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Free radical chain mechanism in the acetone-sensitized photolysis of 5-bromouracil and derivatives in alcohol–water mixtures

Helmut Görner

Max-Planck-Institut für Strahlenchemie, D-45413 Mülheim an der Ruhr, Germany

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Abstract

The acetone-sensitized photolysis of 5-bromouracil (BrU), 1-methyl-5-bromouracil (MeBrU) and 5-bromouridine (BrUd) in deoxygenated aqueous solution in the presence of alcohols was studied on irradiation at 254 nm. BrU is converted to uracil with the release of Br^-, as shown by UV analysis and high performance liquid chromatography. The quantum yield of the BrU$\rightarrow$uracil photoconversion, using 0.1 M acetone and approximately 1 M 2-propanol, increases linearly with the inverse square root of the intensity (I_{254}) and approaches values of 15 at pH 13 for $I_{254}=0.06$ mW cm^{-2}. Comparable results were obtained for MeBrU and BrUd; the maximum Φ values are 12 and 4 respectively. $\Phi(-\mathrm{BrU})$ decreases significantly with decreasing pH and decreasing concentrations of either acetone or 2-propanol (at a given pH). No evidence for the involvement of the triplet state of BrU in the photoreduction was found. $\Phi(-\mathrm{BrU})$ is reduced when 2-propanol is replaced by methanol and significantly reduced in the presence of tert-butanol. To account for the chain reaction in neutral solution, it is suggested that the $(CH_3)_2{}^{\bullet}COH$ radical is initially formed by H-atom abstraction by triplet acetone from 2-propanol; propagation occurs by electron transfer to BrU, Br^- release and H-atom abstraction by the uracil-5-yl radical from 2-propanol. The enhancement of $\Phi(-\mathrm{BrU})$ in the alkaline pH range is ascribed to a lower reactivity of the termination reaction with respect to the competing electron transfer from the $(CH_3)_2{}^{\bullet}CO^-$ radical to BrU.

Keywords: 5-Bromouracil; Acetone-sensitization; 254 nm Irradiation; Laser flash photolysis

1. Introduction

The photoreactions of pyrimidines in aqueous solution, e.g. the formation of hydrates and specific photoadducts, mainly cyclobutane-type dimers, are accompanied by changes in the near-UV absorption spectra [1–5]. The effects of chromophore loss, induced by the irradiation at 254 nm of monomers of uracil derivatives at low concentrations or in the presence of oxygen, are mainly ascribed to hydrates [1–4]. 5-Halouracils, with the exception of 5-fluorouracils, yield virtually no photohydrates [1–3]. The current interest in the photochemistry of bromopyrimidines and iodopyrimidines, e.g. 5-bromouracil (BrU), 5-iodouracil (IU) and their derivatives [6–17], is due to the fact that the sensitivity of DNA and its biological activity in cells can be enhanced by the incorporation of BrU or 5-iodocytidine instead of thymine or cytosine respectively [18–22]. Photochemical studies are complemented by those on the radiation chemistry of 5-halouracils in aqueous solution in the presence of alcohol or OH radicals [23–29].

The major effect during the irradiation of BrU or IU (in the absence of a sensitizer) is photohomolysis (Eq. (1), Scheme 1) [10–12]. The precursor is the excited singlet state of the 5-halouracil (XU^{1*}) rather than the lowest triplet state (XU^{3*}). The uracil-5-yl radical ($U^{\bullet}$) converts into uracil by H-atom abstraction from a suitable donor (e.g. an alcohol HROH, Eq. 3, Scheme 1). The ketyl radical ($^{\bullet}$ROH) is also formed via reaction (2).

For BrU, in addition to the free radical pathway from the excited singlet state (Eqs. (1)–(3), Scheme 1), another mechanism has been discussed. This second mechanism, which is ionic in character, involves the lowest triplet state (BrU^{3*}) [14–17]; it is based on studies in acetone–2-propanol mixtures, i.e. in the absence of water [14]. Accordingly, on photolysis at 254 nm of BrU, approximately two-thirds of uracil should result from BrU^{1*} via Eqs. (1) and (3) and one-third from BrU^{3*} [14,17]. This raises the question as to the properties of BrU^{3*}.

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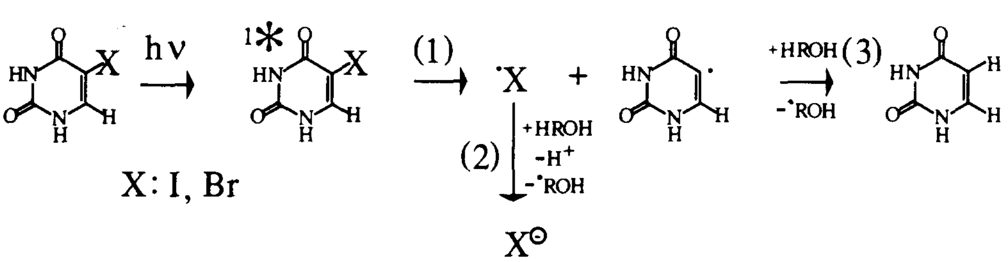


Scheme 1. Photolysis of BrU and IU on direct irradiation in neutral deoxygenated aqueous solution.

The present work aims to understand the photochemistry of BrU and two derivatives (1-methyl-5-bromouracil (MeBrU) and 5-bromouridine (BrUd)) in aqueous solution. Phosphorescence measurements in glassy media and laser flash photolysis at room temperature were carried out. The acetone-sensitized photolysis of the 5-bromopyrimidines was studied in the presence of alcohols as H-atom donors, and the quantum yields using continuous irradiation at 254 nm were determined under various conditions.

2. Experimental details

BrU, BrUd, MeBrU, IU and 5-iodouridine (IUd) were used as received (Sigma); the purity was found by high performance liquid chromatography (HPLC) to be better than 99.4%. The solvents (Merck) were purified by distillation (acetone, 2-propanol, methanol) or used as received (tert-butanol); water was from a millipore (Milli Q) system. Phosphate and borate buffers were used to keep the pH constant in the range 5–10. HPLC measurements were carried out using a Nucleosil 5-C18 column (4.6 mm × 125 mm) [12]. Absorption and emission spectra were recorded on a spectrophotometer (Perkin–Elmer 554) and spectrofluorometers (Perkin–Elmer LS-5 and Spex-Fluorolog) [30]. All measurements, except for phosphorescence, were carried out at 24 ± 2 °C. For irradiation at 254 nm, a low-pressure mercury lamp (Gräntzel) and a cut-off filter to discriminate against the 185 nm line were used. Potassium iron(III) oxalate [31] and the chromophore loss of uridine in aerated aqueous solution at pH 6 ($\Phi = 0.017$ [1,4]) (due to hydrate formation) were used as actinometers. The maximum intensity (photon fluence rate) was 2.2 mW cm^{-2}. Laser excitation was performed with a KrF excimer laser (Lambda Physik, EMG 201 MSC); the energy was 100 mJ per pulse or less, corresponding to an intensity of 5 MW cm^{-2} or less [5,12].

