Oxidation of Lipids. XVI. Inhibition of Autoxidation of Methyl Linoleate by Diarylamines

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The inhibition of oxidation of methyl linoleate in homogeneous solution and in aqueous dispersions by diarylamines has been studied. Diphenylamines with electron-donating substituents exhibited antioxidant activities, while those having electron-withdrawing substituents such as niflumic acid and flufenamic acid did not suppress the oxidation appreciably. When both 4,4′-dimethoxydiphenylamine and α-tocopherol were present as antioxidants, only α-tocopherol was consumed at first and when α-tocopherol was almost completely depleted, 4,4′-dimethoxydiphenylamine began to be consumed to give nitroxide radical, which further acted as antioxidant.

Oxygen is a double-edged sword: various useful and important chemicals are synthesized from the oxidations of organic compounds by molecular oxygen, but at the same time, oxygen is responsible for oxidative degradation and deterioration of plastics, rubber, oils, foods and even biological membranes and tissues.[1,2] Various kinds of both natural and synthetic antioxidants have been explored and used to protect biological, chemical, and industrial molecules from the oxygen toxicity.[3] Especially, phenols and amines have been used extensively as antioxidants.[4] The inhibition of oxidation by secondary amines has been studied by several groups.[4] Amines scavenge the chain-carrying peroxyl radicals to interrupt the chain propagation reaction giving N-centered aminyl radicals, which may dimerize, disproportionate, react with a second peroxyl radical on nitrogen to give a nitroxide or on carbon to give quinonoid products.[4-10] The diaryl nitroxides may react not only with carbon radical but also with peroxyl radicals to give quinone imine *N*-oxides.[11] Diaryl nitroxides react also with hydroperoxides. Thus, the amines are transformed by several competing reactions into various products which may also act as antioxidants. The relative importance of the above competing reactions depends on the reaction conditions and variables and hence the mechanism for antioxidant action by amines and stoichiometry also depends on the conditions.

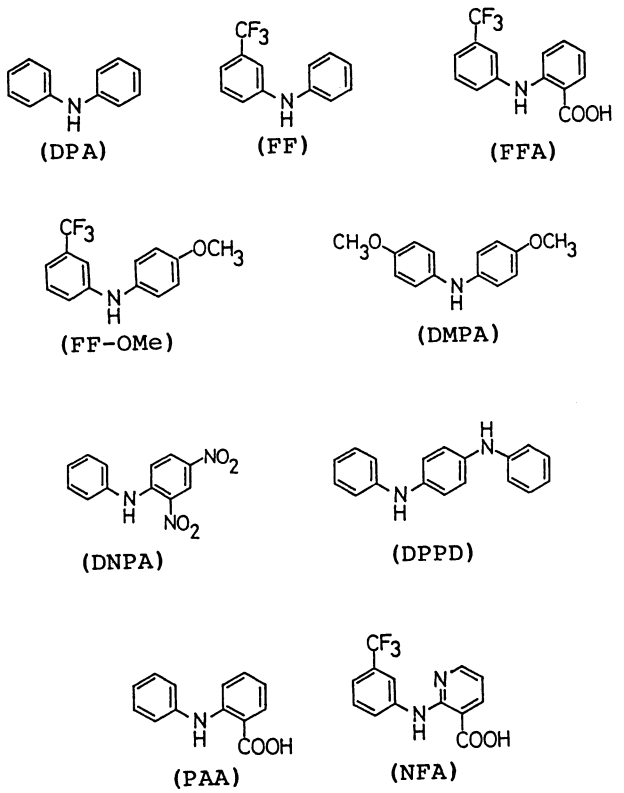


Fig. 1. Diarylamines used in this study and their abbreviations.

