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A Comparative Photomechanistic Study (Spin Trapping, EPR Spectroscopy, Transient Kinetics, Photoproducts) of Nucleoside Oxidation (dG and 8-oxodG) by Triplet-Excited Acetophenones and by the Radicals Generated from α-Oxy-Substituted Derivatives through Norrish-Type I Cleavage

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Abstract: The photooxidation of 2′-deoxyguanosine (dG) and its derivative 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) by a series of acetophenones (AP-X) and benzophenone (BP) has been studied.The favorable absorption characteristics of the benzoyl chromophore enables time-resolved spectroscopy of the triplet ketones to assess their quenching kinetics by dG and 8-oxodG. Whereas the photolysis of acetophenone (AP), 2-acetoxyacetophenone (AP-OAc), and benzophenone (BP) does not produce radicals (group A ketones), the oxymethyl-substituted derivatives 2-hydroxyacetophenone (AP-OH) and 2-*tert*-butoxyacetophenone (AP-O^{t}Bu) lead to carbon-centered radicals by α cleavage (group B ketones). For the latter ketones, this was confirmed by EPR studies with the spin trap 5,5-dimethylpyrroline *N*-oxide (DMPO) and by their triplet lifetimes that were shorter than those for the unsubstituted acetophenone. Both groups of ketones photooxidize dG and 8-oxodG; the oxidation products are spiroiminodihydantoin and guanidine-releasing products (GRP) in the case of dG and AP-OH also 8-oxodG. In the presence of O_2, the photooxidation by the group A ketones is efficient at high dG or 8-oxodG concentrations, whereas the group B ketones photooxidize dG and 8-oxodG also at low substrate concentrations. These results imply that peroxyl radicals are responsible for the photooxidation by the group B ketones, which are formed by α cleavage of the triplet ketone and subsequent O_2 trapping of the carbon-centered radicals. At higher dG concentrations, direct electron transfer from dG to the triplet ketone, as observed for the group A ketones, competes with the radical activity.

Introduction

The relevance of DNA damage by reactive oxygen species (ROS) in mutagenesis, carcinogenesis, and aging has motivated the current interest in oxidative DNA damage.[1−4] The reactive oxygen species include oxyl radicals (in particular hydroxyl, alkoxyl, and peroxyl derivatives, the superoxide ion, and triplet ketones) but also nonradical species such as singlet oxygen and hydrogen peroxide which are all involved in the phenomenon known as oxidative stress. Oxidative stress may be induced upon exposure of cells to solar irradiation, but also by lipid peroxidation and inflammatory processes.

In this context, we have been studying the oxidation of DNA and its nucleoside 2′-deoxyguanosine (dG) by electronically excited states, mainly of carbonyl species, because the latter may be generated thermally through the decomposition of dioxetanes or photochemically by the direct irradiation of the corresponding carbonyl compounds.[5−8] In the case of photocleavable ketones, we have recently shown the importance of radical production in oxidative efficiency.[9,10] In the past, acetone derivatives have primarily been used, for which it is known that the excited acetyl chromophore in addition to undergoing

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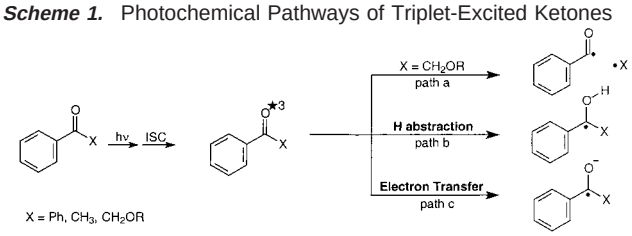
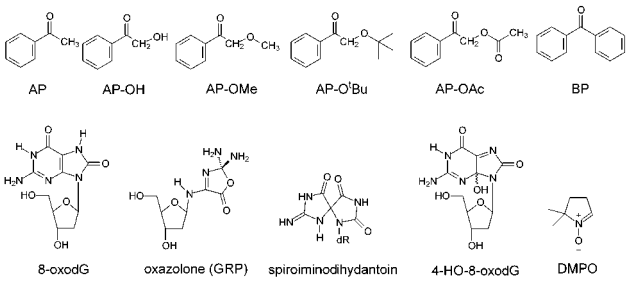
Scheme 1. Photochemical Pathways of Triplet-Excited Ketones



energy transfer also undergoes chemical quenching (electron transfer or hydrogen abstraction) by the guanine, rather than α cleavage into radicals.[11] In view of the unfavorable absorption characteristics of acetone-type ketones (low extinction coefficients in the UVA region), it has been difficult to assess the relative efficiencies of the chemical quenching versus radical-type oxidative activity by transient absorption spectroscopy.

For this purpose, we chose the benzoyl chromophore as contained in the hydroxy (AP-OH), methoxy (AP-OMe), *tert*-butoxy (AP-O^{t}Bu), and acetoxy (AP-OAc) derivatives of acetophenone (AP).



For these oxymethyl-substituted acetophenone derivatives, α cleavage (and thus radical production) may be expected and has been reported for AP-OH.[12−14] To confirm the involvement of radicals for the photocleavable ketones, 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) was to be employed as radical scavenger. Detection of the expected carbon-centered radicals in the α cleavage of these triplet ketones was to be achieved through spin trapping by DMPO, coupled with EPR spectroscopy. For comparison, we chose acetophenone (AP) and benzophenone (BP),[6] which are known to react with DNA and its nucleoside dG by one-electron transfer, and thus, these serve as typical type I photosensitizers,[11] since no Norrish-type I cleavage into radicals takes place.[6,12]

The acetophenone derivatives possess the advantage of higher extinction coefficients at longer wavelengths, which enables short irradiation times and low ketone concentrations to minimize undesirable side reactions. Moreover, favorable triplet absorption characteristics (high ϵ, near UV) allow the direct assessment of the excited-state reactivity toward dG by means of transient absorption spectroscopy. With such data, the distinction between alternative oxidative mechanisms by the intermediary triplet-excited ketone should be accessible and fundamental mechanistic knowledge of the photochemical properties of the triplet-excited states becomes available (Scheme 1). In addition to undergoing α cleavage (path a), the triplet ketone may directly interact with the substrate by H abstraction (path b) or by electron transfer (path c),[15−18] as evidenced by the oxidation of the base guanine,[11,19,20] the most easily oxidizable base in DNA.[21]

Table 1. EPR Data of the DMPO Adducts Obtained in the AP-OH and AP-O^{t}Bu Photolyses[a]

substrate	DMPO adduct of	α_N [G]	α_H [G]	g factor
	•COPh[b]	15.3	17.9	2.0056
		(15.3)	(18.7)	(2.0055)
	•CH$_2$OH[c]	16.0	22.6	2.0055
		(15.7)	(22.7)	(2.0056)
	•COPh[b]	15.3	17.9	2.0055
		(15.3)	(18.7)	(2.0055)
	•CH$_2$O^{t}Bu[c]	16.0	21.1	2.0055
		(16.1)	(21.6)	(2.0052)

[a] 2.00 mM ketone in a 9:1 H_2O:CH_3CN mixture at 300 nm for 30 min and 20 °C in a Rayonet photoreactor [sixteen 300-nm lamps (24 W)], [DMPO] = 50.0 mM. [b] In parentheses are given the data for an analogous acyl-radical adduct in H_2O:CH_3CN (9:1), see ref 10. [c] In parentheses are given the literature data, see ref 10.

For these studies on the mechanism of the photooxidation, the nucleoside 2′-deoxyguanosine (dG) was selected as the model for damage of DNA, since guanine is the most vulnerable base in DNA toward photooxidation. To monitor the photooxidative reactivity, dG conversion as well as the detection of the oxidation products 8-oxodG, spiroiminodihydantoin, and guanidine-releasing products (GRP) were to be employed. Since 8-oxoGua is considered to be one of the most important base lesions in DNA, special attention was focused on the formation and the fate of the dG oxidation product 8-oxodG.

