

PHOTOINDUCED REACTIONS—XXI

PHOTOSENSITIZED OXYGENATION OF N-UNSUBSTITUTED HYDROXYPURINES¹ *

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Abstract—In order to contribute to the elucidation of the photodynamic degradation of deoxyribonucleic acids, the photosensitized oxygenation of some *N*-unsubstituted hydroxypurines in aqueous alkaline solution in the presence of rose bengal was investigated. Under these conditions, xanthine gave allantoin and triuret, uric acid gave triuret, sodium oxonate and allantoxaidin, and 8-methylxanthine resulted in the formation of a complex mixture from which acetamide and sodium oxonate were isolated. Possible mechanisms for these reactions, including peroxide intermediates, are discussed.

IN CONNECTION with the photodynamic inactivation of deoxyribonucleic acid²⁻⁴ the photosensitized oxygenation of guanine and related purine derivatives has been investigated. Irradiation of guanine and guanosine by visible light in the presence of sensitizing dyes and oxygen results in destruction of the molecule to give various products,⁵⁻⁷ but details of the reaction pathway remain unknown. Other hydroxylated purine derivatives, such as uric acid, xanthine, and hypoxanthine, are also known to consume oxygen under similar conditions.⁸⁻¹⁰ Recently Anmann and Vynch¹¹ have reported that uric acid, when submitted to the methylene blue-sensitized photooxygenation in aqueous ethanol, yields cyanuric acid, allantoin, parabanic acid, urea, and other three unidentified compounds, although all these compounds were identified only on paperchromatograms. Without a sensitizer, triuret was obtained in the photooxidation of uric acid under the influence of UV light.¹² Since the nature of these reactions still remains unknown, we initiated a systematic investigation on the photosensitized oxygenation of various purine derivatives. This paper describes the photosensitized oxygenation of three representative *N*-unsubstituted hydroxypurines, i.e. xanthine (I), uric acid (IV), and 8-methylxanthine (VII), in alkaline media using rose bengal as a sensitizer.

When an aqueous alkaline solution of xanthine (I) was irradiated in the presence of rose bengal under bubbling oxygen, 1.05 mole equivalent of oxygen was consumed. Acidification of the reaction mixture liberated CO₂ (1.0 mole equiv), which was determined by conversion into barium carbonate. From the acidified mixture triuret II (5%) and allantoin III (41%) were obtained as major products. Triuret was identified by synthesis¹³ and conversion into cyanuric acid.

Uric acid (IV) also consumed 1.1 mole equivalent of oxygen under similar conditions. In this case, the yields of products were dependent upon pH during isolation of the products. Isolation of the photoproducts at pH 2 yielded triuret (II), sodium oxonate

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(V), allantoxaidin (VI), and CO₂, in 20, 30, 15 and 85% yields, respectively. When the isolation was made at pH 5.0, the yield of sodium oxonate (V) was increased to 40% at the expense of CO₂ (25%) and no allantoxaidin (VI) was obtained. On the other hand, photooxygenation of uric acid in the presence of a large excess of alkali followed by the isolation of the products at pH 5.0, afforded triuret (II, 8%), sodium oxonate (V, 69%), and CO₂ (10%). Since it is known that sodium oxonate (V) is converted to allantoxaidin (VI) by treatment with a strong acid,^{14, 15} it is concluded that allantoxaidin formed from uric acid is a secondary product formed in the course of isolation.

Photosensitized oxygenation of 8-methylxanthine (VII) in the presence of 1.1 mole equivalent of alkali resulted in consumption of 0.95 mole equivalent of oxygen to give a complex mixture, which was found, by paper chromatography, to consist of at least six compounds. From the mixture, acetamide (6%), sodium oxonate (V, 25%), and CO₂ (53%) were obtained. All these results are summarized in Table 1.

TABLE I. PHOTSENSITIZED OXYGENATION OF HYDROXYPURINES

Substrate	NaOH added (mole)	O ₂ consumed (mole)	CO ₂ liberated (mole)	pH change	Products (% yield)
Xanthine (I)	0.5 ^a	1.0	1.0	11.7-8.8	II (5)
					III (41)
Uric acid (IV)	1.6 ^a	1.1	0.85	13.0-12.7	II (20)
					V (30)
	1.6 ^b	1.0	0.25	13.0-12.6	VI (15)
					II (29)
13.5 ^b	1.0	0.1	13.2-13.1	V (40)	
				II (8)	
					V (69)
8-Methyl-xanthine (VII)	1.1 ^a	0.95	0.53	12.8-12.4	V (25)
					VIII (6)

