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that is a simple function of the number of protic hydrogens, and hydrophobic solvation which is a simple function of the number of alkyl hydrogens.

By use of gas-phase data, a model emerges that accounts for the complexities of ion solvation in terms of simple, regular trends as a function of ion structure.

Acknowledgment. I thank Drs. George Ford and Robert Wood for helpful discussions and Dr. C. A. Deakyne for making ab initio results available.

Reactions of Oxidizing Radicals with 4,6-Dihydroxypyrimidines as Model Compounds for Uracil, Thymine, and Cytosine

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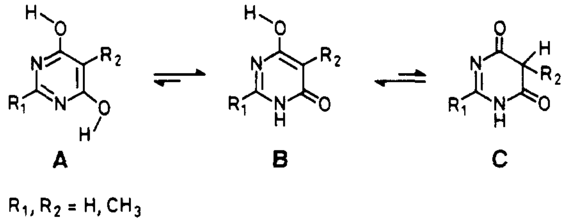
The reactions of the oxidizing radicals OH, O^-, SO_4^-, and Br_2^- with 4,6-dihydroxypyrimidine (4,6-DHP) and with 2- and 5-methyl-4,6-DHP were investigated by using in situ radiolysis and photolysis ESR and pulse radiolysis techniques. In the pH range 1–6 the OH radical reacts with these compounds ($k = (4\text{–}7) \times 10^9$ M^{-1} s^{-1}) selectively by addition at C-5 to produce radicals characterized by two pairs of magnetically equivalent nitrogens and exchangeable protons. On the basis of the coupling constants and from the increased pK_a value (8.3) of the radical from 4,6-DHP relative to that (5.3) of the parent compound, a diketo-type structure is suggested for the OH adducts. At pH 8–12 a base-catalyzed dehydration of the radical anions takes place which leads to the formation of species formally derived from the ionized parent compound by one-electron oxidation followed by deprotonation. These radicals can also be produced by reaction with SO_4^- or Br_2^-. Those from 4,6-DHP and from 2-Me-4, 6-DHP were seen in two different protonation states: as a neutral radical between pH 3 and 5, and as the radical anion above pH $\approx$ 5. Methyl groups on the 4,6-DHP molecule increase the rate of dehydration of the OH adducts. The O^- radical reacts with the methylated 4,6-DHP's by H abstraction from the methyl groups. The structures of all the radicals from the 4,6-DHP's are compared with those from naturally occurring dioxopyrimidines such as the uracils.

Introduction

The interactions of the naturally occurring oxopyrimidines such as uracil, thymine, and cytosine with the radicals from the radiolysis of water such as OH, H, and e_{aq}^- have been intensively studied.[2] These radicals react by addition to the heterocyclic system. With respect to this addition reaction, the OH radical, in spite of its high reactivity, exhibits a remarkable selectivity,[3-5] which results from and reflects the electrophilicity[6] of OH. With uracil, thymine, and cytosine OH addition takes place predominantly at C-5 to give 6-yl radicals which have reducing properties,[4,5] due to α-substitution of the carbon-centered 6-yl radical by nitrogen.[7] This radical undergoes a base-catalyzed dehydration reaction which changes its redox character; i.e., it converts from a reducing radical to an oxidizing one.[4,5]

Unlike uracil and thymine, with the 4,6-dihydroxypyrimidines the molecular structure is symmetric. Unlike uracil and thymine, in aqueous solution the predominant form is not the diketo structure C, (eq 1) but the monoketo/monoenol form B,[8,9] characterized by pK_a values of 5–6.[8-10] In this respect the 4,6-dihydroxypyrimidines are analogous to cytosine, which exists as a keto/enamine. On the basis of this analogy an investigation of the reactions of the 4,6-dihydroxypyrimidines with oxidizing radicals appeared appropriate, especially in view of the recent demonstration[10] of the usefulness of these compounds as models for the uracils in reactions with reducing radicals.



(1)

Experimental Section

All solutions were deoxygenated for at least 30 min by bubbling with N_2O or argon. The pH of the solutions was adjusted by addition of NaOH, $HClO_4$, or phosphate (in the pH range 6–8). 4,6-Dihydroxypyrimidine was obtained from Fluka and 2-methyl-4,6-dihydroxypyrimidine from Aldrich and both were used without further purification. 5-Methyl-4,6-dihydroxypyrimidine was synthesized from formamidine hydrochloride (Fluka) and diethyl methylmalonate (Fluka) according to literature.[11] 4,5,6-Trihydroxypyrimidine was synthesized by a similar method, using as reagents formamidine hydrochloride and diethylhydroxymalonate; this reagent was prepared from diethyl malonate and lead tetraacetate to give diethyl acetoxymalonate which was then cleaved by dry hydrogen chloride in absolute ethanol in analogy with the preparation of the corresponding dimethyl ester.[12]

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(1) (a) Instituto Superior Tecnico. (b) Max-Planck-Institut.

(2) For reviews see: (a) Scholes, G. In *Effects of Ionizing Radiation on DNA*; Bertinchamps, A. J., Ed.; Springer: Berlin 1978; p 153. Teoule, R.; Cadet, J. *Ibid.*, p 171. (b) Cadet, J.; Berger, M. *Int. J. Radiat. Biol.* **1985**, *47*, 127. (c) von Sonntag, C.; Schuchmann, H.-P. *Int. J. Radiat. Biol.* **1986**, *49*, 1.

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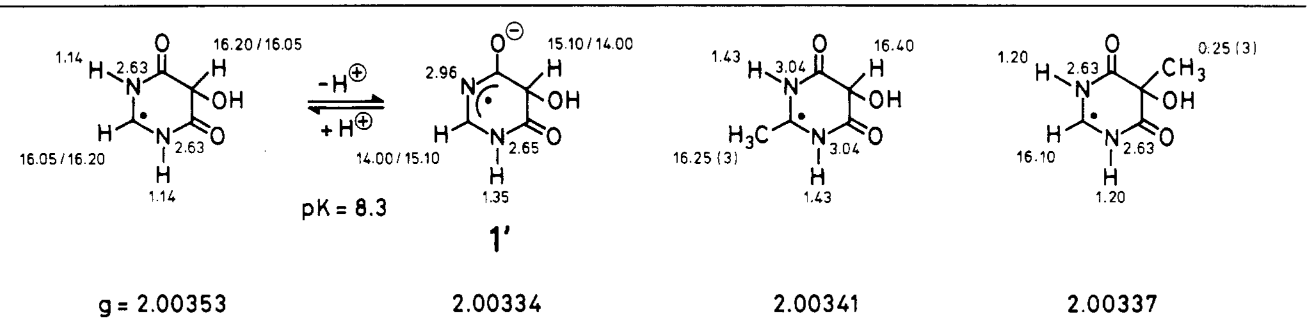
TABLE I: Structures and ESR Parameters[a] of the Radicals 1 Produced by Addition of OH to C-5 of 4,6-DHP's



[a] The coupling constants are in gauss. The g factors have been measured by the NMR method and are corrected for second-order effects. A slash between two numbers means "or".

