Hydroxyl-Radical-Induced Decomposition of 2′-Deoxycytidine in Aerated Aqueous Solutions

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Abstract: The stable products resulting from γ-irradiation of 2′-deoxycytidine in aerated aqueous solutions have been purified to homogeneity and characterized by MS and NMR analyses. The main group of products, which are similar in structure to those that arise from the •OH-mediated decomposition of thymidine, included four diastereomers of 5,6-dihydroxy-5,6-dihydro-2′-deoxyuridine (**2**−**5**), 5-hydroxy-2′-deoxycytidine (**6**), two diastereomers of N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-hydroxyhydantoin (**7** and **8**), and N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)formamide (**9**). These products are likely formed by way of either intermediate 5(6)-hydroxy-6(5)-peroxyl radicals or the corresponding hydroperoxides. In labeling experiments, $^{18}O_2$ was incorporated into products **2**−**9** with about 30% label at C_5 and 60% at C_6. Several other products were observed in the γ-irradiation of 2′-deoxycytidine in aerated aqueous solutions. They included four isomeric nucleosides of biuret (**10**−**13**), two diastereomers of N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1-carbamoyl-2-oxo-4,5-dihydroxyimidazolidine (**14**, **15**), and aminocarbonyl[2-(2-deoxy-β-D-*erythro*-pentofuranosyl)amino]-2-oxo-ethylcarbamic acid (**16**). The first step in the formation of these products is probably intramolecular addition of the 5-hydroxy-6-hydroperoxide to C_4 of the pyrimidine ring, resulting in a 4,6-endoperoxide which decomposes into an open-chain α-hydroxyaldehyde by C_4-C_5 cleavage. The formation of products **10**−**13** can be explained by subsequent fragmentation of the intermediate α-hydroxyaldehyde, whereas cyclization gives rise to products **14** and **15**. In the case of product **16**, a series of reactions have been proposed, starting with the rearrangement of the α-hydroxyaldehyde. The above pathways are supported by $^{18}O_2$-labeling experiments. Finally, we characterized two diastereomers of 5′,6-cyclo-5-hydroxy-5,6-dihydro-2′-deoxyuridine (**17**, **18**) in the γ-irradiation of 2′-deoxycytidine in aerated aqueous solutions. These products likely arise from initial •OH abstraction of a proton from $C_{5'}$ of the sugar moiety, followed by intramolecular addition of the resulting radical to C_6 of the cytosine moiety. The •OH-mediated decomposition of 2′-deoxycytidine in DNA is discussed.

Introduction

The deleterious effects of ionizing radiation may largely be attributed to DNA damage induced by both the reaction of hydroxyl radicals (•OH) and direct ionization of DNA.[1,2] In some respects, ionizing radiation is similar to oxidative metabolism. The latter generates copious amounts of superoxide ions and H_2O_2, which can produce •OH upon reaction with transition metal ions such as Fe^{2+} and Cu^{+}. Oxidative DNA damage resulting from oxidative metabolism contributes to the natural processes of aging and cancer.[3,4]

Hydroxyl-radical-induced damage to DNA leads to a multitude of sugar and base modifications. The majority of biological studies have focused on 8-oxo-7,8-dihydro-2′-deoxyguanosine, a major oxidation product of 2′-deoxyguanosine in DNA.[5] The level of 5-hydroxy-2′-deoxycytidine, an oxidation product of 2′-deoxycytidine, in cellular DNA was found to be comparable to that of 8-oxo-7,8-dihydro-2′-deoxyguanosine.[6] In addition, several oxidative modifications of cytosine within DNA have been shown to be substrates for *Escherichia coli* endonuclease III, an enzyme involved in the repair of oxidative DNA damage by base excision.[7] Using synthetic oligonucleotides, the efficiency of strand cleavage by endonuclease III was determined for three oxidative lesions of cytosine: 5,6-glycols > 5-hydroxycytosine > 5-hydroxyuracil.[8] It should also be noted that there is evidence for similar repair activity in human cells.[9]

Both 5-hydroxy-2′-deoxycytidine and 5-hydroxy-2′-deoxyuridine lesions appear to be mutagenic. They have been shown to miscode during DNA synthesis in vitro such that C is incorporated opposite 5-hydroxy-2′-deoxycytidine, while A goes

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opposite 5-hydroxy-2′-deoxyuridine.[10] This suggests that the formation of 5-hydroxy-2′-deoxycytidine and 5-hydroxy-2′-deoxyuridine in DNA may lead to C-to-G transversions and C-to-T transitions, respectively. Recently, the specific incorporation of 5-hydroxy-2′-deoxycytidine, 5-hydroxy-2′-deoxyuridine, and the 5,6-glycol of 2′-deoxyuridine into *E. coli* was found to cause C-to-T transitions with a frequency of 0.05%, 83%, and 80% respectively.[11] In general, we expect that the oxidation products of 2′-deoxycytidine have a good chance of giving rise to mutations in cellular DNA because oxidation induces deamination that directly affects base pairing. This may explain the bias toward C-to-T transitions in the spectrum of mutations induced by ionizing radiation, as well as exogenous and endogenous reactive oxygen species.[12]

The main products that arise from the •OH-mediated decomposition of uracil, thymine, and cytosine under aerated conditions have been characterized.[13−15] These studies have been extended to pyrimidine nucleosides. For example, 22 modified nucleosides have been characterized after γ-irradiation of thymidine in aerated aqueous solutions.[16] In addition, the decomposition of the individual thymidine 5(6)-hydroxy-6(5)-hydroperoxides, including eight diastereomers, has been studied in detail by kinetics and product analysis.[17] These studies have been extended to 5-methyl-2′-deoxycytidine, and recently the corresponding 5,6-glycols and methyl oxidation products were reported.[18,19] In contrast, it has been difficult to study the stable products from the •OH-mediated decomposition of dCyd because of problems associated with their preparation and purification from product mixtures. Further complications arise from the involvement of labile products including the 5,6-glycols and 5(6)-hydroxy-6(5)-hydroperoxides of dCyd.[20]

A number of recent studies has focused on the decomposition of 2′-deoxycytidine (dCyd) induced by various free radical- or oxidant-generating systems. The γ-irradiation of dCyd in frozen aqueous solution was investigated and shown to produce 5,6-dihydro-2′-deoxyuridine and 5′,6-cyclo-5,6-dihydro-2′-deoxyuridine, presumably by way of intermediate dCyd radical anions and cations.[21] The exposure of dCyd to ozone in aqueous solutions was recently shown to result in the formation of several radiation-like nucleoside decomposition products.[22] In another study, the Fenton-reaction-mediated decomposition of dCyd, d-CpC, d-CpCpC, dCMP, and DNA was examined.[23] Eleven modified bases along with an equal number of modified nucleosides were tentatively identified by FAB-MS. However, it is difficult to draw any definite conclusions about the mechanism of decomposition of cytosine derivatives in this study because of the high concentration of Fenton reagents, i.e., 1 mM of Fe^{2+} or Fe^{3+} and 2 mM of H_2O_2, which may interfere with the fate of intermediate species. For example, transition metal ions have been shown to induce the reduction or oxidation of initial •OH adducts of cytosine derivatives.[24]

In the present work, the separation of dCyd oxidation products was achieved by a combination of normal- and reversed-phase HPLC. Photosensitization with menadione and UVA light was utilized as a means to prepare large quantities of the products for chemical analyses. Together, the above procedures have permitted the separation and identification of 17 nucleoside products that arise from γ-irradiation and photosensitization of dCyd in aerated aqueous solutions. This work provides the mechanistic and analytical aspects necessary to explore the consequences of •OH-mediated oxidation products of dCyd in more complex biological systems.

Experimental Section

2′-Deoxycytidine (dCyd; **1**) was obtained from Sigma (St. Louis, MO). High-performance liquid chromatography (HPLC) was performed with dual piston pumps (model M6000, Millipore, Mildford, MA), equipped with flow cell spectrophotometers (model 441, Millipore) and refractive index detectors (model 401, Millipore), using either home-packed silica gel columns with CH_3Cl/MeOH/H_2O (4:2:1, 5% CH_3OH was added to the organic phase; solvent A), $CH_3CH_2COCH_3$/CH_3CH_2-$OHCH_3$/H_2O (75:16:9; solvent B), or octadecylsilyl silica gel columns (Ultrasphere ODS 2) using bidistilled H_2O without or with CH_3OH as the mobile phases. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker AM400 spectrometer (Wissembourg, France) in Fourier transform mode, equipped with an Aspect 3000 computer. Chemical shifts were calibrated in D_2O with reference to 3-(trimethylsilyl)propionate-2,2,3,3-d_4 as internal standard. ^{1}H NMR spectra were simulated for best fit by LAOCOON III software with a precision of 0.05 Hz. Mass spectrometry analyses were performed on a Kratos MS 50 instrument equipped with both electron impact (EI) and fast atom bombardement (FAB) attachments. Gycerol was used as the matrix for FAB analysis. Direct analysis of modified nucleosides was done by desorption chemical ionization (DCI) with $CH_3CH(CH_3)CH_3$ and NH_3 using a NERMAG apparatus. High-resolution mass spectrometry (HRMS) was carried out on a ZabSpec-T instrument (Micromass, Manchester, UK) with nanospray ionization in H_2O/CH_3OH/CH_3COOH (50:50:1).

Preparation of dCyd Oxidation Products. The preparation of dCyd oxidation products in sufficient quantities for spectroscopic analyses was carried out by menadione photosensitization. The procedure involved photolysis of aqueous unbuffered solution (2 L) containing menadione (0.8 mM) and dCyd (10 mM) using a Rayonet photochemical reactor (Southern New England Ultraviolet Co., Hawden, CT) fitted with 16 RPR-3500A lamps emitting a total of 1.5×10^{16} photons cm^{-2} s^{-1}, as determined by ferrioxalate actinometry of UVA light (λ_{max} = 350 nm). During photolysis, the solutions were bubbled with O_2, and their temperature was maintained at 10 °C by circulation of cold tap water. The mixture of dCyd oxidation products was evaporated to dryness, first by rotary evaporation and then by extensive lyophilization. Subsequently, the products were fractionated by HPLC using a normal-phase semipreparative column and solvent A. Each of the fractions was subsequently purified to homogeneity by reversed-phase HPLC with H_2O as the mobile phase. Hydroxyl-radical-induced oxidation products were prepared by γ-irradiation (^{60}Co) of continuously aerated aqueous solutions of dCyd (1 mM). The solutions were irradiated for

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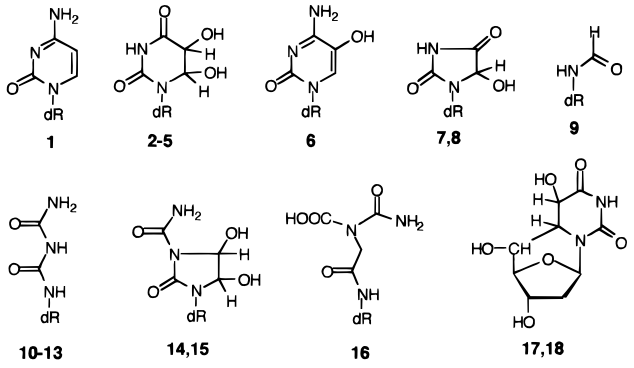
Chart 1. Stuctures of Stable Radiation-Induced Products of dCyd (**1**)



20 min at a dose rate of 80 Gy/min, as determined by Fricke dosimetry (total dose = 1.6 kGy). The structures of **1** and its radiation-induced products (**2**−**18**) are shown in Chart 1.

