unique polarization pattern (Figures 3 and 4). The *tert*-butyl(dimethylamino)fulvenes, on the other hand, show evidence for geometric isomerization under electron transfer conditions. However, the two isomers show opposite polarization (Figure 5). This result indicates clearly that the reactant and the product are (re)generated by different pathways. Such a finding precludes the involvement of a fully perpendicular radical cation as the key intermediate.

Conclusions

The results presented here fail to support the existence of perpendicular (nonvertical) radical cations in the systems studied. The lack of isomerization observed for the di-*tert*-butylfulvenes is not surprising in view of the fact that a partially or fully twisted structure would contain a poorly stabilized positive charge. The isomerization observed in the *tert*-butyl(dimethylamino)fulvenes may indicate some degree of twisting, though certainly far short of the perpendicular conformation. Diaryl substitution in the 6-position would offer more extended delocalization of the positive charge. However, this possibility does not lend itself as readily to experimental verification. We are actively exploring other molecules as precursors to this elusive nonvertical structure type.

Acknowledgment. The authors thank F. C. Schilling for obtaining 500-MHz ^{1}H NMR spectra of the di-*tert*-butylfulvenes.

Registry No. 6,6-Dimethylfulvene, 2175-91-9; 6,6-diphenylfulvene, 2175-90-8; (*E*)-2,6-di-*tert*-butylfulvene, 98678-03-6; (*Z*)-2,6-di-*tert*-butylfulvene, 98678-04-7; (*Z*)-*tert*-butyl(dimethylamino)fulvene, 62667-49-6; (*E*)-*tert*-butyl(dimethylamino)fulvene, 62667-50-9; chloranil, 118-75-2; anthraquinone, 84-65-1.

Reaction of 6-yl Radicals of Uracil, Thymine, and Cytosine and Their Nucleosides and Nucleotides with Nitrobenzenes via Addition To Give Nitroxide Radicals. OH^--Catalyzed Nitroxide Heterolysis

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Abstract: The 6-yl radicals produced by addition of OH to C(5) of the C(5)/C(6) double bond of naturally occurring pyrimidine bases, nucleosides, and nucleotides, or those formed by H-abstraction from C(6) of 5,6-dihydropyrimidines, react with para-substituted nitrobenzenes by addition ($k \approx 6 \times 10^6$ to 2×10^9 M^{-1} s^{-1}) to yield nitroxide-type radicals which were characterized by electron spin resonance and optical detection techniques. In basic solution the nitroxide radicals deprotonate: with the radicals derived from the free bases deprotonation occurs at N(1), with those from the nucleosides and nucleotides of uracil deprotonation at N(3) is observed. The ionized 6-yl radicals react with the nitrobenzenes also by addition with rate constants considerably higher ($k = 1 \times 10^8$ to 1×10^9 M^{-1} s^{-1}) than those for the case of the neutral radicals. The neutral nitroxide radicals react with OH^- to give the nitroxide radical anion which is able to undergo a unimolecular heterolysis reaction which results in the formation of the nitrobenzene radical anion. In the case of the nitroxide radicals having N(1)–H (the radicals derived from the free bases), the OH^- catalysis of nitroxide heterolysis proceeds via deprotonation at N(1) and the unimolecular rates are comparatively high (2.4×10^5 s^{-1} for the 5-hydroxy-6-yl radical adduct from uracil/4-nitroacetophenone). On substitution of N(1) by a methyl or (deoxy)ribosyl(phosphate) group the site of OH^- attack is changed to N(3)–H (with the uracils) or to N′(4)–H (with the cytosines). With radicals from N(1)-alkylated pyrimidines the rates of heterolysis of the nitrobenzene adducts are considerably lower (e.g., 4.5×10^3 s^{-1} for that from deoxyuridylic acid) than if N(1) is ionized. The addition/OH^- catalyzed elimination sequence of interaction of pyrimidin-6-yl radicals and nitrobenzenes results in the ultimate transfer of an electron from the pyrimidine radical to the nitrobenzene and is therefore an example of a one-electron redox reaction.

Nitro compounds have received a large amount of attention as a result of their (potential) use as sensitizers in the radiotherapy of cancer.[2-6] In the course of these investigations numerous radicals, derived from molecules of importance in biological systems and also from model compounds, have been reacted with representative nitro compounds[7-11] with the aim of contributing to the understanding of in vivo radiation sensitization. An important result of these studies is that it is the redox potential of

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the nitro compound which determines to a large extent both its sensitization efficiency and its reactivity with radicals.[2] This may be (and has been) taken to imply that the nitro compounds react with radicals by an (outer sphere) electron transfer. However, there is evidence from pulse radiolysis,[8,10] and, more specifically, from electron spin resonance,[12] that radicals may react with nitro compounds by addition rather than by electron transfer. In fact, it has recently been shown[13] that even strong one-electron reductants[14] such as α-hydroxyalkyl radicals react with nitrobenzenes *predominantly* by addition to give nitroxide-type radicals. These nitroxide radicals may undergo a unimolecular heterolysis reaction to give an oxoalkane, H^+, and the nitrobenzene radical anion.[13] A similar reaction mechanism has also been found for the oxidation of α-alkoxyalkyl radicals by tetranitromethane.[15] For nitroxides of the semi-acetal type a second and more general mode of heterolysis involves OH^- catalysis.[12a,13]

In the case of radicals produced by reaction of OH with pyrimidines, reactions with nitro compounds via a similar mechanism are in principle possible. Addition reactions have indeed been proposed;[3,5,10] however, the evidence presented was not sufficient to identify the hypothetical adducts or to specify in a more general way the conditions of their formation and disappearance. In particular, the effects of structure of the reactants or that of the medium have not been studied. With the α-hydroxyalkyl radical/para-substituted nitrobenzene system, these factors are of great importance in determining the overall reactivity.[13] It was therefore decided to study in a systematic way the consequences of varying the structure of the pyrimidine radical, the pH of the solutions, and, to some extent, the structure of the nitrobenzene. From the results it is evident that the 6-yl radicals of the naturally occurring pyrimidines and pyrimidine nucleosides and nucleotides react with nitrobenzenes exclusively by addition and that the nitroxide radicals thus formed are susceptible to OH^--catalyzed decomposition thus mimicking an electron-transfer mechanism.

Experimental Section

The nitrobenzenes and the pyrimidines were obtained from Aldrich, Fluka, Pfaltz and Bauer, and Sigma and were used as received. The aqueous solutions (using water purified with a Millipore-Milli-Q system) typically contained 2 mM pyrimidine and 0.1–0.5 mM nitrobenzene and they were saturated with N_2O in order to convert e_{aq}^- into ·OH. With the pulse radiolysis experiments a 3 MeV van de Graaff accelerator was used that delivered pulses of 0.1–0.4 μs duration with doses such that 0.2–1 μM radicals were produced. The optical and conductance (from a 10 MHz AC bridge) signals were fed into a Biomation 8100 transient recorder interfaced with a VAX 11/38 computer via a PDP 11/10. The digitized data were stored and analyzed with the VAX. For optical detection, dosimetry was performed with N_2O-saturated 0.1 M formate solutions that contained 0.2 mM of the nitrobenzene derivative studied, and the absorption of the corresponding radical anion at λ_{max} (for λ_{max} and ϵ values see ref 13) was monitored. This absorption represents the OH (G = 6.0) and H (G = 0.6) radicals. With conductance detection, N_2O-saturated 0.1 M 2-propanol solutions containing 0.2 mM nitrobenzene at pH 4–6 or 8–11 were used. Since OH and H produce from 2-propanol only 85% of the reducing $(CH_3)_2\dot{C}OH$[16] and since this radical reacts with the nitrobenzenes studied by quantitative formation of nitrobenzene radical anion and H^+,[13] the conductance signals measured correspond to 0.85 (G(OH) + G(H)).

