Free Radical Research, Vol. 35, pp. 895–905
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Antioxidative Activity of Hydroxylamines. ESR Spectra of Radicals Derived from Hydroxylamines

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Accepted by Professor B. Kalyanaraman

(Received 25 February 2001; In revised form 5 April 2001)

The antioxidative activity of hydroxylamines was evaluated for the oxidation of tetralin at 61°C and linoleic acid micelles in an aqueous dispersion at 37°C, induced by an azo initiator. The antioxidative efficacy of the hydroxylamines for the oxidation of tetralin was smaller than that of α-tocopherol. However, the hydroxylamines showed more potent antioxidative activity than that of the α-tocopherol against the oxidation of linoleic acid micelles. On the basis of the results of an ESR study and the oxidation product obtained, it is suggested that active position in hydroxylamines depend not only on hydroxyl hydrogen-atom, but also on the allylic hydrogen atom.

Keywords: Antioxidant; Hydroxylamine; ESR; Radicals

INTRODUCTION

All organic substances, such as oils, plastics, and rubber products are degraded by autoxidation with reactive oxygen-derived species. The oxidation of polyunsaturated fatty acids in lipids has received as much attention as organic substances recently in connection with its pathological effects such as cancer[1] and aging.[2] As a result, a great deal of research has been devoted to kinetic and mechanistic studies on the antioxidative effects of natural and synthetic antioxidants. For example, phenols such as vitamin E (tocopherol) are a major lipid-soluble peroxyl radical-trapping antioxidant in human blood.[3] There have been a number of studies concerning the antioxidative mechanism and synergistic effects.[4,5] The antioxidative properties of tocopherols have been ascribed to their capacity to rapidly transfer hydrogen atom to peroxyl radicals. The high activities of tocopherols have been interpreted by Ingold *et al.* in terms of stereo-electronic effects stabilizing the tocopheryl radical formed in rate-controlling inhibition reactions.[6]

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It is known that nitrones are excellent spin trapping reagents.[7] For example, phenyl-*N*-*t*-butylnitrone (PBN) traps not only carbon radical but also peroxyl radical to give an extremely stable aminoxyl (nitroxide). On this basis, we expect *N*-phenyl- and *N*-*t*-butylhydroxylamines to act as antioxidants, since hydroxylamines have a reactive hydrogen atom and must act first as a radical scavenger by donating the hydrogen to give aminoxyl. More recently, hydroxylamines and their oxidized forms (aminoxyl radical) has been found to play a variety of roles in biological systems, and extensive biological and physiological research has come to be carried out.[8,9]

In this paper, we report the antioxidant activity of PBN model compounds, hydroxylamines (R_1R_2NOH) in the oxidation of tetralin. Furthermore, ESR technique is used in this study to determine the active species for the autoxidation.

MATERIALS AND METHODS

Reagents

The antioxidants examined in this study were divided into four classes as shown in Fig. 1: *N*-*tert*-butyl-*N*-(1-phenylethyl) hydroxylamine **1**,[10] *N*-phenyl-*N*-benzylhydroxylamine **2**, *N*-phenyl-*N*-(1-phenylethyl)hydroxylamines **3**, *N*-phenyl-*N*-(2,2-dimethylpropyl-1-phenyl)hydroxylamines **4**. These hydroxylamines (R_1R_2NOH) were prepared by the methods depicted in Fig. 2.[10] Accordingly, secondary amines underwent tungstate-catalyzed oxidation with hydrogen peroxide to give nitrones **5, 6**. The reaction of these nitrones with the Grignard reagent[10] or $LiAlH_4$[11] gave the corresponding *N*-hydroxylamines **1, 3**, and **4**, and **2**, respectively.

General Procedure for Preparation of Amine *N*-oxides 6

Sodium tungstate (0.2 g, 0.6 mmol) dihydrate was added to a three-necked flask. After the flask was flushed with nitrogen, 30 ml of methanol and 2.7 g (15 mmol) of *N*-phenylbenzylamine were added. To an ice-cooled mixture of amine was added dropwise, 4 ml of 30% aqueous hydrogen peroxide solution and the mixture was stirred for 30 min. After removal of the cooling bath, the mixture was stirred for 4 h. The excess hydrogen peroxide was decomposed by adding *ca.* 2 g of sodium hydrogen sulfite with ice cooling. After the usual work-up, the residue was chromatographed on silica (methylene chloride:hexane = 2:1) to give a crude product. The crystallization of the crude product from a mixture of methylene chloride and hexane (2:1) provided a colorless power of *N*-benzylideneaniline *N*-oxide **6a** (1.4 g, 48%): mp 116–117°C. ^{1}H NMR ($CDCl_3$) δ: 7.47–7.49 (m, 6H), 7.77–7.79 (m, 2H), 7.93 (s, 1H), 8.39–8.41 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 121.8, 128.7, 129.0, 129.2, 129.9, 130.7, 130.9, 134.6. MS *m/z* (rel. intensity, %): 197 (M^+, 67), 196 (100), 181 (20), 180 (35), 169 (9), 168 (19), 105 (9), 91 (40), 77 (27).

