[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGY, TUFTS UNIVERSITY SCHOOL OF MEDICINE]

Photochemistry of Nucleic Acids and Related Compounds. I. The First Step in the Ultraviolet Irradiation of 1,3-Dimethyluracil[1,2]

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The first isolated product of the ultraviolet irradiation of 1,3-dimethyluracil was identified by synthesis as 6-hydroxy-1,3-dimethylhydrouracil. 5-Hydroxy-1,3-dimethylhydrouracil was eliminated as a possible intermediate in this process. A mechanism for this process was proposed.

Introduction

Owing to the current interest in the effects of irradiation on biological systems in general, a systematic study of the effects of the ultraviolet irradiation of nucleic acids and related compounds has been undertaken in this Laboratory. Nucleic acids were chosen not only because of their biological importance but also because of the possibility that they are immediate receptors of radiant energy.[3,4]

In the past decade, the results of many spectroscopic studies of the irradiation of pyrimidines, purines, nucleosides and nucleotides have been published.[5] In addition, the identification of many of the extensive breakdown irradiation products from pyrimidine and purine derivatives[6] has been accomplished. However, we feel that in order to obtain insight into these complicated reactions, the stepwise isolation and identification of the products involved and the actual mechanism of their formation are of prime importance. It is hoped to provide from these results the basis for an interpretation of the irradiation effects on the polymeric nucleic acids.

We have been studying the effects of ultraviolet irradiation on a wide variety of pyrimidines and purines. These include the derivatives of thymine, cytosine, uracil, nicotinamide and others. The isolation and identification of the irradiation products involved are now in progress. The paper of Moore and Thomson,[7] the first of its kind, has described the isolation of an irradiation product of 1,3-dimethyluracil and suggested the product to be 6-hydroxy-1,3-dimethylhydrouracil. We have published our own results relating to this in a previous communication,[8] which serves as the basis of this article.

Irradiation Apparatus and Measurements.—A bank of seven lights (General Electric Germicidal tubes, G15T8) are mounted horizontally and parallel to each other on the back panel of an apparatus (1 × 2 × 1¼ ft.). Quartz tubes (16; 30 × 1 cm.), topped with refluxing bulbs, are put in two holders and placed against the lights. A piece of filter paper is placed between the inlet of a fan and wire screen on one side panel in order to dissipate air flow for cooling and eliminate the oil vapor from the fan motor. The other side panel is perforated and has attached louvres. The back panel and the doors are covered with aluminum foil as reflectors.

Various types of irradiators are used in this Laboratory for different purposes. The one described above is mainly for controlled chemical studies. We have found that the shape of containers, temperature, concentration of the solution, etc., could affect the reaction significantly. Above all, the variation in the intensity of light gave varying results, *i.e.*, low intensity light for a longer period of time did not produce the same effect as did high intensity light for a shorter period. We have, however, noted the light intensity and expressed it in terms of milliwatts per square foot. The intensity was measured with a germicidal ultraviolet intensity meter (General Electric Co.). The distance between the bank of lights and the lens of the meter was eight feet. Both the lights and the meter were elevated to minimize the reflection from floor surface.

Experimental[9]

1,3-Dimethyluracil (I) was prepared according to the method of Davidson and Baudisch[10]; $\lambda_{max}^{H_2O}$ 267 mμ, ε 8.67 × 10³; $\lambda_{min}^{H_2O}$ 235 mμ, ε 1.68 × 10³; λ250/λ260, λ280/λ260; 0.62, for *p*H 2–12; ν_{CO}^{KCl} 1701 cm.$^{-1}$.

