# The Radiolysis of Uracil in Oxygenated Aqueous Solutions. A Study by Product Analysis and Pulse Radiolysis

**Man Nien Schuchmann and Clemens von Sonntag ***
*Max-Planck-Institut für Strahlenchemie, Stiftstrasse 34—36, D-4330 Mülheim a.d. Ruhr, Germany*

Hydroxyl radicals are generated by the radiolysis of $N_2O$–$O_2$ (4 : 1 v/v)-saturated aqueous solutions of uracil. They add to the 5,6-double bond of the substrate [80% at C(5), 20% at C(6)]. These radicals are converted by oxygen into the corresponding peroxyl radicals (I) and (II), respectively. Peroxyl radical (I) undergoes a base-induced $O_2^{-\cdot}$ elimination ($k_{obs}$ 8 × $10^3$ $s^{-1}$ at pH 10.5). As an intermediate 5-hydroxyisopyrimidine is formed which rearranges into isobarbituric acid and adds water forming 5,6-dihydro-5,6-dihydroxyuracil. Competing with this base-induced reaction of radical (I) there is a bimolecular decay of radicals (I) and (II). These processes become predominant at low pH. For this reason a strong pH dependence of *G* (products) is observed. The major products are (*G* values at pH 3 and 10 in parentheses) 5,6-dihydroxy-5,6-dihydrouracil (1.1; 2.4), isobarbituric acid (0; 1.2), *N*-formyl-5-hydroxyhydantoin (1.6; 0.2), 5-hydroxybarbituric acid (0.9; 0.2). 5-Hydroxybarbituric acid is formed in its keto form. Its deprotonation (*k* 4.4 $s^{-1}$) has been followed by pulse conductometry. Details of the reaction mechanism, *e.g.* the involvement of oxyl radicals in the bimolecular decay of (I) and (II), are discussed.

The radiolysis of uracil in aqueous solutions has been the subject of extensive studies [1–6] as a model for the effect of radiation on nucleic acids.

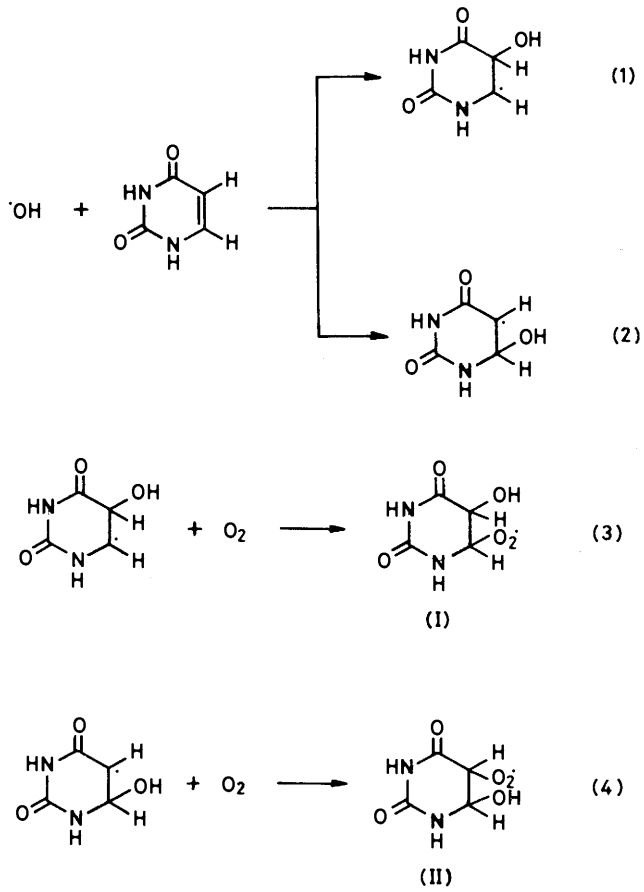
Hydroxyl radicals from the radiolysis of water react with uracil by addition to the 5,6-double bond. The preferential site of attack is at C-5 (82%) [6] [reaction (1)], with the attack at C-6 making up the rest [reaction (2)].

In $N_2O$-saturated dilute aqueous solutions of uracil the effective scavenging of OH radicals is *G* † (uracil ·OH adducts) 5.4 in 1mM-uracil solutions and 5.3 in 2 × $10^{-4}$M solutions.[7]

In the presence of oxygen, the 6-yl radicals formed in reaction (1) add oxygen at near diffusion-controlled rates [8] to give the corresponding peroxyl radicals (I) [reaction (3)]. There is evidence that the 5-yl radicals formed in reaction (2) do not add oxygen at such a fast rate. These radicals are electron-deficient (oxidising) radicals due to the α-keto function. The same type of radicals can be generated by hydrogen abstraction from acetone. Oxygen consumption measurements and product analysis studies of γ-irradiated $N_2O$–$O_2$-saturated aqueous solutions of acetone [9] clearly show that such radicals also react with oxygen albeit with a rate less than diffusion controlled and hence reaction (4) has to be considered as well. Hydrogen atoms from the radiolysis of water are also scavenged by oxygen to give $HO_2$· [$G(HO_2\cdot) \approx G(H\cdot) \approx 0.55$], since in these experiments [$O_2$] ≈ [uracil] and $k(H\cdot + O_2) \gg k(H\cdot$ + uracil).

