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Oxidative degradation of nucleic acids

D G Knorre, O S Fedorova, E I Frolova

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Abstract. The chemical mechanisms of the oxidative degradation of nucleic acids are surveyed and described systematically. The bibliography includes 141 references.

I. Introduction

The oxidative degradation of nucleic acids, induced by active forms of oxygen, is of interest from several points of view.

Firstly, in aerobic organisms biochemical processes and various physical and chemical influences (ionising radiation, UV light, etc.) result in the formation of free radicals and activated oxygen species: the superoxide radical-ion $O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and the free hydroxyl radical ($^{\cdot}OH$).[1–4] The damage to DNA induced by them plays an important role in mutagenic, carcinogenic, ageing, and other processes.

Secondly, many oxygen-dependent antitumour preparations (bleomycin) and low-molecular-weight complexes of variable-valence metal ions (Cu-1,10-phenanthroline, Fe-EDTA, Fe- and Mn-porphyrins, etc.) catalyse effectively the cleavage of nucleic acids with participation of active forms of oxygen.[5–9]

Thirdly, investigators have devoted increasing attention recently to designing synthetic molecules capable of binding specifically to DNA and cleaving the sugar phosphate skeleton like natural antibiotics of the type of bleomycin. Such compounds might find applications in the creation of the so called "chemical restrictases", i.e. reagents which, like restriction endonucleases, cleave nucleic acids in the vicinity of definite sequences, and of chemotherapeutic preparations which cause selective damage to nucleic acids.[10]

D G Knorre, O S Fedorova, E I Frolova Novosibirsk Institute of Bioorganic Chemistry, Siberian Division of the Russian Academy of Sciences, prosp. akad. Lavrent'eva 8, 630090 Novosibirsk, Fax (7-383-2) 35 16 65

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II. Activated oxygen species

Molecular oxygen is a four-electron oxidant and its stepwise reduction to water may be represented schematically as follows:[11–13]

$$E^0_{O_2/O_2^{\cdot-}} = -0.16\,V; \quad E^0_{HO_2^{\cdot}/HO_2^-} = 0.74\,V;$$

$$E^0_{HO^{\cdot}/HO^-} = 1.9\,V;$$

$$E^0_{O_2/HO_2^{\cdot}} = 0.13\,V; \quad E^0_{HO_2^{\cdot}/H_2O_2} = 1.43\,V;$$

$$O_2 \xrightarrow{e^-,H^+} HO_2^{\cdot}(H^+ + O_2^{\cdot-}) \xrightarrow{e^-,H^+} H_2O_2(H^+ + HO_2^-) \xrightarrow{e^-} HO^{\cdot} + OH^-$$

$(E^0)'' = 0.3$ V; e^-, H^+; $(E^0)' = 0.815$ V; $2H_2O$

In reduction to both H_2O and to H_2O_2, molecular oxygen in aqueous solution is a powerful oxidant—the values of $(E^0)'$ are respectively 0.815 and 0.3 V. However, direct oxidation by oxygen, both two-electron and four-electron oxidation, hardly occurs. This is associated primarily with the fact that the O_2 molecule has a triplet ground state whereas the majority of organic substrates and their oxidation products have singlet ground states. The direct reaction of triplet O_2 with the substrate molecule is therefore spin-forbidden.

Several ways of overcoming this limitation are known: (1) successive one-electron reduction of the oxygen molecule to H_2O in conformity with a scheme such that each individual elementary reaction is spin-allowed; (2) activation of oxygen by enzymes or by the formation of complexes with transition metal ions; (3) transition of O_2 molecules to the excited singlet state.

The successive one-electron reduction of O_2 can be most simply achieved using variable-valence metal ions. Iron and copper ions are usually employed for this purpose. The process can be described by the following scheme:

$$M^{(n-1)+} + O_2 \rightarrow M^{n+} + O_2^{\cdot-}(HO_2^{\cdot}) \quad (1)$$

$$M^{(n-1)+} + O_2^{\cdot-}(HO_2^{\cdot}) \rightarrow M^{n+} + HO_2^-(H_2O_2) \quad (2)$$

$$M^{(n-1)+} + H_2O_2 \rightarrow M^{n+} + HO^{\cdot} + OH^- \quad (3)$$

$$M^{(n-1)+} + HO^{\cdot} \rightarrow M^{n+} + OH^- \quad (4)$$

The involvement of the metal ion is particularly significant in the first stage, because the reduction of O_2 to $O_2^{\cdot-}$ is characterised by a low oxidation–reduction potential $E^0 = -0.16$ V, i.e. is thermodynamically unfavourable.[11] Since the use of small amounts of metal ions is preferable, the occurrence of these reactions requires the presence of a coupled, readily oxidisable substrate H_2A (ascorbic acid, thiols, etc.) for the reduction of the metal ion to the lowest oxidation state:

$$M^{n+} + H_2A \text{ (or } HA^{\cdot}) \rightarrow M^{(n-1)+} + HA^{\cdot} \text{ (or } A + H^+) \quad (5)$$

Reaction (1) is reversible and $M^{(n-1)+}$ can be partly regenerated by the reaction

$$M^{n+} + O_2^{\cdot-} \rightarrow M^{(n-1)+} + O_2 \quad (6)$$

The rate constants for the competing reactions (2) and (6) are as a rule similar and are determined to a large extent[14] by the values of E^0 for the $M^{n+}/M^{(n-1)+}$ couple. Apart from reaction (2), the superoxide radical can be converted into hydrogen peroxide also via the disproportionation reaction (7) or via a reaction with an auxiliary reductant [reaction (8)]:

$$2O_2^{\cdot-} + 2H^+ \rightarrow O_2 + H_2O_2 \quad (7)$$

$$O_2^{\cdot-}(HO_2^{\cdot}) + H_2A \rightarrow HO_2^-(H_2O_2) + HA^{\cdot} \quad (8)$$

The hydrogen peroxide formed subsequently enters into reaction (5). The OH radical is the strongest oxidant among all the oxygen species examined and not only can it be reduced by metal ions [reaction (4)] but it can also accept an electron or can abstract a hydrogen atom from many organic substrates:

$$HO^{\cdot} + HX \rightarrow OH^- + H^+ + X^{\cdot} \quad (9)$$

Molecular oxygen reduction processes with participation of variable-valence metal ions have been described in greater detail in various other reviews, for example, those of Aust et al.[15] and Halliwell and Gutteridge.[16]

The one- and two-electron reduction of molecular oxygen in living organisms takes place in the course of various biochemical processes.[4] The formation of the superoxide radical is catalysed by the majority of NAD(P)H-dependent oxidases functioning within the composition of membrane complexes[17] and by flavin-dependent oxidases such as xanthine oxidase.[18] In the normal functioning of the mitochondrial respiratory chain, $O_2^{\cdot-}$ is formed in small amounts, but under the conditions of various disturbances in the operation of mitochondria the generation of $O_2^{\cdot-}$ as well as other active oxygen species increases sharply.[19]

Oxygen can be reduced to $O_2^{\cdot-}$ also by certain long-lived organic radicals (for example, semiquinone radicals) formed in the enzymic or photochemical reduction of anthraquinone antibiotics, riboflavin, and certain other compounds.[20]

Hydrogen peroxide can be formed via the disproportionation reaction (7), which can be both spontaneous or can be catalysed by special enzymes—superoxide dismutases. The direct enzymic reduction of O_2 to H_2O_2 without the intermediate formation of $O_2^{\cdot-}$ is characteristic of metabolic processes occurring in neutrophils and peroxisomes[21] and occurs, for example, in reactions catalysed by aminooxidases.[22]

The generation of hydroxyl radicals *in vivo* is apparently due primarily to the reduction of O_2 or H_2O_2 by transition metal ions.[6, 23, 24]

Active oxygen species are also formed under various external influences on living organisms, for example when the latter are acted upon by ionising radiation. The radiolysis of water[25–27] results in the formation of the following products:[27]

$H_2O \rightarrow$	$HO^{\cdot}$,	e_{aq}^-,	$H^{\cdot}$,	H_2O_2,	H_2	$H^+(H_3O^+)$
G	2.7	2.7	0.55	0.7	0.45	2.7

where G is the radiation-chemical yield (in dilute solutions) equal to the number of species formed per 100 eV of absorbed energy.

In aerated aqueous solutions, the solvated electron e_{aq}^- and $H^{\cdot}$ interact very rapidly with O_2, affording the superoxide radicals $O_2^{\cdot-}$ or $HO_2^{\cdot}$. Both H_2O_2 and $O_2^{\cdot-}$ can be reduced subsequently by the transition metal ions to OH radicals.

As a result of all these factors, the principal species in aerated solutions responsible for the oxidation of various substrates under the action of ionising radiation are OH radicals (>90%). In the study of reactions with participation of OH radicals, solutions are radiolysed in the presence of nitrous oxide N_2O, which promotes the conversion of e_{aq}^- into the OH radical.

Among the intermediates in the reduction of O_2 examined, OH radicals play the main role in the oxidative degradation of DNA. Under physiological conditions, the superoxide radical-ion is incapable of oxidising organic substrates. With the use of various systems for the generation of $O_2^{\cdot-}$, it has been shown that[28] it does not participate in the oxidative degradation of nucleic acids. Likewise H_2O_2 does not oxidise DNA under physiological conditions.[29–32] However, the introduction into the same systems of transition metal ions, which catalyse the reduction to the OH radicals of both the superoxide radical-ion and hydrogen peroxide via reactions (2), (3), and (7), leads to the degradation of nucleic acids.[28–30]

The OH radical is a powerful oxidant and is highly reactive. Under physiological conditions, it is able to abstract the hydrogen atom from the C–H bonds [reaction (10)] or it adds to unsaturated C=C double bonds [reaction (11)]:

$$R\text{-}H + \dot{O}H \longrightarrow \dot{R} + H_2O \quad (10)$$

$$\text{>C=C<} + \dot{O}H \longrightarrow \text{>}\dot{C}\text{–}C(OH)\text{<} \quad (11)$$

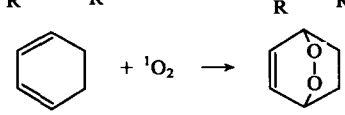
The rates of addition to the double bonds are usually higher than the rates of abstraction of the hydrogen atom from C–H bonds.[33]

The OH radicals interact with nucleic acids and model compounds such as nucleosides and nucleotides, the rate constant for the corresponding reactions being close to the diffusion-controlled values (Table 1).[25, 26, 33]

The dominant processes are addition to the double bonds of the heterocycles and the abstraction of hydrogen atoms from the C–H bonds of the pentose with formation of the corresponding free radicals. In aerated solutions, these radicals interact rapidly with oxygen O_2 to form peroxyl radicals. The subsequent reactions, leading ultimately to the modification of the heterocycles or to the cleavage of the pentose phosphate skeleton, will be examined in the later sections of this review.

Singlet oxygen can be formed as a result of photosensitisation and also as a side product in many biological oxidation processes.[34] Despite its considerable reactivity, singlet oxygen cannot react with alkanes and many aromatic systems. Interaction with alkenes to form allyl peroxides [reaction (12)] or endoperoxides [reaction (13)] is most characteristic of this species:[35]

$$R_2C{=}CR\text{–}CHR_2 + {}^1O_2 \longrightarrow R\text{–}C(OOH)(R)\text{–}C(CR_2)\text{–}R \quad (12)$$



$$+ {}^1O_2 \longrightarrow \quad (13)$$

It has been shown that singlet oxygen does not oxidise the sugar phosphate skeleton in nucleic acids,[36, 37] but does react selectively with guanine residues, forming as a result modified fragments in which the sugar phosphate skeleton may be cleaved on treatment under alkaline conditions.[28]

The reduction of oxygen by variable-valence metal ions can occur with preliminary coordination of O_2 or species corresponding to its partial reduction. This results in the formation of oxo-complexes of metals, which are in high-valence states (Fe or Mn in the +4 or +5 oxidation states, Cu in the +3 state, etc.).[38] Such complexes can behave directly as oxidants or can generate OH radicals with transition of the metal to a lower oxidation state. The latter version has been postulated, for example, for the Fe·ATP complexes:[39]

$$Fe^{II} \cdot ATP + H_2O_2 \rightarrow \overset{O}{\underset{\|}{Fe^{IV}}} \cdot ATP + H_2O \quad (14)$$

$$\overset{O}{\underset{\|}{Fe^{IV}}} \cdot ATP + H^+ \rightarrow Fe^{III} \cdot ATP + {}^{\cdot}OH \quad (15)$$

Such oxo-complexes have been isolated and characterised by various chemical and physical methods for iron-porphyrins.[40-46] It has been shown that they can be obtained by the two-electron oxidation of iron(III)-porphyrins by peracids, hydroperoxides, or iodosobenzene (Por = porphyrin):

$$Fe^{III} \cdot Por + PhIO \rightarrow \overset{O}{\underset{\|}{Fe^{IV}}} \cdot Por^{\cdot +} + PhI$$

(RCO$_3$H, ROOH) (RCOOH, ROH)

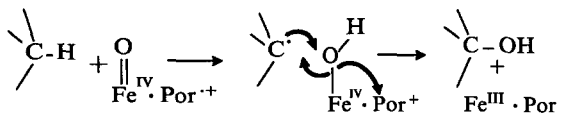
The structure of the oxo-complex containing iron in the +4 oxidation state and a porphyrin radical-cation stabilised by delocalisation in the π-system of the macrocycle has been generally accepted for iron-porphyrins.[42, 46]

The formation of high-valence metal oxo-species has been observed also for the porphyrin complexes of other transition metals. However, in the manganese and chromium complexes[47] the macrocyclic ligand does not participate in the oxidation and the metal is in the +5 oxidation state:

$$Mn^{III} \cdot Por + PhIO \rightarrow \overset{O}{\underset{\|}{Mn^{V}}} \cdot Por + PhI$$

(RCO$_3$H, ROOH) (RCOOH, ROH)

Such oxo-complexes of high-valence metals are apparently weaker oxidants than OH radicals.[48] The following types of reactions are characteristic of them: the insertion of an oxygen atom in C–H bonds (hydroxylation) or addition to C=C bonds (epoxidation). It is believed that the first stage of the hydroxylation of alkanes involves the abstraction of the H atom from a C–H bond with subsequent transfer of the OH radical from the oxo-complex to the alkyl radical via the following mechanism:[49-51]

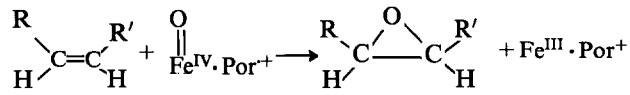


The selectivity of the reaction is achieved mainly as a result of the orientation of the oxygen atom in the complex relative to particular C–H bonds in the molecule being oxidised.[52] The hydroxylation of aldehydes with formation of carboxylic acids takes place in accordance with the same mechanisms.[53, 54]

Table 1. Rate constants for the reactions of OH radicals with nucleic acids and their components under neutral conditions.

Component	10^9 k/litre mol^{-1} s^{-1}
Heterocycles	
Thymine	5.6
Cytosine	4.7
Uracil	5.2
Adenine	4.4
Pentoses	
Ribose	1.6
Ribose-5′-phosphate	1.3
Nucleosides	
Thymidine	4.8
Cytidine	4.8
Uridine	4.3
Adenosine	4.0
Nucleotides	
TMP	5.3
CMP	4.5
dCMP	4.4
AMP	3.0
dAMP	3.5
dGMP	5.0
Polynucleotides	
poly (C)	1.2
poly (U)	1.25
poly (A)	0.9
poly (U+A)	0.5
DNA	0.3–0.8

The epoxidation of alkenes is achieved by the insertion of an oxygen atom in the C=C double bond:[47]



The oxidative degradation of DNA constitutes the basis of the action of the group of antibiotics of the class of bleomycin (Blm). It has been suggested[7, 55-56] that species of the type Blm·Fe^{V}=O participate in this process.

