Properties of the OH Adducts of Hydroxy-, Methyl-, Methoxy-, and Amino-Substituted Pyrimidines: Their Dehydration Reactions and End-Product Analysis

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Reactions of hydroxyl radicals (•OH) with 2-amino-4-methyl pyrimidine (AMP), 2-amino-4,6-dimethyl pyrimidine (ADMP), 2-amino-4-methoxy-6-methyl pyrimidine (AMMP), 2-amino-4-hydroxy-6-methyl pyrimidine (AHMP), 4,6-dihydroxy-2-methyl pyrimidine (DHMP), 2,4-dimethyl-6-hydroxy pyrimidine (DMHP), 6-methyl uracil (MU), and 5,6-dimethyl uracil (DMU) have been studied by pulse radiolysis and steady-state radiolysis techniques at different pH values. The second-order rate constants of the reaction of •OH with these systems are of the order of $(2-9) \times 10^9$ dm^3 mol^{-1} s^{-1} at near neutral pH. The difference in the spectral features of the intermediates at near neutral pH and at higher pH (10.4) obtained with these pyrimidines are attributed to the deprotonation of the OH adducts. The G(TMPD•$^+$) obtained at pH ∼ 6, from the electron-transfer reactions of the oxidizing intermediates with the reductant, *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD), are in the range $(0.2-0.9) \times 10^{-7}$ mol J^{-1} which constituted about 3−16% oxidizing radicals. These yields were highly enhanced at pH 10.5 in the case of AHMP, DHMP, DMU, and MU (G(TMPD•$^+$) = 3.8−5.5 ≅ 66−95% oxidizing radical). On the basis of these results, it is proposed that a nonoxidizing C(6)-ylC(5)OH radical adduct is initially formed at pH 6 which is responsible for the observed transient spectra. The high yield of TMPD•$^+$ at higher pH is explained in terms of a base-catalyzed conversion (via a dehydration reaction) of the initially formed C(6)-ylC(5)OH adduct (nonoxidizing) to C(5)-ylC(6)OH adduct which is oxidizing in nature. Among the selected pyrimidines, such a dehydration reaction was observed only with those having a keto (or hydroxy) group at the C(4) position of the pyrimidine ring. Qualitative analyses of the products resulting from the OH adducts of DHMP (at pH 4.5) and DMHP (at pH 6) were carried out using HPLC-ES-MS and a variety of products have been identified. Glycolic and dimeric products were observed as the major end-products. The product profiles of both DHMP and DMHP have shown that the precursors of the products are mainly the C(6)-ylC(5)OH and the H adduct radicals. The identified products are formed mainly by disproportionation and dimerization reactions of these radicals. The mechanistic aspects are discussed.

Introduction

Reactions of primary free radicals such as hydroxyl radical (•OH), hydrogen atom (•H), and hydrated electron (e^-_{aq}), generated by the radiolysis of water, with nucleic acid components have been a subject of increased attention for the last many decades using pulse and steady-state radiolysis techniques.[1−4] The reactions of •OH with both purines and pyrimidines are of particular interest because •OH is the major damaging agent that leads to DNA lesions due to ionizing radiation.[3] •OH reacts with both purines and pyrimidines at almost diffusion controlled rate ($\geq 10^9$ dm^3 mol^{-1} s^{-1}).[5] It generally undergoes addition at C(4), C(5), and C(8) positions of the purine ring. The resulting OH adducts in the case of 2′-deoxyguanosine were reported to have both oxidizing [*G*(4)OH] and reducing [*G*(5)OH and *G*(8)-OH] properties with respect to some known oxidants and reductants.[6,7] A more or less similar distribution of both oxidizing and reducing OH adducts was reported in the case of adenine.[7] In purine nucleosides and nucleotides, 25% of the products were reported to be formed by the primary attack of •OH at the sugar moiety.[3]

In the case of pyrimidines, the main reaction is also the addition at C(5)−C(6) double bond while the double bond between N(3) and C(4) of cytosine is a potential additional site.[8−12] The major OH radical adducts are, therefore, C(5)-ylC(6)-hydroxy and C(6)-ylC(5)-hydroxy radicals. The former has oxidizing properties with respect to *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) and the latter has reducing properties with respect to tetranitromethane (TNM). The ratio of the oxidizing to reducing radicals depends on the nature of the substituents present in the C(5) and C(6) position of the pyrimidine ring whereas such substituent effect is not predominant with pyrimidines substituted at N(1) position.[9,10] The deprotonation of the OH adduct normally occurs at N(1) when there is a hydrogen at N(1) position. On the bases of the redox properties of the radicals, it has been identified that the distribution of C(6)-yl radical in uracil is 80% while that of thymine, cytosine and 1-methyluracil are 56, 87, and 65%

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respectively.[11] A third type of radical derived by a H-abstraction from the methyl group of pyrimidine has also been reported.[11] An interesting property of the reducing radicals (C(6)-ylC(5)-OH) of pyrimidines is their base-catalyzed dehydration reaction to yield oxidizing radicals (C(5)-ylC(6)OH) which can oxidize TMPD to $TMPD^{\bullet+}$.[9,10] To look at this important transformation reaction in basic pHs more closely, a series of pyrimidines substituted by methyl, hydroxy, amino, and methoxy groups at different positions have been selected and investigated the effect of these various substituents as well as their positions in the pyrimidine ring on the dehydration reaction. TMPD has been utilized to determine the ratio of oxidizing to non-oxidizing radicals and to demonstrate the transformation reaction. The selected pyrimidines include 2-amino-4-methyl pyrimidine (AMP), 2-amino-4,6-dimethyl pyrimidine (ADMP), 2-amino-4-methoxy-6-methyl pyrimidine (AMMP), 2-amino-4-hydroxy-6-methyl pyrimidine (AHMP), 4,6-dihydroxy-2-methyl pyrimidine (DHMP), 2,4-dimethyl-6-hydroxy pyrimidine (DMHP), 6-methyl uracil (MU). and 5,6-dimethyl uracil (DMU). Both the kinetics of the reaction and the spectral nature of the intermediates were also investigated at different pH values.

