involves peroxyl radicals produced after H abstraction reactions from methyl groups, is suggested to contribute to the production of $TMPD^{+}\cdot$. E.g., in the case of thymine:

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(36) Balkas, T. I.; Fendler, J. H.; Schuler, R. H. *J. Phys. Chem.* **1970**, *74*, 4497.

(37) Neta, P.; Fessenden, R. W. *J. Phys. Chem.* **1974**, *78*, 523.

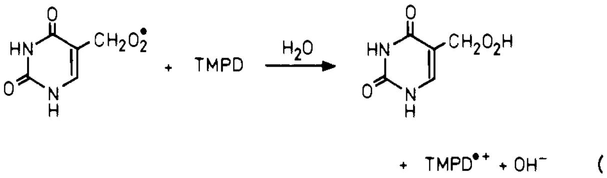
(38) Adams, G. E.; Willson, R. L. *Trans. Faraday Soc.* **1969**, *65*, 2981. Willson, R. L. *Int. J. Radiat. Biol.* **1970**, *17*, 349.

(39) In the case of the corresponding oxygenated H adduct of uracil, there is evidence that elimination of $O_2^{-}\cdot$ yields a compound that contains a double bond between N(1) and C(6) (Scholes, G.; Sonntag, C. v., private communication).

(40) Rabani, J.; Klug-Roth, D.; Henglein, A. *J. Phys. Chem.* **1974**, *78*, 2089.

(41) Bothe, E.; Behrens, G.; Schulte-Frohlinde, D. *Z. Naturforsch.* **1977**, *32b*, 886.

(42) This value is characteristic for simple alkylperoxyl radicals (Steenken, S., unpublished data).

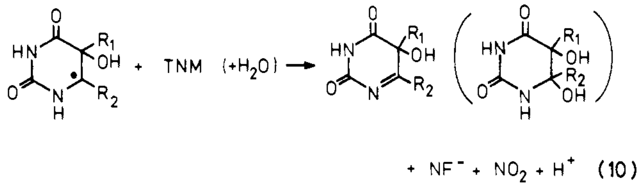


(9)

The fact that with thymine $G(\mathrm{TMPD}^{+}\cdot)$ is larger by 0.5 in the presence of O_2 than in its absence is thus explained by reaction 9. H abstraction from methyl groups in the uracil series has previously been deduced from ESR measurements.[2,8-10]

In solutions containing O_2 the yield of $\mathrm{TMPD}^{+}\cdot$ at high pH is the same as that at pH ~8 in the absence of O_2 (Figure 4). The rate constants for formation of $\mathrm{TMPD}^{+}\cdot$ are also similar ($\sim 2 \times 10^9$ M^{-1} s^{-1}). It is thus reasonable to assume that under both conditions the same radical (U-6-OH) is involved in the production of $\mathrm{TMPD}^{+}\cdot$. It is also possible that TMPD is oxidized by oxygenated U-6-OH (U-6-OH-$O_2\cdot$). However, this is considered unlikely since the rate constants for reaction of TMPD with ordinary organic peroxyl radicals seem to be considerably less than 10^9 M^{-1} s^{-1}.[28]

c. Reduction of Tetranitromethane (TNM). In N_2O-saturated solutions containing 0.5 mM substituted uracil and 0.05–0.08 mM TNM >97% of e_{aq}^- reacts with N_2O to yield OH. The formation of NF^- thus occurs via OH and possibly H adducts. As seen in Table III, the yields of reduced TNM, as measured by the concentration of NF^-, are less than that of OH and depend on the position of the substituent in a way which is opposite to that observed with $G(\mathrm{TMPD}^{+}\cdot)_{\mathrm{initial}}$; in the case of substitution at C(5) the yield of NF^- is considerably less than if the substituent is at C(6). This dependence on the pattern of substitution is expected if NF^- measures the concentration of U-5-OH. U-5-OH should, in fact, be a reducing radical (reaction 10) since the carbon carrying the unpaired electron is substituted by the electron-donating amino group. In comparison, U-6-OH, where the unpaired electron is conjugated with the electron-withdrawing carbonyl group, has been shown to be an oxidizing radical.



(10)

Column 4 of Table III contains the sum of the yields of U-5-OH and U-6-OH, measured at pH 6–8 via NF^- and $(\mathrm{TMPD}^{+}\cdot)_{\mathrm{initial}}$. Except for thymine, the values are equal to the yield of OH. The material balance is thus very satisfactory. This is also true for thymine, if the ~10% yield of H abstraction from the methyl group is taken into account.

Conclusions

The OH radical reacts with uracil and substituted uracils by addition to the C(5)–C(6) double bond. The radicals formed by attachment to C(6) (U-6-OH) have oxidizing properties with respect to TMPD; those produced by addition to C(5) (U-5-OH) are able to reduce TNM. These differences in redox properties were utilized to determine the distribution of OH attachment between C(5) and C(6). With unsubstituted uracil the ratio of attachment to C(5) as compared to C(6) is between 5 and 8, depending on the exact amount of $\mathrm{TMPD}^{+}\cdot$ formed by direct reaction with OH. Substitution by methyl or carboxyl groups at C(5) or C(6) decreases to approximately the same extent the probability of OH attack at the site of substitution and favors addition at the adjacent carbon.

The radicals U-5-OH undergo a base-catalyzed dehydration reaction by which they lose their reducing properties and become oxidizing in nature. This type of conversion is analogous to those shown by OH adducts of phenol, which are reductive,[43] to yield phenoxyl radical, which is an oxidant.[31,44] Further analogies with respect to conversion by dehydration of a reducing into an oxidizing radical exist with the OH adducts of anisole[45] and of 4-pyridone[17] and, in the aliphatic series, with α,β-dihydroxyalkyl radicals.[18]

The rates of dehydration of U-5-OH increase with increasing pH. At the same pH the rates increase on introducing methyl substituents into the uracil system. Substitution by carboxyl groups results in a drastic decrease in the rates of dehydration. The rate constants for dehydration, as determined by the formation of $\mathrm{TMPD}^{+}\cdot$, are very similar to the values previously measured[14] by direct observation of the OH adducts. However, these first-order changes in the optical spectra were interpreted in terms of ring opening of U-6-OH. The ring-opening hypothesis[10] has recently been questioned on the basis of ESR results.[15,16] The results described in the present paper do not exclude ring opening in the case of the U-6-OH's since the ring-opened radicals are still of the oxidizing[18] β-oxoalkyl type. However, from the results it is clear that the observed first-order changes involve the U-5-OH's which are the main products of the reaction of OH with the uracils. There does not appear to be a reasonable way by which the U-5-OH's can undergo ring opening and become oxidizing in nature. However, the results presented and also the previously reported data[2,3,11-14,16] on the properties of uracil OH adducts are in agreement with a mechanism involving dehydration. In conclusion, and in support of the ESR results,[16] the radical observed by Neta[10] at high pH and assigned to ring-opened U-6-OH is identified as dehydrated U-5-OH.

Acknowledgment. We thank Professor D. Schulte-Frohlinde and Dr. E. Bothe for valuable discussions.

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(43) Raghavan, N. V.; Steenken, S. *J. Am. Chem. Soc.* **1980**, *102*, 3495.
(44) Schuler, R. H. *Radiat. Res.* **1977**, *69*, 417.
(45) Steenken, S.; Raghavan, N. V. *J. Phys. Chem.* **1979**, *83*, 3101.