The hydrogen peroxide–Cu-1,10-phenanthroline complex system has found extensive applications in the oxidative degradation of DNA. There are two points of view on the mechanism of the oxidative cleavage of DNA in this system. According to one of them, a species bound to the metal and equivalent to the OH radical is responsible for the oxidation.[57, 58] According to the other, more widely adopted point of view, the Cu-1,10-phenanthroline complex is bound to DNA and interacts with H_2O_2 in the presence of reductants, generating OH radicals[8, 59, 60] via a mechanism analogous to that described by reactions (14) and (15) for the Fe·ATP complexes.[39] As a result, both free OH radicals and such radicals bound to the metal function in the system.

Thus, summarising all the factors described above, one may say that the free hydroxyl radicals, which are the products of the three-electron reduction of molecular oxygen, and, in certain systems, coordinated oxygen species in the form of oxo-complexes of metals in the highest oxidation states are primarily responsible for the degradation of nucleic acids under various oxidative conditions.

III. Oxidation of the heterocycles in nucleic acids

The oxidative modification of nucleic acid heterocycles by hydroxyl radicals has been studied in greatest detail under the conditions of γ-radiolysis, because this method of generation of OH radicals makes it possible to eliminate the involvement of other active species. These studies led to the isolation of a large number of products of the modification of the heterocycles and the mechanisms of the formation of some of them have been established. These investigations were carried out mainly on the free heterocycles and nucleosides. Further studies on nucleic acids showed that the data obtained on model compounds can be to a large extent transferred to the oxidation of polymeric molecules. The greatest progress has been achieved in the study of the oxidation of pyrimidine heterocycles.[25, 26, 61]

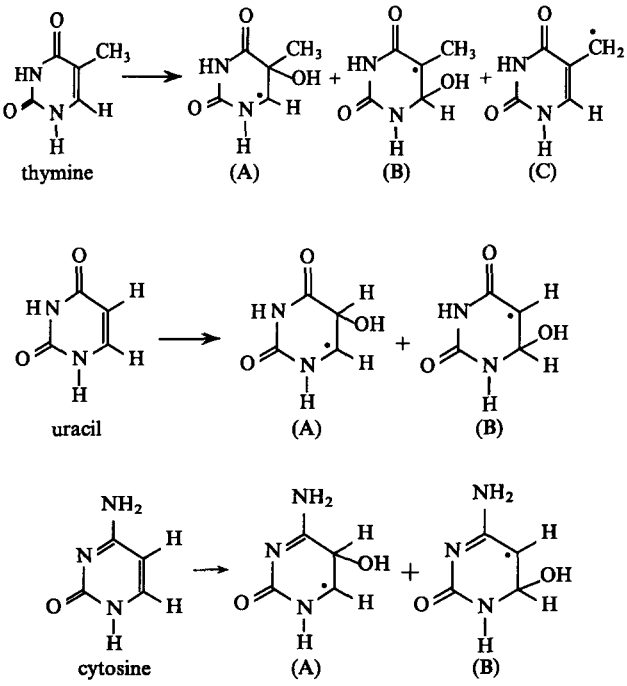
1. Anaerobic oxidation of pyrimidine heterocycles

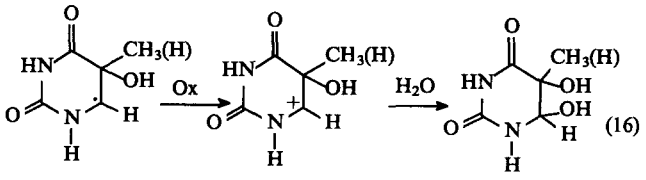
Hydroxyl radicals are electrophiles and attack preferentially sites with a high electron density, forming free radicals derived from the heterocycles. The structures of these radicals have been largely characterised on the basis of the structures of the products formed on γ-radiolysis in the presence of spin trapping agents—2-methyl-2-nitrosopropane or α-(1-hydroxy-4-pyridyl)-*N*-*tert*-butyl nitrone.[62-66] It was established that, as a result of the reaction with pyrimidines, the OH radicals add to the C(5) or C(6) carbon atoms, forming type (A) and type (B) radicals, while for thymine the abstraction of a H atom from the methyl group at the C(5) atom with formation of a type (C) radical is also observed.

The three types of free radicals formed differ in their oxidation–reduction properties. The type (A) radicals, which are α-aminoalkyl derivatives, have the properties of reductants and can be determined quantitatively by reaction with oxidants such as benzoquinone, tetranitromethane, and tetracyanoferrate(III). The type (C) radical of thymine is also a reductant, although a weaker one, but it can be determined by reactions with strong oxidants. On the other hand, the type (B) radicals have oxidising properties by virtue of the presence of the mesomeric form—the oxyl radical—and can be determined with the aid of reductants such as *N,N,N′,N′*-tetramethylphenylenediamine and ascorbic acid. Such differences in the oxidation–reduction properties of the radicals formed made it possible to devise a method for their quantitative determination based on pulse radiolysis in the presence of the corresponding oxidants and reductants with the subsequent determination of their consumption.[67] It has been established with the aid of this method[68-70] that the preferential addition of the OH radicals to the C(5) position in the heterocycles is characteristic of all pyrimidines: to the extent of 87% for cytosine, 80% for uracil, and 56% for thymine. This is due to the greater electron density on the C(5) carbon atom. Only 10% of the OH radicals add to C(6) in the case of cytosine, 20% for uracil, and 35% for thymine. Less than 9% of the OH radicals participate in the abstraction of a hydrogen atom from the methyl group of thymine (Table 2).

In the oxidation of type (A) radicals of thymine or uracil, the carbonium ion **1** is formed, the rapid solvolysis of which leads to the quantitative formation of glycols, namely the *cis*- or *trans*-form of 5,6-dihydroxy-5,6-dihydrothymine **2** or 5,6-dihydroxy-5,6-dihydroxyuracil **3**, by the following reaction:

The equality of the amount of type (A) radicals, estimated by the method of pulse radiolysis, and of the amount of the corresponding thymine or cytosine glycols, isolated chromatographically (Table 2), permitted the conclusion that the pyrimidine glycols are formed exclusively by the reaction involving the oxidation of type (A) radicals [reaction (16)].[68]



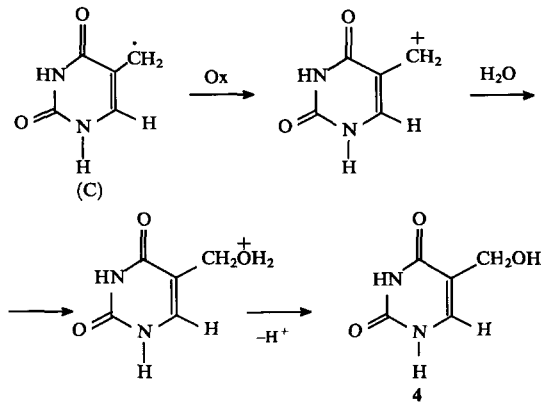


(16)

Table 2. The yields and oxidation-reduction properties of free radicals of the pyrimidines formed on reaction with hydroxyl radicals.[67-69]

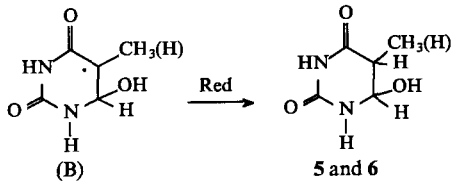
Heterocycle	Radical	Oxidation–reduction properties	Yield/(%[OH])	
			pulse radiolysis	analysis of products
Thymine	(A)	reductant	56	57
	(B)	oxidant	35	–
	(C)	weak reductant	9	–
Uracil	(A)	reductant	80	77
	(B)	oxidant	20	–
Cytosine	(A)	reductant	87	–
	(B)	oxidant	10	–

The oxidation of the type (C) thymine radical leads to the formation of 5-hydroxymethyluracil **4**.[71, 72]



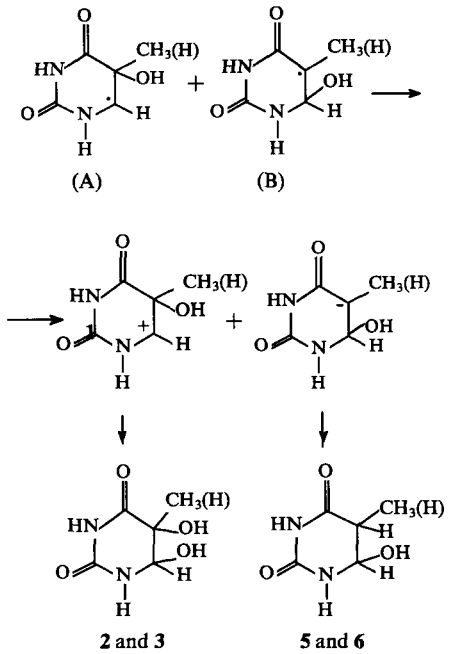
(C) **4**

The reduction of the type (B) thymine or uracil radicals leads to the formation of 6-hydroxy-5,6-dihydrothymine **5** or 6-hydroxy-5,6-dihydrouracil **6** respectively:



(B) **5** and **6**

Since the oxidation–reduction properties of the radicals differ, it follows that, in the absence of exogenic oxidants or reductants, a type (A) radical may be oxidised by type (B) radicals to a carbonium ion with the subsequent formation of the glycols **2** or **3** and the 6-hydroxy-derivatives **5** or **6**.

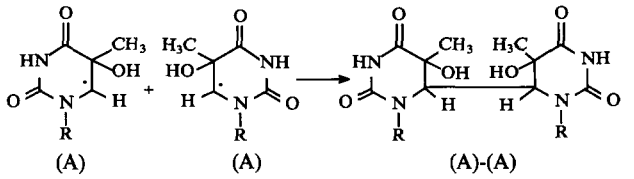


(A) (B)

2 and **3** **5** and **6**

Under these conditions, the yield of glycols is appreciably lower and amounts to one third of the yield of type (B) radicals, which are consumed also in the dimer formation reaction. The dimerisation reaction is likewise characteristic of other types of radicals [type (A) and type (C) radicals]:[73]

(B) + (B) → dimer (B),
(A) + (A) → dimer (A),
(C) + (C) → dimer (C),

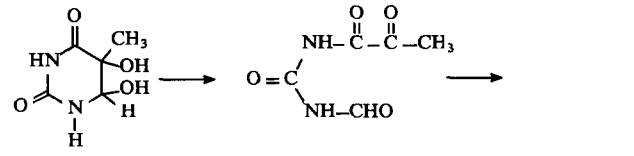


(A) (A) (A)-(A)

The cytosine radicals behave similarly.[68] The *cis*- and *trans*-glycols of cytosine **7**, C–C cytosine dimers, 5-hydroxycytosine **8**, and, to a lesser extent, 6-hydroxycytosine **9** are formed in the course of further reactions of the cytosine radicals. Furthermore, the deamination of cytosine is observed, which results in the formation of uracil and the uracil glycols **3**.

Thus the main products of the oxidation of pyrimidines are glycols, hydroxyhydrates, and dimers, while in the oxidation of thymine 5-hydroxymethyluracil is also formed. The formation of these products in the γ-radiolysis of deaerated solutions of pyrimidines has been noted in studies by different investigators, although with some differences in their estimated amounts (Tables 3–5).[74–80]

Apart from these products, the radiolysis of pyrimidines in deaerated solutions (particularly for high doses) involves the formation of various urea derivatives: N^1-formyl-N^2-pyruvylurea **10**, *N*-formylurea **11**, and urea **12**. Their formation can be accounted for by the further oxidative degradation of the glycols.[78]



2 **10**

11 **12**

Table 3. The products of the γ-radiolysis of thymine in N_2O-saturated aqueous solutions.

Product	G^{79}	G^{25}
cis-5,6-Dihydroxy-5,6-dihydrothymine	1.45	–
trans-5,6-Dihydroxy-5,6-dihydrothymine	0.81	0.32
5-Hydroxy-5,6-dihydrothymine	–	0.08
6-Hydroxy-5,6-dihydrothymine	0.13	0.10
5-Hydroxymethyluracil	0.22	0.27
5-Hydroxy-5-methylbarbituric acid	0.11	–
N^1-Formyl-N^2-pyruvylurea	0.12	0.14
Urea	0.19	–
Dimers	0.26	–
Degradation of thymine	3.9	2.71

Table 4. The products of the γ-radiolysis of uracil in N_2O-saturated aqueous solutions.

Product	G^{75}		G^{76}		
	pH 5	pH 8.1	pH 4	pH 6	pH 8
cis-5,6-Dihydroxyl-5,6-dihydrouracil	0.2	0.4	0.32	0.30	0.18
trans-5,6-Dihydroxy-5,6-dehydrouracil	0.5	0.4			
6-Hydroxy-5,6-dihydrouracil	0.5	0.3	–	–	–
Isobarbituric acid	0.1	0.1	0.44	0.29	0.18
N^1-Formylurea	–	–	0.12	0.52	0.16
Dimers	3.1	0.7	3.30	4.38	1.60
Dehydration of uracil	4.4	1.9	4.25	5.51	2.11

2. Aerobic oxidation of pyrimidine heterocycles

Under aerobic conditions, the type (A), (B), and (C) radicals combine with oxygen, the reaction having a diffusion-controlled rate constant ($k \approx 2 \times 10^9$ litre mol^{-1} s^{-1}), forming the corresponding peroxyl radicals [reaction (17)], the decomposition of which leads to the formation of stable products.[25, 26]

Studies carried out in recent years on simple model compounds made it possible to formulate general principles governing the decomposition of organic peroxy-radicals.[25, 26] It usually takes place via a bimolecular mechanism with formation of a short-lived intermediate—a tetroxide, which can redissociate at low temperatures into two peroxy-radicals, while at room temperature it decomposes rapidly to stable products. The number of reactions involved in the decomposition of the tetroxide is small. Four main processes have been distinguished:

(1) the formation of an alcohol molecule, a carbonyl compound, and an oxygen molecule [reaction (18)]—Russell's concerted mechanism;

(2) the formation of two molecules of a carbonyl compound and a H_2O_2 molecule [reaction (19)];

(3) dissociation into two molecules of a carbonyl compound with a carbon atom having a lower degree of substitution, one oxygen molecule, and two alkyl radicals, which recombine with O_2 to form peroxy-radicals [reaction (20)];

(4) the products of all these reactions can be obtained also via a free-radical pathway through the formation of alkoxyl radicals [reaction (21)].

