Study on the Effect of Antioxidants in the Autoxidation of Methyl Nonconjugated Octadecadienoates

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Abstract

Many studies have been published on the effect of antioxidants on unsaturated fatty acid esters but the differences of the effects of antioxidants on geometric isomers have never been investigated. In this study, methyl *cis*-9, *cis*-12-octadecadienoate and its *trans* isomer methyl *trans*-9, *trans*-12-octadecadienoate were used as methyl nonconjugated dienoates, and BHA, BHT, PG, NDGA, 4,4′-dihydroxy-3,5,3′,5′-tetra-tert-butyl diphenyl methane, L-thyroxine sodium salt, α-tocopherol and sesamol were used, as antioxidants. The differences of the effects of antioxidants on both geometric isomers were investigated by determining the induction period using the weighing method. Also determined were the infrared and ultraviolet spectra, peroxide values, conjugated diene contents, isolated *trans* double bond contents and molecular weights for the controls and the samples containing antioxidants. The *cis,cis* isomer was more easily autoxidized and had a shorter induction period than the *trans,trans* form. By the end of the induction period, no isolated *trans* double bond forms in the *cis,cis* isomer, but a considerable amount of isolated *trans* double bond decreased in the *trans,trans* isomer. In general, the effects of antioxidants, except NDGA, on the *cis,cis* isomer were larger than the *trans,trans* form.

Introduction

Many studies have been made on the effect of antioxidant in autoxidation, but details are still obscure (1–14). There have been no papers on the differences of the effects of antioxidants on geometric isomers. In this study, methyl *cis*-9, *cis*-12-octadecadienoate and its geometric isomer methyl *trans*-9, *trans*-12-octadecadienoate were used as methyl nonconjugated dienoates, and butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), nordihydroguaiaretic acid (NDGA), 4,4′-dihydroxy-3,5,3′,5′-tetra-tert-butyl diphenyl methane, L-thyroxine sodium salt, α-tocopherol, and sesamol were used as antioxidants. The relative effectiveness of antioxidants for both geometric isomers was estimated by determining the induction period by the weighing procedure. Infrared and ultraviolet spectra, peroxide values, conjugated diene contents, isolated *trans* double bond contents and molecular weights for the controls and samples containing antioxidants were determined, and the component change for each sample at the end point of the induction period in the autoxidation was estimated.

Experimental Procedure

Sample

Preparation of Methyl Cis-9, Cis-12-Octadienoate (methyl linoleate). Methyl linoleate was obtained by treating safflower oil fatty acids with methanol (in quantity equivalent to twice the fatty acids) containing 2% of sulfuric acid and by fractionating them by twice the urea-adduct formation at room temperature. The second filtrate was further fractionated by urea-adduct formation at −20 C to obtain methyl linoleate as the urea-adduct and to remove natural antioxidants. Thus obtained, methyl linoleate was of 99% purity as determined by GLC. The removal of natural antioxidants from methyl linoleate was confirmed by colorimetry using ferric chloride and α,α′-dipyridyl alcohol solutions as reagents (15).

Preparation of Methyl Trans-9, Trans-12-Octadecadienoate. Ninety-nine per cent of linoleic acid was converted to the *trans,trans* isomer by treatment with nitric acid and sodium nitrite (16). The *trans,trans* isomer was refined by the elution chromatography with silica gel adsorbent and by low temperature recrystallization with acetone. Then *trans*-9, *trans*-12-octadecadienoic acid [mp found: 28.3–28.7 C; expected: 28–29 C (17,18)] was obtained, and converted to the methyl ester by using methanol containing sulfuric acid.

Antioxidant. BHA, BHT, PG, NDGA, α-tocopherol, L-thyroxine sodium salt, and sesamol were commercial products. 4,4′-Dihydroxy-3,5,3′,5′-tetra-tert-butyl diphenyl methane [mp 154.2–154.6 C; 154 C (19)] was a synthetic product. These products were used as antioxidants.

Autoxidation of Methyl Nonconjugated Octadecadienoate and the Sample Containing the Antioxidant (20, 21)

Each 1.5000 ± 0.0020 g of methyl nonconjugated octadecadienoate and 0.15 ml of alcohol 1.0×10^{-1}% (g/100 ml) antioxidant solution were added to a beaker of 4.1 cm in diameter. The solvent was carefully removed in a vacuum desiccator at constant temperature (30 ± 1 C) for 1 hr, at 1 mm Hg pressure. Each beaker was accurately weighed, then placed in an oven at a constant temperature of 36.5 ± 0.5 C. At given intervals each beaker was weighed to estimate weight increase. The depth of the oil layer for each sample was maintained constant.