The dilute solutions were freshly prepared and carefully deoxygenated either by the freeze–thaw method or, in most cases, by purging with argon for 30 min prior to and during the whole irradiation procedure in order to avoid the penetration of traces of oxygen [12]. Both methods were applied in several cases where $\Phi > 1$, and no discernible difference was found (overall error, smaller than ±30%). The initial absorbance (A) of BrU was adjusted to 2.0–2.5 (1 cm path length) at λ_{max}. The absorption spectra of BrU and uracil in aqueous solution at pH 7 are characterized by $\lambda_{max} = 278$ nm, $\epsilon = 7.1 \times 10^3$ M^{-1} cm^{-1} and $\epsilon_{259} = 8.2 \times 10^3$ M^{-1} cm^{-1} respectively. The absorption maxima of BrU and uracil at pH 12 are red shifted: $\epsilon_{303} = 6.9 \times 10^3$ and $\epsilon_{283} = 6.2 \times 10^4$ M^{-1} cm^{-1} respectively.

The relative quantum yield from optical detection was obtained from the linear decrease in $\log\{(A_o - A_t)/(A_o - A_{end})\}$, at an appropriate wavelength longer than λ_{max} (e.g. at 290 nm at pH 7 or 305 nm at pH 9–10), with the fluence (dose) or the irradiation time (t); here the two other subscripts o and end refer to $t = 0$ and long times respectively. In the presence of 0.1 M acetone, 60%–90% of the 254 nm light is absorbed by the sensitizer. The above plots were found to be linear up to conversions of typically 40%–60%; the decomposition of uracil became noticeable at longer times. The experimental standard condition was 5-bromopyrimidine : acetone : 2-propanol of $3 \times 10^{-4} : 0.12 : 1.4$ M. The quantum yield is expressed by Φ_{HU} in those cases where nearly complete photoconversion is established and by $\Phi(-XU)$ in the others. It was verified that the spectroscopically measured BrU → uracil, MeBrU → 1-methyluracil and BrUd → uridine conversions are the same within experimental error as obtained by HPLC analysis.

3. Results and discussion

3.1. Optical transients

Excitation at 248 nm of BrU in aqueous solution at pH 7 yields the hydrated electron (e_{aq}^-) as a major transient, similar to the case of IU [12]. It was not possible to observe the triplet–triplet (T–T) absorption spectrum of BrU in argon-saturated water or acetonitrile at room temperature either in the absence or presence of high-energy sensitizers (acetone, xanthone). The rate constant for the quenching of triplet acetone (for its characterization [12,32,33]) by BrU (concentration range, 0.1–0.5 mM) was found to be 1.5×10^9 M^{-1} s^{-1} (for quenching by IU, a value of $k_5 = 1.6 \times 10^9$ M^{-1} s^{-1} has been reported [12]). Assuming that quenching of triplet acetone occurs quantitatively by energy transfer to BrU, a lifetime for BrU^{3*} of shorter than 50 ns is estimated, using a BrU concentration of 4 mM or more (a longer lifetime would require an extinction coefficient of less than 10^3 M^{-1} cm^{-1}).

3.2. Phosphorescence

Phosphorescence was recorded for BrU, IU, BrUd and IUd in glassy ethanol (or a water–ethylene glycol mixture (1 : 2) [30]) at −196 °C. The emission spectrum of BrU consists of fluorescence and phosphorescence; the latter is unstructured with $\lambda_{max}=480$ nm and a half-width of approximately 80 nm. The emission spectrum of IU which consists, due to a much weaker fluorescence, only of phosphorescence is similar to that of BrU (Fig. 1); the maxima are red shifted relative to those of other pyrimidines. A triplet energy of approximately 300 kJ mol^{-1} may be estimated from the onset of the phosphorescence signal at around 400 nm. The phosphorescence lifetime is typically $\tau_p=60$–80 μs for IU or IUd and 200–300 μs for BrU or BrUd; the quantum yield ranges from $\Phi_p=2\times10^{-3}$ for BrU in ethanol to 0.03 for IUd in a water–ethylene glycol mixture. This indicates that the population of the triplet state of the 5-halopyrimidines is low even in frozen media. Attempts to extend the measurements to markedly higher temperatures failed; this is due to a strong reduction (more than 100-fold) in τ_p in a relatively small temperature range, e.g. −160 to −140 °C for ethanol.

3.3. Acetone-sensitized photolysis of BrU in neutral aqueous solution

The absorbance of an aqueous solution of BrU (0.3 mM) at pH 7 ($\lambda_{max}=278$ nm) increases, especially in the 220–280 nm range, when acetone is added. Typically, 60%–80% of the incident 254 nm light, depending on the pH, is absorbed by the sensitizer, whereas the presence of 2-propanol has no influence on the non-irradiated spectra. Examples of the absorption spectra of BrU and BrU plus acetone (0.12 M) are shown in Figs. 2(a) and 2(b) respectively.

On irradiation of BrU in the presence of 1 M tert-butanol, chromophore loss occurs; $\Phi(-BrU)$ is small and almost the same (less than 0.003) in the absence or presence of acetone. In the presence of both acetone and 2-propanol, however, $\Phi(-BrU)$ is significantly larger (Table 1) and uracil and Br^- were detected by HPLC; irradiation also leads to the formation of protons. To maintain a constant pH 7, the samples were irradiated in the presence of phosphate buffer ($\leqslant 1$ mM); otherwise a conversion of 50% corresponds to about pH 4. Examples of the changes in concentration of BrU and uracil as a function of the (incident) fluence are shown in Fig. 3. The photoconversion and absence of secondary reactions are supported by isosbestic points at 207, 233 and 256 nm (Fig. 2(b)). Quantum yields of up to three (Table 2) and their dependence on intensity (Section 3.5) demonstrate a chain reaction for the BrU–acetone–2-propanol system (3×10^{-4} : 0.12 : 1.4 M) at pH 7.

