Oxidative DNA Damage by Radicals Generated in the Thermolysis of Hydroxymethyl-Substituted 1,2-Dioxetanes through the α Cleavage of Chemiexcited Ketones

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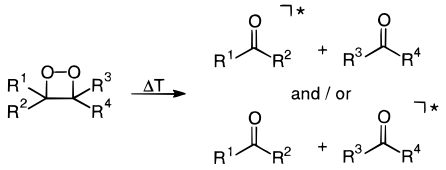
Abstract: The 3-(hydroxymethyl)-3,4,4-trimethyl-1,2-dioxetane (HTMD) highly efficiently damages DNA compared to the merely alkyl-substituted derivative 3,3,4,4-tetramethyl-1,2-dioxetane (TMD). To elucidate this difference in oxidative reactivity, two additional hydroxymethyl-substituted 1,2-dioxetanes, namely *cis*/*trans*-3-(hydroxymethyl)-3,4-dimethyl-4-(phenylmethyl)- (**1**α/**1**β) and 3-(hydroxymethyl)-4,4-dimethyl-3-(phenylmethyl)-1,2-dioxetane (**2**), were investigated in regard to their photochemical and photobiological properties. The high genotoxic effects of the hydroxymethyl-substituted 1,2-dioxetanes, which are reflected in the significant formation of single-strand breaks in plasmid pBR 322 DNA and the efficient oxidation of guanine in calf thymus DNA and the nucleoside 2'-deoxyguanosine (dGuo), are for the first time understood in terms of radical chemistry. The reactivity order of the dioxetanes **1**α/**1**β > HTMD > **2** ≫ TMD to damage DNA parallels the propensity of these dioxetanes to generate radicals. These reactive species are formed in the thermolysis of the dioxetanes through α cleavage of the intermediary triplet-excited α-hydroxy- and α-phenyl-substituted carbonyl products. The presence of radicals was confirmed by spin-trapping experiments with 5,5-dimethyl-1-pyrroline *N*-oxide and by laser-flash photolysis. These carbon-centered radicals are efficiently scavenged by molecular oxygen to produce peroxyl radicals, which are proposed as the active DNA-damaging species in the thermal decomposition of the hydroxymethyl-substituted 1,2-dioxetanes HTMD, **1**α/**1**β, and **2**.

Introduction

1,2-Dioxetanes have been postulated as labile intermediates in a variety of biologically relevant processes[1] such as in the enzymatic generation of electronically excited states in oxidative stress and in the induction of spontaneous mutations.[2] The most characteristic property of such high-energy cyclic peroxides is their efficient generation of electronically excited carbonyl fragments on thermolysis (Scheme 1).[1,3] Therefore, 1,2-dioxetanes have been extensively used in the past to study the genotoxicity of excited carbonyl compounds in the dark.[1] The thermal generation of excited states from dioxetanes has the advantageous feature that it circumvents the direct exposure of biological systems, in particular cells, to UV radiation. Thereby, valuable mechanistic insight into the complex process of DNA photogenotoxicity and its mutagenic consequences may be assessed. This has paved the way for extensive studies on an important bioorganic topic, namely the *photobiology without light*,[4−11] a subject primarily propagated by the late Professor G. Cilento (São Paulo).

Scheme 1. Generation of Electronically Excited Ketones in the Thermolysis of 1,2-Dioxetanes



The use of dioxetanes as thermal source of excited ketones has demonstrated their efficiency to damage DNA in cell-free

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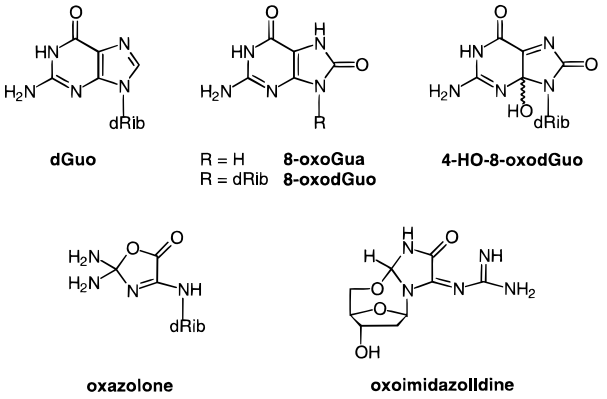
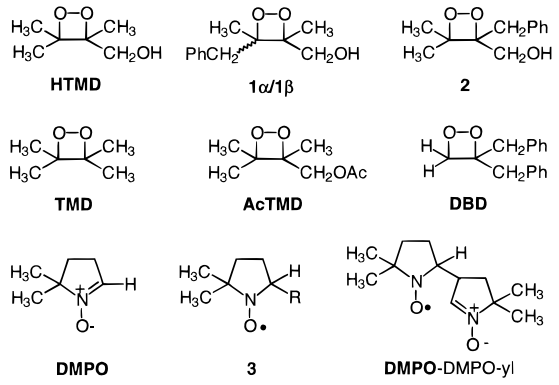
and cellular systems.[4] Besides the formation of pyrimidine dimers,[6] we have shown by means of the formamidopyrimidine DNA-glycosylase (FPG protein) that the majority of the induced DNA lesions constitute oxidative guanine modifications [e.g., 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxodGuo), formamidopyrimidines (Fapy)] and AP sites.[7]



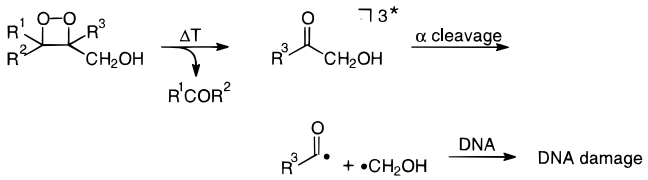
Recently, the chemical nature of the dioxetane-induced DNA modifications has been evaluated.[8−11] By using acetylated guanine as model compound, we have shown that the nucleophilic attack of the guanine base on the peroxide bond of 3,3-disubstituted 1,2-dioxetanes leads to the oxidation of guanine.[8] Investigations on calf thymus DNA have established that 1,2-dioxetanes, in particular alkyl-substituted ones, oxidize efficiently and almost exclusively the guanine base to form the mutagenic 7,8-dihydro-8-oxoguanine (8-oxoGua)[12−15] and the 2,2-diamino-[(2-deoxy-β-D-*erythro*-pentofuranosyl)-4-amino]-5(*2H*)-oxazolone (oxazolone)[16] in high yields.[9] The generation of these well-known DNA-photooxidation products was explained by the involvement of triplet-excited carbonyl compounds as reactive species, formed in the thermolysis of the dioxetanes.

3-Hydroxymethyl-3,4,4-trimethyl-1,2-dioxetane (HTMD) was hitherto found to be the most reactive 1,2-dioxetane compared to various derivatives without hydroxymethyl substitutents, e.g.



3,3,4,4-tetramethyl- (TMD) or 3-(acetoxymethyl)-3,4,4-trimethyl-1,2-dioxetane (AcTMD).[9] Our previous studies with the nucleoside 2'-deoxyguanosine (dGuo)[11] demonstrated that HTMD generates, besides the characteristic type-I photooxidation products oxazolone[16] and 2-(*S*)-2,5'-anhydro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-guanidinylidene-2-hydroxy-4-oxoimidazolidine[17] (oxoimidazolidine), also the type-II photooxidation products 4*R** and 4*S** diastereomers of 4-hydroxy-8-oxo-4,8-dihydro-2'-deoxyguanosine[18,19] (4-HO-8-oxodGuo) and 8-oxodGuo.[20] The type-I/type-II product ratio of approximately unity was explained in terms of the simultaneous involvement of a direct electron-transfer process between the triplet-excited ketone and guanine (type-I photooxidation)[21] and energy transfer to molecular oxygen with the formation of singlet oxygen (type-II photooxidation)[21] as oxidizing species.

Scheme 2. DNA Damage Induced by Radicals Generated in the Thermal Decomposition of Hydroxymethyl-Substituted 1,2-Dioxetanes



The remarkable reactivity of the hydroxymethyl-substituted 1,2-dioxetane HTMD to induce efficient oxidative DNA damage was tentatively attributed to its possible association to DNA or dGuo through hydrogen bonding. Our present study with HTMD and the two additional hydroxy-substituted dioxetanes **1**α/**1**β and **2** now demonstrates that the enhanced reactivity and distinct selectivity of the hydroxymethyl-substituted dioxetanes in the oxidative damage of DNA are due to radicals. The latter derive from the efficient and fast α cleavage of the electronically excited α-hydroxy-substituted ketone intermediates[22,23] (Scheme 2). The efficiency of radical formation and their identity was established by spin-trapping experiments with 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) coupled with EPR spectroscopy, by time-resolved UV spectroscopy, and by the inhibitory effect of radical scavengers. Our results reveal for the first time that hydroxymethyl-substituted dioxetanes serve as effective thermal sources of radicals, which provides a new, valuable tool for the investigation of radical-induced oxidative DNA damage (strand breaks and guanine oxidation) under nonphotolytic conditions.

