Mechanism and Stoicheiometry in the Radiolytic Oxidation of Purines and Aminopurines in Aqueous Solution

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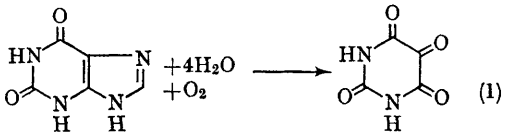
WE recently found that action of γ-rays on the typical purine bases, xanthine, hypoxanthine, and uric acid in oxygenated solution results in a preferential oxidation at the 4,5 carbon–carbon double bond.[1] Oxidation of the base, B, may be represented in terms of OH addition to the 4,5-position, $B + OH \rightarrow \dot{B}(OH)$, 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 stoicheiometry for the radiolytic oxidation of xanthine and hypoxanthine is given by equations (1) and (2) respectively.

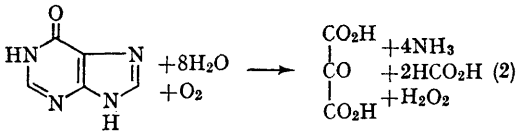
The 100ev yield (*G*-value) for carbonyl production in these systems depends on the chemical

composition of the purine in question. With xanthine, the addition of OH at the 4,5-position is essentially quantitative, *i.e.*, G(alloxan) ~2 which value approximates the 100ev yield of OH radicals formed in water under γ-rays, $G_{OH} = 2{\cdot}5$.[2] With the aminopurine, adenine, the carbonyl yield is quite low, G(mesoxalic acid) = 0·45; other purine bases give intermediate G(carbonyl) values. It is clear that there is an alternative path for oxidation of the purine nucleus that does not yield carbonyl products.



(1)

$+2NH_3+HCO_2H+H_2O_2$



(2)

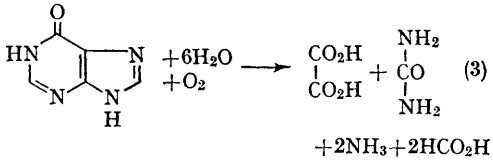
We have just completed a quantitative study of reaction stoicheiometry in the γ-radiolysis of oxygenated solutions of hypoxanthine and adenine and find in these systems that a second mode of oxidation at the 4,5-position yields oxalic acid and urea as characteristic products. The irradiated solutions were subjected to mild acid hydrolysis (2N-HCl, 90°, 2 hr.) to effect the quantitative release of the various product species. Oxalic acid was identified and determined by colorimetric[3] and gas-chromatographic[4]† methods. Urea was assayed colorimetrically;[5] preliminary identification and assay involved the use of urease.[6] Ammonia was measured according to the method of Conway.[6] Methods used in the identification and determination of carbonyl products have been described.[1] Typical data are summarized in the Table.

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(OH)OOH as represented in equations 1 and 2 occurs in parallel and in competition with a more extensive degradation which is typified below for hypoxanthine and adenine.‡

TABLE. *Product yields in the γ-ray-induced oxidation of hypoxanthine and adenine in oxygenated solution*[a]

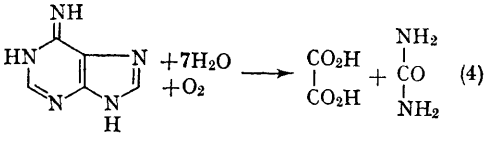
Product	Hypoxanthine	Adenine
	G (mol. 100 ev)	
—Base	2·4[b]	2·1[c]
Ammonia	8·8	9·6
Urea	0·4	0·5
Oxalic acid	0·75	1·2
Mesoxalic acid ..	1·1	0·45[d]

[a] 10^{-3} M, pH 1·2 (adjusted with H_2SO_4).
[b] Independent of hydrogen ion concentration over the range pH 1—7.
[c] Decreases to $G(\pm B) = 1{\cdot}2$ at pH 7.
[d] Represents a combined yield of mesoxalic acid plus a lesser amount of glyoxylic acid.



(3)

$+2NH_3+2HCO_2H$



(4)

$+3NH_3+2HCO_2H$

For both hypoxanthine and adenine, the data given in the Table give the value $G(-B)-[G(\text{oxalic}) + G(\text{mesoxalic})] \sim 0{\cdot}5$ which represents an upper limit for the yield of OH reaction at sites other than the 4,5-position.

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† We are indebted to Mr. H. A. Sokol for the gas-chromatographic determinations.
‡ 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 stoicheiometrically equivalent reactions of the radical intermediate $B(OH)\dot{O}_2$ may also be involved.

[1] J. Holian and W. M. Garrison, *J. Phys. Chem.*, 1967, **71**, 462.
[2] C. J. Hochanadel and R. Casey, *Radiation Res.*, 1965, **25**, 198.
[3] M. Paget and R. Berger, *Bull. Biol. Pharm.*, 1938, 70.
[4] E. T. Oakley, L. Weissbecker, and F. E. Resnick, *Analyt. Chem.*, 1965, **37**, 380.
[5] R. M. Archibald, *J. Biol. Chem.*, 1945, **157**, 507.
[6] E. J. Conway, "Microdiffusion Analysis and Volumetric Error", Crosby Lockwood, London, 1962.