Tertiary peroxyl radicals do not undergo concerted decomposition via reactions of type (18)–(20), but do decompose via reaction (21), forming O_2 and tertiary alkoxyl radicals, which rapidly undergo β-elimination:

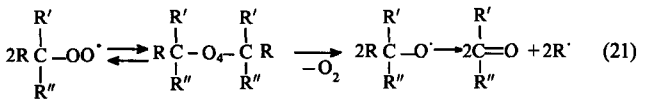
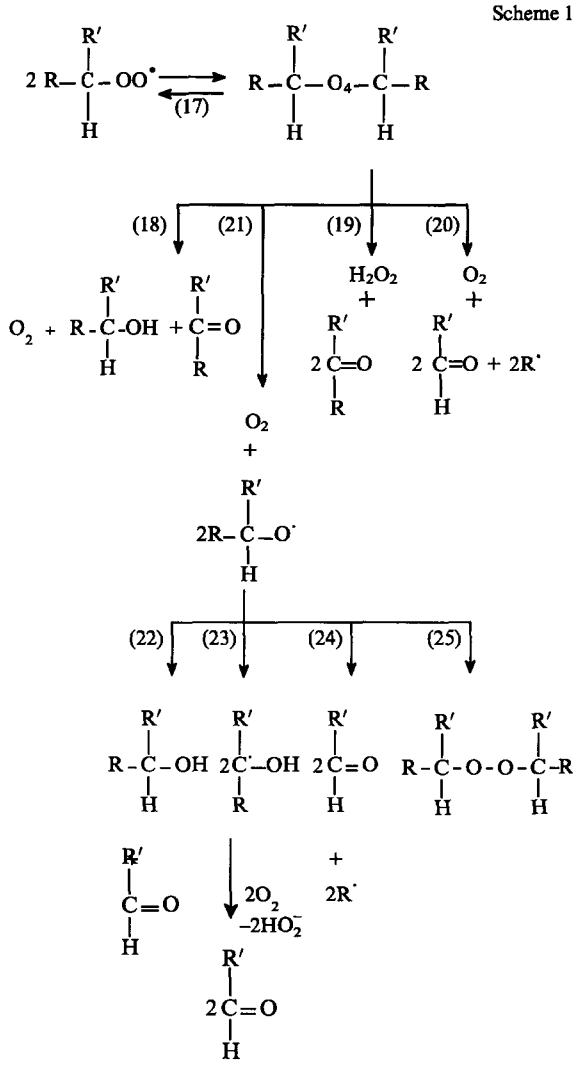


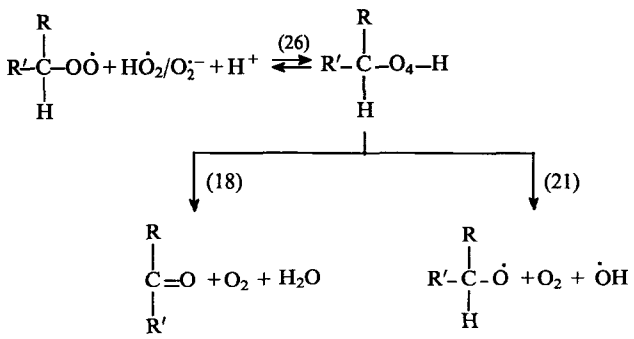
Table 5. The products of the γ-radiolysis of cytosine in N_2O-saturated aqueous solutions.

Product		G
cis-5,6-Dihydroxy-5,6-dihydrocytosine	}	0.05
trans-5,6-Dihydroxy-5,6-dihydrocytosine		
cis-5,6-Dihydroxy-5,6-dihydrouracil	}	0.15
trans-5,6-Dihydroxy-5,6-dihydrouracil		
6-Hydroxycytosine		0.07
5-Hydroxycytosine		1.4
5^6-Dihydroxycytosine		0.2
Dimers		3.2
Uracil		0.02

Scheme 1

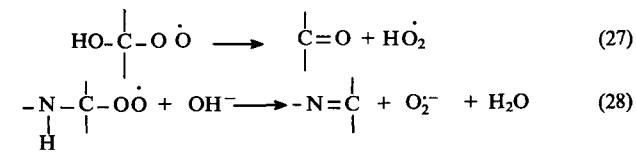


The rates of reaction of various peroxyl radicals, leading to the formation of tetroxides, differ greatly. Certain peroxyl radicals interact with rate constants close to the diffusion-controlled values, whilst others, particularly tertiary peroxyl radicals can have rate constants several orders of magnitude smaller.[33] However, usually more than one type of peroxyl radical is formed and tertiary peroxyl radicals, which interact with one another very slowly, are involved in reactions with primary or secondary peroxyl radicals. A similar situation occurs also with superoxide radical-ions. Owing to their comparatively low rate of disproportionation, they probably interact with other peroxyl radicals present [reaction (26)].



The resulting hydrotetroxide can decompose to an alkoxyl radical, molecular oxygen, and a OH radical via a reaction of type (21) or to a carbonyl compound, molecular oxygen, and water via a reaction of type (18).

If the peroxyl radical contains a OH or NH group in the α-position, then there is a possibility in this case of unimolecular decomposition with formation of the superoxide radical-ion. In the presence of the OH substituent, $O_2^{\cdot-}$ is formed spontaneously [reaction (27)], while in the presence of the substituent NH base catalysis is necessary [reaction (28)]:



On the basis of the mechanisms described, one may predict the pathways leading to the formation of the majority of the products identified in the γ-radiolysis of uracil in a N_2O/O_2-saturated aqueous solution (Table 6).

Since the OH radical adds preferentially to the C(5) position in uracil (80%), the main peroxyl radical is 5-hydroxy-6-peroxy-5,6-dihydrouracil **13**. The unimolecular reaction (28), involving the decomposition of the peroxyl radical **13** accompanied by the evolution of O_2 and requiring catalysis by OH^- ions, leads to the formation of 5-hydroxyisouracil **14** which can afford the *cis*- or *trans*-glycol **3** on interaction with H_2O and at pH 7 it can rearrange to isobarbituric acid **15**. In conformity with the foregoing, four mechanisms are possible for the bimolecular reaction (17) involving the decomposition of the peroxy-radical **13**. The first [mechanism (18)] leads to the formation of the *cis*- or *trans*-glycol **3** and dialuric acid **16** and is accompanied by the evolution of O_2. Two molecules of dialuric acid **16** and H_2O_2 are obtained via the second pathway [mechanism (19)]. In the third case [mechanism (20)], the C(5)–C(6) bond is ruptured

Scheme 2

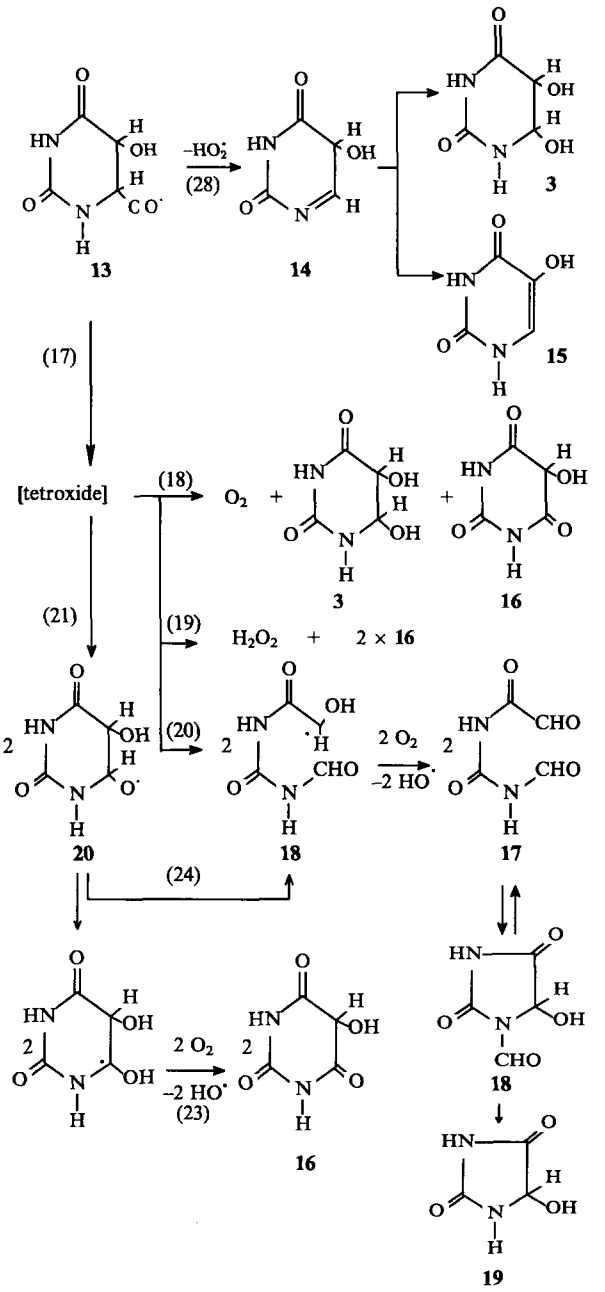


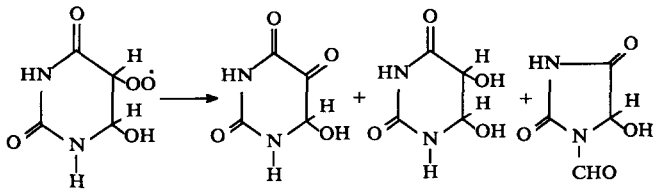
Table 6. The products of the γ-radiolysis of uracil in N_2O/O_2-saturated aqueous solutions.

Product	G^{82}		G^{81}	
	pH 6.0	pH 3.0	pH 6.5	pH 10
cis-5,6-Dihydroxy-5,6-dihydrouracil	0.42	0.6	0.9	1.4
trans-5,6-Dihydroxy-5,6-dihydrouracil	0.62	0.5	1.1	1.0
Isobarbituric acid	0.10	0	0.2	1.2
N^1-Formyl-N^2-glyoxylurea	0.72	–	–	–
N^1-Formyl-5-hydroxyhydantoin	–	1.6	1.4	0.2
5-Hydroxyhydantoin	0.18	0.4	0.4	0.3
Dialuric acid	–	0.9	0.4	0.2
Isodialuric acid	–	0.1	0.2	0.1
Alloxan	0.06	–	–	–
N^1-Formylurea	0.27	–	–	–
Urea	0.02	–	–	–
Oxalic acid and its ureides	0.13	–	–	–
Degradation of uracil	2.49	4.9	5.3	5.2

and the radical formed recombines with a O_2 molecule and, after the formation of HO_2^-, N^1-formyl-N^2-glyoxylurea **17** or its cyclic isomer N^1-formyl-5-hydroxyhydantoin **18** is produced. The latter readily loses the formaldehyde group and is converted into 5-hydroxyhydantoin **19**. The fourth pathway [mechanism (21)] leads to the formation of two alkoxyl radicals **20**, which are subsequently converted into dialuric acid **16** via pathway (23) or into N^1-formyl-5-hydroxyhydantoin **18** via pathway (24) [Scheme 2].

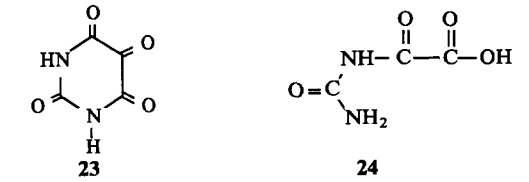
The unimolecular reaction (28) and the bimolecular reaction (17) compete. As shown in Table 6, the composition of the product varies with the pH of the medium and the power of the radiation.[81] At high pH, base catalysis renders the unimolecular reaction preferable, which results in isobarbituric acid (**15**) becoming the main product together with the glycols **3**, while the amount of N^1-formyl-5-hydroxyhydantoin **18** and dialuric acid **16** which are the main products of the bimolecular reaction, diminishes appreciably. At acid pH, there is a sharp change in the proportions of the product involving their inversion: N^1-formyl-5-hydroxyhydantoin and dialuric acid become the main products, while isobarbituric acid is not detected at all.

Evidently, only the bimolecular decomposition reaction is characteristic of the minor 6-hydroxy-5-peroxy-5,6-dihydrouracil **21** peroxyl radical. The main products are in this case the glycols **3** and N^1-formyl-5-hydroxyhydantoin **18**, as for the C(6)-peroxyl radical, and isodialuric acid **22** is formed instead of dialuric acid (Table 6).

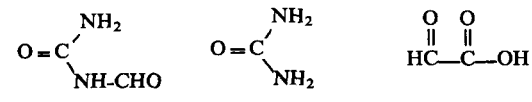


21 **22** **3** **18**

For high irradiation dose rates and doses,[82] alloxan **23**, *N*-formyl-urea **11**, urea **12**, oxalic acid and its ureide **24**, and glyoxalic acid **25** are detected to the products listed above. These compounds are the products of more far-reaching oxidation of uracil and become the main ones for high irradiation doses.



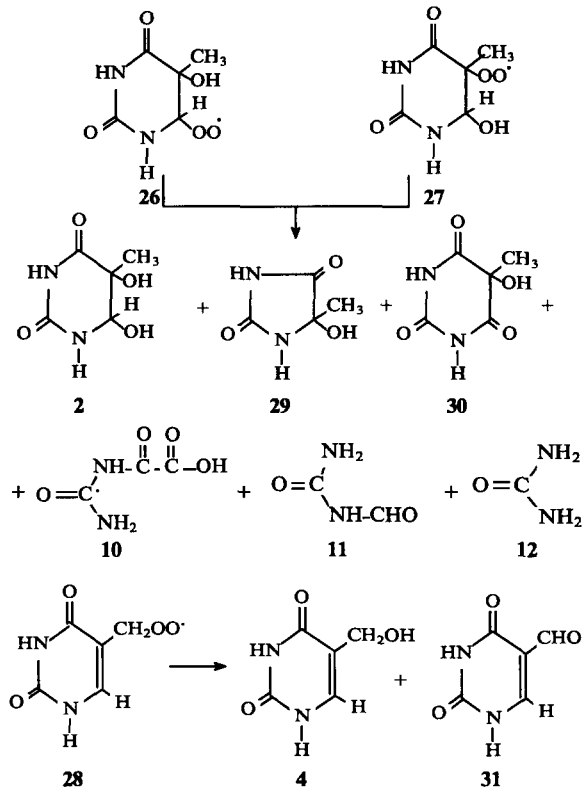
23 **24**



11 **12** **25**

In aerated aqueous thymine solutions, type (A), (B), and (C) radicals combine with O_2 and form respectively three types of peroxyl radicals: 5-hydroxy-6-peroxy-5,6-dihydrothymine **26** and 6-hydroxy-5-peroxy-5,6-dihydrothymine **27**, which are analogous to the uracil peroxyl radicals **13** and **21**, as well as 5-peroxymethyluracil **28**.

Overall, the mechanisms of the decomposition of the peroxyl radicals **26** and **27** do not differ from those described above for uracil peroxyl radicals. Table 7 presents the yields of different products of the anaerobic γ-radiolysis of thymine.[25, 79, 83–85] As in the γ-radiolysis of uracil, the oxidation of thymine entails the formation of the *cis*- and *trans*-glycols of thymine **2**, 5-hydroxy-5-methylhydantoin **29**, 5-hydroxy-5-methylbarbituric acid **30**,



26 **27**

2 **29** **30**

10 **11** **12**

28 **4** **31**

Table 7. The products of the γ-radiolysis of thymine in N_2O/O_2-saturated aqueous solutions.[86]

Product	*G*
cis-6-Hydroperoxy-5-hydroxy-5,6-dihydrothymine	0.3
trans-6-Hydroperoxy-5-hydroxy-5,6-dihydrothymine	0.7
cis-5-Hydroperoxy-6-hydroxy-5,6-dihydrothymine	0.08
trans-5-Hydroperoxy-6-hydroxy-5,6-dihydrothymine	0.17
cis-5,6-Dihydroxy-5,6-dihydrothymine	0.12
trans-5,6-Dihydroxy-5,6-dihydrothymine	0.12
cis-6-Hydroperoxy-5,6-dihydrothymine	0.03
trans-6-Hydroperoxy-5,6-dihydrothymine	0.03
5-Hydroperoxy-5,6-dihydrothymine	0.06
5-Hydroperoxymethyluracil	0.05
5-Hydroxy-5,6-dihydrothymine	0.02
6-Hydroxy-5,6-dihydrothymine	0.01
5-Hydroxymethyluracil	0.02
5-Hydroxy-5-methylhydantoin	0.15
5-Hydroxy-5-methylbarbituric acid	–
N^1-Formyl-N^2-pyruvylurea	0.5
N^2-Formylurea	0.07
Urea	0.08
Degradation of thymine	2.6

N^1-formyl-N^2-pyruvylurea **10**, *N*-formylurea **11**, and urea **12**. The decomposition of 5-peroxymethyluracil **28** leads to two main products: 5-hydroxymethyluracil **4** and 5-formyluracil **31**.