The in situ radiolysis (using 3-MeV electrons) and photolysis ESR experiments were performed as described.[13,14] The pulse radiolysis set-up has also been described.[15] For dosimetry, N_2O saturated 10 mM KSCN solutions were irradiated and the optical density was monitored at 480 nm. $G((SCN)_2^{\bullet-})$ was taken to be 6.0 and $\epsilon((SCN)_2^{\bullet-})$ at 480 nm to be 7600 M^{-1} cm^{-1}.[16]

Results and Discussion

Radiolysis of water leads to the radicals e_{aq}^-, OH, and H, as shown in eq 2. The values in parentheses indicate the yields

$$H_2O \rightsquigarrow e_{aq}^-(2.7),\ H(0.6),\ OH(2.8) \qquad (2)$$

expressed in terms of G values (molecules/100 eV of absorbed radiation). The reducing e_{aq}^- can be converted into the oxidizing OH by reaction with N_2O:

$$e_{aq}^- + N_2O + H_2O \rightarrow OH^- + N_2 + OH \qquad (3)$$

In N_2O-saturated solution the half-life for reaction 3 is ≤5 ns. This means that the e_{aq}^- are quantitatively scavenged to yield OH the G value of which then corresponds to 6.0.[16] Thus in these solutions the ratio of the primary radicals OH and H is 10 to 1; i.e. the radical chemistry is dominated by the presence of OH.

1. Production of OH Adducts. ESR Studies. When N_2O-saturated aqueous solutions containing, e.g., 0.5 mM 4,6-DHP were irradiated with 3-MeV electrons, lines from a radical (with a strongly polarized spectrum) were seen (Figure 1) that consisted of a doublet (16.20 G) of doublets (16.05 G) of 1:2:3:2:1 quintets (2.63 G) of 1:2:1 triplets (1.14 G) with a g factor of 2.00353. The quintet is due to two equivalent nitrogens, and the triplet to two equivalent hydrogens. This radical was found to be detectible in the pH range 1–10.

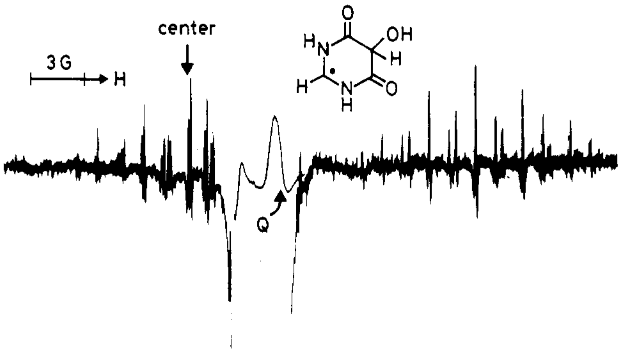
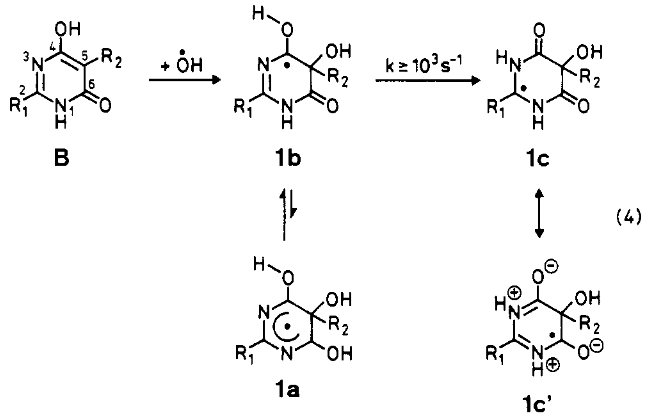


Figure 1. ESR spectrum recorded on electron irradiation of N_2O-saturated 0.5 mM 4,6-DHP at pH 4.6 and ≈5 °C. The spectrum is highly polarized; i.e., only the center and high-field groups are visible. Q denotes the quartz signal.

When water was substituted by D_2O as a solvent[17] (at pH 4.5), the same radical was seen; however, the 1:2:1 triplet of 1.14 G was replaced by a 1:2:3:2:1 quintet of 0.175 G (ratio $a(H):a(D) = 6.52$). This shows that the two equivalent protons in the radical are exchangeable. A radical structure that is consistent with these data is obtained by addition of the OH radical to C-2 or to C-5 of 4,6-DHP. A way to distinguish between these alternatives is to replace H at C-2 and C-5 by a substituent, e.g., a methyl group. It was found that in going from 4,6-DHP to 2-Me-4,6-DHP one of the 16-G doublet splittings is replaced by a 16.25-G quartet splitting, whereas with 5-Me-4,6-DHP the quartet splitting is only 0.25 G (see Table I), indicating that in this case the methyl group interacts quite weakly with the unpaired electron. On the basis of these results it is evident that the unpaired spin is localized mainly on C-2, i.e., that addition of OH has occurred at C-5, as indicated in eq 4.



(4)

Addition of OH to C-5 (which carries the highest electron density[8,9]) is expected also on the basis of the electrophilic character of the OH radical. Finally, attachment of OH at C-5 can be concluded also from the similarity of the coupling constants of the OH adduct of 4,6-DHP with that[10] of the H adduct, where the OH group at C-5 is replaced by H. With this radical the existence of the methylene configuration at position 5 (and not 2) was proved by isotopic exchange experiments.[10]

Both the diketo structure **1c** and the dienol form **1a** satisfy the experimental data with respect to equivalence of the nitrogens and the exchangeable protons. However, structure **1c**, where the unpaired electron is essentially localized on C-2, is in much better agreement with the large splitting (16 G) of the hydrogen or the methyl group at C-2 than in structure **1a**, where the electron is delocalized from C-4 to C-6. A further argument in favor of **1c**

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(17) Such experiments were performed in several instances in order to either facilitate spectrum analysis or to decide about possible positions of ring attack. By using NMR detection of 4,6-DHP dissolved in Me_2SO-d_6 with added deuteriated formic or acetic acid, it was seen that a rapid (≤1 min) exchange with deuterium took place, not only with N–H and O–H protons, but also with the C–H proton bonded to the 5-position. This exchange must proceed via the diketo form C of 4,6-DHP in equilibrium with the other tautomeric forms. However, in aqueous solution at low concentrations of acid or base the exchange at C-5 is quite slow (≥2 h).