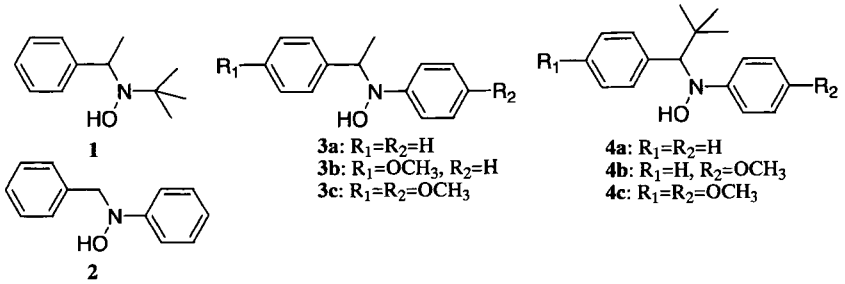

FIGURE 1 Antioxidants used in this study.

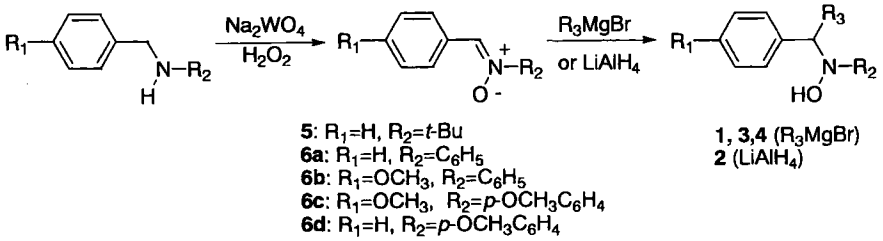

FIGURE 2 Synthetic route of hydroxylamines *via* nitrones.

N-(4-methoxybenzylidene)aniline N-oxide (6b)

Yield, 37%. mp 121–122°C. ^{1}H NMR ($CDCl_3$) δ: 3.87 (s, 3H), 6.99 (d, J = 9.1 Hz, 2H), 7.45–7.47 (m, 3H), 7.76–7.78 (m, 2H), 7.86 (s, 1H), 8.41 (d, J = 9.1 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ: 55.4, 114.1, 121.7, 123.8, 129.1, 129.6, 131.2, 134.1, 148.9, 161.5.

N-(4-methoxybenzylidene)-N-(4′-methoxyaniline) N-oxide (6c)

Yield, 81%. mp 154–155°C. ^{1}H NMR ($CDCl_3$) δ: 3.68 (s, 3H), 3.88 (s, 3H), 6.95 (d, J = 9.1 Hz, 2H), 6.99 (d, J = 9.1 Hz, 2H), 7.72 (d, J = 9.1 Hz, 2H), 7.81 (s, 1H), 8.38 (d, J = 9.1 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ: 55.4, 55.6, 114.0, 122.9, 123.9, 130.9, 133.3, 142.4, 160.4.

N-benzylidene-N-(4-methoxyaniline) N-oxide (6d)

Yield, 67%. mp 136–137°C. ^{1}H NMR ($CDCl_3$) δ: 3.85 (s, 3H), 6.95 (d, J = 9.1 Hz, 2H), 7.45–7.47 (m, 3H), 7.72 (d, J = 9.1 Hz, 7.88 (s, 1H), 8.37–8.39 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 55.6, 114.0, 122.9, 128.6, 128.9, 130.7, 130.9, 133.6, 142.5, 160.6.

Preparation of Hydroxylamines 3 and 4

To a solution of the above *N*-benzylideneaniline *N*-oxide **6a** (0.5 g, 2.6 mmol) in 60 ml of THF was added dropwise, 3 M solution of methylmagnesium bromide (1.3 ml, 3.8 mmol) in ether at room temperature. After stirring at the same temperature for 2 h, saturated aqueous ammonium chloride and water were added. The resulting mixture was extracted with ether. After the usual work-up, the residue was recrystallized from a mixture of methylene chloride and hexane (2:1) to give *N*-phenyl-*N*-(1-phenylethyl)hydroxylamine **3a** (0.4 g, 74%) as colorless crystals: mp 70–71°C. ^{1}H NMR ($(CD_3)_2CO$) δ: 1.48 (d, J = 6.6 Hz, 3H), 4.82 (q, J = 6.6 Hz, 1H), 6.82–6.85 (m, 1H), 7.15–7.27 (m, 7H), 7.44–7.46 (m, 2H), 7.63 (s, 1H). ^{13}C NMR ($(CD_3)_2CO$) δ: 16.4, 64.9, 117.7, 121.5, 125.7, 127.5, 128.6, 129.0, 129.2, 129.3, 129.6, 143.2, 153.7. Calcd for $C_{14}H_{15}NO$: C, 78.84; H, 7.09; N, 6.57. Found: C, 78.45; H, 7.04; N, 6.57.

