Photosensitized Reaction of 8-Oxo-7,8-dihydro-2′-deoxyguanosine: Identification of 1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)cyanuric Acid as the Major Singlet Oxygen Oxidation Product

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Abstract: The main photosensitized oxidation product in aqueous solution of 8-oxo-7,8-dihydro-2′-deoxyguanosine (**1**) has been isolated by HPLC and characterized as 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-cyanuric acid (**7**) and its precursor, 1,3,5-triazine-1(2*H*)-carboximidamide, 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)tetrahydro-2,4,6-trioxo- (**6**). This was achieved by carrying out extensive spectroscopic measurements including FAB mass spectrometry, ^{1}H and ^{13}C NMR analyses. The formation of the photooxidized nucleoside **7** is accounted for by a type II mechanism through initial [2+2] cycloaddition across the C-4−C-5 ethylenic bond of **1**. The 1,2 bond cleavage of the transient dioxetanes gives rise to the modified nucleoside **6** through a nine-membered ring oxidized intermediate nucleoside. The derivative **6** further decomposes into the stable cyanuric acid nucleoside **7** by the hydrolysis of the guanidino residue with the concomitant release of urea **8**. Photosensitizers such as methylene blue and rose Bengal were found to efficiently photooxidize 8-oxodGuo **1** *via* a type II mechanism ($^1O_2$), whereas riboflavin and benzophenone, which act mostly by a type I mechanism, were far less efficient.

Introduction

Biological and chemical systems generating reactive oxygen species[1] are responsible for a variety of damage to cellular DNA. This includes strand breaks, crosslinks to proteins together with oxidation of the sugar and base residues.[2,3] The resulting lesions of the genetic material play an important role in aging, mutagenesis, and carcinogenesis.[4−6] Among the modified DNA bases, 8-oxo-7,8-dihydro-2′-deoxyguanosine (**1**) (8-oxodGuo) has been widely studied. Recent investigations have focused on its mutagenic potential[7−9] and its repair by enzymes such as the formamidopyrimidine-DNA glycosylase (the Fpg protein)[10,11] and the UvrABC nuclease complex.[12] It should be added that 8-oxodGuo **1** is widely used as a sensitive indicator of oxidative damage to DNA and in a more general way as a biomarker of oxidative stress.[13−16]

The 8-oxo modification of guanine can be produced by hydroxyl radicals •OH[17,18] but also by one-electron oxidation[19] and by singlet oxygen ($^1O_2$).[20] The two latter processes are usually involved in photosensitization reactions. An endogenous or exogenous excited photosensitizer exposed to either UV-A radiation or visible light may return to the ground state *via* two major types of reaction.[21] Type I involves either electron transfer or hydrogen abstraction from the substrate to the excited photosensitizer. On the other hand, type II consists in energy transfer from triplet excited state of sensitizer to ground state oxygen, resulting in the formation of singlet oxygen. However, the formation of 8-oxo-7,8-dihydroguanine (8-oxoGua) damage upon photosensitization of either DNA or 2′-deoxyguanosine is not linear with irradiation time and quickly reaches a plateau.[22,23] This strongly suggests that, once it is produced, 8-oxoGua becomes a better substrate than guanine for further photooxidation. Support for the oxidation of 8-oxoGua derivatives by $^1O_2$ comes from recent studies of Sheu and Foote[24,25] involving, in particular, time-resolved infrared luminescence experiments. In addition, the transient formation of dioxetanes and their subsequent conversion to 4-hydroperoxy-8-oxo-4,8-

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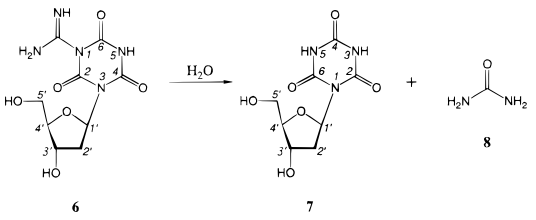
Scheme 1



dihydroguanosine derivatives were followed by ^{1}H NMR analysis at low temperature.[24]

So, it becomes important to elucidate the mechanism of such a further photooxidation: is either type I photosensitized reaction or singlet oxygen-mediated reaction responsible for subsequent oxidation process of 8-oxoGua? Furthermore, the structural determination of the products arising from the oxidation of 8-oxodGuo **1** may be of biological importance to understand why, for example, G → C transversions are observed in single-stranded M13mp2 bacteriophage DNA exposed to methylene blue (MB) + light.[26] Indeed, since the expected G → T transversions induced by the presence of 8-oxoGua[27−29] are not observed, another more potent mutagenic event must occurred.

In a recent study, we have shown that some of the primary products of the MB-photosensitized 8-oxodGuo **1** are an oxazolone **11** and its imidazolone precursor **10** together with the 4*R** and 4*S** diastereoisomers of 4-hydroxy-8-oxo-4,8-dihydro-2′-deoxyguanosine (**4**).[30] Herein, we wish to report the isolation and the structural determination of 1-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)cyanuric acid (**7**) and its precursor 1,3,5-triazine-1(2*H*)-carboximidamide, 3-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)tetrahydro-2,4,6-trioxo- (**6**), as the major $^1O_2$-induced decomposition products of 8-oxodGuo **1** (see Scheme 1). Mechanistic aspects of the $^1O_2$-mediated oxidation of **1** are also presented, and a complete decomposition pathway of the latter nucleoside is proposed.