***cis*-(5*R*,6*S*)-5,6-Dihydroxy-5,6-dihydro-2′-deoxyuridine (2).** HPLC (silica gel, solvent A): $k' = 13.6$ (octadecylsilyl silica gel, H_2O), $k' = 1.08$, where $k' =$ (retention volume − column void volume)/column void volume. Yield (photolysis) of **2** and **3**: 20 mg. ^{1}H NMR (400.13 MHz, D_2O, TSP): δ 6.17 (dd, 1H, $H_{1'}$, $J_{1',2'} = 8.5$ Hz, $J_{1',2''} = 6.2$ Hz), 5.35 (d, 1H, H_6, $J_{5,6} = 3.8$ Hz), 4.60 (d, 1H, H_5), 4.41 (m, 1H, $H_{3'}$, $J_{2',3'} = 6.4$ Hz, $J_{2'',3'} = 3.0$ Hz), 3.94 (m, 1H, $H_{4'}$, $J_{3',4'} = 3.1$ Hz, $J_{4',5'} = 3.8$ Hz, $J_{4',5''} = 5.2$ Hz), 3.77 (m, 1H, $H_{5'}$, $J_{5',5''} = -12.3$ Hz), 3.70 (m, 1H, $H_{5''}$), 2.40 (m, 1H, $H_{2'}$, $J_{2',2''} = -14.0$ Hz), 2.20 (m, 1H, $H_{2''}$). ^{13}C NMR (100.62 MHz, D_2O, TMS): δ 171.0 (C_4), 152.1 (C_2), 85.5 ($C_{4'}$), 84.3 ($C_{1'}$), 75.4 (C_6), 71 ($C_{3'}$), 69.0 (C_5), 61.8 ($C_{5'}$), 36.8 ($C_{2'}$). FAB-MS (positive mode, glycerol mull): *m*/*z* (relative intensity) 377 (25, M^+ + glycerol + Na), 355 (25, M^+ + glycerol), 285 (45, MNa^+), 263 (30, MH^+), 245 (50, $MH^+ - H_2O$). FAB-MS (negative mode, glycerol mull): *m*/*z* (relative intensity) 283 (10, $M^- - H + Na$), 261 (40, $M^- - H$). DCI-MS ($CH_3CH(CH_3)CH_3$ and NH_3): *m*/*z* (relative intensity) 280 (15, $M + NH_4^+$), 263 (20, MH^+), 245 (60, $MH^+ - H_2O$), 177 (100), 117 (100, 2-deoxyribose$^+$). HRMS (ES, positive mode): calcd for $C_9H_{14}N_2O_7$ ($[M + Na]^+$) 285.0699, found 285.0699.

***cis*-(5*S*,6*R*)-5,6-Dihydroxy-5,6-dihydro-2′-deoxyuridine (3).** HPLC (silica gel, solvent A): $k' = 13.6$ (octadecylsilyl silica gel, H_2O), $k' = 1.08$. Yield (photolysis) of **2** and **3**: 20 mg. ^{1}H NMR (400.13 MHz, D_2O, TSP): δ 6.15 (pst, 1H, $H_{1'}$, $J_{1',2'} = 7.3$ Hz, $J_{1',2''} = 6.7$ Hz), 5.34 (d, 1H, H_6, $J_{5,6} = 3.8$ Hz), 4.63 (d, 1H, H_5), 4.42 (m, 1H, $H_{3'}$, $J_{2',3'} = 6.9$ Hz, $J_{2'',3'} = 4.2$ Hz), 3.92 (m, 1H, $H_{4'}$, $J_{3',4'} = 4.3$ Hz, $J_{4',5'} = 3.6$ Hz, $J_{4',5''} = 4.9$ Hz), 3.79 (m, 1H, $H_{5'}$, $J_{5',5''} = -12.3$ Hz), 3.73 (m, 1, $H_{5''}$), 2.34 (m, 1H, $H_{2'}$, $J_{2',2''} = -14.1$ Hz), 2.24 (m, 1H, $H_{2''}$). ^{13}C NMR (100.62 MHz, D_2O, TMS): δ 172.3 (C_4═O), 153.0 (C_2═O), 85.4 ($C_{4'}$), 84.9 ($C_{1'}$), 75.8 (C_6), 70.4 ($C_{3'}$), 69.0 (C_5), 61.2 ($C_{5'}$), 37.3 ($C_{2'}$). DCI-MS ($CH_3CH(CH_3)CH_3$ and NH_3): *m*/*z* (relative intensity) 280 ($M + NH_4^+$, 13), 263 (MH^+, 17), 245 ($MH^+ - H_2O$, 60), 177 (100), 117 (100). HRMS (ES, positive mode): calcd for $C_9H_{14}N_2O_7$ ($[M + Na]^+$) 285.0699, found 285.0699.

***trans*-(5*S*,6*S*)-5,6-Dihydroxy-5,6-dihydro-2′-deoxyuridine (4).** HPLC (silica gel, solvent A): $k' = 7.75$ (octadecylsilyl silica gel, H_2O), $k' = 0.80$. Yield (photolysis): 17 mg. ^{1}H NMR (400.13 MHz, D_2O, TSP): δ 6.26 (dd, 1H, $H_{1'}$, $J_{1',2'} = 7.7$ Hz, $J_{1',2''} = 6.2$ Hz), 5.36 (d, 1H, H_6, $J_{5,6} = 2.8$ Hz), 4.47 (m, 1H, $H_{3'}$, $J_{2',3'} = 7.2$ Hz, $J_{2'',3'} = 3.4$ Hz, $J_{3',4'} = 2.9$ Hz), 4.24 (d, 1H, H_5), 3.99 (m, 1H, $H_{4'}$, $J_{4',5'} = 4.3$ Hz, $J_{4',5''} = 5.0$ Hz,), 3.80 (m, 2H, $H_{5',5''}$, $J_{5',5''} = -11.5$ Hz), 2.45 (m, 1H, $H_{2'}$, $J_{2',2''} = -14.0$ Hz), 2.30 (s, 1H, $H_{2''}$). DCI-MS ($CH_3CH(CH_3)CH_3$ and NH_3): *m*/*z* (relative intensity) 280 ($M + NH_4^+$, 13), 263 (MH^+, 17), 245 ($MH^+ - H_2O$, 60), 177 (100), 117 (100).

***trans*-(5*R*,6*R*)-5,6-Dihydroxy-5,6-dihydro-2′-deoxyuridine (5).** HPLC (silica gel, solvent A): $k' = 5.25$ (octadecylsilyl silica gel, H_2O), $k' = 0.92$. Yield (photolysis): 16 mg. ^{1}H NMR (400.13 MHz, D_2O, TSP): δ 6.30 (pst, 1H, H_1), 5.34 (d, 1H, H_6, $J_{5,6} = 3.0$ Hz), 4.48 (m, 1H, $H_{3'}$), 4.25 (d, 1H, H_5), 3.98 (m, 1H, $H_{4'}$), 3.82 (m, 2H, $H_{5',5''}$), 2.42 (m, 2H, $H_{2',2''}$). DCI-MS ($CH_3CH(CH_3)CH_3$ and NH_3): *m*/*z* (relative intensity) 280 ($M + NH_4^+$, 13), 263 (MH^+, 17), 245 ($MH^+ - H_2O$, 60), 177 (100), 117 (100).

5-Hydroxy-2′-deoxycytidine (6). HPLC (silica gel, solvent A): $k' = 15.5$ (octadecylsilyl silica gel, H_2O), $k' = 6.33$. Yield (photolysis): 9 mg. UV (λ_{max}): 289 nm. ^{1}H NMR (250 MHz, D_2O, TSP): δ 7.37 (s, 1H, H_6), 6.29 (t, 1H, $H_{1'}$), 4.44 (m, 1H, $H_{3'}$), 4.04 (m, 1H, $H_{4'}$), 3.85 (dd, 1H, $H_{5'}$), 3.76 (dd, 1H, $H_{5''}$), 2.39 (m, 1H, $H_{2'}$), 2.27 (m, 1H, $H_{2''}$). ^{1}H NMR (250 MHz, DMSO-d_6,TSP): δ 7.30 (s, 1H, NH), 6.74 (s, 1H, NH). ^{13}C NMR (100.62 MHz, D_2O, TMS): δ 161.3 (C_2), 155.6 (C_4), 128.1 (C_5), 123.1 (C_6), 86.6 ($C_{1'}$), 86.0 ($C_{4'}$), 70.6 ($C_{3'}$), 61.3 ($C_{5'}$), 39.2 ($C_{2'}$). LC-MS: *m*/*z* (relative intensity) 244 (24, MH^+), 177 (20), 128 (100), 117 (44, deoxyribose$^+$). HRMS (ES, positive mode): calcd for $C_9H_{13}N_3O_5$ ($[M + H]^+$) 244.0934, found 244.0934.

(5*S* or 5*R*)-*N*$_1$-(2-Deoxy-*β*-D-*erythro*-pentofuranosyl)-5-hydroxyhydantoin (7). HPLC (silica gel, solvent A): $k' = 3.62$ (octadecylsilyl silica gel, H_2O), $k' = 1.83$. Yield (photolysis): 9 mg. ^{1}H NMR (400.13 MHz, D_2O, TSP): δ 5.99 (dd, 1H, $H_{1'}$, $J_{1',2'} = 8.1$ Hz, $J_{1',2''} = 6.4$ Hz), 5.54 (s, 1H, H_6), 4.39 (m, 1H, $H_{3'}$, $J_{2',3'} = 6.1$ Hz, $J_{2'',3'} = 3.5$ Hz), 3.92 (m, 1H, $H_{4'}$, $J_{3',4'} = 3.1$ Hz, $J_{4',5'} = 4.1$ Hz, $J_{4',5''} = 5.5$ Hz), 3.73 (m, 1H, $H_{5'}$, $J_{5',5''} = -12.3$ Hz), 3.65 (m, 1H, $H_{5''}$), 2.57 (m, 1H, $H_{2'}$, $J_{2',2''} = -13.8$ Hz), 2.20 (m, 1H, $H_{2''}$). DCI-MS ($CH_3CH(CH_3)CH_3$ and NH_3): *m*/*z* (relative intensity) 250 (30, $M + NH_4^+$), 233 (30, MH^+), 134 (60, base NH_4^+), 117 (50, deoxyribose$^+$). HRMS (ES, positive mode): calcd for $C_8H_{12}N_2O_6$ (M^+) 232.0696, found 232.0695.