The in situ radiolysis ESR experiments were performed with use of the method described by Eiben and Fessenden.[17]

With both the optical and conductance detection methods the temperature of the solutions studied could be kept constant within ±0.1 °C with cells that are an integral part of a heat exchanger.[18]

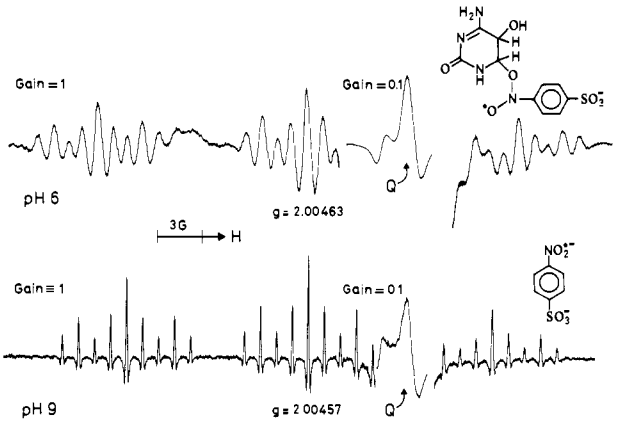
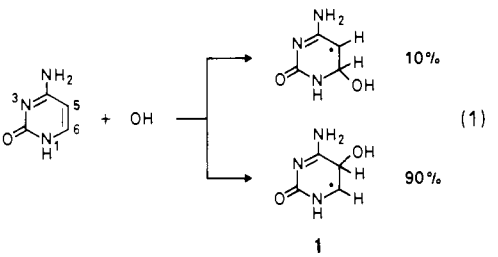


Figure 1. ESR spectra observed on reaction of 5-hydroxycytosine-6-yl with 4-nitrobenzenesulfonate (0.5 mM) at pH 6 and 9. [Cytosine] = 2 mM, $[N_2O] \approx 20$ mM, ≈5 °C.

Results and Discussion

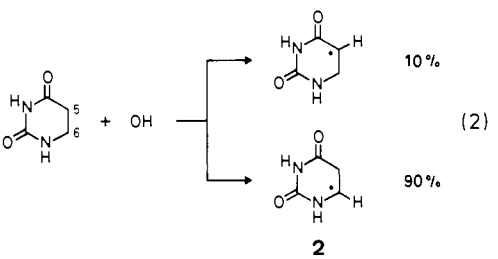
OH radicals were produced by irradiating with 3 MeV electrons N_2O-saturated aqueous solutions containing, e.g., 2 mM pyrimidine and 0.5 mM para-substituted nitrobenzene. Due to the larger concentration of the pyrimidine and to the higher rate constant[19] for its reaction with OH, under these conditions the majority of the OH radicals are scavenged by the pyrimidine. In this reaction, the OH radicals add to the pyrimidine C(5)/C(6) double bond, with a pronounced preference for addition at C(5) to give 5-hydroxy-6-yl radicals **1**,[20,21] cf. eq 1 for the case of cytosine. The analogous 5-hydro-6-yl radicals **2** can be produced



(1)

1

most conveniently by H abstraction by OH from 5,6-dihydropyrimidines. In this reaction OH also reacts very selectively, i.e., by preferential abstraction from C(6),[22] cf. eq 2 for the case of 5,6-dihydrouracil. In contrast to the 5-yl radicals, which have



(2)

2

been shown to be weak oxidants,[20–22] the 6-yl radicals have reducing properties (with respect to tetranitromethane[20–22] and quinones[23]), due to the presence of the amido substituent at C_α.

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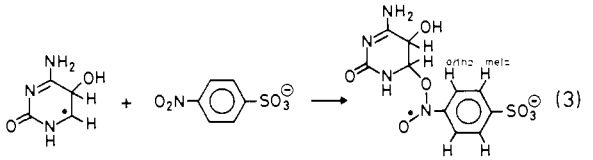
Table I. Coupling Constants (Gauss) and g Factors of Nitroxide Radicals Produced by Addition of Pyrimidin-6-yl Radicals to Substituted Nitrobenzenes[a]

parent pyrimidine	nitrobenzene	a(N)	$a(H)_{ortho}$[b]	$a(H)_{meta}$[b]	g[c]
uracil	4-nitrobenzenesulfonate (4-NBS)	15.0	3.2 (2)	1.1 (2)	2.0045
	4-NBS$^{-\cdot}$[d]	12.81	3.38 (2)	1.14 (2)	2.00457
5,6-dihydrouracil	4-NBS	14.66	3.14 (2)	1.09 (2)	2.00462
5,6-dihydrouracil	4-nitrobenzoate (4-NBA)	14.89	3.15 (2)	1.12 (2)	2.0046
	4-NBA$^{-\cdot}$[d]	13.02	3.35 (2)	1.14 (2)	2.00458
5,6-dihydrouracil	5-nitroisophthalate (5-NPH)	15.6	3.1 (2)	3.1[e]	2.0046
	5-NPH$^{-\cdot}$[f]	13.71	3.38 (2)	3.57[e]	2.00457
1-methyl-5,6-dihydrouracil	4-NBS	14.85	3.22 (2)	1.12 (2)	2.0046
1-methyl-5,6-dihydrouracil	4-NBA	14.95	3.20 (2)	1.12 (2)	2.0046
cytosine	4-NBS	14.75	3.14 (2)	1.10 (2)	2.00463
cytosine	4-nitrobenzenemethylsulfonate (4-NBMS)	14.75	3.12 (2)	1.10 (2)	2.0046
	4-NBMS$^{-\cdot}$[d]	11.75	3.32 (2)	1.03 (2)	2.00461
cytosine	4-NBA	14.95	3.14 (2)	1.14 (2)	2.0046
cytosine	5-NPH	15.7	3.05 (2)	3.05[e]	2.0046
1-methylcytosine	4-NBS	14.91	3.12 (2)	1.11 (2)	2.0045
cytidine	4-NBS	14.89	3.19 (2)	1.13 (2)	2.0046
cytidylic acid (cytidine-5-phosphate)	4-NBS	14.65	3.1 (2)	1.1 (2)	2.0046

[a] N_2O saturated aqueous solutions, 1–2 mM pyrimidine, 0.2–0.5 mM nitrobenzene, pH 4–6, ≈5 °C. [b] Defined relative to the nitroxide group. [c] The numbers with 4 digits after the decimal point were measured relative to the quartz signal (g = 2.00044, ref 17). The numbers with 5 digits were determined by the NMR method. [d] Parameters of the nitrobenzene radical anion, from ref 13. [e] Splitting of the para proton. [f] Parameters of the 5-NPH radical anion.

These radicals are therefore expected to be able to react with weak oxidants such as nitrobenzenes, whereas the oxidizing 5-yl radicals should show no reactivity.

(1) Uracil, 5,6-Dihydrouracil, Thymine, Cytosine, and N(3)-Substituted Derivatives. (a) In Situ Radiolysis ESR Experiments. Figure 1 shows the ESR spectrum observed on producing the C(5)–OH adduct of cytosine **1** (by reaction of OH with cytosine) in the presence of 4-nitrobenzenesulfonate at pH 6. The spectrum consists of a 1:1:1 triplet (a(N) = 14.75 G) of 1:2:1 triplets ($a(H)_{ortho}$ = 3.14 G) of 1:2:1 triplets ($a(H)_{meta}$ = 1.10 G) with g = 2.00463. On the basis of the similarity of these parameters with those of the nitroxide radicals formed by addition of $\dot{C}H_2OH$,[13] $\dot{C}H_2OCH_3$, $CH_2(CH_2)_2\dot{C}HO$, or $CH_2CH_2OCH_2\dot{C}HO$[24] to the nitro group of 4-nitrobenzenesulfonate, the spectrum is assigned to the nitroxide **3**, produced according to eq 3. The observed splittings are due to the nitroxide



3

nitrogen and to the ortho and meta protons on the benzene ring. Attempts to resolve the splitting of the proton at C(6) of the cytosine were unsuccessful. This splitting is therefore smaller than the line width of **3** (0.4 G). The splitting of C(6)–H can, however, be seen with a nitroxide formed by reaction of **1** with 5-nitro-2-furoic acid.[24]

When the pH of the solutions containing cytosine and 4-nitrobenzenesulfonate was increased above ≈7.5, the lines due to **3** disappeared and lines originating from the radical anion of 4-nitrobenzenesulfonate appeared. At pH 9 the nitroxide had disappeared completely and the radical anion was the only radical present (Figure 1). This indicates that the radical anion is produced from the nitroxide by reaction with OH^-.

A nitroxide radical was also produced on reaction at pH 3–6 of **2** with 4-nitrobenzenesulfonate (Figure 2), or with 4-nitrobenzoate or 5-nitroisophthalate or 4-nitrobenzenesulfonic acid methyl ester. In Table I the coupling constants and g-factors of

(24) Steenken, S., unpublished material.