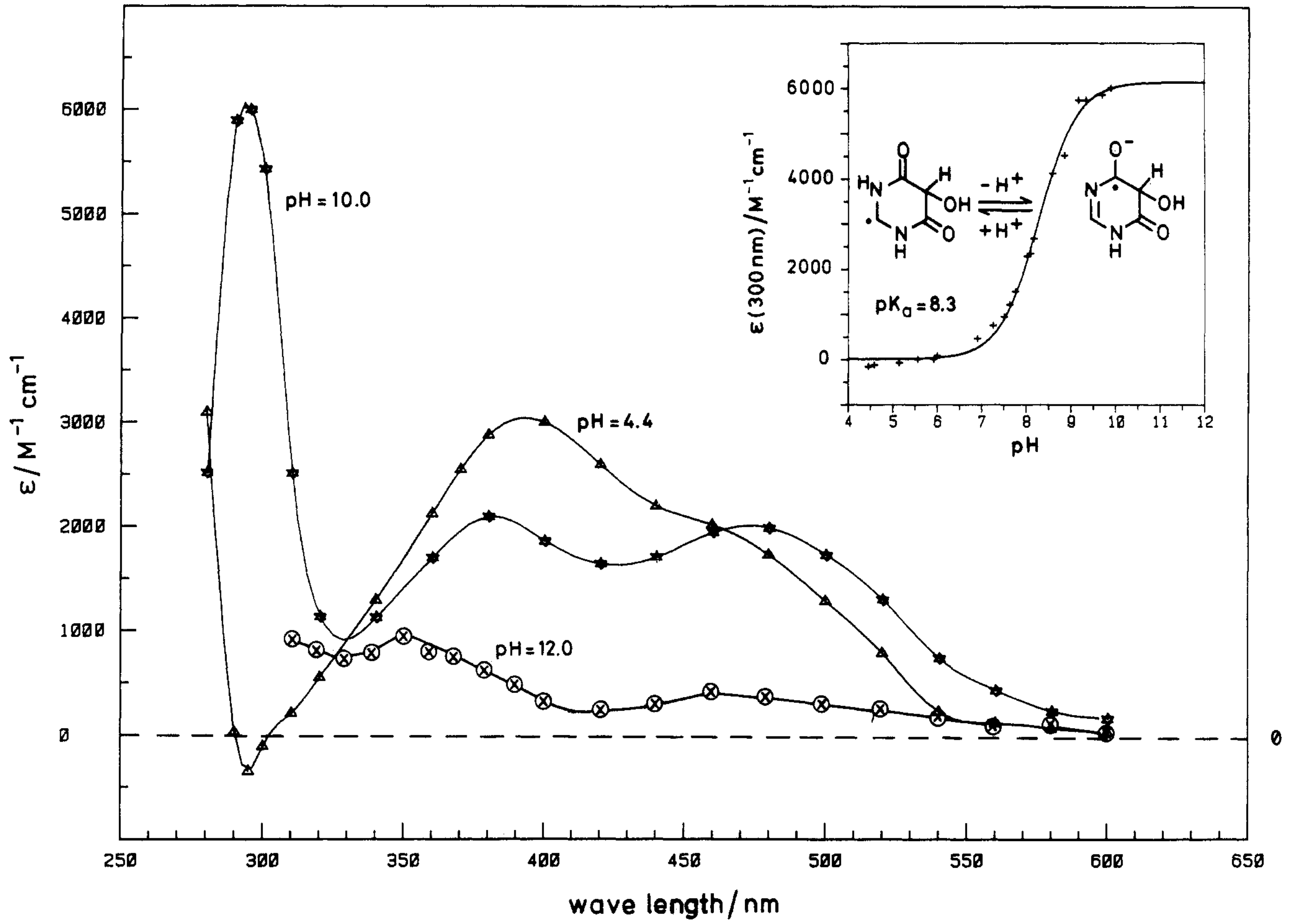


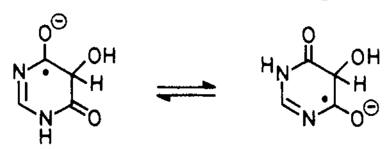
Figure 2. Absorption spectra measured on reaction of OH with 0.5 mM 4,6-DHP at 2–5 μs (pH 4.4), 1–2 μs (pH 10.0), and 10 μs after the pulse (pH 12.0). The spectrum at pH 10 is recorded *before* the occurrence of significant dehydration, that at pH 12 *after* completion of the dehydration reaction. The ε-values are based on G(radical) = G(OH) = 6.0. The spectra are *not* corrected for depletion of substrate. Insert: The dependence on pH (with 1 mM phosphate) of the OD at 300 nm measured at 3–20 μs after the pulse. The sigmoidal curve is from a computer fit which yields a pK of 8.3.

are the coupling constants for the exchangeable hydrogens: values of the order 1 G are expected for protons attached to the ring nitrogens whereas the values for (external) OH groups seem to be much smaller (≤0.1 G).[3b] A final support for **1c** is the pK_a value of this radical: with 4,6-DHP ($R_1 = R_2$ = H) the pK_a value was determined by pulse radiolysis (see later) to be 8.3, i.e., three units *larger* than that[8-10] of the (enolic) parent compound. If the radical existed in the dienol form **1a**, a pK_a value *smaller* than that of the parent would be expected.

A note may be added concerning the ionic resonance forms **1c′**. These have been introduced to account for the fact that the coupling constants for the hydrogen at C-5 are relatively large, indicating a nonnegligible interaction with the unpaired electron. A similar phenomenon has previously been seen with hydrogen adducts to the 4,6-DHP system, in which case an interaction with a pseudo-π orbital was invoked.[10] The ionic resonance structures **1c′** symbolize an alternative spin-transfer mechanism.

An estimate can be made for the rate constant for ketonization of the monoketo/monoenol type adduct originally formed by addition of OH to C-5 of the parent compound. Based on a typical lifetime for radicals under steady-state conditions of the order of milliseconds, the rate constant $k(\mathbf{1b}\rightarrow\mathbf{1c})$ must be $\geq 10^3$ s^{-1}, a value similar to that for the corresponding reaction on hydrogen addition to C-5 of the DHP's.[10]

2. *Ionization of the OH Adducts. a. ESR Studies.* When the pH of solutions containing 4,6-DHP was raised above ≈7, the intensity of the lines due to the neutral OH adduct **1** ($R_1 = R_2$ = H) decreased and lines from a new radical (**1′**) showed up. The coupling constants of **1′** (Table I) are similar, but somewhat smaller than those of **1**. In particular, **1′** has one doublet splitting less than **1** and the two nitrogens are no longer equivalent. By working in D_2O (pD = 9.4) instead of H_2O, the smallest doublet splitting of **1′** (a(H) = 1.37 G) is converted into a 1:1:1 triplet with a(D) = 0.21 G (ratio a(H)/a(D) = 6.52) which shows the exchangeability of this nucleus. All the other splittings were unaffected. Radical **1′** is obviously the conjugate base of **1**. The decrease in the coupling constants on ionization of **1** reflects the increased delocalization of the unpaired spin over a system which is enolic in nature and which can exist in two tautomeric forms. Since the two nitrogens are magnetically nonequivalent [a(N-1) − (a(N-3 = 0.3], the rate of tautomerization must be $\leq 0.3 \times 2\pi \times 2.8 \times 10^6$ s^{-1} = 5×10^6 s^{-1} (slow exchange).



