Clemens von Sonntag and the early history of radiation-induced sugar damage in DNA

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Abstract

Purpose: This article reviews the early history of ionizing radiation-induced sugar damage in DNA in dedication to Prof. Clemens von Sonntag, who recently passed away. It covers the time between 1968 and 1978, during which most of the work on the ionizing radiation-induced damage to polyalcohols, carbohydrates and the 2'-deoxyribose moiety in DNA was performed. Methodologies using gas chromatography-mass spectrometry (GC-MS) were developed to identify and quantify the radiation-induced products that had previously remained elusive. Products were identified by GC-MS either directly or after reduction of samples with $NaBH_4$ or $NaBD_4$. Incorporation of deuterium atoms by $NaBD_4$-reduction facilitated the identification of aldehyde, keto, carboxyl and deoxy groups in the molecules. Numerous products of a polyalcohol and carbohydrates were identified and quantified. Mechanisms of product formation were proposed. Several products of the 2'-deoxyribose moiety in DNA were identified, indicating that they were released from DNA strand, not bound to it. Alkali labile sites and products still remaining within DNA or bound to DNA as end groups were also elucidated by first reducing irradiated samples with $NaBD_4$ followed by alkali treatment and GC-MS analysis. *Conclusion:* The knowledge of the products of the 2'-deoxyribose moiety in DNA led to the first mechanistic understanding of various pathways of hydroxyl radical-induced DNA strand breakage. To this date, some of these mechanisms still remain the most-widely studied mechanisms of DNA damage. Prof. von Sonntag's contributions to the understanding of the radiation chemistry of carbohydrates and DNA helped shape this field of science for years to come.

Introduction

Professor Clemens von Sonntag passed away last year after a brief illness (Schuchmann et al. 2013). He was a great scientist and a teacher to many of us who worked with him or to those who learned from his great scientific contributions published in his papers and books. It is out of the scope of this paper to review all the scientific accomplishments of Prof. von Sonntag. My intention is to give a brief review of the early work on the elucidation of the ionizing radiation-induced products of the sugar moiety in DNA and the mechanisms of DNA strand breakage. I will also briefly discuss the preceded works on polyalcohols and carbohydrates because they formed the foundation for the subsequent work on DNA. I met Clemens toward the end of 1968, when I started my doctoral work at the Nuclear Research Center (Kernforschungszentrum) in Karlsruhe, Germany. I was a student at the University of Karlsruhe (now called "Karlsruhe Institute of Technology"); however, I worked at the Institute for Radiation Technology (Institut für Strahlentechnologie) within the Kernforschungszentrum. Clemens was located nearby at the Institute for Radiation Chemistry (Institut für Strahlenchemie) along with the director of the institute Prof. Dietrich Schulte-Frohlinde. With the help of Prof. J. F. Diehl, who was the director of the Institut für Strahlentechnologie, Prof. Schulte-Frohlinde graciously accepted me as a doctoral student and became my official "Doktorvater" (doctoral adviser). However, I closely worked with Clemens and occasionally met with Prof. Schulte-Frohlinde at the laboratory meetings or to discuss my work. At the Institut für Strahlentechnologie, I worked under the supervision of Dr. Heimo Scherz in his laboratory. The aim of my doctoral work was to investigate ionizing radiation-induced damage to polyalcohols as model compounds for carbohydrates in general and for 2'-deoxyribose in DNA in particular. The product analysis and elucidation of mechanisms of product formation were among the aims of the work. As a

polyalcohol, we chose *meso*-erythritol, which possesses four carbon atoms and four OH groups. During my work, I was introduced to the technique of gas chromatography-mass spectrometry (GC-MS), which I am still using to this date. Product analyses were performed using this technique. I will briefly discuss the product analysis and mechanisms of product formation in γ-irradiated N_2O-saturated aqueous solutions of *meso*-erythritol (Dizdaroglu et al. 1972), since the methodologies developed in this work subsequently helped elucidate the radiation-induced products of carbohydrates and 2'-deoxyribose within DNA.

Products of *meso*-erythritol and mechanisms of product formation

The analysis by GC-MS of γ-irradiated samples of *meso*-erythritol revealed the formation of butandiol-(3,4)-one-(2) (also called 1-deoxyerythrulose), butandiol-(1,4)-one-(2) (also called 3-deoxyerythrulose), α-erythrose, β-erythrose and erythrulose. However, some of the products could not be analyzed because they did not pass through a so-called Biemann-separator between the GC-column and the ion source of the mass spectrometer, most likely due to the presence of an aldehyde or a keto group. To overcome this problem, we developed a new procedure. Irradiated samples were treated with KBH_4 to reduce aldehyde and keto groups to alcohol groups in order to facilitate the analysis of the products by GC-MS. This turned out to be an excellent way for the proper analysis of the compounds containing aldehyde, keto and deoxy groups resulting from polyalcohols, carbohydrates and 2'-deoxyribose within DNA. Trimethylsilyl (TMS) derivatives of reduced compounds with alcohol groups were much more amenable to GC-MS analysis than their precursors. Besides the confirmation of the aforementioned products, 2-deoxytetrose, glycerol aldehyde, glycol aldehyde and dimers were identified additionally. The reduction by $NaBD_4$ was also employed to see whether the presence of aldehyde, keto and deoxy

groups could unequivocally be confirmed by the mass spectra. Indeed, the insertion of a deuterium atom clearly revealed the presence of an aldehyde group or the presence and position of a keto group and deoxy groups in the precursor molecules. As examples, Figure 1 illustrates the mass spectra of the TMS derivatives of butanetriol-(2,3,4) and butanetriol-(2,3,4)-2-d_1, which resulted from the reduction of butanediol-(3,4)-on-(2) by KBH_4 and $NaBD_4$, respectively. The inserts illustrate the fragmentation pathways of the molecules.

(insert Figure 1)

Typical ions resulting from the cleavage of the C-C bonds were present at *m/z* 103, 117, 205 and 219 (Figure 1A). The intense ion at *m/z* 117 showed the presence of a methyl group. The shift in the mass of this ion by 1 Da leading to the intense ion at *m/z* 118 (Figure 1B) pointed to a deuterium atom at carbon-2, and thus the position of the keto group in the precursor molecule. This was also confirmed by the ion at *m/z* 220 (Figure 1B), which represented a shift by 1 Da in the mass of the ion at *m/z* 219 (Figure 1A). The lack of shifts in the masses of the ions at *m/z* 103 and *m/z* 205 pointed to one deuterium atom only in the molecule. The high abundance of the ions at *m/z* 117 (Figure 1A) or at *m/z* 118 (Figure 1B) proved the facile cleavage of the C2-C3 bond, most likely due to the presence of the CH_3 group. It should be pointed out that the ions at *m/z* 73 and *m/z* 147 in Figures 1A and 1B, and in other mass spectra presented here are typical ions of TMS derivatives and serve no diagnostic purpose (White et al. 1972).

The mass spectra of the TMS derivatives of butanetriol-(1,3,4) and butanetriol-(1,3,4)-1-d_1, which resulted from the reduction of 2-deoxytetrose by KBH_4 and $NaBD_4$, respectively, are shown in Figure 2. In these spectra, the high abundance of the ions at *m/z* 103 (base peak) and *m/z* 219 (Figure 2A) was remarkable, when compared to those in the mass spectra in Figure 1.

Apparently, the presence of the 2-CH_2 group greatly facilitated the cleavage of the C1-C2 bond with the charge remaining on C1. The same was true for the cleavage of the C3-C4 bond leading to the intense ion at *m/z* 219. The shifts in the masses of the ions at *m/z* 103 and *m/z* 219 by 1 Da leading to the ions at *m/z* 104 and *m/z* 220 and no shift in the mass of the ion at *m/z* 205 in Figure 2B provided the evidence for a deuterium atom at C1, and thus the precursor product as 2-deoxytetrose.

(insert Figure 2)

When compared to the mass spectra in Figure 1A, the intensity of the ion at *m/z* 117 along with that of the ion at *m/z* 205 was very low, pointing to the non-preferred cleavage of the C2-C3 bond in contrast to the highly preferred cleavage of the C1-C2 bond. The discovery of this fragmentation behavior of TMS derivatives of polyalcohols containing a CH_2 group or a CH_3 group, and, of course, the idea of employing KBH_4 and $NaBD_4$ reduction, greatly facilitated the elucidation of the products of the 2'-deoxyribose within DNA and those of carbohydrates under a variety of experimental conditions. These will be discussed below.

Figure 3 illustrates the proposed mechanisms of product formation. Abstractions of an H atom ($H^{\bullet}$) from C1 and C2 by radiation-generated hydroxyl radical ($^{\bullet}OH$) and $H^{\bullet}$ (in part) results in the formation of C1- and C2-centered radicals (radicals I and II), respectively.

(insert Figure 3)

Subsequent oxidation of these radicals gives rise to erythrose and erythrulose, respectively, with similar *G*-values. Electron spin resonance studies and product analysis had shown that 1,2-dihydroxyalkyl radicals such as those of polyalcohols and monosaccharides undergo water elimination, leading to a C-centered radical with a vicinal keto or aldehyde group as shown in Supplementary Figure 1 (Buley et al. 1966, Livingston and Zeldes 1966, Norman and Pritchett

1967, Seidler and von Sonntag 1969, von Sonntag and Thoms 1970, von Sonntag 2006). Thus, water elimination from radicals I and II (Figure 3) was proposed, leading to radicals V, VI and VII, with subsequent reduction that produced 2-deoxytetrose, butandiol-(3,4)-one-(2) and butandiol-(1,4)-one-(2), respectively. Mass spectra of the dimers revealed that radicals V and VI reacted with one another to produce three types of dimers (Dizdaroglu et al. 1972).

Products of carbohydrates and mechanisms of product formation

In 1970, Prof. Schulte-Frohlinde received an invitation to become the director of the Radiation Chemistry Division of the Max-Planck-Institute for Coal Research (Abteilung Strahlenchemie, Max-Planck-Institut für Kohlenforschung) in Mülheim a.d. Ruhr. Later, this division became an institute within the Max-Planck-Institute (Institut für Strahlenchemie) and, then, itself a Max-Planck-Institute for Radiation Chemistry (Max-Planck-Institut für Strahlenchemie). Meanwhile, Clemens was promoted to the position of a "Private Dozent" (Associate Professor) at the University of Karlsruhe. He then followed Prof. Schulte-Frohlinde and joined the Max-Planck-Institut für Kohlenforschung in 1970, becoming a group leader. In 1975, he was appointed as an adjunct professor at the Kernforschungszentrum in Karlsruhe. Having completed my dissertation at the University of Karlsruhe, I was invited by both Prof. Schulte-Frohlinde and Clemens to join them. In 1971, I moved to Mülheim a.d. Ruhr and started working in Clemens's group as a post-doctoral fellow. Two years later, I was hired as a regular employee of the Max-Planck-Institut.

Clemens and I started working on the radiation chemistry of carbohydrates, which included monosaccharides, disaccharides and polysaccharides in aqueous solution as well as in the crystalline state. Again, product analyses were accomplished using GC-MS and the methods developed previously, including the reduction of samples with $NaBD_4$ to elucidate the presence

of aldehyde, keto, carboxyl and deoxy groups in the products. We performed the GC-MS measurements with the help of Dr. Gerhard Schomburg and Dr. Dieter Henneberg, who were the leaders of the gas chromatography and mass spectrometry departments, respectively, and their co-workers. This led to the elucidation of structures of many radiation-induced products of carbohydrates in the crystalline state and in aqueous solution. Previously discovered fragmentation mechanisms of TMS derivatives of KBH_4- or $NaBD_4$-reduced products of *meso*-erythritol were confirmed with larger polyalcohols that resulted from the reduction of carbohydrate products (Dizdaroglu et al. 1974).