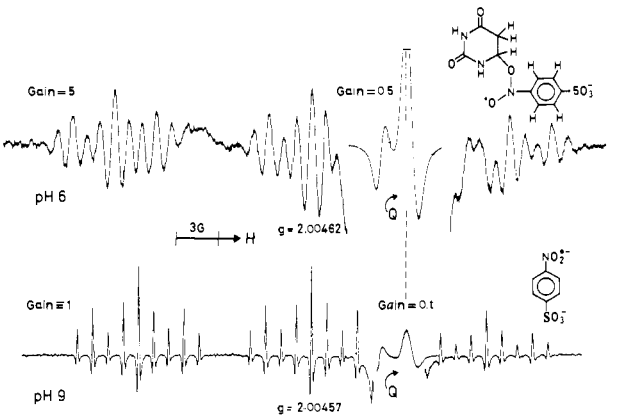


Figure 2. ESR spectra observed on reaction of 5-hydrouracil-6-yl with 4-nitrobenzenesulfonate (0.5 mM) at pH 6 and 9. [5,6-Dihydrouracil] = 2 mM, $[N_2O]$ ≈ 20 mM, ≈5 °C.

the experimentally observed nitroxide radicals are collected together with the data from the radical anions that are formed from the nitroxides by OH^--catalyzed decomposition. As observed with analogous systems,[13] the radical anions are characterized by smaller values for a(N) and larger ones for the ring protons, as compared to the corresponding nitroxide radicals. This phenomenon indicates transfer of unpaired spin density from the nitrogen to the ring in going from the nitroxide to the more highly delocalized radical anion.

The OH^--catalyzed conversion of the nitroxide into the radical anion was observed also with 3-methyluracil, 3-methyl-5,6-dihydrouracil, and 3-methylcytosine. As judged by the pH dependence of the reaction, the presence of the methyl group at N(3) did not seem to alter the sensitivity of the nitroxide toward its decomposition.

(b) Time-Resolved Experiments. In Figure 3 are shown the time-dependent changes in optical density (OD) and the optical absorption spectra observed on reaction of OH with 2 mM uracil in the presence of 0.5 mM 4-nitroacetophenone (4-NAP). At pH 6 there is a rapid buildup of OD at 400 nm followed by a slow exponential decrease, whereas an increase in OD is seen at 330 nm, which is also exponential in nature and is characterized by exactly the same rate as the decrease at 400 nm. The absorption spectrum measured 16 μs after initiation of the reaction with OH represents 5-hydroxyuracil-6-yl (≈ 71%), 6-hydroxyuracil-5-yl

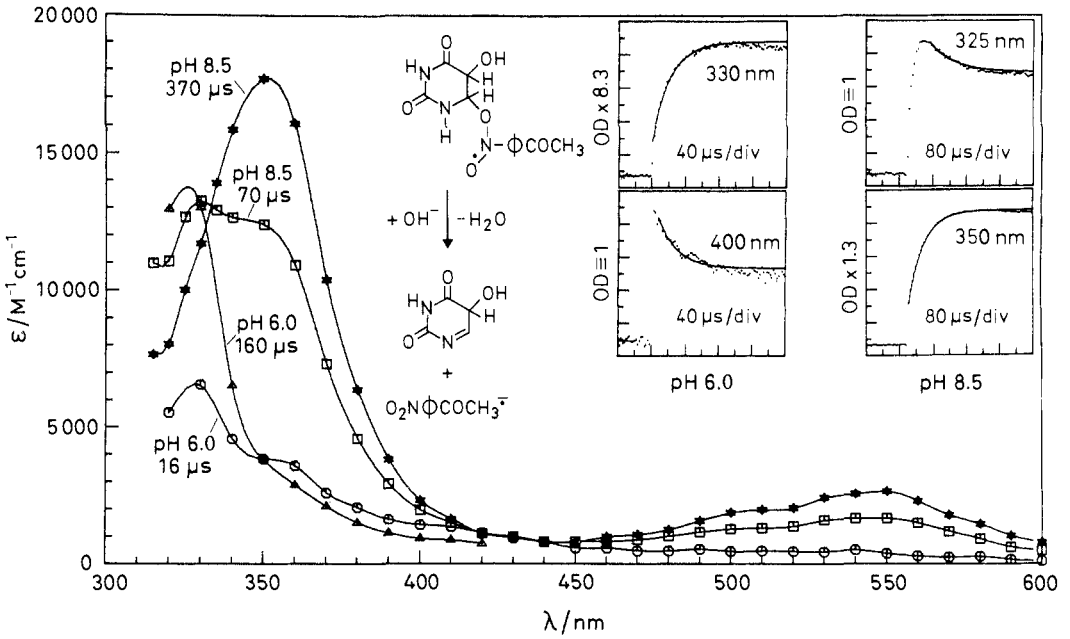


Figure 3. Time-dependent optical absorption spectra observed after reaction of OH with 2 mM uracil in the presence of 0.5 mM 4-nitroacetophenone at pH 6 and 8.5. Insets show the time dependence of the optical density at 325, 330, 350, and 410 nm for two pH values. $[N_2O] \approx 20$ mM, 20 °C. The ϵ values are based on G(nitroxide) = 4.4.

Table II. Rate Constants for Reaction of 5-Hydroxypyrimidin-6-yl Radicals with 4-Nitroacetophenone (4-NAP) and 4-Nitrobenzonitrile (4-NBN)[a]

pyrimidine	nitrobenzene	k/M^{-1} s^{-1}
uracil	4-NAP	4.4×10^7
	4-NAP	1.2×10^9 [b]
1-methyluracil	4-NAP	5.2×10^7
	4-NBN	5.1×10^7
uridine	4-NAP	2.6×10^7
deoxyuridine	4-NAP	5.4×10^7
uridylic acid	4-NAP	6.1×10^6
deoxyuridylic acid	4-NAP	1.0×10^7
	4-NAP	1.1×10^8 [b]
3-methyluracil	4-NAP	6.4×10^7
	4-NBN	7.0×10^7
1,3-dimethyluracil	4-NAP	6.5×10^7
	4-NBN	5.0×10^7
5-methyluracil (thymine)	4-NAP	3.1×10^7
5-carboxyuracil (isoorotic acid)	4-NAP	5.4×10^7
6-methyluracil	4-NAP	3.2×10^8
	4-NBN	4.4×10^8
cytosine	4-NAP	1.2×10^8
1-methylcytosine	4-NAP	2.1×10^8
cytidine	4-NAP	4.4×10^7
cytidylic acid	4-NAP	3×10^7
3-methylcytosine	4-NAP	4.8×10^7

[a] [Pyrimidine] = 2 mM, [nitrobenzene] = 0.5–1 mM, N_2O saturated aqueous solutions, pH 4.5–6.5, λ(observation) = 330 nm, 20 °C. [b] Value refers to the ionized 6-yl radical (pH 11). [c] Measured at λ = 340 nm.

(≈18%), and the OH adduct of 4-NAP (≈11%).[25] The exponential decrease in OD at 400 nm is suggested to be due to the reaction of 5-hydroxyuracil-6-yl with 4-NAP, in which the nitroxide radical shown in Figure 3 is produced. This radical has a λ_{max} of ≈325 nm, i.e., 25 nm lower than the λ_{max} value[13] of the radical anion of 4-NAP. The rate of removal of 5-hydroxyuracil-6-yl and that for the formation of the nitroxide increased with increasing concentration of 4-NAP, and from these dependencies the rate constant for formation of the nitroxide by reaction

(25) These numbers are calculated from the concentrations of uracil and 4-NAP, the rate constants for reaction with OH (ref 10 and 19), and the percentages of OH attack at C(5) and C(6) of uracil (ref 20).