(5*R* or 5*S*)-*N*$_1$-(2-Deoxy-*β*-D-*erythro*-pentofuranosyl)-5-hydroxyhydantoin (8). HPLC (silica gel, solvent A): $k' = 4.37$ (octadecylsilyl silica gel, H_2O), $k' = 1.50$. Yield (photolysis): 9 mg. ^{1}H NMR (400.13 MHz, D_2O, TSP): δ 5.98 (dd, 1H, $H_{1'}$, $J_{1',2'} = 8.4$ Hz, $J_{1',2''} = 6.3$ Hz), 5.43 (s, 1H, H_6), 4.43 (m, 1H, $H_{3'}$, $J_{2',3'} = 6.4$ Hz, $J_{2'',3'} = 3.1$ Hz), 3.92 (m, 1H, $H_{4'}$, $J_{3',4'} = 2.6$ Hz, $J_{4',5'} = 4.4$ Hz, $J_{4',5''} = 5.6$ Hz), 3.69 (m, 1H, $H_{5'}$, $J_{5',5''} = -12.1$ Hz), 3.64 (m, 1H, $H_{5''}$), 2.53 (m, 1H, $H_{2'}$, $J_{2',2''} = -13.8$ Hz), 2.16 (m, 1H, $H_{2''}$). DCI-MS ($CH_3CH(CH_3)CH_3$ and NH_3): *m*/*z* (relative intensity) 250 (30, $M + NH_4^+$), 233 (20, MH^+), 117 (70), 160 (25). HRMS (ES, positive mode): calcd for $C_8H_{12}N_2O_6$ (M^+) 232.0696, found 232.0695.

***N*$_1$-(2-Deoxy-*β*-D-*erythro*-pentofuranosyl)formamide (9).** HPLC (silica gel, solvent A): $k' = 9.60$ (octadecylsilyl silica gel, H_2O), $k' = 1.08$. Yield (photolysis): 9 mg. ^{1}H NMR (400.13 MHz, D_2O, TSP): δ (major) 8.12 (s, 1H, H_2, $J_{2,1'} = 0.9$ Hz), 5.89 (t, 1H, $H_{1'}$, $J_{1',2'} = 7.2$ Hz, $J_{1',2''} = 6.0$ Hz), 4.39 (m, 1H, $H_{3'}$, $J_{2',3'} = 6.2$ Hz, $J_{2'',3'} = 3.2$ Hz, $J_{3',4'} = 3.0$ Hz), 3.96 (m, 1H, $H_{4'}$, $J_{4',5'} = 4.2$ Hz, $J_{4',5''} = 5.3$ Hz), 3.65 (m, 1H, $H_{5'}$, $J_{5',5''} = -12.2$ Hz), 3.62 (m, 1H, $H_{5''}$), 2.27 (m, 1H, $H_{2''}$, $J_{2',2''} = -14.0$ Hz), 2.14 (m, 1H, $H_{2'}$); δ (minor) 8.22 (s, 1H, H_2, $J_{2,1'} = 0.2$ Hz), 5.62 (spt, 1H, $H_{1'}$, $J_{1',2'} = 7.7$ Hz, $J_{1',2''} = 3.0$ Hz), 4.38 (m, 1H, $H_{3'}$, $J_{2',3'} = 6.2$ Hz, $J_{2'',3'} = 3.2$ Hz, $J_{3',4'} = 3.0$ Hz), 3.95 (m, 1H, $H_{4'}$, $J_{4',5'} = 4.2$ Hz, $J_{4',5''} = 5.3$ Hz), 3.65 (m, 1H, $H_{5'}$, $J_{5',5''} = -12.2$ Hz), 3.62 (m, 1H, $H_{5''}$), 2.31 (m, 1H, $H_{2''}$, $J_{2',2''} = -14.0$ Hz), 2.16 (m, 1H, $H_{2'}$). ^{13}C NMR (100.62 MHz, D_2O, TMS): δ (major) 165 (C_2), 86.5 ($C_{4'}$), 79.2 ($C_{1'}$), 71.9 ($C_{3'}$), 62.3 ($C_{5'}$), 39.1 ($C_{2'}$); δ (minor) 168.1 (C_2), 86.5 ($C_{4'}$), 84.7 ($C_{1'}$), 71.9 ($C_{3'}$), 62.3 ($C_{5'}$), 39.4 ($C_{2'}$). FAB-MS (positive ion, glycerol): *m*/*z* (relative intensity) 162 (30, MH^+), 184 (15, $M + Na^+$), 254 (20), 117 (40, 2-deoxyribose$^+$). HRMS (ES, positive mode): calcd for the diacetylated derivative $C_6H_{11}NO_4$ ($[M + H]^+$) 246.0973, found 246.0993.

***N*$_1$-(2-Deoxy-*β*-D-*erythro*-pentofuranosyl)biuret (10).** HPLC (silica gel, solvent A): $k' = 6.87$ (octadecylsilyl silica gel, H_2O), $k' = 3.66$. Yield (photolysis): 6 mg. ^{1}H NMR (400 MHz, D_2O, TSP): δ 5.86 (pst, 1H, $H_{1'}$, $J_{1',2'} = 6.2$ Hz, $J_{1',2''} = 7.5$ Hz), 4.45 (m, 1H, $H_{3'}$, $J_{2',3'} = 3.2$ Hz, $J_{2'',3'} = 6.0$ Hz), 4.00 (m, 1H, $H_{4'}$, $J_{3',4'} = 2.6$ Hz, $J_{4',5'} = 4.3$ Hz, $J_{4',5''} = 4.9$ Hz), 3.70 (m, 2H, $H_{5'}$, $H_{5''}$, $J_{5',5''} = -12.1$ Hz), 2.32 (m, 1H, $H_{2'}$, $J_{2',2''} = -14.0$ Hz), 2.20 (m, 1H, $H_{2''}$). ^{1}H NMR (250 MHz, DMSO-d_6, TMS): δ 8.80 (s, 1H, NH), 8.04 (d, 1H, NH, $J_{NH,1'} = 9.6$ Hz), 6.97 (s, 2H, NH). FAB-MS (positive mode, glycerol): *m*/*e* (relative intensity) 220 (10, MH^+), 242 (20, $MH + Na^+$), 207 (90), 185 (100). FAB-MS (negative mode, glycerol mull): *m*/*z* (relative intensity) 218 (10, $M - H^-$), 191 (60), 183 (70).

***N*$_1$-(2-Deoxy-α-D-*erythro*-pentofuranosyl)biuret (11).** HPLC (silica gel, solvent A): $k' = 6.87$ (octadecylsilyl silica gel, H_2O), $k' = 6.16$. Yield (photolysis): 8 mg. ^{1}H NMR (400.13 MHz, D_2O, TSP): δ 5.96 (dd, 1H, $H_{1'}$, $J_{1',2'} = 7.0$ Hz, $J_{1',2''} = 2.5$ Hz), 4.49 (m, 1H, $H_{3'}$, $J_{2',3'} = 5.6$ Hz, $J_{2'',3'} = 2.5$ Hz), 4.22 (m, 1H, $H_{4'}$, $J_{3',4'} = 3.5$ Hz, $J_{4',5'} = 4.0$ Hz, $J_{4',5''} = 5.3$ Hz), 3.66 (m, 2H, $H_{5'}$, $H_{5''}$), 2.48 (m, 1H, $H_{2'}$, $J_{2',2''} = -14.5$ Hz), 2.10 (m, 1H, $H_{2''}$). DCI-MS ($CH_3CH(CH_3)CH_3$ and NH_3):

m/*e* (relative intensity) 220 (25, MH^+), 237 (10, $M + NH_4^+$), 117 (100, $MH^+ - CONH$), 160 (70, MH^+ − urea), 117 (50, 2-deoxyribose$^+$).

***N*$_1$-(2-Deoxy-*β*-D-*erythro*-pyranosyl)biuret (12).** HPLC (silica gel, solvent A): $k' = 6.87$ (octadecylsilyl silica gel, H_2O), $k' = 2.77$. Yield (photolysis): 6 mg. ^{1}H NMR (400.13 MHz, D_2O, TSP): δ 5.41 (dd, 1H, $H_{1'}$, $J_{1',2'} = 8.0$ Hz, $J_{1',2''} = 2.9$ Hz), 4.20 (m, 1H, $H_{3'}$, $J_{2',3'} = 2.8$ Hz, $J_{2'',3'} = 3.5$ Hz), 3.90 (m, 1H, $H_{4'}$, $J_{3',4'} = 6.3$ Hz, $J_{4',5'} = 4.3$ Hz, $J_{4',5''} = 8.0$ Hz), 3.82 (m, 2H, $H_{5'}$, $H_{5''}$, $J_{5',5''} = -11.3$ Hz), 2.14 (m, 1H, $H_{2'}$, $J_{2',2''} = -13.4$ Hz), 1.95 (m, 1H, $H_{2''}$). FAB-MS (positive mode, glycerol mull): *m*/*e* (relative intensity) 220 (20, MH^+), 242 (15, $M + Na^+$), 312 (10, M^+ + glycerol).

***N*$_1$-(2-Deoxy-α-D-*erythro*-pyranosyl)biuret (13).** HPLC (silica gel, solvent A): $k' = 6.87$ (octadecylsilyl silica gel, H_2O), $k' = 4.55$. Yield (photolysis): 6 mg. ^{1}H NMR (400.13 MHz, D_2O, TSP): δ 5.12 (dd, 1H, $H_{1'}$, $J_{1',2'} = 2.5$ Hz, $J_{1',2''} = 10.0$ Hz), 4.06 (m, 1H, $H_{3'}$, $J_{2',3'} = 4.5$ Hz, $J_{2'',3'} = 10.0$ Hz), 3.95 (dd, 1H, $H_{5'}$, $J_{5',5''} = -12.0$ Hz, $J_{4',5'} = 1.8$ Hz), 3.87 (m, 1H, $H_{4'}$, $J_{3',4'} = 3.0$ Hz, $J_{4',5''} = 3.5$ Hz), 3.71 (dd, 1H, $H_{5''}$), 2.03 (m, 1H, $H_{2'}$, $J_{2',2''} = -13.0$ Hz), 1.90 (m, 1H, $H_{2''}$). FAB-MS (negative mode, glycerol mull): *m*/*e* (relative intensity) 218 (25, $M - H^-$), 310 (10, $M - H^-$ + glycerol), 184 (20), 183 (100).

