

# Formation of Aminoxy Radicals in Alkaline Water Solutions from Spin Traps and Short-Lived Radicals Derived from Some CH-Acidic Compounds

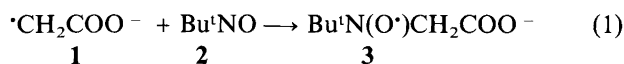
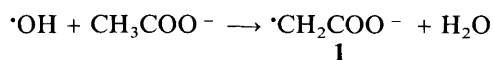
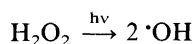
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Lagercrantz, C. and Ebersson, L., 1995. Formation of Aminoxy Radicals in Alkaline Water Solutions from Spin Traps and Short-Lived Radicals Derived from Some CH-Acidic Compounds. – Acta Chem. Scand. 49: 399–404 © Acta Chemica Scandinavica 1995.

Aminoxy radicals Bu<sup>t</sup>N(O<sup>•</sup>)R or Ph-CH(R)N(O<sup>•</sup>)-Bu<sup>t</sup> were formed in alkaline water solution (0.01–1 M NaOH) of the spin traps 2-methyl-2-nitrosopropane (Bu<sup>t</sup>NO) or phenyl-*N-tert*-butylnitron (PBN), and substances (RH) such as dimethyl sulfoxide, acetate, propionate or malonate. The radicals appeared subsequently on keeping the reaction mixtures between 90–100°C for 30–60 s, or by UV irradiation of the samples. The reaction took place without any added hydrogen peroxide. The yield of aminoxy radicals increased with increasingly alkaline pH. The same radicals were obtained by UV irradiation of non-alkaline water solutions of the spin traps and parent substances after the addition of sodium nitrite. The reaction mechanism is suggested to involve the formation of carbanions R<sup>-</sup> derived from the parent substances RH. These anions are oxidized to short-lived radicals R<sup>•</sup> in an ET reaction with the spin traps. Subsequently the radicals R<sup>•</sup> are trapped to form the observed aminoxy radicals. However, the direct formation of R<sup>•</sup> by abstraction of hydrogen atoms could not be completely ruled out.

The abstraction of hydrogen atoms from a large number of substances has been extensively studied by the spin trapping technique and EPR spectroscopy.<sup>1–7</sup> Hydroxyl radicals (<sup>•</sup>OH) have been used as the abstractor species in many of these experiments. The <sup>•</sup>OH radicals were generally produced by cleavage of hydrogen peroxide or hydroperoxides by UV irradiation or in Fenton reactions. The short-lived secondary radicals produced by hydrogen abstraction were trapped by nitron or nitroso spin traps with the formation of stable aminoxy radicals detectable by EPR spectroscopy. The result obtained with acetate and the spin trap 2-methyl-2-nitrosobutane (Bu<sup>t</sup>NO) **2** is given as an example:



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The stable aminoxy radical **3** exhibited a 3 × 3 (1:2:1) line EPR spectrum due to the interaction of the unpaired electron with one <sup>14</sup>N and two equivalent hydrogen nuclei.

It has now been observed that the aminoxy radical **3** is also obtained in reaction mixtures containing only the spin trap **2** and acetate without any added hydrogen peroxide when dissolved in an alkaline water solution. In this case the radicals **3** appeared subsequently on keeping the reaction mixture between 90 and 100°C for 30–60 s, or by UV irradiation of the samples *in situ* in the EPR cavity. Analogous aminoxy radicals were obtained with parent substances such as dimethyl sulfoxide, propionate and malonate. The corresponding aminoxy radicals were obtained with the nitron trap phenyl-*N-tert*-butylnitron (PBN). However, it remains to elucidate the actual reaction mechanism leading to the observed aminoxy radicals.

