Oxidation *vs.* fragmentation in radiosensitization. Reactions of α-alkoxyalkyl radicals with 4-nitrobenzonitrile and oxygen. A pulse radiolysis and product analysis study

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α-Monoalkoxyalkyl radicals produced from 1,4-dioxane (100%), 1,3-dioxane (56%), tetrahydrofuran (92%) and dimethyl ether (100%) by H-abstraction by hydroxyl radicals generated in the radiolysis of water were found to react with 4-nitrobenzonitrile (NBN) by *addition* to give *N*-alkoxyaminoxyl-type radicals, which have absorption maxima at about 310 nm and decay very slowly ($k = 0.4 - 1.0\ s^{-1}$). On the other hand, the reaction of the α-dialkoxyalkyl radical, 1,3-dioxan-2-yl **3** [from the reaction of hydroxyl radicals with 1,3-dioxane (32%)] with NBN leads to the rapid formation of the radical anion $NBN^{\bullet -}$.

The *N*-alkoxyaminoxyl-type radicals (**A** in the case of 1,4-dioxane and **D** in the case of dimethyl ether) react with ascorbate ($k \approx 2 \times 10^4\ dm^3\ mol^{-1}\ s^{-1}$). They have a very low reactivity with oxygen ($k < 10^3\ dm^3\ mol^{-1}\ s^{-1}$ in the case of tetrahydrofuran). On the other hand, they are rapidly reduced by $NBN^{\bullet -}$ ($k \approx 10^9\ dm^3\ mol^{-1}\ s^{-1}$ as observed with **A** and with **B** derived from 1,3-dioxane).

The products [*G* values (in parenthesis) in units of 10^{-7} mol J^{-1}] in the γ-radiolysis of N_2O-saturated solution of 1,4-dioxane in the presence of NBN are 1,4-dioxan-2-one (0.3), 2-hydroxy-1,4-dioxane (2.5), ethane-1,2-diol monoformate (2.1), ethane-1,2-diol diformate (0.7), formaldehyde (2.1), 4-nitrosobenzonitrile and other reduction products of 4-nitrobenzonitrile. These products are accounted for as resulting from the fragmentation of the aminoxyl radical **A** by (*a*) heterolysis of the C–O bond (45%, leading to the one-electron oxidation of the 1,4-dioxan-2-yl radical) and (*b*) homolysis of the N–O bond (55%, leading to the formation of the 1,4-dioxan-2-oxyl radical which undergoes further fragmentation).

The products from the reaction of methoxymethyl radicals with NBN under γ-radiolysis conditions are formaldehyde (5.7), methanol (2.5) and methyl formate (1.3). It is concluded that also in this case the decay of the aminoxyl radical **D** occurs by two pathways: the heterolysis route (46%) and the homolysis route (54%).

In the presence of oxygen the 1,4-dioxan-2-yl radicals are converted into the corresponding peroxyl radicals. Their bimolecular decay ($2k = 2.0 \times 10^8\ dm^3\ mol^{-1}\ s^{-1}$) yields the same products as in the case of NBN (albeit with a different product distribution and the formation of some peroxides): 1,4-dioxan-2-one (0.4), 2-hydroxy-1,4-dioxane (0.4), ethane-1,2-diol monoformate (0.6), ethane-1,2-diol diformate (2.8) and formaldehyde (0.6).

These results indicate that fragmentation reactions involving the carbon-skeleton of organic radicals are important not only in the case of peroxyl radicals but they can also be induced by nitroaromatic sensitizers. In cells, reduction of the long-lived sensitizer adduct radicals by reducing agents such as ascorbate to give (toxic) hydroxylamine type products may compete with the homolytic or heterolytic fragmentation of the *N*-alkoxyaminoxyl radicals.

Introduction

In the presence of oxygen, living cells are more sensitive to ionizing radiation than in its absence. This oxygen effect is best explained by competition for the radiation-induced radicals in the cell between reducing substances, such as thiols (*e.g.* glutathione), which have the capacity to repair the cell material, and oxygen, which typically enhances the damage. In radiotherapy, hypoxia may prevent tumour cells from being effectively destroyed by the radiation and tumor regrowth may start from the surviving fraction. To counteract the increased radiation resistance under hypoxic conditions, drugs have been developed which are capable of mimicking the oxygen effect, *i.e.* in the absence of oxygen they sensitize the cells with respect to the effects of ionizing radiation.[1-3] Most of these radiation sensitizers are nitro compounds. In general, their sensitizing efficiency increases with increasing reduction potential,[4,5] from which it has been concluded that these radiosensitizers react with the relevant substrate radicals (*e.g.* DNA radicals) by electron transfer.

On the other hand, it is well known that the more powerful oxidant oxygen mainly *adds* to radicals and reacts rarely, if at all, by electron transfer to directly yield $O_2^{\bullet -}$.[6,7] In the few cases where prompt $O_2^{\bullet -}$ formation was observed upon reaction of carbon-centred radicals with oxygen,[8-12] peroxyl radicals may have been the short-lived precursors. It is thus likely that the radiation-sensitization properties of oxygen are related to its tendency to *add* to radicals rather than to be reduced. A legitimate question is whether this is also true for other sensitizers, *i.e.* whether the sensitization is due to *addition* rather than to electron transfer reactions. Indeed, it has been shown that many carbon-centred radicals react with nitroaromatics by *addition* to the nitro group to give *N*-alkoxyaminoxyl-type radical intermediates.[6,13-16] Although there is no doubt that *strongly* reducing radicals are eventually oxidized by the nitro compounds (*i.e.* the adduct formation is followed by heterolysis of the adducts leading to one-electron oxidation of the radicals and the formation of radical anions of the nitroaromatics),[6,14-17] the question arises whether this is also the route of decomposition of *weakly* reducing radicals. We therefore decided to investigate weakly reducing radicals, such as those derived from 1,4-dioxane, tetrahydrofuran and dimethyl ether. These radicals have been chosen on the basis of

their similarity to those generated at the sugar moiety of DNA by H-abstraction. Goldberg *et al.*[18,19] have described a product derived from the DNA 5'-radical generated by the radiomimetic drug neocarzinostatin in the presence of nitroaromatics. This product can be understood as resulting from fragmentation of the sugar moiety in a series of processes starting with homolysis of the N–O bond of the radical adduct with the nitroaromatic. Here we will present additional evidence for this type of fragmentation reaction. It was considered of interest to compare the effect of a nitroaromatic sensitizer with that of oxygen. We therefore included a study of the corresponding peroxyl radicals.

Experimental

Analytical grade 1,4- and 1,3-dioxane and tetrahydrofuran were distilled twice from sodium under argon. Triply distilled water was presaturated with a 4:1 (v/v) mixture of N_2O–O_2 and an aliquot amount of 1,4-dioxane was injected to give a concentration of 0.1 mol dm^{-3}. In the case of experiments with sensitizers, 10^{-4}–10^{-3} mol dm^{-3} solutions of 4-nitrobenzonitrile were saturated with N_2O prior to injecting the required amount of 1,4-dioxane. For product analysis, irradiations were performed with a ^{60}Co γ-source at a dose rate of 0.35 Gy s^{-1}.

