Oxidation of Nucleic Acid Related Compounds by the Peroxodisulfate Ion

Toshio ITAHARA,* Takashi YOSHITAKE, Sunao KOGA, and Akihiro NISHINO
Institute of Chemistry, College of Liberal Arts, Kagoshima University, Korimoto, Kagoshima 890
(Received March 8, 1994)

The treatment of nucleic acid bases, nucleosides, and nucleotides with peroxodisulfate ion in a phosphate buffer solution at pH 7.0 or water at 70—75 °C was investigated. The reaction of thymine and 5-methylcytosine nucleosides and nucleotides resulted in the oxidation of the 5-methyl groups. The oxidation products from 1,3-dimethyluracils and the time-course of the reaction of uracils led to two plausible reaction mechanisms for the oxidation of uracils.

DNA in γ-irradiated cells contains some oxidized nucleic acid bases, such as 5-hydroxymethyluracil[1] and thymine glycols,[2] instead of thymine. The 5-hydroxymethyluracil and thymine glycols in DNA are eliminated by 5-hydroxymethyluracil DNA glycosylase[3] and thymine glycol DNA glycosylase (endonuclease III),[4] respectively. It was reported that the amount of 5-hydroxymethyluracil in normal urine is ca. twice as much as that of thymine glycols.[5] The conversion of thymine into 5-hydroxymethyluracil is also performed by thymine 7-hydroxylase.[6] Furthermore, increasing interest is being shown to the role of the 5-methylcytosine residue in DNA,[7] and 5-hydroxymethylcytosine, which is formed from 5-methylcytosine in a reaction that is mechanistically similar to the formation of 5-hydroxymethyluracil, and is also eliminated by 5-hydroxymethylcytosine DNA glycosylase.[8] The oxidative damage of DNA by an active oxygen species and γ-radiation has been considered to be caused by the reaction of DNA with the hydroxyl radical.[9] However, although the reaction of thymine with the hydroxyl radical mainly results in an addition at the 5,6-double bond of thymine, hydrogen-abstraction from the methyl group by the hydroxyl radical occurs only to an extent of 10%.[10] This observation indicates that the formation of 5-hydroxymethyluracil in DNA may be caused by not only the hydrogen-abstract from the methyl group, but also another mechanism. On the other hand, the γ-radiation of DNA and its components as a solid state or in a frozen solution is known to result in the formation of those cation radicals.[11] Although the reaction of nucleic acid-related compounds by the peroxodisulfate ion has been investigated by several groups as a model for the oxidative damage of nucleic acids,[10,12] the great majority of work has dealt almost exclusively with spectroscopic studies, such as the detection of radicals by means of ESR spectroscopy, except for the reports by Moschel and Behrmann.[12a,12b] We also reported on the oxidation of thymines with sodium peroxodisulfate from the viewpoint of isolation of the products, i.e., the oxidation led to the formation of the corresponding 5-hydroxymethyluracils and 5-formyluracils.[13] In this paper, the oxidation of nucleic acid bases, nucleosides, nucleotides, and 1,3-dimethyluracils is described.[14]

Results and Discussion

Treatment of uracil (**1a**), thymine (**1b**), cytosine (**2a**), and adenine (**3**) with the peroxodisulfate ion in a phosphate buffer solution at pH 7.0 at 70 °C was investigated by HPLC analysis, even though the reaction of guanine was not studied because of its poor solubility. The reaction of **1b** predominantly gave 5-hydroxymethyluracil (**1c**) and 5-formyluracil (**1d**).[13] The oxidation of **1a** gave 5-hydroxyuracil (**1e**) together with a trace amount of parabanic acid (**4**), and that of **2a** gave a small amount of **1a** (Chart 1).

Similar treatments of nucleosides, such as uridine

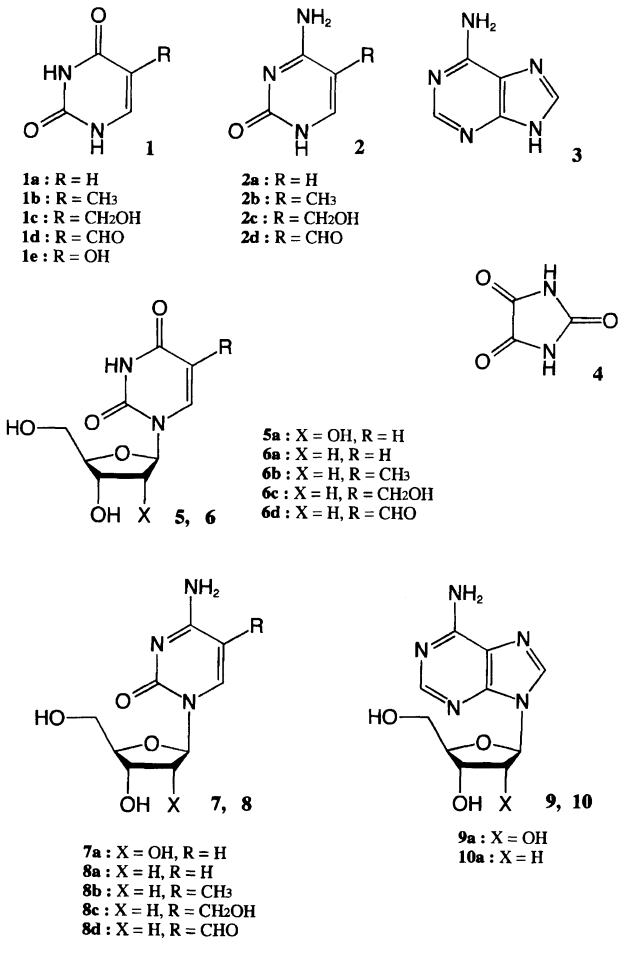


Chart 1.

Table 1. Oxidation of Nucleic Acid Bases, Nucleosides, and Diribonucleotides by Peroxodisulfate Ion[a)]

Substrate	Recovery and Product: Yield/%[c)]	Eluent[c)]	Standard[c)]
1a	**1a**/84; **1e**/4; **4**/trace	Water	**7a**
1b	**1b**/70; **1c**/20; **1d**/7	1% CH_3CN/water[d)]	**6a**
2a	**2a**/82; **1a**/1	Water	**1b**
3	**3**/97	3% CH_3CN/buffer[e)]	**2b**
5a	**5a**/73; **1a**/22	Water	**7a**
6a	**6a**/90; **1a**/8	Water	**7a**
6b	**6b**/71; **6c**/20; **6d**/5	5% CH_3CN/water	**9a**
7a	**7a**/74; **2a**/20	1% CH_3CN/water	**1b**
8a	**8a**/90; **2a**/4	Water	**7a**
9a	**9a**/84; **3**/7	3% CH_3CN/buffer	**2b**
10a	**10a**/96; **3**/trace	3% CH_3CN/buffer	**2b**
14	**14**/**34**;	5% CH_3CN/buffer	**9a**
	1a/50; **5a**/trace; **17**/15;	Buffer	**7a**
	18/23; **19**/1; **20**/1	Buffer	**7a**
14[b)]	**14**/38;	5% CH_3CN/buffer	**9a**
	1a/44; **5a**/trace; **17**/19;	Buffer	**7a**
	18/33; **19**/2; **20**/1	Buffer	**7a**
15	**15**/28;	5% CH_3CN/buffer	**9a**
	1a/48; **5a**/trace; **17**/8;	Buffer	**7a**
	18/25; **19**/43; **20**/4	Buffer	**7a**
16	**16**/80; **3**/4; **9a**/4;	8% CH_3CN/buffer	**f)**
	1a/11; **5a**/trace; **18**/4;	Buffer	**7a**
	20/1; **21**/12	Buffer	**7a**

