Antioxidant Activities of Aminophenols against Oxidation of Methyl Linoleate in Solution

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The antioxidant activities of 48 kinds of aminophenols, chromanols, indoles, carbazoles, aromatic amines, and related compounds were assessed in the oxidations of methyl linoleate in acetonitrile solution induced by azo radical initiator. Chromanols, aminochroman and *p*-phenylenediamines exhibited strong antioxidant activity. Indole and carbazole did not act as antioxidant *per se*, but those having either amino or hydroxy group as substituent at the *para* position acted as potent antioxidant. Amino-substituted compounds were in general more potent than hydroxy-substituted ones. These results were interpreted by both polar and resonance effect.

The free radical-mediated oxidation by molecular oxygen is one of the most important of all chemical processes and many important industrial processes in petrochemical industry are based on the controlled oxidation of hydrocarbons. At the same time, however, oxidation is the main cause of deterioration of food stuffs, oils, and polymers.[1] Furthermore, the recent studies suggest that the uncontrolled oxidations of lipids, proteins and DNA in biological systems are important in the progression of various diseases, cancer, and aging.[2] Consequently, the inhibition of these oxidations has received much attention and various natural and synthetic antioxidants have been explored. Phenols and amines have been used as antioxidants extensively and vitamin E is known as one of the most important antioxidants *in vivo*.[3] We have been studying the action of various phenols and aromatic amines as antioxidant[4] and in the present study we have extended to various aminophenols and related compounds.

Experimental

Materials. The antioxidants used in this study are shown in Fig. 1. Vitamin E and its analogues **1** to **8** were kindly provided by Eisai Co. (Tokyo, Japan). Ubiquinol-10, **46** was prepared by reduction with sodium borohydride of ubiquinone-10 which was also provided by Eisai Co. Carazostatin **23** and probucol **45** were kind gifts from Dr. Kato at Kirin Brewery Co. (Takasaki, Japan) and from Daiichi Pure Chemicals (Tokyo, Japan) respectively. 6-Hydroxy-1, 4-dimethylcarbazole **19** and α-tocopheramine **33** were kind gift from Dr. Willson at Brunel University and Dr. Mukai at Ehime University, respectively. Other antioxidants were those commercially available.

Methyl linoleate used as a substrate was obtained from Sigma Chemical Co. (St. Louis, MO) and purified before use by a silica-gel column. Lipid-soluble and water-soluble azo compounds, 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) and 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) used as radical initiators, were obtained from Wako Pure Chemical Ind. (Osaka, Japan). Sodium dodecyl sulfate (SDS) used as a surfactant was purchased from Wako Pure Chemical Industries (Osaka).

Procedures. The oxidation of methyl linoleate was induced by AMVN in acetonitrile solution in the absence and presence of antioxidant at 37 °C in air and the formation of conjugated diene hydroperoxides was followed with a UV-detector (JASCO 875-UV). The reaction mixture was directly injected onto an HPLC (JASCO 880-PU); a reverse phase LC18 (particle size 5 μm, 4.6 mm×25 cm, Supelco, Tokyo) and methanol/water (95/5, v/v, 1.0 ml min^{-1}) were used as a column and eluent respectively. Four isomeric hydroperoxides eluted as a single peak at 4.6 min were measured at 234 nm, ε being 28000 M^{-1} cm^{-1}.[5] The rate of oxidation of methyl linoleate SDS micelle initiated by AAPH in the presence and absence of water soluble antioxidant was measured with an oxygen monitor equipped with a Clark-type oxygen electrode (YSI model 5300). The interactions of galvinoxyl and antioxidants were measured with a spectrophotometer (Shimadzu UV-2200) equipped with a rapid-mixing stopped-flow apparatus (Applied Photophysics, RX1000). The rate of consumption of galvinoxyl was followed by measuring the decrease of its maximum absorption at 428 nm.