N-(4-methoxy-1-phenylethyl)-N-phenylhydroxylamine (3b)

Yield, 48%. mp 72–73°C. ^{1}H NMR ($(CD_3)_2CO$) δ: 1.46 (d, J = 7.0 Hz, 3H), 3.75 (s, 3H), 4.78 (q, J = 7.0 Hz, 1H), 6.79–6.84 (m, 3H), 7.15–7.21 (m, 4H), 7.33–7.35 (m, 2H), 7.52 (s, 1H). ^{13}C NMR ($(CD_3)_2CO$) δ: 16.5, 55.3, 64.4, 113.8, 117.6, 121.4, 129.1, 130.1, 134.9, 153.7, 159.5. Calcd for $C_{15}H_{17}NO_2$: C, 74.05; H, 7.04; N, 5.76. Found: C, 73.61; H, 6.70; N, 5.72.

N-(4-methoxy-1-phenylethyl)-N-(4′-methoxyphenyl)-hydroxylamine (3c)

Yield, 10%. mp 83°C (decompd). ^{1}H NMR ($(CD_3)_2CO$) δ: 1.39 (d, J = 6.6 Hz, 3H), 3.72 (s, 3H), 3.75 (s, 3H), 4.49 (q, J = 6.6 Hz, 1H), 6.77–6.81 (m, 4H), 7.09 (d, J = 8.4 Hz, 2H), 7.29 (d, J = 8.4 Hz, 2H), 7.42 (s, 1H). ^{13}C NMR ($(CD_3)_2CO$) δ: 17.4, 55.3, 55.5, 66.1, 113.7, 114.2,

120.7, 130.1, 135.4, 147.2, 155.9, 159.4. Calcd for $C_{16}H_{19}NO_3$: C, 70.31; H, 7.01; N, 5.12. Found: C, 69.92; H, 6.91; N, 4.97.

N-(2,2-dimethylpropyl-1-phenyl)-N-phenylhydroxylamine (4a)

Yield 65%. mp 79–80°C. ^{1}H NMR ($(CD_3)_2CO$) δ: 1.18 (s, 9H), 4.42 (s, 1H), 6.62–6.69 (m, 1H), 7.06–7.07 (m, 7H), 7.50–7.52 (m, 2H), 7.69 (s, 1H). ^{13}C NMR ($(CD_3)_2CO$) δ: 36.5, 78.5, 116.7, 120.5, 127.8, 128.9, 131.9, 139.3, 154.8. Calcd for $C_{17}H_{21}NO$: C, 79.96; H, 8.29; N, 5.49. Found: C, 79.57; H, 8.08; N, 5.51.

N-(2,2-dimethylpropyl-1-phenyl)-N-(4-methoxyphenyl)-hydroxylamine (4b)

Yield, 27%. mp 72–73°C. ^{1}H NMR ($(CD_3)_2CO$) δ: 1.16 (s, 9H), 3.64 (s, 3H), 4.18 (s, 1H), 6.64 (d, $J = 9.0$ Hz, 2H), 6.96 (d, $J = 9.0$ Hz, 2H), 7.12–7.15 (m, 3H), 7.40–7.48 (m, 2H), 7.53 (s, 1H). ^{13}C NMR ($(CD_3)_2CO$) δ: 36.4, 55.4, 80.3, 114.0, 118.9, 127.3, 127.7, 132.1, 139.1, 148.6, 154.8. Calcd for $C_{18}H_{23}NO_2$: C, 75.76; H, 8.12; N, 4.91. Found: C, 75.40; H, 8.03; N, 4.84.

N-[2,2-dimethylpropyl-1-(4-methoxyphenyl)]-N-(4′-methoxyphenyl)hydroxylamine (4c)

Yield, 27%. mp 74–75°C. ^{1}H NMR ($(CD_3)_2CO$) δ: 1.15 (s, 9H), 3.64 (s, 3H), 3.71 (s, 3H), 4.11 (s, 1H), 6.64 (d, $J = 8.8$ Hz, 2H), 6.71 (d, $J = 8.8$ Hz, 2H), 6.96 (d, $J = 8.8$ Hz, 2H), 7.36 (d, $J = 8.8$ Hz, 2H), 7.42 (s, 1H). ^{13}C NMR ($(CD_3)_2CO$) δ: 36.5, 55.1, 55.4, 79.8, 112.9, 114.0, 118.9, 130.1, 131.9, 130.9, 133.1, 148.8, 154.8, 159.3. Calcd for $C_{19}H_{25}NO_3$: C, 72.35; H, 7.99; N, 4.44. Found: C, 71.94; H, 7.80; N, 4.39.

