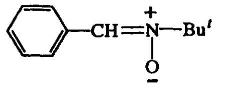
Generation of α-aminoxylcarbenium ions by electron-transfer oxidation of *N-tert*-butyl-3-phenyloxazirane and their role in nitrone spin trapping chemistry

Valentin E. Zubarev and Ortwin Brede
Max Planck Society, Research Unit 'Time Resolved Spectroscopy' at the University of Leipzig, Permoserstr. 15, D-04303 Leipzig, Germany

Using low-temperature EPR and optical absorption spectroscopy the α-aminoxylcarbenium ion $1^{\cdot +}$ was directly observed in frozen (77 K) halogenated matrices as a result of electron-transfer oxidation of *N-tert*-butyl-3-phenyloxazirane **4** by matrix radical cations. The aminoxylcarbenium ion $1^{\cdot +}$ reacts with nucleophilic anions Cl^- to form an α-chloro-substituted aminoxyl radical **3**.

Introduction

Recently (ref. 1), we have shown that in the electron-transfer oxidation [eqn. (1)] of the spin-trap compound *N-tert*-butyl-*C*-phenylnitrone **1** (PBN), aminoxyl radicals **2** appeared as a result of the addition of nucleophiles [eqn. (2)] to the initially formed α-aminoxylcarbenium ions ($1^{\cdot +}$) and not *via* the simple trapping by PBN of the oxidised nucleophile-derived radicals ($Nu^{\cdot}$) according to eqn. (3).



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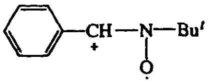
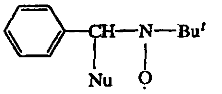


$1^{\cdot +}$



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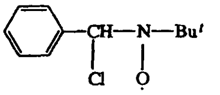

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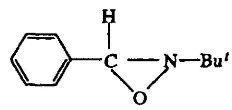
$$1 - e^- \longrightarrow 1^{\cdot +} \qquad (1)$$

$$1^{\cdot +} + Nu^- \longrightarrow 2 \qquad (2)$$

$$1 + Nu^{\cdot} \longrightarrow 2 \qquad (3)$$

Structural, kinetic and chemical evidence was presented in ref. 1 to describe the formation and reactivity of the aminoxylcarbenium ion $1^{\cdot +}$ in solutions of different polarity [water, MeCN, 1,1,2-trichloro-1,2,2-trifluoroethane (Freon-113) and butyl chlorides] at room temperature and in frozen glassy halogenated matrices. In order to gain further insight into the chemistry of the aminoxylcarbenium ion $1^{\cdot +}$, in this work we report on the formation of the chloro-substituted aminoxyl radicals **3** from carbenium ion $1^{\cdot +}$ under conditions excluding any (competitive) radical trapping reactions like that shown in eqn. (3).

The aminoxylcarbenium ion $1^{\cdot +}$ was generated by γ-irradiation of *N-tert*-butyl-3-phenyloxazirane **4** dissolved in frozen glassy matrices of Freon-113 and *sec*-butyl chloride (Bu^sCl). The oxazirane **4** is known [2,3] as an isoelectronic compound of PBN and cannot trap reactive free radicals. By photoexcitation of PBN, the oxazirane **4** is formed (*via* the singlet excited state) [3] as the primary photoisomerisation product of PBN. The thermally and photochemically (*via* the triplet excited state) activated isomerisation of the oxazirane **4** results [2,3] in the formation of PBN. By means of low-temperature EPR and UV–visible optical absorption spectroscopy we have observed the (matrix-isolated) aminoxylcarbenium ion $1^{\cdot +}$ as a stable, electron-transfer oxidation product of the oxazirane **4**.



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Experimental

EPR studies

First-derivative X-band EPR measurements and γ-irradiation (dose 5 kG) of the frozen, degassed solutions of the spin trap PBN or oxazirane **4** in halogenated matrices were performed as described in our previous work (ref. 1). A copper cryostat was used for the annealing of the irradiated samples at various temperatures; after annealing of the samples for 10 min they were recooled to 77 K for EPR measurements.

Matrix isolation

Upon γ-irradiation, matrix-isolated radical cations are formed as a result of electron-transfer oxidation of the solute by matrix radical cations, while secondary electrons are completely scavenged by matrix molecules, resulting in the formation of matrix-derived radicals and anions Cl^- (for a review see refs. 4 and 5). In Freon-113, the intensity of the EPR signal of the matrix-derived $CF_2ClCFCl^{\cdot}$ radicals is very low because of the anisotropic line broadening and the EPR spectrum of the solute cation radicals could be clearly observed, while in *sec*-butyl chloride the EPR spectrum of matrix-derived *sec*-butyl radicals strongly overlaps that of the solute cation radicals [see, *e.g.*, Fig. 4(*a*) and 4(*b*) in ref. 1].

