Photolysis of 5-Bromouracil and Some Related Compounds in Solution

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The steady state photolysis of 5-bromouracil (BU) in aqueous solution has been studied as a function of wavelength, pH, temperature, and hydrogen-donor concentration. Under all conditions studied, the primary reaction is shown to be C–Br bond cleavage followed by abstraction from the hydrogen-donor to give uracil and HBr. At pH > 12 further products are formed. In deoxygenated aqueous solution at pH 6, 20 °C, and 254 nm, the quantum yield of BU consumption, \( \Phi (-BU) \), is \( 1.8 \times 10^{-5} \) independent of hydrogen-donor type or concentration (e.g., \( 3 \times 10^{-2} \) to 2 M MeOH). With increasing pH, \( \Phi (-BU) \) increases stepwise to 0.012 at pH 10 and to 0.28 at pH 14. pK-values calculated from these data are the same as ground state pK values. \( \Phi (-BU) \) increases with temperature with an activation energy of approx. 3.4 kcal/mol. \( \Phi (-BU) \) increases with photon energy. Above 2 M MeOH \( \Phi (-BU) \) increases reaching \( \Phi (-BU) = 0.025 \) in neat MeOH. Similar high, solvent dependent, values are obtained for other hydrogen-donor solvents. In neat organic solvents an additional reaction with BU induced by solvent radicals was observed.

These results have been explained in terms of a homolytic dissociation of the C–Br bond of the excited BU followed by recombination or H atom abstraction by the radicals. At high hydrogen-donor concentration H atom abstraction can compete with cage recombination. A comparison has been made between BU photolysis in organic, hydrogen-donor solvents and BU photolysis within the DNA of bacteria or phages. It has been concluded that the much higher quantum yields observed for chain breaks in the photolysis of DNA containing BU compared to photolysis of BU in aqueous solution is due to the high local concentration of hydrogen-donors (sugar molecules) within the DNA molecule, even in dilute aqueous solution.

Introduction

5-Bromo-uracil (BU) can replace thymine in the DNA of phages, bacteria, and other cells without substantially altering their biological activity. The sensitivity of BU substituted DNA (BU-DNA) towards UV radiation is however markedly increased. It has been found that on UV irradiation of BU-DNA one single strand break is introduced per BU consumed while BU is changed predominantly to uracil (U). These results have been explained assuming that the primary process in the destruction of BU and BU-DNA is the homolytic splitting of the C–Br bond giving rise to an uracilyl radical and a bromine atom (reaction 1). The uracilyl radical and the bromine atom formed were expected to abstract hydrogen from a hydrogen-donor (RH) (reactions 2 and 3).

\[
\begin{align*}
\text{Bu} & \text{H}^+ \rightarrow \text{HBr} + \text{Br}^- \quad (1) \\
\text{Bu} & \text{H}^+ \rightarrow \text{HBr} + \text{Br}^- \quad (2) \\
\text{Bu} & \text{H}^+ \rightarrow \text{HBr} + \text{Br}^- \quad (3)
\end{align*}
\]

The uracilyl radical is particularly reactive. Its rate constant for the abstraction reaction from...
hydrogen-donors (H-donors) such as alcohols and 2-deoxy-D-ribose is only one to two orders of magnitude less than its rate constant of its reaction with oxygen.\(^2\). The reaction with oxygen is probably diffusion controlled\(^1\). In BU–DNA it was believed that the H-donor is the neighbouring 2-deoxy-D-ribose moiety. This sugar radical in turn is believed to lead to a strand break in the DNA chain.

The quantum yield of strand breaks, \(\Phi\) (s.s.b.), for the photolysis of BU–DNA in T3 phage\(^8\) at 265 nm in dilute aqueous solution is \(3 \times 10^{-11}\), and \(3.9 \times 10^{-2}\) for the disappearance of BU from E. coli BU–DNA photysed at 280 nm\(^2\). The photolysis of the simple analogue BU, in aqueous solution at 254 nm, however, gives a quantum yield for the loss of BU\(^4\), \(\Phi(-BU)\), of \(2 \times 10^{-3}\). This order of magnitude discrepancy induced us to investigate the UV photolysis of BU under a variety of conditions in the hope of understanding more clearly the reactions occurring within the BU–DNA.