As reference material, mono- and di-formates of ethane-1,2-diol were synthesized by azeotropic removal of water from the equilibrium mixture of ethane-1,2-diol and formic acid in diisopropyl ether.[20] After *ca.* 3 h of refluxing, diisopropyl ether was removed from the reaction mixture by fractional distillation. A mixture of mono- (64%) and di-formates (4%) and unreacted ethane-1,2-diol (32%) was obtained. The products in an ether extract were identified by GC–MS: ethane-1,2-diol monoformate [M_w 90, *m/z* (%): 29 (100), 31 (54), 43 (25), 44 (17) and 60 (34)]; and ethane-1,2-diol diformate [M_w 118, *m/z* (%): 29 (100), 31 (39), 43 (25), 44 (28), 60 (21) and 72 (4)].

The products were analysed either by GC or high-performance ion-chromatography (HPIC). Formaldehyde and (2-hydroxyethoxy)acetaldehyde (tautomer of 2-hydroxy-1,4-dioxane) were converted into the corresponding oximes with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride at pH 3.[21] After saturation with sodium chloride and acidification with H_2SO_4, the oximes were extracted with cyclohexane. The oxime of formaldehyde was analysed directly by GC (25 m OV-225 column, carrier gas H_2, flame ionization detection). After the removal of cyclohexane by rotary evaporation, the oximes of (2-hydroxyethoxy)acetaldehyde (two stereoisomers) were trimethylsilylated with bis(trimethylsilyl)trifluoroacetamide (BSTFA) in dry pyridine and analysed by GC–MS (50 m OV1 column, temperature 50–280 °C, 0.5 °C min^{-1}). Their identical mass spectra (M_w = 371) are characterized by *m/z* (%) 73 (90), 103 (30), 117 (35), 181 (100), 190 (11), 238 (4), 255 (5), 299 (8), 356 (0.1) and 371 (0.1).

1,4-Dioxan-2-one was determined as (2-hydroxyethoxy)-acetate by HPIC (Dionex, column HPIC AS4; eluent 4 × 10^{-4} mol dm^{-3} $NaHCO_3$, 1.5 cm^3 min^{-1}, retention time 13 min) after the pH of the irradiated solutions had been brought to > 10 with aq. NaOH whereby the lactone opens up into its corresponding acid, (2-hydroxyethoxy)acetic acid. Collected fractions from the HPIC analyses were rotatory evaporated to dryness, the residue trimethylsilylated and identified by GC–MS. The mass spectrum of the trimethylsilylated derivative of (2-hydroxyethoxy)acetic acid (M_w = 264) is characterized by *m/z* (%) 73 (100), 103 (24), 117 (27), 133 (6), 147 (73), 191 (15), 249 (16) and 264 (0.5).

The yields of mono- and di-formates of ethylene glycol could not be determined directly by GC as these esters hydrolyse substantially in water as observed with authentic samples. In alkaline media complete hydrolysis occurs. Hence, formic acid so released was measured by HPIC after adjusting the pH of the irradiated solutions to 10 with NaOH.

Products derived from 4-nitrobenzonitrile were identified by GC–MS of an ether extract: 4-nitrosobenzonitrile [M_w 132; *m/z* (%): 132 (61), 102 (100), 75 (13)]; 4-aminobenzonitrile [M_w 118; *m/z* (%): 118 (100) and 91 (35)]; 4,4'-dicyanoazoxybenzene [M_w 248; *m/z* (%): 248 (16), 232 (8), 220 (3), 130 (13), 116 (18), 102 (100), 90 (11) and 75 (16)].

Total hydroperoxide formation was determined by the iodide method.[22] To differentiate between organic peroxide and hydrogen peroxide, the latter was destroyed with catalase and the remaining organic peroxide determined.

Pulse radiolysis experiments were performed at room temperature with 0.4–2 μs pulses from a 2.8 MeV van de Graaff generator[23] with doses ranging from 5 to 20 Gy per pulse, as determined by KSCN dosimetry (10^{-2} mol dm^{-3} KSCN in N_2O-saturated aqueous solution, taking $G \times \varepsilon(480\ nm) = 4.7 \times 10^{-3}$ Gy^{-1} cm^{-1}).[24]

In experiments with dimethyl ether the solutions were saturated with a gas mixture of N_2O–dimethyl ether (4:1 v/v) prior to and during irradiation to maintain the concentration of dimethyl ether at 0.2 mol dm^{-3}.

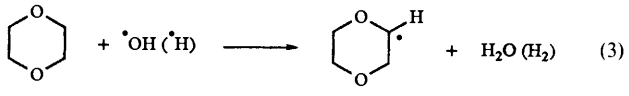
Results and discussion

Radiolysis of N_2O-saturated aqueous solutions leads to OH-radicals and H-atoms with *G* values of 5.6 × 10^{-7} and 0.6 × 10^{-7} mol J^{-1}, respectively [reactions (1) and (2)]. Hydrogen

$$H_2O \xrightarrow{\text{Ionizing radiation}} {}^{\bullet}OH,\ e_{aq}^{-},\ H^{\bullet},\ H^{+},\ H_2O_2,\ H_2 \qquad (1)$$

$$e_{aq}^{-} + N_2O \longrightarrow {}^{\bullet}OH + N_2 + OH^{-} \qquad (2)$$

atoms and hydroxyl radicals react with 1,4-dioxane by abstracting H-atoms [$k(^{\bullet}OH + 1,4\text{-dioxane}) = 2.8 \times 10^9$ dm^3 mol^{-1} s^{-1}; $k(H^{\bullet} + 1,4\text{-dioxane}) = 10^7$ dm^3 mol^{-1} s^{-1}][25] thereby forming 1,4-dioxan-2-yl **1** as the only radical [reaction (3)].



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In the case of dimethyl ether, the methoxymethyl radical is produced (for rate constants see ref. 25). With tetrahydrofuran, H-abstraction by hydroxyl radicals occurs predominantly at the C(2) position, giving 92% tetrahydrofuran-2-yl radicals.[17]

Reactions of alkoxyalkyl radicals with 4-nitrobenzonitrile

In the present study 4-nitrobenzonitrile (NBN) was chosen as a model for nitroaromatic sensitizers because it is sufficiently soluble in water and its reactions with a number of α-oxyalkyl radicals are well known.[6,14] The rate constant for reaction of NBN with the OH-radical can be estimated as ≈4 × 10^9 dm^3 mol^{-1} s^{-1}, based on those for nitrobenzene and benzonitrile.[25] Hence at a 1,4-dioxane concentration of 0.1 mol dm^{-3} and a NBN concentration of (1–5) × 10^{-4} mol dm^{-3}, >99% of the OH-radicals (and most of the H-atoms) react with 1,4-dioxane [reaction (3)].