a) Reaction conditions: In the case of nucleic acid bases and nucleosides: substrate (0.5 mmol), $Na_2S_2O_8$ (0.5 mmol), 0.05 mol dm^{-3} sodium phosphate buffer solution at pH 7.0 (100 ml), 70 °C, 4 h reaction. In the case of diribonucleotides: substrate (1 mg), $Na_2S_2O_8$ (equimolar), 0.05 mol dm^{-3} sodium phosphate buffer solution at pH 7.0 (2 ml), 70 °C, 4 h reaction. b) Potassium peroxodisulfate $K_2S_2O_8$ was used instead of $Na_2S_2O_8$. c) Yield was determined on the basis of HPLC analysis (TOSOH CCPE, flow rate: 1.0 ml min^{-1}) with TOSOH ODS-80 TM column (4.6 mmID×250 mm or 4.6 mmID×180 mm). In the case of nucleic acid bases and diribonucleotides, a Shimadzu SPD-6A UV spectrometric detector was used for the detector. In the case of nucleosides, a TOSOH RI 8012 differential referentive index detector was used. The area of an elution band in comparison with that of a standard is used for the quantitative purpose (Shimadzu C-R6a chromatopac). The standards and eluents for the analysis are shown in Table 1. d) distilled water. e) 0.05 mol dm^{-3} sodium phosphate buffer solution at pH 7.0. f) 5-Fluoro-1-(tetrahydro-2-furyl)uracil (Ftorafur).

(**5a**), 2′-deoxyuridine (**6a**), thymidine (**6b**), cytidine (**7a**), 2′-deoxycytidine (**8a**), adenosine (**9a**), 2′-deoxyadenosine (**10a**), were also investigated by HPLC analysis. These results are summarized in Table 1. When **6b** and 5-methyl-2′-deoxycytidine (**8b**) were treated with peroxodisulfate ion in water, the oxidation of the 5-methyl groups occurred, i.e., 5-hydroxymethyl-2′-deoxyuridine (**6c**) (30%), 5-formyl-2′-deoxyuridine (**6d**) (17%), 5-hydroxymethyl-2′-deoxycytidine (**8c**) (34%), and 5-formyl-2′-deoxycytidine (**8d**) (16%) were obtained. However, the reaction of the other nucleosides almost resulted in a release of the bases.

The oxidation of the 5-methyl groups of **6b** and **8b** was applicable to nucleotides. A solution of thymidine 5′-monophosphate (**11b**) and sodium peroxodisulfate in the phosphate buffer solution at pH 7.0 was heated at 75 °C for 4 h under an argon atmosphere. The reaction mixture was submitted to chromatography on an ODS column. Elution with a mixture of sodium phosphate buffer solution at pH 7.0 and acetonitrile led to the isolation of 5-hydroxymethyl-2′-deoxyuridine 5′-monophosphate (**11c**) (15%) and 5-formyl-2′-deoxyuridine 5′-monophosphate (**11d**) (10%) (Chart 2). The reaction of 5-methyl-2′-deoxycytidine 5′-monophosphate (**12b**) also gave 5-hydroxymethyl-2′-deoxycytidine 5′-monophosphate (**12c**) (12%) and 5-formyl-2′-deoxycytidine 5′-monophosphate (**12d**) (8%). Similar reactions of dinucleotides, such as thymidilyl-(3′→5′)-2′-deoxyadenosine (**13b**), with sodium peroxodisulfate in the buffer solution at 70 °C for 3 h gave 5-hydroxymethyl-2′-deoxyuridilyl(3′→5′)-2′-deoxyadenosine (**13c**) (12%) and 5-formyl-2′-deoxyuridilyl(3′→5′)-2′-deoxyadenosine (**13d**) (8%). Figure 1 shows a chromatogram for the isolation of **13c** and **13d**. The ^{1}H NMR spectra of **13c** and **13d** at 24 and 55 °C are shown in Fig. 2. The assignments shown in Fig. 2 were determined on the basis of their ^{1}H–^{1}H COSY NMR and the reported data for **13d**.[15)] In Fig. 2, there were differences of ca. 0.15 ppm between the chemical shifts of $U_{H-2'}$ at 24 °C and those at 55 °C, even though a similar shielding effect of the H-2′ protons of the oligonucleotides by a stacking interaction is known.[16)] Furthermore, the 5-methylene protons of the uracil residue in **13c** appeared as two doublets, as shown in Fig. 2(C),

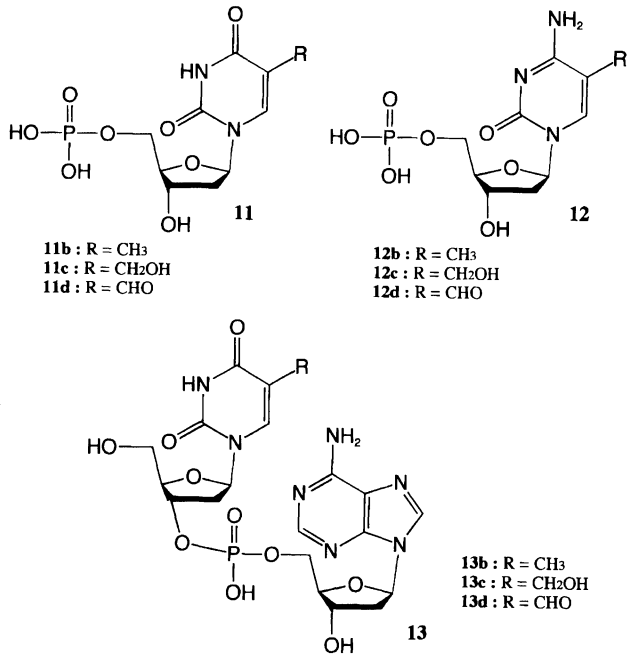


Chart 2.

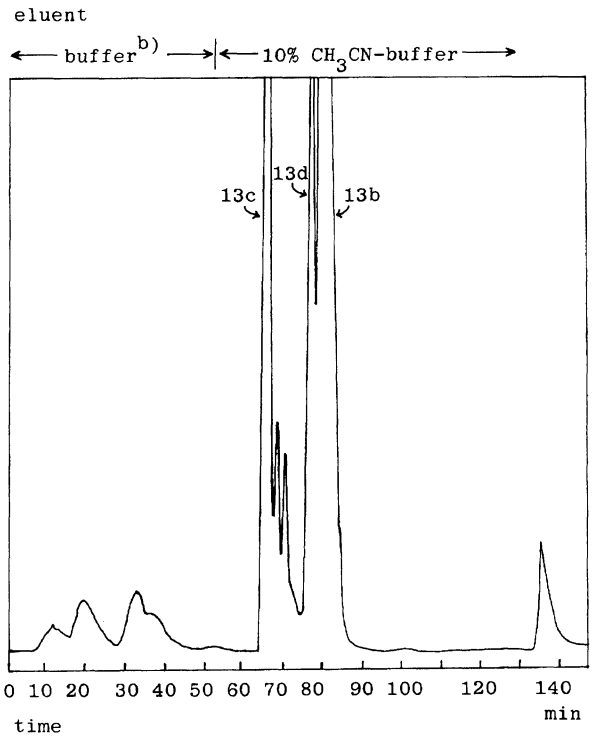


Fig. 1. Chromatogram for the separation of the reaction mixture of **13b** and sodium peroxodisulfate.[a)] a) Reaction conditions: **13b** (10 mg), $Na_2S_2O_8$ (5 mg), 0.2 mol dm^{-1} sodium phosphate buffer solution at pH 7.0 (25 ml), 70 °C, argon atmosphere, 3 h reaction. Separation conditions: flow rate; 3.0 ml min^{-1} chart speed; 0.1 cm min^{-1}. b) 0.05 mol dm^{-1} sodium phosphate buffer solution at pH 7.0.

while those of **1c**, **6c**, and **11c** exhibited singlet peaks.

