EPR Study of Radical Intermediates from the Oxidation of 6-Ethoxy-2,2,4-trimethyl- and 6-Ethoxy-2,2,4,8-tetramethyl-1,2-dihydroquinoline

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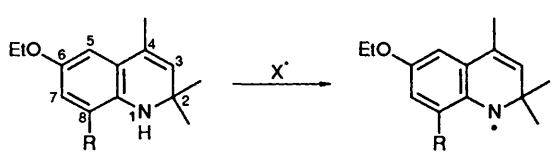
The EPR spectra of 1,2-dihydro-6-ethoxy-2,2,4-trimethyl- and 1,2-dihydro-6-ethoxy-2,2,4,8-tetramethyl-quinolin-1-yl radicals were observed in heptane solution. The hyperfine splittings showed that this class of radical is extensively delocalised with significant spin density at C(8). Both radicals decayed by second-order processes, the rate constants being 5×10^6 and 4×10^2 dm^3 mol^{-1} s^{-1}, respectively at 273 K. The latter reaction is much slower because the 8-methyl substituent blocks the formation of the 1,8'-dimer. Both radicals reacted with oxygen to give the corresponding nitroxides, although reaction was very slow for the 8-methyl derivative. A mechanism is proposed to rationalise product formation from 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline when used as an antioxidant.

6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (ethoxyquin) **1a** is used as an antioxidant in fish meal and various animal feeds, and as a post-harvest dip for apples to prevent scald.[1]



Diarylamines are the most common type of secondary amine antioxidant, but **1a** differs from them in that it possesses a dihydroquinoline ring system and a 6-ethoxy substituent. To compare its mode of action with that of other secondary amines we have undertaken a study of its oxidation and antioxidant activity in detail. Most secondary amines function as antioxidants by transferring hydrogen from their NH groups to the radicals which propagate the autoxidation chains.[2] However, ethoxyquin contains allylic hydrogens (4-CH_3) and hydrogens adjacent to oxygen in the ethoxy group which are easily abstracted and might contribute to the efficiency of this compound as an antioxidant. We have used EPR spectroscopy to identify the main species formed on hydrogen abstraction from ethoxyquin, 8-methylethoxyquin and related compounds, and the results obtained by this method are reported in this paper. A previous EPR study of ethoxyquin[3] showed the presence of a radical which was tentatively identified as the aminyl **2a**, however, subsequent work showed that this was the aminoxyl, **6**.[4]

Results and Discussion

Examination of commercial ethoxyquin by EPR spectroscopy showed that it contained significant amounts of **6**. The substrate **1a** was therefore carefully purified by column chromatography and by preparative HPLC. Solutions of pure **1a** (0.1 to 5 mol dm^{-3}) in heptane or toluene were carefully deoxygenated; no EPR signals were present prior to photolysis, but on illumination with broad spectrum UV light the spectrum shown in Fig. 1 was obtained. This spectrum was quite different in total width and splitting pattern from that of the aminoxyl **6** and it

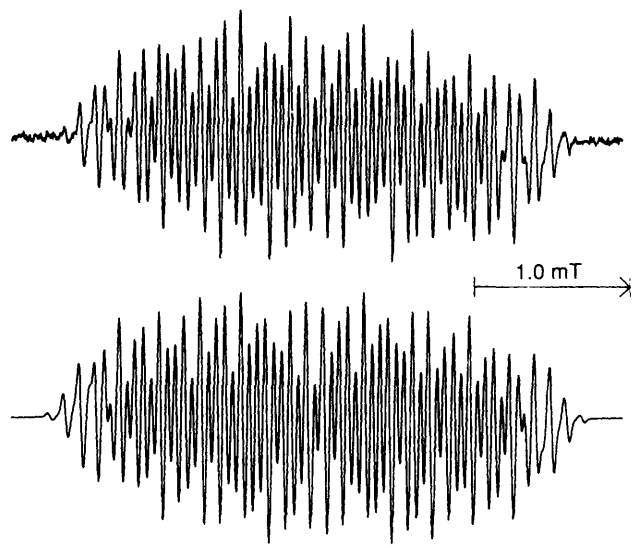


Fig. 1 9.4 GHz EPR spectrum of 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinolin-1-yl radicals **2a** at 305 K in heptane. Upper spectrum, experimental; lower spectrum, computer simulation.