Determination

The above mentioned weight increase was determined by the weighing procedure, which is the most convenient method for estimating autoxidation (20,21). The point of gaining weight chosen as the end point of the induction period was 10 mg, since at this point most samples began to gain weight rapidly. Based on the weight of each sample, the weight increase at this point was found to be 0.67%. Determination of infrared spectra (cell: 0.1 mm NaCl, solvent: carbon tetrachloride, carbon disulfide), ultraviolet spectra, peroxide values [m.eq./kg, iodometry (22)], conjugated diene contents (estimated from the result of the ultraviolet spectra), isolated trans double bond contents (estimated from the result of infrared spectra), and molecular weights (with the Hitachi Perkin-Elmer 115 type apparatus for molecular weight determination) was made for the samples.

TABLE I

Effect of Antioxidants on the Induction Period in the Autoxidation of Methyl *Cis*-9, *Cis*-12- and *Trans*-9, *Trans*-12-Octadecadienoates

Antioxidant	Methyl *cis*-9, *cis*-12-octadecadienoate (C)		Methyl *trans*-9, *trans*-12-octadecadienoate (T)	
	Induction period (hr)	Effect (ratio)[a]	Induction period (hr)	Effect (ratio)[a]
Blank control	3.5×10	1	5.2×10	1
BHA	2.0×10^2	5.7	2.6×10^2	5.0
BHT	2.9×10^2	8.3	1.7×10^2	3.3
PG	5.6×10^2	15.9	2.2×10^2	4.2
NDGA	7.0×10	2.0	3.2×10^2	6.1
D[b]	2.0×10^2	5.7	1.3×10^2	2.5
L-Thyroxine sodium salt	1.2×10^2	3.4	1.4×10^2	2.7
α-Tocopherol	4.5×10	1.3	7.5×10	1.4
Sesamol	3.2×10^2	9.1	5.5×10	1.1

[a] Ratio of the induction period of the sample containing antioxidant to that of C or T.
[b] 4,4'-Dihydroxy-3,5,3',5'-tetra-tert-butyl diphenyl methane.

Results and Discussion

The autoxidation induction period was estimated for methyl *cis*-9, *cis*-12-octadecadienoate (C) and methyl *trans*-9, *trans*-12-octadecadienoate (T) and for each sample containing the antioxidant. In one case, determination was performed for 10 samples and the significant figure had two units (Table I).

Infrared spectra for the samples gaining weight in the early stage were almost the same regardless the kind of antioxidant. Infrared spectra for the samples and for those gaining 10 mg and 50 mg (containing BHT) are shown in Figure 1 for *cis,cis* isomers and in Figure 2 for *trans,trans* isomers.

Peroxide values (m.eq./kg) for the blank controls and the samples (containing antioxidants) gaining 10 mg are given in Table II.

It has been confirmed that the main product in the early stage of the autoxidation for unsaturated compounds is a hydroperoxide having –OOH group at the methylenic group adjacent to double bonds (1,6). However, it is not absolutely certain whether the reaction of producing the hydroperoxide begins by abstracting hydrogen at the α-methylenic group (6) (molecule-induced homolysis may promote the reaction), or by direct oxygen attacking extremely small numbers of double bonds (1). In the autoxidation reaction at the steady state, the attack by the hydroperoxyl radical at the methylenic group adjacent to double bonds occurs, after all (1).

Comparing C with T in Table I, the induction period in the autoxidation for T is longer than for C. That is, T is more stable against autoxidation than C. This would be owing to whether the abstract of hydrogen in C occurs more easily than in T, or whether the direct attack of oxygen at an extremely small number of double bonds takes place more easily in C than in T. The effects of the antioxidants, except NDGA, on C are in general larger than on T. Figure 1 shows the absorption band for the hydroperoxyl group at 3450 cm^{-1} (23) in samples C

TABLE II

Peroxide Values for the Blank Controls and the Samples (Containing Antioxidants) Gaining 10 mg/1.5 g Weight

Antioxidant	Peroxide value (m.eq./kg)	
	C	T
Sample	1.2	2.3
Blank control	5.0×10^2	4.3×10^2
BHA	5.0×10^2	4.5×10^2
BHT	4.8×10^2	4.0×10^2
PG	3.8×10^2	4.0×10^2
NDGA	5.3×10^2	4.3×10^2
D	5.3×10^2	3.5×10^2
L-Thyroxine sodium salt	7.0×10^2	3.8×10^2
α-Tocopherol	4.5×10^2	4.8×10^2
Sesamol	5.0×10^2	3.8×10^2

