Chemistry of 4'-Hydroperoxy Nucleosides as a Model for the Intermediate in Bleomycin-Induced Degradation of DNA

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The reaction of 1-(3-*O*-benzoyl-2,5-dideoxy-β-D-*glycero*-pent-4-enofuranosyl)thymine (**5**) with anhydrous hydrogen peroxide in the presence of trifluoroacetic acid gave two isomeric 4'-hydroperoxides (**6** and **7**). The structure of the major product was assigned as 3'-*O*-benzoyl-5'-deoxy-4'-hydroperoxythymine (**6**) on the basis of spectral data. It has been demonstrated that the 4'-hydroperoxide **6** is capable of undergoing a Criegee-type rearrangement in an aqueous buffered solution to give *trans*-3-(thymin-1'-yl)propenal (**10**) stereospecifically, whereas the more stable isomer **7** does not produce **10** under the same conditions. These model experiments suggest that once a 4'-hydroperoxy intermediate of β-D-deoxyribo configuration is formed by action of the activated bleomycin on DNA, it spontaneously decomposes to afford DNA strand scission and trans base propenal. Reduction of these 4'-hydroperoxy nucleosides with dimethyl sulfide in methanol gave thymine and γ-keto aldehyde **14** quantitatively. The result indicates that the 4'-hydroxy nucleoside might be the precursor of alkali-labile lesion in DNA.

Bleomycins are a family of glycopeptide-derived antitumor antibiotics, and the oxidative DNA degradation mediated by certain metallobleomycins is thought to be responsible for their therapeutic effects.[1] Current interest in the chemistry of bleomycin (BLM) is concerned with the mechanistic nature of its oxidative DNA strand scission.[2] The DNA degradation by BLM requires metal ions, such as Fe(II), and molecular oxygen to give free nucleic acid bases and base propenals (**3**) as monomeric products.[2] The base propenal formation has been suggested to result from abstraction of C-4' hydrogen of the deoxyribose moiety by activated BLM followed by the formation of the unstable 4'-hydroperoxide **1** which would then decompose to give base propenals and cause DNA strand scission.[2f,4b] Free base formation which also requires activated BLM but no additional oxygen affords alkali-labile site on DNA.[3] Based on the site-specific tritium release from a labeled polynucleotide, Wu et al.[4] have demonstrated that free bases and base propenals formation are the outcome of a single BLM-induced event and proposed a mechanism involving 4'-hydroperoxy (**1**) and 4'-hydroxy (**4**) intermediates for the formation of base propenals (path a) and a free base (path b), respectively (Scheme I).

The 3'-termini of DNA strand breakage mediated by the BLM–Fe(II)–O_2 system have been determined and found to consist of glycolic acid esterified through its hydroxy group to the phosphate termini, i.e., 3'-phosphoglycolate termini (**2**).[2f,5] It has been shown that base propenals produced in BLM–Fe(II)-mediated degradation of DNA are exclusively of the trans configuration.[5] By the use of a self-complementary dodecanucleotide, Hecht et al.[6] have recently shown the structure and chemistry of the alkali-labile site, which are predominant products together with the free base formation when oxygen is limiting in BLM–Fe(II)-mediated DNA degradation as illustrated in Scheme I (path b). Hertzberg and Dervan[7] have demonstrated that a synthetic DNA cleaving molecule, methidiumpropyl–EDTA(MPE)–Fe(II), produces roughly equal proportion of 3'-phosphate and 3'-phosphoglycolate termini at the sites of DNA strand scission. A similar cleavage leading to 3'-phosphoglycolate termini was also observed in γ-irradiation of DNA.[8] In either case, free hydroxyl radical or its equivalent has been suggested to be implicted in the DNA cleavage reaction,[7,8] while the reaction sequence leading to the formation of 3'-phosphoglycolate termini is not well understood. It seems clear that oxidation of the C-4' position of the deoxyribose moiety of DNA is indispensable to the formation of these 3'-phosphoglycolate termini. In fact, 4'-hydroperoxy intermediate **1** has been proposed to account for the formation of **2** in the BLM–Fe(II) reaction of DNA.[2f,4,5]

While the 4'-hydroperoxy intermediate **1** has been suggested to play a key role in the oxidative degradation of the deoxyribose moiety in these systems,[4-8] the chemistry of the 4'-hydroperoxy intermediate itself or its model

Table I. ^{13}C NMR Chemical Shifts of 4'-Hydroperoxy Nucleosides 6 and 7

	hydroperoxide			hydroperoxide	
carbon	6[a]	7[b]	carbon	6[a]	7[b]
C-2	152.8	153.3	C-1'	89.1	87.5
C-4	168.3	167.4	C-2'	38.3	39.3
C-5	112.7	112.7	C-3'	77.6	78.3
C-6	140.4	138.6	C-4'	112.5	116.2
C-5 methyl	13.0	13.4	C-5'	21.6	18.7

[a] In $CDCl_3$. [b] In acetone-d_6.

