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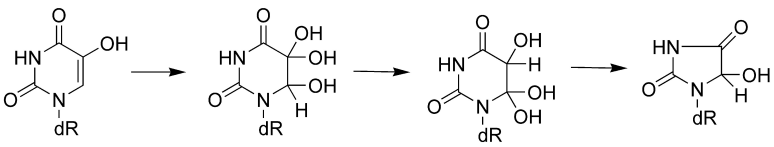

dR= 2-deoxy-β-D-*erythro*-pentofuranose (2-deoxyribose)

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Oxidation of 5-Hydroxy-2′-deoxyuridine into Isodialuric Acid, Dialuric Acid, and Hydantoin Products

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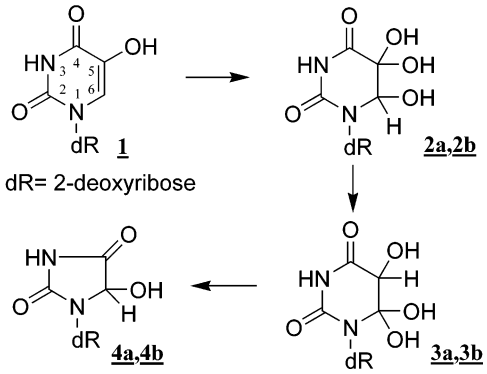
The biological consequences of DNA damage induced by ionizing radiation and endogenous free radicals are ultimately determined by their structure and chemistry.[1] The oxidation of guanine derivatives has received much attention particularly since it is the base with the lowest ionizing potential in DNA.[2] For this reason, guanine is the most sensitive and sometimes the only component to undergo oxidation in DNA; for example, guanine is the most sensitive DNA base to riboflavin photosensitization, singlet oxygen, and oxidizing metal complexes.[3] Interestingly, 8-oxo-7,8-dihydroguanine, one of the main products of guanine oxidation has a lower oxidation potential than guanine, making it highly susceptible to secondary oxidation. Indeed, the oxidation of 8-oxo-7,8-dihydroguanine is greatly favored over that of guanine under identical conditions.[4] Cytosine is also an important target of oxidation in DNA; for example, it is particularly sensitive to oxidation by 2-methyl-1,4-naphthoquinone photosensitization, ozone, and potassium permanganate.[5] Similar to the case of guanine in DNA, the oxidation of cytosine gives products that have a relatively low oxidation potential, e.g., 5-hydroxyuracil and 5-hydroxycytosine.[6] Although the sensitivity of 5-hydroxypyrimidines to $KMnO_4$ and one-electron oxidants has been reported, there has been little effort to untangle the complicated chemistry of oxidation.[7] Here, we report the characterization of six products in the oxidation pathway of 5-hydroxy-2′-deoxyuridine (5-oh-dUrd).

Oxidation of 5-oh-dUrd (**1**) by either Br_2 (1 equiv) or Na_2IrBr_6 (5 equiv) led to the quantitative formation of two polar products (**2a** and **2b**). These products were also formed in good yields by photosensitization using near-UV light and 2-methyl-1,4-naphthoquinone (50%) but less efficiently by treatment with $KMnO_4$ (10%). When the purified compounds were dissolved in neutral aqueous solution, they were observed to undergo a series of decompositions. The decomposition of **2a** and **2b** led to **3b** and **3a**, respectively, and the decomposition of either **3a** or **3b** led to a mixture of **4a** and **4b** (Scheme 1; Figure 1). The above products were identified by NMR and MS analyses. Each product is represented by a pair of diastereomers (e.g., **2a** and **2b**) as inferred by the close similarities in their chromatography, decomposition, and spectral features (see Supporting Information). Products **4a** and **4b** were identified as diastereomers of 5-hydroxyhydantoin nucleoside by comparison of their ^{1}H NMR and MS features with those of authentic standards. On the other hand, products **2a**, **2b**, **3a**, and **3b** have not previously been reported, and their identity was based on more detailed analyses of the most abundant diastereomer (**2b** and **3a**).