decayed rapidly on removal of the light source. Thus, it corresponds to a transient radical generated from **1a**. The spectrum was eventually analysed by correlation analysis using the AUTO, SEEK and MULTIPEAK routines developed for this purpose,[5] and the maximum entropy method.[6] The computer-simulated spectrum, obtained with the hyperfine splittings (hfs) determined in this way, is also shown in Fig. 1. The number and magnitude of the hfs (Table 1) show that this species cannot be an allyl radical or a radical generated by hydrogen abstraction adjacent to oxygen. We attribute the spectrum to the ethoxyquin aminyl radical **2a**. No cyclic aminyls of this type have been observed by EPR spectroscopy before so the hfs cannot be directly compared with those of model radicals. The N hfs of **2a** is larger than that of the structurally related 1,2-dihydropyridin-1-yl radical ($a\{N\}$ = 0.51 mT),[7] but is comparable to that of the 9,9-dimethyl-9,10-dihydroacridin-10-yl radical ($a\{N\}$ = 0.80 mT).[8]

The hfs of **2a** were computed by means of the INDO semiempirical method[9] (Table 1). The geometry of **2a** used in the INDO calculations was obtained from AM1 SCF MO

Table 1 EPR hyperfine splittings for 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinolin-1-yl and related radicals **2**[a]

Radical	T/K	N	2-$(CH_3)_2$	3-H	4-CH_3	5-H	CH_2O	7-H	8-H(CH_3)
2a	305	0.795	0.100	0.312	0.007[c]	0.255	0.014[c]	0.167	0.525
2a	INDO[b]	1.27	−0.08	−0.36	−0.27	0.31	0.05	0.30	−0.51
2b	300	0.84	0.16	0.42	0.00	0.26	0.10	0.10	(0.26)

[a] hfs in mT. [b] Semiempirical INDO computed values (see the text), $\langle S^2 \rangle = 0.831$. [c] Not resolved; best estimate from simulations.

Table 2 Kinetics of bimolecular termination reactions of 2,2,4-trimethyl-1,2-dihydroquinolin-1-yl and related radicals

Radical	Solvent	T/K	$2k_t$/dm^3 mol^{-1} s^{-1}	$[R^2]^a$	Ref.
3	C_6H_6	293	4.4×10^7	—	11
2a	C_7H_{16}	273	$(5 \pm 3) \times 10^6$	0.994	*b*
2b	C_7H_{16}	273	$(4 \pm 2) \times 10^2$	0.992	*b*

[a] Correlation coefficient of second-order plot. [b] This work.

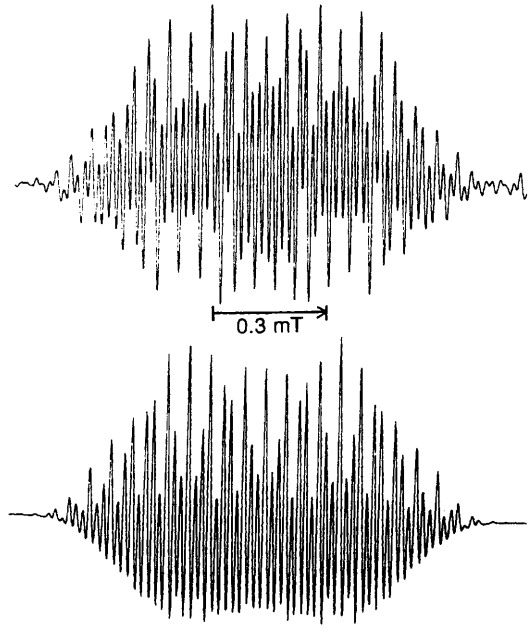


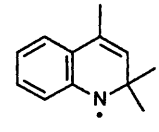
Fig. 2 Low-field component of the ^{14}N triplet of the 9.4 GHz EPR spectrum of ethoxyquin aminoxyl **6** in benzene 300 K. Upper spectrum, experimental; lower spectrum, computer simulation (both second-derivative presentation).

