Free Radical Research, Volume 38 Number 1 (January 2004), pp. 67–72

Taylor & Francis
healthsciences

Hydrogen Abstraction Ability of Different Aromatic Nitroxides

ELISABETTA DAMIANI[a], PAOLA ASTOLFI[a], MASSIMO BENAGLIA[b], ANGELO ALBERTI[b] and LUCEDIO GRECI[a,*]

[a]*Dipartimento di Scienze dei Materiali e della Terra, Università Politecnica delle Marche, Via Brecce Bianche, I-60131 Ancona, Italy;* [b]*ISOF-CNR Area della Ricerca, Via P. Gobetti 101, I-40129 Bologna, Italy*

Accepted by Prof. B. Kalyanaraman

(Received 23 June 2003; In revised form 1 October 2003)

Indolinonic aromatic nitroxides have been shown to efficiently inhibit free radical mediated oxidation reactions in biological systems. Since all antioxidants also possess pro-oxidant activity, possibly through a hydrogen abstraction process from suitable substrates, the relative hydrogen abstraction abilities of these compounds were evaluated. Different hydrogen donors were reacted with an indolinic and two indolinonic nitroxides and the rates of hydrogen abstraction were determined using UV–Vis spectroscopy. From the data obtained, a structure–activity relationship was found. In addition, the hydrogen abstraction ability of these compounds was found to be much greater than that of the aliphatic nitroxide TEMPO, despite existing reports indicating that these two classes of compounds show similar antioxidant activities in biological systems.

Keywords: Nitroxides; Hydrogen abstraction; Antioxidants; Prooxidants; Free radicals; Hydrogen donors

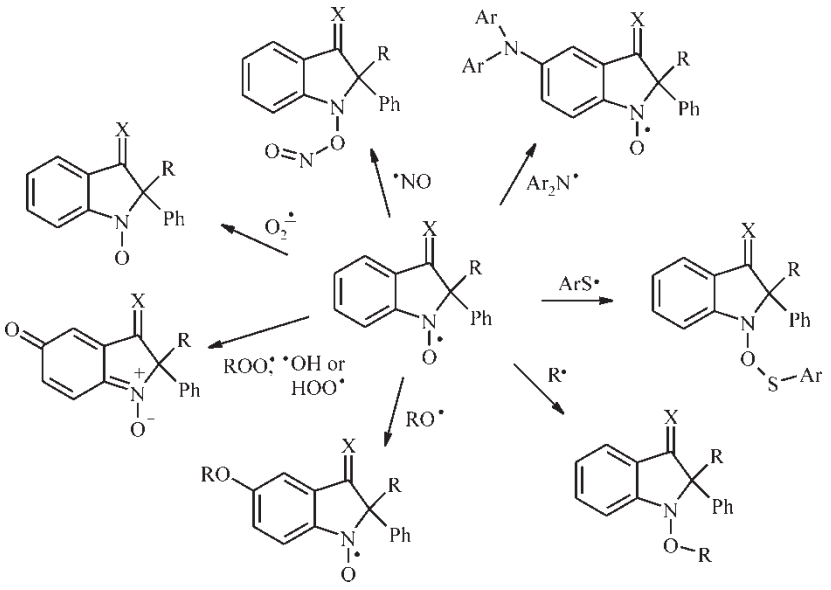
INTRODUCTION

Life scientists are becoming increasingly aware of the participation of active oxygen species and free radicals in the pathogenesis of various diseases, including cancer. As a consequence, the role of antioxidants in preventing free radical-mediated damage is currently receiving considerable attention. Our interest in this field has been mainly focused on aromatic indolinonic and quinolinic nitroxides, a series of nitroxides that exhibit a different behaviour towards radical species with respect to other nitroxides such as 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and 4-hydroxy-TEMPO. This more variegated reactivity is due to their ability to react not only with *C*-centred[1,2] or *S*-centred radicals (thiyls)[3] but also with *O*-centred radicals (peroxyl,[4] alkoxyl,[5] hydroxyl,[6] superoxide[6,7]) and nitrogen monoxide,[8] as outlined in Scheme 1.