Low-temperature UV-visible optical absorption measurements

Optical absorption spectra in a frozen glassy matrix of Bu^sCl at 77 K were measured in Suprasil quartz cells ($1 \times 10 \times 30\ mm^3$) using a Shimadzu UV–VIS Scanning Spectrophotometer UV-2101PC according to the procedure outlined in ref. 5. Optical absorption measurements were performed in the butyl chloride matrix only, because this matrix results in good glasses upon freezing. The experimental error in the absorbance measurements was estimated to be 10–15%.

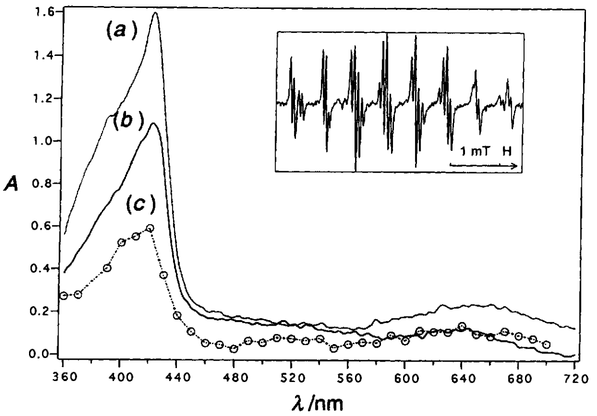


Fig. 1 Absorption spectra of aminoxylcarbenium ion $1^{\bullet +}$ derived from (*a*) PBN and (*b*) the oxazirane **4** in an irradiated *sec*-butyl chloride matrix at 77 K. The absorption spectrum (*c*) of the aminoxylcarbenium ion $1^{\bullet +}$ in *sec*-butyl chloride at room temperature taken from ref. 1 is shown for comparison (corrected for the different optical pathlengths and multiplied by 10 for ease of comparison). The insert shows the EPR spectrum of aminoxyl radical **3** in *sec*-butyl chloride at 193 K.

Chemicals

PBN **1** and *N*-*tert*-butyl-3-phenyloxazirane **4** were prepared according to ref. 2. Freshly prepared solutions of the oxazirane were used, to avoid the formation of PBN as a result of the thermally activated isomerisation of oxazirane **4** into PBN (refs. 2 and 3). $CF_2ClCFCl_2$ (Freon-113, *puriss.* 'Genetron® 113', Fluka) and *sec*-butyl chloride (Bu^sCl), both from Aldrich, were used as described in ref. 1.

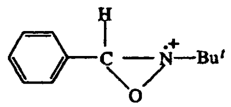
Results

Owing to its low ionisation potential (E_i),† γ-irradiation of the oxazirane **4** in the frozen halogenated matrices (RHal) should lead to its ionisation[4,5] as a result of electron transfer to the matrix radical cations $RHal^{\bullet +}$

Low-temperature EPR and optical absorption studies in *sec*-butyl chloride matrices

After irradiation of the oxazirane **4** or PBN (both 0.05 mol dm^{-3}) in a frozen glassy *sec*-butyl chloride matrix the samples were intensely green in colour due to the ionisation of the solute. In the case of PBN the aminoxylcarbenium ion $1^{\bullet +}$ is formed as a result of electron-transfer oxidation by matrix radical cations (ref. 1). Fig. 1 shows the absorption spectra of ionised PBN (dotted line) and the oxazirane **4** (bold line) in a frozen *sec*-butyl chloride matrix. For comparison, the absorption spectrum (taken from ref. 1) of the aminoxylcarbenium ion $1^{\bullet +}$ obtained in pulse radiolysis of PBN in *sec*-butyl chloride at room temperature is shown by a broken line in Fig. 1. The absorption spectra of ionised PBN (at 77 K and at room temp.) and the oxazirane **4** are quite similar and exhibit two bands at λ_{max} 420 and 640 nm (*cf.* ref. 1) with the ratio (*r*) of the extinction coefficients $r = \varepsilon_{420}/\varepsilon_{640}$ measured as 6 ± 1. Hence, upon ionisation of the oxazirane **4** in a *sec*-butyl chloride matrix, aminoxylcarbenium ion $1^{\bullet +}$ is formed as a stable intermediate.