***trans*-(4S*,5S*)-*N*$_1$-(2-Deoxy-*β*-D-*erythro*-pentofuranosyl)-1-carbamoyl-3,4-dihydroxy-2-oxoimidazolidine (14).** HPLC (silica gel, solvent A): $k' = 6.12$ (octadecylsilyl silica gel, H_2O), $k' = 2.25$. Yield (photolysis): 10 mg. ^{1}H NMR (400.13 MHz, D_2O, TSP): δ 5.95 (dd, 1H, $H_{1'}$, $J_{1',2'} = 8.3$ Hz, $J_{1',2''} = 6.1$ Hz), 5.54 (s, 1H, H_5), 5.23 (s, 1H, H_4), 4.47 (m, 1H, $H_{3'}$, $J_{2',3'} = 6.0$ Hz, $J_{2'',3'} = 3.1$ Hz), 4.01 (m, 1H, $H_{4'}$, $J_{3',4'} = 3.0$ Hz, $J_{4',5'} = 4.2$ Hz, $J_{4',5''} = 5.5$ Hz), 3.75 (m, 2H, $H_{5'}$, $H_{5''}$, $J_{5',5''} = -12.2$ Hz), 2.59 (m, 1H, $H_{2'}$, $J_{2',2''} = -14.0$ Hz), 2.31 (m, 1H, $H_{2''}$). DCI-MS ($CH_3CH(CH_3)CH_3$ and NH_3): *m*/*z* (relative intensity) 295 (5, $M + NH_4^+$), 278 (15, MH^+), 177 (70), 160 (30), 117 (25, 2-deoxyribose$^+$), 98 (100, 2-deoxyribose$^+ - H_2O$). HRMS (ES, positive mode): calcd for $C_9H_{15}N_3O_7$ ($[M + Na]^+$) 300.0808, found 300.0808.

***trans*-(4R*,5R*)-*N*$_1$-(2-Deoxy-*β*-D-*erythro*-pentofuranosyl)-1-carbamoyl-3,4-dihydroxy-2-oxoimidazolidine (15).** HPLC (silica gel, solvent A): $k' = 5.25$ (octadecylsilyl silica gel, H_2O), $k' = 2.42$. Yield (photolysis): 10 mg. ^{1}H NMR (400.13 MHz, D_2O, TSP): δ 5.94 (dd, 1H, $H_{1'}$, $J_{1',2'} = 8.2$ Hz, $J_{1',2''} = 6.3$ Hz), 5.54 (s, 1H, H_5), 5.25 (s, 1H, H_4), 4.52 (m, 1H, $H_{3'}$, $J_{2',3'} = 6.3$ Hz, $J_{2'',3'} = 3.2$ Hz), 4.02 (m, 1H, $H_{4'}$, $J_{3',4'} = 3.2$ Hz, $J_{4',5'} = 4.0$ Hz, $J_{4',5''} = 4.8$ Hz), 3.76 (m, 2H, $H_{5'}$, $H_{5''}$, $J_{5',5''} = -12.2$ Hz), 2.57 (m, 1H, $H_{2'}$, $J_{2',2''} = -13.9$ Hz), 2.30 (m, 1H, $H_{2''}$). ^{13}C NMR (100.62 MHz, D_2O, TMS): δ 155 (CO), 154.5 (CO), 85.8 ($C_{4'}$), 83.1 ($C_{1'}$), 81.5 (CHOH), 81.2 (CHOH), 73.3 ($C_{3'}$), 61.7 ($C_{5'}$), 35.4 ($C_{2'}$). DCI-MS ($CH_3CH(CH_3)CH_3$ and NH_3): *m*/*z* 295 (10, $M + NH_4^+$), 278 (10, MH^+), 260 (10, $MH^+ - H_2O$). HRMS (ES, positive mode): calcd for $C_9H_{15}N_3O_7$ ($[M + Na]^+$) 300.0808, found 300.0808.

Aminocarbonyl-[2-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)amino]-2-oxomethylcarbamic Acid (16). HPLC (silica gel, solvent A): $k' = 10.3$ (octadecylsilyl silica gel, H_2O), $k' = 6.0$. Yield (photolysis): 15 mg. ^{1}H NMR (400.13 MHz, D_2O, TSP): δ 5.96 (dd, 1H, $H_{1'}$), 4.80 (s, 2H, CH_2), 4.50 (m, 1H, $H_{3'}$), 4.06 (m, 1H, $H_{4'}$), 3.75 (m, 2H, $H_{5'}$, $H_{5''}$), 2.32 (m, 1H, $H_{2'}$), 2.31 (m, 1H, $H_{2''}$). ^{1}H NMR (400.13 MHz, DMSO-d_6, TMS): δ 8.40 (d, 1H, NH), $J_{NH,1'} = 9$ Hz), 7.2 (s, 2H, NH_2). ^{13}C NMR (100.62 MHz, D_2O, TMS): δ 171.1 (CONH), 154.8 (COOH), 142.7 ($CONH_2$), 87.0 ($C_{4'}$), 81.0 ($C_{1'}$), 72.3 ($C_{3'}$), 64.4 (CH_2), 62.9 ($C_{5'}$), 39.4 ($C_{2'}$). DCI-MS ($CH_3CH(CH_3)CH_3$ and NH_3): *m*/*z* (relative intensity) 295 (10, $M + NH_4^+$), 278 (20, MH^+), 235 (95, $MH^+ -$ CONH), 192 (95). FAB-MS (positive mode, glycerol mull): *m*/*z* (relative intensity) 370 (20, M^+ + glycerol), 300 (20, $M + Na^+$), 278 (10, MH^+), 162 (20, base H^+); diacetylated derivative (positive mode) *m*/*z* 362 (MH^+), (negative mode) *m*/*z* 360 ($M^- - H$).

(5′*S*,5*S*,6*S*)-5′,6-Cyclo-5-hydroxy-5,6-dihydro-2′-deoxyuridine (17). Yield (γ-irradiation): 2 mg. ^{1}H NMR (400.13 MHz, D_2O, TSP): δ 6.30 (dd, 1H, $H_{1'}$, $J_{1',2'} = 0.4$ Hz, $J_{1',2''} = 6.3$ Hz), 4.70 (m, 1H, $H_{3'}$, $J_{2',3'} = 7.4$ Hz, $J_{2'',3'} = 2.9$ Hz, $J_{3',4'} = 0.4$ Hz), 4.35 (dd, 1H, H4′, $J_{4',5'} = 5.2$ Hz, $J_{3',4'} = 0.4$ Hz), 4.26 (d, 1H, H_5, $J_{5,6} = -10.8$ Hz), 4.04 (dd, 1H, $H_{5'}$, $J_{5',6} = 8.8$ Hz, $J_{4'5'} = 5.2$), 3.39 (dd, 1H, H_6, $J_{5,6} = 10.8$ Hz, $J_{5',6} = 8.8$ Hz), 2.50 (m, 1H, $H_{2'}$, $J_{2',2''} = -14.6$ Hz, $J_{2'3'} = 7.4$ Hz), 2.16 (m, 1H, $H_{2''}$, $J_{1',2''} = 6.3$ Hz, $J_{2',2''} = -14.6$ Hz, $J_{2'',3'} = 2.9$ Hz). EI-MS (triacetylated derivative, positive mode): *m*/*z* (relative intensity) 310 (10, $MH^+ - CH_3COOH$), 268 (20, $MH^+ - CH_2CO$), 251 (10, $MH^+ - COOCH_3$), 208 ($MH^+ - COCH_3$).

(5′*R*,5*R*,6*R*)-5′,6-Cyclo-5-hydroxy-5,6-dihydro-2′-deoxyuridine (18). Yield (γ-irradiation): 2 mg. ^{1}H NMR (400.13 MHz, D_2O, TSP): δ 6.30 (dd, 1H, $H_{1'}$, $J_{1',2'} = 0.4$ Hz, $J_{1',2''} = 6.1$ Hz), 4.63 (m, 1H, $H_{3'}$, $J_{2',3'} = 7.2$ Hz, $J_{2'',3'} = 2.8$ Hz, $J_{3',4'} = 0.4$ Hz), 4.51 (dd, 1H, $H_{4'}$, $J_{4',5'} = 2.7$ Hz, $J_{3',4'} = 0.4$ Hz), 4.45 (d, 1H, H_5, $J_{5,6} = -11.5$ Hz), 3.88 (dd, 1H, $H_{5'}$, $J_{5',6} = 3.2$ Hz, $J_{4'5'} = 2.7$), 3.55 (dd, 1H, H_6, $J_{5,6} = -11.5$ Hz, $J_{5',6} = 3.2$ Hz), 2.50 (m, 1H, $H_{2'}$, $J_{2',2''} = -15.0$ Hz, $J_{2',3'} = 7.2$ Hz), 2.12 (m, 1H, $H_{2''}$, $J_{1',2''} = 6.1$ Hz, $J_{2',2''} = -15.0$ Hz, $J_{2'',3'} = 2.8$ Hz). EI-MS (triacetylated derivative, positive mode): *m*/*z* (relative intensity) 310 (10, $MH^+ - CH_3COOH$), 268 (20, $MH^+ - CH_2CO$) 251 (10, $MH^+ - COOCH_3$) 208 ($MH^+ - COCH_3$).

2-Deoxy-1,4-ribonolactone. HPLC (silica gel, solvent A): $k' = 1.87$. Yield (photolysis): 9 mg. ^{1}H NMR (400.13 MHz, D_2O, TSP): δ 4.58 (m, 1H, H_4), 4.55 (m, 1H, H_3, $J_{2,3} = 3$ Hz, $J_{2',3} = 6$ Hz), 3.88 (dd, 1H, H_5), 3.79 (dd, 1H, $H_{5'}$), 2.78 (dd, 1H, H_2, $J_{2',2'} = 9$ Hz), 2.59 (dd, 1H, $H_{2'}$). ^{13}C NMR (100.62 MHz, D_2O, TMS): δ 181.7 (C═O), 91.2 (C_4), 70.5 (C_3), 63.2 (C_5), 39.9 (C_2). IR (KBr pellet): λ_{max} 1770 (C═O), 1070 cm^{-1} (COH). MS (EI, positive mode): *m*/*z* (relative intensity) 133 (15, MH^+), 114 (20, $M^+ - H_2O$), 104 (25, $M^+ -$ CO), 101 (30, $M^+ - CH_2OH$).

