Endoperoxide Formation in a Guanosine Derivative

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Photodynamic action (the damage caused by photosensitizers upon exposure to visible light and air to organisms, cells, and biomolecules) has been studied extensively.[1-12] Photodynamic therapy has been used for certain skin diseases and several types of malignant tumors.[13-15] However, photodynamic action also has some genotoxic effects. These photocarcinogenic or photomutagenic effects are believed to result from photosensitized oxidative modifications of DNA.[4,16-19]

Early observations[20-23] and recent direct determination[24] of the deactivation rate constants of singlet oxygen by purines and pyrimidines showed that purines are more readily photooxidized than pyrimidines and that guanine (as the free base or in nucleosides and nucleotides) is the most reactive. To develop a better understanding of the chemical processes occurring in the photodynamic effect, many attempts have been made during the past 2 decades to isolate and characterize intermediates and final products of photooxidation of guanine derivatives. However, the extremely low solubility of purine derivatives in most solvents, the instability of the products, and analytical difficulties in the separation of relatively polar and unstable photoproducts from guanine and its nucleosides have retarded progress.

The three main photooxidation products from the singlet oxygenation of 3′,5′-di-*O*-acetyl-2′-deoxyguanosine (**1**) are reported to be the 4*R* and 4*S* diastereomers of 9-(3′,5′-di-*O*-acetyl-2′-deoxy-β-D-*erythro*-pentofuranosyl)-4,8-dihydro-4-hydroxy-8-oxoguanine (**2**) and *N*-(3′,5′-di-*O*-acetyl-2′-β-D-*erythro*-pentofuranosyl)cyanuric acid (**3**) (Scheme I).[8-10,25]

It has been suggested[8,9] that the two diastereomers of **2** are formed from unstable diastereomeric intermediate endoperoxides **4** from 1,4-cycloaddition of $^1O_2$. Product **3** has been proposed to occur via [2 + 2] cycloaddition of $^1O_2$ at the C-4,C-5 double bond of the purine ring to give an unstable dioxetane (**5**), which decomposes to the cyanuric acid derivative.[8] However, neither the endoperoxide nor the dioxetane has been directly observed in a purine.

We now report preparation and characterization of diastereomeric endoperoxides in the low-temperature photooxygenation of 2′,3′,5′-*O*-(*tert*-butyldimethylsilyl)-8-methylguanosine (**6**). We photooxygenated a number of 2′,3′,5′-*O*-substituted guanosines and 8-substituted guanosines at low temperature and found that only **6** gave a product with long enough lifetime to allow spectroscopic characterization. The use of the *tert*-butyldimethylsilyl (TBDMS) group as the protecting group gives enough low-temperature solubility in organic solvents to allow spectroscopic characterization, and the 8-methyl group presumably prevents the rearrangement which occurs in guanine peroxides bearing an 8-H substituent.[26]

Photosensitized oxygenations of **6** were carried out in 5-mm NMR tubes in CD_2Cl_2 at −78 °C with tetraphenylporphyrin as sensitizer and a Cermax 300-W xenon lamp as the light source. A 1% potassium dichromate filter solution was used to cut off wavelengths below 500 nm, and an 18-cm-long water filter was placed in front of the sample tube to eliminate heating. The reaction was complete after 48 h, and the starting material was quantitatively converted to the two diastereomeric endoperoxides **7a** and **b** (Scheme II). Control experiments showed that no reaction took place in the absence of light or sensitizer. When the reaction was carried out in protiated solvent under the same condition, only a small amount of the endoperoxide was detected. This result is consistent with the well-known solvent deuterium isotope effect, which leads to an increase in the yields of the singlet oxygen-mediated oxidation.[27-29]

The ^{1}H NMR spectrum (CD_2Cl_2) of the solution at −20 °C showed two well-separated sets of resonances for **7a** and **b** in a ratio A:B = 56:44.[30] The ^{13}C NMR spectrum of the unstable endoperoxides is fully consistent with the structure of the diastereomers **7a** and **b**.[31] Further supporting evidence for quantitative incorporation of 1 mol of oxygen into the purine base came from the low-temperature FAB mass spectrometric analysis.[32] Characteristic peaks at *m/e* (rel intensity) = 672.7 (21, M + 1), 656.4 (16, M + 1 − 16), and 640.3 (100, M + 1 − 32) are strongly indicative of the incorporation of molecular oxygen into the guanine moiety and the loss of one and two oxygens in the mass spectrum.

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(30) Product A showed resonances at δ 6.25 (2H, s, NH_2), 5.24 (1H, d, J = 2.8 Hz, 1′-H), 4.99 (1H, br, 2′-H), 4.62 (1H, dd, J = 4.9, 5.0 Hz, 3′-H), 3.95 (1H, m, J = 4.3 Hz, 5.0 Hz, 4′-H), 3.78 (1H, m, 5′-H), 3.67 (1H, m, 5′-H), and 1.80 (3H, s, 8-CH_3). Product B showed resonances at δ 6.36 (2H, s, NH_2), 5.27 (1H, d, J = 3.6 Hz, 1′-H), 5.09 (1H, dd, J = 3.6 Hz, 4.1 Hz, 2′-H), 4.48 (1H, dd, J = 4.1 Hz, 4.7 Hz, 3′-H), 4.02 (1H, q, J = 4.7, 4.7, 4.7 Hz, 4′-H), 3.78 (1H, m, 5′-H), 3.67 (1H, m, 5′-H), and 1.81 (3H, s, 8-CH_3).
(31) The spectrum at −60 °C showed two sets of 11 carbon peaks at δ = 19.73, 19.88, 60.55, 61.37, 70.11, 70.42, 71.12, 72.03, 82.66, 82.80, 87.48, 88.09, 116.35, 116.92, 151.37, 151.52, 158.36, 159.08, 163.58, 163.60, 167.54, and 167.90.
(32) Low-temperature FAB was done by transferring the sample to the precooled matrix and probe.