A number of products formed after the decay reactions of these peroxyl radicals have been identified.[2,4,5] The precise mechanisms involved were however not adequately understood. In the meantime further research into the general reactions of aqueous organic peroxyl radicals,[10–13] combined with studies on the closely related peroxyl radicals derived from 5,6-dihydrouracil,[14] now permits a better understanding of the processes involved.

In the case of 5,6-dihydrouracil [14] the peroxyl radical at C-6, which has an α-N–H group similar to that in radical (I), has been shown to eliminate $O_2^{-\cdot}$ in basic solutions [reaction (5)]. The second step of reaction (5) is very fast ($k \approx 8 \times 10^4$ $s^{-1}$). Thus at low $OH^-$ concentrations the first step is rate determining.

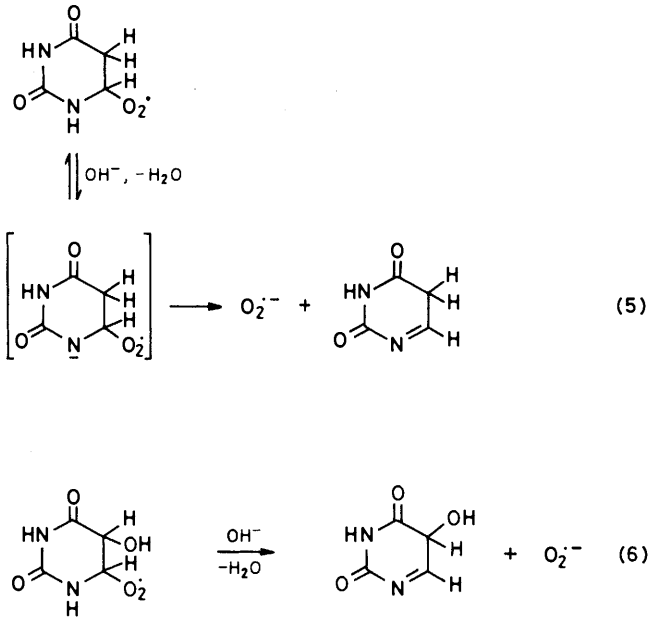



It can be reasonably expected that radical (I) can also undergo a similar reaction besides the usual bimolecular reactions (*cf.* ref. 12). Indeed it has been suggested from evidence obtained by pulse radiolysis that radical (I) can eliminate $O_2^{-\cdot}$ in basic solutions [reaction (6)].[6]

## Experimental

Uracil (Merck), [2-$^{14}$C]uracil (Amersham), isobarbituric acid, and parabanic acid (imidazolinetrione) were commerci-

† The *G* value is defined as molecules formed per 100 eV of absorbed energy. *G* = 1 corresponds to a concentration of 0.1036 μM after a dose of 1 Gy.



(5)



(6)

ally available. The following compounds were synthesized for use as authentic material to be compared with the irradiation products.

1-Formyl-5-hydroxyhydantoin (4) (m.p. 162 °C) was synthesized by the action of ozone on uracil.[15] The 270 MHz $^1$H n.m.r. spectrum of this compound in DMSO confirmed its structure with one proton (5-H) at δ 5.6 (d, $J \approx 8$ Hz), one exchangeable proton (5-OH) at 7.7 (d, $J \approx 8$ Hz), one proton (CHO) at 9.05 (s), and one exchangeable proton (3-H) at 11.85 (s). The mass spectrum of this compound showed its molecular ion at *m/z* 144 and that of its trimethylsilyl derivative showed $M - 15$ at *m/z* 273. The material appears to be somewhat unstable and both by t.l.c., and g.l.c. after silylation, 5-hydroxyhydantoin and to a lesser extent parabanic acid hydrate were always observed as decomposition products. Clearly this instability should also apply to the radiolytically formed 1-formyl-5-hydroxyhydantoin.

Dialuric acid (5-hydroxybarbituric acid) (5) was prepared [16] from alloxan by reduction with $SnCl_2$ (m.p. 215—218 °C with reddening >180 °C). This compound is not simple to deal with because of its keto–enol tautomerisation. The u.v. spectrum of this compound in aqueous solution has an absorption maximum at 273 nm whose extinction coefficient depends on the keto–enol equilibrium. In freshly prepared DMSO solutions its 270 MHz $^1$H n.m.r. spectrum suggested the keto conformation with one proton (5-H) at δ 6.75 and two protons (2 NH) at 11.35. The spectrum degenerated into a broad OH peak after standing for a few days. Its trimethylsilyl derivative showed a single g.l.c. peak. Its mass spectrum corresponds to the enol structure (tetrakistrimethylsilyl) derivative, *M* 432).

