PHOTOCHEMISTRY OF IODOURACIL. I

PHOTOPRODUCTS OBTAINED IN WATER*

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Although the replacement of DNA-thymine by halogenated pyrimidines generally leads to an increased sensitivity to the lethal effects of UV irradiation, there are occasions where the sensitivity to UV is unchanged. Howard-Flanders et al. (1962) have found that in the absence of enzymatic repair, T1 phage substituted with 5-bromo-2'-deoxyuridine (BUdR) showed the same survival as did unsubstituted phage. Hotz (1963) has shown that T1 phage substituted with BUdR were protected by the presence of cysteamine during irradiation and under these conditions showed the same sensitivity to UV as did control phage. These results have been confirmed in T1 phage substituted with 5-iodo-2'-deoxyuridine (IUdR) and have been extended to show in addition that combination of irradiation in the presence of cysteamine and lack of enzymatic repair resulted in the IUdR-T1 phage being killed less rapidly than were the unsubstituted control phage (Rupp and Prusoff, 1964). The photochemical reactions of iodouracil (IU) were investigated in an attempt to understand the chemical basis for these biological observations. It has been

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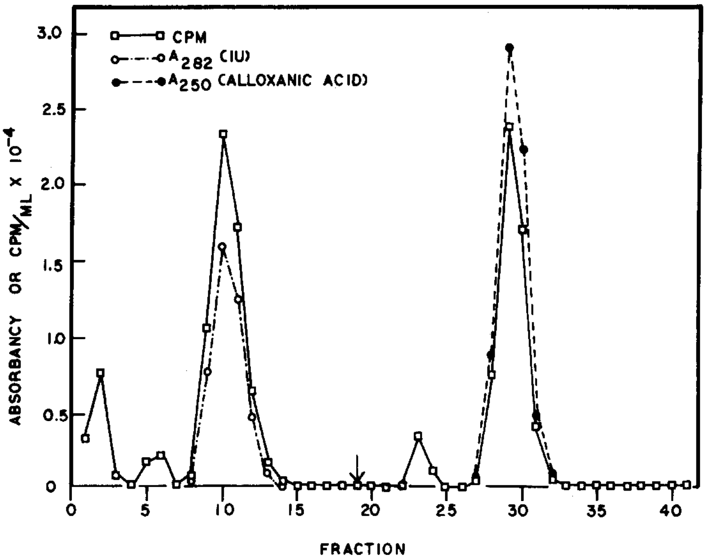

Fig. 1. Anion exchange analysis of UV-irradiated IU-2-C^{14}. The IU-2-C^{14} (2.85 ml, 0.14 μmole, 3.5 mc/mmole, Calbiochem), purified by prior anion exchange chromatography, was irradiated for 20 minutes with stirring at a distance of 4.5 cm from a GE 15W germicidal lamp partially shielded to give an opening of 3 cm. The absorbancy at 282 mμ was reduced to 50% by the irradiation. Following irradiation 10 μmoles of IU and 200 μmoles of alloxanic acid were added, pH was adjusted to 11-12 by addition of NaOH, and the solution passed through a Dowex formate column (Ag 1 x 8, 200-400 mesh, 1 x 10 cm). Formic acid (0.01 N) was passed through the column until IU (absorbancy measured at 282 mμ) had been eluted. A gradient of ammonium formate (1 M, pH 5.6) into a reservoir of water (200 ml) was begun at fraction 19 (see Canellakis and Cohen, 1955); and alloxanic acid was measured by absorbancy at 250 mμ. Radioactivity was measured in a liquid scintillation counter with a recovery of 106%. The volume of each fraction was 12 ml.

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observed that aqueous solutions of IU are very sensitive to UV radiation under conditions where bromouracil is unaltered (Wacker, 1963).

UV irradiation of unbuffered aqueous solutions of IU resulted in the decrease of UV absorption above 250 mμ. The possibility of a water addition product of either IU or uracil was ruled out since heating in dilute acid (10 min, 100°C, 0.1 N HCl) resulted in no spectral changes and prolonged heating in 1 N HCl (1 hr, 100°C) resulted in spectral changes attributed solely to degradation of IU.

