Reactivity of 2,2-diphenyl-1,2-dihydro-4-ethoxyquinolin-1-yloxyl towards oxygen- and carbon-centred radicals

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2,2-Diphenyl-1,2-dihydro-4-ethoxyquinolin-1-yloxyl reacts efficiently with both carbon and oxygen centered (peroxyl, alkoxyl, hydroxyl) radicals leading to products which arise mainly from coupling of radicals with the N–O$^{\bullet}$ function or with the conjugated benzene ring. The reaction with superoxide radical is, however, not very effective, as predicted on the basis of the low reactivity of this radical in typical radical reactions.

Introduction

There is accumulating evidence in the literature on the role exerted by free radicals and active oxygen species in a variety of disorders *in vivo*.[1] Consequently, much attention has been recently given to the role and the mechanisms of action of antioxidants which inhibit free radical-induced oxidative stress.[2,3] An unusual class of synthetic antioxidants are aminoxyl radicals, which includes the commercial aliphatic tetramethylpiperidinics and -pyrrolidinics. These have been thoroughly investigated and applied as antioxidants in many different biological systems;[4–6] their activity is well documented and has been attributed to their capacity to scavenge carbon-centered radicals[7] and to maintain metal ions in their oxidized state.[8] However, their reactivity with oxygen-centered radicals is still under discussion and not yet confirmed.[9]

Our research group has focused its attention on a different class of aminoxyls, namely the aromatic indolinonic and quinolinic aminoxyls.[10] Our interest in the antioxidant activity of indolinonic aminoxyls stems from the fact that these compounds were shown in chemical systems to scavenge effectively aroyloxyl,[11] phenoxyl,[12] alkyl,[13,14] aminyl,[15] peroxyl[16] and alkoxyl[14] radicals. Based on this reactivity, studies were undertaken to determine their antioxidant activity toward biologically relevant molecules exposed to free radical-mediated damage. The results obtained showed that they are efficient inhibitors of oxidative damage to lipids,[17–20] proteins[18,19,21,22] and nucleic acids.[23,24] The antioxidant activity of the quinolinic aminoxyl 2,2-diphenyl-1,2-dihydro-4-ethoxyquinolin-1-yloxyl (**1**) in these systems was also tested and the compound proved to be as efficient as the indolinonic ones.[17,19–21,24] However, its chemical reactivity towards free radical species has never been investigated previously. Therefore, the purpose of this study was to elucidate the chemistry of this quinolinic aminoxyl, by studying its reactivity with peroxyl, alkoxyl, alkyl, hydroxyl and superoxide radicals generated by different means.

Results and discussion

Quinolinic aminoxyl **1** reacts efficiently with both carbon- and oxygen-centered radicals leading to a number of products which arise mainly from coupling of radicals with the N–O$^{\bullet}$ function or with the conjugated benzene ring (Scheme 1). All new compounds were identified through their spectroscopic data (see Table 1) while the others were identified by comparison with authentic samples.[25]

The reaction with *tert*-butylperoxy radicals, generated by hydrogen abstraction with lead dioxide from *tert*-butyl hydroperoxide in acetone at room temperature, gave rise to compound **3** along with minor amounts of aminoxyl **2** and compounds **4**–**6** (Scheme 1).

The identification of aminoxyl **2** was based on its mass spectrum and was substantiated by the finding that its EPR spectrum was identical with that exhibited by the species formed when **1** is reacted with *tert*-butoxyl radicals (see below). The presence of the *tert*-butoxy group on C6 was confirmed by the Fe(III) oxidation of **2** to quinoneimine **3**.[14,26] The formation of **2**, **3** and **4** can be accounted for through the mechanism outlined in Scheme 2, which parallels the one already proposed for the similar reaction of indolinonic aminoxyls,[16] while **5** may result from elimination of a *tert*-butyl alcohol molecule from the hydroxylamine corresponding to aminoxyl **2**. The formation of amine **6** is believed to involve hydrolysis of amine **10** formed in the disproportionation of **1**, a process typical of aromatic aminoxyls.[27,28] However, in an experiment performed with a commercially available water-free solution of *tert*-butyl hydroperoxide in anhydrous benzene, amine **10** was not detected, but only amine **6**, together with the typical reaction products. Therefore, at present we have no plausible explanation for the cleavage of the ethyl group at C4 which is part of an enol ether fragment. Secondly, when aminoxyl **1** was dissolved in "wet" acetone, amine **6** was not detected during the typical reaction time (2 h). Therefore, the non-spontaneous disproportionation of the aminoxyl to give amine **6** is thought to be catalyzed by some unknown species present in the reaction mixture.