calculations[10] on ethoxyquin; attempts to obtain the optimum geometry of **2a** by the AM1 method were unsuccessful. The computed hfs of the CH_2O hydrogens of the ethoxy group depended on their orientation and varied from 0.00 to 0.10 mT for torsion angles about the O–C(6) bond of 180 and 90°, respectively; an average value is given in Table 1. Where there was doubt about the assignment of the experimental hfs to specific hydrogens in **2a** the INDO results were used for guidance. Thus, the largest doublet hfs was assigned to H(8) and the remaining doublet hfs were assigned to H(3), H(5) and H(7) in decreasing magnitude; obviously, these assignments cannot be regarded as incontrovertible.

Photolysis of a solution of 8-methylethoxyquin **1b** in heptane produced an EPR spectrum of the same general character as that obtained from **1a**. The hfs deduced from the spectral analysis are in Table 1. The large doublet of 0.525 mT, assigned to H(8) in **2a**, was replaced by a quartet splitting of 0.26 mT in **2b** which confirms these assignments. The other hfs are of similar size in **2a** and **2b**; the slightly greater $a\{N\}$ value in **2b** may indicate that the proximity of the 8-methyl substituent to the radical centre causes marginally less spin delocalisation. Possibly steric hindrance from this methyl group induces slightly more bending at the nitrogen radical centre which reduces resonance delocalisation.

The intensity of the UV illumination of **1a** was varied with calibrated gauzes and the EPR signal height was recorded at each intensity. From a plot of log (signal height) *vs.* log (relative intensity) the exponent of the light intensity was found to be 0.62 ± 0.1. The experimental exponent exceeds the theoretical value for a second-order reaction (0.50) only marginally, which indicates that radical **2a** decays mainly by a second-order process; any first-order component must be minor. The decay of the signal could be satisfactorily recorded as a function of time at 273 K by using the spectrometer fast-scan facility at a fixed magnetic field. The initial radical concentration was estimated by using a known concentration of 2,2-di(4-*tert*-octylphenyl)-picrylhydrazin-1-yl free radical (DPPH) under similar spectrometer conditions. The data were not sufficiently accurate to attempt to resolve out first- and second-order components. A second-order plot was satisfactorily linear; the correlation coefficient and rate constant are given in Table 2. The decay of 8-methylethoxyquin aminyl **2b** was very much slower. The data were in best accord with a second-order decay (Table 2) but a minor contribution from a first-order process cannot be ruled out.

The termination rate constant for **2a** compares very favourably with that for 2,2,4-trimethyl-1,2-dihydroquinolin-1-yl radicals **3** in benzene determined by Nekipelova *et al.*[11]