Isodialuric acid (6-hydroxyisobarbituric acid) (6) was synthesized [17] by oxidation of isobarbituric acid with bromine (m.p. 157—158 °C). The $^1$H n.m.r. spectrum of this compound in freshly prepared DMSO solution suggests the keto structure in the hydrate form [one proton (6-H) at δ 4.4 (d, $J \approx 5$ Hz), three exchangeable protons (3 OH) at 6.0, 6.5, and 6.8, one exchangeable proton (1-H) at 8.2 (d, $J \approx 5$ Hz), and another exchangeable proton (3-H) at 10.1 (s)]. The trimethylsilyl derivative of this compound is identical to the trimethylsilyl derivative of dialuric acid.

5,6-*cis*-Dihydroxy-5,6-dihydrouracil (1) with the *trans*-isomer (2) as a minor by-product were synthesized from uracil.[18,19] They are readily converted into isobarbituric acid by acids or heat, a characteristic which can be used for their identification on t.l.c.

**Table 1.** T.l.c. of products from the radiolysis of uracil in $N_2O$–$O_2$ (4 : 1 v/v)-saturated aqueous solutions

| | Solvent A $R_u$ | Solvent B $R_u$ |
|---|---|---|
| 5-Hydroxyhydantoin [a] | 0.14 | 0.18 |
| 5,6-*cis*-Dihydroxy-5,6-dihydrouracil [a] | 0.45 | 0.47 |
| Isodialuric acid [a,b] | 0.75 | 0.54 |
| 5,6-*trans*-Dihydroxy-5,6-dihydrouracil [a] | 0.89 | 0.90 |
| Isobarbituric acid [a,b] | 0.90 | 0.95 |
| Uracil [b] | 1.00 | 1.00 |
| Dialuric acid [a,b] | 1.42 | 1.21 |
| 1-Formyl-5-hydroxyhydantoin [a] | 1.66 | 1.62 |

[a] Developed with NaOH–$AgNO_3$–$NH_4OH$.[2] [b] U.v.

For g.l.c. analysis, dried samples were silylated with a mixture of BSTFA (0.5 ml; Serva), dried pyridine (0.1 ml), and TMCS (20 μl; Fluka) at 80 °C for 1 h. The silylated sample (0.2 μl) was analysed with a Varian 1700 IIA gas chromatograph equipped with a flame ionization detector. Separation was achieved on a 26 m, 0.25 mm i.d. glass capillary column coated with OV-330. The column was operated with a temperature gradient from 60—260 °C at 4 °C min$^{-1}$. The carrier gas was hydrogen.

Mass spectra were taken with an Atlas CH-4 spectrometer linked to a Varian 1400 gas chromatograph.

T.l.c. was carried out on Kieselgel $F_{254}$ thin-layer plates (Merck) and paper chromatography on Whatman 3 MM paper. The same two solvent systems were used for both media, namely (A) ethyl acetate–methanol–water (75 : 16 : 20, upper layer enriched with 2% methanol [19] and (B) ethyl acetate–propan-2-ol–water (75 : 16 : 9). The $R_u$ values (distance travelled by a substance relative to that by uracil) are given in Table 1.

For quantitative determinations samples (5 ml) of a $2 \times 10^{-4}$M $N_2O$–$O_2$ (4 : 1 v/v)-saturated uracil solution containing [2-$^{14}$C]uracil with 2.5 μCi activity were adjusted to the desired pH value and irradiated to total absorbed doses of 17—86 Gy. After irradiation the solutions were neutralised and rotary evaporated. The residues were redissolved in water (~50 μl), from which samples were streaked on strips of Whatman paper and developed (descending solvent) with solvent A. The developed chromatograms were scanned with a Berthold scanner LB 2723 with a gas flow detector to locate the various products. The sections on the chromatograms corresponding to the product peaks were cut up into small pieces and added to individual counting vials. The liquid scintillation fluid Unisolve 100 (10 ml; Zinsser) was added to each vial and the activities were counted for 10 min with a Betaszint 5003 Counter (Berthold and Frieseke).

For γ-irradiations a $^{60}$CO γ-source (Nuclear Engineering Ltd.) was used. Aqueous solutions of uracil ($2 \times 10^{-4}$—$10^{-2}$M) were saturated with a mixture of $N_2O$–$O_2$ (4 : 1 v/v) prior to and during irradiation. The dose rate used was 0.3 Gy s$^{-1}$ as determined by Fricke dosimetry. For pulse radiolysis experiments a 2.8 MeV van de Graaff accelerator delivering 1 μs pulses with doses ranging from 3 to 30 Gy was used. The optical and conductivity detection set-ups have been described elsewhere.[20,21] Doses were determined for optical measurements by KSCN dosimetry (0.01 M-KSCN in $N_2O$-saturated solutions, taking $G \times \varepsilon$ of $(SCN)_2^{\cdot-}$ at 472 nm to be 46 400 (molecules/100 eV) × M$^{-1}$ cm$^{-1}$).[22] For conductometric measurements a $CH_3Cl$-saturated (0.1M) solution containing