Prompted by this interesting reactivity towards different radical species and the type of radicals produced during oxidative processes, we have studied these nitroxides in various biological systems ranging from lipids,[9–11] proteins[10,11] and DNA[12] to intact cells,[13] with the aim of testing their potential as biological antioxidants. The results obtained so far have been very promising, and in every case the extent of oxidation of the system studied was notably reduced. It is, however, common knowledge that under appropriate conditions antioxidants also exert a prooxidant activity,[14] that may possibly involve a hydrogen abstraction process from suitable substrates. As an example, Bowry and Ingold have suggested that α-tocopherol may mediate oxidation in low-density lipoproteins through its α-tocopheroxyl radical.[15] Some time ago, we observed[9] that C-3 phenylimino indolinonic nitroxides are better than C-3 carbonyl indolinonic nitroxides at inhibiting the oxidation of low-density lipoproteins. These results, obtained by monitoring the extent of conjugated diene formation as an index of oxidation, suggested that this could be due to different hydrogen-abstracting abilities of the two types of nitroxides. In this light it would appear that nitroxides with a greater hydrogen-abstracting

*Corresponding author. Tel.: +39-071-2204408. Fax: +39-071-2204714. E-mail: greci@univpm.it

ISSN 1071-5762 print/ISSN 1029-2470 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/10715760310001636355



SCHEME 1 Reactivity of indolinonic nitroxides towards different radical species.

ability are less efficient antioxidant compounds or, from a different point of view, may act as better prooxidants.

Although it is not established that the hydrogen-abstracting ability of nitroxides is the sole factor determining their prooxidant activity, a qualitative relationship between these two properties is likely to exist. Therefore, we thought it interesting to evaluate the hydrogen-abstracting ability of the indolinonic nitroxides **1a** and **1b** and indolinic nitroxide **2** with three different hydrogen-donors **4**–**6**. The behaviour of the very popular TEMPO was also investigated for comparative purposes. At the same time, the study was also aimed at establishing the relationship, if any, between the structure of the nitroxides and their hydrogen abstraction ability.

MATERIALS AND METHODS

Chemicals

1,2-Dihydro-2-methyl-2-phenyl-3*H*-indole-3-one-1-oxyl **1a**,[16] 1,2-dihydro-2-methyl-2-phenyl-3*H*-indole-3-phenylimino-1-oxyl **1b**,[16] 5,7-di-*tert*-butyl-2,3-dihydro-3,3-dimethyl-2,2-diphenyl-1*H*-indole-1-oxyl **2**[17] and *N*,*N*-dimethyl-*N*-(2-phenyl-1*H*-indol-3yl)-benzene-1,4-diamine **6**[18] were synthesised according to the literature, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) **3** and *N*,*N*-diethylhydroxylamine **5** were purchased from Aldrich in the purest grade available, while 5,7-di-*tert*-butyl-3-(3,4-dimethyl-phenyl)-3*H*-benzofuran-2-one **4** was supplied by CIBA and was used without further purification, although it was a mixture of 5,7-di-*tert*-butyl-3-(3,4-dimethyl-phenyl)-3*H*-benzofuran-2-one (90%) and 5,7-di-*tert*-butyl-3-(2,3-dimethyl-phenyl)-3*H*-benzofuran-2-one (10%). Solvents were also of the purest grades commercially available and were deoxygenated by bubbling with argon.

Kinetic Measurements

UV–Vis measurements were carried out on a UVIKON 941 Plus or a Perkin Elmer Lambda 20 spectrophotometer equipped with a PTP-6 Peltier system to control the temperature.

Kinetic Measurements with Hydrogen Donor (4)

A 1×10^{-2} M stock solution of compound **4** and 1×10^{-3} M stock solutions of nitroxides **1** and **2** in deoxygenated acetonitrile were prepared. Three hundred microlitres of the nitroxide solution were diluted with 2400 μl of acetonitrile, then 300 μl of the hydrogen donor solution were added to reach final concentrations of 1×10^{-4} M for the nitroxide and 1×10^{-3} M for the lactone **4**. The absorption decay of nitroxides at 293 K was monitored at their appropriate λ_{max}, 289 (**1a**), 293 (**1b**) and 316 nm (**2**).

Kinetic Measurements with Hydrogen Donor (5)

A 1×10^{-2} M stock solution of *N*,*N*-diethylhydroxylamine **5** and 1×10^{-3} M stock solutions of nitroxides **1** and **2** were prepared using deoxygenated tetrahydrofuran as solvent. Three hundred microlitres of the nitroxide solution were diluted with 2670 μl of tetrahydrofuran, then 30 μl of the hydrogen donor solution were added to reach a final concentration of 1×10^{-4} M for both the reactants. The absorption decays of nitroxides at 293 K were monitored at their λ_{max}

(see above: there are no significant differences in λ_{max} in acetonitrile or tetrahydrofuran).