Results

EPR-Spectral Spin-Trapping Studies. The spin-trapping experiments with DMPO (Scheme 2) were performed in aqueous solution (10% acetonitrile as cosolvent), to provide EPR-spectral evidence for radical production in the photolysis of the α-alkoxymethyl-substituted acetophenone derivatives. Upon photolysis of the ketones AP-OH and AP-O^{t}Bu, characteristic doublet-of-triplet EPR signals (Table 1) were observed for the DMPO adducts of the benzoyl radical (g = 2.0056, α_N = 15.3 G, α_H = 17.9 G), the hydroxymethyl radical (g = 2.0055, α_N = 16.0, α_H = 22.6 G), and the *tert*-butoxymethyl radical (g = 2.0055, α_N = 16.0 G, α_H = 21.1 G). At this point, it should be mentioned that some DMPO-adduct signals do not persist at room temperature (ca. 20 °C) for more than 30 min; the *tert*-butoxymethyl adduct is especially labile ($\tau \approx 5$ min). For AP, AP-OMe, AP-OAc, and BP, as well as in the irradiation of DMPO alone, no EPR signals were observed under the same experimental conditions. The formation of benzoyl radicals was further confirmed by spin trapping with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO, cf. Scheme 2); thus, during the photolysis of AP-OH and AP-O^{t}Bu in the presence of TEMPO, the benzoyl-radical adducts were observed by HPLC/UV detection.

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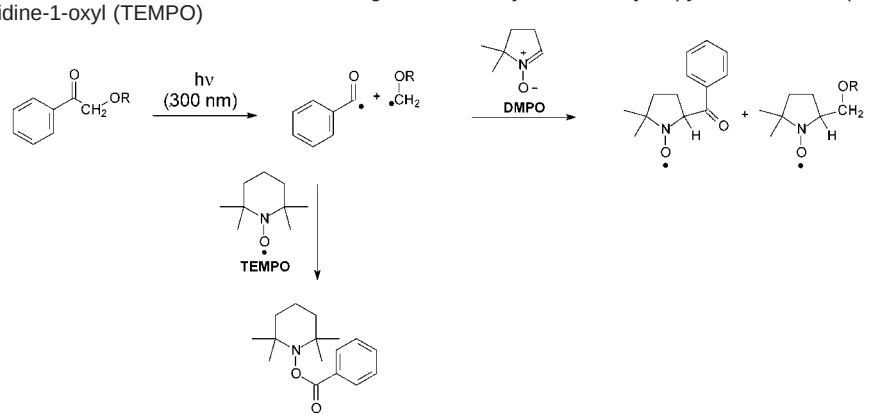
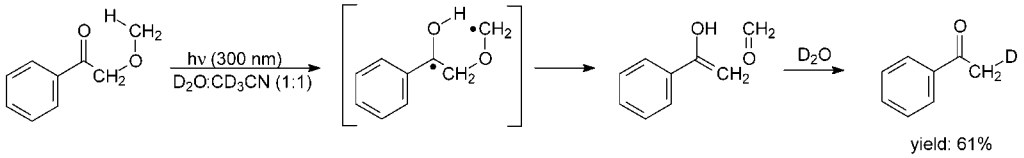
Scheme 2. Trapping of the Radicals Produced in the α Cleavage of AP-OR by 5,5-Dimethyl-1-pyrroline *N*-oxide (DMPO) and 2,2,6,6-Tetramethylpiperidine-1-oxyl (TEMPO)



Scheme 3. Norrish-Type II Cleavage in the AP-OMe Photolysis



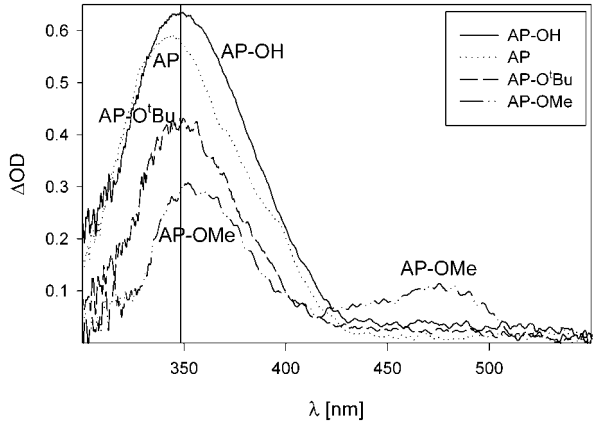


Figure 1. Triplet absorption spectra in the laser-flash photolysis (308 nm) of the ketones AP-R [4.00 mM in H_2O:CH_3CN (9:1)].

Norrish-Type II Cleavage of AP-OMe. To assess whether the methoxy-substituted acetophenone undergoes the Norrish-Type-II cleavage in aqueous solution (Scheme 3), the AP-OMe was photolyzed in a 1:1 mixture of D_2O and CD_3CN. Indeed, by means of ^1H NMR analysis of the photolyzate, the deuterated acetophenone was detected as a cleavage product in 61% yield. The hydrate of formaldehyde was detected in trace amounts as well.

Laser-Flash-Photolysis Studies. To determine the rate constants for the quenching of the excited ketone by dG and 8-oxodG, the absorption spectra of the transients (Figure 1) were recorded directly (approximately 20 ns) after the laser flash in thoroughly degassed (three "freeze−pump−thaw" cycles) aqueous solution (10% acetonitrile as cosolvent). All ketones possess an absorption maximum around 340−345 nm, except AP-OMe, which has its maximum at 355 nm and a second band at 480 nm.

The fact that intersystem crossing in aromatic ketones proceeds in the picosecond range[22] and that there is similarity with the literature-known triplet absorption spectrum of acetophenone in aqueous solution ($\lambda_{max} \approx 340$ nm)[23] indicates that the observed transients for the acetophenone derivatives, except AP-OMe, belong to the absorption of the triplet state. Thus, a wavelength of 340 nm was chosen for the detection of the triplet state. For benzophenone, instead of the literature-known absorption maximum of the triplet state ($\lambda = 525$ nm), the wavelength of 600 nm was chosen for detection because of less overlap with the absorption of its ketyl radical at this wavelength;[24] the latter was formed in low amounts already at 1.00 mM BP.

The decay of triplet transients was measured by using 1.00 mM ketone solutions in water:acetonitrile (9:1) at the wavelength of 340 nm, and the triplet decay times (τ_T) were obtained from the transient absorption curves by monoexponential fitting (Table 2).

The quenching of these triplet transients by the ground-state ketone (self-quenching), molecular oxygen, dG, and 8-oxodG was determined by varying the quencher concentrations. The rates of the individual experiments were obtained by using monoexponential fitting for the pseudo-first-order decay of the triplet state, and the quenching-rate constants were determined by linear regression. The quenching process also produced ketyl radicals, which were observed as long-lived transients that decay by second-order processes.[25] Thus, in addition to the monoexponential function, a less intense second-order process needed to be included in the fitting function to account for the formation of ketyl radicals in the kinetics, but only the monoexponential part was used in the calculation of the triplet lifetimes and rate constants (Table 2). The triplet lifetimes for AP, AP-OAc, and BP lie between 6 and 10 μs, while lifetimes around 1 μs were obtained for AP-OH and AP-O^{t}Bu.

In the case of AP and AP-OAc, quenching-rate constants for the ketone itself take values between 1 to 2×10^8 M^{-1} s^{-1}; for

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Table 2. Lifetimes and Quenching Rate Constants of the Triplet States Formed during the Ketone (AP-R) Photolysis for Various Quenchers

AP-R	τ_T [μs][a,b]	k_q [10^9 $M^{-1}s^{-1}$] ketone[c]	O_2[b,d]	dG	8-oxodG
	6.6	0.12 ± 0.01	3.2 (4.0[e])	3.3 ± 0.1	2.2 ± 0.1
	1.1	-[f]	4.0	3.3 ± 0.2	1.9 ± 0.2
	-[g]	-	-	-	-
	0.54	-[f]	1.9	3.9 ± 0.3	3.4 ± 0.2
	6.7	0.12 ± 0.02	2.4	3.3 ± 0.2	2.0 ± 0.2
	9.2	0.30 ± 0.01	4.8	2.9 ± 0.1	1.9 ± 0.2

[a] Value given for [AP-R] = 1.00 mM. [b] Only one point measured, error ±10%. [c] Self-quenching by AP-R. [d] $[O_2]$ = 0.265 mM under atmospheric conditions. [e] Measured in H_2O. [f] Self-quenching not measured due to short lifetime. [g] Detected transient is not the triplet.

BP a value of 3.0×10^8 M^{-1} s^{-1} was determined. The k_q rate constants for the deactivation by molecular oxygen fall between 2 and 5×10^9 M^{-1} s^{-1}. The results for the addition of dG show for all ketones a fast deactivation by dG on the order of $3-4 \times 10^9$ M^{-1} s^{-1}. Quenching by 8-oxodG is less efficient by a factor of approximately 1.5.

For AP-OMe a different absorption spectrum has been measured. More strikingly, photolysis of AP-OMe in acetonitrile (lifetime of the transient 10.0 μs) did not result in triplet energy transfer to 1-methylnaphthalene (10.0 μM). This behavior is different from that of the triplet states of the other ketones, and it is concluded that the observed transient is *not the triplet state*. Thus, AP-OMe is not considered in the dG oxidation experiments (see Supporting Information).