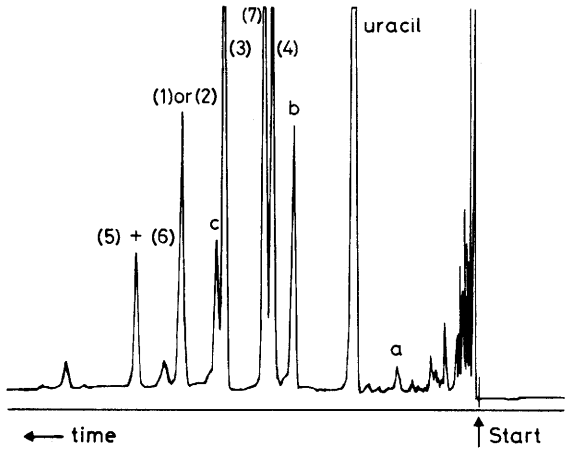



**Figure 1.** G.l.c. of trimethylsilyl derivatives of products from the γ-radiolysis of uracil in $N_2O$–$O_2$ (4 : 1 v/v)-saturated aqueous solutions ($10^{-2}$M). Compounds (1)—(7) are products listed in Table 1: a, urea; b, parabanic acid; c, parabanic acid hydrate (for operating conditions see Experimental section)

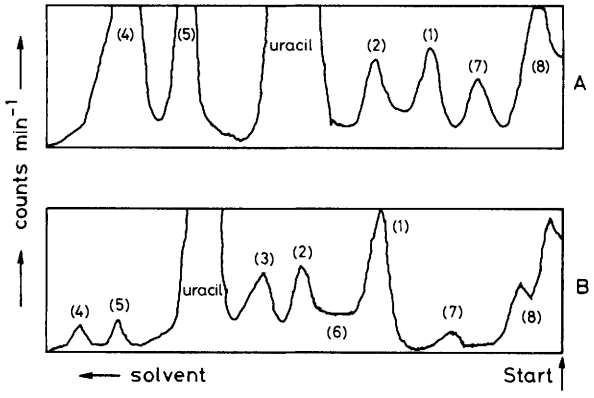



**Figure 2.** Paper chromatograms of products from the γ-radiolysis of [2-$^{14}$C]uracil in $N_2O$–$O_2$ (4 : 1 v/v)-saturated aqueous solutions. (A) Uracil solution irradiated at pH 3.0, chromatogram developed once in solvent A. (B) Solution irradiated at pH 10.0, chromatogram developed twice in solvent A for better resolution of isobarbituric acid (3)

0.1M-t-butyl alcohol at pH 4 was used for dosimetry taking $G$(HCl) 3.0.[23]

Oxygen consumption was determined by means of an oxygen-specific membrane electrode (Wiss.-Techn. Werkst., Weilheim). Hydrogen peroxide was determined iodometrically.[24]

## Results and Discussion

*Product Analysis.*—The products from the γ-radiolysis of $N_2O$–$O_2$-saturated uracil solutions are listed in Table 1. With the exception of $H_2O_2$ the products were identified by g.c.–m.s. as trimethylsilyl derivatives (Figure 1) and by comparison with authentic material on thin layer or paper chromatography (p.c.). The g.c. method, despite producing excellent separation, was not applicable for quantitative determinations because some of the products were either indistinguishable as trimethylsilyl derivatives (dialuric acid and isodialuric acid), or were unstable under the derivatization conditions [the glycols (1) and (2)]. In addition, other products failed to give reproducible g.c. response factors. Therefore it was necessary to carry out quantitative determinations by paper chromatography using $^{14}$C-labelled uracil (Figure 2). 5-Hydroxyhydantoin (7) found as a radiation product both as its trimethylsilyl derivative (Figure 1) and directly by t.l.c. and p.c. (Figure 2), is probably a decomposition product of 1-formyl-5-hydroxyhydantoin (4) and is not a primary radiation product (see Experimental section). Likewise urea, parabanic acid, and parabanic acid hydrate may also be decomposition products (Figure 1). In the t.l.c. and p.c. analysis of radiation products some material always remained at the origin [(8) in Figure 2]. This material has not been identified. Although this material may consist of well defined products, the possibility also exists that some products are degraded during the work-up procedure and the resultant fragments bind strongly to the chromatographic medium at the origin. This appears to be a common source of error encountered in these analytical methods.[25]

**Table 2.** Products and their $G$ values from the γ-radiolysis of $N_2O$–$O_2$ (4 : 1 v/v)-saturated uracil ($2 \times 10^{-4}$M) solutions under various pH conditions