Results and Discussion

Isolation of the Main Photosensitization Products of 8-oxodGuo 1. The separation of the MB-mediated oxidation products of **1** was achieved on a polar amino column. Indeed, such a silica gel NH_2 column was shown to be very efficient to separate polar modified nucleosides.[31] Whereas most of the oxidation products of photosensitized **1** are eluted in the void volume of the reverse-phase C18 column, the use of the amino column allowed the isolation of the main $^1O_2$ oxidation product **6**. The latter polar photooxidation product has the longer retention time (capacity factor[32] $k' = 12.3$) under the present semipreparative conditions, in agreement with the presence of the two amino exocyclic groups. On the other hand, its decomposition product which was characterized as cyanuric acid nucleoside **7** (*vide infra*) was much less retained on the NH_2 column ($k' = 1.3$). The urea **8** was eluted within the void volume of the column. The other oxidation products, which were produced in lower yields (*vide infra*), were identified[30] and characterized as the oxazolone derivative **11** and its precursor, the imidazolone derivative **10**,[33,34] together with the 4*R** and 4*S** diastereoisomers of **4**.[23] Under such HPLC conditions, the latter oxidized nucleosides exhibit a capacity factor of 4.1, 1.2, 8.0, and 8.7, respectively. In addition, 8-oxodGuo **1** has a capacity factor of 2.2. It should be noted that an unstable unidentified product, which decomposes within a few hours, has a $k' = 0.7$.

Characterization of 7 and Its Precursor 6. The FAB mass spectrum of **6**, recorded in the positive mode, exhibits two peaks at *m/z* 288 ($[M + H]^+$) and *m/z* 172 ($[BH + H]^+$). The specific fragmentation of the pseudomolecular ion at *m/z* 288 reveals a single characteristic daughter ion at *m/z* 172 which corresponds to the aglycon of **6**. However, exact mass measurement was only possible with the derivatized molecule obtained upon a short period of acetylation (25 min). Interestingly acetylation occurred preferentially within the base moiety of **6** instead of the sugar moiety as inferred from the presence of the characteristic fragment of 2-deoxy-D-*erythro*-pentose at *m/z* 117 ($[S]^+$). No degradation of the molecule during acetylation was observed as inferred from the observation of characteristic peaks at *m/z* 372 ($[(M + 2Ac) + H]^+$) and *m/z* 394 ($[(M + 2Ac) + Na]^+$). Exact mass measurement obtained from the fragment corresponding to $[(BH + 2Ac) + H]^+$ (*m/z* = 256.0656) reveals a molecular formula of $C_8H_9N_5O_5$ for the acetylated base moiety. Therefore, relative to **1**, which has a molecular weight of 283 and a molecular formula of $C_{10}H_{13}N_5O_5$, **6** had lost 4 mass units. This corresponds to the addition of one molecule of oxygen (32 amu) and the loss of one molecule of carbon monoxide (28 amu). It should be added that the oxidative modification occurred within the base moiety of **6**. In addition, the aglycon has amino groups very reactive toward acetic anhydride. Furthermore a positive test for the release of a guanidine residue from **6** was observed upon strong alkali treatment (pH 14, 65 °C, 10 min). The UV spectrum of **6** in aqueous solution at pH 7 exhibits a shift in the absorption maximum toward shorter wavelengths, with a shoulder at 215 nm. The absence of strong absorption bands at longer wavelengths is indicative of a considerable loss of aromaticity with respect to **1**.

The milder alkali treatment (pH 13, room temperature) of **6** was monitored by detecting the UV absorbance as a function of time. Compound **6** decomposes very rapidly (half-life time of *ca.* 1 min) to give two stable products, in equimolar amounts, identified as the cyanuric acid nucleoside **7** and urea **8** (Scheme 1). The hydrolysis reaction of **6** occurred by a single decomposition pathway as inferred from the observation of an isosbestic point at 227 nm. The guanidine test was negative with **7**, indicating that no guanidine residue was present in the molecule. The positive FAB mass spectrum of **7** reveals a pseudomolecular ion at *m/z* 246 ($[M + H]^+$), a quasimolecular ion at *m/z* 268 ($[M + Na]^+$) together with the corresponding glycerol adducts at *m/z* 338 ($[M + \text{glycerol} + H]^+$) and *m/z* 360 ($[M + \text{glycerol} + Na]^+$). The exact mass measurement of the monoacetylated cyanuric acid nucleoside **7** is indicative of an empirical formula of $C_{10}H_{13}O_7N_3$. It should be noted that under the mild acetylation conditions used, only one acetyl group is incorporated within the base moiety of **7**. The presence of urea **8** was inferred from its specific detection after spraying the silica gel TLC plates with the (*p*-dimethylamino)benzaldehyde dyeing reagent.[35]

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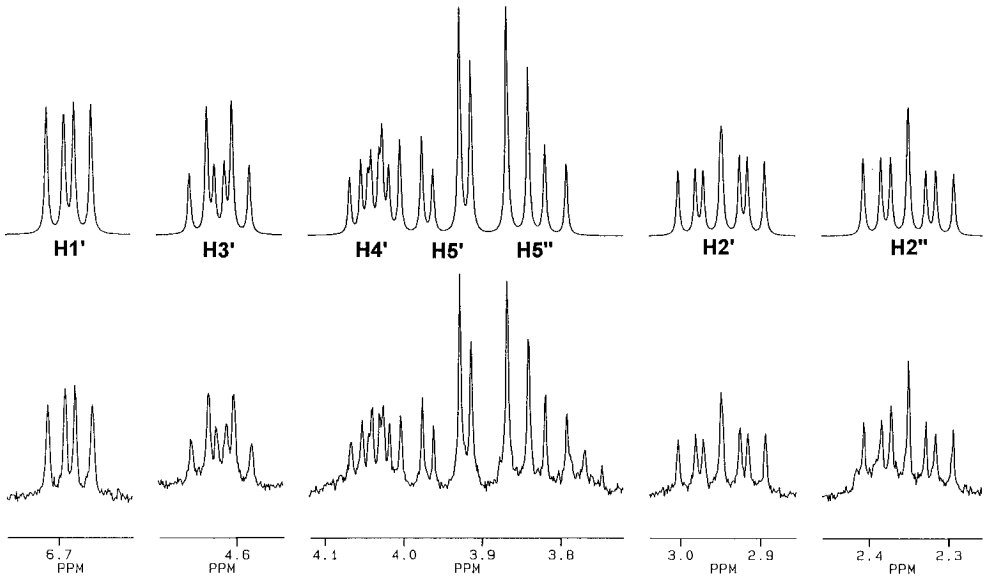