Aminoxyl **1** was reacted with *tert*-butoxyl radicals, generated from the decomposition of *tert*-butyl hydroperoxide with Fe(II) [eqn. (1)] in acetonitrile, to give *tert*-butoxyl substituted aminoxyl **2** and compounds **3**, **5**, **6** (Scheme 1).

$$Bu^tOOH + Fe^{2+} + 4H_2O \longrightarrow Bu^tO^{\bullet} + Fe(OH)_3\downarrow + 2H_3O^+ \quad (1)$$

The mechanisms of formation of the substituted aminoxyl **2**, and of compounds **5** and **6**, are the same as those mentioned above for the reaction with peroxyl radicals. However, aminoxyl **2** was only detectable at the beginning of the reaction (see Experimental section) since it rapidly evolved to compound **3**. The substituted aminoxyl **2** can be oxidised to the corresponding oxoammonium ion by Fe(III) (formed during the reaction) and then it can eliminate a carbocation to give compound **3**.

Hydroxyl radicals were generated by the classical Fenton reaction according to eqn. (2); the reaction was carried out in acetonitrile, at room temperature.

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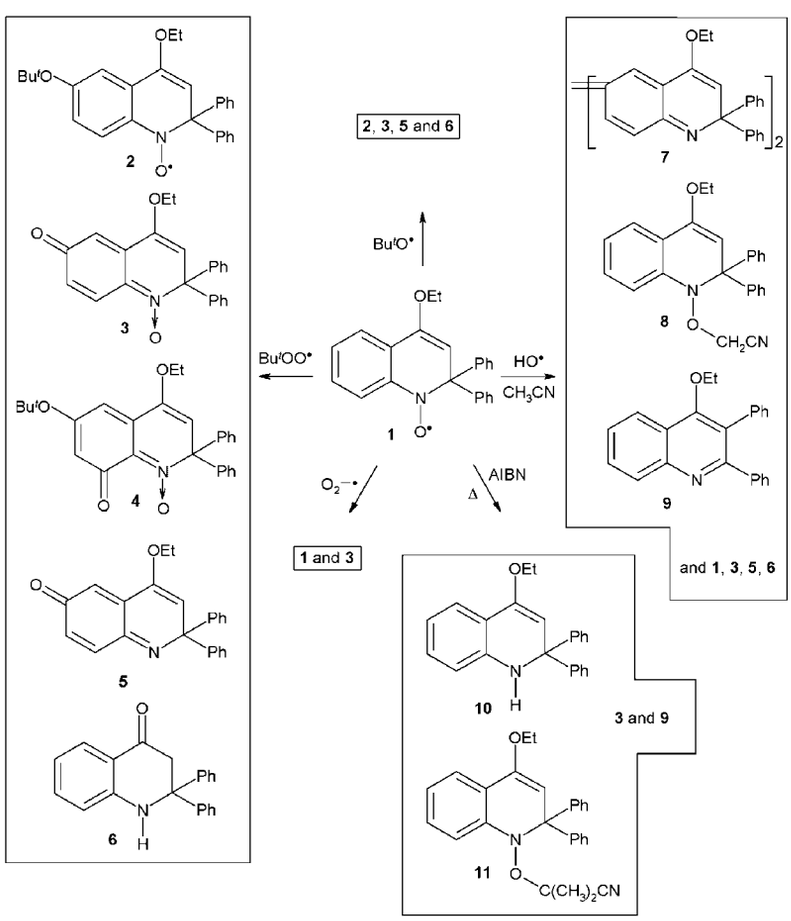
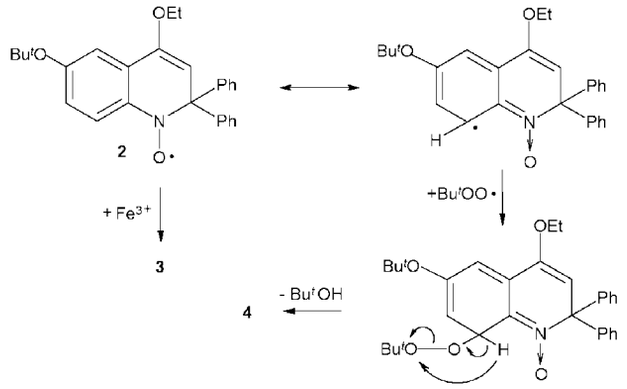
Table 1 Analytical and spectroscopic data of compounds **2**, **4**, **5**, **7**, **8**, **10** and **11**