The oxidation of diribonucleotides, such as uridilyl-(3′→5′)uridine (**14**), uridilyl(2′→5′)uridine (**15**), and uridilyl(3′→5′)adenosine (**16**), was also studied. A solution of the diribonucleotides and equimolar amounts of sodium peroxodisulfate in a buffer solution was heated at 70 °C for 4 h. The yields of the oxidation products, such as **1a**, **3**, **5a**, **9a**, uridine 5′-monophosphate (**17**), uridine 3′-monophosphate (**18**), uridine 2′-monophosphate (**19**), uridine 2′:3′-cyclic monophosphate (**20**), adenosine 5′-monophosphate (**21**), were determined on the basis of an HPLC analysis. The results are summarized in Table 1. In spite of the identification of many products from the HPLC analysis, the data reported in Table 1 indicate that the oxidation may give rise to a preferential decomposition of the sugar moiety at the uracil residue. Schulte-Frohlinde et al.[12i,12k] reported that a fast transfer of the radical site from the base to the sugar was observed on the reaction of $SO_4^{-\bullet}$ with nucleosides and nucleotides. On the basis of the ESR spectroscopic parameters of the sugar radical of poly(U), the hydrogen-abstraction by base radicals was reported to occur at the 2′-position of the sugar.[12i,12k] Our results may be compatible with their interpretation.

In order to elucidate the reaction mechanism, the products from the oxidation of 1,3-dimethyluracils, such as 1,3-dimethyluracil (**22a**), 1,3-dimethylthymine (**22b**),[13] 5-bromo-1,3-dimethyluracil (**22f**), 5-fluoro-1,3-dimethyluracil (**22g**), and 1,3,6-trimethyluracil (**23**) with sodium peroxodisulfate in water, were investigated (Chart 3). The results are shown in Table 2. When **22a** was allowed to react with $Na_2S_2O_8$, 5-hydroxy-1,3-dimethyluracil (**22e**), 1,3-dimethylparabanic acid (**24**), 5,5′-oxybis(1,3-dimethyluracil) (**25**), and 5,5′-dihydroxy-1,1′,3,3′-tetramethyl-[5,5′-bipyrimidine]-2,2′,4,4′,6,6′ (1*H*, 1′*H*, 3*H*, 3′*H*, 5*H*, 5′*H*)-hexone (amalic acid) (**26**) were isolated. Compound **24** was reported to be formed by a benzilic acid rearrangement of **26**.[17] While the oxidation of **22b** gave **22c**, **22d**, and **27**,[13] the reaction of **23** did not result in the oxidation of the 6-methyl group, and gave 1,6-dihydro-6-hydroxy-1,3,6-trimethyl-2,4,5(3*H*)-pyrimidinetrione (**29**). Furthermore, the reaction of **1f** interestingly led to the formation of 6,6′-bis(5-hydroxy-1,3-dimethyluracil) (**28**) as a main product, together with **24** and **26**, which were also isolated from the oxidation of **22a**, **22f**, and 5-hydroxy-1,3-dimethyluracil (**22e**).

Heating and photo-irradiation of peroxodisulfate ion $S_2O_8^{2-}$ are known to result in the formation of the sulfate anion radical $SO_4^{-\bullet}$, which abstracts an electron from aromatic compounds[18] and olefins[19] to yield the corresponding cation radicals. Since it is now well known that the oxidation of the alkyl side chains attached to aromatic compounds proceeds by cation radical intermediates,[18] the oxidation of the 5-methyl group of thymines and 5-methylcytosines may proceed via their cation radicals. However, the formation of **28** from **22g** is more reasonably explained in terms of the for-

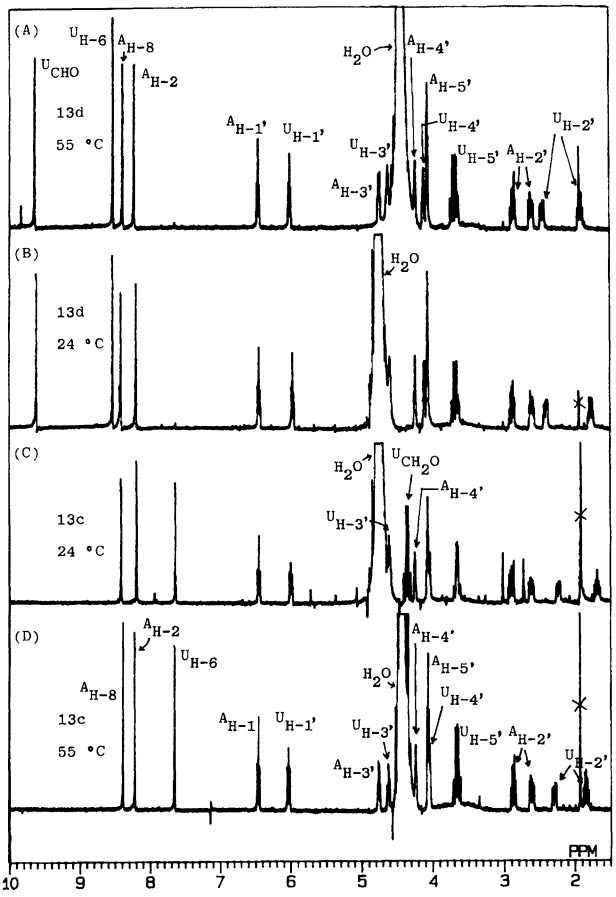


Fig. 2. ^{1}H NMR spectra of **13c** and **13d** in D_2O.

