Photooxidative Damage of Guanine in DG and DNA by the Radicals Derived from the α Cleavage of the Electronically Excited Carbonyl Products Generated in the Thermolysis of Alkoxymethyl-Substituted Dioxetanes and the Photolysis of Alkoxyacetones

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On thermolysis of the methoxy (**MeO-TMD**), *tert*-butoxy (**^{t}BuO-TMD**), and hydroxy (**HO-TMD**) derivatives of 3,3,4,4-tetramethyl-1,2-dioxetane (**TMD**) in the presence of **dG** and calf-thymus DNA, the guanine is oxidized considerably more efficiently than the parent **TMD**. The same trend in the oxidative reactivity is observed for the photolysis of the corresponding oxy-substituted ketones versus acetone. The oxidative reactivity order in the dioxetane thermolysis, as well as in the ketone photolysis, parallels the ability of the excited ketones to release radicals (determined by spin trapping with **DMPO** and EPR spectroscopy) upon α cleavage (Norrish-type-I reaction). In the presence of molecular oxygen, the carbon-centered radicals are scavenged to produce peroxyl radicals, which are proposed as the reactive species in the oxidation of the guanine in **dG** and calf-thymus DNA.

Introduction

In oxidative stress, reactive oxygen species such as oxyl radicals (hydroxyl, alkoxyl, and peroxyl), superoxide radical anion, singlet oxygen, hydrogen peroxide or alkyl peroxides are responsible for the damage of cellular constituents through oxidation.[1] The consequences of the oxidative damage in the case of DNA may be mutagenesis and carcinogenesis.[2] As for the origin of these oxidants, they are formed during oxygen metabolism[3] or by exposure to UV radiation (sunlight). The latter involves electronically excited states, which may be generated by excitation of endogenous sensitizers, but also by lipid peroxidation (Russell mechanism).[4]

The induction of DNA damage by electronically excited compounds directly (e.g., triplet ketones) is well established.[5] Since 1,2-dioxetanes produce electronically excited ketones upon thermolysis, they are ideally suited to assess their excited-state reactivity without the need of exposing the biological target directly to UV irradiation. Another advantage is the selective generation of triplet-excited ketones on thermal decomposition of dioxetanes.[6] The propensity of various dioxetanes to damage DNA in cell-free as well as in cellular systems has been demonstrated.[7,8]

In our group, the oxidative damage of **dG** and DNA in the thermolysis of 3-(hydroxymethyl)-3,4,4-trimethyl-1,2-dioxetane (**HO-TMD**) has been studied intensively since it is significantly more effective than the merely alkyl-substituted **TMD**.[9] The products detected in the oxidation of calf-thymus DNA have been 7,8-dihydro-8-oxoguanine (**8-oxoGua**) and guanidine-releasing products (**GRP**), e.g. oxazolone. The oxidative damage was attributed to

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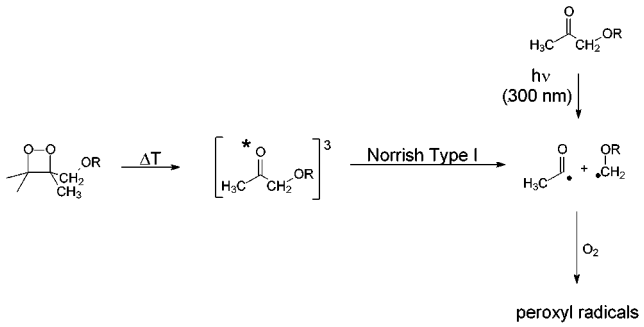
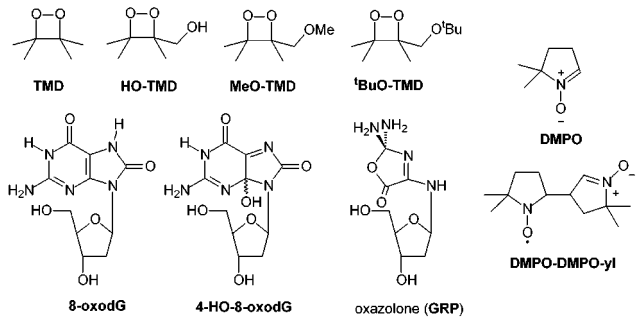
Scheme 1. Generation of Radicals in the Thermolysis of 1,2-Dioxetanes and in the Photolysis of the Corresponding Ketones



the radical species released upon α cleavage (Norrish type I) of the initially formed excited ketone (Scheme 1). The



carbon-centered radicals are trapped by molecular oxygen, and the resulting peroxyl radicals are made responsible for the observed DNA oxidation products. The higher efficiency of **HO-TMD** to oxidize DNA compared to **TMD** relates to the fact that the latter generates triplet acetone, which is well established to undergo reluctantly α cleavage[10] relative to the triplet hydroxyacetone[11] derived from **HO-TMD**.

The large difference in the capacity of **HO-TMD** versus **TMD** to oxidize guanine by means of the in-situ-generated radicals raised the question how efficiently alkoxy-substituted dioxetanes would cause such damage. For this purpose, the alkoxy-substituted derivatives **MeO-TMD** and **^{t}BuO-TMD** were chosen, both of which are unknown, but readily prepared from **HO-TMD** by alkylation. Since these alkoxymethyl-substituted dioxetanes release triplet-excited ketones, namely methoxyacetone from **MeO-TMD** and *tert*-butoxyacetone from **^{t}BuO-TMD**; for comparison, it was important to examine the oxidation of **dG** and DNA by these ketones on photoexcitation. In this comparative study, we present the results on the oxidation of **dG** and DNA in the thermolysis of dioxetanes and the photolysis of the respective ketones. The efficiency of the oxidative damage is correlated with the release of radicals (determined by spin trapping with **DMPO** and EPR spectroscopy) through α cleavage of the electronically excited ketones.

