Acta Chemica Scandinavica, 1994: 48: 670–674
Printed in Belgium – all rights reserved

Copyright © Acta Chemica Scandinavica 1994
ACTA
CHEMICA SCANDINAVICA
ISSN 0904-213X

An EPR Study of the Alkylhydrazyl Radicals Formed at Room Temperature in the Photochemical Reaction of 2,2′-Azobis(2,4-dimethylvaleronitrile) or 2,2′-Azobis(propane-2-carboxamidine) Dihydrochloride in the Presence of Sensitizers

Carl Lagercrantz[a] and Johanna Deinum[b]

[a] Department of Medical Physics, University of Göteborg, Medicinaregatan, 11, S-413 90 Göteborg, Sweden and [b] Astra-Hässle AB, S-431 83 Mölndal, Sweden

Lagercrantz, C. and Deinum, J., 1994. An EPR Study of the Alkylhydrazyl Radicals Formed at Room Temperature in the Photochemical Reaction of 2,2′-Azobis(2,4-dimethylvaleronitrile) or 2,2′-Azobis(propane-2-carboxamidine) Dihydrochloride in the Presence of Sensitizers. – Acta Chem. Scand. 48: 670–674 © Acta Chemica Scandinavica 1994.

Alkylhydrazyl radicals R–NH–$\dot{\text{N}}$–R are formed in the photochemical reaction of 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) and 2,2′-azobis(propane-2-carboxamidine) dihydrochloride (APCH) in methanol in the presence of sensitizers such as *p*-hydroquinone. The radicals from AMVN exhibited a nine-line EPR spectrum due to interaction with two non-equivalent ^{14}N nuclei ($a_{\mathrm{N1}} = 1.22$ mT; $a_{\mathrm{N2}} = 0.854$ mT; $g = 2.0038$). The radicals from APCH exhibited an 18-line spectrum by interaction with two non-equivalent ^{14}N nuclei and one hydrogen nucleus ($a_{\mathrm{N1}} = 1.20$ mT; $a_{\mathrm{N2}} = 0.83$ mT; $a_{\mathrm{H}} = 0.21$ mT). The addition of HCl changed the spectra to five-line patterns, indicating an interaction with two equivalent ^{14}N nuclei ($a_{\mathrm{N}} = 1.04$ mT). At the same time, the doublet splitting of the spectrum of APCH disappeared. The reactions involve an equilibrium between two identical states **I** and **II**.

$$\underset{\textbf{I}}{\text{R–N}^1\text{H–}\dot{\text{N}}^2\text{–R}} \rightleftharpoons \underset{\textbf{II}}{\text{R–}\dot{\text{N}}^1\text{–N}^2\text{H–R}}$$

As long as the interconversion is slow, the spectrometer shows two non-equivalent ^{14}N nuclei. When the exchange rate is increased (acid), the two ^{14}N nuclei are registered as equivalent. The sensitization is believed to involve a one-electron transfer to the azo compounds from an excited state of the sensitizer, followed by the addition of a proton to the azo anion radical.

The thermal or UV light induced cleavage of azo compounds R–N=N–R (R = alkyl), i.e., substances such as azoisobutyronitrile, into alkyl radicals and N_2, eqn. (1),

$$\text{R–N=N–R} \xrightarrow[\text{or UV}]{\Delta} 2\,\text{R}\cdot + \text{N}_2 \qquad (1)$$

is a general method for initiating radical reactions.[1] The radicals R· which are very reactive and not observable directly by the EPR technique, start further radical reactions by the abstraction of hydrogen atoms from substrate molecules.

In connection with some experiments performed on lipid peroxidation initiated by the azo compounds 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) or 2,2′-azobis(propane-2-carboxamidine) dihydrochloride (APCH), and a study of the antioxidative properties of a number of indole and carbazole derivatives,[2] it was found that radicals are formed which are derived from the azo compounds. The EPR spectra of these radicals which appeared when the reactions were initiated by UV irradiation at room temperature, indicated an interaction of the unpaired electron with two ^{14}N nuclei. It is suggested that the radicals are alkylhydrazyl radicals with the general structure R–NH–$\dot{\text{N}}$–R (R = alkyl). The hydrazyl radicals derived from the azo compounds were formed only in the presence of the carbazole/indole derivatives. No radicals were observed in the dark. Most of the experiments were carried out with 8-methoxy-6-methyl-4b,5,9b,10-tetrahydroindeno[1,2-*b*]indole (H 290/51). However, further experiments showed that a number of other substances were effective as photo-sensitizers, especially *p*-hydroquinone (see Table 1).