One of the difficult areas in the field of free radical chemistry of biomolecules is the end product analyses. This is mainly due to low concentrations of the products ($\approx 1 \times 10^{-6}$ mol dm^{-3} or less) and hence cannot be easily analyzed using the normal analytical techniques. GC-MS analysis after rotary evaporation followed by silylation of the dry products has made some progress in this area.[12] However, such studies are restricted only to few DNA model systems. In the present study, we have used a more sophisticated analytical technique, HPLC connected to electrospray mass spectrometer (HPLC-ES-MS), and analyzed the end products directly in aqueous medium resulting from the reaction of $^{\bullet}$OH with DHMP and DMHP. It will be shown that HPLC-ES-MS is an ideal choice for the direct detection of such radiolytic products and that this technique may have advantages over GC-MS analysis in which the products have to undergo lengthy and rigorous steps before being analyzed.

Experimental Section

Commercially available AMP, ADMP, AHMP, AMMP, DMHP, DHMP, DMU, MU, and TMPD (Aldrich) were used without further purification. The radiolysis of water produces three highly reactive species (e^-_{aq}, $^{\bullet}$H, and $^{\bullet}$OH) in addition to the formation of inert or less reactive molecular products (H_2, H_2O_2, etc.) (reaction 1).

$$H_2O \rightsquigarrow e_{aq}^-, H^{\bullet}, H_2, H_2O_2, H_3O^+ \qquad (1)$$

In N_2O-saturated solutions, e_{aq}^- are quantitatively converted to $^{\bullet}$OH (reaction 2), and therefore, the transient species available for reaction are $^{\bullet}$OH ($G(^{\bullet}OH) = 5.3-6.8 \times 10^{-7}$ mol J^{-1})[13] and $^{\bullet}$H ($G(^{\bullet}H) = 0.6 \times 10^{-7}$ mol J^{-1}) (where G value represents the radiolytic yield).

$$N_2O + e_{aq}^- \rightarrow {}^{\bullet}OH + OH^- + N_2 \qquad (2)$$

N_2O-saturated aqueous solutions typically contained 10^{-3} mol dm^{-3} of the substrate were pulse irradiated using a linear accelerator delivering 7 MeV electron pulses of 50 ns duration. KSCN dosimetry was used to determine the dose per pulse using $G\epsilon_{(500\ nm)} = 2.15 \times 10^{-4}$ m^2 J^{-1} in aerated solutions,[14] and the dose per pulse was normally kept at 15 Gy. Low dose per pulse of 5 Gy was used for the study of electron-transfer reaction between the OH adducts and TMPD. The details of the pulse radiolysis set up have been described elsewhere.[15,16] The pH of the selected pyrimidines was found to be around 6.0 when dissolved in water and was maintained the same for all of the experiments at near neutral pH. However, a pH of 4.5 was maintained in the case of DHMP in order to keep it in its neutral form as the pK values are 0.21, 6.35, and 12.9.[17]

N_2O-saturated aqueous solutions of DHMP (1×10^{-3} mol dm^{-3}) and DMHP (1×10^{-3} mol dm^{-3}) were irradiated in a ^{60}Co-γ-source (maximum dose $\sim$2 kGy and the dose rate was determined by ceric sulfate dosimetry[18]) and then injected into a Hewlett-Packard 1100 HPLC system with UV detection and coupled to a mass spectrometer through an electrospray interface (ES-MS). The mass specta were recorded for both positive and negative ions in the range 50−1000 m/z units at 3 s/scan and a skimmer voltage of 3.5 kV. A 3 m, 100 × 4 mm Hypersil BDS−C18 column (Hewlett-Packard) was used. The eluent was Millipore-Milli-Q water and the flow rate was fixed at 0.5 mL/min.

TABLE 1: Second-Order Rate Constants[a] and the Absorption Maxima of the Intermediates Obtained for the Reaction of OH Radicals with the Selected Pyrimidines

	pH 6		
compound	$k_2/10^9$ (dm^3 mol^{-1} s^{-1})[b]	λ_{max} (nm)	pH 10.4 λ_{max} (nm)
AMP	2.0	315	320
		550	550
ADMP	7.2	310	320
		550	565
AHMP	5.8	320	300
		455	460
			750
AMMP	6.5	300	300
		515	520
DHMP	5.6,[c] (3.7)[d]	420	290
			390
			480
DMHP	6.2	325	330
		450	455
DMU	5.9	435	310
			425
MU	9.0	410	310
			440

[a] These were determined from the average value of slopes of k_{obs} vs concentration plots for intermediates having two maxima (these two values were nearly the same). [b] The deviation in the calculation of these values was between 5 and 10%. [c] Measured at pH 4.5. [d] From reference 19.

Results and Discussion

I. Pulse Radiolysis. The second-order rate constants of the reaction of $^{\bullet}$OH with the selected pyrimidines were found to be diffusion controlled ($(2-9) \times 10^9$ dm^{3}mol^{-1}s^{-1}) at near neutral pH and the values are tabulated in Table 1. These were determined from the plot of the pseudo first-order formation of intermediates (k_{obs}) versus the concentration of the substrate. The k_{obs} was monitored at the respective absorption maxima of the intermediates (see Table 1). Such plots gave straight line graphs with good correlation coefficient ($\geq$0.99) with all the compounds. The absorption build-up of the intermediate at 320 nm and a typical k_{obs} versus concentration plot obtained with AHMP (at pH 6) are shown in Figure 1. The high rate constants obtained for these compounds are in good agreement with other pyrimidines reported earlier.[5]

The transient absorption spectra showed two absorption maxima at pH 6 with the selected pyrimidines except for DHMP which has only a single peak at 420 nm. The transient absorption