Results

Preparation of the Hydroxymethyl-Substituted 1,2-Dioxetanes. The hydroxymethyl-substituted 1,2-dioxetanes **1**α/**1**β,[24] **2**,[24] and HTMD[25] were prepared according to the literature procedures. The relative configurations of the cis and trans isomers of dioxetane **1**α/**1**β could not be determined by NOE

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Table 1. Activation Parameters and Excitation Yields (Φ^S, $\Phi^T_{acetone}$) of the 1,2-Hydroxymethyl-Substituted 1,2-Dioxetanes HTMD, **1**α/**1**β, and **2** and of TMD for Comparison [a]

dioxetane	$t_{1/2}$ (h) 37 °C	$t_{1/2}$ (h) 50 °C	log A	E_a (kcal/mol)	$\Delta H^{\ddagger}$ (kcal/mol)	$\Delta S^{\ddagger}$ (eu)	$\Phi^T_{acetone}$ [b,c] (%)	$\Phi^S \times 10^2$ [b] (%)
HTMD[c]	39 ± 11	7.8 ± 0.5	11.9 ± 0.4	24.1 ± 0.5	23.4 ± 0.5	−6.3 ± 0.3	9.0 ± 0.9[d]	1.0 ± 0.2
1α	44 ± 4	8.2 ± 0.2	12.3 ± 0.1	24.7 ± 0.4	23.6 ± 0.3	−6.2 ± 0.1	*d*	1.8 ± 0.2
1β	37 ± 1	7.7 ± 0.2	12.1 ± 0.2	24.5 ± 0.5	24.1 ± 0.6	−4.7 ± 0.2	*d*	3.0 ± 0.3
2	46 ± 5	8.1 ± 0.4	14.2 ± 0.2	27.5 ± 0.8	27.1 ± 0.8	+4.5 ± 0.1	14.6 ± 2.1	2.2 ± 0.2
TMD[e]	45 ± 2		*f*	25.0 ± 1.0	24.9 ± 0.7	−2.8 ± 2.3	35 ± 4	25 ± 5

[a] CH_3CN as solvent, temperature constant within ± 0.1 °C, mean value of at least three independent runs, experimental data are listed in the Supplementary Information in Table S-1. [b] Determined at 50 °C in CH_3CN/toluene (80/20), experimental data are listed in the Supplementary Information in Table S-2. [c] Values referring to triplet-excited acetone only (cf. text). [d] Triplet quantum yields for the short-lived ketones 1-phenyl-2-propanone and 1-hydroxy-2-propanone were determined to be ca. 1% (cf. text). [e] Literature values[27] in toluene. [f] Not known.

experiments nor by X-ray crystallography since for the latter no suitable crystals were obtained.

Activation Parameters and Excitation Yields of the Dioxetanes. Although the dioxetanes **1**α/**1**β and **2** are known for some time,[24] their photophysical data have not been reported. Therefore, the activation parameters and excitation yields of these dioxetanes were determined. The rates of the dioxetane thermolysis were monitored by the decay of the chemiluminescence intensity in acetonitrile over a temperature range of 30 °C and were found to follow clean first-order kinetics. The activation parameters of **1**α/**1**β and **2** were determined according to standard isothermal kinetics.[26] The results are summarized in Table 1 together with the literature results for HTMD and TMD[27] for comparison. The activation enthalpy ($\Delta H^{\ddagger}$) of the dioxetane **2** was found to be by ca. 3 kcal/mol higher than those for HTMD and the two isomers of dioxetane **1**α/**1**β. The $\Delta S^{\ddagger}$ terms are negative and close to zero, as is usually the case for simple dioxetanes.[28] An exception is dioxetane **2**, for which $\Delta S^{\ddagger}$ is positive and offsets its higher $\Delta H^{\ddagger}$ value compared to the other dioxetanes.

Without added fluorescers, the thermolyses of the dioxetanes **1**α, **1**β, and **2** were only weakly chemiluminescent. Addition of 9,10-diphenylanthracene (DPA) and 9,10-dibromoanthracene (DBA) resulted in an increase in the chemiluminescence intensity without affecting the rate of dioxetane decomposition. The singlet and triplet excitation yields were determined by established chemiluminescence methods[29] with DPA for singlet[30] and DBA[30,31] for triplet counting (variation of the concentration of added fluorescers at constant dioxetane concentration and temperature). As expected,[3,28] the thermal decomposition of the dioxetanes HTMD, **1**α, **1**β, and **2** produced greater yields of triplet-excited carbonyl products than of excited singlets (Table 1). The singlet yields (Φ^S) for all hydroxymethyl-substituted dioxetanes were ≤0.1%.

The measured triplet yields must be interpreted with some caution since the experimental method, which relies on efficient bimolecular energy transfer between the triplet-excited ketone and the fluorescer, works reliably only for relatively long-lived (>10 ns) triplet states. Therefore, the triplet excitation yields ($\Phi^T_{acetone}$) of HTMD and **2** (ca. 15%) only reflect the fraction of triplet acetone (the hydroxy-substituted ketones are too short-lived, see below) and are about one-half that for the TMD (35%), which in view of its symmetrical nature releases exclusively the rather long-lived triplet-excited acetone.[27] The much smaller Φ^T values determined for the dioxetanes **1**α and **1**β (ca. 1%) represent, of course, a lower limit since these dioxetanes generate only the quite short-lived (efficient α cleavage) excited ketones 1-phenyl-[32] or 1-hydroxy-2-propanone.[22]

EPR Studies of the Dioxetanes and Their Ketone Products. The α cleavage of triplet-excited α-hydroxy- and α-phenyl-substituted ketones to generate radicals is a well-known photochemical reaction.[22,23,32] Since such excited ketones are formed in the thermolysis of the hydroxymethyl- and phenylmethyl-substituted 1,2-dioxetanes HTMD, **1**α/**1**β, and **2**, spin-trapping experiments with DMPO were performed to provide spectroscopic evidence for the generation of radicals in the thermal decomposition of such reactive cyclic peroxides. The results of the EPR investigations are shown in Table 2.

On thermolysis of HTMD in the presence of DMPO in aqueous acetonitrile, two characteristic doublet-of-triplets EPR signals were observed (Table 2, entry 1) for the DMPO adducts of the acetyl[33] and the hydroxymethyl radicals.[34] A control experiment revealed that no EPR signal was detected in the thermolysis of DMPO at 37 °C without dioxetane. For comparison, 1-hydroxy-2-propanone (1 mM) was irradiated at 300 nm for 30 min in the presence of DMPO (45 mM). The EPR spectrum was a superposition of the two DMPO-radical adducts (Table 2, entry 7), described above for the thermolysis of HTMD, which confirms that a pair of α-hydroxymethyl and acyl radicals is formed in the thermolysis of HTMD from triplet-excited 1-hydroxy-2-propanone through its α cleavage.[22]

The thermolysis of the simple alkyl-substituted dioxetane TMD, which only releases triplet-excited acetone as decomposition product, afforded a doublet-of-triplets signal pattern in its EPR spectrum (Table 2, entry 2). This EPR pattern is assigned to the known DMPO-DMPO-yl radical adduct, generated by hydrogen-atom abstraction from the hetero-allylic position of the nitrone by triplet-excited acetone and subsequent addition of the resulting DMPO-yl radical to a further molecule of DMPO.[35] The same EPR spectrum (Table 2, entry 8) was

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Table 2. EPR-Spectral Data of the Nitroxyl-Radical Adducts **3** Generated in the Thermolysis of Hydroxymethyl- and Phenylmethyl-Substituted Dioxetanes and the Photolysis of the Corresponding Ketone Fragmentation Products in the Presence of the DMPO as Radical Trap[a]

entry	substrate	conc. [mM]	cond.[b]	**3** R	g factor[c,d]	a_H[d,e,f] [Gauss]	a_N[d,e,f] [Gauss]
1	HTMD	20	A	**CH_2OH**	2.0058 (2.0055)	22.6 (22.6)	15.8 (15.9)
				$COCH_3$	2.0056 (2.006)	18.5 (18.5)	14.9 (14.9)
2	TMD	100	A	**DMPO-yl**	2.0059	23.0 (22.8)	15.7 (15.6)
3	AcTMD	100	A	no EPR signal detected			
4	1α/1β	20	A	**C radical**	2.0056[g]	22.8[g]	15.8[g]
				$COCH_3$	2.0057 (2.006)	18.8 (18.5)	15.4 (14.9)
5		20	A	no EPR signal detected			
6	2	100	A	**C radical**	2.0058[g]	22.6[g]	15.9[g]
7		1	B	**CH_2OH**	2.0053 (2.0053)	22.6 (22.6)	15.8 (15.9)
				$COCH_3$	2.0056 (2.006)	18.9 (18.5)	15.3 (14.9)
8		10	B	**DMPO-yl**	2.0058	22.8 (22.8)	15.6 (15.6)
9		1	B	no EPR signal detected			
10		1	B	**$COCH_3$**	2.0056 (2.006)	18.9 (18.5)	15.3 (14.9)
11		1	B	**COX**[h]	2.0056	18.9	15.5