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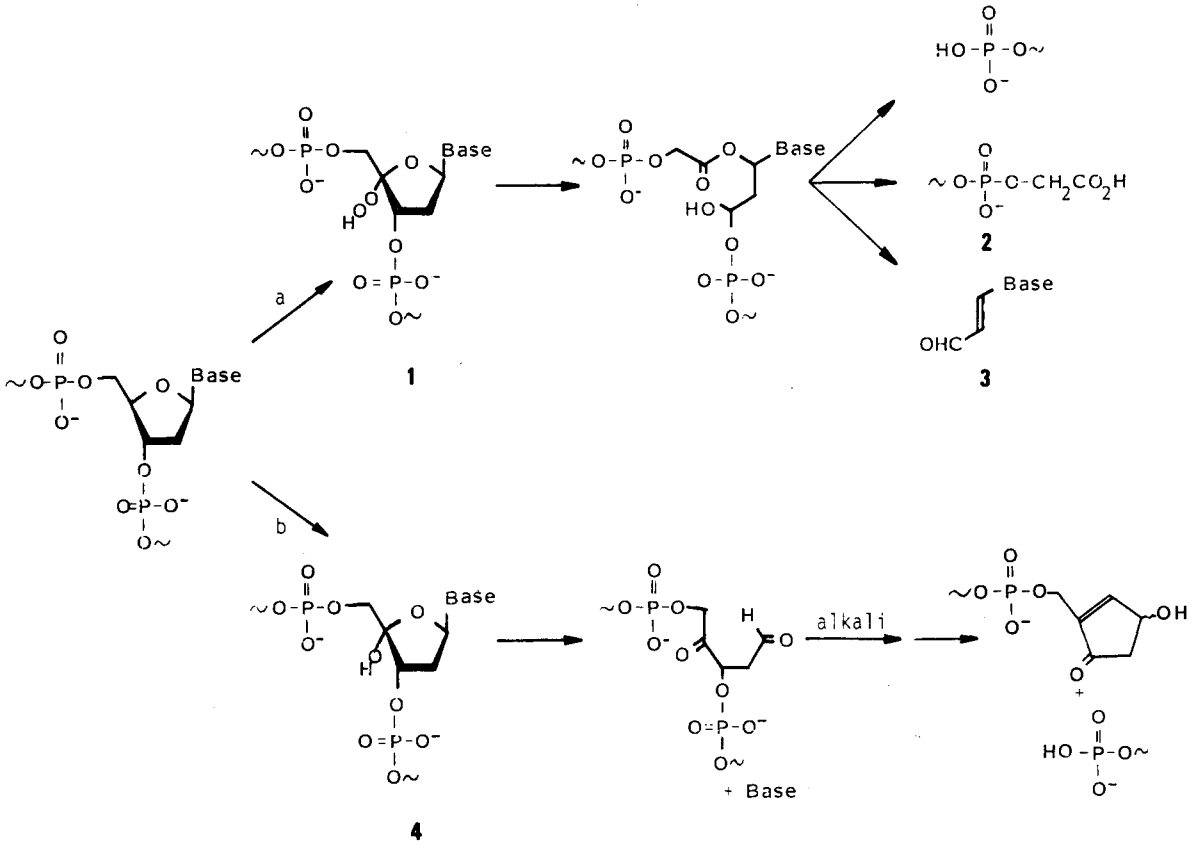
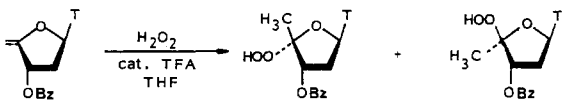
Scheme I



compound has totally been unknown. We describe herein the synthesis of isolable 4′-hydroperoxy nucleosides,[9] as a model for **1**, and the structural elucidation of two isomers at C-4′ position to demonstrate that only the 4′-hydroperoxy nucleoside of β-D-ribo configuration is capalbe of undergoing a Criegee-type rearrangement to lead ultimately to trans base propenal stereospecifically, whereas reduction of either 4′-hydroperoxides affords free base via 4′-hydroxide. Moreover, the present model studies provide the chemical support to the formation of the 3′-phosphoglycolate termini from the postulated 4′-hydroperoxy intermediate **1**.