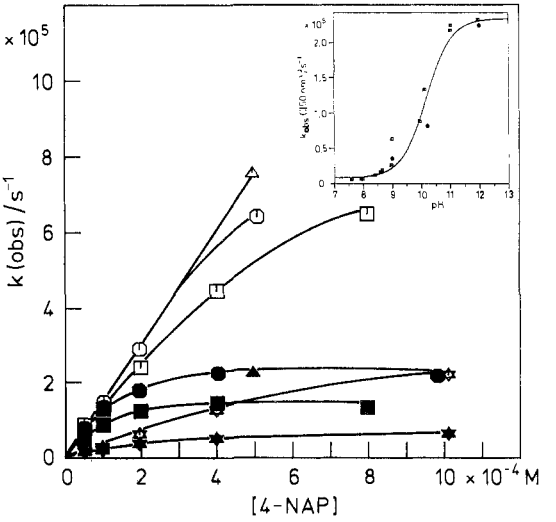


Figure 4. Dependence on pH and [4-NAP] of the rates of formation of nitroxide (measured at 330 nm, open symbols) and 4-NAP radical anion (measured at 350 nm, full symbols): (*) pH 9; (□) pH 10; (○) pH 11; (△) pH 12. [Uracil] = 2 mM, $[N_2O] \approx 20$ mM, 20 °C. The inset shows the dependence on pH of the rate of production of 4-NAP radical anion for solutions containing 2 mM uracil and 0.4–1 mM 4-NAP. The inflection point is at pH 10.2. The upper plateau corresponds to a rate of 2.4×10^5 s^{-1}.

of 5-hydroxyuracil-6-yl with 4-NAP was obtained as 4.4×10^7 M^{-1} s^{-1} (Table II). The spectrum recorded at pH 6 160 μs after the beginning of the reaction is that of the nitroxide.

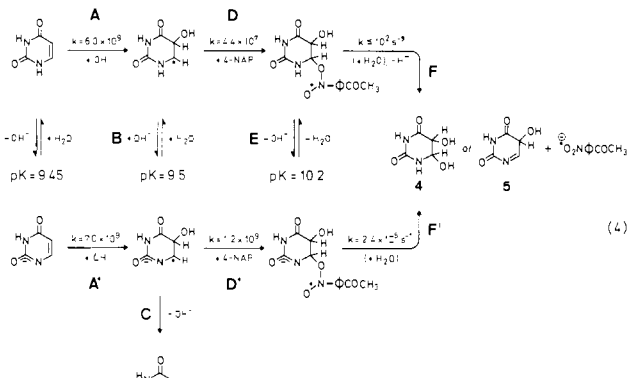
When the pH of the solution was raised above pH 7.5 changes occurred in the buildup and decay kinetics as well as in the absorption spectra of the transients. As seen in Figure 3, at pH 8.5, after a rapid increase in OD at 325 nm (due to the formation of nitroxide) there is a slower (exponential) decrease, which indicates that the nitroxide is decomposed. Synchronous with the nitroxide decay, there is a buildup of absorption at 350 nm, where the radical anion of 4-NAP has a maximum.[8,10,13] The rates of nitroxide decomposition and radical anion production are identical (Figure 3, inset). The base-catalyzed transformation of the nitroxide into the radical anion can also be seen from the time-dependent absorption spectra at pH 8.5: 70 μs after the production of 5-hydroxyuracil-6-yl the spectrum reflects an approximately 1:1

mixture of nitroxide and radical anion, 300 μs later (at 370 μs) the observed spectrum is that of the pure radical anion, produced with 100% yield from 5-hydroxyuracil-6-yl.

It can also be seen from the insets of Figure 3 that not only the rate of *decomposition* of the nitroxide but also the rate of its *production* (as observed at 325-330 nm) increases with increasing pH. In Figure 4 this is demonstrated in a more general way. Figure 4 contains, for the system uracil + 4-NAP, the rates of formation of nitroxide and of 4-NAP radical anion (monitored at 330 and 350 nm, respectively) as a function of [4-NAP] for different pH values from pH 8 to 12. As can be seen, the initial slopes of the k_{obsd} vs. [4-NAP] plots increase with increasing pH for both wavelengths of observation. However, there is a pronounced difference between the two with respect to the dependence of the rates on [4-NAP]: the rates of formation of radical anion (observed at 350 mm) become independent of [4-NAP] at quite low concentrations, whereas the rates of formation of nitroxide, as monitored at 330 nm, show what may be the beginning of a curvature only at the highest accessible 4-NAP concentrations. Due to the presence of a plateau in the k_{obsd} (350 nm) vs. [4-NAP] relation, the dependence of this plateau value on pH can be investigated: at sufficiently high concentrations of 4-NAP (i.e., ≥0.5 mM) the k_{obsd} (formation of radical anion) vs. pH relationship follows a titration curve (see Figure 4, inset, which also contains data not shown in the main figure), with an inflection point at 10.2. The k_{obsd} value corresponding to the upper plateau of this pK curve is 2.4×10^5 s^{-1}.

The reaction between OH and uracil in the presence of 4-NAP was also studied by using conductance detection. At pH 4–6 only a small increase of conductance was seen that could be entirely attributed to the reaction of the hydrated electron with 4-NAP in competition with its reaction with N_2O. This result, which is in agreement with earlier data,[10] shows that in the reaction between the uracil radical(s) and 4-NAP ions are not produced, and this supports the observations by ESR and optical spectroscopy.

(c) Reaction Scheme. The results presented in sections 1.a and 1.b are interpreted in terms of reaction scheme 4, in which 4-NAP is used as a model for all the nitrobenzenes studied.



(4)

Addition of OH to uracil or its anion occurs predominantly at C(5) to produce (reactions A/A') the 6-yl radical[20] which has a pK of 9.5.[26] The neutral and the ionized 6-yl radicals react with 4-NAP by addition (reactions D/D') to give a nitroxide radical, whose absorption spectrum is characterized by a maximum at ≈325 nm. The bimolecular rate constant for the addition process with 4-NAP is 4.4×10^7 M^{-1} s^{-1} for the neutral radical, and that for the conjugate radical anion is much higher, i.e., 1.2×10^9 M^{-1} s^{-1} (obtained from the plot k_{obsd} vs. [4-NAP] at pH 11, see Figure 4). Since the rate constants for reaction of nitrobenzenes with reducing radicals increase with increasing reducing ability of the radical,[13,27] the increase in rate constant caused by ionization of the 6-yl radical indicates a pronounced enhancement of its reducing power. Amplification of reductive capacity by deprotonation is a frequently observed phenomenon, e.g., with α-hydroxyalkyl radicals.[14]

It is interesting that the ionized 6-yl radical does not react with 4-NAP by electron transfer,[28] as do the radical anions of α-hydroxyalkyl radicals.[13,27] The rate constant for reaction 4D' (1.2×10^9 M^{-1} s^{-1}) is lower than those[13,27] ($(3-5) \times 10^9$ M^{-1} s^{-1}) for the α-hydroxyalkyl radical anions. On the basis of a Marcus[29]-type argument, this may be taken to indicate that ionized 5-hydroxyuracil-6-yl is a weaker reductant than the least powerful reductant among the simple ketyl radicals, i.e., $\dot{C}H_2O^-$. The lower reducing capacity of 5-hydroxyuracil-6-yl is probably caused by transfer of electron density from N(1) to the adjacent carbonyl group. This transfer away from the reaction site is relatively more efficient in the radical anion than in the neutral radical, i.e., the increase in reducing power on ionization is insufficient to raise the rate constant to the diffusion-controlled limit, in contrast to the case of $\dot{C}H_2OH/\dot{C}H_2O^-$.[13,27]

Ionized 5-hydroxyuracil-6-yl may undergo elimination of OH^- (eq 4C) to produce an N-centered radical.[20,30] By this reaction, a strong reductant is converted into an oxidant.[20] However, at pH 10–11, the rate[20] of this redox inversion reaction is only $(1-2) \times 10^4$ s^{-1} and therefore, as long as [4-NAP] ≥ 0.1 mM, it does not compete efficiently with reaction D', the addition of ionized 5-hydroxyuracil-6-yl to 4-NAP.