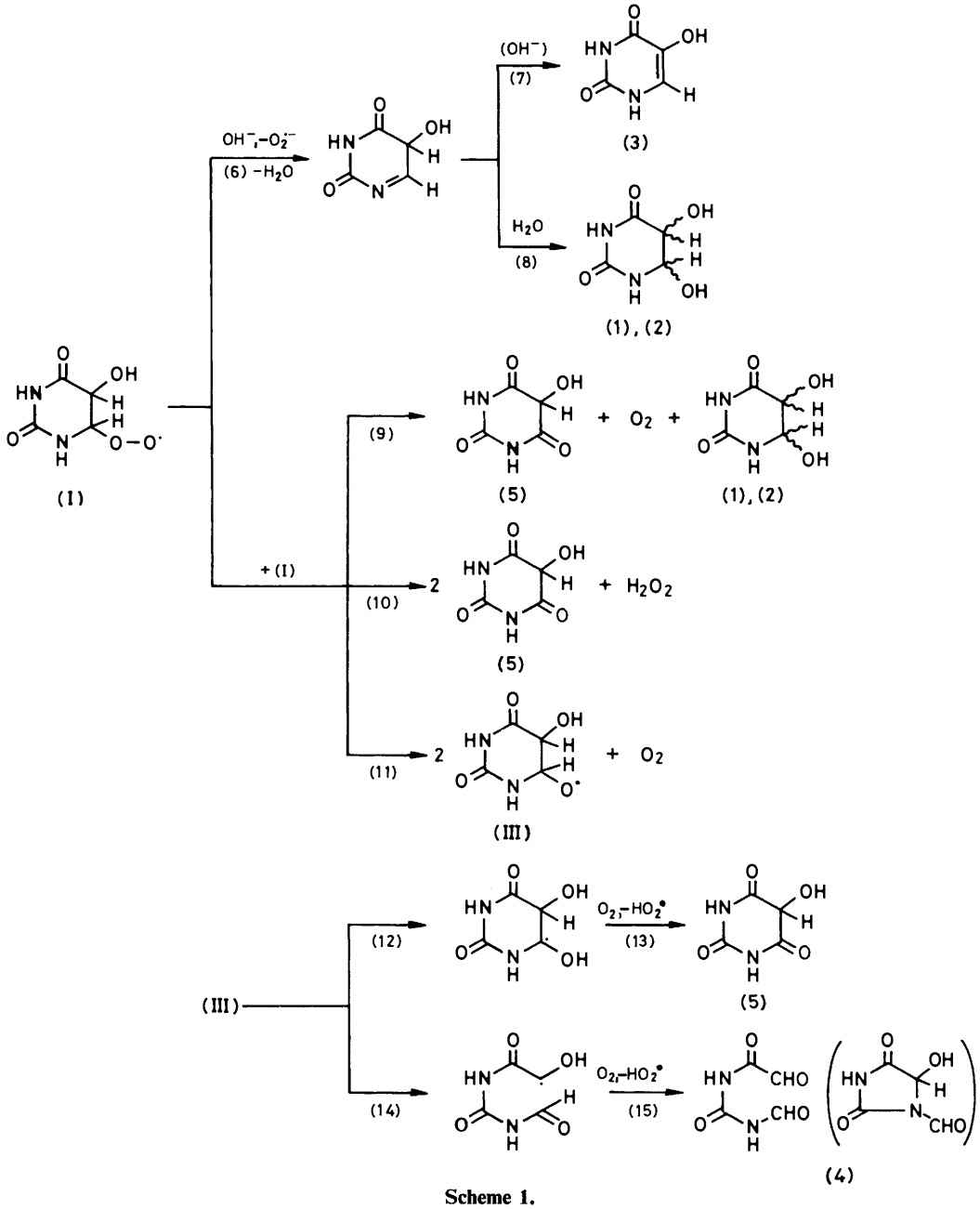
| Product | $G$ Values | | |
|---|---|---|---|
| pH | 3.0 | 6.5 | 10.0 |
| 5,6-*cis*-Dihydroxy-5,6-dihydrouracil (1) | 0.6 | 0.9 | 1.4 |
| 5,6-*trans*-Dihydroxy-5,6-dihydrouracil (2) | 0.5 | 1.1 | 1.0 |
| Isobarbituric acid (3) | 0 | <0.2 | 1.2 |
| 1-Formyl-5-hydroxyhydantoin (4) | 1.6 | 1.4 | 0.2 |
| Dialuric acid (5) | 0.9 | 0.4 | 0.2 |
| Isodialuric acid (6) | ~0.1 | ~0.2 | ~0.1 |
| 5-Hydroxyhydantoin (7) | 0.4 | 0.4 | 0.3 |
| Unidentified product(s) at starting point (8) | 0,9 | 0.6 | 0.9 |
| Hydrogen peroxide | n.d. | 3.0 | n.d. |
| Oxygen consumption | n.d. | 5.0 | n.d. |
| Uracil consumption | 4.9 | 5.3 | 5.2 |

N.d. = not determined.

The $G$ values of the products were calculated from linear yield–dose plots at <25% conversion under three pH conditions and are given in Table 2. It becomes apparent from Table 2 that the $G$ values of isobarbituric acid, and the glycols (1) and (2) increase with increasing pH. On the other hand, 1-formyl-5-hydroxyhydantoin (4) and dialuric acid (5) become the major products at pH 3.

*Reaction Mechanism.*—To explain the formation of the products as well as the pH dependence of their distribution two schemes are proposed starting either from radical (I) (Scheme 1) or (II) (Scheme 2).

