benzene at the low thione concentrations employed, excited-state deactivation by irreversible hydrogen abstraction would appear not to be dominant despite the possibility of efficient solvent cage recombination.

On the assumption that (1) photodissociation, atom abstraction, and exciplex formation do not occur in perfluoroalkane solvents[10] and (2) internal conversion to S_1 is the major S_2 radiationless decay process, k_{nr} of **1**, **2**, and **4** may be correlated with the S_2–S_1 electronic energy difference (Table I) according to the "energy-gap law".[14] A plot of ln k_{nr} vs. $(E_{S_2} - E_{S_1})$ for the three thiones is linear if data taken in perfluoroalkane solvents are used. This supports the proposals that S_2 decays primarily by internal conversion and that perfluoroalkane solvents are sufficiently chemically inert to permit the characterization of the photophysical properties of these excited thiones in fluid solutions at room temperature.

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Radiation-Induced Reduction of Thymidine in Aqueous Solution: Isolation and Characterization of a Novel Dimeric Product

Sei-ichi Nishimoto, Hiroshi Ide, Kikumi Nakamichi, and Tsutomu Kagiya*

Department of Hydrocarbon Chemistry
Faculty of Engineering, Kyoto University
Kyoto 606, Japan
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The radiation chemistry of DNA constituents has been a subject for intensive studies, because the DNA is considered to be one of the most vital targets of ionizing radiation that leads to lethal damage of bacterial and mammalian cells.[1,2] It is suggested by a number of workers that radiolyses of pyrimidine bases and related nucleosides in aqueous solution under deaerated conditions give the possible dimeric products[3-7] as well as those from the C5–C6 double-bond saturation with hydrogen and/or hydroxyl groups.[2,5,8] Although chemistries of the hydrogenation and hydroxylation were well established, only a few attempts[3] have been made to characterize the dimeric products, which appears to be of not only radiation chemical but also biological interest. We report here the successful characterization of a novel dimeric product of 5,5′-bi-5,6-dihydrothymidine (**2**) produced on γ-irradiation of a deaerated aqueous solution of thymidine (**1**) in the presence of formate. The reported dimer **2** is apparently distinct from typical cyclobutane-type dimers produced in the photochemical reaction of pyrimidine derivatives.[9]

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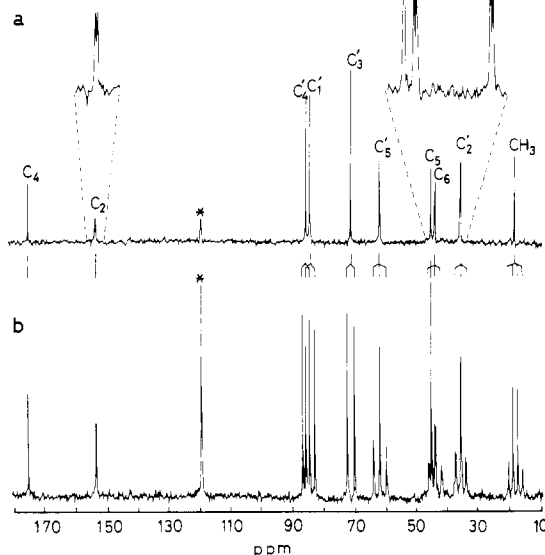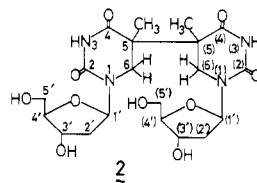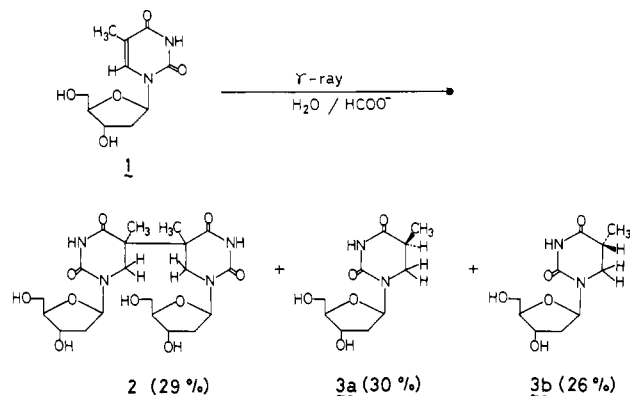

Figure 1. Observed critical temperatures plotted against values calculated through eq 5.

Scheme I

Typically, a solution of **1** (1 mM) in 3 mL of triply distilled water containing sodium formate (0.1 M) was buffered at pH 7.0 ± 0.1 with sodium phosphate (2 mM) and deaerated (<3 × 10⁻³ mmHg). Irradiation was performed at room temperature with a ⁶⁰Co γ-ray source (0.38 kGy h⁻¹).

