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Photochemistry of 5-Bromouracil in Aqueous Solution*

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ABSTRACT: 5,5′-Diuracil, uracil, glyoxaldiurene, barbituric acid, oxalic acid, isoorotic acid, parabanic acid, urea, ammonia, and glyoxal formed by the ultraviolet irradiation (mainly 254 mμ) of 5-bromouracil in aqueous solution were quantitatively isolated and identified. The photochemical process is therefore a free radical reaction and both 5,5′-diuracil and uracil are formed through the uracil radicals as in the case of photolysis of 5-bromo-1,3-dimethyluracil, albeit their secondary products are different. 5,5′-Diuracil type of coupled products may be of importance in radiation and photobiology.

In a previous paper (Ishihara and Wang, 1966a), we have reported the isolation and identification of 5,5′-diuracils as photoproducts from the irradiation of 5-bromouracil derivatives. This type of coupled product results from the formation of a single bond between two uracil radicals. The possible importance of the formation of coupled products between purines and pyrimidines in deoxyribonucleic acid (DNA) molecules was discussed in relation to radiation and photobiology.

The preceding paper (Ishihara and Wang, 1966b) deals with studies on the photochemistry of 5-bromo-1,3-dimethyluracil (BDMU)[1] in aqueous medium, including isolation, identification, and quantitation of nine compounds from this irradiation reaction. Also reported was evidence for the possible absence of any aldehydes or 5-hydroxy-1,3-dimethyluracil as photoproducts. Actually, BDMU has been used as a model compound in order to facilitate our further work with biologically important compounds such as 5-bromouracil (BU), its nucleosides, and its nucleotides.

This paper describes our studies of the photochemistry of BU in aqueous solution, including the isolation and identification of 11 compounds in the irradiation mixture. In addition, there is evidence for the possible absence of four conceivable photoproducts which are of importance in considering the photochemical mechanisms. In general, both BDMU and BU form free radi-

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[1] Abbreviations used in this work: BDMU, 5-bromo-1,3-dimethyluracil; BU, 5-bromouracil; U-U, 5,5′-diuracil; DMU-DMU, 5,5′-di-1,3-dimethyluracil.

cals upon irradiation, but there are significant differences in their secondary processes. This difference must be attributed to the difference in the electronic effects of the methyl and hydrogen groups upon the uracil moiety. Although this has no direct bearing on radiation and photobiology, it is of some interest in the study of chemical mechanisms.

Experimental Procedures

Melting points were determined on a Fisher-Johns block and are uncorrected. Infrared spectra were measured on a Perkin-Elmer Model 21 recording spectrophotometer. Ultraviolet spectra were measured on a Beckman Model DK-1 recording spectrophotometer. Microanalyses were performed by Mr. J. Walter at The Johns Hopkins University. Paper chromatography was carried out on Whatman No. 1 filter paper unless otherwise stated and the ratios given for the eluents are by volume. Evaporation of the solvents was carried out by a rotary evaporator at <40° unless otherwise specified.

Irradiation apparatus has been described previously (Wang, 1958a). General Electric germicidal tubes (light source) (G15T8), which emit mainly 253.7-mμ wavelength light, were used. Chromatographically pure samples (BU) were prepared according to the method of Wang (1959); λ_{max}^{HOH} 276 mμ (ϵ 7.30 × 10^3), λ_{min}^{HOH} 241 mμ (ϵ 1.76 × 10^3).

Irradiation of BU in Aqueous Solution

A solution of BU (1 mM) was irradiated in quartz tubes with germicidal lamps for 1.5 hr at room temperature. During irradiation, the pH of the solution changed from 6.0 to *ca.* 3.0 and the absorbancy at 276 mμ decreased by *ca.* 50% of the original. Either the irradiated solutions or the dried residues obtained by evaporating the irradiated solution were used for the following experiments.

Separation and Identification of the Principal Photoproducts

This was carried out essentially according to the procedure of Cohn (1949). The dried residue (1.220 g) from 10 l. of irradiated solution was dissolved in 30 ml of ammonium chloride–ammonium hydroxide buffer (pH 10.6, 0.025 M NH_4Cl) with the aid of a minimum volume of concentrated NH_4OH and was chromatographed on a column of Dowex 1-X8 (Cl^-, 100–200 mesh, 2.2 × 27 cm). The column was eluted successively with the different solvents shown in Table I. At a flow rate of *ca.* 1 ml/min, fractions of 25 ml were collected. On the basis of ultraviolet absorbancy between 210 and 360 mμ, nine large fractions were obtained by combining 25-ml fractions.