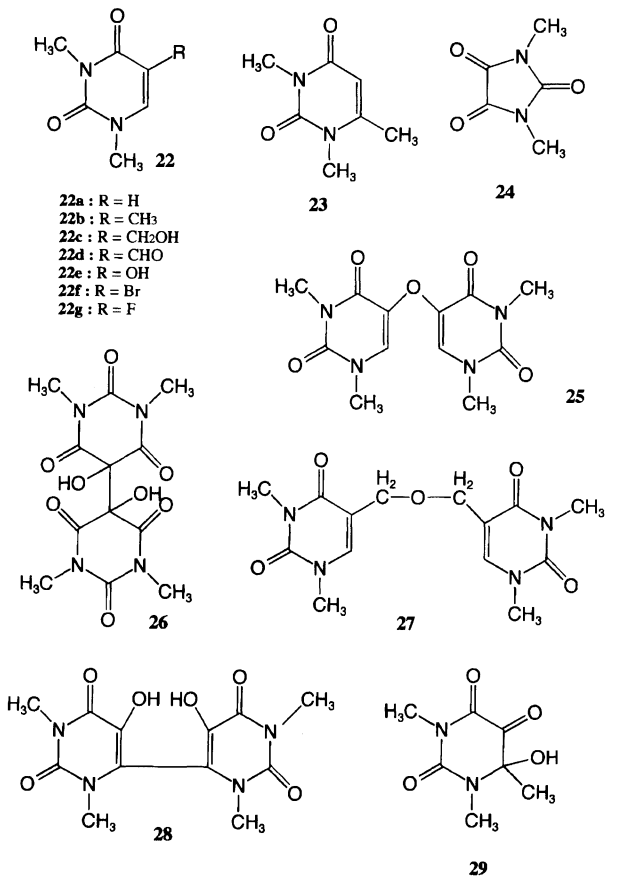


Chart 3.

Table 2. Oxidation of 1,3-Dimethyluracils by Sodium Peroxodisulfate in Water[a)]

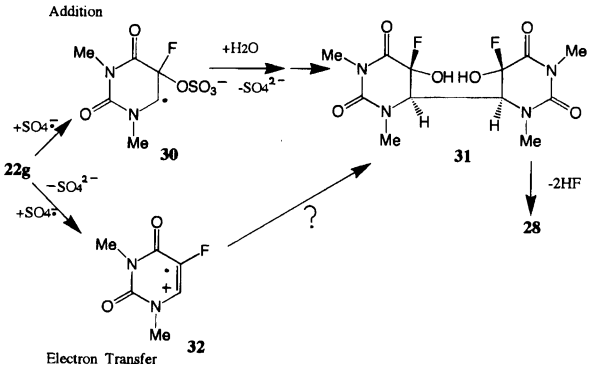
Substrate	Recovery and Products: Yield/%[b)]
22a	**22a**/53; **22e**/18; **24**/2; **25**/13; **26**/2
22b	**22b**/35; **22c**/30; **22d**/17; **27**/8
22e	**22e**/25; **24**/21; **26**/5
22f	**22f**/43; **24**/11; **26**/10
22g	**22g**/68; **24**/3; **26**/3; **28**/18
23	**23**/85; **29**/12

a) Reaction conditions: substrate (1 mmol), $Na_2S_2O_8$ (1 mmol), water (50 ml), 75 °C, 5 h, under argon atmosphere. b) Isolated yield based on substrate or $Na_2S_2O_8$ used.

mation of a radical (**30**) rather than a cation radical (**32**) (Scheme 1). The dimeric compounds similar to the intermediate (**31**) were obtained in the γ-radiation of thymine[20)] and cytosine.[21)]

In an effort to determine the intermediates on the oxidation of uracils, we further studied the time-course of the reaction in D_2O at 75 °C by means of NMR spectroscopy. The ^{1}H NMR spectra of the reaction mixture of **1a**, **22a**, **1b**, and **22b** in D_2O at 75 °C are shown in Fig. 3. The reaction of **22a** was reported to give uracil glycol (**34a**),[12f)] and the result in Fig. 3 (C,D) also suggests that the reaction of **1a** and **22a** first yielded uracil glycols (**33a**) and (**34a**), respectively, whose structures were estimated on the basis of their ^{1}H NMR spectra.[22)] On the other hand, the reaction of thymines almost resulted in the oxidation of the methyl group, even though a small amount of thymine glycol (**33b**)[13)] was observed in Fig. 3(B). In view of the above mentioned results, it is concluded that there may be two reaction mechanisms with regard to the oxidation of uracils by $SO_4^{-\cdot}$: i.e., the reaction of thymines is explained in terms of the formation of thymine cation radicals which are easily converted into the radical (**35**) by an elimination of a proton, while that of uracils and 5-fluorouracils results in the addition of $SO_4^{-\cdot}$ at the 5-position of the pyrimidine ring. Scheme 2 is a schematic illustration. In Scheme 2, the formation of the radical (**35**, X=CH_3) from the reaction of **22b** with $SO_4^{-\cdot}$ and the radical (**36**, X=H) from that of **22a** is known.[12m)] Furthermore, it was reported that 1,3,5,6-tetramethyluracil reacted with $SO_4^{-\cdot}$ to give the corresponding cation radical.[12k,12l)] There may be two reasons for the difference in the two reaction pathways: (1) the relationship between the oxidation-reduction potentials of uracils and the substituents at the 5-position and (2) the steric effect of the methyl group against an addition at the 5-position.

When the γ-radiation of **1b** and **6b** was studied, the yields of **1c** and **6c** were very low.[1a,1b)] On the other hand, the γ-radiation of DNA resulted in the formation



Scheme 1.

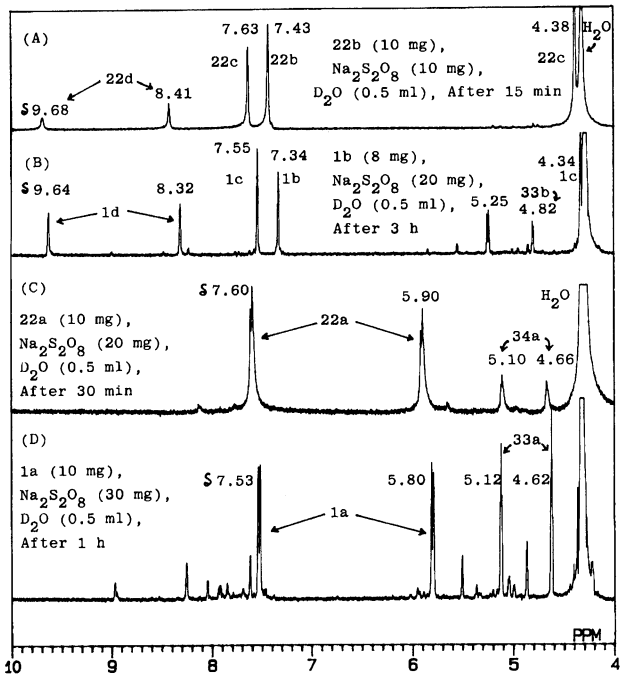
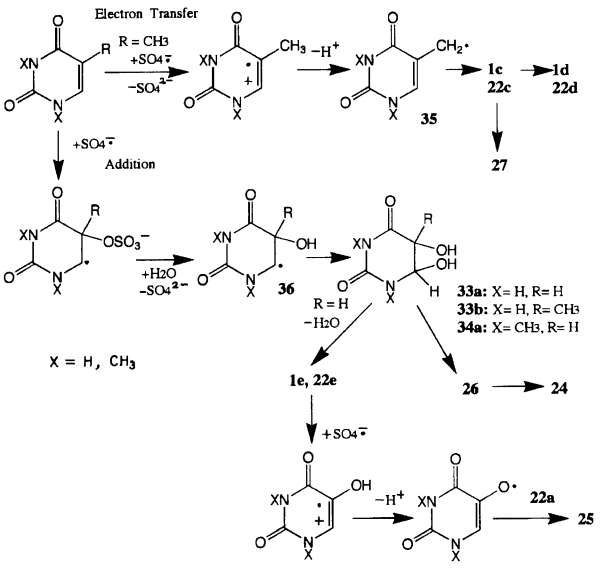