We have recently found that disodium 4-chloro-2,2′-iminodibenzoate and disodium 4-chloro-3′,6′-dimethyl-2,2′-iminodibenzoate, the water-soluble derivatives of diphenylamine, function as chain-breaking antioxidants in the oxidations of methyl linoleate micelles and phosphatidylcholine liposomes, oxidative hemolysis of red blood cells and in free radical-mediated damage of biological tissues.[12] The objective of the present work is to study the inhibition of oxidation of methyl linoleate by several types of lipophilic diarylamines. Figure 1 shows the diarylamines used in this study.

Experimental

Commercial methyl linoleate was purified by silica-gel column before use. 2,2′-Azobis(2,4-dimethylvaleronitrile) (AMVN) and 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) were obtained from Wako Pure Chemical Ind. and used as lipid-soluble and water-soluble radical initiators respectively. Di-*t*-butyl diperoxyoxalate (DBPO), used as a *t*-butoxyl radical source, was prepared and purified as described in the literatures.[13,14] Commercial diarylamines, galvinoxyl and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were used as received. RRR-α-tocopherol was kindly supplied by Eisai Co., Ltd.

Methyl linoleate micelles were prepared as reported previously[15] by mixing methyl linoleate and aqueous solution of 10 mM Triton X-100 vigorously with Vortex mixer for 2 minutes. The oxidation was carried out at 37 °C under air. The rate of oxygen uptake was followed with either a

pressure transducer or an oxygen electrode.[15] The rates of consumption of α-tocopherol and diarylamines were followed with a high-performance liquid chromatography. Electron spin resonance (ESR) spectra were recorded on a X-band JEOL FE1X spectrometer.

Results and Discussion

Inhibition of Oxidation of Methyl Linoleate by Diarylamines. The oxidation of methyl linoleate by molecular oxygen induced by a radical initiator proceeds by a free radical chain mechanism and gives four isomeric, conjugated diene hydroperoxides quantitatively as primary products.[16-19] Therefore, the rate of oxidation can be measured quantitatively by following the rate of either oxygen uptake or conjugated diene hydroperoxides formation.[19] In the presence of AMVN but absence of diarylamine, the oxidation proceeded without induction period and a constant rate of oxygen uptake was observed. As observed previously,[15] α-tocopherol (vitamin E) suppressed the oxidation markedly and after some inhibition period, the oxidation proceeded at the similar rate as that without α-tocopherol. α-Tocopherol was consumed linearly with time and when it was depleted the inhibition period was over. Similarly, DPPD, DPA, FF-OMe, and DMPA suppressed the oxidation, but FF, FFA, PAA, DNPA, and NFA did not suppress the oxidation. The pertinent results are summarized in Table 1. It can be seen that electron-donating substituent such as methoxy group on the aromatic ring promotes the antioxidant activity of diarylamine while electron-withdrawing substituent such as trifluoromethyl and nitro group reduces its antioxidant activity. Table 1 shows that DMPA, DPPD, and α-tocopherol act as strong antioxidants and suppress the oxidation markedly, while FF-OMe and DPA function less efficiently and reduce the rate of oxidation (R_{inh}/R_p) to one half to one fourth. Niflumic acid (NFA)[20] and flufenamic acid (FFA)[21] known as anti-inflammatoires had little antioxidant activities.

Table 1. Inhibition of Oxidation of Methyl Linoleate (MeLH) in Benzene by Diarylamines (IH) at 37 °C under Air

MeLH	AMVN	IH		t_{inh}[a]	10^7R_{inh}[b]	10^7R_p[c]
mM	mM		μM	s	M s^{-1}	M s^{-1}
159	10.5	None	0	0		3.04
151	16.1	α-TOC	50.0	1620	0.62	3.16
151	16.1	DPPD	50.0	1950	0.19	3.00
159	10.5	DMPA	50.0	2940	0	2.78
151	16.1	DMPA	50.0	1800	0	2.81
159	10.5	DPA	31.1	1800		2.50
159	10.5	DPA	50.1	3663	1.21	2.66
151	16.1	DPA	50.0	2220	1.70	3.05
159	10.5	FF-OMe	32.5	1800	0	2.28
159	10.5	FF-OMe	100	5280	0.87	3.60
152	10.1	FF-OMe	50.0	3120	0.82	3.12
151	16.1	FF-OMe	50.0	2040	0.34	3.09
159	10.4	FF	50.0	0		2.79
159	10.5	FFA	50.0	0		2.84
151	16.1	FFA	50.0	0		2.81
116	12.4	PAA	115	0		1.78
151	16.1	PAA	50.0	0		3.09
116	12.4	NFA	115	0		2.30
151	16.1	NFA	50.0	0		2.96
159	10.5	DNPA	50.0	0		2.78