Results and Discussion

Our approach to the synthesis of 4′-hydroperoxy nucleosides is based on the addition of the hydrogen peroxide to 5′-deoxy-4′,5′-unsaturated nucleoside.[9] Treatment of 1-(3-*O*-benzoyl-β-D-*glycero*-pent-4-enofuranosyl)thymine (**5**) prepared by the known method[10] with an ether solution of anhydrous hydrogen peroxide[11] in dry THF in the presence of a catalytic amount of trifluoroacetic acid at ambient temperature produced a mixture of two isomeric hydroperoxide. These hydroperoxides gave a rust-red colored spot on TLC plate by spraying with $FeSO_4$–NH_4SCN solution.[12] The isomers were separated by flash column chromatography followed by preparative HPLC to give **6** and **7** in 54% and 25% yields, respectively. Assignment of the structure including the configuration at C-4′ was made on the basis of the spectroscopic data, especially ^{13}C NMR (Table I). It is well-established that a cis relationship between the C-5′ carbon and the C-3′ hydroxy group in furanose sugar systems exhibits the resonance of C-5′ carbon at higher field than that in corresponding compound possessing a trans configuration due to the sheilding effect of the vicinal oxygen substituent.[13] Examination of the ^{13}C NMR of **6** and **7** revealed that the C-5′ methyl carbon of **7** appeared at 2.9 ppm upfield relative to that of **6**, indicating a cis relationship between the C-5′ methyl and C-3′-*O*-benzoyl group in **7**. Accordingly, the major product **6** has a trans orientation and is assigned to have β-D-deoxyribo configuration.



5 **6** **7**

While both hydroperoxides **6** and **7** were considerably stable in dry organic solvent at ambient temperature, hydroperoxide **6** gradually decomposed in aqueous solution. In order to know whether the hydroperoxides **6** and **7** could produce thyminylpropenal, thermal decomposition of **6** and **7** in aqueous media was examined at various pH. The hydroperoxide **6** in a mixed solution of phosphate buffer and acetonitrile (5:3) at pH 6.0 decomposed with the half-life of 3 h at 25 °C to give *trans*-3-(thymin-1′-yl)propenal (**10**) stereospecifically, which was confirmed by the presence of vinylic coupling (14 Hz)[5] in the ^1H NMR of the isolated compound. The progress of the reaction was monitored by UV spectroscopy as shown in Figure 1. The absorption at 303 nm due to **10** gradually appeared

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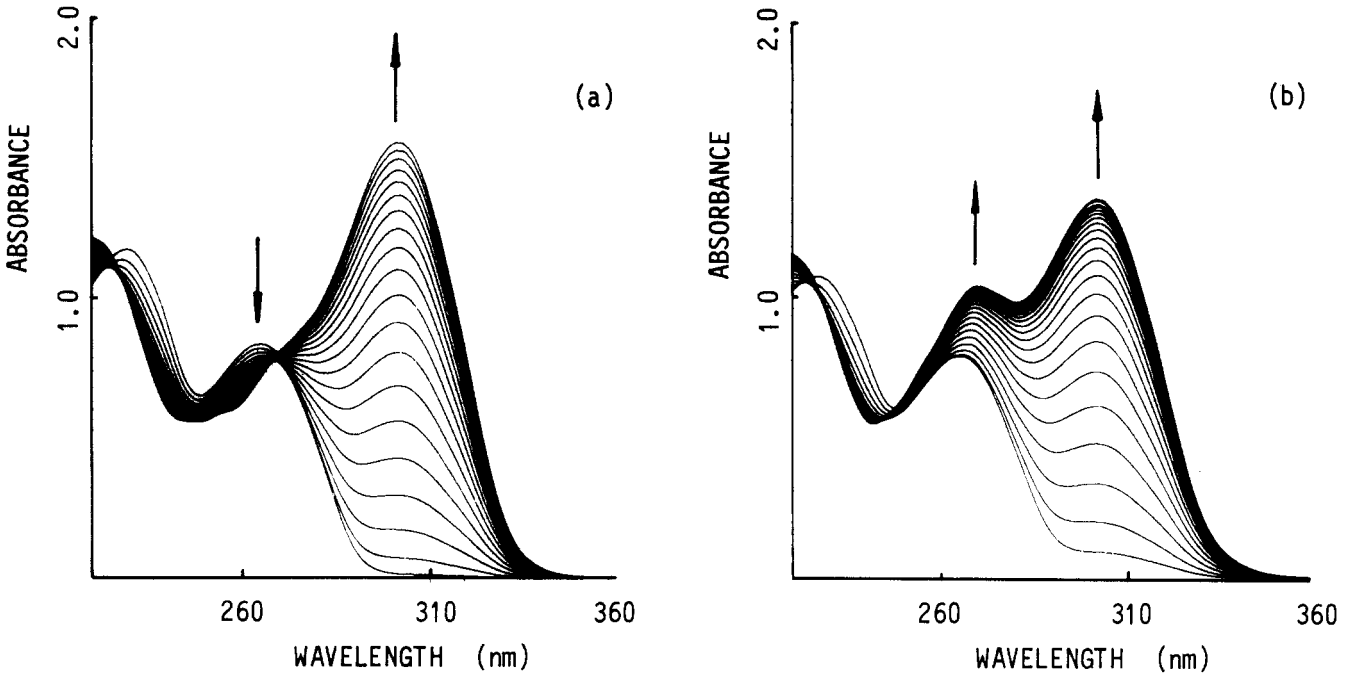