Fig. 3. ^{1}H NMR spectra of the reaction mixture of **1a**, **1b**, **22a**, and **22b** with sodium peroxodisulfate in D_2O at 75 °C.

of considerable amount of 5-hydroxymethyluracils.[1c] However, almost no reasonable explanation concerning the conflicting fact is known, although Teebor et al.[23] reported that ionizing radiation of [methyl-^3H]-thymidine caused an increasing amount of 5-hydroxymethyl-2′-deoxyuridine. In this paper, we describe that the oxidation of the methyl group of thymines and 5-methylcytosines proceeds via their cation radicals. Therefore, the oxidation of the 5-methyl groups of thymine and 5-methylcytosine residues in DNA may be explained in terms of the formation of 5-methylpyrimidine cation radicals in DNA by electron transfer between the 5-methylpyrimidines in DNA and the hydroxyl radical. This is because the stacking interaction on DNA may cause a certain condition similar to the solid state or frozen solution of nucleic acid related compounds.[11]



Scheme 2.

Experimental

The melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. The ^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz) were obtained with a JEOL GSX400 spectrometer using tetramethylsilane ($CDCl_3$) or sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 (D_2O) as an internal standard. The mass spectra were obtained with a JEOL JMS-D300 spectrometer. The elemental analyses were performed by the Analytical Center of Kyoto University. The starting materials such as uracil (**1a**), thymine (**1b**), cytosine (**2a**), adenine (**3**), uridine (**5a**), 2′-deoxyuridine (**6a**), thymidine (**6b**), cytidine (**7a**), 2′-deoxycytidine (**8a**), 5-methyl-2′-deoxycytidine (**8b**), adenosine (**9a**), 2′-deoxyadenosine (**10a**), thymidine 5′-monophosphate sodium salt (**11b**), 5-methyl-2′-deoxycytidine 5′-monophosphate sodium salt (**12b**), thymidilyl(3′→5′)-2′-deoxyadenosine sodium salt (**13b**), uridilyl(3′→5′)uridine sodium salt (**14**), uridilyl(2′→5′)-uridine sodium salt (**15**), and uridilyl(3′→5′)adenosine sodium salt (**16**) and the products such as 5-hydroxymethyluracil (**1c**), 5-hydroxyuracil (**1e**), 5-methylcytosine (**2b**), 5-hydroxymethylcytosine (**2c**), parabanic acid (**4**), 5-hydroxymethyl-2′-deoxyuridine (**6c**), uridine 5′-monophosphate sodium salt (**17**), uridine 3′-monophosphate sodium salt (**18**), uridine 2′-monophosphate sodium salt (**19**), uridine 2′:3′-cyclic monophosphate sodium salt (**20**), and adenosine 5′-monophosphate sodium salt (**21**) were obtained commercially. 5-Formyluracil (**1d**), 1,3-dimethyluracil (**22a**), 1,3-dimethylthymine (**22b**), 5-hydroxymethyl-1,3-dimethyluracil (**22c**), 5-formyl-1,3-dimethyluracil (**22d**), and 5,5′-[oxybis(methylene)]bis(1,3-dimethyluracil) (**27**) were prepared as in the foregoing experiment.[13] 5-Bromo-1,3-dimethyluracil (**22f**) was prepared according to a method described by Wong.[24]

Oxidation of Nucleosides 6b and 8b with Sodium Peroxodisulfate in Water. A solution of thymidine (**6b**) (1 mmol) and $Na_2S_2O_8$ (2 mmol) in water (100 ml) was heated at 75 °C for 4 h under an argon atmosphere. The reaction mixture was concentrated to a volumn of ca.

10 ml with an aspirator at room temperature, and submitted to chromatography on LiChroprep RP-18 (Merck Co. 25—40 μm, 16 mmϕ×500 mm) with a pump (Pharmacia P-500) and UV-monitor (Pharmacia UV-1, 254 nm). By elution with a mixture of water and acetonitrile, 5-hydroxymethyl-2′-deoxyuridine (**6c**) (0.30 mmol) and 5-formyl-2′-deoxyuridine (**6d**) (0.17 mmol) were obtained. Under similar conditions, the treatment of 5-methyl-2′-deoxycytidine (**8b**) (0.5 mmol) and $Na_2S_2O_8$ (0.5 mmol) in water (100 ml) at 75 °C for 3 h gave 5-hydroxymethyl-2′-deoxycytidine (**8c**) (0.17 mmol), 5-formyl-2′-deoxycytidine (**8d**) (0.08 mmol), 5-hydroxymethylcytosine (**2c**) (0.01 mmol), and recovered (**8b**) (0.53 mmol). The spectral data of **6d**, **8c**, and **8d** are as follows.

6d: Mp 172—175 °C (lit,[25] 175—175.5 °C); ^{1}H NMR (D_2O) δ=2.36—2.48 (1H, m, H-2′), 2.51—2.59 (1H, m, H-2′), 3.80 (1H, dd, J=13 and 4.5 Hz, H-5′), 3.91 (1H, dd, J=13 and 3 Hz, H-5′), 4.11 (1H, ddd, J=5, 4.5, and 3 Hz, H-4′), 4.48 (1H, q, J=5 Hz, H-3′), 6.26 (1H, t, J=6Hz, H-1′), 8.79 (1H, s, H-6), and 9.64 (1H, s, CHO); ^{13}C NMR (D_2O) δ=28.10, 49.13, 58.34, 75.47, 75.76, 99.79, 138.78, 139.73, 151.30, and 177.47.

8c: Decomp 198—203 °C (lit,[26] decomp 203 °C); ^{1}H NMR (D_2O) δ=2.31 (1H, m, H-2′), 2.45 (1H, m, H-2′), 3.77 (1H, dd, J=12.5 and 5 Hz, H-5′), 3.87 (1H, dd, J=12.5 and 3 Hz, H-5′), 4.07 (1H, m, H-4′), 4.44 (1H, m, H-3′), 4.46 (2H, s, CH_2), 6.27 (1H, t, J=6.5 Hz, H-1′), and 7.88 (1H, s, H-6); ^{13}C NMR (D_2O) δ=42.22, 60.62, 63.96, 73.20, 88.99, 89.48, 109.53, 143.32, 160.18, and 167.90.

8d: Mp 187—191 °C; ^{1}H NMR (D_2O) δ=2.38 (1H, m, H-2′), 2.59 (1H, m, H-2′), 3.81 (1H, dd, J=13 and 5 Hz, H-5′), 3.93 (1H, dd, J=13 and 3.5 Hz, H-5′), 4.15 (1H, m, H-4′), 4.45 (1H, m, H-3′), 6.21 (1H, t, J=6 Hz, H-1′), 8.82 (1H, s, H-6), and 9.53 (1H, s, CHO); ^{13}C NMR (D_2O) δ=42.98, 63.57, 72.69, 90.08, 90.35, 108.58, 157.51, 158.15, 165.55, and 193.23.