Formation of the *N*-alkoxyaminoxyl radicals and their decay. The reaction of the 1,4-dioxan-2-yl radicals **1** with NBN was studied by pulse radiolysis of an N_2O-saturated solution of 1,4-dioxane (0.1 mol dm^{-3}) in the presence of (1–5) × 10^{-4} mol dm^3 NBN. At the end of the electron pulse a strong build-up of absorption with a maximum at 308 nm was observed [Fig. 1 and inset (*a*)]. The spectrum of this transient is very similar

Table 1 Rate constants of the reactions of 4-nitrobenzonitrile with α-alkoxyalkyl radicals, spectral parameters and rate constants of the decay of the so-formed *N*-alkoxyaminoxyl-type radicals in aqueous solution

	Aminoxyl radical			
Radical	λ_{max}/nm	ε/dm^3 mol^{-1} cm^{-1}	k(formation)/dm^3 mol^{-1} s^{-1}	k(decay)/s^{-1}
$^{\bullet}CH_2$–O–CH_3	310	5.2×10^3	2.0×10^8	0.6
$^{\bullet}CH$(–O–CH_2–CH_2–CH_2)	310	2.0×10^4	1.5×10^9 [a]	1.0
$^{\bullet}CH$(–O–CH_2–CH_2–O–CH_2) **1**	308	1.4×10^4	1.0×10^8	0.4
$^{\bullet}CH$(–O–CH_2–O–CH_2–CH_2) **2**	310	2.0×10^4	1.2×10^8 [b]	0.4
$^{\bullet}CH$(–O–CH_2–CH_2–CH_2–O) **3**	c	c	9.0×10^8 [d]	$> 5 \times 10^5$

[a] In agreement with the value 1.3×10^9 dm^3 mol^{-1} s^{-1} measured by Jagannadham and Steenken.[6] [b] Measured at 310 nm. [c] Only NBN$^{\bullet -}$ (λ_{max} = 330 nm) is observed. [d] Formation of NBN$^{\bullet -}$ measured at 330 nm.

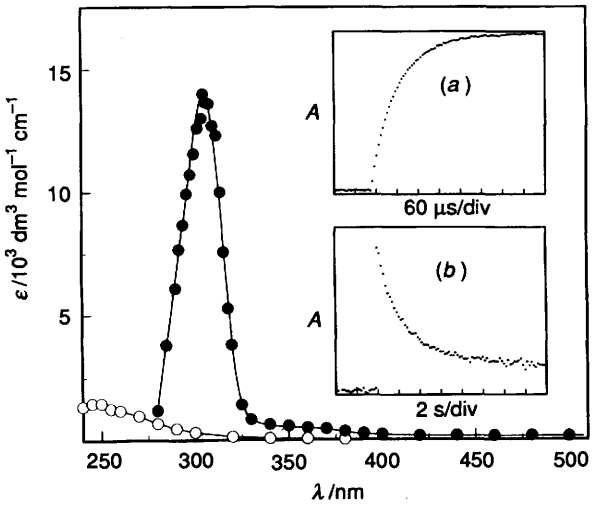
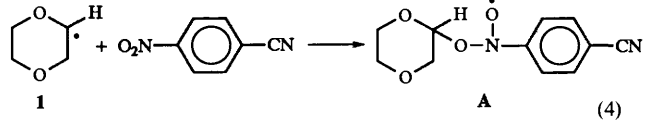


Fig. 1 Absorption spectrum of the 1,4-dioxan-2-yl radical **1** (○) taken 2 μs after the pulse (≈6 Gy/pulse) in N_2O-saturated solution of 1,4-dioxane (2×10^{-3} mol dm^{-3}). Absorption spectrum of the aminoxyl radical **A** (●) obtained 300 μs after the pulse (≈5 Gy/pulse) in N_2O-saturated solution of 1,4-dioxane (0.1 mol dm^{-3}) containing 10^{-4} mol dm^{-3} 4-nitrobenzonitrile. Insets (*a*) absorption build-up of radical **A** at 310 nm, (*b*) its decay at 310 nm.

to those observed on reaction of NBN with $^{\bullet}CH_2OH$ or $CH_3{}^{\bullet}CHOH$, which were identified as *N*-alkoxyaminoxyl radicals.[14] On this basis, the transient absorbing at 308 nm is identified as the *N*-alkoxyaminoxyl radical **A** formed in reaction (4). From the comparison of the absorption at 308 nm after



1 **A** (4)

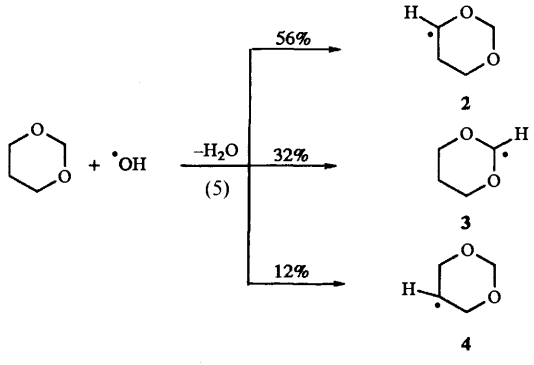
completion of reaction (4) with that from the KSCN dosimetry, the ε value of **A** is 1.4×10^4 dm^3 mol^{-1} cm^{-1} (see Table 1).

The observed rates of the exponential build-up of optical density [*cf.* inset (*a*) in Fig. 1] increased linearly with increasing concentration of NBN. From this dependency the rate constant $k_4 = 1.0 \times 10^8$ dm^3 mol^{-1} s^{-1} was obtained for the formation of the aminoxyl radical **A** (Table 1), a value that is similar to that measured for the reaction of the hydroxymethyl radical with NBN.[14]

Radical **A** is very long-lived (its bimolecular decay rate constant is $< 10^5$ dm^3 mol^{-1} s^{-1}). It decays by first-order kinetics with a rate constant of 0.4 s^{-1} at 20 °C [inset (*b*) in Fig. 1], with no product visible above 320 nm. Surprisingly, there is little change in the rate of decay with increasing temperature up to 70 °C.

The reactions of NBN with the methoxymethyl radical and the tetrahydrofuran-2-yl radical were investigated under similar conditions as described above. The methoxymethyl radical behaves similar to radical **1**, *i.e.* it forms an adduct radical **D** with NBN [see reaction (20)] with an absorption maximum at 310 nm which decays with a rate constant of 0.6 s^{-1} (see Table 1). Somewhat surprisingly, the tetrahydrofuran-2-yl radical reacts with NBN much faster than the other α-monoalkoxyalkyl radicals (see Table 1). Here an adduct with an absorption maximum at 310 nm is formed. This species decays with a rate constant of 1.0 s^{-1}.