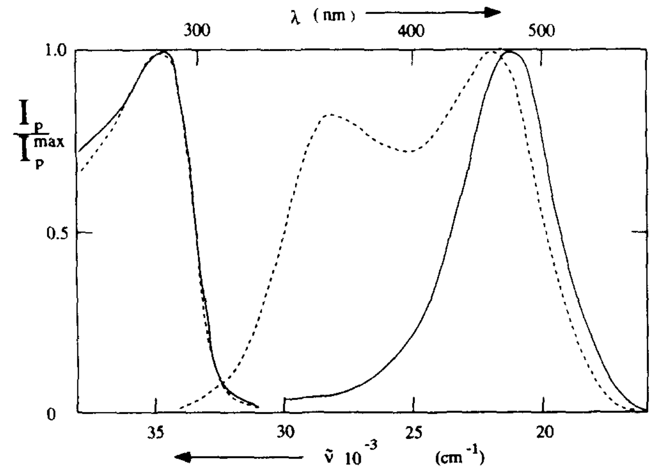


Fig. 1. Luminescence excitation and emission spectra of BrU (broken lines) and IU (full lines) in ethanol at −196 °C; $\lambda_{exc}=280$ nm; $\lambda_{em}=480$ nm.

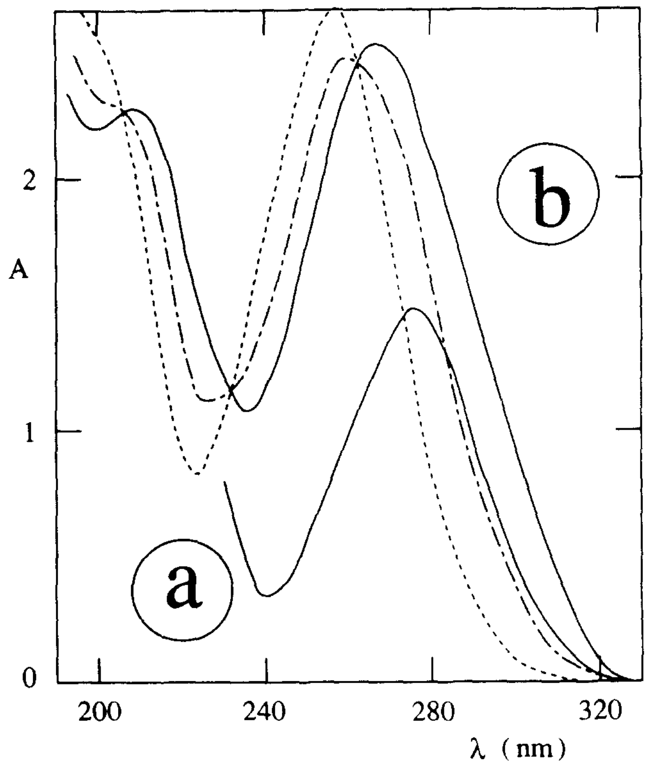


Fig. 2. Absorption spectra of BrU in deoxygenated aqueous solution at pH 7 (plus 1.4 mM 2-propanol and 1 mM K_2HPO_4–KH_2PO_4) in the absence (a) and presence (b) of 0.12 M acetone, prior to (full lines) and after (broken lines) 254 nm irradiation.

Table 1
Quantum yield of decomposition of BrU in aqueous solution in the presence and absence of alcohols and/or acetone [a]

pH	Additive [b]	$\Phi(-\mathrm{BrU})$	
		No acetone	Acetone (0.12 M)
2	None		<0.005
2	2-Propanol	0.002	0.2
7	None	<0.002	0.002
7	tert-Butanol	0.002	<0.003
7	Methanol	0.002	0.05
7	2-Propanol	0.003 (0.001)	0.8 (0.02) [c]
9.5	2-Propanol		1.8
11	2-Propanol	0.015	1.9
12	2-Propanol	0.03	2.6
13	None	0.005	0.02
13	tert-Butanol	0.03	0.03
13	Methanol		0.3
13	2-Propanol	0.08 (0.01)	3.3 (0.01)
14	2-Propanol		2

[a] Using 254 nm irradiation, $I_{254} = 2.2$ mW cm^{-2}; initial BrU concentration, 0.3 mM; in argon-saturated solution unless otherwise indicated.

[b] 10 vol.%, e.g. 1.4 M 2-propanol.

[c] Values in parentheses refer to O_2-saturated solution.

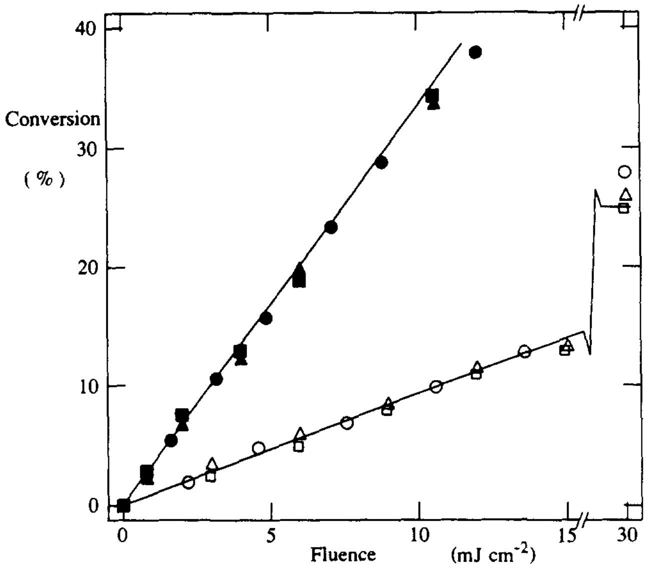


Fig. 3. Changes in the relative concentrations during irradiation at 254 nm of a deoxygenated aqueous solution of BrU–acetone–2-propanol (3×10^{-4} : 0.12 : 1.4 M) at pH 7 (open symbols) and pH 13 (filled symbols): BrU (□, ■, 100 − %, using HPLC); uracil, obtained by HPLC (△, ▲) and optical detection (○, ●); $I_{254} = 2.2$ mW cm^{-2}.