Synthesis of *trans*-5-Bromo-6-hydroxy-5,6-dihydro-2′-deoxyuridine. The cis diastereomers of the 5,6-glycols of 2′-deoxyuridine (**2** and **3**) cannot be separated by either normal-phase or reversed-phase HPLC, and thus they were prepared in two steps from the corresponding trans diastereomers of the bromohydrins of 2′-deoxyuridine. The latter were prepared by the addition of bromine (dropwise until coloration) to a solution of 2′-deoxyuridine (500 mg/5 mL of H_2O) at 4 °C. After the reaction, excess bromine was eliminated by bubbling with N_2. The solution was neutralized by the addition of sodium acetate and taken to dryness on a rotary evaporator. Two products were obtained after purification by HPLC using an octadecylsilyl silica gel semipreparative column and 10% methanol in water as the mobile phase. The first fraction ($k' = 1.1$) gave 100 mg (yield 25%) of *trans*-(5*S*,6*S*)-5-bromo-6-hydroxy-5,6-dihydro-2′-deoxyuridine. ^{1}H NMR (400.13 MHz, D_2O, TSP): δ 6.32 (dd, 1H, $H_{1'}$, $J_{1',2'} = 8.6$ Hz, $J_{1',2''} = 6.1$ Hz), 5.62 (d, 1H, H_6, $J_{5,6} = 2.4$ Hz), 4.63 (d, 1H, H_5), 4.52 (m, 1H, $H_{3'}$, $J_{2',3'} = 5.7$ Hz, $J_{2'',3'} = 3.1$ Hz), 4.04 (m, 1H, $H_{4'}$, $J_{3',4'} = 3.2$ Hz, $J_{4',5'} = 4.3$ Hz, $J_{4',5''} = 5.0$ Hz), 3.84 (m, 2H, $H_{5'5''}$, $J_{5',5''} = -12.6$ Hz), 2.46 (m, 1H, $H_{2'}$, $J_{2',2''} = -14.5$ Hz), 2.30 (m, 1H, $H_{2''}$). FAB-MS (negative mode, glycerol mull): *m*/*z* (relative intensity) 325 (10, MH^+), 243 (10). The second fraction ($k' = 1.75$) gave 200 mg (yield 50%) of *trans*-(5*R*,6*R*)-5-bromo-6-hydroxy-5,6-dihydro-2′-deoxyuridine. ^{1}H NMR (400.13 MHz, D_2O, TSP): δ 6.32 (pst, 1H, $H_{1'}$, $J_{1',2'} = 7.1$ Hz, $J_{1',2''} = 6.5$ Hz), 5.61 (d, 1H, H_6, $J_{5,6} = 3.2$ Hz), 4.64 (d, 1H, H_5), 4.50 (m, 1H, $H_{3'}$, $J_{2',3'} = 6.4$ Hz, $J_{2'',3'} = 4.2$ Hz), 4.03 (m, 1H, $H_{4'}$, $J_{3',4'} = 4.0$ Hz, $J_{4',5'} = 2.4$ Hz, $J_{4',5''} = 5.2$ Hz), 3.86 (m, 2H, $H_{5'5''}$, $J_{5',5''} = -12.2$ Hz), 2.43 (m, 1H, $H_{2'}$, $J_{2',2''} = -14.5$ Hz), 2.30 (m, 1H, $H_{2''}$). FAB-MS (negative mode, glycerol mull): *m*/*z* (relative intensity) 325 (15, MH^+), 307 (10, $-H_2O$), 243 (10, −BrH), 111 (15, uracil).

Synthesis of 5,6-Dihydroxy-5,6-dihydro-2′-deoxyuridine. The purified bromohydrins (*trans*-(5*S*,6*S*)- or *trans*-(5*R*,6*R*)-5-bromo-6-hydroxy-5,6-dihydro-2′-deoxyuridine; 45 mg) were dissolved in 4 mL of H_2O and 4 mL of 30% H_2O_2, and 100 mg of Ag_2O was added to the mixture, maintained for 2 min at 4 °C. Hydroperoxides formed in the reaction were reduced to the alcohol by bubbling with H_2S. The solution was filtered to eliminate the black precipitate, neutralized with sodium carbonate, and evaporated to dryness. The product mixtures were purified by HPLC with silica gel and solvent B ($k' = 13.4$), giving 25 mg of each of the corresponding 5,6-glycols (50% yield): *cis*-(5*R*,6*S*)-5,6-dihydroxy-5,6-dihydro-2′-deoxyuridine (**2**) and *cis*-(5*S*,6*R*)-5,6-dihydroxy-5,6-dihydro-2′-deoxyuridine (**3**).

Quantitation of dCyd Oxidation Products. dCyd (10 mM) and 0.1 mCi of [$^{14}C_2$]-labeled dCyd (New England Nuclear, Boston, MA) were subjected to either γ-irradiation or menadione photosensitization using a procedure similar to that described for the preparation of oxidation products, except that the reactions were done on a smaller scale (2 mL). The resulting mixture was analyzed by two-dimensional thin-layer chromatography (2D-TLC) with silica gel and solvent A, followed at right angles with solvent B. The products which contained

Table 1. Yield of Stable Products from γ-Irradiation and Photosensitization of dCyd in Aerated Aqueous Solutions[a]

product (dR = 2-deoxy-β-D-*erythro*-pentofuranosyl)	γ-irradiation[b]	UVA/menadione photosensitization[c]
5,6-dihydroxy-5,6-dihydro-2′-deoxyuridine (**2**−**5**)	28	27
5-hydroxy-2′-deoxycytidine (**6**)	12	7
N_1-(dR)-5-hydroxyhydantoin (**7**,**8**)	3	2
N_1-(dR)-formamide (**9**)	12[d]	2[d]
N_1-(dR)-biuret (**10**−**13**)	3	2
N_1-(dR)-1-carbamoyl-3,4-dihydroxy-2-oxoimidazolidine (**14**, **15**)	17	6
aminocarbonyl[2-(dR)-amino]-2-oxomethylcarbamic acid (**16**)	3	2
5′,6-cyclo-5-hydroxy-5,6-dihydro-2′-deoxyuridine (**17**, **18**)	∼2	nd[e]
2′-deoxyuridine (**19**)	nd[e]	36
cytosine	12	4
2-deoxyribono-1,4-lactone	ta[f]	4[g]
total	92	92

[a] Yields from normal-phase 2D-TLC and HPLC analyses with $^{14}C_2$-labeled dCyd. [b] Total loss of substrate (15%); $G = -85 \times 10^{-9}$ J mol^{-1}. [c] Total loss of substrate (22%). [d] Estimated by comparison of refractive index signal with that of **16**. [e] Not detected. [f] Trace amounts. [g] Produced in equal yield with cytosine.

radioactivity were localized by autoradiography and later quantitated by liquid scintillation counting. Most of the products were well resolved by 2D-TLC and were identified on the basis of comigration with authentic standards.[20] Since dCyd (**1**), 5-hydroxy-2′-deoxycytidine (**6**), and cytosine are not well separated by 2D-TLC, they were quantitated by HPLC using an octadecylsilyl silica gel column with water as the mobile phase. Finally, the yield of N_1-(2-deoxy-β-D-*erythro*-pentofuranosy)formamide (**9**), which does not retain radioactivity from the parent molecule, was estimated by HPLC (silica gel and solvent A) on the basis of its refractive index signal compared with that of product **16**.

Labeling Experiments with $^{18}O_2$: γ-Irradiation. dCyd (1 mM) was irradiated in unbuffered aqueous solutions (100 mL) for 20 min (total dose 1.6 kGy). Prior to irradiation, the solution was saturated with 99.9% $^{18}O_2$ (CEA, Saclay, France) by two cycles of degassing (rotary evaporation) and bubbling with $^{18}O_2$ for 10 min. The last cycle was completed in the irradiation vessel (1 L), and the vessel was closed under $^{18}O_2$. After 10 min of irradiation, the solution was resaturated with $^{18}O_2$. The resulting mixtures were evaporated to dryness, and the products were initially separated by HPLC using silica gel phase with solvent A and then with octadecylsilyl silica gel phase with water. The percentage of ^{18}O label in dCyd oxidation products was determined by DCI-MS.

Results

The •OH-mediated decomposition of 2′-deoxycytidine (dCyd; **1**) in aerated aqueous solutions gave a complex mixture of products. It was possible to separate 17 nucleoside products from this mixture using a combination of normal- and reversed-phase HPLC. The radiation yields of individual decomposition products were determined by 2D-TLC and/or HPLC analyses of reaction mixtures containing [$^{14}C_2$]-labeled dCyd (Table 1). The products may be separated into three groups: (1) nucleosides which are similar in structure to products that arise from the •OH-mediated decomposition of thymidine (**2**−**9**; 55% of the total decomposition of dCyd); (2) nucleosides which are unique to •OH-mediated decomposition of dCyd derivatives (**10**−**16**; 23%); and (3) novel cyclic products (**17**, **18**; ∼2%), cytosine (12%), and 2-deoxy-1,4-ribonolactone (trace amounts). Taken together, the above products account for 92% of the overall •OH-induced decomposition of dCyd.

Common Pyrimidine Base Modifications. The major product of the •OH-mediated decomposition of dCyd was 5,6-dihydroxy-5,6-dihydro-2′-deoxyuridine including four diastereomers (**2**−**5**; 28%). The FAB-MS analyses of **2**−**5** exhibited a molecular ion at 263 *m*/*z* (MH^+) with a major ion fragment at 245 *m*/*z*, indicating the loss of H_2O from the molecular ion. The ^{1}H NMR spectra of **2**−**5** displayed two midrange resonances at 4.60 ppm for H_5 and at 5.35 ppm for H_6 with proton−proton coupling constants in the range of 2.8−3.8 Hz. The remaining seven protons may be assigned to an intact 2-deoxyribose moiety. $H_{2'}$ and $H_{2''}$ protons were distinguished on the basis of proton−proton coupling constant arguments.[25] The lower field component of the $H_{5'}$ and $H_{5''}$ pattern was attributed to $H_{5'}$, in line with studies of specifically deuterated nucleosides.[26] The stereospecific synthesis of 2′-deoxyuridine bromohydrins, followed by their conversion into 5,6-glycols, confirmed the absolute configuration of cis diastereomers (**2** and **3**). The absolute configuration of *trans*-2′-deoxyuridine bromohydrins is based on the difference between 6*R* and 6*S* diastereomers of 6-hydroxy-substituted 5,6-dihydrothymine derivatives with respect to the conformation of the sugar moiety. As shown for thymine derivatives, the conversion of pyrimidine bromohydrins into pyrimidine 5,6-glycols involves a shift of the hydroxyl group from C_6 to C_5, leading to inversion of the configuration at C_5.[27] The conversion preferentially leads to *cis*-5,6-glycol products because H_2O preferentially attacks the same side of the zwitterionic intermediate due to anchimeric assistance.[28] Thus, *trans*-(5*S*,6*S*)-5-bromo-6-hydroxy-5,6-dihydro-2′-deoxyuridine transforms into *cis*-(5*R*,6*S*)-5,6-dihydroxy-5,6-dihydro-2′-deoxyuridine (**2**), and similarly *trans*-(5*R*,6*R*)-5-bromo-6-hydroxy-5,6-dihydro-2′-deoxyuridine transforms into *cis*-(5*S*,6*R*)-5,6-dihydroxy-5,6-dihydro-2′-deoxyuridine (**3**). The absolute configuration of products **4** and **5** was established by the observation that they transform into cis diastereomers by ring−chain tautomerism, leading to epimerization of C_6 (**4** into **3** and **5** into **2**). This is analogous to the isomerization of thymidine 5,6-glycols in aqueous solution.[29]