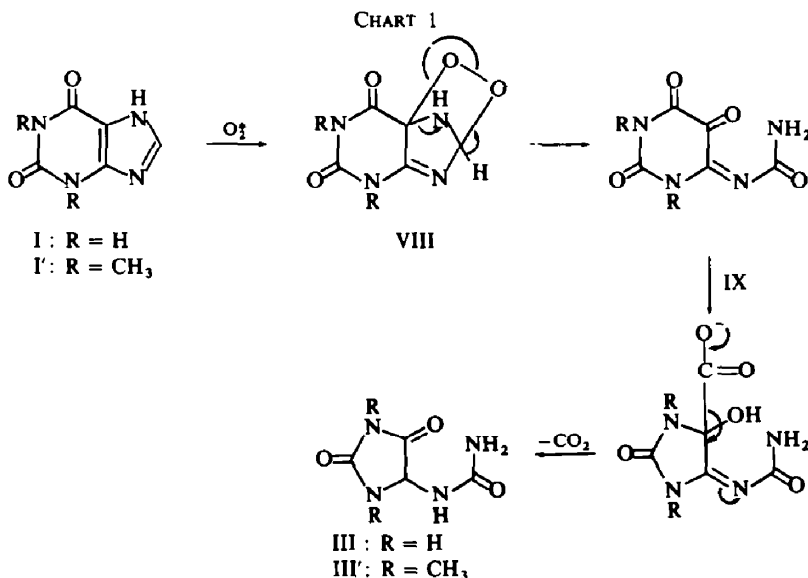
^a The reaction mixture was adjusted to pH 2.0 with 2N HCl before the isolation of the products.

^b The reaction mixture was adjusted to pH 5.0 with acetic acid before the isolation of the products.

In the absence of the sensitizer, none of the substrates consumed oxygen upon irradiation and the starting materials were recovered quantitatively, indicating that the presence of a sensitizer is a requisite condition for the present photooxygenation. Although Weber has shown that some purine derivatives form a complex with riboflavin in solution,¹⁶ no spectroscopic evidence was obtained for the formation of a charge-transfer complex between rose bengal and any of the substrate. The above results demonstrate that the excited state, possibly a triplet state,¹⁷ of the sensitizer may be involved in the present reaction. Recently the suggestion that singlet excited oxygen is involved in dye-sensitized photooxygenation of certain organic compounds was strengthened by several workers.¹⁸ Although mechanisms involving an energy transfer⁵ and an electron transfer¹⁹ between triplet excited sensitizer and purine derivatives have been postulated, recent results obtained in the oxygenation of N-alkylated purine derivatives with singlet oxygen²⁰ strongly suggest that, in the present

photosensitized oxygenation, singlet oxygen may attack the ground state molecule of the substrates.* Thus, it appears that the attack of singlet oxygen may result in the initial formation of a peroxide intermediate, either a cyclic peroxide or a hydroperoxide, depending upon the nature of the starting material. Possible sequences for the photosensitized oxygenation of the hydroxypurines, I, IV, and VII, are illustrated in Charts 1, 2 and 3.

The formation of allantoin (III), the major product from xanthine (I), is well rationalized by a pathway involving a cyclic peroxide VIII (Chart 1). Such a cyclic peroxide is known to be an initial product in the photosensitized oxygenation of cyclic dienes,²¹ and it has been postulated as an intermediate in the photosensitized oxygenation of certain types of 5-membered N-heteroaromatics, i.e. pyrroles²² and oxazoles.^{23, 24} Peroxide VIII then rearranges to form an alloxanimide derivative IX which is, on benzylic acid-type rearrangement† and subsequent decarboxylation, converted to allantoin (III). The formation of 1,3-dimethylallantoin (III') in the photosensitized oxygenation of theophylline (I')⁹ is quite analogous to our results obtained with xanthine (I).