### Experimental

**Chemicals:** 5-chlorouracil (CU) was obtained from Pfaltz and Bauer, BU from Fluka, 5-iodouracil (IU) from Schuchardt, and 1-methyl-5-bromouracil (MBU) from Cyclochem. All but MBU were recrystallized twice in triple distilled water before use. The UV absorption spectra measured on a Cary 17 spectrograph agreed with the published spectra\(^10\).

Thin layer chromatography (TLC) showed BU and IU free of other UV absorbing compounds and CU with less than 2% uracil. Water was triply distilled and the organic solvents, analytical grade from Pfaltz and Bauer, BU from Fluka, 5-iodouracil (IU) from Schuchardt, and 1-methyl-5-bromouracil (MBU) from Cyclochem. All but MBU were recrystallized twice in triple distilled water before use. The UV absorption spectra measured on a Cary 17 spectrograph agreed with the published spectra\(^10\).

**Apparatus and procedure**

Photolysis was carried out in a 1.0 cm path length quartz cell equipped with a degassing head which allowed the solution to be purged with Ar. For photolysis, the cell was held in a thermostatted block with a magnetic stirrer. The cell could be removed for optical density measurements in a Zeiss spectrophotometer and returned to the block for further exposure. The 254 nm and 214 nm radiation sources were a Hg low pressure arc (Gräntzel, Karlsruhe) and a zinc low pressure arc (Osram), respectively. A cutoff filter was included to eliminate shorter wavelength radiation. Longer wavelength radiation did not interfere (254 nm lamp) or were taken into account by measuring the residual photolysis by determining their photochemical effect after filtering off the 214 nm light (zinc lamp). An Osram high pressure xenon 450 watt XBO lamp with a SEM Brückel UGM 500 monochromator with 3 mm slits (5 nm half width) was used for the photolysis with 282 nm radiation. Actionometry was done in the same cell using potassium ferrioxalate for 282 nm and uranyl oxalate for 254 and 214 nm\(^11\).

Chloride and bromide ions were measured with Orion specific electrodes and H\(^+\) yield was determined with a Metrohm recording titrator.

BU and U were measured quantitatively by TLC and after silylation by gas chromatography. Cellulose TLC-plates from Merck were developed in ethyl acetate – formic acid – H\(_2\)O (60:5:35) and gave good separation of the UV absorbing compounds. Quantitative measurements were made with a Zeiss spectrophotometer with a TLC scanner and recorder. Uraeil was also identified by GC as having the same retention time as the authentic material when both were silylated and gas chromatographed on a 2 m glass column containing 2% SE 52 (temperature programmed from 70 °C to 270 °C at 6 °C/min).

For silylation, 140 ml solutions of BU (3 \(\times 10^{-4}\) to 1.2 \(\times 10^{-3}\) m) were saturated with argon and photolysed at 254 nm in a round quartz cell 20 mm thick. The solvent was evaporated to dryness, dissolved in 0.5 ml pyridine, and silylated by adding 0.3 ml either bis(trimethylsilyl)-trifluoroacetamide containing a few \(\mu\)l of trimethylchlorosilane (TMCS)\(^12\) or 0.2 ml hexamethyldisilazane and 0.1 ml TMCS\(^13\), and heating in a sealed tube at 70 °C for 4 hours. Quantitative measurements by GC agreed with those from TLC.