Preparation of Hydroxylamines 2

A solution of *N*-benzylideneaniline *N*-oxide **6a** (1.97 g, 0.01 mol) in dry ether (10 ml) was added dropwise to a suspension of excess lithium aluminum hydride (1.52 g, 0.04 mol) in ether solution (10 ml) at 0°C. The whole mixture was stirred at room temperature for 4 h. After usual work-up, the residue was purified by recrystallization to give *N*-phenyl-*N*-benzylhydroxylamine **2** (0.8 g, 0.004 mol) in 40% yield. Mp 72–73°C. ^{1}H NMR δ: 4.47 (s, 2H), 6.86–6.92 (m, 1H), 7.21–7.42 (m, 7H), 7.44–7.49 (m, 2H), 7.99 (s, 1H). ^{13}C NMR δ: 64.2, 116.7, 127.3, 128.2, 128.5, 128.9, 136.8, 152.2.

General

Melting point (mp) data were measured with Yanaco MP-J3 micro-melting apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded with a Jeol GSX-400 spectrometer operated at 400 MHz for ^{1}H and at 100.6 MHz for ^{13}C in $CDCl_3$ unless otherwise noted, and chemical shift data are with reference to $(CH_3)_4Si$. Electron spin resonance measurements were performed on a Jeol-FE-1X (X-band) with 100 kHz field modulation at room temperature. Mass spectra were recorded with a Perkin–Elmer model 910 gas chromatograph–mass spectrometer at 70 eV.

Assay of Antioxidant Activity

The rate of oxygen absorption was measured either by following the oxygen concentration in the solution or by measuring the volume of oxygen consumption during oxidation. The rate of oxygen absorption of linoleic acid micelle initiated by 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) in the presence and absence of antioxidant was measured with a Biological Oxygen Monitor, Model YSI 5300 (Yellow Springs Instrument Co., Inc., Yellow Springs, OH) at 37°C. The volume of oxygen consumption was measured as a function of time under 760 Torr (1 Torr = 133.322 Pa) of O_2 with tetralin containing an antioxidant and α,α'-azobisisobu-

tyronitrile (AIBN) as the initiator. The oxidation temperature was maintained at 61±0.1°C. The induction period (t_{inh}) was graphically determined from the length of time between initiator injection and the point of intersection of the tangents to the oxidation curve corresponding to the initial inhibited and final uninhibited rates of oxidation.

RESULTS AND DISCUSSION

Scavenging Effect of Hydroxylamines on the AIBN-Induced Peroxidation in Tetralin Solution

Figure 3 shows examples of oxygen-uptake curves for the oxidation of tetralin initiated by AIBN at 61°C. In the absence of an antioxidant (control), the oxidation proceeded with a very brief initiation period at a constant rate of oxygen uptake. In the presence of α-Toc, which is a well-known radical scavenger, the rate of oxygen uptake was suppressed. When the induction period was over, the oxidation proceeded at the same rate as that in the absence of a radical scavenger, and there was a measurable induction period. Similarly, hydroxylamines **1–4** suppressed the oxidation. The addition of hydroxylamine **1** significantly decreased the rate of oxygen absorption. However, the value of t_{inh} with **1** was shorter than that of α-Toc. The data regarding the antioxidative activities of **1–4**, evaluated by t_{inh}, rate constant k_{inh}/k_p, and stoichiometric number (n) of the peroxyl radicals trapped by each antioxidant, are listed in Table I.

At first, the stoichiometric factors (n) were determined using the induction-period method relative to α-tocopherol (α-Toc), for which $n = 2$ was assumed.[6,12] Less hindered hydroxylamine