[a] [DMPO] = 45 mM, H_2O/CH_3CN (95:5). [b] **A**: 37 °C, 40 min, **B**: $h\nu$ (300 nm, Rayonet photoreactor), 30 min. [c] Landé g factor, error ± 0.0001. [d] The literature values[33−35] are given in parentheses. [e] Hyperfine coupling constants a_H (with α-H) and a_N (with N) of the nitroxyl radical **8** of DMPO, error ± 0.0001 G. [f] In all EPR spectra, traces of the DMPO-OH trapping product [$a_H = a_N = 14.8$ G, $g = 2.0054$, literature values:[35a,40] $a_H = a_N = 14.9$ G, $g = 2.0053$] were detected. [g] Mean values since the EPR signals of the DMPO-CH_2OH, DMPO-DMPO-yl, and DMPO-CH_2Ph adducts overlap. [h] X = CH_2OH versus CH_2Ph are not clearly distinguishable, no literature data available.

obtained when acetone (10 mM) was irradiated at 300 nm for 40 min in the presence of DMPO (45 mM). Since HTMD leads to both triplet-excited acetone and 1-hydroxy-2-propanone on thermolysis, besides spin trapping of the released hydroxymethyl radicals from triplet-excited 1-hydroxy-2-propanone, also the DMPO-DMPO-yl radical adduct from the triplet-excited acetone is expected. Unfortunately, the similar hyperfine coupling constants of the DMPO-CH_2OH and DMPO-DMPO-yl radical adducts (cf. Table 2, entries 1 and 2) do not allow a definitive EPR-spectral confirmation. Nevertheless, the formation of the adduct between DMPO and the acyl radical in the thermolysis of HTMD (Table 2, entry 1) demonstrates unequivocally its propensity to produce radicals through α cleavage of the triplet-excited 1-hydroxy-2-propanone.

In the thermal decomposition of the acetoxymethyl-substituted 1,2-dioxetane AcTMD in the presence of DMPO, no EPR signal was detected even at a 5-fold higher concentration of the dioxetane (Table 2, entry 3). This was further supported by a control experiment in which 1-acetoxy-2-propanone was irradiated at 300 nm for 30 min, but again no EPR signal of a DMPO adduct was obtained (Table 2, entry 9). Thus, acylation of the hydroxy group in 1-hydroxy-2-propanone reduces the ease of α cleavage[36] (otherwise the acetyl radical should have been trapped with DMPO).

For the thermolysis of the dioxetanes **1**α and **1**β (20 mM) in the presence of DMPO (45 mM) also two characteristic doublet-of-triplets EPR signals were observed (Table 2, entry 4). The main paramagnetic species was attributed to the acetyl-radical product of DMPO, but the expected hydroxymethyl- and phenylmethyl-radical adducts of DMPO could not be definitively assigned because their hyperfine coupling constants and the Landé factor are quite similar[34,37] to the DMPO-yl radical adduct (cf. Table 2, entries 2 and 8). A control experiment, in which 1-phenyl-2-propanone (1 mM) was irradiated at 300 nm to generate the authentic pair of phenylmethyl and acetyl radicals through α cleavage,[32] yielded only the trapping product of the acetyl radical (Table 2, entry 10). The DMPO adduct of the phenylmethyl radicals was not observed, presumably due to its lower reactivity to add to double bonds compared to acyl radicals.[38,39]

In the thermolysis of the dioxetane **2**, hydroxymethyl as well as phenylmethyl radicals may be generated from the α cleavage of the electronically excited 1-hydroxy-3-phenyl-2-propanone and subsequent decarbonylation. In the presence of DMPO, only a very weak and noisy EPR signal with a doublet-of-triplets pattern was observed at a relatively high dioxetane concentration (Table 2, entry 6), the structure of the spin adduct could not be definitively assigned. Besides alkyl-radical adducts, the main EPR-active species was the DMPO-OH adduct,[35a,40] which was also observed in traces in the thermolysis of all the other dioxetanes. Its formation is explained in terms of the direct oxidation of DMPO by the dioxetanes to generate the DMPO radical cation and subsequent addition of water, analogous to what is described in the literature for other oxidizing agents.[41]

A control experiment with authentic 1-hydroxy-3-phenyl-2-propanone showed that the direct irradiation of this ketone at 0 °C in the presence of DMPO leads to an acyl radical adduct of DMPO (Table 2, entry 11), which was not found in the thermolysis (37 °C) of the dioxetane **2**. The structure of the acyl radical (hydroxyacetyl or phenylacetyl) could not be unequivocally assigned. Although the photolysis of 1-hydroxy-3-phenyl-2-propanone affords the phenylacetyl radical in nearly quantitative yield (proven by laser flash studies, see below), its fast decarbonylation (k_{CO} ca. 5×10^6 s^{-1}, this work)[42,43] renders

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(42) Läufer, M.; Dreeskamp, H. *J. Magn. Res.* **1984**, *60*, 357−365.
(43) (a) Maouf, A.; Lemmetyinen, H.; Koskikallio, J. *Acta Chem. Scand.* **1990**, *44*, 336−338. (b) Tsentalovich, Y. P.; Fischer H. *J. Chem. Soc., Perkin Trans. 2* **1994**, 729−733.

spin trapping of this shorter-lived acyl radical unlikely. In contrast, the less efficient produced hydroxyacetyl radical may be trapped, since its rate of decarbonylation is slow enough ($k_{CO} = 1 \times 10^5$ s^{-1}).[44]

Photochemical Studies of the Dioxetane-Derived Ketone Products. Conventional Photolysis of 1-Hydroxy-3-phenyl-2-propanone. This α,α'-disubstituted ketone was photolyzed at 300 nm in a Rayonet photoreactor, and the product distribution and the photolysis rate were monitored by ^{1}H NMR spectroscopy. The photolysis led to 2-phenylethanol (43%) and 1,2-diphenylethane (49%) as major products; furthermore, 1,2-dihydroxyethane (6%) and traces of methanol (<2%) were detected, the total mass balance was 78%. The photolytic consumption of 1-hydroxy-3-phenyl-2-propanone was slightly higher compared to the well-investigated[32,42] 1-phenyl-2-propanone, i.e. 85 ± 4% versus 78 ± 4% in 2 h.

Laser-Flash Photolysis of the Ketones. The direct laser-flash photolyses (λ = 351 nm) of the dioxetanes HTMD and TMD gave no observable radical transients. Therefore, the kinetic fate of the electronically excited ketones acetone, 1-hydroxy-3-phenyl-2-propanone, 1-hydroxy-2-propanone, and 1-phenyl-2-propanone was investigated by time-resolved (nanosecond) UV spectroscopy.[45,46] Since the singlet-excited states of aliphatic ketones are very short-lived (<5 ns) due to quite fast intersystem crossing to their triplet states,[47] only the lifetimes and reactions of the latter may be determined with this technique. Moreover, since the simple dioxetanes produce quite selectively triplet states upon thermolysis (cf. Table 1), only the triplet reactivity is relevant in the context of the present study.

Acetone. The triplet state of acetone is long-lived[48] in acetonitrile (50 μs) and water (20 μs), the solvents which were employed for the photobiological investigations, and does not undergo significant α cleavage.[47] To demonstrate the ability of triplet acetone to engage efficiently in hydrogen abstraction, the quenching rate constant with diphenylmethanol was determined through the kinetics of the time-resolved growth of the resulting diphenylhydroxymethyl radical as a function of hydrogen-donor concentration. A value of $k_q = 4.7 \times 10^7$ M^{-1} s^{-1} (cf. Table S-3) was found for hydrogen abstraction by triplet acetone from diphenylmethanol.

1-Hydroxy-3-phenyl-2-propanone Compared to 1,3-Diphenyl-2-propanone. The triplet state of 1,3-diphenyl-2-propanone is too short-lived (<1 ns)[43,49] to allow direct detection in the nanosecond time regime, since it undergoes very fast and efficient ($\Phi_\alpha = 0.84 \pm 0.06$)[49c] α cleavage to produce a pair of phenylmethyl and phenylacetyl radicals. The latter undergoes fast decarbonylation (k_{CO} ca. 5×10^6 M^{-1} s^{-1}) to produce a second phenylmethyl radical. In accordance with previous studies,[43,49−51] laser-flash photolysis produced a transient absorption at 317 nm characteristic for phenylmethyl radicals (Figure 1). The absorption is characterized by a step-and-rise pattern, in which the initial sudden rise corresponds to the fast α cleavage to produce the first phenylmethyl radical. The time-resolved slow growth pertains to the decarbonylation of the phenylacetyl radical to produce the second phenylmethyl radical. The ratio of the absorbances of the time-resolved and sudden rise is approximately unity. The rate of decarbonylation is solvent dependent, and the measured values (Table 3) are in good agreement with the absolute values reported by Turro[50] and Scaiano[51] and with the trend in solvent effects observed by Fischer,[43b] namely, k_{CO} (acetonitrile) < k_{CO} (methanol) < k_{CO} (hexane).

