

γ -Radiolyses of DNA in Oxygenated Aqueous Solution. Structure of an Alkali-Labile Site *

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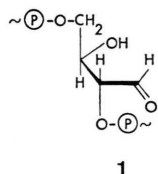
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Peroxy Radicals, DNA Strand Breaks, *G*-Value

Erythritol-1- d_1 has been isolated from γ -irradiated aqueous oxygenated solution of DNA after reduction with NaBD_4 , alkali and phosphatase treatment. It is concluded that this product stems from a D-erythrose 2,4-diphosphate unit in the DNA which is formed *via* a sequence of reactions following H-abstraction at C-2' by OH radicals..

Irradiation of DNA with ionizing radiation leads to strand breaks¹. The treatment of irradiated DNA with alkali increases the yield of strand breaks^{2–4}. The effect of alkali is due to alkali-labile sites which are produced as a result of alterations of either the base or the sugar moiety⁵. Recently we have isolated a sugar from γ -irradiated DNA after alkali and phosphatase treatment and determined its structure (2-deoxy-D-erythro-pentonic acid)⁶. The latter is different from the sugars which have been isolated without alkali treatment^{7, 8}.

In this paper we report the identification of *meso*-erythritol-1- d_1 (**7**) from which we infer the structure of a further alkali-labile site (**1**) in DNA, γ -irradiated in oxygenated aqueous solution.

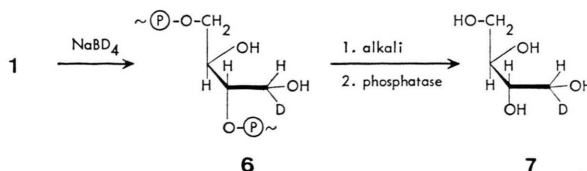
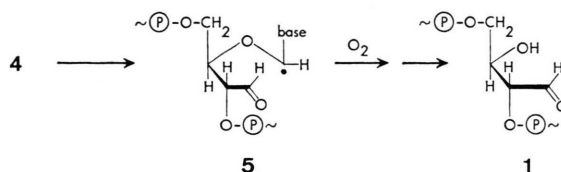
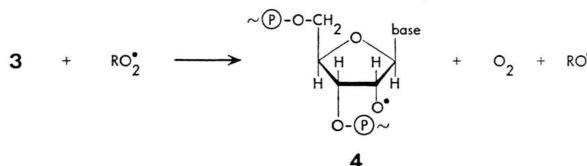
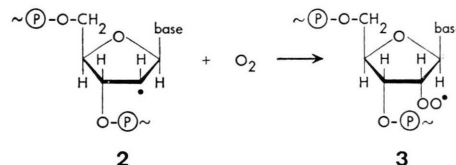


1 is a modified section of DNA structurally equivalent to the 2,4-diphosphate of D-erythrose. The free OH group next to the phosphate ester group causes alkali-lability^{9, 10}.

In the γ -irradiated DNA **1** was identified by excision of the D-erythrose unit as follows:

Aqueous solutions of DNA from calf thymus (Merck; 0.25 mg/ml) were saturated with $\text{N}_2\text{O}/\text{O}_2$ (80/20; v/v) and irradiated with ^{60}Co - γ -rays (dose

range: 10^{18} to $4 \times 10^{18} \text{ eV} \cdot \text{g}^{-1}$; dose rate: $3 \times 10^{18} \text{ eV} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$). After irradiation the samples were reduced with NaBD_4 leading to **6**. The sodium ions were removed with an ion exchanger (Dowex 50 WX2, H^+ -form) and the boric acid evaporated as its methyl ester. The residue was dissolved in water (2 mg DNA/ml) and adjusted to pH 12 with NaOH. The solution was kept at 37°C for 48 h, adjusted to pH 8 with formic acid, and incubated with alkaline phosphatase (0.4 U/ml; Boehringer) at 37°C for 12 h. The freeze dried material was trimethylsilylated with BSTFA/TMCS (100/3) in pyridine at room temperature, concentrated *in vacuo* to remove the excess of the silylating agent, and analysed by GC-MS using a 123 m Dexsil 300 glass capillary column¹¹ at 170°C . From the GC-peak corresponding to the TMS ether of *meso*-erythritol a mass spectrum was taken. Its typical fragment ions were m/e 73 (100%), 103 (22%), 104 (10%), 205 (17%), 206 (14%), 217 (17%), 218 (10%), 232 (M-90-89; 1%), 307 (3%), 308 (4%) and 321

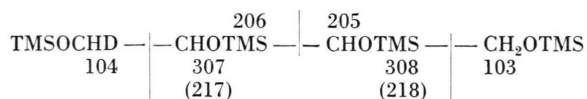


* Part IV of the series: Radiation Chemistry of DNA. For part III see ref. 6.

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(M-90; 1%). This spectrum corresponds to the TMS ether of *meso*-erythritol-1-d₁ (**7**).



The reduction of D-erythrose to *meso*-erythritol was necessary to avoid the degradation of D-erythrose on alkaline treatment. The G value of *meso*-erythritol-1-d₁ is ca. 0.005. This small yield is likely due to both a low propability of H abstraction by OH at C-2' and to side reactions in the reaction sequence leading from radical at C-2' to the isolated product.

The formation of **7** from γ -irradiated DNA may be explained analogous to the mechanism proposed for the formation of D-erythrose in the γ -radiolysis of 2-deoxy-D-ribose in oxygenated aqueous solution¹² and for the formation of *erythro*-tetrodialdose in the γ -radiolysis of D-ribose-5-phosphate¹³: OH radicals produced by γ -irradiation of N₂O saturated

aqueous solutions abstract H atoms from the sugar moiety of DNA. Abstraction at C-2' gives rise to the formation of **2**. Radical **2** is scavenged by molecular oxygen to give **3**.

The peroxy radical **3** reacts with another peroxy radical leading to the oxyl radical **4**, molecular oxygen and another oxyl radical^{14, 15}. The oxyl radical **4** undergoes β -fragmentation^{14, 15} to give **5**. Radical **5** reacts with molecular oxygen and is expected to lead to product **1**. Reduction of **1** with NaBD₄ yields **6**. Treatment with alkali and phosphatase converts **6** into **7**.

Köhnlein and Hutchinson¹⁶ postulated that in the photolysis of bromouracil-containing DNA, radicals at C-2' are formed in high yields and are subsequently converted into strand breaks. In the present paper a mechanism is described which leads from the C-2' radical to strand breaks. We believe, however, that starting from bromouracil-containing DNA other reactions leading to strand breaks may occur in addition.

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