Characterization of the IU photoproducts was carried out with IU-2-C^{14} since in parallel experiments with IU-2-C^{14} and IU-I^{125}, no photoproducts detected contained both isotopes. When an irradiated solution of IU was adjusted to pH 11-12 and chromatographed on an anion exchange column, the major product had a much higher affinity for the resin than IU. This technique was used to obtain the major radioactive product from 1 g of UV-irradiated IU. This compound is believed to be alloxanic acid for the following reasons:

1. The product eluted from an anion exchange column with alloxanic acid (see also Fig. 1). Alloxanic acid was prepared by treating alloxan with alkali (Seligson and Seligson, 1951) and subsequent conversion to the acid form with Bio-Rad AG50X8 (H^+ form).

2. Titrimetric determination of pK's of 1.9 and 8.4 were in agreement with those determined for alloxanic acid.

3. Thin layer chromatography indicated identical R_f values in several systems.

4. Both compounds gave identical color formation with dinitrophenylhydrazine following basic hydrolysis by the method of Seligson and Seligson (1951). Ketomalonic acid was qualitatively identified by thin layer chromatography of the dinitrophenylhydrazone.

5. End absorption of UV above 220 mμ was similar to that of alloxanic acid.

An alternate procedure for characterization of the photoproducts involved the application of the irradiated IU-2-C^{14} directly onto Whatman #1 paper (Fig. 2). The major photoproduct (R_f 0.10-0.20) does not correspond with alloxanic acid. This area (R_f 0.10-0.20) was eluted and subjected to anion exchange chromatography (conditions as in Fig. 1). About 67% of the radioactivity was eluted with carrier alloxanic acid. When the eluate from the paper chromatogram (R_f 0.10-0.20) was exposed to 0.01N NaOH for 30 min and rechromatographed in 86% aqueous n-butanol, the major peak appeared at

R_f 0.00, the R_f of alloxanic acid. These observations indicated that the initial product observed under these experimental conditions was converted to alloxanic acid in base. The possibility that the major photoproduct was alloxan, whose lability to alkali is similar to that of the photoproduct, was ruled out by chromatography in a butanol-acetic acid system (n-butanol/acetic acid/water: 80/12/30, (Smith, 1963)) where alloxan had an R_f of 0.43 and the major photoproduct had an R_f of 0.26.

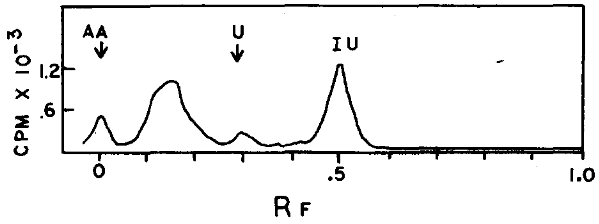

Fig. 2. Radiochromatogram of IU-2-C^{14} irradiated in water. Iodouracil (0.1 ml, 0.5 x 10^{-4} M, 3.5 mc/mmole) was irradiated for twenty min at a distance of 5 cm from a GE 15W germicidal lamp. The entire reaction mixture was applied to a 5 x 4 cm area on a 4 cm wide Whatman #1 paper strip and concentrated into a narrow band with H_2O as described by Wulff (1963). The chromatograms were developed by the descending technique with 86% aqueous butanol and scanned with a Packard radiochromatogram scanner. The position of IU, uracil (U), and alloxanic acid (AA) are indicated on the graph. Alloxan streaks badly with this solvent system and is found at R_f 0.10-0.30. Alloxan and alloxanic acid were detected by spraying with 0.050 M $AgNO_3$ in 2 M NH_4OH as described by Seligson and Seligson (1951). Evidence that the major unknown peak is not alloxan has been presented in the text.

The method used in the preparation of the IU for these experiments was an important factor in determining the nature of the photoproducts. IU-2-C^{14} used directly or purified by anion exchange chromatography gave the results above. However, if the IU-2-C^{14} was chromatographed on a Whatman #1 paper strip developed in 86% aqueous butanol, eluted, and then irradiated, uracil was the major product (identified by anion exchange and paper chromatography). The experiment described in Fig. 3 indicates that dilution with water resulted in a decrease in the proportion of uracil formed and that addition of a blank paper eluate corresponding to the iodouracil area produced an increase in the proportion of uracil formed. This suggests that an impurity eluted from the paper is responsible for the observed differences. This may account for the apparent discrepancy of our results with those of Wacker where uracil has been reported to be the major photoproduct of IU (Wacker, 1963).