In addition, thymine hydroperoxides, obtained by the reduction of the peroxyl radicals, were isolated and characterised (Table 7).[86] The half-reaction time of the thymine hydroperoxides in aqueous solutions can reach 5 days at 37 °C.[25, 26] Four possible isomeric forms were isolated: the *trans*- and *cis*-forms of 6(5)-hydroxy-5(6)-peroxy-5,6-dihydrothymine and derivatives of type (A) and (B) radicals, the *trans*-isomers of the latter being formed preferentially. Hydroperoxymethyluracil—a derivative of the radical (C)—was also isolated.

Estimation of the products of the γ-radiolysis of cytosine in aerated aqueous solutions showed that 40% of the products isolated are the same as in the radiolysis of uracil (Table 8).[87, 88] This is apparently associated with the fact that the free-radical product formed from cytosine undergoes deamination in one of the first stages of the radiolysis. The free-radical products derived from uracil obtained as a result react via the mechanisms described for them above. The mechanism of the deamination has not yet been finally established.

In the decomposition of the peroxyl radicals derived from cytosine **32** and **33** which have not yet undergone deamination, the main products formed are *trans*-1-carbamoyl-2-oxoimidazolidine-4,5-diol **35** and 4-amino-N^1-formyl-5-hydroxy-2-oxo-Δ^3-imidazoline **36**. Compound **36** is apparently obtained via a mechanism similar to that of the formation of 5-hydroxyhydantoin and is subsequently converted on deamination into parabanic acid **37**. The formation of compound **35** can be explained by the dissociation of the C(4)–C(5) bond with formation of compound **34** which subsequently either cyclises to form compound **35** or undergoes further degradation to the biuret **38**. It is of interest to note that glycols are not formed in the radiolysis of cytosine in the presence of O_2 (Table 8). However, 5-hydroxycytosine, which may be a product of the dehydrogenation of the glycols, has been detected (Scheme 3):

Scheme 3

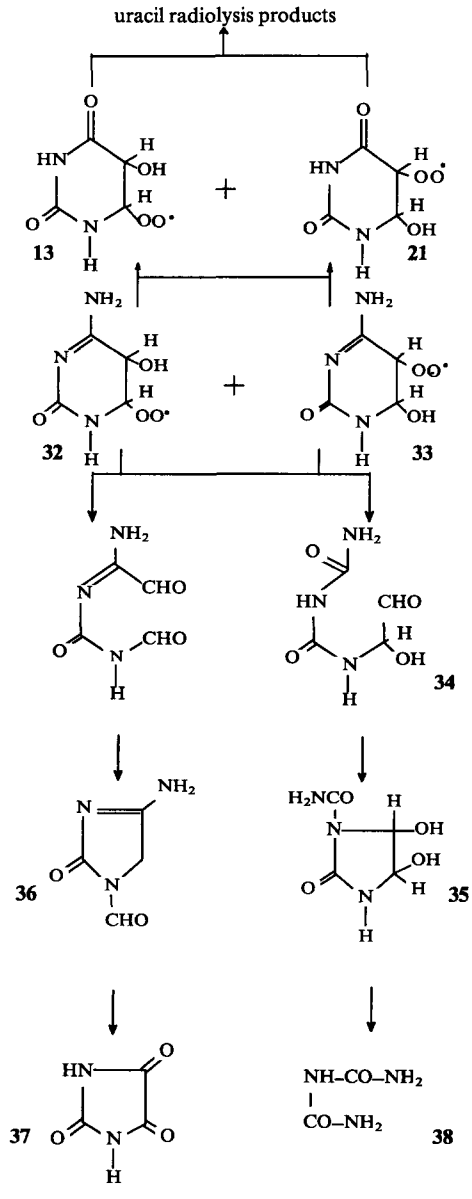


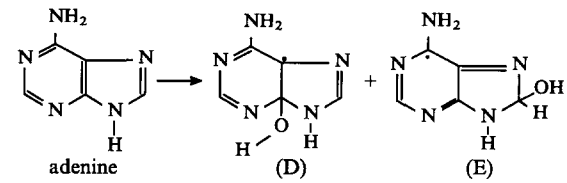
Table 8. The products of the γ-radiolysis of cytosine in N_2O/O_2-saturated aqueous solutions.[87, 88]

Product	*G*
cis-5,6-Dihydroxy-5,6-dihydrouracil	0.03
trans-5,6-Dihjydroxy-5,6-dihyrouracil	0.10
5-Hydroxyhydantoin	0.10
4-Amino-N^1-formyl-5-hydroxy-2-oxo-Δ^3-imidazoline	0.20
trans-1-Carbamoyl-2-oxoimidozilidine-4,5-diol	0.61
N^1-Formylurea	0.06
Parabanic acid	0.03
Biuret	0.06
Oxalic acid and its ureides	0.21
Degradation of cytosine	2.49

3. Oxidation of purine heterocycles

The advances in the study of the oxidation of purines by OH radicals on γ-radiolysis are not quite so considerable. This is because the methods for the investigation of the reactions of OH radicals, which have proved very useful in studies involving pyrimidines, encounter certain difficulties when applied to purine heterocycles. The use of EPR methods in combination with spin-trapping agents did not permit the unambiguous identification of the purine radicals formed on interaction with OH radicals.[89] Pulse radiolysis experiments combined with the study of the oxidation-reduction properties of the radicals obtained yield much information but their interpretation is also difficult. This is associated with the fact that the radicals formed in the reaction between free hydroxyls and purine heterocycles undergo rapid intramolecular rearrangements such as ring opening, dehydration, or dehydrogenation with formation of secondary radicals.

By means of pulse radiolytic methods it has been established[90–93] that, in its reaction with adenine, the OH radical adds preferentially to the C(4) carbon atom of the C(4)=C(5) double bond and to a lesser extent to the C(8) atom of the N(7)=C(8) double bond, forming respectively type (D) and (E)



radicals.

The type (D) radical is a weak oxidant, but as a result of dehydration it is converted into a type (F) radical, which is a powerful oxidant. The amount of radicals (F), determined by reaction with a reductant, was 81% for free adenine (Table 9). The reduction of the radical (F) leads to the formation of the initial adenine, which explains the observed low level of its degradation (30% relative to the amount of OH radicals).[93]

The type (E) radical is a reductant. Its yield, determined by reaction with an oxidant (Methyl Viologen), is 15% (Table 9). The oxidation of the radical (E) leads to the formation of 8-hydroxy-adenine **39**. When the γ-radiolysis was carried out in the presence of the oxidant $Fe(CN)_6^{3-}$, the yield of 8-hydroxyadenine was 18%, which agrees with the amount of the radical (E). However, the intramolecular reaction involving the opening of the imidazole ring with formation of the radical (G), which is an oxidant, is also characteristic of the radical (E). The reduction of the radical (G) leads to the formation of 4,6-diamino-5-formamidopyrimidine **40**.

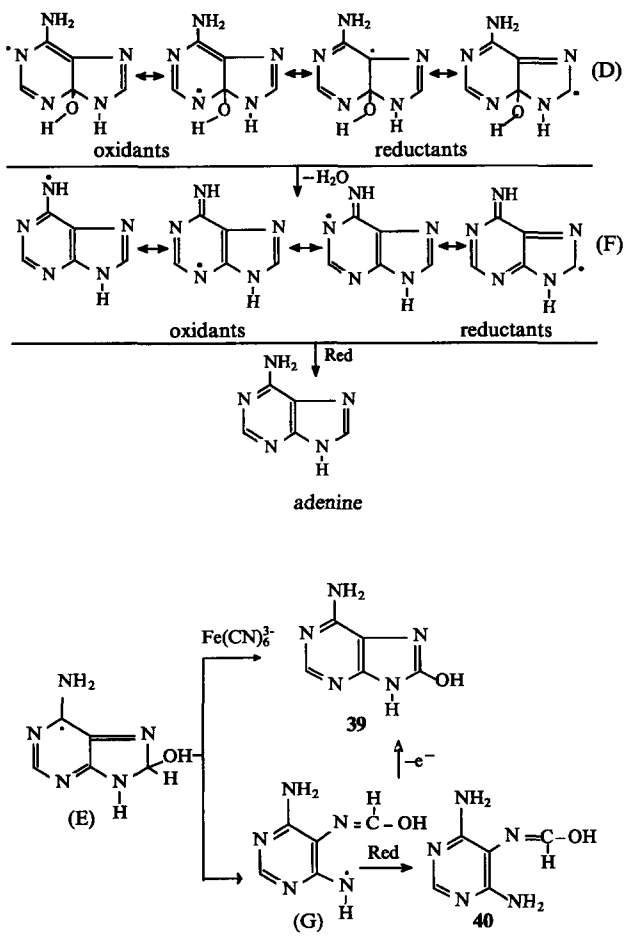
In the absence of exogenic oxidants, the yield of 8-hydroxyadenine **39** exceeds that of the derivative of **40** by a factor of 3. This confirms that the oxidation reaction is the main reaction pathway to the radical (E), which can be oxidised in this instance

Table 9. The yields and oxidation – reduction properties of the free radicals of purines formed by reaction with hydroxyl radicals.

Heterocycle	Radical	Oxidation–reduction properties	Yield, %[OH]$^{-1}$	Method of estimation
Adenine	(D)	oxidant	81	by reaction with TMPD
	(E)	reductant	15	by reaction with MV
	(E)	reductant	18	yield of 8-hydroxyadenine
Guanosine	(D)	oxidant	50	by reaction with TMPD
	(E)	reductant	25	yield of 8-hydroxyguanosine
	(H)	reductant	25	difference between the overall yield of the radicals formed by reaction with MV and the yield of the radical (E)

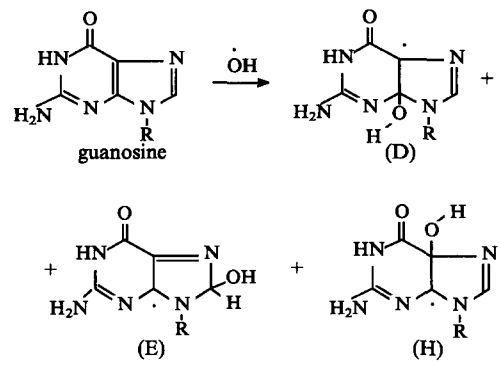
Notation: MV = Methyl Viologen;
TMPD = *N,N,N',N'*-tetramethylphenylenediamine.

by the radical (F) with formation of 8-hydroxyadenine and adenine. Compound **40** is apparently formed only as a result of the disproportionation of the radical (G).

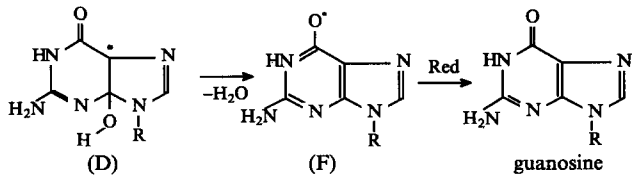


The addition of the OH radical to the C(5) carbon atom of adenine has been detected only in the case of *N*(6)-methyladenine and its contribution does not exceed 10–15%. Further reaction pathways for the radicals formed have not been characterised.

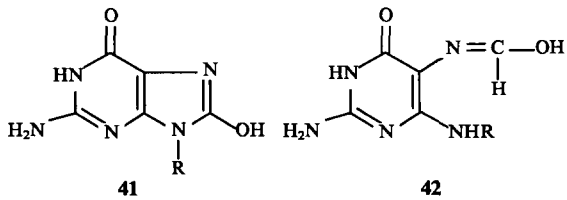
As a result of the interaction of OH radicals with guanosine or its 9-substituted analogues, the formation of approximately equal amounts of radicals of two types has been detected. One of these radicals has oxidising properties and the other has reducing properties (Table 9).[92] As in the case of adenine derivatives, OH radicals add to the C(4), C(8), and also, with appreciable yields, C(5) carbon atoms of guanosine, forming type (D), (E), and (H) radicals respectively:



Like the adenine radical (D), the type (D) guanosine radical is rapidly converted as a result of dehydration into a type (F) radical, which is a powerful oxidant. The yield of the radical (D) was determined by reaction with a powerful reductant and was found to be 50%. The reduction of the radical (F) leads to the formation of the initial guanosine,[93] lowering thereby the overall yield in the degradation of guanosine.



The radicals (E) and (H) have reducing properties. The same reaction pathways as for the adenine radical (E) are characteristic of the type (E) guanosine radical. Its oxidation and the reduction of its radical product (F), in which the imidazole ring has been opened, lead to the formation of 8-hydroxyguanosine **41** and 6-*N*-(2'-deoxy-D-*erythro*-pentofuranosyl)-2,6-diamino-5-formamido-4-hydroxypyrimidine **42** respectively. The amount of the radical (E), estimated from the yield of 8-hydroxyguanosine, was 25% (Table 9). The remaining 25% of radical products derived from guanosine were assumed to comprise a type (H) radical, the subsequent reaction pathways of which have not yet been established.



The interaction of purine radicals with molecular oxygen has been investigated very poorly. The rates of these reactions are lower than for the pyrimidine radicals. Furthermore, the type (D) radical is rapidly converted into a type (F) radical, which does not react with oxygen. The reactions of purine peroxyl radicals have not been characterised.