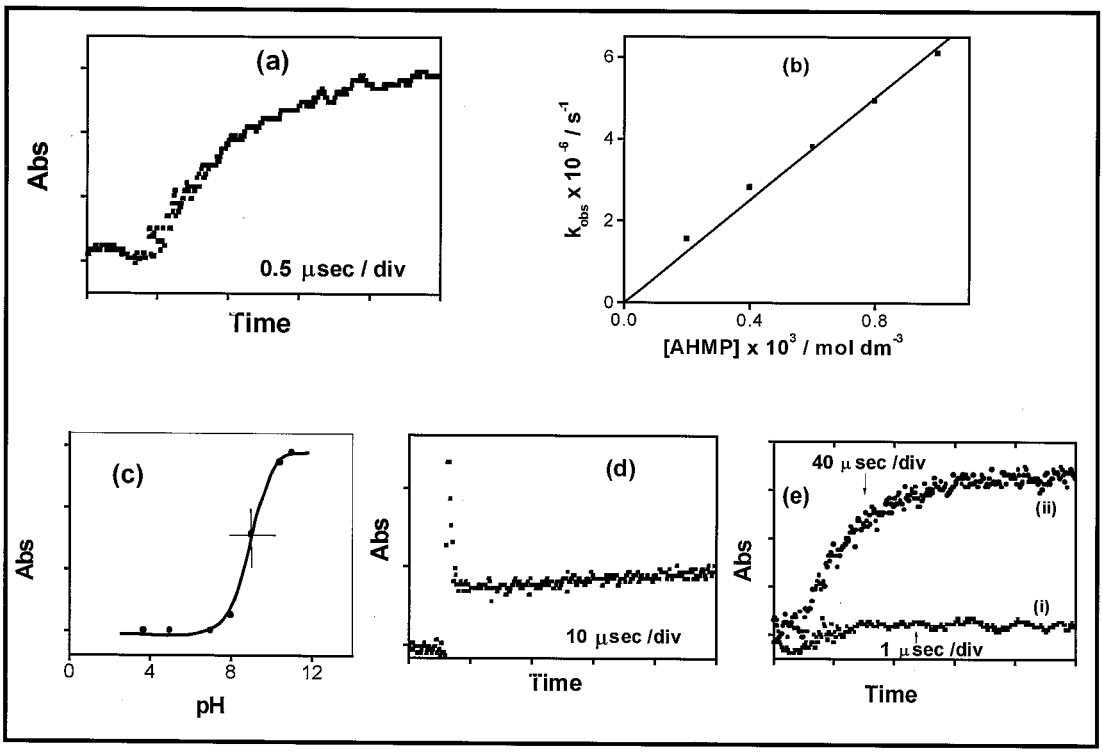


Figure 1. (a) A build-up of the intermediate at 320 nm ([AHMP] = 2×10^{-4} mol dm^{-3}) at pH 6. (b) k_{obs} vs concentration plot at 320 nm at pH 6. (c) Absorbance measured at 2 μs after the pulse versus pH at 700 nm. (d) decay trace of the intermediate at 300 nm at pH 10.4. (e) The $TMPD^{\bullet+}$ build-up at 565 nm ([AHMP] = 2×10^{-3} mol dm^{-3} and [TMPD] = 5×10^{-5} mol dm^{-3}) at pH (i) 6.0 and (ii) 10.4, obtained in N_2O-saturated aqueous solutions of 2-amino-4-hydroxy-6-methyl pyrimidine (AHMP)

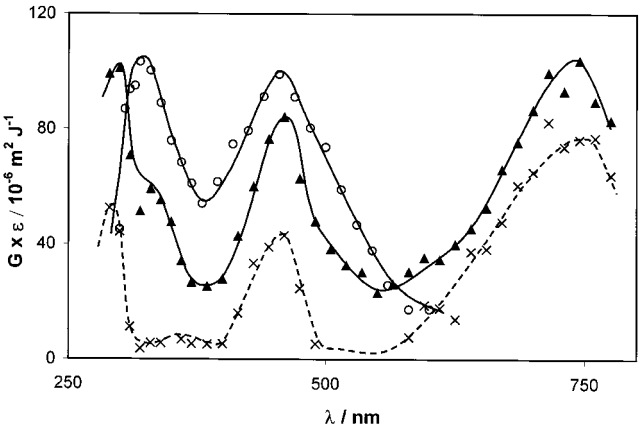


Figure 2. Transient absorption spectra obtained in N_2O saturated aqueous solutions of 2-amino-4-hydroxy-6-methyl pyrimidine (AHMP) (1×10^{-3} mol dm^{-3}) at 2 μs after the pulse at pH 6 (○) and at 2 μs (▲) and 40 μs (×) after the pulse at pH 10.4 (dose per pulse = 15 Gy).

spectrum obtained with AHMP at pH 6 has maxima around 320 and 455 nm (Figure 2). The initial spectrum was found to undergo a second-order decay. However, when the pH was raised to 10.5, the spectrum showed three maxima. An additional peak appeared around 750 nm while the lower λ_{max} is blue shifted to 300 nm with a shoulder around 330 nm (Figure 2). The initial spectrum undergoes a fast decay, (first-order type, $k \sim 10^6$ s^{-1}) with a slow absorption build-up around 300 nm region. This is found to continue over 300 μs and then undergoes a bimolecular decay. A typical trace obtained at 300 nm is also shown in Figure 1. An absorbance versus pH plot measured at 2 μs after the pulse at 700 nm, shown in Figure 1, gave an inflection point at pH 9. The time-resolved spectra obtained for ADMP at pH 6 have absorption maxima at 310 and 550 nm. The spectrum at 2.5 μs after the pulse was found to decay by second-order kinetics. The spectrum at higher pH (~10.4) also has similar characteristics with the same maxima but with higher absorbance values (Figure 1S, see Supporting Information). In the case of AMMP, the spectrum has λ_{max} at 300 and 515 nm at pH 6. At higher pH (10.4) there is a slight change in the maxima which are at 300 and 520 nm. This spectrum was found to decay by second-order kinetics. The intermediate obtained with AMP has absorption maxima around 315 and 550 nm at pH 6. Identical spectra were obtained at higher pH except there is a slight difference in the lower λ_{max}, which is at 320 nm.

The transient spectra obtained with DHMP is different from that obtained for the other pyrimidines. In this case the spectrum is characterized by its single λ_{max} at around 420 nm at pH 4.5. The spectrum obtained at 2 μs after the pulse at higher pH (10.4) showed three absorption maxima at 290, 390, and 480 nm (Figure 3). These spectral features are in line with an earlier report on the OH adduct of DHMP.[19] The initial spectrum undergoes a very fast first-order type decay ($k = 6 \times 10^5$ s^{-1}) followed by a slow build up of absorption around 290−300 nm. The spectrum with DMHP showed two absorption maxima at 325 and 450 nm at pH 6 and at pH 10.4 with nearly same absorbance values (Figure 2S, see Supporting Information). The transient spectra obtained with DMU and MU have two maxima (295 and 410 nm for DMU and 285 and 410 nm for MU) at pH 6. The transients at their lower abosorption maxima (at 295 and 285 nm, respectively, from DMU and MU) were found to undergo a very fast decay. We have recently identified these peaks as due to the deprotonated DMU and MU (at N(1)) which result from the reaction of radiolytically formed OH^- in N_2O-saturated solution (reaction 2) even at this short time scale and the fast decay is only a (re)protonation.[20] In the present case too, a similar deprotonated DMU and MU (at N(1)) could be formed and therefore, the peaks at 295 and 285 nm are very unlikely to be due to the OH adducts. The time-resolved