Compound	Formula	δ_H/ppm in $CDCl_3$	ν_{max}/cm^{-1}	*m*/*z*	HRMS Found (Calculated)
4	$C_{27}H_{27}NO_4$	1.37 (12H, s, -OCH$_2$C*H*$_3$ and -O*Bu*^{t}); 3.99 (2H, q, *J* 7.0, -OC*H*$_2$CH$_3$); 5.52 (1H, s, 3-H); 6.19 (1H, d, *J* 2.2, arom); 6.71 (1H, d, *J* 2.2, arom); 7.34 (10H, m, arom)	1730, 1626, 1251	429 (M$^+$, 4%); 373 (37); 328 (23); 57 (100)	429.5201 (429.5205)
5	$C_{23}H_{19}NO_2$	1.41 (3H, t, *J* 7.0, -OCH$_2$C*H*$_3$); 3.99 (2H, q, *J* 7.0, -OC*H*$_2$CH$_3$); 6.14 (1H, s, 3-H); 6.66 (1H, dd, *J* 10.2 and 2.2, arom); 6.76 (1H, dd, *J* 2.2 and 0.7, arom); 7.30 (11H, m, arom)	1735, 1641, 1490	341 (M$^+$, 76%); 312 (59); 266 (100)	341.4129 (341.4134)
7	$C_{46}H_{38}N_2O_2$	1.42 (3H, t, *J* 7.0, -OCH$_2$C*H*$_3$); 3.99 (2H, q, *J* 7.0, -OC*H*$_2$CH$_3$); 6.14 (1H, s, 3-H); 6.67 (1H, bdd, *J* 12.3 and 2.3, arom); 6.77 (1H, bd, *J* 2.6, arom), 7.3 (11H, m, arom)	1741, 1649, 1599, 1448	650 (M$^+$, 42%); 621 (32); 573 (100)	650.8288 (650.8280)
8	$C_{25}H_{22}N_2O_2$	1.41 (3H, t, *J* 7.0, -OCH$_2$C*H*$_3$); 3.64 (2H, s, -C*H*$_2$CN); 3.92 (2H, q, *J* 7.0, -OC*H*$_2$CH$_3$); 5.38 (1H, s, 3-H); 6.92 (1H, td, *J* 7.3 and 1.3, arom); 7.29 (9H, m, arom); 7.47 (4H, m, arom)	2750, 2200, 1941, 1603, 1484, 1446	382 (M$^+$, 23%); 342(16); 298 (33); 222 (100)	382.4657 (382.4663)
10	$C_{23}H_{21}NO$	1.41 (3H, t, *J* 7.0, -OCH$_2$C*H*$_3$); 3.95 (2H, q, *J* 7.0, -OC*H*$_2$CH$_3$); 4.32 (1H, br, NH); 4.97 (1H, s, 3-H); 6.51 (1H, dd, *J* 7.8 and 0.8, arom); 6.64 (1H, dd, *J* 7.5 and 1.1, arom); 7.04 (1H, td, *J* 7.8 and 1.4, arom); 7.34 (11H, m, arom)	3344, 1722, 1649, 1606, 1479	327 (M$^+$, 13%); 298 (41); 222 (100)	327.4293 (327.4299)
11	$C_{27}H_{26}N_2O_2$	1.12 (6H, br, -C(C*H*$_3$)$_2$CN); 1.44 (3H, t, *J* 7.0, -OCH$_2$C*H*$_3$); 4.01 (2H, bq, *J* 7.0, -OC*H*$_2$CH$_3$); 5.58 (1H, s, 3-H); 6.93 (1H, td, *J* 7.5 and 1.1, arom); 7.34 (13H, m, arom)	2618, 1640, 1600	410 (M$^+$, 3%); 342 (10); 314 (20); 265 (100)	410.5208 (410.5205)
EPR/hfccs, Gauss in ethyl acetate					
2	$C_{27}H_{28}NO_3$	a^N (NO$^\bullet$) = 9.47, a^H (H-3) = 1.07, a^H (H-5,H-7) = 0.89, a^H (H-8) = 2.92 G	1770, 1660, 1493	414 (M$^+$, 6%); 266 (100)	414.5288 (414.5291)