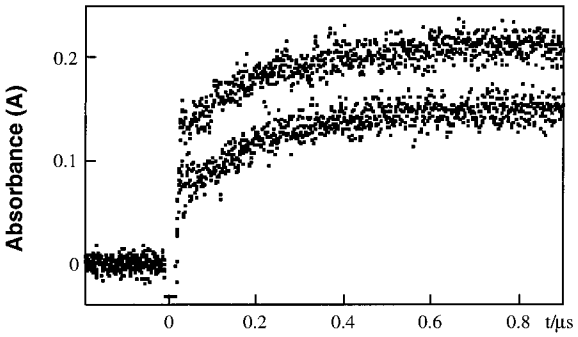

Figure 1. Transient-absorption traces (λ_{mon} = 317 nm) produced upon laser-flash photolysis (λ_{exc} = 308 nm, corrected for concomitant fluorescence) of optical-density-matched (OD = 0.40) solutions of 1,3-diphenyl-2-propanone (upper trace) and 1-hydroxy-3-phenyl-2-propanone (lower trace).

Table 3. Solvent Effects on the Decarbonylation Rate of Phenylacetyl Radicals and the Relative Quantum Yields of Their Formation in the Laser-Flash Photolysis of 1-Hydroxy-3-phenyl-2-propanone Compared to 1,3-Diphenyl-2-propanone[a]

	solvent[d]	k_{CO} [10^6 s^{-1}][e]	OD ($PhCH_2$•)[c] (λ = 317 nm)	ratio Φ_{rel} [b] ($PhCH_2CO$•)	Φ_α[f]
PhCH$_2$C(=O)CH$_2$OH	CH_3CN	4.2	0.043	1.25	1.0
	CH_3OH	5.4	0.054	1.11	0.93
	hexane	8.2	0.050	1.08	0.90
PhCH$_2$C(=O)CH$_2$Ph	CH_3CN	4.5	0.034	-	0.84[f]
	CH_3OH	5.4	0.050	-	0.84[f]
	hexane	8.1	0.045	-	0.84[f]

[a] Optical-density-matched (OD = 0.4) solutions of 1-hydroxy-3-phenyl- and 1,3-diphenyl-2-propanone; direct excitation with XeCl-excimer laser pulse (λ = 308 nm, 25-ns laser pulse, 75 mJ). [b] Maximum absorbance of the time-resolved rise is equated to the amount of generated phenylacetyl radicals; ratio defined as quotient of OD($PhCH_2$•) for 1-hydroxy-3-phenyl-2-propanone and 1,3-diphenyl-2-propanone, error ± 10%. [c] Error ± 10%. [d] Degassed by two freeze−pump−thaw cycles. [e] Determined by monoexponential fitting of the time-resolved increase in the absorbance at λ_{mon} = 317 nm due to phenylmethyl radical, error ± 10%. [f] Reference 49, error ± 0.06.

For the flash photolysis of the 1-hydroxy-3-phenyl-2-propanone, we also have observed a transient step-and-rise absorption trace similar to that of 1,3-diphenyl-2-propanone, except that the ratio of the absorbances for the slow and sudden rise was not unity but rather about 2-fold higher for the time-resolved growth (Figure 1). The latter could be unambiguously assigned to the decarbonylation of a phenylacetyl radical to produce phenylmethyl radicals on the following basis: (a) the absorption

(44) Vollenweider, J.-K., Paul, K. *Int. J. Chem. Kinet.* **1986**, *18*, 791−800.

(45) For example: (a) Leyva, E.; Platz, M. S.; Persy, G.; Wirz, J. *J. Am. Chem. Soc.* **1986**, *108*, 3783- 3790. (b) Burnett, M. N.; Boothe, R.; Clark, E.; Gisin, M.; Hassaneen, H. M.; Pagni, R. M.; Persy, G.; Smith, R. J.; Wirz, J. *J. Am. Chem. Soc.* **1988**, *110*, 2527−2538.

(46) For example: (a) Paul, H.; Small Jr., R. D.; Scaiano, J. C. *J. Am. Chem. Soc.* **1978**, *100*, 4520- 4527. (b) Baignée, A.; Howard, J. A.; Scaiano, J. C.; Stewart, L. C. *J. Am. Chem. Soc.* **1983**, *105*, 6120−6123.

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(48) (a) Porter, G.; Dogra, S. K.; Loutfy, R. O.; Sugamori, S. E.; Yip, R. W. *J. Chem. Soc., Faraday Trans. 1* **1973**, *69*, 1462−1474. (b) Encinas, M. V.; Lissi, E. A.; Scaiano, J. C. *J. Phys. Chem.* **1980**, *84*, 948−951.

(49) (a) Engel, P. S. *J. Am. Chem. Soc.* **1970**, *92*, 6074−6076. (b) Robbins, W. K.; Eastman, R. H. *J. Am. Chem. Soc.* **1970**, *92*, 6076−6077. (c) Noh, T. H.; Step, E.; Turro, N. J. *J. Photochem. Photobiol. A: Chem.* **1993**, *72*, 133−145.

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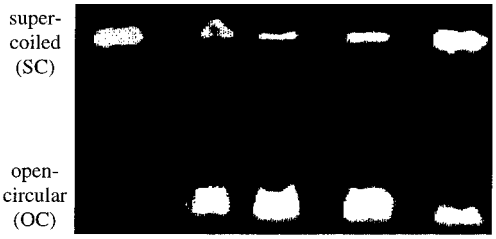
(51) Lunazzi, L.; Ingold, K. U.; Scaiano, J. C. *J. Phys. Chem.* **1983**, *87*, 529−530.

spectrum matched that of the phenylmethyl radicals, (b) the kinetics of the growth was, within the error limit, the same as for 1,3-diphenyl-2-propanone, and (c) the solvent effects on the growth rate (cf. Table 3).

Optical-density-matched solutions of 1-hydroxy-3-phenyl-2-propanone and 1,3-diphenyl-2-propanone were employed (at constant pulse energy of the incident laser flash) to determine the relative efficiencies (Φ_α) of phenylacetyl formation of these ketones (Table 3). For this purpose, the absorbance due to the time-resolved rise was set equal to the amount of the phenylacetyl precursor. In general, the relative quantum efficiency of phenylacetyl-radical formation from 1-hydroxy-3-phenyl-2-propanone was higher (ca. 10−25%); the actual ratios are listed in Table 3. From these data and the known α-cleavage quantum yield ($\Phi_\alpha = 0.84 \pm 0.06$) for 1,3-diphenyl-2-propanone,[49c] the quantum efficiency of phenylacetyl-radical formation from the triplet-excited 1-hydroxy-3-phenyl-2-propanone may be estimated to be unity (within a 15% error) and its α cleavage slightly more efficient than for 1,3-diphenyl-2-propanone.[49c] Since the triplet lifetime of 1,3-diphenyl-2-propanone has been estimated to be <1 ns,[49] we may conclude that the lifetime of 1-hydroxy-3-phenyl-2-propanone is even less and, hence, the α cleavage for the hydroxymethyl is faster than for the phenylmethyl group.

1-Hydroxy- and 1-Phenyl-2-propanone. No time-resolved growth of triplet absorption signals could be observed in the laser-flash photolysis of 1-hydroxy- and 1-phenyl-2-propanone. This is a consequence of the very fast α cleavage of the phenylmethyl and hydroxymethyl groups, which is corroborated by the above experiments with 1,3-diphenyl-2-propanone and 1-hydroxy-3-phenyl-2-propanone. In addition, no hydrogen abstraction was found for these triplet-excited ketones to afford the diphenylhydroxymethyl radical from diphenylmethanol (0.3 M), which implies a triplet lifetime of less than 20 ns. The flash photolysis of 1-hydroxy-2-propanone afforded immediately (within the 25-ns pulse time of the laser pulse) a long-lived (μs range) transient at ca. 320 nm (trace not shown), presumably due to the acetyl radical. In the flash photolysis of 1-phenyl-2-propanone, an initial fast rise in the absorption was obtained within the laser pulse at the detection wavelength of 317 nm. The latter is ascribed to the formation of phenylmethyl and acetyl radicals, which disappear by biexponential kinetics due to recombination. By taking the estimated lifetimes of the triplet-excited 1,3-diphenyl-2-propanone (<1 ns)[49] and 1-hydroxy-3-phenyl-2-propanone (<1 ns, this work) as a measure of the ease of α cleavage to produce phenylmethyl and hydroxymethyl radicals, we may conclude that the lifetimes of triplet-excited 1-hydroxy- and 1-phenyl-2-propanone are also within this range (<1 ns).

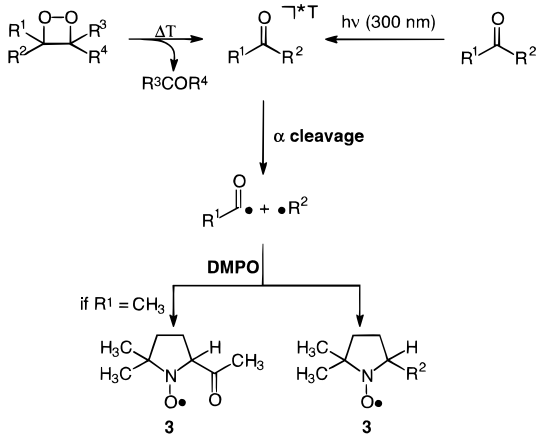
Photobiological Studies with DNA and dGuo. Formation of Strand Breaks in pBR 322 DNA. The DNA-cleaving properties of these dioxetanes were assessed in supercoiled pBR 322 DNA by gel electrophoresis. The thermolysis (37 °C) of the dioxetanes **1**α and **1**β (2 mM) for 5 h in the presence of supercoiled pBR 322 DNA (c = 10 mg/L) resulted in the significant formation (80%) of open-circular (OC) DNA (Figure 2, lanes 3 and 4). This should be compared with about 15% OC DNA (blank value) detected in the thermolysis of pBR 322 DNA under the same conditions without dioxetane (Figure 2, lane 1). Only 40% open-circular form was observed for the thermal decomposition of dioxetane **2** (Figure 2, lane 5) in the presence of pBR 322 DNA. For comparison, HTMD was thermolyzed with pBR 322 DNA and 69% of open-circular DNA was found. The efficiency of the hydroxymethyl-substituted 1,2-dioxetanes in inducing DNA strand breaks follows the order **1**α/**1**β > HTMD > **2**, which qualitatively parallel their propensity to generate active radicals, namely 3:2:1 per decomposed dioxetane molecule (see Scheme 4 below).