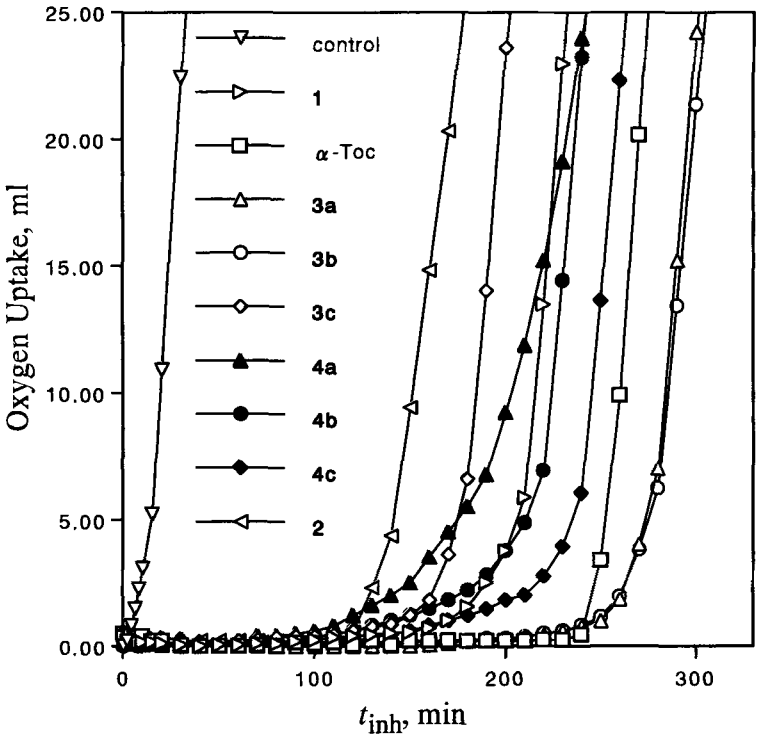

FIGURE 3 Rate of oxygen uptake in the oxidation of tetralin initiated by 10 mM AIBN in the absence (control) and presence of 1 mM antioxidant at 61°C under oxygen.

TABLE I Inhibition of the oxidation of tetralin by 1 mM of an antioxidant initiated by 10 mM α,α′azobisisobutyronitrile (AIBN) at 61°C

Compound number	t_{inh} (min)	R_{inh} ($\times 10^8$ M/s)	k_{inh}/k_p ($\times 10^3$)	$k_{inh}(R_1R_2NOH)/k_{inh}$ (α-Toc)	n
1	196	8.41	7.21	0.17	1.6
2	121	2.48	39.6	0.94	1.0
3a	262	3.31	13.7	0.33	2.2
3b	262	2.37	19.1	0.46	2.2
3c	166	10.5	6.82	0.16	1.4
4a	213	57.7	0.96	0.02	1.8
4b	204	15.1	3.85	0.09	1.7
4c	225	16.4	3.22	0.08	1.9
α-Toc	238	1.19	41.9	1.00	(2.0)

2 showed a low n value, presumably being related to an $R_1R_2NO^{\cdot}$ wasting reaction that resulted from a chain-transfer reaction with the substrate and a bimolecular self-reaction.[12] However, the stoichiometric factor for hydroxylamines **1**, **3**, and **4** was similar except for **3c**. This finding indicated that hydroxylamines can trap two peroxyl radicals. The t_{inh}, or n values for compounds **1**, **3** and **4** were almost the same in contrast to the remarkable difference observed for the k_{inh}/k_p values. That is, the antioxidative activities as measured by the ratio k_{inh}/k_p decreasing in the order, α-Toc > **2** > **3b** > **3a** > **1**, **3c** > **4b**, **4c** > **4a**. From this data, the relative efficiency of hydroxylamines as an inhibitor with respect to α-Toc, $k_{inh}(R_1R_2NOH)/k_{inh}$(α-Toc), can be worked out as 0.1–0.9. It is clear that α-Toc has a higher antioxidative activity evaluated by the $k_{inh}(R_1R_2NOH)/k_{inh}$(α-Toc) values. α-Toc was found to scavenge the peroxyl radical generated from tetralin more quickly than hydroxylamines **1**–**4**. By comparing the substituents on the benzyl position between **3a** and **4a**, **3c** and **4c**, a methyl group was found to give a higher k_{inh}/k_p value than a *t*-butyl group. From these results, the reduction in antioxidative activity of hydroxylamines **4** compared with **1**–**3** suggests that a bulky *t*-butyl group attached to a benzyl position can cause a noticeable steric hindrance to approach of a peroxyl radical. However, this hindrance decreases in R_1R_2NOH **1**–**3**. Similar observations have been reported.[12] In other words, phenols having bulky *t*-butyl group on both the *o*-positions and said to be the most effective, show a lower antioxidative activity than phenols having one vacant *o*-position.[13]

As expected from antioxidative mechanisms, t_{inh} would be proportional to the concentration of the antioxidant.[14] Figure 4 shows a plot of an induction period as a function of $[R_1R_2NOH]/[AIBN]$, where R_1R_2NOH is an antioxidant. The t_{inh} produced by the addition of **3a** for the oxidation of tetralin initiated with AIBN is proportional to $[R_1R_2NOH]/[AIBN]$.