Conversion of **1** increased linearly with irradiation dose up to 1.52 kGy, from which the *G* value[10] for the conversion was derived as *G*(−1) = 2.97. An HPLC analysis (monitored by UV absorption at 210 nm) showed that the dimer **2** (relative retention volume:[11] *k′* = 3.79) and hydrogenated products[12] of 5(*S*)-(−)-5,6-dihydrothymidine (**3a**, *k′* = 1.34) and 5(*R*)-(+)-5,6-dihydrothymidine (**3b**, *k′* = 1.61) are among the major products. The hydrogenated products **3a,b** are analogous to 5,6-dihydrothymine as a major product in the radiolytic reduction of thy-

(10) The number of molecules produced or changed per 100 eV of energy absorbed by the reaction system.

(11) The relative retention volume is defined by $k' = (V - V_0)/V_0$ where *V* is the retention volume at a given peak and V_0 is the void volume of the system. The analysis was carried out on an ODS-type column and 90% water/10% methanol was delivered at a flow rate of 0.6 mL min⁻¹. Under these conditions **1** was eluted with *k′* = 2.87.

(12) **3a** and **3b** were confirmed by comparison of their retention volumes with those of authentic samples (see also ref 7, 14).

Scheme II
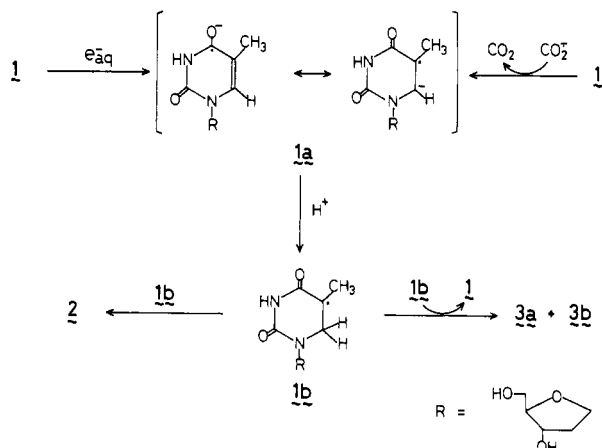
mine.[13] The formation of **3a,b** has been also observed in the radiolysis of **1** in the presence of alcohols.[7] Using HPLC data for a series of irradiated samples by reference to isolated **2** and authentic samples[7,14] of **3a,b**, the *G* values of these major products were evaluated as $G(2) = 0.87$ based on monomer unit (29% yield based on consumed **1**), $G(3a) = 0.89$ (30%), and $G(3b) = 0.76$ (26%) (Scheme I).

In a preparative work, a deaerated solution of **1** (10 mM) in 500 mL of triply distilled water containing sodium formate (0.2 M) (pH 7.0) was irradiated up to the quantitative conversion, condensed, and then chromatographed.[15] Evaporation of the fraction containing **2** and recrystallization from ethanol/petroleum ether gave a colorless crystal: mp 140–150 °C; IR (KBr) 3375, 1685 cm^{-1}. Anal. (C$_{20}$H$_{32}$N$_4$O$_{11}$, as monohydrate) C, H, N. The four hydroxyl groups of the sugar moieties (C3′, C(3′), C5′, and C(5′)) in **2** were trimethylsilylated[16] followed by measurement of mass spectrum: m/e 775 (M$^+$, C$_{32}$H$_{62}$N$_4$O$_{10}$Si$_4$ requires 775), 760 ((M – CH$_3$)$^+$).

The ^{13}C NMR (D$_2$O, 25 MHz) spectra[17] of **2** (Figure 1a,b) are consistent with the linkage of two monomer units, **1**, at their pyrimidine C5 positions. Thus, the ^{13}C chemical shifts assigned to the sugar moieties of **2** were substantially identical with those of **1** and other deoxyribonucleosides[18] and the hydrogenated products **3** as well.[19] In contrast, the pyrimidine C5 and C6 signals of **1** shifted to upfield by more than 65 ppm due to the dimerization to **2**, as was also the case for the hydrogenation to **3a,b**.[19] Moreover, the partially decoupled ^{13}C NMR spectrum (Figure 1b) shows that signals characteristic of the dimer pyrimidine C5,C(5) and C6,C(6) are singlet and triplet, respectively. It is also interesting to note the observation of ^{13}C NMR shielding differences, although small ($\Delta\delta_C$ 0.2 for all cases, see C2,C(2), C6,C(6), and C2,C(2′)).[17] Potentially, unhindered rotation around the linkage between asymmetric C5 and C(5) seems to account for such a magnetic nonequivalence.[20]

In accord with the structural evidence from ^{13}C NMR, the ^{1}H

NMR (D$_2$O, 300 MHz) spectrum[21] of **2** showed the absence of protons at C5 and C(5) positions, while **3b** gave the corresponding C5 proton signal at δ_H 2.820 (1 H, m, $J = 10.7, 6.0, 7.2$ Hz).