### Results and Discussion

#### a. BU photolysis in neutral aqueous solution

The photolysis (254 nm) of 1.25 \(\times 10^{-2}\) m BU in argon saturated neutral (starting pH = 6) aqueous solution containing 0.05 m isopropanol as a radical scavenger is shown in Fig. 1. Uraeil (U), H\(^+\), and Br\(^-\) were the major products observed. The BU consumption is linear with dose and is equal to the formation of H\(^+\), Br\(^-\), and (at BU conversion less than about 5%) uracil. At higher doses the \(\Phi\) (U) dropped off due to U photolysis, leading predominantly to the water adduct 6-hydroxy-5,6-dihydrouracil. This compound was identified by its back-reaction to U when being heated to 80 °C for 1 h\(^14\). Under these conditions \(\Phi(-BU)\) was \(1.6 \times 10^{-2}\).
Fig. 1. Photolysis of 5-bromouracil (1.25 \times 10^{-3} \text{ m}) in neutral, deoxygenated aqueous solution at 254 nm and 20 °C in the presence of 5 \times 10^{-2} \text{ M isopropanol}: \text{O}, loss of BU; \text{△}, H\textsuperscript{+} yield; \text{X}, Br\textsuperscript{-} yield; \text{●}, uracil yield.

This value of \( \Phi (\text{-BU}) \) is in agreement with other work under similar oxygen free conditions\textsuperscript{4} and in the presence of oxygen\textsuperscript{7}.

Since BU and U are the only UV absorbing species in this system, the same results could be obtained by measuring the optical density of the solution at intervals during the photolysis (Fig. 2).

Fig. 2. Photolysis of 5-bromouracil (3.2 \times 10^{-4} \text{ m}) in neutral, deoxygenated aqueous solution containing \textit{t}-butanol (1.5 \times 10^{-1} \text{ m}) at 254 nm and 10°: \text{O}, loss of BU; \text{●}, uracil; \text{△}, effect of heating 1 hour at 80°.

The amount of light absorbed by the BU was calculated, taking into account the fraction of incident light absorbed by U (inner filter effect) and the fraction not absorbed in the solution. The concentration of BU and U determined by this method were in agreement with those determined by TLC and GC. The effect of H-donor type and concentration is shown in Table I. Within experimental error \( \Phi (\text{-BU}) \) is independent of the nature of the H-atom donor when present in low concentration (<1 m).

Reduction of BU by the alcohol radicals as proposed for the 2-hydroxy-isopropyl radical anion\textsuperscript{15} in a chain reaction is not indicated in our system.

Table I. Photolysis of 5-bromouracil (3 \times 10^{-4} \text{ m}) at 254 nm and 20 °C in neutral deoxygenated aqueous solution. Quantum yields for the loss of BU in the presence of various radical scavengers.

<table>
<thead>
<tr>
<th>Scavenger</th>
<th>Concentration</th>
<th>( \Phi (\text{-BU}) \times 10^{2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0.03–2.0 m</td>
<td>1.8</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>0.05</td>
<td>1.9</td>
</tr>
<tr>
<td>tert-Butanol</td>
<td>0.15–0.5</td>
<td>1.6</td>
</tr>
</tbody>
</table>

b. The effect of pH

Since BU has two acidic protons, it can exist in three different ionic forms with different absorption spectra\textsuperscript{10} (Fig. 3). In Fig. 4, \( \Phi (\text{-BU}) \) is shown for the BU photolysis at 254 nm as a function of pH in argon saturated aqueous solution containing \textit{t}-butanol as the H-donor. U, H\textsuperscript{+}, and Br\textsuperscript{-} are the only primary products observed at pH <12. Photolysis at pH >12 leads to U and another UV-absorbing product (\( R_{f} = 0.29 \) compared to \( R_{f}(U) = 0.39 \). Since \( \Phi (\text{Br}^-) \) equalled \( \Phi (\text{-BU}) \), this unknown product cannot contain Br\textsuperscript{-}.

The solid line in Fig. 4 represents a computed total quantum yield \( \Phi (\text{-BU})_{\text{total}} \) where

\[
\Phi (\text{-BU})_{\text{total}} = \frac{\sum_{n=1}^{3} (f_{n} \epsilon_{n} \Phi_{n})}{\sum_{n=1}^{3} (f_{n} \epsilon_{n})}
\]

Fig. 3. Absorption spectra of the three forms of 5-bromouracil: ---, neutral form, pH = 4; -----, monoanion, pH = 10.1; ----, dianion, 1.0 m NaOH. BU conc. \sim 1 \times 10^{-4} \text{ m}.