A number of other products were also characterized, including 5-hydroxy-2′-deoxycytidine (**6**; 12%), two diastereomers of N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-hydroxyhydantoin (**7** and **8**; 3%), and 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)formamide (**9**; 12%). Identification of **7** and **8** was based on a comparison of their spectroscopic features with those of analogous compounds obtained by the oxidation of thymidine: N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-hydroxy-5-methylhydantoin.[30] The absolute configuration of the two diastereomers was recently addressed by detailed ^{1}H NMR and molecular

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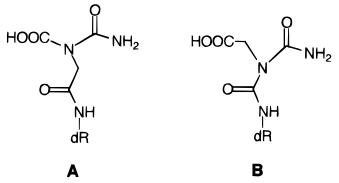
modeling studies.[31] Similarly, 5-hydroxy-2′-deoxycytidine (**6**) and N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)formamide (**9**) were identified by comparison with authentic compounds.[32,33] As a diagnostic feature of product **9**, the ^{1}H NMR and ^{13}C NMR measurements depict the presence of minor (40%) and major (60%) species because of hindered rotation about the amide bond.

Modifications Unique to dCyd. The •OH-mediated decomposition products of dCyd led to several products that are not produced in the related reaction of thymidine. These included four isomeric nucleosides of biuret (**10**−**13**; 3%), two diastereomers of N_1-(2-β-D-*erythro*-pentofuranosyl)-1-carbamoyl-2-oxo-4,5-dihydroxyimidazolidine (**14** and **15**; 17%) and aminocarbonyl[2-(2-deoxy-β-D-*erythro*-pentofuranosyl)amino]-2-oxoethylcarbamic acid (**16**; 3%). Together, these products represented about 23% of the overall •OH-mediated decomposition of dCyd.

MS and ^{1}H NMR analyses of products **10**−**13** revealed that they were modified nucleosides with biuret attached to α-furanose, β-furanose, α-pyranose, or β-pyranose moieties. The molecular ions of all four products appeared at 220 *m*/*z*, as observed by FAB- and DCI-MS. Each of the products contained a biuret moiety, indicated by the presence of two intense fragments, one at 177 *m*/*z* (MH^+ − CONH) and the other at 160 *m*/*z* (MH^+ − urea). To determine the exact structure of the sugar moiety required close examination of the proton−proton coupling constants. The most stable conformations of the 2-deoxy-β-D-*erythro*-pentofuranose moiety are 2′-*exo*-3′-*endo* and 2′-*endo*-3′-*exo*, which restrict the orientation of sugar protons such that $J_{1',2'} + J_{3',4'} \approx 10$ Hz, $J_{2'',3'} \approx J_{3',4'}$ and $J_{1',2'} \approx J_{2',3'}$. In contrast, the pyranose ring system of nucleosides is more flexible, leading to unusually large or small coupling for $J_{1',2'}$ or $J_{1',2''}$ and $J_{4',5''}$.[34] Thus, products **10** and **11** contain a furanose moiety, whereas products **12** and **13** contain a pyranose moiety. The distinction between α and β furanose anomers was based on the proton−proton coupling between $H_{1'}$ and $H_{2''}$, $H_{3'}$ and $H_{2''}$, and $H_{3'}$ and $H_{4'}$.[35] In the case of the pyranose anomers (α or β), the differences in their structure lead to changes in the coupling between $H_{1'}$ and $H_{2'}$ and $H_{1'}$ and $H_{2''}$. Since the α-pyranose anomer preferentially assumes a $^1C_4$ conformation, the coupling between diaxial $H_{1'}$ and $H_{2'}$ is strong ($J_{1',2''} = 10$ Hz). On the other hand, the β-pyranose anomer preferentially assumes a $^4C_1$ conformation, leading to strong coupling between $H_{1'}$ and $H_{2'}$ ($J_{1',2'} = 8$ Hz). It should be noted that, in both cases, the biuret moiety likely adopts a preferential pseudoequatoral orientation.

Products **14** and **15** were identified as the trans diastereomers of N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1-carbamoyl-3,4-dihydroxy-2-oxoimidazolidine. This product is analogous to *trans*-1-carbamoyl-3,4-dihydroxy-2-oxoimidazolidine, which is the major •OH-mediated decomposition product of cytosine in aerated aqueous solutions and which has been well characterized by various measurements, including X-ray crystallography analysis.[15,36,37] Thus, identification of the related modified nucleosides was straightforward. The ^{1}H NMR in D_2O indicated the presence of an intact 2-deoxyribose moiety as well as two singlet resonance at 5.5 and 5.2 ppm, which may be assigned to H_4 and H_5, respectively. The weak coupling between H_4 and H_5 (0.25 Hz) implies that these protons are diequatorial, and thus the C_4 and C_5 hydroxyl substituents must be diaxial. The conformation of the imidazolidine ring may be described as a half-chair in which C_5 is puckered with respect to a plane formed by $N_1-C_2-N_3-C_4$ atoms of the imidazolidine ring.[36] It was not possible to determine the absolute configuration of these diastereomers on the basis of ^{1}H NMR analyses since the coupling constants of sugar protons were similar for both diastereomers (in contrast, the stereochemistry of C_6 for 5,6-saturated pyrimidine derivatives leads to significant changes in the conformation of the sugar moiety). Last, **14** was observed to transform into **15** over a period of several days in D_2O. For this to occur, **14** must undergo *two* ring−chain tautomerism cycles with epimerization at C_4 *and* C_5 positions of the imidazolidine ring.

Chart 2. Possible Stuctures of Aminocarbonyl[2-(2-deoxy-β-D-*erythro*-pentofuranosyl)amino]-2-oxomethylcarbamic Acid (**16**)



Structural elucidation of product **16** required extensive spectroscopic measurements. The ^{1}H NMR spectrum in D_2O revealed an isolated methylene resonance in midrange field ($\delta = 6$ ppm) as well as the characteristic protons of an intact 2-deoxyribose moiety. The spectrum in DMSO displayed two nonexchangeable protons at low field which may be assigned to the terminal NH_2 group (2H, singlet, 8.4 ppm) and the central NH group (1H, doublet, 7.2 ppm). The NH proton signal and its coupling to the anomeric $C_{1'}$ proton confirmed the presence of an open-ring structure. The ^{13}C NMR spectrum displayed nine signals: one secondary carbon signal (64.4 ppm), three quartenary carbon signals (142.7, 154.8, and 171.1 ppm), and five signals which may be assigned to an unmodified 2-deoxyribose moiety (39.4 ($C_{2'}$), 62.9 ($C_{5'}$), 72.3 ($C_{3'}$), 81.0 ($C_{1'}$), and 87.0 ($C_{4'}$)). Product **16** was subjected to various mass spectrometry analyses. Although the compound was not directly suitable for analysis by electron impact ionization, the derivative with two acetyl groups on the 2-deoxyribose moiety (plus 84 *m*/*z*) gave a molecular ion peak at 362 *m*/*z* (MH^+) and at 360 *m*/*z* (M−H) in the positive and negative modes, respectively. In addition, FAB-MS in the positive mode depicted a molecular ion at 278 *m*/*z* (MH^+, 10%) accompanied by characteristic satellite peaks at 300 *m*/*z* (MH^+ + Na, 20%) and 370 *m*/*z* (MH^+ + glycerol, 20%), and DCI-MS analyses showed a molecular ion at 278 *m*/*z* (MH^+, 20%) with the satellite peak ($M + NH_4^+$).

The NMR and MS analyses of product **16** were consistent with two structures (Chart 2). At first, we were reluctant to assign structure A because it comprises a carbamic acid group which is not usually stable. For example, the lifetime of *N*-carboxyimidazolidone is approximately 2 min in neutral solution at 25 °C.[38] In contrast, we did not observe any decomposition of product **16** in aqueous solutions throughout the entire study. Thus, we looked for alternatives (structure B).

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(32) Eaton, M. A.; Hutchinson, D. W. *Biochim. Biophys. Acta* **1973**, *319*, 281−287.
(33) Cadet, J.; Nardin, R.; Voituriez, L.; Remin, M.; Hruska, F. E. *Can. J. Chem.* **1981**, *59*, 3313−3318.
(34) Mariaggi, N.; Téoule, R.; Cadet, J.; Dickie, H.; Hughes, E. *Radiat. Res.* **1979**, *79*, 431−438.
(35) Cadet, J.; Taieb, C.; Remin, M.; Niemczura, W. P.; Hruska, F. E. *Biochim. Biophys. Acta* **1980**, *608*, 435.
(36) Hahn, B. S.; Wang, S. Y.; Flippen, J. L.; Karle, I. L. *J. Am. Chem. Soc.* **1973**, *95*, 2711−2712.
(37) Leonard, N. L.; Wiemer, D. F. *J. Am. Chem. Soc.* **1976**, *98*, 8218−8221.
(38) Caplow, M.; Yager, M. *J. Am. Chem. Soc.* **1967**, *89*, 4513.

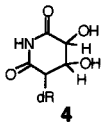
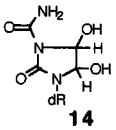
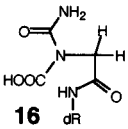
However, it should be noted that structure B was not completely compatible with the ^{13}C NMR analyses, since the chemical shifts of the carbonyl signals for the ureid residues were distinctly different (142.7 and 171.1 ppm). The assignment of the carboxylic acid group at 154.8 ppm is consistent with shielding of this group within carbamic acid derivatives. Nevertheless, ongoing studies of an analogous product obtained from the •OH-mediated decomposition of 5-methyl-2′-deoxycytidine support the carbamic acid structure.[39] The structure of the corresponding methyl-substituted derivative has been proven by $^{1}H-^{13}C$ hetereonuclear NMR analyses, which indicated long-range coupling between the downfield carbonyl group of CONH and the $CHCH_3$ protons of this derivative. Thus, product **16** is likely a carbamic acid derivative, as depicted in structure A. The stability of this carbamic acid derivative is unprecedented but may be a result of strong intramolecular H-bonding with the terminal amide group.