Figure 1. Progressive UV spectral change in the decomposition of **6** in 0.1 M phosphate buffer–acetonitrile (5:3) at 25 °C: (a) at pH 6.0; (b) at pH 7.2. The spectra were recorded every 30 min for (a) and 5 min for (b). The direction of the spectral change is indicated by arrows.

Table II. pH Dependency in the Decomposition of 4′-Hydroperoxy Nucleoside 6

hydroperoxide	pH	half-life[a]	product (yield, %)[b]	
6	6.0	3 h	**10** (84)	thymine (15)
6	7.2	27 min	**10** (56)	thymine (32)
6 + BLM–Fe(II)[c]	7.2	ca. 20 min	**10** (48)	thymine (47)
6	8.9	a few s		thymine (100)

[a] At 25 °C. [b] Yields determined by HPLC. [c] 0.67 mM BLM and 0.50 mM ferrous sulfate.

during the incubation with the isosbestic point at 273 nm at pH 6.0 (Figure 1a). HPLC analysis of the reaction mixture indicated the presence of **10** and benzoic acid in 84% and 93% yields, and the formation of acetic acid was confirmed by GC analysis.

A similar mode of the decomposition was observed in the mass spectral fragmentation of **6** where major intense fragment ions consist of **10** (*m/e* 180), benzoic acid (*m/e* 122), and acetic acid (*m/e* 60). The decomposition rate and the product distribution are highly dependent on the solution pH. At pH 1.2, **6** decomposed with a half-life of only 27 min at 25 °C to give a mixture of **10** and thymine in a ratio of ca. 5:3 (Table II and Figure 1b). The time course of the decomposition of **6** at pH 6.0 and 7.2 is shown in Figure 2. With increasing pH, half-lives of **6** become shorter with the enhancement of thymine production as shown in Table II. Interestingly, the trend in this pH dependency on the ratio of **10** vs. thymine has a similarity to that observed in the BLM–Fe(II)-induced degradation of DNA in which the ratios of base propenals vs. free bases were 5:2 at pH 6.4 and 1:1 at pH 8.2,[2f] although the decomposition rate was much faster in the case of BLM-induced reaction. Addition of the BLM–Fe(II) complex to the reaction system at pH 7.2 did not alter the decomposition rate and the product ratio appreciably (Table II).

In marked contrast, the isomeric hydroperoxide **7** was considerably stable at neutral pH as shown in Figure 2 and only slowly decomposed to give a complex mixture of products including a small amount of thymine with no

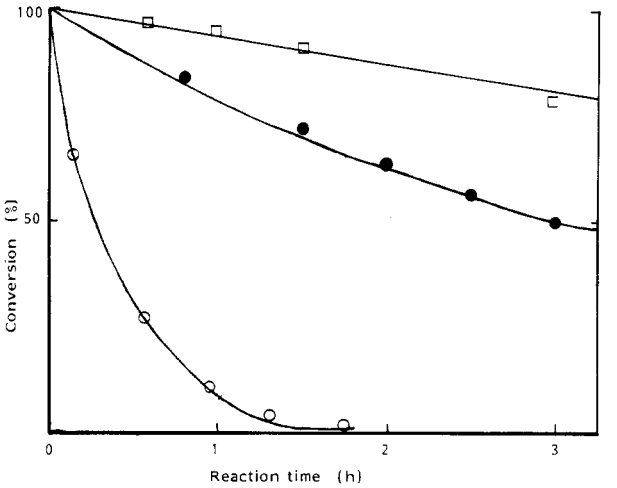
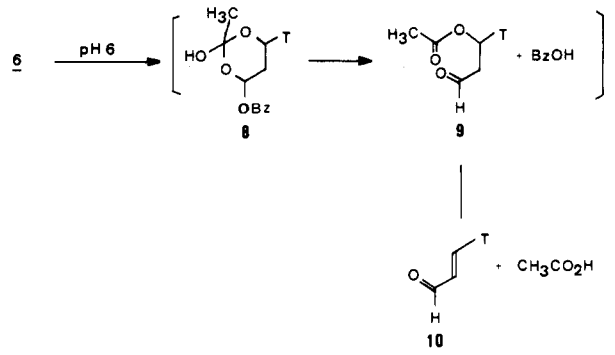


Figure 2. Time course of the decomposition of 4′-hydroperoxides **6** and **7** in 0.1 M phosphate buffer–acetonitrile (5:3) at 25 °C: ●, **6** at pH 6.0; ○, **6** at pH 7.2; □, **7** at pH 7.2.