The Dependence of the Nucleoside Photooxidation as a Function of Nucleoside Concentration. When the dG conversion in the ketone photolysis is measured for different dG concentrations at similar ketone:dG ratios and under similar irradiation conditions, the five ketones display different types of photochemical reactivity toward dG, as is evident from the plots of dG conversion versus [dG] in Figure 2. In fact, these ketones fall into two distinct groups in regard to their photoreactivities: The derivatives AP-OH and AP-O^{t}Bu (left-hand plots) oxidize dG even at low (0.1 mM) dG concentrations; indeed, although the extent of dG oxidation remains quite constant over the employed [dG] range, it actually slightly decreases (notice the negative slopes) with increasing [dG]. In this context, for the AP-OH photolysis, the 8-oxodG formation also follows this pattern (Figure 3), that is, an approximately constant photochemical activity is observed over the employed [dG] range, with a slightly lower amount of 8-oxodG produced at the higher (0.5 mM) [dG]. In contrast, dG is unreactive toward the ketones AP, AP-OAc, and BP (right-hand plots) at low [dG], but is oxidized with increasing efficiency as [dG] is raised.

Similar 8-oxodG concentration profiles were observed for the oxidation of 8-oxodG (Figure 4). Thus, AP-OH and AP-O^{t}Bu (left-hand plots) oxidize 8-oxodG already at low [8-oxodG], and the conversion of 8-oxodG remains relatively constant over the employed [8-oxodG] range. In contrast, AP, AP-OAc, and BP (right-hand plots) are only active at high [8-oxodG], and the activity increases with increasing [8-oxodG].

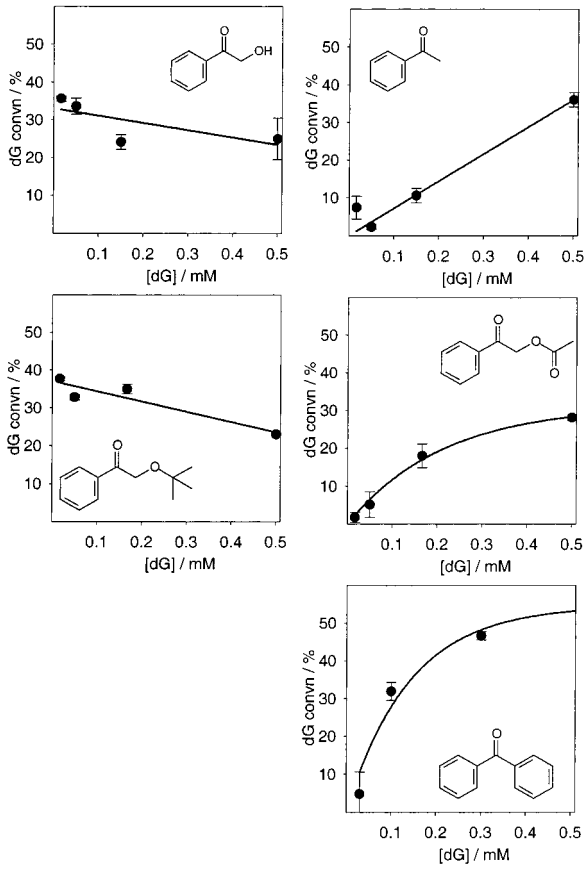

Figure 2. Dependence of the dG conversion on the dG concentration in the ketone photolysis [2.00 equiv of ketone, 10.0 equiv of phosphate buffer (pH 7.0) in H_2O:CH_3CN (9:1), irradiated at 300 nm for 45 min].

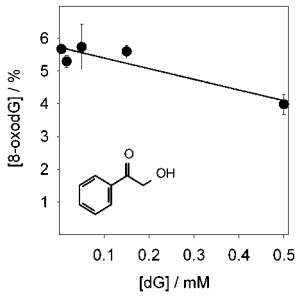

Figure 3. Dependence of the 8-oxodG formation on the dG concentration in the photolysis of AP-OH [2.00 equiv of ketone, 10.0 equiv of phosphate buffer (pH 7.0) in H_2O:CH_3CN (9:1), irradiated at 300 nm for 45 min].

The Photoreactivity of the Ketones AP and AP-OAc Induced by the Nucleosides dG and 8-oxodG. The ketones AP and AP-OAc were irradiated with and without dG or 8-oxodG, and the conversion of the ketone was monitored by HPLC/UV. Without dG or 8-oxodG, neither the ketone AP nor AP-OAc was photolyzed in detectable amounts. In contrast, upon addition of dG or 8-oxodG, the ketones were converted to the extent of 30−60% on photolysis (Figure 5). The photoreactivity was more effectively induced by dG than 8-oxodG for both ketones.

The Photooxidation Products of dG. To assess the oxidation products of dG in the photolysis of the ketones, dG (0.200 mM) was irradiated in the presence of the ketones (2 equiv, 0.400 mM) for 45 min. The conversion of dG was monitored as a measure of the extent of dG oxidation, as well as of the formation of the oxidation products spiroiminodihydantoin,

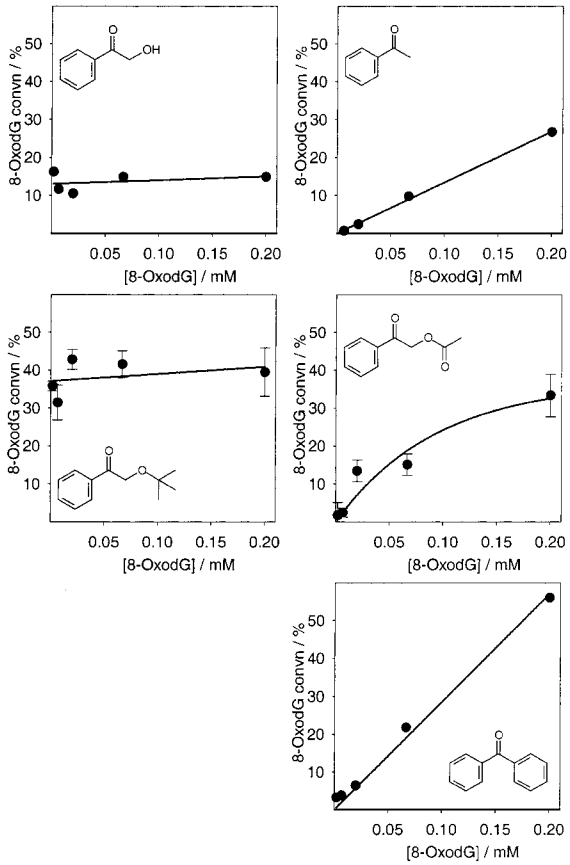

Figure 4. Dependence of the 8-oxodG conversion on the 8-oxodG concentration in the ketone photolysis [2.00 equiv of ketone, 10.0 equiv of phosphate buffer (pH 7.0) in H_2O:CH_3CN (9:1), irradiated at 300 nm for 45 min].

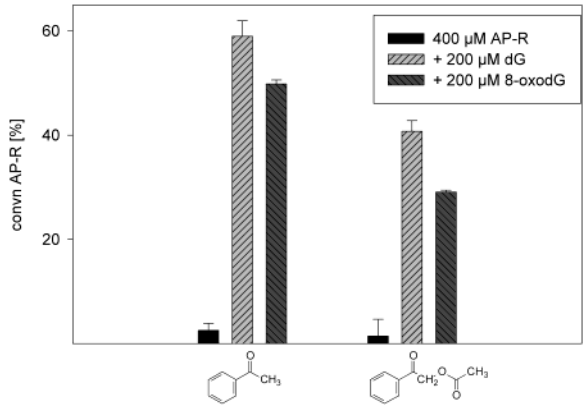

Figure 5. Enhancement of the AP-R conversion by the presence of dG or 8-oxodG in the ketone photolysis [irradiated at 300 nm for 45 min at 0 °C in 2.00 mM 9:1 phosphate buffer (pH 7.0):CH_3CN].