Figure 1. 250.13 MHz ^{1}H NMR spectra of the modified nucleoside **6** in D_2O. Bottom: experimental spectrum. Top: simulated spectrum. An almost similar spectrum was obtained for the cyanuric acid nucleoside **7**, with the exception of the H-1′ chemical shift which is 0.06 ppm upfield shifted.

Table 1. ^{1}H NMR Chemical Shifts (ppm/TSP) and Coupling Constants[a] (Hz) of 8-oxodGuo **1** and Its Main $^1O_2$ Oxidation Products **6** and **7** in D_2O

$J_{i-j\,(Hz)}$	$J_{1'-2'}$	$J_{1'-2''}$	$J_{1'-3'}$	$J_{2'-2''}$	$J_{2'-3'}$	$J_{2''-3'}$	$J_{3'-4'}$	$J_{4'-5'}$	$J_{4'-5''}$	$J_{5'-5''}$
1	7.8	6.9	0.3	−13.9	6.5	3.3	3.2	3.5	5.1	−12.3
6	5.5	8.6	0.3	−13.9	8.0	5.6	5.7	3.6	6.8	−12.0
7	5.4	8.5	0.3	−13.9	8.2	5.6	5.6	3.6	6.8	−12.2

δ (ppm)	H-1′	H-2′	H-2″	H-3′	H-4′	H-5′	H-5″
1	6.31	3.12	2.37	4.73	4.16	3.94	3.89
6	6.68	2.95	2.35	4.62	4.03	3.94	3.84
7	6.62	2.94	2.35	4.62	4.01	3.92	3.83

[a] Estimated errors ±0.1 Hz.

Inspection of the ^{1}H NMR spectra of both **6** and **7**, recorded in D_2O, confirmed that the sugar moiety was not modified upon photosensitization of 8-oxodGuo **1** (Figure 1). H-2′ and H-2″ proton signals were further assigned on the basis of coupling constant arguments.[36,37] The H-5′ signal was assigned downfield to the H-5″ signal according to Remin and Shugar.[38] Interestingly, **6** and **7** exhibit similar ^{1}H NMR features (Table 1). The main difference is a slight upfield shift (0.06 ppm) of the anomeric proton of **7** with respect to that of **6**. This is indicative of a similar structure within the base moiety, at least in the close proximity of the anomeric centre (*e.g.*, two bond apart from the C-1′). The presence of two carbonyl groups in *ortho* positions to the *N*-glycosidic bond mimics a *syn* conformation for both **6** and **7** as already reported for 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-hydroxy-5-methylbarbituric acid and 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-6-methyluracil.[39] The relatively large value for the *cis* coupling constants $J_{1'-2''}$ and $J_{2'-3'}$ also denotes that the related protons are more eclipsed as the result of the decrease in the puckering of the sugar moiety. The similar value for the *trans* coupling constants $J_{2''-3'} \approx J_{3'-4'} \approx$ 5.6 Hz and the pattern $J_{1'-2'} + J_{3'-4'} \approx 11$ Hz[40] are indicative of a significant contribution of a C3′ *endo* puckered conformation in the dynamic equilibrium C3′ *endo* ⇌ C2′ *endo*.[41,42] In addition, the high value for the sum $J_{4'-5'} + J_{4'-5''} = 10.4$ Hz is indicative of a strong destabilization of the *gauche*−*gauche* rotameric population of the 5′-hydroxymethyl group.[43] This is interpreted in terms of electrostatic repulsions between the carbonyl group and the hydroxyl group at C-5′.[44] Furthermore, the diamagnetic anisotropy induced by the carbonyl group in *ortho* to the *N*-glycosidic bond leads to a pronounced downfield shift of the H-2′ and to a slighter extent of the H-3′.[45]

Observation of the exchangeable protons of modified nucleosides **6** and **7** was achieved by carrying out ^{1}H NMR experiments in DMSO-d_6. A broad resonance signal is noted in the low field region of the spectrum of **6** ($\delta = 9.35$ ppm; half-height line width $W_{1/2} = 109$ Hz). The integration of the latter signal is indicative of the presence of four proton atoms. Moreover, addition of D_2O leads to the collapse of the signal. Such a broadening of exchangeable proton resonances may be accounted for by amino groups.[46] In addition, the chemical

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Table 2. ^{13}C NMR Chemical Shifts (ppm) of **6** and **7**[a]: Comparison with Starting 8-oxodGuo **1**

δ (ppm)	C-6	C-2	C-4	C-8	C-5	C-4′	C-1′	C-3′	C-5′	C-2′
1[b]	151.2	153.1	147.1	151.6	98.5	87.3	81.1	71.3	62.2	35.6
6	153.9	149.8[c]	150.9[c]	155.3[d]		87.1	82.0	71.1	62.3	36.7
7	149.6	149.6	148.9			87.2	81.7	70.8	62.1	36.6

[a] All spectra were obtained in DMSO-d_6 at 50.3 MHz. [b] Taken from Cho.[47] [c] The assignment of these two carbon atoms may be reversed. [d] Resonance signal of the C═NH. The broadening of the signal is due to the electric quadrupole moment of the ^{14}N nuclei.

exchange of the latter protons with residual water in the NMR tube may contribute to the broadening of the signals.