The general procedure of desalting was as follows. The combined solution was evaporated to dryness and the residue was dissolved in a small amount of water. The solution was then passed through a column of Dowex 50-X8 (H^+, 100–200 mesh). The amount of the resin used was *ca.* 5 mole equiv (calculated from the exchange capacity of *ca.* 2.0 mequiv/ml of wet resin) of the NH_4^+ present. The column was washed with water until the washings were free of any product. The residue from the eluate and washings was treated as specified.

TABLE I: Separation of Compounds from Irradiated BU (10^{-2} M) on Dowex 1 (Cl^-) Column.

Fraction	Eluent[a]	Test Tube No.	Indentfn	Amt (mg)
1	A	3–16	Glyoxaldiurene	48
2	A	17–76	Unidentified	100
3	A	77–150	Uracil	78
4	B	151–189	Unidentified	27
5	B	190–290	U-U	4.5
			Oxalic acid	23[b]
			Others	100
6	B	291–320	U-U	14.5
			Others	10
7	B	321–380	U-U	10
			BU	425
8	B	381–480	BU	
8	C	481–540	BU	
9	D	541–740	Unidentified	134

[a] Solutions used for elution: A, NH_4Cl–NH_4OH (0.025 M chloride, pH 10.6); B, NH_4Cl–NH_4OH (0.1 M, pH 10.0); C, NH_4Cl–NH_4OH (0.5 M, pH 7.0); D, HCl (0.001, 0.003, 0.01, and 0.1 N). [b] Ca salt.

Fraction 1. The residue obtained by the general procedure was dissolved in 30 ml of water and was allowed to pass through a column of Dowex 1-X8 (OH^-, 100–200 mesh, 0.6 × 8 cm) to remove the color. The column was washed with 50 ml of water. Eluate and washings were concentrated to 1.5 ml, and the solution was kept in a refrigerator overnight. The product, which crystallized as colorless needles, was collected by filtration, washed with water, ethanol, and ether, and then air dried. It weighed 39 mg (mp >300°). This compound was identified as glyoxaldiurene by comparing its ultraviolet and infrared spectra with those of the authentic sample prepared by two methods: condensation of urea and glyoxal in the presence of HCl (Beilstein, 1937), and reduction of allantoin with sodium amalgam (Beistein, 1937).

Anal. Calcd for $C_4H_6N_4O_2$: C, 33.80; H, 4.26; N, 39.42. Found: C, 33.79; H, 4.55; N, 39.56.

Fraction 3. In an alkaline solution (pH 10.6), this fraction showed a characteristic ultraviolet absorption spectrum of uracil, λ_{max} 282 mμ (further details, see Discussion). After desalting, the residue was dissolved in 30 ml of hot water and was decolorized with Norit. Upon evaporation, the filtrate gave 78 mg of the residue which on recrystallization from water gave 67 mg of colorless needles. Both ultraviolet and infrared spectra were identical with those of uracil (mp >300).

Fraction 5. This fraction was concentrated to 50 ml and kept in ice water for 2 hr. Colorless needles appeared (4.5 mg), which were collected by filtration. [The product was retained for identification, see below, U-U.] The filtrate was passed through the cation exchanger according to the general procedure. Eluate and washings were concentrated to 10 ml and the solution (pH <1) was extracted continuously with ether for 3 days. Ether and aqueous layers were treated separately as follows.

A. ETHER LAYER. To the ether extract, 2 ml of 2% acetic acid solution was added, and ether was evaporated off on a steam bath. The remainder was diluted with 4 ml of 95% ethanol, and the solution was mixed with 2 ml of 10% $CaCl_2$ solution. After stirring for 2 hr, the solution was allowed to stand at 5° for 3 days. The needlelike crystals were collected and washed with 3% $CaCl_2$ solution, water, ethanol, and finally ether. After drying, the crystalline product weighed 23 mg. The infrared spectrum of this compound was identical with that of an authentic sample of calcium oxalate (Lewis and Weinhouse, 1957).

