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SPIN TRAPPING BY USE OF *N-TERT*-BUTYLHYDROXYLAMINE. INVOLVEMENT OF FENTON REACTIONS

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Spin trapping of short-lived R· radicals is done by use of *N-tert*-butylhydroxylamine (**1**) and H_2O_2. The hydroxylamine is oxidized to the radical *t*-BuṄ(O)H (**2**) which is converted into the spin trap 2-methyl-2-nitrosopropane (**3**). Simultaneously, hydroxyl radicals ·OH are formed from H_2O_2. The latter radical species abstracts hydrogen atoms from suitable molecules HR to give R· radicals, which are trapped with the formation of aminooxyl radicals, i.e., *t*-BuṄ(O)R (**4**), detectable by EPR spectroscopy. The reaction is enhanced by the presence of iron ions. The cleavage of H_2O_2 into ·OH radicals is considered to involve both a radical-driven (*t*-BuṄ(O)H **2**) and an iron-driven Fenton reaction.

KEY WORDS: EPR spectroscopy, spin trapping, Fenton reactions, *N-tert*-butylhydroxylamine, 2-methyl-2-nitrosopropane.

INTRODUCTION

In connection with experiments on some aminooxyl radicals formed from oximes,[1] it was observed that the EPR spectra of these radicals, i.e., R′Ṅ(O)H, were partially replaced by other spectral systems after some time. An analysis of the spectra indicated that radicals of the type R′Ṅ(O)R had been formed by trapping R· radicals. This paper describes the results obtained with *N-tert*-butylhydroxylamine as the parent compound.

EXPERIMENTAL

N-tert-Butylhydroxylamine hydrochloride, *tert*-butylhydroperoxide, cumene hydroperoxide, methanol-d_4, 2,4-dihydroxypyrimidine-5-carboxylic acid (isoorotic acid), DETAPAC, desferrioxamine, and other chemicals used were obtained from Fluka AG, Aldrich or Sigma Chemical Company. Chelex 100 was from Bio-Rad Laboratories.

Preparation of Radicals

N-tert-Butylhydroxylamine hydrochloride was dissolved in H_2O, 0.1 M NaOH or in the liquid substrate to a concentration between 50 and 100 mM. The substrates were dissolved in water solutions of *N-tert*-butylhydroxylamine to a concentration between 50 and 100 mM. To 0.4 ml of *N-tert*-butylhydroxylamine/substrate solution, about 25 to 50 μl of 3% H_2O_2 was added.

Electron Spin Resonance Measurements

The EPR spectra were recorded using a Varian E-9 spectrometer, as described elsewhere.[1]

Quantitative estimation of the contents of iron and copper in solvent water and reaction mixtures was made by Mikro Kemi AB, Uppsala.

RESULTS AND DISCUSSION

A large number of radicals (R·) derived from substances (substrates) such as methanol, ethanol, dimethyl sulfoxide (DMSO), amino acids, and pyrimidine derivatives, could be trapped by the use of *N-tert*-butylhydroxylamine. The aminooxyl radicals were obtained after the addition of a small amount of a hydroperoxide such as H_2O_2, *t*-BuOOH or cumene hydroperoxide to water solutions of *N-tert*-butylhydroxylamine and substrates, or solutions of *N-tert*-butylhydroxylamine in liquid substrates. The radicals were obtained in the dark with no previous irradiation of the samples with light, or any addition of metal ions.

The EPR spectra of the aminooxyl radicals formed were identical to, or very similar to, the spectra obtained by the use of the spin trap 2-methyl-2-nitrosopropane.[2–11]

It is obvious that the reaction includes the transformation of *N-tert*-butylhydroxylamine (**1**) to the spin trap *t*-BuNO (**3**), i.e., 2-methyl-2-nitrosopropane, and that ·OH radicals are formed from the hydroperoxides. Further, it was noted that the reaction was catalyzed by iron ions, which are present in small amounts as impurities in the chemicals and solvent water used. The question is whether the reaction mechanism involves a radical-driven or a conventional iron-driven Fenton reaction.

Radical-Driven Fenton Reaction

Disregarding any catalytic activity of metal ions, the reactions are suggested to be as follows:

$$\underset{\mathbf{1}}{t\text{-BuN(OH)H}} \longrightarrow \underset{\mathbf{2}}{t\text{-BuN(O)H}} \qquad (1)$$

$$\underset{\mathbf{2}}{t\text{-BuN(O)H}} + H_2O_2 \longrightarrow t\text{-BuN(O)H}^+ + {\cdot}OH + OH^- \qquad (2)$$

$$t\text{-BuN(O)H}^+ \longrightarrow \underset{\mathbf{3}}{t\text{-BuNO}} + H^+ \qquad (3)$$

The parent hydroxylamine **1** is oxidized by the hydroperoxide to the corresponding radical **2**. A further molecule of hydroperoxide is then cleaved in a Fenton reaction by the primary radical **2** with formation of the nitroso compound **3**, i.e., 2-methyl-2-nitrosopropane, and an ·OH radical, which is able to perform homolytic abstraction of a hydrogen atom from suitable substrates.

$$HR + {\cdot}OH \longrightarrow R{\cdot} + H_2O \qquad (4)$$

The radical R· is then trapped by the nitroso compound **3** to form the aminooxyl radical **4**

$$\underset{\mathbf{3}}{t\text{-BuNO}} + \text{R}\cdot \longrightarrow \underset{\mathbf{4}}{t\text{-BuṄ(O)R}} \qquad (5)$$

All reaction paths take place in the dark. Therefore, no ·OH radicals formed by photolytic cleavage of the hydroperoxides are involved.