It should be pointed out that a closer study of these hydrazyl radicals is of interest from the point of general radical chemistry, and also for studies of lipid peroxidation initiated by UV irradiation of azo compounds.

Experimental

The EPR spectra were recorded using a Varian E-9 EPR spectrometer as previously described.[3] The samples were irradiated with UV light (no filters) from a mercury lamp (Osram HBO-200) *in situ* in the EPR cavity.

The *g*-value of the radicals was evaluated by comparison with the EPR spectrum of *p*-semiquinone ($g = 2.00468$)[4] when *p*-hydroquinone was used as the sensitizer. In samples containing a very small amount of alkali, the *p*-semiquinone spectrum was present in the dark. On irradiation with UV light, this spectrum disappeared and was replaced by the nine- or 18-line spectra.

The linewidths Γ were evaluated by measuring peak-to-peak widths of the recorded derivative curves, i.e., ΔH_{pp}, and the relation $\Gamma = \Delta H_{pp}\sqrt{3}/2$.

The chemicals used were obtained from Aldrich, Fluka AG, or Polyscience Inc. H 290/51 was synthesized at Astra-Hässle AB, Mölndal, Sweden. Removal of O_2 was achieved by bubbling the reaction mixtures with N_2.

Results and discussion

Fig. 1(a) shows the EPR spectrum obtained by irradiation with UV light *in situ* in the EPR cavity, of a solution of AMVN in methanol (0.1 M) together with *p*-hydroquinone (0.1 M). The spectrum consists of nine lines of equal intensity, which indicates an interaction of the unpaired electron with two non-equivalent ^{14}N nuclei. The coupling constants are: $a_{N1} = 1.22$ mT; $a_{N2} = 0.854$ mT; $g = 2.0036$. The radicals appeared after 10–20 s of irradiation, and disappeared when the light was shut off. Identical nine-line spectra were observed in the presence of substances such as carbazole and a number of indole derivatives and anthraquinone ('sensitizers'). The radicals were observed with ethanol, acetone or dimethyl sulfoxide, in addition to methanol, as solvents. Table 1 lists the sensitizers which gave rise to radicals.

Fig. 1(b) shows the EPR spectrum obtained with AMVN (0.1 M) and *p*-hydroquinone (0.1 M) in methanol containing 0.12 M of HCl. The spectrum still consists of nine lines. However, the three spectral components with the magnetic quantum numbers $M_I = +1, +1;\ 0;\ -1, -1$ are sharp, whereas the remaining six components are broadened.

Fig. 1(c) shows the spectrum observed with a 0.4 M concentration of HCl. The spectrum exhibits five lines due to the interaction with two equivalent ^{14}N nuclei. However, the spectral components $M_I = \pm 1$ are broadened, whereas $M_I = \pm 2;\ 0$ are sharp.

Fig. 2(a) shows the EPR spectrum obtained with APCH dissolved in methanol (0.1 M), together with H290/51 (0.05 M) when irradiated *in situ* in the EPR cavity. The spectrum consists of 18 lines of equal intensity, which indicates an interaction with two non-equivalent ^{14}N nuclei and one hydrogen nucleus ($3 \times 3 \times 2$). The coupling constants are: $a_{N1} = 1.20$ mT; $a_{N2} = 0.83$ mT; $a_H = 0.21$ mT; $g = 2.0038$. Identical spectra were obtained with sensitizer substances such as *p*-hydroquinone. When the reaction was performed in 10:1 ethanol-d_1–D_2O, a nine-line spectrum was observed. Evidently, hydrogen had been exchanged for deuterium.