Product **2b** was identified as the nucleoside derivative of isodialuric acid (Scheme 1). Reduction of **2b** with $NaBH_4$ gave a single peak that coeluted and had the same mass as *cis*-5,6-glycol

Scheme 1. Proposed Mechanism of Oxidation of 5-oh-dUrd (**1**)



of dUrd, indicating that **2b** contains a carbonyl group. It should be noted, though, that the carbonyl of isodialuric acid exists as a hemiacetal as shown by the presence of two exchangeable OH protons at 6.6 and 6.9 ppm. Similarly, a hydrated structure was proposed in the case of the modified nucleobase.[8] Additional evidence was provided by the presence of a quatenary carbon at 89 ppm in ^{13}C NMR analysis, which can be assigned to C5 of the hemiacetal. Such a structure is consistent with the initial loss of H_2O from the molecular ion in MS analysis. The position of the CHOH group at C6 was ascertained by additional NMR analyses. The carbon at 78 ppm was assigned to the CHOH group on the basis of DEPT and HETCOR experiments. The chemical shift of this carbon (78 ppm) is very close to the δ value of the C6 carbon

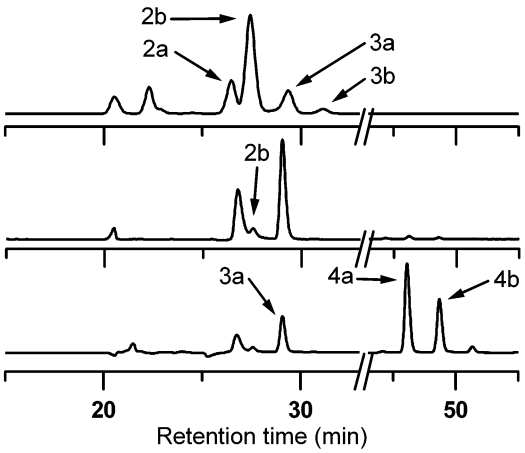


Figure 1. HPLC analysis of 5-oh-dUrd oxidation products. (Top) Semi-preparative separation of a partially decomposed mixture in water. (Middle) Decomposition of purified **2b** (2 h; pH 7; 37 °C). (Bottom) Decomposition of purified **3a** (24 h; pH 7; 37 °C). Amplitude of the peaks corresponds to their relative absorption at 210 nm.

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of dUrd 5,6-glycols (76 ppm); in contrast, the chemical shift of the C5 carbon of dUrd 5,6-glycols is 10 ppm lower (69 ppm).[5a] In addition, HMBC experiments suggest that the CHOH group is at C6 because the CH proton shows approximately equal long-range coupling with equidistant carbons at C2 and C4.

Two other products were characterized in the mixture of decomposition products (peaks 21 and 22 min; Figure 1). The product eluting at 21 min was identified as isodialuric acid nucleobase by comparison with authentic standard.[8] In contrast, the product at 22 min gave the same mass and nearly identical NMR characteristics as isodialuric acid nucleoside (**2b**) except for changes in the profile of osidic protons.[9] The formation of these products indicates that **2b** undergoes N1−C6 ring-chain tautomerism leading to an intermediate ureid. An analogous intermediate has been proposed in the interconversion of furanosyl and pyranosyl forms of biruret-containing nucleosides.[5a]

