Phenothiazine Derivatives as New Antioxidants for the Autoxidation of Methyl Linoleate and Their Reaction Mechanisms[1]

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ABSTRACT

The effect of new antioxidants, such as phenothiazine derivatives, on the autoxidation of methyl linoleate was evaluated by estimating the induction period using the weighing method. Peroxide values (iodometry and IR), refractive indices, mol wts, and UV and IR spectra were measured to investigate the extent of the autoxidation. The induction period evaluated from the weighing method gives almost the same value as that from the peroxide values. It was shown that some new phenothiazine derivatives are remarkably effective antioxidants, and, besides, the mechanism for the autoxidation of methyl linoleate containing phenothiazine derivatives as antioxidants is probably of the same type as that for the substrate alone. This study also investigated the reaction mechanism of phenothiazine derivative antioxidants by determining the electron spin resonance spectra for the antioxidants in the autoxidation of methyl linoleate. Then, the following mechanism was proposed. That is, within the induction period, these inhibitors hold stable nitroxide radicals (>NO•) in the reaction between the antioxidant amino radical (>N•), produced by the reaction of the antioxidant with ROO• or O_2, and the peroxy radical (ROO•). Besides, the more superior the phenothiazine derivative antioxidant, the more inactive the antioxidant makes oxygen and the peroxy radical for the methyl linoleate autoxidation and also for the antioxidant oxidation.

INTRODUCTION

Some phenothiazine derivatives are useful as antioxidants for a wide variety of materials, e.g., lubricants (1-3), rubbers (4), polymers (5), and detergents (6). Phenothiazine and its derivatives are so nonpoisonous as to be used as insecticides for domestric animals. They have never been studied on the oxidative inhibitor effect for fatty acid esters. Therefore, this study took place, and phenothiazine derivatives are evaluated as antioxidants in the autoxidation of methyl linoleate by the determination of the induction period using the weighing method. Also determined were peroxide values (iodometry and IR), refractive indices, mol wts, and UV and IR spectra for the controls and for samples containing antioxidants, and component change for each sample was studied. The induction period was also estimated from peroxide values.

Only a few investigations have been performed on the reaction mechanism of phenothiazine derivative antioxidants (7,8). In studies of the antioxidant action of phenothiazine, Murphy et al. (7) have suggested the formation of free radicals by the homolytic fission of the N–H bond, i.e., that of the phenothiazinyl radical (>N•). But the reaction of phenothiazine with tertiary-butyl hydroperoxide in benzene either at room temperature or under irradiation conditions produced free radicals assigned to the phenothizine nitroxide radical (>NO•) (8). Besides, Coppinger and Swalen (9) have observed that several amines produced nitroxide radicals when treated with tertiary-butyl hydroperoxide. But, as far as is known, the reaction mechanism of the antioxidant action of phenothiazine derivatives for fatty acid esters has not previously been studied. Therefore, this study has been performed by measuring the electron spin resonance (ESR) spectra for phenothiazine derivative antioxidants in the autoxidized methyl linoleate.

[1]Presented at the 12th World Congress of ISF, Milan, 1974.

EXPERIMENTAL PROCEDURES

Materials

Methyl linoleate: It was obtained by treating safflower oil (obtained from Linol Fat Co., Nagoya, Japan) fatty acids with methanol containing sulfuric acid and by fractionating them by the urea adduct formation. Thus obtained methyl linoleate has a purity of 99% by GLC. The removal of natural antioxidants and metallic ions from methyl linoleate was confirmed by colorimetry (10) and atomic absorption spectrophotometric analysis, respectively.

Antioxidants: Phenothiazine was a commercial product. Phenoxazine, naphthothiazine, and naphthoxazine were synthesized in our laboratory by known methods. 3-Aminophenothiazine, 3-hydroxyphenothiazine, N-(2,4-dichloro-6-pyrimidinyl)-N-(3-phenothiazinyl)-urea, and N-(2,4-dichloro-6-pyrimidinyl)-carbamic acid-3-phenothiazinyl ester were obtained from Toyama Chemical Co., Toyama, Japan. The data for the elementary analysis for these products agree with the theoretical values. These substances were used as antioxidants.