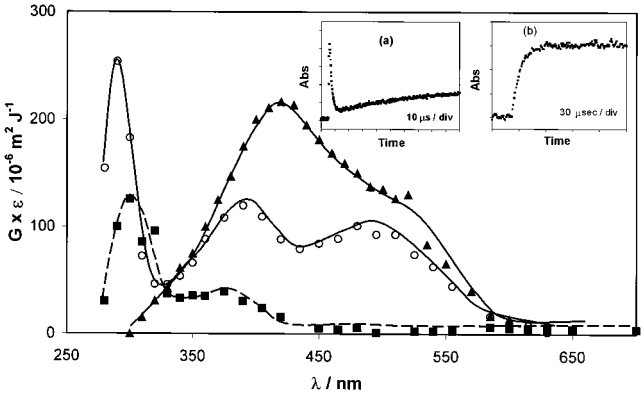


Figure 3. Transient absorption spectra obtained in N_2O saturated aqueous solutions of DHMP (1×10^{-3} mol dm^{-3}) at 1 μs after the pulse at pH 6 (▲) and at 2 μs (○) and 90 μs (♦) after the pulse at pH 10.5 (dose per pulse = 15 Gy). Inset: (a) intermediate trace obtained at 290 nm at pH 10.4. (b) The TMPD$^{\bullet+}$ build-up at 565 nm obtained with DHMP (2×10^{-3} mol dm^{-3}) in the presence of TMPD at pH 10.4 (5×10^{-5} mol dm^{-3}) (dose per pulse = 5 Gy).

TABLE 2: The *G*(TMPD$^{\bullet+}$) ($\times 10^7$/mol J^{-1}) Values and the Percentage of Oxidizing Radicals Obtained for the Selected Pyrimidines at pH 6 and 10.4

	G(TMPD$^{\bullet+}$) at pH		percentage of oxidizing radicals[a] at pH	
compound	6.0	10.4	6.0	10.4
AMP	0.72	0.68	12.5	11.8
ADMP	0.91	0.75	15.7	13.0
AHMP	0.37	5.49	6.4	94.6
AMMP	0.72	0.74	12.5	12.8
DHMP	0.44[b]	5.18	7.5	89.3
DMHP	0.37	0.32	6.3	5.5
DMU	0.34	3.83	5.9	66.0
MU	0.19	4.76	3.2	82.0

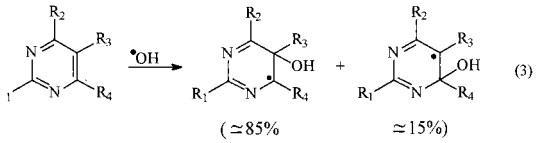
[a] Percentages are calculated based on a maximum yield of the intermediates, *G*(intermediates) $\approx 5.7 \times 10^{-7}$ mol J^{-1}. [b] At pH 4.5.

transient spectra obtained with MU at 10.4 showed absorption maxima at 310 and 440 nm (Figure 3S, see Supporting Information) and that of DMU at 10.4 has maxima at 310 and 425 nm (Figure 4S, see Supporting Information). In both cases, the spectra recorded at higher time scales (>40 μs) have only a single λ_{max} around 440 nm.

The reaction of $^{\bullet}$OH was carried out at low doses in the presence of TMPD which is an effective reductant and can be oxidized to TMPD$^{\bullet+}$ by transferring one electron to an oxidizing intermediate.[9−11] The build-up of TMPD$^{\bullet+}$ has been monitored with solutions contained 2×10^{-3} mol dm^{-3} pyrimidine and 5×10^{-5} mol dm^{-3} TMPD at 565 nm. The yields of the radical cation, *G*(TMPD$^{\bullet+}$), were calculated for all these pyrimidines at near neutral pH and at higher pH (~10.4), and are summarized in Table 2. The percentage of oxidizing radicals were thus calculated for all the pyrimidines based on a maximum *G*(TMPD$^{\bullet+}$) as 5.7×10^{-7} mol J^{-1} and are also tabulated in Table 2. The *G*(TMPD$^{\bullet+}$) values are almost the same at pH 6 and pH 10.4 for AMP, ADMP, AMMP, and DHMP ((0.3−0.9) $\times 10^{-7}$ mol J^{-1}, Table 2). However, in the cases of AHMP, DHMP, DMU, and MU, a much higher yield of TMPD$^{\bullet+}$ ((3.8−5.5) $\times 10^{-7}$ mol J^{-1}) is obtained at higher time scales (Table 2). A well-defined build-up of TMPD$^{\bullet+}$ was obtained at pH 10.5 against the feeble absorbance at pH 6 in all these cases and the typical traces obtained in the case of AHMP and DHMP are shown in Figures 1 and 3, respectively (also in Figures 3S and 4S for MU and DMU, respectively: see Supporting Information).

$^{\bullet}$OH generally undergoes addition reaction at C(5)−C(6) double bond of the pyrimidines according to earlier reports.[8−11] The resulting transient spectra are characterized by their absorption maxima around 320 and 430 nm. On the bases of the reactions of the OH adducts with TMPD, a comparatively high yield of the reducing radicals (C(5)OH−C(6)yl radicals) were reported to be formed with a number of uracil derivatives.[10] In agreement with these reports we propose a similar OH addition at the C(5) and C(6) positions of the selected pyrimidines at pH 6 as well as at pH 10.4 (reaction 3). The transient spectra obtained with these compounds are also having two absorption maxima in the same region except for AMP, ADMP, and AMMP where the second λ_{max} is in the 520−550 nm region. This slight difference in the second λ_{max} could be due to the substituent effect.