## Experimental

The EPR spectra were recorded using a Varian E-9 EPR spectrometer at 20°C with a microwave power of 1–5 mW and a 100 kHz modulation amplitude of 0.01–0.05 mT. The samples were contained in a flat aqueous

solution cell. Hyperfine splitting constants were measured by comparison with the splittings of Fremy's radical ( $a_N = 1.3$  mT). The samples were kept in small test tubes placed in a heated water bath, and were subsequently filled into the EPR solution cell. Quantitative estimations of the radical yield were made by measuring the relative peak-to-peak amplitudes of the actual spectra (Fig. 1). Degassing of the reaction mixtures was performed by bubbling with  $N_2$ . Irradiation of the samples with UV was performed *in situ* in the EPR cavity by use of a mercury lamp (Osram HBO-200).

The chemicals used were from Aldrich Chemical Co. or Sigma, and were used as supplied.

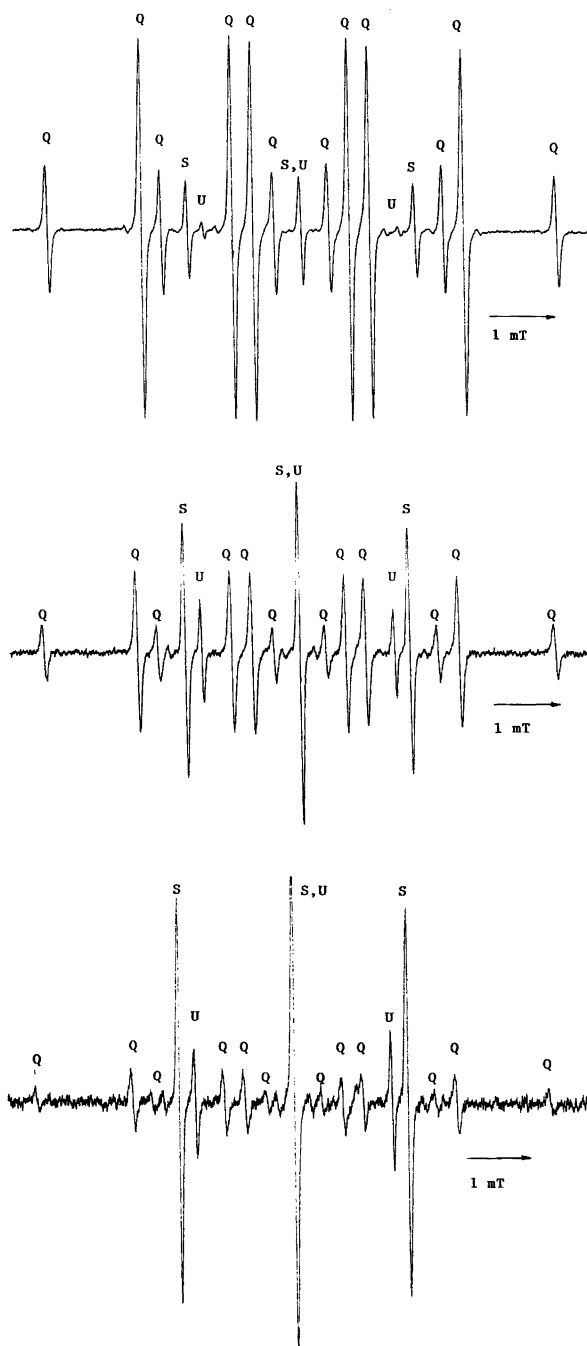
## Results

Table 1 gives the coupling constants of the aminoxyl radicals  $Bu^1N(O^{\cdot})R$  obtained with the parent substances dimethyl sulfoxide (DMSO), acetate, propionate and malonate when  $Bu^1NO$  was the spin trap together with the trapped radical  $R^{\cdot}$ . Most of the recordings were made in 0.2 M NaOH. The coupling constants are in conformity with those observed in reaction mixtures in non-alkaline solutions, in which hydrogen abstraction had been obtained by hydroxyl radicals derived from added hydrogen peroxide.<sup>1-6</sup> The aminoxyl radicals of this series appeared subsequently on keeping the alkaline reaction mixtures between 70 and 100°C for about 30–60 s, or by UV irradiation *in situ* in the EPR cavity. No radicals were observed in neutral solutions except the symmetrical aminoxyl radical  $(Bu^1)_2NO^{\cdot}$  formed by trapping  $Bu^1$  radicals derived from the spin trap. No radicals were observed

**Table 1.** Aminoxyl radicals  $Bu^1N(O^{\cdot})R$  formed in the reaction mixtures of 2-methyl-2-nitrosopropane ( $Bu^1NO$ ) and a number of parent substances dissolved in alkaline water solutions (0.2–0.5 M NaOH) subsequent to heating at 90°C for about 30 s. Earlier values of the coupling constants of the actual aminoxyl radicals are given in parenthesis.