Results

Dioxetane Decomposition Kinetics: Relative Triplet-Excitation Efficiency. The rates of the dioxetane (1 mM) thermolysis were determined by monitoring the decay of the chemiluminescence intensity at 50 °C in CH_3CN (Table 1). The decomposition rate constants (k_{dec}) for the alkoxymethyl-substituted dioxetanes were determined by fitting the data to an exponential function (first-order kinetics) and were found to be slightly smaller than for the unsubstituted 3,3,4,4-tetramethyl-1,2-dioxetane (**TMD**) or the hydroxymethyl-substituted (**HO-TMD**) ones. For **MeO-TMD** and **^{t}BuO-TMD** also the decay at 37 °C (5 mM) and 65 °C (1 mM) was measured to calculate the activation parameters of these two dioxetanes from these data (**MeO-TMD**: $\Delta H^{\ddagger} = 24.6 \pm 1.0$ kcal/mol, $\Delta S^{\ddagger} = 4.3 \pm 3.2$ cal/mol K, **^{t}BuO-TMD**: $\Delta H^{\ddagger} = 24.4 \pm 0.5$ kcal/mol, $\Delta S^{\ddagger} = -4.6 \pm 1.6$ cal/mol K, the data for **TMD** and **HO-TMD** may be found in ref 12).

Table 1. Triplet-Excitation Parameters of the Dioxetanes and Relative Efficiencies of the DG Oxidation

dioxetane	k_{dec} (10^{-6} s^{-1})	Φ^T [b] (%)	E^T_p [c] (10^{17} s^{-1} L^{-1})	rel efficiency of **dG** oxidn [a] dioxetane	ketone
TMD	4.3 ± 0.2	35 ± 3 [d]	9.1 ± 1.1	0.1 ± 0.1	0.0 ± 0.1
HO-TMD	4.9 ± 1.2	14 ± 3 [d]	4.1 ± 0.9	1.0 ± 0.3	1.0 ± 0.4
MeO-TMD	3.4 ± 0.5	17 ± 4	3.5 ± 0.8	1.5 ± 0.6	0.1 ± 0.1
^{t}BuO-TMD	3.2 ± 0.3	8 ± 8	1.6 ± 0.3	1.2 ± 0.2	1.2 ± 0.1

[a] The values for **HO-TMD** and hydroxyacetone were set to unity. [b] Calculated according to eq 1 (T = 37 °C). [c] Relative to **HO-TMD** in CH_3CN. [d] Data from ref 12.

To determine the amount of triplet-excited ketones formed during dioxetane thermolysis, the triplet quantum yield (Φ^T) was measured by chemiluminescence with 9,10-dibromoanthracene (**DBA**) as triplet-energy acceptor (variation of the concentration of **DBA** at a constant temperature and a constant dioxetane concentration);[13] the data are given in Table 1. From the triplet quantum yields (Φ^T) and the decomposition rate constants (k_{dec} at 37 °C), the triplet excitation flux (E^T_P) was calculated according to the established relation in eq 1. The E^T_p parameter is a useful measure of the effective number of triplet-excited species generated in the thermolysis of the dioxetanes per unit time and volume.[14] Significant is the fact that the E^T_p values (Table 1) for the alkoxymethyl-substituted dioxetanes were all smaller than that for the unsubstituted **TMD**.

$$E^T_p = \Phi^T \times k_{dec} \times N_A\ [\text{dioxetane}] \qquad (1)$$

E^T_p triplet excitation flux
Φ^T triplet excitation quantum yield
k_{dec} decomposition rate constant of the dioxetane at 37 °C
N_A Avogadro constant

Relative Efficiency of dG Oxidation. To assess the relative efficiency of the various dioxetanes to oxidize **dG,** the amount of **dG** consumed (% conversion) in the thermolysis of the particular dioxetane (20.0 mM) with **dG** (0.500 mM) was determined in a 9:1 mixture of phosphate buffer (5.00 mM, pH 7.0) and acetonitrile at

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Table 2. Absorption Characteristics of the Ketones[a]

CH_3COCH_2X, X =	λ_{max} (nm)	ϵ_{max}[b] (L/mol cm)
H	265	8
OH	270	8
OMe	269	11
O^{t}Bu	268	19

[a] 40 mM in 9:1 H_2O:CH_3CN, 20 °C. [b] Error ± 10%.

50 °C and 15 h. For ease of comparison, the extent of **dG** oxidation induced by the triplet-excited ketones generated in the dioxetane thermolysis was calculated relative to that of **HO-TMD**, which was set to unity (Table 1). As the data in Table 1 display, the alkoxymethyl-substituted dioxetanes are slightly more efficient than **HO-TMD** in oxidizing **dG**, but all oxymethyl-substituted dioxetanes are as much as five to seven times more efficient than the parent derivative **TMD**. Comparison of the relative efficiency (% conversion) of the **dG** oxidation with the relative efficiency of excited-state production (E^T_P) for the various dioxetanes reveals that there is no direct correspondence (Table 1). Thus, while the E^T_P values follow the order **TMD** > **HO-TMD** > **MeO-TMD** > **^{t}BuO-TMD**, their efficiency of **dG** oxidation follows essentially the opposite trend, i.e., **TMD** < **HO-TMD** ~ **MeO-TMD** ~ **^{t}BuO-TMD**.

Since α cleavage has been proposed[15] as the major radical-generating process to account for the enhanced **dG** oxidation in the thermolysis of **HO-TMD** compared to **TMD**, the **dG**-oxidation experiments were also performed by direct irradiation of the respective ketone derived from the dioxetane thermolysis [20.0 mM ketone, 0.500 mM dG in a 9:1 mixture of phosphate buffer (5.00 mM, pH 7.0) and acetonitrile at 300 nm and 0 °C for 5 h]. The extent of **dG** conversion has been corrected for the molar extinction coefficient of the ketone chromophore to normalize the damage relative to the number of excited species formed (Table 2).