Scheme 1



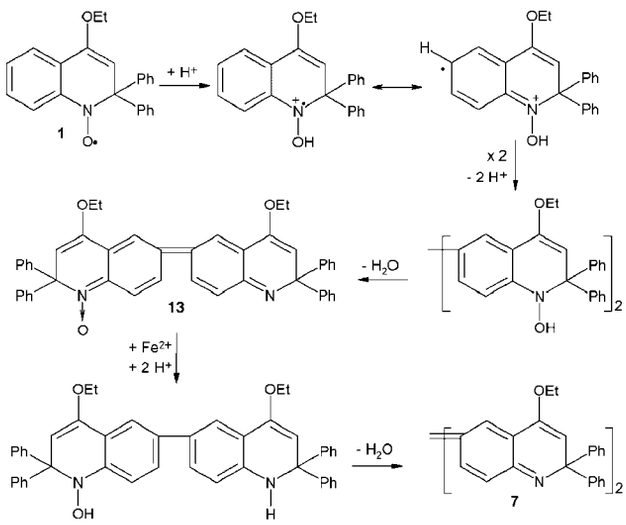
Scheme 2

$$H_2O_2 + Fe^{2+} + 4H_2O \longrightarrow HO^{\bullet} + Fe(OH)_3\downarrow + 2H_3O^{+} \quad (2)$$

Once again, the main reaction product was the quinoneimine *N*-oxide **3**, even though in this case a certain amount of starting aminoxyl was recovered (25%). The other products isolated were compounds **5**, **6**, the dimer **7**, the alkoxyamine **8** and quinoline **9**. Blank reactions were also performed to see whether hydrogen peroxide alone or Fe(II) alone could react with the aminoxyl in the same conditions employed for the Fenton reaction; in both cases, no products were observed (see Experimental section).

In this reaction, the hydroxyl radical could attack the conjugated benzene ring of the aminoxyl **1** at C6 giving rise to hydroxylamine **12** (Scheme 3). This intermediate can be oxidized to quinoneimine *N*-oxide **3** by Fe(III) through a mechanism similar to the one already discussed (Scheme 2) for the reaction with *tert*-butoxyl radicals, or it could lose a water molecule to give the deoxygenated quinoneimine **5** (Scheme 3). This proposed mechanism may be supported by our previous observation that indolinonic quinoneimine *N*-oxides upon reduction, give rise to an intermediate similar to **12** which easily loses a water molecule.[29] Amine **6** may arise through the disproportionation reaction as previously mentioned.

The dark blue dimer **7** was identified by comparing its ^{1}H NMR and mass spectra with those of the yellow compound **5**. In fact, the ^{1}H NMR spectrum of the former shows the same pattern as the latter but its mass spectrum gives the expected molecular ion peak. A possible explanation for the formation of this dimer (Scheme 4), could come from the acidic medium generated by the Fenton reaction: aminoxyl **1** can be protonated and the mesomeric form of the radical cation may undergo dimerization at C6, followed by elimination of a water molecule to give intermediate **13**. This may subsequently undergo reduction (by the excess Fe(II) present in the reaction medium), protonation and elimination of a second water molecule to give dimer **7**. Protonation and dimerization of aminoxyl **1** has been previously observed under acid treatment;[25] furthermore, radical cations of this type easily undergo dimerization on the