The reaction of OH-radicals with 1,3-dioxane leads to the formation of three types of radical [reaction (5)] (for



justification of the respective assignments see below). The 1,3-dioxan-4-yl radical **2** is an α-monoalkoxyalkyl radical similar to radical **1**. The 1,3-dioxan-2-yl radical **3** is activated by two alkoxy functions and is thus more strongly reducing than radical **2** (*cf.* refs. 12 and 17). In a pulse-irradiated N_2O-saturated solution of 1,3-dioxane (0.1 mol dm^{-3}) containing 10^{-4} mol dm^{-3} NBN, two separate processes, a fast absorption build-up at 330 nm [Fig. 2 inset (*b*)] and a relatively slow build-up at 310 nm [Fig. 2 inset (*a*)] are observed. Both processes have rates proportional to the NBN concentration. The build-up at 310 nm is attributable to the formation of the aminoxyl radical **B** from the reaction of radical **2** with NBN [reaction (6)]. The rapid build-up at 330 nm is ascribed to the formation of the radical anion of NBN (NBN$^{\bullet -}$) [reaction (7)]. Since its rate of formation is proportional to the NBN concentration, the reaction of radical **3** with NBN thus leads to the rapid formation of NBN$^{\bullet -}$ without an adduct intermediate being observed. If an *N*-alkoxyaminoxyl-type radical was an intermediate, then its heterolysis decay is very fast ($k > 5 \times 10^5$ s^{-1}).

From the linear relationships of the observed rate constants of absorption build-up at 310 and at 330 nm with the NBN

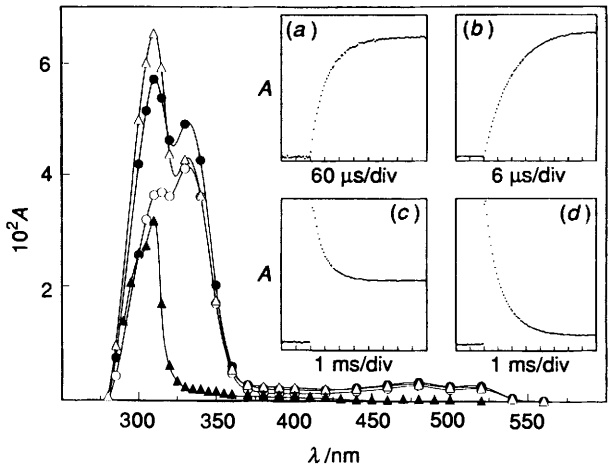
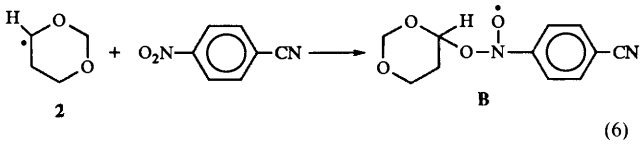


Fig. 2 Absorption spectra obtained in the pulse-irradiated (≈5 Gy/pulse) N_2O-saturated solution of 1,3-dioxane (0.1 mol dm^{-3}) containing 10^{-4} mol dm^{-3} 4-nitrobenzonitrile (○) 18 μs, ● 72 μs, △ 300 μs, and ▲ 3 ms after the pulse). Insets (*a*) absorption build-up at 310 nm, (*b*) absorption build-up at 330 nm, (*c*) decay at 310 nm, and (*d*) decay at 330 nm.



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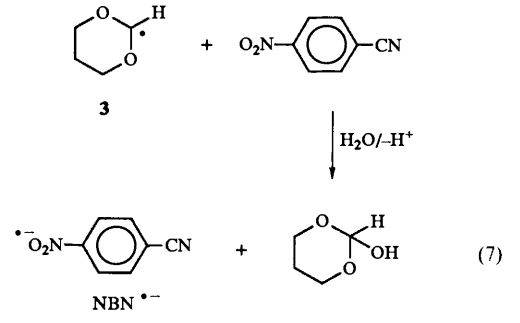
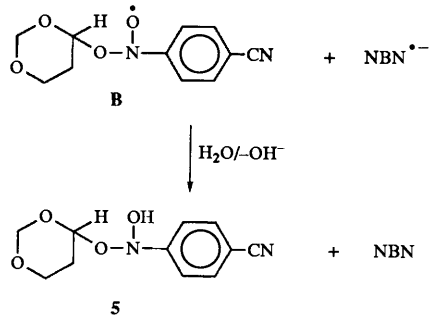


(7)

concentrations, the bimolecular rate constants of the reactions of NBN with radicals **2** and **3** were calculated (see Table 1). Using the reported value[14] of ε(330 nm) = 2.06 × 10^4 dm^3 mol^{-1} cm^{-1} for $NBN^{\bullet-}$, the *G* value of radical **3** is calculated to be 2.1 × 10^{-7} mol J^{-1}, which corresponds to 32% of OH-radical attack. The sum of the radicals **2** and **3** was determined independently using their reactions with tetranitromethane (TNM) (*cf.* ref. 17). Unlike NBN, TNM is reduced quantitatively by both radicals **2** and **3** to the nitroform anion (NF^-) at a diffusion-controlled rate (4 × 10^9 dm^3 mol^{-1} s^{-1}). $G(NF^-)$ = 5.9 × 10^{-7} mol J^{-1} was calculated from the absorbance at 350 nm. Hence G(**2**) = 3.7 × 10^{-7} mol J^{-1} (56% of OH-radical yield). This leaves G(**4**) = 0.8 × 10^{-7} mol J^{-1} (12% of OH-radical yield).

The radical anion of nitrobenzene is known to be rather stable in alkaline solution (life time of several seconds).[26] When $NBN^{\bullet-}$ was generated independently from the reaction of 2-hydroxyprop-2-yl radical with NBN in neutral solution,[14,26] we found its bimolecular rate constant for decay to be $2k \approx 10^6$ dm^3 mol^{-1} s^{-1}. However, in the case of 1,3-dioxane, where both the aminoxyl radical **B** and $NBN^{\bullet-}$ are formed, the absorption at 310 nm [*ca.* 60%, see Fig. 2 inset (*c*)] and at 330 nm [Fig. 2 inset (*d*)] decay by first-order kinetics with the same rate constant (k_{obs} = 1.4 × 10^3 s^{-1} at 4.6 Gy/pulse). This observed rate constant increases with increasing dose rate, *e.g.* k_{obs} = 2.5 × 10^3 s^{-1} at 12.7 Gy/pulse. Following this decay, the rest of the absorption at 310 nm (*ca.* 40% of the initial absorption) decays with a rate constant of 0.4 s^{-1}, typical of the unimolecular decay of aminoxyl-type radicals.