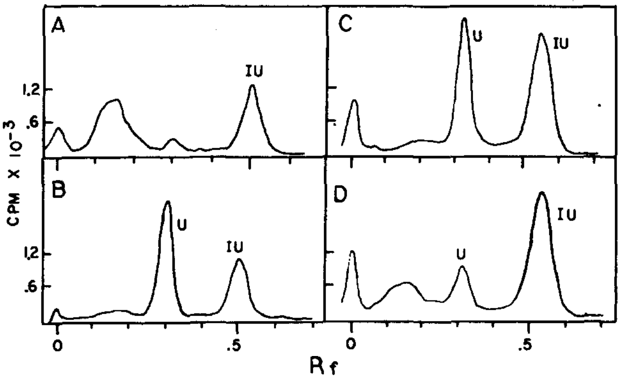

Fig. 3 Effect of substances present in paper chromatograms on the nature of the IU photoproducts. The IU used in each of these experiments was purified by anion exchange chromatography. For experiments A and B, IU was used without additional treatment. For experiments C and D the IU in addition was chromatographed on Whatman #1 paper (solvent: 86% aqueous butanol) and eluted. The "paper extract" used in (B) was prepared by developing a 4 inch wide blank strip of Whatman #1 paper in 86% aqueous butanol and eluting that area corresponding to the R_f of IU with 4 ml of H_2O. The conditions of irradiation and chromatography are described in Figure 2.

A. 0.1 ml of IU (0.5 - 1.0 x 10^{-4} M)
B. Same as A plus 1.0 ml of "paper extract"
C. 0.1 ml of IU (0.5 - 1.0 x 10^{-4} M) eluted from paper
D. Same as C plus 1.0 ml distilled H_2O.

Since the conversion of IU to alloxanic acid results in the net addition of 3 oxygen atoms, the uptake of O_2 during irradiation was measured manometrically in a Vycor Warburg vessel (Corning Glass #7910, % transmission: 254 mμ, >80%, 185 mμ, <1%). During irradiation of 2 x 10^{-3} M IU, 0.55-0.85 mole of O_2 was consumed for each mole of IU degraded. Control experiments ruled out the possibilities of ozone formation or oxidation of iodide, processes that would have resulted in an apparent O_2 uptake. If iodide had been formed as the initial product and then oxidized completely to iodine, only 0.25 moles of O_2 would have been consumed for each mole of IU destroyed. Further evidence in support of a role for O_2 in this process is presented in the following paper.

The oxidation state of the photochemically released iodine is of interest in considering the possible mechanisms of this reaction. I_2 was determined by conversion to the I_3^- ion (Hochanadel, 1952) and subsequent measurement of the absorbancy at 353 mμ, the molar extinction coefficient of I_3^- being 26,400 (Awtrey and Connick, 1951). The I_2 found in the studies of the uptake of oxygen described above was 73-99% of that expected based on the quantity of IU destroyed. Although this method was originally presented for the determination of peroxides, the material measured here is presumably I_2 since prior extraction of the irradiated solution with an equal volume of chloroform removes 90% of the reacting material. The extraction with chloroform removes a slight brown color present in the aqueous layer with a simultaneous development of a pink-violet color in the chloroform layer, an observation consistent with the presence of I_2. Experiments with irradiated IU-2-C^{14} indicate that less than 1% of the radioactivity is extracted into the chloroform layer. If I_2 is estimated following irradiation of IU in an open vessel, the yield is much lower than that presented here. This is presumably a result of the volatility of I_2 under these conditions, since in control experiments a rapid loss of I_2 was observed when an aqueous solution of iodine was stirred in an open vessel.

In the subsequent paper, these observations together with the effects of sulfur compounds, ethanol and oxygen on the nature of the IU photoproducts are considered in terms of a possible mechanism of the photolytic reaction.

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