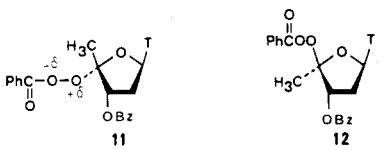
detectable amount of thyminylpropenal (**10**). For example, prolonged incubation of **7** at pH 7.2 at 25 °C gave thymine in only 22% yield even after 22 h. Thyminylpropenal (**10**) was undetectable in this mixture. The reason for this striking difference in the stabilities between **6** and **7** is not clear. However, it is conceivable that the hydroperoxy group of **6** is destabilized by the interaction with an adjacent *cis*-3′-benzoyl group probably through the hydrogen bonding. Such effects may also be expected in the case of DNA where the benzoyl group is replaced by the phosphate group. The thermal reaction of 4′-hydroperoxides observed here clearly indicates that only the 4′-hydroperoxide of β-D-deoxyribo configuration, namely **6**, can be a precursor of *trans*-thyminylpropenal (**10**) at neutral pH.

An efficient formation of **10** at slightly acidic pH, e.g., pH 6.0, suggests a proton-assisted Criegee-type rearrangement of **6** to the six-membered hemiketal **8** which would then undergo ring-opening and subsequent β-elimination leading to the formation of **10** via **9** (Scheme II).

Scheme II

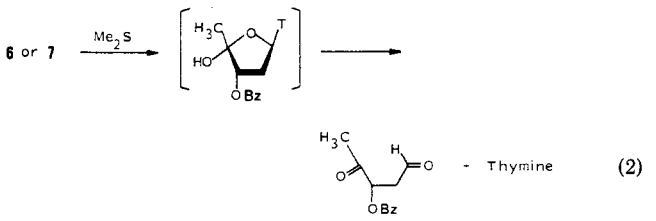


In order to probe this possibility we next prepared the benzoyl derivatives **11** and **12** which would be more susceptible to such rearrangement due to the more polarized peroxide bond induced by the electron-withdrawing benzoyl group.[14] Benzoylation of **6** with benzoic anhydride and *N,N*-diisopropylethylamine in dichloromethane at 0 °C gave unstable peroxy ester **11** which on purification by silica gel flash column chromatography resulted in a rapid decomposition to give **10** as the major product. In contrast, peroxy ester **12** prepared similarly from **7** was stable enough to be purified by silica gel column chromatography. Unlike the parent hydroperoxide **7**, the peroxy ester **12** underwent a smooth decomposition upon heating in acetonitrile to produce **10** in 70% yield. With all of these observations taken together, it seems reasonable to assume that the formaton of *trans*-thyminylpropenal (**10**) is the outcome of a proton-assisted Criegee-type rearrangement of 4′-hydroperoxide **6** to hemiketal **8** which would then decompose to **10**, benzoic acid, and acetic acid by ring-opening followed by successive β-elimination as shown in Scheme II. It is of interest to note here that thyminylpropenal (**10**) formed from **6** exclusively with trans geometry at neutral pH. The BLM-mediated degradation of DNA was also shown to produce only trans base propenals as primary reaction products with specific loss of α-2′ hydrogen of deoxyribose.[4a,c,5] The reason for this specificity has been assumed to relate constraints on conformation of the damaged DNA duplex.[5] However, the present finding that a simple model like **6** can specifically produce *trans*-thyminylpropenal suggests a more simple mechanism, i.e., anti β-elimination from a most favorable conformer possessing acidic α-2′ hydrogen which is adjacent to aldehyde and anti to the leaving group.



Free base release and the formation of alkali-labile lesions were predominantly observed in the reaction of activated BLM and DNA when oxygen was limiting (Scheme I, path b).[4a,c,6] Under aqueous alkaline conditions the alkali-labile lesions would result in the strand scission of DNA. The 4′-hydroxy intermediate **4** has been proposed to form the free base and alkali-labile lesion.