Iron-Driven Fenton Reaction

The catalytic effect of iron ions is considered to involve a redox cycle in which the parent hydroxylamine (**1**) is oxidized by Fe^{III} ions to the radical **2**. This radical is further oxidized by Fe^{III} ions to the nitroso spin trap **3**, i.e.,

$$\underset{\mathbf{1}}{t\text{-BuN(OH)H}} \longrightarrow \underset{\mathbf{2}}{t\text{-BuṄ(O)H}} \longrightarrow \underset{\mathbf{3}}{t\text{-BuNO}} \qquad (6)$$

This mechanism implies that one reaction path leads to the spin trap **3**, whereas another path is involved in the formation of ·OH radicals in a Fenton reaction brought about by cleavage of the hydroperoxides mediated by Fe^{II} ions formed in the oxidation of the parent hydroxylamine (**1**),

$$Fe^{II} + H_2O_2 \longrightarrow Fe^{III} + \cdot OH + OH^- \qquad (7)$$

The reactions, Eqs. 1–7, leading to the formation of aminooxyl radicals detectable by EPR spectroscopy, were studied for a number of systems previously known to give rise to short-lived R· radicals, which could be trapped by the use of 2-methyl-2-nitrosopropane. References to these experiments are given in the text.[2-11]

The Aminooxyl Radicals Formed by Trapping of $CH_3\dot{C}HOH$ Radicals

The aminooxyl radical $t\text{-BuṄ(O)CH(CH}_3\text{)OH}$, formed by trapping the radical $CH_3\dot{C}HOH$ derived from ethanol after abstraction of one of the methylene hydrogen atoms, gives rise to a simple 3 × 2 line spectrum, indicating the interaction of the unpaired electron with one ^{14}N nucleus (a_N = 1.48 mT) and one hydrogen nucleus (a_H = 0.182 mT). This reaction system was found to be especially suitable for studying the reaction mechanism proposed above.

A. Oxidation of N-tert-butylhydroxylamine. Figure 1 shows the EPR spectrum of the radicals formed at room temperature in a reaction mixture consisting of 0.4 ml of a 100 mM solution of *N-tert*-butylhydroxylamine hydrochloride in H_2O (pH ~3.7), 50 μl of ethanol and 50 μl of a 3% solution of sodium wolframate. The four-line spectrum with intensities 1 : 2 : 2 : 1 indicated the presence of the radical $t\text{-BuṄ(O)H}$ (**2**) formed by oxidation of **1**, Eqn (1). The interaction with one ^{14}N nucleus and one hydrogen nucleus is expected to give rise to a six-line spectrum. However, the coupling constants a_N and a_H are very close to each other (~1.35 mT), which leads to overlap among the four central lines of the six-line spectrum. No aminooxyl radical (**4**) formed by the trapping of primary radicals derived from ethanol could be observed. Thus, the oxidation of **1** to the radical $t\text{-BuṄ(O)H}$ (**2**) does not *per se* lead to the formation of $t\text{-BuṄ(O)CH(CH}_3\text{)OH}$ in the presence of ethanol.

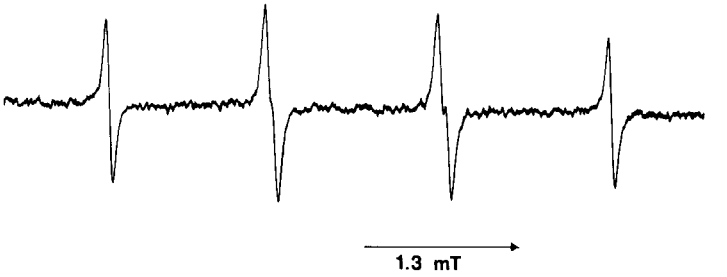


FIGURE 1 EPR spectrum of the radical *t*-BuṄ(O)H (**2**) obtained with 0.4 ml of a 100 mM solution of *N-tert*-butylhydroxylamine hydrochloride in H_2O (pH 3.7), 50 μl of ethanol and 50 μl of a 3% solution of Na_2WO_4.

Oxidation of the parent hydroxylamine (**1**) to radical **2** with H_2O_2 is described below (1).