The 8-hydroxy-derivatives **39** and **41** and compounds **40** and **42**, in which the imidazole ring has been opened, have been detected as the main products of the oxidation of nucleic acid purines in different oxidation systems (Tables 10 and 11).[26, 94–98] Furthermore, in the oxidation of the dinucleotide d(CG) by the Cu-1,10-phenanthroline complex, the formation of *N*-(2′-deoxy-D-*erythro*-pentofuranosyl)formamide was detected. This compound is apparently the product of more far reaching oxidation of guanosine.[99]

Table 10. The products of the γ-radiolysis of adenine in N_2O-saturated aqueous solutions.[97]

Product	*G*
4,6-Diamino-5-formamidopyrimidine	0.2
8-Hydroxyadenine	0.35
6-Amino-8-hydroxy-7,8-dihydropurine	0.1
Degradation of adenine	1.0

Table 11. The products of the γ-radiolysis of guanosine in N_2O-saturated aqueous solutions.[96]

Product	*G*
6-*N*-(2′-Deoxy-α-D-*erythro*-pentofuranosyl)-2,6-diamino-5-formamido-4-oxopyrimidine	0.09
6-*N*-(2′-Deoxy-α-L-*erythro*-pentofuranosyl)-2^6-diamino-5-formamido-4-oxopyrimidine	0.25
5′,8-Cyclo-2′,5′-dideoxyguanosine	0.06
8-Hydroxyguanosine	0.24
Guanine	0.38
9-(2′-Deoxy-α-D-*erythro*-pentofuranosyl)guanine	0.03
9-(2′-Deoxy-β-D-*erythro*-pentofuranosyl)guanine	0.02
9-(2′-Deoxy-α-D-*erythro*-pentofuranosyl)guanine	0.02
9-(2′-Deoxy-α-L-*threo*-pentofuranosyl)guanine	0.03
9-(2′-Deoxy-β-D-*erythro*-pento-1,5-dialdo-1,4-furanosyl)guanine	0.08

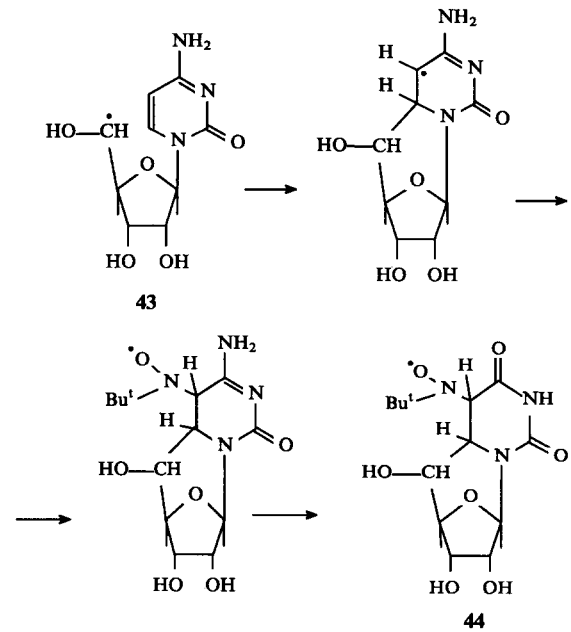
The majority of the products of the oxidation of heterocycles, characterised in terms of model compounds, have been detected also in the oxidation of DNA both *in vitro* and *in vivo* in different oxidation systems, namely on γ-radiolysis[100, 101] in the hydrogen peroxide–(Fe-EDTA)[31] or the superoxide radical ion–(Fe-EDTA)[29, 30] systems and also on oxidation by Cu-1,10-phenanthroline or Fe-bleomycin.[60, 102]

IV. Oxidation of the carbohydrate fragments in nucleic acids

1. General characteristics of the oxidation of the carbohydrate fragments of nucleic acids

The rate of reaction of hydroxyl radicals with ribose and 2′-deoxyribose is several times lower than for the heterocycles (Table 1). The reaction involves the abstraction of a H atom from all five carbon atoms in the pentose residue with not very different probabilities: approximately 25% each from the C(1′) and C(2′) carbon atoms, 20% each from the C(3′) and C(4′) atoms, and only 10% from C(5′).[103] This leads to the formation of a large number of modification products, which hinders the determination of the reaction pathways for particular primary radicals.

The use of spin-trapping agents made it possible to detect some of the ribose and deoxyribose free radicals formed in nucleic acids and their model compounds.[62, 63, 65] The stable products of the interaction of the C(4′), C(5′), and C(1′) radicals with 2-methyl-2-nitrosopropane have been isolated and characterised on γ-radiolysis of aqueous solutions of derivatives of uracil (uridine 3′-monophosphate and polyuridylic acid) and thymine (thymidine 5′-monophosphate). For cytosine derivatives (cytidine, deoxycytidine, cytidine 5′-monophosphate, cytidine 3′-monophosphate, and deoxycytidine 5′-monophosphate), it has been possible to detect by the reaction with 2-methyl-2-nitrosopropane only the C(4′) radical **43** and the radical product resulting from the further reaction of the C(5′) radical **44**:

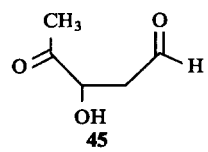
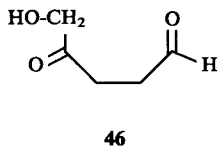
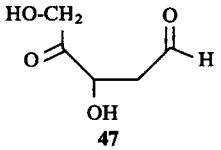
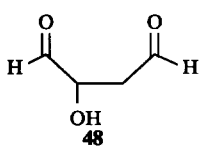


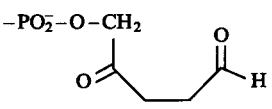
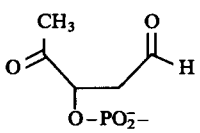
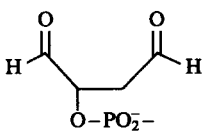
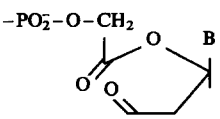
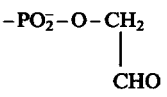
For purine nucleosides (deoxyadenosine, adenosine, guanosine, deoxyguanosine inosine) and their homopolymers poly(A) and poly(G), only the product derived from the C(4′) radical was identified.[58]

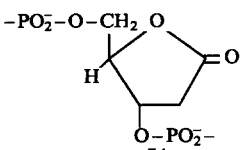
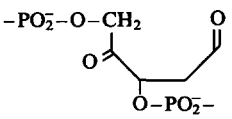
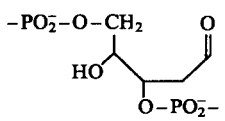
All the final products of the modification of the sugar residue which have been established (Table 12), formed on γ-radiolysis of aqueous DNA solutions, have been arbitrarily divided into 4 types:[25, 26, 61] (1) the pentose oxidation products **45–48** obtained on dissociation of both phosphoester bonds and on splitting off the unmodified heterocycles; (2) modification of the pentose residues linked to only one terminal phosphate group and containing or not containing the heterocycles **49–53**; (3) products in which both phosphate bonds are retained but the heterocyclic base has been split off and the pentose has been modified [compounds **54–56**]; (4) the product **57** with a non-purine/non-pyrimidine site formed as a result of the modification of the heterocycles leading to the cleavage of the glycosidic bond. The formation of type (1) and (2) products is accompanied by the rupture of the sugar phosphate skeleton of DNA and the isolation of intact heterocycles. Products of types (3) and (4) represent the so called alkali-labile sites, giving rise to breaks in the DNA chain after heating in an alkaline medium. Their formation is not always accompanied by the simultaneous splitting off of intact heterocycles, for example in the case of the product **52**.

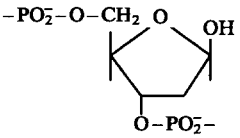
Table 12. Products of the modification of the hydrocarbon residue on γ-radiolysis of aqueous solutions of DNA.

Product	Precursor radical	Without O_2	With O_2	Chain breaks	Alkali-labile sites
2'-Deoxyribolactone	C(1')	+	-	-	+
Erythrose bound to DNA	C(2')	-	+	-	+
2^5-Dideoxypentos-4-ulose	C(4')	+	-	+	-
2^5-Dideoxypentos-4-ulose bound to DNA	C(4')	+	-	+	-
2^3-Dideoxypentos-4-ulose	C(4')	+	-	+	-
2^3-Dideoxypentos-4-ulose bound to DNA	C(4')	+	-	+	-
2-Deoxypentos-4-ulose	C(4')	+	+	-	+
2-Deoxypentos-4-ulose bound to DNA	C(4')	+	+	-	+
Glycolic acid bound to DNA	C(4')	-	+	+	-
Compound abstracting malonodialdehyde	C(4')	-	+	+	-
2-Deoxytetrodialdose	C(5')	-	+	+	-
2-Deoxytetrodialdose bound to DNA	C(5')	-	+	+	-
Non-purine/non-pyrimidine sites	heterocycle	+	+	-	+

1.  **45**
 **46**
 **47**
 **48**

2.  **49**
 **50**
 **51**
 **52**
 **53**

3.  **54**
 **55**
 **56**

4.  **57**

Studies on the mechanisms of the oxidation of the simplest DNA analogues made it possible to establish certain characteristic features in the reactions of the free radicals derived from sugar phosphate esters, on the basis of which the mechanisms of the OH-radical-induced oxidation of ribose and deoxyribose in nucleic acids to known final products were postulated. The principal features are listed briefly below. They were described in greater detail in other reviews.[25, 26, 61]

(1) α-Alcoholic and α-alkoxyalkyl radicals, containing a phosphate group in the β-position, are capable of splitting off the latter rapidly:

$$R\dot{C}OH-CH_2-O-PO_2^- \longrightarrow R-CO-\dot{C}H_2 + H^+ + PO_3^{2-} \quad (29)$$

$$RO\dot{C}H-CH_2-O-PO_2^- \longrightarrow \left[R-O-\overset{+}{\dot{C}}H-CH_2 \leftrightarrow R-O-CH-\overset{+}{\dot{C}}H_2\right] + PO_3^{2-} \quad (30)$$

$$\left[\quad\right] \xrightarrow{OH^-} RO-\dot{C}H-CH_2OH + RO-\underset{OH}{CH}-\dot{C}H_2$$

(2) Radicals of the type $RCHOH-\dot{C}O$ rapidly split off a CO molecule:

$$R-CH(OH)-C\dot{O} \rightarrow R-\dot{C}H-OH + CO \quad (31)$$

(3) The oxidation of the radicals by various oxidants leads to the formation of carbonium ions:

$$R\dot{H} + Ox \rightarrow R^+H + Ox^-, \quad (32)$$

(4) In the presence of oxygen, alkyl radicals rapidly combine with an oxygen molecule to form peroxyl radicals:

$$RR'R''C^{\cdot} + O_2 \rightarrow RR'R''C-OO^{\cdot} \quad (33)$$

(5) The peroxyl radicals decompose via the unimolecular or bimolecular mechanisms, described in the previous section.

A series of important data on the mechanism of the oxidation of the carbohydrate fragments have been obtained with the use of antitumour antibiotics (bleomycin, neocarzinostatin) and metal complexes (Cu-1,10-phenanthroline, Mn-porphyrin) which bind to DNA and oxidise pentose selectively in a specific position (subsections 2, 4, and 5 of the present section).

The oxidation of pentoses in the C(4') position has been studied in greatest detail. This is due to two causes. Firstly, the oxidation in the C(4') position is the main pathway leading to the degradation of nucleic acids by the antitumour antibiotics of the bleomycin group.[7] Secondly, the dominant formation of the ribose C(4') radical on γ-radiolysis in aqueous solutions as a result of the additional abstraction of a H atom from the C(4') position

in the sugar by the free radical of a heterocycle in a neighbouring monomer unit is characteristic of certain homopolynucleotides—poly(U) and poly(dT).[104] The known pathways leading to the oxidation of the sugars in nucleic acids in different positions in ribose or deoxyribose by both free OH radicals and certain antibiotics and metal complexes are described below.

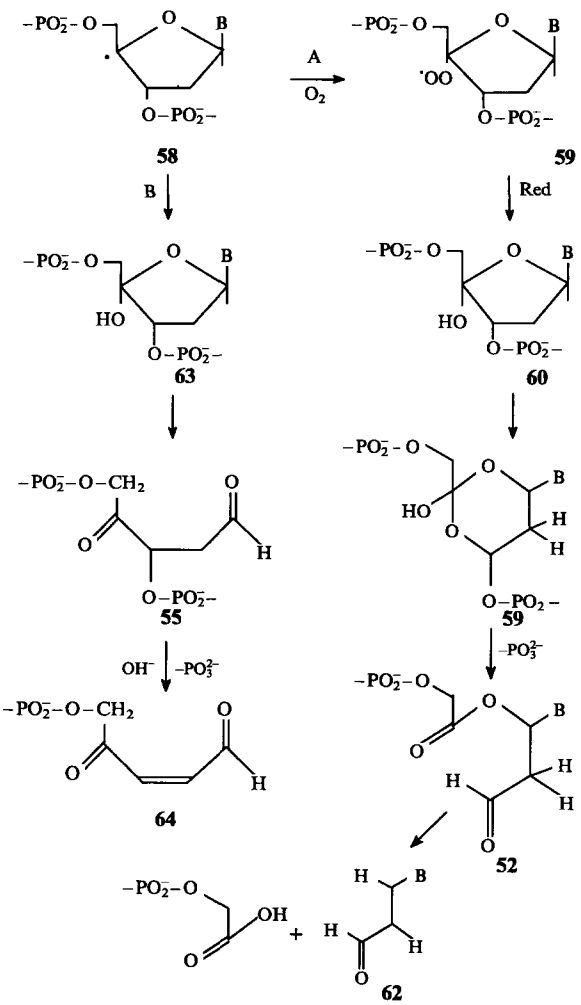
2. Oxidation of deoxyribose in the C(4′) position in the presence of bleomycin

Bleomycins are a group of glycopeptide antitumour antiobiotics inducing the degradation of DNA in the presence of Fe^{2+} ions and molecular oxygen.[7, 55, 105, 106]

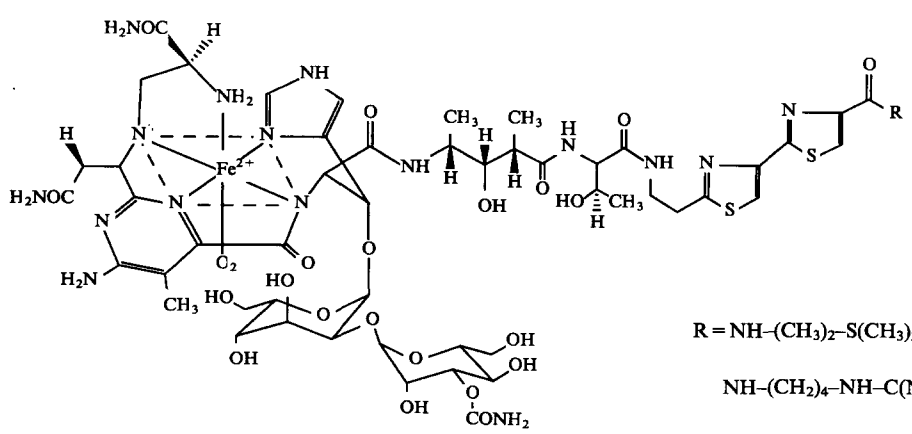
The oxidising unit is the so called 'activated bleomycin', which is a product of the two-electron reduction of the ternary complex Blm · Fe^{2+} · O_2, as already noted in Section II of this review, which is an oxo-complex of iron[50]. As a result of the degradation of DNA by the activated bleomycin, two types of monomeric products are detected: *trans*-propenals derived from the heterocycles (pathway A) and free heterocycles (pathway B). The formation of propenals requires the involvement of yet another oxygen molecule in addition to that used to activate bleomycin. This process is accompanied by the cleavage of DNA into two fragments, one of which contains a 5′-terminal phosphate and the other a 3′-terminal phosphoglycolate. The splitting off of the heterocycles does not require an additional oxygen molecule and leads to the formation of non-pyrimidine/non-purine sites in the oxidised deoxyribose residue, at which cleavage takes place only under alkaline conditions. At the normal oxygen pressure, the amount of the free heterocycles formed is virtually equal to the amount of heterocycle propenals. When the oxygen pressure is raised, reaction A takes place preferentially; under anaerobic conditions [Fe(III) and H_2O_2], pathway B is observed exclusively.[7, 55, 105, 106]

Experiments with different DNA containing tritium in the 4′-positions showed that the liberation of tritiated water is correlated with the formation of the sum of both monomeric products—propenals and free heterocycles.[55, 107] This shows that the first stage in both pathways is the abstraction of a hydrogen atom from the 4′-carbon atom of deoxyribose. The considerable isotope effect associated with the replacement of 4′-H by tritium or deuterium (by a factor of 7–11 and 4–5 respectively) permits the conclusion that the abstraction of a H atom from the C(4′) position in the pentose is the rate-limiting stage in both reactions.