To test the hypothesis, we next examined the chemical nature of the 4′-hemiketal **13**, which would be obtained by the reduction of the 4′-hydroperoxide **6** or **7**. Reduction of **6** with dimethyl sulfide in methanol gave thymine and γ-keto aldehyde **14** quantitatively. The result indicates



(2)

that the formation of 4′-hemiketal **13** readily results the release of thymine; in case of BLM–DNA reaction, hydroxylation at the C4′ position of deoxyribose would release the free base. The γ-keto aldehyde obtained in this model study may correspond to the alkali-labile lesion in DNA. Unlike the thermal decomposition, both hydroperoxides **6** and **7** equally produced thymine and **14** upon dimethyl sulfide reduction under the neutral conditions. It should be pointed out that the formation of thymine from **6** at alkaline pH most likely arises from the same 4′-hemiketal **13** which might be formed by a nucleophillic attack of hydroxide ion on the hydroperoxy group of **6**.[15]

In summary, the present model experiments clearly demonstrate that the 4′-hydroperoxide of β-D-deoxyribo configuration is capable of undergoing a rearrangement to give *trans*-thyminylpropenal, whereas more stable 4′-hydroperoxide **7** of the opposite configuration at C-4′ position gives no thynimylpropenal (**10**). Our results suggest that (i) the BLM-induced degradation of double-stranded DNA would produce 4′-hydroperoxide of only β-D-deoxyribo configuration, e.g., **1**, by steric reason, and (ii) once the 4′-hydroperoxy intermediate **1** is formed by the action of activated BLM on DNA, it spontaneously decomposes to afford DNA strand scission at the site and base propenal as a monomeric product.

The formation of acetic acid from **6** in the thermal decomposition may correspond to the production of 3′-phosphoglycolate termini from 4′-hydroperoxy intermediate **1** in DNA. Therefore, it is conceivable that the hydroperoxidation of the deoxyribose moiety at C-4′ position plays a key role in the DNA strand scission induced by oxygen-requiring drugs such as BLM–Fe(II)[2f,4] and MPE–Fe(II)[7] as well as the ionizing radiaiton[8] where the 3′-phosphoglycolate termini are produced. However, it has also been pointed out that the formation of 3′-phosphoglycolate termini does not necessarily accompany the production of the base propenal, as actually being observed in MPE–Fe(II) reaction[7] or γ-irradiaiton[8] of DNA. In any event further studies including metal-catalyzed or radiation-induced decomposition of 4′-hydroperoxy nucleosides are apparently necessary to elaborate this point.

Experimental Section

Microanalyses were carried out at Analytical Center of Kyoto University. Mass spectra were recorded on a JEOL-JMS-DX300 spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Varian FT-80A or JEOL JNM-GX400 spectrometer with tetramethylsilane as a internal standard. High-performance liquid chromatography (HPLC) was performed on a Waters ALC/204 model equipped with a 254- or 214-nm fixed-wave-length detector. Reverse phase NOVA-pak and YMC-S343 ODS columns (20 mm × 25 cm) were used for analytical and preparative purposes, respectively. Column chromatography and TLC were performed on Wako silica gel C-200 and Merck 60 PF_{254}, respectively.

Materials. 1-(3-*O*-Benzoyl-2,5-dideoxy-β-D-*glycero*-pent-4-enofuranosyl)thymine (**5**) was prepared in 61% yield from 3′-*O*-

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benzoyl-5′-deoxy-5′-iodothymidine according to the published procedure.[10] The ether solution of anhydrous hydrogen peroxide was prepared by the extraction of 30% aqueous hydrogen peroxide with ether.[11] The ether solution was dried over anhydrous $MgSO_4$ and titrated iodometically before use. The buffer solution was purified with Amberlite IRC-718 to remove heavy metal ions. Bleomycin hydrochloride was purchased from Nihonkayaku Co. Ltd and contains approximately 55–70% bleomycin A_2 and other bleomycins.