Scheme 4

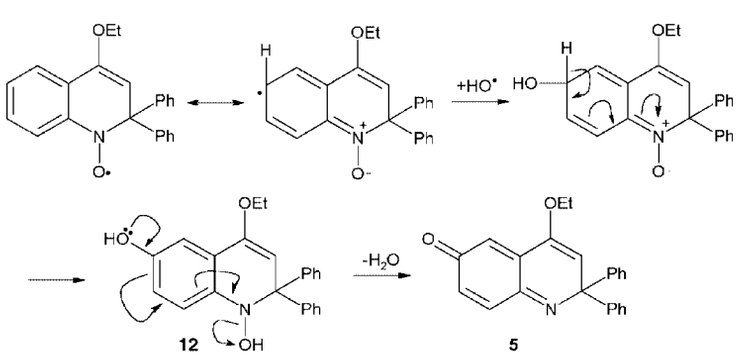
conjugated benzene ring.[30] The last steps of this mechanism are not unusual for this type of molecule since a similar behavior has been observed during the reaction of conjugated bisnitrones in the presence of reducing agents.[31]

The formation of the alkoxyamine **8** arises from coupling of the N–O$^{\bullet}$ function with $^{\bullet}CH_2CN$, formed by hydrogen abstraction of the hydroxyl radical on the reaction solvent; a similar compound was observed on reacting an indolinonic aminoxyl with hydroxyl radicals in the same reaction solvent[32] and it represents the typical reaction product of carbon-centered radicals with aminoxyls.[7]

Quinoline **9**, which had already been observed upon acid treatment of quinolinic aminoxyls,[25] might originate through a Wagner–Meerwein migration of a phenyl group in alkoxyamine **8**, which, in acidic media, readily loses an alcohol molecule.[33]

With superoxide radical generated by dissolving KO_2 in benzene in the presence of a crown ether, only a small conversion of aminoxyl **1** took place: in fact, 70% of the aminoxyl was recovered. This is not surprising since superoxide radical is itself not very reactive in typical radical reactions.[34] Besides the unreacted **1**, only quinoneimine **3** was isolated in small amounts and its formation could be envisaged according to the same reaction mechanism described for peroxyl radicals, by considering that the hydroperoxyl radical, formed from protonation of superoxide (promoted by traces of water in the reaction solvent) could attack the benzene ring.

Aminoxyl **1** was reacted with cyanoisopropyl radicals generated by thermal decomposition of AIBN in refluxing benzene and in a nitrogen atmosphere.[35] The main product of the reaction, which was isolated in high yields (70%), was the alkoxyamine **11** formed by scavenging of the cyanoisopropyl radical on the N–O$^{\bullet}$ function. The other minor products isolated from the reaction between aminoxyl **1** and AIBN were the amine **10** and the quinoneimine *N*-oxide **3** formed through the dis-



Scheme 3

proportionation reaction favoured by the reaction temperature. However, the amine **10** does not undergo hydrolysis at C4 as previously observed, since no water is present in the reaction mixture. The mechanism proposed above for the formation of quinoline **9** could be used to explain the achievement of the compound in this reaction: the exit of the RO- group may be promoted by the high temperature.

Conclusion

Aminoxyl **1**, as stated in the introduction, has been recently exploited as antioxidant for the protection of biological systems from oxidation induced by free radicals. The fact that the protection exerted on the experimental systems studied was remarkable prompted us to study its chemical reactivity toward those radicals which are usually involved in peroxidation processes.

From this study it can be confirmed that this aminoxyl can scavenge different types of radicals—both oxygen and carbon centered ones—and therefore works better as antioxidant than the commercial aliphatic aminoxyls, which react only with alkyl radicals and are commonly employed in the biological field. In fact, the quinolinic aminoxyl studied, efficiently reacts by homolytic substitution on the conjugated benzene ring with electrophilic radicals such as peroxyl and alkoxyl radicals and, to a lesser extent, with hydroxyl and superoxide radicals, to mainly give non-paramagnetic species. Furthermore, the coupling reaction at the N–O$^{\bullet}$ function with alkyl radicals is extremely favourable.

In conclusion, the results obtained give a chemical and mechanistic explanation of the specific activity of this aminoxyl as antioxidant. Furthermore, kinetic studies on the actions of aminoxyl **1** and indolinonic aminoxyls in the oxidation of lipids induced by peroxyls, showed that aminoxyl **1** is slightly more reactive toward peroxyl radicals than indolinonic aminoxyls,[36] and this could account for the slightly higher antioxidant activity observed for this aminoxyl in previous studies.[17,19,22] Taken together, these results further stimulate the application of the aromatic aminoxyls (quinolinic and indolinonic) in the prevention of free radical mediated oxidative processes.