The ^{1}H NMR spectrum of **7** exhibits three exchangeable signals including a singlet at $\delta = 11.42$ ppm, in addition to the resonance of the two OH groups of the sugar moiety. The former signal corresponds to the two equivalent NH protons of the aglycon. This was confirmed by comparison with the resonance signal of the three equivalent NH protons ($\delta = 11.28$ ppm) of cyanuric acid, the free base of **7**.

The ^{13}C resonance signals of the sugar moiety of **6** and **7** were assigned by selective proton decoupling experiments. The respective chemical shifts are similar to those of the sugar moiety of the starting 8-oxodGuo **1** (Table 2). Additional structural information was gained from the analysis of the ^{13}C resonance signals of the aglycon of **6** and **7**. The two symmetrical C═O in *ortho* positions to N-1 were assigned to the signal at 149.6 ppm (compound **7**) by comparison with cyanuric acid. Indeed, the ^{13}C NMR spectrum of cyanuric acid, which has three similar carbon atoms, exhibited a single resonance signal at 149.9 ppm. The remaining signal at 148.9 ppm was attributed to the carbonyl C-4. It should be noted that the signal at 149.6 ppm was twice as intense as that at 148.9 ppm. The ^{13}C NMR spectrum of the aglycon of **6** showed four resonances, characteristic of quaternary sp^2 carbon atoms.

In conclusion, the chemical and spectroscopic data described above are consistent with a cyanuric acid nucleoside for **7**. The assignment of nucleoside **6** is not so straightforward. In fact two structures may be inferred from the consideration of the spectrometric data and chemical properties of **6**. A major relevant information deals with the fact that the sugar moiety of **6** and of its transformation product **7** exhibits almost identical ^{1}H and ^{13}C NMR features. As already mentioned this is characteristic of 2′-deoxyribonucleosides which mimic a *syn* conformation. Among the two possible structures, 1,3,5,7-tetrazocine-2,4,6(1*H*,3*H*,5*H*)-trione, 8-amino-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)- has an eight-membered ring, whereas 1,3,5-triazine-1(2*H*)-carboximidamide, 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)tetrahydro-2,4,6-trioxo- (**6**) exhibits a six-membered ring. The similarities in the NMR properties of the furanose ring of **6** and **7** is better rationalized in terms of a six-membered ring structure for **6**. This should allow a similar spacial orientation of the two C═O in the *ortho* positions to the *N*-glycosidic bond in **6** and **7**. A higher flexibility would be expected with an eight-membered ring structure, and therefore this should affect the relative orientation of the keto groups with respect to the sugar moiety. The assignment of the ^{13}C NMR resonances is also in agreement with the proposed structure. The broadest signal at 155.3 ppm may be assigned as the C═NH. The broadening of the latter signal, even in a proton-decoupled spectrum, is due to electric quadrupole moment of the three vicinal nitrogen atoms as already observed for similar structures.[34] The signals at 150.9 and 149.8 ppm were assigned as the two C═O in the *ortho* positions to N-3. The slight difference in the chemical shifts of the latter signals (1.1 ppm) did not allow the exact attribution of the two carbons. The remaining signal at 153.9 ppm was attributed to C-6. Additional relevant support for the six-membered ring structure is provided

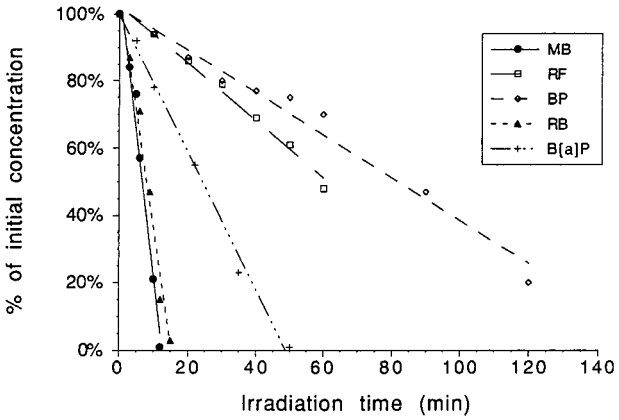

Figure 2. Consumption of 8-oxodGuo **1** as a function of irradiation time in the presence of different photosensitizers (MB and RB which produce large amount of $^1O_2$ (type II); BP, and RF, as mainly type I sensitizers; B[a]P, as both type I and type II sensitizer). The initial concentration of 8-oxodGuo **1** in water was 1 mM.

by the conversion of **6** into the cyanuric acid nucleoside **7** in aqueous solutions at pH 7 at room temperature. Within a few days **6** is quantitatively converted into the stable cyanuric acid nucleoside **7** with the concomitant release of urea **8**. Interestingly, **6** is much less stable in DMSO which should catalyze the hydrolysis reaction. The half-life time was estimated to be 15 h, as inferred from quantitative measurements of the H-1′ resonance signal of **6** in the ^{1}H NMR spectra.