Synthesis of 4′-Hydroperoxy Nucleosides 6 and 7. To a stirred solution of **5** (160 mg, 0.5 mmol) in dry THF (10 mL) was added etherial anhydrous hydrogen peroxide (2 mL, 5 mmol). To this solution was added trifluoroacetic acid (0.01 mL) at 0 °C. The solution was stirred overnight at room temperature. Ethyl acetate (50 mL) was added to the solution, and the solution was washed twice with cold brine[16] and then dried over anhydrous $MgSO_4$. The solvent was removed to dryness by rotary evaporation under reduced pressure below 10 °C. The residue was subjected to silica gel column flash chromatography; elution with methanol–chloroform (5:95) gave two major fractions which were separated to give unreacted **5** (72 mg) and a mixture of peroxides (85 mg). The peroxidic mixture was then subjected to preparative HPLC (methanol–water, 58:42) followed by the evaporation in vacuo to yield **6** (49 mg, 58%) and **7** (25 mg, 29%.). **6**: UV λ_{max} (CH_3CN) 262 nm (log ϵ 3.90); 400-MHz ^1H NMR ($CDCl_3$) δ 1.65 (s, 3 H), 1.94 (d, 3 H, J = 1 Hz), 2.64 (ddd, 1 H, J = 9, 9, 14 Hz), 2.85 (ddd, 1 H, J = 3.5, 10, 14 Hz), 5.60 (dd, 1 H, J = 9, 10 Hz), 6.50 (dd, 1 H, J = 3.5, 9 Hz), 6.89 (d, 1 H, J = 1 Hz), 7.40–7.60 (m, 3 H), 8.12 (m, 2 H), 8.90 (br s, 1), 9.20 (br s, 1 H); 100-MHz ^{13}C NMR ($CDCl_3$) δ 13.0, 21.6, 38.3, 77.6, 89.1, 112.5, 112.7, 130.4, 131.7, 131.8, 135.3, 140.4, 152.8, 167.2, 168.3; MS (70 eV), m/e (relative intensity) 180 (7.4, thyminylpropenal), 126 (31.1), 122 (66.7) 105 (100), 60 (8.1). Anal. Calcd for $C_{17}H_{18}N_2O_7 \cdot H_2O$: C, 53.70; H, 5.30; N, 7.36. Found: C, 54.07; H, 5.10; N, 7.36. **17**: UV λ_{max} (CH_3CN) 261 nm (log ϵ 3.95); 400-MHz ^1H NMR (acetone-d_6) δ 1.59 (s, 3 H), 1.88 (d, 3 H, J = 1 Hz), 2.60–2.72 (m, 2 H), 5.69 (dd, 1 H, J = 1.5, 5.0 Hz), 6.63 (dd, 1 H, J = 6.8, 8.0 Hz), 7.55 (dd, 1 H, J = 7.5 Hz), 7.68 (d, 1 H, J = 7.5 Hz), 7.79 (d, 1 H, J = 1 Hz), 8.10 (dd, 2 H, J = 1,7.5 Hz), 10.08 (br s, 1 H), 11.45 (br s, 1 H); 100-MHz ^{13}C NMR (acetone-d_6) δ 13.4, 18.7, 39.3, 78.3 87.5, 112.7, 116.2, 130.6, 131.4, 131.7, 131.8, 135.6, 153.3, 167.1, 167.4; MS (70 eV), m/e (relative intensity) 180 (3.0), 126 (21.5), 122 (20.4), 105 (100), 60 (4.4). Attempts to isolate analytically pure **7** were unsuccessful. However, the sample is homogeneous on HPLC and has more than 90% peroxide content as revealed by iodometric titration.

Thermal Decomposition of 4′-Hydroperoxy Nucleosides 6 and 7. (a) HPLC Analysis of the Decomposition Products. HPLC analysis of the decomposition products was carried out on a NOVA-pak C_{18}ODS column eluted with acetonitrile–water (4:6) at a flow rate of 1.5 mL/min. Retention times of thyminylpropenal (**10**), thymine, and **6** were 1.9, 2.3, and 4.6 min, respectively. Quantitative analysis was performed by a Waters Data Module 730. Identification of the decomposition products was done in a preparative scale run. The products were identified by comparison of their chromatographic behaviors (TLC, HPLC) and ^1H NMR spectra after collection of each HPLC peaks with those of the authentic samples. (*E*)-3-(Thymin-1′-yl)propenal (**10**):[2f] ^1H NMR (methanol-d_4,) δ 1.94 (d, 3 H, J = 1.5 Hz), 6.35 (dd, 1 H, J = 8, 14 Hz), 7.77 (d, 1 H, J = 1.5 Hz), 8.14 (d, 1 H, J = 14 Hz), 9.48 (d, 1 H, J = 8 Hz); UV λ_{max} (H_2O) 303 nm (log ϵ 4.40).

Two solutions of **6** (each 8.95×10^{-5} M) in 0.1 M phosphate buffer–acetonitrile (5.3) were prepared and adjusted to pH 6.0 and 7.2, respectively. Each solution was incubated at 25 °C until **6** had completely disappeared (22 h at pH 6.0, 1.8 h at pH 7.2). The products were analyzed by HPLC as described above. A buffered solution (5 mL) of bleomycin (0.67 mM) and $FeSO_4$ (0.50 mM) was added to the solution (5 mL) of **6** (0.56 mM) in 0.1 M phosphate buffer–acetonitrile (5:3). The solution was incubated at 25 °C for 2 h and analyzed by HPLC. For the decomposition at a higher pH, 0.1 mL of an acetonitrile solution of **6** (9 mM) was added to 10 mL of aqueous $NaHCO_3$ (pH 8.9). The solution was immediately analyzed by HPLC. The results are shown in Table II.