First, Clemens and I studied γ-radiolysis of crystalline α-lactose•H_2O to elucidate the mechanisms of formation of acids that had been found with unusually high *G*-values in γ-irradiated crystalline carbohydrates (Phillips and Baugh 1963, Phillips et al. 1966). Chain reactions restricted to the crystalline state had been proposed for the formation of acids; however, product analyses had been limited. GC-MS analyses of trimethylsilylated samples of γ-irradiated crystalline α-lactose•H_2O revealed the presence of 5-deoxylactobionic acid (*G*-value = 40), 2-deoxylactobionic acid lactone (*G*-value = 20), galactonic acid lactone (*G*-value = 4.5) and 4-deoxyglucose (*G*-value = 4.5) (von Sonntag and Dizdaroglu 1973). The structures were confirmed by $NaBH_4$- and $NaBD_4$-reduction of the samples followed by GC-MS analysis. As an example, Supplementary Figure 2A illustrates the mass spectrum of the TMS derivative of 2-deoxyglucitol that resulted from $NaBH_4$-reduction of 2-deoxylactobionic acid lactone (Dizdaroglu et al. 1974). The abundant ion at *m/z* 103 (base peak) clearly indicated the presence of a 2-CH_2 group in the molecule as had been shown in the case of the reduced products of *meso*-erythritol and other polyalcohols (Dizdaroglu et al. 1972, Dizdaroglu et al. 1974). The mass spectrum after $NaBD_4$-reduction pointed to a mass shift by 2 Da in the mass of the *m/z* 103 ion

(Supplementary Figure 2B), meaning that two deuterium atoms were present at C1, thus providing the evidence for a carboxyl group in the precursor molecule. The other ions at *m/z* 221, 323 and 335 (produced by loss of HOTMS from *m/z* 425) also showed the presence of two deuterium atoms in the molecule. In contrast, the mass spectrum of the TMS derivative of 5-deoxyglucitol-1,1-d_2 that resulted from $NaBD_4$-reduction of 5-deoxylactobionic acid lactone (Supplementary Figure 2C) exhibited an *m/z* 105 ion with low intensity, but an abundant ion at *m/z* 103. These ions clearly showed the presence of a 5-CH_2 group and two deuterium atoms at C1. The identified products provided evidence for three types of chain reactions in α-lactose•H_2O. For the chain propagation in a carbohydrate crystal, a readily abstractable $H^{\bullet}$ and sufficient proximity of two reacting molecules are necessary. The most available H atoms in α-lactose•H_2O are those at C1- and C1'-positions. In the proposed mechanism (von Sonntag and Dizdaroglu 1973) (Supplementary Figure 3), $H^{\bullet}$ abstraction from C1 produces a C1-centered radical of lactose (**A**). The rearrangement of radical **A** leading to a C5-centered radical and a carboxyl group produces the main product 5-deoxylactobionic acid upon $H^{\bullet}$ abstraction from the C1 of a neighboring lactose molecule and regenerates radical **A**. Water elimination from radical **A**, as was described above, leads to the formation of a C2-centered lactone radical (**B**). $H^{\bullet}$ abstraction by radical **B** from the C1 of a neighboring lactose molecule produces 2-deoxylactobionic acid lactone, regenerating radical **A**. Regenerated radical **A** then continues the chain reaction. On the other hand, the C1'-centered radical of lactose undergoes glycosidic bond scission generating galactonic acid lactone and a C4-centered radical, which abstracts an $H^{\bullet}$ from a neighboring lactose molecule to yield 4-deoxyglucose (not shown) (von Sonntag and Dizdaroglu 1973).

Next, we studied γ-radiolysis of 2-deoxyribose in the crystalline state as a model compound for the 2'-deoxyribose moiety in DNA. This is because DNA possesses a well-ordered structure, and solid state reactions and products may occur, which may not be observed in aqueous solution. The radiation chemistry of 2-deoxyribose in the crystalline state had previously been studied; however, product analysis had been limited to the detection of H_2 and CO_2 only (Hüttermann and Müller 1969a, Hüttermann and Müller 1969b). Using GC-MS following $NaBH_4$- and $NaBD_4$-reduction, and trimethylsilylation, we identified 2,5-dideoxy-erythropentonic acid with a large yield (*G*-value > 650) (von Sonntag et al. 1974). Supplementary Figure 4 illustrates the mass spectrum of the TMS derivative of 2,5-dideoxy-erythropentitol-1,1-d_2 that resulted from $NaBD_4$-reduction of this product. The ion at *m/z* 105 pointed to two deuterium atoms at C1, providing the evidence for a carboxyl group in the precursor molecule. The high abundance of this ion indicated an adjacent CH_2 group. On the other hand, the presence of a CH_3 group at the other end of the molecule was clearly shown by the abundant ion at *m/z* 117. 2-Deoxyribonic acid was also identified, but to a much lesser extent (*G*-value = 1.5). The high yield of 2,5-dideoxy-erythropentonic acid, in contrast to its much lower yield in aqueous solution (Hartmann et al. 1970), indicated an efficient chain reaction. The mechanism given in Supplementary Figure 5 was proposed. 2-Deoxyribose was present in its β-pyranose form in the crystalline state. γ-Irradiation produces a series of radicals including H atoms, which can abstract an $H^{\bullet}$ from 2-deoxyribose. Since the $H^{\bullet}$ at C1 is bound with the least bond energy, its abstraction from C1 is the most likely event leading to the C1-centered radical **A**. The rearrangement of radical **A**, as was previously observed with the same type of radicals (see e.g., von Sonntag and Dizdaroglu 1973), leads to a C5-centered radical (**B**), which then abstracts an $H^{\bullet}$ from a neighboring 2-deoxyribose molecule to produce 2,5-dideoxy-

erythropentonic acid and to regenerate radical **A**. The chain reaction would be propagated by radical **A**, as was described above.

Further work investigated γ-radiolysis of cellobiose, glucose, fructose, ribose and cyclic oligosaccharides in aqueous solution and in crystalline state (Dizdaroglu and von Sonntag 1973, Stelter et al. 1974, Dizdaroglu et al. 1975a, Dizdaroglu et al. 1976a, von Sonntag et al. 1976, Baugh et al. 1976, Stelter et al. 1976, Dizdaroglu et al. 1977a, von Sonntag and Dizdaroglu 1977, von Sonntag and Schulte-Frohlinde 1978, von Sonntag 2006). Many products were identified and reaction mechanisms were elucidated. Again, the reduction of irradiated samples with $NaBH_4$ and $NaBD_4$ with subsequent trimethylsilylation and GC-MS analysis was the key to the successful elucidation of the most product structures. Supplementary Figure 6 illustrates the proposed mechanisms of reactions of C1- and C5-centered radicals of glucose leading to the identified products.

Radiation-induced damage to the 2'-deoxyribose moiety in aqueous solutions of DNA

Products released from DNA

DNA in living organisms is constantly damaged by normal intracellular metabolism and by exogenous sources such as ionizing radiations, UV radiation, redox-cycling drugs, carcinogenic compounds, environmental pollutants, etc. (von Sonntag 2006, Halliwell and Gutteridge 2007). Oxygen metabolism generates $^{\bullet}OH$, superoxide radical ($O_2^{\bullet-}$) and non-radical H_2O_2. The interaction of ionizing radiations with cellular water produces these species as well, and, in addition, hydrated electron (e_{aq}^-) and $H^{\bullet}$ (von Sonntag 2006, Halliwell and Gutteridge 2007). Superoxide radical and H_2O_2 do not react with most biological molecules or with each other at a

considerable reaction rates, unless transition metal ions catalyze this reaction, generating •OH (Haber-Weiss reaction) (Halliwell and Gutteridge 2007). Hydroxyl radical reacts with the heterocyclic bases of DNA near or at diffusion-controlled rates, leading to a plethora of products by a variety of mechanisms (reviewed in von Sonntag 2006, Dizdaroglu and Jaruga 2012). The reactions of •OH with 2'-deoxyribose in DNA amount to approximately 20% (von Sonntag 2006); however, this rate may be different in living cells. Radical transfer from a base radical to 2'-deoxyribose is also likely, because DNA strand breakage is greater than expected from the amount of •OH attack on 2'-deoxyribose (Lemaire et al. 1984, Deeble et al. 1986, Karam et al. 1988, Hildenbrand et al. 1993, Jones and O'Neill 1991). In dilute aqueous solutions of DNA, ionizing radiation-induced damage occurs by reactions of free radicals (indirect effect of radiation). In living cells with high concentrations of radical scavengers, however, the direct effect of ionizing radiations leading to DNA ionization may also play a major role in DNA damage (von Sonntag 2006).

The effects of ionizing radiations on DNA and its constituents have been investigated since 1950s (see reviews in Hüttermann et al. 1978). Until early 1970s, radiation-induced damage to 2'-deoxyribose in DNA and mechanisms of DNA strand breaks had not been well understood. Clemens and I started working on radiation-induced DNA damage with the aim of the identification of products of 2'-deoxyribose in DNA and the elucidation of formation mechanisms of products and DNA strand breaks. For product identification, we used GC-MS and the methods previously developed by us for product analysis of polyalcohols and carbohydrates. However, the polymeric structure of DNA presented a significant challenge for the analysis. First, we identified 2,5-dideoxypentos-4-ulose, 2,3-dideoxypentos-4-ulose and 2-

deoxypentos-4-ulose in γ-irradiated N_2O-saturated aqueous solutions of DNA (Dizdaroglu et al. 1975b) (Figure 4).

(insert Figure 4)

These compounds were found in irradiated solutions of DNA, meaning they were released from DNA strand, not bound to it. Figures 5A and 5B illustrate the mass spectra of the TMS derivatives of 2,5-dideoxypentitol and 2,5-dideoxypentitol-1,4-d_2. The abundant ion at *m/z* 103 (base peak) (Figure 5A) pointed to a 2-CH_2 group. The shift by 1 Da in the mass of this peak (*m/z* 104 ion in Figure 5B) indicated an aldehyde group next to the CH_2 group in the precursor molecule.

(insert Figure 5)

The presence of a 5-CH_3 group and a keto group at C4 was shown by the intense ions at *m/z* 117 (Figure 5A) and at *m/z* 118 (Figure 5B). These mass spectra revealed the precursor compound as 2,5-dideoxypentos-4-ulose. 2,3-Dideoxypentos-4-ulose was identified by the mass spectra of its reduced forms 2,3-dideoxypentitol and 2,3-dideoxypentitol-1,4-d_2 (Supplementary Figure 7A,B). In contrast to those in Figures 5A and 5B, these mass spectra were dominated by the ions at *m/z* 233 and *m/z* 143 (*m/z* 233 – HOTMS) (base peak), and *m/z* 235 and *m/z* 145 (*m/z* 235 – HOTMS) (base peak), with the ions at *m/z* 103 and *m/z* 104 with negligible intensity, indicating two adjacent CH_2 groups, a keto group at C4 and an aldehyde group in the precursor molecule. The mass spectra of 2-deoxypentitol and 2-deoxypentitol-1,4-d_2 in Figures 6A and 6B, respectively, identified the precursor molecule as 2-deoxypentos-4-ulose.

(insert Figure 6)

The abundant ion at *m/z* 103 (base peak) pointed to a 2-CH_2-group. The shift in its mass by 1 Da leading to *m/z* 104 showed the presence of an aldehyde group. The shift by 1 Da in the masses of

m/z 307 and *m/z* 205 leading to *m/z* 308 and *m/z* 206, respectively, pointed to another deuterium atom in the molecule. The shift by 2 Da in the masses of *m/z* 321 and *m/z* 231 (*m/z* 321 – HOTMS) resulting in *m/z* 323 and *m/z* 233 (*m/z* 323 – HOTMS) and the shift by 1 Da in the mass of *m/z* 205 (to *m/z* 206) were a clear evidence for the presence of a keto group at C4 in the precursor molecule.

In the presence of oxygen, we identified two products, 2-deoxypentos-4-ulose and 2-deoxytetrodialdose released from DNA (Dizdaroglu et al. 1975c). The former had also been identified in the absence of oxygen (see above). 2,5-Dideoxypentos-4-ulose and 2,3-dideoxypentos-4-ulose were not found, indicating that oxygen prevented their formation. After reduction of irradiated DNA samples, followed by trimethylsilylation and GC-MS analysis, we identified butantriol-(1,3,4) and butantriol-(1,3,4)-1,4-d_2, which provided the evidence for 2-deoxytetradialdose. The mass spectra (not shown) pointed to two aldehyde groups and a 2-CH_2 group in the precursor molecule. This was based on the fact that the masses of the ions at *m/z* 103, *m/z* 205 and *m/z* 219 in the mass spectrum of the TMS derivative of butantriol-(1,3,4) were shifted by 1 Da to the ions at *m/z* 104, *m/z* 206 and *m/z* 220 in the mass spectrum of the TMS derivative of butantriol-(1,3,4)-1,4-d_2. In addition, there was a shift by 2 Da in the masses of *m/z* 307 [molecular ion ($M^{+\bullet}$) – $^{\bullet}CH_3$] and *m/z* 232 ($M^{+\bullet}$ – HOTMS) leading to *m/z* 309 and *m/z* 234. This also indicated the presence of two deuterium atoms in the molecule.

In a subsequent work, we identified most of the aforementioned DNA products in aqueous solutions of thymidine γ-irradiated in the absence or presence of oxygen (Dizdaroglu et al. 1976b). This work not only confirmed the products observed in DNA, but also the release of an unaltered base from a 2'-deoxynucleoside in DNA due to the sugar damage.

