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

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Isopyrimidines are formed as a result of the oxidation of the 6-yl radicals derived by $^{\bullet}OH$ 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 × 10^7 l mol^{-1} s^{-1}). Around pH 7 a spontaneous reaction, k 3 000 s^{-1}, is observed. On increasing the pH the isouracil deprotonates at N(3) (pK_a *ca.* 9.4). The spontaneous rearrangement of the isouracil anion is considerably slower ($k \leq 50$ s^{-1}). At pH > 10.5 an OH^--catalysed reaction sets in (k 4.9 × 10^5 l mol^{-1} 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 OH^--induced rearrangement sets in at a much lower pH (pH ≤ 9.5), *i.e.* the rearrangement is faster (k 2.7 × 10^7 l mol^{-1} s^{-1}) than that observed in the other two systems.

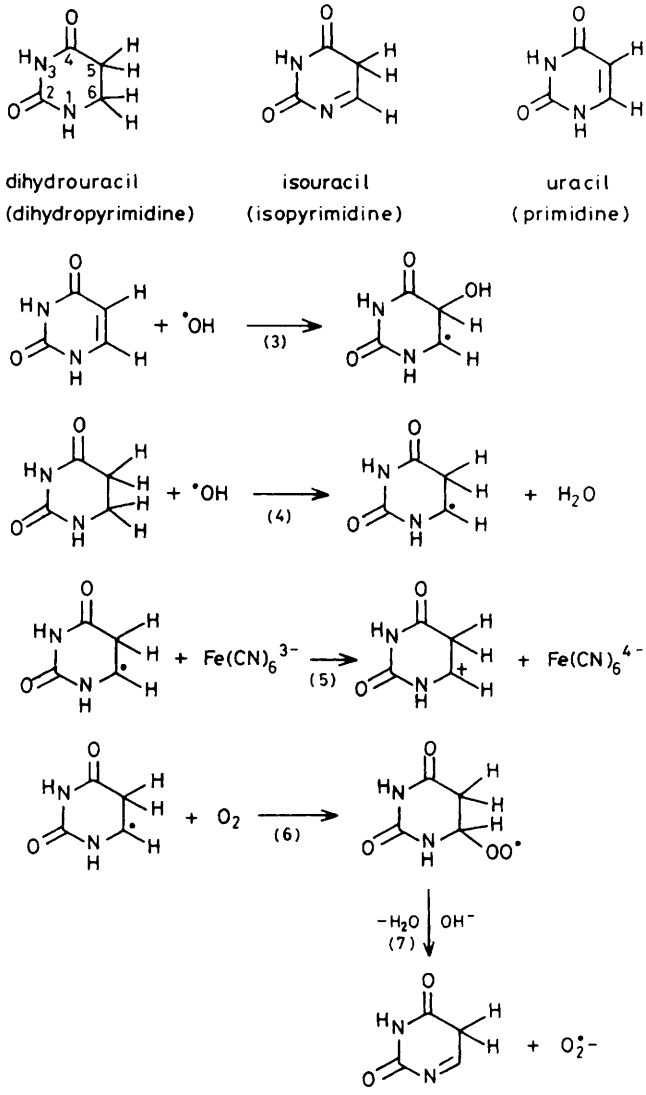
Isopyrimidines are unstable intermediates which play an important role in the free radical chemistry of pyrimidines and dihydropyrimidines in aqueous systems.

Hydroxyl radicals generated in the radiolysis of N_2O-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)][1] whereas they react with dihydropyrimidines predominantly by abstracting hydrogen atoms from the C(6) position [reaction (4)].[2] In both cases pyrimidine radicals with a free spin at C(6) (6-yl radicals) are formed. Such

$$H_2O \xrightarrow{\text{radiolysis}} {}^{\bullet}H, e^-_{eq}, H^{\bullet}, H_3O^+, H_2O_2, H_2 \qquad (1)$$