GRP, and 8-oxodG (Figure 6) by established HPLC methods.[26,27] The previously assigned 4-HO-8-oxodG[28] structure for the dG oxidation product has been reassessed, and the recently suggested spiroiminodihydantoin[29] was confirmed by NMR

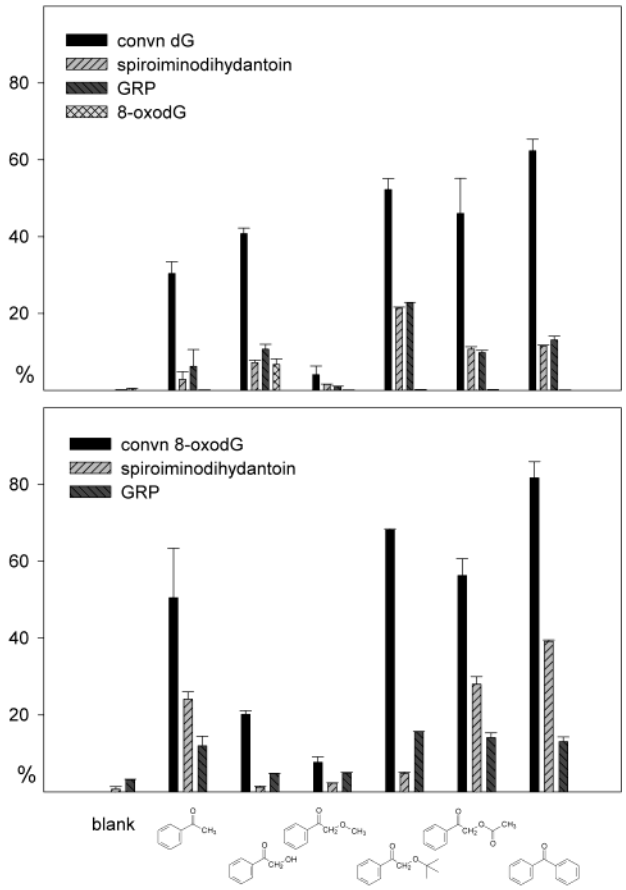

Figure 6. Conversion of dG or 8-oxodG and formation of the oxidation products in the photooxidation [400 μM ketone, 2.00 mM phosphate buffer (pH 7.0), 45 min $h\nu$, 300 nm, 0 °C] of dG (200 μM, upper) and 8-oxodG (200 μM, lower) in H_2O:CH_3CN (9:1) during the ketone photolysis.

Inadequate experiments (see Supporting Information).[30] The structurally definitive NMR-spectral characteristic is the carbon atom at δ 80 ppm, which is coupled to two direct carbon neighbors. This resonance is assigned to the C-5 spiroatom in spiroiminodihydantoin, since the corresponding C-4 atom in the 4-HO-8-oxodG structure has only one neighboring carbon atom.

In the photolysis of dG without ketone (blank), only traces of these oxidation products were observed, whereas upon addition of the ketones AP, AP-OH, AP-OAc, and BP substantial dG conversion was obtained.[31] The main oxidation products were spiroiminodihydantoin and GRP, while appreciable amounts of 8-oxodG (7% at 40% conversion) are only detected for AP-OH. A comparison of the amounts of detected photooxidation products with the extent of dG conversion reveals that for the derivatives AP-OH and AP-O^{t}Bu ca. 70−80% of the consumed dG is accounted for. In contrast, with the ketones AP, AP-OAc, and BP the quantified photooxidation products amounted to only approximately 30% of the dG conversion.

The Photooxidation Products of 8-oxodG. In the photolysis of 8-oxodG without ketone (blank), also only traces of oxidation products were observed (Figure 6), whereas upon addition of the ketones substantial conversion of 8-oxodG was induced.[31] For the ketones AP, AP-OAc, and BP more spiroiminodihydantoin than GRP was observed, whereas with AP-OH and AP-

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(27) Ravanat, J.-L.; Berger, M.; Benard, F.; Langlois, R.; Ouellet, R.; van Lier, J. E.; Cadet, J. *Photochem. Photobiol.* **1992**, *55*, 809−814.
(28) Ravanat, J.-L.; Cadet, J. *Chem. Res. Toxicol.* **1995**, *8*, 379−388.
(29) Luo, W.; Muller, J. G.; Rachlin, E. M.; Burrows, C. J. *Org. Lett.* **2000**, *2*, 613−616.
(30) Adam, W.; Arnold, M. A.; Grüne, M.; Nau, W. M.; Pischel, U.; Saha-Möller, C. R. *Org. Lett.* **2002**, *4*, 537−540.
(31) For the methoxy derivative AP-OMe, only low conversion of both dG and 8-oxodG was found.

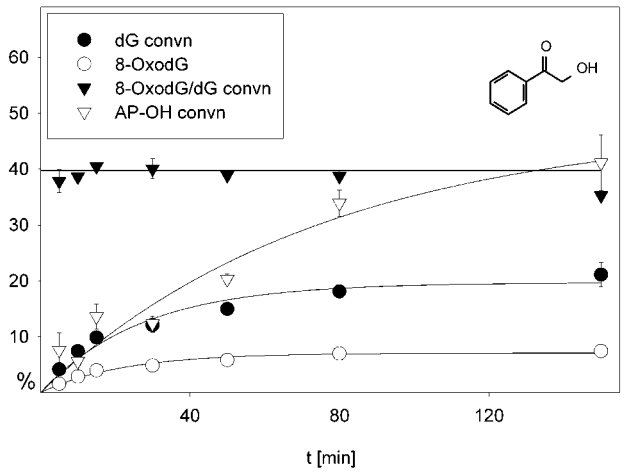

Figure 7. Time profile of the photoinduced (300 nm, 0 °C) oxidation of dG (500 μM) by the ketone AP-OH (1.00 mM) in 5.00 mM phosphate buffer (pH 7.0): CH_3CN (9:1).

O^{t}Bu the quantity of GRP exceeded that of spiroiminodihydantoin. Comparison of the amounts of detected photooxidation products with the extent of 8-oxodG conversion indicates that with the ketones AP, AP-OAc, and BP ca. 70% of the consumed 8-oxodG is accounted for; however, in the case of the derivatives AP-OH and AP-O^{t}Bu only approximately 30% of the converted 8-oxodG is accounted for by the quantified photooxidation products spiroiminodihydantoin and GRP. A comparison of the efficacy of the 8-oxodG photooxidation versus dG (cf. % conversion of 8-oxodG and dG in Figure 6) disclosed that the ketones AP, AP-O^{t}Bu, AP-OAc, and BP are slightly (10−20%) more reactive toward 8-oxodG, whereas AP-OH is approximately 2 times less reactive.

Time Profiles of the dG Photooxidation by AP−OH. The time dependence of the conversion of dG and of the formation of the oxidation product 8-oxodG during the photooxidation by the ketone AP-OH are shown in Figure 7. Comparison of the formation of 8-oxodG (open circles) with the conversion of dG (solid circles) reveals that the curves run parallel over the time period. This is also reflected in a constant ratio of the 8-oxodG:dG conversion with time (solid triangles). After 1 h of irradiation, the time profiles for the dG conversion (solid circles) as well as for the formation of 8-oxodG (open circles) level off, whereas AP-OH (open triangles) is further consumed. Although the ketone AP-OH is further consumed, the dG conversion stops at approximately 20%.

The Effect of dG on the Photooxidation of 8-oxodG in the Ketone Photolysis. The photooxidative persistence of 8-oxodG during the photooxidation of dG in the ketone photolysis was determined by measuring a time profile for the consumption of 8-oxodG with (open circles) and without (solid circles) dG (Figure 8). For the ketones AP-OH and AP-O^{t}Bu (left-hand plots), in the absence of dG (solid circles), the 8-oxodG is photooxidized, but by AP-OH (upper left-hand plot) less efficiently than by AP-O^{t}Bu (lower left-hand plot). In the presence of dG (open circles), for the ketone AP-OH (upper left-hand plot) the amount of 8-oxodG actually grows with time instead of the expected decrease. The growth (open circles) parallels the formation of 8-oxodG in the photooxidation of dG (solid triangles). For the ketone AP-O^{t}Bu (lower left-hand plot) the photooxidation of 8-oxodG proceeds in the presence of dG

