

## The Kinetics of the Rearrangement of Some Isopyrimidines to Pyrimidines studied by Pulse Radiolysis

Man Nien Schuchmann, Mohamed Al-Sheikhly, and Clemens von Sonntag\*

Max-Planck-Institut für Strahlenchemie, Stiftstr. 34-36, D-4330 Mülheim a.d. Ruhr, West Germany

Anthony Garner

Department of Biochemistry, Brunel University, Kingston Rd, Uxbridge UB8 3PH

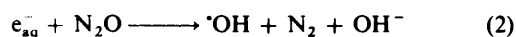
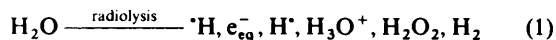
George Scholes\*

Radiation and Biophysical Laboratory, School of Chemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU

Isopyrimidines are formed as a result of the oxidation of the 6-yl radicals derived by  $\cdot\text{OH}$  attack on pyrimidines and dihydropyrimidines. The kinetics of the rearrangement of the isopyrimidines into the corresponding pyrimidines has been followed by pulse radiolysis. The rearrangement of isouracil into uracil is proton-catalysed ( $k\ 1.8 \times 10^7\ \text{l mol}^{-1}\ \text{s}^{-1}$ ). Around pH 7 a spontaneous reaction,  $k\ 3\ 000\ \text{s}^{-1}$ , is observed. On increasing the pH the isouracil deprotonates at N(3) ( $\text{p}K_a\ \text{ca.}\ 9.4$ ). The spontaneous rearrangement of the isouracil anion is considerably slower ( $k \leq 50\ \text{s}^{-1}$ ). At pH > 10.5 an  $\text{OH}^-$ -catalysed reaction sets in ( $k\ 4.9 \times 10^5\ \text{l mol}^{-1}\ \text{s}^{-1}$ ) which involves a second deprotonation, at C(5). Similar results have been obtained for the rearrangement of 5-hydroxyisouracil into isobarbituric acid. On blocking the N(3) position as in 3-methylisouracil, the  $\text{OH}^-$ -induced rearrangement sets in at a much lower pH (pH  $\leq 9.5$ ), *i.e.* the rearrangement is faster ( $k\ 2.7 \times 10^7\ \text{l mol}^{-1}\ \text{s}^{-1}$ ) than that observed in the other two systems.

Isopyrimidines are unstable intermediates which play an important role in the free radical chemistry of pyrimidines and dihydropyrimidines in aqueous systems.

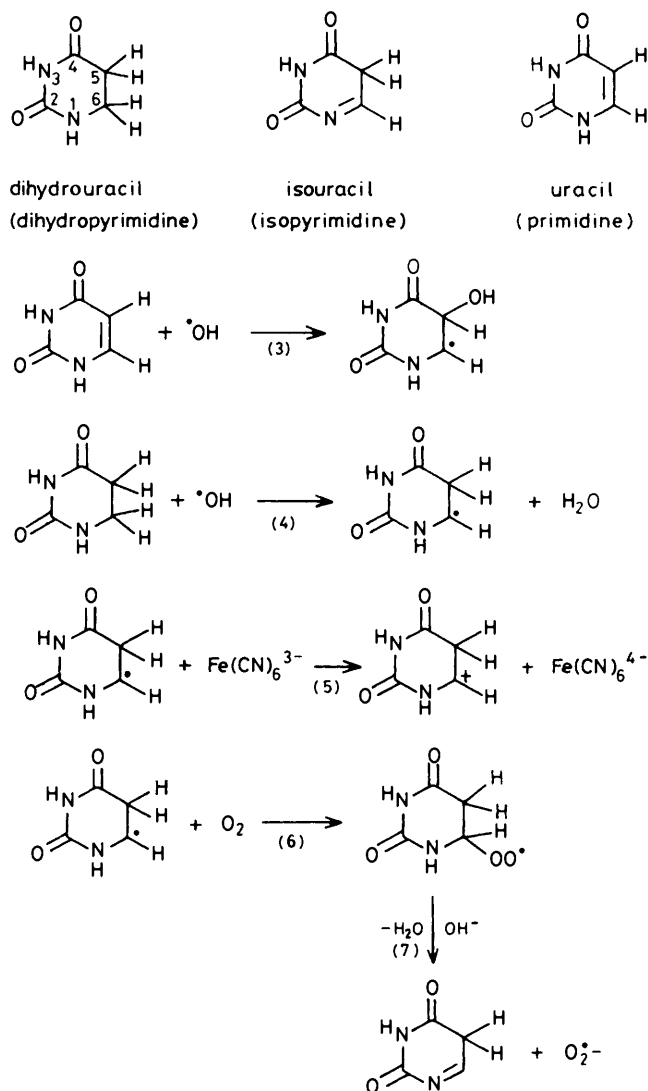
Hydroxyl radicals generated in the radiolysis of  $\text{N}_2\text{O}$ -saturated water [reactions (1) and (2)] react with pyrimidines mainly by adding to the 5-position of the C(5)-C(6) double bond [reaction (3)]<sup>1</sup> whereas they react with dihydropyrimidines predominantly by abstracting hydrogen atoms from the C(6) position [reaction (4)].<sup>2</sup> In both cases pyrimidine radicals with a free spin at C(6) (6-yl radicals) are formed. Such