a) Induction period. b) Rate of oxidation during the induction period. c) Rate of oxidation without antioxidant or after induction period.

Figure 2 shows the plot of inhibition period as a function of [IH]/[AMVN], where IH is an antioxidant such as diarylamine and α-tocopherol. As observed previously, the inhibition period was proportional to the antioxidant concentration and inversely proportional to the rate of chain intiation.[15,22-27]

Figure 3 shows the rates of disappearance of α-tocopherol and FF-OMe during the oxidation of methyl linoleate in benzene in the presence of both antioxidants. Interestingly, α-tocopherol was consumed first, while FF-OMe remained constant at first and it began to be consumed after substantially all of α-tocopherol disappeared. Fast oxidation took place when both antioxidants were depleted.

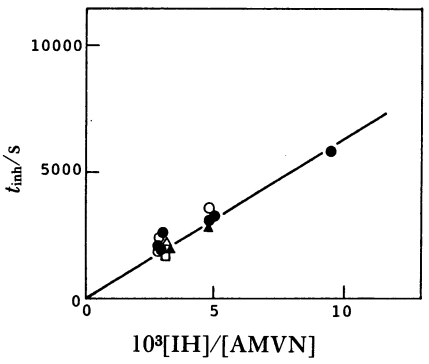


Fig. 2. Plot of inhibition period as a function of [IH]/[AMVN] in the oxidation of methyl linoleate in benzene at 37 °C initiated with AMVN and inhibited by α-tocopherol and diarylamines.
□: α-Tocopherol, ○: DPA, ●: FF-OMe, ▲: DMPA, △: DPPD.

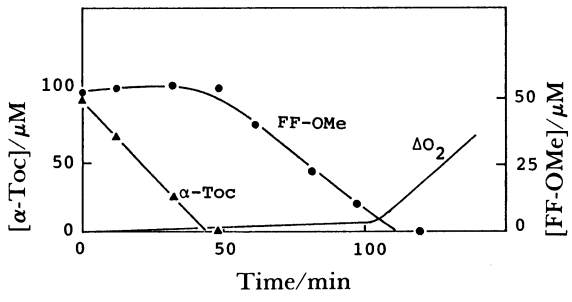


Fig. 3. Rates of disappearance of α-tocopherol and FF-OMe during the oxidation of 151 mM methyl linoleate in benzene initiated with 16.1 mM AMVN at 37 °C under air in the presence of both antioxidants. [α-TOC]=100 μM, [FF-OMe]=50 μM.

Inhibition of Oxidation of Methyl Linoleate Micelles. The oxidation of methyl linoleate micelles in Triton X-100 aqueous dispersions proceeds similarly and gives conjugated diene hydroperoxides quantitatively.[15] AAPH dissolved in water produces free radicals in the aqueous phase and they attack methyl linoleate and induce free radical chain oxidation. Table 2 shows some pertinent results of the oxidation of methyl linoleate micelles induced by AAPH in the presence of diarylamines or α-tocopherol. DPA, DPPD, DMPA, and α-tocopherol inhibited the oxidation, but FFA and PAA did not. As shown in Fig. 4, the inhibition period was again proportional to the antioxidant concentration and inversely proportional to the rate of chain initiation.