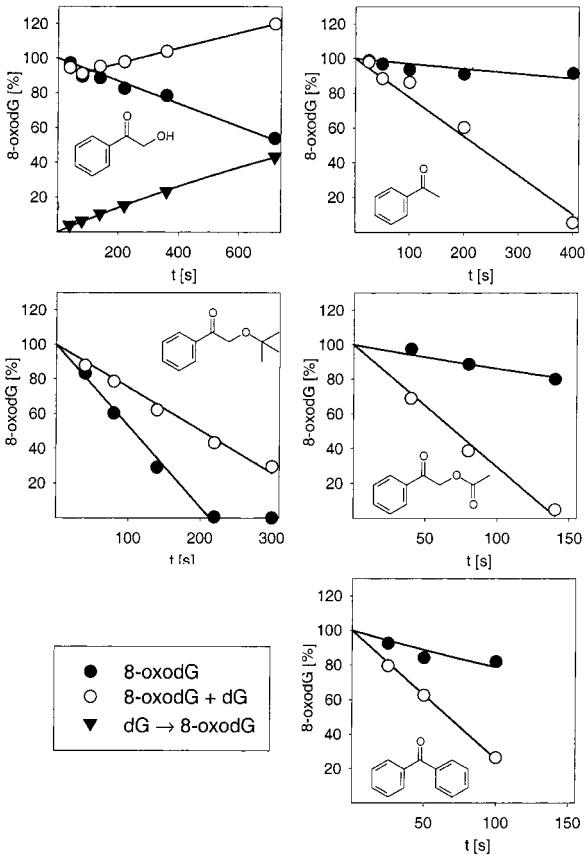

Figure 8. Time profile for the 8-oxodG photooxidation in the ketone photolysis [25 μM 8-oxodG, 1.00 mM ketone, 300 nm, 0 °C, 5.00 mM phosphate buffer (pH 7.0): CH_3CN (9:1)] with and without dG (500 μM); the starting concentration of 8-oxodG (25 μM) was set to 100%.

(open circles) less efficiently than in the absence of dG (solid circles) by a factor of approximately 2. For AP, AP-OAc, and BP (right-hand plots), slow photooxidation of 8-oxodG is observed in the absence of dG (solid circles), which in the presence of dG (open circles) is drastically accelerated for all three ketones.

Discussion

To rationalize the present dG and 8-oxodG photooxidation by the acetophenone-derived ketones mechanistically, the pertinent qualitative trends are compared in Table 3. It becomes evident that the ketones may be divided into two groups: The first one (photochemically inactive: AP, AP-OAc, and BP) shows no photochemical activity in the absence of the dG or 8-oxodG substrate; the second group (photochemically active: AP-OH and AP-O^{t}Bu) generates radical species upon irradiation as confirmed by DMPO trapping and EPR-spectral detection. For convenience, we designate the *photochemically inactive* ketones AP, AP-OAc, and BP as the group A ketones and the *photochemically active* AP-OH and AP-O^{t}Bu as the group B ketones. Since the direct oxidation of dG or 8-oxodG substrates by the triplet ketone plays a role even for the group B ketones, the oxidative reactivity of the group A ketones will be discussed first.

The Oxidative Reactivity of the Triplet-Excited Group A Ketones (No Radical Release). Spin-trapping experiments with DMPO (Table 1) reveal that no radicals are formed upon irradiation, and thus, no α cleavage occurs for these ketones.

Scheme 4. Electron Transfer between 8-oxodG and the $dG^{\bullet+}$ Radical Cation and the $dG^{\bullet}$ Radical

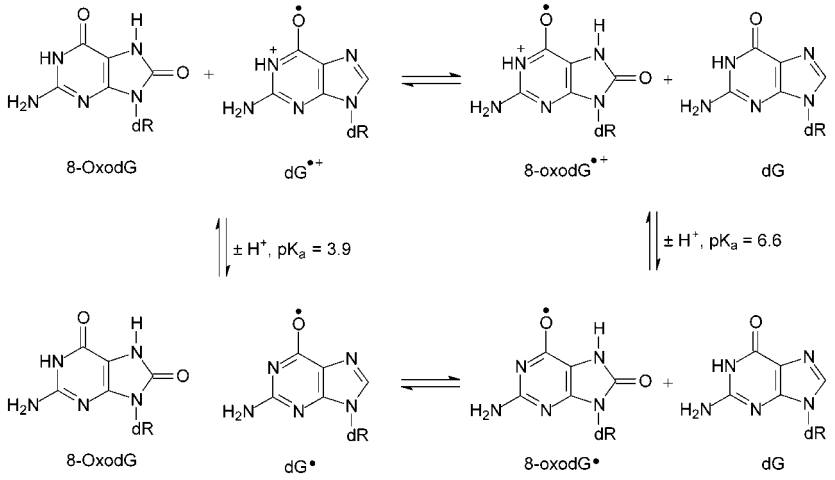

Table 3. Reactivity Trends in the Photochemical and Photobiological Activity of Triplet-Excited Ketones

reactivity	AP-OH	AP-OtBu	AP	AP-OAc	BP
AP-R decomposition without substrate[a]	+	++	-	-	-
Norrish-Type-I cleavage[b]	+	++	-	-	-
photooxidation at low [dG][c]	++	++	-	-	-
dG convn to 8-oxodG[d]	++	-	-	-	-
8-oxodG convn to spiroiminodihydantoin[d]	+	+	++	++	++
dG increases rate of 8-oxodG oxidation[e]	-	-	++	++	++
D_2O effect (dG oxidation)[a]	-	-	-	-	-
dG oxidation without O_2 [a,f]	-	-	-	++	-
O_2 (excess) quenching of dG oxidation [a,g]	-	-	+	++	+

[a] Data not shown. [b] Table 1. [c] Figure 2. [d] Figure 6. [e] Figure 8. [f] Upon three freeze−pump−thaw cycles. [g] Upon saturation with O_2.

In the absence of dG or 8-oxodG, only negligible conversion of the ketone was observed upon irradiation (Figure 5). Thus, deactivation of the triplet ketone proceeds by photophysical processes (phosphorescence, quenching) rather then chemical transformations. In the presence of dG or 8-oxodG the triplet ketones directly interact with these substrates. Indeed, the triplet ketones are quenched with rate constants around 3×10^9 M^{-1} s^{-1} for dG and 2×10^9 M^{-1} s^{-1} for 8-oxodG (Table 2), which is more than an order of magnitude faster than the interaction of the ketone with itself (self-quenching). That the direct interaction of the triplet-excited ketone with the substrate is responsible for the dG or 8-oxodG oxidation was confirmed by varying the dG and 8-oxodG concentrations. Thus, at low substrate concentrations, a linear increase of substrate conversion with substrate concentration was observed for dG (Figure 2) and 8-oxodG (Figure 4). The possible participitation of singlet oxygen for the photooxidation of dG is unlikely because D_2O, in which singlet oxygen possesses a 10-fold longer lifetime,[32] displayed no effect on the dG conversion. Thus, we conclude that for the group A ketones, their triplet states interact directly with the substrate as the principal step in the photooxidation.

The question whether 8-oxodG, although not detected as an oxidation product of dG (Figure 6), may be involved as an intermediate in the photooxidation by the group A ketones was assessed by kinetic measurements of its oxidation with these triplet ketones (Figure 8). For AP, AP-OAc, and BP, slow direct photooxidation of 8-oxodG was observed but had 8-oxoG been formed during the photooxidation of dG, enough would have remained for detection. To simulate the conditions under which the initially formed 8-oxodG is further photooxidized by excess dG, an authentic mixture of 8-oxodG and dG was submitted to photolysis (Figure 8). Compared to the results obtained in the absence of dG, for the group A ketones AP, AP-OAc, and BP, the disappearance rates of 8-oxodG increased drastically. Even when most of the 8-oxodG has been consumed, its rate of disappearance (slope of the curve in Figure 8) does not level off. Thus, an intermediate is formed in the photooxidation of dG, which consumes 8-oxodG. We propose that this intermediate is the $dG^{\bullet+}$ radical cation or the $dG^{\bullet}$ radical, which oxidizes 8-oxodG by electron transfer (Scheme 4). On the basis of the oxidation potentials of dG (assumed to be similar to that of guanosine: 1.29 V/NHE[33]) and 8-oxodG (0.74 V/NHE[34]), the equilibrium should be displaced to the right side. An analogeous oxidation of 8-oxodG by the guanosine radical with a rate constant of $4.6 \cdot 10^8$ M^{-1} s^{-1} has been described by Steenken et al.[35] However, if a fast equilibrium operates between the $dG^{\bullet+}$ radical cation and the $dG^{\bullet}$ radical due to deprotonation, a mechanistic distinction between the upper and the lower branches of Scheme 4 is difficult, and presumably both $dG^{\bullet+}$ and $dG^{\bullet}$ oxidize the 8-oxodG.