Irradiation of 1,3-Dimethyluracil in Aqueous Solution.—1,3-Dimethyluracil (I), 1.4 g., was dissolved in 300 cc. of distilled water (λ^{H_2O} 260 mμ, ε 8 × 10³; *p*H 5, T 32°) and was irradiated for 27 hr. at a light intensity of 55 m.w./sq. ft. The final solution (λ^{H_2O} 260 mμ, ε 0.8 × 10³; *p*H 4), which had lost 90% of its absorption at 260 mμ, was lyophilized, and a white fluffy product was obtained, comprising about 90% of the original weight. This was then dissolved in 30 cc. of chloroform, filtered, and the filtrate concentrated under a stream of nitrogen to about 10 cc. To this solution petr. ether (30–60°) was added until it was just cloudy and it was allowed to stand at room temperature for a half-hour. The solution, in which small crystalline clusters had begun to form, was allowed to stand in the cold room. The resulting crystals were collected by suction filtration and were washed thoroughly with chloroform–petr. ether (3:7), m.p. 101–103°, weight about 0.9

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(3) J. R. Loofbourow, *Growth*, **12**, 75 (1948).

(4) A. C. Giese, *Physiol. Revs.*, **30**, 431 (1950).

(5) (a) R. L. Sinsheimer and R. Hastings, *Science*, **110**, 525 (1949); (b) R. L. Sinsheimer, *Radiation Res.*, **1**, 505 (1954); (c) R. L. Sinsheimer, *ibid.*, **6**, 121 (1957); (d) D. Shugar and K. Wierzchowski, *Biochim. et Biophys. Acta*, **23**, 657 (1957); (e) A. M. Moore and C. H. Thomson, 4th Intern. Conf. on Radiobiology, Cambridge, England, 1955.

(6) (a) A. Canzanelli, R. Guild and D. Rapport, *Am. J. Physiol.*, **167**, 364 (1951); (b) W. E. Conrad, *Radiation Res.*, **1**, 523 (1954); (c) J. Fellig, *Science*, **119**, 3082 (1954).

(7) A. M. Moore and C. H. Thomson, *ibid.*, **122**, 594 (1955); *Can. J. Chem.*, **35**, 163 (1957).

(8) S. Y. Wang, M. Apicella and B. R. Stone, THIS JOURNAL, **78**, 4180 (1956).

(9) All the melting points are uncorrected and were taken with a Fisher–Johns melting point apparatus. Infrared spectra were determined with a Baird spectrophotometer upon potassium chloride disks and the analyses were carried out by Dr. S. M. Nagy and his associates, Microchemical Laboratory, M.I.T. Ultraviolet spectra were determined with the Beckman spectrophotometer, model DU.

(10) Davidson and Baudisch, THIS JOURNAL, **48**, 2382 (1926).

g. Mother liquor and washings gave more product. After two additional crystallizations, 6-hydroxy-1,3-dimethylhydrouracil (II), which had a m.p. 105–106°, was obtained and the yield was about 1 g. for each run[7]; ν_{OH}^{KCl} 3344 cm.$^{-1}$, ν_{CO}^{KCl} 1704 cm.$^{-1}$.

Anal. Calcd. for $C_6H_{10}O_3N_2$: C, 45.58; H, 6.33; N, 17.72. Found: C, 45.61; H, 6.49; N, 18.00.

Alkaline Reconstitution of 6-Hydroxy-1,3-dimethylhydrouracil (II).—A 10^{-4} *M* solution of II at various alkaline *p*H's was allowed to stand at room temperature. The data obtained were

*p*H	Time, min.	$\epsilon_{260}^{H_2O}$	λ250/λ260	λ280/λ260
9	1	0.31×10^3		
9	160	7.22	0.61	0.62
10	1	1.05		
10	20	7.42	.61	.62
11	1	4.53		
11	6	7.77	.61	.63
12	1	8.18	.61	.62

Isolation of Reconstituted I by Acid Treatment of II.—6-Hydroxy-1,3-dimethylhydrouracil (II) (100 mg.) was dissolved in 10 cc. of dil. HCl solution (*p*H 1.58) and was allowed to stand overnight at room temperature. The solution was then lyophilized, and the residue was taken up with ether. Petroleum ether (30–60°) was added for recrystallization; 80.9 mg. of material gave m.p. 121–122°, m.m.p. 121–122° with 1,3-dimethyluracil. Infrared spectra were identical.