The rate constant for reaction of OH^- with the neutral nitroxide to give the 4-NAP radical anion (reaction E) is $\approx 5 \times 10^9$ M^{-1} s^{-1}. This value is obtained from the initial slopes of k_{obsd}(350 nm) vs. [OH^-] plots (measured in the pH range 7.5–8.7 with [4-NAP] = 0.5–1 mM). The value is similar in magnitude to the rate constants for reaction of OH^- with α-hydroxyalkyl,[31] α-hydroxyperoxyl,[32] or (β-hydroxy)alkoxynitroxide[13] radicals. The pK value for the equilibrium between the neutral and the ionized nitroxide is obtained from the dependence on pH of the rate of formation of radical anion (as measured at 350 nm) under conditions of high enough concentrations of 4-NAP (0.5–1 mM) such that the rate of formation of the nitroxide is not rate determining. The pK value obtained (see inset to Figure 4) is 10.2, which is higher than the pK value of uracil (9.45) or of 5-hydroxyuracil-6-yl (9.5),[26] but it is not unreasonable, since with the nitroxide the uracil 5,6 bond is saturated, which should result in a decrease of the acidity of N(1)–H.

From the upper plateau of the k_{obsd}(formation of radical anion) vs. pH curve, the rate constant for the unimolecular heterolysis of the *ionized* nitroxide (eq F') is obtained as 2.4×10^5 s^{-1}. The mere presence of the plateau indicates that the decomposition of the *ionized* nitroxide is not catalyzed by OH^-. This is not unexpected since, with respect to the uracil C(6) position, the nitroxide is of the acetal/amidal type, which are usually resistant to base-catalyzed decomposition. The heterolysis of the ionized nitroxide may thus be identified as an S_N1 reaction. An S_N1 mechanism was also observed[15] in the heterolysis of acetal-type nitroxide radicals formed by addition of α-alkoxyalkyl radicals to tetranitromethane.

The rate constant for heterolysis of the neutral nitroxide, eq F, is $\leq 10^2$ s^{-1}. This is concluded from the absence at pH ≤6.5 of radical anion or H^+ formation (by conductance detection) even at initial concentrations of nitroxide as low as ≈ 0.2 μM, where the lifetime with respect to bimolecular decay is ≥10 ms.

The nonradical product of the decomposition of the nitroxide is suggested to be the glycol **4**, or the 5-hydroxyisopyrimidine derivative **5** (for simplicity, only the latter is shown in Figure 3). In the case of reaction of OH adducts of thymine with nitro compounds, the corresponding glycol has been identified[11] as the

(26) Hayon, E.; Simic, M. *J. Am. Chem. Soc.* **1973**, *95*, 1029. Shragge, P. C.; Hunt, J. W. *Radiat. Res.* **1974**, *60*, 233.
(27) Asmus, K.-D.; Wigger, A.; Henglein, A. *Ber. Bunsenges. Phys. Chem.* **1966**, *70*, 862.
(28) If ionized 5-hydroxyuracil-6-yl did react by electron transfer, the dependence of the rates of formation of radical anion on [4-NAP] would not show a plateau region and they would be independent of λ(observation).
(29) Marcus, R. A. *Annu. Rev. Phys. Chem.* **1964**, *15*, 155.
(30) Bansal, K. M.; Fessenden, R. W. *Radiat. Res.* **1978**, *75*, 497.
(31) Laroff, G. P.; Fessenden, R. W. *J. Phys. Chem.* **1973**, *77*, 1283.
(32) Bothe, E.; Schulte-Frohlinde, D. *Z. Naturforsch.* **1977**, *32b*, 886.

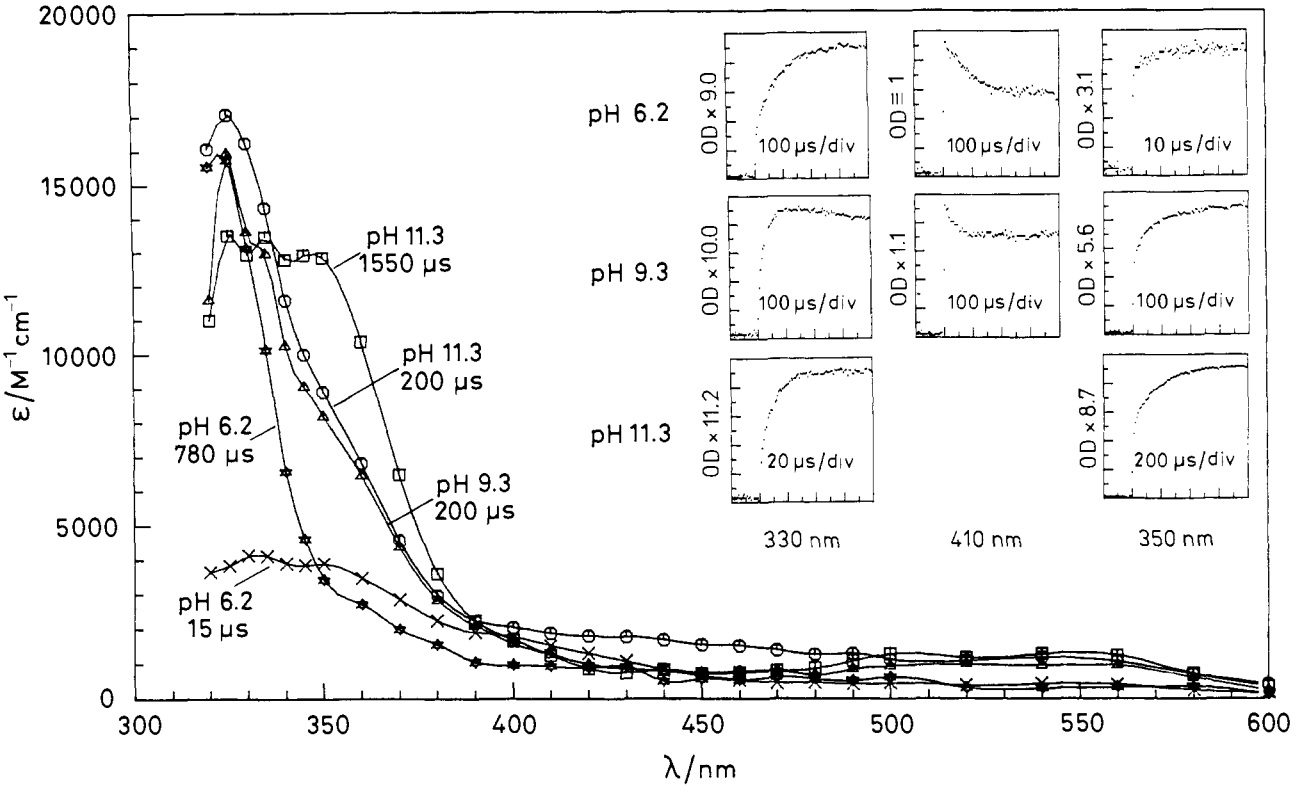
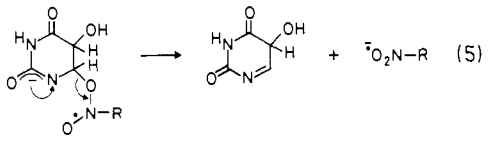


Figure 5. Time-dependent absorption spectra observed after reaction of OH with 2 mM deoxyuridylic acid in the presence of 0.5 mM 4-NAP at pH 6.2, 9.3, and 11.3. Insets show the time dependence of the optical density at 330, 350, and 410 nm for the different pH values. The ϵ values are based n G(nitroxide) = 4.4.

main radiation chemical product at pH 7. Production of the glycol **4** involves reaction with a molecule of water of the hypothetical C(6) carbocation in the course of eq F or F′, whereas the hydroxyisopyrimidine **5** would result directly from the heterolysis of the ionized nitroxide, cf. eq 5. The isopyrimidine **5** has been



shown[33] to rearrange to give isobarbituric acid, which in addition to the glycol **4** is a major product from the reaction of OH with uracil.[33a,34] In the presence of the oxidant ferricyanide there is a pronounced pH dependence of the ratio **5**/**4**: from pH 3–4 to 5–6 it increases from 0.2 to 0.9.[33a]

As a conclusion, it can be stated that reaction scheme 4 describes in a consistent way the results presented and also results described in the literature[10,11] on the reactions of 5-hydroxyuracil-6-yl and analogous radicals with nitro compounds. The absence at pH 5.5 of electron transfer has previously been documented in a very careful investigation;[10] however, the effect of pH was not studied and it was thus not seen that the nitroxide is able to undergo a base-catalyzed heterolysis which may mimic an electron-transfer reaction since it results in the formation of a one-electron reduced nitro compound.