Table 1. Radicals observed in the photochemical reaction of the azo compounds AMVN and APCH at room temperature in the presence of sensitizers.

Azo compound	Sensitizer	No. of lines in the EPR spectrum	Solvent
AMVN	Carbazole	9	Methanol
	2-Hydroxycarbazole	9	Methanol
	3-Methylindole	9	Methanol
	Indol-3-ylacetic acid	9	Methanol
	Phenazine	9	Methanol
	H 290/51	9	Methanol
	p-Hydroquinone	9	Methanol
	p-Hydroquinone	5	Methanol–HCl
	Anthraquinone	9	Methanol
	2,6-Dimethylpiperidine	9	Methanol
APCH	H 290/51	18	Methanol
	H 290/51	9	Ethanol-d_1–D_2O
	p-Hydroquinone	18	Methanol
	p-Hydroquinone	5	0.1 M HCl
	No sensitizer	No radicals	Methanol
AMVN	No sensitizer	No radicals	Methanol

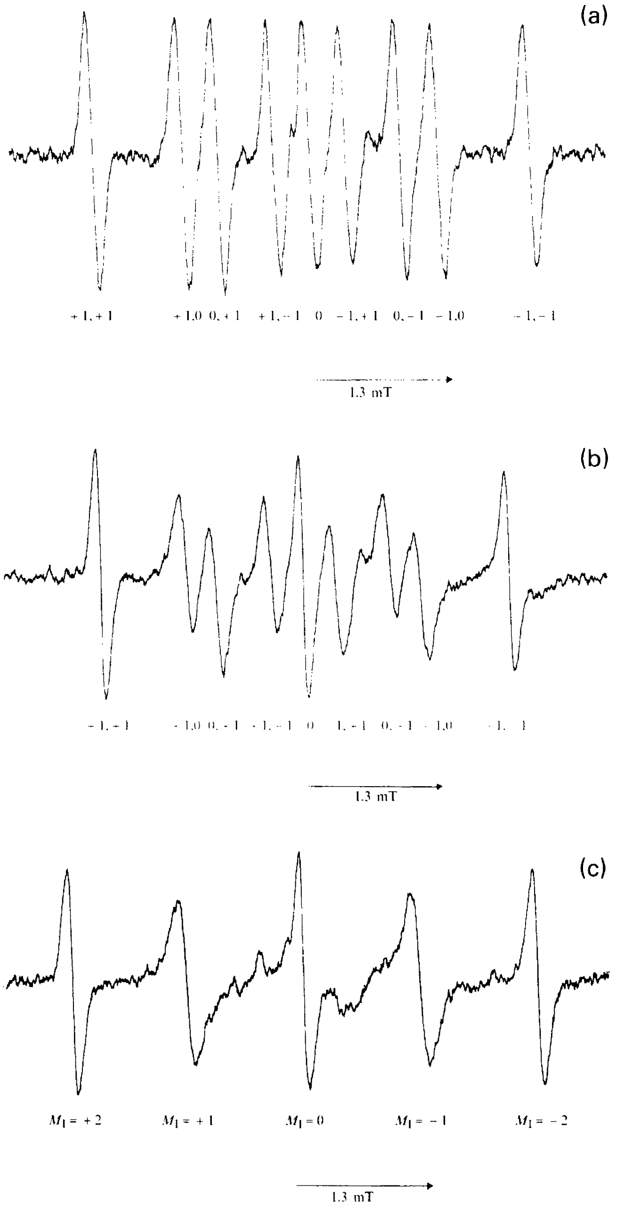


Fig. 1. The EPR spectra obtained by irradiation with UV light *in situ* in the EPR cavity of a solution of 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) 0.1 M in methanol containing 0.1 M *p*-hydroquinone. The magnetic quantum numbers are given in the figures. (a) No HCl. (b) 0.12 M HCl. (c) 0.4 M HCl.

However, the deuterium triplet expected to appear in place of the hydrogen doublet could not be resolved ($a_D = 0.21/6.5 = 0.033$ mT).