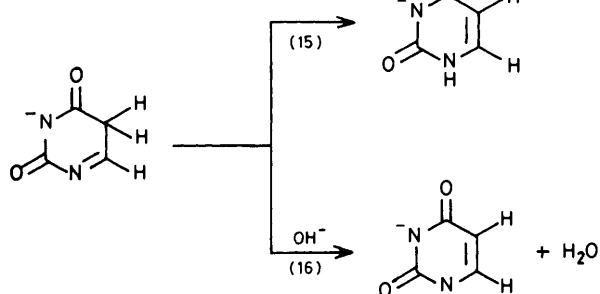
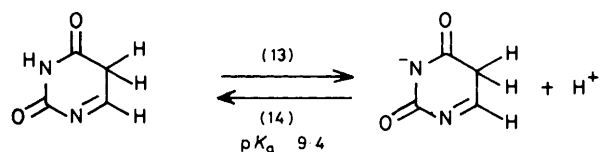
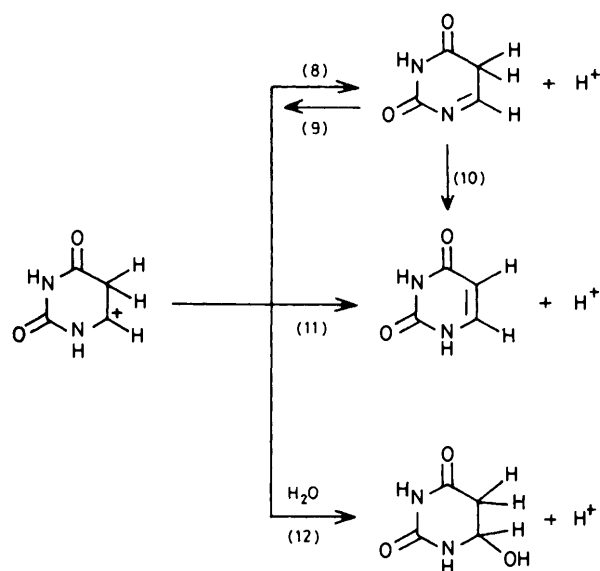


radicals are known to be readily oxidized by transition metal ions or their complexes [*e.g.*  $\text{Fe}(\text{CN})_6^{3-}$ ] to carbocations [*cf.* reaction (5)] which can then lead, in the case of dihydrouracil as substrate, to the formation of both the parent pyrimidine (the major product at pH > 4) and the corresponding 5,6-dihydro-6-hydroxypyrimidine (the major product at pH < 4).<sup>3</sup> In a similar manner oxidation of the OH adduct formed according to reaction (3) leads to formation of isobarbituric acid and uracil glycol, respectively. Pulse-radiolysis studies<sup>4-6</sup> showed the involvement of isopyrimidines as intermediates. The addition of oxygen to the 6-yl radicals followed by a base-induced  $\text{O}_2^{\cdot-}$  elimination also leads to such an intermediate [*e.g.* reactions (6) and (7)].<sup>7,8</sup> Furthermore, it has been suggested that isopyrimidines are formed in disproportionation reactions of pyrimidine-derived radicals, possibly *via* electron-transfer processes.<sup>6</sup> There is evidence that they have to be considered as intermediates in the photohydration of pyrimidines.<sup>6,9</sup> In the present study an attempt has been made to elucidate some of the properties of isopyrimidines and to determine the kinetics of their rearrangement to the corresponding pyrimidines.