The radical **2** was not detected when sodium wolframate was replaced by $Fe^{III}(NO_3)_3$. This might indicate the oxidation of *t*-BuN(OH)H (**1**) to the spin trap **3** directly, according to Eqn. 6. However, irradiation of the reaction mixtures with UV light, at pH 3.7, in the EPR cavity did not give rise to any radicals of the type $(t\text{-Bu})_2\dot{N}O$, a species which is expected to be formed when a solution of *t*-BuNO is irradiated with UV light. When a solution of *t*-BuN(OH)H, was irradiated by UV light at pH 7.4 radicals of the type $(t\text{-Bu})_2\dot{N}O$ were formed in small amounts. Addition of Fe^{III} ions to the reaction mixture did not increase the yield of radicals. Consequently, iron ions do not seem to participate directly in the oxidation of the parent hydroxylamine (**1**) to the radical **2**, or to the spin trap **3**.

B. The Fenton reaction. Figure 2a shows the EPR spectrum obtained with 0.4 ml of a 100 mM solution of *N-tert*-butylhydroxylamine hydrochloride in H_2O (pH $\sim$3.7), 50 μl of ethanol and 25 μl of a 3% solution of H_2O_2. The spectrum is dominated by the 3 × 2 line system of the aminooxyl radical *t*-BuṄ(O)CH(CH_3)OH (**4**). A small amount of the radical *t*-BuṄ(O)H is also present, a species that gives rise to an incompletely resolved six-line system. The maximum amplitude of the 3 × 2 line system was obtained in less than 5 min after mixing the reagents. The radicals persisted for more than one hour at room temperature.

The experiment illustrated the cleavage of H_2O_2 into ·OH radicals, the formation of the spin trap (**3**) and the aminooxyl radical *t*-BuṄ(O)CH(CH_3)OH. The transformation of the radical *t*-BuṄ(O)H (**2**) into the spin trap (**3**) also manifested itself by the relatively low concentration of **2** (Figure 2a), which further decreased with the time of observation. The formation of the spin trap (**3**) is visualized by the appearance of a faint blue colour, characteristic of the monomeric form of 2-methyl-2-nitrosopropane.

Figure 2b shows the center lines of the three main groups under higher resolution. Each line of the doublet is incompletely resolved into at least five narrow lines brought about by interaction with hydrogen nuclei of the four methyl groups of the radical *t*-BuṄ(O)CH(CH_3)OH.

Very similar results were obtained when H_2O_2 was replaced by a small amount of *t*-BuOOH, or with a solution of *t*-BuN(OH)H – HCl in ethanol, together with a small amount of cumene hydroperoxide dissolved in cumene.

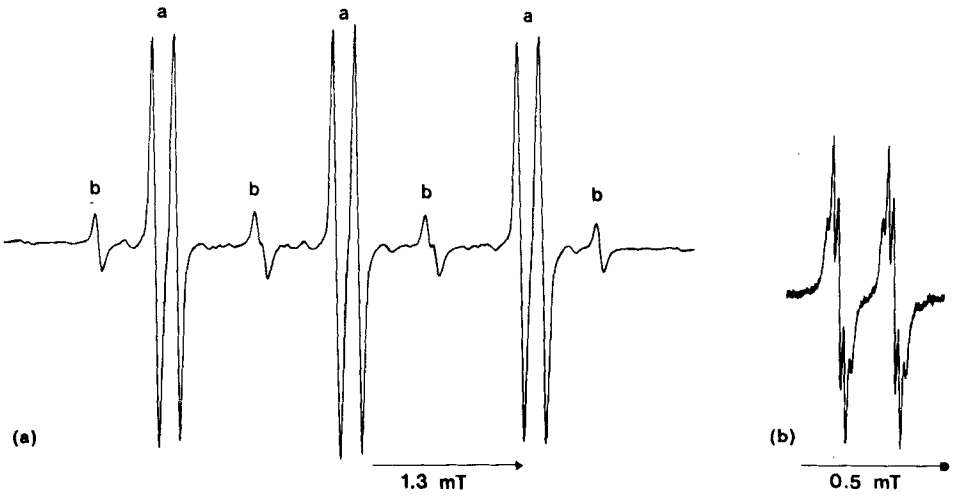


FIGURE 2 (a) The radical spectrum obtained with 0.4 ml of a 100 mM solution of *N-tert*-butylhydroxylamine hydrochloride in H_2O (pH ~3.7), 50 μl of ethanol and 25 μl of 3% H_2O_2: *a*. 3 × 2 line system: *t*-BuN̈(O)CH(CH_3)OH: a_N = 1.53 mT; a_H = 0.20 mT. *b*. four-line system: *t*-BuN̈(O)H: $a_N \simeq a_H$ = 1.33 mT. The spectrum was recorded about 5 min after mixing the reagents. (b) The ^{14}N component $M_I = 0$ (center group) of the spectrum of *t*-BuN̈(O)CH(CH_3)OH (Figure 2a) under higher resolution: a_H = 0.20 mT; narrow splittings ~0.02 mT.

C. Catalytic activity of iron. Radical-driven Fenton reactions which involve substances such as semiquinones, imino-semiquinones or paraquat radicals have been found to be catalyzed by small amounts of iron.[12-15] In view of these findings and the fact that the reaction mixtures in the present experiments contained about 0.4 ppm of iron (solvent water ≤0.05 ppm of Fe; ≤0.01 ppm of Cu), the reaction leading to the aminooxyl radicals described above (*B*) was performed in the presence of two different types of iron chelators: the imino diacetate chelator DETAPAC (diethylenetriamino pentaacetic acid), and the trihydroxamic acid desferrioxamine.