The otherwise atypical rapid decay of the absorption at 310 nm and at 330 nm can be explained by electron transfer from $NBN^{\bullet-}$ to the aminoxyl radical **B** [reaction (8)] to



(8)

produce NBN and a hydroxylamine-type product **5**. If this is the case, it means that the reduction potential of the aminoxyl is larger than that of NBN. Compound **5** possibly decomposes into nitrosobenzonitrile, formaldehyde, and 3-hydroxypropanal. Since there is an excess of **B**, $NBN^{\bullet-}$ and **B** decay exponentially with the rate dependent on the initial concentration of **B**. In order to test this concept, the aminoxyl radical **A** and $NBN^{\bullet-}$ were independently generated by pulse-irradiating an N_2O-saturated solution of 1,4-dioxane (0.1 mol dm^{-3}), propan-2-ol [(2–4) × 10^{-2} mol dm^{-3}] and NBN (10^{-4} mol dm^{-3}; the 2-hydroxyprop-2-yl radical reduces NBN to $NBN^{\bullet-}$). Analogous observations were made as in the case of the 1,3-dioxane solution described above, *i.e.* the lifetime of **A** was dramatically shortened by the presence of $NBN^{\bullet-}$. It was found that under these conditions the rate (but not the fraction) of absorption decay of **A** at 310 nm is independent of the concentration of propan-2-ol added. From the linear dependence of the observed first-order rate of absorption decay at 330 nm on initial aminoxyl radical concentration, the rate constant for the reaction of **A** with $NBN^{\bullet-}$ was calculated to be k = 1.0 × 10^9 dm^3 mol^{-1} s^{-1}. The same rate constant was found for reaction (8).

Concerning the unimolecular decay of the *N*-alkoxyaminoxyl radicals, in no case was there any product radical seen. If $NBN^{\bullet-}$ was the product of this decay [*e.g.* reaction (9), also see below], its formation could not possibly be observed due to its rapid reaction with the aminoxyl radicals [*cf.* reaction (8)].

Reactions of the *N*-alkoxyaminoxyl radicals with ascorbate and with oxygen. As shown above, the *N*-alkoxyaminoxyl-type adduct radicals are long-lived intermediates. Since they have a high spin density at oxygen (for the EPR parameters, see ref. 14), they are likely to have oxidizing properties. This idea was tested by adding ascorbate [(1–8) × 10^{-4} mol dm^{-3}] to the solutions. As a result, the observed first-order rate constant of absorption decay at 310 nm of radical **A** was found to increase proportionally with ascorbate concentration. From such a linear dependence of k_{obs} on ascorbate concentration, the rate constant 2 × 10^4 dm^3 mol^{-1} s^{-1} for the reaction of the NBN adduct radical **A** with ascorbate was obtained. Similar experiments were carried out with dimethyl ether instead of 1,4-dioxane. The rate constant for the reaction of the aminoxyl radical with ascorbate is also about 2 × 10^4 dm^3 mol^{-1} s^{-1}. The corresponding formation of the ascorbyl radical anion ($Asc^{\bullet-}$) with its well-characterized[27] absorption maximum at 360 nm was not observed in either of the above cases. This is understandable since the bimolecular decay of $Asc^{\bullet-}$ is fast

Table 2 Products and their G values in the γ-radiolysis (0.35 Gy s^{-1}) of N_2O-saturated aqueous 1,4-dioxane solutions (0.1 mol dm^{-3}) in the presence of 4-nitrobenzonitrile (10^{-4} mol dm^{-3}) or oxygen [N_2O–O_2 (4:1)]-saturated solution

Product	$G/10^{-7}$ mol J^{-1}	
	N_2O–4-Nitrobenzonitrile	N_2O–O_2
Formaldehyde	2.1 (3.7; 4.5)[a]	0.6
1,4-Dioxan-2-one	0.3	0.4
2-Hydroxy-1,4-dioxane	2.5	0.4
1,2-Ethanediol monoformate[b]	2.1	0.6
1,2-Ethanediol diformate[b]	0.7	2.8
Formic acid (after hydrolysis)	3.5	6.2
4-Nitrosobenzonitrile	present[c]	n.a.
Organic (hydro)peroxides	n.a.	1.1
Hydrogen peroxide	n.d.	0.9
Oxygen consumption	n.a.	6.2

[a] Yields in 50% and 90% (v/v) 1,4-dioxane, respectively. [b] Identified by GC–MS, quantified *via* the measured products formic acid and formaldehyde (see text). [c] Yield not quantified. n.d. = not determined, n.a. = not applicable.

compared to its formation under present experimental conditions.

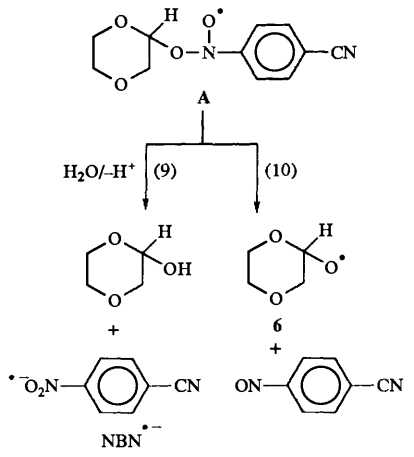
A number of aminoxyl radicals have previously been shown to be able to oxidize ascorbic acid, albeit very slowly. The reduction of the radiosensitizer triacetone-amine-*N*-oxyl (TAN) by ascorbic acid has been suggested as a thermal reaction.[28] More quantitative data are available for the reduction of piperidine and pyrrolidine aminoxyl radicals by ascorbate.[29] The enhanced reactivity of *N*-alkoxyaminoxyl radicals towards ascorbate observed in this work may be explained by the much lower electron density of these aminoxyl radicals due to presence of the alkoxyl function as compared to the other aminoxyl radicals.

In cells the corresponding sensitizer-radical adducts may similarly react with reductants such as ascorbate in competition with their unimolecular decay. In such a reaction, (toxic) hydroxylamine-type products are likely to be formed.

To test for the possible reaction of aminoxyl radicals with oxygen, an N_2O–O_2 (9:1 v/v, $[O_2] = 1.4 \times 10^{-4}$ mol dm^{-3}) saturated solution of tetrahydrofuran (0.1 mol dm^{-3}) and NBN (10^{-3} mol dm^{-3}) was pulse-irradiated. Under these conditions, > 80% of tetrahydrofuran-2-yl radicals react with NBN to give the aminoxyl radical. The rate of the unimolecular decay of the aminoxyl radical monitored at 315 nm was found to be the same as in the absence of oxygen ($k \approx 1.0\ s^{-1}$). We conclude that the aminoxyl radical has a very low reactivity towards oxygen ($k < 10^3$ dm^3 mol^{-1} s^{-1}).