Products bound to DNA

The release of products from DNA and proposed mechanisms (see below) led us to hypothesize that altered sugars should be present within DNA with two phosphate groups still attached or as end groups with one phosphate group in a broken DNA strand. Such products bound to DNA would not be found in irradiated solutions of DNA. Previously, additional strand breaks had been reported to occur in irradiated DNA upon treatment with alkali (Bopp and Hagen 1970, Ullrich and Hagen 1971, Kessler et al. 1971, Kay and Ward 1977). The effect of alkali was thought to result from either base damage or sugar damage in DNA (Ward and Kuo 1973). Altered sugars within DNA or as end groups must have a free OH group at C4' to be alkali-labile sites. Our rationale for this notion was that RNA, but not DNA, is readily hydrolyzed with alkali because of the free OH group at C2' of the ribose (Brown and Todd 1952, Tamm et al. 1953). Hydrolysis occurs via cyclic phosphate esters, which are then converted into nucleoside 2'- and 3'-phosphates (Brown et al. 1953). In analogy to RNA, alkali-labile sites in DNA would release altered sugars with phosphate groups upon alkali treatment. In order to identify alkali-labile sites, we treated irradiated DNA samples with alkali and then with alkaline phosphatase to remove any phosphate groups still attached to altered sugars. Following trimethylsilylation and GC-MS analysis, 2-deoxy-erythropentonic acid was identified (Dizdaroglu et al. 1977b). The authentic material was synthesized to help with the identification. This product was formed in the absence and presence of oxygen, having a greater yield in the latter case. We also searched for other products by first reducing irradiated DNA samples with $NaBD_4$ and then treating them by alkali and alkaline phosphatase. This was done to avoid a possible destruction by alkali of possible aldehyde or keto groups in altered sugars. GC-MS analysis following trimethylsilylation identified erythritol-d_1, whose mass spectrum showed the presence of an aldehyde group in the

precursor molecule, thus identifying D-erythrose in DNA (Dizdaroglu et al. 1977c). The authentic material was also available. D-Erythrose was identified only in DNA samples irradiated in the presence of oxygen.

We also hypothesized that the products identified in irradiated solutions of DNA (Figure 10) would be highly likely to exist as end groups in broken DNA strands as well. The same procedure used for the identification of D-erythrose was applied. Indeed, we identified 2,5-dideoxypentos-4-ulose, 2,3-dideoxypentos-4-ulose and 2-deoxypentos-4-ulose (Figure 4) (Beesk et al. 1979). Their yields were significantly greater than those found directly in the case of irradiated solutions of DNA without alkali treatment. As examples, Figure 7 illustrates the identification pathway of two products bound to DNA.

(insert Figure 7)

Later on, 2-deoxytetradialdose has also been identified as an end group after alkali treatment (Isildar et al. 1981, von Sonntag et al. 1981, Schulte-Frohlinde and von Sonntag 1990). These findings provided the evidence for the formation of these four products as end groups in broken DNA strands. In the case of 2-deoxypentos-4-ulose, three different end groups have been proposed (Beesk et al. 1979). This product may also be present within DNA with two phosphate groups attached. The structures of the products bound to DNA are shown in Figure 8.

(insert Figure 8)

Mechanisms of formation of DNA products and strand breaks

In the absence of oxygen

For the formation of the products and strand breaks in the absence of oxygen, we proposed the mechanism illustrated in Figure 9. This illustration is the same as the one in the original paper published by us in 1975 (Dizdaroglu et al. 1975b).

(insert Figure 9)

Hydroxyl radical can abstract an H$^{\bullet}$ from all five carbons of 2'-deoxyribose, leading to five C-centered radicals (von Sonntag et al. 1981, von Sonntag 2006, Dizdaroglu and Jaruga 2012). The proposed mechanism starts with the C4'-centered radical formed by H$^{\bullet}$ abstraction from the C4'. Later work in 1990s and 2000s showed that the H$^{\bullet}$ abstraction is most probable from H4' and H'5 of 2'-deoxyribose in DNA (Pardo et al. 1992, Miaskiewicz and Osman 1994, Sy et al. 1997, Begusova et al. 2001, Toure et al. 2002). Moreover, H4' and H5' were found to be more exposed to solvent and thus more accessible to $^{\bullet}$OH attack than the other H atoms, when the solvent accessibility in DNA was taken into account (Balasubramanian et al. 1998, Pogozelski and Tullius 1998). Experimental results and calculations also suggested the C4'- and C5'-centered radicals to cause strand breaks to approximately equal extents.

The C4'-centered radical (radical **4** in Figure 9) is an alkoxyalkyl radical with a phosphate group at the β-position on both sites of the DNA strand bound to the C3' and C5' of the 2'-deoxyribose moiety. Studies on model systems such as glycerophosphates and ribose-5-phosphate showed that such radicals undergo heterolytic elimination of the phosphate group (Stelter et al. 1974, Stelter et al. 1976, Behrens et al. 1978, Behrens et al. 1982). The phosphate elimination at C3' of 2'-deoxyribose should be more favorable than that at C5', because the C3' is a secondary C-atom, whereas the C5' is a primary one. The original mechanism in Figure 9

shows the elimination by β-fragmentation of the phosphate group at C5', giving rise to a carbocation radical **5**. Subsequent reaction with water (HO^- addition) generates radical **6** or radical **7**. Reduction of radical **6** gives rise to **8**, which is unstable and undergoes ring opening leading to a hemiaminal (**9)**. Hydrolysis of this unstable compound releases the unaltered base accompanied with the phosphate elimination generating 2,5-dideoxypentos-4-ulose (**1**). The elimination of the phosphate group from radical **7** generates a carbocation radical (**10**), which reacts with water (HO^- addition) to give rise to a C3'-radical (**11**). The reduction of radical **11** leads to an hydrate (**12**), which is unstable and releases the unaltered base resulting in the formation of 2,3-dideoxypentos-4-ulose (**2**). In agreement with this mechanism, the release of unaltered bases from DNA and 2'-deoxynucleosides had already been reported in earlier works (Hems 1960, Ullrich and Hagen 1971) (also see Ward and Kuo 1976, Dizdaroglu et al. 1976b). Figure 10 illustrates the proposed mechanism for the formation of free 2-deoxypentos-4-ulose (**3** in Figure 4) (Beesk et al. 1979).

(insert Figure 10)

This involves the elimination of the phosphate group from the C4'-centered radical by β-fragmentation, leading to a strand break and the formation of a carbocation radical. Reaction of the carbocation radical with H_2O (HO^- addition), and then the elimination of the phosphate group with the DNA strand leads to a free C4'-centered nucleoside radical. The oxidation of this radical and subsequent reaction with water (HO^- addition) generates an unstable hemiaminal. Free 2-deoxypentos-4-ulose is then formed by the release of the unaltered base from this unstable hemiaminal. On the other hand, the oxidation of the C4'-radical as an end group (Figure 10) would cause the formation of 2-deoxypentos-4-ulose still attached to a broken DNA strand (not shown) (Beesk et al. 1979). The formation of 2-deoxypentos-4-ulose within DNA should involve

the oxidation of the C4'-radical, followed by reaction with water (HO^- addition) and elimination of the unaltered base (Figure 11).

(insert Figure 11)

The mechanism starting with the C4'-centered radical of DNA (Figure 9) was the first to describe the chemistry of strand breakage and products of 2'-deoxyribose formed in DNA by $^{\bullet}OH$ attack. In the latest edition of his book (page 382) (von Sonntag 2006), Clemens states: "*Concerning DNA strand breakage in the absence of O_2, the sequence of reactions resulting from the C(4') radical were the first understood mechanistically (Dizdaroglu et al. 1975), and this reaction still remains the most-widely studied mechanism of DNA strand breakage*." The details of this mechanism leading to identified altered sugars have subsequently been dealt with in review articles by Clemens and in his book (von Sonntag and Schulte-Frohlinde 1978, von Sonntag et al. 1981, von Sonntag 1984, Schulte-Frohlinde and von Sonntag 1990, von Sonntag 2006).

The formation of 2-deoxypentonic acid within DNA was explained *via* $H^{\bullet}$ abstraction by $^{\bullet}OH$ attack at C1' leading to a C1'-radical (Figure 12) (Dizdaroglu et al. 1977b). Oxidation of this radical gives rise to a carbocation, which reacts with water (HO^- addition) generating a hemiaminal. 2-Deoxypentonic acid is then formed by hydrolytic elimination of the unaltered base from this unstable hemiaminal.

(insert Figure 12)

In the presence of oxygen

Oxygen reacts with C-centered radicals at diffusion-controlled rates generating peroxyl radicals (Ross et al. 1992). 2,5-Dideoxypentos-4-ulose and 2,3-dideoxypentos-4-ulose are not formed in

the presence of oxygen because of its rapid addition to the C4'-radical of 2'-deoxyribose in DNA, which prevents the reactions shown in Figure 9. On the other hand, this reaction generates the formation of 2-deoxypentos-4-ulose in the presence of oxygen, too, *via* reactions of the C4'-centered peroxyl radical (von Sonntag 2006). Later, this peroxyl radical has been reported to also lead to the formation of 3'-phosphoglycolate as an end group (Henner et al. 1983, Balasubramanian et al. 1998, Pogozelski and Tullius 1998, von Sonntag 2006). In addition, the reaction of oxygen with other C-centered radicals of 2'-deoxyribose gives rise to peroxyl radicals. The C5'-peroxyl radical reacts with another peroxyl radical, leading to the C5'-oxyl radical, molecular oxygen and another oxyl radical (Dizdaroglu et al. 1975c) (Figure 13).

(insert Figure 13)

The β-fragmentation of the C5'-oxyl radical gives rise to a strand break resulting in two DNA fragments, one with a formyl phosphate end group and another one with a sugar fragment radical missing one C-atom. Oxygen addition followed by elimination of the unaltered base leads to 2-deoxytetradialdose as an end group. Additional phosphate elimination may generate free 2-deoxytetradialdose. Both forms of this compound have been identified in DNA irradiated in aqueous solution in the presence of oxygen (see above). Later work reported that the C5'-peroxyl radical leads to the formation of the altered 2-deoxyribose with a 5'-aldehyde as an end group (Balasubramanian et al. 1998). Figure 14 illustrates the formation of erythrose within DNA *via* addition of O_2 to the C2'-radical.

(insert Figure 14)

The thus-formed C2'-peroxyl radical is converted into an C2'-oxyl radical, the β-fragmentation of which leads to erythrose after loss of C1' and unaltered base (Dizdaroglu et al. 1977c). The formation of 2-deoxypentonic acid within DNA can be envisioned by addition of O_2 to the C1'-

centered radical leading to a peroxyl radical and then to an oxyl radical. The β-fragmentation of the latter with release of a base radical would give rise to 2-deoxypentonic acid lactone within DNA (Figure 15).

(insert Figure 15)

Conclusions

This article is dedicated to the memory of Prof. Clemens von Sonntag, whom I had the honor to work with for a total of ten years, first for three years as a doctoral student at the Kernforschungzentrum in Karlsruhe and then for another seven years as a member of his group at the Max-Planck-Institut für Strahlenchemie in Mülheim a.d. Ruhr. In this article, I attempted to briefly cover the research performed during those ten years under Clemens's supervision and leadership with the emphasis on radiation- and free radical-induced damage to polyalcohols and carbohydrates in general, and to the sugar moiety of DNA in particular. Methodologies were developed using GC-MS for the positive identification of many radiation- and free radical-induced products that had remained elusive in previous years. The knowledge of the products of 2'-deoxyribose in DNA led to the first mechanistic understanding of various pathways of $^{\bullet}$OH-induced DNA strand breakage. To this date, some of these pathways still remain the most-widely studied mechanisms of DNA damage. Throughout his illustrious career, Clemens greatly contributed to science in other areas as well. His contributions helped shape the field of DNA damage and will no doubt shape careers of future generations of scientists in this field of science for years to come.

Declaration of interest

The author reports no conflicts of interest.

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Figure legends

Figure 1. Mass spectra of the TMS derivatives of butanetriol-(2,3,4) (**A**) and butanetriol-(2,3,4)-2-d_1 (**B**) (from Dizdaroglu et al. 1972).

Figure 2. Mass spectra of the TMS derivatives of butanetriol-(1,3,4) (**A**) and butanetriol-(1,3,4)-1-d_1 (**B**) (from Dizdaroglu et al. 1972).

Figure 3. Mechanisms of product formation from *meso*-erythritol (from Dizdaroglu et al. 1972).

Figure 4. Products identified in γ-irradiated N_2O-saturated aqueous solutions of DNA (from Dizdaroglu et al. 1975b).

Figure 5. Mass spectra of the TMS derivatives of 2,5-dideoxypentitol (**A**) and 2,5-dideoxypentitol-1,4-d_2 (**B**) (from Beesk et al. 1979).

Figure 6. Mass spectra of the TMS derivatives of 2-deoxypentitol (**A**) and 2-deoxypentitol-1,4-d_2 (**B**) (from Beesk et al. 1979).