Results

The antioxidant activities of various aminophenols and related compounds were assessed in the oxidation of methyl linoleate in homogeneous solution induced by a radical initiator. This is a convenient and appropriate system since this oxidation proceeds by a free radical chain mechanism with long kinetic chain length and methyl linoleate gives four conjugated diene hydroperoxides quantitatively.[6,7] Thus, the rate of oxidation and hence the antioxidant activity can be mea-

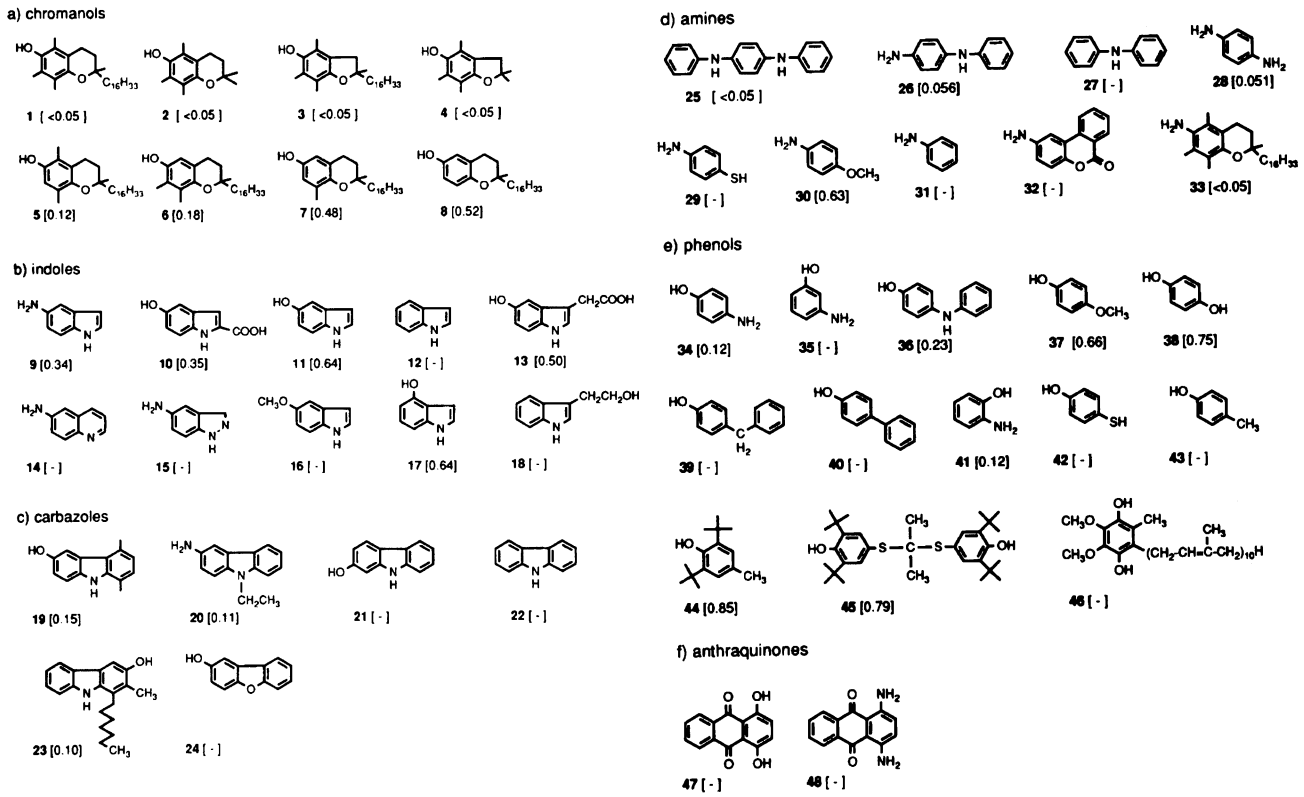


Fig. 1. Antioxidants used in this study. The numbers in the brackets show the ratio of the rate of oxidation inhibited by respective antioxidant to that of uninhibited oxidation, see text.

sured quantitatively by following either oxygen uptake, substrate consumption, conjugated diene or hydroperoxide formation. In the present study, the accumulation of hydroperoxides was followed with an HPLC as described above. The example of the oxidations in the absence and presence of several antioxidants is shown in Fig. 2. It shows that the oxidation of methyl linoleate induced by AMVN in the absence of antioxidant proceeded at a constant rate without any noticeable induction period. As observed previously,[8,9] α-tocopherol suppressed the oxidation markedly and produced a clear induction period, or a lag phase. *p*-Aminophenol **34** and *p*-phenylenediamine **28** also suppressed the oxidation efficiently, while hydroquinone **38** retarded the oxidation only slightly. *p*-Aminophenol **34** suppressed the oxidation dose-dependently (Fig. 3) and the induction period increased with its concentration proportionally (Fig. 4). As shown in Fig. 3, the rate of oxidation after the induction period was similar to that in the absence of antioxidant. In Fig. 4 is also plotted the induction period produced by α-tocopherol in the oxidation of methyl linoleate under similar conditions. *p*-Aminophenol produced a longer induction period than α-tocopherol, indicating that it can scavenge more radicals than α-tocopherol.