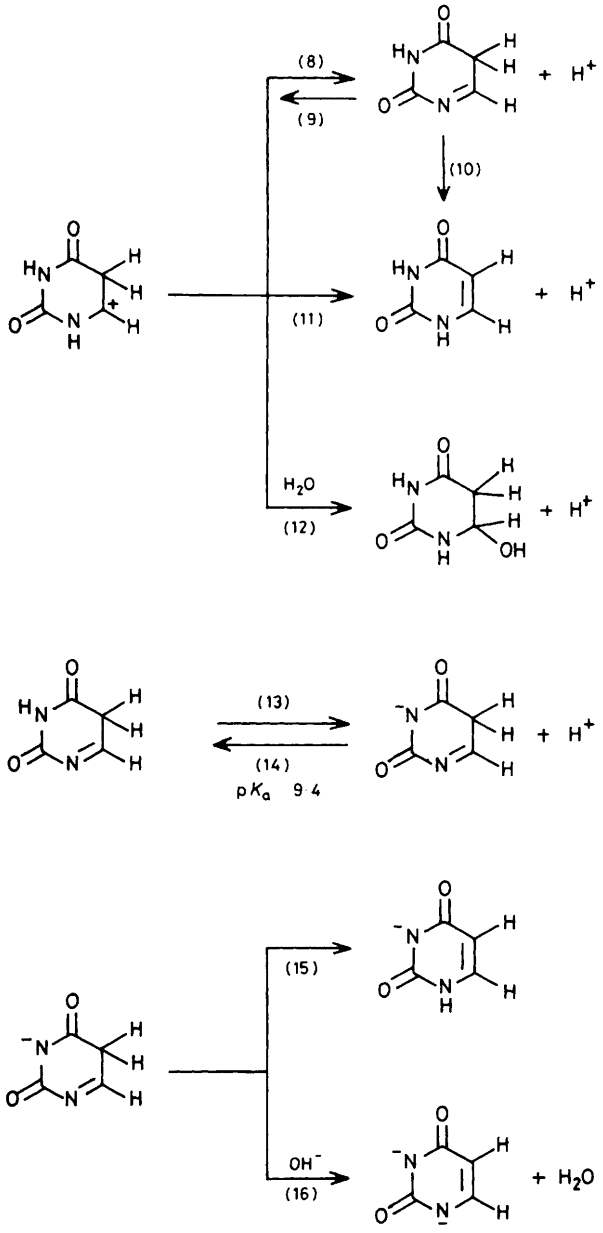
$$e^-_{aq} + N_2O \longrightarrow {}^{\bullet}OH + N_2 + OH^- \qquad (2)$$

radicals are known to be readily oxidized by transition metal ions or their complexes [*e.g.* $Fe(CN)_6^{3-}$] 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).[3] 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[4–6] showed the involvement of isopyrimidines as intermediates. The addition of oxygen to the 6-yl radicals followed by a base-induced $O_2^{-\bullet}$ elimination also leads to such an intermediate [*e.g.* reactions (6) and (7)].[7,8] Furthermore, it has been suggested that isopyrimidines are formed in disporportionation reactions of pyrimidine-derived radicals, possibly *via* electron-transfer processes.[6] There is evidence that they have to be considered as intermediates in the photohydration of pyrimidines.[6,9] 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 N_2O-saturated solutions of dihydrouracil, 3-methyldihydrouracil, or uracil (0.5mM). Hydroxyl radicals are generated by the



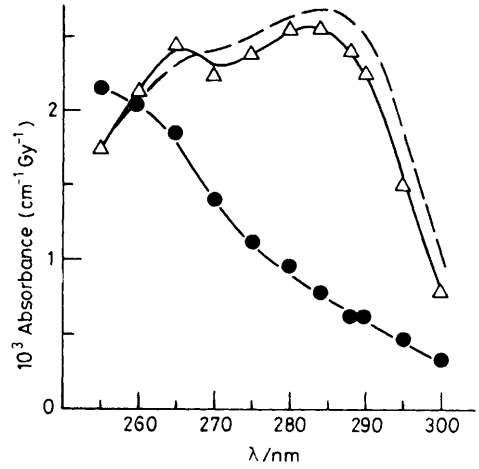


Figure 1. Optical absorption spectra of the intermediate isouracil (filled circles; 30 μs after pulse) and of the final product uracil (triangles; 16 ms after pulse) in N_2O-saturated 5,6-dihydrouracil solution (1mM) containing $K_3Fe(CN)_6$ (4×10^{-5}M) at pH 10.6 following a 1 μs electron pulse of *ca.* 14 Gy. Corrections have been made for the disappearance of $K_3Fe(CN)_6$ and the formation of $K_4Fe(CN)_6$. The dashed line represents the spectrum of uracil–uracilate taking *G*(uracil) 4.8

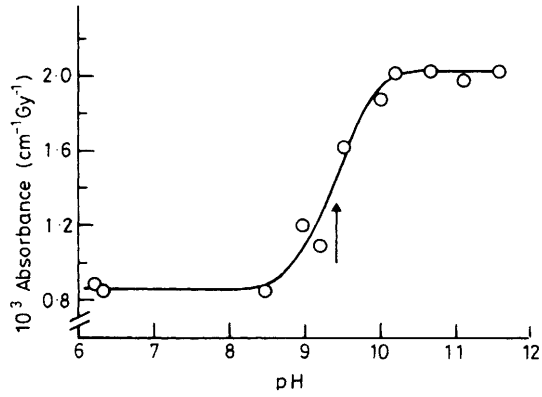


Figure 2. pH-Dependence of the optical absorbance at 260 nm of the intermediate isouracil (conditions as given for Figure 1)