Figure 7. Identification pathway of two products bound to DNA: erythrose within DNA and 2,3-dideoxypentos-4-ulose as and group of a broken DNA strand (adapted from Dizdaroglu et al. 1977c).

Figure 8. Structures of the products bound to DNA.

Figure 9. Mechanisms of product formation and strand breaks in DNA γ-irradiated in aqueous solution (from Dizdaroglu et al. 1975b).

Figure 10. Mechanism of formation of free 2-deoxypentos-4-ulose (adapted from Beesk et al. 1979).

Figure 11. Mechanism of formation of 2-deoxypentos-4-ulose within DNA in the absence of oxygen.

Figure 12. Mechanism of formation of 2-deoxypentonic acid within DNA in the absence of oxygen (adapted from Dizdaroglu and Jaruga 2012).

Figure 13. Mechanism of formation of free 2-deoxytetradialdose and 2-deoxytetradialdose bound to DNA as an end group in the presence of oxygen (adapted from Dizdaroglu et al. 1975c).

Figure 14. Mechanism of formation of erythrose within DNA in the presence of oxygen (adapted from Dizdaroglu et al. 1977c).

Figure 15. Mechanism of formation of 2-deoxypentonic acid within DNA in the presence of oxygen.

Supplementary Figure legends

Supplementary Figure 1. Mechanism of water elimination from 1,2-dihydroxyalkyl radicals.

Supplementary Figure 2. Mass spectra of the TMS derivatives of 2-deoxyglucitol (**A**), 2-deoxyglucitol-1,1-d_2 (**B**) and 5-deoxyglucitol-1,1-d_2 (**C**) (from Dizdaroglu et al. 1974).

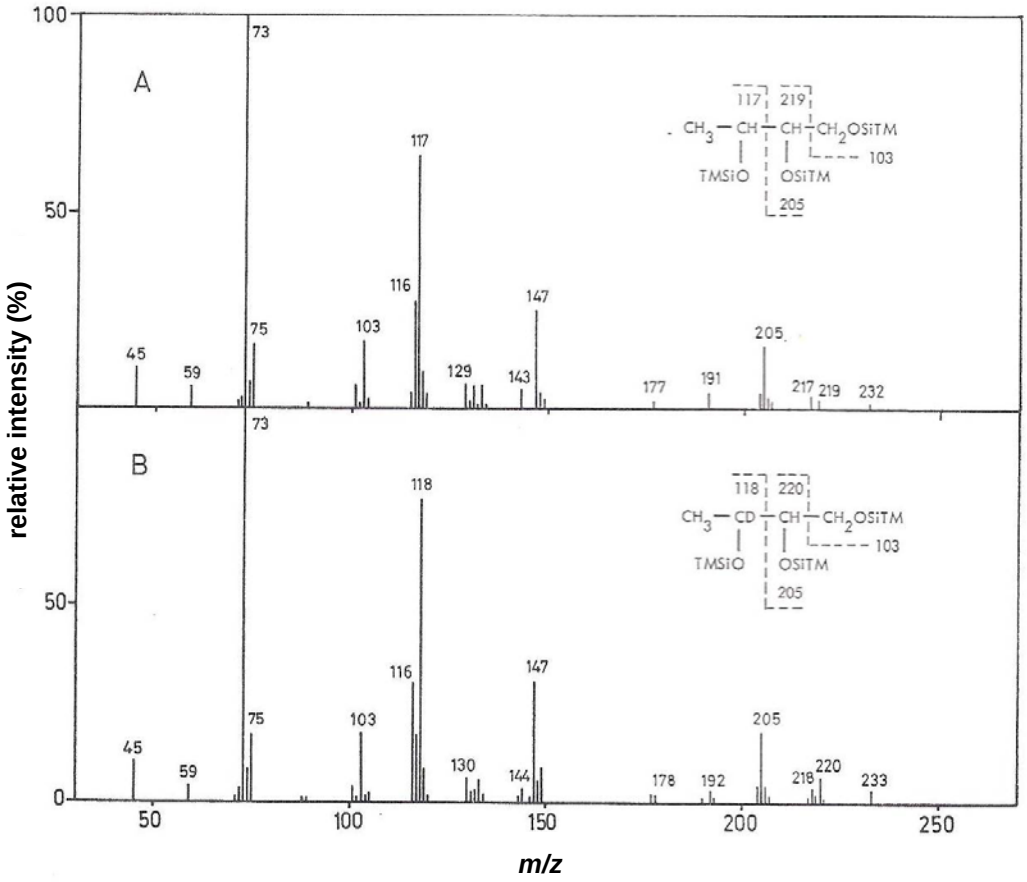
Supplementary Figure 3. Mechanisms of formation of 5-deoxylactobionic acid and 2-deoxylactobionic acid lactone in crystalline α-lactose•H_2O upon γ-irradiation (from von Sonntag and Dizdaroglu 1973).

Supplementary Figure 4. Mass spectrum of the TMS derivative of 2,5-dideoxy-erythropentitol-1,1-d_2 (from von Sonntag et al. 1974).

Supplementary Figure 5. Mechanism of formation of 2,5-dideoxy-erythropentonic acid in γ-irradiated crystalline 2-deoxyribose (from von Sonntag et al. 1974).

Supplementary Figure 6. Mechanisms of product formation from C1- and C5-centered radicals of glucose (from Dizdaroglu et al. 1975a).

Supplementary Figure 7. Mass spectra of the TMS derivatives of 2,3-dideoxypentitol (**A**) and 2,3-dideoxypentitol-1,4-d_2 (**B**) (from Beesk et al. 1979).



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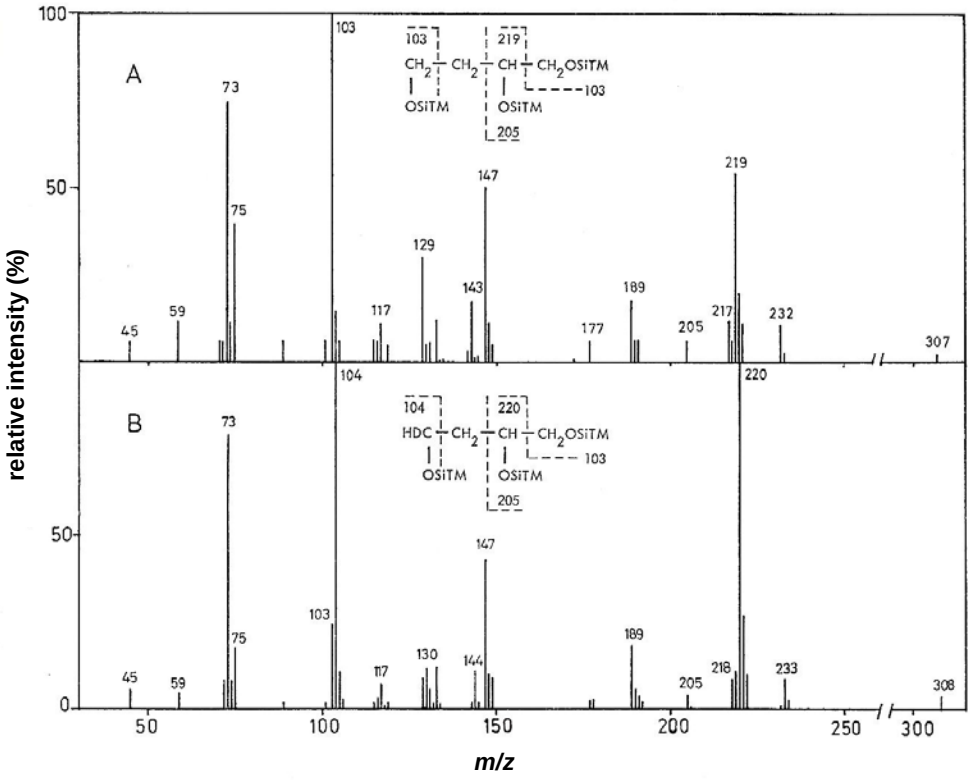
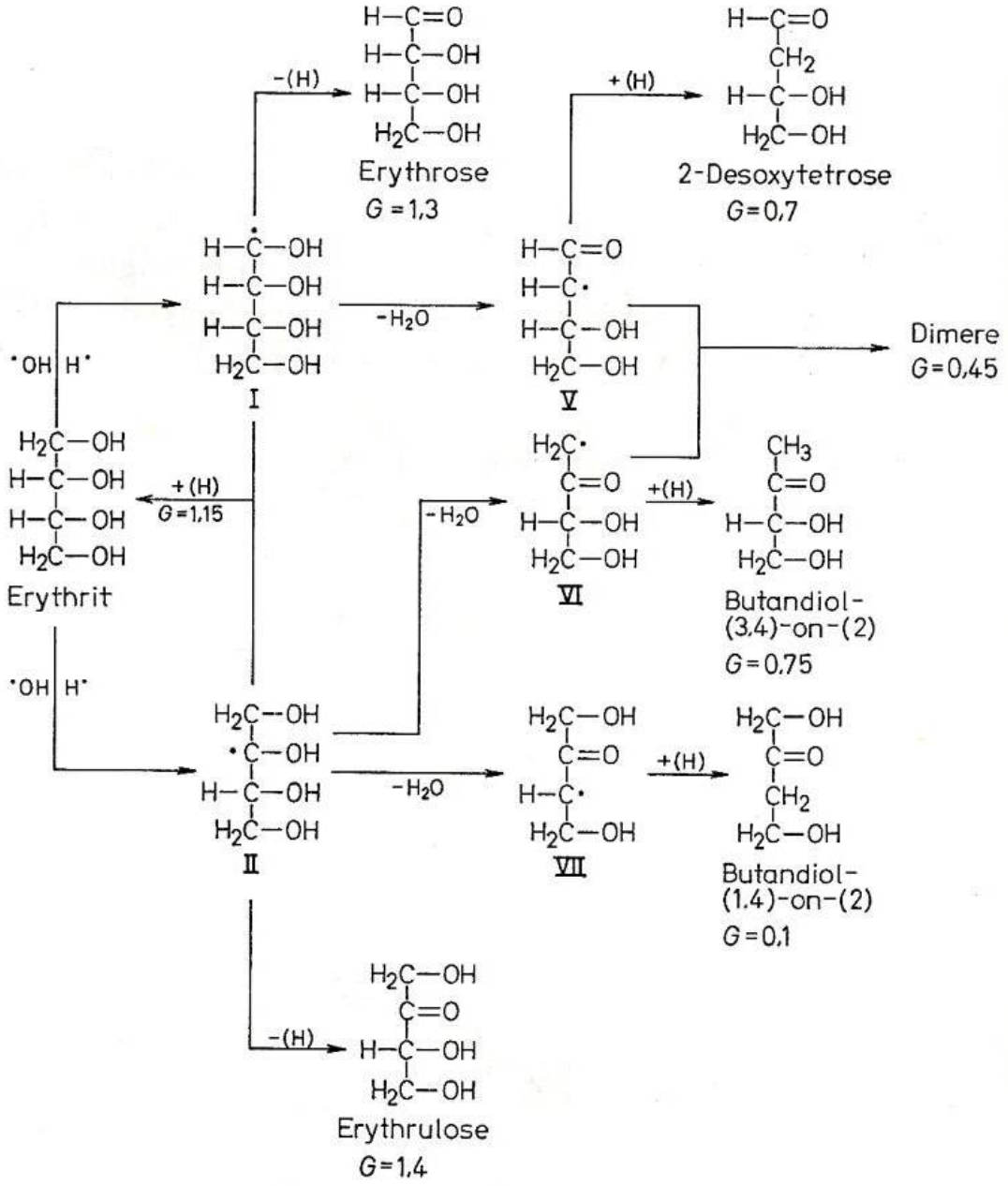


Figure 2. Mass spectra of the TMS derivatives of butanetriol-(1,3,4) (**A**) and butanetriol-(1,3,4)-1-d_1 (**B**) (from Dizdaroglu et al. 1972).