Isolation of Reconstituted I by Alkaline Treatment of II.—6-Hydroxy-1,3-dimethylhydrouracil (II) (100 mg.) was dissolved in sodium hydroxide solution (*p*H 12.08) and was allowed to stand at room temperature for 20 min. The product then was extracted six times with 10-cc. portions of chloroform, and the organic extracts were filtered through anhydr. sodium sulfate. The filtrate was evaported to dryness, and 78 mg. of product, m.p. 120–122°, was obtained, m.m.p. 121–122° with 1,3-dimethyluracil. Infrared spectra were identical.

5-Bromo-1,3-dimethyluracil (IV) was prepared according to Johnson and Clapp.[11] A purified product was obtained after repeated crystallization from absolute ethanol, m.p. 184–185°; $\lambda_{max}^{H_2O}$ 283 mμ, ε 8.57×10^3; $\lambda_{min}^{H_2O}$ 246 mμ, ε 1.57×10^3; ν_{CO}^{KCl} 1684 cm.$^{-1}$.

5-Hydroxy-1,3-dimethyluracil (V).—To a solution of 1.15 g. of 1,3-dimethyluracil in 30 cc. of water bromine was added dropwise with stirring until the color was permanent. Four and six-tenths g. of litharge was added, and the solution was then boiled for one hour. The reaction mixture was evaporated to about 15 cc. and was treated with 30 cc. of ethyl alcohol. It was kept for some time at room temperature and then filtered through Celite. The lead in the solution was removed by the dropwise addition of dilute sulfuric acid (1 cc. of concd. H_2SO_4 diluted to 50 cc.). After most of the alcohol was removed by a stream of nitrogen, the solution was lyophilized. The lyophilized material was dissolved in boiling abs. alcohol for crystallization, and 0.76 g. of the material was obtained, m.p. 192–194°. Repeated crystallization from absolute alcohol gave colorless needles, m.p. 198–199°[12]; $\lambda_{max}^{H_2O}$ 285 mμ, ε 7.35×10^3; $\lambda_{min}^{H_2O}$ 248 mμ, ε 1.78×10^3; ν_{OH}^{KCl} 3215 cm.$^{-1}$, ν_{CO}^{KCl} 1669 cm.$^{-1}$.

Anal. Calcd. for $C_6H_8O_3N_2$: C, 46.15; H, 5.16; N, 17.95. Found: C, 46.15; H, 5.38; N, 17.83.

6-Hydroxy-1,3-dimethylhydrouracil (II) by Hydrogenolysis.—One-half gram of I was dissolved in 20 cc. of water, and 0.19 ml. of bromine (slight excess) was added to the solution; 6 ml. of *p*H 7 buffer (Beckman) and about 250 mg. of 10% Pd–C were added. The solution was stirred and was immediately hydrogenated. About 90% of the theoretical amount of hydrogen was taken up in less than 30 min. The resulting solution was washed twice with 2 cc. of chloroform and the aqueous phase was lyophilized. The material from lyophilization was extracted once with 30-cc. and three times with 10-cc. portions of chloroform. The combined chloroform extracts were evaporated to dryness with nitrogen. The residue was redissolved in chloroform. Petroleum ether (30–60°) was added for crystallization and 0.26 g. of product was obtained, m.p. 98–99°. Repeated crystallization from chloroform–petr. ether gave small clusters of needles, m.p. 105–106°, m.m.p. 105–106° (with irradiated product). Infrared and ultraviolet spectra were identical with the irradiation product.

Anal. Found: C, 45.46; H, 6.49; N, 17.71.

5-Hydroxy-1,3-dimethylhydrouracil (VI).—5-Hydroxy-1,3-dimethyluracil (0.96 g.) and about 0.48 g. of 10% of Pd–C were suspended in 50 cc. of water. The hydrogenation was carried out at room temperature with a Paar hydrogenator and under a 35-lb. pressure. The hydrogenation was completed in 20 hours as indicated by the ultraviolet spectrum. After the removal of the catalyst, the filtrate was lyophilized; 0.86 g. of the product was obtained, m.p. 108–110°. Repeated crystallization from ether–petr. ether (30–60°) gave rod-like prisms, m.p. 109–110°, m.m.p. with 6-hydroxy isomer 70°; ν_{OH}^{KCl} 3390 cm.$^{-1}$, ν_{CO}^{KCl} 1704 cm.$^{-1}$.