Because the oxazirane **4** has two π electrons localised on the sp^3-hybridised (pyramidal) nitrogen,[3b] the primary ionisation of compound **4**, eqn. (4), is expected to give a cyclic nitrogen-centred radical cation like that shown by the structure $4^{\bullet +}$ and similar to that assigned earlier[7] to the cyclic nitrogen-centred aziridine radical-cation in Freon matrices. Subsequent intramolecular rearrangement of the parent radical-cation $4^{\bullet +}$ into the more stable aminoxylcarbenium ion $1^{\bullet +}$ (ref. 1) proceeds, therefore, as a result of the ring opening at the C–O bond as is shown in eqn. (5).



$4^{\bullet +}$

$$4 + RHal^{\bullet +} \longrightarrow 4^{\bullet +} + RHal \qquad (4)$$

$$4^{\bullet +} \longrightarrow 1^{\bullet +} \qquad (5)$$

From matrix isolation studies it is known (refs. 7 and 8) that ionisation of three-membered heterocycloalkane compounds results in the formation of ring-opened distonic-type radical cations.

Upon melting (transfer of the sample from the liquid nitrogen-bath into the cooled heptane-bath at 183 K) of the irradiated *sec*-butyl chloride matrix containing the oxazirane **4** the well resolved EPR spectrum of aminoxyl radical **3** (see ref. 1 for parameters) was observed at 193 K (see insert in Fig. 1). The formation of aminoxyl radicals **3** can only be understood as resulting from an ion–molecule addition reaction of anions Cl^- to the aminoxylcarbenium ions $1^{\bullet +}$ according to eqn. (6).

$$1^{\bullet +} + Cl^- \longrightarrow 3 \qquad (6)$$

The observed yield of aminoxyl radicals **3** was estimated as being 10% of the initial concentration of the paramagnetic species detected immediately after irradiation at 77 K. This low yield of aminoxyl radicals can be explained by assuming that *sec*-butyl radicals as well as Cl^- anions can compete for the aminoxylcarbenium ion $1^{\bullet +}$ upon warming of the matrix. Furthermore, the oxazirane **4** is known as an oxidant[2] and, as was demonstrated in the earlier spin trapping studies,[9] could react with radicals.

Low-temperature EPR studies in Freon-113 matrix

Based on the optical absorption measurements in a *sec*-butyl chloride matrix (above) the EPR spectrum of ionised oxazirane **4** in Freon-113 matrix shown in Fig. 2(*a*) may be attributed to the aminoxylcarbenium ion $1^{\bullet +}$. For comparison, the EPR spectrum of ion $1^{\bullet +}$ (from ref. 1) derived from PBN under the same experimental conditions is shown in Fig. 2(*b*). These EPR spectra are very similar, although in the case of aminoxylcarbenium ion $1^{\bullet +}$ derived from compound **4** the high-field ^{14}N parallel feature is not clearly observed. From matrix-isolation low-temperature EPR studies[4,7,8] it is known that in some cases intramolecular rearrangement of the primary solute radical cations proceeds readily upon warming (ΔT 10–30 K) of the matrices. Additional rearrangement of the surrounding matrix molecules near the newly formed species should be expected also. The differences in the EPR spectra of radical cation $1^{\bullet +}$ derived from oxazirane **4** and spin trap PBN immediately after irradiation at 77 K [*cf.* Figs. 2(*a*) and 2(*b*)] is, therefore, attributed to intramolecular and/or matrix effects.

Upon annealing at 107 K the colour of the samples was not changed appreciably and a broad ^{14}N high-field feature could be recognised in the EPR spectrum of cation radical $1^{\bullet +}$ derived from compound **4**. Further annealing of irradiated samples at 121 K resulted in a loss of colour and simultaneously irreversible changes in the EPR spectra were observed [Fig.

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† In this work we assume that compound **4** has nearly the same E_i (8.3 eV) as its nearest analogue, *N*-methyl-3-phenyloxazirane (ref. 6).