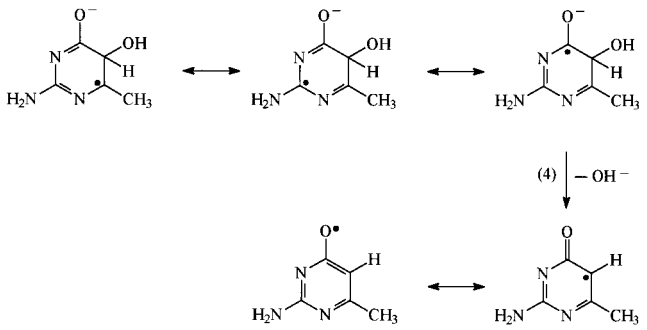
The G(TMPD$^{\bullet+}$) values at near neutral pH, given in Table 2, show a clear account of the yield of oxidizing radicals. Such low yield of oxidizing radicals is in agreement with the earlier reports on the OH adducts of thymine and uracil derivatives.[10,11] The oxidizing property of the C(5)-ylC(6)OH radical can be understood as its mesomeric structure is either an oxygen-centered radical (at C(4)−O) or a nitrogen centered (at N(3)). Therefore, this gives an additional support for the assignment of the spectra to the formation of C(6)-ylC(5)OH adduct in the case of all the selected pyrimidines which could act as reducing radical as was reported in the case of uracil and thymine.[11] Furthermore, there can be a possibility of H-abstraction reaction from the methyl group of these compounds leading to allyl type radicals as reported in the case of thymine.[11] However, these radicals cannot be reducing unlike that of thymine. Therefore, if formed, these radicals could also contribute to the TMPD$^{\bullet+}$ yield. But this is expected to be a minor process.



The transient absorption spectra at higher pHs (~10.4) were different from those at lower pHs either by their higher absorption coefficient or by the shift in their λ_{max} (including additional peaks such as those in the cases of AHMP and DHMP). Such differences in the case of uracil, thymine, and cytosine were explained in terms of the formation of deprotonated OH adducts at higher pHs.[8,18] In the present case too, such an interpretation is logical. The absorbance versus pH plot in the case of AHMP (Figure 1) gave a clear p*K* type curve with inflection point around pH 9. AHMP has pK_a values as 4.1 and 9.4. Therefore, it can be concluded that the species existing at pH 10.4 is the deprotonated form of the OH adduct of AHMP. On the bases of these observations and on the previous reports,[8,19] the spectral differences at lower and higher pHs, are attributed to the protonation−deprotonation reaction of the OH adducts. However, in the case of DMHP, there was no observable difference between the absorption spectra at pH 6 and 10.5 and hence it must be assumed that the pK_a value of the OH adduct is higher than pH 10.5 and that the spectrum existing at both the pH are the neutral OH adduct of DMHP.

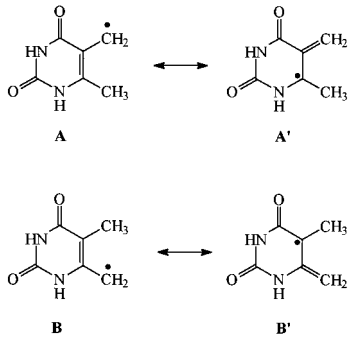
As shown in Table 2, the percentage of oxidizing radicals calculated from the G(TMPD$^{\bullet+}$) values were almost constant for AMP, ADMP, AMMP, and DMHP at lower and higher pH values, but for AHMP, DHMP, DMU, and MU the calculated percentage of oxidizing radicals were 95, 89, 66 and 82%, respectively, at higher pH. The high difference in the *G*(T-

SCHEME 1



MPD$^{\bullet+}$) value at lower and higher pHs gives an indication of the change in the oxidizing property of the intermediate radicals. A two component build-up of TMPD$^{\bullet+}$ absorption has been reported with uracil at pH>9 where the initial component depended on the concentration of TMPD.[10] The second component, on the other hand, was dependent only on the pH (and not on the concentration) indicating a slow transformation (in the pulse scale) of the initial non-oxidizing species into an oxidizing species. Therefore, in the present case, we have monitored the build-up of TMPD$^{\bullet+}$ at a higher time scale with the pyrimidines at pH $\sim$ 10.4 and the G(TMPD$^{\bullet+}$) values were determined (Table 2). The mechanism of the change in the oxidizing properties at higher pH values, observed in the case of AHMP, DHMP, DMU, and MU, is proposed on the basis of the conversion of the initially formed (deprotonated) OH adduct (C(6)-ylC(5)OH) to C(5)-ylC(6)OH. In the case of cytosine, this occurs by the dehydration of C(5)OH adducts (reducing in nature) which ultimately leads to the formation of a radical site at C(5) or oxygen at C(2) position.[9,10] Therefore, a similar conversion of the initially formed C(6)-ylC(5)OH to an oxidizing radical at basic pHs is proposed as shown in the case of AHMP (Scheme 1).

A similar type of dehydration reaction of the C(6)-ylC(5)-OH radical in basic medium is proposed for DHMP, DMU, and MU. In the case of DHMP, EPR evidence is available for the formation of an oxyradical at basic pHs[19] and this assignment is in good agreement with our observation of a high G(TMPD$^{\bullet+}$) value at pH 10.5 (Table 2). The relatively low value of G(TMPD$^{\bullet+}$) obtained with DMU could be understood on the basis of possible formation of two kinds of allyl type radicals (**A** and **B**) from a H-abstraction from the methyl groups. It could be probable that the radical **A** is formed in high yield compared to the radical **B**. The radical **A** could be reducing, but cannot be transformed into an oxidizing radical. The distribution of



these radicals, on the other hand, cannot be determined using the common oxidants or reductants as both the C(6)-ylC(5)OH and the allyl type radical **A** (due to its mesomeric form **A′**) can be reducing in nature while C(5)-ylC(6)OH and the allyl radical **B** (due to its mesomeric form **B′**) can be oxidizing in nature. The redox properties of similar radicals such as from thymine and 1-methyl uracil are already well documented.[11]

The fast first-order decay of the transients at higher pHs is, therefore, proposed as the dehydration of the initially formed reducing radicals in the cases of AHMP, DHMP, DMU, and MU. It is also important to note that such dehydration of the reducing radicals and the transformation reactions have been observed only with AHMP, DHMP, DMU, and MU. The major structural difference of these compounds with others is that these contain either a keto or hydroxy group at the C(4) of the pyrimidine ring. It is therefore obvious that the chemical environment in the cases of other pyrimidines, such as ADMP, AMP, AMMP, and DMHP, is not conducive for an OH^- elimination reaction.