With 2-Me- and 5-Me-4,6-DHP the radical anions corresponding to **1′** were not detected, probably because these anions undergo dehydration too rapidly (see subsection 3).

b. Pulse Radiolysis Studies. In Figure 2 are shown the absorption spectra measured after reaction of OH with 0.5 mM 4,6-DHP at pH 4.4, 10.0, and 12.0. The former two spectra are assigned to the neutral radical and, respectively, its conjugate base, formed by deprotonation from one of the amido groups. The dependence of OD at 310 nm (see inset, Figure 2) and at 440 nm (not shown) follows a titration curve and from this dependence the pK_a value for the radical is obtained as 8.3, which is three units larger than that (5.3)[8-10] for the parent compound, and it is in agreement with the qualitative data from ESR (see subsection 2a). The rate constants for reaction of OH with the 4,6-DHP's

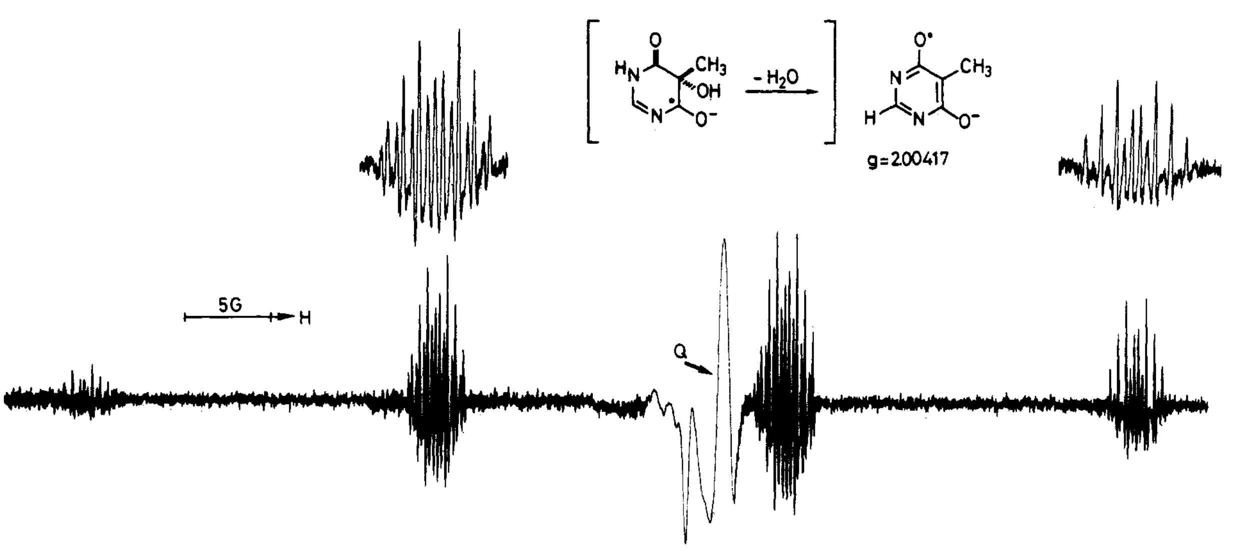
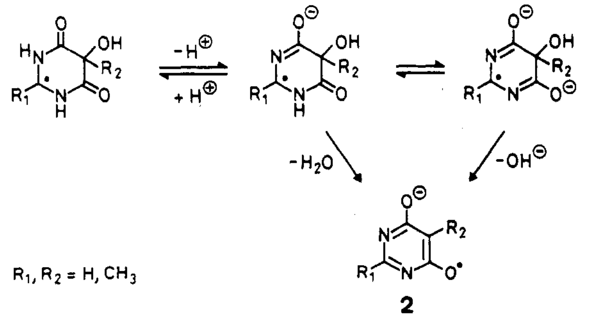


Figure 3. ESR spectrum recorded on reaction of OH with 0.5 mM 5-Me-4,6-DHP at pH 10 and ≈5 °C. With the insets the field axis is expanded by the factor 2.5.

were measured by monitoring the buildup of OD in the wavelength region 300–400 nm after the electron pulse. The values (see Table II) are ≈6 × 10^9 M^{-1} s^{-1} for reaction with the neutral pyrimidines, similar to the rate constants[18] for reaction of OH with the naturally occurring pyrimidines. The values for the ionized compounds are a factor ≈1.7 higher.

3. Dehydration of the OH Adducts. a. ESR Studies. Lines from radical **1′** ($R_1 = R_2 = H$) could be seen between pH 7.5 and 10 (in the presence of phosphate buffer only between 7.5 and 8.5). The lower limit is reasonable on the basis of the pK_a of **1** (=8.3). That there is an upper limit indicates that **1′** undergoes an OH^--catalyzed rearrangement reaction. In fact, at pH ≈ 11.5 very weak lines from a new radical (type **2**) with a doublet splitting of 17.45 and another one of 0.70 G and two equivalent nitrogens (a(N-1,N-3) = 0.35 G) were detected. With 2-Me- and 5-Me-4,6-DHP analogous radicals (see Table III) were produced in basic solution from the OH adducts; however, they were much more easily detectible (see Figure 3) since their lines are narrower than those of the radical from 4,6-DHP. From the fact that with 5-Me-4,6-DHP the large splitting is a quartet ($a(CH_3)$ = 20.44 G) while with 2-Me-4,6-DHP it is a doublet (a(H) = 17.80 G) it is evident that the unpaired spin is essentially localized on C-5. From the facts that the nitrogens are equivalent (a(N-1) = a(N-3) = 0.3–0.5 G) and that the coupling constants do not change with pH between 9 and 13 it can be concluded that none of the nitrogens is protonated. A structure that is in line with these observations is the semiquinone-type radical **2** that is formed by elimination of H_2O or OH^- from the ionized OH adduct(s), as shown in eq 5.



(5)

An analogous reaction has been shown to occur with the OH adducts of uracil,[4,19] thymine,[4] and cytosine.[5] The products of these reactions have oxidizing properties,[4,5] probably due to the unpaired spin at N-1.[4,5,19,20]

TABLE II: Rate Constants for Reaction of OH and $Br_2^{\bullet-}$ with 4,6-DHP's[a]

substrate	$k^b/M^{-1}\ s^{-1}$ OH	$k^b/M^{-1}\ s^{-1}$ $Br_2^{\bullet-}$
4,6-DHP	5.7 × 10^9 (4.0)	<10^7 (3–4)
4,6-DHP anion	7.9 × 10^9 (9.2)	6.0 × 10^8 (7.4)
2-Me-4,6-DHP	7.4 × 10^9 (3.7)	<10^7 (3–4)
2-Me-4,6-DHP anion	1.2 × 10^{10} (7.7)	7.5 × 10^8 (7.5)
5-Me-4,6-DHP	4.2 × 10^9 (4.3)	<10^7 (3–4)
5-Me-4,6-DHP anion	8.8 × 10^9 (7.5)	9.6 × 10^8 (7.7)

[a] 20 °C, [DHP] = 0.05–0.5 mM. [b] The numbers in parentheses are the pH of measurement.

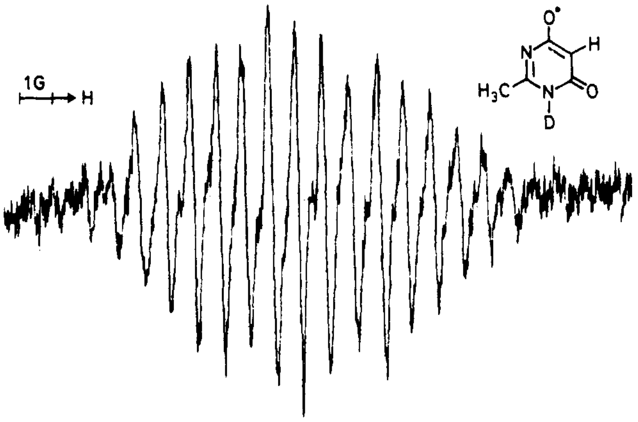


Figure 4. ESR spectrum measured on UV photolysis of 20 mM $K_2S_2O_8$ in the presence of 3 mM 2-Me-4,6-DHP in D_2O at pD 6.4 (1 mM phosphate buffer) and ≈2 °C.