ESR Spectra of Radicals Derived from Hydroxylamines

To determine the active species for the antioxidative activity of hydroxylamines, the reaction of hydroxylamine and stable aminyl radical, 1,1′-diphenyl-2-picrylhydrazyl (DPPH) was carried out in benzene with electron spin resonance (ESR) spectroscopy. When 530 μM of hydroxylamine **1** was added to a solution containing 53 μM of DPPH at room temperature, the ESR signal of DPPH disappeared rapidly and a new radical signal appeared and the solution was colored to yellow. The ESR signal showed six lines based on hyperfine splitting by the nitrogen ($g = 2.0070$, $a_N = 1.18$ mT) and the hydrogen atoms ($a_H = 0.28$ mT). The hyperfine structures and *g*-value indicated in the figure are consistent with the generation of the aminoxyl (Fig. 5).[15]

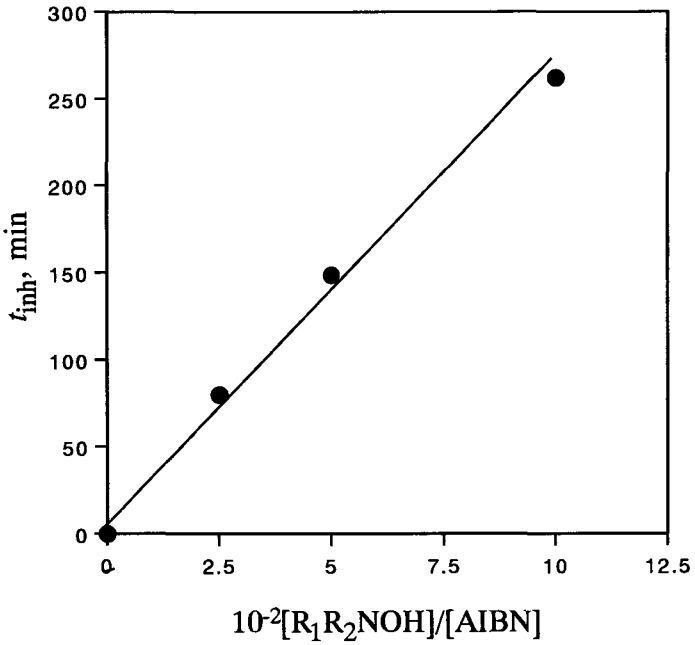

FIGURE 4 Induction period produced by **3a** for the oxidation of tetralin induced by AIBN at 61°C.

Reactions of DPPH with other hydroxylamines **2–4** were also carried out under the same reaction conditions. In these cases, a new ESR signal was also observed as shown in Figs. 6 and 7. An ESR simulation attempt was made to determine the spectral parameters of the radical **3a** and **4a**. The best fit between experimental (Fig. 6a) and simulated spectra

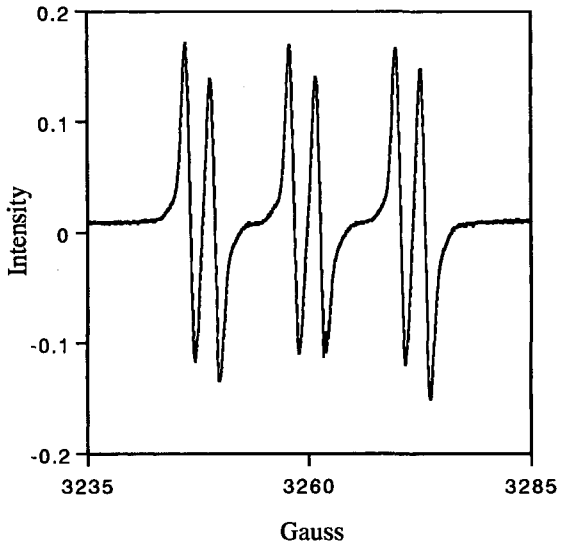

FIGURE 5 ESR spectrum of an aminoxyl obtained by exposing a mixture of 530 mM 1 and 53 mM DPPH in benzene under vacuum at room temperature.

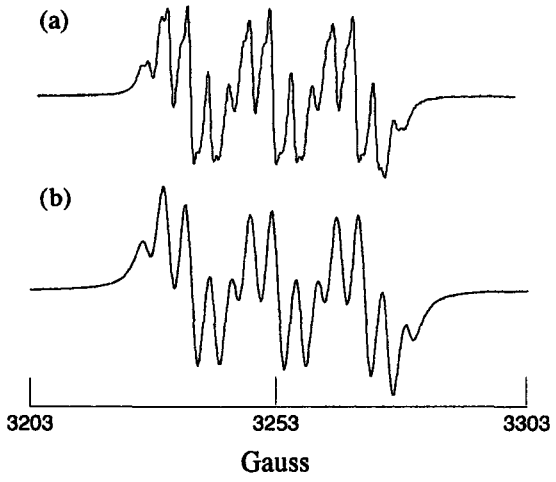

FIGURE 6 (a): ESR spectrum observed after hydroxylamine **4a** was dissolved in benzene containing DPPH under vacuum at room temperature. (b): Computer simulation of the ESR spectrum shown in Fig. 6a. The best fit was obtained using the following parameters: $a_N = 0.87$, $a_H = 0.27$ and 0.21 mT.