Quantitative Analysis. The disappearance of 8-oxodGuo **1** upon photosensitization was followed by using a sensitive HPLC electrochemical detection assay. Different rates of photooxidation were observed depending on the photosensitizer which was used (Figure 2). In this respect, rose Bengal (RB) and methylene blue (MB) were much more efficient than riboflavin (RF) or benzophenone (BP) to photosensitize the decomposition of **1**. These observations strongly suggested that 8-oxodGuo **1** reacts mostly and efficiently with singlet oxygen. Indeed, RB and MB are known to produce singlet oxygen in large amount[48−50] by a type II mechanism, as defined by Foote.[21] On the other hand, RF and BP, which act mainly by type I photosensitized oxidation, do not produce significant amount of $^1O_2$.[51,52] The rate of photooxidation of 8-oxodGuo **1** was considerably lowered by using the latter type I photosensitizers. Photosensitization experiment performed with benzo[*a*]pyrene (B[a]P) reveals an intermediate rate of disappearance of **1** between those of MB or RB experiments (type II process) and RF or BP experiments (type I process). This result is consistent with a previous study which has established that B[a]P acted on 2′-deoxyguanosine by both type I and type II mechanisms

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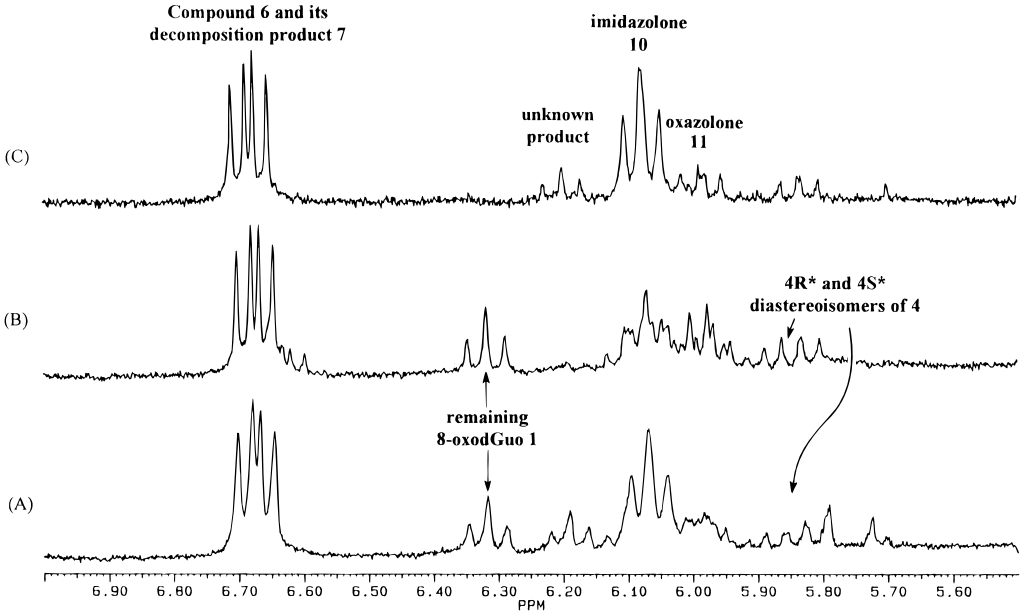

Figure 3. Region of the resonance signals of the anomeric protons of the 250.13 MHz ^{1}H NMR spectra in D_2O of MB-photosensitized 1mM 8-oxodGuo **1**: (A) immediately after 35 min of visible light irradiation in H_2O: (B) the same irradiated sample after being left 20 h in the dark at room temperature, and (C) immediately after 10 min MB-photosensitized 1 mM 8-oxodGuo **1** in D_2O.

in an approximately 1:1 relative ratio.[52] It should be noted that under the conditions of irradiation used, the photodynamic effect mediated by the latter photosensitizers on 2′-deoxyguanosine was similar whatever the sensitizer used. In addition, MB-photosensitization experiments performed in D_2O instead of H_2O led to a 5-fold increase in the decomposition rate of **1**. The latter result provided additional support for the involvement of $^1O_2$ in the photooxidation of 8-oxodGuo **1**, since deuteriated solvents are known to increase the lifetime of singlet oxygen.[53] However, it is important to note that deuteriated solvents may also increase the lifetime of triplet excited state of photosensitizers.[54] Hence, the isotopic effect associated with the use of D_2O does not constitute an unambiguous proof for the involvement of $^1O_2$ in photosensitization reactions.

Interestingly, whatever the photosensitizer used, the final distribution of the oxidation products was similar as inferred from the HPLC elution profiles. In this respect, similar HPLC elution profiles were obtained upon either MB or RB mediated photosensitization of 8-oxodGuo **1** after 20 min of illumination. It may be added that similar HPLC degradation profiles of **1** were also generated by photoexcited riboflavin and benzophenone. However, this required a 160 min irradiation period. Further qualitative and quantitative information was obtained by using ^{1}H NMR spectroscopy as the analytical tool, as illustrated in Figure 3. Immediately after visible light irradiation, the MB-photosensitized solution of 1 mM 8-oxodGuo **1** was concentrated to dryness and redissolved in D_2O prior to ^{1}H NMR analysis. The 250.13 MHz spectrum shows in the region of the anomeric protons ($7.0 < \delta$ (ppm) < 5.5) several resonance signals including a doublet of doublet at 6.68 ppm (spectrum A) which corresponds to **6**. It should be noted that after 20 h in the dark at room temperature (spectrum B), **6** was barely decomposed into **7** as inferred by the increase of the H-1′ signal of **7** at 6.62 ppm. The determination of the relative yields of **4**, **7**, and **11** was achieved by ^{1}H NMR analysis of the crude mixture. This was further confirmed by the integration of the related HPLC peaks upon separation on the silica gel NH_2 column. The relative integration of the H-1′ resonance signal of each nucleoside indicates that **6** represents more than 50% of the final stable oxidation products of 8-oxodGuo **1**. The second modified nucleoside by decreasing importance, which is produced in a yield of *ca.* 35%, was identified as the imidazolone derivative **10**. It further decomposes into the oxazolone derivative **11** (spectrum B) with the half-life of 24 h at 20 °C. The other previously identified products,[30] the 4*R** and 4*S** diastereoisomers of **4** are present in a relatively lower yield (<10%). The formation of an unidentified product which exhibits a H-1′ signal at $\delta = 6.19$ ppm was also observed (spectrum A). Nevertheless, the latter minor product (yield of *ca.* 5%) is unstable and decomposes rapidly (spectrum B). It is interesting to note that the final distribution of the oxidation products arising from MB-photosensitized 8-oxodGuo **1** performed in either D_2O (spectrum C) or H_2O is the same. Heavy water increases only the rate of decomposition of **1** without any detectable effect on the relative yield of the final oxidation products. This provides further evidence for the involvement of singlet oxygen in the photooxidation of 8-oxodGuo **1**.