Kinetic Measurements with Hydrogen Donor (6)

Stock solutions, 1.5×10^{-3} M, of nitroxides **1** and **2** and a 7.5×10^{-4} M stock solution of **6** were prepared using acetonitrile as the solvent. Three hundred and thirty three microlitres of the nitroxide solution was diluted with 1834 μl of acetonitrile, then 333 μl of the hydrogen donor solution were added to reach final concentrations of 2×10^{-4} M for the nitroxide and 1×10^{-4} M for the donor **6**. In the experiment with TEMPO **3**, where more concentrated solutions were used, 400 μl of a 1.875×10^{-3} M TEMPO stock solution were diluted with 1700 μl of the solvent, then 400 μl of the 7.5×10^{-4} M stock solution of **6** were added. The final concentrations were, in this case, 3×10^{-3} M for TEMPO and 1.5×10^{-3} M for compound **6**. The increase in absorption of the hydrogen donor during its oxidation was monitored at its λ_{max}, i.e. 564 nm.

RESULTS AND DISCUSSION

The reaction of nitroxides with hydrogen donors leads to the corresponding hydroxylamines, which are characterized by UV–Vis spectra different from those of the starting nitroxides.

When **1a** was reacted with an excess of **5** in tetrahydrofuran, the colour of the solution rapidly changed from red to yellow, and at the end of the reaction, hydroxylamine **7** was isolated as the only reaction product. In a similar way, when **1b** was treated with an excess of lactone **4**, the hydroxylamine **8** was isolated along with the dimeric compound **9** (Fig. 1). Both hydroxylamines have UV–Vis spectra very different from those of the starting nitroxides as shown in Fig. 2. Hence, the different absorbance spectra allowed this UV-spectrophotometric study of the H-abstraction reactions to be carried out.

When 100 μM nitroxides **1a** and **1b** were allowed to react with a ten-fold excess of hydrogen-donor **4** in acetonitrile solution, the disappearance of the nitroxides could be followed by monitoring the decrease of their absorbance at the appropriate λ_{max}, i.e. 289 nm for **1a** and 293 nm for **1b**. At the concentrations used, the absorbance of dimer **9** does not significantly affect the absorbance at these