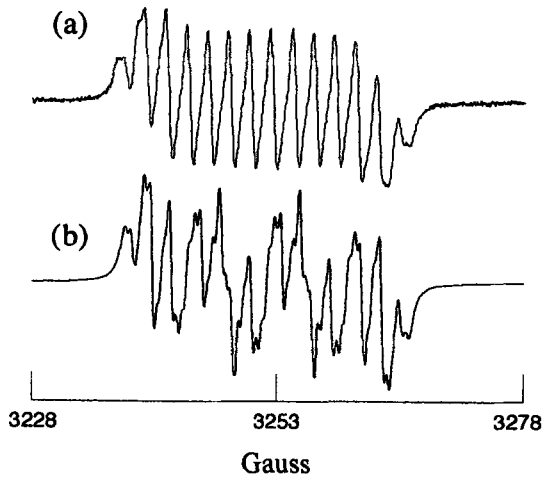

FIGURE 7 (a): ESR spectrum observed after hydroxylamine **3a** was dissolved in benzene containing DPPH under vacuum at room temperature. (b): Computer simulation of the ESR spectrum shown in Fig. 7a: $a_N = 1.07$, $a_H = 0.68, 0.26$, and 0.09 mT.

(Fig. 6b) was obtained with the following parameters: $a_N = 0.87$, $a_H = 0.27$ and 0.21 mT. These results provide evidence for the generation of the aminoxyl. However, the spectral line shape between experimental (Fig. 7a) and simulated (Fig. 7b) from coupling constants reported in literature[16] was clearly different, suggesting the mixture with another type of radical species. Therefore, further study is required to analyze the ESR signal observed from hydroxylamines **3**.

To confirm the formation of aminoxyl for the oxidation of tetralin in the presence of hydroxylamines, ESR spectra of the reaction mixture were measured under the same reaction conditions as those used in Fig. 3. Figure 8 shows the radical concentration observed in the oxidation of tetralin initiated with AIBN in the presence of hydroxylamine **3a**. Figure 8 also shows the ESR spectrum after 5 min from the start of the oxidation reaction, whose coupling pattern is consisted with those for the hydroxylamine aminoxyl (data not shown). The resulting spectrum increased gradually (which was superimposed to the original peak) and after passing through a maximum, it decreased. After the induction period was over ($t_{inh} = 262$ min), the signal of the aminoxyl decreased to almost complete disappearance. On the other hand, no ESR signal was observed for the oxidation of

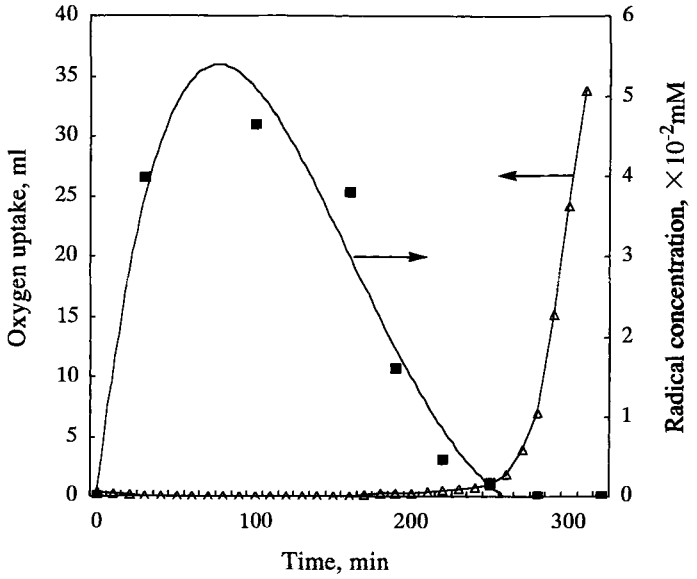

FIGURE 8 Relationships between oxygen uptake and radical concentration during the oxidation of tetralin by 1 mM **3a** initiated by 10 mM AIBN at 61°C.

tetralin initiated by AIBN in the absence of hydroxylamines.

Competitive Hydrogen-atom Abstraction from Hydroxylamines by a Peroxyl Radical

From the results of an ESR study, hydroxylamines were found to scavenge a peroxyl radical by donating hydroxyl hydrogen-atom to give an intermediate aminoxyl, which gives the ESR signal and then reacts with another radical to give a stable product. However, the mechanism for the antioxidative behavior of this aminoxyl remains unresolved, and we consider that it is important to determine the second active position. We therefore expect the benzylic hydrogen of hydroxylamine to play an important role in the antioxidative activity. That is, hydroxylamines may be ascribed to the formation of the benzyl radical from the abstraction of a benzylic hydrogen.[17]