b. Pulse Radiolysis Studies. Above pH ≈8.5 the absorptions of the OH adducts at, e.g., ≈300 nm were found to decrease with time to very low values of optical density. The approximately first-order rates of decrease of OD increased with increasing [OH^-] (measured up to pH ≈12), whereby the rates were roughly proportional to $[OH^-]^{1/2}$. This type of relationship has previously been found with OH adducts of uracils and cytosines,[4,5] where it was interpreted in terms of dehydration from both the mono- and the dianion radicals, analogous to scheme 5. The slopes (intercepts) of plots of k_{obsd} (dehydration) vs. $[OH^-]^{1/2}$ were found to be 1.7 × 10^6 (≈0 s^{-1}), 4.7 × 10^6 (1.5 × 10^5 s^{-1}), and 5.3 × 10^6 s^{-1} $[OH^-]^{-1/2}$ (1.2 × 10^5 s^{-1}) for 4,6-DHP, 2-Me-4,6-DHP, and 5-Me-4,6-DHP, respectively, which shows that elimination of H_2O or OH^- occurs much more readily from the methylated systems.

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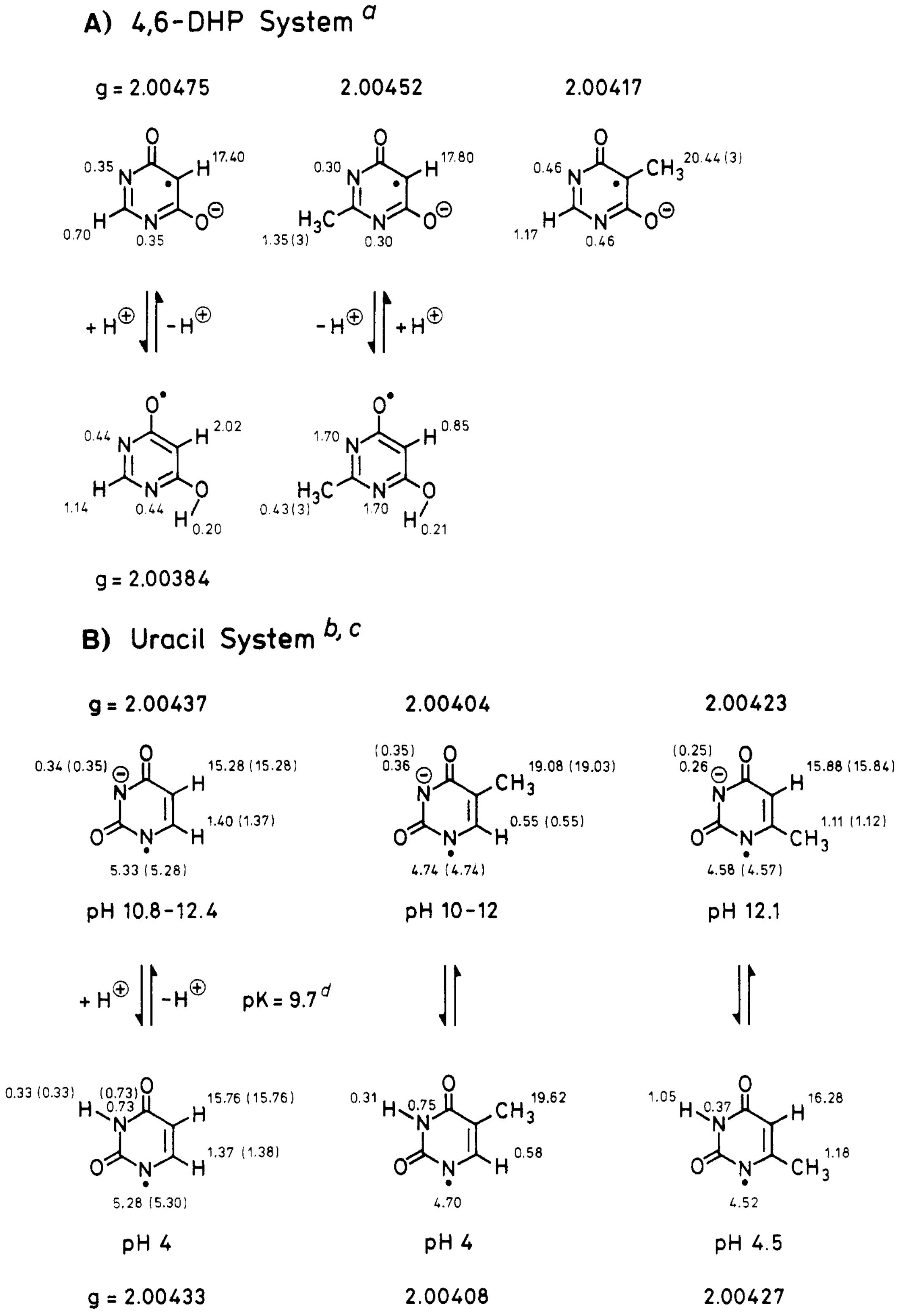
(20) Chipman, D. M. *Radiat. Res.* **1981**, *85*, 257.

TABLE III: Structures and ESR Parameters of the Radicals Produced by One-Electron Oxidation from (A) 4,6-DHP's and (B) Uracils



[a] Measured at ≈5 °C and pH 8–13 using OH, $Br_2^{\bullet -}$ or $SO_4^{\bullet -}$ radicals for initiation. [b] The parameters of the radicals in the upper row are from Neta's work (ref 23). The structures have been written according to (or in analogy to) the assignment given by Fessenden (ref 19). The assignment of the splittings to N-1 and N-3, which differs from those in refs 19 and 23, is based on the spin density distribution for the radical from uracil as calculated by ref 20. The values in parentheses are from measurements (this work) using photochemically produced $SO_4^{\bullet -}$ at pH 10.2. [c] The parameters of the radicals in the lower row were measured using photochemically produced $SO_4^{\bullet -}$. The values for uracil-1-yl are from ref 19 and from this work (the values in parentheses). The values for 5- and 6-methyluracil-1-yl are from this work (1–2 mM substrate, 5 mM $S_2O_8^{2-}$, 3–5% acetone, ≈4 °C). [d] From ref 4.

A similar rate-enhancing effect of methylation has been observed in the case of uracils[4] and cytosines.[5]

The optical absorption spectrum measured at pH 12 (Figure 2) is due to radical **2** ($R_1 = R_2 = H$). At this pH the rate of dehydration of the OH adduct is $\approx 4 \times 10^5$ s^{-1}. Exactly the same spectrum was observed on replacement of OH by $Br_2^{\bullet -}$ (see subsection 4).