Fig. 4. Absorption spectra of the three forms of 5-bromouracil: ---, neutral from, pH = 4; -----, monoanion, pH = 10.1; ----, dianion, 1.0 m NaOH. BU conc. \sim 1 \times 10^{-4} \text{ m}.
are known. From this the $f_n$ values have been calculated. The calculated values of $\Phi(-\text{BU})_{\text{total}}$ are obtained by using $\Phi_n$ values obtained at a pH range where only one species $n$ absorbs light. The contribution of the $f_n \cdot \Phi_n$ values to $\Phi(-\text{BU})_{\text{total}}$ are shown as the broken lines in Fig. 4. The $\Phi_n$ values for photolyses at 254 nm or 282 nm are given in Table II.

The good fit of the computed curve to the experimental data using the known ground state pK values is surprising. The use of ground state pK values suggests that either:

a) the excited level from which dissociation occurs has the same pK values (within about 0.4 units) as the ground state or

b) the rate determining step of the dissociation reaction proceeds before ionic equilibrium is established.

The first alternative seems unlikely since the excited state pK values which are known for other pyrimidines differ strongly from those in the ground state. The pK value of the excited singlet of U (derived from the photohydration reaction of U) is approx. 5 units less than that of the ground state. The pK value of the thymine triplet state (derived from the dimer yield) is about 11 compared to 9.8 in the ground state and that of orotic acid is shifted by 2.8 units toward higher values.

From this follows that case b) applies. Since the multiplicity of the reacting state is not known further conclusions cannot be drawn.

A possible explanation for the much higher BU anion photoreactivity could be enhanced internal charge transfer in the excited state, similar to the influence of substituents on the naphthalene sensitized decomposition of carbon tetrachloride.

c. Photolysis of 1-methyl-5-bromouracil (MBU)

It is known from substitution effects on the absorption spectra that the BU monoanion exists in two forms in the ratio of about 1:1.8 for 1:2.

Since 1 and 2 have different absorption spectra they may have different reactivities as well. In this
context the photolysis (254 nm) of MBU in aqueous, 
argon saturated solution containing 0.16 M tert-
butanol as a radical scavenger was studied as a 
function of pH (Fig. 5). At low conversion (<5% 
MBU) and pH < 8 the main product is 1-methyl-
uracil (MU) in more than 80% yield.

![Fig. 5. Photolysis of 1-methyl-5-bromouracil 
(2.2 \times 10^{-4} \text{ M}) in deoxygenated aqueous solution con-
taining tert-butanol (1.6 \times 10^{-1} \text{ M}) at 254 nm and 20 °C. 
Quantum yield for the loss of MBU \( \Phi (-\text{MBU}) \): , experimental; —— calculated \( \Phi (-\text{MBU}) \)total; ---, 
neutral form; ----, anion.]

The \( \Phi (-\text{MBU}) \)total values show a single step as a 
function of pH (Fig. 5). By treating the data similarly 
to those in the BU photolysis, with pK = 8.3, 
the solid line in Fig. 5 was generated. Again, as 
in BU, the pK calculated from \( \Phi (-\text{MBU}) \)total 
experiments agrees with the ground state value.19 
The quantum yield for the neutral form of MBU was 
\(2.6 \times 10^{-3}\) and \(1.8 \times 10^{-2}\) for the basic form.