OC [%] [a]	15	69	80	80	40
lane	1	2	3	4	5
dioxetane	-	**HTMD**	**1α**	**1ß**	**2**

Figure 2. Gel-electrophoretic detection of single-strand breaks in pBR 322 DNA [c = 10 mg/L in 0.5 mM KH_2PO_4 buffer (pH 7.4) with 10% CH_3CN as cosolvent] induced in the thermolysis of the hydroxymethyl-substituted dioxetanes HTMD, **1**α/**1**β, and **2** (2 mM) at 37 °C for 5 h in the dark. Data shown are estimated from the light intensities of the spots for supercoiled (SC) and open-circular (OC) forms of pBR 322 DNA and are mean values of at least three independent runs, error ± 10% of the stated value.

Scheme 3. Mechanistic Pathways for the Formation of Radicals in the α Cleavage of Electronically Excited Ketones Generated in the Thermolysis of Hydroxymethyl- and Phenylmethyl-Substituted Dioxetanes and the Photolysis of Their Carbonyl Products[a]

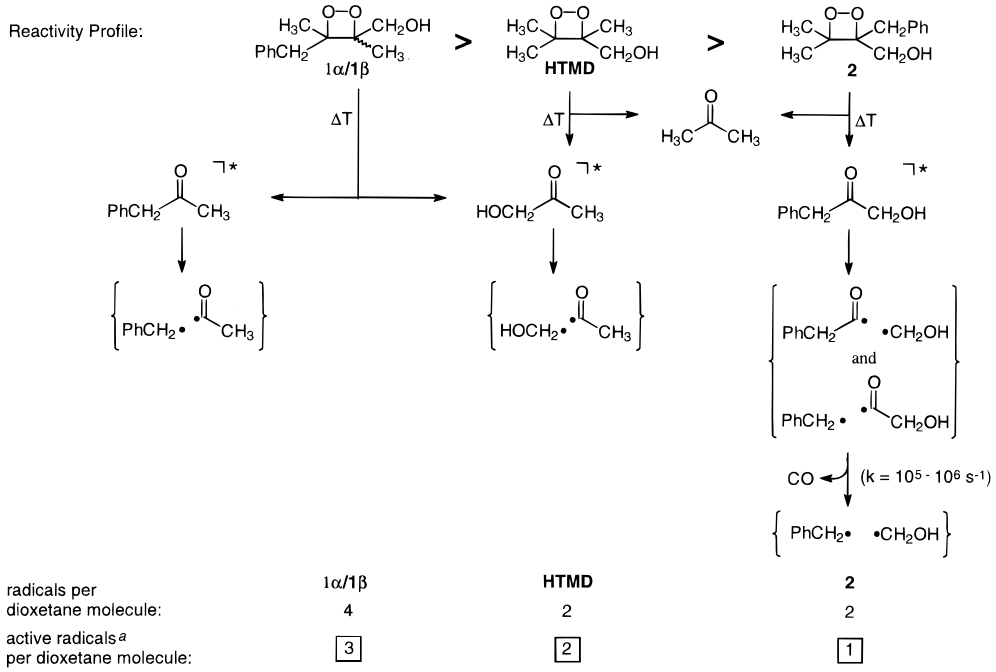


[a] Also the R^3COR^4 product may be triplet-excited, but for mechanistic simplicity of the scheme, only the electronically excited R^1COR^2 fragment and its subsequent transformations are shown.

Oxidation of Calf Thymus DNA. Isolated calf thymus DNA at a concentration of 0.1 mg/mL (62.5 μM guanine concentration) was treated at 37 °C in the presence of the hydroxymethyl-substituted 1,2-dioxetanes **1**α/**1**β and **2** (concentration range 1−10 mM) for 21 h in the dark to establish their efficacy to generate oxidative DNA-base modifications. The well-examined HTMD[9−11] was also applied for comparison. Besides the established oxidation product 8-oxoGua,[12−15] substantial amounts of oxazolone[16] were detected by the fluorescence-labeling HPLC assay with 1,2-naphthoquinone-4-sulfonic acid.[16−18a,52] Former studies[9,10] have established that the oxazolone yield rises with increasing dioxetane concentration, whereas the guanine oxidation product 8-oxoGua is prone to further oxididation by excess dioxetane and goes through a maximum.[10] Thus, under these

(52) Kobayashi, Y.; Kinoshita, T.; Kubo, H. *Anal. Biochem.* **1987**, *160*, 392−398.

Scheme 4. Reaction Pathways for the Electronically Excited Carbonyl Products in the Thermolysis of the Hydroxymethyl-Substituted 1,2-Dioxetanes HTMD, **1**α/**1**β, and **2** as a Mechanistic Rationale for Their Reactivity Profile in the Type-I Photooxidation of Guanosine in DNA and DGuo[a]



[a] Phenylmethyl radicals are ineffective in the damage of DNA.

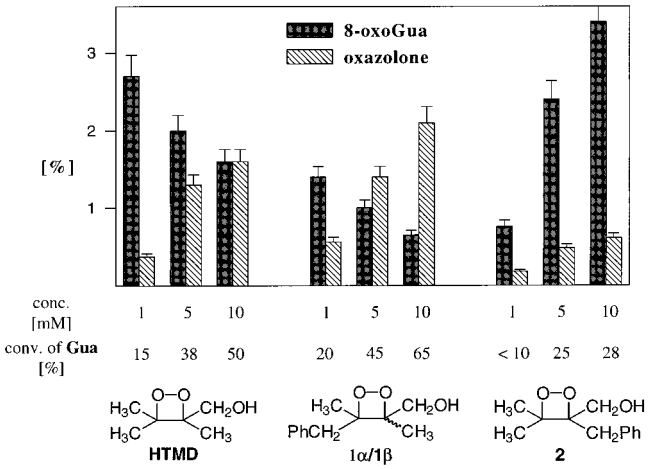


Figure 3. Concentration profiles for the oxidation of calf thymus DNA (0.1 mg/mL, 62.5 μM guanine) by the hydroxymethyl-substituted dioxetanes HTMD, **1**α/**1**β, and **2** in the dark and in acetonitrile (10 vol %) at 37 °C for 20 h in 5 mM phosphate buffer (pH 7.0, 90 vol %). The ordinate values are averages of at least three independent runs, error ± 10% of the stated value.

reaction conditions, the yield of oxazolone constitutes a more reliable measure of the oxidative reactivity of the dioxetanes.

The concentration profiles are displayed in Figure 3. For the dioxetanes **1**α and **1**β, which were used as a 40:60 diastereomeric mixture (the isomerically pure dioxetanes **1**α and **1**β gave exactly the same results, data not shown), an increase in the dioxetane concentration from 1 to 10 mM enhanced the oxazolone yield ca. 4-fold. As a second marker for the oxidative reactivity of the dioxetanes, the conversion of guanine also rises at higher dioxetane concentration (Figure 3). In contrast, the yield of 8-oxoGua decreased ca. 3-fold when the dioxetane concentration was increased from 1 to 10 mM. Compared to the well-investigated HTMD, the dioxetanes **1**α/**1**β are more effective, which is clearly reflected in the conversion of guanine as well as in the yield of oxazolone (Figure 3), but not in the total yield of guanine oxidation products. Guanine was selectively oxidized due to its low oxidation potential,[53] whereas the other bases remained mainly intact (data not shown).

The concentration profile for the DNA oxidation by dioxetane **2** clearly shows (Figure 3) that this hydroxymethyl-substituted 1,2-dioxetane is the least reactive in regard to guanine conversion and oxazolone formation, which parallels the findings with plasmid pBR 322 DNA. This is demonstrated by a lower consumption of guanine together with a lower yield of oxazolone (Figure 3). The formation of 8-oxoGua dominates with increasing concentration of the dioxetane, presumably because under these conditions no significant further oxidation takes place and it accumulates. Therefore, the qualitative reactivity order for the dioxetanes **1**α/**1**β > HTMD > **2** ≫ TMD applies.