B. AQUEOUS LAYER. After being concentrated to a small volume, the sample was chromatographed with *t*-butyl alcohol–methyl ethyl ketone–formic acid–water (40:30:15:15) by ascending technique to reveal five ultraviolet-absorbing bands (R_F 0.20, 0.42, 0.58, 0.70, and 0.84) and a fluorescent one (near the origin) under ultraviolet light (253.7 mμ). From its R_F value, the major product may be tentatively identified as isoorotic acid (R_F 0.42; Alcántara and Wang, 1965b). This band was eluted with hot water and its ultraviolet spectrum proved to be identical with that of isoorotic acid, but the amount was insufficient for isolation.

Fraction 6. Colorless needles (14.5 mg), formed after concentration of this fraction to 25 ml followed by cooling in ice–water for 2 hr, were collected by suction filtration. The filtrate was desalted and then evaporated to dryness to yield 10 mg of unidentified product(s).

Fraction 7. After concentration of the fraction to 30 ml, colorless needles appeared (10 mg), which were collected by filtration. The filtrate was desalted and retained for identification with fraction 8.

Identification of 5,5′-Diuracil (U-U)

Colorless needles isolated from fractions 5 (4.5 mg), 6 (14.5 mg), and 7 (10 mg) as a sparingly soluble material were combined and were dissolved in 100 ml of concentrated NH_4OH. The solution was filtered through a Norit pad and the filtrate was concentrated to 10 ml. After standing at 5° overnight, colorless needles (27 mg) were collected by filtration (mp >300°); λ_{max} 292 mμ (ϵ 16 × 10^3), λ_{min} 260 mμ (ϵ 4.38 × 10^3) (in 0.1 M NaOH); λ_{max} 269 mμ, λ_{min} 244 mμ (in H_2O and 0.1 N HCl).

Anal. Calcd for $C_8H_6N_8O_4$: C, 43.25; H, 2.72; N, 25.22. Found: C, 43.30; H, 3.16; N, 25.41.

This compound (20 mg) was pulverized and suspended in 50 ml of absolute methanol. The mixture was cooled with ice water and 10 ml of an ether solution of diazomethane (200 mg/ml) was then added. The methylation was allowed to proceed at 20° for 6 days with occasional shaking. During this period, 10 ml of diazomethane solution was added once a day. The reaction mixture was filtered through a sintered glass funnel, the filtrate was evaporated to dryness, and the residue was dissolved in a small amount of chloroform. The chloroform solution was then chromatographed on paper with isoamyl alcohol saturated with water by ascending technique. The major band (R_F 0.4) on the chromatogram was eluted three times with 10-ml portions of chloroform, and the chloroform was evaporated off. Sublimation of the residue at 170° (0.05 mm) for 2 hr yielded 5 mg of a product (mp 281–282°). Its ultraviolet and infrared spectra are identical with those of an authentic sample of 5,5′-di-1,3-dimethyluracil and mixture melting point showed no depression (Ishihara and Wang, 1966a).

Fraction 8. As indicated in Table I, the identification of this fraction was carried out with the filtrate from fraction 7. The starting material (BU), 425 mg, was recovered after desalting according to the general procedure. After the elution of fraction 8, the column was washed with 2 l. of water; however, no significant amount of product was obtained.

Fraction 9. After washing with water, the column was consecutively eluted with 1250-ml portions of 0.001, 0.003, 0.01, and 0.1 N HCl solutions. A total of 134 mg of dark brown residue was collected from all the eluates. However, their identification has not been accomplished.

Isolation of Parabanic Acid

The residue (605 mg) from 5 l. of irradiated solution was suspended in 15 ml of water and was extracted continuously with ether for 2 days. After removal of the ether, the dried residue (335 mg) was extracted three times with 50-ml portions of hot ethyl acetate. The combined extract was cooled to room temperature and the solids formed (mainly BU) were removed by filtration. Fresh ethyl acetete was added to the filtrate to bring the volume to 150 ml, and 300 ml of petroleum ether (bp 30–60°) was added just as the solution became turbid. After 10 min, the precipitate (110 mg) was filtered off, and the filtrate was allowed to pass through a column of 10 g of silica gel (0.6 × 15 cm, Davidson Chemicals) according to the procedure of Conrad (1954). The residue (6.1 mg) remaining after evaporation of the solvent from the eluate (mp 227–232°, reported 251–254°) gave an infrared spectrum identical with that of the authentic parabanic acid.