Autoxidation

Methyl linoleate (1.5000 ± 0.0020 g) and 3.76×10^{-6} mol of the antioxidant were put into a beaker 4.1 cm in diameter. Each beaker was accurately weighed and then placed in an oven at a constant temperature of 36.5 ± 0.5 C. Each beaker was weighed in order to estimate weight

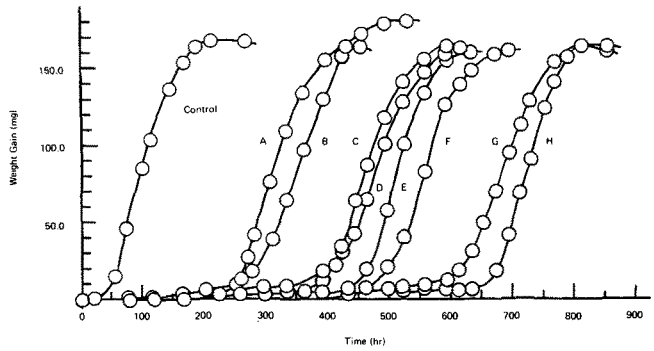

FIG. 1. Weight gain in the autoxidation of methyl linoleate and samples containing antioxidants. Temperature, 36.5 ± 0.5 C; antioxidants, 3.76×10^{-6} mol/1.5 g of methyl linoleate. (A) Aminophenothiazine. (B) Phenoxazine. (C) Phenothiazine. (D) Hydroxyphenothiazine. (E) N-(2,4-dichloro-6-pyrimidinyl)-N-(3-phenothiazinyl)-urea. (F) Naphthothiazine. (G) Naphthoxazine. (H) N-(2,4-dichloro-6-pyrimidinyl)-carbamic acid-3-phenothiazinyl ester.

TABLE I

Effect of Antioxidants on the Induction Period in the Autoxidation of Methyl Linoleate

Antioxidant (3.76 x 10^{-6} mol/1.5 g)	Methyl linoleate						
	Induction period[a] (hr)	Effect[d] (ratio)	Induction period[b] (hr)	Effect[d] (ratio)	Induction period[c] (hr)	Effect[d] (ratio)	Effective order
Control	49	1.0	48	1.0	49	1.0	-
A[e]	249	5.1	240	5.0	230	4.7	8
B	255	5.2	250	5.2	255	5.2	7
C	350	7.1	360	7.5	365	7.4	6
D	380	7.8	380	7.9	390	8.0	5
E	447	9.1	430	9.0	430	8.8	4
F	472	9.6	460	9.6	470	9.6	3
G	550	11.2	540	11.3	560	11.4	2
H	659	13.4	640	13.3	640	13.1	1
BHA	690	14.1	-	-	-	-	-

[a]Induction period determined by the weighing method.
[b]Induction period determined by the peroxide values (iodometry).
[c]Induction period determined by the peroxide values (IR).
[d]Ratio of the induction period of the sample containing an antioxidant to that of methyl linoleate.
[e]A, B, C, D, E, F, G, and H represent the antioxidants shown in Figure 1, respectively.