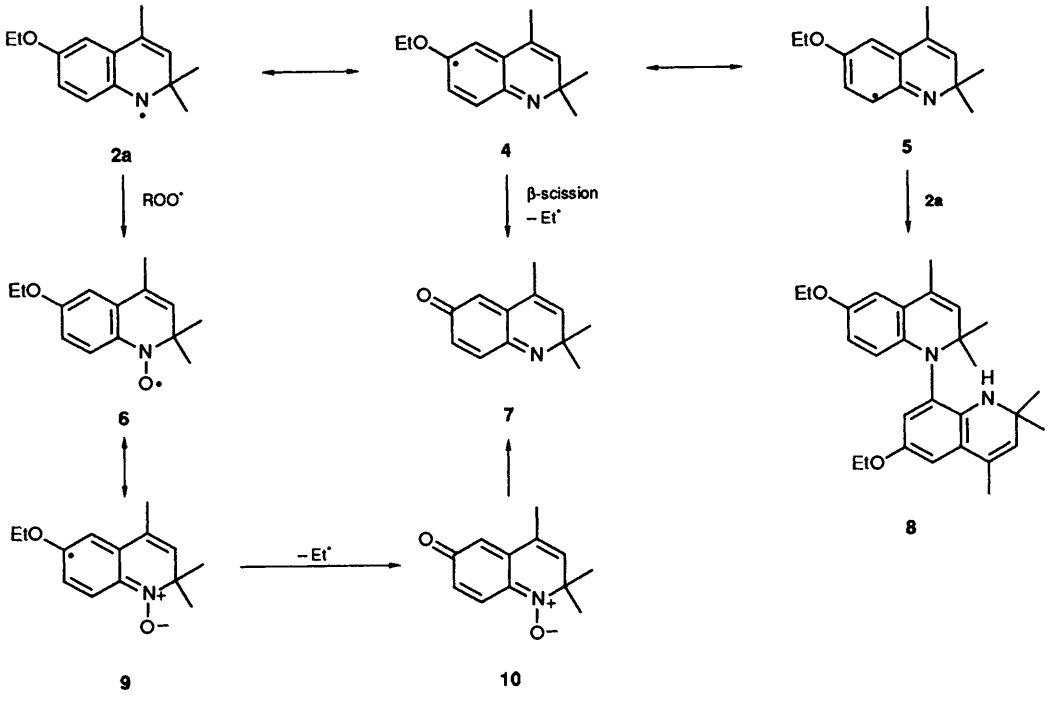


3

at a somewhat higher temperature (Table 2). First-order decay of **3** took over as the main termination at $T > 383$ K.[11] That $2k_t$ for the 8-methyl substituted radical is far smaller is not surprising because the 8-methyl substituent prevents formation of the 1,8′-dimer **8**.

Models of **2b** suggest that steric hindrance to formation of the *N*,*N*-dimer would be very great. The kinetic data show that the termination of **2b** remains second-order, so this may involve formation of the 1,5′-dimer, or even the 5,5′-dimer. Disproportionation is not a likely alternative to second-order termination because of the absence of β-hydrogens in any low-energy canonical form of **2**. The lack of an unhindered dimer causes a decrease of *ca.* four orders of magnitude in the termination rate constant for this type of cyclic aminyl radical. The dimerisation is so slow that the reverse reaction, *i.e.* dimer homolysis, may be important, particularly at higher temperatures.

When oxygen was admitted to the solution of **1a** the EPR spectrum changed completely to that of the ethoxyquin aminoxyl **6a** (Fig. 2) having $a\{N\} = 1.06$, $a\{2\text{-}(CH_3)_2\} = 0.048$, $a\{3\text{-H}\} = 0.101$, $a\{5\text{-H}\} = 0.129$, $a\{7\text{-H}\} = 0.129$ and $a\{8\text{-H}\} = 0.361$ mT at 305 K. This aminoxyl has been



Scheme 1

observed before,[3,4] as have a number of other substituted 2,2-dimethyl-1,2-dihydroquinoline aminoxyls.[12] The $a\{N\}$ and $a\{8\text{-}H\}$ hfs were in reasonable agreement with those reported previously, but our spectra in dilute benzene solution (*ca.* 10^{-4} mol dm^{-3}) were so much better resolved that complete analysis was possible to give the hfs of all the other hydrogens (see Fig. 2 for the simulation). The identity of the aminoxyl was confirmed by observation of the same spectrum from authentic aminoxyl isolated from the oxidation of ethoxyquin with hydrogen peroxide and sodium tungstate and purified by preparative HPLC.[13] Photolysis of a mixture of **1a** and di-*tert*-butyl peroxide showed mainly the aminoxyl **6a**. The intensity of the EPR signal from **6a** remained unchanged indefinitely which indicated that the aminoxyl has a long lifetime.