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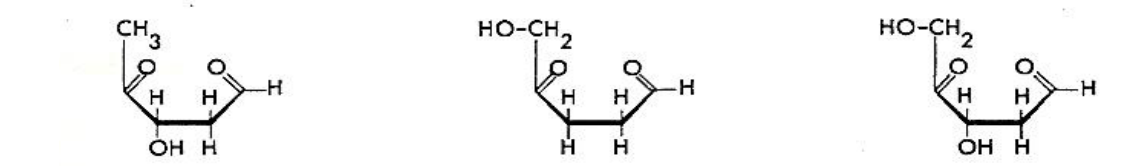
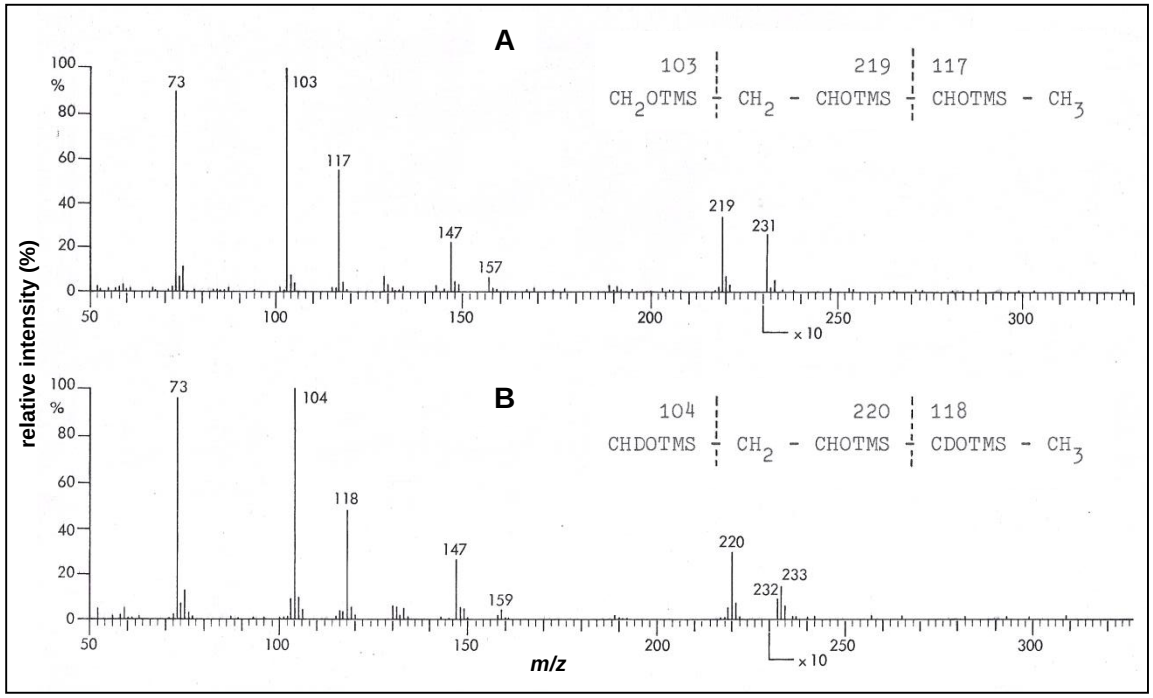


2,5-dideoxypentos-4-ulose **2,3-dideoxypentos-4-ulose** **2-deoxypentos-4-ulose**

1 **2** **3**

Figure 4. Products identified in γ-irradiated N_2O-saturated aqueous solutions of DNA (from Dizdaroglu et al. 1975b).



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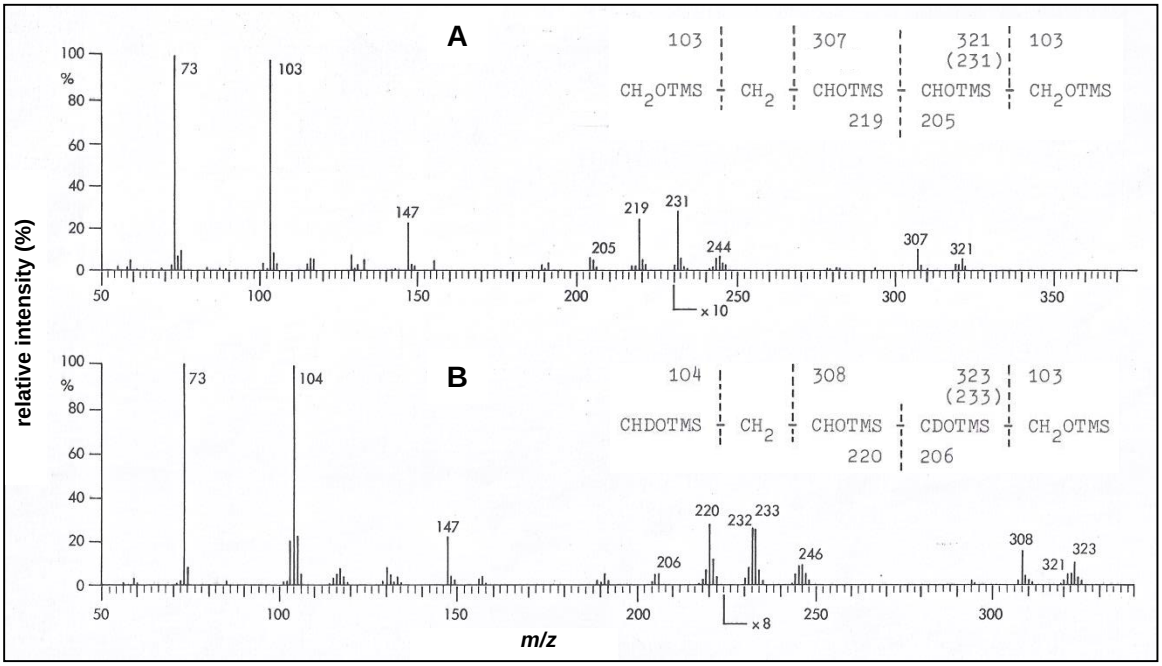

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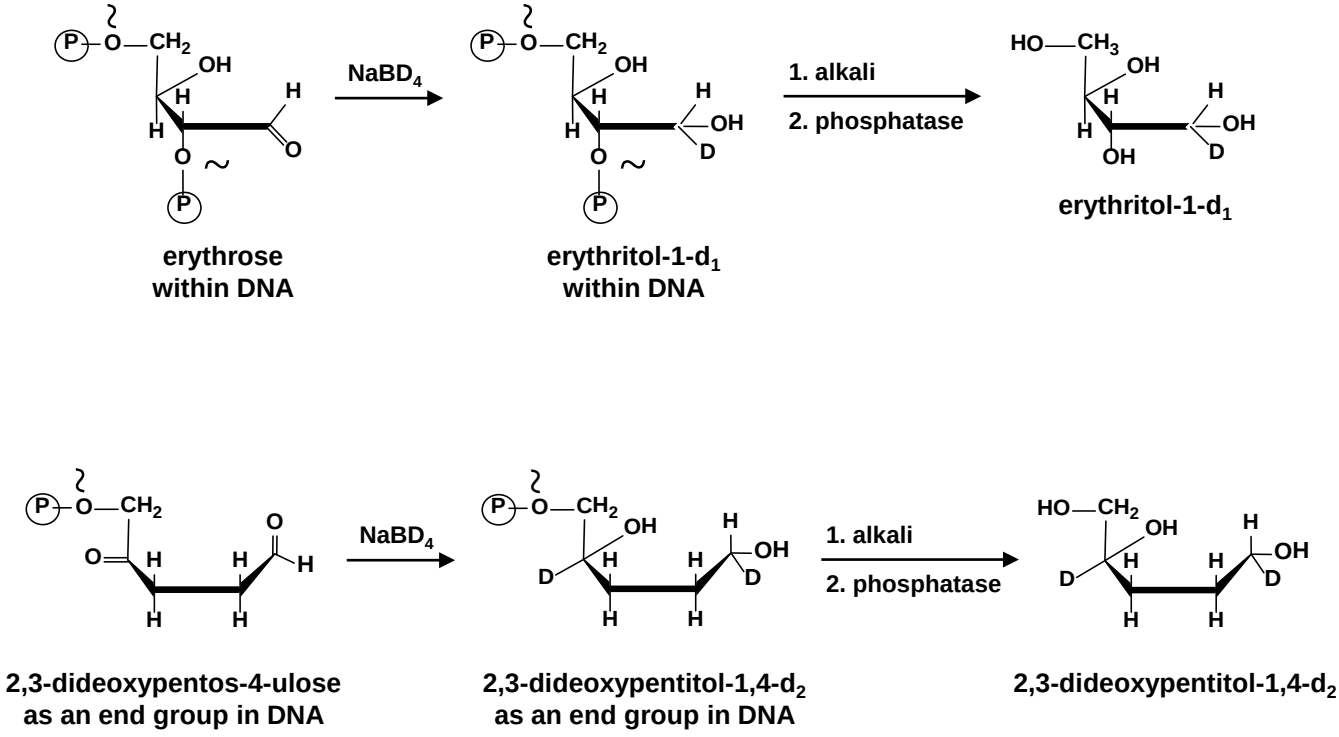
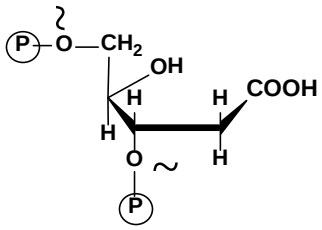
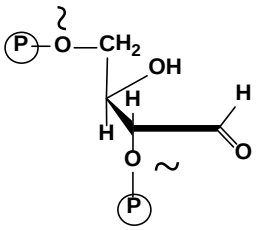


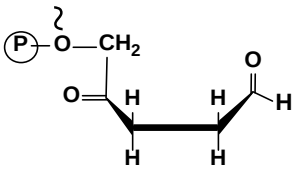
Figure 7. Identification pathway of two products bound to DNA: erythrose within DNA and 2,3-dideoxypentos-4-ulose as and group of a broken DNA strand (adapted from Dizdaroglu et al. 1977c).



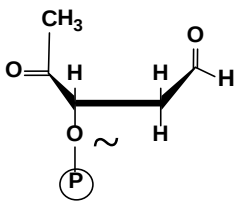
2-deoxypentonic acid within DNA



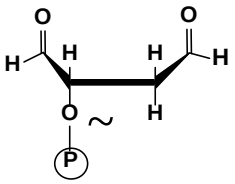
erythrose within DNA



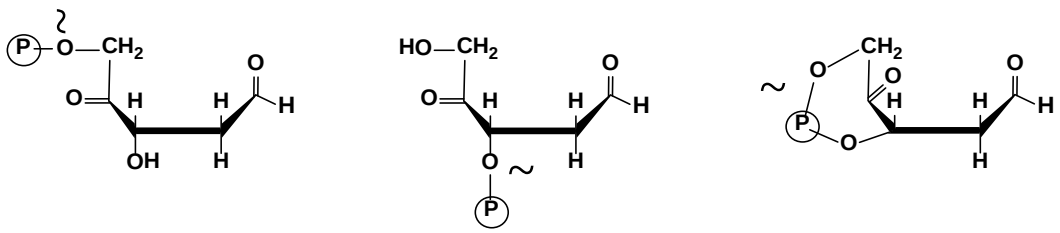
2,3-dideoxypentos-4-ulose as an end group



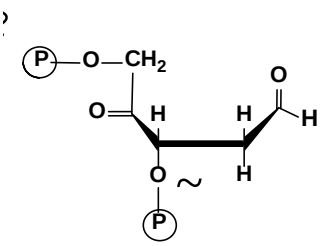
2,5-dideoxypentos-4-ulose as an end group



2-deoxytetradialdose as an end group



2-deoxypentos-4-ulose as an end group



2-deoxypentos-4-ulose within DNA

Figure 8. Structures of the products bound to DNA.

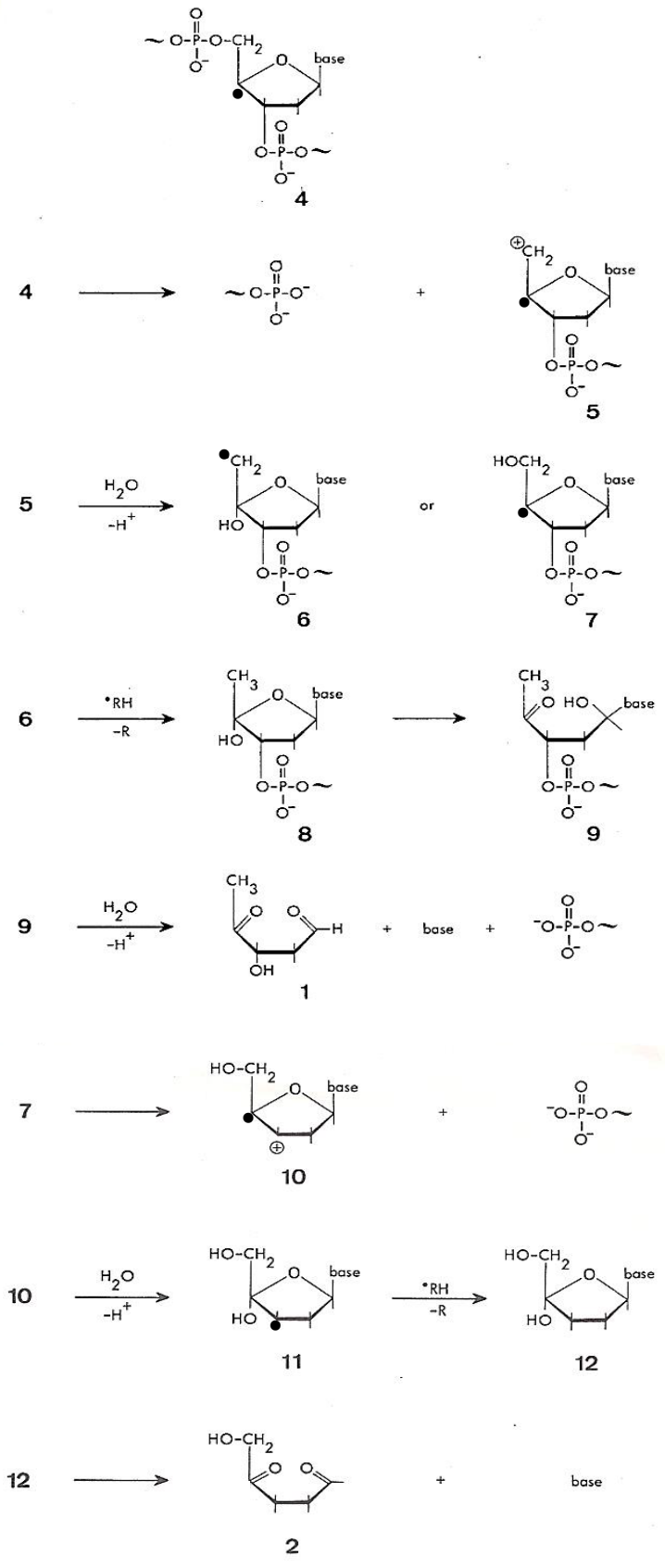


Figure 9. Mechanisms of product formation and strand breaks in DNA γ-irradiated in aqueous solution (from Dizdaroglu et al. 1975b).