Fig. 2(b) shows the EPR spectrum observed with APCH (0.1 M) dissolved in 0.1 M HCl together with *p*-hydroquinone (0.1 M). The spectrum exhibits five lines with the intensity distribution 1:2:3:2:1 due to the interaction with two equivalent ^{14}N nuclei; $a_{N1} = a_{N2} = 1.04$ mT. When the pH was brought back to about 5, the 18-line spectrum reappeared.

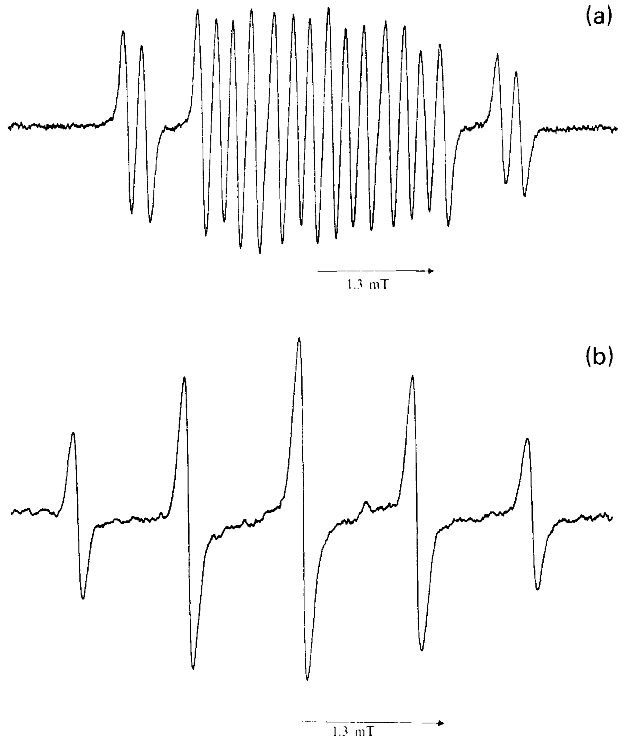
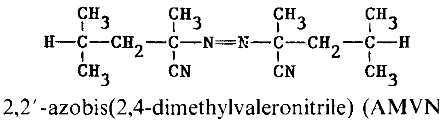


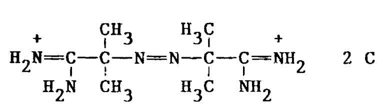
Fig. 2. The spectra obtained with 2,2′-azobis(propane-2-carboxamidine) dihydrochloride (APCH) 0.1 M. (a) In methanol and H 290/51 (0.05 M). (b) In 0.1 M HCl and *p*-hydroquinone 0.1 M.

It is considered that the general structure of the radicals that give rise to the spectra of Figs. 1 and 2 is R-NH-$\dot{N}$-R (**1**), where R is the alkyl part of AMVN and APCH, i.e., H-C(CH_3)$_2$-CH_2-C(CH_3)(CN)- and $H_2\overset{+}{N} = C(NH_2)-C(CH_3)_2-$. The structural formulae of AMVN and APCH are given below. The reaction leading to the observed radicals is suggested to involve a one-electron reduction of the azo compounds, followed by protonation of the radical anion [eqn. (2)].



$$H-\overset{CH_3}{\underset{CH_3}{C}}-CH_2-\overset{CH_3}{\underset{CN}{C}}-N{=}N-\overset{CH_3}{\underset{CN}{C}}-CH_2-\overset{CH_3}{\underset{CH_3}{C}}-H$$

2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN)



$$\overset{+}{H_2N}{=}\underset{H_2N}{C}-\overset{CH_3}{\underset{CH_3}{C}}-N{=}N-\overset{H_3C}{\underset{H_3C}{C}}-\underset{NH_2}{C}{=}\overset{+}{NH_2}\qquad 2\,Cl^-$$

2,2′-azobis(propane-2-carboxamidine) dihydrochloride (APCH)

$$R\text{-}N{=}N\text{-}R \xrightarrow{+e^-} [R\text{-}N = N\text{-}R]\cdot \xrightarrow{+H^+} \underset{\mathbf{1}}{R\text{-}NH\text{-}\dot{N}\text{-}R} \qquad (2)$$

The spectrum of the radical from APCH [Fig. 2(a)] is consistent with this mechanism, i.e., an interaction with two non-equivalent ^{14}N nuclei and one hydrogen nucleus.