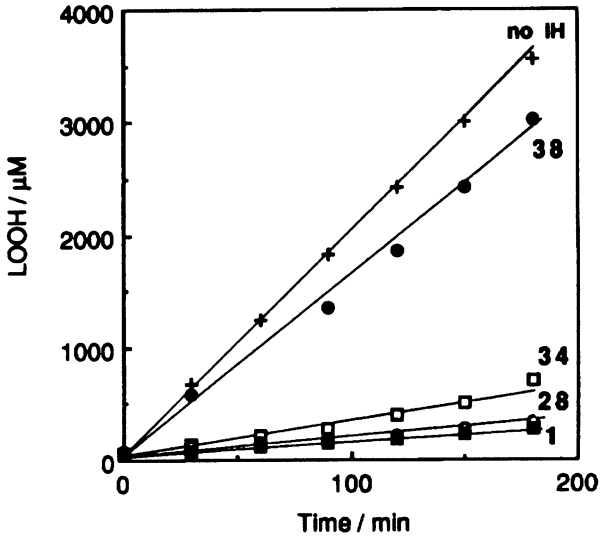


Fig. 2. Oxidation of methyl linoleate (453 mM) in acetonitrile in the absence (+) and presence of antioxidant (5 μM) induced by AMVN (0.20 mM) at 37 °C in air. The accumulation of methyl linoleate hydroperoxide (LOOH) is plotted against time. The numbers donate the antioxidant shown in Fig. 1.

The ratio of the rate of oxidation inhibited by antioxidant (R_{inh}) to that of uninhibited oxidation (R_o) is a measure for the antioxidant activity; that is, the value R_{inh}/R_o gives how much does the antioxidant reduce the rate of oxidation. The oxidations of methyl linoleate were carried out in the presence of anitoxidant shown in Fig. 1 and the ratio R_{inh}/R_o was measured for each antioxidant. The results are shown in the brack-

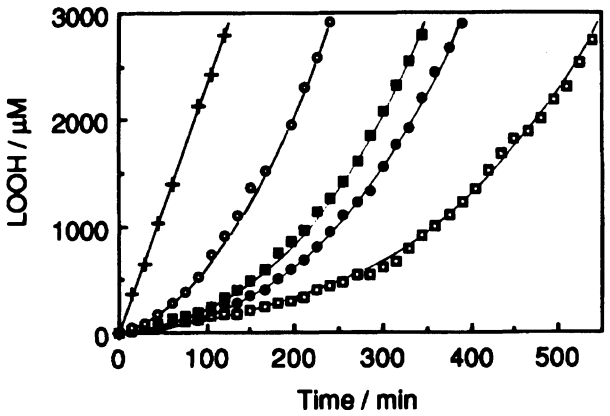


Fig. 3. Inhibition of oxidation of methyl linoleate (453 mM) by *p*-aminophenol induced by AMVN (0.20 mM) in acetonitrile at 37 °C in air. The concentrations of *p*-aminophenol were: +=0; ○=2; ■=3; ●=5; □=8 μM.

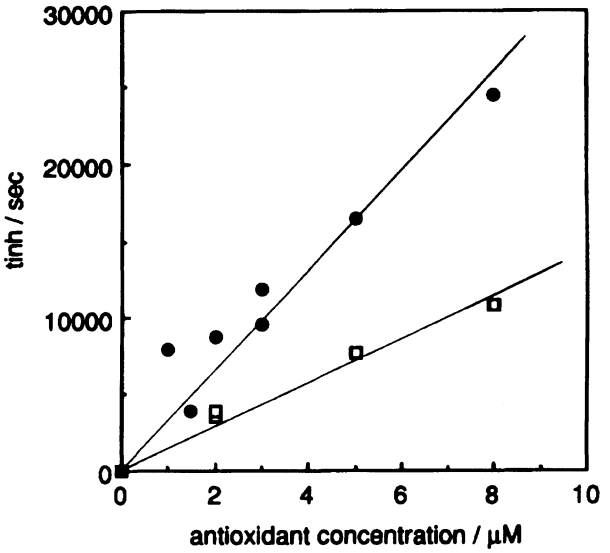


Fig. 4. Induction period observed in the oxidation of methyl linoleate (453 mM) in acetonitrile induced by AMVN (0.20 mM) at 37 °C in air in the presence of *p*-aminophenol (●) or α-tocopherol (□).

ets of Fig. 1. It shows that some antioxidants inhibited the oxidation quite efficiently, some moderately, while others did not suppress the oxidation appreciably.