The 8-methylethoxyquin radical **2b** was not converted so readily into the aminoxyl. On prolonged photolysis, oxygen saturated solutions of **1b** eventually showed a very weak EPR spectrum with $a\{N\}$ = 1.01 mT. The large hfs from H(8) was absent, of course, but well resolved spectra could not be obtained. Attempts to synthesise 8-methylethoxyquin aminoxyl by the standard catalysed oxidations with H_2O_2 failed, but traces of the aminoxyl developed when **1b** was exposed to air over a period of months. It is evident that the 8-methyl group greatly hinders approach to the nitrogen radical centre in **2b**.

The products obtained from oxidation of **1a** with air or oxygen are:[13] 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinolin-1-yloxyl **6a**, 2,2,4-trimethyl-6-quinolone **7** and 1,8′-di(6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline) **8**.[14] Our EPR observation of **2a** confirms that it is a primary intermediate in free-radical reactions of ethoxyquin. In this work **2a** formed on photolysis in the absence of $Bu^tO^•$, or any other added initiator. It is probable that minute traces of peroxides present in the solvent provide the photochemically produced radicals which abstract hydrogen from ethoxyquin. Because the concentration of peroxyl radicals is so low the conversion of **2a** into the aminoxyl (see Scheme 1) is slow and this permits the spectroscopic observation of the cyclic aminyl radicals. When oxygen or a peroxide is present the peroxyl radical concentration becomes much greater and this causes a rapid build up of the persistent aminoxyl **6a** which then prevents observation of **2a**. Formation of **6a** is first order in **2a** whereas the coupling reaction to give the 1,8′-dimer **8** is second order in **2a**. The kinetic results confirmed that under the EPR conditions **2a** mainly reacts by the coupling route. Of course, in the presence of autoxidising lipids, the peroxyl radical concentration will be much higher and a greater proportion of the ethoxyquin radicals will be converted into aminoxyl. The EPR hfs of **2a** show that the unpaired electron is extensively delocalised around the rings. Table 1 shows that C(8) has the highest spin density of all the C-atoms. The exclusive formation of the 1,8′-dimer is therefore explained because C(8) is favoured electronically as well as being sterically the least congested site. The other major oxidation product, the quinolone **7**, could be formed by β-scission and loss of an ethyl radical from resonance form **4** (see Scheme 1). Alternatively, it could be formed *via* the aminoxyl. β-Scission of **9**, with or without assistance from a peroxyl radical, could lead to the quinolone *N*-oxide **10**. Analogous reactions of diaryl aminoxyls are well documented.[15] The quinolone *N*-oxide is then readily converted into the quinolone; authentic pyrrolone *N*-oxide was shown to be converted into **7** in autoxidising lipids.[13]

Experimental

EPR spectra were recorded on a Bruker ER 200D spectrometer operating at 9.4 GHz with 100 kHz modulation. Samples were degassed by several freeze–pump–thaw cycles, or by being bubbled with nitrogen for *ca.* 10 min, sealed in 4 mm o.d. Spectrosil tubes and were irradiated in the cavity of the spectrometer with light from a 500 W super-pressure mercury lamp. Radical concentrations were measured by double integration of appropriate peaks in the sample and DPPH (known concentration *ca.* 10^{-4} mol dm^{-3}). Both sample and DPPH signals were normalised relative to the constant signal from a ruby disc.

6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline **1a** *and 6-Ethoxy-2,2,4,8-tetramethyl-1,2-dihydroquinoline* **1b**.—These were available from previous work.[13] Samples of each were purified by preparative HPLC on silica (Li Croprep Si 60, 5–20

μm, Merck) columns (25 cm × 22 mm i.d.) using 7% isopropyl alcohol in hexane at 7 cm^3 min^{-1}.

6-*Ethoxy*-2,2,4-*trimethyl*-1,2-*dihydroquinolin*-1-*yloxyl* **6** was prepared by the oxidation of **1a** with sodium tungstate, hydrogen peroxide and ethylenediaminetetraacetic acid (EDTA) as described in the literature.[3,4] The aminoxyl underwent conversion into the 6-quinolone *N*-oxide **10** when purification by column chromatography or TLC on silica was attempted. Small pure samples were obtained by preparative HPLC (conditions as above); m.p. 67–69 °C.

Acknowledgements

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