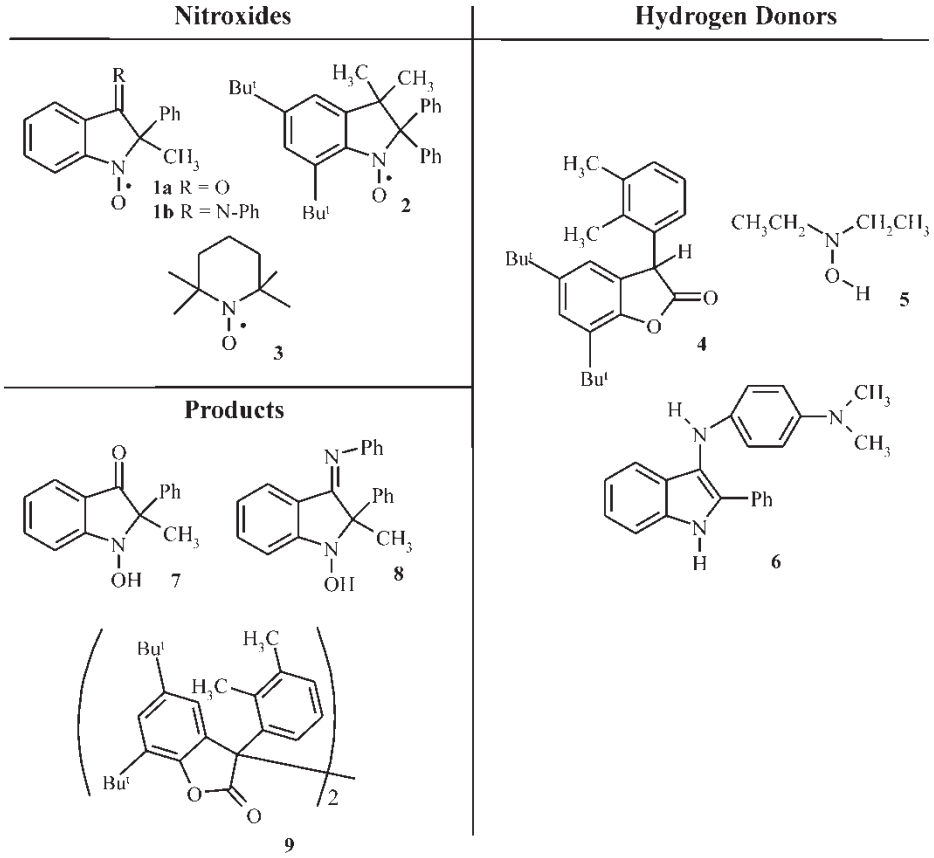


FIGURE 1 Chemical structures of compounds studied.

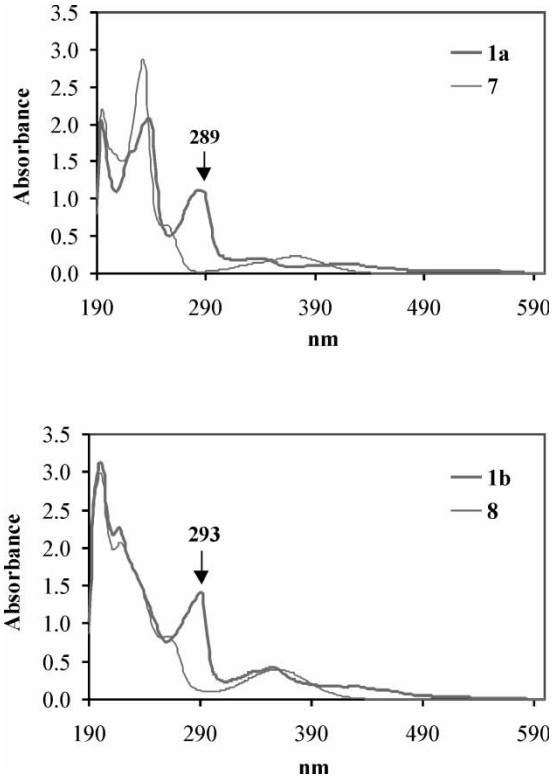


FIGURE 2 UV–Vis spectra of 100 μM nitroxides and their corresponding hydroxylamines in acetonitrile.

wavelengths. In the case of compound **5**, side reactions occurred between the hydroxylamine and the solvent,[19] and for this reason acetonitrile had to be replaced with tetrahydrofuran. For all of the examined nitroxides, changing the solvent did not lead to a significant shift in the λ_{max}.

When reacting **1a** and **1b** with **4** the pseudo first order rate constants were obtained from the plot of ln [**1a**, **1b**] vs. time (see Fig. 3) and by dividing these values by the average concentration of **4** the absolute rate constants were determined at 293 K as $3.55\,dm^3\,mol^{-1}\,s^{-1}$ for **1a** and $2.61\,dm^3\,mol^{-1}\,s^{-1}$ for **1b**. A similar study was not possible in the case of nitroxide **2** because its reaction with **4** was too slow.

In the case of diethylhydroxylamine **5** as the hydrogen donor it was not possible to use an excess

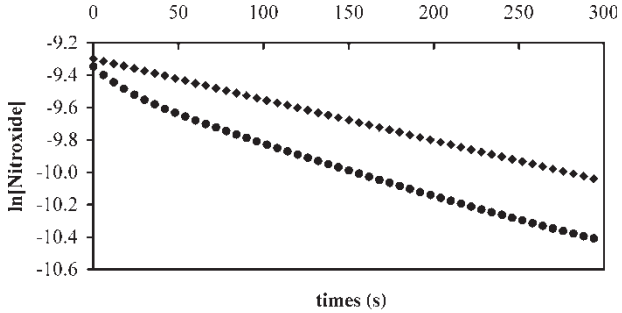


FIGURE 3 Plot of ln [nitroxide] vs. time for the reaction of nitroxides **1a** (●) and **1b** (◆) with compound **4**.