Parent substance	Trapped radical	Coupling constants/mT			Ref.
		$a_N$	$a_{H1}$	$a_{H2}$	
Dimethyl sulfoxide	$\cdot CH_3$	1.69	1.41		7
		(1N) (1.59)	(3H) (1.35)		
Acetate	$\cdot CH_2COO^-$	1.56	0.862		6
		(1N) (1.60)	(2H) (0.85)		
Propionate	$CH_3\dot{C}HCOO^-$	1.61	0.47	0.03	6
		(1N) (1.61)	(1H) (0.53)	(3H) (0.042)	
Malonate	$\cdot CH_2COO^-$	1.69	0.73		6
		(1N) (1.55)	(2H) (0.436)		
Malonate	$\cdot CH(COO^-)_2$	1.51	0.40		6
		(1N) (1.55)	(1H) (0.436)		

prior to heating at 90°C or irradiation of the reaction mixtures. Figure 1 shows the EPR spectra of the aminoxyl radicals obtained with DMSO. The 3 × 4 (1:3:3:1)



**Fig. 1.** The EPR spectra of the aminoxyl radicals formed in the reaction between  $Bu^1NO$  (5 mg) and dimethyl sulfoxide (DMSO) (100  $\mu$ l) dissolved in 400  $\mu$ l of (A) 1 M; (B) 0.1 M, (C) 0.01 M NaOH subsequent to keeping the samples at 90°C for about 30 s. The following species are present:  $Bu^1N(O^{\cdot})CH_3$  formed by trapping of  $\cdot CH_3$  radicals derived from DMSO; 3 × 4 (1:3:3:1) line spectrum, **Q**; the symmetric aminoxyl radicals  $(Bu^1)_2NO^{\cdot}$  formed by trapping of  $Bu^1$  radicals derived from the spin trap; 3 × 1 line spectrum, **S**; unidentified species which gives rise to a 3 × 1 line spectrum, **U**.

line system (Q) represents the aminoxy radical  $\text{Bu}^t\text{N}(\text{O}')\text{CH}_3$  formed by trapping of  $\cdot\text{CH}_3$  radicals derived from DMSO.<sup>7</sup> These radicals were observed with an NaOH concentration between 1 and 0.01 M (Figs. 1A–1C). The yield of  $\text{Bu}^t\text{N}(\text{O}')\text{CH}_3$  radicals increases with increasing  $\text{OH}^-$  concentration (Fig. 1). In addition to the radical species  $\text{Bu}^t\text{N}(\text{O}')\text{CH}_3$  and  $(\text{Bu}^t)_2\text{NO}'$ , ( $3 \times 1$  line system,  $a_N = 1.68$  mT, S in Fig. 1), a further species is present (U) in Fig. 1 that gave rise to a  $3 \times 1$  line system with  $a_N = 1.43$  mT. This species is more prominent at low alkaline concentration (Fig. 1C).

Figure 2 shows the EPR spectrum obtained subsequently to heating at  $90^\circ\text{C}$  of a solution of  $\text{Bu}^t\text{NO}$  in 0.2 M NaOH without added parent substances. Two radical species are present:  $(\text{Bu}^t)_2\text{NO}'$  (S) and the species denoted U (cf. Fig. 1). No radicals were observed with ethanol or acetone as parent substances.