Analogous to the dioxetanes with **HO-TMD** as reference, the relative oxidation efficiency has been taken versus hydroxyacetone as reference (set to unity). The ketone photolysis data show (Table 1, last column) that while the 1-hydroxy-2-propanone and 1-*tert*-butoxy-2-propanone possess the same relative efficiency to oxidize **dG** as the corresponding triplet-excited ketones formed in the dioxetane thermolysis (Table 1, fifth column), 1-methoxy-2-propanone is much less efficient (only ca. one tenth). Acetone, which does not generate radicals upon photolysis, did not induce any **dG** oxidation.

Chemiluminescence Measurements during the dG Oxidation. To assess whether the electronically excited species generated from the dioxetanes are involved in the observed oxidation of **dG**, the latter was exposed to **^{t}BuO-TMD** thermolysis at 50 °C and the chemiluminescence emission measured [monitored continuously on a Mitchell-Hastings photometer (Figure 1)] in parallel with the rate of **dG** oxidation (at each point in Figure 1, a sample was taken and the **dG** conversion determined by HPLC analysis). The decomposition rate of the dioxetane was within the experimental error the same in the presence [$t_{1/2}$(50 °C, H_2O/CH_3CN 9:1) = 740 ± 100 min] and absence [$t_{1/2}$(50 °C, CH_3CN) = 580 ± 100 min] of **dG**. After 1080 min (cf. arrow in Figure 1), a further equivalent of dioxetane was added to the solution

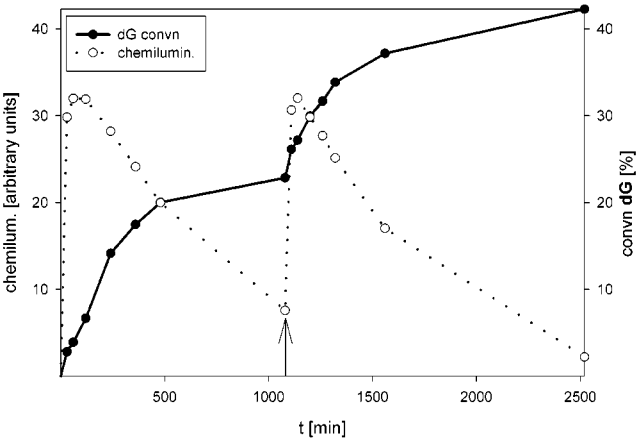
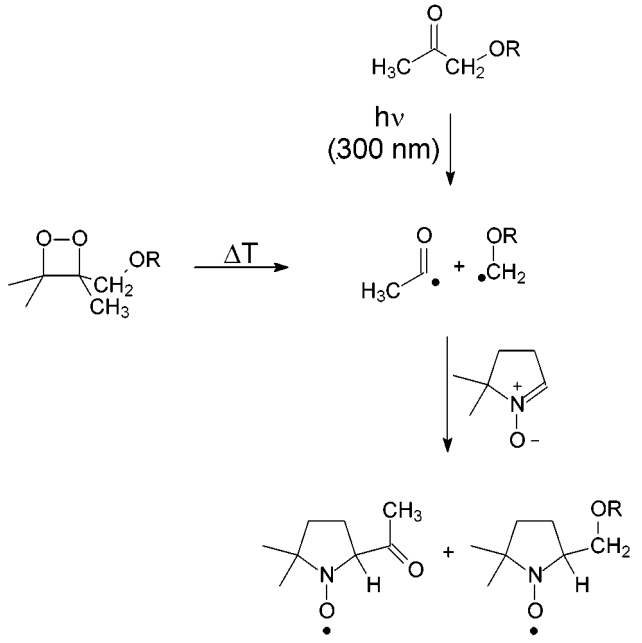


Figure 1. Time profiles of the dependence of the **dG** (0.500 mM) conversion and dioxetane chemiluminescence in a 9:1 mixture of phosphate buffer (5.00 mM, pH 7.0) and acetonitrile; [**^{t}BuO-TMD**] = 20.0 mM, after 1080 min (see arrow) a further 1 equiv was added.

Scheme 2. Formation of Spin Adducts with 5,5-Dimethyl-1-pyrroline *N*-Oxide (DMPO) in the Thermolysis of the Dioxetanes and Photolysis of the Ketones



to check whether the **dG** oxidation could be induced once more. Indeed, an increase in **dG** oxidation (% conversion) was observed. Thus, the time profile of the **dG** oxidation parallels that of the formation of excited carbonyl species in the thermolysis of the dioxetanes: The more excited species that are formed, the more **dG** is oxidized.

Spin-Trapping EPR Studies in the Dioxetane Thermolysis and Ketone Photolysis. The α cleavage of triplet-excited α-alkoxymethyl-substituted ketones to generate radicals is a well-known photochemical reaction.[15] Since such excited ketones are formed in the thermolysis of alkoxymethyl-substituted dioxetanes, spin-trapping experiments with **DMPO** (Scheme 2) were performed to provide spectroscopic evidence for the intervention of radicals in the thermal decomposition of the dioxetanes examined here. On thermolysis of all the alkoxymethyl-substituted dioxetanes in aqueous solution

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Table 3. EPR Data for the DMPO Adducts Formed in the Thermolysis of the Dioxetanes or Photolysis of the Ketones[a]

substrate	DMPO adduct	g factor	α_N (G)	α_H (G)
TMD	DMPO-yl• [b]	2.0056	15.8	23.2
HO-TMD	$HOCH_2$•	2.0056	15.7	22.7
	Ac•	2.0056	15.4	18.5
^{t}BuO-TMD	^{t}BuOCH_2•	2.0052	16.1	21.6
	Ac•	2.0053	15.3	19.0
MeO-TMD	$MeOCH_2$•	2.0056	16.0	22.6
	Ac•	2.0056	15.3	18.8
CH_3COCH_2OH	$HOCH_2$•	2.0054	15.9	22.6
	Ac•	2.0054	15.2	18.7
$CH_3COCH_2O^tBu$	Ac• [c]	2.0055	15.2	18.7

[a] Thermolysis of the dioxetanes (20 mM in 19:1 H_2O/acetone) at 37 °C, photolysis of the ketones (100 mM in 9:1 H_2O/CH_3CN) at 300 nm in the Rayonet photoreactor, [**DMPO**] = 45 mM. [b] See structures in the Introduction. [c] Besides acyl radicals, no other carbon-centered radical adducts have been detected.