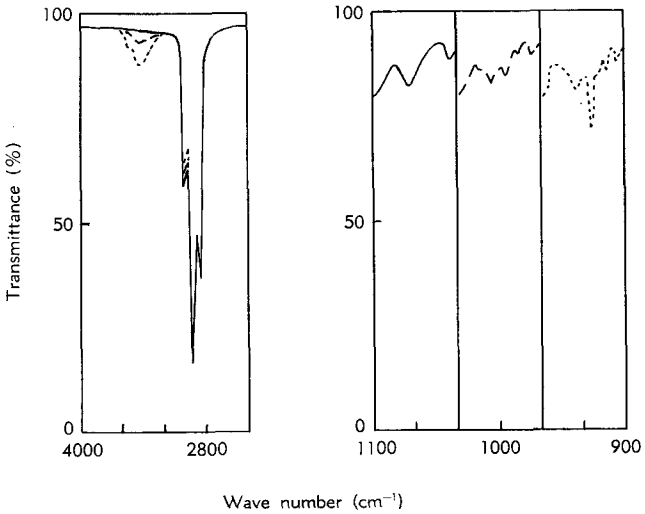

FIG. 1. Infrared spectra for methyl *cis*-9, *cis*-12-octadecadienoate and the methyl esters (containing BHT) gaining weight, 10 mg and 50 mg/1.5 g. ——— The sample, — — — 10 mg (weight increase), - - - - - 50 mg (weight increase).

gaining 10 mg. In samples T gaining 10 mg, the above mentioned absorption band although smaller than C, can be also recognized in Figure 2. This result agrees in general with the peroxide values in Table II.

In samples C gaining 10 mg, the absorption bands at 982 cm^{-1} and 948 cm^{-1} ascribed to *cis,trans* conjugated diene (17) and that at 988 cm^{-1} due to *trans,trans* conjugated diene (17) (absorption bands at 988 cm^{-1} and 982 cm^{-1} overlap) appear (Fig. 1). [In samples C gaining over 50 mg, the isolated *trans* double bond absorption (17) is observed as a shoulder at 968 cm^{-1}.] In samples T gaining 10 mg, the *cis, trans* conjugated diene bands at 982 cm^{-1} and 948 cm^{-1} scarcely appear, and only *trans,trans* conjugated diene exhibits a band at 988 cm^{-1} as a shoulder, as shown in Figure 2. (In the spectra for the samples T gaining over 50 mg, the *trans,trans* conjugated diene band at 988 cm^{-1} appears clearly.) The conjugated diene contents of the blank controls and of the samples gaining 10 mg/1.5 g, for samples C are generally larger than for samples T (4–7 and 3–5, respectively).

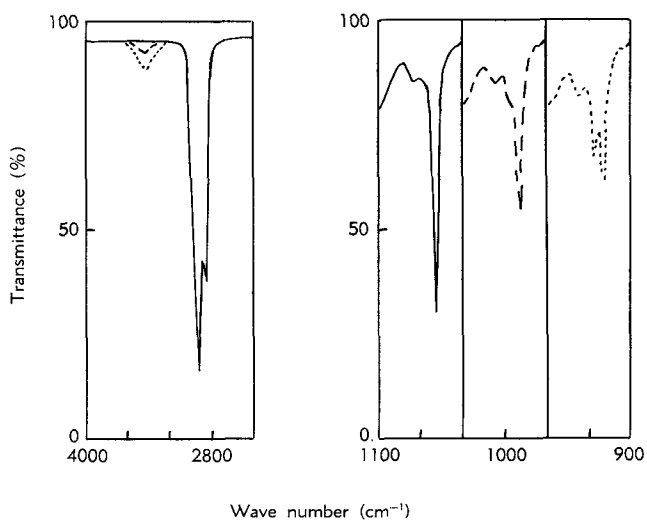

FIG. 2. Infrared spectra for methyl *trans*-9, *trans*-12-octadecadienoate and the methyl esters (containing BHT) gaining weight, 10 mg and 50 mg/1.5 g. ——— The sample, — — — 10 mg (weight increase), - - - - - 50 mg (weight increase).