A solution of an equimolar amount of hydroxylamine **2** and AIBN (0.01 mol) in tetralin (5 ml) was stirred at 61°C under oxygen, and oxidation products were monitored by GLC at appropriate time intervals. At an earlier stage of the reaction, an oxidation product was produced as the major product and after passing through a maximum (0.5 h), it decreased. The structure of the main oxidation product was determined by GC–MS spectroscopy. The GC–MS gave *m/z* 197 as an M^+ ion and a fragmentation pattern agreed with the authentic sample of *N*-benzylideneaniline *N*-oxide **6a** (see experimental section). From the results of the present study and those reported in literature,[18–21] a plausible mechanism for the formation of *N*-benzylideneaniline *N*-oxide is shown in Fig. 9. The hydroxylamine has a reactive hydrogen and must act first as a peroxyl radical scavenger by donating the hydrogen to give aminoxyl. Another peroxyl radical abstracts the benzylic hydrogen of the resulting aminoxyl to give a *N*-benzylideneaniline *N*-oxides (nitrone).

Antioxidant Activity of Hydroxylamines for the Oxidation of Linoleic Acid in Aqueous Dispersions

Using the above procedure, the auto-oxidation of linoleic acid micelles in Tween 20 aqueous dispersions was carried out under oxygen at 37°C. Figure 10 shows results of the oxidation of linoleic acid micelles induced by AAPH in the presence of hydroxylamines 1, 3–4, and α-Toc. One of the most striking results from our study becomes apparent when the k_{inh} (R_1R_2-NOH)/k_{inh}(α-Toc) value is compared with the tetralin and linoleic acid micelles. The k_{inh}(R_1-R_2NOH)/k_{inh}(α-Toc) value for the hydroxylamines is 3–80-fold higher in an aqueous system relative to a tetralin system. The reduction in the antioxidative activity of α-Toc in the aqueous micelles is attributed to the effect of hydrogen bonding by water with a lone pair on a *para* ether oxygen atom. This inhibits the stereo electronic effect, which raises the antioxidative activity in the tetralin system.

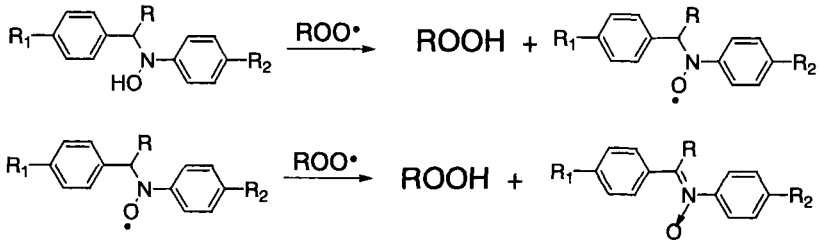

FIGURE 9 Mechanism of the free radical scavenging reaction of hydroxylamines.

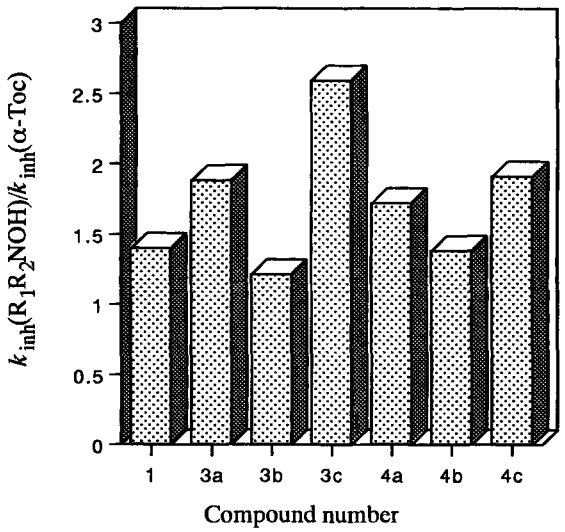

FIGURE 10 Antioxidative activity denoted by $k_{inh}(R_1R_2NOH)/k_{inh}(\alpha\text{-Toc})$ for the oxidation of 62.5 mM linoleic acid in Tween 20 aqueous micelle dispersions initiated by 0.67 mM AAPH in the presence of 0.5 mM antioxidant at 37°C.

CONCLUSION

In conclusion, the overall efficiency of hydroxylamines can be determined by the induction period, as well as by the ratio of $k_{inh}(R_1R_2NOH)/k_{inh}(\alpha\text{-Toc})$ for the oxidation of tetralin and linoleic acid micelles in aqueous dispersions. The antioxidative activity for hydroxylamines was found to be lower than that for α-Toc in a tetralin system. However, the hydroxylamines had a higher antioxidative activity than the α-Toc, when evaluated by the $k_{inh}(R_1R_2NOH)/k_{inh}(\alpha\text{-Toc})$ value in linoleic acid micelles. On the basis of the results of an ESR study and oxidation product, it was suggested that active positions in hydroxylamines depends not only on hydroxyl hydrogen-atom, but also on the allylic hydrogen atom.

Acknowledgements

This work was partially supported by the Kansai University Grant-in-Aid for the Faculty Joint Research Program, 2001.

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