Similar to the dehydrated OH adducts of the uracils and cytosines, the dehydrated OH adducts of the 4,6-DHP's have oxidizing properties, e.g., with respect to *N,N,N′,N′*-tetramethyl-*p*-

TABLE IV: Rate Constants for Oxidation of TMPD by the Semiquinone-Type Radicals 2[a]

parent compd	$k/M^{-1}\ s^{-1}$
4,6-DHP	2.7×10^9
2-Me-4,6-DHP	2.8×10^9
5-Me-4,6-DHP	2.7×10^9

[a] [DHP] = 2 mM, [TMPD] = 0.02–0.1 mM, N_2O-saturated solutions, pH 12.3–12.5, 20 °C.

phenylenediamine (TMPD) which is transformed to the radical cation. With solutions containing, e.g. 2 mM of a DHP and 0.2 mM TMPD the dependences on pH of the rates of formation of $TMPD^{\bullet+}$ (monitored at 565 nm) were equal (within 10%) from pH 8 to 11.5 with those for dehydration of the OH adducts (monitored at ≈300 nm). TMPD can thus be used to specifically monitor the dehydration of the OH adducts. The rate constants for one-electron oxidation of TMPD by the radicals **2** are $\approx 3 \times 10^9\ M^{-1}\ s^{-1}$ (see Table IV), similar to the values[4,5] for the corresponding reaction involving uracil and cytosine derived radicals.

4. Reactions of 4,6-DHP's with $Br_2^{\bullet-}$ and $SO_4^{\bullet-}$. The former radical was produced from OH via the reaction sequence (6) and (7), the latter from $S_2O_8^{2-}$ by e_{aq}^- (eq 8) or by photolysis (eq 9).

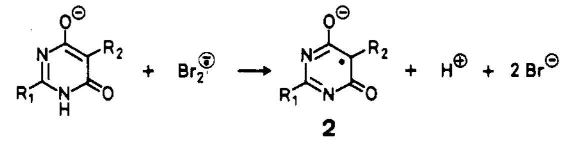
$$OH + Br^- \rightarrow OH^- + Br^\bullet \quad (6)$$

$$Br^\bullet + Br^- \rightarrow Br_2^{\bullet-} \quad (7)$$

$$e_{aq}^- + S_2O_8^{2-} \rightarrow SO_4^{2-} + SO_4^{\bullet-} \quad (8)$$

$$S_2O_8^{2-} \xrightarrow{h\nu} 2SO_4^{\bullet-} \quad (9)$$

$Br_2^{\bullet-}$ was used in radiolysis experiments. The N_2O-saturated solutions contained 20–50 mM Br^- (to scavenge OH) and 0.2–1 mM DHP (to react with $Br_2^{\bullet-}$). No radicals were seen when the pH of the solutions was below the pK_a values of the pyrimidines; i.e., the nonionized molecules do not react with $Br_2^{\bullet-}$ (see pulse radiolysis results below). At pH > pK_a, however, the same radicals were observed as those produced on reaction with OH in basic solution. The reaction between $Br_2^{\bullet-}$ and the ionized DHP's thus leads to their one-electron oxidation, as shown in eq 10. Since



2

(10)

$R_1, R_2 = H, CH_3$

with **2** there are no splittings assignable to exchangeable hydrogens, since the ESR spectra do not change with pH in the range 7–13, and since the nitrogens are equivalent, it is concluded that radicals **2** are ionized above pH ≈ 7, in agreement with the properties measured on producing **2** by the OH-addition/OH^--elimination sequence, eq 4 and 5.

The rate constants for reaction of $Br_2^{\bullet-}$ with the DHP's (see Table II) were determined by monitoring the absorption of $Br_2^{\bullet-}$ at 370–380 nm. Up to concentrations of 1 mM the DHP's had no influence on the lifetime of $Br_2^{\bullet-}$ when the DHP's were nonionized (pH 3–4). The rate constants for reaction of $Br_2^{\bullet-}$ with the neutral DHP's must therefore be $\leq 10^7\ M^{-1}\ s^{-1}$. However, at pH > pK_a (DHP's) (pH 7–8) increasing concentrations of DHP led to increasing decay rates of $Br_2^{\bullet-}$ and from these dependences the rate constants for reaction 10 were obtained as $(6–10) \times 10^8\ M^{-1}\ s^{-1}$ (Table II). After completion of the reaction with $Br_2^{\bullet-}$ the remaining absorptions are very weak; i.e., the extinction coefficients in the range 300–600 nm of the radicals **2** are quite low. This is in agreement with the observation reported in subsection 2 that the elimination of OH^- (or H_2O) from the OH adducts leads to a decrease in the optical density to very low values.

The radicals of type **2** were also formed at pH ≥ 8 on reaction with $SO_4^{\bullet-}$, produced according to eq 8 or 9. In the latter case the solutions contained 30 mM $S_2O_8^{2-}$, 1 mM DHP, and 1% acetone[21] and they were buffered with borate.

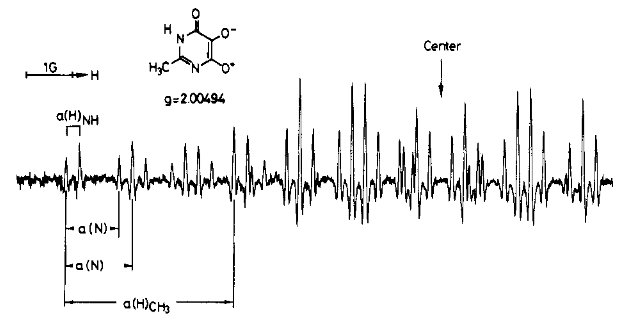
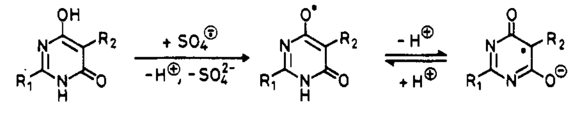


Figure 5. ESR spectrum recorded on electron irradiation of an N_2O-saturated aqueous solution containing 1 mM 2-Me-4,6-DHP at pH 8.2 and ≈5 °C. The lines are from the secondary radical shown.

$SO_4^{\bullet-}$ was found to be able to oxidize also the neutral DHP's (eq 11). With 4,6-DHP, in the pH range 4–7 (1 mM phosphate)

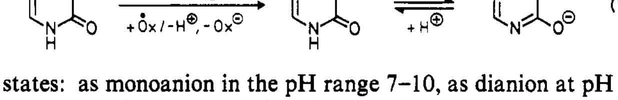


(11)

$R_1, R_2 = H, CH_3$ pK = 5-7

lines were seen from a radical characterized by three doublet splittings (2.02, 1.14, and 0.20 G), of which the smallest one was absent in D_2O, and by two equivalent nitrogens (a(N-1) = a(N-3) = 0.44 G) and g = 2.003 84. A similar radical was observed from 2-Me-4,6-DHP (see Figure 4 and Table III). These radicals (observed at pH < 7) are identified as the one-electron-oxidation products of the DHP's in the undissociated state. This assignment is based on the fact that the same radicals were produced on reaction with photochemically formed $HPO_4^{\bullet-}$. The possiblity of the pyrimidine radicals being SO_4^- adducts is thereby excluded. Besides, the coupling constants of SO_4^- adducts should be similar to those of the OH adducts. The experimentally observed splittings are, however, very different (see Table III).