Some additional spectral features obtained with AHMP, DHMP, DMU, and MU at high pHs further gave some indication of the optical absorption spectra of the oxidizing radicals. The absorption trace obtained with AHMP at 300 nm, given in Figure 1, showed a slow build-up of absorption after a very fast first-order decay (corresponding to the dehydration) of the transient. As this is not due to the formation of a stable product (it started a second-order decay after about 300 μs), this is likely due to the absorption of the oxidizing radical which is formed after the dehydration reaction. A similar absorption build-up was observed in the case of DHMP as well (Figure 3). While the initial spectra had undergone a fast decay at the maxima 290, 390, and 480 nm, the later spectrum (90 μs after the pulse) appears to have a single prominent peak at 300 nm. The absorption trace at 290 nm (in the inset of Figure 3) has also shown a build-up similar to AHMP. This trace was found to undergo a second-order decay after about 500 μs (data not shown). Therefore, in this case, too, it is likely that the radical which is formed after the dehydration reaction of C(6)-ylC(5)-OH of DHMP, contribute to the absorption build-up around 300 nm. Such a difference in the time-resolved absorption spectra in the case of both DMU and MU was also prominent. The initial spectra were found to have two absorption maxima where the later spectra (85 and 40 μs respectively after the pulse) have only a single λ_{max} (Figures 3S and 4S, see Supporting Information) which undergo a bimolecular decay. These spectral features also give some indication of the contribution of absorption from the oxidizing radicals of DMU and MU.

II. Product Analysis. Qualitative HPLC-ES-MS analysis of N_2O-saturated solution of DHMP (pH$\sim$4.5) and DMHP (pH$\sim$6) after γ-irradiation gave a large number of mass peaks with varying *m*/*z* values.[21] Among these mass peaks a number of *m*/*z* values were selected for the most probable products (protonated or deprotonated under mild ionization conditions). The identified products along with their *m*/*z* values are summarized in Tables 3 and 4. Figure 5S, see Supporting Information, illustrates a typical mass spectra obtained from a given chromatographic peak in the case of DMHP. The probable mechanism of the formation of the identified products resulting from the proposed radical intermediates from DHMP and DMHP, is shown in Schemes 2 and 3, respectively. As discussed in section I**,** C(5)OH adduct is the major radical intermediate in the case of DHMP and DMHP at near neutral pH. Therefore, the products are likely to result from the disproportionation and dimerization reactions of these radicals.

Although, the presence of C(6)OH adduct is evidenced by the formation of TMPD$^{\bullet+}$ (see section I) no products which has a likely precursor as C(6)OH were obtained from the product profile at this pH (pH 4.5). This could be due to the very low

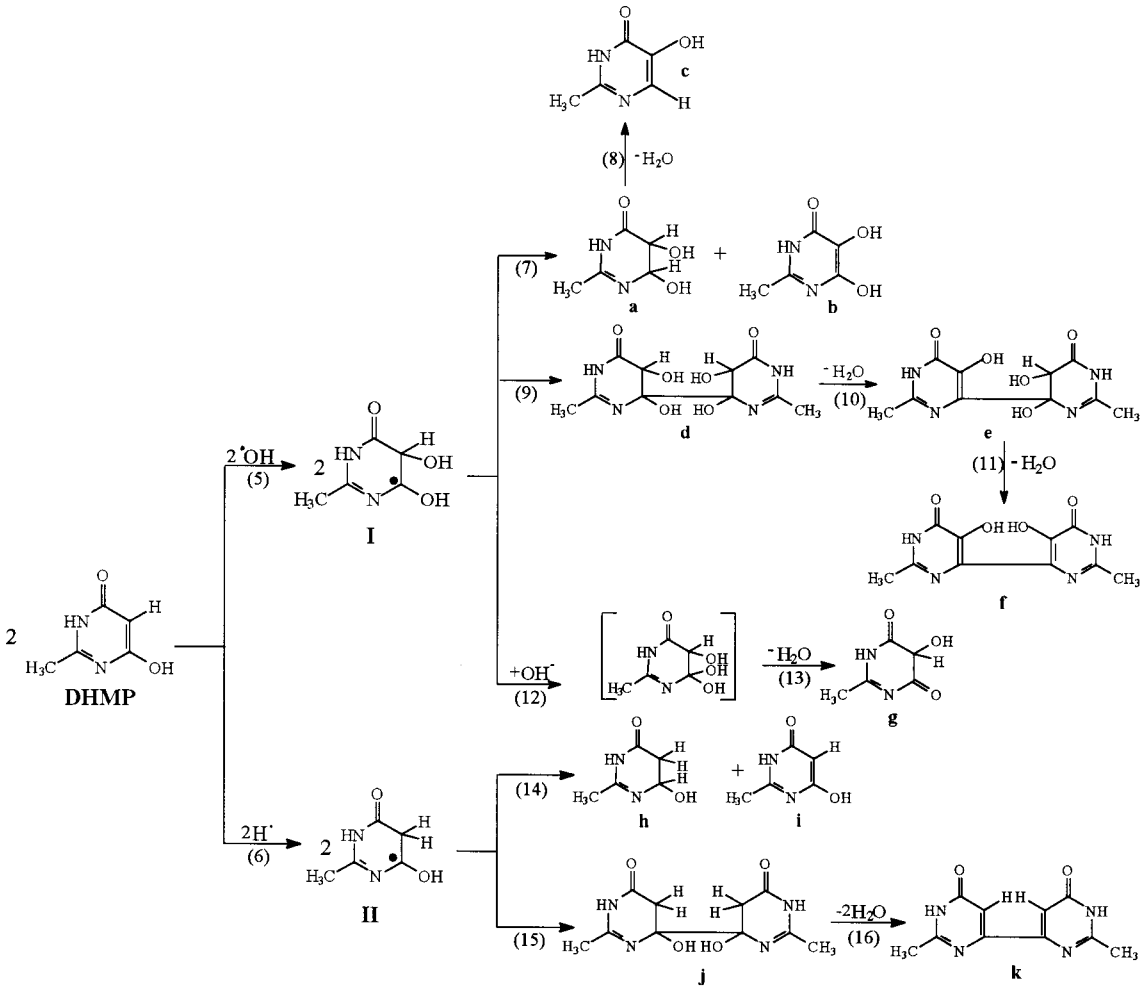
TABLE 3: Products Identified from the Reaction of •OH with DHMP Using HPLC-ES-MS Analysis at pH 4.5