3.4. Reaction scheme for acetone-sensitized photolysis of BrU

After generation of the acetone triplet in aqueous solution in the presence of XU and an alcohol, two photoreactions must be considered. One is the formation of two ketyl radicals via H-atom abstraction

Table 2
Quantum yield of decomposition of BrU, MeBrU and BrUd in the presence of alcohols during acetone-sensitized irradiation in aqueous solution [a]

Compound	pH	Φ_{HU} at [2-propanol] (M)					
		0.01	0.04	0.14	0.4	1.4	4
BrU	7	0.005	0.02	0.08	0.3	0.8 (3) [b]	0.6
	9.5	0.04	0.1	0.3	0.8	1.8 (10)	1.5
	11	0.05	0.1	0.3	0.8	1.9 (11)	1.8
	13	0.07	0.2	0.6	1.4	3.3 (15)	3.0
MeBrU	7	0.003	0.01	0.03	0.1	0.4	0.3
	13	0.02	0.05	0.2	0.8	4 (12)	4
BrUd	13	0.02	0.06		0.6	1.8 (4)	2

Compound	pH	Φ_{HU} at [methanol] (M)				
		0.07	0.2	0.7	2.2	7
BrU	13	<0.03	0.04	0.1	0.3	0.5
BrUd	13	0.02		0.1	0.2	0.4

[a] Initial pyrimidine concentration, 0.3 mM; 0.12 M acetone; in argon-saturated solution; $I_{254} = 2.2$ mW cm^{-2}.

[b] Values in parentheses refer to $I_{254} = 0.06$ mW cm^{-2}.

$$^{3*}(CH_3)_2C{=}O + HROH \longrightarrow (CH_3)_2{}^{\bullet}COH + {}^{\bullet}ROH \qquad (4)$$

For 2-propanol, $^{\bullet}$ROH is the 2-hydroxy-2-propyl radical ($k_4 \approx 1 \times 10^6$ M^{-1} s^{-1} [32,34]). The competing photoreaction is the quenching of triplet acetone by XU. This could lead to ground state XU (Eq. (5a), Scheme 2) or, via energy transfer, to its triplet state (Eq. (5b))

$$^{3*}(CH_3)_2C{=}O + XU \longrightarrow (CH_3)_2C{=}O + XU^{3*} \qquad (5b)$$

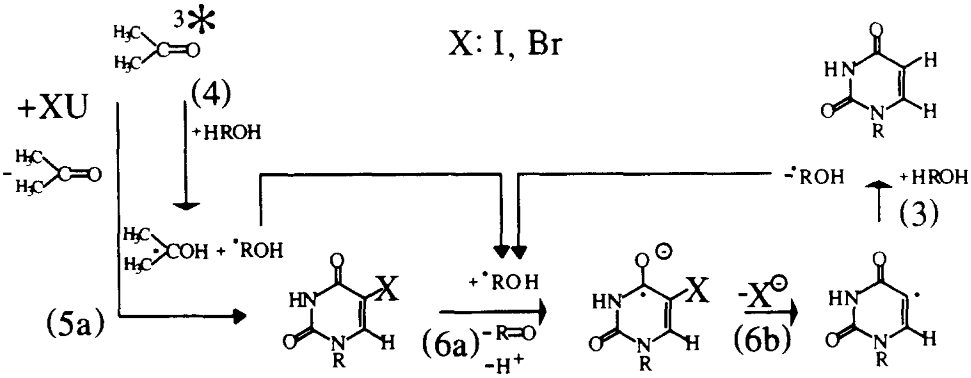
For aqueous IU in the presence of 2-propanol, it has recently been shown [12] that electron transfer occurs from the $(CH_3)_2{}^{\bullet}COH$ radical to IU

$$^{\bullet}ROH + XU \longrightarrow XU^{\bullet -} + R{=}O + H^+ \qquad (6a)$$

$$XU^{\bullet -} \longrightarrow U^{\bullet} + X^- \qquad (6b)$$

Reaction (6a) yields R=O (i.e. acetone in the case of 2-propanol), the radical anion $XU^{\bullet -}$ and a proton (Scheme 2). From pulse radiolysis, it is known that $IU^{\bullet -}$ and $BrU^{\bullet -}$ rapidly eliminate X^- (Eq. (6b)), the half-lives being 1.7 and 7 ns respectively [27]. Reactions (6a), (6b) and (3) constitute a chain propagation and account for the large Φ_{HU} values under acetone-sensitized conditions (Table 2). A chain reaction involving the radiolytically generated $(CH_3)_2{}^{\bullet}COH$ radical has already been reported for BrU [26].

The reactivity of methanol is much lower; typical rate constants for H-atom abstraction by triplet ketones are $k_4 = 1 \times 10^5$ M^{-1} s^{-1} [34]. The concentration of primary ketyl radicals is therefore expected to be much lower than with 2-propanol. Correspondingly, the Φ



Scheme 2. Acetone-sensitized photolysis of BrU, MeBrU and BrUd in neutral deoxygenated aqueous solution.

values are much smaller in the presence of methanol than in the presence of 2-propanol (Tables 1 and 2); it should be noted that, at very large alcohol concentrations, the properties of the aqueous medium itself change quite drastically. tert-Butanol has a much lower reactivity (the rate constants are typically less than 10^4 M^{-1} s^{-1} [34]), but no chain reaction can be expected since the $^{\cdot}CH_2C(CH_3)_2OH$ radical is non-reducing.

Radical–radical termination in the absence of oxygen occurs via the reactions (7a) and (7b)

$$^{\cdot}ROH + {}^{\cdot}ROH \longrightarrow \text{products} \qquad (7a)$$

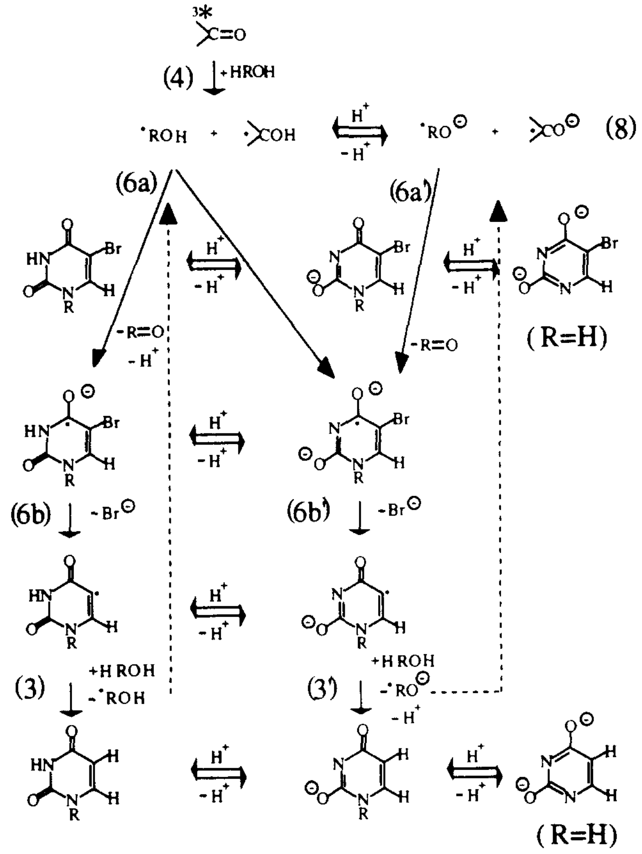
$$U^{\cdot} + U^{\cdot} \longrightarrow \text{products} \qquad (7b)$$

and cross terminations. Other reactions of the ketyl radicals could involve quenching by trace impurities (Section 3.9).