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_2O–O_2 (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 $Fe(CN)_6^{3-}$ (4×10^{-5}M) is fast (k_5 4×10^9 l mol^{-1} s^{-1}),[2] 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.[5] Thus, with $Fe(CN)_6^{3-}$ as oxidant the isopyrimidine can be formed within a few microseconds. On the other hand, formation of the isopyrimidine through oxidation by O_2 [reactions (6) and (7)] is considerably slower. Although oxygen adds to the 6-yl radicals at near diffusion-controlled rate (k_6 1.9×10^9 l mol^{-1} s^{-1})[10] the elimination of $O_2^{-\bullet}$ is base-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 8×10^4 s^{-1} (probably the rate constant for the unimolecular decay of the anionic form of the peroxyl radical).[7]

The isopyrimidines do not show strong optical absorptions in the accessible wavelength region (λ > 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 μs after the pulse) as a function of pH (Figure 2). At pH ≥ 9 the isouracil–isouracilate ion equilibrium [reactions (13) plus its OH^--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, $\lambda_{max.}$ 260 nm (ε 8 200 l mol^{-1} cm^{-1}); uracilate ion, $\lambda_{max.}$

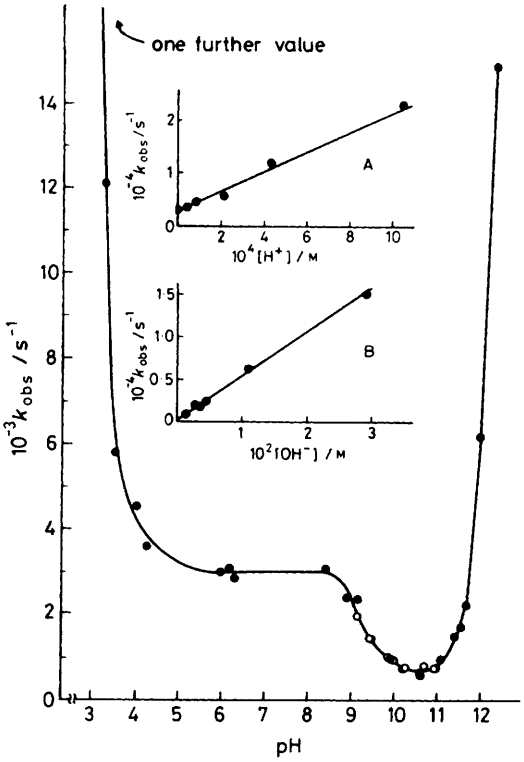


Figure 3. pH-Dependence of the observed first-order rate constant (k_{obs}) of the formation of uracil from isouracil in electron-pulse-irradiated 0.5mM-dihydrouracil solutions either saturated with N_2O and containing 4×10^{-5}M-$K_3Fe(CN)_6$ (filled circles) or only saturated with N_2O–O_2 (4:1 v/v) (open circles). Insets: A, plot of k_{obs} *versus* [H$^+$]; B, plot of k_{obs} *versus* [OH$^-$]

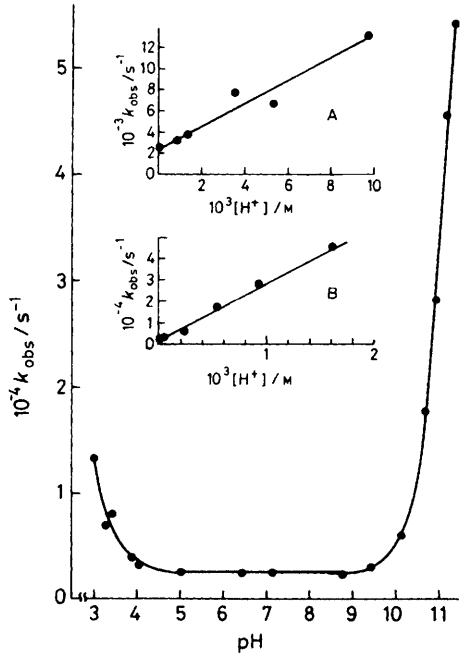


Figure 4. pH-Dependence of the observed first-order rate constant of the formation of 3-methyluracil from 3-methylisouracil in electron-pulse-irradiated 0.5mM-3-methyl-5,6-dihydrouracil solution containing 4×10^{-5}M-$K_3Fe(CN)_6$ saturated with N_2O. Insets: A, plot of k_{obs} *versus* [H$^+$]; B, plot of k_{obs} *versus* [OH$^-$]