Isolation of Urea and Ammonia

A dried residue from 10 l. of irradiated solution was dissolved in 75 ml of hot water. After cooling to room temperature, the insoluble materials were removed by filtration. The filtrate was passed through the column of Dowex 50-X8 (H^+, 100–200 mesh, 2.2 × 25 cm). Both the top of the column and the receiver were equipped with tubes containing moist Dowex 50 (H^+) in order to prevent contamination by atmospheric ammonia. The column was washed with water until

the optical density reading of the eluate became <0.03 at 260 mμ (*ca.* 2.5 l. of water was required). The column was then eluted with 2 l. of 4 N HCl. The HCl eluate was evaporated to dryness and the hygroscopic brown residue thus obtained was used for the isolation and identification of urea and ammonia.

A. UREA. The brown residue was dissolved in 2.4 ml of water. To this solution, 0.2 ml of 10% sodium acetate solution, 5 ml of glacial acetic acid, and then 0.5 ml of 5% xanthydrol in methanol were added. The mixture was allowed to stand at room temperature for 30 min, and an additional 1 ml of water was added. After standing overnight, the crystalline product was collected by filtration and washed with 60% acetic acid followed by water. It weighed 35 mg after drying over KOH *in vacuo*. Recrystallization from 3.5 ml of glacial acetic acid gave 25 mg of small silky needles which were identified as dixanthylurea by the comparison of melting point (262–264° dec), of mixture melting point (no depression), and of infrared spectrum with the authentic sample which was prepared from urea and xanthydrol by the same procedure (Korn, 1957).

B. AMMONIA. The method used here was essentially the same as described by Archibald (1943) and Varner *et al.* (1953) and was employed in our previous study (Ishihara and Wang, 1966b).

The residue, from 10 l. of irradiated solution which was treated with the resin as above, was dissolved in 10 ml of water. The solution (0.1) consumed 1.335 ml of 0.01 N HCl. The titrated solutions were combined and subjected to microKjeldahl distillation. The distillate was collected in 0.1 N HCl. The dried residue gave an infrared spectrum identical with that of authentic NH_4Cl.

Identification of Glyoxal

The irradiated solution (1 l.) was mixed with 1.2 l. of hot 2,4-dinitrophenylhydrazine solution (1.67% in 2 N HCl) immediately after irradiation, and the mixture was heated for 30 min on a steam bath. The precipitate was collected by filtration while hot and washed with hot 2 N HCl and then with water. After drying at 120°, it weighed 25 mg. That the product did not sublime at 110° (1 mm) indicates the absence of 2,4-dinitrophenylhydrazone of formaldehyde (Alcántara and Wang, 1965a). The product was recrystallized from nitrobenzene, yielding a total of 19.9 mg of orange-red crystals (mp >300°). The infrared spectrum of the product was identical with that of a synthetic sample of glyoxal bis-2,4-dinitrophenylhydrazone (Neuberg and Simon, 1932).

Experiments Related to Mechanistic Study

The Absence of 5-Hydroxyuracil (OH-U). The dried residue from 10 l. of the irradiated solution was extracted three times with 75-ml portions of hot water. The combined extract was concentrated to *ca.* 20 ml and the filtrate was chromatographed on Whatman No. 3MM with *n*-butyl alcohol–acetic acid–water (80:12:30) by descending technique. Areas corresponding to OH-U (R_F 0.39) were eluted three times with 200-ml portions of hot water. The combined extract was concentrated to 3 ml and rechromatographed with *n*-butyl alcohol–water (86:14), and areas of OH-U (R_F 0.31) were extracted three times with 20 ml of hot water. After concentration to a very small volume, the extract was chromatographed on a paper strip, which was developed with the first solvent system. However, the dried chromatogram gave a negative ferric chloride test (Ishihara and Wang, 1966b). In the control experiments, 5 l. of irradiated solution containing 100 μg of OH-U gave a positive ferric chloride test.