The action of tryptophan and 5-hydroxytryptophan was also measured. Since they were hydrophilic, the activity was assessed against the oxidation of methyl linoleate in SDS micelles induced by a water-soluble azo initiator, AAPH. The values R_{inh}/R_o for tryptophan and 5-hydroxytryptophan were 0.98 and 0.41 respectively. Under the same conditions, 2-carboxy-2,5,7,8-tetramethyl-6-hydroxychroman, a water-soluble analogue of vitamin E, suppressed the oxidation completely, that is $R_{inh}/R_o \approx 0$.

It has been known that a strong antioxidant which scavenges oxygen radicals by donating hydrogen atom reacts rapidly with a stable phenoxyl radical such as galvinoxyl.[10] The reactions of *p*-aminophenol **34**, *p*-phenylenediamine **28**, and hydroquinone **38** with galvinoxyl were measured with a stopped-flow spectrophotometer. The results shown in Fig. 5 indicate that the rates decreased in the order of *p*-phenylenediamine **28** > *p*-aminophenol **34** ≫ hydroquinone **38**, in accordance with the results shown in Fig. 2. Galvinoxyl was stable without antioxidant.

Discussion

The above results show that the antioxidant activity varies markedly with the compound tested. Chromanols (Vitamin E) acted as potent antioxidant and the potency decreased in the order of α-tocopherol **1** > β-tocopherol **5** > γ-tocopherol **6** > δ-tocopherol **7** as observed previously by our group[11] and others[12,13] and tocol **8** exhibited only moderate activity. Indole **12** did not act as an antioxidant. 5-Aminoindole **9** and 5-hydroxyindole **11** suppressed the oxidation, but 5-methoxyindole **16** and 6-aminoquinoline **14** did not. Carbazole **22** itself did not act as an antioxidant but those having either amino group or hydroxy group at the para position **19**, **20**, **23** were good antioxidant. On the other hand, 2-hydroxycarbazole **21** and 2-hydroxybenzofuran **24** did not suppress the oxidation. *N*,*N*′-Diphenyl-*p*-phenylenediamine **25**, 4-aminodiphenylamine **26**, *p*-phenylenediamine **28** are potent antioxidants. Aniline **31**, diphenylamine **27**, and *p*-aminothiophenol **29** are not good antioxidant. *o*-Aminophenol **41** and *p*-aminophenol **34**, are potent antioxidants, but *m*-aminophenol **35** is not. Under the present conditions, 2,6-di-*t*-butyl-4-methylphenol **44**, probucol **45**, and ubiquinol-10 **46** were poor antioxidants. The poor antioxidant activity of ubiquinol-10 may be ascribed to the formation of hydroperoxyl radical from the interaction of oxygen and ubisemiquinone radical which is formed when ubiquinol-10 scavenges peroxyl radicals

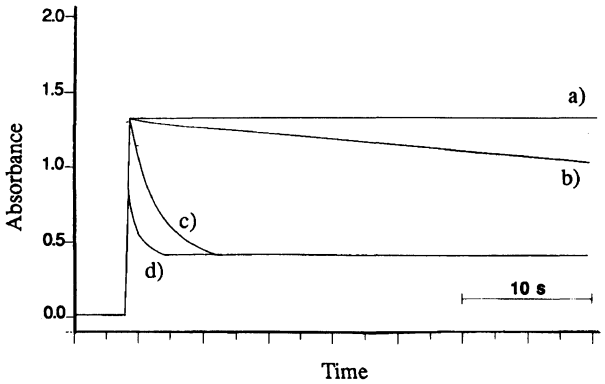
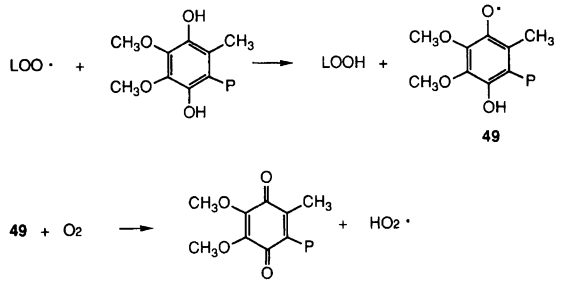


Fig. 5. Consumption of galvinoxyl as measured by its absorption at 428 nm when it was reacted with equimolar concentration (20 μM) of antioxidant in methanol at 37 °C. a): without antioxidant; b): hydroquinone; c): *p*-aminophenol; d): *p*-phenylenediamine.