From the calculation made by Wimper and Fox20 
on the BU absorption spectra, the two BU species 1 
and 2 absorb about equally strongly at 254 nm. 
The anion of MBU has the same structure as form 1 
of BU. \( \Phi (-\text{MBU}) \) is 1.5 times larger than \( \Phi (-\text{BU}) \) 
in the neutral as well as in the monoanionic form. 
It appears, therefore, that both BU monoanionic 
forms, 1 and 2, show the same photoreactivity, if 
the enhancement of photoreactivity caused by the 
methylation on N-1 in the neutral form persists in 
the anion.

d. The effects of wavelength and halide substitution

The effect of wavelength on the photolysis of 
5-chlorouracil (CU), BU, and 5-iodouracil (IU) in 
aqueous deoxygenated solution has been studied.

The products in all cases are U, H+Cl-, and Cl-, Br- or 
I- in yields equal to the loss of CU, BU, or IU, respectively. At 282 nm and 214 nm the U yield 
remains linear with dose to at least 20% conversion 
of the parent compound since its absorption 
coefficient at these wavelengths is much less than 
that of the parent compounds.

Table III compares the photolysis of BU with 
CU and IU at 254 nm and 214 nm. At a given 
wavelength the quantum yield increases markedly 
from CU to IU reflecting the strength of the halide-
carbon bond.

<table>
<thead>
<tr>
<th></th>
<th>( \lambda = 254 \text{ nm} )</th>
<th>( \lambda = 214 \text{ nm} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Chlorouracil</td>
<td>0.7</td>
<td>30</td>
</tr>
<tr>
<td>5-Bromouracil</td>
<td>1.9</td>
<td>40</td>
</tr>
<tr>
<td>5-Iodouracil</td>
<td>38</td>
<td>72</td>
</tr>
</tbody>
</table>

The effect of wavelength on \( \Phi (-\text{BU}) \) for the 
different ionic forms of BU have also been studied. 
Similar to photolysis at 254 nm, \( \Phi (-\text{BU}) \) at 282 nm 
was found to increase stepwise with increasing pH. 
Ground state pK values again gave the best fit of 
the data (Table II). Within experimental error, \( \Phi (-\text{BU}) \) for the dianion appears to be independent 
of wavelength.

Decomposition of the neutral and monoanionic 
forms depend on wavelength even within the first 
absorption band (\( \lambda = 282 \) and 254 nm, Table II). 
Assuming predissociation the strong wavelength 
dependence of \( \Phi (-\text{BU}) \) indicates that the rate 
determining step must be fast and can compete 
with relaxation processes of the excited state. 
The same effect was observed with the other halouracils 
(Table III). Pronounced wavelength dependences 
are also reported for other pyrimidines and were 
attributed to a wavelength dependent crossover to 
the triplet manifold18.

e. The effect of temperature

\( \Phi (-\text{BU}) \) was found to increase with increasing 
temperature using deoxygenated aqueous solutions 
of BU (Fig. 6). Since a chain reaction involving 
tert-butanol is not indicated, temperature must 
effect the BU dissociation in a direct way.
In view of the fast reaction required above and the scavenging experiments described below and elsewhere we suggest that dissociation of **BU** occurs within a solvent cage. Most of the initially formed radical pairs recombine and only a fraction of those formed diffuse apart (reaction 4-6 where the bar represents radicals within the cage).

\[
\begin{align*}
BU + \text{hv} & \rightarrow Br^- + U^+ \quad (4) \\
Br^- + U^+ & \rightarrow BU \quad (5) \\
& \rightarrow Br^+ + U^- \quad (6)
\end{align*}
\]

The observable reaction would be reaction 6 since these radicals would live long enough to be scavenged by the H-donor present in the aqueous solution. Increasing the temperature decreases the solvent viscosity and results in easier separation of the radical pair (reaction 6). In terms of a cage reaction, the activation energy difference of 3.4 kcal/mol would represent the difference between viscous flow and the recombination process. This can be compared with a value of 3.8 kcal/mol calculated from the viscosity of water between 0 °C and 90 °C. However, it is also possible that the activation energy is associated with electronic conversion processes in the molecule.