(10% acetonitrile as cosolvent) in the presence of **DMPO**, two characteristic doublet-of-triplet EPR signals were observed for the **DMPO** adducts of the acetyl ($g = 2.0055 \pm 0.0002$, $\alpha_N = 15.3 \pm 0.1$ G, $\alpha_H = 18.7 \pm 0.2$ G) and a carbon-centered radical ($g = 2.0054 \pm 0.0002$, $\alpha_N = 15.9 \pm 0.2$ G, $\alpha_H = 22.1 \pm 0.5$ G, cf. Table 3). Without dioxetane or the corresponding ketone, no EPR signals were observed under the same experimental conditions of the thermolysis (or photolysis). Analogous spectra have been obtained upon irradiation of 1-hydroxy-2-propanone and 1-*tert*-butoxy-2-propanone (data for acetyl and other carbon-centered radicals see Table 3). The fact that the acetyl radical has been detected in the α cleavage of the triplet-excited carbonyl products, generated either in the thermolysis of the dioxetanes or in the photolysis of the corresponding ketones, implies that the other observed carbon-centered radicals, should be the respective α-alkoxymethyl species. The EPR parameters (g, α_N, α_H) for these species in Table 3 are consistent with this supposition. For the photolysis of 1-methoxy-2-propanone, no radicals were detected in the spin-trapping experiments.

Oxidation of dG. To assess the oxidation products of **dG** in the thermolysis of the alkoxymethyl-substituted dioxetanes, **dG** was treated with the dioxetanes at 50 °C for 15 h. For this purpose, the (4*R**)- and (4*S**)-**4-HO-8-oxodG** diastereomers and **8-oxodG** were determined by established HPLC methods[16] and the guanidine-releasing products (**GRP**) were quantified by a fluorescence-labeling HPLC assay with 1,2-naphthoquinone-4-sulfonic acid).[17] The results are shown in Figure 2 and exhibit that the extent of **dG** oxidation (given as % convn) decreases in the order **MeO-TMD** (71.7 ± 0.9%), **HO-TMD** (49.0 ± 0.4%), **^{t}BuO-TMD** (24.0 ± 0.2%) and **TMD** (6.8 ± 0.2%). Except acetone, this order correlates within the experimental error with the efficiency of the formation of triplet-excited carbonyl compounds in the thermolysis of the alkoxymethyl-substituted dioxetanes (Table 1).

The main oxidation products are the (4*R**)- and (4*S**)-**4-HO-8-oxodG** diastereomers and guanidine-releasing products (**GRP**). Note that the values for **8-oxodG** in Figure 2 have been multiplied by 100 and, thus, this product is formed only in trace amounts. For the most efficient dioxetane, **HO-TMD**, at most only 0.2% **8-oxodG** has been detected.

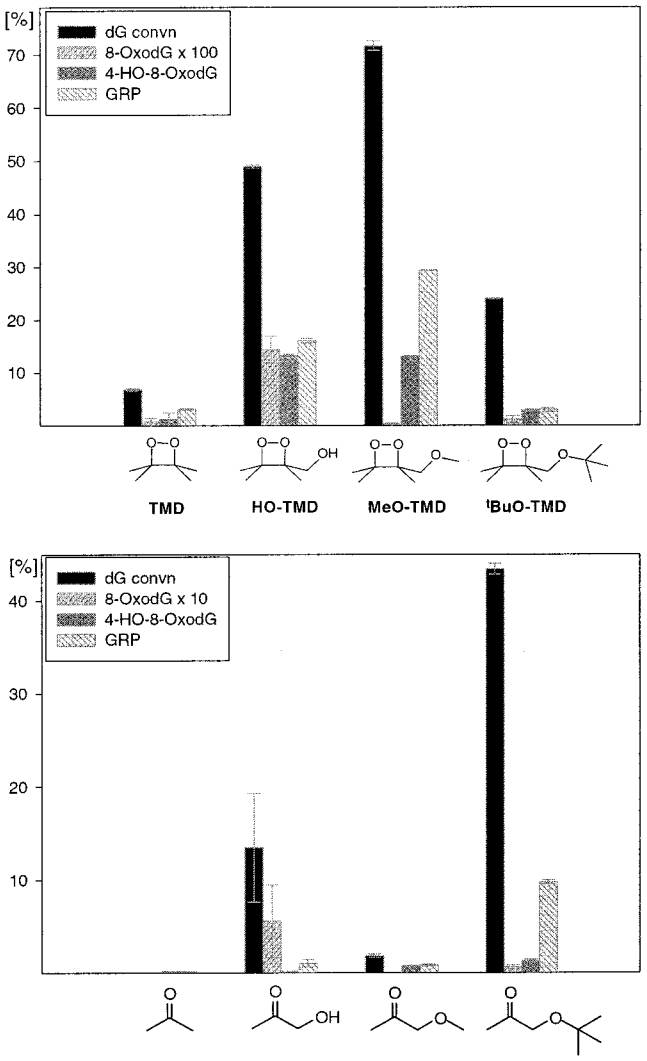

Figure 2. dG (0.500 mM) oxidation (upper) in the thermolysis (37 °C for 15 h) of the substituted dioxetanes (20.0 mM) and (lower) in the photolysis (300 nm at 0 °C for 5 h) of the substituted ketones (20.0 mM) in a 9:1 mixture of phosphate buffer (5.00 mM, pH 7.0) and acetonitrile.