Experimental

IR spectra were recorded on a Nicolet Fourier Transform Infrared 20-SX spectrophotometer equipped with a Spectra Tech "Collector" for DRIFT measurements. ^{1}H NMR spectra were recorded at room temperature in $CDCl_3$ on a Varian Gemini 200 spectrometer (δ in ppm are relative to $(CH_3)_4Si$). Mass spectra were recorded on a Carlo Erba QMD 1000 spectrometer in EI$^+$ mode. High resolution mass spectra were recorded on a VG7070-E 5000 spectrometer with PFK as the resolution and calibration standard, whereas EPR spectra were recorded on a Varian E4 spectrometer interfaced with a computer. 2,2-Diphenyl-1,2-dihydro-4-ethoxyquinolin-1-yloxyl (**1**) was prepared as reported in the literature.[37] All experiments were performed twice and the yields reported are the average of the two individual runs.

Reaction of 1 with *tert*-butyl peroxyl radicals

An aqueous solution (70%) of *tert*-butyl hydroperoxide (1.2 mmoles) in 20 ml anhydrous acetone was added dropwise to a mixture of aminoxyl **1** (0.6 mmoles) dissolved in 100 ml anhydrous acetone and PbO_2 (2.3 mmoles), under magnetic stirring. The reaction was monitored by TLC eluting with cyclohexane–ethyl acetate, 8:2. After 2 h, when all the aminoxyl had been consumed, PbO_2 was filtered off and the reaction was concentrated to a small volume. The residue was purified by chromatography on silica gel preparative plates, eluting with cyclohexane–ethyl acetate, 85:15. The following compounds were isolated from top to bottom with their corresponding percentage yields: 2,2-diphenyl-1,2-dihydro-4-ethoxy-6-*tert*-butoxyquinolin-1-yloxyl **2** (6%); 2,2-diphenyl-4-ethoxy-2,6-dihydroquinolin-6-one **5** (3%); 2,2-diphenyl-1,2,3,4-tetrahydroquinolin-4-one **6** (2%); 2,2-diphenyl-4-ethoxy-6-oxo-2,6-dihydroquinoline 1-oxide **3** (61%); 2,2-diphenyl-4-ethoxy-8-oxo-6-*tert*-butoxy-2,8-dihydroquinoline 1-oxide **4** (6%).

Reaction of 1 with *tert*-butoxyl radicals

Powdered $FeSO_4 \cdot 7H_2O$ (0.9 mmoles) in 10 ml distilled water was added dropwise to a mixture of aminoxyl **1** (0.3 mmoles) dissolved in 30 ml anhydrous acetonitrile and *tert*-butyl hydroperoxide (aqueous solution 70%; 0.9 mmoles), under vigorous magnetic stirring. After 15 mins, the reaction was worked up by extraction with chloroform (3 × 50 ml), washed with distilled water (3 × 50 ml), dried over anhydrous Na_2SO_4, filtered and concentrated to a small volume. The residue was purified by chromatography on silica gel preparative plates eluting with benzene. The following compounds were isolated from top to bottom with their corresponding percentage yields: **6** (12%); **5** (8%); **3** (55%).

From the same reaction, 1 ml of reaction mixture was withdrawn after 2 min following complete addition of $FeSO_4 \cdot 7H_2O$ and chromatographed on TLC eluting with cyclohexane–ethyl acetate, 9:1, in order to isolate the substituted aminoxyl **2**. A red-brown spot appeared below the starting aminoxyl which was extracted with ethyl acetate and analyzed by EPR and mass spectroscopy. The analyses confirmed the assigned structure.