In the case of AMVN [Fig. 1(a)], it is believed that the expected doublet splitting is too small to be resolved, possibly due to a steric arrangement that gives rise to an unfavourable interaction with this nucleus. Alternatively, the doublet might be obscured by a very fast exchange of the proton.

Owing to the high symmetry of the molecules involved, it is expected that the protonation of N^1 and N^2 will take place with equal probability, i.e., two identical states **I** and **II** are present [eqn. (3)]. As long as the exchange rate of the proton attached to N^1 or N^2 is slow, the EPR spectrometer will record two non-equivalent ^{14}N nuclei and the doublet splitting from the hydrogen nucleus [Figs. 1(a) and 2(a)]. When the exchange rate increases in more acidic media, the spectrometer recognizes the two ^{14}N nuclei as equivalent [Figs. 1(c) and 2(b)]. At the same time, the hydrogen doublet disappears due to the short time the proton spends attached to each of the nitrogen atoms.[5]

$$\underset{\mathbf{I}}{\mathrm{R{-}N^1H{-}\dot{N}^2{-}R}} \rightleftharpoons \underset{\mathbf{II}}{\mathrm{R{-}\dot{N}^1{-}N^2H{-}R}} \qquad (3)$$

The spectra of Figs. 1(b) and 1(c) represent transitory states between slow and fast exchange. Fig. 1(b) still shows nine lines but exhibits linewidth alternations due to an increasing rate of exchange. The five-line spectrum of Fig. 1(c) also shows linewidth alternations, since the two ^{14}N nuclei are not recognized as wholly equivalent.[6] Fig. 3 gives the ratio between the amplitudes of the spectral components $M_I = +1, -1$ and $M_I = 0$, i.e., $A(+1, -1)/A(0)$ of the nine-line spectra plotted against the concentration of HCl in the samples. The derivative amplitude and the ratio $A(+1, -1)/A(0)$ are a sensitive measure of the *relative* variations of the mean lifetime of the states **I** and **II**, since this amplitude is inversely proportional to the square of the linewidth. Fig. 3 shows that rather small amounts of HCl have a large influence on the ratio $A(+1, -1)/A(0)$. At about 0.6 M HCl, the ratio approaches zero, a concentration at which the nine-line spectra are converted into five-line patterns.

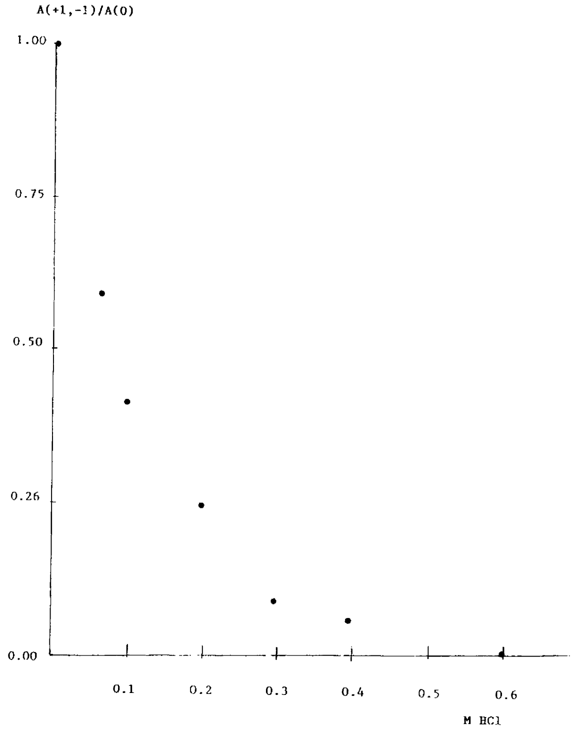


Fig. 3. The ratio between the amplitudes of the spectral components $M_I = +1, -1$ and $M_I = 0$ of nine-line spectra obtained with AMVN plotted against the concentration of HCl in the samples. The reaction mixtures consisted of AMVN (0.2 M) + *p*-hydroquinone (0.2 M) in methanol, 400 μl + 100 μl of varying amounts of HCl in H_2O.