The photolysis of the corresponding ketones in the presence of **dG** displays similarities in the oxidation efficacy to the dioxetane thermolysis in that the oxyfunctionalized ketones are much more effective than acetone. Thus, the oxidation of **dG** (% convn) decreases in the order 1-*tert*-butoxy-2-propanone (43.4 ± 0.6%), 1-hydroxy-2-propanone (13.5 ± 5.8%), 1-methoxy-2-propanone (1.8 ± 0.2%), and acetone (<0.5%); the latter is ineffective for the oxidation of **dG**. For all ketone photolyses, except the photoinert acetone, the major oxidation product is of the guanine-releasing type (i.e. **GRP**), while **4-HO-8-oxodG** is formed in small amounts, and **8-oxodG** only in traces.

Since the (4*R**)- and (4*S**)-**4-HO-8-oxodG** diastereomers have been described in the literature[18] as typical type-II photoproducts, the **dG** oxidation was conducted

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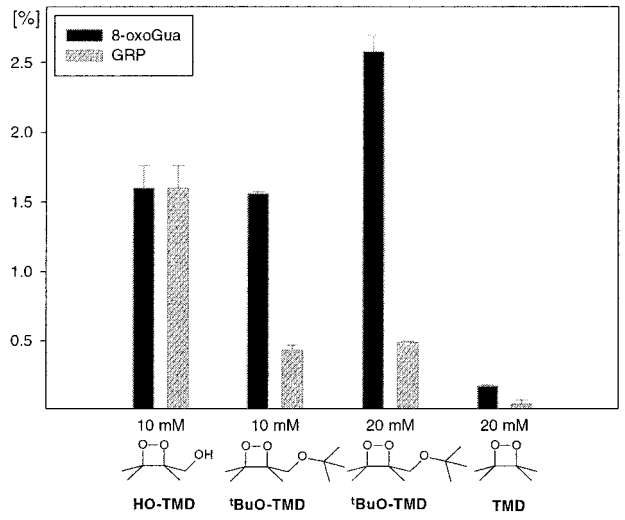

Figure 3. Oxidation products of *CT* DNA (100 mg/mL) for the thermolysis of the dioxetanes in a 9:1 mixture of phosphate buffer (5.00 mM, pH 7.0) and acetonitrile at 37 °C and 12 h.

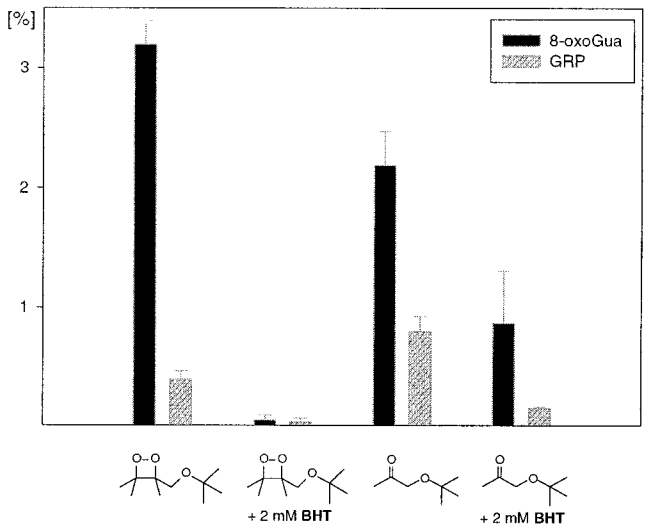

Figure 4. Effect of radical scavengers on the oxidation of *CT* DNA (100 mg/mL) for the **tBuO-TMD** (20.0 mM) thermolysis (37 °C for 12 h) and for the ketone (20.0 mM) photolysis (300 nm) in a 9:1 mixture of phosphate buffer (5.00 mM, pH 7.0) and acetonitrile.

in D_2O, in which singlet oxygen has a 10-fold longer lifetime.[19] However, the amounts of the (4*R**)- and (4*S**)-**4-HO-8-oxodG** diastereomers were the same within the experimental error in D_2O and H_2O; thus, singlet oxygen is not directly involved in these photooxidations of **dG**. Addition of 2 mM **BHT** (2,6-Di-*tert*-butyl-4-methylphenol) to the **dG** oxidation experiment with **tBuO-TMD** caused a reduction of **dG** conversion by 59% whereas 1 mM **BHT** reduced the **dG** conversion by 28%.

Oxidation of Calf-Thymus DNA by the Dioxetane tBuO-TMD. Isolated calf-thymus DNA (0.100 mg/mL which corresponds to a 62.5 μM guanine concentration) was treated at 37 °C for 21 h with the alkoxymethyl-substituted dioxetane **tBuO-TMD** to determine the guanine oxidation products. For comparison, the dioxetanes **TMD** (does not release radicals)[9a] and **HO-TMD** (generates carbon-centered radicals)[9a] have been also employed (Figure 3). For all these dioxetanes, the 8-oxoguanine (**8-oxoGua**) and guanidine-releasing products (**GRP**) were determined. Comparison of the yields of oxidation products for **HO-TMD** (1.60 ± 0.16% **8-oxoGua**, 1.60 ± 0.16% **GRP**) versus **tBuO-TMD** (1.56 ± 0.01% **8-oxoGua**, 0.43 ± 0.04% **GRP**) discloses that both dioxetanes are approximately of equal oxidative reactivity toward DNA, while **TMD** is inactive (20 mM: 0.17 ± 0.01% **8-oxoGua**, 0.04 ± 0.03% **GRP**). Again, this does not correspond with the fact that the yield of triplet-excited ketone follows the order **TMD** > **HO-TMD** > **tBuO-TMD** (Table 1). With increasing concentration of **tBuO-TMD** it was found that the amount of **8-oxoGua** increases as well, i.e., at 20 mM there are formed 2.58 ± 0.12% of **8-oxoGua** and at 10 mM 1.56 ± 0.01% of **8-oxoGua** (Figure 3).