5. Secondary Radicals Seen in ESR Experiments. With 4,6-DHP and 2-Me-4,6-DHP at pH ≥ 7 additional radicals to those already described were observed. Lines from these radicals were particularly strong when OH was the initiating radical (see Figure 5), but they were also seen with $SO_4^{\bullet-}$ as the oxidant. The spectra of these radicals are characterized by one proton splitting less than with the semiquinone-type radicals **2** and by considerably higher g factors, indicating that there is more spin density on oxygens. In the case of 4,6-DHP the most prominent secondary radical observed[22] was identified as the one-electron-oxidation product of 4,5,6-trihydroxypyrimidine, on the basis of its identity with that produced from authentic material, applying either OH addition/$H_2O(OH^-)$ elimination, or reaction with $Br_2^{\bullet-}$ or $SO_4^{\bullet-}$ (eq 12). Depending on pH, this radical exists in two ionization



(12)

states: as monoanion in the pH range 7–10, as dianion at pH > 10. The radicals observed under similar conditions in the case of 2-Me-4,6-DHP (see Figure 5) are identified in analogy to 4,6-DHP, i.e., in terms of the one-electron-oxidation product of 2-methyl-4,5,6-trihydroxypyrimidine. The coupling constants of the 4,5,6-trihydroxypyrimidine type radicals are presented in Table V.

The 4,5,6-trihydroxypyrimidines that give rise to the above radicals are suggested to be produced by disproportionation of

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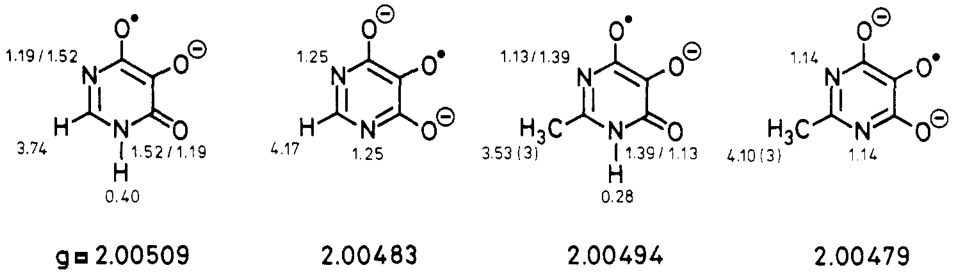
(21) The addition of acetone increased the line intensities without influencing the nature of the radicals.

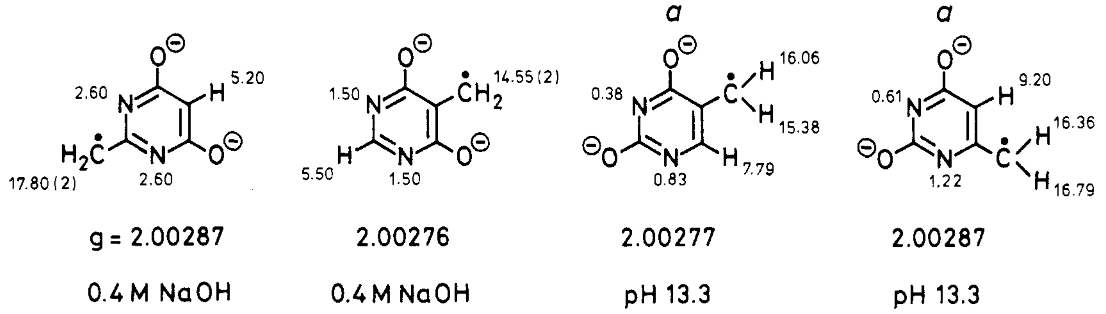
(22) Lines from this secondary radical have been erroneously interpreted as due to a radical formed by OH addition to C-2 (Planinic, J. *Radiat. Eff. Lett.* **1980**, *57*, 63).

TABLE V: Structures and ESR Parameters of (A) Secondary Radicals and (B) Radicals Produced by H Abstraction by O⁻

(A) Secondary Radicals from the 4,6-DHP System

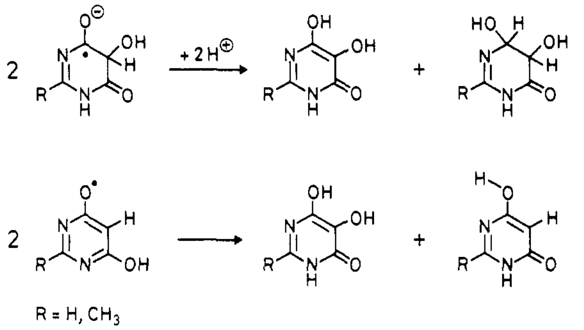


(B) Radicals Formed by H Abstraction



[a] These data relating to thymine and 6-Me-uracil are from ref 23.

the C-5-OH adducts of the 4,6-DHP's or of the semiquinone-type radicals:



(13)

This mechanism requires the presence of a hydrogen at C-5. On this basis secondary radicals are not expected with 5-Me-4,6-DHP, in agreement with experiment.

It may be interesting that with uracil a secondary radical was observed[23-26] totally analogous to that described with 4,6-DHP and 2-Me-4,6-DHP. Its mechanism of formation is probably similar to that of eq 13.[23]

6. H-Abstraction Reactions of $O^{\bullet-}$. This radical is the conjugate base of OH ($pK_a(OH) = 11.9$). On radiolysis of 0.4 M NaOH solutions saturated with N_2O and containing 0.2–1 mM 2- or 5-Me-4,6-DHP only the radicals formed by H abstraction from the methyl groups were observed, but not the semiquinone radicals of type **2**. The structures and ESR parameters of these allyl-type radicals are presented in Table V, together with the values[23] for the corresponding radicals from thymine and 6-methyluracil, which were also produced by reaction with $O^{\bullet-}$. It is interesting that $O^{\bullet-}$ reacts with these (doubly ionized) pyrimidines not by electron transfer (as it does with phenolate and aniline)[27] but by H abstraction.

7. Summary and Comparison of the 4,6-DHP with the Uracil System. It has been shown that the OH radical reacts with 4,6-DHP and 2-Me- and 5-Me-4,6-DHP by addition to C-5 of the C-5/C-6 double bond. The resulting OH adducts undergo ketonization with $k \geq 10^3$ s^{-1} to give diamido-type radicals with the unpaired spin essentially localized on C-2. In comparison, with uracil OH addition also takes place predominantly at C-5.[28]

In basic solution, the OH adducts eliminate H_2O or OH^- to give semiquinone-type radicals (with oxidizing properties) which can also be produced by one-electron oxidation of the parent compounds with the oxidants $Br_2^{\bullet-}$ or $SO_4^{\bullet-}$. Also in this respect the 4,6-DHP system is totally analogous to the uracil system which yields an oxidizing radical on dehydration.[4,19] In both the 4,6-DHP and the uracil[4] systems replacement of H by methyl at C-2, C-5, or C-6 leads to an increase in the rate of dehydration.

Concerning the unpaired spin distribution in the dehydrated OH adducts (=radicals of type **2**), replacement of H by CH_3 is of little effect. The same situation exists with the corresponding radicals from uracil, thymine, and 6-methyluracil, as shown in Table III, which summarizes the data obtained in this study and those from previous[19,23] investigations. The data on uracil, thymine, and 6-methyluracil[23] have been reinterpreted in terms of the ring-closed radical anions, as suggested for uracil by Bansal and Fessenden.[19] The assignment of the larger ^{14}N splitting to N-1 rather than to N-3 is based on MO spin density calculations.[20]

As seen in Table III, with the radicals from the uracils the coupling constants change only very slightly on protonation of the radical anion; i.e., the distribution of the unpaired spin over the N-1/C-5 system is rather independent of the protonation state of N-3. Very much in contrast to this the situation with the 4,6-DHP system: with the radical anion the spin density at C-5 is considerably larger than with the neutral radical, which indicates that protonation is accompanied by a pronounced electronic change.[29]

The last point of comparison refers to the reactivities of the 4,6-DHP and uracil systems with the radical $O^{\bullet-}$: in both cases H abstraction from methyl groups seems to be much preferred over electron transfer to $O^{\bullet-}$ from the dianions.