Figure 3a gives the results obtained with DETAPAC, and shows the relative amplitudes of the four-line and the 3 × 2 line radical systems plotted against the reaction time. The mixture consisted of 0.4 ml of a 100 mM solution of *t*-BuN(OH)H – HCl in H_2O containing 1.3 mg of DETAPAC/ml, and with pH adjusted to 7.4, 50 μl of ethanol, and 50 μl of 3% H_2O_2. The 3 × 2 line system originating from the aminooxyl radical *t*-BuN̈(O)CH(CH_3)OH increased slowly with time to a maximum that was reached after about 1.5 h. After this time the amplitude was found to decrease. The amplitude of the four-line system reached its maximum value almost immediately after mixing the reagents, and remained almost constant during the time of observation. The maximum ratio between the amplitudes of the 3 × 2 line and the four-line systems was about 3 : 1 (85 min).

When 25 μl of $Fe^{III}(NO_3)_3$ (1 g/l) was added to 0.4 ml of the *t*-BuN(OH)H/DETAPAC solution, 50 μl of ethanol and 50 μl of 3% H_2O_2, a radical spectrum very similar to that of Figure 2a was immediately obtained after mixing the reagents. The 3 × 2 line system was the dominant species, with an amplitude at least an order of magnitude larger than the maximum value of the corresponding species of Figure 3a. The ratio between the 3 × 2 line and the four-line systems was about 14 : 1.

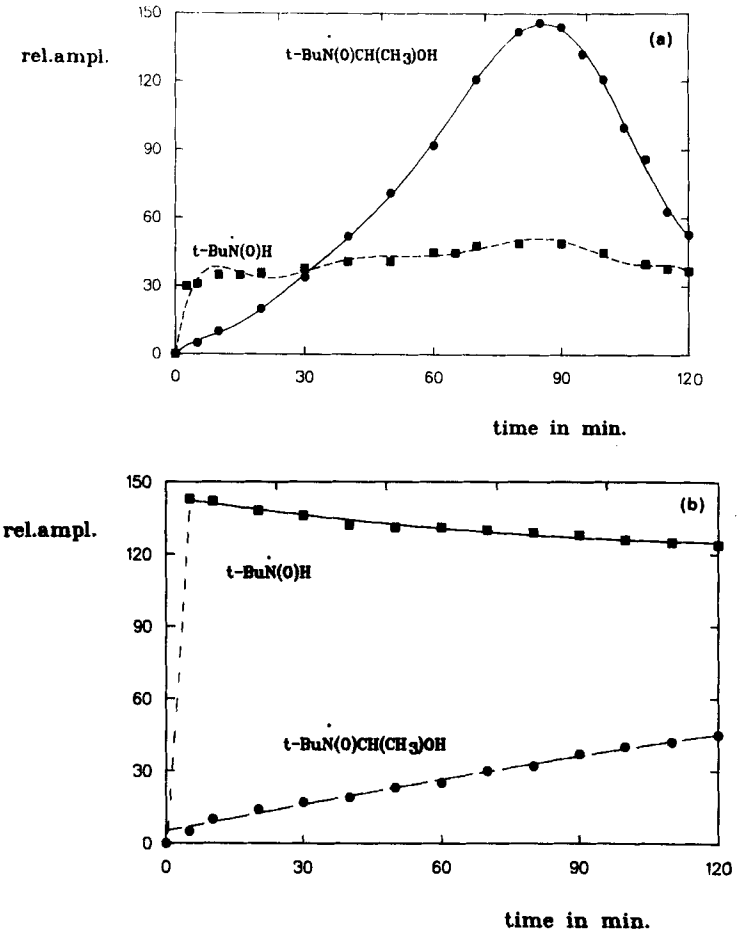


FIGURE 3 The relative amplitudes of the spectra from the four-line system (*t*-BuṄ(O)H) and the 3 × 2 line system (*t*-BuṄ(O)CH−(CH_3)OH) plotted against the reaction time. Reaction mixtures: 0.4 ml of *N-tert*-butylhydroxylamine hydrochloride (adjusted to pH 7.3), 50 μl of ethanol and 50 μl of 3% H_2O_2. (a): with 1.3 mg of DETAPAC/ml, (b): with 1 mg of desferrioxamine/0.4 ml.

Figure 3b shows the results obtained with 1 mg of desferrioxamine dissolved in 0.4 ml of a 100 mM solution of *t*-BuN(OH)H−HCl, pH 7.3, after the addition of 50 μl of ethanol and 50 μl of 3% H_2O_2. The four-line system reached its maximum value after less than 5 min. After 2 h, the four-line system had decreased to a value of about 87% of the maximum value. The 3 × 2 line system increased very slowly, and after the 2 h of observation, it had reached to a value about 64% of the four-line system. The spectrum observed after 24 h for the same sample indicated that the four-line system had further decreased to about 64% of its maximum value, whereas the 3 × 2 line system had increased to value twice of that recorded after 2 h.