If molecular oxygen is excluded from the reaction mixture, irradiation of dG in the presence of AP and BP does not photooxidize dG. The first step of the oxidation mechanism (electron transfer) must be independent of oxygen. For this reason, the $dG^{\bullet+}$ radical cation or the $dG^{\bullet}$ radical (formed upon

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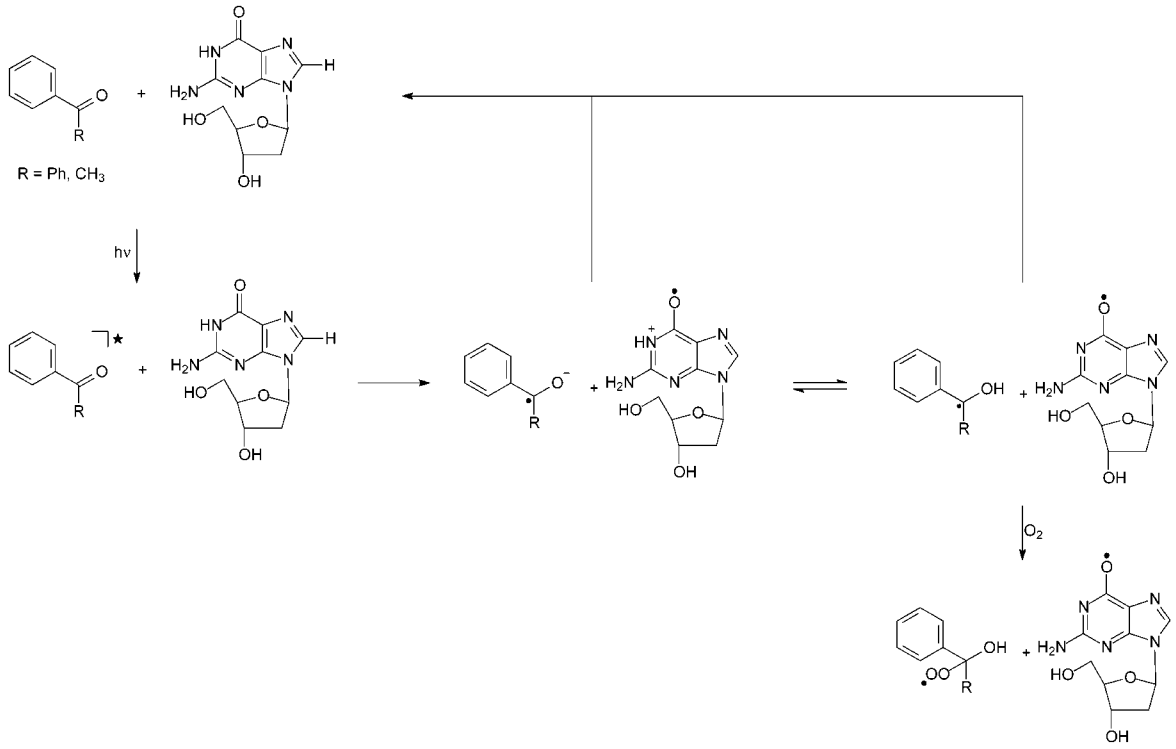
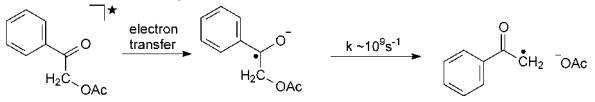
Scheme 5. Reversible Formation of Ketyl Radicals, $dG^{\bullet+}$ Radical Cations, and dG• Radicals for the Ketone Photolysis in the Presence of dG



Scheme 6. Heterolytic Fragmentation of the Ketyl Radicals Formed in the Photolysis of AP-OAc.



deprotonation of the former) must be reduced to regenerate the substrate dG; thus, no photooxidation of dG would be observed. The ketyl radical or the protonated ketyl radical (formed from the ketyl radical at pH 7) may act as the reducing agent (Scheme 5). Such a reversible electron transfer was already observed by Schuster et al. between DNA and triplet-excited anthraquinone derivatives in the absence of molecular oxygen.[35] In the presence of molecular oxygen, the protonated ketyl radicals will be scavenged in terms of peroxyl radicals, and thus, the amount of both the protonated and unprotonated ketyl radicals would be decreased. Consequently, under these conditions, less reduction of the dG• radical to dG should occur and more dG oxidation is expected, as observed.

Nevertheless, AP-OAc photooxidizes dG even in the absence of O_2. Since the ketyl radical formed upon electron transfer to AP-OAc is known to undergo fast heterolytic scission to the benzoylmethyl radical and the acetate anion,[36] it is concluded that this fate of the AP-OAc ketyl radical is responsible for the dG photooxidation. Thus, in the photolysis of AP-OAc, the ketyl radical is consumed by unimolecular scission and is not available for protonation and subsequent regeneration of dG from the $dG^{\bullet+}$ radical cation by reduction (Scheme 6).

For all three group A ketones, the amount of dG oxidation is decreased in an oxygen atmosphere compared to that under normal aerobic conditions. This is rationalized in terms of oxygen quenching of the triplet ketones and thereby a decrease of the triplet lifetime and a lower probability of dG photooxidation. In addition to the lack of a D_2O effect, this also indicates that the involvement of singlet oxygen is negligible in this photooxidation, because oxygen quenching would generate singlet oxygen, and an increase in the photooxidation of dG should be observed, which is not the case.

In accordance with previous results for BP,[6] the formation of the photooxidation products may be explained by electron transfer from dG to the triplet-excited ketone (type I photooxidation, Scheme 7). In the latter, the predominant formation of oxazolone was rationalized in terms of rapid deprotonation of the guanine radical cation in aqueous solution (Scheme 7, path a). The fact that no 8-oxodG could be observed was attributed to this rapid deprotonation of the dG radical cation (path a), and thus, no nucleophilic attack of water (as is the case in DNA)[37−40] occurs (path b). Our studies (Figure 8) clearly demonstrate that 8-oxodG does not persist in the presence of dG and is presumably oxidized by the $dG^{\bullet+}$ radical cation (Scheme 4). Consequently, the fact that no 8-oxodG is detected in the oxidation of dG does not necessarily mean that it is not formed during the oxidation process. However, we cannot exclude direct oxidation of dG to the spiroiminodihydantoin, which may proceed along path c.

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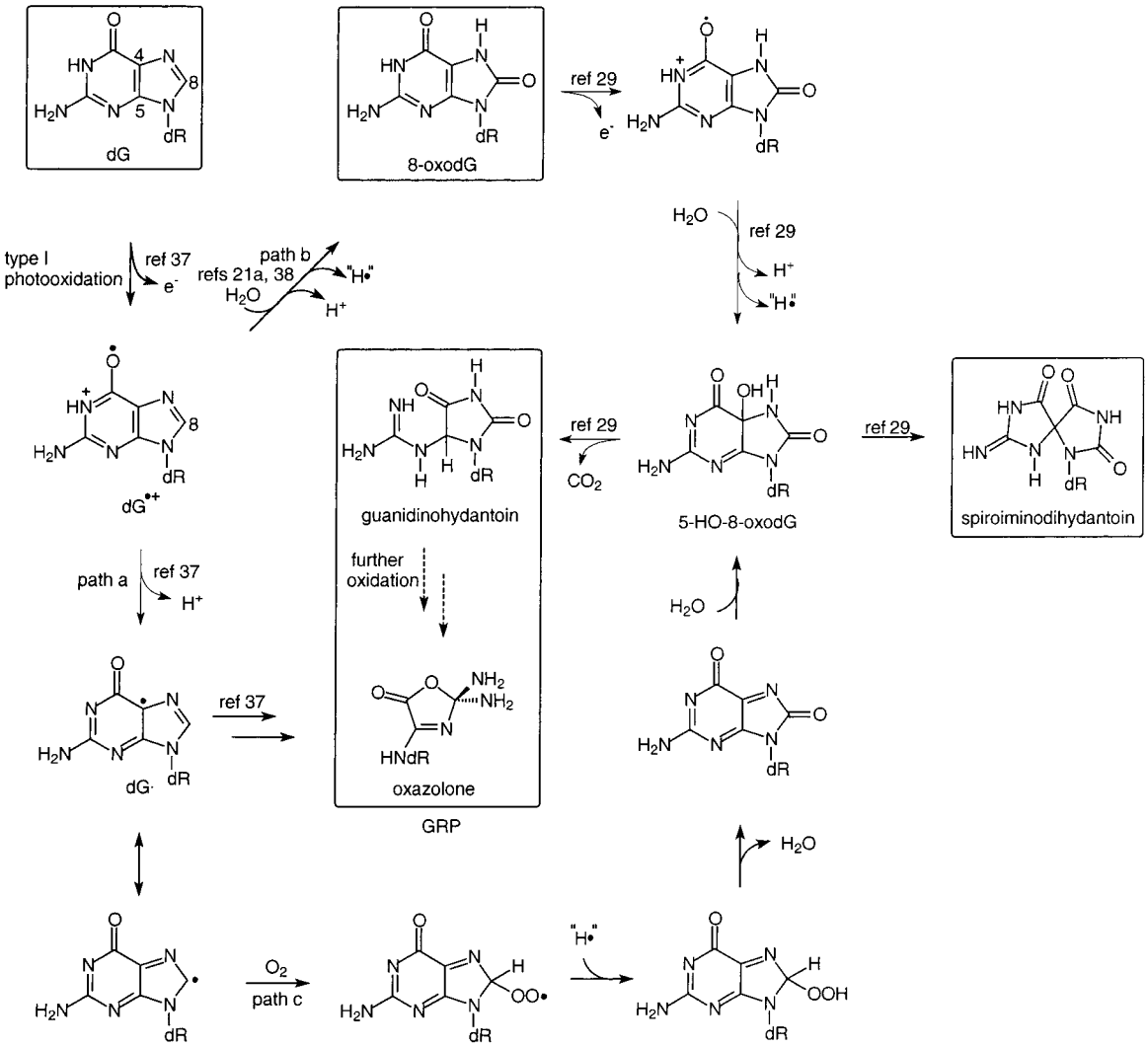
(39) Dizdaroglu, M. *Free Radical Biol. Med.* **1991**, *10*, 225−242.
(40) Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1993**, *115*, 2437−2440.