**f. The effect of H-donor and oxygen concentration**

The \( \Phi (-BU) \) values in deoxygenated aqueous solution are independent of the nature of the H-donor when present in low concentration (Table I, Fig. 7). The effect of increasing the methanol concentration is shown in Figure 7. At about 4 \( \text{m} \) methanol \( \Phi (-BU) \) begins to increase rapidly and reaches a value of \( 2.5 \times 10^{-2} \) in 100% methanol. Similarly, high values were found for several other H-donors (Table IV). The effect of wavelength and temperature on the methanol solution photolysis are shown in Table V. The rapid increase of \( \Phi (-BU) \) at high H-donor concentration (e.g. [methanol] > 4 \( \text{m} \)) has been discussed in terms of cage scavenging (cf. also section e). Similar results obtained in the photolysis of cystein were also attributed to cage scavenging reactions.

![Fig. 7. Quantum yield of 5-bromouracil (2 \( \times 10^{-4} \text{m} \)) disappearance at 254 nm and 20 °C in aqueous solution as a function of the concentration of radical scavengers: \( \times \), methanol; \( \triangle \), isopropanol; \( \circ \), tert-butanol.](image)

**Table IV. Photolysis of 5-bromouracil (2 \( \times 10^{-4} \text{m} \)) in deoxygenated solution at 254 nm. Quantum yields of **BU** loss and initial **U** formation.**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>( \Phi (-BU) ) ( \times 10^{2} )</th>
<th>( \Phi (U) ) ( \times 10^{3} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>H(_2)O*</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>tert-Butanol</td>
<td>5.5</td>
<td>5.4</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Methanol</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>45</td>
<td>28</td>
</tr>
<tr>
<td>BU-DNA in H(_2)O</td>
<td>30**</td>
<td>21***</td>
</tr>
</tbody>
</table>

* Containing H-donors (10\(^{-1}\) m).
** Quantum yield for strand breaks induced through energy absorbed by BU\(^{8}\).
*** Uracil constitutes 80% of products\(^{2,3}\).

In all cases \( \Phi (-BU) \) was linear with dose when corrected for product absorption. Also \( \Phi (Br^-) \) equalled \( \Phi (-BU) \). Further, U was formed in at least 60% yield suggesting the same reaction pre-
Table V. Photolysis of 5-bromouracil (2 \times 10^{-4} \text{ M}) in deoxygenated solution at 282 and 254 nm. Quantum yields for the loss of BU and initial formation of U.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$T$ [°C]</th>
<th>$\lambda = 282$ nm</th>
<th>$\Phi (-\text{BU})$</th>
<th>$\Phi (\text{U})$</th>
<th>$\lambda = 254$ nm</th>
<th>$\Phi (-\text{BU})$</th>
<th>$\Phi (\text{U})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{H}_2\text{O}^*$</td>
<td>20</td>
<td>2.9 \times 10^{-4}</td>
<td>2.2 \times 10^{-4}</td>
<td>1.6 \times 10^{-3}</td>
<td>1.6 \times 10^{-3}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{H}_2\text{O}^*$</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MeOH</td>
<td>20</td>
<td>5.9 \times 10^{-3}</td>
<td>4.0 \times 10^{-3}</td>
<td>2.5 \times 10^{-2}</td>
<td>1.5 \times 10^{-2}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MeOH</td>
<td>55</td>
<td></td>
<td></td>
<td>3.2 \times 10^{-2}</td>
<td>2.0 \times 10^{-2}</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Containing 0.16 \text{ M tert-butanol.}

dominates. Above about 5\% conversion of BU, the yield of U was strongly reduced due to its photolysis. The difference between $\Phi (-\text{BU})$ and the initial $\Phi (\text{U})$ varies between 5\% and 40\% depending on solvent (Table IV). In neat methanol, three additional peaks appear in the TLC ($R_f = 0.09$, 0.19, and 0.42, $R_f (\text{U}) = 0.35$). Gas chromatography of the silylated photolysis mixture also gave three peaks at longer retention times than BU.