Product **3a** was identified as the nucleoside derivative of dialuric acid (Scheme 1). The reduction of **3a** with $NaBH_4$ was less efficient than that of **2b**, and the reaction not only gave *cis*-5,6-glycol but also traces of 5-hydroxyhydantoin nucleoside. The presence of exchangeable protons at 6.6 and 6.9 ppm, as well as the corresponding carbon signal at 89 ppm, indicated that **3a** comprises a hydrated carbonyl moiety. The greater downfield shift of CH and OH protons of CHOH by 0.7 and 1.0 ppm downfield, respectively, in **3a** compared to **2b**, is consistent with the presence of two strongly electron-withdrawing groups, i.e., a carbonyl at C4 and a hydrated carbonyl at C6, next to CHOH. The ^{13}C NMR spectrum of **3a** was extracted as a minor component from the spectrum of **2b**. Again, the main difference between isodialuric acid (**2b**) and dialuric acid (**3a**) derivatives was the position of the CHOH carbon, which was shifted 11 ppm farther downfield for **3a** compared to **2b** (78 ppm compared to 89 ppm). An alternative structure, in which dialuric acid transforms into an ureid with a carboxylic acid substituent, was ruled out because there was no indication for the presence of a carboxylic acid in ^1H NMR, ^{13}C NMR, or MS/MS analyses. Nevertheless, the difficulties encountered with the analysis of **3b** by NMR are likely related to its conversion into a complex mixture of products under certain conditions, depending on the concentration and pH of the solution. Taken together, our results indicate that compound **3a** is the nucleoside derivative of dialuric acid, with an OH group at C5 and a hydrated carbonyl group at C6.

The oxidation of 5-oh-dUrd (**1**) into final stable products includes three steps (Scheme 1). The initial reaction involves nucleophilic substitution of BrOH to give a C5-substituted bromohydrin, similar to the reaction of other pyrimidines.[10] In contrast to other pyrimidines, however, the bromohydrins of 5-oh-dUrd are not at all stable. The decomposition of pyrimidine bromohydrins under slightly alkaline conditions involves transfer of the hydroxyl group from C6 to C5 and elimination of Br anion giving pyrimidine 5,6-glycols.[10d] A similar pathway for the unstable bromohydrins of 5-oh-dUrd directly leads to isodialuric acid products (**2a** and **2b**). The conversion of **2a** and **2b** into dialuric acid (**3a** and **3b**) involves a stereospecific reaction that transfers a proton from C6 to C5, such as α-hydroxyketone isomerization. Finally, it is reasonable to propose that the formation of 5-hydroxyhydantoin products (**4a** and **4b**) involves ring opening of **3a** or **3b**, and decarboxylation of an intermediate ureid bearing an α-hydroxycarboxylic acid. For thymine derivatives, an analogous reaction is not possible, presumably because the electron-donating methyl group at C5 stabilizes the corresponding ureid, i.e., *N*-methyltartronoylurea.[10b]

To explore the oxidation of 5-oh-dUrd (**1**) in DNA, we subjected d(Tp5ohUpT) to oxidation by Na_2IrBr_6 and examined the products by HPLC and ion trap MS. These analyses gave a single product with a mass of 885 *m*/*z*, consistent with the formation of either hydrated isodialuric or dialuric acid derivatives (addition of 34 *m*/*z* to d(Tp5ohUpT)). In addition, subsequent fragmentation of the molecular ion peak gave a peak at 867 *m*/*z*, indicating the initial loss of H_2O, similar to monomers (**2a**, **2b**, **3a**, and **3b**). These analyses indicate that the same products are formed by oxidation of 5-oh-dUrd (**1**) and d(Tp5ohUpT) in aqueous solution. Similarly, our studies with longer oligonucleotides containing 5-oh-dUrd (**1**) point to the same pathway of oxidation, with the formation of products with M + 34 *m*/*z*.

It is well-known that 5-oh-dUrd (**1**) and 5-hydroxy-2′-deoxycytidine are susceptible to chemical oxidation.[7] Treatment of synthetic oligonucleotides containing 5-oh-dUrd with $KMnO_4$ generates single-strand breaks at the modified site.[7a] Indeed, incorporation of 5-oh-dUrd, and other easily oxidizable nucleoside analogues, followed by treatment with $KMnO_4$, has been proposed as a new strategy to detect single-nucleotide polymorphism.[7b] On the other hand, RNA containing 5-oh-dUrd catalyzes the oxidoreduction of NADH, suggesting a role of these modifications in ribozymes.[7c] 5-oh-dUrd and 5-hydroxy-2′-deoxycytidine are potentially mutagenic and substrates for DNA repair enzymes.[11] The ease of secondary oxidation of 5-hydroxy-2′-deoxycytidine may explain some of the discrepancies in its reported mutagenesis.[11b] It is reasonable to propose 5-hydroxypyrimidines as relevant targets for further oxidation.