Reaction of $^1O_2$ with 8-oxodGuo 1: Mechanism of Formation of the Oxidation Products. It has been clearly established that the predominant structure of 8-oxodGuo **1** is the 6,8-diketo tautomeric form.[55−57] Hence, a [2 + 2] cycloaddition of $^1O_2$ across the C-4−C-5 double bond on both sides of the purine ring of **1** is the most likely process, as recently suggested for 8-oxoGua derivative.[24] However, one cannot exclude a [4 + 2] cycloaddition of $^1O_2$ with the small fraction of the 8-enol form which is in equilibrium with the 8-keto tautomer in aqueous solution.[58] Then, the decomposition of the unstable endoperoxides may explain, at least partly, the formation of the unknown product. However, it should be noted that this pathway does represent only a minor process.

The formation of the bulk of photooxidized decomposition products of **1** may be rationalized in terms of initial formation

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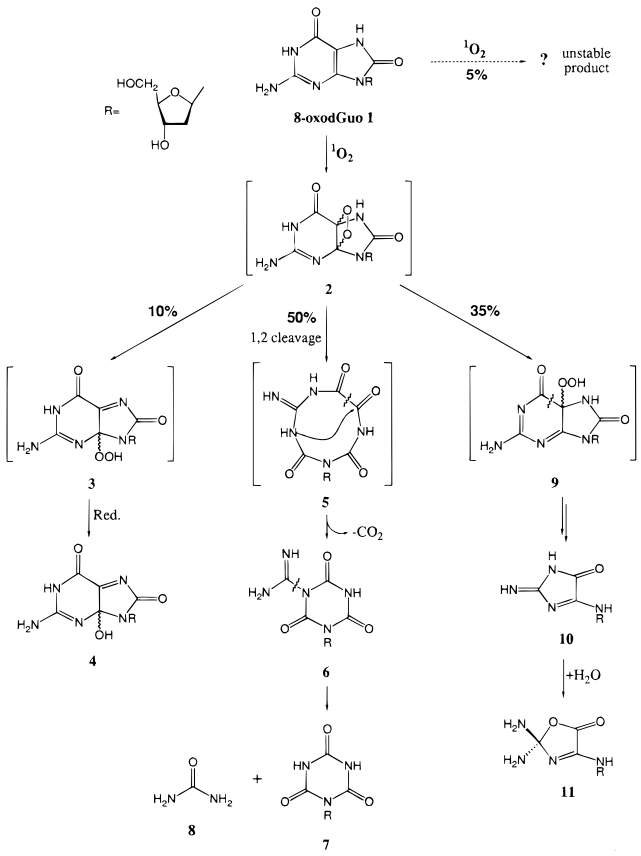
Scheme 2



of the unstable dioxetanes **2**. These may decompose according to three main pathways as depicted in Scheme 2. The thermal decomposition of the 1,2-dioxetanes is the predominant one and leads to the formation of the transient nine-membered ring nucleoside **5**. The intramolecular cyclization between N-3 and the carbonyl C-5 gives rise to the formation of **6** after the release of carbon dioxide from C-6.[59,60] The latter nucleophilic addition is in agreement with a recent study[24] in which the formation of cyanuric acid was suggested to occur after the N-3′ → C-5 cyclization of the nine-membered ring nucleoside **5**. The mild hydrolysis of **6** leads to the formation of urea **8** and the cyanuric acid nucleoside **7**. The two other decomposition pathways of **2** involve the formation of the transient hydroperoxides **3** and **9**. However, they can also be produced directly from 8-oxodGuo **1**. Indeed with compounds presenting electron-rich-donating atoms, such as nitrogens, adjacent to a double bond and with an hydrogen in the α position, singlet oxygen can react to give both unstable dioxetanes by a [2 + 2] cycloaddition[61] and also allylic hydroperoxides by an ene-type reaction.[62] The hydroperoxide intermediates **3** are the likely precursors of the two stable 4*R** and 4*S** diastereoisomers of **4**[23,24] *via* a reduction step. This pathway represents only a minor route (relative yield of **4**: 10%). The second process of importance involves the opening of the pyrimidine ring at the C-5−C-6 bond of the hydroperoxides **9** to give rise, after further rearrangements, to the formation of imidazolone **10** which hydrolyses into the oxazolone **11**. It should be noted that similar reactions from the derivative **9** are likely to take place when 2′-deoxyguanosine is exposed to either •OH radicals or photoexcited type I photosensitizers.[33,63]

Conclusion

Singlet oxygen, which was produced by type II photosensitization, reacts with 8-oxodGuo **1** to yield the modified nucleoside **6** and its primary decomposition product 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)cyanuric acid (**7**) as the major oxidation products. This result clearly establishes that **7** is a secondary photooxidation product of 2′-deoxyguanosine. When 2′-deoxyguanosine or DNA solutions are irradiated for a long period of time with photosensitizers producing singlet oxygen, the formation of **7** *via* $^1O_2$ oxidation of 8-oxodGuo **1** rapidly becomes an important process. Indeed, the formation of cyanuric acid nucleoside **7** was already observed in the past but described as the $^1O_2$-induced oxidation of 2′-deoxyguanosine when more than 50% of the latter nucleoside was decomposed.[51,64] It should be added that the measurement of the rate of consumption of 8-oxodGuo **1** constitutes an interesting alternative approach for determining whether a photosensitizer is an efficient generator of $^1O_2$ (type II mechanism).