### Results and Discussion

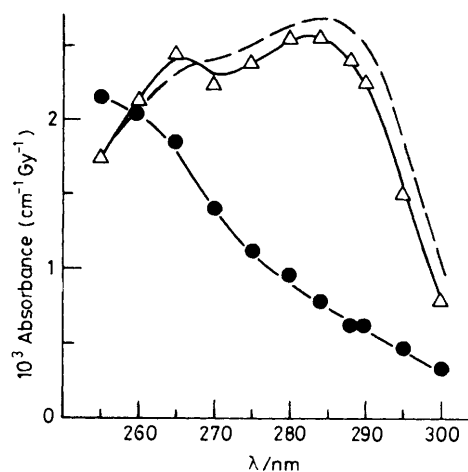
Electron pulses from a Van de Graaff generator were delivered to  $\text{N}_2\text{O}$ -saturated solutions of dihydrouracil, 3-methyldihydrouracil, or uracil (0.5mM). Hydroxyl radicals are generated by the



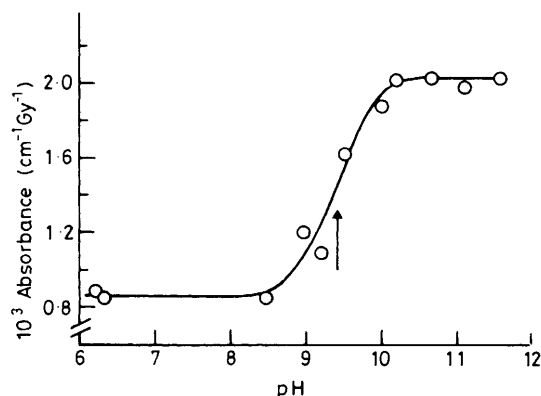


electron pulse according to reactions (1) and (2). The H atoms, the yield of which is only *ca.* 10% of that of the OH radicals, are expected to react with pyrimidines and dehydropyrimidines in a similar manner. In N<sub>2</sub>O–O<sub>2</sub> (4:1 v/v)-saturated solutions, a system used to generate the isopyrimidines according to reactions (6) and (7), the H atoms are largely scavenged by oxygen.

Under the experimental conditions reactions (3) and (4) occur practically within the pulse duration and subsequent oxidation of the substrate radicals by Fe(CN)<sub>6</sub><sup>3-</sup> ( $4 \times 10^{-5}$  M) is fast ( $k_5$   $4 \times 10^9$  l mol<sup>-1</sup> s<sup>-1</sup>),<sup>2</sup> as is the proton loss at N(1) of the intermediate carbocation [reaction (8)]. In fact such an intermediate has not been detected in a view of the very short life time.<sup>5</sup> Thus, with Fe(CN)<sub>6</sub><sup>3-</sup> as oxidant the isopyrimidine can be formed within a few microseconds. On the other hand, formation of the isopyrimidine through oxidation by O<sub>2</sub> [reactions (6) and (7)] is considerably slower. Although oxygen adds to the 6-yl radicals at near diffusion-controlled rate ( $k_6$   $1.9 \times 10^9$  l mol<sup>-1</sup> s<sup>-1</sup>)<sup>10</sup> the elimination of O<sub>2</sub><sup>-•</sup> is base-



**Figure 1.** Optical absorption spectra of the intermediate isouracil (filled circles; 30  $\mu$ s after pulse) and of the final product uracil (triangles; 16 ms after pulse) in N<sub>2</sub>O-saturated 5,6-dihydrouracil solution (1 mM) containing K<sub>3</sub>Fe(CN)<sub>6</sub> ( $4 \times 10^{-5}$  M) at pH 10.6 following a 1  $\mu$ s electron pulse of *ca.* 14 Gy. Corrections have been made for the disappearance of K<sub>3</sub>Fe(CN)<sub>6</sub> and the formation of K<sub>4</sub>Fe(CN)<sub>6</sub>. The dashed line represents the spectrum of uracil–uracilate taking  $G(\text{uracil})$  4.8