Scheme 7. Proposed Mechanism for the Oxygen-Dependent (Path a, ref 37) and Oxygen-Independent (Path b, ref 21a, 38) Type-I Photooxidation of dG



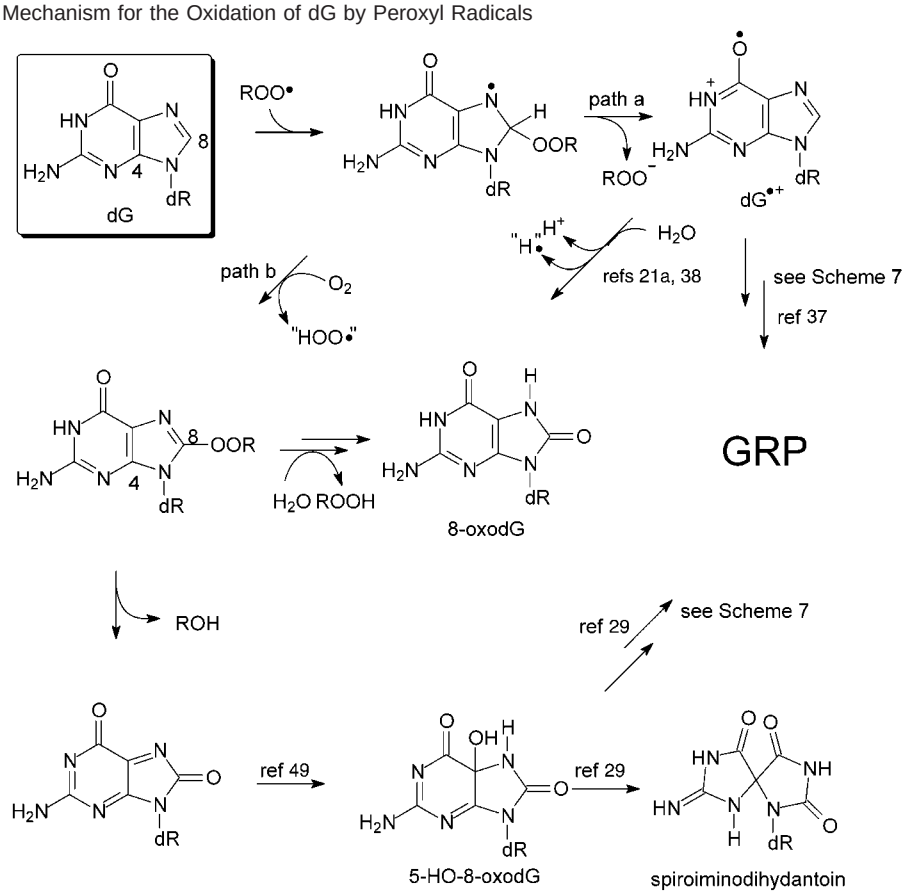
The oxidation of 8-oxodG by AP, AP-OAc, and BP yields the spiroiminodihydantoin (Figure 6) and GRP (see Scheme 7). The yields of these two products in the oxidation of 8-oxodG are higher than in the direct oxidation of dG. Burrows et al.[29] describe the type I oxidation of 8-oxodG as proceeding through the 8-oxodG$^{\bullet+}$ radical cation, which upon water attack at the C-5 position and subsequent one-electron oxidation yields 5-HO-8-oxodG (Scheme 7). In aqueous solution, the 5-HO-8-oxodG is transformed to spiroiminodihydantoin and guanidinohydantoin; the latter is eventually transformed to oxazolone by reported steps.[37,41] On the basis of our results that no $^1O_2$ is involved and that 8-oxodG is rapidly oxidized in the presence of dG to its radical cation 8-oxodG$^{\bullet+}$, we propose that the spiroiminodihydantoin is formed in the dG oxidation from the intermediary 8-oxodG (generated in path b) through one-electron oxidation, as described by Burrows et al.[29] The product ratios of spiroiminodihydantoin and GRP observed in the photooxidation of dG (ca. 1:1) and 8-oxodG (ca. 2:1) by group A ketones (Figure 6) provide support, because in the dG photooxidation, GRP may be produced directly from dG through path a in Scheme 7.

In this context, the 4-HO-8-oxodG (actually spiroiminodihydantoin[30,42]) has been previously considered as a characteristic type II (singlet oxygen) photooxidation product of dG.[38c] Now that the spiroiminodihydantoin structure has been established in the one-electron oxidation of acetylated 8-oxodG,[29] the erroneously assigned 4-HO-8-oxodG serves no longer as a characteristic monitor for type II ($^1O_2$) photooxidation. Indeed, we have observed the formation of spiroiminodihydantoin in the oxidation of dG by excited benzophenone[6] and by peroxyl radicals[9,10,43] under conditions at which no appreciable amounts of singlet oxygen are produced.

Oxidative Reactivity of Triplet-Excited Group B Ketones (Radical Release). For the ketones AP-OH and AP-O^{t}Bu, spin-trapping experiments with DMPO revealed that α cleavage occurs, since for both ketones a benzoyl radical and the corresponding oxymethyl radical was trapped and identified by EPR spectroscopy. Thus, the group B ketones are consumed through α cleavage even in the absence of dG and 8-oxodG,

(41) Vialas, C.; Claparols, C.; Pratviel, G.; Meunier, B. *J. Am. Chem. Soc.* **2000**, *122*, 2157−2167.
(42) Niles, J. C.; Wishnok, J. S.; Tannenbaum, S. R. *Org. Lett.* **2001**, *3*, 963−966.
(43) Adam, W.; Kurz, A.; Saha-Möller, C. R. *Chem. Res. Toxicol.* **2000**, *13*, 1199−1207.
(44) Ingold, K. U. *Acc. Chem. Res.* **1969**, *2*, 1−9.

Scheme 8. Proposed Mechanism for the Oxidation of dG by Peroxyl Radicals



which contrasts the photochemical behavior of the group A ketones.

The triplet states of AP-OH and AP-O^{t}Bu possess lifetimes of 1 and 0.5 μs and are, thus, by approximately a factor of 10 shorter-lived than those of the group A ketones. The shorter lifetimes are attributed to the efficient α cleavage of the triplet ketone. The dG quenching constant for triplet-excited AP-OH, assessed by time-resolved laser-flash spectroscopy (Table 2), does not differ significantly from those of the group A ketones; AP-O^{t}Bu exhibits a slightly greater reactivity toward dG and 8-oxodG. Since the α cleavage is a unimolecular process and the reaction of the triplet ketone with dG or 8-oxodG is bimolecular, the latter will be of minor importance at low substrate concentrations; however, at higher dG or 8-oxodG concentrations, oxidation of these substrates may compete with α scission. By lowering the dG and 8-oxodG concentrations, instead of a decrease in the substrate conversion even a slight increase was observed (Figures 2 and 4); also this photooxidative activity contrasts the behavior of the group A ketones. This suggests that a long-lived species with a lifetime much longer than for the triplet ketones is responsible for the observed dG and 8-oxodG photooxidation. Analogous to the group A ketones, the participation of singlet oxygen was excluded because of the lack of D_2O effect on the dG conversion. Consequently, we propose peroxyl radicals as the active species, which are formed by O_2 trapping of the carbon-centered radicals derived from the α cleavage of the triplet ketones. Peroxyl radicals possess the necessary long lifetimes,[44] since no effective unimolecular deactivation channels are available as for the triplet ketones. Also, the formation of 8-oxodG in the concentration-dependent photooxidation of dG by AP-OH follows a similar behavior, and thus, 8-oxodG as well is attributed to arise from the direct oxidation by the long-lived peroxyl radicals.