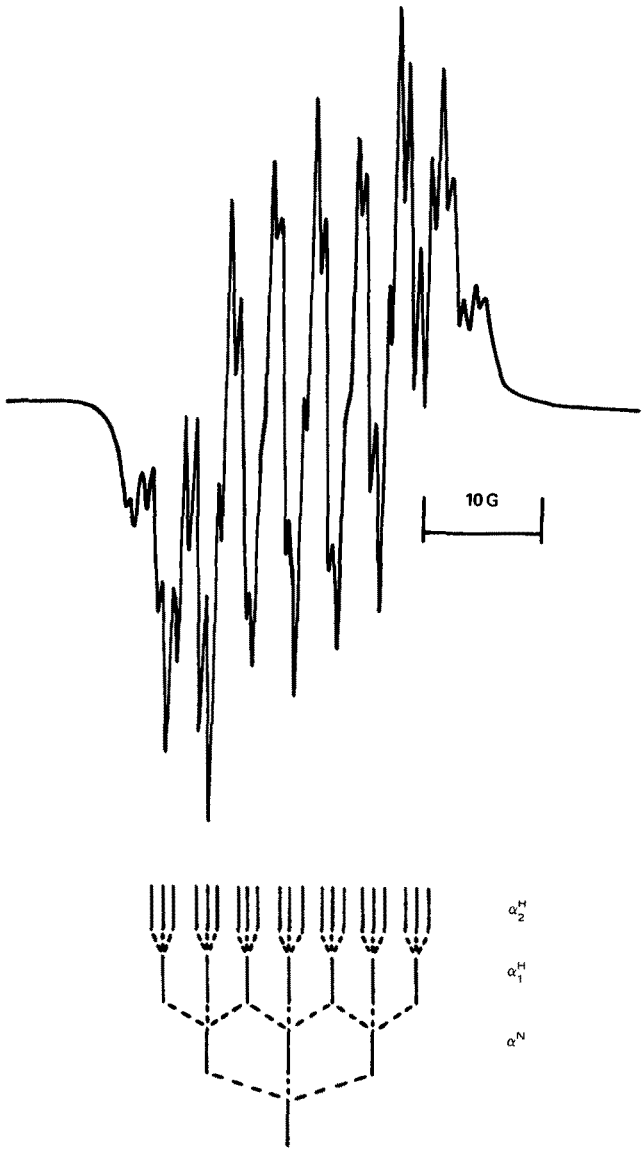

FIG. 2. Electron spin resonance spectrum of the free radical produced from phenothiazine of which the oxidation is carried out by PbO_2 in benzene solution (phenothiazine: 2.00 x 10^{-3} mol/liter) at 36.5 ± 0.5 C under the evacuation of air.

increase at given intervals, as described in previous papers (11-13). Of course, the depth of the oil layer for each sample was kept constant.

Oxidation

Each antioxidant and tertiary-butyl hydroperoxide were dissolved in benzene by 2.00 x 10^{-3} mol/liter and 7.61 x 10^{-3} mol/liter, respectively. The obtained solution was kept at 36.5 ± 0.5 C to measure produced antioxidant radicals by ESR. Besides, the benzene solution of each antioxidant (2.00 x 10^{-3} mol/liter) in the presence of PbO_2 was maintained at 36.5 ± 0.5 C under the evacuation of air, in order to investigate the oxidation of each antioxidant by PbO_2. The reason why PbO_2 was used instead of iron or copper compounds is so that good ESR spectra could be obtained. Moreover, each antioxidant benzene solution (2.00 x 10^{-3} mol/liter) in an ESR cylinder was kept at a constant temperature of 36.5 ± 0.5 C to examine the air oxidation of each antioxidant.

Decomposition of Autoxidized Methyl Linoleate by Antioxidants

Autoxidized methyl linoleate (peroxide value: 2.10 x 10^3 meq/kg) and the antioxidant were dissolved in benzene at a concentration of 27.26 g/liter and, for example, 6.243 x 10^{-2} mol/liter, respectively, at 36.5 ± 0.5 C under the evacuation of air. The ability of each antioxidant to decompose the autoxidized methyl linoleate was estimated by measuring the peroxide value of the autoxidized methyl linoleate at given intervals.

Determination

The weighing method is the most convenient method for estimating autoxidation (14,15). The point at which most samples began to gain weight rapidly was chosen as the end point of the induction period. It was 10 mg/1.5 g as in the previous papers (11-13). Peroxide values (meq/kg, iodometry, and IR [16]), refractive indices, mol wt (with a Hitachi-Perkin-Elmer 115 type apparatus), and UV and IR (with a spectrophotometer having NaCl prism) spectra were measured to investigate the extent of the autoxidation.

The free radicals produced from phenothiazine derivative antioxidants and from autoxidized methyl linoleate were measured at 20 C with a Nihon-Denshi-JES-1 X type ESR spectrometer using a quartz cylinder of 5 mm in diameter or a capillary of 1 mm.

RESULTS AND DISCUSSION

The weight increase in the autoxidation of samples was

TABLE II

Coupling Constants (α^N, α_1^H, and α_2^H) and g-Values of the Electron Spin Resonance Spectra of the Free Radicals Produced from Phenothiazine Derivative Antioxidants

Phenothiazine derivative antioxidant		α^N	α_1^H	α_2^H	g-Value	Effect[a] (ratio)
Phenothiazine	Autoxidized methyl linoleate	8.9	2.2	-	2.0041	8.0
	t-BuOOH in benzene	8.8	2.3	0.8		
	PbO_2 in benzene	7.0	3.5	0.9		
	Air oxidation in benzene	7.0	3.3	-		
N-(2,4-dichloro-6-pyrimidinyl)-N-(3-phenothiazinyl)-urea	Autoxidized methyl linoleate	9.0	2.4	-	2.0075	8.8
N-(2,4-dichloro-6-pyrimidinyl)-carbamic acid-3-phenothiazinyl ester	Autoxidized methyl linoleate	8.8	-	-	2.0051	13.1

[a]Ratio of the induction period of the sample containing an antioxidant to that of methyl linoleate.