Comparing samples C with T, the peroxide values for C are in general somewhat larger than T, as indicated in Table II. Therefore, samples T may absorb oxygen from and except from hydroperoxide since the initial stage. In infrared spectra for samples C, there is a difference between the absorption bands for the –OOH group and the conjugated diene in the original sample and bands appearing in samples gaining 10 mg; in general there is no difference for the other groups. However, for samples T, besides the differences of the –OOH group and conjugated diene, the isolated *trans* double bond decreases considerably in the sample gaining weight in comparison with the original sample. The formation of conjugated diene is little and, as mentioned above, usually less in samples T than in C. This indicates that the migration of the position of double bonds is small, especially in samples T. Nevertheless, the contents of isolated *trans* double bonds decrease considerably from 78% to 44–63% in samples T. Namely, some *trans* double bonds convert to *cis* isomers at the position they are. The geometric isomerization without the migration of double bond occurs by the reversible addition of a free radical (24). So, the addition of oxygen, a diradical, to double bond may occur reversibly. In contrast to samples T, in samples C gaining 10 mg no isolated *trans* isomers form. Therefore, in this case, the geometric isomerization without the migration of double bond does not occur. Namely, in samples C the reversible addition of oxygen to double bond is not probable. The above mentioned oxygen absorption, except by hydroperoxide in samples T, might be owing to this reversible addition of oxygen. Consequently, when the induction period ends, in samples C the absorbed oxygen is used only to the formation of hydroperoxides accompanied by the geometric isomerization due to the migration of double bonds. But in samples T besides this change the reversible addition of oxygen to double bond may occur, and the geometric isomerization not accompanied by the migration of double bond, is considerable. In either case, samples C or T, the molecular weight is ca. 290, and has almost the same value as in each original sample. Namely, no samples gaining 10 mg polymerize. Peroxide values, conjugated diene contents, isolated *trans* double bond contents, and molecular weight for samples containing antioxidant are generally little different from the blank control, in samples C and T, respectively. Consequently, it is confirmed that the action of these antioxidants only delays the induction period of the substrate, and does not change fundamentally the mechanism of the autoxidation of the substrate.

REFERENCES

1. Wexler, H., Chem. Rev. *64*, 591 (1964).
2. Morawetz, H., Ind. Eng. Chem. *41*, 1442 (1949).
3. Rosenwald, R. H., J. R. Hoatson and J. A. Chenicek, Ibid. *42*, 162 (1950).
4. Bickoff, E. M., JAOCS *28*, 65 (1951).
5. Cosgrove, S. L., and W. A. Waters, J. Chem. Soc. *1951*, 288.
6. Campbell, T. W., and G. M. Coppinger, J. Am. Chem. Soc. *74*, 1469 (1952).
7. Bickel, A. F., and E. C. Kooyman, J. Chem. Soc. *1953*, 3211.
8. Miller, G. J., and F. W. Quackenbush, JAOCS *34*, 404 (1957).
9. Privett, O. S., Ibid. *36*, 507 (1959).
10. Ingold, K. L., J. Phys. Chem. *64*, 1636 (1960).
11. Kim, D. H., and F. A. Kummerow, JAOCS *39*, 150 (1962).
12. Holman, R. T., W. O. Lundberg and T. Malkin, "Progress in the Chemistry of Fats and Other Lipids," Vol. 2, Pergamon Press, London, *1954*, p. 81.
13. Pryor, W. A., "Free Radicals," McGraw-Hill Book Company, New York, 1966, p. 293.
14. Emanuel, N. M., and Yu. N. Lyaskovskaya, "The Inhibition of Fat Oxidation Processes," Pergamon Press, London, 1967, p. 3, 121.
15. Mehlenbacher, V. C., "The Analysis of Fats and Oils," The Garrard Press, 1960, p. 586.
16. Harlow, R. D., C. Litohfield and R. Reiser, JAOCS *40*, 505 (1963).
17. Jackson, J. E., R. F. Paschke, W. Tolberg, H. M. Boyd and D. H. Wheeler, Ibid. *29*, 229 (1952).
18. Kass, J. P., and G. O. Burr, J. Am. Chem. Soc. *61*, 1062 (1939).
19. Kharasch, M. S., and B. S. Jochi, J. Org. Chem. *22*, 1437 (1957).
20. Olcott, H. S., and E. Einset, JAOCS *35*, 159 (1958).
21. Olcott, H. S., and E. Einset, Ibid. 161 (1958).
22. Privett, O. S., W. O. Lundberg and C. Nickell, Ibid. *30*, 17 (1953).
23. Privett, O. S., W. O. Lundberg, N. A. Khan, W. E. Tolberg and D. H. Wheeler, Ibid. *30*, 61 (*1953*).
24. Pryor, W. A., "Free Radicals," McGraw-Hill Book Company, New York, 1966, p. 275.

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