Reaction of 1 with hydroxyl radicals

To a mixture of aminoxyl **1** (0.6 mmoles) dissolved in 20 ml acetonitrile and H_2O_2 (6 mmoles), powdered $FeSO_2 \cdot 7H_2O$ (6 mmoles) dissolved in 10 ml distilled water was added dropwise, everything thoroughly degassed under nitrogen using teflon needles. The reaction mixture darkened upon addition of the ferrous salt. By monitoring the reaction using TLC, the starting aminoxyl almost completely disappeared after 15 min from the end of the addition. The mixture was extracted with dichloromethane (2 × 50 ml), washed with distilled water (3 × 50 ml), dried over anhydrous Na_2SO_4, filtered and concentrated to a small volume. The mixture was purified by chromatography on silica gel preparative plates eluting with ethyl acetate–cyclohexane, 1:9. From top to bottom the following compounds were obtained with their corresponding percentage yields: 1-cyanomethoxy-2,2-diphenyl-1,2-dihydro-4-ethoxyquinoline **8** (6%); 2,3-diphenyl-4-ethoxyquinoline **9** (7%); **1** (25%); **5** (7%); **6** (8%); **3** (20%); 4,4′-diethoxy-2,2,2′,2′-tetraphenyl-2,2′,6,6′-tetrahydro-6,6′-biquinolinylidene **7** (2%).

Reaction of 1 with superoxide radical

KO_2 (3 mmoles) was added under magnetic stirring to a solution of 18-crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecane; 3 mmoles present to facilitate dissolution of KO_2 in the organic solvent) in 25 ml benzene. To this mixture, aminoxyl **1** (0.3 mmoles) was added. The reaction was monitored by TLC every 15 mins. After 3 h, when no more changes in the reaction were verified, the mixture was worked up by neutralizing with 1% HCl, extracted with dichloromethane (2 × 50 ml), washed with distilled water (2 × 50 ml), dried over anhydrous Na_2SO_4, filtered and concentrated to a small volume. The reaction mixture was purified by chromatography on silica gel preparative plates eluting with ethyl acetate–cyclohexane, 1:9. Aminoxyl **1** (70%) and quinoneimine **3** (15%) were recovered from top to bottom.

Reaction of 1 with AIBN

Aminoxyl **1** (0.6 mmoles) and α,α′-azoisobutyronitrile (AIBN, 1.2 mmoles) in 30 ml anhydrous benzene were refluxed for 30

mins under a stream of nitrogen: the reaction mixture turned from red to yellow. The reaction mixture was then concentrated to a small volume and then purified by chromatography on silica gel preparative plates eluting with cyclohexane–ethyl acetate, 85:15. The following compounds were isolated from top to bottom with their corresponding percentage yields: 2,2-diphenyl-1,2-dihydro-4-ethoxyquinoline **10** (5%); 1-(cyano-dimethylmethoxy)-2,2-diphenyl-1,2-dihydro-4-ethoxyquinoline **11** (70%); **9** (4%); **3** (5%).

Reaction of 1 with hydrogen peroxide

To a solution of aminoxyl **1** (0.3 mmoles) dissolved in 30 ml acetonitrile, H_2O_2 (3 mmoles) was added dropwise under nitrogen atmosphere and under magnetic stirring. The reaction was monitored by TLC (cyclohexane–ethyl acetate, 8:2) and after 1.5 h it was interrupted as there was no reaction.

Reaction of 1 with Fe(II)

To a solution of aminoxyl **1** (0.15 mmoles) dissolved in 30 ml acetonitrile, $FeSO_4 \cdot 7H_2O$ (0.45 mmoles) in 5 ml water was added dropwise under nitrogen atmosphere and under magnetic stirring. The reaction was monitored by TLC (cyclohexane–ethyl acetate, 8:2) and after 10 h the reaction was worked up by extraction with dichloromethane (3 × 20 ml), washed with distilled water (3 × 20 ml), dried over anhydrous Na_2SO_4, filtered and concentrated to a small volume. The reaction mixture was purified by chromatography on silica gel preparative plates eluting with cyclohexane–ethyl acetate, 9:1 where 90% of the aminoxyl **1** was recovered.

Reaction of 2 with Fe(III)

$FeCl_3$ (30 mg in 0.5 ml of H_2O) was added to a solution of **2** (10 mg in 2 ml of acetonitrile) at room temperature and with stirring. By monitoring the reaction on TLC (ethyl acetate–cyclohexane, 2:8) there was immediate and complete transformation of the aminoxyl **2** into the yellow quinoneimine **3** (added on the TLC for reference).

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