

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 solvents¹⁰ 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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(14) Engleman, R.; Jortner, J. *Mol. Phys.* **1970**, *18*, 145-164.

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

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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 products³⁻⁷ 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 attempts³ 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

(1) Altman, K. I., Gerber, G. B., Okada, S., Eds. "Radiation Biochemistry"; Academic Press: New York/London, 1970; Vol. I, Chapter 3.

(2) Hüttermann, J., Köhnlein, W., Teoule, R., Bertinchamps, A. J., Eds. "Effects of Ionizing Radiation on DNA. Physical, Chemical and Biological Aspects"; Springer-Verlag: Berlin/Heidelberg/New York, 1978.

(3) (a) Shragge, P. C.; Verghese, A. J.; Hunt, J. W.; Greenstock, C. L. *J. Chem. Soc., Chem. Commun.* **1974**, 736; (b) *Radiat. Res.* **1974**, *60*, 250.

(4) Ali, K. M.; Scholes, G. *J. Chem. Soc., Faraday Trans. 1* **1980**, *76*, 449.

(5) (a) Infante, G. A.; Jirathana, P.; Fendler, J. H.; Fendler, E. J. *J. Chem. Soc., Faraday Trans. 1* **1973**, *69*, 1585; (b) *Ibid.* **1974**, *70*, 1162.

(6) Cadet, J.; Teoule, R. *Int. J. Appl. Radiat. Isot.* **1971**, *22*, 273.

(7) Cadet, J.; Balland, A.; Berger, M. *Int. J. Radiat. Biol.* **1981**, *39*, 119.

(8) (a) Ekert, B. *Nature (London)* **1962**, *194*, 278. (b) Scholes, G. *Prog. Biophys. Mol. Biol.* **1963**, *13*, 59. (c) Khattak, M. N.; Green, J. H. *Int. J. Radiat. Biol.* **1966**, *11*, 131; (d) *Ibid.* **1966**, *11*, 577. (e) Wada, T.; Ide, H.; Nishimoto, S.; Kagiya, T. *Chem. Lett.* **1982**, 1041.

(9) Wang, S. Y., Ed. "Photochemistry and Photobiology of Nucleic Acids"; Academic Press: New York, 1976; Vol. 1, p 169.

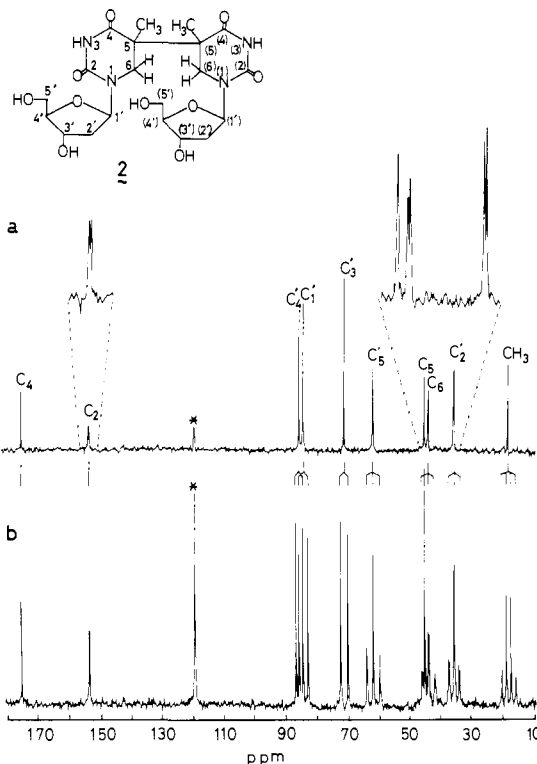
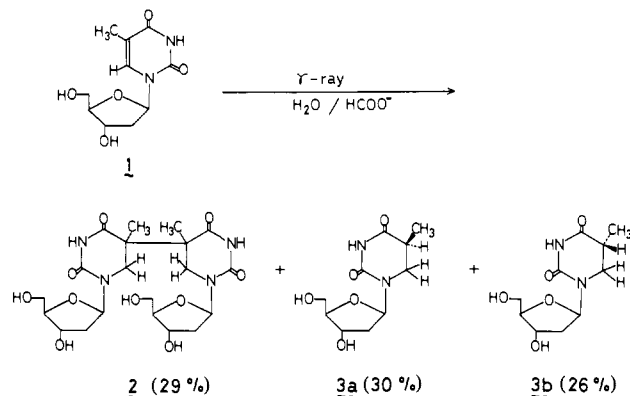


Figure 1. Observed critical temperatures plotted against values calculated through eq 5.

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 \pm 0.1 with sodium phosphate (2 mM) and deaerated ($<3 \times 10^{-3}$ mmHg). Irradiation was performed at room temperature with a ^{60}Co γ -ray source (0.38 kGy h⁻¹).