Similar results were obtained by use of the spin trap phenyl-*N-tert*-butylnitron  $\text{Ph}-\text{CH}=\text{N}(\text{O}^-)-\text{Bu}^t$  (PBN) with DMSO, acetate and propionate when dissolved in 0.1 M NaOH followed by incubation of the reaction mixtures at about  $90^\circ\text{C}$  for 1/2 min, or by UV irradiation. However, the yield of aminoxy radicals was rather low for acetate. Figure 3 shows the EPR spectrum obtained with DMSO. The coupling constants are given in Table 2. They indicate that the radicals  $\cdot\text{CH}_3$ ,  $\cdot\text{CH}_2\text{COO}^-$  or  $\text{CH}_3\dot{\text{C}}\text{HCOO}^-$  had been trapped. No radicals were detected in reaction mixtures containing only PBN in 0.1 M NaOH. It was also found that solutions of alkaline concentration higher than about 0.1 M NaOH exhibited no radicals, probably owing to decomposition of the nitron trap.

Removal of dissolved oxygen by bubbling the samples with  $\text{N}_2$  prior to incubation at elevated temperature had almost no influence on the yield of aminoxy radicals. Bubbling the samples with  $\text{NO}'$  did not increase the yield of aminoxy radicals.

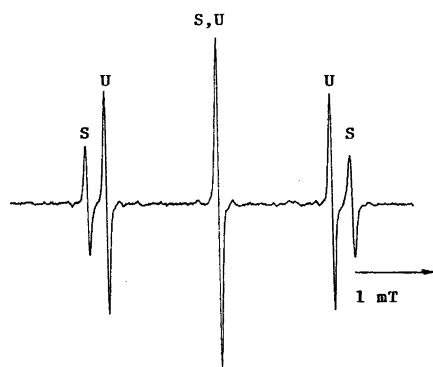


Fig. 2. The EPR spectrum of the aminoxy radicals formed in the reaction of  $\text{Bu}^t\text{NO}$  dissolved in 0.2 M NaOH. No parent substances. The radical species S and U are present. The sample was kept at  $90^\circ\text{C}$  for about 30 s.

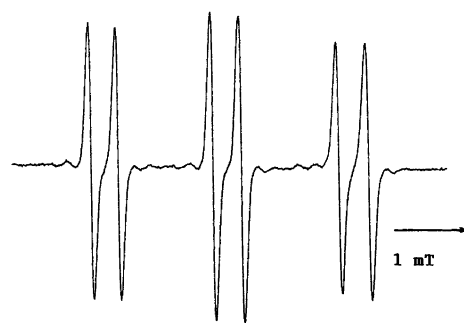


Fig. 3. The spectrum of the aminoxy radicals formed in the reaction between the nitron spin trap PBN and DMSO dissolved in 0.1 M NaOH. The sample was incubated as described for Fig. 1 and 2.

Table 2. Aminoxy radicals  $\phi-\text{CH}(\text{R})\text{N}(\text{O}')-\text{Bu}^t$  formed in the reaction mixtures of  $\phi-\text{CH}=\text{N}(\text{O}^-)-\text{Bu}^t$  and a number of parent substances dissolved in alkaline water solutions (0.1 M NaOH) subsequently to heating at  $90^\circ\text{C}$  for 30 s. Earlier values of the coupling constants of one of the actual aminoxy radicals are given in parenthesis.

Parent substance	Trapped radical	Coupling constants/mT		Ref.
		$a_N$	$a_H$	
Dimethyl sulfoxide	$\cdot\text{CH}_3$	1.56 (1N) (1.52)	0.351 (1H) (0.347)	15
Acetate	$\cdot\text{CH}_2\text{COO}^-$	1.56 (1N)	0.403 (1H)	
Propionate	$\text{CH}_3\dot{\text{C}}\text{HCOO}^-$	1.56 (1N)	0.403 (1H)	