	products	molecular formula	molecular weight	*m/z*
a	5,6-dihydro-5,6-dihydroxy-2- methyl-(3H)-pyrimidin-4-one	$C_5N_2O_3H_8$	144	+144,−143
b	5,6-dihydroxy-2-methyl-(3H)-pyrimidin-4-one	$C_5N_2O_3H_6$	142	−141, +143
c	5-hydroxy-2-methyl-(3H)- pyrimidin-4-one	$C_5N_2O_2H_6$	126	−126
d	bis(5-hydro-5,6-dihydroxy-2-methyl-(3H)-pyrimidin-4-one-6yl)	$C_{10}N_4O_6H_{14}$	286	−286
e	5-hydroxy-2-methyl-(3H)-pyrimidin-4-one-6-(5-hydro-5,6-dihydroxy-2-methyl-(3H)-pyrimidin-4-one)	$C_{10}N_4O_5H_{12}$	268	−267.5,+268.5
f	bis(5-hydroxy-2-methyl-(3H)-pyrimidin-4-one-6-yl)	$C_{10}N_4O_4H_{10}$	250	+250
g	5-hydroxy-2-methyl-(3H)- pyrimidin-4,6-dione	$C_5N_2O_3H_6$	142	−141,+143
h	5,5-dihydro-6-hydro-6-hydroxy-2-methyl-(3H)-pyrimidin-4-one	$C_5N_2O_2H_8$	128	+129
i	6-hydroxy-2-methyl-(3H)- pyrimidin-4-one	$C_5N_2O_2H_6$	126	−125,+127
j	bis(5,5-dihydro-6-hydroxy-2-methyl-(3H)-pyrimidin-4-one-6-yl)	$C_{10}N_4O_4H_{14}$	254	−254
k	bis(2-methyl-(3H)-pyrimidin-4-one-6-yl)	$C_{10}N_4O_2H_{10}$	218	−217

TABLE 4: Products Identified from the Reactionof •OH with DHMP Using HPLC-ES-MS Analysis at pH 6

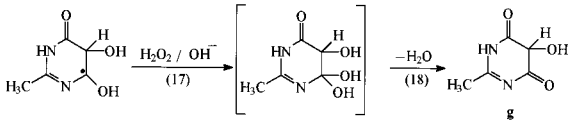
	products	molecular formula	molecular weight	*m/z*
l	5,6-dihydro-5,6-dihydroxy-2,4-dimethyl pyrimidine	$C_6N_2O_2H_{10}$	142	−142,+142
m	5,6-dihydroxy-2,4-dimethyl pyrimidine	$C_6N_2O_2H_8$	140	−139, +141
n	5-hydroxy-2,4-dimethyl pyrimidine	$C_6N_2OH_8$	124	−124
o	bis(5-hydro-5,6-dihydroxy-2,4-dimethyl pyrimidine-6-yl)	$C_{12}N_4O_4H_{18}$	282	+283
p	5-hydroxy-2,4-dimethyl-6-(5-hydro-5,6-dihydroxy-2,4-dimethyl pyrimidine) pyrimidine	$C_{12}N_4O_3H_{16}$	264	−264
q	bis(5-hydroxy 2,4-dimethyl pyrimidine-6-yl)	$C_{12}N_4O_2H_{14}$	246	−245
r	5-hydro-5-hydroxy-2,4-dimethyl pyrimidin-6-one	$C_6N_2O_2H_8$	140	−139, +141
s	5,5-dihydro-6-hydro-6-hydroxy-2,4-dimethyl pyrimidine	$C_6N_2OH_{10}$	126	−125, +126
t	6-hydroxy-2,4-dimethyl pyrimidine	$C_6N_2OH_8$	124	−123, +125
u	bis(5,5-dihydro-6-hydroxy-2,4-dimethyl pyrimidine-6-yl)	$C_{12}N_4O_2H_{18}$	250	+250
v	bis(2,4-dimethyl pyrimidine-6-yl)	$C_{12}N_4H_{14}$	214	−213, +215

SCHEME 2



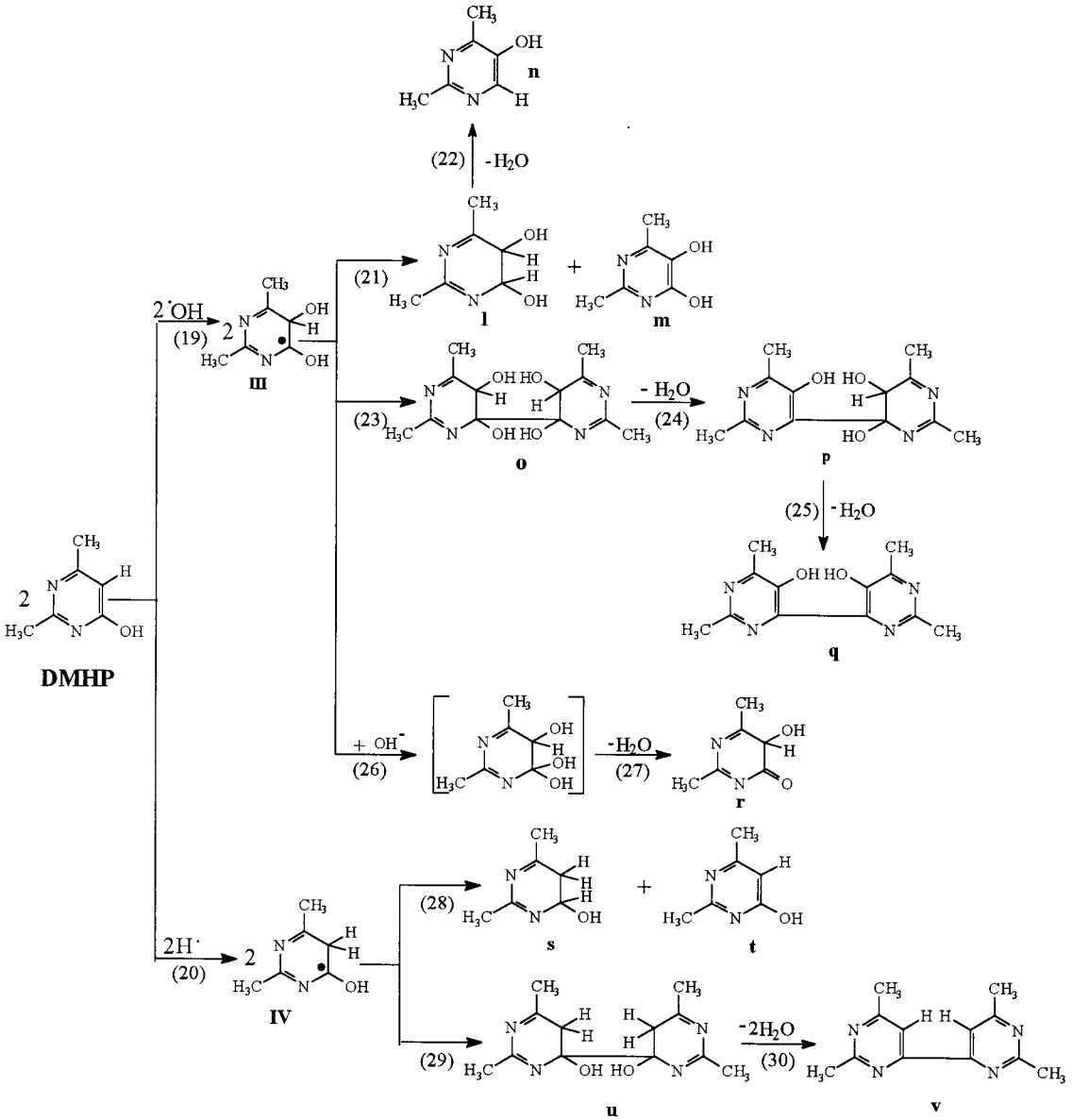
yield of C(6)OH adduct as evidenced by the low yield of $TMPD^{\bullet+}$ (see section I). On the other hand, the product mass peaks clearly gave evidence for the formation of products resulting from the H adducts of both DHMP and DMHP (Schemes 2 and 3).