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Supporting Information Available: Details of reaction conditions. ^1H NMR, ^{13}C NMR, and HMBC of products **2b** and **3a**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Cadet, J.; Delatour, T.; Douki, T.; Gasparutto, D.; Pouget, J. P.; Ravanat, J. L.; Sauvaigo, S. *Mutat. Res.* **1999**, *424*, 9−21. (b) Wallace, S. S. *Free Radical Biol. Med.* **2002**, *33*, 1−14.

(2) (a) Steenken, S.; Jovanovic, S. V. *J. Am. Chem. Soc.* **1997**, *119*, 617−618. (b) Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 7063−7068.

(3) (a) Douki, T.; Cadet, J. *Int. J. Radiat. Biol.* **1999**, *75*, 571−581. (b) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109−1151.

(4) (a) Ravanat, J. L.; Saint-Pierre, C.; Cadet, J. *J. Am. Chem. Soc.* **2003**, *125*, 2030−2031. (b) Duarte, J. V.; Gasparutto, D.; Yamaguchi, L. F.; Ravanat, J. L.; Martinez, G. R.; Medeiros, M. H. G.; Di Mascio, P.; Cadet, J. *J. Am. Chem. Soc.* **2000**, *122*, 12622−12628. (c) Luo, W. C.; Muller, J. G.; Rachlin, E. M.; Burrows, C. J. *Org. Lett.* **2000**, *2*, 613−616. (d) Tretyakova, N. Y.; Niles, J. C.; Burney, S.; Wishnok, J. S.; Tannenbaum, S. R. *Chem. Res. Toxicol.* **1999**, *12*, 459−466.

(5) (a) Wagner, J. R.; Decarroz, C.; Berger, M.; Cadet, J. *J. Am. Chem. Soc.* **1999**, *121*, 4101−4110. (b) Bienvenu, C.; Wagner, J. R.; Cadet, J. *J. Am. Chem. Soc.* **1996**, *118*, 11406−11411. (c) Girault, I.; Fort, S.; Molko, D.; Cadet, J. *Free Radical Res.* **1997**, *26*, 257−266. (d) Hayatsu, H. *J. Biochem.* **1996**, *119*, 391−395.

(6) Wagner, J. R.; Hu, C. C.; Ames, B. N. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 3380−3384.

(7) (a) Fujimoto, J.; Tran, L.; Sowers, L. C. *Chem. Res. Toxicol.* **1997**, *10*, 1254−8. (b) Wolfe, J. L.; Kawate, T.; Sarracino, D. A.; Zillmann, M.; Olson, J.; Stanton, V. P.; Verdine, G. L. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11073−11078. (c) Yanagawa, H.; Ogawa, Y.; Ueno, M. *J. Biol. Chem.* **1992**, *267*, 13320−6.

(8) (a) Schuchmann, M. N.; von Sonntag, C. *J. Chem. Soc., Perkin Trans. 2* **1983**, 1525−1531.

(9) ^1H NMR analyses suggest that isodialuric acid is attached to an α-pranosyl moiety because of the chemical shift of H1′ (5.5 ppm) and H4′ (<4 ppm) and large coupling between H1′−H2′ (9.7 Hz).

(10) (a) Bienvenu, C.; Cadet, J. *J. Org. Chem.* **1996**, *61*, 2632−2637. (b) Wagner, J. R.; van Lier, J. E.; Berger, M.; Cadet, J. *J. Am. Chem. Soc.* **1994**, *116*, 2235−2242. (c) Harayama, T.; Yanada, R.; Iio, K.; Yoneda, F. *Nucleic Acids Symp. Ser.* **1986**, *17*, 25−82. (d) Ryang, H. S.; Wang, S. Y. *J. Org. Chem.* **1979**, *44*, 1191−1192.

(11) (a) Feig, D. I.; Sowers, L. C.; Loeb, L. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 6609−6613. (b) Kreutzer, D. A.; Essigmann, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3578−3582.

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