Radical-scavenging experiments (Figure 4) revealed that in the presence of 2,6-di-*tert*-butyl-4-methylphenol (**BHT**, 2 mM) or **DMPO** (2 mM) most of the DNA oxidation was inhibited [**8-oxoGua** drops from 3.20 ± 0.20% (0.40 ± 0.07% **GRP**) to 0.05 ± 0.04% (0.04 ± 0.03% **GRP**) with **BHT** and to 0.36 ± 0.02% (0.02 ± 0.02% **GRP**) with **DMPO**]. 2-Propanol (100 mM) was less effective and reduced the amount of the oxidation products down to 2.16 ± 0.27% for **8-oxoGua** and 0.19 ± 0.03% for **GRP**. Similar results were obtained in the photolysis of 1-*tert*-butyl-2-propanone in the presence of calf-thymus DNA. The radical scavenger **BHT** inhibited also efficiently the formation of oxidation products [**8-oxoGua** was reduced from 2.19 ± 0.28% (0.79 ± 0.14% **GRP**) to 0.87 ± 0.44% (0.15 ± 0.01% **GRP**)], as shown in Figure 4. The main oxidation product was **8-oxoGua**, but also substantial amounts of guanidine-releasing products (**GRP**) were observed (**8-oxoGua**/**GRP** ratio ca. 2:1). Photolysis of acetone was again ineffective in the formation of DNA oxidation products (0.14 ± 0.12% **8-oxoGua** and 0.01 ± 0.02% **GRP**).

Discussion

The similar qualitative trends, with the exception of the methoxy derivatives (shall be discussed later), in the oxidative reactivity toward **dG** for the photolysis of the ketones (tBuO ~ HO > H) and for the thermolysis of the dioxetanes (tBuO ~ HO > H) imply that electronically excited ketones are responsible for the oxidation of **dG** in *both* processes. Indeed, the time profile in Figure 1 reflects that the **dG** conversion parallels the chemiluminescence intensity, which is taken as a measure of excited-state formation during the thermolysis of **tBuO-TMD**. For the dioxetane thermolysis, the trend in the efficiency of **dG** oxidation (with the exception **TMD**) parallels the triplet excitation flux (Table 1), while for the ketone photolysis this qualitative trend coincides reasonably well (notably acetone is out of line) with the efficiency of singlet excitation (the molar extinction coefficients in Table 2 are taken as measure). The disparity in the present data, i.e., the inefficiency of excited acetone (generated by direct photoexcitation or by **TMD** photolysis) and methoxyacetone (generated photochemically) to oxidize **dG** suggests that excited states (singlet or triplet) are not directly involved in the **dG** oxidation. Presumably other species generated by or from the excited ketones serve as oxidants, whether produced by photoexcitation of the ketones or by thermolysis of the dioxetanes.

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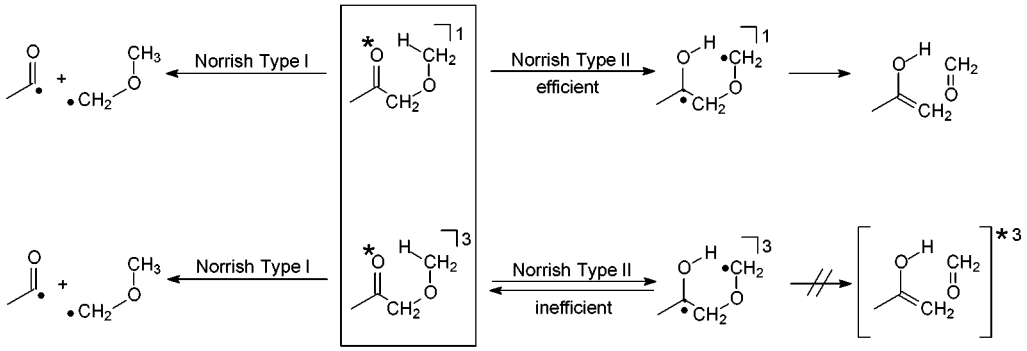
Scheme 3. Norrish-Type-I and Type-II Photochemical Transformations of Singlet- and Triplet-Excited Methoxyacetone



A possibility could be singlet oxygen ($^1O_2$), since it is well established that excited ketones (generated by photoexcitation of ketones or by chemiexcitation from dioxetanes) afford this oxidant by energy transfer.[20] However, this is unlikely to be a significant pathway because control experiments in D_2O for **HO-TMD** as well as **^{t}BuO-TMD** gave the same extent of **dG** oxidation as in H_2O. Since the lifetime of $^1O_2$ in D_2O is more than 10-fold[21] longer, the conversion of **dG** should have been notably higher in this medium. Additionally, for **HO-TMD** it was shown that this dioxetane is quite inefficient in producing singlet oxygen,[22] hardly sufficient to oxidize **dG** to the extent observed in Figure 2.

In view of the photochemical property of appropriate n,π^*-excited ketones to undergo efficient α cleavage into a pair of acyl and alkyl radicals (Scheme 1),[23] these reactive intermediates are likely to serve as oxidants, especially in the presence of molecular oxygen. In aqueous media, sufficient dioxygen is present to trap such radicals quite efficiently and generate oxygen-centered derivatives (peroxyl, alkoxyl, hydroxyl), which effectively oxidize **dG**.[24] Indeed, experimental evidence for radicals formed through α cleavage of the excited ketone (Scheme 2) has been provided through spin-trapping experiments with **DMPO** and EPR-spectral detection (Table 1), as well as the inhibition of **dG** and DNA oxidation (Figure 4) by **BHT** as radical scavenger. Certainly, the observed qualitative trends in the **dG** oxidation by the photoexcited ketones (^{t}BuO ∼ HO > MeO ∼ H) and by the dioxetanes (^{t}BuO ∼ HO ∼ MeO > H) are in reasonable accord with the propensity of radical release by the excited ketones generated in the ketone photolysis (^{t}BuO ∼ HO > MeO ∼ H) and in the dioxetane thermolysis (^{t}BuO ∼ HO ∼ MeO > H). Thus, we conclude that radical formation from the excited ketones is responsible for the **dG** oxidation; nevertheless, for both the ketone and the dioxetane series, excited acetone is inefficient in oxidizing **dG**. But it is an established fact that excited acetone suffers negligible α cleavage;[25] our present results confirm this, since we have failed to detect spin-trapping products in the photolysis of acetone and thermolysis of **TMD**. In contrast, the oxyfunctionalized dioxetanes all gave spin-trapped radicals with **DMPO** (Table 1) in nearly the same efficiency and, thus, also nearly the same extent of **dG** oxidation has been observed for **^{t}BuO-TMD**, **HO-TMD** and **MeO-TMD**. This correspondence is also displayed for the ketone photolysis, except methoxyacetone. For this oxyfunctionalized ketone, no spin-trapped radicals with **DMPO** were observed in its photolysis and, correspondingly, only negligible **dG** conversion was found (Table 1). This discord in the photochemical reactivity of methoxyacetone requires mechanistic rationalization.