Oxidation of dGuo. To gain mechanistic insight into which processes may be responsible for the high reactivity of hydroxymethyl-substituted dioxetanes **1**α, **1**β, **2**, and HTMD in the formation of oxidative DNA-base modifications, further studies with the nucleoside 2'-deoxyguanosine (dGuo) were performed. The dGuo (0.5 mM) was treated with the respective dioxetanes at various concentrations and 50 °C for 15 h. The four major oxidation products of the guanine base, namely the diastereomeric mixture of (4*R**)- and (4*S**)-4-HO-8-oxodGuo (type-II photooxidation),[18,19] 8-oxodGuo[20] (formed in dGuo only by type-II process), and the guanidine-releasing products oxazolone[16] and oxoimidazolidine[17] (type-I photooxidation), were observed and quantified as previously described.[9−11] For all dioxetanes an almost linearly dependent degradation of dGuo was observed with increasing dioxetane concentration (Figure 4). The product balance was 50 ± 8% and nearly the same for all dioxetanes.

Mechanistically more significant, the concentration profiles for the dGuo oxidation in Figure 4 reflect the reactivity trend of the hydroxymethyl-substituted dioxetanes obtained in the

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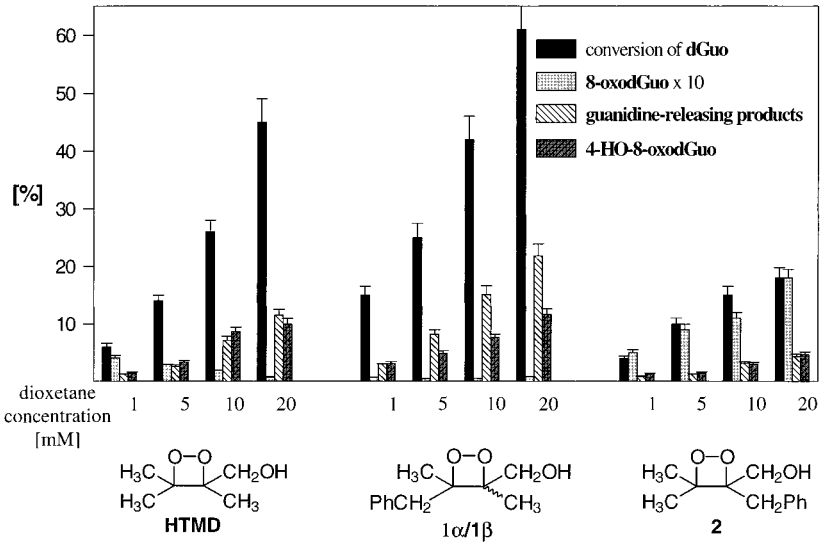

Figure 4. Concentration profiles for the thermal photooxidation of dGuo by hydroxymethyl-substituted 1,2-dioxetanes in the dark with 0.5 mM dGuo at 50 °C for 15 h in 5 mM phosphate buffer (pH 7.0) and acetonitrile (10 vol %) as cosolvent. The ordinate values are averages of at least three independent runs, error ± 10% of the stated value.

studies with DNA (strand cleavage and oxidative damage). A control experiment under the same reaction conditions confirmed the low reactivity of the merely alkyl-substituted derivative TMD (20 mM, only 2−3% conversion of dGuo).

In this context and for comparison purposes, it was of interest to determine the efficacy of the photochemically excited ketones, namely 1-phenyl-2-propanone, 1-hydroxy-3-phenyl-2-propanone, and 1,3-diphenyl-2-propanone (the thermolysis products of the various dioxetanes), to cause dGuo oxidation. Therefore, 0.25 mM dGuo was irradiated at 300 nm and 0 °C for 30 min in the presence of 10 mM of these ketones[54] and the conversion of dGuo was measured. For the 1,3-diphenyl-2-propanone, an efficient source of phenylmethyl radicals,[43,47,49−51] only 15% of dGuo conversion compared to 85% in the case of 1-phenyl-2-propanone,[54] which releases acetyl as well as phenylmethyl radicals, was observed.[32,42] This reflects the higher propensity of acetyl versus phenylmethyl radicals to degrade dGuo oxidatively. The hydroxymethyl radical, which is generated in the photolysis of 1-hydroxy-3-phenyl-2-propanone along with the phenylmethyl radical (after decarbonylation), shows a moderate tendency to oxidize dGuo (50% conversion of dGuo). The low reactivity of the phenylmethyl radicals was independently confirmed by the rather ineffective oxidation of dGuo (4% conversion of dGuo compared to 40% for HTMD) when 3,3-bis(phenylmethyl)-1,2-dioxetane (DBD, 20 mM) was employed as efficient thermal source of such species.[55]

Significant differences in the product balances of type-I and type-II photooxidation products were found for the dioxetanes **1**α/**1**β and **2**. The predominant oxidative modifications in the thermolysis of dioxetane **1**α and **1**β are the type-I products oxazolone and oxoimidazolidine, which were generated in ca. 2-fold higher amounts compared to the characteristic type-II product 4-HO-8-oxodGuo (Figure 4). The other type-II photooxidation product, namely the easily oxidized 8-oxodGuo, was generated in negligible yields (<0.1%). A completely different situation applies in the oxidation of dGuo by dioxetane **2**, for which the ratio of type-I to type-II products is even smaller than unity and significant amounts of 8-oxodGuo (up to 1.8%) were detected. A quite similar trend was found for HTMD, for which maximally 0.4% 8-oxodGuo and a type-I to type-II ratio of about unity were obtained.[9,11]

Table 4. Inhibitory Effect of Additives on the Oxidation of dGuo Induced in the Thermolysis of the Hydroxymethyl-Substituted 1,2-Dioxetanes HTMD, **1**α/**1**β, and **2**[a] or in the Irradiation of Benzophenone (BP)[a]

		inhibitory effect[b] (%)			
additive[c]	concentration	HTMD	**1**α/**1**β	**2**	BP
cresol[d]	20 mM	34	37	33	47
^{i}PrOH	8 vol %	70	80	70	88
DBH	20 mM	58	27	60	nd[e]
galvinoxyl	5 mM	37	45	42	87
DMPO[f]	2 mM	90	90	90	94

[a] 0.5 mM dGuo, 5 mM phosphate buffer (pH 7.0), 10% acetonitrile as cosolvent, 10 mM dioxetane (thermolyzed at 50 °C for 14 h) or 1 mM benzophenone (photolyzed at 350 nm in the Rayonet photoreactor at 0 °C for 30 min). [b] Mean values determined from the conversion of dGuo with and without additives according to the following: inhibitory effect (%) = [1 − (conv. dGuo with additive/conv. dGuo without additive)] × 100, mean values of three independent experiments, standard deviation ± 10% of the stated value. [c] Cresol: 2,6-di-*tert*-butyl-4-methylphenol. DBH: 2,3-diazabicyclo[2.2.1]hept-2-ene. [d] 0.1 mM dGuo, 5 mM phosphate buffer (pH 7.0), 10% acetonitrile as cosolvent, 2 mM dioxetane (thermolyzed at 50 °C for 7 h) or 1 mM benzophenone (photolyzed at 350 nm in the Rayonet photoreactor at 0 °C for 30 min). [e] nd: not determined. [f] 0.1 mM dGuo, 5 mM phosphate buffer (pH 7.0), 10% acetonitrile as cosolvent, 2 mM dioxetane (thermolyzed at 50 °C for 14 h) or 0.5 mM benzophenone (photolyzed at 350 nm in the Rayonet photoreactor at 0 °C for 30 min).

To assess the nature of the oxidizing species derived from the dioxetanes **1**α/**1**β, **2**, and HTMD upon thermolysis, the inhibitory effect of a number of additives was tested in the dioxetane-mediated photooxidation of dGuo (Table 4) and compared to photoexcited benzophenone (BP) triplets. DMPO,[33−35] cresol,[55a] and galvinoxyl[55a] were representatively examined as well-established radical scavengers. Such radical scavengers do not only intercept radicals, but also influence the triplet-excited states. Triplet states may be deactivated through hydrogen abstraction (DMPO and cresol) or by assisted intersystem crossing (paramagnetic galvinoxyl). Indeed, a strong inhibitory effect was observed for these additives with

(54) Carried out in 5 mM phosphate buffer (pH 7.0) with acetonitrile (10 vol %) as cosolvent. The values for the conversion of dGuo are averages of at least three independent runs, error ± 10%. The least effective ketone, namely 1,3-diphenyl-2-propanone, has the highest absorption coefficient [ϵ (308 nm, CH_3CN) = 120] compared to 1-phenyl-2-propanone and 1-hydroxy-3-phenyl-2-propanone [ϵ (308 nm, CH_3CN) = 40].

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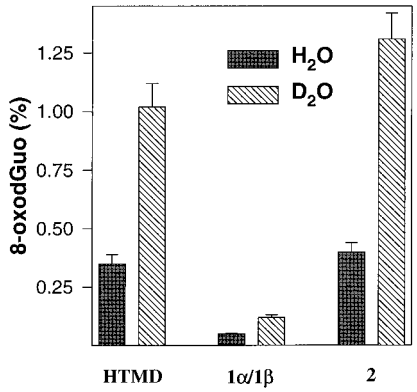

Figure 5. Effect of D_2O on the 8-oxodGuo yield in the photooxidation of dGuo induced by the thermolysis of the hydroxymethyl-substituted 1,2-dioxetanes HTMD, **1**α/**1**β, and **2** in the dark at 50 °C for 15 h with 0.25 mM dGuo and 1.25 mM dioxetane in 5 mM phosphate buffer [pH(D) 7.0] and acetonitrile as cosolvent (10 vol %). Absolute yields are derived from the mean value of at least three independent runs, error ± 10% of the stated value.