(a) $OH^-$-*Induced elimination of* $O_2^{-\cdot}$ *from radical* (I). α-Hydroxyalkylperoxyl radicals are known to undergo spontaneous as well as base-induced $HO_2/O_2^{-\cdot}$ elimination (*cf.* ref 10). Although radical (I) is not an α-hydroxyalkylperoxyl radical, there is an NH substituent in the position α to the peroxyl group which in basic solutions can also be deprotonated to facilitate the elimination of $O_2^{-\cdot}$ [reaction (6)]. The resulting intermediate 5-hydroxyisouracil then undergoes either base-induced isomerisation to isobarbituric acid [reaction (7)] or water addition leading to the glycols (1) or (2) [reaction (8)]. This is reflected by the enhanced yields of isobarbituric acid and to a lesser extent that of the glycols in basic solutions (Table 2). Under such conditions reaction (6) successfully competes with the bimolecular decay of the peroxyl radicals (see below). That reaction (8) is more favoured over reaction (7) at lower pH has been suggested [26] based on evidence obtained from the radiolysis of uracil in the presence of an

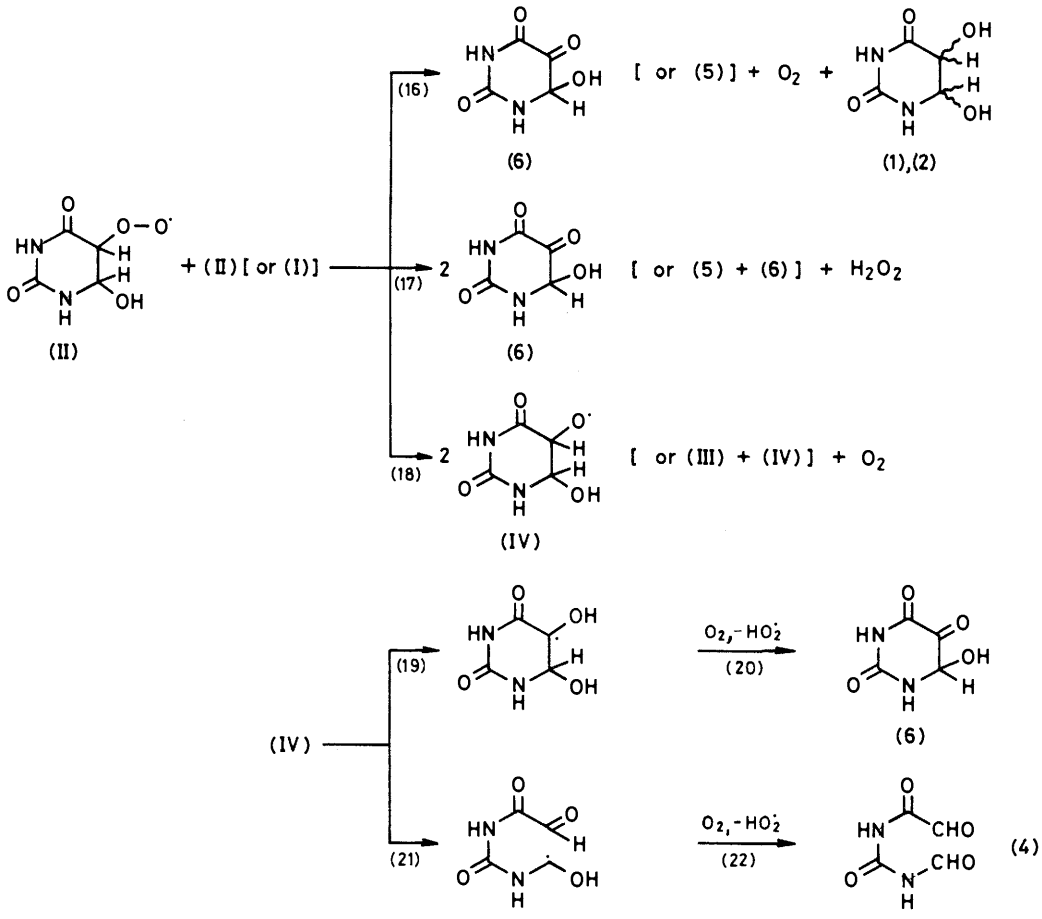


Scheme 1.

oxidant where the intermediate 5-hydroxyisouracil can be independently produced. Our pulse radiolysis observations also provide evidence that more isobarbituric acid is formed at higher pH (see below). The pulse radiolytic results also indicate that even at pH 10.5 there is considerable formation of glycol from the intermediate 5-hydroxyisouracil [reaction (8)]. The present system differs in this respect from the isouracil system [14] which at this pH rearranges practically only to uracil.

(b) *Bimolecular decay of the peroxyl radicals* (I) *and* (II). In competition with the unimolecular decay of peroxyl radical (I) discussed above is the bimolecular decay of this radical. The bimolecular decay route is favoured at low pH where reaction (6) becomes of little importance. The bimolecular decay of the peroxyl radicals most likely leads initially to the formation of a short-lived tetraoxide (not shown in Scheme 1). This tetraoxide could then decompose to products either by concerted processes, reactions (9) (*cf.* ref. 27) and (10) (*cf.* refs. 11, 28, and 29), or by a free radical process [reaction (11), *cf.* ref. 30]. Reaction (9) produces dialuric acid (5) and the glycols [the sum of (1) and (2)] in equal yields.