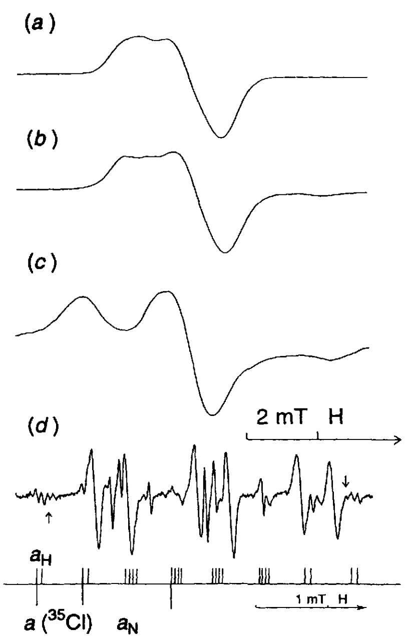


Fig. 2 First-derivative EPR spectra of aminoxylcarbenium ion **1**$^{\cdot+}$ in a frozen Freon-113 matrix derived from (*a*) the oxazirane **4** and (*b*) PBN (taken from ref. 1) immediately after irradiation at 77 K. EPR spectrum (*c*) of aminoxyl radical **3** at 77 K after annealing of the irradiated oxazirane **4** in Freon-113 at 121 K, and (*d*) shows solution EPR spectrum (at 243 K) after melting of sample (*c*). The contribution of aminoxyl radical **3** in spectrum (*d*) is indicated by a stick diagram, which shows lines only for the most abundant (75.4%) isotope of chlorine, ^{35}Cl[a_N 1.224, $a(^{35}Cl)$ 0.638 and a_H 0.069 mT], for clarity of presentation. For the ^{37}Cl-substituted aminoxyl **3** the positions of the outer lines are indicated with two arrows.

2(*c*)]. The signal from cation radical **1**$^{\cdot+}$ disappeared, and instead an EPR signal of the chloro-substituted aminoxyl radical **3** appeared with $A_{\parallel} = 3.24$ mT and $g_{\parallel} = 2.0038$ (ref. 1). As in the *sec*-butyl chloride matrix (above), the formation of aminoxyl radical **3** can be understood only if we assume that upon softening of the Freon-113 matrix the aminoxylcarbenium ion **1**$^{\cdot+}$ reacts with anions Cl$^-$ in an ion–molecule reaction (6). Nearly quantitative conversion of radical-cation **1**$^{\cdot+}$ into radical **3** in the temperature range 107–121 K reflects the high diffusion mobility of Cl$^-$ anions compared with matrix-derived $CF_2ClCFCl^{\cdot}$ radicals which do not decay below 121 K.

Upon further warming of the Freon-113 matrix, the aminoxyl radicals **3** decayed in the temperature range below the mp of Freon-113 (234 K); simultaneously, the signal from secondary aminoxyl radicals grew in the EPR spectrum. Only trace amounts of radical **3** could therefore be observed in the solution EPR spectrum above the mp in Freon-113 together with at least two other, unidentified aminoxyl radicals, which strongly contribute to the combined EPR signal in Fig. 2(*d*).

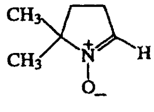
These observations are in contrast to the results obtained (above) in a *sec*-butyl chloride matrix under the same experimental conditions. The decay of aminoxyl radicals **3** and simultaneous formation of the secondary aminoxyl radicals upon annealing of the Freon-113 matrix above 121 K could be attributed to the thermally activated reactions of matrix-derived $CF_2ClCFCl^{\cdot}$ radicals (which do not decay below 121 K) with both aminoxyls **3** and oxazirane **4** in the softened solid matrix still below the mp.

The optical absorption measurements as well as the EPR results allow us to conclude that ionisation of the oxazirane **4** by halogenated matrix(solvent) radical cations results in the formation of the aminoxylcarbenium ion **1**$^{\cdot+}$ *via* rearrangement of the metastable transient species **4**$^{\cdot+}$ according to eqn. (5). The same species **1**$^{\cdot+}$ appeared as the primary electron-transfer product of the spin trap PBN (ref. 1).

Discussion

α-Aminoxylcarbenium ions as key intermediates in the formation of aminoxyl radicals in spin trapping applications of nitrones under oxidative conditions