A part of the identified products, summarized in Table 3 in the case of DHMP, are proposed to be mainly resulted from the disproportionation and dimerization reactions of radicals I and II (as shown in Scheme 2). From the quantitative analysis of the products in the case of 1,3-dimethyluracil (1,3-DMU) using GC-MS it is known that the major products resulting from C(5)OH adducts are the glycol and the dimer.[12] A detailed description of the formation of glycolic products from the reaction of OH radicals in the radiolysis as well as in the fenton reaction of 1,3-DMU using GC-MS has been reported recently.[22] The HPLC-ES-MS data in the present case shows a similar trend. The immediate product after the disproportionation (reaction 7) of I is the 5,6-dihydro-5,6-dihydroxy-2-methyl-(3H)-pyrimidin-4-one (**a**) and 5,6-dihydroxy-2-methyl-(3H)-pyrimidin-4-one (**b**). The pyrimidine glycols are known to be acid-labile,[12] and therefore, can undergo a water elimination as shown in reaction 8. The product **c**, therefore, is a result of a dehydration reaction of **a**. Another important reaction that normally occurs with the OH adducts of pyrimidine is the dimerization reaction.[12,22−27] Reaction 9 leads to a dimer of the type **d** and the subsequent water elimination reaction leads to the products **e** and **f** as shown in reaction 10 and 11. The formation of **e** and **f** through water elimination reactions is supported by the report by K. M. Idriss Ali[27] where the OH adduct of uracil undergoes a similar water elimination reaction. A probable product with the structure **g** is also proposed, resulting from the dehydration of a trihydroxy derivative, as shown in reactions 12 and 13. This product must have a *m*/*z* value of 142 and the mass peaks showed *m*/*z* values as −141 and +143. However, the product **b** (from reaction 7) has also similar *m*/*z* value and hence the existence of **g** could not be



confirmed. An alternative mechanism for the formation of the product **g** could be the reaction of radiolytically formed H_2O_2

SCHEME 3



which acts as an oxidizing agent as shown in reaction 17 and forms an unstable trihydroxy-substituted derivative. The immediate water elimination reaction might lead to a product like **g**.

Other identified products include *m*/*z* values corresponding to **h**−**k** which can be explained only through an initial attack of H• with DHMP. It is also formed in the reaction mixture with a *G* value of 0.6×10^{-7} mol J^{-1} under the reaction conditions that we used. The most likely mechanism for the formation of the product **h** is the disproportionation of the H adduct radical, **II**. The attack of H• at the C(5)−C(6) double bond of pyrimidines, a reaction similar to •OH, is well understood.[28] In a recent study, it is demonstrated that C(6)-ylC(5)-H radical is the major H adduct (**II**) in the case of DHMP.[29] It may be further noted that the product **i** is the starting compound, DHMP, but is considered as the product partner of **h** from the disproportionation reaction. The dimerization of the radical **II** may lead to the product **j**. Although a single water elimination is possible from **j**, *m*/*z* value corresponds to only **k** was observed in the mass peak. On the other hand, our assignment of the product **k** can be rationalized on the basis of the report on the water elimination from a dimeric hydroxy methyl radical resulted from the OH attack on methyl uracil where such water-eliminated products were identified using chromatographic techniques.[27] It must be further noted that though a substantial details of OH reaction products is available in the literature, practically little information is available on the end products from the reaction of H• with pyrimidines. The product profile obtained with DMHP shows a similar reaction route as in the case of DHMP. The identified products and the mechanism of their formation from the C(5)OH adduct and from the H adducts are presented in Table 4 and Scheme 3, respectively.

In conclusion, a detailed investigation of the important pH-dependent transformation of a nonoxidizing to oxidizing OH adduct radicals from hydroxy, methyl-, methoxy-, and amino-substituted pyrimidines is presented. Among the selected pyrimidines the transformation of the nonoxidizing to oxidizing radical reaction was observed only with pyrimidines having a keto or hydroxy group at the C(4) position. A variety of new stable products have been identified using HPLC-ES-MS analysis and a detailed degradation pathway is proposed. The product analysis from DHMP and DMHP gave indications that these products mainly arise from the dispropotionation and dimerization of the initially formed C(5)OH adduct as well as the H adducts. To our knowledge, the identification of the products resulted from the H adducts in N_2O-saturated aqueous solutions, is the first report of this kind.

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Supporting Information Available: The absorption spectra of the OH adducts of ADMP, DMHP, MU, and DMU at pH 6 and 10.4 (Figures 1S−4S), and a typical ES-MS from DMHP (Figure 5S). This material is available free of charge via the Internet at http://pubs.acs.org.

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