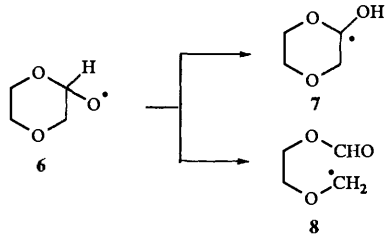
Product analysis. The products identified from the decay of the 1,4-dioxane-derived aminoxyl radical **A** under ^{60}Co-γ-radiolysis conditions (see Experimental section) are compiled in Table 2. All these products showed a linear yield-dose dependence from which the G values given in Table 2 were calculated.

One of the products is 2-hydroxy-1,4-dioxane ($G = 2.5 \times 10^{-7}$ mol J^{-1}). Its formation can be understood in terms of oxidation of 1,4-dioxan-2-yl radical (**1**) to the carbocation followed by hydroxylation at C-2 [reaction (9)]. The oxidation occurs by heterolysis of the C–O bond of the alkoxyaminoxyl radical **A**. The heterolysis route constitutes the actual electron-transfer step which is common among α-hydroxyalkyl radicals.[6,14] In agreement with this concept, on production of radical **1** in the presence of the potent one-electron oxidant $[Fe(CN)_6]^{3-}$ (*cf.* refs. 30 and 31), 2-hydroxy-1,4-dioxane was the only product observed with near-quantitative yield ($G = 5.6 \times 10^{-7}$ mol J^{-1}). Since in the present case G(2-hydroxy-1,4-dioxane) $= 2.5 \times 10^{-7}$ mol J^{-1} constitutes only 45% of the

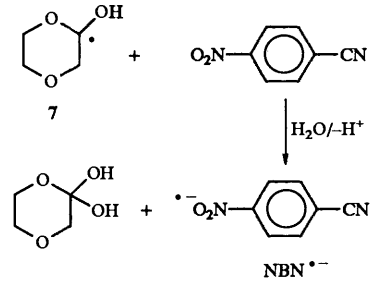


expected yield of **A**, it is clear that heterolysis of the C–O bond [reaction (9)] is not the only decay route of **A**. The other 55% of **A** are accounted for by 1,4-dioxan-2-one and the products of fragmentation of the 1,4-dioxane ring. This route is suggested to be started off by homolysis of the N–O bond in **A** giving 4-nitrosobenzonitrile and the oxyl radical **6** [reaction (10)]. This type of reaction has previously been suggested to explain products observed in the radical-induced degradation of DNA in the presence of nitroaromatics.[18,19]

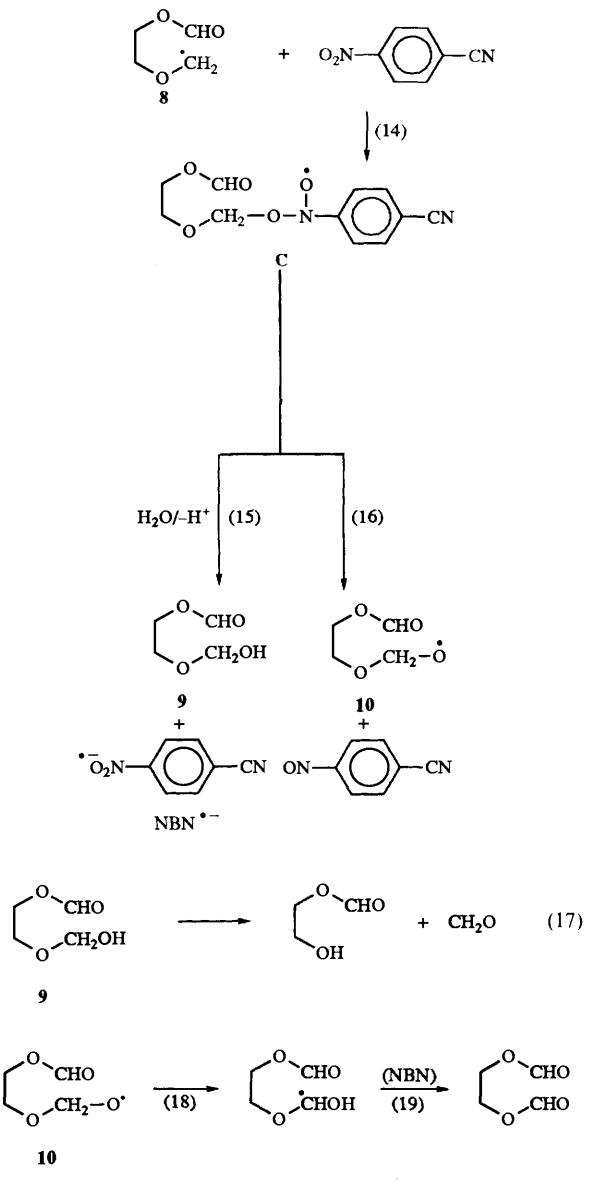
Oxyl radicals in aqueous solution are known (for reviews see refs. 7 and 32) to undergo mainly two types of reactions: 1,2-H-shift[33–36] [reaction (11)] and β-C–C fragmentation[37] [reaction



(12)]. Radical **7** from reaction (11) is activated by an α-hydroxyl function in addition to an α-alkoxyl function and is therefore considerably more reducing than radical **1**. Hence it is reasonable to assume that it is quantitatively oxidized by NBN to 1,4-dioxan-2-one [reaction (13)].



Further fragmentation of radical **8** formed in reaction (12) is unlikely (*cf.* ref. 38) to compete with the scavenging of this radical by NBN [reaction (14)]. Very similar reactions as outlined above may be suggested to follow [reactions (15)–(19)]. The resulting products are formaldehyde, ethane-1,2-diol formate [reaction (17)] and ethane-1,2-diol diformate [reaction (19)]. The formate esters have been identified by GC–MS of an ether extract of the irradiated solution (see Experimental). The total yields of these two esters were determined by measuring



the formate ions yields by HPIC after hydrolysis of these esters. The yield of ethane-1,2-diol formate is then taken to be the same as that of formaldehyde. Under this assumption a reasonable material balance is obtained [*i.e.* G(ethane-1,2-diol mono- and di-formates) + G(2-hydroxy-1,4-dioxane) + G(1,4-dioxan-2-one) = 5.6×10^{-7} mol J^{-1}], which means that more than 80% of the 1,4-dioxane-derived products are accounted for.

The G value of 1,4-dioxan-2-one is 0.3×10^{-7} mol J^{-1}, which is low compared to the combined yields of the fragmentation products, the ethanediol formates ($G = 2.8 \times 10^{-7}$ mol J^{-1}). This result, which reflects the fact that the 1,2-H-shift [reaction (11)] is less favoured than β-fragmentation [reaction (12)], is in agreement with expectation based on previous experience with similar systems.[39]

It is thus concluded that the reaction of NBN with radical **1** yields first an adduct which decays with a rate constant of 0.4 s^{-1} *via* two pathways: heterolysis resulting in one-electron oxidation of radical **1** (45%) and homolysis leading to oxidative fragmentation of the carbon–carbon skeleton (55%).