determined by the weighing procedure. These results are given in Figure 1. Thus estimated autoxidation induction periods are shown in Table I. In each sample, the induction period almost corresponds to the interval from the starting point to the point giving a sharp curvature in Figure 1. The effect of the antioxidants was evaluated by the ratio of the induction period of the sample containing an antioxidant to that of methyl linoleate. Phenothiazine and its derivatives are effective as antioxidants; in particular, the eighth antioxidant, N-(2,4-dichloro-6-pyrimidinyl)-carbamic acid-3-phenothiazinyl ester, is excellent. But even this antioxidant is inferior to BHA, as shown in Table I. Peroxide values for the samples were determined by iodometry and by IR (by using a 10 mm cell) (16). In the IR method, monomeric monohydroperoxides can be determined, but dimeric or polymeric hydroperoxides cannot (16). In peroxide values, refractive indices, and conjugated diene contents evaluated from the UV spectra for the samples, the points giving sharp curvatures were observed at almost the same hours as that in the case of weight increases (Fig. 1), though in mean mol wts the point somewhat shifted to the longer by ca. 50 hr. Active methylene (CH_2 group in $=CHCH_2CH=$ estimated from the absorption band at 3020 cm^{-1} with an infrared spectrophotometer having NaCl prism) (17) and trans-trans (18) and cis-trans (18) conjugated dienes for samples were estimated from the IR spectra. In these cases, the points giving sharp curvatures are found at almost the same hours as in the case of the corresponding weight increases (Fig. 1), but the point in the case of isolated trans double bond content determined by the IR spectra shifted a little to the longer by ca. 50 hr.

From results mentioned above on weight increases, peroxide values (by iodometry and by IR), refractive indices, mol wts, total, cis-trans, and trans-trans conjugated diene contents, and also isolated trans double bond contents, it was concluded that the mechanism for the autoxidation of methyl linoleate containing phenothiazine derivatives as antioxidants is probably of the same type as that for the substrate alone.

Within the induction period, these samples are gradually autoxidized to yield hydroperoxides having trans-trans and cis-trans conjugated dienes with a gradual decrease of active methylene groups. From the end point of the induction period onwards, these samples are rapidly autoxidized to yield the hydroperoxides and also oxidized polymers with isolated trans double bonds, and are accompanied by a rapid decrease in active methylene groups.

The free radicals from phenothiazine by its oxidation with PbO_2 in benzene solution at 36.5 ± 0.5 C under the evacuation of air were measured by ESR, as shown in Figure 2. In Table II were given coupling constants and *g* values of the ESR spectra of radicals produced from some phenothiazine derivative antioxidants. As described above, the reaction of phenothiazine with 2.5% v/v tertiary-butyl hydroperoxide in benzene at room temperature produced

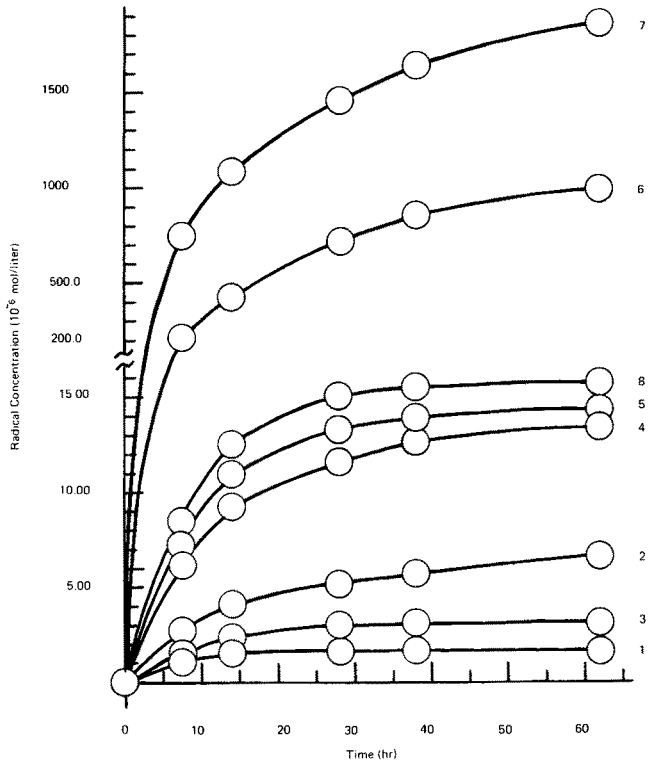

FIG. 3. Change in phenothiazine derivative radical concentration during the oxidation of each antioxidant benzene solution (2.00 x 10^{-3} mol/liter) in the presence of PbO_2 at 36.5 ± 0.5 C under the evacuation of air.