The Absence of Oxamide. The irradiated solution (5 l.) was concentrated to 10 ml. After cooling in ice water for 2 hr, the solid was collected and washed with hot water. It was then taken up in 50 ml of concentrated NH_4OH and filtered to collect a small amount of the residue. The infrared spectrum of the residue did not show the presence of oxamide. A control experiment was carried out in the same manner with the addition of 2 mg of oxamide to the irradiated solution. The infrared spectrum of the residue showed the presence of oxamide.

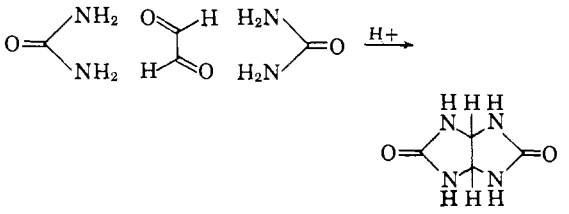
The Absence of Alloxanic Acid. The dried residue from 5 l. of the irradiated solution was extracted three times with 15-ml portions of hot ethanol. The combined extract was concentrated to 10 ml and was allowed to stand in a refrigerator overnight. After removal of the solids by filtration, the filtrate was evaporated to dryness. The residue was dissolved in 5 ml of water and was treated with 2 ml of saturated $Ba(OH)_2$ solution. The reaction mixture was kept in a refrigerator overnight. The white precipitate was collected, washed with water, and dried over P_2O_5 *in vacuo*. Its infrared spectrum was identical with that of barium oxalate and therefore indicated the absence of barium alloxanate. In a control experiment with the addition of 3 mg of alloxanic acid [prepared according to the procedure of Biltz *et al.* (1916)] to the irradiated solution, the final infrared spectrum showed the presence of barium alloxanate in addition to the barium oxalate.

Results and Discussion

Although the knowledge acquired in our study of BDMU (Ishihara and Wang, 1966a,b) could serve as a guide for our present investigation, the method could not be applied directly to the separation of the photoproducts of BU. BDMU and its products are readily soluble in most organic solvents and have characteristic melting points. BU and its products, however, are sparingly soluble in most solvents and their melting points are generally >300°. Therefore, the experimental approach employed was, to a large extent, different from that used for BDMU.

Glyoxaldiurene

The separation of the principal photoproducts was accomplished on a Dowex 1 (Cl^-) column. Fraction 1 was identified as glyoxaldiurene. One conceivable mechanism for the formation of this compound would be the condensation of urea and glyoxal in acid medium as follows



This conjecture is supported by the fact that both urea and glyoxal are present as photoproducts (see below), and the irradiation medium was acidic. Furthermore, one of the two methods used to prepare this compound for identification purposes was the condensation of urea and glyoxal in the presence of HCl.

Uracil and Barbituric Acid

Fraction 3 (test tube fractions 77–150) showed the characteristic ultraviolet absorption spectrum of uracil (λ_{max} 282 mμ, pH 10.6). Near λ_{max} the spectra were unsymmetrical and gradually developed into a shoulder at 261 mμ with test tube fraction 111. Afterwards, it became a distinct maximum at 256 mμ until test tube fraction 150. [A known mixture of barbituric acid and uracil (0.5:99.5) gave a similar elution pattern.] A similar technique of cation-exchange chromatography has been used for the identification of these compounds by Hayaishi and Kornberg (1952). The major product from this fraction was isolated and characterized by infrared and ultraviolet spectra as uracil. Although isolation of the minor product was not achieved, the chromatographic pattern and the ultraviolet spectrum both indicated its identity as barbituric acid. It should be emphasized that barbituric acid derivatives had been suggested as unstable intermediates during the irradiation of uracil derivatives in aqueous solution (Wang, 1958b).

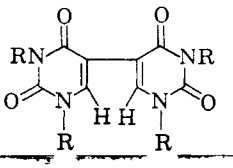
Oxalic Acid and Isoorotic Acid

Oxalic acid was isolated from the ether layer of fraction 5 and identified as its calcium salt. This compound could be originated from the 4, 5, and 6 positions of the pyrimidine ring and should have resulted from hydrolysis and oxidation processes.