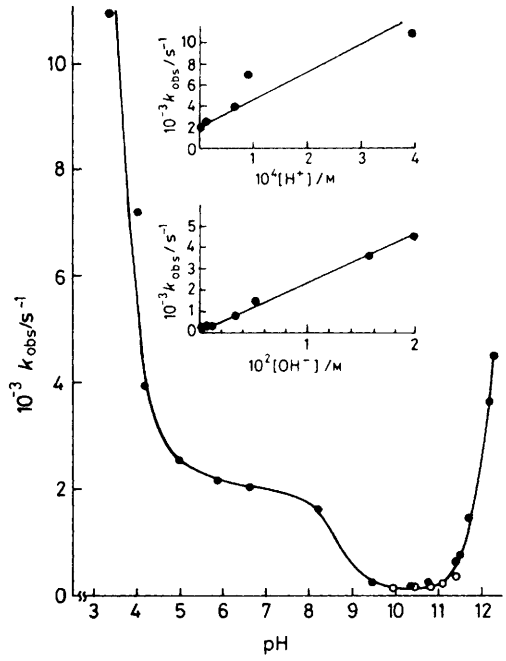


Figure 5. pH-Dependence of the observed first-order rate constant of the formation of isobarbituric acid from 5-hydroxyisouracil in electron-pulse-irradiated 0.5mM-uracil solutions either saturated with N_2O and containing 4×10^{-5}M-$K_3Fe(CN)_6$ (●) or saturated with N_2O–O_2 (4:1 v/v) (○). Insets: A, plot of k_{obs} *versus* [H$^+$]; B, plot of k_{obs} *versus* [OH$^-$]

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

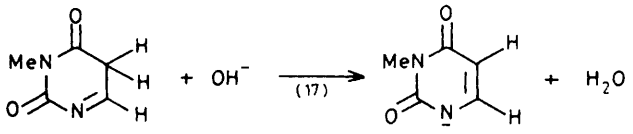
In Figure 3 the observed first-order rate constants for the formation of uracil–uracilate ion in solutions containing either $Fe(CN)_6^{3-}$ or O_2 as oxidant are plotted as a function of pH. It can be seen that the two methods of forming the isopyrimidine gave identical results. Figures 4 and 5 give 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 s^{-1} in all systems investigated (Table). Given that the pK_a value of the isouracil is *ca.* 9.4 (and assuming k_{14} 10^{10} l mol^{-1} s^{-1}) the rate constant of the spontaneous deprotonation at N(3), k_{13}, is *ca.* 4 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 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.[6] 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 occurrance 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_{obs} *versus* [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^+-catalysed	1.8×10^7 l mol^{-1} s^{-1}	1.1×10^7 l mol^{-1} s^{-1}	2.6×10^7 l mol^{-1} s^{-1}	(9), (11)
Neutral spontaneous	3 000 s^{-1}	2 500 s^{-1}	2 000 s^{-1}	(10)
Neutral OH^--catalysed	Absent	2.7×10^7 l mol^{-1} s^{-1}	Absent	(17)
Anion spontaneous	$\leq$ 50 s^{-1}	Absent	$<$ 50 s^{-1}	(15)
Anion OH^--catalysed	4.9×10^5 l mol^{-1} s^{-1}	Absent	2.2×10^5 l mol^{-1} s^{-1}	(16)



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

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^--catalysed reaction sets in [reaction (17)]. For reaction (17) a rate constant of 2.7×10^7 l mol^{-1} 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^--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[11] was a gift of Dr. J. Wroblewski.[2] 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.[12,13]

The solutions of 0.5—1mM-5,6-dihydrouracil, 3-methyl-5,6-dihydrouracil, or uracil containing 4×10^{-5}M-$K_3Fe(CN)_6$ (Merck; p.a.) in triply distilled water were saturated with oxygen-free N_2O and were irradiated at 20 ± 1 °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 $HClO_4$ or NaOH. In experiments where oxidation by O_2 was required the solutions were saturated with a 4:1 v/v mixture of N_2O and O_2. Dosimetry was performed with N_2O-saturated 10mM-KSCN solutions assuming $G(SCN)_2^{-\bullet}$ 6.0 molec. (100 eV)$^{-1}$ and ε(480 nm) 7 600 l mol^{-1} cm^{-1}.

Dihydropyrimidines are not stable in alkaline solutions.[14] 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.

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