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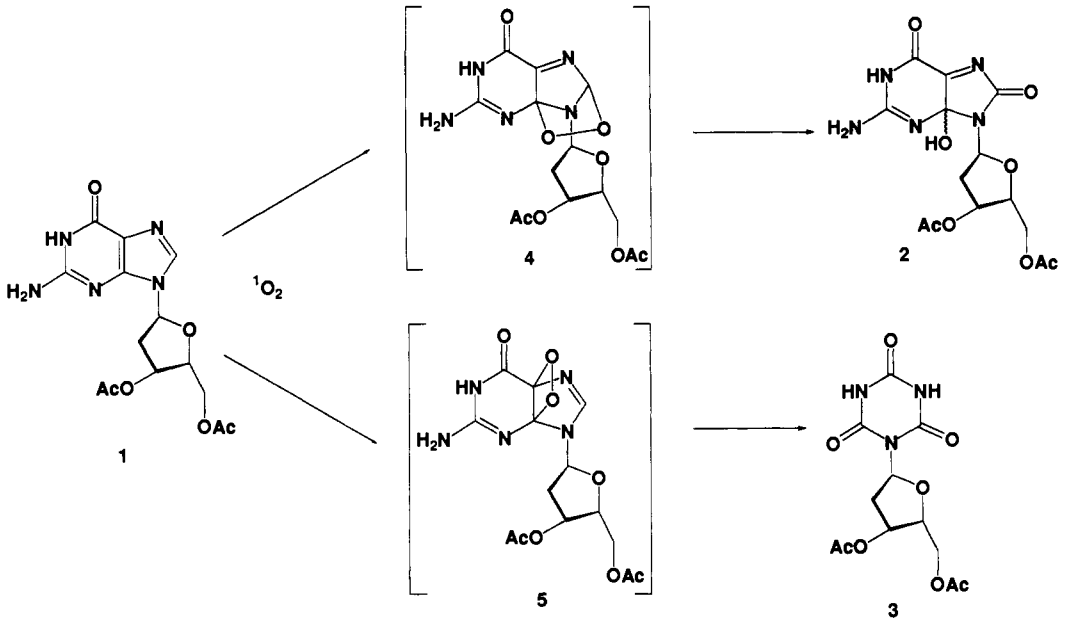
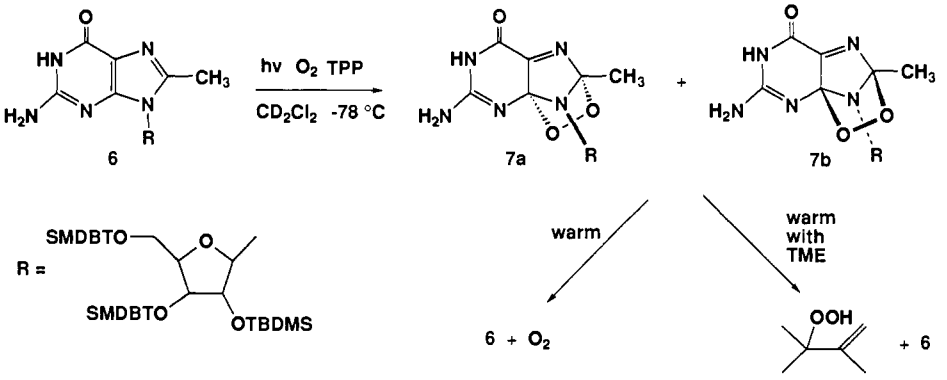
Scheme I



Scheme II



The endoperoxides gradually decompose back to **6** at - 30 °C; however, they undergo no significant decomposition in more than 16 h at –60 °C. Starting material **6** was the only organic product detectable by ^{1}H NMR, ^{13}C NMR, IR, and TLC; the absolute yield was typically over 95%. If tetramethylethylene (TME) was added to a solution of endoperoxides and the solution was warmed to room temperature in the dark, the corresponding hydroperoxide from reaction of $^1O_2$ with TME was the only detectable product by NMR analysis (Scheme II). As determined from the amount of hydroperoxide formed, the yield of singlet oxygen was 21%. The quantitative conversion of **7** to starting material on heating is similar to the reaction of many other endoperoxides[33-35] and is further evidence for the endoperoxide structure.

Photooxygenation of 2′,3′,5′-*O*-(*tert*-butyldimethylsilyl)guanosine or other 8-substituted guanosines such as 2′,3′,5′-*O*-(*tert*-butyldimethylsilyl)-8-methoxy- or -8-(benzyloxy)guanosine at low temperature did not produce endoperoxides detectable by NMR spectroscopy. Thus an alkyl group at the 8 position of guanosine is important for the stabilization of the endoperoxide.

We have no evidence for [2 + 2] cycloaddition to form a dioxetane; [4 + 2] cycloaddition appears to be more favorable in this system. Similar results were observed in the photosensitized oxidations of imidazoles.[34] To our knowledge, this is the first direct observation of an endoperoxide in a purine nucleoside. Further investigations of photooxygenation of guanosine derivatives are in progress and will be reported in the near future.

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Supplementary Material Available: ^{1}H NMR and ^{13}C NMR spectra for **6**, **7a**, and **7b**; H-COSY spectrum of **7a** and **7b** (6 pages). Ordering information is given on any current masthead page.

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