The formaldehyde yield may be taken as a rough measure for the efficiency of the fragmentation route. As can be seen from Table 3, its yield increases significantly with increasing temperature. It is at present not clear why the increase in formaldehyde yield is not paralleled by an increase in the rate of decay of the aminoxyl radical **A** (see pulse radiolysis section).

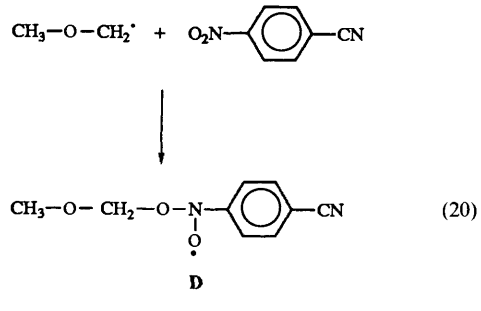
Table 3 Effect of temperature on G(formaldehyde) in the γ-radiolysis (0.35 Gy s^{-1}) of N_2O-containing solutions of 1,4-dioxane in the presence of 4-nitrobenzonitrile (10^{-3} mol dm^{-3}) or oxygen [N_2O–O_2 (4:1)-saturated].

	G(HCHO)/10^{-7} mol J^{-1}	
T/°C	4-Nitrobenzonitrile	Oxygen
20	2.1	0.6
30	4.1	1.2
50	4.5	1.5
70	5.0	1.7

The fragmentation (*i.e.* homolysis) reaction [reaction (10)] is not restricted to 4-nitrobenzonitrile; it was also observed in the case of 4-nitroanisol [G(formaldehyde) = 1.9×10^{-7} mol J^{-1}], and with nitrobenzene itself. In the latter case nitrosobenzene was found as a product which is in support of the homolysis concept. It is to be expected that the rate of heterolysis [reaction (9)] decreases with decreasing polarity of the solvent, and hence the relative importance of the competing route, the homolysis, should increase. Experiments were performed using 1,4-dioxane–water ratios of 1:1 (v/v) and 9:1 and the formaldehyde yields were determined. They were found at 3.6×10^{-7} and 4.4×10^{-7} mol J^{-1}, respectively, compared to 2.0×10^{-7} mol J^{-1} in purely aqueous solution. These data thus support this concept, although at high 1,4-dioxane concentration the radiation chemistry is not that of dilute aqueous solutions.

In reactions (9), (10), (15) and (16), 1,4-dioxane is oxidized while NBN is reduced, either to its radical anion [reactions (9) and (15)] or to 4-nitrosobenzonitrile [reactions (10) and (16)]. The radical anion is likely to undergo a complex redox chemistry (*cf.* refs. 25 and 40). In the present case, attempts failed to determine the products derived from NBN quantitatively and thereby to correlate the 1,4-dioxane-derived products with those derived from NBN, as these products, identified as 4-nitrosobenzonitrile, 4,4′-dicyanoazoxybenzene and 4-aminobenzonitrile (see Experimental) are formed in non-reproducible yields.

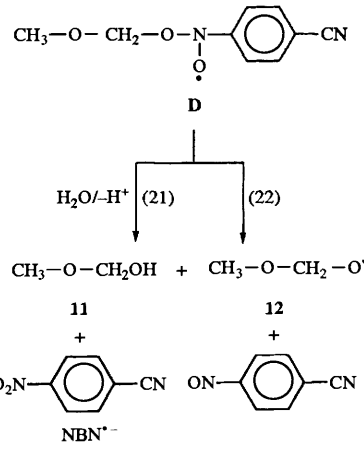
For comparison with the 1,4-dioxane system, the products from the reaction of the methoxymethyl radical (from the reaction of hydroxyl radicals with dimethyl ether) with NBN [reaction (20)] were also determined under γ-radiolysis

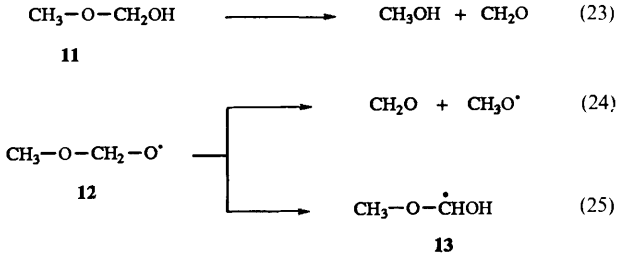


conditions and are given in Table 4. These products, which account for about 80% of the expected yield of the aminoxyl radical, can be accounted for by reactions analogous to those of the aminoxyl radical **A** [*cf.* reactions (9) and (10)]. The heterolysis of the aminoxyl radical **D** leads to the formation of methanol and formaldehyde [reactions (21) and (23)]. On the other hand, the homolysis of radical **D** leads to the formation of the oxyl radical **12** [reaction (22)]. Radical **12** can undergo either β-C–O-fragmentation giving formaldehyde and the methoxyl radical [reaction (24), k_{24} in the order of 10^6 s^{-1}],[37] or 1,2-H-shift giving the α-hydroxy-α-methoxymethyl radical **13** [reaction

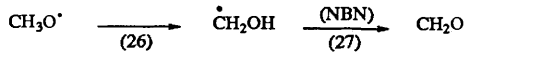
Table 4 Products and their G values in the γ-radiolysis of N_2O-saturated aqueous solution of dimethyl ether [0.2 mol dm^{-3}, N_2O–dimethyl ether (4:1 v/v)] containing 4-nitrobenzonitrile (10^{-3} mol dm^{-3})

Product	$G/10^{-7}$ mol J^{-1}
Formaldehyde	5.7
Methanol	2.5
Methyl formate	1.3

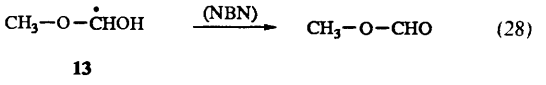




(25), k_{25} is probably close to the value of 5×10^5 s^{-1} estimated for the 1,2-H-shift of the methoxyl radical, reaction (26)].[36,41]



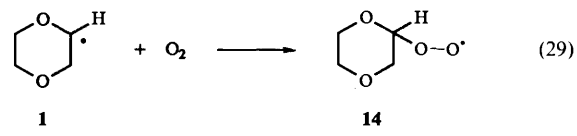
Judging by the reaction of the methoxyl radical with methanol ($k = 2.6 \times 10^5$ dm^3 mol^{-1} s^{-1}),[42] H-abstraction reactions of radical **12** and the methoxyl radical with the parent compound are negligible at the given concentration of dimethyl ether (0.2 mol dm^{-3}). The hydroxymethyl radical and radical **13** are subsequently oxidized quantitatively by NBN to formaldehyde [reaction (27)], and to methyl formate [reaction (28)]. From the



above considerations, it is concluded that the homolysis decay route of radical **D** [reaction (22), $G = \frac{1}{2}G$(CH_2O from reaction 28) + G(methyl formate) = 2.9×10^{-7} mol J^{-1}] is slightly more favoured than the heterolysis route [reaction (21), $G = G(CH_3OH) = 2.5 \times 10^{-7}$ mol J^{-1}].