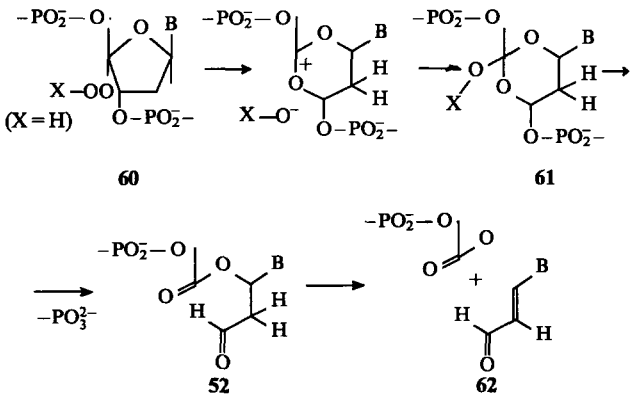
Under aerobic conditions, the 4′-radical **58** rapidly interacts with oxygen, forming the 4′-peroxyl radical **59**. In order to demonstrate directly the occurrence of this process a series of experiments were carried out[108], in which the ^{16}O and ^{18}O oxygen isotopes were used. When $^{18}O_2$ was incorporated in the activated bleomycin and DNA was oxidised directly in the presence of $^{16}O_2$, no incorporation of the label in the product was detected. In the opposite situation, where the reaction medium contained $^{18}O_2$, the label was found to be incorporated in one of the oxidation products—3′-phosphoglycolate. Under these conditions, only one ^{18}O atom was incorporated in 3′-phosphoglycolate, while the other oxygen atom in this product originated from deoxyribose. The second oxygen atom of the peroxide group should apparently be included in the heterocycle propenal. However, so far it has not been possible to demonstrate this.



The subsequent stages, leading to the dissociation of the C(3′)–C(4′) bond, the cleavage of the sugar phosphate residue, and the formation of heterocycle propenals cannot yet be regarded as finally established. It appears that the 4′-peroxyl radical **59**



subsequently accepts yet another electron from the reductant present and is converted into the fairly unstable 4′-peroxide **60**. It has been suggested that the peroxide **60** subsequently undergoes the Criegee rearrangement. During this rearrangement, the C(3′) atom migrates from the C(4′) atom to the O atom of the peroxide with the simultaneous heterolytic dissociation of the O–O bond. The C(2′) atom, activated by the appearance of the carbonyl group in the C(3′) positon in the intermediate **52**, can be deprotonated, initiating elimination via the C(2′)–C(1′) bond and the formation of the heterocycle propenal **62** and the phosphoglycolate product. In this mechanism, the appearance of the label in the phosphoglycolate product should be observed when DNA is oxidised in the presence of $^{18}O_2$, which has in fact been done:[108]

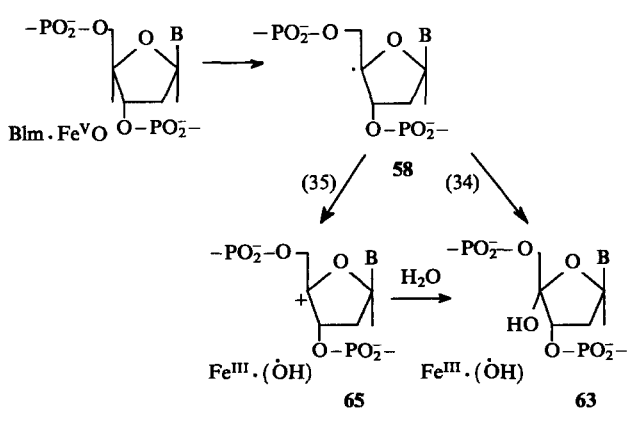


Confirmation has been obtained for this mechanism in a study[109] where it was shown, with 3′-^{18}O-labelled poly(dA-dT) as the substrate, that the 5′-phosphate products obtained on degradation by bleomycin contain the ^{18}O-label in the phosphate group. This indicates that the reaction proceeds via the pathway accompanied by the cleavage of the C(3′)–O(3′) bond.

Data in support of the existence of a precursor of the propenals **52** have been obtained.[110] The existence of a long-lived precursor of the heterocycle propenal, coprecipitated with DNA, was detected. This made it possible to demonstrate that the rate of formation of the propenals is an order of magnitude lower than the rate of appearance of single-strand breaks: $t_{1/2} \simeq 40$ min and $t_{1/2} \simeq 2.5-5$ min, respectively.

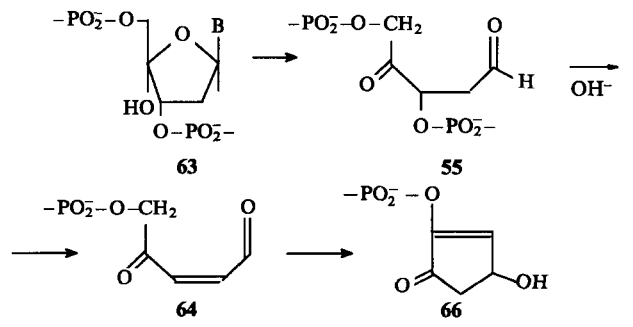
In a more detailed study of the rupture of the C(2′)–H bond, DNA selectively labelled with tritium was used:[111] poly(dA-[2′-proS-^{3}H]dU) and poly(dA-[2′-proR-^{3}H]dU). It was shown that the rigorously stereospecific elimination of 2′-proR-H takes place via anti-elimination with the exclusive formation of *trans*-propenals.

As noted previously, in the presence of a low O_2 content the oxidation of DNA proceeds via pathway B. After the abstraction of hydrogen from the C(4′) position, the splitting off of the heterocycles requires the addition of some kind of analogue of the OH radical. Two C(4′) hydroxylation mechanisms can be postulated.



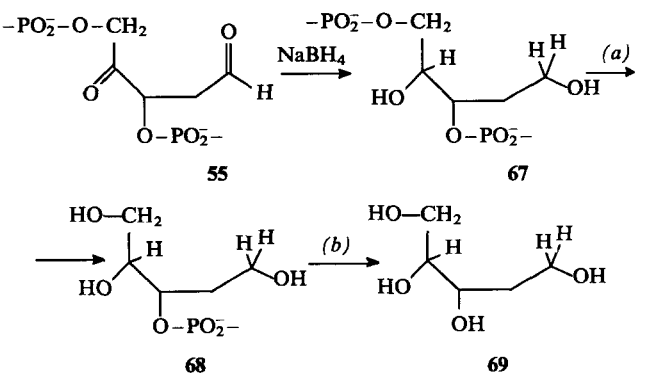
In the first case [reaction (34)], the C(4′) radical recombines with the hydroxyl radical bound to the antibiotic, resembling the mechanism proposed for cytochrome P-450 and metalloporphyrins.[111] In the second case [reaction (35)], the C(4′) radical can be oxidised by a second equivalent of the activated antibiotic, affording a carbonium ion, which rapidly interacts with water. The results of experiments using oxygen isotopes, which will be discussed below, support the second pathway.

The compound **63** formed is cleaved, giving rise to the free heterocycles. Under physiological conditions, the oxidised deoxyribose residue **55** remains bound to the DNA chain. Under alkaline conditions, the 3′-terminal fragment is split off in the form of a phosphomonoester. In the second fragment, the sugar oxidation product—3-hydroxyl-5-oxo-1-cyclopentenyl **66**— remains at the 3′-phosphate end:



Certain aspects of this mechanism have been confirmed experimentally. Sugiyama et al.,[112] using the model substrate d(CGCTTTAAAGCG) containing a single site in the third deoxycytidine, which is cleaved by bleomycin, isolated the product CpGpx and determined its structure by direct comparison with synthetic analogues. This compound corresponded to the product **66** postulated in the mechanism. However, a correlation was not traced in the above study between the amount of heterocycles isolated and the amount of alkali-labile products of the modification of deoxyribose.

An alternative approach to the identification of the product **55** has been proposed.[113, 114] After incubating the model substrate d(CGCGCG) under anaerobic conditions with Fe(III)·Blm and H_2O_2, the product of the oxidation of compound **55** was stabilised by reduction with $NaBH_4$. After the enzymic degradation of the polymer, the deoxyribose residue **69** was identified with the aid of chromatograph-linked mass spectrometry by comparison with synthetic analogues of 2-deoxy-D-*erythro*-pentitol and 2-deoxy-D-*threo*-pentitol:



(*a*) treatment with P1-nuclease and alkaline phosphatase;
(*b*) treatment with phosphodiesterase I and alkaline phosphatase.

The product **69** isolated corresponded to a mixture of diastereoisomers and its amount was correlated to the amount of heterocycles formed.

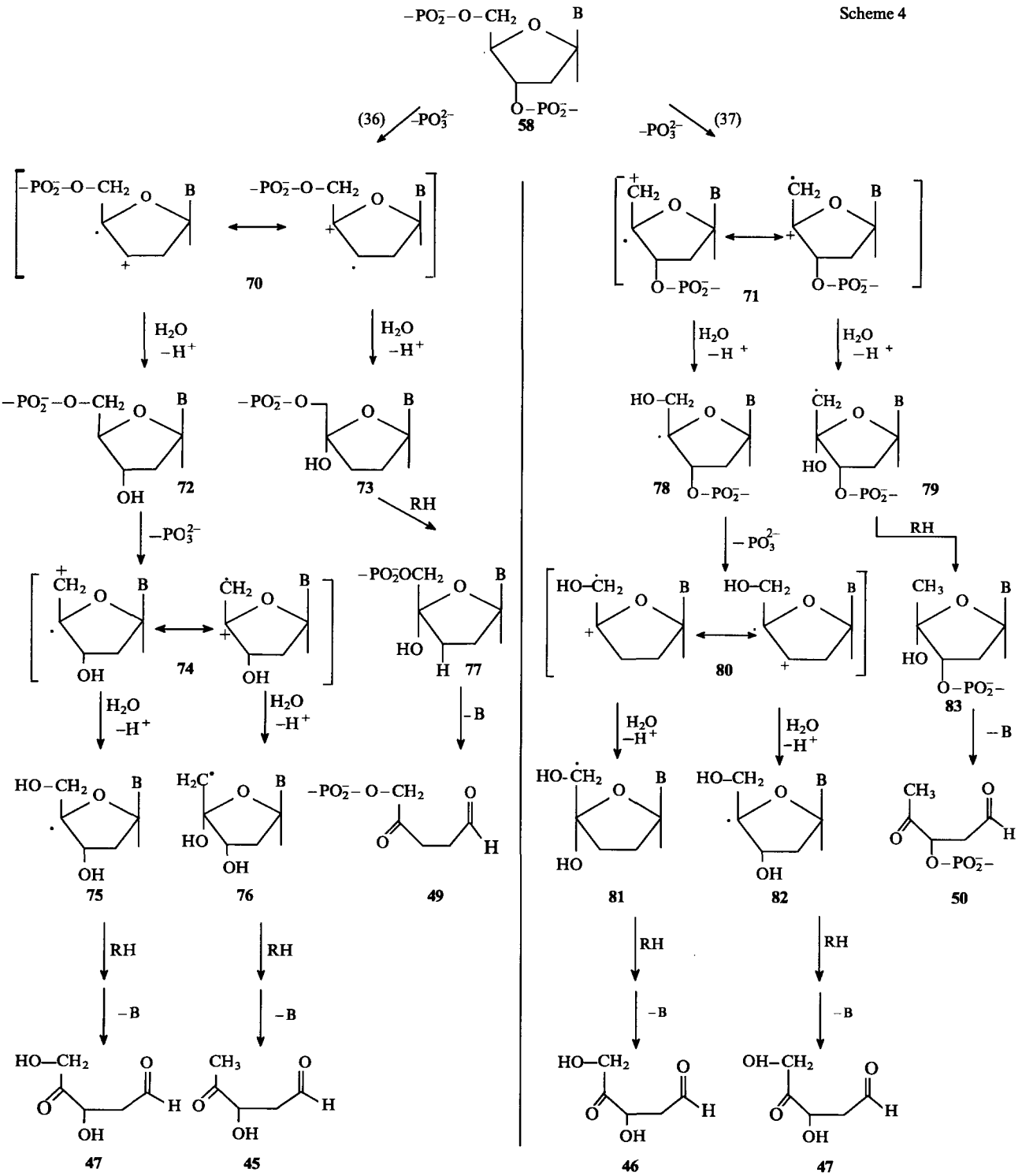
Using the approach described, the same workers[114] established the mechanism of the hydroxylation of the C(4') position. Using ^{18}O-labelled O_2 or H_2O, they showed that the source of oxygen in the 4'-keto-group of compound **64** is the solvent H_2O and the C(4') position is therefore hydroxylated via the second of the proposed pathways.

Thus wide scale investigations carried out in various laboratories made it possible to establish the mechanism of the oxidative fragmentation of DNA by bleomycin *in vivo*, although individual stages require further evidence.

3. Oxidation of pentose in the C(4') position by free OH radicals (according to γ-radiolytic data)

By analogy with bleomycin, the abstraction of a H atom by the OH radical from the C(4') carbon atom on γ-radiolysis leads to the splitting off of the free heterocycles and different modifications of the pentose. However, whereas the chain rupture under anaerobic conditions occurs only after treatment with alkali during oxidation with bleomycin, the splitting off of the heterocycle on γ-radiolysis is accompanied by rapid chain rupture.[25, 26]

Scheme 4



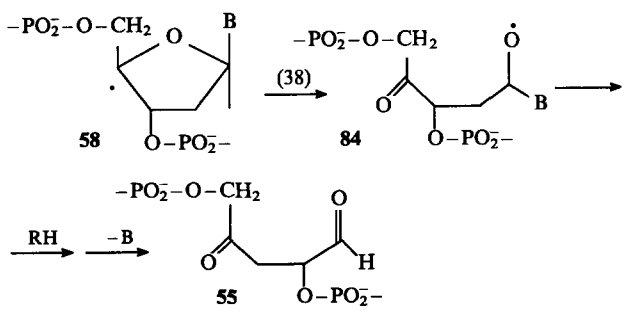
Consequently, the reactions of the C(4′) radicals leading to the final products are different in γ-radiolysis and in oxidation by bleomycin.

The C(4′) radical is a β-phospho-α-alkoxyalkyl radical, a characteristic feature of which has been shown, in simple models,[26] to involve the rapid elimination of the phosphate group. Since the ribose and deoxyribose C(4′) radicals contain two phosphate groups in the β-positions relative to the free valence, two competing reactions should be observed for them: the elimination of the phosphate group from the C(3′) atom [reaction (36)] and the C(5′) atom [reaction (37)]. It follows from the results of the study of model compounds that the elimination from the C(3′) atom should be faster, which is in fact reflected in the ratio of the final products (Table 12).

The postulated main reaction pathway for the deoxyribose C(4′) radical is presented in scheme 4. The radical-cation **70**, obtained when the phosphate group is split off from the C(3′) carbon atom [reaction (36)], can exist in two mesomeric forms with the positive charge located on the C(3′) or C(5′) atom. This radical-cation combines rapidly with a water molecule at one of the positively charged centres, forming respectively the C(4′)-β-phospho-α-alkoxyalkyl radical **72** again or the C(3′)-alkyl radical **73**. The radical **72** eliminates a second phosphate group and is converted into the radical-cation **74**. One nucleoside unit is fully eliminated from DNA at this stage. The intermediate **74** rapidly combines with a water molecule at the C(4′) or C(5′) position. The resulting alkyl radicals **75** and **76** are converted into the identified final products **45** and **47** respectively after reduction via a disproportionation reaction with any other radical and after the elimination of the intact heterocycle. The C(5′) radical is converted similarly via a disproportionation reaction into the unstable compound **77**, which is converted, after the heterocycle has been split off, into the detected product **49**. Although DNA is in fact cleaved at this stage, the oxidised nucleoside residue remains bound to the 3′-phosphate end of one of the DNA fragments.