Anal. Calcd. for $C_6H_{10}O_3N_2$: C, 45.58; H, 6.33; N, 17.72. Found: C, 45.57; H, 6.40; N, 17.92.

5-Hydroxy-1,3-dimethylhydrouracil (VI) in Acids. A. Hydrochloric Acid.—A 10^{-4} *M* of VI at a *p*H 1.98 was allowed to stand at room temperature for over 20 hr. During this time there were no appreciable changes in ultraviolet spectra.

B. **50% Sulfuric Acid (w./w.).**—A 10^{-4} *M* solution of VI in 50% sulfuric acid was maintained at room temperature overnight and then refluxed for one hour. There were no appreciable changes in ultraviolet spectra under these conditions.

5-Hydroxy-1,3-dimethylhydrouracil (VI) in Alkali.—A 10^{-4} *M* solution of VI at *p*H 12 was allowed to stand at room temperature for 5 minutes. These readings were obtained

	$\epsilon_{initial}$	ϵ_{final}
230 mμ	3.93×10^3	0.02×10^3
260 mμ	0.18	.06

Irradiation of 5-Hydroxy-1,3-dimethylhydrouracil (VI).—Compound VI (0.0984 g.) was dissolved in 300 cc. of distilled water (*p*H 6.28) and was irradiated for 2 hr. at a light intensity of 55 m.w./sq. ft. The optical density was 0.022 at 260 mμ before irradiation and 0.013 subsequently. The final *p*H value was 5.62. The irradiated material was lyophilized and the solid residue collected. A 10^{-4} *M* solution of the solid residue (according to the mol. weight of II) was prepared. After treating with base (*p*H 11.98) or acid (*p*H 1.98) there were no appreciable changes in ultraviolet spectra.

Hydrogenolysis of 5-Bromo-1,3-dimethyluracil (IV).—Compound IV (0.219 g.) was dissolved in 20 cc. of absolute alcohol and was reduced in the presence of 0.1 g. of 10% Pd–C. Theoretical amount of hydrogen was taken up in about 20 min. A 75% theoretical yield of crude 1,3-dimethyluracil was obtained by concentrating the filtrate to 2 cc. after the catalyst was removed. Two more recrystallizations gave a product with m.p. 121–122°. It had a m.m.p. 121–122° with authentic I and gave identical ultraviolet and infrared spectra.

Results and Discussions

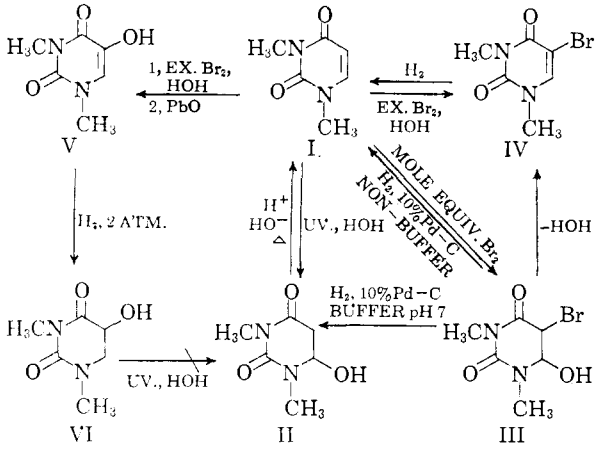
1,3-Dimethyluracil (I), a model compound, was irradiated under optimal conditions in order to obtain a good yield of 6-hydroxy-1,3-dimethylhydrouracil (II) and to avoid the complications of subsequent reactions.[13]

On treating II with acid or heat the ultraviolet spectrum of I was reconstituted. Alkaline treatment of II at *p*H 9, 10, 11 or 12 also reconstituted the spectrum the optical density ratios agreeing with standard values. This alkaline reconstitution differs from that of irradiated uridylic acid which gives a spectrum other than that of the original.[5b] For further substantiation, compound I was isolated and identified from these reconstitution reactions.