Results analogous to those described for uracil were obtained for 3-methyluracil, thymine, 6-methyluracil,[35] 5,6-dihydrouracil, 5,6-dihydrothymine, isoorotic acid, cytosine, and 3-methylcytosine. The rate constants for formation of nitroxide by reaction of the 6-yl radicals are listed in Tables II and III. The rate constants tend to be larger for the cytosine radicals than for the corresponding uracil radicals, and they are still larger for the 6-yl radicals produced by H abstraction from the 5,6-dihydrouracils. These differences in rate constants probably reflect differences in reducing power of the radicals. With respect to uracil/cytosine, it has previously been inferred[21] that the cytosin-6-yl radical is more electron-rich than that from uracil and the former should therefore be the better reductant. Concerning 5-*hydroxy*uracil-6-yl/5-*hydro*uracil-6-yl, the considerable difference in rate constant between the two, which corresponds to a factor of 7.5, is due to the substituent at the β-position: the lower rate constant (=reducing power) for 5-*hydroxy*uracil-6-yl originates from the

Table III. Rate Constants for Reaction of 5,6-Dihydropyrimidin-6-yl Radicals with 4-Nitroacetophenone[a]

pyrimidine	k/M^{-1} s^{-1}
5,6-dihydrouracil (DHU)	3.3×10^8
1-Me-DHU	1.7×10^8
5,6-dihydrouridine	5.3×10^7
5-Me-DHU (5,6-dihydrothymine)	2.7×10^8
6-Me-DHU	1.7×10^9

[a] [Pyrimidine] = 2 mM, [nitrobenzene] = 0.1–1 mM, N_2O saturated aqueous solutions, pH 4.5–6.3, λ(observation) = 330 nm, 20 °C.

(33) (a) Al-Yamoor, K. Y.; Garner, A.; Idriss Ali, K. M.; Scholes, G. "Proceedings of the 4th Tihany Symposium Radiation Chemistry", Hedvig, P., Schiller, R., Eds.; Akademiei Kiado: Budapest 1977, p 845. (b) Schuchmann, M. N.; von Sonntag, C. *J. Chem. Soc., Perkin Trans. 2* **1983**, 1525.

(34) By using HPLC with electrochemical detection, the yield per OH of isobarbituric acid from the reaction of OH with 2 mM uracil in the presence of 0.2 mM 4-nitrobenzenesulfonate was found to increase with pH from 4.3% at pH 4.9 to a plateau value corresponding to 53% at pH 9.6–11.

(35) With this compound there is a spontaneous, i.e., uncatalyzed, heterolysis of the nitroxide (Jagannadham, V.; Steenken, S., manuscript in preparation).

(36) Experiments with tetranitromethane as an oxidant for reducing radicals and *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine as a reductant for oxidizing radicals indicate that the ratio of reducing to oxidizing radicals from the reaction of OH with N(1)-substituted uracils is independent of the nature of the substituent (from H and CH_3 to ribose or deoxyribose (phosphates)). In addition, the ratio is also invariant with pH (from 7 to 11). From this it is concluded that ≥80% of the OH radicals react with uridines and uridinephosphates by addition to C(5) of the uracil moiety, using an argument similar to that applied (ref 21) to analogous cytosine derivatives.

(electron-withdrawing) inductive effect of the OH group at C(5) (≡C_β to C(6), the radical and reaction site).

The nitroxide radicals formed from the 6-yl radicals of the pyrimidines mentioned above react with OH^- to give the nitrobenzene radical anions with k values of the order $\approx 5 \times 10^9$ M^{-1} s^{-1}, as calculated from the rates of production of radical anion as a function of [OH^-] in the pH range 7.5–8.7. However, in contrast to the other pyrimidines, with cytosine and 3-methylcytosine the *yield* of nitrobenzene radical anion does not reach 100% at pH >8.5. This is due to the fact that with 5-hydroxycytosin- and 5-hydroxy-3-methylcytosin-6-yl the dehydration reaction[21] (=analogous to step 4C) competes very efficiently with the addition reaction (analogous to step 4D′).

It is reasonable to assume that in all cases the mechanism of the OH^- induced radical anion formation involves deprotonation of N(1)–H followed by $S_N(1)$ type heterolysis of the ionized nitroxide, analogous to the mechanism discussed with the uracil system (eq 4).

(2) N(1)-Substituted Pyrimidines. (a) Deoxyuridylic Acid (2′-Deoxyuridine-5′-phosphate). In Figure 5 are presented kinetic traces and optical absorption spectra which show the reaction between 4-NAP and essentially the *N*(1)-deoxyribosyl-5-hydroxyuracil-6-yl radical (=d-UMP-6-yl, see eq 7). The absorption spectrum measured 15 μs after production of the OH radicals at pH 6.2 is predominantly due to this radical.[36] At the same pH, 765 μs later the observed spectrum has grown in intensity and it has a distinct peak at 325 nm, a region where nitroxide radicals from 4-NAP seem to absorb.[13] The insets (first horizontal row in Figure 5) show the buildup of the nitroxide at 330 nm, and the decrease of the concentration of d-UMP-6-yl at 410 nm, which occurs with exactly the same rate as the production of the nitroxide at 330 nm. From the dependence on [4-NAP] of these rates the rate constant for reaction of d-UMP-6-yl with 4-NAP was obtained as 1.0×10^7 M^{-1} s^{-1}. The third picture in the upper horizontal row shows that at 350 nm there is a very rapid buildup of optical density followed by a further increase. This further growth is approximately ten times faster than the buildup at 330 nm. If it is assumed that it is due to formation of the radical anion of 4-NAP, the yield of the additional increase can be calculated to correspond to 3% of the OH radicals. It is tentatively suggested that the radical responsible for this low yield but fast reaction[37] is C(1)′-yl, i.e., that produced by H abstraction from C(1) of the deoxyribose unit.[38] This radical should be a good reductant, since it is substituted at C_α by *two* heteroatoms.[15]

As seen from the kinetic traces in the second horizontal row of the insets in Figure 5, at pH 9.3 the rate of formation of nitroxide, as observed at 330 nm, is considerably accelerated compared to that at pH 6.2. At the same time, the rate of decrease of the absorption at 410 nm, which is assigned to the d-UMP-6-yl radical, is faster than that at pH 6.2 by exactly the same factor as the increase at 330 nm. At 350 nm, however, where the radical anion absorbs, the buildup of optical density is much slower. Moving on to pH 11.3 (third horizontal row), the buildup at 330 nm is still faster, and the decrease at 410 nm (not shown) appears to be instantaneous, whereas the slow rate of increase in the absorption of the radical anion at 350 nm is still the same as that at pH 9.3. The increase with pH in the rate of reaction between d-UMP-6-yl and 4-NAP is thus clearly evident from the first two vertical rows, as is the *in*variance with pH of the slow increase at 350 nm from the two bottom traces in the third vertical row. The pH dependence of the rates is shown in a more systematic way in Figure 6: the rates of formation of nitroxide at 330 nm follow a dissociation curve with the inflection point at pH 10.3

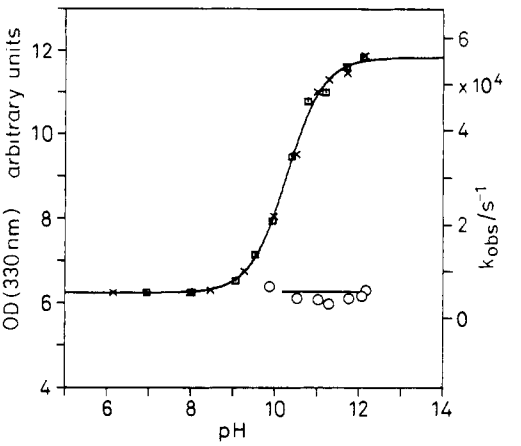


Figure 6. The dependence on pH of the rates of production of (a) the nitroxide from the reaction d-UMP-6-yl with 4-NAP (observed at 330 nm (×)) and of (b) the radical anion of 4-NAP (monitored at 350 nm (○)). [d-UMP] = 2 mM, [4-NAP] = 0.5 mM, [N_2O] ≈ 20 mM, 20 °C. (c) The squares (□) represent the OD values measured at 330 nm in the *absence* of 4-NAP (see also Figure 7).