Figure 5 shows the rates of consumption of antioxidants during the oxidations of methyl linoleate micelles in aqueous dispersions inhibited by both α-tocopherol and DMPA. As observed in the homogeneous solution, α-tocopherol was consumed first and after all of α-tocopherol disappeared, DMPA was consumed. When both antioxidants were depleted, a fast oxidation proceeded.

Table 2. Oxidation of Methyl Linoleate (MeLH) Micelles in 10 mM Triton X-100 Aqueous Dispersions at 37 °C in the Presence of Diarylamines and α-Tocopherol

MeLH	AAPH	IH		t_{inh}	$10^7 R_{inh}$
mM	mM		μM	s	M s^{-1}
151	10.1	α-TOC	50.0	8040	0.24
151	9.9	DPPD	10.0	1800	0
151	10.0	DPPD	50.0	8400	0
137	17.2	DMPA	10.0	960	1.03
151	10.0	DMPA	10.0	2110	0.65
137	8.54	DMPA	10.0	2180	0.71
151	10.1	DMPA	50.0	8940	0
151	10.2	DPA	10.0	550	3.3
151	10.1	FFA	10.0	0	
151	10.9	PAA	50.0	0	

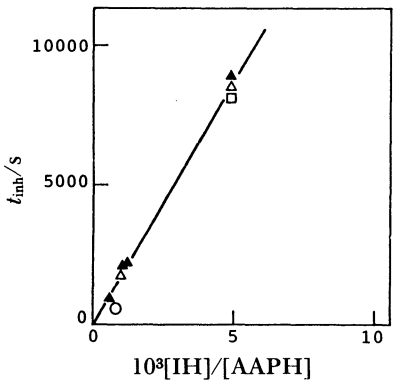


Fig. 4. Plot of inhibition period as a function of [IH]/[AAPH] in the oxidation of methyl linoleate micelles in 10 mM Triton X-100 aqueous dispersions initiated with AAPH and inhibited by α-tocopherol and diarylamines at 37 °C under air.
□: α-Tocopherol, ○: DPA, ▲: DMPA, △: DPPD.

ESR Spectra of Radicals Derived from Diarylamines. Figure 6 shows the ESR spectra observed in the oxidations of methyl linoleate in benzene and in aqueous dispersions inhibited with DMPA. Figure 7 shows the relative amount of radicals observed in the oxidation of methyl linoleate micelles initiated with AAPH and inhibited by both α-tocopherol and DMPA. The ESR spectrum of α-tocopheroxyl radi-

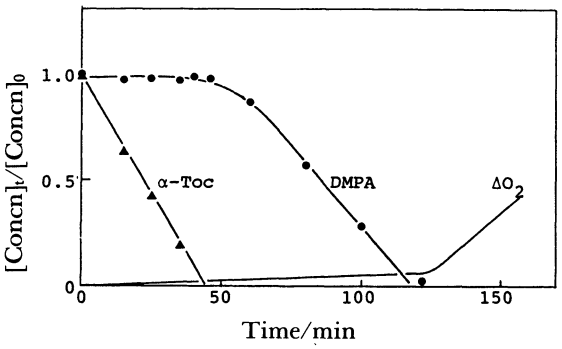


Fig. 5. Rates of disappearance of α-tocopherol and DMPA during the oxidation of 116 mM methyl linoleate micelles in aqueous dispersions initiated with 21.5 mM AAPH and inhibited by 50 μM α-tocopherol and 50 μM DMPA at 37 °C.

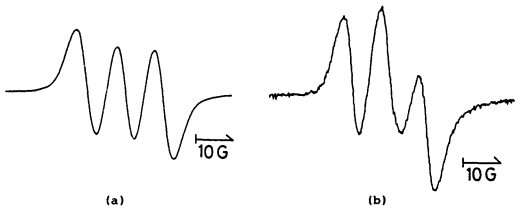


Fig. 6. ESR spectra observed (a) in the oxidation of 151 mM methyl linoleate in benzene at 37 °C under air initiated with 72 mM AMVN in the presence of 204 μM DMPA and (b) in the oxidation of 302 mM methyl linoleate micelles in 10 mM Triton X-100 aqueous dispersions at 37 °C under air initiated with 92.3 mM AAPH in the presence of 600 μM DMPA.