The aqueous layer of fraction 5 on paper chromatography revealed several dark bands and one fluorescent band. However, these compounds were present in minute quantities. The major band has the same R_F value as isoorotic acid, and the ultraviolet spectrum of the eluate of this region from the paper chromatogram was identical with that of isoorotic acid. The quantity was *ca.* 100 μg/10 l. of irradiated solution; therefore, its isolation was impractical. Similarly, 5-carboxy-1,3-dimethyluracil was shown to be a photoproduct of BDMU. The possible mechanism of its formation was conjectured to be CO_2 fixation on the uracil radicals (Ishihara and Wang, 1966b).

5,5′-Diuracil

The sparingly soluble material of fractions 5–7 was obtained simply by concentration of the eluates. The material from all these fractions was combined because their infrared spectra were identical. The elemental analyses of the purified product agree with the empirical formula of 5,5′-diuracil (R = H, U-U).

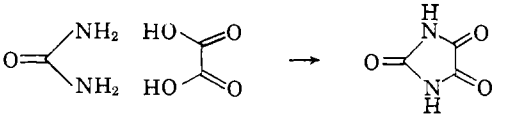


All attempts to prepare U-U according to our method of preparing 5,5′-di-1,3-dimethyluracil (R = CH_3, DMU-DMU) were unsuccessful (Ishihara and Wang, 1966a). Upon exhaustive methylation, however, U-U gave DMU-DMU as the major product which was identical in every respect with the synthetic material. The identification of this coupled product again demonstrates the possibility of the formation of homocoupled products and heterocoupled products in the irradiated DNA. These may be of significance in photobiology and radiobiology.

The identification of 5,5′-diuracil and uracil suggests that uracil radicals are formed as intermediates when BU is irradiated. Further reactions on these two primary photoproducts result in the formation of various degradation products.

Parabanic Acid

The identification of parabanic acid as a photoproduct has confirmed an earlier report that this acid was isolated from extensively irradiated uracil (Conrad, 1954). It was conjectured that ring contraction must have occurred in this step which consists of reduction from a six-membered ring (uracil derivative), to a five membered ring (parabanic acid). However, the identification of oxalic acid and urea suggests that parabanic acid may be the condensation product of these two compounds.



The isolation of glyoxaldiurene as a photoproduct (described above) lends support to this belief, since this product is most probably formed from a condensation reaction.

Urea and Ammonia

Urea was separated from the neutral and the acidic photoproducts by first retaining it on a cation exchanger, and then eluting it with 4 N HCl. The identification of urea was carried out by the preparation of the crystalline derivative dixanthylurea. The amount of urea present in 10 l. of the irradiated solution was found to be 5.0 or 16 mg of dixanthylurea; its yield was shown to be quantitative (Korn, 1957). Ammonia was also separated and identified as NH_4Cl.

Glyoxal and the Absence of Formaldehyde

Previous experience in this laboratory indicated that

aldehydes in the irradiated solution of 1,3-dimethylthymine, namely formaldehyde and 5-formyl-1,3-dimethyluracil, can be converted quantitatively to their 2,4-dinitrophenylhydrazones by the addition of an excess of 2,4-dinitrophenylhydrazine, and that formaldehyde is a photoproduct of thymine derivatives (Alcántara and Wang, 1965a). In the present case, however, formaldehyde was shown to be absent in the irradiated solution. This discrepancy may yield some knowledge as to the origin of formaldehyde.

Glyoxal, on the other hand, was identified as the only aldehyde present in the irradiated solution. Considering the formation of both glyoxaldiurene and oxalic acid, glyoxal is more likely to be the precursor of both. The presence of glyoxal in the irradiation mixture renders the idea of condensation reaction mechanisms more convincing.

The Absence of OH-U

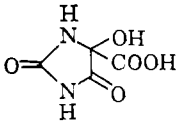
In control experiments, it was shown that OH-U was so strongly retained on the Dowex 1 column used in this study that it could be eluted from the column neither with NH_4OH–NH_4Cl buffers, nor water, nor 4 N HCl. Therefore, the ferric chloride color reaction (Ishihara and Wang, 1966b) was employed to determine whether OH-U is present in the irradiated solution of BU. This method, developed specifically for this purpose, had been used in our study of BDMU. On the basis of the experimental findings, we concluded that OH-U is not a final photoproduct, and that, therefore, the participation of solvent water in this chain reaction is most unlikely. Generally, abstraction of H from organic molecules is greatly favored over abstraction from water.