A solution of **7** (8.14×10^{-5} M) in 0.1 M phosphate buffer–acetonitrile (5:3) at pH 7.2 was incubated at 25 °C for 22 h. HPLC analysis of the mixture revealed the presence of thymine (22%) and several unidentified products. The formation of **10** could not be detected by HPLC analysis.

(b) Time Course of the Thermal Decomposition. A solution of **6** (8.95×10^{-5} M) in 0.1 M phosphate buffer–acetonitrile (5:3) at pH 6.0 was incubated in a UV cell at 25 °C. The progess of the decomposition was monitored by UV spectroscopy and HPLC periodically. The results are shown in Figure 1a and 2. In a separate run, solutions of **6** and **7** (each 8.95×10^{-5} M) in 0.1 M phosphate buffer–acetonitrile (5:3) were prepared and adjusted to pH 7.2. Each solution was incubated at 25 °C and analyzed by HPLC. The results are shown in Figure 2. In the case of **6**, the reaction was monitored by UV spectroscopy (Figure 1b).

Reduction of 4′-Hydroperoxy Nucleosides 6 and 7. To a dry methanol solution of **6** (28 mg, 0.08 mmol) was added dimethyl sulfide (0.5 mL) under stirring at ambient temperature. The solution was stirred for additional 12 h. Solvent was removed under reduced pressure and the residue was subjected to silica gel preparative TLC (5% methanol–chloroform). Elution of the upper band gave 3-benzoxy-4-oxopentanal (**14**) (16 mg, 95%), whereas lower band gave thymine (9 mg, 92%). **14**: ^1H NMR ($CDCl_3$) δ 2.10 (s, 3 H), 2.85 (dd, 2 H, J = 1, 5 Hz), 5.40 (t, 1 H, J = 5 Hz), 7.40–7.55 (m, 3 H), 7.85–8.10 (m, 2 H), 9.65 (t, 1 H, J = 1 Hz).

Treatment of **7** (31 mg, 0.08 mmol) with excess dimethyl sulfide in methanol as described above produced **14** (15 mg, 92%) and thymine (10 mg, 93%).

Benzoylation of 4′-Hydroperoxy Nucleoside 6. A solution of benzoic anhydride (25 mg, 0.11 mmol) in dry dichloromethane (5 mL) was added to the ice-cooled, stirred solution of **6** (40 mg, 0.11 mmol) and *N*,*N*-disopropylethylamine (0.02 mL) in dry dichloromethane (10 mL). The mixture was allowed to warm to room temperature and stirred for 4 h. The mixture was washed with cold brine and the organic layer was dried over Na_2SO_4. The solvent was evaporated and the residue was subjected to silica gel flash chromatography. Elution with methanol–chloroform (5:95) resulted in the decomposition of the perester **11** to give **10** and thymine. Attempts to isolate pure **11** were unsuccessful.

Benzoylation of 4′-Hydroperoxy Nucleoside 7. A solution of benzoic anhydride (25 mg, 0.11 mmol) in dry dichloromethane (5 mL) was added to the ice-cooled stirred solution of **7** (40 mg, 0.11 mmol) and 4-(dimethylamino)pryidine (14 mg, 0.11 mmol) in dichloromethane (10 mL). The reaction mixture was stirred at room temperature for 4 h. The mixture was washed with cold brine and dried over anhydrous Na_2SO_4, and the solvent was evaporated to dryness. The residue was subjected to silica gel column chromatography. Elution with methanol–chloroform (5:95) gave **12** (38 mg, 70%). **12**: ^1H NMR ($CDCl_3$) δ 1.80 (s, 3 H), 1.92 (d, 3 H, J = 1 Hz), 2.55–2.75 (m, 2 H), 5.75 (br d, 1 H, J = 4 Hz), 6.88 (t, 1 H, J = 8 Hz), 7.30–7.60 (m, 6 H), 7.75 (d, 1 H, J = 1 Hz), 7.80–8.20 (m, 4 H), 9.60 (br s, 1 H); MS (70 eV), m/e (relative intensity) 466 (0.1), 180 (7.4), 126 (14.8), 122 (40.0), 105 (100), 60 (6.7).

Thermal Degradation of Perester 12. A solution of **12** (25 mg, 0.054 mmol) in acetonitrile (10 mL) was heated at 50 °C for 1 h. After evaporation, the residue was subjected to silica gel preparative TLC, eluting with methanol-chloroform (5:95). Elution of the major band gave **10** (8 mg, 70%).

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(16) In a small-scale preparation, repeated washing with cold brine was sufficient to remove excess hydrogen peroxide from the organic layer completely.