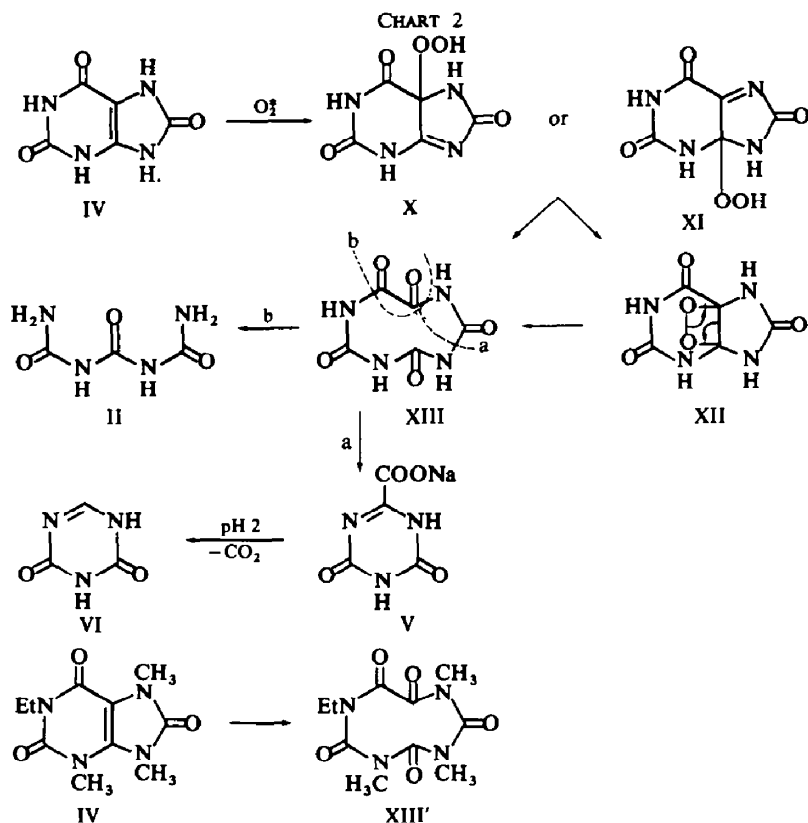
In the photosensitized oxygenation of uric acid (IV), the attack of singlet oxygen would form a hydroperoxide X or XI (Chart 2). This type of hydroperoxide is generally accepted as an intermediate in the photooxygenation of certain N-heterocycles having a moiety $-C=C-NH-$, i.e. imidazoles,^{26, 27} pyrroles,^{28, 29} and indoles.³⁰ The 5,6-bond of hydroperoxide X or XI is cleaved, concertedly or via a 4-membered cyclic peroxide XII,‡ to form a 9-membered intermediate XIII, which could be

* Oxidation with singlet oxygen generated from hydrogen peroxide and hypochlorite¹⁸ would not be applied to these hydroxypurines which are soluble in the solvent system employed only in the presence of alkali. Because these hydroxypurines are known to be easily oxidized with hydrogen peroxide alone under alkaline conditions to give various products.^{14, 15}

† An aqueous alkaline solution of alloxan decomposes to give alloxanic acid, 5-hydroxyhydantoin-5-carboxylic acid.²⁵

‡ Direct formation of XII from IV can be also considered as an alternative pathway.

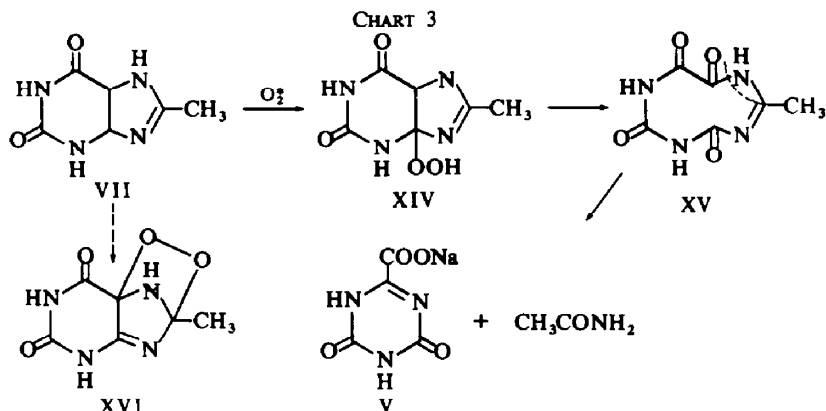
hydrolyzed to give sodium oxonate (V) via path *a* and triuret (II) via path *b*. 9-Membered compounds analogous to XIII are known to be obtained in the photosensitized oxygenation of fully N-alkylated uric acids, e.g. XIII' from 1-ethyl-3,7,9-trimethyluric acid (IV).²⁰ As already described, sodium oxonate (V) when treated with hydrochloric acid is easily decarboxylated to form allantoxaidin (VI).



Finally, a hydroperoxide intermediate XIV may also account for the photooxygenation of 8-methylxanthine (VII; Chart 3). Hydroperoxide XIV would give analogously a cyclic intermediate XV which is then hydrolyzed to acetamide and sodium oxonate (V). However, the possibility of a cyclic peroxide XVI analogous to VIII being formed cannot be eliminated. Peroxide XVI should yield the intermediate XV and other unidentified products.