For α-methoxy-substituted ketones, the Norrish-Type-II photoreaction may be expected as a competitive process for α cleavage.[26] Particularly for aliphatic ketones, this process occurs efficiently from the singlet state.[27,28] When the ketone contains a β-oxygen atom, as is the case for methoxyacetone, the Norrish-Type-II reaction is especially accelerated[29] (AM1 calculations).[30] Consequently, the fact that no radicals have been detected by spin-trapping in the direct irradiation of methoxyacetone and, thus, no **dG** oxidation was found under these consitions, implicates preferential Norrish-Type-II rather than α cleavage (Scheme 3). Indeed, the expected acetone and formaldehyde (its hydrate) as the photolysis products were observed. In contrast, in the thermolysis of the dioxetane **MeO-TMD**, in which the formation of the triplet-excited methoxyacetone prevails, α cleavage dominates, as manifested by the EPR-spectrally observed spin-trapped radicals and the concomitant **dG** oxidation.

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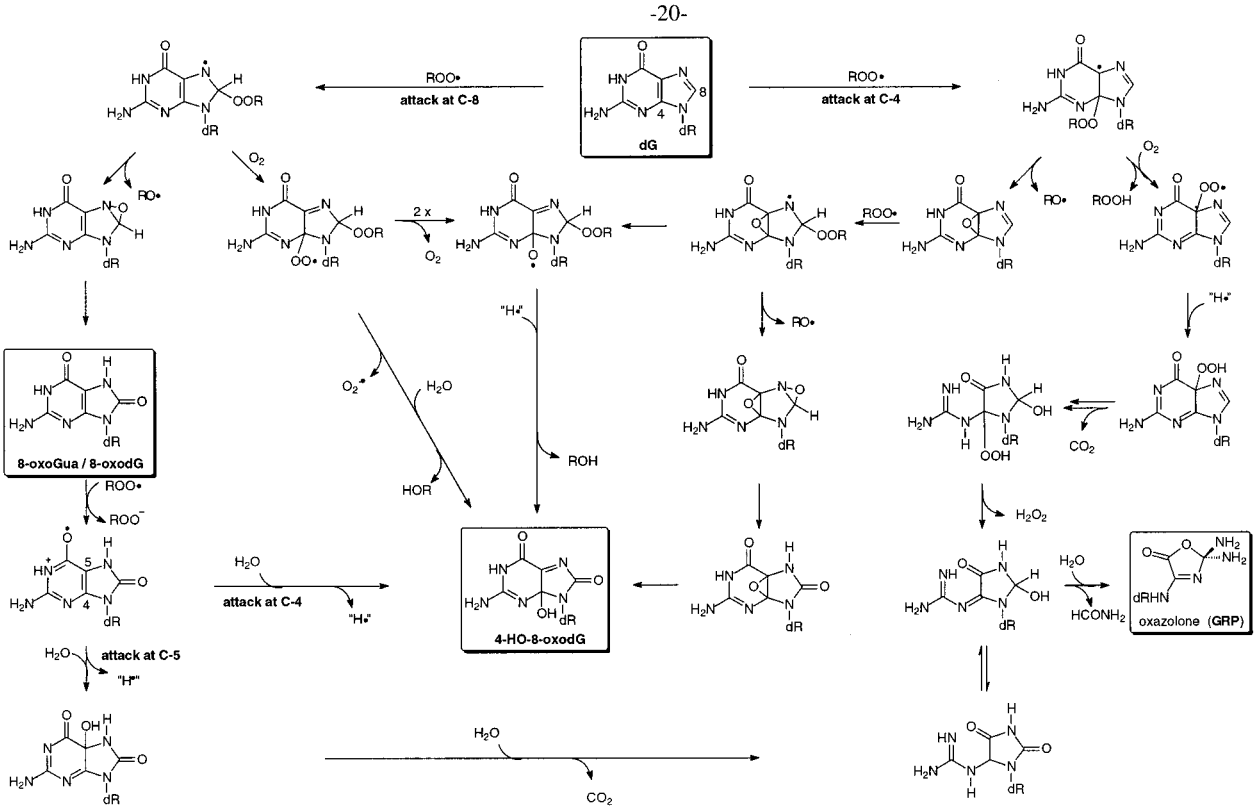
(29) The Norrish-Type-II cleavage depends on the bond dissociation energy of the γ-CH bond: For >95 kcal/mol, intersystem crossing is faster than Norrish-Type II, while for <94 kcal/mol the Norrish-Type II competes with intersystem crossing. Since a β-oxygen atom is expected to lower γ-CH bond energy by ca. 9−12 kcal/mol (value reported for CH_2OH versus CH_3; see: Benson, S. W. *J. Chem. Educ.* **1965**, *42*, 502−518, Kerr, J. A. *Chem. Rev.* **1966**, *66*, 465−500), a value <90 kcal/mol is estimated for 1-methoxy-2-propanones (compared to 98 kcal/mol for 2-pentanone) and, thus, the Norrish-Type-II reaction from the singlet state is likely.
(30) Sengupta, D.; Bhattacharyya, A.; Sumathi, R.; Chandra, A. K. *J. Photochem. Photobiol. A: Chem.* **1995**, *86*, 161−170.