free radicals, such as phenothiazine nitroxide (8). The coupling constant (α^N) of the ESR spectra for the phenothiazine nitroxide radical and that of the phenothiazinyl were 9.18 and 7.05, respectively. Though coupling constant changes, due to solvent effects, arose—e.g., the coupling constant for phenothiazinyl radical in benzene and that in ethanol were 7.05 and 7.20, respectively—the mutual difference was small (8). Moreover, there was almost no difference among the coupling constants (α^N) of the ESR spectra for radicals produced from phenothiazine in autoxidized methyl linoleates containing different amounts of hydroperoxides measured in the usual cylinders and those in the capillary. From the results, the influence of hydroperoxides on the coupling constant (α^N) for radicals obtained from phenothiazine in this case is negligible. Therefore, in Table II, the coupling constant (α^N) of the ESR spectra for radicals produced from phenothiazine in autoxidized methyl linoleate and that from phenothiazine with tertiary-butyl hydroxperoxide in benzene solution, estimated at 8.9 and 8.8, respectively, are derived from the same free radical, i.e., the phenothiazine nitroxide radical.

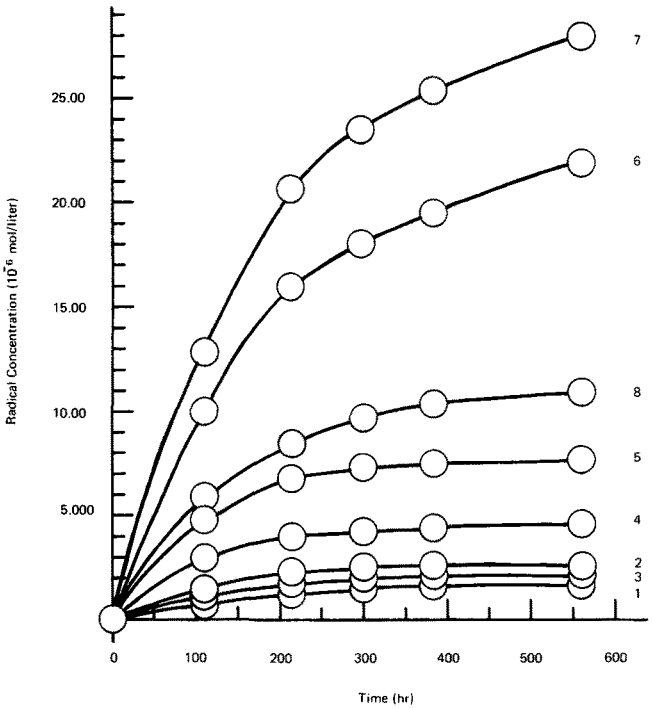

FIG. 4. Change in each phenothiazine derivative antioxidant radical concentration during the air oxidation of each antioxidant benzene solution (2.00 x 10^{-3} mol/liter) at 36.5 ± 0.5 C.

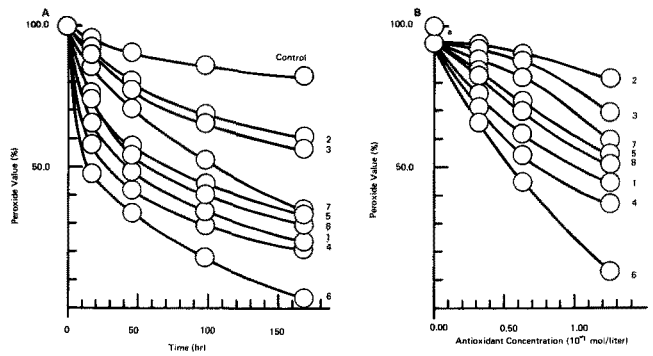

FIG. 5. Effects of each phenothiazine derivative antioxidant on the decomposition of the hydroperoxides in autoxidized methyl linoleate, referring to time (A) and to the antioxidant concentration (B).

Besides, the coupling constant (α^N) of radicals produced from phenothiazine with PbO_2 in benzene solution and that from phenothiazine by air oxidation, estimated at 7.0, are due to the same free radical, i.e., the phenothiazinyl radical. Similar results were obtained in the case of phenoxazine. Therefore, the radical produced from phenothiazine in autoxidized methyl linoleate and that from phenoxazine are nitroxide radicals. It may be confirmed, then, that in the other phenothiazine derivative antioxidants as well, the nitroxide radicals exists in autoxidized methyl linoleates containing these antioxidants, respectively, from the results on α^N shown in Table II.