Peroxyl radical reactions

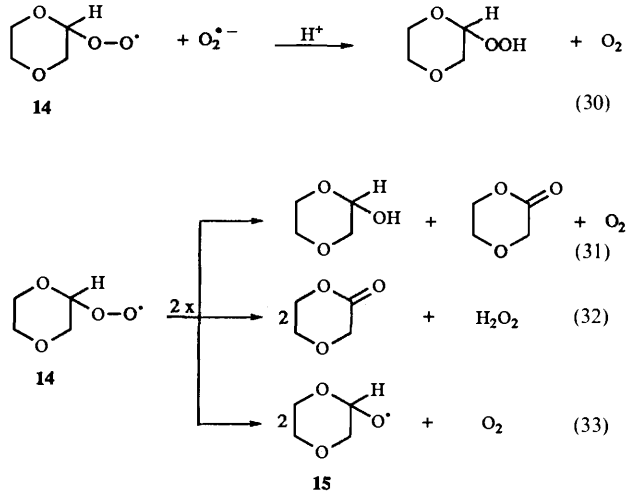
Pulse radiolysis. To compare the effect of oxygen to that of NBN, 1,4-dioxane solutions were saturated with N_2O–O_2 (4:1 v/v) to convert the 1,4-dioxan-2-yl radicals **1** into the corresponding peroxyl radicals **14** [reaction (29)]. The absorption



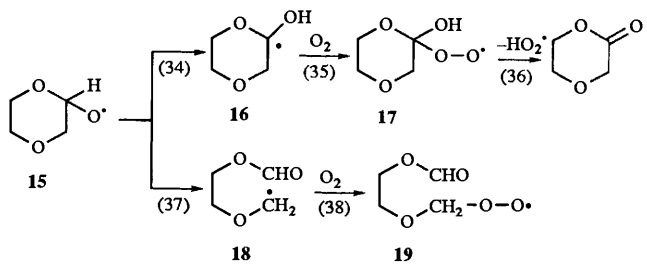
spectrum of radical **14** is very similar to that of the 1,4-dioxan-2-yl radical **1** (see Fig. 1). For this reason the rate constant of reaction (29) cannot be determined optically. We assume that k_{29} is about 2×10^9 dm^3 mol^{-1} s^{-1} as has been found for similar radicals.[43] Radical **14**, similar to the α-ethoxyethylperoxyl radical derived from diethyl ether,[39] is expected to decay only bimolecularly. The rate constant of its bimolecular decay was determined by monitoring its absorption decay at 260 nm to be 2.0×10^8 dm^3 mol^{-1} s^{-1}.

Pulse radiolysis of N_2O–O_2 (4:1 v/v)-saturated solution of 1,3-dioxane (2×10^{-3} mol dm^{-3}) showed that the peroxyl radicals corresponding to radicals **2** to **4** have relatively weak absorptions around 250 nm ($\varepsilon = 10^3$ dm^3 mol^{-1} cm^{-1}) and decay bimolecularly with an overall rate constant of about 10^8 dm^{-3} mol^{-1} s^{-1}. It is of interest to note that the 1,3-dioxan-2-peroxyl radical (the peroxyl radical corresponding to radical **3**) does not undergo unimolecular elimination of $O_2^{\bullet-}$ as the $CH_3C(OCH_3)_2O_2^{\bullet}$ radical does (derived from hydroxyl radical reaction with acetaldehyde dimethyl acetal).[12] This was confirmed by conductance measurements (formation of H^+ and $O_2^{\bullet-}$), as well as by optical measurement in the presence of tetranitromethane (TNM) (nitroform anion formation monitored at 350 nm from the reaction of $O_2^{\bullet-}$ with TNM).[44,45]

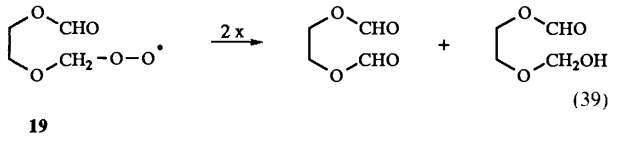
Product studies. The products and their G values from the γ-radiolysis of 1,4-dioxane in N_2O–O_2 (4:1 v/v)-saturated solution are given in Table 2. In addition to the products also observed in the presence of NBN, organic hydroperoxides are found but their structures have not been identified. They probably arise from the reactions of $O_2^{\bullet-}$ with the peroxyl radicals **14** or **19** [*cf.* reaction (30)].



In the 1,4-dioxane system, all the products are derived from the peroxyl radical **14**. Its bimolecular termination reactions, reactions (31)–(33) are well known peroxyl radical reactions (for a review see ref. 7). It is evident that products possibly formed in oxidation reactions, such as 1,4-dioxan-2-one and 2-hydroxy-1,4-dioxane [reactions (30) and (31)], are of minor importance, *i.e.* the fragmentation reaction (37) which follows the formation of the oxyl radical **15** in reaction (32) dominates. This implies that under steady-state conditions the (secondary) peroxyl radical **19** is of about equal importance as the primarily formed 1,4-dioxan-2-peroxyl radical **14**. As a consequence,



cross-termination reactions between the two peroxyl radicals are favoured over the self-termination reactions (30)–(32) and (39). This complexity makes it difficult to come up with a



detailed reaction scheme as was possible for many single peroxyl radical systems.[7] However, it is noticeable that a good material balance [G('OH) *vs.* G(products)] is obtained and that there is practically no increase observed when the dose rate is lowered, *i.e.* no chain reaction of any significance occurs under these conditions. As can be seen from Table 3, G(formaldehyde) increases with increasing temperature. Thus the importance of the various routes must change with temperature. No attempt was made to investigate this point in more detail.

Conclusions

Nitroaromatic sensitizers are able to react with organic radicals by addition to give α-alkoxyaminoxyl-type radicals. In the case of 'strongly' reducing radicals, the aminoxyl-type adducts decay by *hetero*lysis leading to the oxidation of the radicals (*cf.* ref. 14). In the case of 'weakly' reducing radicals, *homo*lysis is an additional mode of decay of the aminoxyls. This reaction leads to further homolytic fragmentation reactions of the type known from peroxyl radical chemistry (*cf.* ref. 7). In these respects the reactivity of nitroaromatic sensitizers is very similar to that of oxygen. This behaviour may be of great radiation-biological significance.

In cells where reductants such as ascorbate are abundant the long-lived nitroaromatic adduct radicals will be readily reduced. The hydroxylamine-type products of these reactions can produce nitroso products upon intramolecular transformation. The latter have been implicated in the cytotoxicity of nitroaromatics.

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