Scheme 4. Postulated Mechanism for the Oxidation of DG by Peroxyl Radicals to the 4-HO-8-oxodG and GTP Products



Thus, it is concluded that in the direct photolysis experiments of methoxyacetone, the observed Norrish-Type-II cleavage proceeds from the singlet state, which competes effectively with intersystem crossing and subsequent α cleavage from the triplet state. For all the other photoexcited ketones a similar α cleavage and oxidative reactivity as for dioxetane thermolysis was observed. *These results make evident the advantage of dioxetanes for photobiological studies in that their thermolysis allows to generate selectively triplet-excited ketones without trespassing the singlet state.*

The major products in the radical induced oxidation of **dG**, both for the ketone photolysis and dioxetane thermolysis (Figure 2), are **4-HO-8-oxodG** and the guanidine-releasing products (**GRP**), while **8-oxodG** is formed in only minor amounts. This is unusual, because **4-HO-8-oxodG** has been assigned as a characteristic singlet-oxygen photoproduct of **dG**.[31] However, we have ruled out that sufficient singlet oxygen is formed under the present conditions to account for the large amounts of observed **4-HO-8-oxodG**. More likely, the **4-HO-8-oxodG** product is formed by oxidation of **dG** with radical species, presumably by the peroxyl radicals generated in the oxygenation of the carbon-centered radicals (Scheme 1), which are derived from the α cleavage of the electronically excited ketones. The production of **4-HO-8-oxodG** has also been observed in the oxidation of **dG** by enzymatically generated peroxyl radicals.[32] Since the C-8 and C-4 positions of guanine are known to be the major sites of attack by hydroxyl radicals,[33] presumably the same two sites are also involved in the oxidation by peroxyl radicals. We speculate (Scheme 4) that the initial attack of a peroxyl radical at the C-8 position of **dG** would generate a carbon-centered radical, which has two possibilities for further reaction: The first one is addition of dioxygen,[34] the second one formation of an oxaziridine by loss of an alkoxyl radical, as known for peroxyl adducts to simple alkenes.[35] The latter oxaziridine intermediate may rearrange rapidly to form **8-oxodG**,[36] while the dioxygen adduct may be reduced to **4-HO-8-oxodG**. We conjecture that the peroxyl-radical adduct at the C-4 site of **dG** has also at least two options: The oxirane intermediate may be transformed by a peroxyl radical to **4-HO-8-oxodG**, while the dioxygen adduct leads eventually by a complex sequence of transformations, similar to those postulated in the Type-I oxidation of **dG**, to the oxazolone (**GRP**).

As for the **8-oxodG** product, proposed to be generated from **dG** by C-8 attack (Scheme 4), it is not expected to survive the present oxidative conditions in view of its low oxidation potential.[37] Thus, we postulate electron transfer from **8-oxodG** to a peroxyl radical to generate the corresponding radical cation. The latter is subsequently transformed to **4-HO-8-oxodG** and **GRP** (Scheme 4), for

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which there exist precedents.[38,39] It is, therefore, not surprising that only traces of **8-oxodG** have been observed under the present oxidative conditions. Indeed, a control experiment with authentic **8-oxodG** confirmed that it was oxidized in the dioxetane thermolysis. Had **8-oxodG** been formed in our oxidations, it would not have accumulated and, thus, its intervention is uncertain.

In contrast to **dG**, the oxidation of DNA affords the **8-oxoGua** as the major product, but also **GRP**. As for **4-HO-8-oxoGua**, this oxidation product is difficult to quantify in DNA, and to date no data for **4-HO-8-oxoGua** in DNA have been reported. It is a general fact that in the oxidation of DNA, the **8-oxoGua** is formed in substantial amount, whereas in **dG** only in traces. For example, in the hydroxyl-radical-induced oxidation of DNA, the main oxidation product is **8-oxoGua,** but for **dG** it is the **GRP**.[33] This reactivity trend applies also in the case of one-electron oxidants, which has been rationalized in terms of the intermediacy of the stabilized guanine radical cation in the DNA matrix; subsequently, the radical cation is hydrolyzed selectively to **8-oxoGua** prior to deprotonation and formation of **GRP**.[40] Since under our conditions the **8-oxoGua** in DNA accumulates but the **8-oxodG** in **dG** is oxidized rapidly, the high levels of **8-oxoGua** in DNA are due to its protection in the DNA matrix against further oxidation. As an alternative to the above-mentioned electron-transfer process, the **8-oxoGua** in DNA might be formed by the same mechanism as already postulated for the formation of the **8-oxodG** in the **dG** oxidation in Scheme 4, that is, by the addition of a peroxyl radical to the C-8 position, generation of an oxaziridine, and rearrangement of the latter to **8-oxoGua**. Besides **8-oxoGua**, the large amount of **GRP** is in accord with radical-type oxidation of DNA.[41]

Conclusion

This study reveals that the major radical-induced oxidation products of **dG** are **GRP** and **4-HO-8-oxodG**, while **8-oxodG** is formed in only minor amounts. The major oxidation product in DNA is **8-oxoGua**, but also significant amounts of **GRP** are found. The observed guanine oxidation in **dG** and in DNA is attributed to radical species, in particular peroxyl radicals. The latter are formed by dioxygen addition to the carbon-centered radical species, which are generated upon α cleavage of the excited ketones derived from photoexcitation (ketone photolysis) or chemiexcitation (dioxetane thermolysis).

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Supporting Information Available: Experimental details for the preparation of the key compounds and their spectral data as well as the procedures for the quantitative determination of the **dG** and DNA oxidation products. This material is available free of charge via the Internet at http://pubs.acs.org.

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