3.5. *Effects of pH and intensity*

The different states (neutral or deprotonated) of the substrates and their radicals have certain consequences on the behaviour of the direct and sensitized photolysis. The first and second pK_a values of BrU are 8.05 and 13 respectively [10]; for uracil, the pK_a values are 9.5 and 12.5 respectively. The pK_a values for the ketyl radicals of 2-propanol and methanol are 12.2 and 10.7 respectively (Scheme 3). The absorption spectra of the BrU–acetone–2-propanol system at pH 9.5 and 13 (full lines) and the photochemical changes (broken lines) are shown in Figs. 4(a) and 4(b) respectively. At pH 9.5, the maxima are red shifted due to the monoanionic nature of BrU and uracil. Reactions (8, forward), (6') and (3') (Scheme 3) constitute the corresponding chain reaction at pH 12 or above (note that 3' and 6' refer to the alkaline pH range).

The light intensity was mostly kept constant at $I_{254} = 2.2$ mW cm^{-2}. The largest quantum yield, $\Phi_{HU} = 3.3$, was measured at pH 13 in the presence of 1.4 M 2-propanol (Tables 1 and 2). However, when I_{254} was decreased, Φ_{HU} was found to increase. Linear



Scheme 3. Photolysis of BrU, MeBrU and BrUd during acetone-sensitized irradiation in deoxygenated aqueous solution at various pH values.

plots of Φ_{HU} as a function of the inverse square root of I_{254}, as shown in Fig. 5, were observed at several pH values. This and the quantum yields larger than unity are indicative of a chain reaction. The following dependences of the aqueous BrU–acetone–2-propanol system were studied with $I_{254} = 2.2$ mW cm^{-2}.

Decreasing the pH (from pH 7 to pH 2) has no marked effect on the product pattern or the absorption spectrum, which shows isosbestic points in the presence of acetone plus 2-propanol. Under these conditions,

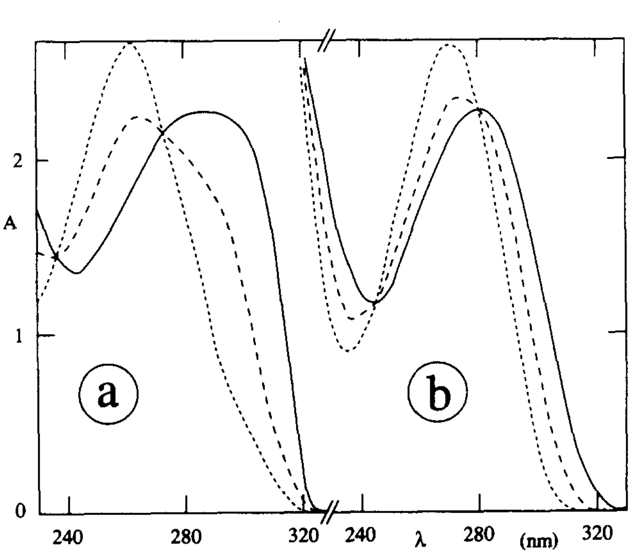


Fig. 4. Absorption spectra of a deoxygenated aqueous solution of BrU–acetone–2-propanol (3×10^{-4} : 0.12 : 1.4 M) prior to (full lines) and after (broken lines) 254 nm irradiation at pH 9.5 (a) and pH 13 (b).

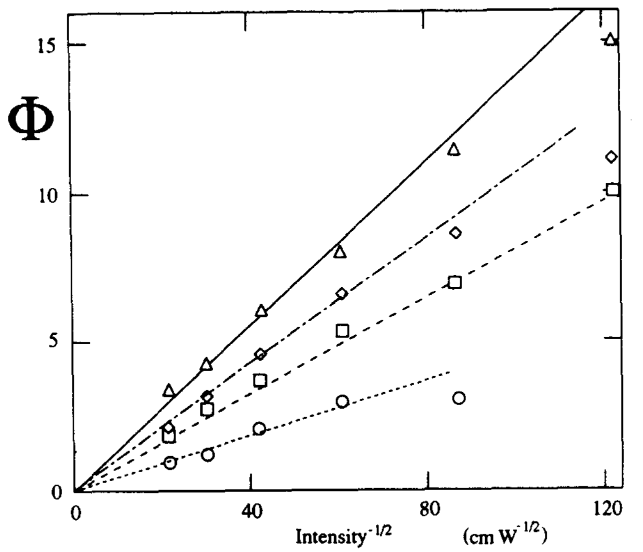


Fig. 5. Dependence of Φ_{HU} on the inverse square root of the incident light intensity for a deoxygenated aqueous solution of BrU–acetone–2-propanol (3×10^{-4} : 0.12 : 1.4 M) at pH 7 (○), 9.5 (□), 11 (◇) and 13 (△).

Φ_{HU} increases only slightly on going from pH 2 to pH 7. However, shifting the pH into the alkaline range leads, besides the corresponding spectral changes, to generally much larger Φ_{HU} values (Table 1). Φ_{HU} increases with increasing pH (the largest change is around pH 11) and reaches a maximum at pH 13 (Fig. 6).

The larger quantum yields of IU, BU and their derivatives in the alkaline pH range are ascribed to two differences in the reactivity: (a) the larger reactivity of the anion radical of 2-propanol with respect to the neutral form; (b) the more efficient radical termination at pH 9 or less with respect to the overall chain reaction [12,28,35].