These experiments indicated that the radicals *t*-BuṄ(O)H and *t*-BuṄ(O)CH(CH_3)OH are formed even in the presence of the iron-chelators DETAPAC and desferrioxamine. However, the rate of formation of *t*-BuṄ(O)CH(CH_3)OH is much slower compared

with the very rapid appearance of this radical in the presence of free iron ions. The yield of this radical species is also much greater in the presence of free iron ions.

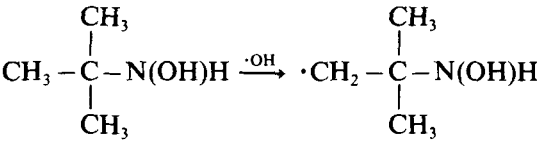
When the iron ions are bound to the chelators, it seems reasonable to assume that the *t*-BuṄ(O)CH(CH_3)OH radicals are formed according to Eqs. 1–5, including the radical-driven Fenton reaction. The very slow increase of the 3 × 2 line system observed with desferrioxamine (Figure 3b) might be connected with the extremely tight binding of iron ions to this chelator[17] leading to a more effective withdrawal of iron ions available for the conventional Fenton reaction than obtained with DETAPAC (Figure 3a). This would mean that the formation of the 3 × 2 line system is exclusively dependent on the radical-driven Fenton reaction in the presence of desferrioxamine.†

In the presence of free iron ions, the formation of the 3 × 2 line system is greatly enhanced, very probably due to ·OH radicals formed in the conventional iron-driven Fenton reaction (Eq. 7), which induced increased formation of $CH_3\dot{C}HOH$ radicals derived from ethanol. It is also possible that the oxidation of the parent hydroxylamine is mediated by the combined action of Fe^{III} ions and H_2O_2, a reaction in which Fe^{II} ions that participate in the iron-driven reaction are formed. The problem of the role of iron is further complicated by the finding that chelator-bound iron ions, even when tight bound in ferrioxamine, might have a catalytic effect in Fenton reactions.[15,16]

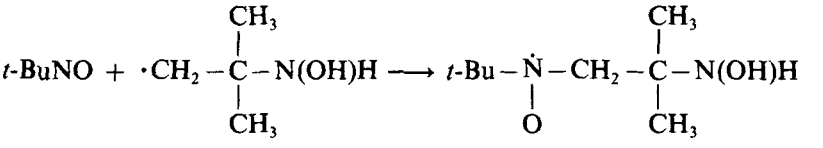
The use of *N-tert*-butylhydroxylamine for trapping short-lived radicals is further illustrated as follows.

1. *The Radicals Derived from N-tert-butylhydroxylamine*

Oxidation of *N-tert*-butylhydroxylamine (**1**), Eq. 1, gave rise to the *t*-BuṄ(O)H radical (**2**) as described above. When the reaction was performed by the use of H_2O_2 as the oxidizing species, and with a somewhat higher concentration of *N-tert*-butylhydroxylamine, and at low pH (~ 3), the reaction mixture was found to contain an additional radical species that gave rise to six lines of equal intensity (Figure 4). This species was formed by trapping a radical derived from *t*-BuN(OH)H according to Eq. 8:



1 **5**



5 **6**

†Some experiments were done by use of reagent solutions prepared with H_2O treated with Chelex 100 for removing iron contaminations.[18] However, these efforts failed, very probably depending on the fact that the main part of the iron contaminations was in sample of *N-tert*-butylhydroxylamine–HCl used (cf. above: iron and copper analysis).

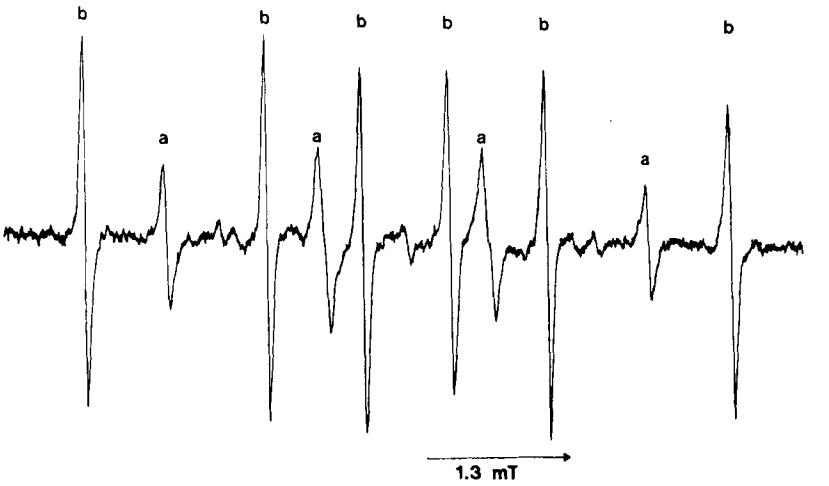


FIGURE 4 EPR spectrum of the radicals observed with *N-tert*-butylhydroxylamine–HCl dissolved in H_2O (pH 3.7) after the addition of a small amount of H_2O_2. *a*. Four-line system: *t*-BuṄ(O)H: $a_N \simeq a_H = 1.39$ mT. *b*. Six-line system: *t*-BuṄ(O)$CH_2C(CH_3)_2N(OH)H$: $a_N = 1.59$ mT; $a_{H1} + a_{H2} = 2.50$ mT.