(Scheme 1). Since the hydroperoxyl radical is active and capable of initiating chain oxidation, this lowers the overall antioxidant activity of ubiquinol-10. In agreement with this discussion, it was found that hydrogen peroxide was formed in the oxidation of ubiquinol-10. Finally, α-tocopheramine **33** was a very potent antioxidant.

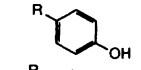
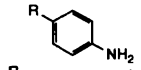
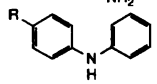
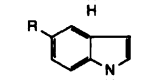
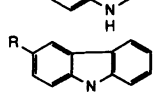
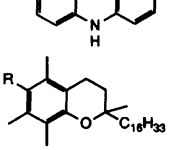
The correlation between structure and antioxidant activity is summarized in Table 1. It shows that phenol, aniline, diphenylamine, indole, and carbazole do not act as antioxidant *per se*, but become active when they have either hydroxy or amino group at the *para* position. In every case, amino group was more effective than hydroxy group, the only exception being α-tocopherol which was slightly more potent than α-tocopheramine. Sulfanyl group does not produce antioxidant activity. Coromanol ring with methyl group was stronger than carbazole, which was stronger than indole. The antioxidant activities of indole derived compounds have been reported previously.[14—17]

The above results suggest that the antioxidant activities of aminophenols and related compounds are determined by both resonance effect and polar effect.



Scheme 1.

Table 1. Inhibition by Antioxidant (5 μM) of Oxidation of Methyl Linoleate (453 mM) in Acetonitrile Induced by AMVN (0.20 mM) at 37 °C in air[a)]

R=	H	OH	NH_2	SH
		0.75	0.12	1.0
	0.96	0.12	0.051	0.92
	0.87	0.23	0.056	
	1	0.64	0.34	
	1	0.15[b)] 0.10[c)]	0.11	
		<0.05	<0.05	

a) Numbers show the ratio of the rate of inhibited oxidation to that of uninhibited oxidation, R_{inh}/R_o. b) For **19**. c) For **23**.

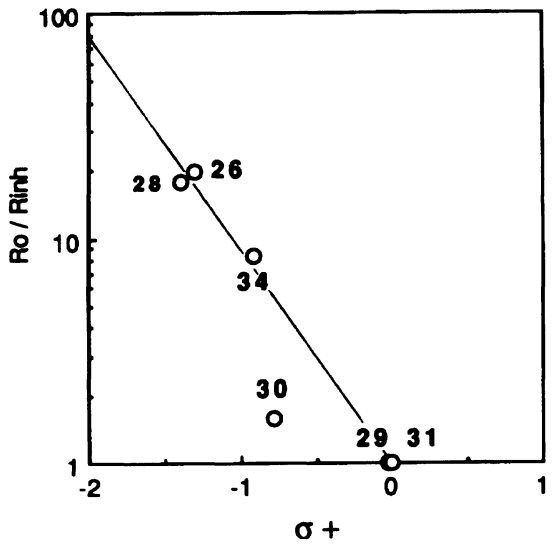


Fig. 6. Plot of R_o/R_{inh} as a function of Hammett σ^+ for the oxidation of methyl linoleate induced by AMVN inhibited by *p*-aminodiphenylamine **26**, *p*-phenylenediamine **28**, *p*-aminothiophenol **29**, aniline **31**, *p*-aminophenol **34**, *p*-anisidine **30** at 37 °C in air.

The previous study[4)] has shown a profound polar effect on the antioxidant activity of diphenylamines, that is, the electron-donating substituents increased the antioxidant activity, whereas the electron-withdrawing substituents diminished the antioxidant potency as expected from the electrophilic nature of the peroxyl radical.

Figure 6 shows the Hammett plot for the compounds **26**, **28**, **29**, **30**, **31**, and **34**. Since the rate of oxidation inhibited by antioxidant is inversely proportional to the rate constant kinh for the scavenging of peroxyl radical by an antioxidant,[9)] the ratio R_o/R_{inh} is a good measure for kinh and hence plotted in Fig. 6 against Hammett σ^+. It gives fairly good straight line with a slope $\rho=-1.1$ ($r^2=0.86$). However, the activity of *p*-anisidine **30** was markedly smaller than that considering only a polar effect. This probably implies the importance of resonance effect as well as polar effect. In other words, the resonance stabilization for the radical derived from the antioxidant when it scavenges active radicals is important in determining the antioxidant activity. Apparently, the formation of conjugated 1,4-cyclohexadiene derivatives is a structural requirement for high potency as an antioxidant.

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