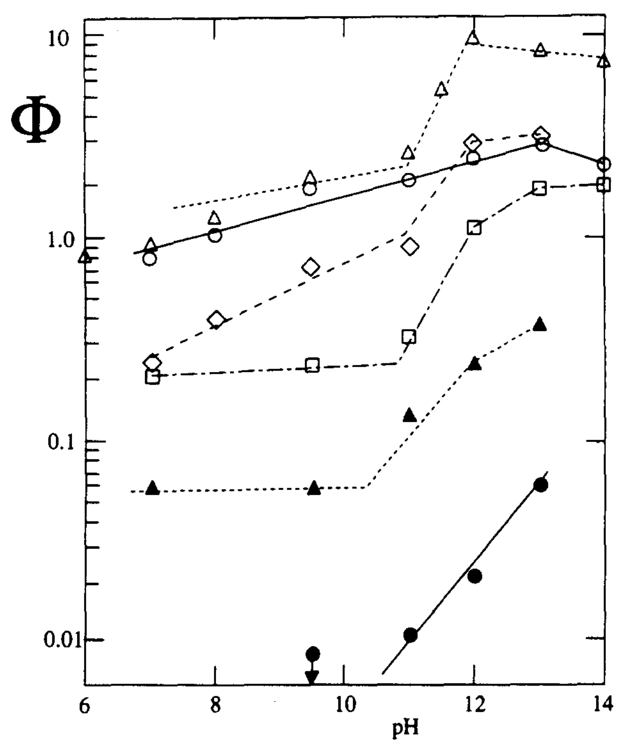


Fig. 6. Effect of pH on Φ (logarithmic scale) for BrU (circles), MeBrU (◇), BrUd (□) and IU (triangles) in deoxygenated aqueous solution in the presence of 0.12 M acetone (open symbols) and 1.4 M 2-propanol; filled symbols refer to the absence of acetone; $I_{254}=2.2$ mW cm^{-2}.

3.6. Effects of concentration

At low 2-propanol concentrations (e.g. less than 0.2 M), reaction (5) is the major quenching process of triplet acetone due to the rate constants $k_5=1.5\times10^9$ M^{-1} s^{-1} (Section 3.1) and k_4 (a value of 1.4×10^6 M^{-1} s^{-1} was used [34]). Under the standard condition of 0.3 mM XU, about 20% and 80% of the acetone triplets react via Eq. (4) for 0.1 and 1.4 M 2-propanol respectively. When the 2-propanol concentration is increased at a fixed pH and for 0.12 M acetone, Φ_{HU} becomes significantly larger. The plot of Φ_{HU} vs. the logarithm of [2-propanol] is initially sigmoidal; it reaches a maximum Φ_{HU} value at a concentration of approximately 1 M and then decreases (Fig. 7). This optimum 2-propanol concentration, for which reaction (4) competes efficiently with reaction (5), is roughly the same in the alkaline pH range. (The broken line in Fig. 7 indicates only competiton kinetics without chain propagation.) Φ_{HU} also increases with the methanol concentration, but a maximum was not found. The Φ_{HU} values are significantly smaller than in the presence of 2-propanol (Table 2), e.g. for an alcohol concentration of 1 M, $\Phi_{HU}=0.05$ and 0.5 at pH 7 with methanol and 2-propanol respectively. This is due to the different rate constants for the reaction of alcohols with triplet acetone.

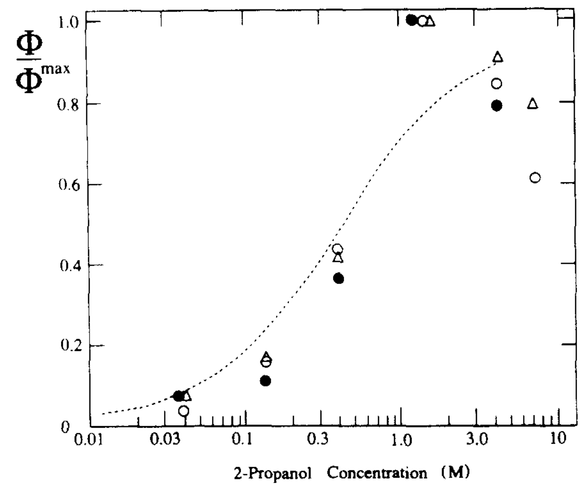


Fig. 7. Relative quantum yields as a function of the logarithm of the 2-propanol concentration for BrU in deoxygenated aqueous solution in the presence of 0.12 M acetone at pH 7 (●), pH 9.5 (○) and pH 13 (△); I_{254} = 2.2 mW cm^{-2}.

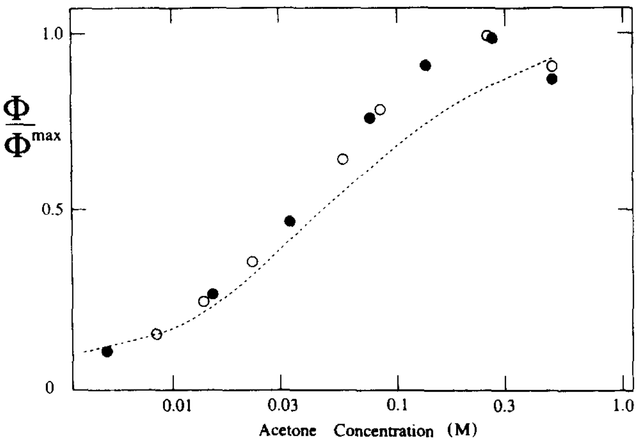


Fig. 8. Relative quantum yields as a function of the logarithm of the acetone concentration for BrU at pH 7 (●) and 13 (○) in deoxygenated aqueous solution in the presence of 1.4 M 2-propanol; I_{254} = 2.2 mW cm^{-2}.

The dependence of Φ/Φ^{max} on the acetone concentration is shown in Fig. 8 for a fixed 2-propanol concentration of 1.4 M at pH 7 and 13. Φ_{HU} = 0.2 at pH 13 ([acetone] = 0.01 M); it initially increases as more light is absorbed by acetone relative to BrU. After reaching a maximum, Φ_{HU} = 3 at [acetone] ≈ 0.2 M, it decreases slightly at 0.5 M acetone. Self-quenching of triplet acetone in competition with the reactions with 2-propanol and BrU may account for the decrease in Φ_{HU}. As noticed previously [12], the relative quantum yields cannot follow directly the broken line in Fig. 8, which is proportional to the amount of light yielding excited acetone. Instead, larger values are expected, increasing with decreasing steady state radical concentrations.

The effect of the concentration of BrU (as well as IU) on Φ_{HU} was studied for 1.4 and 7 M 2-propanol at two fixed pH values using 10, 1.0 and 0.10 mm cells keeping the relative absorbance of acetone constant (Table 3). The quantum yield is nearly constant for XU concentrations below about 0.5 mM, but decreases with increasing [XU]. Examples of Φ/Φ^{max} at pH 9.5 and 12 show a similar trend with smaller values for 1.4 M than for 7 M 2-propanol (Fig. 9). The curves refer to the simple competition of reactions (4) and (5b) without chain propagation. Because of the chain reaction, the relative quantum yield is not expected to be only determined by the branching ratio k_4/k_5. Nonetheless, the trend in the XU and 2-propanol concentration dependences is striking.

