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MECHANISM AND STOICHIOMETRY IN THE RADIOLYTIC OXIDATION
OF PURINES AND AMINOPURINES IN AQUEOUS SOLUTION

John Holian and Warren M. Garrison

May 1967

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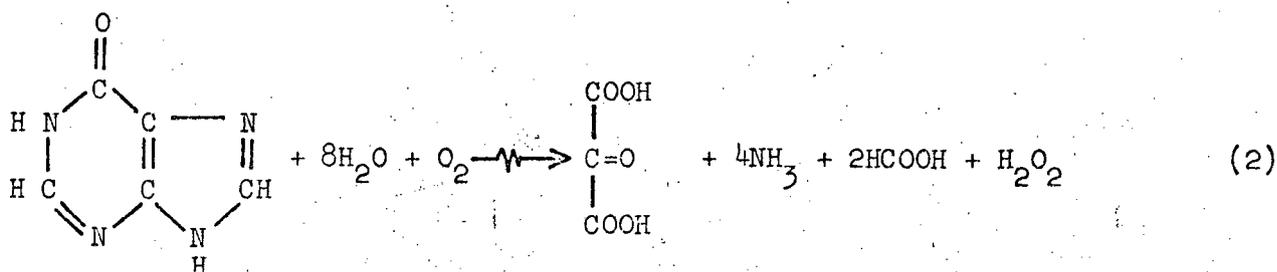
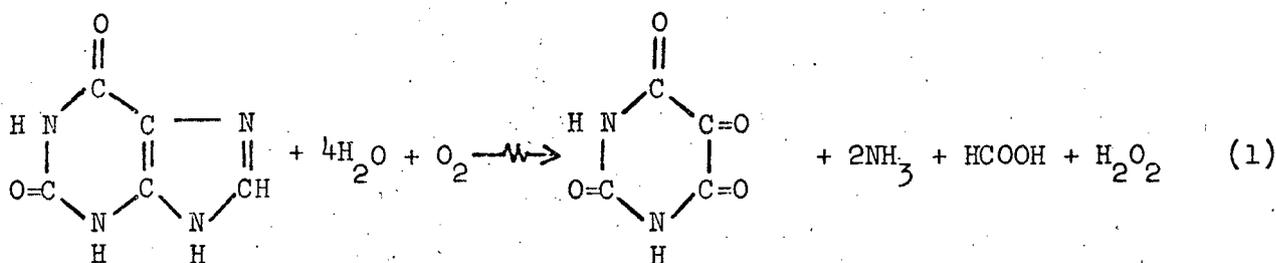
MECHANISM AND STOICHIOMETRY IN THE RADIOLYTIC OXIDATION OF PURINES AND
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We recently found that a principal action of γ -rays on the typical purine bases, xanthine, hypoxanthine, and uric acid in oxygenated solution results in a preferential oxidation at the 4,5 carbon-carbon double bond.¹ Oxidation of the base, B, may be represented in terms of OH addition to the 4,5 position, $B + OH \rightarrow \dot{B}(OH)$, followed by $\dot{B}(OH) + O_2 \rightarrow B(OH)\dot{O}_2$, $HO_2 + B(OH)\dot{O}_2 \rightarrow B(OH)OOH + O_2$ where, $B(OH)OOH$, represents a labile hydroxyhydroperoxide intermediate which undergoes hydrolytic degradation to yield characteristic carbonyl products; the overall stoichiometry for the radiolytic oxidation of xanthine and hypoxanthine is given by equations 1 and 2 respectively.

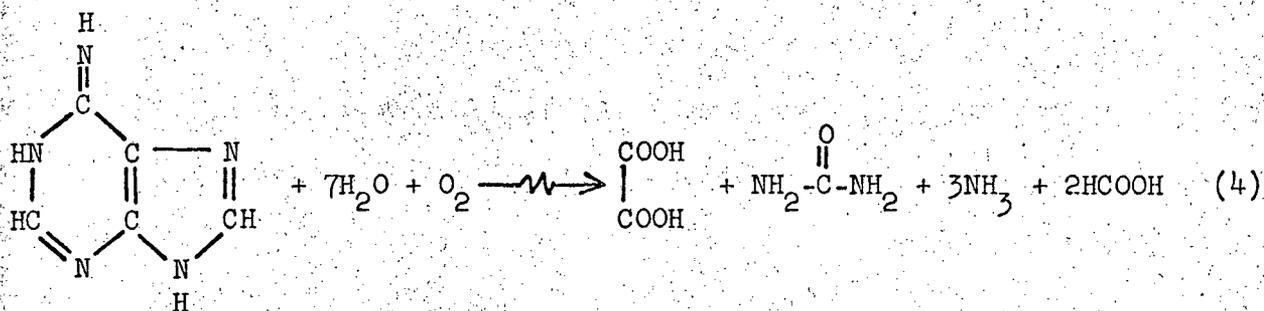
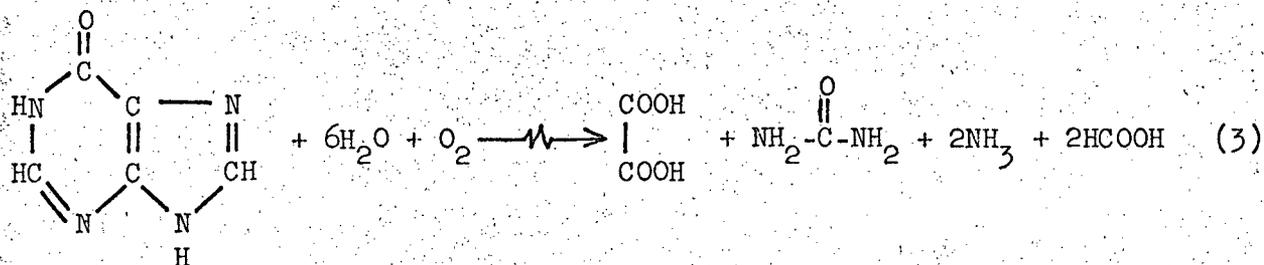