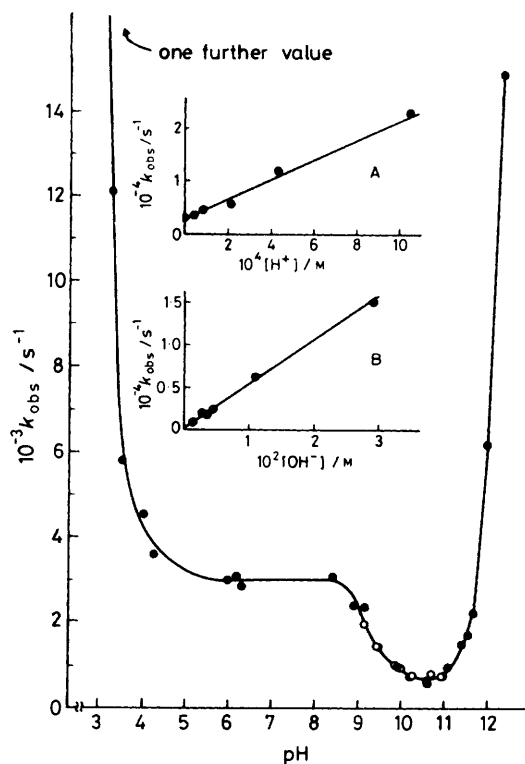
**Figure 2.** pH-Dependence of the optical absorbance at 260 nm of the intermediate isouracil (conditions as given for Figure 1)

catalysed and therefore is only fast at high pH. At pH > 10.5 the first-order rate constant for elimination approaches a limiting value of  $8 \times 10^4$  s<sup>-1</sup> (probably the rate constant for the unimolecular decay of the anionic form of the peroxy radical).<sup>7</sup>

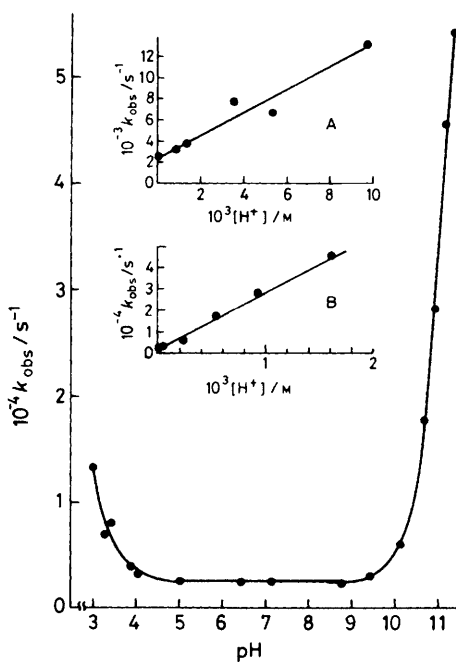
The isopyrimidines do not show strong optical absorptions in the accessible wavelength region ( $\lambda > 250$  nm). The spectra of their anions, however, can be measured. Figure 1 shows that of the isouracilate anion formed according to reaction (13).

The  $pK_a$  value of isouracil has been determined by measuring the absorbance at 260 nm immediately after oxidation of the 6-yl radical (30  $\mu$ s after the pulse) as a function of pH (Figure 2). At pH  $\geq 9$  the isouracil–isouracilate ion equilibrium [reactions (13) plus its OH<sup>-</sup>-induced equivalent (14)] is expected to be practically attained within this time which is short compared with that for the subsequent rearrangement into uracil–uracilate (see below). From Figure 2 a  $pK_a$  of *ca.* 9.4 is obtained; this value is very similar to that of uracil ( $pK_a$  9.5).

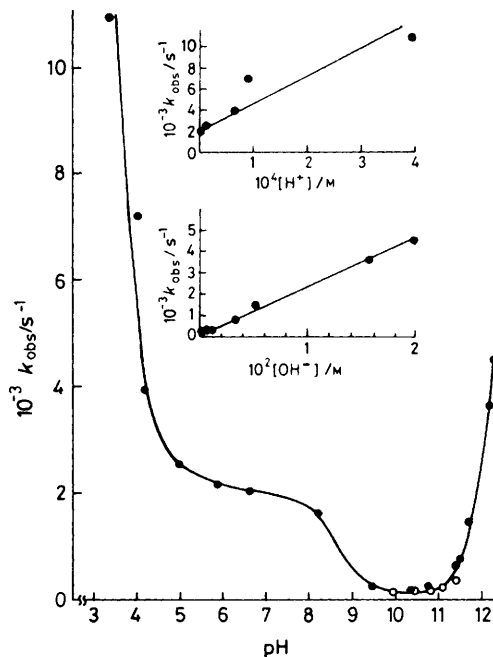
The rearrangement of the isopyrimidines into the corresponding pyrimidines can be followed spectrophotometrically [uracil,  $\lambda_{\text{max}}$  260 nm ( $\epsilon$  8 200 l mol<sup>-1</sup> cm<sup>-1</sup>); uracilate ion,  $\lambda_{\text{max}}$