A quantitative estimate of the mean lifetime τ of the radicals in the states **I** and **II** in samples with varying concentrations of HCl was made by measuring the linewidth (in Gauss) of the spectral components with $M_I = +1, -1$ and $M_I = 0$, i.e. $\Gamma(+1, -1)$ and $\Gamma(0)$, using the formula in eqn. (4), valid in the limit of slow conversion.[5]

$$\Gamma(+1, -1) = \Gamma(0) + 1/(2\,\tau\gamma_e) \qquad (4)$$

In this equation γ_e is the magnetogyric ratio of an electron, and equal to 1.76×10^7 rad s^{-1} G^{-1}. The results are shown in Table 2. By use of the field shift of the lines and eqn. (4), the lifetime τ at the coalescence of the line $M_I = +1, -1$ with $M_I = 0$ was evaluated and found to be equal to 0.24×10^{-7} s.[5]

$$(\delta H_o^2 - \delta H_e^2)^{\frac{1}{2}} = \sqrt{2}/(\tau\gamma_e) \qquad (5)$$

In eqn. (5), δH_o is the line separation in the absence of interconversion and δH_e the separation when the conversion is taking place, in this case $\delta H_o = 3.38$ G and $\delta H_e = 0$ G.

The doublet splitting of the OH proton in the radical $\cdot CH_2OH$ is clearly resolved at high pH, but as the pH is lowered, the doublet lines broaden, coalesce, and finally collapse into a single line. The OH proton exchanged rapidly with H^+ ions in solution.[7]

The lifetime at the pH value (1.04) of collapse of the doublet was found to be 0.95×10^{-7} s evaluated from

Table 2. The mean lifetime τ and the second-order rate constant k_2 for the radical derived from AMVN in the presence of different concentrations of HCl. τ was evaluated from eqn. (4), k_2 from eqn. (6).

HCl M	$\tau/10^{-7}$ s	$k_2/10^8$ l mol^{-1} s^{-1}
0	∞	0
0.1	1.25	0.80
0.3	0.34	0.79
0.4	0.32	0.78
0.5	0.24[a]	0.83
	Mean value	0.80

[a] Evaluated from eqn. (5).

eqn. (4). The second-order rate constant k_2 was estimated from the relation in eqn. (6),[7] and found to be $1.75 \times 10^8\ \mathrm{l\ mol^{-1}\ s^{-1}}$.

$$k_2 = 1/(\tau \cdot [\mathrm{H_3O^+}]) \qquad (6)$$

Here $[\mathrm{H_3O^+}]$ is the concentration of hydrogen ions.[7] By use of eqn. (6) the rate constant k_2 of the protonation of the radicals derived from AMVN was evaluated and found to be $0.80 \times 10^8\ \mathrm{l\ mol^{-1}\ s^{-1}}$ (see Table 2). Thus, there is rather good agreement between the constants k_2 found for protonation of $\cdot \mathrm{CH_2OH}$ and the radicals derived from AMVN.

It should be emphasized that the hydrazyl radicals of this series have only been obtained, so far, in the photochemical reaction in the presence of sensitizer substances given in Table 1. No radicals were observed with rather weak reductive reagents sodium dithionite ($\mathrm{Na_2S_2O_4}$) or ascorbate in the dark or with UV irradiation.

An alternative interpretation of the EPR spectra of this series involves the formation of diazenyl radicals of the type $\mathrm{R{-}N{=}N\cdot}$ hitherto observed in the photoirradiation of cyclopropane solutions of 1-aryl-2-(arylthio)diazenes, 1-aryl-3,3-dimethyltriazenes and 1,2,3-benzothiadiazoles at low temperature. However, such radicals are too unstable to be observed above about − 100°C. A characteristic of diazenyl radicals is their very low g-value, about 2.0003. The two nitrogen coupling constants are very different: one 2.5 mT and the other 0.95 mT.[8]

A further alternative might involve the formation of hydrazoyl radicals, $\mathrm{R^1(R^2)N{-}N(O\cdot)R^3}$ ($\mathrm{R^1}$, $\mathrm{R^2}$, $\mathrm{R^3}$, aryl, alkyl).[9] However, the unsymmetrical structure of such radicals, in this case $\mathrm{R^1NH{-}N(O\cdot)R^1}$, is not consistent with the finding of alternating linewidths and equalization of the two $^{14}\mathrm{N}$ nuclei described here.