(11) T. B. Johnson and S. H. Clapp, *J. Biol. Chem.*, **5**, 62 (1890).

(12) H. Biltz and H. Paetzold, *Ann.*, **452**, 67 (1927).

(13) S. Y. Wang, THIS JOURNAL, **80**, 6199 (1958).

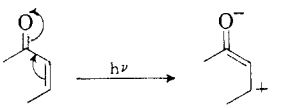


The disappearance of the spectrum at 260 mμ after irradiation, together with the appearance of an OH band in the infrared spectrum of the irradiation product, indicated the loss of the 5,6-double bond by the addition of water. The formation of I from II, therefore, is a dehydration reaction. However, the above data appear to be consistent with the formation of an irradiation product of either 6-hydroxy- (II) or 5-hydroxy-1,3-dimethylhydrouracil (VI) or both. In order to identify the product, both II and VI were synthesized.

5-Bromo-1,3-dimethyluracil (IV) was prepared by boiling I with an excess of bromine water. The addition of lead oxide before boiling with bromine water produced 5-hydroxy-1,3-dimethyluracil (V) from I. Hydrogenation of V at a pressure of two atmospheres yields 5-hydroxy-1,3-dimethylhydrouracil (VI) which behaved entirely different from the irradiation product in the case of treatment with either acid or base. Reconstitution was not observed either with ordinary acidic conditions or with 50% sulfuric acid. The ultraviolet spectrum of VI after alkali treatment was flat with complete disappearance of the end absorption. The inability to obtain I from either alkali or acid treatment of VI strongly suggests that VI is not a product of irradiation. Compound VI was irradiated under similar conditions and then treated with acid or alkali; no change in the ultraviolet spectrum occurred. Therefore it is not likely that VI is an intermediate on the irradiation pathway to II.

When Compound I was treated with one mole equivalent of bromine water, 5-bromo-6-hydroxy-1,3-dimethylhydrouracil (III) was obtained in solution. The ultraviolet spectrum of I disappeared, with only end absorption remaining. After standing for several hours there was no detectable change in the spectrum. This indicated the formation of bromohydrin at the 5,6-double bond. However, when the solution was allowed to stand overnight or crystallized from a chloroform extract, only 5-bromo-1,3-dimethyluracil (IV) was obtained by dehydration. Hydrogenolysis of III in a non-buffered solution with 10% Pd–C as catalyst gave only 1,3-dimethyluracil (I) as the product, which resulted from the dehydration of II. However, hydrogenolysis of III in a phosphate buffered solution gave II as the product. The very low yields obtained by Moore and Thomson[7] might be explained by the possibility that the hydrogenolysis of 5,5-dibromo-6-hydroxy-1,3-dimethylhydrouracil gave the intermediate *cis* form of III, which would permit the ready *trans* elimination of water with a tertiary hydrogen to yield IV.[14] Compound IV, as we have found, readily was reduced catalytically to I. The synthetic product from hydrogenolysis was found to be identical with the irradiated product; II was thus identified by synthesis as 6-hydroxy-1,3-dimethylhydrouracil.

As shown above, VI was not an intermediate in this photoreaction; II was shown to be the only product from the addition of water. Therefore, a 1,4-addition mechanism seems likely. Apparently, the addition of water at the 5,6-double bond was the net result.[15–17] The primary process, however, is probably the polarization of the α,β-unsaturated ketone to the ionic intermediate. The possible existence of this intermediate may be of significance in a biochemical situation.



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(14) Observation made on thymine derivatives in this Laboratory.
(15) R. L. Sinsheimer, *Radiation Res.*, **6**, 121 (1957).
(16) D. Shugar and K. Wierchowski, *Biochim. et Biophys. Acta*, **23**, 657 (1957); **25**, 335 (1957).
(17) F. F. Heyroth and J. R. Loofbourow, THIS JOURNAL, **53**, 3441 (1931).