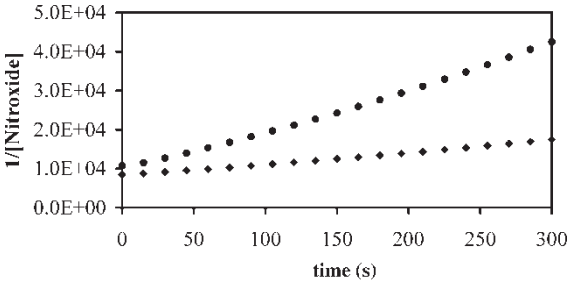


FIGURE 4 Plot of 1/[nitroxide] vs. time for the reaction of nitroxides **1a** (•) and **1b** (◆) with compound **5**.

of nitroxide because the reaction was too fast to be measured. The nitroxides and **5** were, therefore, reacted in a 1:1 molar ratio and in Fig. 4 the plot is shown of the reciprocal of the nitroxide concentration vs. time. The second order rate constants at 293 K were obtained from the slope of the straight lines as $107.19\,dm^3\,mol^{-1}\,s^{-1}$ for **1a** and $30.11\,dm^3\,mol^{-1}\,s^{-1}$ for **1b**. Even in this case, the reaction with nitroxide **2** was too slow to be monitored.

With the aim to compare the hydrogen abstraction ability of nitroxides **1a**, **1b** and **2** with that of functionally related aliphatic compounds, attempts were made to study the reactivity of TEMPO **3** towards compounds **4** and **5**. This study proved, however, impossible, because the UV–Vis spectrum of the hydroxylamine resulting from the hydrogen abstraction process strongly overlapped that of TEMPO, thus making it unfeasible to adequately follow the changes in its absorbance. In order to overcome this impasse, the hydrogen-abstraction ability of nitroxides **1**–**3** was investigated using the hydrogen donor **6**. After undergoing hydrogen abstraction, this compound assumes an indolinic structure which leads to a new absorption with λ_{max} at 564 nm. The increase in absorbance at this wavelength, therefore, provides a means to follow its reaction with the nitroxides. The possibility of the occurrence of electron transfer between compound **6** and the nitroxides is highly unlikely on the basis of their redox potentials.[7,20] Figure 5 shows the time profile of A_{564} during the reactions with the different

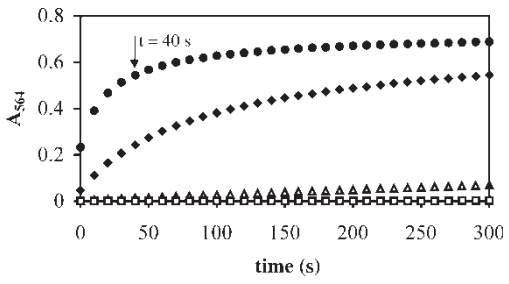


FIGURE 5 Time profile of the absorbance at 564 nm for the reaction between nitroxides **1a** (●), **1b** (◆), **2** (□) and **3** (△) (200 μM) and hydrogen donor **6** (100 μM) in acetonitrile at 293 K.

TABLE I Absorbance at 564 nm observed for hydrogen donor **6** (100 μM) in the presence of **1–3** (200 μM) in CH_3CN at 293 K at $t = 40$ s from mixing

Nitroxide	A_{564}
1a	0.545
1b	0.243
2	0.001
3	0.013

nitroxides. In the case of **2** and **3** the reaction was extremely slow and a reliable kinetic analysis was impossible. The relative reactivity of the nitroxides towards donor **6** was, therefore, evaluated by comparing the absorbance values collected in Table I that were measured for the four nitroxides at a given time, i.e. 40 s.