Treatment of **8d** (10 mg) with NaOH (100 mg) in water (10 ml) at 75 °C for 4 h gave 5-formylcytosine (**2d**) (2 mg): Mp >300 °C; ^{1}H NMR (DMSO-d_6) δ=7.9 (2H, broad, NH_2), 8.40 (1H, s, H-6), 9.45 (1H, s, CHO), and 11.6 (1H, broad, NH); ^{13}C NMR (DMSO-d_6) δ=103.91, 154.15, 156.99, 162.96, and 188.61; Mass m/z 139 (M^+, 100%). Found: m/z 139.0376. Calcd for $C_5H_5N_3O_2$: M, 139.0381.

Oxidation of Nucleotides 11b, 12b, and 13b. A solution of thymidine 5′-monophosphate (**11b**) (0.5 mmol) and $Na_2S_2O_8$ (0.5 mmol) in 0.2 mol dm^{-3} sodium phosphate buffer solution at pH 7.0 (100 ml) was heated at 75 °C for 4 h under an argon atmosphere. The reaction mixture was concentrated to a volumn of ca. 10 ml by an aspirator at room temperature and submitted to chromatography on a LiChroprep RP-18 column with a pump and a UV-monitor. By elution with a mixture of 0.05 mol dm^{-3} sodium phosphate buffer solution at pH 7.0 and acetonitrile, two fractions containing products were obtained. The first fraction was concentrated to a volumn of ca. 10 ml at room temperature and further chromatographed on the same gel (eluted with a mixture of water containing 1% acetic acid and CH_3CN) to give 5-hydroxymethyl-2′-deoxyuridine 5′-monophosphate (**11c**) (15%). The second fraction was treated in a similar manner as above to give 5-formyl-2′-deoxyuridine 5′-monophosphate (**11d**) (10%). Similar treatment of 5-methyl-2′-deoxycytidine 5′-monophosphate (**12b**) gave 5-hydroxymethyl-2′-deoxycytidine 5′-monophosphate (**12c**) (12%), 5-formyl-2′-deoxycytidine 5′-monophosphate (**12d**) (8%) and **12b** recovered (20%). Treatment of thymidilyl-(3′→5′)-2′-deoxyadenosine (**13b**) (10 mg) with $Na_2S_2O_8$ (5 mg) in 0.2 mol dm^{-3} phosphate buffer solution at pH 7.0 (25 ml) at 70 °C for 3 h under an argon atmosphere gave 5-hydroxymethyl-2′-deoxyuridilyl(3′→5′)-2′-deoxyadenosine (**13c**) (12%) and 5-formyl-2′-deoxyuridilyl(3′→5′)-2′-deoxyadenosine (**13d**) (8%). The chromatogram on the separation is shown in Fig. 1. The yields of **11c**, **11d**, **12c**, **12d**, **13c**, and **13d** were determined by means of a comparison of the protons at the 6-position of the isolated products with that (δ=7.47) of 1,3-dimethylthymine (**22b**) as an internal reference on ^{1}H NMR spectra (in D_2O) and the yield of **12b** recovered was determined in a similar manner by using 5-formyl-1,3-dimethyluracil (**22d**) (δ=8.48) as the reference, because salts such as phosphate and acetate could not be completely removed from the isolated nucleotides. The spectral data of **11c**, **11d**, **12c**, **12d**, **13c**, and **13d** are as follows.

11c: ^{1}H NMR (D_2O) δ=2.40—2.46 (2H, m, H-2′), 3.94—4.06 (2H, m, H-5′), 4.19—4.21 (1H, m, H-4′), 4.39 (2H, s, CH_2), 4.58—4.60 (1H, m, H-3′), 6.34 (1H, t, J=6.6 Hz, H-1′), and 8.01 (1H, s, H-6); ^{13}C NMR (D_2O) δ=42.06, 59.42, 67.47 (d, J_{C-P}=4 Hz), 73.88, 88.47, 88.74 (d, J_{C-P}=9.5 Hz), 116.73, 142.69, 154.56, and 168.04.

11d: ^{1}H NMR (D_2O) δ=2.40—2.60 (2H, m, H-2′), 4.05—4.20 (2H, m, H-5′), 4.27—4.29 (1H, m, H-4′), 4.56—4.58 (1H, m, H-3′), 6.27 (1H, t, J=6.2 Hz, H-1′), 8.72 (1H, s, H-6), and 9.63 (1H, s, CHO); ^{13}C NMR (D_2O) δ=42.87, 67.32 (d, J_{C-P}=4 Hz), 73.56, 89.33 (d, J_{C-P}=9 Hz), 90.16, 114.61, 153.21, 155.97, 165.20, and 192.59.

12c: ^{1}H NMR (D_2O) δ=2.30—2.38 (1H, m, H-2′), 2.43—2.50 (1H, m, H-2′), 4.03—4.17 (2H, m, H-5′), 4.20—4.25 (1H, m, H-4′), 4.50 (2H, s, CH_2), 4.55—4.60 (1H, m, H-3′), 6.30 (1H, t, J=6 Hz, H-1′), and 8.08 (1H, s, H-6); ^{13}C NMR (D_2O) δ=42.71, 60.38, 67.35 (d, J_{C-P}=4 Hz), 73.71, 88.88 (d, J_{C-P}=9 Hz), 89.36, 109.58, 144.23, 162.35, 166.16, and 181.50.

12d: ^{1}H NMR (D_2O) δ=2.35—2.42 (1H, m, H-2′), 2.53—2.61 (1H, m, H-2′), 4.05—4.12 (1H, m, H-5′), 4.17—4.24 (1H, m, H-5′), 4.25—4.30 (1H, m, H-4′), 5.53—5.58 (1H, m, H-3′), 6.24 (1H, t, J=6 Hz, H-1′), 8.86 (1H, s, H-6), and 9.62 (1H, s, CHO); ^{13}C NMR (D_2O) δ=43.29, 66.98, (d, J_{C-P}=4 Hz), 73.03, 89.10 (d, J_{C-P}=9 Hz) 90.45, 108.79, 157.56, 165.58, 178.02, and 194.03.

13c: ^{1}H NMR (D_2O, 24 °C) δ=1.80—1.90 (1H, m, H-$U_{2'}$), 2.26—2.34 (1H, m, H-$U_{2'}$), 2.58—2.66 (1H, m, H-$A_{2'}$), 2.84—2.93 (1H, m, H-$A_{2'}$), 3.64 (1H, dd, J=12.5 and 5 Hz, H-$U_{5'}$), 3.69 (1H, dd, J=12.5 and 3.5 Hz, H-$U_{5'}$), 4.03—4.06 (1H, m, H-$U_{4'}$), 4.06—4.10 (2H, m, H-$A_{5'}$), 4.23—4.26 (1H, m, H-$A_{4'}$), 4.33 (1H, d, J=13 Hz, CH_2), 4.38 (1H, d, J=13 Hz, CH_2), 4.61—4.65 (1H, m, H-$U_{3'}$), 4.75—4.80 (1H, m, H-$A_{3'}$), 6.04 (1H, dd, J=8 and 6.5 Hz, H-$U_{1'}$), 6.47 (1H, t, J=6.5 Hz, H-$A_{1'}$), 7.66 (1H, s, H-U_6), 8.23 (1H, s, H-A_2), and 8.40 (1H, s, H-A_8).