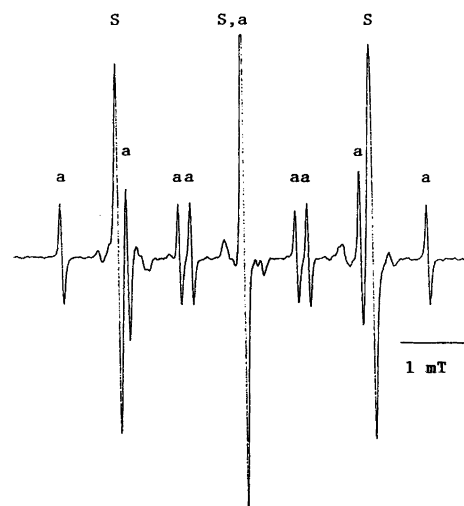
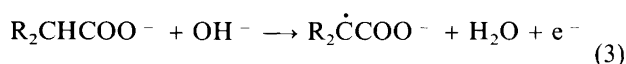


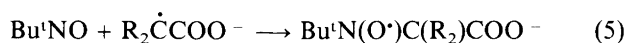
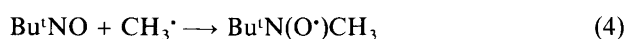
Fig. 4. EPR spectrum of the aminoxy radicals formed by UV irradiation *in situ* in the EPR cavity of a water solution of  $\text{Bu}^t\text{NO}$ , acetone and sodium nitrite. The radical  $\text{Bu}^t\text{N}(\text{O}')\text{CH}_2\text{COCH}_3$  is formed by trapping of the radical  $\cdot\text{CH}_2\text{COCH}_3$ .

### Reaction mechanism

The general principle of the reactions studied here involves a thermal or UV-mediated oxidation of DMSO or the compounds  $R_2CHCOO^-$  ( $R = H$ , alkyl) in alkaline solutions, which leads to dissociation of carbon-sulfur or carbon-hydrogen bonds with formation of short-lived radicals. Subsequently, the latter are trapped by  $Bu^1NO$  or PBN to give the observed aminoxyl radicals, i.e. reactions (2)–(5):



and with  $Bu^1NO$



In determining the details of these reactions, it is at first assumed that an abstraction of hydrogen atoms from the parent substances is involved, in analogy with the reactions of eqn. (1). Under such conditions, the problem is to identify the abstractor species.

The formation of the aminoxyl radical  $(Bu^1)_2NO\cdot$  (S) in Figs. 1 and 2 indicated that some proportion of the spin trap  $Bu^1NO$  is split when the samples are kept at elevated temperature, or irradiated with UV:

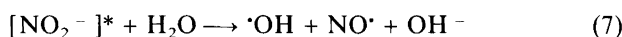


Nitric oxide  $NO\cdot$  can be excluded as an abstractor, since  $NO\cdot$  is a rather unreactive species. The  $Bu^{1*}$  radical might be the abstractor. In such a case, it is expected that abstraction would be observed in non-alkaline or neutral solutions too. However, no aminoxyl radicals derived from the parent substances were observed under such conditions. Therefore, it seems rather unlikely that  $Bu^{1*}$  radicals are the abstractor species.

In view of these considerations, and the finding that the yield of aminoxyl radicals increased with increasing alkaline pH (Fig. 1), the abstracting species might be  $\cdot OH$  radicals formed in some way from  $OH^-$  ions of the reaction mixtures by a one-electron oxidation. For this purpose, the reaction system has to include an oxidant able to oxidize  $OH^-$  ions to  $\cdot OH$  radicals, a reaction with a standard potential  $E^\circ(\cdot OH/OH^-)$  of about +1.9 V ( $H_2O$ ).<sup>8</sup> The  $Bu^{1*}$  radicals could be excluded as the oxidant, since the standard potential  $E^\circ(Bu^{1*}/Bu^{1-})$  is about -2 V (tetrahydrofuran).<sup>8</sup> Consequently,  $Bu^{1*}$  is a reducing agent and not the requested oxidant. However, an agent able to oxidize  $OH^-$  to  $\cdot OH$  radicals could not be identified for the present reaction system. Nitric oxide  $NO\cdot$  formed from  $Bu^1NO$  or PBN,  $NO_2\cdot$  produced by its

oxidation with dissolved  $O_2$ , or peroxyxynitrite anions  $ONOO^-$  possibly formed from  $NO\cdot$  and superoxide  $O_2^{\cdot -}$  are presumably not sufficiently strong as ET oxidants [ $E^\circ(NO_2\cdot/NO_2^-) = 1.0$  V;  $E^\circ(ONOO^-/NO_2^-, H_2O) = 1.4$  V].<sup>9</sup>

It has recently been suggested that UV excitation of nitrite anion  $NO_2^-$  gives rise to  $\cdot OH$  radicals in its reaction with  $H_2O$ ,<sup>10</sup> i.e.