At pH 3 the sum of the *G* values of the glycols is approximately equal to that of dialuric acid [plus isodialuric acid, see reaction (16), Scheme 2] as predicted by reaction (9). Reaction (10) then can play only a minor role. The reaction sequence (11), (14), (15) leading to the formation of *N*-formyl-*N'*-glyoxylurea consists of well established reaction routes (*cf.* ref. 13). The latter was found in the more stable ring-closed form of (4). The formation of (4) was also found to be highest at low pH in accordance with Scheme 1. An alternative route is the rearrangement of the alkoxyl radical to an α-hydroxyalkyl radical [reaction (12)].[12,31-34] This would lead to the formation of dialuric acid [reaction (13), *cf.* ref. 10]. However, since the *G* value of dialuric acid, even



**Scheme 2.**

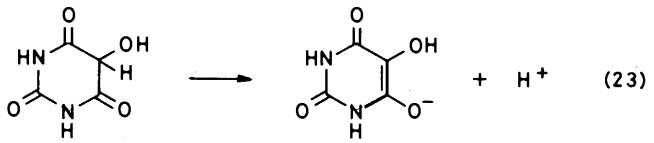
in acid solution, does not exceed that of the glycols, reaction (14) is preferred to reaction (12). Radical (II), having neither an OH nor an NH group α to the peroxyl function, can only decay bimolecularly as depicted in Scheme 2, which is otherwise analogous to Scheme 1.

*Kinetic Measurements.*—Pulse radiolytic investigations in the present system are considerably restricted by the properties of uracil. The high absorbance of uracil in the u.v. masks the absorption of the peroxyl radicals. The buffering effect of uracil in basic solutions hampers conductivity measurements around its p$K$ of 9.5. In this respect the 5,6-dihydrouracil system [14] which otherwise is very similar to the uracil system is much superior. In the interpretation of our data we therefore are guided by the information obtained from the dihydrouracil system. Despite these difficulties some useful information can still be gained by the application of pulse radiolytic techniques to the uracil system.

(a) *Pulse conductometry.* When a $N_2O$–$O_2$ (4 : 1 v/v)-saturated 0.5mM-uracil solution at pH 6.5 was subjected to an electron pulse, the build-up of transient conductivity was observed. The build-up followed approximately second-order kinetics (*ca.* $10^9$ l mol$^{-1}$ s$^{-1}$). $G$($H^+$/anion) at the maximum was 1.1. After reaching its maximum the conductivity change declined to a very low level in *ca.* 10 ms before a second slow build-up was observed, which was derived from a permanent conducting species. The latter process followed a first-order rate law with $k$ 7.7 s$^{-1}$ and $G$($H^+$/anion) 1.0. The transient conductivity change was reduced to $G$($H^+$/anion) 0.6 when the uracil solution was made more acidic (pH 5.0) but the $G$ value of the permanent conductivity did not change although the build-up occurred with a somewhat slower rate ($k$ 4.4 s$^{-1}$).

The formation of a transient conductivity change by a second-order rate process can be explained by the bimolecular reactions of radicals (I) and (II), which, *via* radicals (III) and (IV), lead to the elimination of $HO_2$· and the formation of 1-formyl-5-hydroxyhydantoin (4) [reactions (15) and (22)]. At pH ≥ 5, $HO_2$· is largely dissociated into $H^+$ and $O_2^{-}$· (p$K_a$ 4.7) and $O_2^{-}$· disappears by reacting with $HO_2$· ($k_{obs}$ $10^6$ l mol$^{-1}$ s$^{-1}$ at pH 6.5).[35] Since the scavenging of the radiolytically formed hydrogen atoms yields $G$($HO_2$·/$O_2^{-}$·) ≈ 0.5 and since $G$[(4) + (7)] was found to be *ca.* 2 under conditions where bimolecular reactions prevail (see Table 2), the expected $G$($O_2^{-}$·) totals *ca.* 2.5. The low yield of $O_2^{-}$· observed [$G$($H^+$/$O_2^{-}$·) 1.1 at maximum], however, does not fully reflect the expected yield. It is therefore suggested that the observed fast $O_2^{-}$· build-up ($2k$ ≈ $10^9$ l mol$^{-1}$ s$^{-1}$) is due to the bimolecular reaction involving the minor radical (II) while $O_2^{-}$· from the slower reaction of (I) + (I) disappears as fast as it is formed by reacting with $HO_2$· or (I). The peroxyl radical at C-6 of 5,6-dihydrouracil has been shown [14] to react bimolecularly rather slowly, $2k$ 2 × $10^7$ l mol$^{-1}$ s$^{-1}$. Radicals (I) probably also react with one another with a similarly slow rate.