In conclusion, it appears that the 4,6-dihydroxypyrimidines are very good model compounds for the isomeric uracil systems with respect to radiation-induced radical reactions. This refers particularly to the selective addition of OH to C-5 of the dioxypyrimidine systems and the subsequent elimination of OH^- to give

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(28) See ref 4 and references therein.
(29) On the basis of the coupling constants the radical anions of the DHP's may be considered to be carbon-centered (at C-5), whereas their conjugate acids appear to be heteroatom-centered.

oxidizing radicals. From an ESR point of view, the 4,6-DHP's are easier to study due to their higher symmetry as compared to the uracils.

Acknowledgment. We thank Dr. J. Wroblewski for the synthesis of 5-methyl-4,6-dihydroxy- and 4,5,6-trihydroxypyrimidine. H.M.N. is very grateful to the Deutscher Akademischer Austauschdienst, the Max-Planck-Gesellschaft, and the Instituto Nacional de Investigacao Cientifica for supporting three short-term visits to Mülheim.

Registry No. **1c** ($R_1 = R_2 =$ H), 105163-90-4; **1c** ($R_1 = R_2 =$ H), 105163-91-5; **1c** ($R_1 =$ Me, $R_2 =$ H), 105163-92-6; **1c** ($R_1 =$ H, $R_2 =$ Me), 105163-93-7; OH, 3352-57-6; O^-, 14337-01-0; SO_4^-, 12143-45-2; Br_2^-, 12595-70-9; 4,6-DHP, 1193-24-4; 2-Me-4,6-DHP, 1194-22-5; 5-Me-4,6-DHP, 18337-63-8; 4,6-DHP radical anion, 105163-94-8; 2-Me-4,6-DHP radical anion, 105163-95-9; 5-Me-4,6-DHP radical anion, 105163-96-0; 4,6-DHP radical, 105163-97-1; 2-Me-4,6-DHP radical, 105163-98-2; uracil radical anion, 105163-99-3; 5-Me-uracil radical anion, 105164-00-9; 6-Me-uracil radical anion, 105164-01-0; uracil radical, 51446-22-1; 5-Me-uracil radical, 63031-49-2; 6-Me-uracil radical, 63221-84-1; 4,5,6-trihydroxypyrimidine radical anion, 105164-02-1; 4,5,6-trihydroxypyrimidine radical dianion, 105182-67-0; 4,5,6-trihydroxy-2-methylpyrimidine radical anion, 105164-03-2; 4,5,6-trihydroxy-2-methylpyrimidine radical dianion, 105164-04-3; 4,6-DHP-2-ylmethyl radical dianion, 105164-05-4; 4,6-DHP-5-ylmethyl radical dianion, 105164-06-5; 4,6-DHP anion, 14341-11-8; 2-Me-4,6-DHP anion, 105164-07-6; 5-Me-4,6-DHP anion, 105164-08-7.

Production of Cl_2O_2 from the Self-Reaction of the ClO Radical

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The species Cl_2O_2 has been generated in a gaseous flow system at 220–240 K by reacting Cl atoms with one of three different ClO precursors: O_3, Cl_2O, or OClO. The infrared spectra of the reactive mixture indicate that at least two different dimers are produced: a predominant form with bands centered at 1225 and 1057 cm^{-1} attributed to ClOOCl, and a second form with a band at 650 cm^{-1} attributed to ClOClO. The UV spectrum of the predominant form shows a maximum absorption cross section of $\sim 6.5 \times 10^{-18}$ cm^2/molecule around 270 nm, with a wing extending beyond 300 nm. The implications of these results for the chemistry of the stratosphere are discussed.

Introduction

The self-reaction of the ClO radical has been the subject of numerous kinetic investigations, which have been reviewed on several occasions.[1,2] The different studies have shown that the reaction exhibits second-order kinetic behavior with respect to the ClO concentration, but they have yielded conflicting results in terms of the relative importance of the various reactive pathways:

$$\mathrm{ClO + ClO \rightarrow Cl_2 + O_2} \tag{1}$$

$$\mathrm{\rightarrow Cl + ClOO} \tag{2}$$

$$\mathrm{\rightarrow Cl + OClO} \tag{3}$$

$$\mathrm{ClO + ClO + M \rightarrow Cl_2O_2 + M} \tag{4}$$

Evidence for the formation of a dimer, as in reaction 4, comes from the studies of Cox and co-workers[3,4] and of Basco and Hunt,[5] who observed a transient spectrum in the ultraviolet attributed to Cl_2O_2.

The self-reaction of the ClO radical has been neglected in stratospheric modeling calculations due to the slow overall rate to yield final products (Cl or Cl_2, reactions 1–3).[2,4] However, the rate of formation of the dimer (reaction 4) is likely to be considerably faster, especially at the higher pressures and lower temperatures prevailing in the lower stratosphere, and the reaction might be of importance depending on the subsequent fate of the dimer. In particular, the following cycle might play a role in the Antarctic stratosphere, where the total atmospheric ozone content in the spring has fallen remarkably over the past 10 years:[6,7]

$$\mathrm{ClO + ClO \xrightarrow{M} Cl_2O_2} \tag{4}$$

$$\mathrm{Cl_2O_2} + h\nu \rightarrow \mathrm{Cl + ClOO} \tag{5}$$

$$\mathrm{ClOO \xrightarrow{M} Cl + O_2} \tag{6}$$

$$\mathrm{2(Cl + O_3 \rightarrow ClO + O_2)} \tag{7}$$

$$\text{net:}\ \mathrm{2O_3 \rightarrow 3O_2}$$

In this paper we present the results of our studies of IR and UV spectroscopic characterization of the ClO dimer produced in reaction 4.

Experimental Section

The infrared analyses were obtained using a Teflon-coated absorption cell wrapped with a copper cooling coil and fitted with internal optics (57 cm long, 36 passes, 300 mL volume) interfaced to a Nicolet 7199A or a Nicolet 20SX Fourier-transform infrared (FTIR) spectrometer and a liquid N_2 cooled HgCdTe detector. Routine spectra were recorded at 1 cm^{-1} resolution in order to achieve rapid data collection. The temperature inside the cell was controlled by circulating cooled methanol through the copper coil and monitored with copper–constantan thermocouples.

The ultraviolet spectra were recorded with a Cary 219 double beam spectrophotometer interfaced to a Nova 3 computer, and using a 50 cm long, 3.5 cm diameter jacketed quartz cell fitted with Suprasil windows and with folded optics giving an optical path length of 100 cm. Low-temperature spectra were recorded by pumping cooled methanol through the outer jacket.

Chlorine atoms were generated by flowing a 1–5% Cl_2/N_2 mixture (Matheson, Certified Standard) through a quartz capillary tube wrapped with Nichrome heating wire. The effluent gas was

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