*This work was done under the auspices of the U.S. Atomic Energy Commission.

The 100-eV yield (G value) for carbonyl production in these systems depends on the chemical composition of the purine in question. With xanthine, the addition of OH at the 4,5 position is essentially quantitative, i.e., $G(\text{alloxan}) \sim 2$ which value approximates the 100-eV yield of OH radicals formed in water under γ -rays, $G_{\text{OH}} = 2.5$.² With the amino purine, adenine, the carbonyl yield is quite low, $G(\text{mesoxalic acid}) = 0.45$; other purine bases give intermediate $G(\text{carbonyl})$ values. It is clear that there is an alternative path for oxidation of the purine nucleus that does not yield carbonyl products.

We have just completed a quantitative study of reaction stoichiometry in the γ -radiolysis of oxygenated solutions of hypoxanthine and adenine and find in these systems that a second mode of oxidation at the 4,5 position yields oxalic acid and urea as characteristic products. The irradiated solutions were subjected to mild acid hydrolysis (2N HCl, 90°C, 2 hr) to effect the quantitative release of the various product species. Oxalic acid was identified and determined by colorimetric³ and by gas-chromatographic^{4,5} methods. Urea was assayed colorimetrically⁶; preliminary identification and assay involved the use of urease.⁷ Ammonia was measured after Conway.⁷ Methods used in the identification and determination of carbonyl products have been described.¹ Typical data are summarized in table 1.

The formation of these observed products in the indicated yields is consistent with a formulation in which all reaction is initiated by OH attack at the 4,5 position. That is, the hydrolytic degradation of $B(\text{OH})\text{OOH}$ as represented in equations 1 and 2 occurs in parallel and in competition with a more extensive overall degradation which is typified below for hypoxanthine and adenine⁸



For both hypoxanthine and adenine, the data of table 1 give the value $G(-B) - [G(\text{oxalic}) + G(\text{mesoxalic})] \sim 0.5$ which represents an upper limit for the yield of OH reaction at sites other than the 4,5 position.

REFERENCES AND FOOTNOTES

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2. C. J. Hochanadel and R. Casey, Rad. Research 25, 198 (1965).
3. M. Paget and R. Berger, Bull. Biol. Pharm. 70 (1938).
4. E. T. Oakley, L. Weissbecker, and F. E. Resnick, Anal. Chem. 37, 380 (1965).
5. We are indebted to Mr. H. A. Sokol for the gas-chromatographic determinations.
6. R. M. Archibald, J. Biol. Chem. 157, 507 (1945).
7. E. J. Conway, Microdiffusion Analysis and Volumetric Error (Crosby Lockwood and Son, Ltd., London (1962)).
8. Although the radiolytic oxidation of the purine bases can be satisfactorily represented in terms of the degradations of the labile product $B(OH)OOH$, it is to be noted that stoichiometrically equivalent reactions of the radical intermediate $B(OH)\dot{O}_2$ may also be involved.

Table 1. Product yields in the γ -ray induced oxidation of hypoxanthine and adenine in oxygenated solution.^a

product	G (mol/100 eV)	
	hypoxanthine	adenine
-base	2.4 ^b	2.1 ^c
ammonia	8.8	9.6
urea	0.4	0.5
oxalic acid	0.75	1.2
mesoxalic acid	1.1	0.45 ^d

^a 10^{-3} M, pH 1.2 (adjusted with H_2SO_4).

^b Independent of hydrogen ion concentration over the range pH 1 to 7.

^c Decreases to $G(-B) = 1.2$ at pH 7.

^d Represents a combined yield of mesoxalic acid plus a lesser amount of glyoxylic acid.

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