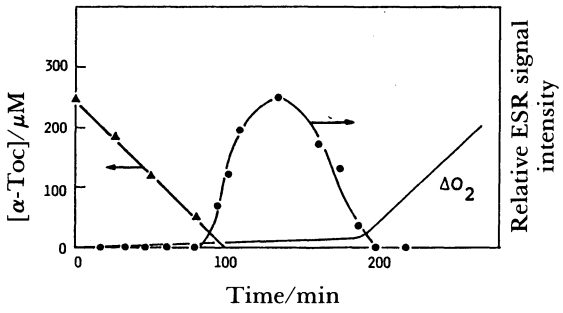
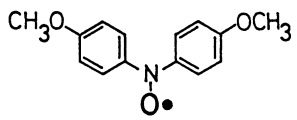


Fig. 7. Relative amount of radical observed during the oxidation of 302 mM methyl linoleate micelles in aqueous dispersions initiated with 92.3 mM AAPH and inhibited by both 204 μM DMPA and 244 μM α-tocopherol at 37 °C under air.

cal (phenoxyl radical from α-tocopherol) is well characterized,[28] but the ESR signal for α-tocopheroxyl radical was not observed, probably because it reacted rapidly with peroxyl radical to give a stable adduct.[29] As Fig. 7 shows, little ESR signal was observed at the initial stage, when only α-tocopherol was consumed. After α-tocopherol was depleted and when DMPA began to be consumed, ESR signal intensity increased and after passing a maximum it decreased. When most of ESR signal disappeared, the rapid oxidation took place.

These results suggest that DMPA scavenges peroxyl radical to give intermediate radical which gives ESR signal and then reacts with another radical to give a stable product. The identification or assignment of the ESR signal observed is difficult at present. It has been reported that 4,4′-dimethoxydiphenylaminyl radical and its *N*-oxyl radical have coupling constants of $a^N=0.849$ mT and $a^N=1.006$ mT, respectively.[30] Since the coupling constant for the radicals observed in this study was 1.0 mT, the radicals shown in Fig. 6 may be ascribed to *N*-oxyl radical **1**.



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DPA, FFA, and PAA do not react with galvinoxyl nor with DPPH, suggesting that the diarylaminyl radicals derived therefrom are less stable than galvinoxyl and DPPH. However, when these amines were dissolved in benzene containing DBPO, ESR signals were observed and the solution was colored to brown. Figure 8 shows the ESR spectra observed for DPA, FFA, and PAA, respectively.

$$(CH_3)_3COO\overset{O}{\overset{\|}{C}}\overset{O}{\overset{\|}{C}}OO(CH_3)_3 \rightarrow 2(CH_3)_3CO^{\cdot}+2CO_2 \quad (1)$$

$$(CH_3)_3CO^{\cdot}+Ar_2NH \rightarrow (CH_3)_3COH+Ar_2N^{\cdot} \quad (2)$$

$$(CH_3)_3CO^{\cdot} \rightarrow (CH_3)_2C{=}O+CH_3^{\cdot} \quad (3)$$

$$CH_3^{\cdot}+O_2 \rightarrow CH_3O_2^{\cdot} \quad (4)$$

$$CH_3O_2^{\cdot}+Ar_2N^{\cdot} \rightarrow CH_3O^{\cdot}+Ar_2NO^{\cdot} \quad (5)$$

When these amines were dissolved in benzene containing both DBPO and *t*-butyl hydroperoxide, the same ESR spectra were observed and the solution became brown similarly. This system generates *t*-butylperoxyl radical[31] and the *N*-oxyl radical must be formed by a sequence of reactions 1, 6, 7, and 8.