13d: ^{1}H NMR (D_2O, 24 °C) δ=1.88—1.96 (1H, m, H-$U_{2'}$), 2.42—2.50 (1H, m, H-$U_{2'}$), 2.58—2.64 (1H, m, H-$A_{2'}$), 2.84—2.91 (1H, m, H-$A_{2'}$), 3.66 (1H, dd, J=12.5 and 4.5 Hz, H-$U_{5'}$), 3.73 (1H, dd, J=12.5 and 3 Hz, H-$U_{5'}$), 4.06—4.10 (2H, m, H-$A_{5'}$), 4.10—4.14 (1H, m, H-$U_{4'}$), 4.23—4.27 (1H, m, H-$A_{4'}$), 4.61—4.64 (1H, m, H-$U_{3'}$), 4.75—4.78 (1H,

m, H-$A_{3'}$), 6.02 (1H, t, J=7 Hz, H-$U_{1'}$), 6.47 (1H, t, J=7 Hz, H-$A_{1'}$), 8.24 (1H, s, H-A_2), 8.40 (1H, s, H-A_8), 8.54 (1H, s, H-U_6), and 9.65 (1H, s, CHO).

Oxidation of 1,3-Dimethyluracils 22a, b, f, g and 23. A solution of **22a** (1 mmol) and $Na_2S_2O_8$ (1 mmol) in water (50 ml) was heated at 75 °C for 5 h under an argon atmosphere. The mixture was evaporated and chromatographed on silica gel with a pump (Chemco Low-prep 81-M-2) and a UV-detector (Shimadzu SPD-6A). Elution with a mixture of ethyl acetate and hexane resulted in the formation of 5-hydroxy-1,3-dimethyluracil (**22e**), 1,3-dimethylparabanic acid (**24**), 5,5′-oxybis(1,3-dimethyluracil) (**25**), and amalic acid (**26**). The reaction of 5-bromo-1,3-dimethyluracil (**22f**) gave **24** and **26**. A similar treatment of 5-fluoro-1,3-dimethyluracil (**22g**) gave 6,6′-bis(5-hydroxy-1,3-dimethyluracil) (**28**) together with **24** and **26**. The oxidation of 1,3,6-trimethyluracil (**23**) gave 1,6-dihydro-6-hydroxy-1,3,6-trimethyl-2,4,5(3*H*)-pyrimidinetrione (**29**). The yields are shown in Table 2 and their spectral data are given bellow.

22e: Mp 198—200 °C (lit,[27] 198—199 °C); ^{1}H NMR ($CDCl_3$) δ=3.38 (3H, s, CH_3), 3.41 (3H, s, CH_3), 6.01 (1H, s, OH, D_2O exchangeable), and 6.88 (1H, s, H-6); ^{13}C NMR ($CDCl_3$) δ=28.48, 36.96, 121,57, 131,76, 150.00, and 161.35; Mass m/z 156 (M^+, 100%).

24: Mp 151—152 °C (lit,[28] 151—153 °C); ^{1}H NMR ($CDCl_3$) δ=3.34 (6H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ=24.94, 29.00, 154.16, and 157.03.

25: Mp 247—251 °C; ^{1}H NMR ($CDCl_3$) δ=3.39 (6H, s, CH_3), 3.46 (6H, s, CH_3), and 8.41 (2H, s, H-6); ^{13}C NMR ($CDCl_3$) δ=28.35, 37.58, 104.09, 143.00, 150.73, and 162.62; Mass m/z 294 (M^+, 37%) and 278 (100%). Found: m/z 294.0949. Calcd for $C_{12}H_{14}N_4O_5$: M, 294.0962.

26: Mp 240—243 °C (lit,[29] 241—243 °C); ^{1}H NMR ($CDCl_3$) δ=3.35 (12H, s, CH_3); ^{13}C NMR δ=29.11, 84.65, 149.71, and 167.18.

28: Mp 290—296 °C; ^{1}H NMR (DMSO-d_6) δ=3.15 (6H, s, CH_3), 3.26 (6H, s, CH_3), and 9.30 (2H, s, OH, D_2O exchangeable); (D_2O) δ=3.27 (6H, s, CH_3) and 3.39 (6H, s, CH_3); (D_2O+NaOH, pD 13) δ=3.22 (6H, s, CH_3) and 3.37 (6H, s, CH_3); ^{13}C NMR (DMSO-d_6) δ=28.35, 32.47, 123.05, 130.74, 149.91, and 159.91; MS m/z 310 (M^+, 16%) and 309 (100%). Found: m/z 310.0907. Calcd for $C_{12}H_{14}N_4O_6$: M, 310.0912. Anal. Found: C, 46.39; H, 4.59; N, 17.78%. Calcd for $C_{12}H_{14}N_4O_6$: C, 46.45; H, 4.55; N, 18.06%.

29: Oil; ^{1}H NMR ($CDCl_3$) δ=2.26 (3H, s, CH_3), 2.81 (3H, s, CH_3), 3.11 (3H, s, CH_3), and 5.10 (1H, s, OH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$) δ=23.39, 24.75, 25.35, 87.99, 156.22, 168.79, and 199.66; Mass m/z 186 (M^+, 4%) and 143 (100%). Found: m/z 186.0641. Calcd for $C_7H_{10}N_2O_4$: M, 186.0641.

Uracil Glycol (33a). A solution of **1a** (10 mg) and $Na_2S_2O_8$ (30 mg) was heated at 75 °C in D_2O (0.5 ml) for 1 h. The ^{1}H NMR spectrum of the reaction mixture showed the presence of **33a** as a main product. The spectrum is shown in Fig. 3 (D). The compound **33a** was not isolated but the structure was estimated by the following NMR spectral data; ^{1}H NMR (D_2O, 25 °C) δ=4.65 (1H, d, J=4 Hz) and 5.11 (1H, d, J=4 Hz); ^{13}C NMR (D_2O, 25 °C) δ=71.01, 76.55, 168.61, and 175.71.

The authors wish to express their thanks to Professors Tsunao Hase, Munehiro Nakatani, and Ken-ichi Somekawa of Kagoshima University for valuable support throughout the study.

References

1) a) R. Teoule, A. Bonicel, C. Bert, J. Cadet, and M. Polvrelli, *Radiat. Res.*, **57**, 46 (1974); b) L. S. Myers, J. F. Ward, W. I. Tsukamoto, D. E. Holms, and J. R. Juka, *Science*, **148**, 1234 (1975); c) K. Frenkel, A. Cummings, J. Solomon, J. Cadet, J. J. Steinberg, and G. W. Teebor, *Biochemistry*, **24**, 4527 (1985).

2) a) P. V. Hariharan and P. A. Cerutti, *J. Mol. Biol.*, **66**, 65 (1972); b) E. A. Furlong, T. J. Jorgensen, and W. D. Henner, *Biochemistry*, **25**, 4344 (1986).

3) a) M. C. Hollstein, P. Brooks, S. Linn, and B. N. Ames, *Proc. Natl. Acad. Sci. U. S. A.*, **81**, 4003 (1984); b) R. J. Boorstein, D. D. Levy, and G. W. Teebor, *Mutat. Res.*, **183**, 257 (1987).

4) a) B. Demple and S. Linn, *Nature*, **287**, 203 (1980); b) L. H. Greimer and T. Lindahl, *J. Biol. Chem.*, **259**, 5543 (1984).