The radical-cation **71** formed via the minor pathway (37) undergoes similar reactions, which result in the formation of the products **46**, **47**, and **50** (Table 12). They have also been detected as the final products of anaerobic γ-radiolysis of nucleic acids.[115]

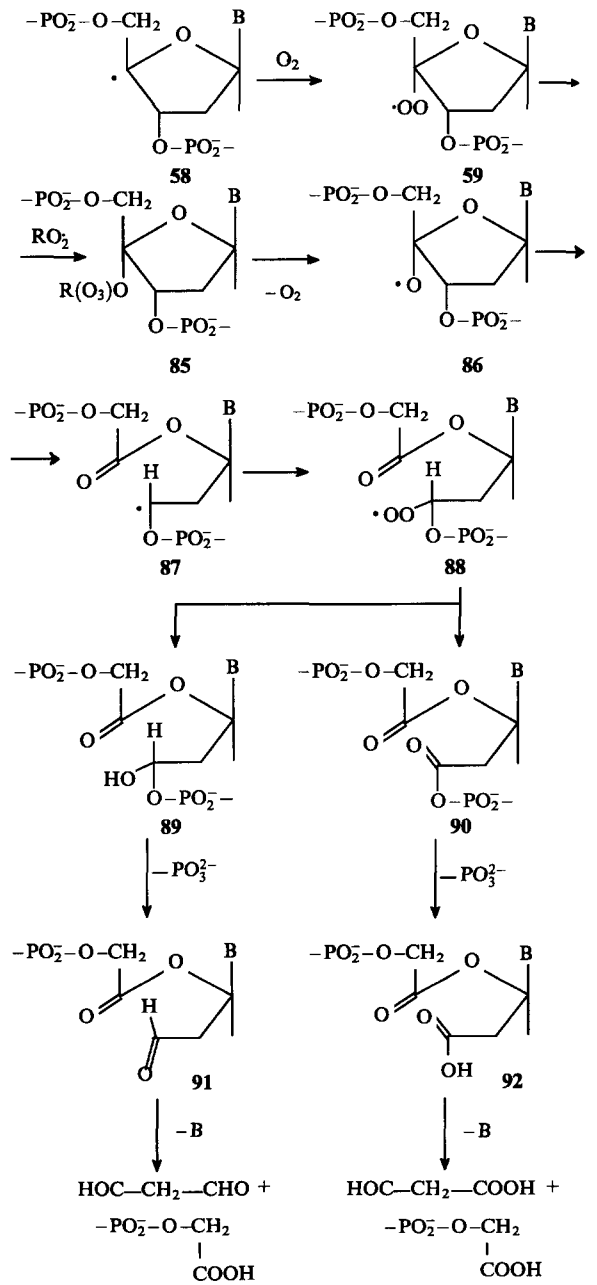
The isomerisation of the C(4′) radical to the C(1′) radical **84** [reaction (38)], accompanied by the opening of the furanose ring, may compete with the elimination reactions (36) and (37).[116] Reaction (38) prevents the cleavage of DNA. The alkyl radical **84** formed eliminates the heterocycle after reduction and is converted into the product **55**, analogous to the product of the anaerobic oxidation by bleomycin.



In the aerobic γ-radiolysis, the reactions of the C(4′) radical are much more complex than in the oxidation by bleomycin despite the fact that certain products are identical in both cases. Molecular oxygen, which adds to the C(4′) radical with a diffusion-controlled rate constant $k = 10^9$ litre mol^{-1} s^{-1}, fully suppresses the phosphate group elimination reactions (36) and (37). The peroxyl radicals formed were detected by EPR. They have an unusually long lifetime[117] (~0.2–3.3 s). The subsequent decomposition of the C(4′) peroxyl radicals leads to the cleavage of nucleic acids. The main products detected were fragments of nucleic acids containing the phosphate group at the 5′-end and fragments containing either the phosphate or the phosphoglycolate group at the 3′-end. In addition, heterocycle propenals and unidentified products, undergoing a reaction with thiobarbituric acid characteristic of malonaldehyde, were also detected.

The mechanism of the decomposition of the peroxyl radical **59** on γ-radiolysis most consistent with the experimental data has been proposed by Schulte-Frohlinde and Bothe.[104] According to this mechanism, the peroxyl radical decomposes via a bimolecular reaction with formation of the tetroxide **85**. The intermediate **59** is a tertiary peroxyl radical and, in conformity with the foregoing, decomposes solely via the radical mechanism (21) with formation of two oxyl radicals and an oxygen molecule. The rearrangement with selective cleavage of the C(3′)–C(4′) bond and the formation of the radical **87**, which again interacts with O_2, is characteristic of such oxyl radicals **86**. Being a secondary peroxyl radical, the radical **88** decomposes via the formation of a tetroxide intermediate in accordance with Russell's concerted bimolecular

Scheme 5



mechanism (18) described above. An hydroxy- or carbonyl group (compounds **89** and **90** respectively) appears with equal probability at the C(3′) carbon atom as a result of this reaction. The phosphoester bond in the C(3′) position undergoes rapid hydrolysis in the compounds **89** and **90** formed, which leads to the rupture of the sugar phosphate skeleton and the formation of the fragments **91** and **92**. The subsequent decomposition of these fragments is accelerated in an acid medium or on heating and leads to the formation of the intact heterocycle and the 5′-phosphoglycolate end group. According to some hypotheses, the hydrolysis of compound **91** can involve the formation of heterocycle propenals (see scheme 5).

As noted above, free OH radicals are formed also during the reduction of molecular oxygen by transition metal ion complexes, for example, Fe-EDTA. One might expect that nucleic acids are oxidised in such systems via the same mechanisms as in γ-radiolysis. The products of the degradation of DNA by the iron complex of methidiumpropyl-EDTA (MPE) in the presence of O_2 and a reductant or H_2O_2 have been most thoroughly characterised.[118] It was shown that the pentose phosphate skeleton is cleaved. For each break, one heterocycle is split off with formation of 5′-phosphate end groups and of 3′-phosphates and 3′-phosphoglycolates in approximately equal amounts. The mechanism of the degradation of DNA was not discussed in the above study.[118] Nevertheless, the formation of the products is consistent with the sequence of the reactions of the pentose C(4′) radical proposed for γ-radiolysis, where the heterocycle is liberated in the presence of a deficiency of oxygen together with free 5′- and 3′-phosphate end groups, while in the presence of oxygen the 5′-phosphate and 3′-phosphoglycolate end groups and the heterocycle are formed. Indeed, if MPE · Fe(III) · H_2O_2 is used instead of MPE · Fe(II) · O_2, then 3′-phosphoglycolates are formed in significantly smaller amounts.

4. Oxidation in the C(5′) position in the pentose

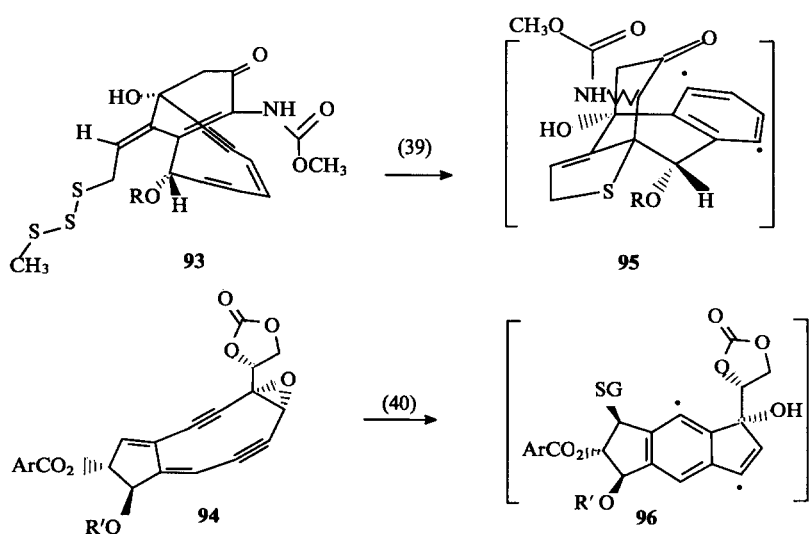
The preferential oxidation in the C(5′) position in deoxyribose has been observed when DNA was cleaved by antibiotics of the diene-ene type such as neocarzinostatin **93** and calcomycin **94**.[119, 120] These antibiotics contain a bicyclodiene-ene structure, capable of being converted via reactions (39) and (40) into the biradicals **95** and **96**, which attack deoxyribose and abstract a H atom preferentially from the C(5′) carbon atom.

The mechanism and the cleavage of DNA by neocarzinostatin has been investigated in greatest detail.[121–124] The neocarzinostatin biradical **95**, formed as a result of reduction by thiols, abstracts as a rule an H atom from the C(5′) position in the pentose, which has been demonstrated in experiments with nucleic acids containing atoms of the ^2H or ^3H hydrogen isotope in different positions in deoxyribose. When the H atom at the C(5′) atom was replaced, both covalent incorporation of a deuterium or tritium atom in the antibiotic molecule and an isotope effect in the DNA cleavage reaction were observed.[122, 125] The abstraction of a H atom from the C(4′) or C(1′) position is observed to a much lesser extent. The specificity of the reaction in relation to a particular position in deoxyribose is apparently due to the different accessibility of the H atoms of deoxyribose in different sections of DNA.

In the next stage, only covalent adducts between DNA and the antibiotics are formed under anaerobic conditions. On the other hand, under aerobic conditions, DNA is cleaved and the formation of two sets of cleavage products is observed. The main product (80%) involves single-strand breaks (T > A ≫ C > G) with formation at the cleavage site of a fragment with the 5′-phosphate end group and a fragment containing a nucleoside-5′-aldehyde at the 3′-end. Less than 20% of the breaks occur in one nucleoside with phosphates at both the 5′- and 3′-ends and accompanied by the spontaneous liberation of the heterocycles.

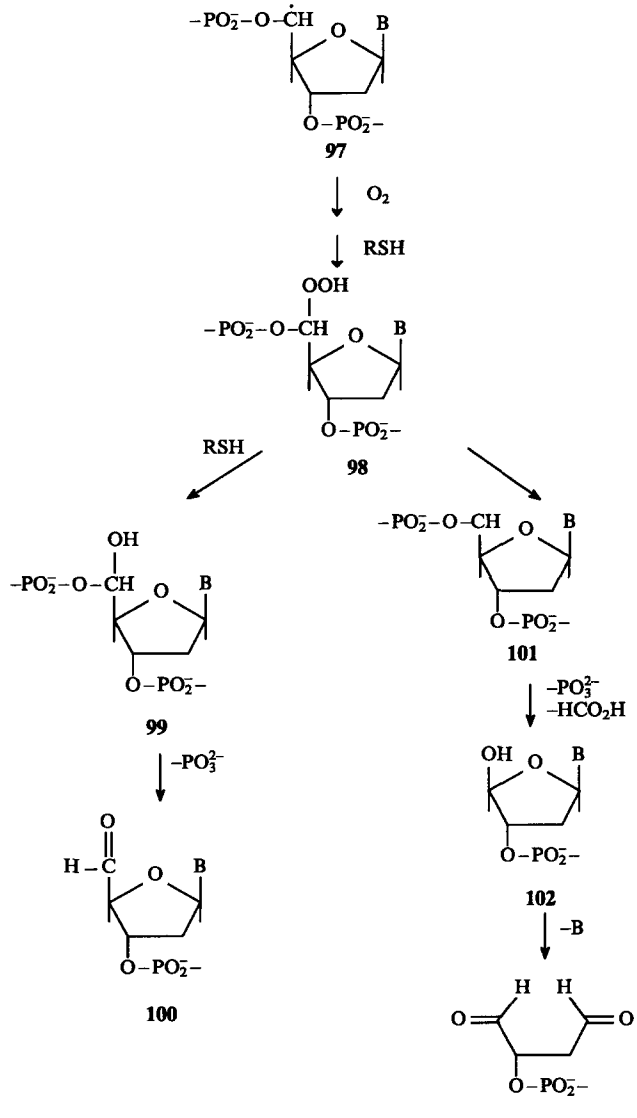
It has been shown [126] that under aerobic conditions the activation of the antibiotic is followed by the rapid absorption of an oxygen molecule via each antibiotic molecule and the subsequent utilisation of at least yet another reductant molecule. These results agree with the reaction in which the oxygen molecule adds to the radical **97**, generating a peroxyl radical, which is rapidly reduced by thiols to the hydroperoxide **98**. The use of $^{18}O_2$ made it possible to detect the incorporation of the oxygen isotope in one of the end products of the oxidation—nucleoside-5′-aldehyde.[127] The formation of the 5′-aldehyde end group has been demonstrated[124, 125] by reaction with $NaBH_4$, which leads to the reduction of the aldehyde group to the alcoholic group, and this was in its turn detected by phosphorylation catalysed by the T4 polynucleotidekinase. The use of a self-complementary short oligonucleotide,[124] having one site for oxidation by neocarzinostatin, made it possible to isolate all the final oxidation products and to establish their structures.

Two pathways leading to the decomposition of the hydroperoxide **98** have been postulated on the basis of the sum total of the results. Since the system contains a reductant (thiols), the peroxide group in the hydroperoxide **98** can be reduced to the hydroxy-group. The resulting compound **99** eliminates the phosphate group from the 5′-end, which ultimately leads to the rupture of the DNA chain and the formation of the 3′-phosphate and 5′-aldehyde (compound **100**) end groups. In addition, the hydroperoxide **98** can apparently undergo the Criegee rearrangement, which results in the formation of compound **101**. Compound **101** eliminates from the 5′-end a fragment containing the 3′-formyl

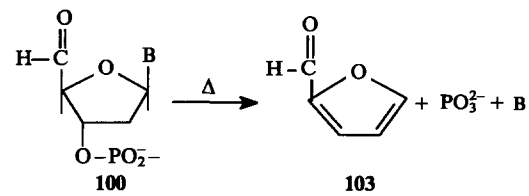


R and R′ are monosaccharides

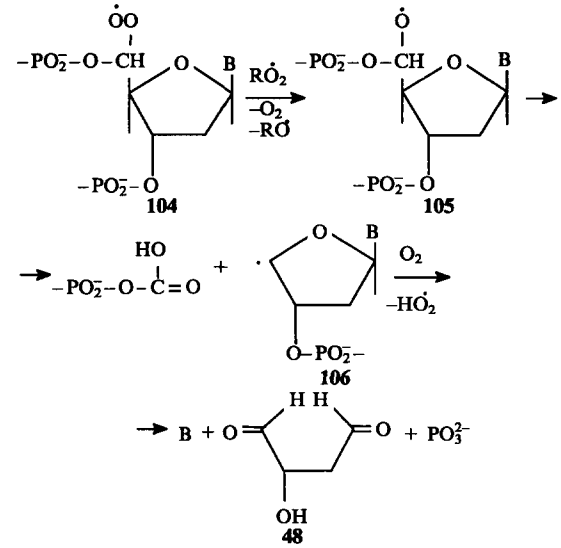
phosphate group, which hydrolyses spontaneously with the formation of the free 5′-phosphate end group. The second fragment contains the unstable compound **102**, which decomposes to compound **51**, liberating the heterocycle. It has been shown[127] that the amount of the nucleoside-5′-aldehyde increases with increase in the concentration of thiols, while the liberation of the free heterocycle diminishes. This confirms that the pathways leading to the formation of these products do indeed start from a single precursor—the hydroperoxide **98**—and that they compete with one another.