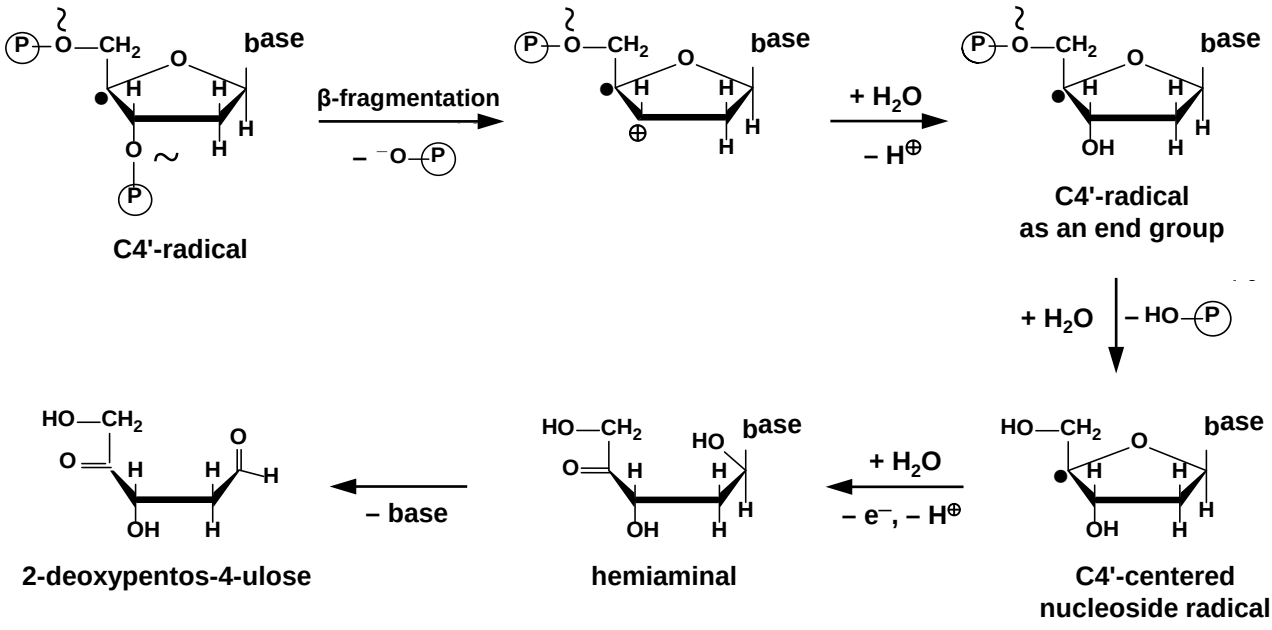


Figure 10. Mechanism of formation of free 2-deoxypentos-4-ulose (adapted from Beesk et al. 1979).

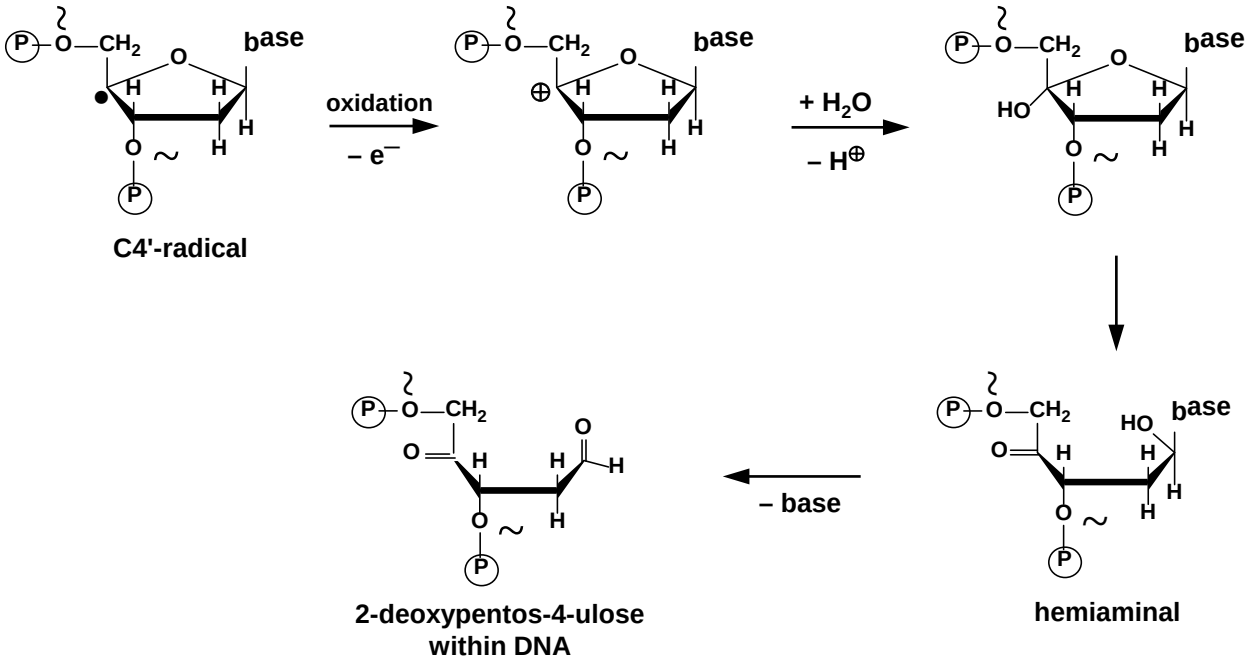


Figure 11. Mechanism of formation of 2-deoxypentos-4-ulose within DNA in the absence of oxygen.

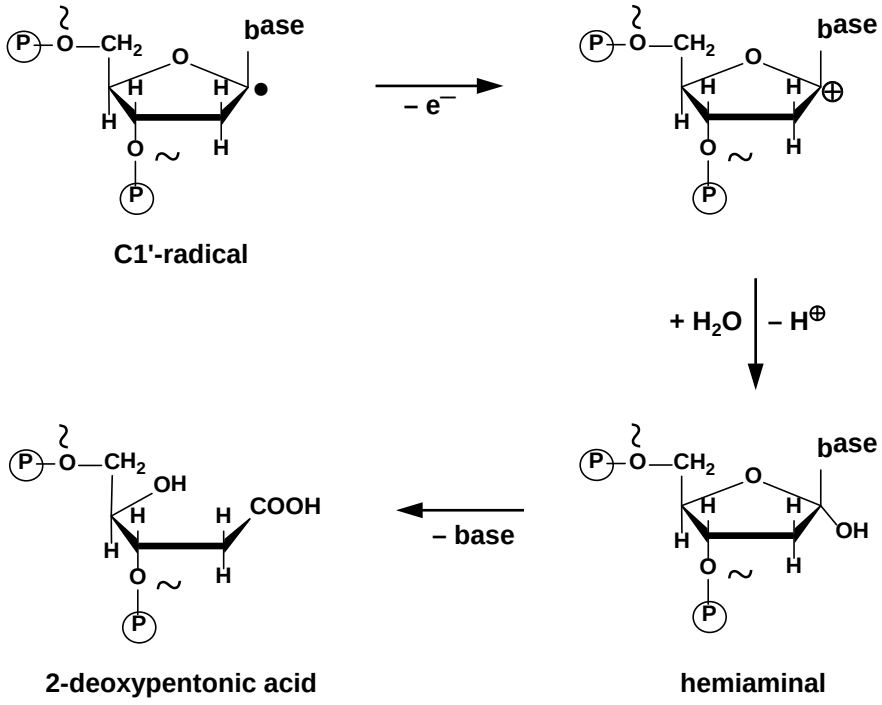


Figure 12. Mechanism of formation of 2-deoxypentonic acid within DNA in the absence of oxygen (adapted from Dizdaroglu and Jaruga 2012).

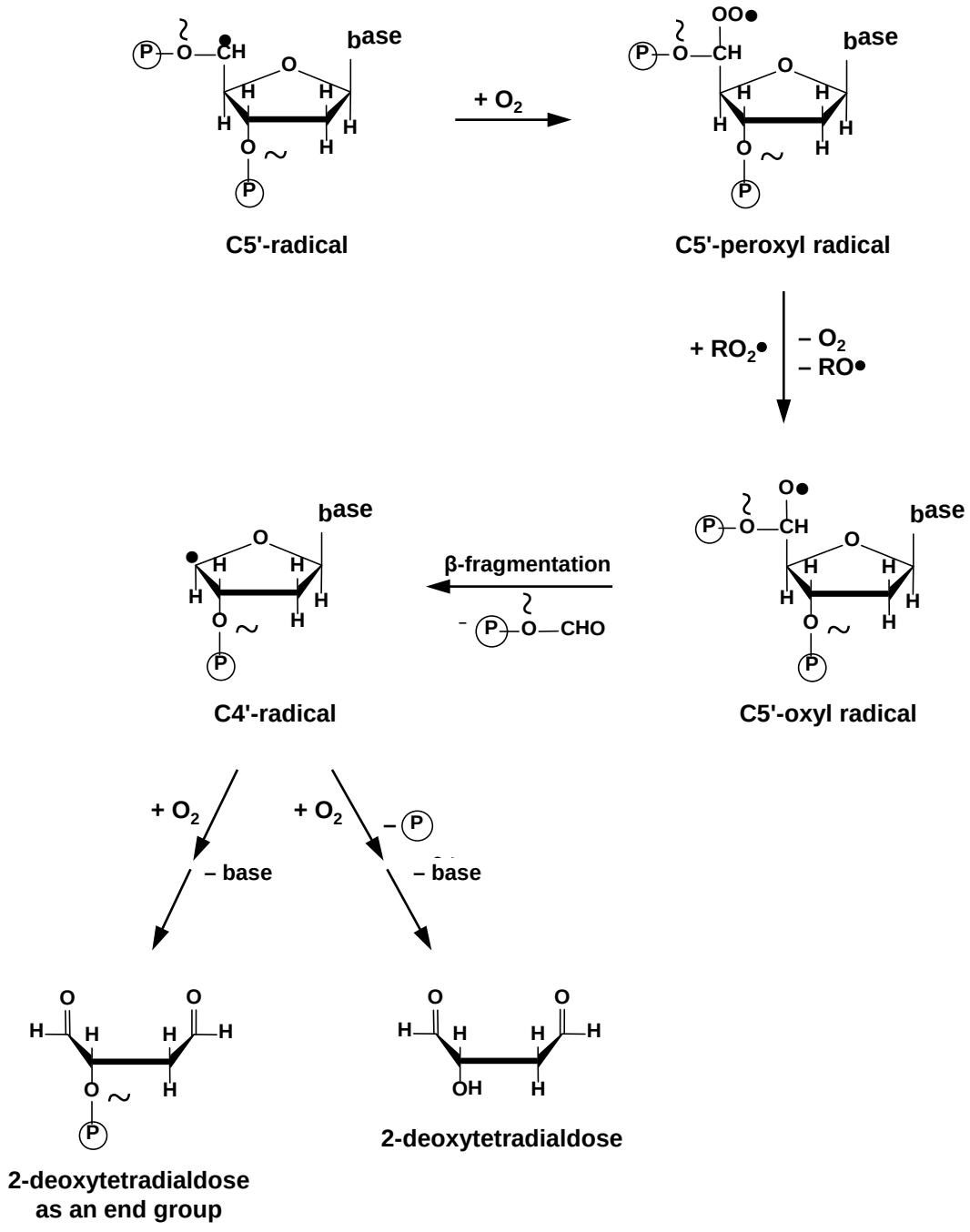


Figure 13. Mechanism of formation of free 2-deoxytetradialdose and 2-deoxytetradialdose bound to DNA as an end group in the presence of oxygen (adapted from Dizdaroglu et al. 1975c).