5) R. Cathcart, R. Schwiers, R. L. Saul, and B. N. Ames, *Proc. Natl. Acad. Sci. U. S. A.*, **81**, 6533 (1984).

6) L. D. Thornburg and J. Stubbe, *J. Am. Chem. Soc.*, **111**, 7632 (1989).

7) R. L. Adams and R. H. Burden, "Molecular Biology of DNA Methylation," Springer, New York (1985).

8) S. Cannon, A. Cumming, and G. W. Teebor, *Biochem. Biophys. Res. Commun.*, **151**, 1173 (1988).

9) C. von Sonntag, "Chemical Basis of Radiation Biology," Taylor and Francis, London (1987), and references therein.

10) S. Fujita and S. Steenken, *J. Am. Chem. Soc.*, **103**, 2540 (1981).

11) a) A. D. Lenherr and M. G. Ormerod, *Nature*, **225**, 547 (1970); b) A. Dulcic and J. N. Herak, *J. Chem. Phys.*, **57**, 2537 (1972); c) M. D. Sevilla, C. Van Paemel, and C. Nichols, *J. Phys. Chem.*, **76**, 3571 (1972); d) M. D. Sevilla, C. Van Paemel, and G. Zorman, *J. Phys. Chem.*, **76**, 3577 (1972); e) M. D. Sevilla, *J. Phys. Chem.*, **80**, 1898 (1976); f) S. Gregoli, M. Olast, and A. Bertinchamps, *Radiat. Res.*, **65**, 202 (1976); g) S. Gregoli, M. Olast, and A. Bertinchamps, *Radiat. Res.*, **72**, 201 (1977); h) M. D. Sevilla, D. Suryanarayana, and K. M. Morehouse, *J. Phys. Chem.*, **85**, 1027 (1981); i) W. I. Bernhard, *Adv. Radiat. Biol.*, **9**, 199 (1981).

12) a) R. C. Moschel and E. J. Behrman, *J. Org. Chem.*, **39**, 1983 (1974); b) R. C. Moschel and E. J. Behrman, *J. Org. Chem.*, **39**, 2699 (1974); c) K. M. Bansal and R. W. Fessenden, *Radiat. Res.*, **75**, 497 (1978); d) D. K. Hazra and S. Steenken, *J. Am. Chem. Soc.*, **105**, 4380 (1983); e) J. N. Herak and G. Behrens, *Z. Naturforsch., C*, **41c**, 1062 (1986); f) H. Schuchmann, D. J. Deeble, G. Olbrich, and C. von Sonntag, *Int. J. Radiat. Biol.*, **51**, 441 (1987); g) P. O'Neil and S. E. Davies, *Int. J. Radiat. Biol.*, **52**, 577 (1987); h) H. M. Novais and S. Steenken, *J. Phys. Chem.*, **91**, 426 (1987); i) G. Behrens, K. Hildenbrand, D. Schulte-Frohlinde, and J. N. Herak, *J. Chem. Soc., Perkin Trans. 2*, **1988**, 305; j) L. P. Candeias and S. Steenken, *J. Am. Chem. Soc.*, **111**, 1094 (1989); k) D. Schulte-Frohlinde and K. Hildenbrand,

"Free Radicals in Synthesis and Biology," ed by F. Minisci, Kluwer Academic, Dordrecht (1989), pp. 335—359; *Chem. Abstr.*, **111**, 174580q; l) K. Hildenbrand, G. Behrens, D. Schulte-Frohlinde, and J. N. Herak, *J. Chem. Soc., Perkin Trans. 2*, **1989**, 283; m) E. Bothe, D. J. Deeble, D. G. E. Lemaire, R. Rashid, M. N. Schuchmann, H. P. Schuchmann, D. Schulte-Frohlinde, S. Steenken, and C. von Sonntag, *Radiat. Phys. Chem.*, **36**, 149 (1990).

13) a) T. Itahara, R. Ebihara, Y. Fujii, and M. Tada, *Chem. Lett.*, **1986**, 1319; b) T. Itahara, Y. Fujii, and M. Tada, *J. Org. Chem.*, **53**, 3421 (1988).

14) Preliminary letters: a) T. Itahara and S. Koga, *Chem. Lett.*, **1991**, 85; b) T. Itahara, *Chem. Lett.*, **1991**, 1591; c) T. Itahara and A. Nishino, *Chem. Lett.*, **1991**, 2203.

15) D. J. Weber, G. P. Mullen, and A. S. Mildvan, *Biochemistry*, **30**, 7425 (1991).

16) J. Mellema, A. K. Jellema, C. A. G. Haasnoot, J. H. Van Boon, and C. A. Altona, *Eur. J. Biochem.*, **141**, 165 (1984).

17) H. Kwart, R. W. Spavd, and C. J. Collings, *J. Am. Chem. Soc.*, **83**, 2579 (1961).

18) a) F. Minisci, A. Citterio, and C. Giordano, *Acc. Chem. Res.*, **16**, 27 (1983), and references therein; b) C. Walling, C. Zhao, and G. M. El-Taliaw, *J. Org. Chem.*, **48**, 4910 (1983); c) C. Walling, G. M. El-Taliaw, and C. Zhao, *J. Org. Chem.*, **48**, 4914 (1983); d) F. Minisci, E. Vismara, G. Morini, F. Fontana, S. Levi, M. Serravalle, and C. Giordano, *J. Org. Chem.*, **51**, 476 (1986); e) D. M. Camaioni and J. A. Franz, *J. Org. Chem.*, **49**, 1607 (1984); f) D. M. Camaioni and M. S. Alnajjar, *J. Org. Chem.*, **50**, 4456 (1985).

19) a) C. Arnoldi, A. Citterio, and F. Minisci, *J. Chem. Soc., Perkin Trans. 2*, **1983**, 531; b) A. Citterio, C. Arnoldi, C. Giordano, and G. Castaldi, *J. Chem. Soc., Perkin Trans. 1*, **1983**, 891; c) P. Dobson, J. A. Norman, and C. B. Thomas, *J. Chem. Soc., Perkin Trans. 1*, **1986**, 1209.

20) M. Dizdaroglu and M. G. Simic, *Int. J. Radiat. Biol.*, **46**, 241 (1984).

21) M. Dizdaroglu and M. G. Simic, *Radiat. Res.*, **100**, 41 (1984).

22) B. S. Hahn and S. Y. Wang, *Biochem. Biophys. Res. Commun.*, **77**, 947 (1977).

23) G. W. Teebor, K. Frenkel, and M. S. Goldstein, *Proc. Natl. Acad. Sci. U. S. A.*, **81**, 318 (1984).

24) S. Y. Wang, *J. Org. Chem.*, **24**, 11 (1959).

25) M. P. Mertes and M. T. Shipchandler, *J. Heterocycl. Chem.*, **8**, 133 (1971).

26) R. Brossmer and E. Roehm, *Angew. Chem.*, **75**, 1105 (1963).

27) S. Y. Wang, M. Apicella, and B. R. Stone, *J. Am. Chem. Soc.*, **78**, 4180 (1956).

28) Y. Hoyano, V. Bacon, R. E. Summons, W. E. Pereira, B. Halpern, and A. M. Duffield, *Biochem. Biophys. Res. Commun.*, **53**, 1195 (1973).

29) H. Bredereck and W. Pfleiderer, *Chem. Ber.*, **87** 1119 (1954).