The selective oxidation of deoxyribose in the C(5′) position has been observed also for cationic metalloporphyrins.[128] However, as mentioned previously, in the case of metalloporphyrins the active oxidising species is apparently a high-valence metal oxocomplex, the most characteristic reaction of which is hydroxylation. Therefore, in the oxidation of DNA by metalloporphyrins, the first stage most probably involves the hydroxylation of the C(5′) carbon atom of the deoxyribose with the immediate formation of compound **99**. After the elimination of the phosphoester fragment from the C(5′) position, the nucleoside-5′-aldehyde fragment **100** is also formed. On further heating, it eliminates furfural **103**, splitting off the 5′-phosphate group.



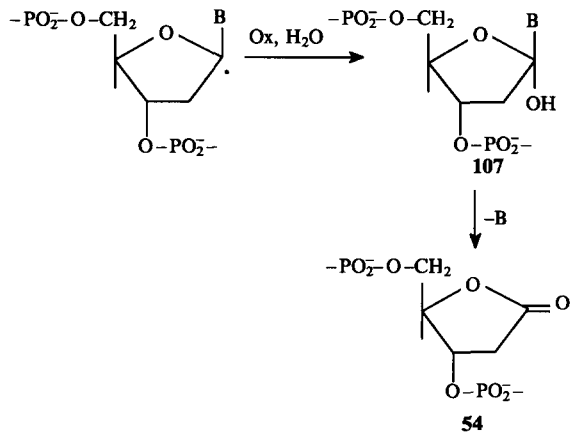
The formation of nucleoside-5′-aldehyde, accompanied by the splitting off of the phosphate, has been observed in the γ-radiolysis of aqueous solutions of D-ribose-5′-phosphate and guanosine 5′-phosphate under both anaerobic and aerobic conditions.[129] However, on γ-irradiation of aqueous DNA solutions, the formation of the nucleoside-5′-aldehyde was not observed. As regards the four-carbon fragment of deoxyribose of type **51**, linked to the 5′-phosphate end group of DNA, under aerobic conditions an analogous 2′-deoxytetradialdose is formed, but it is either linked to the 3′-phosphate end groups, as in **49**, or is present in the free state **96**.[30] The formation of such tetradialdose can be represented as a result of the conversion of the 5′-peroxyl radical **104**. The latter, being a secondary radical, can decompose via Russell's mechanism with formation of the oxyl radical **105**, which undergoes β-fragmentation, affording one DNA fragment with a 3′-formyl phosphate end group and another with the radical **106** linked to the 5′-phosphate group. At this stage, the DNA chain is cleaved betweren the C(4′) and C(5′) carbon atoms of deoxyribose. The radical **106**, which is a β-phospho-α-alkoxyl-akyl radical, rapidly eliminates the phosphate group from C(3′), recombines with an oxygen molecule, and the resulting peroxyl radical decomposes via a unimolecular mechanism with formation of the tetradialdose **48** and the liberation of the intact heterocycle.



5. Oxidation in the C(1′) position of the pentose

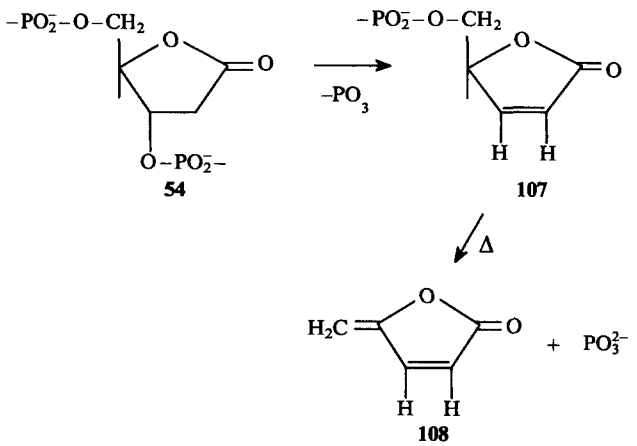
The oxidation in the C(1′) position leads to the formation of only one final product both on γ-radiolysis (under aerobic and anaerobic conditions) and on selective oxidation by certain 'chemical nucleases' (Cu-1,10-phenanthroline, Mn-porphyrins) although apparently via different mechanisms.

On γ-radiolysis under anaerobic conditions, the C(1′) radical can be oxidised with formation of a carbonium ion, the rapid solvolysis of which leads to the formation of compound **107**. The latter is unstable and rapidly splits off the heterocycle. The resulting lactone **54** remains bound to the sugar phosphate skeleton of DNA, as shown by Urata et al.[131] The presence of such a site can lead to the cleavage of DNA on heating or under acid conditions.



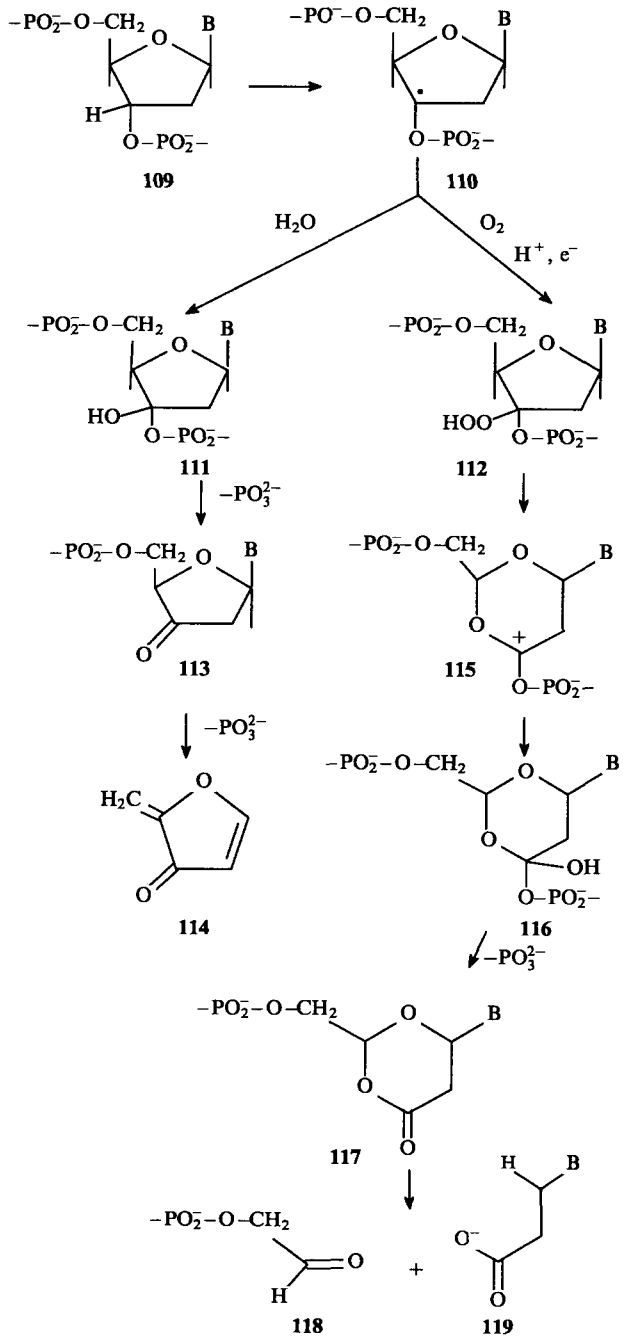
In the presence of molecular oxygen, the C(1′) radical should rapidly combine with oxygen to form a peroxyl radical, the subsequent reaction of which, apparently via Russell's mechanism, also leads to the formation of the lactone **54** bound to DNA.

The selective oxidation of DNA in the C(1′) position in deoxyribose is characteristic of certain metal complexes with affinity for DNA. This applies in the first place to Cu-1,10-phenanthroline[8, 132, 133] and Mn-tetra(4-methylpyridinium)porphyrin.[128, 134–136] The formation of coordinated active forms of oxygen is characteristic of such complexes and nucleic acids are oxidised via the hydroxylated C(1′) positions of deoxyribose, also with formation of compound **107**, which decomposes to the lactone **54**. However, it has been shown that compound **54** is unstable, so that its decomposition is also accompanied by the rupture of the DNA chain with liberation of the 3′-phosphate fragment and the 5′-intermediate. The latter gives rise to 5-methylene-2-5*H*-furanone **108** on heating with liberation of the 5′-phosphate end group. This difference between the stabilities of compound **54** in various oxidation systems has not so far been explained. It may be associated with the catalysis of the elimination of the phosphate group from the C(3′) position in the vicinity of the site where modification by metal complexes takes place. Such complexes may behave as Lewis acids.



6. Oxidation in the C(3′) position of the pentose

The oxidation in the C(3′) and C(2′) positions of the pentose has been investigated to a lesser extent because the majority of compounds catalysing the oxidative cleavage of DNA are bound to a small groove, whilst the C(3′) and C(2′) atoms are located in a large groove. Compounds have been discovered quite recently, which, on binding to DNA, are located in a large groove and catalyse the photochemical oxidative cleavage of DNA. These are mixed rhodium(III) complexes with two 9,10-phenanthrenequinone diimine molecules and one 2,2′-bipyridyl molecule or with two 1,10-phenanthroline molecules and one 9,10-phenanthrenequinone diimine molecule.[137] These complexes bind to DNA from the side of the large groove as a result of the intercalation of the 9,10-phenanthrenequinone diimine ligand. On irradiation, the latter is converted into a radical-cation,[138] which is capable of abstracting a hydrogen atom from deoxyribose. In conformity with crystallographic studies, on



intercalation from the side of the large groove the intercalator comes close to the C(3′)–H bond.[139]

The products of the oxidation of a short oligonucleotide duplex by such rhodium complexes have been isolated and characterised.[140] The mechanism of the oxidation of DNA in the C(3′)-position under both aerobic and anaerobic conditions has been proposed on the basis of the results. The radical **110** obtained is apparently oxidised by the rhodium(II) ion and then combines with water molecule. The compound **111** formed is unstable and as a result of several β-elimination processes splits off the 3′- and 5′-phosphate end groups and the free heterocycle. The oxidised deoxyribose residue is isolated in the form of 2-methylene-3-2*H*-furanone **114**.

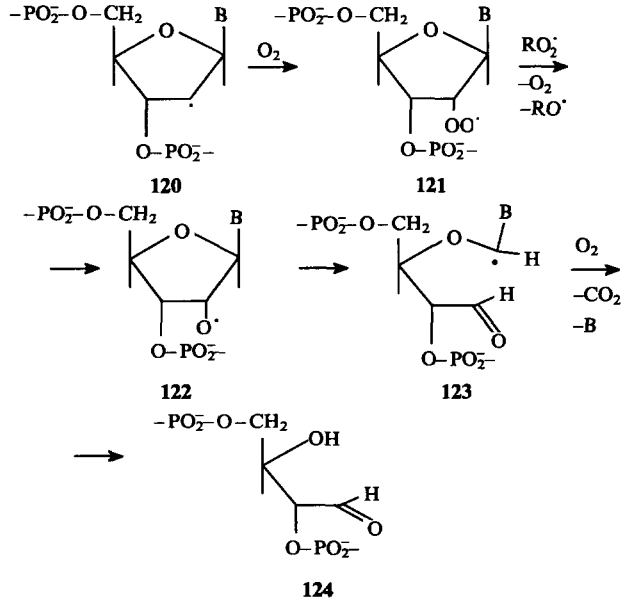
An alternative pathway is observed under aerobic conditions. In the first stage, an oxygen molecule adds rapidly to the C(3′) position and the resulting peroxyl radical is reduced. The hydroperoxide **112** undergoes the Criegee rearrangement, which leads to the rupture of the DNA chain with formation of 5′-phosphate and 3′-phosphoglycolaldehyde and groups (compound **118**). The heterocycles liberated contain a deoxyribose residue—propionic acid **119**.

Among the products of the γ-radiolysis of nucleic acids, products which might be formed as a result of the oxidation in the C(3′) position of the pentose were not identified. Only in the oxidation of deoxycytidine 5′-monophosphate was a product of the reaction of the C(3′) radical characterised,[117] but these results cannot be transferred to nucleic acids because the 3′-phosphate is absent in the given instance.

7. Oxidation in the C(2′) position of the pentose

Among the DNA oxidation products under aerobic conditions, only one compound was isolated for which a mechanism involving its formation from the C(2′) radicals may be postulated.[141]

Like other deoxyribose radicals, the radical **120** rapidly combines with an oxygen molecule and is converted into the peroxyl radical **121**, which decomposes via Russell's mechanism with formation of the oxyl radical **122**. The oxyl radical **122** undergoes fragmentation with rupture of the C(1′)–C(2′) bond and the resulting radical **123** can again combine with an oxygen molecule, which is followed by the elimination of the heterocycle and a CO_2 molecule. The ultimate formation of the four-carbon deoxyribose fragment (erythrose) **124** is not accompanied by the cleavage of the nucleic acid under physiological conditions.



V. Conclusion

Thus the main species responsible for the degradation of nucleic acids in various oxidising systems are usually free OH radicals. Hydroxyl radicals interact with nucleic acids and model compounds the rate constants of which are close to diffusion-controlled values and generate organic free radicals either by adding to the unsaturated bonds of the heterocycles or by abstracting a hydrogen atom from the pentose. The OH radicals interact preferentially with the heterocycles: in nucleotides and single-stranded nucleic acids, the ratio of the radicals formed from the heterocycles to the pentose radicals is 10–15. For double-stranded DNA, in which the heterocycles become less accessible, this ratio reaches 5–7. In a number of instances, the heterocycle radical may abstract a hydrogen atom from the C(4′)-position in the pentose of the preceding monomer unit, inducing the formation of this radical. In this case, the yield of the pentose radical reaches 40%.

In the oxidation of nucleic acids by a number of antibiotics or transition metal complexes, the oxidising species is a bound analogue of the OH radical—the oxo-complex of a high-valence metal. In this case, a selective interaction with the pentose takes place and its radicals become the main primary product of the oxidation reaction.

The pentose radicals obtained induce in the course of further reactions either direct cleavage of nucleic acids or modifications of pentose which lead to cleavage after treatment with alkali. In the presence of oxygen, only oxidation in the C(3′)-, C(4′)-, and C(5′)-positions in the pentose leads to the rupture of the nucleic acid target. In the absence of oxygen, only oxidation in the C(3′)- and C(5′)-positions is accompanied by the cleavage of the pentose phosphate skeleton. Oxidation in the C(1′)-position can induce the cleavage of nucleic acids only under the conditions of catalysis by transition metal complexes.

The appearance of radicals derived from heterocycles usually leads to irreversible modifications of the heterocycle in the course of further reactions. Some of these modifications may weaken the glycosidic linkage and may induce the formation of non-pyridine/non-purine sites, which is accompanied by the cleavage of the pentose phosphate skeleton under alkaline conditions. The stable modifications of the heterocycles, remaining bound to DNA, can have intrinsic coding properties different from those of the initial nucleoside, inducing mutations.

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