Experimental Section

Chemicals. 2′-Deoxyguanosine was purchased from Pharma-Waldhof (Düsseldorf, Germany). BP and MB were from Merck (Darmstadt, Germany). Cyanuric acid and urea were from Aldrich (Milwaukee, WI) whereas RF, RB, and 1,2-naphthoquinone-4-sulfonic acid were obtained from Sigma Chemical Company (St. Louis, MO). B[a]P was a gift from Dr. D. Averbeck (Institut Curie-Section de Biologie, Paris, France). HPLC grade methanol and acetonitrile were obtained from Carlo Erba (Farmitalia Carlo Erba, Milan, Italy). Deuterium oxide (D_2O, 99.96%D) and deuteriated dimethyl sulfoxide (DMSO-d_6, 99.96%D) used for NMR spectroscopy were purchased from Eurisotop (St. Aubin, France). Water was deionized with a Millipore-Milli-Q system. 8-OxodGuo **1** was synthesized according to Cho *et al.*[65]

HPLC Analysis. The HPLC system consisted of two Model 302 HPLC pumps connected to a Model 811 dynamic mixer (Gilson, Middelton, WI), a Model LP-21 pulse damper (Scientific System Inc., State College, PA), a Sil-9A Shimadzu automatic injector (Kyoto, Japan), and a L-4000 UV variable wavelength spectrophotometer (Merck, Darmstadt, Germany) set at 230 nm. The instruments were connected to an Apple IIe computer which controlled the mobile phase composition and the flow rate, usually set at 1 and 3 mL/min for analytical and semipreparative separations, respectively. HPLC elution profiles were recorded, and the peaks of interest were integrated by using a Data Master Model 621 (Gilson), which analyzed the signal coming from the UV detector. The integrator was interfaced with the computer through the HPLC system manager software model 704 (Gilson). The semipreparative (250 × 10.0 mm i.d.) and the analytical (250 × 4.6 mm i.d.) amino substituted silica gel (mean particle size 5 μm) Hypersil NH_2 column were obtained from Interchim (Montluçon, France). The eluent was a mixture of acetonitrile and water in a [85:15] v/v and [75:25] v/v ratio for the analytical and semipreparative purposes, respectively. Under such HPLC conditions, MB was eluted within the void volume of the column.

The consumption of 8-oxodGuo **1** during photosensitization was monitored by HPLC with electrochemical detection.[13,66] For this

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purpose, 5 μL of the irradiated solution was injected onto an analytical (250 × 4.6 mm i.d.) octadecylsilyl silica gel column (Interchim, Montluçon, France). A Model 2150 LKB pump (Pharmacia LKB Biotechnology, Uppsala, Sweden) equipped with a Sil-9A Shimadzu automatic injector (Kyoto, Japan) was set at a flow rate of 1 mL/min of a [87:13] v/v 50 mM sodium citrate (pH 5) and methanol. The electrochemical detection was performed by amperometry using a Model LC-4B/LC-17A (T) apparatus (Bioanalytical Systems, West Lafayette, IN) using two glassy-carbon electrodes in parallel at a potential of +650 mV with respect to an Ag/AgCl reference electrode. The output signal was recorded and integrated by a Model 730 Data Module (Waters Associates, Milford, MA).

Spectroscopic Measurements. Ultraviolet absorption spectra were obtained in water with a Hewlett-Packard 8452A diode array spectrophotometer (Amsterdam, The Netherlands). Fast atom bombardment (FAB) mass spectra were recorded in the positive mode with a ZAB 2-SEQ spectrometer (Fisons-VG, Manchester, UK) equipped with a LSIMS source. The molecules were dissolved in a glycerol-thioglycerol matrix containing 0.1 N NaI and then desorbed upon exposure to a 35 keV Caesium ions beam. The ^{1}H NMR spectra were recorded in the Fourier transform mode with a WM 250 Brüker apparatus. Conformational studies were performed in D_2O, and spectral assignments of the proton signals were achieved by homonuclear decoupling experiments. To verify the assignments and to obtain accurate chemical shifts and coupling constants, the spectra were computer-simulated using the iterative LAOCOON III and PANIC Brüker programs. The chemical shifts were determined with respect either to 3-(trimethylsilyl)-propionate-*2,2,3,3-d*$_4$ sodium salt (TSP) in D_2O or tetramethylsilane (TMS) in DMSO-d_6 as the internal references (0.00 ppm). The proton-decoupled ^{13}C NMR spectra were obtained in DMSO-d_6 with an AC 200 Brüker spectrometer operating at 50.3 MHz. The signal of the solvent was used as the secondary reference set at 39.5 ppm. All the spectra were recorded at 295 K using a 5 mm probe.