The EPR spectrum of **6** indicates non-equivalence of the methylene hydrogen atoms due to steric hindrance, an arrangement which was expected to give rise to $3 \times 2 \times 2 = 12$ lines of equal intensity. The interconversion between the two conformations that arises due to the steric hindrance introduced alternating line-widths, with a broadening of the two inner lines of the three methylene quartets, which leads to a spectrum with six sharp and six broad lines at room temperature. The sum of the coupling constants of the methylene protons, i.e., $a_{H1} + a_{H2} = 2.50$ mT for radical **6**. Non-equivalence of the hydrogen atoms of methylene groups of aminooxyl radicals that gives rise to similar line-width alternations has previously been studied in more detail.[11]

2. *Methanol-d_4* (cf. Ref. 6)

Figure 5 shows the EPR spectrum obtained with *N-tert*-butylhydroxylamine dissolved in methanol-d_4 after the addition of a small amount of H_2O_2. The spectrum is dominated by a 3×5 line spectrum (1:2:3:2:1) indicating the trapping of the $\cdot CD_2OD$ radical, and the formation of the aminooxyl radical *t*-BuṄ(O)CD_2OD. Two additional radical species are present in small quantities, i.e., the primary radical *t*-BuṄ(O)H, which gives rise to a resolved six-line spectrum, and a 3×3 line system due to the corresponding primary radical *t*-BuṄ(O)D, formed after exchange of protium for deuterium.

3. *Dimethyl Sulfoxide* (cf. Ref. 7)

The spectrum obtained with *N-tert*-butylhydroxylamine dissolved in DMSO together with a small amount of H_2O_2 is shown in Figure 6. The following radical

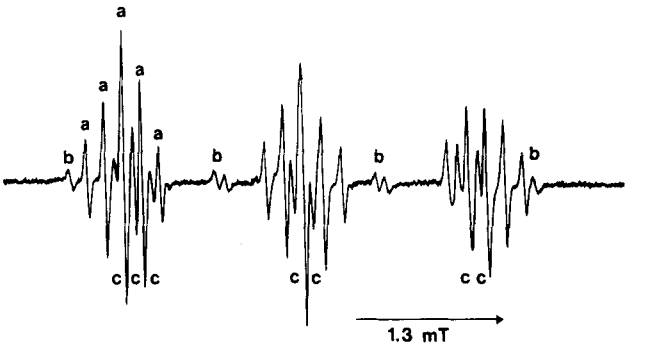


FIGURE 5 The spectrum of the radicals obtained with *N-tert*-butylhydroxylamine–HCl dissolved in methanol-d_4 together with a small amount of H_2O_2. *a*. 3 × 5 line system: *t*-BuṄ(O)CD_2OD; a_N = 1.61 mT; a_D = 0.17 mT. *b*. 3 × 2 line system: *t*-BuṄ(O)H; a_N = 1.38 mT; a_H = 1.30 mT. *c*. 3 × 3 line system: *t*-BuṄ(O)D; a_N = 1.38 mT; $a_D \simeq$ 0.2 mT.

species were present:

1. the primary radical *t*-BuṄ(O)H, which gives rise to a resolved six-line system (*c*);
2. the aminooxyl radical formed by trapping $CH_3 \cdot$ radicals derived from DMSO, giving rise to a 3 × 4 (1 : 3 : 3 : 1) line system (*a*);
3. the aminooxyl radicals formed by trapping $\cdot CH_2S(=O)CH_3$, whose spectrum constitutes a 3 × 3 (1 : 2 : 1) line system with each of the nine lines incompletely split into quartets (1 : 3 : 3 : 1) due to interaction with the hydrogen nuclei of the remaining methyl group of the trapped radical (*b*).