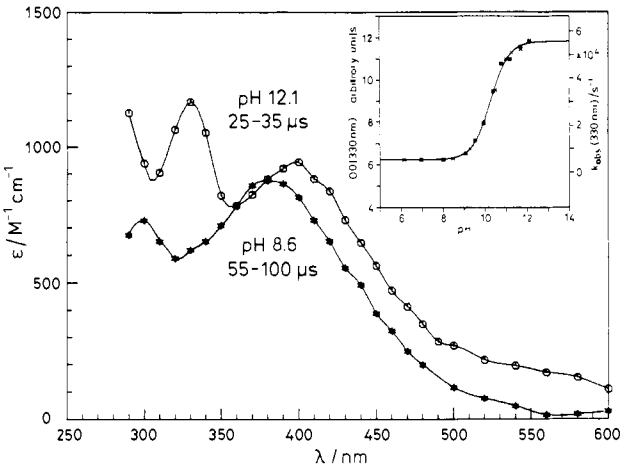
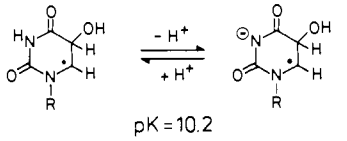


Figure 7. Absorption spectra of the OH adduct(s) of d-UMP (predominantly d-UMP-6-yl) at pH 8.6 and 12.1. The inset shows the dependencies on pH of the OD at 330 nm (squares) and of the rates of production of nitroxide from d-UMP-6-yl plus 4-NAP (crosses). The sigmoidal curve is from a computer fit assuming a pK of 10.2.

whereas the rates of production of 4-NAP radical anion at 350 are constant between pH 10 and 12.3 (traces measured below pH 10 are difficult to analyze accurately due to insufficient separation between the fast (=formation of nitroxide) and slow (=formation of radical anion) components).

The decomposition of the nitroxide at pH 11.3 is also reflected by the time-dependent absorption spectra (Figure 5): at 200 μs after initiation of the reaction the spectrum (λ_{max} = 325 nm) is that of the nitroxide; approximately 1.5 ms later ≈70% have been converted into the radical anion.

In order to obtain information on the mechanism which causes the pH dependence of the rate of nitroxide formation by reaction of d-UMP-6-yl with 4-NAP, the absorption spectra from the reaction of OH with d-UMP in the *absence* of 4-NAP were measured as a function of pH. The results are presented in Figure 7. The spectrum recorded at pH 8.6 is assigned to mainly un-ionized d-UMP-6-yl, that at pH 12.1 to d-UMP-6-yl ionized at N(3) (eq 6).



pK = 10.2 (6)

R = 2′-deoxyribose-5′-phosphate

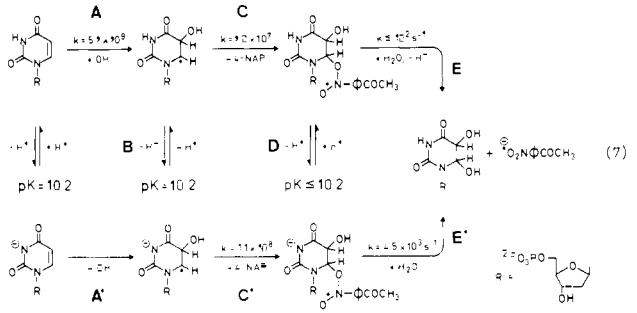
(37) The rate of this increase (=1×10^5 s^{-1}) was found to be independent of [4-NAP] in the range 0.2–1 mM. This indicates that the buildup is due to unimolecular heterolysis of the nitroxide formed by addition of C(1)′-yl to 4-NAP.

(38) With cytidine a similar reaction was seen with $k = 5 \times 10^4$ s^{-1}. In this case the yield of C(1)′-yl corresponds to 2.8% of the OH radicals.

(39) The pK_a value for dissociation of 2′-deoxyuridine-5′-phosphate was determined by spectrophotometric titration, monitoring the OD at λ 230 and 262 nm, to be 10.2 ± 0.1. The pK_a value for uridine-5′-phosphate was measured to be 10.1 ± 0.1.

The inset in Figure 7 contains two sets of parameters as a function of pH: (a) the OD values measured at 330 nm in the *absence* of 4-NAP, and (b) the rate constants for production of nitroxide (in the *presence* of 4-NAP), scaled to fit the range of OD change. It is evident from the figure that both sets of data fit the same dissociation curve, with a pK value of 10.2, which is identical within experimental error with the individual pK values (see, e.g., Figure 6).

The results described are summarized in reaction scheme[39] 7.



The OH radical reacts with d-UMP predominantly by addition to C(5) of the uracil ring, to give the 6-yl radical.[36] This radical is a weak acid. It deprotonates at N(3) (eq 6) with a pK of 10.2. The neutral radical reacts with 4-NAP with a rate constant of 1.0×10^7 M^{-1} s^{-1} (measured at pH 6.2), and the radical anion shows a higher reactivity, with its rate constant of 1.1×10^8 M^{-1} s^{-1} (measured at pH 11–12). In both cases a nitroxide radical is formed, which may exist as a neutral and as an anion radical. The radical anion undergoes a unimolecular heterolysis (step 7E′), which is not accelerated by OH^-, to yield the radical anion of 4-NAP and, presumably, the C(5)–C(6) glycol of d-UMP. The rate constant for this process is 4.5×10^3 s^{-1} (obtained from the pH-independent rate of radical anion formation between pH 10 and 12). From the independence on pH of the rate of heterolysis (step 7E′) in the pH range 10–12 it is concluded that the pK_a for deprotonation of the neutral nitroxide (eq 7D) is ≤10.2.

In order to further check reaction scheme 7, experiments with conductance detection were performed on the reaction of OH with uridine and 2′-deoxyuridine in the presence of 4-NAP at pH 4–6. As with uracil, only very small changes in conductance were seen, which shows that the 6-yl radicals do not react with 4-NAP by electron transfer, in agreement with the ESR and optical data.

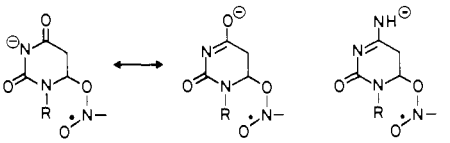
(b) Other N(1)-Substituted Pyrimidines. The N(1)-substituted pyrimidines studied are listed in Tables II and III. In all cases the observations made are similar to those described for the case of d-UMP, i.e., formation of the nitroxide due to reaction of the pyrimidin-6-yl radical with the nitrobenzene was the predominant reaction. In all cases except 1,3-dimethyluracil, the nitroxides could be converted into the corresponding nitrobenzene radical anion by reaction with OH^-, although this conversion was not quantitative at, e.g., pH 11 and initial radical concentrations of ≈1 μM. This is explainable in terms of competition between heterolysis and radical–radical decay. The fact that the nitroxide from 1,3-dimethyluracil is not decomposed even in strong base (pH 13) underlines the importance of N(3)–H in the base-catalyzed decomposition of the nitroxides from uracil nucleosides and nucleotides. It also supports the conclusion arrived at with the d-UMP system, that the nitroxide heterolysis is not of the S_N2 type.

With respect to sensitivity to OH^- catalysis, the nitroxides derived from 5,6-dihydrouracils seem to be more labile and those from cytosines less labile as compared to those from uracils. For an explanation it is suggested that with the dihydrouracils the rate constant for heterolysis of the ionized nitroxide (equivalent to reaction 7E′) is larger than that for the uracil system. Due to the $-I$ effect of the OH group at C(5), 5-*hydroxy*uracil-6-yl radicals are weaker reductants than those of 5-*hydro*-6-yl type and should therefore heterolyze more slowly.

Concerning the N(1)-substituted cytosines, nitrobenzene radical anion formation becomes noticeable only above pH ≈11. This probably means that the amino group at C(4) has to be deprotonated in order to make the rate of heterolysis of the nitroxide become $\geq 10^3$ s^{-1}.