**Figure 3.** pH-Dependence of the observed first-order rate constant ( $k_{\text{obs}}$ ) of the formation of uracil from isouracil in electron-pulse-irradiated 0.5mM-dihydrouracil solutions either saturated with  $\text{N}_2\text{O}$  and containing  $4 \times 10^{-5}\text{M-K}_3\text{Fe(CN)}_6$  (filled circles) or only saturated with  $\text{N}_2\text{O-O}_2$  (4:1 v/v) (open circles). Insets: A, plot of  $k_{\text{obs}}$  versus  $[\text{H}^+]$ ; B, plot of  $k_{\text{obs}}$  versus  $[\text{OH}^-]$



**Figure 4.** pH-Dependence of the observed first-order rate constant of the formation of 3-methyluracil from 3-methylisouracil in electron-pulse-irradiated 0.5mM-3-methyl-5,6-dihydrouracil solution containing  $4 \times 10^{-5}\text{M-K}_3\text{Fe(CN)}_6$  saturated with  $\text{N}_2\text{O}$ . Insets: A, plot of  $k_{\text{obs}}$  versus  $[\text{H}^+]$ ; B, plot of  $k_{\text{obs}}$  versus  $[\text{OH}^-]$



**Figure 5.** pH-Dependence of the observed first-order rate constant of the formation of isobarbituric acid from 5-hydroxyisouracil in electron-pulse-irradiated 0.5mM-uracil solutions either saturated with  $\text{N}_2\text{O}$  and containing  $4 \times 10^{-5}\text{M-K}_3\text{Fe(CN)}_6$  (●) or saturated with  $\text{N}_2\text{O-O}_2$  (4:1 v/v) (○). Insets: A, plot of  $k_{\text{obs}}$  versus  $[\text{H}^+]$ ; B, plot of  $k_{\text{obs}}$  versus  $[\text{OH}^-]$

284 nm ( $\epsilon$  6 200  $\text{l mol}^{-1} \text{cm}^{-1}$ ),  $\text{pK}_a$  9.5; 3-methyluracil:  $\lambda_{\text{max}}$ , 259 nm ( $\epsilon$  7 300  $\text{l mol}^{-1} \text{cm}^{-1}$ ); 3-methyluracilate ion,  $\lambda_{\text{max}}$ , 283 nm ( $\epsilon$   $1.07 \times 10^4$   $\text{l mol}^{-1} \text{cm}^{-1}$ ),  $\text{pK}_a$  10.0; isobarbituric acid,  $\lambda_{\text{max}}$ , 278 nm ( $\epsilon$  6 400  $\text{l mol}^{-1} \text{cm}^{-1}$ ); isobarbiturate ion,  $\lambda_{\text{max}}$ , 304 nm ( $\epsilon$  5 100  $\text{l mol}^{-1} \text{cm}^{-1}$ ),  $\text{pK}_a$  8.0].

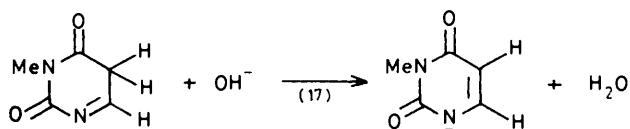
In Figure 3 the observed first-order rate constants for the formation of uracil–uracilate ion in solutions containing either  $\text{Fe(CN)}_6^{3-}$  or  $\text{O}_2$  as oxidant are plotted as a function of pH. It can be seen that the two methods of forming the isopyrimidine gave identical results. Figures 4 and 5 give similar data for the formation of 3-methyluracil–3-methyluracilate ion and isobarbituric acid–isobarbiturate ion, respectively.