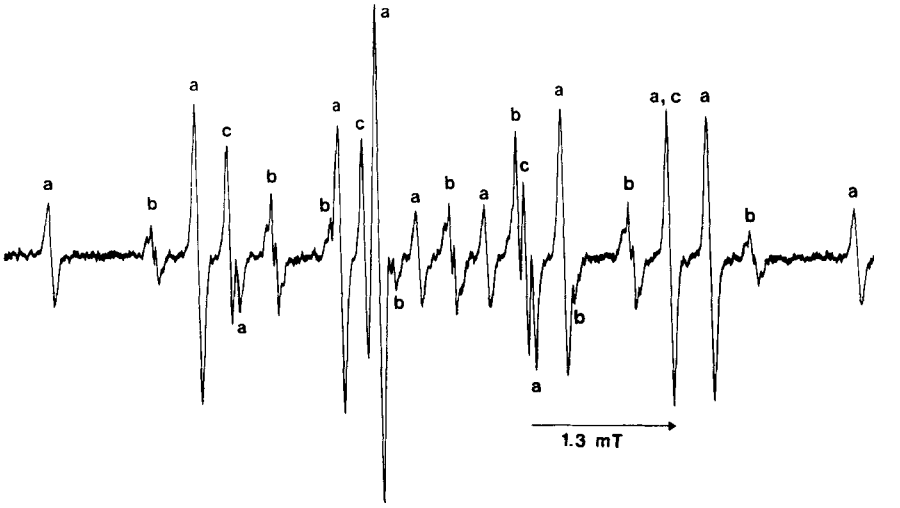


FIGURE 6 The EPR spectrum of the radicals formed with *t*-BuṄ(O)H–HCl dissolved in DMSO together with a small amount of H_2O_2. *a*. 3 × 4 (1 : 3 : 3 : 1) line system: *t*-BuṄ(O)CH_3; a_N = 1.59 mT; a_H = 1.30 mT. *b*. 3 × 3 (1 : 2 : 1) × 4 (1 : 3 : 3 : 1) line system: *t*-BuṄ(O)$CH_2S(=O)CH_3$; a_N = 1.59 mT; a_{H1} = 1.07 mT; $a_{H2} \simeq$ 0.04 mT. *c*. 6 line system: *t*-BuṄ(O)H; a_N = 1.33 mT; a_H = 1.20 mT.

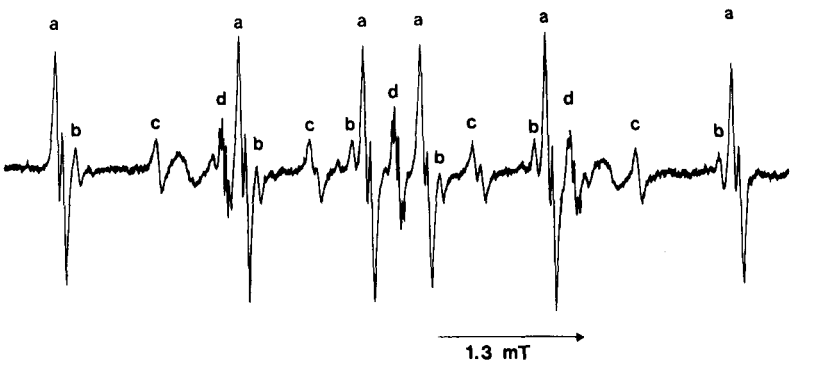


FIGURE 7 The spectrum of the radicals formed in a solution of *t*-Bu(OH)H–HCl and L-alanine in H_2O_2; pH adjusted to about 7; after addition of a small amount of H_2O_2. *a*. 6 × 2 line system: *t*-BuṄ(O)$CH_2CH(NH_3^+)COO^-$; a_N = 1.59 mT. $a_{H1} + a_{H2}$ = 2.70 mT; $a_{H3} \simeq$ 0.05 mT. *b*. 6 line system: *t*-BuṄ(O)*t*-BuṄ(O)$CH_2C(CH_3)_2N(OH)H$; a_N = 1.59 mT $a_{H1} + a_{H2}$ = 2.50 mT. *c*. four-line system: *t*-BuṄ(O)H; $a_N \simeq a_H$ = 1.39 mT. *d*. 3 × 4 line system: *t*-BuṄ(O)$C(CH_3)(NH_3^+)COO^-$; a_N = 1.52 mT. $a_H \simeq$ 0.03 mT.

4. *L-Alanine* (cf. Ref. 9)

Figure 7 shows the EPR spectrum obtained with a solution of *N-tert*-butylhydroxylamine and L-alanine in H_2O adjusted to pH 7 after the addition of a small amount of H_2O_2. The following radical species were present:

a. A six-line system with each of the lines split into a narrow doublet. This species is formed by trapping a radical derived from L-alanine, with the formation of the aminooxyl radical *t*-BuṄ(O)–CH_2–$C(H)(NH_3^+)COO^-$. The hydrogen atoms of the methylene group are non-equivalent due to steric hindrance, which leads to line-width alternations of the same type as described above for radical **6**. The sum of the coupling constants of the methylene hydrogen nuclei, i.e., $a_{H1} + a_{H2}$, is 2.7 mT. The narrow doublet splitting originates from an interaction with the hydrogen atom in the α-position of the L-alanine fragment.

b. The radical species **6** formed by trapping a radical derived from *N-tert*-butylhydroxylamine. This species gives rise to the six-line system described above (**1**).

c. The species *t*-BuṄ(O)H, which gives rise to an incompletely resolved six-line spectrum.

d. A radical species that exhibits a three-line system with each line incompetely split into a number of narrow lines. A tentative structure for this species is: *t*-BuṄ(O)–$C(CH_3)$–$(NH_3^+)COO^-$, with the narrow splittings originating from an interaction with the hydrogen atoms of the methyl group and/or the nuclei of the NH_3^+ group of the alanine fragment.