Table 3
Effect of the concentration of BrU and IU on the quantum yield of decomposition during acetone-sensitized irradiation in the presence of 2-propanol [a]

pH	[XU] (mM)	[2-propanol] (M)	Φ_{HU}	
			BrU	IU
9.5	0.05	1.4	2.0	2.2
9.5	20	1.4	0.4	0.5
9.5	2.5	7	0.9	1.4
9.5	20	7	0.5	0.7
12	0.05	1.4	2.5	9
12	20	1.4	0.4	
12	2.5	7	1.5	
12	20	7	0.8	

[a] In argon-saturated solution; 50%–70% of the incident light was absorbed by acetone; I_{254} = 2.2 mW cm^{-2}.

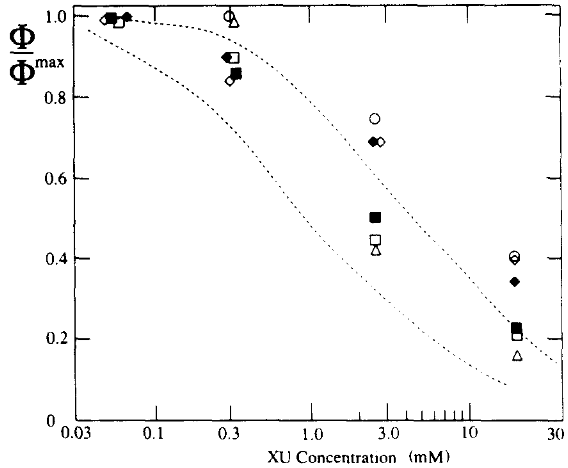


Fig. 9. Relative quantum yields (I_{254} = 2.2 mW cm^{-2}) vs. the XU concentration (BrU, open symbols; IU, filled symbols) using 0.12 M acetone in deoxygenated aqueous solution at pH 9.5 and 1.4 M 2-propanol (squares), pH 9.5 and 7 M 2-propanol (diamonds), pH 12 and 1.4 M 2-propanol (△) and pH 12 and 7 M 2-propanol (○); the lower and upper lines refer to 1.4 and 7 M 2-propanol respectively (competition between reactions (4) and (5)).

3.7. Acetone-sensitized photolysis of BrU and IU in 2-propanol solution

In order to gain an insight into the mechanism of photolysis of 5-halouracils at reduced water content, measurements were carried out with BrU or IU in mixtures of acetone and 2-propanol. In the absence of water, $\Phi(-\mathrm{XU})$ is rather small (Table 4). The maximum $\Phi(-\mathrm{BrU})$ value of 0.04 is much smaller than that of 0.20, reported for 4.6–6.2 mM BrU and a larger amount of acetone [14]. When the water content is 10 vol.%, the pH has a large effect; $\Phi(-\mathrm{BrU})$ increases tenfold on going from pH 7 to pH 12. The corresponding $\Phi(-\mathrm{IU})$ values are much larger. Under the latter conditions (but not for BrU), a chain was established; Φ_{HU} of up to 30 was obtained when 5–6 M water at pH 12 was added. Increasing the XU concentration tenfold has no marked influence on the behaviour (Table 4).

It may be asked whether or not triplet BrU is involved in the photoreaction. Intersystem crossing to BrU^{3*} is established in glasses at − 196 °C, but its yield is probably low, as concluded from the result $\Phi_{\mathrm{p}} = 2\times10^{-3}$ (Section 3.2). Observation of BrU^{3*} at room temperature, however, has not been reported. From quenching measurements of triplet ketones by BrU in acetonitrile, it is concluded that BrU^{3*} has a lifetime of shorter than 50 ns (Section 3.1). On the other hand, from the quenching of the photoreduction in 2-propanol by *cis*-piperylene, $\tau_{\mathrm{T}} = 400$ ns has been estimated [15].

The key reaction in the acetone-sensitized photoreduction of BrU to uracil is suggested to be electron transfer (Eq. (6a)) from the 2-hydroxy-2-propyl radical to ground state BrU (Scheme 2). This, however, conflicts with another mechanism, proposed in Refs. [14–17]. According to this other ionic mechanism, the photoreduction is initiated by intersystem crossing and the electron should be transferred from 2-propanol to BrU^{3*}. In this case, the radical anion of BrU and the radical cation of 2-propanol should initially be formed

$$\mathrm{BrU}^{3*} + (\mathrm{CH_3})_2\mathrm{CHOH} \longrightarrow \mathrm{BrU}^{\cdot-} + (\mathrm{CH_3})_2\mathrm{CHOH}^{\cdot+} \quad (9)$$

The proposed triplet mechanism [14] then contains a proton transfer from $(\mathrm{CH_3})_2\mathrm{CHOH}^{\cdot+}$ to $\mathrm{BrU}^{\cdot-}$, followed by release of atomic Br and reaction of the $(\mathrm{CH_3})_2\mathrm{CHO}^{\cdot}$ radical with 2-propanol to yield the 2-hydroxy-2-propyl radical. This scheme was based on studies with BrU in acetone–2-propanol mixtures and, if applied to dilute aqueous BrU solution, requires criticism. The radical cation of 2-propanol, if formed at all, should immediately deprotonate. Moreover, release of Br^- (rather than atomic Br) occurs within a few nanoseconds [27]. It is obvious that reaction (4) also operates effectively in acetone–2-propanol mixtures, and the amount of 2-hydroxy-2-propyl radicals can only be reduced via reaction (5) by a high BrU concentration but cannot be completely suppressed. This, together with the lack of any direct evidence of the formation of BrU^{3*} at room temperature (Section 3.1) and the above results in 2-propanol solution in the presence of small amounts of water, does not support the ionic triplet mechanism.

Table 4
Quantum yield of decomposition of BrU and IU during acetone-sensitized irradiation in 2-propanol and mixtures with small amounts of water [a]

Acetone (M)	Water (%)	pH	$\Phi(-\mathrm{XU})$		
			BrU		IU
None	None	–	0.02		0.06
0.12	None	–	0.03	(0.04) [b]	0.07
None	10	7			0.7
0.12	10	7	0.05	(0.06)	0.08 (0.15)
0.12	10	9.5	0.1	(0.2)	0.25
0.12	10	11	0.2	(0.4)	0.5
None	10	12			0.3
0.12	10	12	0.3	(0.5)	8, ≈30 [c]
0.12	10	13	0.2		<4

[a] In argon-saturated solution; XU concentration, 0.3 mM; pH refers to absence of 2-propanol; $I_{254} = 2.2$ mW cm^{-2}.