(3) Summary and Conclusions. Pyrimidin-6-yl radicals, formed by addition of OH to C(5) of uracils and cytosines or by H abstraction from C(6) of dihydrouracils, react with nitrobenzenes by addition to an oxygen of the nitro group to yield nitroxide radicals. The nitroxide radicals were identified by their ESR and optical absorption spectra ($\lambda_{max} \approx 325$ nm for the case of 4-NAP). On the basis of these spectra and also from conductance experiments electron transfer between pyrimidin-6-yl radicals and nitrobenzenes can be excluded, in agreement with earlier results.[9,10]

The nitroxide radicals are able to undergo an OH^--catalyzed heterolysis to yield the radical anion of the nitrobenzene and an oxidized pyrimidine.[34] In the case of the nitroxides substituted at N(1) by H, the OH^- catalysis involves deprotonation at N(1) which is adjacent to the reaction site (=C(6)). If, however, N(1) is alkylated (as with the nucleosides and nucleotides), OH^- catalysis is much less efficient since it now proceeds by deprotonation from N(3) (with the uracils) or from the amino group at C(4) (with the cytosines). In these cases the area of deprotonation is separated from the reaction site by a (hydroxy)methylene group,



which means that the increase in electron density resulting from deprotonation is not transferable efficiently to the reaction site, in contrast to the case of deprotonation from N(1), which is adjacent to the reaction site.

A few remarks may be added relating to the mechanism of action of nitro compounds as radiation sensitizers. It is very well established[2-6] that there is a correlation between sensitizing efficiency of a sensitizer and its one-electron redox potential. To a first approximation, for a compound to be a good sensitizer, its redox potential should be in the range −0.7 to −0.2 V/NHE.[2] The existence of the upper limit is explained by interference of the sensitizer with the biological electron transfer metabolism of oxygen and the lower limit by insufficient ability to scavenge unpaired negative charge (=trapped electrons) or oxidize reducing radicals by electron transfer.

On the basis of the results presented in sections 1 and 2, the ability of nitro compounds to scavenge radicals by *addition* rather than by electron transfer should be considered more thoroughly. In fact, oxygen,[16a,40] which is still the most potent radiosensitizer,[2] and nitroaromatics[13] seem to have in common the pronounced tendency to react with reducing radicals not by electron transfer but by addition, and this in spite of the fact that, on the basis of the redox potentials of the reactants, electron transfer would be strongly exothermic.[41] It may be that it is this ability of nitro compounds to add to radicals that makes nitro compounds good substitutes for oxygen in radiosensitization.

A second analogy between oxygen and nitroaromatics refers to the way the radical adducts may react further: if there is an OH or an NH group at C_α, which carries the $-OO\cdot$ or $-ON(\dot{O})$ group, a base-catalyzed heterolysis of the C–O bond may occur,[13,20,21,32,33b] which results in a one-electron oxidation of the radical. The results presented in section 2.a provide an example for heterolysis of a C–O bond after OH^- catalysis at a site remote from the heterolysis reaction center. A similar mechanism may also be possible for peroxy radicals from 6-yl radicals of nucleosides and nucleotides.

On the basis of the excellent correlations[2-6] between sensitizer redox potential and its radiation-biological sensitizing efficiency it is tempting to conclude that the chemical *mechanism* of sensitization involves electron transfer. However, the correlations are as well explainable in terms of an *addition* mechanism. The

(40) Willson, R. L. *Int. J. Radiat. Biol.* **1970**, *17*, 349.
(41) For the redox potentials of the species involved see, e.g. ref 6 or 14.

only requirement is that the *transition state* of the addition reaction be ionic in nature. If this is the case, a Marcus-type correlation between rate constants for addition and redox potentials of the sensitizers is expected. The reactions of nitrobenzenes with α-hydroxyalkyl radicals[13] and with pyrimidin-6-yl radicals (see Tables II and III) seem to fall into this category, as judged from the dependence on radical structure of the rate constants for the addition reaction.

In conclusion, it appears that the ability of nitro compounds to react with reducing radicals by addition[8,10,12-15] may be important in understanding their radiosensitizing action. Binding of nitro compounds to radicals derived from pyrimidine nucleotides[3] and from DNA[7] has in fact been observed.

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Conformation of Mono- and Dicarboxylic Acids Adsorbed on Silver Surfaces

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Abstract: The geometry and conformation of a number of the ions of monocarboxylic and dicarboxylic acids adsorbed on silver colloid surfaces were deduced from their SERS spectra. Monocarboxylic acids adopt an all-trans conformation on the silver sol surface, as evidenced by the absence of gauche markers in the skeletal C–C stretching region of the spectrum, the presence of the low-frequency "accordion" mode in the C–C–C deformation region, and the alternation of the intensity of the asymmetric CH_3 stretching vibration with the number of carbons in the alkyl chain. We speculate that this conformation is chosen in order to avoid, as much as possible, hydropholic interactions between the alkyl chain and the aqueous ambient. The carboxylate group is responsible for the surface bond, probably chelating to silver surface sites. The dicarboxylate acids bind through both carboxylate groups. The polymethylene chain is therefore arranged in such a way as best to accomplish this. As a result, trans "markers" are absent suggesting the presence of a large number of gauche or nearly gauche bonds. Succinate ion is shown to have a cis conformation so as to "stand" on the surface on its two carboxylate groups.

Surface-enhanced Raman spectroscopy has been used to investigate a large number of molecules adsorbed on specially prepared metal surfaces[1] among which silver is the most prominent. We have recently shown that molecular orientation upon the metal surface may be deduced from the SERS spectra by applying simple arguments regarding the relative intensities of SERS bands or in some cases the absence of bands.[2,3] Creighton[4] has used similar arguments to deduce the orientation of pyridine on the surface of Ag, Au, and Cu sol particles. In this paper we investigate the surface geometry and conformation of a number of the ions of carboxylic and dicarboxylic acids by analyzing their SERS spectra. An investigation of the conformation of a long-chained thiol adsorbed on silver colloid particles precedes ours.[5]

Experimental Section

Silver sols were made according to the recipe reported in previous papers.[2,3] Briefly, a sodium borohydride solution (60 mL of 2×10^{-3} M) was mixed with a silver nitrate solution (22 ± mL of 1×10^{-3} M).

The adsorbate was introduced to the colloid by dissolving the acids either in pure triply distilled water or in NaOH solution of various pH. A single measured drop of these solutions was added to approximately 1.5 mL of colloid. Concentrations of less than 1 mM resulted thereby. Spectra were recorded with a SPEX 1401 monochromater equipped with photon counting and interfaced to a Textronix 4052 computer. The 514.5-nm line of the Ar^+ laser was used for most of the experiments at a power level of approximately 150 mW. Slits were set to 4 cm^{-1} with acquisition times of approximately 0.5 s.

Results

The SERS spectra of a series of carboxylic acids (valeric to decanoic) and of dicarboxylic acids (oxalic to suberic) adsorbed on aqueous silver sol particles are shown in Figures 1 and 2. The spectra of members of each group (apart from oxalic and valeric acids) show great similarity to one another, yet each differs markedly from its ordinary solution or liquid Raman counterpart. From the fact that the Raman spectrum of an equivalent number of moles of adsorbate in solution in the absence of silver was undetectable even at a 1000-fold greater sensitivity, we conclude that the SERS spectra of these compounds exhibit considerable enhancement.

The best spectra were obtained with colloid which had aggregated under the influence of the adsorbate. This aggregation process, which is known to produce assemblies of randomly touching sol particles often containing thousands of colloidal particles, was signalled by a change in the colloid's color following addition of adsorbate, from yellow to blue over a period of several hours. The spectra reported were obtained with blue colloids.

No difference was noted between spectra obtained with base added as opposed to base absent expect that with suberic acid no SERS spectrum was obtained when base was added. Likewise with sebacic acid we could not obtain a SERS spectrum under any pH conditions. With monocarboxylic acids the SERS spectra became undetectable when chain lengths beyond dodecanoic acid were used.

With all the acids studied the dominant band in the spectrum is near 1400 cm^{-1}. This is unequivocally assigned to the symmetric stretching vibration of the carboxylate group, indicating, simultaneously, that the acids bind to the surface as anions and that the COO^- group is almost certainly on the surface and forms the surface bond. One observed, in addition, intense vibrations in the CH stretching region, C–C stretching C–C–C bending, and COO^- deformation regions. With some acids one also sees metal–molecule stretching vibrations suggestive of chelating COO^- groups.

With propionic and butyric acids and newly made colloid one sometimes sees the asymmetric COO^- stretching vibration at 1000 cm^{-1}. At times the intensity of this band even exceeds that of the symmetric COO^- stretch.

Discussion

Monocarboxylic Acids. The high intensity of the COO^- symmetric vibration suggests that the series of carboxylic acids are

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