The difference between $\Phi (-\text{BU})$ and the initial $\Phi (\text{U})$ can be explained assuming an attack of solvent radicals on BU. Evidence for this is the effect of oxygen on the quantum yields. Saturating with $\text{O}_2$ ($\sim 10^{-2} \text{ M}$) decreases $\Phi (-\text{BU})$ in MeOH from 2.5 \times 10^{-2} to 1.6 \times 10^{-2} and reduces the minor peaks of the products observed in the TLC and GC, but does not affect the initial $\Phi (\text{U})$. $\Phi (\text{U})$ also remains constant to 30\% conversion of BU, whereas in deoxygenated solution $\Phi (\text{U})$ decreases with dose because of U photolysis. From these observations it is apparent that oxygen has two effects. First, oxygen in high concentration suppresses the U photolysis by quenching of the U triplet state\(^16\). Second, oxygen can scavenge the hydroxymethyl radical formed in reaction 2 with the result that BU is no longer attacked by the solvent radicals.

Since U formation is not reduced by oxygen in MeOH even at an $\text{O}_2$ concentration of about $10^{-2} \text{ M}$ the primary reaction of BU is not quenched by oxygen. Similar results have been obtained in aqueous solutions, e.g. $\Phi (-\text{BU})$ is the same in aqueous oxygenated\(^7\) and deoxygenated\(^4\) solution (cf. section a). From the $\text{O}_2$ solubility in methanol and water, and assuming a diffusion controlled quenching rate constant\(^17\) of about $4 \times 10^8 \text{ M}^{-1} \text{s}^{-1}$, the excited state lifetime of BU must be less than $2 \times 10^{-7} \text{ s}$.

In MeOH only the minor products are suppressed and $\Phi (-\text{BU})$ is reduced nearly to $\Phi (\text{U})$ by the addition of a small amount of oxygen, the probable fate of the hydroxymethyl radicals in the Ar saturated solution is a slow addition to the 5,6-double bond of BU. This would ultimately lead to loss of BU and formation of the minor products. In accordance with the observed UV-absorption and the quantitative production of Br\(^-\), HBr must have been eliminated and the 5,6-double bond regenerated. Further identification of these minor products (up to 30\% in well deoxygenated methanol solution) is in progress. It is significant that the initial U yield is unaffected by oxygen. This suggests that reaction 4 does not occur under these conditions. The scavenging of the U\(^-\) radical by $\text{O}_2$ is not expected in this range of alcohol concentrations\(^5,6\) and no products attributable to this were found.

**Conclusion concerning the photolysis of BU-DNA**

The present results on the photolysis of BU have some implications to the BU-DNA photolysis. Since $\Phi (-\text{BU})$ is very sensitive to pH changes near pH 7, the pH should be carefully controlled before a comparison of various systems can be made. More important, however, is the fact that within the BU-DNA, even in dilute aqueous solutions, there is a high local concentration of H-donor present in the form of a deoxy-D-ribose moiety which is in Van der Waals contact with the Br atom of the BU\(^4\). This sugar group is next along the DNA backbone and in the same strand as the BU base. Consequently, the photolysis of BU-DNA in aqueous solution, or within its protein in the cell, should be compared to BU photolysis in H-donor solvents since this is a much better model system.

It can be seen from Table IV that, in this comparison, the quantum yields observed for BU-DNA are not unusual. DNA (i.e. the sugar) is simply a good H-atom donor. Further, since $\Phi (-\text{BU})$ and $\Phi (\text{U})$ are fairly high in BU-DNA, the deoxy-D-
ribose moiety must be directly involved in scavenging the primary uracilyl radical. Since the quantum yield for BU disappearance in DNA equals the quantum yield of strand breaks, it is reasonable to expect that the sugar radicals formed in this scavenging reaction leads ultimately to a strand break in the DNA at that sugar linkage.

There must be the formation of two sugar radicals per C–Br bond broken. One is formed by the uracilyl radical, the other by the Br atom. The uracilyl radical most probably abstracts at the deoxy position CH2 of the sugar moiety out of steric reasons. At which position the Br atom reacts is not known at present.

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