[b] In parentheses: XU concentration, 3 mM.

[c] $I_{254} = 0.06$ W cm^{-2}.

3.8. Acetone-sensitized photolysis of MeBrU and BrUd

In order to determine whether the above-described properties of BrU are typical of 5-bromopyrimidines or whether other phenomena, such as the deprotonation of BrU at the N-1 position, are involved, MeBrU and BrUd were studied under the same conditions. Replacing BrU by MeBrU or BrUd reduces the quantum yield of debromination by about twofold (Table 5). For

Table 5
Quantum yield of decomposition of MeBrU and BrUd during acetone-sensitized irradiation in aqueous solution [a]

pH	Gas	Additive [b]	MeBrU	BrUd
2	Ar	2-Propanol	0.2	
7	Ar	2-Propanol	0.25	0.2
7	O_2	2-Propanol	0.001	
9.5	Ar	2-Propanol	0.7 (5) [c]	0.2 (1)
11	Ar	Methanol	0.2	0.1
11	Ar	2-Propanol	0.7	0.3
12	Ar	2-Propanol	3 (10)	0.9 (2)
13	Ar	Methanol	0.2	0.15
13	Ar	2-Propanol	4 (12)	1.8 (4)
14	Ar	2-Propanol	2.4	1.8

[a] Initial pyrimidine concentration, 0.3 mM; in argon-saturated solution in the presence of 0.12 M acetone; $I_{254} = 2.2$ mW cm^{-2}.

[b] About 1.4 M.

[c] In parentheses: $I_{254} = 0.06$ mW cm^{-2}.

the MeBrU–acetone–2-propanol (3×10^{-4} : 0.12 : 1.4 M) system, Φ shows the same trend of pH dependence as BrU, e.g. $\Phi = 0.3$ and 4 at pH 7 and 13 respectively. MeBrU has no second pK_a value due to the methyl group at the N-1 position. Generally, the same photochemical pattern was observed for MeBrU and BrUd as for BrU (Fig. 6). In particular, the chain reaction was also found for the two derivatives, as judged from the Φ values of up to 10–12 (Table 5) and their linear dependence on $I_{254}^{-1/2}$.

3.9. *Effect of oxygen*

Oxygen generally has a significant effect on the spectral changes and strongly reduces $\Phi(-\text{BrU})$. At pH 13, for example, $\Phi(-\text{BrU})$ in the presence of 0.12 M acetone is reduced by 300-fold compared with the oxygen-free solution (Table 1). No uracil is detected on direct irradiation of BrU in oxygen-saturated solution either in the presence or absence of 2-propanol; instead, complete chromophore loss in the 230–320 nm range occurs. For IU, it has been shown that the secondary intermediate is a peroxyl radical and the final photoproduct is essentially isodialuric acid [7,8]. The overall reactivity of the BrU system is strongly reduced when conditions are used that allow oxygen to quench triplet acetone in competition with reactions (6) and (7). Even under argon, the ketyl or uracil-5-yl radicals can be quenched by traces of oxygen. However, care was taken to suppress these reactions.

3.10. *Direct photolysis of BrU*

Control measurements in the absence of acetone showed the following. Irradiation at 254 nm of BrU (0.1–0.4 mM) in argon-saturated aqueous solution at pH 7 leads to a loss of the chromophore (approximately 230–300 nm) in low yield, $\Phi(-\text{BrU}) < 0.002$. Photolysis in the presence of 1–2 M 2-propanol leads to a blue shift in the absorption spectrum ($\lambda_{max} = 255$ nm) of the photoproduct(s), isosbestic points (one at approximately 260 nm) and an increase in $\Phi(-\text{BrU})$ to a value of 0.003 (Table 1). HPLC analysis shows that BrU is converted into uracil, Br^- is formed and protons are released (pH measurements). In the presence of methanol, the spectral properties and photoreaction are the same.

The main pathway for the deactivation of XU^{1*} is internal conversion, as is the case for virtually all other uracil derivatives [2,5]. Other photophysical decay pathways of XU^{1*}, such as fluorescence and intersystem crossing, are negligible. The primary photochemical step of BrU is the splitting of the C–X bond (Scheme 1). It is generally accepted that reaction (1) takes place homolytically and originates from the XU^{1*} state [10,15,16]. On reducing the water content, $\Phi(-\text{BrU})$ increases from 0.002 in aqueous solution at pH 7 to values of 0.0055, 0.018 and 0.026 in neat tert-butanol, methanol and 2-propanol respectively [10]. These effects have been interpreted by a cage effect in reaction (1) [10].

The fate of the radical $^{\bullet}$ROH depends on several parameters, e.g. the nature and concentrations of $^{\bullet}$ROH and the substrates (BrU, uracil, OH^- ions) and the respective reactivities. Irradiation of BrU plus 1.4 M 2-propanol at pH 12 leads to an isosbestic point at approximately 290 nm and to a more efficient conversion with $\Phi(-\text{BrU}) = 0.03$ (Table 1). It has already been shown that $\Phi(-\text{BrU})$ shows an increasing trend with increasing pH. The respective values at pH 10 and pH $\geqslant$ 13 were reported to be roughly 10 and 100 times larger than at pH 5–6 [10]; this trend is also reflected by the data in Table 1 and Fig. 6. α-Hydroxyalkyl radicals in their radical anion forms are stronger reducing agents than their neutral forms [28]. This should account, in part, for the larger Φ values on direct irradiation of IU [12], BU and their derivatives in the alkaline pH range. The corresponding effect on the acetone-sensitized reduction of the 5-halopyrimidines is discussed in Section 3.5.

4. Conclusions

The systems containing acetone–2-propanol–water–OH^- and BrU, MeBrU or BrUd exhibit rather high photoreactivities (in the absence of oxygen). The conversions to uracil, 1-methyluracil or uridine respectively are quantitative and the quantum yields exceed unity at intensities lower than about 1 mW cm^{-2}. The chain reaction is initiated by H-atom abstraction by triplet acetone from 2-propanol at the expense of energy transfer to the bromopyrimidine. No evidence for reaction of the triplet state of BrU was found. While the quantum yields for the direct photoreaction of BrU are much lower than those of IU, the results in the absence of acetone, involving the 2-hydroxy-2-propyl and uracil-5-yl radicals, show close similarities.

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