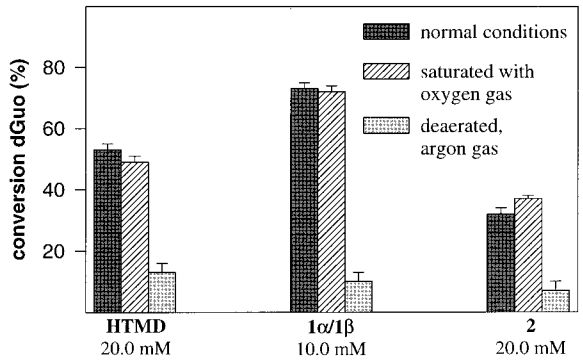

Figure 6. Effect of oxygen on the photooxidation of dGuo (0.5 mM) in the thermal decomposition of hydroxymethyl-substituted 1,2-dioxetanes at 50 °C for 15 h in 5 mM phosphate buffer (pH 7.0) and acetonitrile (10 vol %) as cosolvent. The ordinate values are averages of at least three independent runs, error ± 10% of the stated value. For the saturation with molecular oxygen, the solutions were purged with oxygen gas for 1 min. Deaerated solutions were obtained by two freeze−pump−thaw cycles and saturated with argon gas.

the long-lived (ca. 25 μs) triplet state of benzophenone,[56] which is known to cause dGuo photooxidation.[16c] Therefore, at least one part of the inhibitory effect in the cases of HTMD and **2** may also derive from the deactivation of the relatively long-lived (20-50 μs)[48] triplet-excited acetone as decomposition product of the dioxetanes. The dioxetanes **1**α and **1**β, however, do not yield long-lived triplet ketones upon thermal decomposition, and thus, the observed inhibitory effect in the dioxetane-induced photooxidation of dGuo (Table 4) is attributed to the scavenging of reactive radicals. Analogously, the substantial reduction of the dGuo oxidation by the dioxetanes **1**α and **1**β with 2-propanol as hydrogen donor (Table 4) also implicates a decrease of the active radical concentration. For the dioxetanes HTMD and **2** and for benzophenone 2-propanol may also intercept the triplet-excited ketones and, thus, account for the observed inhibition.

Also mechanistically instructive is the azoalkene DBH, which was employed as an efficient triplet-excited ketone quencher.[55b,57−59] As expected, DBH efficiently suppressed the conversion of dGuo for all dioxetanes which release long-lived ketone triplet states (HTMD and **2**) and, of course, for benzophenone (Table 4). In marked contrast, in the case of the dioxetanes **1**α and **1**β, which do not generate long-lived triplet-excited carbonyl products to allow quenching by a triplet-energy acceptor, the inhibitory effect was quite small and, in fact, may be due to some (less efficient) interception of radicals by DBH through scavenging.

To confirm the participation of singlet oxygen, which is also known to cause oxidative DNA-base modifications, the dioxetane-mediated oxidation of dGuo was examined in deuterium oxide, in which the lifetime of singlet oxygen is prolonged by about 10-fold.[60,61] The results for the dioxetane-induced formation of 8-oxodGuo in H_2O and D_2O are shown in Figure 5. At concentrations of 1.25 mM for the dioxetanes and 0.25 mM for dGuo, the yield of 8-oxodGuo was significantly enhanced in D_2O compared to H_2O. The D_2O effect for the dioxetanes **2** and HTMD (300−320% increase in 8-oxodGuo formation) was much more pronounced than for the dioxetanes **1**α and **1**β (∼150% increase), for which only a very small, if at all significant D_2O effect was observed. This contrast is readily accounted for since HTMD and **2**, but not **1**α and **1**β, release long-lived triplet ketones upon thermolysis, which are capable of undergoing energy transfer to produce singlet oxygen (type-II photooxidation).[62] Note in Figure 5 that the total amount of 8-oxodGuo, the product derived from singlet oxygen with dGuo, is about the same for HTMD and **2**, which release relatively long-lived triplet acetone, but very small for the dioxetanes **1**α and **1**β, which produce exclusively short-lived ketone triplets. These results are in accord with the type-I versus type-II product ratios observed in the oxidation of dGuo by the dioxetanes **1**α/**1**β, **2,** and HTMD.

Influence of Molecular Oxygen on the Photooxidation of dGuo. To assess whether peroxyl radicals, produced by dioxygen trapping of the carbon radicals[63] derived from the α cleavage of the electronically excited α-hydroxy ketones, are responsible for the formation of oxidative DNA base modifications by these highly reactive hydroxymethyl-substituted dioxetanes, the effect of molecular oxygen was examined. For this purpose, the dioxetane-mediated conversion of dGuo was investigated in the presence (saturated with O_2) and absence (degassed with Ar) of molecular oxygen (Figure 6). For the dioxetanes HTMD, **1**α/**1**β, and **2**, the conversion of dGuo in the presence of O_2 was within the error limit the same as under normal aerated conditions (Figure 6). This reveals that under regular conditions the oxygen concentration in water (0.5−1 mM)[64] is sufficient for scavenging the carbon radicals gradually formed during the α cleavage. In the absence of O_2 (degassed with argon), however, a significant reduction (ca. 70−80%) of the dGuo oxidation was observed.

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Mechanistic Discussion

Analogous to HTMD, also the hydroxy-functionalized dioxetanes **1**α, **1**β, and **2** cause DNA damage, principally strand breaks (Figure 2) and guanine oxidation (Figures 3 and 4). Their efficacy follows the reactivity sequence **1**α/**1**β > HTMD > **2** ≫ TMD. While for some time the high efficacy of HTMD to promote DNA modifications was a mystery,[9−11] through the present study we know now that radicals, in particular acetyl radicals, are responsible for this oxidative reactivity and presumably also for the formation of DNA strand breaks in the case of the hydroxymethyl-substituted dioxetanes HTMD, **1**α/**1**β and **2** versus TMD; *the latter does not release radicals!* In view of the large volume of results given here, it should be helpful for the mechanistic discussion to summarize briefly the salient experimental findings and reactivity trends, which are (a) the thermal decomposition of the hydroxymethyl-substituted dioxetanes HTMD, **1**α/**1**β, and **2** leads to acyl and alkyl radicals as shown by spin-trapping experiments with DMPO (Table 2), (b) the laser-flash photolysis of the α-hydroxy- and α-phenyl-functionalized ketones (decomposition products of the dioxetanes HTMD, **1**α/**1**β, and **2**) reveal short lifetimes for their triplet-excited states due to fast and efficient α cleavage, (c) the significant reactivity differences of the dioxetanes in the formation of DNA strand breaks and the oxidation of guanosine are found to correlate qualitatively with a reactivity order of the released radicals

$$H_3CC(O)^\bullet > HOCH_2^\bullet \gg PhCH_2^\bullet$$

and (d) the involvement of reactive peroxyl radicals as the proposed ultimate oxidizing species in the dioxetane-mediated oxidation of DNA in the presence of molecular oxygen, as suggested in the photochemically induced α cleavage of α-substituted ketones in oxygen-saturated solutions.[63e]

The generation of radicals from the hydroxymethyl-substituted dioxetanes may be rationalized in terms of the well-established α cleavage of their α-functionalized, n,π^*-triplet-excited carbonyl products formed upon thermal decomposition (Scheme 3).[22,23,32,42] Such α cleavage of the hydroxy- and phenyl-substituted carbonyl compounds leads to radicals, which have been trapped by DMPO and identified by EPR spectroscopy. Since laser-flash-photolysis studies establish a short triplet lifetime (τ < 1 ns) for these α-hydroxy- and α-phenyl-functionalized ketones, the direct interaction of such reactive, triplet-excited species with the DNA to induce its damage is negligible, in contrast to what has been reported for the longer-lived, triplet-excited benzophenone[65] or acetone.[66] Thus, the efficient α cleavage of the triplet-excited, α-substituted ketones qualifies them as good radical sources in the thermally initiated, dioxetane-induced DNA damage. Indeed, the fact that the addition of radical scavengers significantly inhibits the oxidation of guanosine by the dioxetanes **1**α and **1**β (Table 4) supports that trappable radicals are involved in the formation of oxidative DNA-base modifications by the hydroxymethyl-substituted dioxetanes HTMD, **1**α/**1**β, and **2**.