Novel Cyclic Products. A group of novel 2′-deoxycytidine products was discovered, including two diastereomers of 5′,6-cyclo-5-hydroxy-5,6-dihydro-2′-deoxyuridine (**17**, **18**). These products were characterized by extensive MS and ^{1}H NMR analyses. The salient features of the ^{1}H NMR spectra were the lack of detectable coupling between $H_{1'}$ and $H_{2'}$ and between $H_{3'}$ and $H_{4'}$, implying strong structural constraints within the deoxyribose moiety. The stereochemistry of both diastereomers was determined by comparison of the coupling constants with those of similar products, i.e., 5′,6-cyclo-5,6-dihydrothymidine.[40] Thus, the values of $J_{4',5'}$ (3.2 Hz) and $J_{5',6}$ (2.7 Hz) for **17** were consistent with the 5′*S*,6*S*,5*S* configuration and those of $J_{4',5'}$ (3.2 Hz) and $J_{5',6}$ (2.7 Hz) for **18** with the 5′*R*,6*R*,5*R* configuration. It should be noted that only two of the eight possible diastereomers were detected.

$^{18}O_2$-Labeling Experiments. The OH-induced decomposition of dCyd (**1**) was carried out in $^{18}O_2$-saturated solutions in order to probe the reaction mechanisms. To study the incorporation of $^{18}O_2$, the products were purified by HPLC and then analyzed by DCI-MS. This method gave intense molecular ions accompanied with characteristic satellite peaks occurring at M + 17 *m*/*z*. The results are summarized in Table 2.

γ-irradiation of dCyd (**1**) in $^{18}O_2$-saturated solutions gave unlabeled and singly labeled *trans*-(5*S*,6*S*)-2′-deoxyuridine 5,6-glycol (**4**) in equal amounts (45%), together with a minor amount of doubly labeled molecules. The presence of unlabeled product may be attributed to the exchange of ^{18}OH with H_2O at C_6 by way of ring−chain tautomerism. The percentage of label in the primary MS fragment of 5,6-glycols ($MH^+ - H_2O$ or $H_2{}^{18}O$) allows one to distinguish the position of label (C_5 or C_6) since dehydration takes place with loss of the OH group at C_6 (β-elimination). Thereby, the percentage of label incorporated at C_5 and C_6 in product **5** may be estimated to be about 35% (C_5) and 65% (C_6). The analysis of label in the other 5,6-gylcols of 2′-deoxyuridine gave similar results. In addition, this was consistent with the percentage of unlabeled (70%) and singly labeled (30%) molecules of 5-hydroxy-2′-deoxycytidine (**6**), which is formed by dehydration of intermediate 5,6-glycols of dCyd. Finally, the proportion of unlabeled and singly labeled molecules was 40% and 60% for 2-deoxy-β-D-*erythro*-pentofuranosyl)formamide (**9**). This suggests that most of the label (60%) was incorporated at C_6 since the carbonyl group of the formamide product is derived from C_6 rather than C_2 of pyrimidine, as inferred by studies with radioactive [$^{14}C_2$]-thymidine. Taken together, the results indicate that $^{18}O_2$ was incorporated at C_5 and C_6 of products **2**−**9** with a percentage of 30%−40% and 60%−70%, respectively.

Table 2. Incorporation of $^{18}O_2$ into Stable Radiation-Induced Products of dCyd (**1**)

	MH^+	#^{18}O	%MH^+	% -H_2O
 **4**	263	0	45	65
	265	1	45	35
	267	2	10	0
 **9**	162	0	40	----
	164	1	60	----
 **14**	278	0	10	15
	280	1	15	65
	282	2	65	25
	290	3	10	0
 **16**	278	0	0	----
	280	1	50	----
	282	2	50	----

When dCyd (**1**) was exposed to γ-irradiation in $^{18}O_2$-saturated solutions, the majority of trans N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1-carbamoyl-2-oxo-4,5-dihydroxyimidazolidine (**14**) was doubly labeled (65%). In addition, a small fraction of other molecular ions contained no label (10%), one label (15%), and two labels (10%). The presence of singly labeled molecules may be explained by exchange of ^{18}OH with water by a process involving ring−chain tautomerism. Thus, the initial yield of doubly labeled molecules may be assumed to contain both singly and doubly labeled molecules for a total of 80% (15% + 65%). In addition, the distribution of label within the imidazolidine ring (C_4 and C_5) may be estimated upon examination of the secondary ions resulting from dehydration of the molecular ions. Upon dehydration, the percentage of doubly labeled molecules decreased from 65% to 15% (25% less the percentage of triply labeled molecules (10%)), while the percentage of singly labeled molecules increased from 10% (15% less the percentage of singly labeled molecules (5%)) to 65%. These findings indicate that the majority of label was eliminated upon dehydration. The C_5-OH group is most likely to undergo dehydration since it sticks out of the plane of the imidazolidine ring. Thus, the majority of label resides at the C_5 position of product **14**.

Discussion

The first steps of the •OH-mediated decomposition of dCyd are similar to those for the decomposition thymidine and related pyrimidines. Hydroxyl radicals predominantly react with the 5,6-double bond of the base moiety of pyrimidines, giving neutral OH adduct radicals (Scheme 1). On the basis of pulse radiolysis studies, the addition of •OH to the base moiety of dCyd leads predominantly to reducing 5-hydroxyl radical adducts (**I**). In view of the rapid rate of reaction of oxygen with pyrimidine OH radical adducts,[41] the latter species (**I**) probably quantitatively react with O_2 to give 5-hydroxy-6-hydroperoxyl radicals (**II**). Thus, the formation of stable modifications of the base moiety of dCyd can largely be attributed to subsequent reactions of intermediate 5-hydroxy-6-hydroperoxyl radicals (Scheme 1).

(39) Bienvenu, C. Oxidations Radicalaires de la 5-methyl-2′-désoxycytidine. Thèse de l'Université Joseph Fourier, Grenoble, France, 1996.

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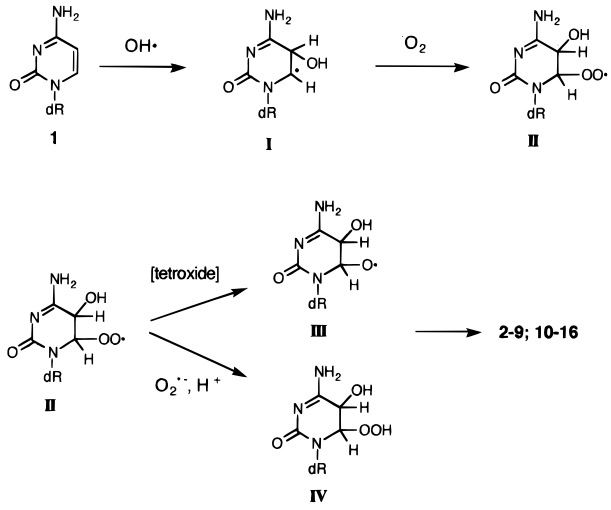
Scheme 1. Initial Steps of •OH-Mediated Decomposition of dCyd (**1**) in Aerated Aqueous Solutions



Initial addition of •OH at C_6 and the formation of 6-hydroxy-5-hydroperoxyl radicals may also contribute to products (not shown).

In general, peroxyl radicals have a relatively long life. They decay by way of a bimolecular reaction involving the formation of transient tetraoxides which rapidly decompose into stable products. Alternatively, peroxyl radicals may be susceptible to reduction by superoxide ion radicals or other reducing agents giving diamagnetic hydroperoxides.[42] The two pathways appear to be of equal importance for the •OH-mediated decomposition of thymidine in aerated aqueous solutions since the initial yield of hydroperoxides is approximately equal to that of stable nonperoxide products.[43] In contrast, γ-irradiation of dCyd in aerated aqueous solutions does not give detectable levels of unstable dCyd hydroperoxides.[44] Thus, it is not possible to make a clear-cut division between tetraoxide- and hydroperoxide-yielding pathways.

The bimolecular decay of peroxyl radicals results in transient tetraoxides which can decompose either by a concerted (Russell) pathway into diamagnetic alcohol and ketone fragments or by cleavage into oxyl radicals (**III**). In the case of dCyd, a concerted pathway of decomposition would result in equal amounts of 5,6-glycol and dialuric acid nucleoside products. However, neither the nucleoside of dialuric acid nor the product resulting from its hydrolysis were observed in product analysis, suggesting that this pathway is negligible. On the other hand, the decomposition of transient tetraoxides by way of oxyl radicals may be an important pathway in the formation of products **2**−**9**. The formation of the 5,6-glycol products (**2**−**5**; 28%) from oxyl radicals can be explained by H atom or electron abstraction. In addition, the most likely pathway to account for the formation of N_1-(2-deoxy-β-D-*erythro*-pentofuranosy)formamide (**9**; 12%) involves β-cleavage of oxyl radicals, giving rise to an open-ring dicarbonyl intermediate which then undergoes fragmentation between N_1 and C_2. Last, it has been proposed that the latter ureid intermediate may recyclize to yield ring contraction products, i.e., 5-hydroxyhydantoin (**7**, **8**; 3%).[16]

In addition to the tetraoxide pathway, the formation of products **2**−**9** may involve intermediate 5(6)-hydroxy-6(5)-hydroperoxides. It should be noted that hydroperoxides are very sensitive to the presence of trace metal ions in the solution. For example, the thermal decomposition of thymidine hydroperoxides results in the formation of 5,6-glycols even in quintuply distilled water.[17] The most sensitive thymidine hydroperoxides were the trans diastereomers of 5-hydroxy-6-hydroperoxides, which decomposed into 5,6-glycols with yields in the range of 30%−40%. Similarly, metal-catalyzed reduction of the 5(6)-hydroxy-6(5)-hydroperoxides of dCyd would result in 5,6-glycol products (**2**−**5**; 28%). This can occur by reduction of the hydroperoxides of dCyd into the corresponding 5,6-glycols, followed by their deamination. However, since the 5,6-glycols of dCyd preferentially undergo dehydration as opposed to deamination in aqueous solutions,[45] it is reasonable to propose an alternative pathway. This would involve initial deamination of the 5(6)-hydroxy-6(5)-hydroperoxides of dCyd, giving the 5(6)-hydroxy-6(5)-hydroperoxides of 2′-deoxyuridine, followed by reduction into 5,6-glycols (**2**−**5**). The formation of 5-hydroxy-2′-deoxycytidine (**6**; 12%) can be explained either by loss of superoxide radical ion from intermediate 5-hydroxy-6-hydroperoxyl radicals or by loss of H_2O_2 from 5-hydroxy-6-hydroperoxides. In addition, product **6** can arise from dehydration of labile dCyd 5,6-glycols in aqueous solution. Likewise, the nucleosides of 5-hydroxyhydantoin (**7** and **8**; 3%) can be explained by decomposition of 6-hydroxy-5-hydroxperoxides of 2′-deoxyuridine, whose yield is minor in the •OH-mediated oxidation of dCyd. As for N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)formamide (**9**), this is a minor product (trace amounts) in the thermal decomposition of thymidine hydroperoxides. In conclusion, dCyd oxidation products, which are analogous to thymidine oxidation products, may arise from the decomposition of either transient tetraoxides or labile 5(6)-hydroxy-6(5)-hydroperoxides.