The kinetic data collected for **1a** and **1b** indicate that these compounds react much more easily with *N*,*N*-diethylhydroxylamine **5** than with compound **4**, this being most certainly a consequence of the lower BDE that characterises the O—H bond in the former compound (i.e. 75 kcal mol^{-1})[21] with respect to that of the C—H bond in the latter (ca. 80 kcal mol^{-1}).[22] Besides, the strength of the O—H bond in the nitroxide-derived hydroxylamines formed following hydrogen abstraction is relatively low (ca. 70 kcal mol^{-1}),[23] which would make the reaction between **1a** and **1b** with **4** more endothermic than that with **5**. The BDE value for the N—H bond in aromatic amines such as **6** is greater than 80 kcal mol^{-1};[24] if the strength of the O—H bond formed in the hydrogen abstraction process were the only factor to determine the easiness of the reaction of hydrogen abstraction by the nitroxides, the behaviour of compounds **1–3** towards **6** should not exhibit any significant difference. In fact, the BDE of TEMPO-H (69.6 kcal mol^{-1})[25] is very similar to the NO—H BDE for hydroxylamines resulting from nitroxides **1** to **2**.[23] Figure 5 clearly shows that this is not the case, and that **6** reacts with **1a** and **1b** relevantly faster than with **2** and TEMPO. Additional factors other than NO—H BDE must therefore play a role in determining the reaction rate constant. Among them, steric hindrance in the proximity of the nitroxidic function may be taken into account. In the case of **6**, its non-univocal reactivity may also be due to the presence of two different labile hydrogen atoms. In any case, it is possible to establish a general trend in the hydrogen abstraction abilities of indolinonic and indolinic nitroxides towards hydrogen donors. The 3-keto derivative **1a** reacts more readily than the 3-phenylimino **1b** that, in turn, is more reactive than **2** bearing an sp^3 carbon in position 3. This trend correlates well with the reduction potentials of these nitroxides that follow the sequence **1a** ($E_{1/2} = -0.28$ V vs. SCE in DMF)[7] > **1b** ($E_{1/2} = -0.35$ V vs. SCE in DMF)[7] > **2** ($E_{1/2} = -0.51$ V vs. SCE in DMF).†

In conclusion, a structure–activity relationship for the indolinonic and indolinic nitroxides does exist; in fact, the hydrogen abstraction ability decreases in the order **1a** > **1b** > **2**. This may explain previous findings according to which in some biological systems 3-keto derivatives were less efficient antioxidants than 3-phenylimino derivatives. However, the results presented here seem to suggest that a greater hydrogen abstraction power, that concurs in determining the pro-oxidant activity of a compound, is not directly correlated with a lower antioxidant efficiency. Indeed, despite the large differences in hydrogen abstraction abilities between indolinonic nitroxides and TEMPO, in biological systems their antioxidant efficiency is in most cases comparable. Therefore, other factors such as lipo/hydrophilicity, steric hindrance, radical scavenging capacity, polarity of the microenvironment must also play a role in determining antioxidant activity.

Acknowledgements

The authors wish to thank Università Politecnica delle Marche for financial support.

References

[1] Stipa, P., Greci, L., Carloni, P. and Damiani, E. (1997) "Synthesis and thermal stability of alkoxyamines", *Polym. Degrad. Stab.* **55**, 323–327.
[2] Carloni, P., Greci, L., Stipa, P. and Eberson, L. (1991) "Electron-transfer reactions. Oxidation of Grignard reagents in the presence of aminoxyl as a radical trapping agent", *J. Org. Chem.* **56**, 4733–4737.
[3] Damiani, E., Carloni, P., Iacussi, M., Stipa, P. and Greci, L. (1999) "Reactivity of sulfur-centered radicals with indolinonic and quinolinic aminoxyls", *Eur. J. Org. Chem.* **1**, 2405–2412.
[4] Carloni, P., Cardellini, L., Greci, L., Stipa, P. and Faucitano, A. (1989) "Homolytic substitution in indolinone nitroxide radicals. Part 5. Reactions with *tert*-butylperoxy radicals", *Gazz. Chim. Ital.* **119**, 621–625.
[5] Greci, L. (1982) "Homolytic substitution in indolinone nitroxide radicals. Part 3. Reactions with terbutoxy and methyl radical", *Tetrahedron* **38**, 2435–2439.
[6] Damiani, E., Carloni, P., Stipa, P. and Greci, L. (1999) "Reactivity of an indolinonic aminoxyl with superoxide anion and hydroxyl radicals", *Free Radic. Res.* **31**, 113–121.
[7] Carloni, P., Damiani, E., Greci, L., Stipa, P., Marrosu, G., Petrucci, R. and Trazza, A. (1996) "Chemical and electrochemical study on the interactions of aminoxyls with superoxide anion", *Tetrahedron* **52**, 11257–11264.
[8] Damiani, E., Greci, L. and Rizzoli, C. (2001) "Reaction of indolinonic aminoxyls with nitric oxide", *J. Chem. Soc. Perkin Trans.* **2**, 1139–1144.
[9] Damiani, E., Paganga, G., Greci, L. and Rice-Evans, C. (1994) "Inhibition of copper mediated low-density lipoprotein peroxidation by quinoline and indolinone nitroxide radicals", *Biochem. Pharmacol.* **48**, 1155–1161.