$$(CH_3)_3CO^{\cdot}+(CH_3)_3COOH \rightarrow (CH_3)_3COH+(CH_3)_3COO^{\cdot} \quad (6)$$

$$(CH_3)_3COO^{\cdot}+Ar_2NH \rightarrow (CH_3)_3COOH+Ar_2N^{\cdot} \quad (7)$$

$$(CH_3)_3COO^{\cdot}+Ar_2N^{\cdot} \rightarrow (CH_3)_3CO^{\cdot}+Ar_2NO^{\cdot} \quad (8)$$

The ESR spectrum shown in Fig. 8(a) obtained for DPA is the same as that of diphenyl nitroxide radical reported by Mukai et al.[32] Furthermore, the same ESR spectrum was observed as that of Fig. 8(a) when phenylazotriphenylmethane was decomposed thermally under vacuum in benzene containing nitrosobenzene, oxygen was added, and then evacuated again: $a^N=0.990$ mT, $a^H_{o,p}=0.184$ mT, $a^H_m=0.078$ mT.

Thus the results of the present study and those reported in the literature suggest that diarylamines such as DPA, FF-OMe, and DMPA suppress the oxidation mainly by the following sequence:

$$LO_2^{\cdot}+Ar_2NH \rightarrow LOOH+Ar_2N^{\cdot} \quad (9)$$

$$LO_2^{\cdot}+Ar_2N^{\cdot} \rightarrow LO^{\cdot}+Ar_2NO^{\cdot} \quad (10)$$

(or ketone + hydroxylamine)

$$L\cdot(LO_2^{\cdot})+Ar_2NO^{\cdot} \rightarrow \text{stable product} \quad (11)$$

On the other hand, *N*,*N*′-dipheyl-1,4-phenylenediamine (DPPD) behaves differently. DPPD donates hydrogen atom to a peroxyl radical and the resulting aminyl radical must donate one more hydrogen atom to another peroxyl radical to give benzoquinone diimines without generating nitroxide radical.

$$LO_2^{\cdot}+Ph\text{-}NH\text{-}Ph\text{-}NH\text{-}Ph \rightarrow LOOH+Ph\text{-}\dot{N}\text{-}Ph\text{-}NH\text{-}Ph \quad (12)$$

$$LO_2^{\cdot}+Ph\text{-}\dot{N}\text{-}Ph\text{-}NH\text{-}Ph \rightarrow LOOH+Ph\text{-}N{=}\langle\bigcirc\rangle{=}N\text{-}Ph \quad (13)$$

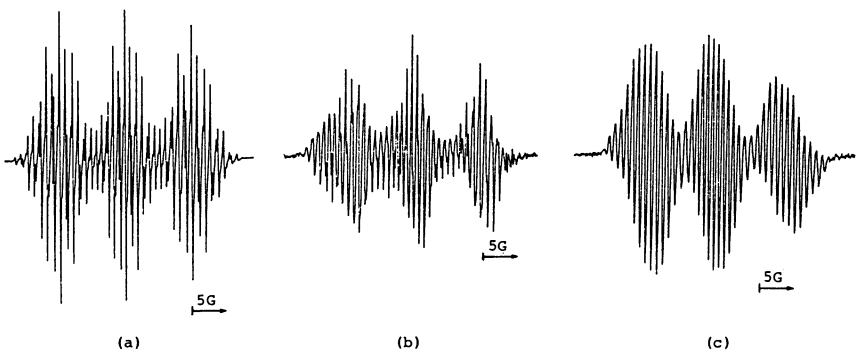

Fig. 8. ESR spectra observed when diarylamines were dissolved in benzene containing DBPO under vacuum at room temperature.
(a) DPA; (b) FFA; (c) PAA.

Under these conditions, each molecule of diarylamines scavenges two molecules of radicals and, as shown in Figs. 2 and 4, they produce similar inhibition period as α-tocopherol which also scavneges two molecules of peroxyl radicals.[22]

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