The reactions leading to the alkylhydrazyl radicals seem to be related to the one-electron reduction of arenediazonium ions (**2**), eqn. (7).

$$\underset{\mathbf{2}}{\mathrm{Ar{-}\overset{+}{N}{\equiv}N}} \xrightarrow{+\,e^-} \underset{\mathbf{3}}{[\mathrm{Ar{-}N{=}N\cdot}]} \longrightarrow \underset{\mathbf{4}}{\mathrm{Ar\cdot}} + \mathrm{N_2} \qquad (7)$$

Radical **3** decomposes to the aryl radical **4** and nitrogen. The intermediate radical **3** has not been observed by the EPR technique, at least not at room temperature (cf. above), whereas the aryl radical **4** has been detected by spin trapping. A large number of reagents are able to partake in the reduction of arenediazonium ions, among others hydroquinones and quinones.[10–12] In these reactions, semiquinone radicals were presumably the species directly responsible for the reduction of the arenediazonium ions.[11,12]

It is possible that similar mechanisms prevail in the formation of the alkyl hydrazyl radicals. The results obtained with p-hydroquinone and anthraquinone as sensitizers are consistent with this suggestion. Semiquinones might be formed from excited states of these sensitizer. The species directly active in the reactions with the indole/carbazole derivatives (see Table 1) are less obvious. It might be radicals formed from these sensitizers, via their excited states, and/or the solvent.

It is considered that the symmetry of the alkylhydrazyl radicals derived from AMVN and APCH, and their protonation will contribute to a higher stability than that of the intermediate radical **3** so that detectable alkylhydrazyl radicals are produced in a steady state before their decay by dediazoniation.

Acknowledgements. The authors are indebted to Professor Lennart Eberson for valuable discussions. This work was supported by grants from *Adlerbertska Forskningsfonden* and The Royal Society of Arts and Sciences in Gothenburg (to C. L.).

References

1. Pryor, W. A. *Free Radicals*. McGraw-Hill, New York 1960, pp. 128–133.
2. Brown, D. W., Graupner, P. R., Sainsbury, M. and Shertzer, H. G. *Tetrahedron 47* (1991) 4383.
3. Lagercrantz, C. *Free Radical Res. Commun. 19* (1993) 387.
4. Wertz, J. E. and Bolton, J. R. *Electron Spin Resonance. Elementary Theory and Practical Applications*, McGraw-Hill, New York 1972, p. 465.
5. Wertz, J. E. and Bolton, J. R. *Electron Spin Resonance. Elementary Theory and Practical Applications*, McGraw-Hill, New York 1972, pp. 192–222.
6. Sullivan, P. D. and Bolton, J. R. In: Waugh, J. S., Ed., *Advances in Magnetic Resonance*, Vol. 4, Academic Press, New York 1970, pp. 39–85.
7. Fischer, H. *Mol. Phys 9* (1965) 149.
8. Suehiro, T., Masuda, S., Tashiro, T., Nakausa, R., Taguchi, M., Koike, A. and Rieker, A. *Bull. Chem. Soc. Jpn. 59* (1986) 1977.
9. Omelka, L., Reinhardt, M., Kluge, E. and Schultz, M. *Collect. Czech. Chem. Commun. 53* (1988) 243.
10. Galli, C. *Chem. Rev. 88* (1988) 765.
11. Reszka, K. J. and Chignell, C. F. *J. Am. Chem. Soc. 115* (1993) 7752.
12. Jirkovsky, J., Fojtik, A. and Becker, H. G. O. *Collect. Czech. Chem. Commun. 46* (1981) 1560.

Received February 7, 1994.