The extent of incorporation of ^{18}O into products **2**−**9** was 35% at C_5 and 65% at C_6 (Table 2). This suggests that the initial addition of •OH radicals to the 5,6-double bond takes place with a percentage of 65% at C_5. In contrast, pulse radiolysis studies indicate that the addition of •OH to cytosine derivatives takes place at C_5 with a strong bias (87%).[46] Thus, the incorporation of $^{18}O_2$ at C_5 in products **2**−**9** is greater (about 20%) than expected from the distribution of C_5−OH and C_6−OH radicals indicated by pulse radiolysis studies. This suggests that the formation of products **2**−**9** from initial C_5−OH and C_6−OH radicals is not the same. Since both of these radicals likely convert quantitatively into the corresponding peroxyl radicals in oxygenated solutions, the difference in the formation of products **2**−**9** can be attributed to the chemistry of 5-hydroxy-6-hydroperoxyl radicals (**II**) and 6-hydroxy-5-hydroperoxyl radicals. In particular, if we assume that half of the 5-hydroxy-6-hydroperoxyl radicals (**II**) are diverted to the endoperoxide pathway (Scheme 2), the percentage of $^{18}O_2$ at C_5 of products **2**−**9** would be compatible with pulse radiolysis studies.

The formation of products **10**−**16** constitutes an unique pathway of •OH-mediated decomposition of pyrimidine nucleosides which does not occur for thymidine derivatives (Scheme 2). The primary sequence of steps to these products likely involves rearrangement of the hydroperoxide to an endoperoxide, followed by C_4−C_5 cleavage into an intermediate α-hydroxyketone (**IV** to **V** to **VI**). The effect of pH on the formation of *trans*-1-carbamoyl-2-oxo-4,5-dihydroxyimidazolidine (**14** or

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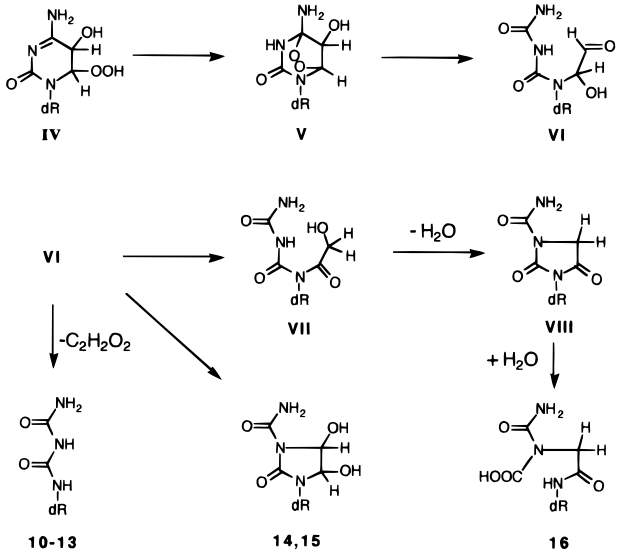
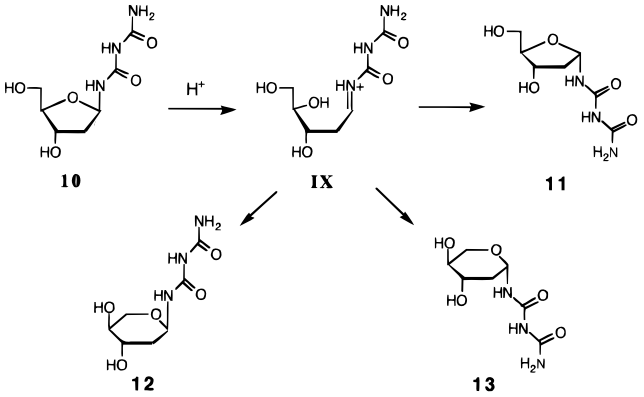
Scheme 2. Proposed Mechanism of Formation of Products **10**−**16**



Scheme 3. Isomerization of N_1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)biuret in Aqueous Solution



15 without the sugar moiety) and N_1-glycolylbiuret (**VII** without the sugar) from the •OH-mediated decomposition of cytosine indicates that both products arise from the same intermediate, namely an α-hydroxyketone (**VI** without the sugar).[47] In contrast, the nucleoside derivative of N_1-glycolylbiuret was not detected in the product mixture, suggesting that it is not stable in aqueous solution. The formation of **10**−**16** is explained by subsequent reactions of an intermediate, α-hydroxyketone (**VI**). The formation of **10**−**13** likely involves initial loss of glyoxal (CHO−CHO) from **VI**. The presence of four isomers implies that the β-furanoid isomerizes into the α-furanoid form and that the furanose derivatives also isomerize into the pyranose derivatives (Scheme 3). The acyclic structure of biuret renders the molecule susceptible to protonation at N_1, leading to isomerization of the sugar moiety and/or anomerization. Furthermore, it should be noted that trace quantities of 2-deoxy-D-*erythro*-pentose and biuret were observed in the reaction mixture. This is strongly indicative of hydrolysis of the nucleoside presumably via a Schiff base intermediate (**IX**). The formation of N_1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1-carbamoyl-2-oxo-4,5-dihydroxyimidazolidine (**14**, **15**) can be explained by cyclization of the intermediate α-hydroxyketone (**VI**). This is supported by $^{18}O_2$-labeling experiments which show the incorporation of two atoms of ^{18}O (Table 2). Finally,

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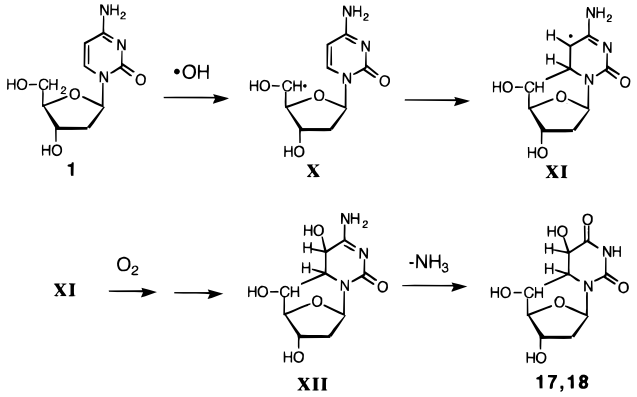
Scheme 4. Proposed Mechanism of Formation of 5′,6-Cyclic Products (**17**, **18**)



rearrangement of the intermediate α-hydroxyketone followed by cyclization and hydrolysis (**VI** to **VII** to **VIII**) explains the formation of aminocarbonyl[2-(2-deoxy-β-D-*erythro*-pentofuranosyl)amino]-2-oxomethylcarbamic acid (**16**). In agreement with this pathway, $^{18}O_2$-labeling experiments indicated the incorporation of two atoms of ^{18}O (Table 2). The presence of singly labeled molecules implies that the hydroxyl group at C_6 of structure **VI** may exchange with bulk water.

Finally, the mechanism for the formation of the cyclic products (**17**, **18**) probably involves initial abstraction of $C_{5'}$−H by •OH radicals, followed by intramolecular addition of the $C_{5'}$-centered radical (**X**) to the C_6 position of the cytosine moiety (Scheme 4). The 5′,6-cyclo base sugar C_5 radicals of cytidine and related nucleosides have previously been characterized by trapping with 2-methyl-2-nitrosopropane and electron spin resonance studies on the X irradiation of the substrates in N_2O-saturated aqueous solutions.[48] The formation of products **17** and **18** from intermediate **XI** requires several steps (**XI** to **XII**). It is likely that oxygen is incorporated at C_5 to give an intermediate peroxyl radical, which in turn converts into intermediate **XII**. In contrast, the $C_{5'}$ radical of dCyd leads to the formation of 5′,6-cyclo-5,6-dihydro-2′-deoxycytidine in frozen aqueous solutions in which competing reactions with oxygen cannot occur. Thus, the identification of products **17** and **18** from γ-irradiation of dCyd in aerated aqueous solutions suggests that intramolecular addition of the $C_{5'}$ radical of the sugar moiety to the C_6 position of the base moiety competes well with the fortuitous addition of oxygen to the $C_{5'}$ radical.

Oxidation of dCyd in DNA. The decay of intermediate 5(6)-hydroxy-6(5)-hydroperoxyl radicals by a bimolecular route will be largely suppressed in DNA, giving way to the formation of hydroperoxides. In fact, the initial yield of DNA hydroperoxides appears to be about 50% of the total base damage immediately following γ-irradiation in aqueous aerated solutions.[49] Of these hydroperoxides, it is estimated that one-third arises from dCyd and two-thirds from thymidine in DNA. Therefore, one can expect that a good part of the stable products of dCyd in DNA derive from initial hydroperoxides. A number of stable products of either cytosine or 2′-deoxycytidine have been detected from DNA exposed to ionizing radiation in aerated aqueous solutions. The radiochemical yields of 5-hydroxy-2′-deoxycytidine, 5-hydroxy-2′-deoxycytidine, and the 5,6-glycols of 2′-deoxyuridine in γ-irradiated DNA under aerated conditions are 2.1×10^{-9},

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2.5×10^{-9}, and 3.6×10^{-9} J mol^{-1}, respectively, as determined by either enzymatic digestion of DNA and HPLC coupled to electrochemical detection or acid hydrolysis of DNA followed by purification of the hydrosylate and then gas chromatography/mass spectrometry analysis with isotopic dilution.[6,50] In addition, the quantitation of some other cytosine modifications in γ-irradiated DNA has also been reported, including 5-hydroxyhydantoin (6.0×10^{-9} J mol^{-1}) and isodialuric acid.[51,52] Together, the yield of known cytosine products in DNA accounts for only about 25% of the total damage to this base in DNA. This suggests that the C_3-C_4 cleavage pathway may be important in DNA. However, *trans*-1-carbamoyl-2-oxo-4,5-dihydroxyimidazolidine is hardly formed in DNA exposed to ionizing radiation in aerated aqueous solutions (<20-fold lower than the formation of 5-hydroxyhydantoin). The analysis of •-OH-mediated products of dCyd in DNA should be a priority in future studies.

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