Around neutrality there is an uncatalysed rearrangement of the isopyrimidine molecule [cf. reaction (10)] the mechanism of which is not yet certain. Its rate constant is *ca.* 2 500  $\text{s}^{-1}$  in all systems investigated (Table). Given that the  $\text{pK}_a$  value of the isouracil is *ca.* 9.4 (and assuming  $k_{14}$   $10^{10}$   $\text{l mol}^{-1} \text{s}^{-1}$ ) the rate constant of the spontaneous deprotonation at N(3),  $k_{13}$ , is *ca.* 4  $\text{s}^{-1}$ . If we accept that deprotonation at N(3) is faster than deprotonation at C(5) then the observed rate constant  $k_{10}$  2 500  $\text{s}^{-1}$  would suggest that the rearrangement does not involve a deprotonation at C(5) followed by reprotonation at N(1). A 1,3-H shift from C(5) to N(1) (by tunnelling or *via* a relay of water molecules?) might be considered as a possible alternative.

In acidic solutions the observed rate constant of rearrangement of the isopyrimidines increases in agreement with earlier observations.<sup>6</sup> Concomitantly the pyrimidine yield decreases and a new product, the pyrimidine hydrate, is formed with increasing yield, as shown for the case of dihydrouracil as substrate, and it has been suggested, that this variation with pH is due to the occurrence of reactions (11) and (12) after protonation of the isopyrimidine [reaction (9)]. Kinetic analysis of this reaction scheme leads to the relationship (i). A plot of  $k_{\text{obs}}$  versus  $[\text{H}^+]$  is indeed linear as is shown in Figure 3.

**Table.** Rate constants of isopyrimidine→pyrimidine rearrangements at 20 °C

Isopyrimidine	Isouracil	3-Methylisouracil	5-Hydroxyisouracil	Cf. reactions
Neutral H <sup>+</sup> -catalysed	$1.8 \times 10^7 \text{ l mol}^{-1} \text{ s}^{-1}$	$1.1 \times 10^7 \text{ l mol}^{-1} \text{ s}^{-1}$	$2.6 \times 10^7 \text{ l mol}^{-1} \text{ s}^{-1}$	(9), (11)
Neutral spontaneous	$3\,000 \text{ s}^{-1}$	$2\,500 \text{ s}^{-1}$	$2\,000 \text{ s}^{-1}$	(10)
Neutral OH <sup>-</sup> -catalysed	Absent	$2.7 \times 10^7 \text{ l mol}^{-1} \text{ s}^{-1}$	Absent	(17)
Anion spontaneous	$\leq 50 \text{ s}^{-1}$	Absent	$< 50 \text{ s}^{-1}$	(15)
Anion OH <sup>-</sup> -catalysed	$4.9 \times 10^5 \text{ l mol}^{-1} \text{ s}^{-1}$	Absent	$2.2 \times 10^5 \text{ l mol}^{-1} \text{ s}^{-1}$	(16)



$$k_{\text{obs}} = \frac{k_9(k_{12} + k_{11})}{k_8 + k_{12} + k_{11}} [\text{H}^+] + k_{10} \quad (\text{i})$$

In the case of 3-methylisouracil the rate constant for the rearrangement increases upon increasing the pH beyond neutrality (Figure 4), *i.e.* an OH<sup>-</sup>-catalysed reaction sets in [reaction (17)]. For reaction (17) a rate constant of  $2.7 \times 10^7 \text{ l mol}^{-1} \text{ s}^{-1}$  is obtained (from inset B, Figure 4). However, in the case of isouracil and 5-hydroxyisouracil (Figures 3 and 5) upon increasing the pH beyond neutrality the observed rate constant for the rearrangement drops. It is conceivable that the rearrangement of the anion [*cf.* reaction (15)] is considerably slower than that of the neutral form [*cf.* reaction (10)]. The OH<sup>-</sup>-induced reaction [*cf.* reaction (16)] sets in at a much higher pH than in the case of 3-methylisouracil. This is to be expected, since deprotonation must now occur from an already negatively charged species, as these two isopyrimidines deprotonate at N(3) in alkaline solution [*e.g.* equilibrium (13)—(14)].