EXPERIMENTAL

General procedure for photosensitized oxygenation. A soln of the substrate and rose bengal in NaOH aq was irradiated using a 100 W high-pressure mercury lamp with a Pyrex cooling jacket at room temp. During irradiation, O_2 was bubbled through the soln in a closed circulating system and the consumption of O_2 was determined manometrically. After the reaction mixture had been acidified with 2N HCl to appropriate pH, N_2 was bubbled through the mixture and CO_2 liberated was trapped with $Ba(OH)_2$ aq.



The yield of CO_2 was determined by weighing $BaCO_3$ precipitated. The products were isolated as described below. The results are summarized in Table 1.

Photooxygenation of xanthine (I)

A soln of xanthine (1.0 g, 6.6 mmoles) and rose bengal (20 mg) in 0.022N NaOH (150 ml, 3.3 mmoles) was photooxidized. After the absorption of O_2 (155 ml) had ceased (1 hr), the mixture was acidified to pH 2.0. CO_2 (6.6 mmoles) was liberated. The acidified mixture was treated with Norite to remove rose bengal, then was concentrated under red press to 30 ml depositing triuret (II) as a yellow solid (50 mg, 5%) which was crystallized from water, m.p. 230–238° (lit.¹³ 231–235°). The IR spectrum was identical to that of an authentic sample.¹³ The identity of the compound was further confirmed by its derivation to cyanuric acid.^{12,13}

The mother liquor was evaporated to dryness to give a residue which was crystallized from water to give III (0.42 g, 41%) as crystals, m.p. 235–236°. The IR spectrum was identical to that of an authentic sample.

Photooxygenation of uric acid (IV)

(a) *Isolation of products at pH 2.0.* A suspension of uric acid (2.0 g, 12 mmoles) in 0.16N NaOH (120 ml, 19.2 mmoles) containing rose bengal (20 mg) was photooxidized (1.5 hr). The mixture became a clear soln as O_2 was consumed. A white solid deposited during irradiation was identified as triuret (50 mg). After O_2 (300 ml) had been consumed, the mixture was acidified to pH 2.0, liberating CO_2 (10.2 mmoles). Removal of the solvent left a solid mass which was crystallized from water (35 ml) to give triuret (0.28 g, 20%). Concentration of the mother liquor to 10 ml afforded crystals (0.63 g, 30%), m.p. > 300°, which were identified as sodium oxonate^{14,31} (by IR); $\lambda_{max}^{H_2O}$ 230 μ (ϵ 4750), $\lambda_{max}^{0.01N NaOH}$ 252 μ (ϵ 6780). Further concentration of the mother liquor gave allantoxaidin (0.21 g, 15%), m.p. 280–282°, which was identical with a sample prepared by treatment of sodium oxonate with 1N H_2SO_4 ^{14,15} (by IR); $\lambda_{max}^{H_2O}$ 232 μ (ϵ 5600), $\lambda_{max}^{0.01N NaOH}$ 250 μ (ϵ 7200), ν_{max}^{NaCl} 1800 and 1730 cm^{-1} .

(b) *Isolation of products at pH 5.0.* The mixture, which was obtained by the photooxygenation of uric acid as described above, was acidified to pH 5.0 with acetic acid. Triuret (0.50 g, 29%), sodium oxonate (0.70 g, 40%), and CO_2 (3 mmoles) were obtained by the similar procedure. No allantoxaidin was detected.

(c) *Photooxygenation in the presence of a large excess of NaOH.* A soln of uric acid (5.0 g, 30 mmoles) and rose bengal (20 mg) in 1.6 N NaOH (250 ml, 0.405 mole) was photooxidized. From the mixture, which was acidified with AcOH to pH 5.0, triuret (0.34 g, 8%), sodium oxonate (3.5 g, 69%), and CO_2 (3 mmoles) were obtained by the similar procedure.

Photooxygenation of 8-methylxanthine (VII)

A soln of 8-methylxanthine³² (2.0 g, 12 mmoles) and rose bengal (20 mg) in 0.088N NaOH (150 ml, 13.2 mmoles) was photooxidized. After O_2 (254 ml) had been consumed, the mixture was acidified to pH 2.0 to liberate CO_2 (6.35 mmoles). A paper chromatographic analysis using 1-BuOH–AcOH–water (5:1:4) as the solvent showed that products consist of at least 6 compounds. After removal of the solvent under red press the residue was extracted with boiling benzene (100 ml). Evaporation of the benzene extract left

crystals (40 mg, 5.5%) which were identified as acetamide (by IR). The residue insoluble in benzene was dissolved in hot water (50 ml) and the soln was treated with Norite. On cooling the soln deposited crystals (0.41 g, 22%), which were identified as V (by IR). No other products could be isolated in a pure form.