Photosensitization Procedure. UV-A irradiations were performed in a Rayonet photoreactor (The Southern New England Ultraviolet Company, Hamden, CT) equipped with 16 "black light" lamps (24 W) emitting about 90% of the light in the 350 nm range. The visible light was generated by a 100 W tungsten lamp. The solution to be irradiated was placed in a Pyrex flask at 20 cm apart from the light source. For both irradiation systems, a continuous air bubbling was provided under stirring. This maintained the solution saturated with molecular oxygen (0.25 mM). The solution was kept at *ca.* 12 °C during irradiation by cooling with a water streamer immersed in the solution. Typically, for the analytical purposes, 2 mL of 1 mM 8-oxodGuo **1** aqueous solution (pH 7) containing 10 μM of MB, RB, or RF was irradiated with the visible light from the tungsten lamp. The UV-A lamps were used when experiments were performed either with BP saturated solution (0.4 mM) or with 20 μL of saturated B[a]P ethanolic solution. To monitor the formation of photoproducts, 100 μL aliquots were removed as a function of time and injected onto the HPLC column.

1,3,5-Triazine-1(2*H*)-carboximidamide, 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)tetrahydro-2,4,6-trioxo- (6). 8-OxodGuo **1** (10 mg, 35.3 μmol) was dissolved in 35 mL of deionized water containing 10 μM MB. After 30 min of visible light irradiation, the solution was concentrated under reduced pressure (30 °C), and the residue was resuspended in the minimum volume of eluent prior to semipreparative HPLC analysis. Lyophilization of the combined fractions ($k' = 12.3$) provided 5.2 mg (yield 51%) of **6** as a powder. Exact mass measurement, see text: UV (H_2O, pH 7) λ_{max}(nm) 215 (shoulder); ^{1}H NMR (250 MHz; D_2O) see Table 1; ^{1}H NMR (250 MHz; DMSO-d_6) δ 9.35 (br, 4H, NH), 6.53 (*pseudo*-t, 1H, H-1′), 5.17 (d, 1H, OH-3′), 4.69 (*pseudo*-t, 1H, OH-5′), 4.37 (m, 1H, H-3′), 3.77 (m, 1H, H-4′), 3.68 (m, 1H, H-5′), 3.53 (m, 1H, H-5″), 2.78 (m, 1H, H-2′), 1.98 (m, 1H, H-2″); ^{13}C NMR (50 MHz; DMSO-d_6) see Table 2; FAB-MS (positive mode, rel intensity) *m/z* 288 (3%, $[M + H]^+$), 172 (16%, $[BH + H]^+$) and 117 (3%, $[S]^+$).

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)cyanuric acid (7). **7** was obtained by the quantitative hydrolysis of a slightly alkaline solution (pH 10) of **6** previously isolated. After complete transformation (48 h at 25 °C), the solution was neutralized by 0.1 N HCl solution, and the solvent was removed by rotary evaporation under reduced pressure (30 °C). The semipreparative HPLC containing fraction ($k' = 1.3$) was concentrated and then lyophilized to yield **7** as a powder (4.4 mg). Exact mass measurement, see text: UV (H_2O, pH 7) λ_{max}(nm) 218 (shoulder); ^{1}H NMR (250 MHz; D_2O) see Table 1; ^{1}H NMR (250 MHz; DMSO-d_6) δ 11.42 (br, 2H, NH), 6.43 (*pseudo*-t, 1H, H-1′), 5.18 (d, 1H, OH-3′), 4.68 (*pseudo*-t, 1H, OH-5′), 4.36 (m, 1H, H-3′), 3.74 (m, 1H, H-4′), 3.64 (m, 1H, H-5′), 3.56 (m, 1H, H-5″), 2.79 (m, 1H, H-2′), 2.03 (m, 1H, H-2″); ^{13}C NMR (50 MHz; DMSO-d_6) see Table 2; FAB-MS (positive mode, rel intensity) *m/z* 360 (4%, $[M + \text{glycerol} + Na]^+$), 338 (3%, $[M + \text{glycerol} + H]^+$), 268 (8%, $[M + Na]^+$), 246 (4%, $[M + H]^+$), 130 (3%, $[BH + H]^+$) and 117 (5%, $[S]^+$).

Acetylation of the Modified Nucleosides 6 and 7. One milligram of either **6** or **7** in the dry state was dissolved in 0.4 mL of acetic anhydride and 1 mL of pyridine. The resulting solution was stirred with a magnetic bar for 25 min at room temperature. Then, the reaction was stopped by addition of 0.5 mL of ethanol. The solvents were removed under vacuum, and the mixture was lyophilized. The crude mixture was analyzed by FAB mass spectroscopy without any purification.

Chemical Detection of Guanidine. The guanidine residue was quantitatively detected by using an adaptation of a method described for postcolumn detection.[67] Typically, 100 μL of the solution to be analyzed was mixed with 50 μL of 1 M sodium hydroxide. After homogenization, the solution was placed in a water bath at 65 °C for 10 min. Then, 10 μL of 0.8 mg/mL 1,2-naphthoquinone-4-sulfonic acid was added, and the resulting solution was placed for 2 min at 65 °C. The sample was neutralized by addition of 50 μL of 1 M hydrochloric acid prior to reverse-phase HPLC analysis. The conditions of detection of the fluorescent aromatic derivative ($k' = 5.0$) are the following: excitation at 355 nm and emission at 405 nm. The eluent was a mixture of 25 mM ammonium formiate and methanol, [90:10] v/v, and the flow rate was set at 1 mL/min.

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Supporting Information Available: Figures of 250.13 MHz ^{1}H NMR spectra of the cyanuric acid nucleoside **7** in D_2O [bottom experimental spectrum and top simulated spectrum] and 50.3 MHz ^{13}C NMR proton decoupled of the cyanuric acid nucleoside **7** in DMSO-d_6 (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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