To rationalize the DNA-damaging efficiency of these dioxetanes, i.e. the reactivity trend **1**α/**1**β > HTMD > **2** ≫ TMD, we shall now assess their capacity to release active radicals upon thermal decomposition, which is depicted in Scheme 4. Comparison of the electronically excited carbonyl products generated on thermolysis of these dioxetanes, only one potential radical source, namely 1-hydroxy-2-propanone (for HTMD) or 1-hydroxy-3-phenyl-2-propanone (for dioxetane **2**), is formed by the less reactive dioxetanes HTMD and **2** (triplet acetone α-cleaves inefficiently[47]), whereas the most reactive dioxetanes **1**α and **1**β release two radical precursors, i.e. 1-phenyl-2-propanone and 1-hydroxy-2-propanone.[22,32,42] Therefore, the number of potential radicals per dioxetane molecule decomposed is 4:2:2 for **1**α/**1**β, HTMD, and **2** and it is tempting to attribute the higher reactivity of the dioxetanes **1**α and **1**β to this enhanced radical formation. However, the mere number of potential radicals per dioxetane molecule decomposed as a criterion for the reactivity of the dioxetanes does not account adequately the difference in the DNA-damaging efficiency of the dioxetanes HTMD and **2** (Scheme 4). Also the capacity of the radicals $RC(O)^\bullet$, $HOCH_2^\bullet$, and $PhCH_2^\bullet$ to induce DNA damage needs to be considered. Indeed, the reactivity profile obtained in photooxidation studies of dGuo with various α-phenyl-substituted ketones reveals the qualitative order $RC(O)^\bullet > HOCH_2^\bullet \gg PhCH_2^\bullet$, with the phenylmethyl radical by far the least reactive. Thus, the number of *active* radicals per dioxetane molecule decomposed capable to induce DNA damage is for HTMD twice that for **2** (Scheme 4). For HTMD a pair of acetyl (active) and hydroxymethyl (active) radicals are obtained from the triplet-excited 1-hydroxy-2-propanone, whereas for the dioxetane **2** the α cleavage of 1-hydroxy-3-phenyl-2-propanone leads to a pair of phenylmethyl (inactive) and hydroxymethyl (active) radicals. Adjustment for the number of *active* radicals formed per dioxetane molecule decomposed, the reactivity order **1**α/**1**β > HTMD > **2** of the dioxetanes to damage DNA is nicely accounted for in terms of the α cleavage of the triplet-excited ketones produced in the dioxetane thermolysis. Consequently, the much lower reactivity of TMD to induce DNA damage than the hydroxy-substituted derivatives is due to the lack of α cleavage of triplet acetone; the latter operates through less effective direct interaction with the DNA by sensitization[66] or/and through $^1O_2$ generation by energy transfer to $^3O_2$.[62]

The carbon radicals produced as the primary reactive species in the α cleavage of α-substituted, triplet-excited ketones are efficiently scavenged by molecular oxygen to form peroxyl radicals ($k > 2 \times 10^9$ s^{-1}).[63] This was previously corroborated in the photolysis of 2,4-diphenylpentan-3-one in oxygen-saturated hexane solution, which provides a clean method for the production of peroxyl radicals.[63e] Since hydroxymethyl-substituted dioxetanes are effective in the oxidation of dGuo under normal aerated but not under argon-deaerated conditions (Figure 6), we suggest that mainly peroxyl radicals are the ultimate oxidizing species;[67] however, the direct participation of carbon-centered radicals in the oxidation of guanine cannot be excluded. Unfortunately, to date, little is known on the oxidative modification of DNA bases by peroxyl radicals.[68]

A closer look at the product distribution in the dioxetane-mediated oxidation of the nucleoside dGuo provides information on the mode of oxidative action of the peroxyl and/or carbon-centered radicals. For this purpose, the ratio of the radical-derived type-I (guanidine-releasing products) versus the $^1O_2$-derived type-II (4-HO-8-oxodGuo, 8-oxodGuo) oxidation products of guanine offers a valuable mechanistic probe.[11] The observed type-I/type-II ratio of approximately 2 (Figure 4) for the dGuo oxidation by the dioxetanes **1**α and **1**β suggests that the oxidation of guanine to the characteristic type-I products oxazolone and oxoimidazolidine may be caused by peroxyl radicals. Carbon-centered radicals are prone to add to the C8

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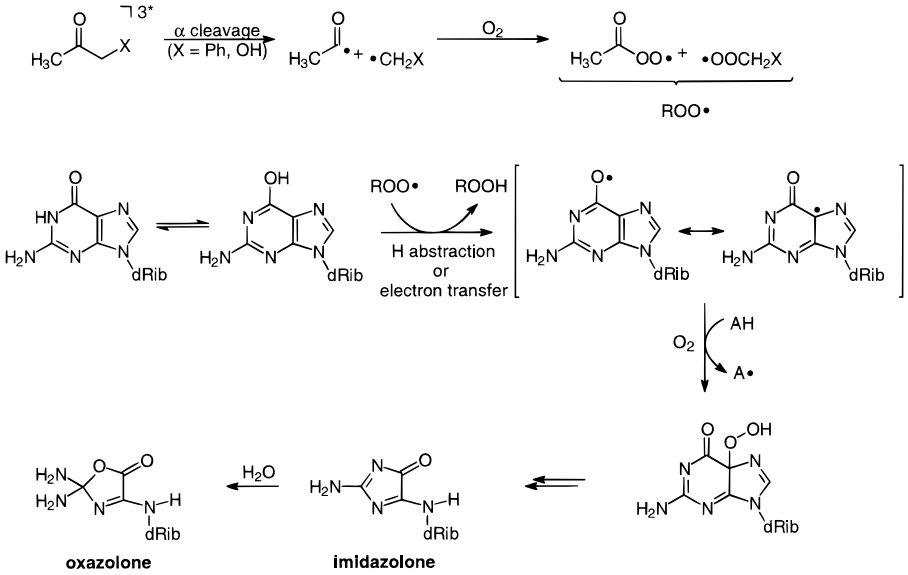
Scheme 5. Proposed Mechanism for the Formation of Guanidine-Releasing Products in the Thermolysis of Hydroxymethyl-Substituted 1,2-Dioxetanes by Radicals[70, 71]



position of guanine;[69] nevertheless, the direct participation of such radicals in the formation of oxazolone and oxoimidazolidine cannot be excluded (cf. Scheme 5). The formation of these guanine oxidation products may be explained in terms of the well-established mechanism[70] with the guanine radical as intermediate (Scheme 5). Therefore, we propose that the active radicals formed in the thermal decomposition of the hydroxymethyl-substituted 1,2-dioxetanes trap molecular oxygen to afford peroxyl radicals, which generate guanine radicals by hydrogen abstraction (Scheme 5).[71]

In contrast, the type-I/type-II ratio of about unity for HTMD and 0.7 for the dioxetane **2** (Figure 4) implies that reactive species other than radicals are involved in the dioxetane-induced guanosine oxidation. The significant increase in the relative amount of the characteristic singlet-oxygen-derived oxidation products 8-oxodGuo[72] (in DNA it may be also formed by a type-I process)[70] and 4-HO-8-oxodGuo[18] for these dioxetanes compared to **1**α and **1**β speaks definitively for the involvement of $^1O_2$ as reactive oxygen species. This was confirmed for HTMD[10] and the dioxetane **2** (Figure 5) by the substantial D_2O effect in the formation of 8-oxodGuo. This is readily accounted for since only HTMD and **2** release triplet-excited acetone, which lives long enough to be quenched by molecular oxygen and to produce significant amounts of singlet oxygen.[62] In the case of the dioxetane **2**, the extent of dGuo oxidation by $^1O_2$ is even more pronounced than for HTMD (Figure 4). Although both dioxetanes afford triplet-excited acetone, **2** produces only the hydroxymethyl radical as active DNA oxidant, whereas HTMD leads also to the acetyl radical as reactive species and, consequently, more singlet oxygen but less radical activity is expected for dioxetane **2** versus HTMD.

Conclusion

The present in-depth study of the DNA damage caused by hydroxymethyl-substituted dioxetanes has allowed us to rationalize their high efficiency compared to merely alkyl-substituted derivatives in terms of radical chemistry. The radicals, which are formed through the efficient α cleavage of the α-substituted, triplet-excited ketones that are generated in the dioxetane thermolysis, have been identified as the reactive species by EPR spin trapping and time-resolved UV spectroscopy. Due to the established fast and efficient O_2 trapping of the initially formed carbon-centered radicals to afford the peroxyl radicals,[63] we suggest that the latter act as the ultimate active oxidizing species. Therefore, we propose that peroxyl radicals cause the oxidative guanine modifications and DNA strand breaks and it seems likely that such oxygen-centered radicals might be involved in the DNA damage in vivo,[67,68] a novel aspect of *oxidative stress* that merits more intensive work. Clearly, the hydroxymethyl-substituted dioxetanes should serve this purpose well, since they constitute efficient sources for carbon-centered radicals as potential precursors for peroxyl radicals at 37 °C in the dark, certainly advantageous mild conditions for biological studies.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 172 "Molekulare Mechanismen kanzerogener Primärveränderungen") and the Fonds der Chemischen Industrie. W.M.N. thanks the latter institution for a Liebig fellowship and the Schweizer Nationalfond. We thank G. N. Grimm for help with the EPR spectroscopy.

Supporting Information Available: Text describing experimental details for the preparation and spectral data of the key compounds, tables with the decomposition rate constants, the chemiluminescence intensities for the determination of the singlet (ϕ^S) and triplet (Φ^T) quantum yields of the dioxetanes **1**α/**1**β and **2**, and the rate constants of hydrogen abstraction by triplet-excited acetone from diphenylmethanol, and selected EPR spectra (20 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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