The slow build-up of the permanent conductivity can be attributed to the slow dissociation of dialuric acid [reaction (23)]. C–H acids are expected to deprotonate slowly, even if they have low p$K_a$ values. As an example barbituric acid (p$K$ 4) in its keto-form has been found [36–38] to deprotonate



(23)

with only $k$ 22 $s^{-1}$ at 19 °C. Dialuric acid is formed [reaction (9)] in the keto-form, which is non-conducting until dissociation ($k_{23}$ 4.4 $s^{-1}$). The $G$ value of conductivity change due to dialuric acid under pulse radiolysis conditions agrees well with the value obtained by product analysis in the γ-radiolysis of uracil in acidic solution (Table 2). The rate measured at pH 6.5 ($k$ 7.7 $s^{-1}$) might include a contribution from the $OH^-$-induced deprotonation.

In basic solutions the $OH^-$-induced $O_2^{-\cdot}$ elimination of radical (I) [reaction (6)] is connected with a decrease in conductivity of the solution since $OH^-$ is replaced by the less conducting $O_2^{-\cdot}$. This decrease in conductivity was measured at pH > 10.5 where the buffering effect of uracil interferes less and less and the bimolecular decay of the radicals is adequately suppressed. Under such conditions a conductivity decrease [$G(-OH^-/+\text{anion})$ 4.4 at pH 10.5, 14 Gy per pulse] was observed. As discussed below the intermediate 5-hydroxyisouracil formed in reaction (6) will be largely deprotonated at this pH. The $G$ value of 4.4 obtained is associated with the replacement of $OH^-$ by $O_2^{-\cdot}$ and the anion of 5-hydroxyisouracil. This would reflect $G$(5-hydroxyisouracil) ≈ 2. Because of the buffering effect of uracil is still present, this value is a lower limit. The range that is accessible to this type of experiment is limited to the pH range 10.5—11 because of buffering effects and background conductivity. For this reason the true value of $k_6$ also cannot be evaluated with accuracy (that is over a large range of $OH^-$ concentrations). At pH 10.5 the observed rate constant was $8 \times 10^3$ $s^{-1}$, which increases to $1.2 \times 10^4$ $s^{-1}$ at pH 10.8. These values indicate that $k_6$ is lower than its equivalent in the dihydrouracil system.[14]

After this $O_2^{-\cdot}$ elimination had essentially come to completion a slight conductivity increase (partial restoration) was observed with a half-life of a few ms [$G(+OH^-/-\text{anion}) \approx 0.7$ at pH 10.5]. It is suggested that this conductivity increase results from the conversion of the intermediate 5-hydroxyisouracil, which will be deprotonated at this pH,[39] into the non-electrolyte glycols (1) and (2) [reaction (8)]. The concomitant reaction that leads to isobarbiturate [reaction (7)] is not connected with a change in conductivity. The latter reaction can, however, be followed optically (see below). It appears that both processes follow the same rate law, although the conductivity signal is too low for accurate kinetic measurements.

(b) *Optical measurements.* The formation of isobarbituric acid in reaction (7) can be followed pulse spectrophotometrically in basic solutions of uracil. At pH > 9 isobarbituric acid ($pK_a$ 8.1) is largely dissociated. Isobarbiturate anion has a maximum absorption at 306 nm with ε 4 500 l $mol^{-1}$ $cm^{-1}$. In $N_2O$–$O_2$ (4 : 1 v/v)-saturated uracil solutions at pH > 9 subjected to an electron pulse of 3—10 Gy, a slow absorption build-up centred at 306 nm could be observed. The end absorbance at 306 nm is pH dependent. Taking $\varepsilon_{306}$ 4 500 l $mol^{-1}$ $cm^{-1}$, this permanent absorption change corresponds to $G$(isobarbiturate) 0.9 at pH 9.4 which increases to $G$ 1.6 at pH 10 and G 3.9 at pH 11.4. This increase in $G$(isobarbituric acid) with increasing pH is largely due to the competition between reactions (7) and (8) whereby at high pH reaction (7) is favoured. This spectrophotometric analysis agrees with the product analysis (see above).

In the present system the keto-form of the isobarbituric acid is not formed. For this reason the slow build-up of the isobarbiturate anion is not due to the slow keto–enol tautomerisation (see however, ref. 37). Instead it is the rearrangement of the 5-hydroxyisouracil [reaction (7)]. The kinetics of this rearrangement are very complex. (For details of isopyrimidine–pyrimidine rearrangements see ref. 39). In acidic solution the reaction is proton catalysed.[39,40] In neutral solution the uncatalysed rate constant is 2 000 $s^{-1}$.[39] The anion of 5-hydroxyisouracil rearranges much more slowly (≤ 100 $s^{-1}$), and only at pH ≥ 11 does an $OH^-$-induced rearrangement of the anion take place. The rate of the latter reaction is slow [$k$(obs) 130 $s^{-1}$ at pH 10.8, 220 $s^{-1}$ at pH 11.1, and 370 $s^{-1}$ at pH 11.4; thus $k(OH^-) \approx 10^5$ l $mol^{-1}$ $s^{-1}$]. This is not unexpected because a carbon-bound proton must be removed from an anion.

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