†The reduction potential for nitroxide **2** reported here was obtained using the same experimental conditions described in Ref. [7].

[10] Antonsiewicz, J., Popinigis, J., Wozniak, M., Damiani, E., Carloni, P. and Greci, L. (1995) "Effects of indolinic and quinolinic aminoxyls on protein and lipid peroxidation of rat liver microsomes", *Free Radic. Biol. Med.* **22**, 249–255.
[11] Damiani, E., Chokri, B., Carloni, P. and Greci, L. (2003) "Comparison of antioxidant activity between aromatic indolinonic nitroxides and natural and synthetic antioxidants", *Free Radic. Res.* **37**, 731–741.
[12] Damiani, E., Kalinska, B., Canapa, A., Canestrari, S., Wozniak, M., Olmo, E. and Greci, L. (2000) "The effects of nitroxide radicals on oxidative DNA damage", *Free Radic. Biol. Med.* **28**, 1257–1265.
[13] Falcioni, G., Gabbianelli, R., Damiani, E., Santroni, A.M., Fedeli, D., Wozniak, M. and Greci, L. (1998) "The effect of indolinonic and quinolinic nitroxide radicals on trout erythrocytes exposed to oxidative stress", *Free Radic. Res.* **28**, 507–516.
[14] Neužil, J., Thomas, S.R. and Stoker, S. (1997) "Requirement for, promotion, or inhibition by α-tocopherol of radical-induced initiation of plasma lipoprotein lipid peroxidation", *Free Radic. Biol. Med.* **22**, 57–71.
[15] Bowry, V.W. and Ingold, K.U. (1999) "The unexpected role of Vitamin E (α-tocopherol) in the peroxidation of human low-density lipoprotein", *Acc. Chem. Res.* **32**, 27–34.
[16] Colonna, M., Greci, L., Berti, C. and Marchetti, L. (1975) "Stable nitroxide radicals from phenyl-isatogen and aryl-imino derivatives with organometallic compounds", *Tetrahedron* **31**, 1745–1753.
[17] Döpp, D., Greci, L. and Nour-el-Din, A.M. (1983) "Reaction of 5,7-di-*tert*-butyl-3,3-dimethyl-3*H*-indole-1-oxide", *Chem. Ber.* **116**, 2049–2057.
[18] Colonna, M., Greci, L. and Bruni, P. (1971) "Preparazione e reazioni redox della 2,2′-difenil-3,3′-bis-indolenina", *Gazz. Chim. Ital.* **101**, 449–452.
[19] Astolfi, P., Greci, L. and Ingold, K.U. unpublished results.
[20] Andruzzi, R. and Trazza, A. (1978) "Anodic oxidation mechanism of 3-aryliminoindoles at a rotating and at a (new type of) pulsing platinum electrode in dipolar aprotic solvents", *J. Electroanal. Chem.* **86**, 201–213.
[21] Bordwell, F.G. and Liu, W.-Z. (1996) "Solvent effect on homolytic bond dissociation energies of hydroxylic acids", *J. Am. Chem. Soc.* **118**, 10819–10823.
[22] Mar'in, A., Greci, L. and Dubs, P. (2002) "Antioxidant activity of benzofuranone stabilizers in polypropylene. The 3-aryl-benzofuran-2-one used is known as Irganox®HP-136", *Polym. Degrad. Stab.* **76**, 489–494.
[23] Amorati, R., Lucarini, M., Mugnaini, V., Pedulli, G.F., Minisci, F., Recupero, F., Fontana, F., Astolfi, P. and Greci, L. (2003) "Hydroxylamines as oxidant catalysts: thermochemical and kinetic studies", *J. Org. Chem.* **68**, 1747–1754.
[24] Denisov, E.T. (1995) *Handbook of Antioxidants* (CRC Press, Boca Raton).
[25] Mahoney, L.R., Mendenhall, G.D. and Ingold, K.U. (1973) "Calorimetric and equilibrium studies on some stable nitroxide and iminoxy radicals. Approximate oxygen-hydrogen bond dissociation energies in hydroxylamines and oximes", *J. Am. Chem. Soc.* **95**, 8610–8614.