The formation of **2** and **3a,b** may be rationalized by the reaction pathway outlined in Scheme II. In the initial step, reducing species of hydrated electron (e$_{aq}^-$, $G = 2.7$) and carbon dioxide radical anion (CO$_2^-\cdot$, $G = 3.25$) are created via radiolysis of water in the presence of excess formate.[22] The key step is one-electron reduction of **1** by both e$_{aq}^-$ and CO$_2^-\cdot$ to the corresponding radical anion intermediate (**1a**), which subsequently undergoes protonation to produce hydrothymidin-5-yl radical (**1b**). Combination of two **1b** leads to **2**, while disproportionation to **3a** and **3b** is accompanied by regeneration of **1**. It is deduced from the *G* values of **2**, **3a**, and **3b** that the disproportionation is favored 3.8-fold over the combination on bimolecular encounter of **1b**.

Support for the CO$_2^-\cdot$-induced reduction of **1** was obtained by irradiation of an N$_2$O-saturated aqueous solution of **1** (1 mM) and sodium formate (0.1 M) at pH 7.0. Under these conditions only CO$_2^-\cdot$ ($G = 5.95$) could participate[22] in the decomposition of **1**, which occurred with efficiency ($G(-1) = 2.65$) comparable to the deaerated system. It is more remarkable that the observed product distribution, i.e., $G(2) = 0.75$ (28%), $G(3a) = 0.70$ (26%), and $G(3b) = 0.68$ (26%), is also almost identical with that of the deaerated system. Thus, comparison of both reaction systems indicates that CO$_2^-\cdot$ has the same reducing ability as e$_{aq}^-$ toward **1**.

In summary we have found that **1** undergoes reduction of the pyrimidine base moiety by e$_{aq}^-$ or CO$_2^-\cdot$ in aqueous solution to give the C5-linked dimer **2** along with **3a,b**.

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(21) The two dihydrothymine moieties: δ 1.406 and 1.431 (s, 5- and (5)-Me), 3.397 and 3.788 (d, $J = -13.8$ Hz, geminal 6- or (6)-H), 3.447 and 3.686 (d, $J = -12.9$ Hz, geminal (6)- or 6-H). Sugar moieties: δ 2.137–2.305 (4 H, 2′- and (2′)-H), 3.691–3.758 (4 H, 5′- and (5′)-H), 3.904 and 3.916 (2 H, 4′- and (4′)-H), 4.398 and 4.736 (2 H, 3′- and (3′)-H), 6.206 and 6.254 (2 H, 1′- and (1′)-H).
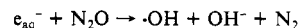
(22) The primary active species in the dilute aqueous solution system (pH 7.0) are derived from radiolysis of water as follows:

$$H_2O \rightsquigarrow \cdot OH \ (G = 2.7) + H\cdot \ (G = 0.55) + e_{aq}^- \ (G = 2.7)$$
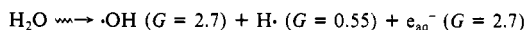
The so-formed ·OH and H· are converted efficiently to the CO$^-\cdot$ in the presence of excess formate.

$$\cdot OH(H\cdot) + HCO_2^- \rightarrow H_2O(H_2) + CO_2^-\cdot$$
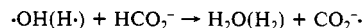
Since N$_2$O converts the e$_{aq}^-$ to ·OH,

$$e_{aq}^- + N_2O \rightarrow \cdot OH + OH^- + N_2$$

the presence of both HCO$_2^-$ and N$_2$O leads to generation of a single active species of CO$_2^-\cdot$.

Linear Solvation Energy Relationships. 26. Some Measures of Relative Self-Association of Alcohols and Water

Mortimer J. Kamlet,* Ruth Doherty, R. W. Taft,* and Michael H. Abraham*

Naval Surface Weapons Center, White Oak Laboratory
Silver Spring, Maryland 20910
Department of Chemistry, University of California
Irvine, California 92717
Department of Chemistry, University of Surrey
Guildford, Surrey GU2 5XH, United Kingdom

In two separate and completely unrelated investigations, we have generated information that allows estimates of bulk solvent properties that are related to the self-association energies of some aliphatic alcohols and water. We believe it to be of fundamental importance that these estimates are in quite good agreement with

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(15) The chromatography was carried out on a column of polystyrene resin (Mitsubishi Kasei HP20) by using methanol/water with linear methanol gradient from 0 to 10 vol %.

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(17) δ 18.2, 35.7, and 35.9 (C2′ and C(2′)), 44.3 and 44.5 (C6 and C(6)), 45.3, 62.1, 71.4, 84.5, 85.8, 153.8, and 154.0 (C2 and C(2)), 175.8. We thank B. Ohtani for the NMR measurements.

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(19) For example, **3b**: ^{13}C NMR (D$_2$O, 25 MHz) δ 12.9 (Me), 35.4 (C5), 36.1 (C2′), 42.7 (C6), 62.4 (C5′), 71.6 (C3′), 86.0 (C4′), 155.0 (C2), 177.4 (C4).

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