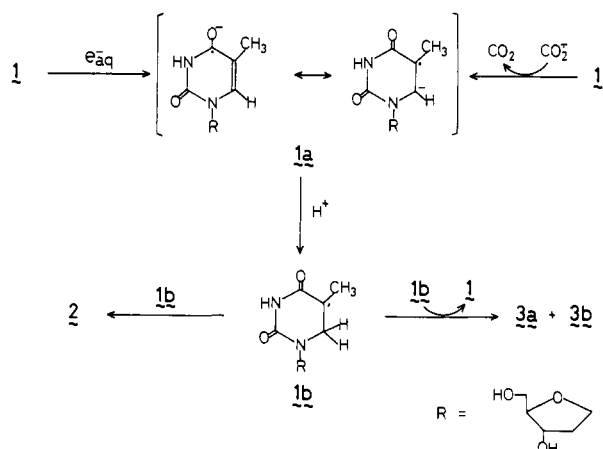
Conversion of **1** increased linearly with irradiation dose up to 1.52 kGy, from which the G value¹⁰ for the conversion was derived as $G(-1) = 2.97$. An HPLC analysis (monitored by UV absorption at 210 nm) showed that the dimer **2** (relative retention volume:¹¹ $k' = 3.79$) and hydrogenated products¹² 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. (C₂₀H₃₂N₄O₁₁, as monohydrate) C, H, N. The four hydroxyl groups of the sugar moieties (C3', C(3'), C5', and C(5')) in **2** were trimethylsilylated¹⁶ followed by measurement of mass spectrum: *m/e* 775 (M⁺, C₃₂H₆₂N₄O₁₀Si₄ requires 775), 760 ((M - CH₃)⁺).

The ¹³C NMR (D₂O, 25 MHz) spectra¹⁷ of **2** (Figure 1a,b) are consistent with the linkage of two monomer units, **1**, at their pyrimidine C5 positions. Thus, the ¹³C chemical shifts assigned to the sugar moieties of **2** were substantially identical with those of **1** and other deoxyribonucleosides¹⁸ 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 ¹³C 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 ¹³C 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 ¹³C NMR, the ¹H

NMR (D₂O, 300 MHz) spectrum²¹ 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).

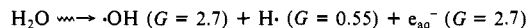
The formation of **2** and **3a,b** may be rationalized by the reaction pathway outlined in Scheme II. In the initial step, reducing species of hydrated electron (e_{aq}^- , *G* = 2.7) and carbon dioxide radical anion (CO₂⁻, *G* = 3.25) are created via radiolysis of water in the presence of excess formate.²² The key step is one-electron reduction of **1** by both e_{aq}^- and CO₂⁻ 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 CO₂⁻-induced reduction of **1** was obtained by irradiation of an N₂O-saturated aqueous solution of **1** (1 mM) and sodium formate (0.1 M) at pH 7.0. Under these conditions only CO₂⁻ (*G* = 5.95) could participate²² 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 CO₂⁻ has the same reducing ability as e_{aq}^- toward **1**.

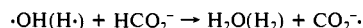
In summary we have found that **1** undergoes reduction of the pyrimidine base moiety by e_{aq}^- or CO₂⁻ in aqueous solution to give the C5-linked dimer **2** along with **3a,b**.

(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).

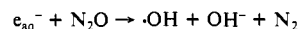
(22) The primary active species in the dilute aqueous solution system (pH 7.0) are derived from radiolysis of water as follows:



The so-formed $\cdot OH$ and $H \cdot$ are converted efficiently to the CO \cdot in the presence of excess formate.



Since N₂O converts the e_{aq}^- to $\cdot OH$,



the presence of both HCO₂⁻ and N₂O leads to generation of a single active species of CO₂⁻.

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

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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

(13) Wada, T.; Ide, H.; Nishimoto, S.; Kagiya, T. *Int. J. Radiat. Biol.* **1982**, *42*, 215.

(14) (a) Kondo, Y.; Witkop, B. *J. Am. Chem. Soc.* **1968**, *90*, 764. (b) Konert, J.; Karle, L.; Karle, J. *Acta Crystallogr., Sect B* **1970**, *B26*, 770.

(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 %.

(16) Butts, W. C. *J. Chromatogr. Sci.* **1970**, *8*, 474.

(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.

(18) (a) Jones, A. J.; Grant, D. M.; Winkley, M. W.; Robins, R. K. *J. Phys. Chem.* **1970**, *74*, 2684; (b) *J. Am. Chem. Soc.* **1970**, *92*, 4079.

(19) For example, 3b: ¹³C NMR (D₂O, 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).

(20) (a) van Gorkom, M.; Hall, G. E. *Quart. Rev.* **1968**, *22*, 14. (b) Kroschwitz, J. A.; Winokur, M.; Reich, H. J.; Roberts, J. D. *J. Am. Chem. Soc.* **1969**, *91*, 5927.