As given in Figure 3, the order of unchangeable property for each phenothiazine derivative antioxidant radical concentration between the origin and a given interval point in the oxidation of each antioxidant benzene solution (2.00 x 10^{-3} mol/liter) in the presence of PbO_2 at 36.5 ± 0.5 C under the evacuation of air, can be shown by 1, 3, 2, 4, 5, 8, 6, and 7, by which the effective order in Table I is denoted. Except for 8, this order on each antioxidant radical concentration in general obeys the effective order given in Table I. Therefore, the more effective the antioxidant, the more persistent the oxidation of the antioxidant by PbO_2. When the air oxidation takes place, the obtained results resemble those in the PbO_2 oxidation, as shown in Figure 4. Consequently, the more effective the antioxidant, the more persistent the air oxidation of the antioxidant.

In general, antioxidants catalyze the decomposition of the hydroperoxides in autoxidized fatty esters, the extent of which is dependent upon antioxidant concentration (19). This plays the role of decreasing the antioxidant effect. The effect of each phenothiazine derivative antioxidant on the decomposition of the hydroperoxides in autoxidized methyl linoleate is given in Figure 5, A and B. The order of the difficulty of decomposing the hydroperoxides in each phenothiazine derivative antioxidant can be shown by 2, 3, 7, 5, 8, 1, 4, and 6, by which the effective order in Table I is denoted. Therefore, in these antioxidants, the difficulty of decomposing the hydroperoxides does not run parallel to the superiority of the antioxidant.

From the results mentioned above, the overall process of phenothiazine derivative antioxidants (>NH) can be represented by the following reaction scheme, which is analogous to the description in the literature (20).

ROO· + >NH → ROOH + >N·	[A]		
O_2 + >NH → ·OOH + >N·	[B]		
ROO· + >N· → RO· + >NO·	[C]		
>NO· + R· → NOR	[D]	(21,22)	
>NO· + ROO· → Nonradical products	[E]	(21)	

Reaction A is popular, as mentioned in many other workers (20). From the result of the air oxidation in benzene solution for the antioxidant, which produces >N· (as recognized from the coupling constant (α^N) in Table II), it may be concluded that Reaction B occurs. As would be expected, Reaction C may occur very fast in comparison with the hydrogen abstraction reaction A (23). Therefore, very few if any radicals, >N·, exist. By Reaction C, the stable radicals >NO· are immediately produced. These nitroxide radicals can be found by the ESR spectra in the autoxidized methyl linoleate containing the antioxidant, as given in Table II. From Figures 3 and 4, the more inferior the antioxidant, the more reactive for oxygen, then the more easily Reaction B may occur. Therefore, the more inferior the antioxidant, the more rapidly the antioxidant radicals are produced by Reaction B. When the oxygen pressure becomes as high as 100 mm (e.g., ordinary air pressure), the majority of the radicals are present as peroxy radicals (ROO·) instead of alkyl radicals (R·) (24). Therefore, Reaction E may practically occur between the two reactions, Reactions D and E.

The most likely process responsible for the spontaneous initiation of autoxidation is the molecule-induced homolysis shown below.

RH + O_2 → R· + ·OOH [F] (24)

But, in other literature, oxygen, excited to its singlet state by a photosensitization process, plays the important role of forming the original hydroperoxides whose presence is necessary before the normal free radical autoxidation process can begin (25). In either case, oxygen can play a role in the spontaneous initiation of autoxidation. As mentioned above, the more superior the antioxidant, the more inactive it can make oxygen. In the following well-known chain reaction,

R· + O_2 → ROO·	[G]
ROO· + RH → ROOH + R·	[H]

peroxy radicals (ROO·) do not practically appear in the ESR spectra until about the end point of the induction

period because peroxy radicals may be consumed by the antioxidant, as represented by Reactions A, C, and E. In other words, until about the end point of the induction period, the antioxidant may be workable as the peroxy radicals' consumer. Therefore, the more effective the antioxidant, the slower the ROO· and the oxygen oxidation of the antioxidant by Reactions A and B, respectively, and, besides, the slower the ROO· oxidation of RH by Reaction H. Thus, some phenothiazine derivative antioxidants play a remarkably excellent role for inhibiting the autoxidation of methyl linoleate.

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[Received June 17, 1976]