5. *Isoorotic Acid* (*2,4-dihydroxypyrimidine-5-carboxylic acid*) (cf. Ref. 10)

The addition of a small amount of H_2O_2 to a solution of *N-tert*-butylhydroxylamine and isoorotic acid in 0.1 M NaOH gave rise to a spectrum with 3 × 3 × 2 × 2 = 36 lines of equal intensity. The aminooxyl radical formed is considered to have

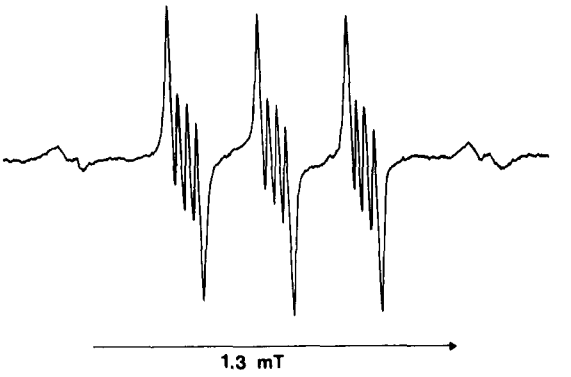
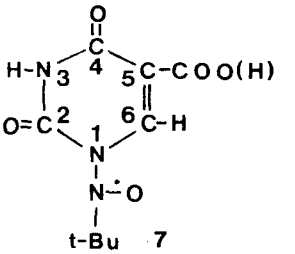


FIGURE 8 The EPR spectrum of the radicals formed in a solution of *t*-BuN(OH)H–HCl and isoorotic acid in 0.1 M NaOH after the addition of H_2O_2. The assumed structure: see **7**. The ^{14}N component $M_1 = 0$ (center group) of the spectrum is shown; $a_{N1} = 1.47\,mT$; $a_{N2} = 0.30\,mT$; $a_{H1} = 0.07\,mT$; $a_{H2} \simeq 0.03\,mT$.

the structure



7

In addition to the large triplet splitting originating from interaction with the ^{14}N nucleus of the nitroxide group of **7** (a_{N1}), there is an interaction with the ^{14}N nucleus of the pyrimidine ring, N1 (a_{N2}). The two non-equivalent doublet splittings are considered to originate from interactions with the hydrogen atom in position **6** on the pyrimidine ring (a_{H1}) and the hydrogen atom attached to nitrogen N3 (a_{H2}).

Figure 8 shows the center group of the three main groups of the EPR spectrum. There is also a small amount of the radical species *t*-BuṄ(O)H, i.e., the two central doublets of the resolved six-line system.

6. Na_2HPO_2 (cf. Ref. 8)

The reaction of *N-tert*-butylhydroxylamine with Na_2HPO_2 dissolved in H_2O gave rise to a six-line spectrum with lines of equal intensity after the addition of a small amount of H_2O_2. The spectrum indicated that the radical $\cdot PO_2^{2-}$ had been trapped with the formation of the aminooxyl radical *t*-BuṄ(O)PO_2^{2-}: $a_N = 1.34\,mT$, $a_P = 1.20\,mT$.

CONCLUSIONS

The aminooxyl radicals formed in the spin trapping experiments using *N-tert*-butylhydroxylamine and hydroperoxides are stable for hours at room temperature, and

give rise to well-resolved spectra with a high signal-to-noise ratio. Generally, the spectra of these radicals are in conformity with the corresponding results obtained by conventional spin trapping performed with 2-methyl-2-nitrosopropane.[2-11]

Spin trapping with the use of *N-tert*-butylhydroxylamine and a hydroperoxide involves the simultaneous formation of both the spin trap 2-methyl-2-nitrosopropane (**3**) and · OH radicals which are able to produce short-lived primary radicals by homolytic abstraction of hydrogen atoms from suitable compounds.

It is obvious that iron ions are involved in the reaction mechanism. Evidently, the spin trapping with the formation of aminooxyl radicals also takes place when the irons ions are inactivated by binding to the chelators DETAPAC or desferrioxamine. Therefore, it is suggested that the mechanism involves both a radical-driven and an iron-driven Fenton reaction.

Irrespective of the exact mechanism, the concentration of iron impurities in the chemicals and solvent water is sufficiently high to mediate fast reactions leading to the observed aminooxyl radicals. A further increase in the concentration of iron ions will certainly increase the yield of aminooxyl radicals, but will impair the resolution of the EPR spectra by the presence of too high a concentration of paramagnetic ions.

t-BuNO is present as a dimer in the solid state, and has to be converted into the monomer by warming when dissolved in H_2O before use in conventional spin trapping experiments. No such problems seem to prevail when *t*-BuNO is generated from *N-tert*-butylhydroxylamine. Disturbing overlaps are sometimes obtained from the four- or six-line spectra of the radical *t*-BuN(O)H. However, these overlaps decrease with the time of observation, or can be minimized by decreasing the amount of *N-tert*-butylhydroxylamine used.

N-tert-Butylhydroxylamine might react with carbonyl compounds with the formation of vinyl hydroxylamines or the tautomeric nitrones (*t*-BuN(OH)–CH=CH–R $\rightleftharpoons$ *t*-Bu$N^+(O^-)$=CH–CH_2–R), which are able to participate in secondary radical reactions.[19] However, no such side reactions have hitherto been observed in the reactions described here.

It is possible that the use of *N-tert*-butylhydroxylamine constitutes a general method for the study of short-lived radicals formed by hydrogen abstraction with the spin trapping technique.

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