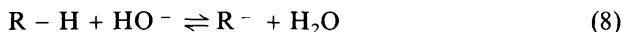
In view of such a reaction, hydroxyl radicals might also be formed in the present systems via  $NO\cdot/NO_2^-$ /nitrite derived from the spin traps [reaction (6)]. Therefore, some experiments were made by UV irradiation of mixtures of  $Bu^1NO\cdot$ , sodium nitrite and the parent substances of this series. The results indicated that aminoxyl radicals were formed, the EPR spectra of which were almost identical to those observed with the alkaline method. The coupling constants, collected in Table 3, were close to those of Table 1. Some radical species were observed with nitrite/UV in these experiments not formed with the alkaline method, e.g.  $Bu^1N(O\cdot)-CH_2CH_2COO^-$ . However, the essential finding was the fact that the radicals of the nitrite/UV method were obtained at neutral pH but not in alkaline media. Consequently, any formation of  $\cdot OH$  radicals from  $NO\cdot/NO_2^-$ /nitrite derived from the spin traps seems to be very unlikely in the alkaline method.

Therefore, other reaction mechanisms than those so far discussed must be considered. The strong dependency of the radical yield upon base concentration (Fig. 1) implies that carbanions of the parent compounds ( $R-H$ ) may be involved, as shown in reaction (8). Subsequently, radicals  $R\cdot$  are assumed to be formed by one-electron oxidation of

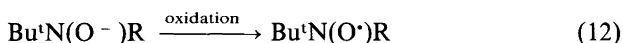
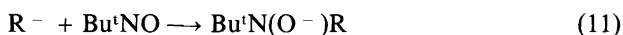
Table 3. Aminoxyl radicals  $Bu^1N(O\cdot)R$  formed in the photochemical reaction between 2-methyl-2-nitrosopropane and a number of parent substances when dissolved in non-alkaline water solutions in the presence of sodium nitrite.

Parent substance	Trapped radical	Coupling constants/mT		
		$a_N$	$a_{H1}$	$a_{H2}$
Dimethyl sulfoxide	$\cdot CH_3$	1.69 (1N)	1.40 (3H)	
Dimethyl sulfoxide	$\cdot CH_2SOCH_3$	1.63 (1N)	1.06 (2H)	
Acetate	$\cdot CH_2COO^-$	1.57 (1N)	0.832 (2H)	
Propionate	$CH_3\dot{C}HCOO^-$	1.56 (1N)	0.481 (1H)	0.03 (3H)
Propionate	$\cdot CH_2CH_2COO^-$	1.64 (1N)	1.22 (2H)	0.07 (2H)
Acetone	$\cdot CH_2COCH_3$	1.50 (1N)	0.83 (2H)	

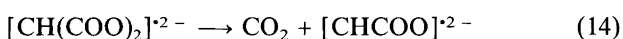
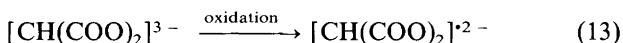
$R^-$  by the spin traps, followed by their trapping to give the observed aminoxyl radicals [reactions (9) and (10)]:



Alternatively, the carbanion will add to the spin trap to give a hydroxylamine anion which is known (cf. Ref. 3) to be oxidized easily to the aminoxyl radical, e.g. as exemplified in reactions (11) and (12):