REFERENCES

- ¹ Part XX, T. Matsuura, Y. Kitaura and R. Nakashima, *Tetrahedron* **24**, 6601 (1968).
- ² For a review, see D. Shugar and A. D. McLaren, *Photochemistry of Proteins and Nucleic Acids*, Pergamon Press Inc., New York (1964).
- ³ E. Seaman, L. Levine and H. Van Vanukis, *Biochem.* **5**, 1216 (1966) and Refs cited therein.
- ⁴ H. Van Vanukis, E. Seaman, L. Kahan, J. W. Kappler and L. Levine, *Biochem.* **5**, 3986 (1966).
- ⁵ J. S. Sussenbach and W. Berends, *Biochem. Biophys. Res. Commun.* **15**, 263 (1964).
- ⁶ J. S. Sussenbach and W. Berends, *Biochim. Biophys. Acta* **95**, 184 (1965).
- ⁷ K. S. Sastry and M. P. Gordon, *Ibid.* **129**, 42 (1966).
- ⁸ C. W. Carter, *Biochem. J.* **22**, 575 (1928).
- ⁹ M. I. Simon and H. Van Vanukis, *Arch. Biochem. Biophys.* **105**, 197 (1964).
- ¹⁰ K. Zenda, M. Saneyoshi and G. Chihara, *Chem. Pharm. Bull.* **13**, 1108 (1965).
- ¹¹ E. C. B. Anmann and V. H. Lynch, *Biochim. Biophys. Acta* **120**, 181 (1966).
- ¹² J. Fellig, *Science* **119**, 129 (1954).
- ¹³ A. Schittenhelm and K. Warnat, *Z. Physiol. Chem.* **171**, 174 (1927).
- ¹⁴ H. Brandenberger and R. H. Brandenberger, *Helv. Chim. Acta* **37**, 2207 (1955).
- ¹⁵ S. C. Hartman and J. Fellig, *J. Am. Chem. Soc.* **77**, 1051 (1955).
- ¹⁶ G. Weber, *Biochem. J.* **47**, 114 (1948).
- ¹⁷ J. S. Sussenbach and W. Berends, *Biochim. Biophys. Acta* **76**, 154 (1963).
- ¹⁸ C. S. Foote, S. Wexler and W. Ando, *Tetrahedron Letters* 4111 (1965) and Refs cited therein.
- ¹⁹ M. Morita and S. Kato, *Symposium on Photochemistry*, Abstracts of Papers, p. 31. Osaka (1966).
- ²⁰ T. Matsuura and I. Saito, *Tetrahedron Letters* 3273 (1968).
- ²¹ For an extensive review, see K. Gollnick and G. O. Schenck, in *1,4-Cycloaddition reactions* (Edited by J. Hamer) p. 255. Academic Press, New York (1967).
- ²² P. de Mayo and S. T. Reid, *Chem. & Ind.* 1576 (1962).
- ²³ H. W. Kurtz and H. Schechter, *Chem. Comm.* 689 (1966).
- ²⁴ H. H. Wasserman and M. B. Floyd, *Tetrahedron Suppl.* No. 7, 441 (1966).
- ²⁵ E. H. Rodd, *Chemistry of Carbon Compounds* 1VB; p. 1297, Elsevier, Amsterdam (1959).
- ²⁶ E. H. White and M. C. J. Harding, *Photochem. Photobiol.* **4**, 1129 (1965).
- ²⁷ C. Dufraisse, G. Rio, A. Ranjon and O. Pouchot, *C.R. Acad. Sci. Paris*, **261**, 3133 (1965).
- ²⁸ G. Rio, A. Ranjon and O. Pouchot, *Ibid.* **263**, 634 (1966).
- ²⁹ H. H. Wasserman and A. Liberles, *J. Am. Chem. Soc.* **82**, 2086 (1960).
- ³⁰ Z. Yoshida and M. Kato, *Ibid.* **76**, 311 (1954); *J. Chem. Soc. Japan* **75**, 112 (1954).
- ³¹ H. Biltz and E. Giesler, *Ber. Dtsch. Chem. Ges.* **46**, 3410 (1913).
- ³² H. Brederick, I. Hennig and W. Pfeleiderer, *Chem. Ber.* **86**, 321 (1953).