### Experimental

5,6-Dihydrouracil (Sigma) was recrystallised three times from triply distilled water. 3-Methyl-5,6-dihydrouracil prepared according to Hotchkiss and Johnson<sup>11</sup> was a gift of Dr. J. Wroblewski.<sup>2</sup> Uracil (Merck) was used without further purification. Pulse radiolysis was carried out with a 2.8 MeV Van de Graaff accelerator. The optical detection system and the computer-assisted data collection have been described previously.<sup>12,13</sup>

The solutions of 0.5–1 mM-5,6-dihydrouracil, 3-methyl-5,6-dihydrouracil, or uracil containing  $4 \times 10^{-5} \text{ M-K}_3\text{Fe}(\text{CN})_6$  (Merck; p.a.) in triply distilled water were saturated with oxygen-free N<sub>2</sub>O and were irradiated at  $20 \pm 1^\circ \text{C}$  with electron pulses of 1 μs duration. Radiation doses which produced 6–10 μM-radicals were used. The pH of the solutions was adjusted with HClO<sub>4</sub> or NaOH. In experiments where

oxidation by O<sub>2</sub> was required the solutions were saturated with a 4:1 v/v mixture of N<sub>2</sub>O and O<sub>2</sub>. Dosimetry was performed with N<sub>2</sub>O-saturated 10 mM-KSCN solutions assuming  $G(\text{SCN})_2^{2-} = 6.0 \text{ molec. (100 eV)}^{-1}$  and  $\epsilon(480 \text{ nm}) = 7\,600 \text{ l mol}^{-1} \text{ cm}^{-1}$ .

Dihydropyrimidines are not stable in alkaline solutions.<sup>14</sup> To avoid hydrolysis in experiments at high pH, neutral solutions of the dihydropyrimidines were mixed with a solution containing NaOH just before reaching the pulse radiolysis cell. The pH was measured in the effluent.

### Acknowledgements

We would like to thank Dipl. Phys. F. Schwörer and Dr. S. Steenken and their staff for the maintenance and improvements of the pulse radiolysis facilities as well as Drs. D. J. Deeble and G. Koltzenburg for valuable suggestions.

### References

- S. Fujita and S. Steenken, *J. Am. Chem. Soc.*, 1981, **103**, 2540.
- M. N. Schuchmann, S. Steenken, J. Wroblewski, and C. von Sonntag, *Int. J. Radiat. Biol.*, in the press.
- H. R. Haysom, J. M. Phillips, and G. Scholes, *J. Chem. Soc., Chem. Commun.*, 1972, 1082.
- H. R. Haysom, J. M. Phillips, J. T. Richards, G. Scholes, and R. L. Willson, in 'Fast Processes in Radiation Chemistry and Biology,' eds. G. E. Adams, E. M. Fielden, and B. D. Michael, Institute of Physics and Wiley, Bristol, 1975, p. 241.
- K.-D. Asmus, D. J. Deeble, A. Garner, K. M. Idriss Ali, and G. Scholes, *Br. J. Cancer*, 1978, **37**, Suppl. 3, 46.
- K. Y. Al-Yamoor, A. Garner, K. M. Idriss Ali, and G. Scholes, Proc. 4th Tihany Symp. Radiation Chem., eds. P. Hedvig and R. Schiller, Akademiai Kiado, Budapest 1977, p. 845.
- M. Al-Sheikhly, A. Hissung, H.-P. Schuchmann, M. N. Schuchmann, C. von Sonntag, A. Garner, and G. Scholes, *J. Chem. Soc., Perkin Trans. 2*, 1984, 601.
- M. N. Schuchmann and C. von Sonntag, *J. Chem. Soc., Perkin Trans. 2*, 1983, 1525.
- A. Garner and G. Scholes, manuscript in preparation.
- R. L. Willson, *Int. J. Radiat. Biol.*, 1970, **17**, 349.
- R. D. Hotchkiss and T. B. Johnson, *J. Am. Chem. Soc.*, 1936, **58**, 525.
- N. Getoff and F. Schwörer, *Radiat. Res.*, 1970, **41**, 1.
- D. K. Hazra and S. Steenken, *J. Am. Chem. Soc.*, 1983, **105**, 4380.
- I. Blagoeva, B. J. Kurtev, and I. G. Pojarlieff, *J. Chem. Soc. B*, 1970, 232.

Received 31st January 1984; Paper 4/173