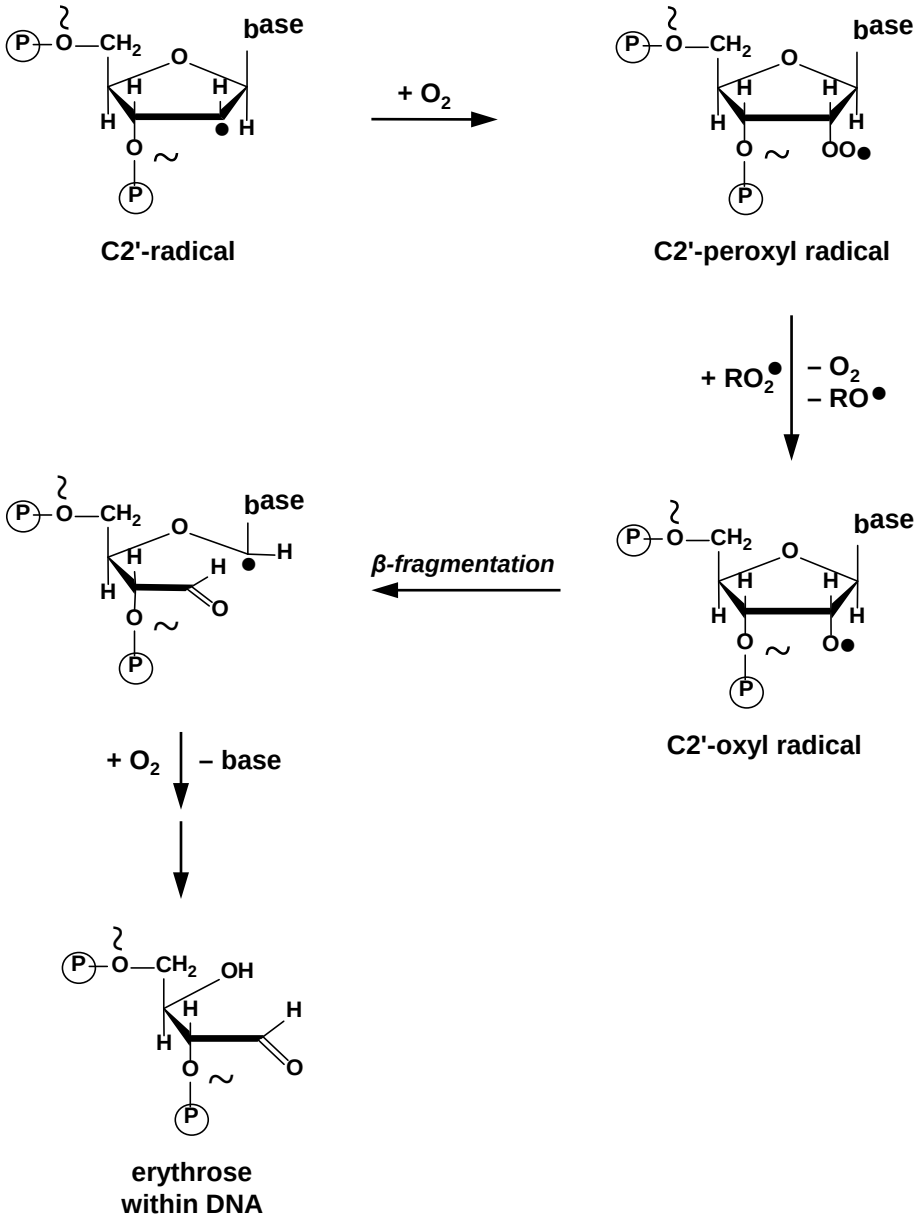


Figure 14. Mechanism of formation of erythrose within DNA in the presence of oxygen (adapted from Dizdaroglu et al. 1977c).

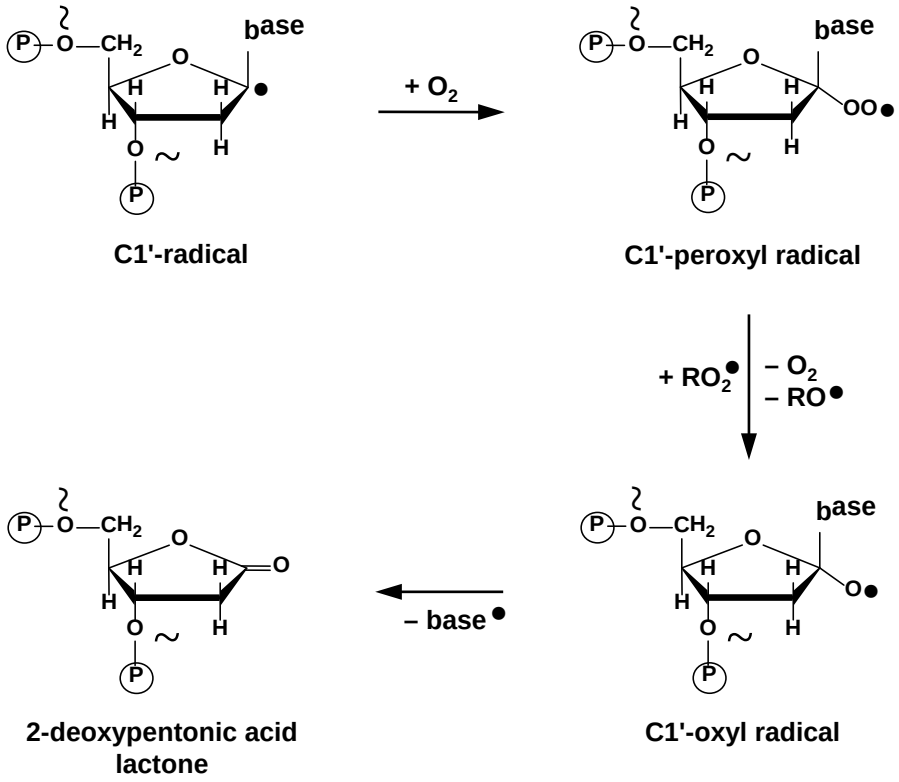
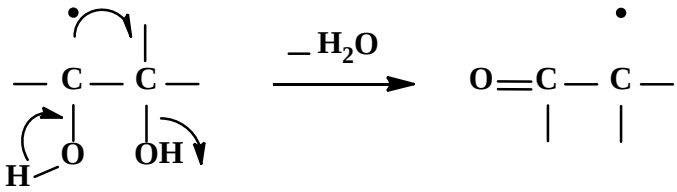
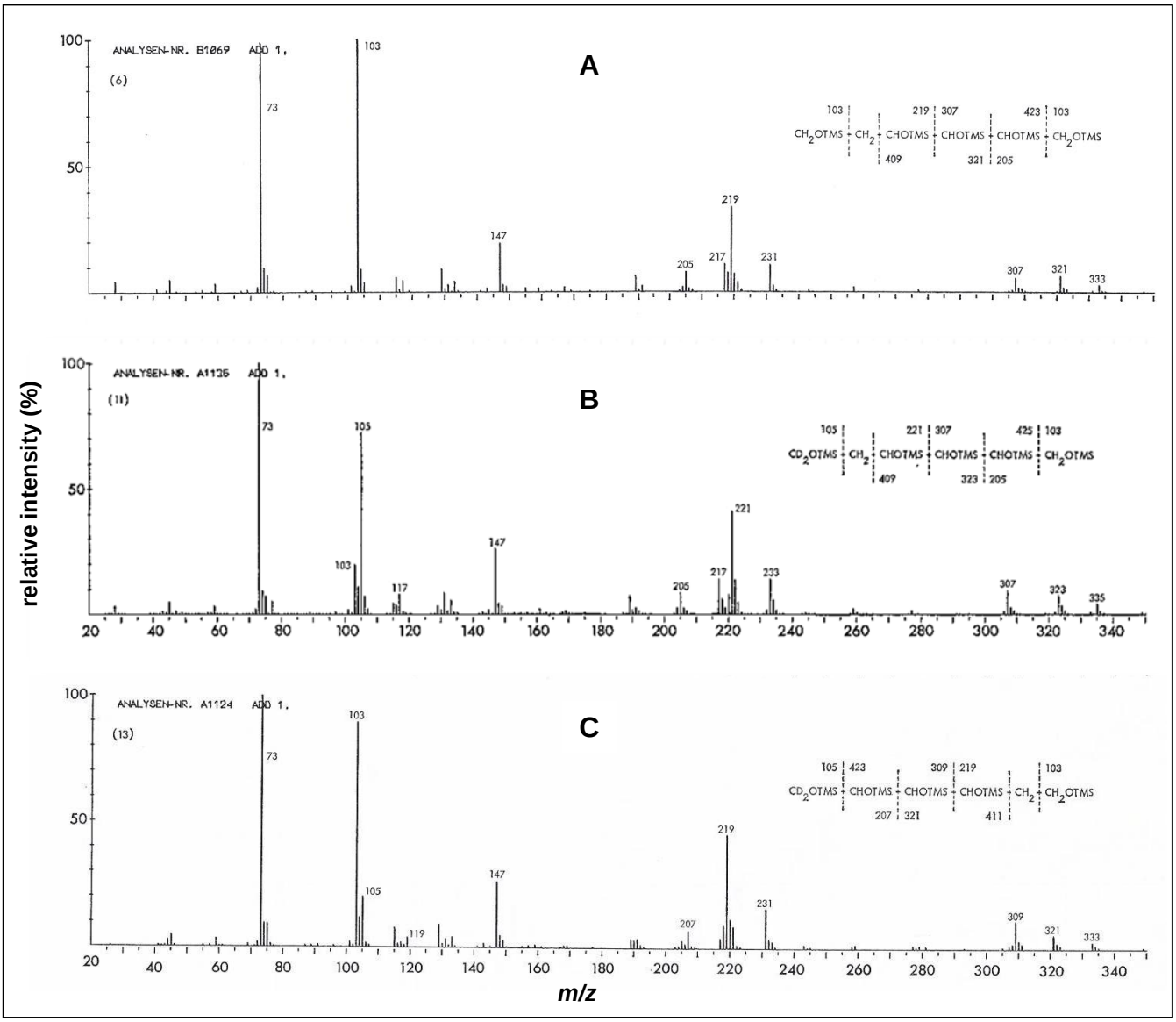


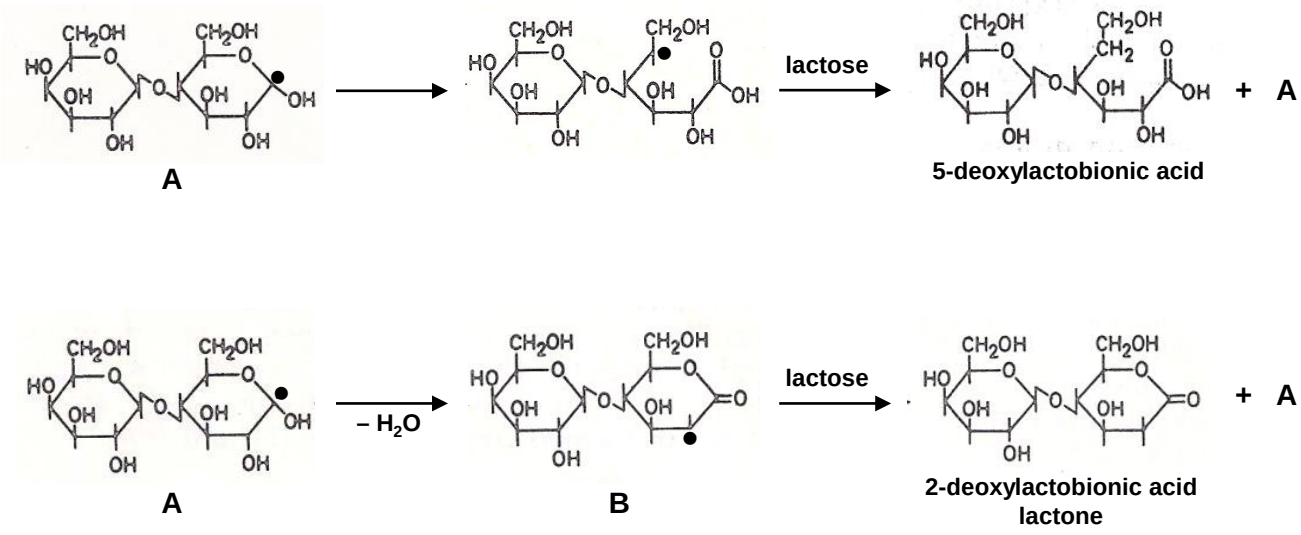
Figure 15. Mechanism of formation of 2-deoxypentonic acid within DNA in the presence of oxygen.