The intermediacy of carbanions is supported by results obtained with malonate. Fig. 5 shows the EPR spectrum obtained from a solution of malonic acid (as the disodium salt) and  $Bu^tNO$  in 0.5 M aqueous NaOH, preheated to 90°C for about 30 s. In addition to  $(Bu^t)_2NO^\cdot$  (labelled S in Fig. 5) two radical species are present, namely  $Bu^tN(O^\cdot)CH_2COO^-$  with a  $3 \times 3$  (1:2:1) line system [(a) in Fig. 5] and  $Bu^tN(O^\cdot)CH(COO^-)_2$  with a  $3 \times 2$  line system [(d) in Fig. 5]. The formation of  $Bu^tN(O^\cdot)CH_2COO^-$  is best explained according to the carbanion hypothesis. The one-electron oxidation of the malonate trianion would give the malonate radical dianion, which is either trapped directly or undergoes competing decarboxylation [reactions (13) and (14)]:



While carbanion formation from malonate ( $pK_3 \approx 13$ ) certainly takes place in 0.5 M aqueous NaOH, it is more questionable to assume the formation of carbanions from

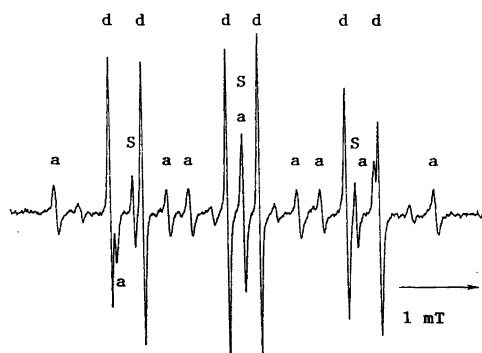


Fig. 5. EPR spectrum of the aminoxyl radicals formed with  $Bu^tNO$  and malonate dissolved in 0.5 M NaOH after heating at 90°C for about 30 s. The following species are present:  $Bu^tN(O^\cdot)CH_2COO^-$  (a) and  $Bu^tN(O^\cdot)CH(COO^-)_2$  (d). Coupling constants are given in Table 1.

acetate and propionate ion. For acetate, a  $pK_2$  of ca. 24 has been given,<sup>11</sup> meaning that  $[CH_2COO]^{2-}$  would be of the order of micromolar. A species at this concentration level would require very fast further reactions for efficient trapping to occur. The problem becomes even more pronounced with DMSO ( $pK \approx 31$ ). Acetone ( $pK \approx 20$ ) does not give any aminoxyl radicals by the alkali method, in spite of the fact that  $Bu^tN(O^\cdot)CH_2COCH_3$  is readily formed by the conventional photolytic  $H_2O_2$  method or the nitrite/UV method (Table 3). However, the latter two methods are run under neutral conditions, whereas the alkali method would be expected to consume acetone rapidly in well known base-catalyzed condensation reactions and thus withdraw the enolate ion from further oxidation [reaction (9)] or addition of the spin trap [reaction (11)]. Moreover, the rather positive oxidation potential of the acetone enolate ion ( $-0.16$  V)<sup>12</sup> militates against the possibility that either of the spin traps can act as an efficient one-electron oxidant ( $Bu^tNO - 1.8$  V; PBN  $-2.4$  V).<sup>13</sup>

In conclusion, this study provides evidence that the aminoxyl radicals formed in alkaline water solutions of nitroso or nitron spin traps and the parent substances of this series are probably formed via their carbanions followed by an ET reaction with the spin traps, even if an abstraction of hydrogen atoms from the parent compounds cannot be completely ruled out. This means that aminoxyl radicals can be produced in the reactions with nitroso or nitron traps by: (a) trapping of short-lived radicals formed by abstraction of hydrogen atoms from the parent compounds;<sup>1-7</sup> (b) inverted spin trapping: addition of a nucleophile, neutral or ionic, to the radical cation form of the spin trap,<sup>14</sup> and (c) ET reaction between the carbanion form of the parent compound and the spin traps.

*Acknowledgement.* This work was supported by grants from *Adlerbertska Forskningsfonden* and the Royal Society of Arts and Sciences in Gothenburg (to C.L.) and the Swedish Natural Science Research Council (to L.E.).

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Received September 26, 1994.