α-Aminoxylcarbenium ions similar to **1**$^{\cdot+}$ are also expected to be the primary products of electron-transfer oxidation of other nitrones, including the most useful cyclic spin trap DMPO **5** (5,5-dimethyl-1-pyrroline *N*-oxide). Thus the aminoxylcarbenium ion **5**$^{\cdot+}$ (radical cation) of the spin trap DMPO was first reported by Chandra and Symons (ref. 10) to be formed in a frozen Freon-11 matrix ($CFCl_3$) and postulated recently (ref. 11) as the primary photoionisation product of DMPO in aq. solutions. An electron-transfer oxidation of substituted *C*-methoxyaryl-*N*-methyl-nitrones and -oxaziranes by photoexcited singlet 9,10-dicyanoanthracene was recently reported[12] to result in the formation of the nitrone radical cations. Hence, electron-transfer-mediated trapping of nucleophiles according to eqns. (1) and (2) or 'inverted spin trapping' (ref. 13) should be operative also in the case of other nitrone compounds used as spin traps.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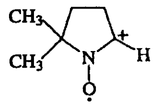
5$^{\cdot+}$

In the case of the spin trap PBN only the addition of nucleophiles to the aminoxylcarbenium ion **1**$^{\cdot+}$ was experimentally observed under various oxidative spin trapping conditions,[1,13] indicating that mesomeric structure **1**$^{\cdot+}$ (see ref. 1 for details) is the dominating electronic structure. Stabilisation of the aminoxylcarbenium ion **1**$^{\cdot+}$ by the loss of a proton and/or rearrangement seems to be impossible. In the case of the aminoxylcarbenium ions derived from nitrones containing β-hydrogen atoms, in competition with the addition of nucleophiles a deprotonation should also occur, resulting directly in vinylaminoxyl radicals, which are known as oxidation products of different acyclic and cyclic nitrones and were studied in depth by Aurich (see ref. 14 for a review). Thus, in the electron-transfer oxidation of some substituted nitrones[15] by PbO_2 both vinylaminoxyl radicals and aminoxyl radicals were directly observed.

In the case of the spin trap DMPO, in competition with reaction[10,13b] with nucleophiles, eqn. (7), aminoxylcarbenium ion **5**$^{\cdot+}$ can deprotonate to give the vinylaminoxyl radical **6**, eqn. (8).

$$\mathbf{5}^{\cdot+} + \mathrm{Nu}^- \longrightarrow \mathbf{7} \qquad (7)$$

$$\mathbf{5}^{\cdot+} \longrightarrow \mathbf{6} + \mathrm{H}^+ \qquad (8)$$

Although postulated,[11,16] vinylaminoxyl radicals **6** have not as yet been directly identified in spin trapping applications of DMPO. Electron-transfer oxidation[15b] of a 2-substituted DMPO analogue **8**‡ provides evidence for such a deprotonation. In this specific case, however, the deprotonation of aminoxylcarbenium ion **8**$^{\cdot+}$ from the side-chain is energetically

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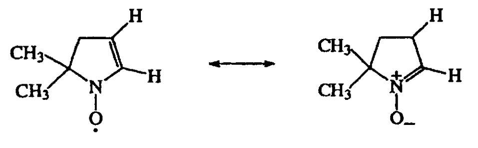
‡ Nitrone **8** is expected (ref. 17) to be formed as a result of the electron-transfer oxidation of the aminoxyl radical 2-(α-cyanobenzyl)-5,5-dimethyl-1-pyrrolidinyl 1-oxide studied in ref. 15(*b*).



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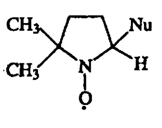

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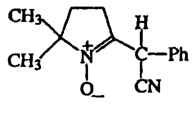

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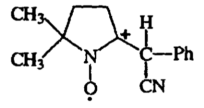
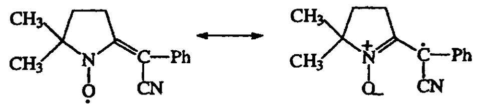


8•+



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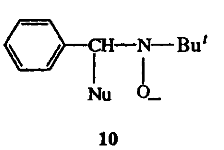
more favourable [*cf.* eqn. (8)] and, therefore, highly planar vinylaminoxyl radicals **9** are formed. Hence, depending on the structure, aminoxylcarbenium ions can be converted into aminoxyl radicals in reactions with nucleophiles or, upon deprotonation, in vinylaminoxyl radicals. These reactions of aminoxylcarbenium ions strongly interfere with the spin trapping of reactive radicals under oxidative conditions.

Addition–oxidation mechanism for the formation of nucleophile-substituted aminoxyl radicals

To explain the formation of a variety of nucleophile-substituted aminoxyl radicals derived from spin traps, an alternative two-step addition–oxidation mechanism [see eqns. (9) and (10)] postulated earlier by Forrester and Hepburn (ref. 18) is often used. This mechanism is based on the fact that aminoxyl radicals like **2** (Nu = CN^-, AcO^-, polymeric anions) were observed in very unusual spin trapping conditions, namely in strongly acidic (2 mol dm^{-3} HCl, acetic acid) or strongly basic [butyllithium in tetrahydrofuran (THF)] solutions of the spin traps PBN or 2-nitrosoisobutane (Bu^tNO).