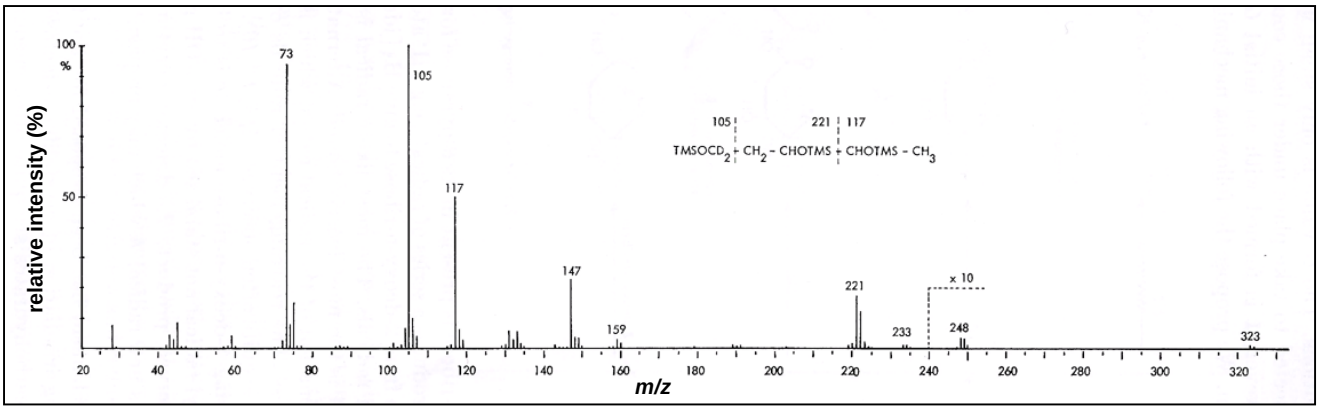
Supplementary Figure 1. Mechanism of water elimination from 1,2-dihydroxyalkyl radicals.



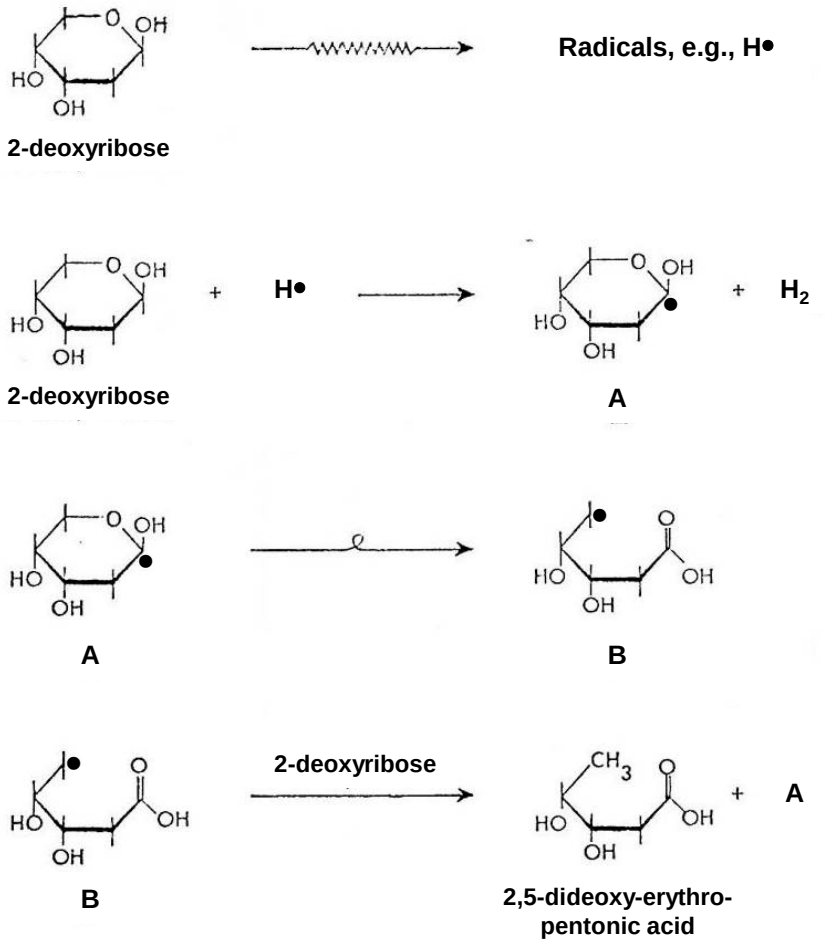
Supplementary Figure 2. Mass spectra of the TMS derivatives of 2-deoxyglucitol (**A**), 2-deoxyglucitol-1,1-d_2 (**B**) and 5-deoxyglucitol-1,1-d_2 (**C**) (from Dizdaroglu et al. 1974).



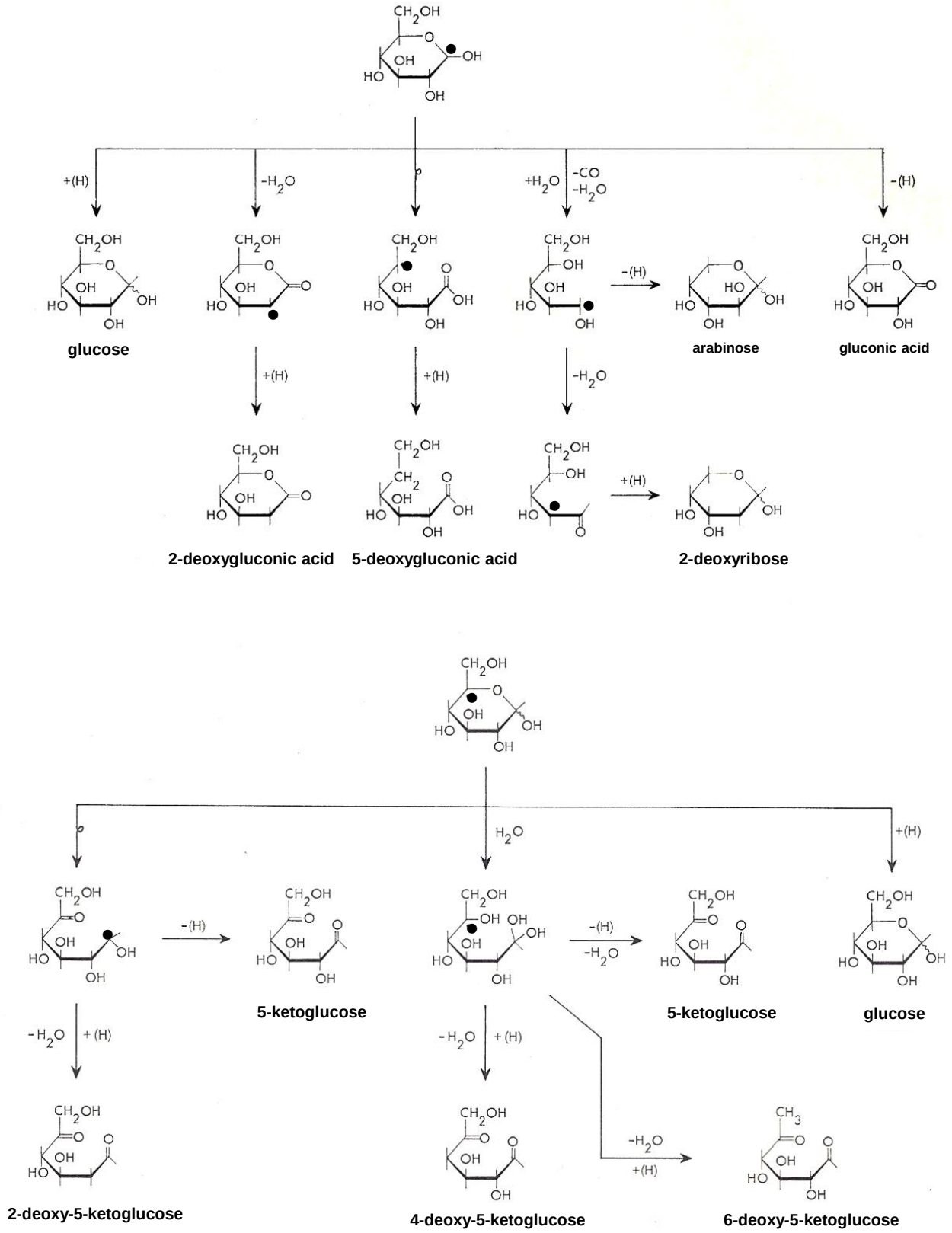
Supplementary Figure 3. Mechanisms of formation of 5-deoxylactobionic acid and 2-deoxylactobionic acid lactone in crystalline α-lactose•H_2O upon γ-irradiation (from von Sonntag and Dizdaroglu 1973).



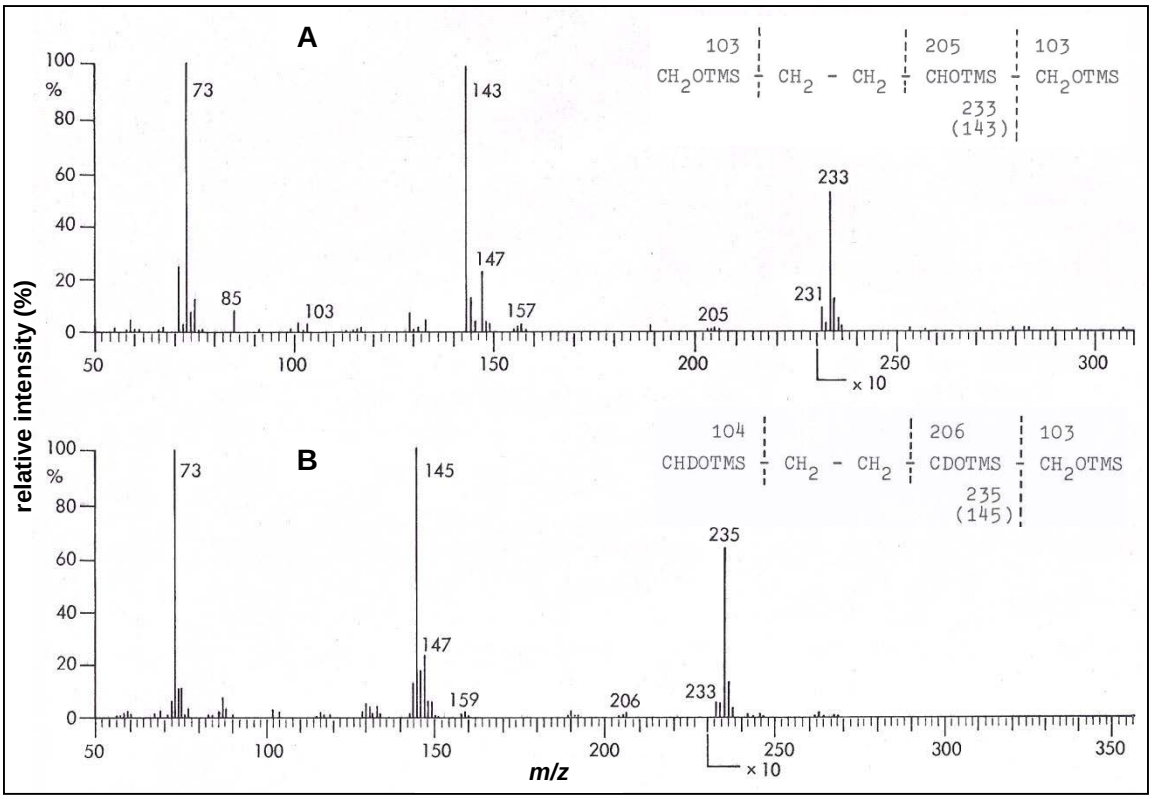
Supplementary Figure 4. Mass spectrum of the TMS derivative of 2,5-dideoxy-erythropentitol-1,1-d_2 (from von Sonntag et al. 1974).



Supplementary Figure 5. Mechanism of formation of 2,5-dideoxy-erythropentonic acid in γ-irradiated crystalline 2-deoxyribose (from von Sonntag et al. 1974).



Supplementary Figure 6. Mechanisms of product formation from C1- and C5-centered radicals of glucose (from Dizdaroglu et al. 1975a).



Supplementary Figure 7. Mass spectra of the TMS derivatives of 2,3-dideoxypentitol (**A**) and 2,3-dideoxypentitol-1,4-d_2 (**B**) (from Beesk et al. 1979).