The Absence of Oxamide

Oxamide has been isolated as one of the photoproducts by the exhaustive irradiation of uracil in an aqueous solution (Conrad, 1954). Furthermore, *sym*-dimethyloxamide has been identified as a photoproduct by the irradiation of BDMU in aqueous solution (Ishihara and Wang, 1966b). Therefore, it was inferred that oxamide should be a photoproduct under this reaction condition. Since oxamide has an extremely low solubility in water (even hot water) and in organic solvents, its isolation would seem to be fairly simple. However, our results indicated that oxamide is not a photoproduct.

The Absence of Alloxanic Acid

Recently, alloxanic acid was suggested as one of the major photoproducts of 5-iodouracil irradiated in the presence of oxygen (Rupp and Prusoff, 1965). This



acid is very soluble in water and in ethanol, and its barium salt is practically insoluble in these solvents. Since alloxanic acid has these characteristics, its presence as a product should not be difficult to demonstrate. However, our experimental evidence suggests that alloxanic acid is not a photoproduct of the irradiation of BU in aqueous solution. It should be mentioned that although no O_2 was added to the solution, no precaution was taken to eliminate O_2 in our experimental conditions.

The results discussed above may be summarized in Table II. The yields of the photoproducts given in the table were obtained from 10 mmoles or 1.910 g of BU. However, the initial amount should be considered as 1.485 g because 425 mg of BU was recovered. Furthermore, the photohomolysis step, resulting in the loss of Br atoms, reduced the recoverable weight to 863 mg. In addition, decarbonylation, condensation, decarboxylation, etc., would further decrease the amount of recoverable material. Therefore, it is difficult to ascertain the actual yields.

TABLE II: Summary of Investigation of Photoproducts of BU.

Method of Sepn	Photoproduct	mg	mequiv
Dowex 1-X8 (Cl^-) column	Glyoxaldiurene	48	0.34
	Uracil	100	0.89
	Barbituric acid[a]		...
	Oxalic acid	23	0.18[b]
	Isoorotic acid[c]		...
	U-U	29	0.13
	BU	425	
Ether extraction	Parabanic acid	12	0.11
Dowex 50-X8 (H^+) column	Urea	35	0.09[d]
	Ammonia	1.5	0.09
2,4-Dinitrophenylhydrazone	Glyoxal	250	0.60
	Formaldehyde	Not detected	
Others	OH-U	Not detected	
	Oxamide	Not detected	
	Alloxanic acid	Not detected	

[a] By chromatography and ultraviolet spectrum. [b] As Ca salt. [c] By R_F and ultraviolet spectrum. [d] As dixanthylurea.

From the results presented in this paper, it may be concluded that upon irradiation with ultraviolet light, BU undergoes photohomolysis to yield free radicals. In turn, these radicals yield U-U and uracil as initial photoproducts. This finding is indeed parallel to that obtained in the study of the irradiation of BDMU as discussed in the preceding articles (Ishihara and Wang, 1966a,b). The isolation of many other products would indicate the photocleavage of the pyrimidine rings under the irradiation condition. Although these results will

be of importance in photochemical mechanistic understanding, their biological significance is undoubtedly questionable. These decomposition products may occur only after a long period of irradiation. As reported in a previous paper (Ishihara and Wang, 1966a), the ultraviolet absorbancy of BU in an aqueous solution which has been irradiated with biological doses of ultraviolet remains unchanged in the neighborhood of λ_{max}, but an increase in ultraviolet absorbancy at around 240 mμ was observed. These changes would suggest that the photoproduct formed may still possess the pyrimidine ring and must have high absorbancy in the 240-mμ region. In this connection, U-U is the most likely candidate. Thus, in biological systems, uracil and bromine radicals produced by irradiation may attack other purines and pyrimidines in the DNA to give a number of products. The terminal step in this chain event is the formation of the coupled products which may be responsible for the lethal effects. Although it is tempting at this stage to form opinions on the biological significance of this finding, we feel that such discussions must await the results of further studies on biological systems.

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