The mechanism postulated by Forrester and Hepburn is in strong contrast with that outlined in eqns. (1) and (2) of this study or in ref. 13. This disagreement should be resolved in order to clarify the spin trapping chemistry of nitrones. According to Forrester and Hepburn, in the case of the spin trap PBN the addition of nucleophiles to PBN should give amino-oxy-anions **10**, eqn. (9), which should be further oxidised as in eqn. (10) by residual traces of oxygen to aminoxyl radicals **2**.



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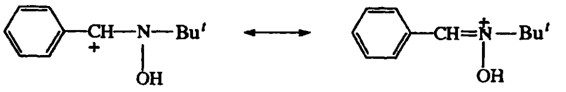
$$\text{PBN} + \text{Nu}^- \longrightarrow \mathbf{10} \qquad (9)$$

$$\mathbf{10} + O_2 \longrightarrow \mathbf{2} + O_2^{\bullet -} \qquad (10)$$

Reaction (9) is very surprising in view of Huisgen's studies (ref. 19) of nitrone chemistry. Thus, according to Huisgen, 1,3-dipolar nitrone molecules show neither electrophilic nor nucleophilic properties. On the other hand, quantitative spin trapping data[20] indicate that, in aq. near-neutral solutions, anionic species such as AcO^- and $HC(O)O^-$ do not react with PBN according to eqn. (9). Hence, the addition–oxidation mechanism [eqns. (9) and (10)] is not operative under neutral conditions.

For us, the mechanism in question is not valid under the acidic conditions used in ref. 18, based on the following reasons. As is known,[21] in strongly acidic solutions not the spin trap PBN but instead only its protonated form **11** is present according to equilibrium (11). The transient carbenium ion **11** reacts further with water resulting in the hydrolysis of PBN, eqns. (12) and (13), *via* the salt-like transients **12** (refs. 2, 21).



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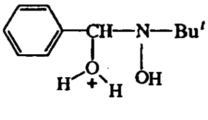

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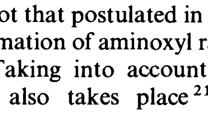
$$\text{PBN} + H^+ \rightleftharpoons \mathbf{11} \qquad (11)$$

$$\mathbf{11} + H_2O \longrightarrow \mathbf{12} \qquad (12)$$

$$\mathbf{12} \xrightarrow{-H^+} PhC(O)H + Bu^tNHOH \qquad (13)$$

It is reasonable to assume that under these conditions nucleophilic anions could compete with water for the carbenium ions **11** [eqns. (12) and (14)] resulting in the formation of the salt-like (*cf.* ref. 2) product **13**, subsequent oxidation of which (by residual oxygen) could result in the formation of nucleophile-substituted aminoxyl radicals **2** as shown in eqn. (15).



13

$$\mathbf{11} + \text{Nu}^- \longrightarrow \mathbf{13} \qquad (14)$$

$$\mathbf{13} + O_2 \longrightarrow \mathbf{2} + HO_2^{\bullet} \qquad (15)$$

Thus, we believe that the reaction mechanism shown in eqns. (11)–(15) and not that postulated in ref. 18 [eqns. (9) and (10)] explains the formation of aminoxyl radicals from nitrones in acidic solution. Taking into account that base-catalysed hydrolysis of PBN also takes place[21] the formation of aminoxyls under the strongly basic conditions in ref. 18 is not surprising.

Conclusions

The α-aminoxylcarbenium ions are key intermediates in the electron-transfer oxidation of both *C*-aryl- and *C*-alkyl-nitrones, including cyclic nitrones like DMPO. Depending on the structure, the parent aminoxylcarbenium ion may react with nucleophiles or deprotonate. In spin trapping applications these reactions are responsible for the appearance of nucleophile-substituted aminoxyl radicals (addition of nucleophiles) or vinylaminoxyl radicals (deprotonation of the parent aminoxylcarbenium ions). Particularly in biological systems *in vivo*, where the spin traps could compete with enzymic scavenging of reactive free radicals (ref. 22), electron-transfer-mediated trapping of nucleophiles according to eqns. (1) and (2) could result in the formation of aminoxyl radicals and not simple radical trapping.

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