AP-O^{t}Bu oxidizes 8-oxodG more efficiently than AP-OH. While for AP-O^{t}Bu all of the 8-oxodG is consumed within 3−4 min, with AP-OH still approximately 80% is left. In the presence of dG, the concentration of 8-oxodG even increases, which means that its formation is more efficient than its consumption. In the presence of dG, the oxidation efficiency of 8-oxodG by AP-O^{t}Bu is diminished, but approximately 70% of the 8-oxodG is consumed after 5 min. Thus, as in the case of the group A ketones, for AP-O^{t}Bu the 8-oxodG is also consumed more rapidly than it is formed.

For AP-OH as well as for AP-O^{t}Bu, no oxidation of dG occurs in the absence of oxygen, because the carbon-centered radicals formed upon α cleavage of the excited ketones cannot generate peroxyl radicals. However, carbon-centered radicals (e.g., hydroxymethyl radicals) are known[45] to add to the C-8 position of dG, and such C-8 radical adducts may be oxidized to modified dG products. In the absence of an oxidant, the addition is reversible, and no dG modification is observed.

The mechanism of the oxygen-dependent photooxidation of dG and 8-oxodG is displayed in Scheme 8, for which peroxyl radicals are held responsible as the efficacious oxidant. The

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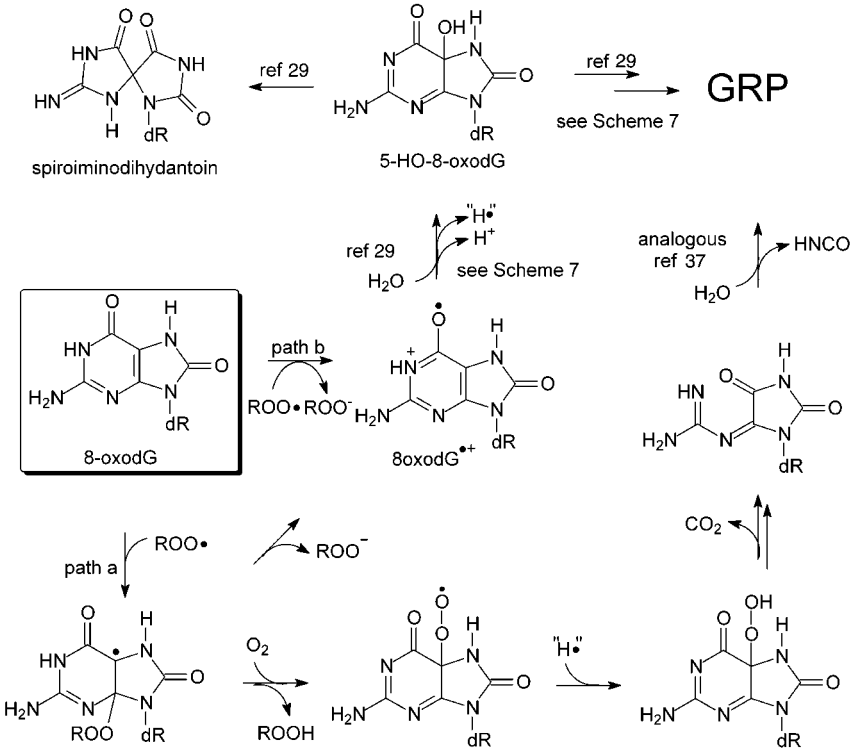
Scheme 9. Proposed Mechanism for the Oxidation of 8-oxodG by Peroxyl Radicals



preference of radical species to add at the C-8 position of the guanine moiety is a quite general phenomenon for carbon-centered[46,47] as well as hydroxyl radicals.[48] Thus, the key step in the proposed mechanism is the oxidation of dG by the addition of peroxyl radicals at the C-8 site of dG. The peroxyl-radical adduct has a number of options to transform chemically, of which we shall focus on the two that appear to us to be the more likely ones: In the first option, the radical adduct eliminates the peroxyl anion and thereby produces the radical cation $dG^{\bullet+}$ (Scheme 8, path a), which may undergo the same reactions as already discussed for the group A ketones (see Scheme 7). The latter may form 8-oxodG by water attack at the C-8 position and subsequent oxidation.[39] In the second option, the radical adduct is oxidized (e.g., by molecular oxygen) to form the dG peroxide product (path b). The latter may be hydrolyzed to 8-oxodG by the attack of water at the C-8 position with the elimination of ROOH. In competition, base-catalyzed elimination of ROH leads to the reactive diimine,[49] which upon hydrolysis affords 5-HO-8-oxodG,[49] the precursor to the spiroiminodihydantoin and GRP (see Scheme 7).

The mechanism for the oxidation of 8-oxodG by peroxyl radicals is quite similar, as shown in Scheme 9. Analogous to dG, for which after the C-8 position the next most reactive position toward oxyl radicals is the C-4 position,[48,50,51] we expect an attack of the peroxyl radical at the C-4 position of 8-oxodG as the key step in the 8-oxodG oxidation (Scheme 9). This peroxyl-radical adduct of 8-oxodG may eliminate the peroxyl anion to form the radical cation $8\text{-oxodG}^{\bullet+}$ (path a). Alternatively, the latter may be formed directly by electron transfer with ROO• (path b), because 8-oxodG possesses a significantly lower oxidation potential than dG (assumed to be similar to that of guanosine).[34] The $8\text{-oxodG}^{\bullet+}$ radical cation may be transformed to spiroiminodihydantoin and GRP, as already described for the group A ketones (see Scheme 7). Finally, the dG peroxyl-radical adduct may react with molecular oxygen, followed by the elimination of hydroperoxide, from which the oxazolone is produced by reported steps.[37,41]

Conclusions

The present results on the photooxidation of dG and 8-oxodG have allowed for the first time the recognition that triplet-excited ketones must be divided into two groups according to their photooxidative activity: Group A ketones (AP, AP-OAc, and BP) do not generate radical species on photoexcitation; thus, such triplets photooxidize the substrate directly, presumably by electron transfer to afford the radical cations $dG^{\bullet+}$ and $8\text{-oxodG}^{\bullet+}$. These radical cations are subsequently transformed to their final oxidation products by conventional steps.[29,37] The group B (AP-OH and AP-O^{t}Bu) ketones additionally undergo competitive α cleavage; thus, these triplet-excited ketones lead to carbon-centered radicals, which upon trapping of molecular oxygen yield peroxyl radicals as effective oxidants of dG and 8-oxodG. The acetoxy derivative AP-OAc is an unusual case in regard to its behavior: Its triplet-excited state is reluctant to undergo α cleavage, and thus, electron-transfer chemistry prevails, but the resulting radical anion readily eliminates the acetate ion and generates the benzoylmethyl radical. Consequently, this ketone possesses the dual character to act as electron-transferer and presumably as radical oxidant.

The principle difference in the photooxidative reactivity of these two types of ketones is that for the group A ketones to be active, the dG or 8-oxodG concentrations must be high enough, since the decisive step entails a bimolecular interaction between the triplet ketones and the substrate. In contrast, the group B ketone triplets operate also at low substrate concentrations

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because they possess the unimolecular α cleavage pathway to produce long-lived radicals for the oxidation of the substrate. However, for the group B ketones to photooxidize dG and 8-oxodG, molecular oxygen must be available to generate the required oxidatively effective peroxyl radicals by trapping of the carbon-centered radicals initially formed in the α cleavage of the triplet-excited ketone. Of course, at high substrate concentrations, in addition to the formation of radical species, the direct interaction of the triplet-excited ketones with the substrate also takes place for the group B ketones, as displayed by the group A ketones. Since we see a distinct difference in the product distribution for the photooxidation of dG and 8-oxodG between the group A and group B ketones (Figure 6), we surmise that at the employed reaction conditions, the radical reactivity of the group B ketones dominates over the direct photooxidation by their triplet states.

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Supporting Information Available: Experimental details for the preparation of the key compounds as well as the procedures for the quantitative determination of dG oxidation products (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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