Technical

Effects of Amino Compounds on the Formation of γ-Tocopherol Reducing Dimers in Autoxidizing Linoleate

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ABSTRACT

The effects of various amino compounds trimethylamine oxide (TMAO), tri-*n*-octylamine (TOA), phosphatidyl choline (PC), and phosphatidyl ethanolamine (PE) on the oxidative decomposition of γ-tocopherol (γ-Toc) and on the formation of its reducing dimers were investigated during the autoxidation of methyl linoleate (ML). In general, γ-Toc diphenyl ether dimer (γ-TED) was formed in preference to two atropisomers of γ-Toc biphenyl dimers [γ-TBD(H) and (L)] in autoxidizing ML. This relationship, however, was reversed when TMAO was added. As the presence of TOA, PC, or PE did not promote the formation of γ-TBD, the preferential formation of γ-TBD was believed to be based on the interaction between TMAO and oxidation products formed from γ-Toc. Effects of TMAO and TOA on the interconversion of γ-Toc reducing dimers were investigated. γ-TED was found to be converted into γ-Toc and γ-TBD(L) in autoxidizing ML. But γ-TBD(H) could not be detected, and the amount of γ-TBD(L) formed was very small. γ-TBD(H) and (L) were formed from their respective atropisomers. In this case, the formations of γ-Toc and γ-TED could not be detected. Therefore, it was concluded that the conversion of γ-TED into γ-TBD and vice versa can be neglected in any event.

INTRODUCTION

Tocopherols (Toc) will be widely used rather than some synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), because Toc are natural antioxidants. However, Toc have a weaker effect on the autoxidation of oil and fat. Proper synergists are added to Toc in oil and fat to achieve marked antioxidative activities of Toc. The following substances have been used as synergists with Toc: TMAO (1), TOA (2), amino acids (3-5), melanoidin (6,7), flavonoids (8), phospholipid (9-11), ascorbic acid (12,13), citric acid (13-15), BHA (16), protein hydrolyzate (17,18), etc.

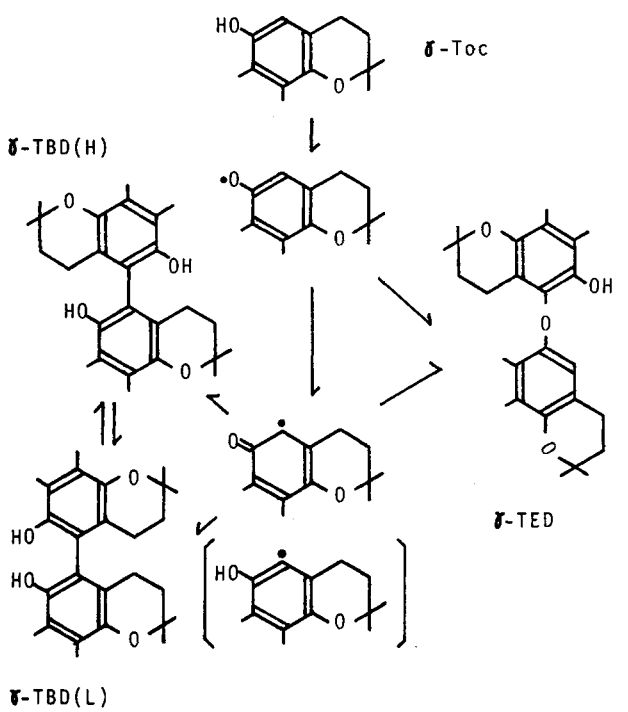
Synergism between Toc and amino compounds TMAO (19,20) and TOA (2) have been investigated in methyl linoleate (ML) autoxidation system. The reducing dimers of Toc were found to play an important role in synergism with TMAO or TOA rather than with their original Toc. In general, the reducing dimers γ-Toc diphenyl ether dimer (γ-TED) and two atropisomers, the γ-Toc biphenyl dimers [γ-TBD(H) and γ-TBD(L)] are formed from γ-Toc when γ-Toc is heated with TMAO under N_2 in liquid paraffin at 180 C (21). γ-TBD(H) and γ-TBD(L) show high and low Rf, respectively, since they are separable by thin layer chromatography (TLC), also, they are formed when γ-Toc is oxidized in autoxidizing oil (2,20). Phenoxy and phenyl radicals of γ-Toc are believed to be formed in these systems. Therefore, we concluded that the phenoxy radicals react with the phenyl radicals to produce γ-TED, and also that two phenyl radicals react with each other to produce γ-TBD. The pathway of their formation is shown in Scheme 1.

γ-TED is superior to γ-TBD with regard to antioxidative activities and the synergistic effects with TMAO (21). Further, the synergists were found to affect both the amounts and the ratios of γ-Toc reducing dimers formed (2,20). The best approach to considering remarkable synergism is to develop better synergists that can form γ-TED preferentially and maintain it for a long time in autoxidizing oil. In order to explain the relationship between the materials added and the kinds of the dimers formed, the present paper deals with the effects of various amino compounds on the oxidation of γ-Toc and on the formation of its reducing dimers.

EXPERIMENTAL PROCEDURES

Materials

Methyl linoleate, commercially available (Tokyo Kasei Co.), was passed through a silica gel column equilibrated with *n*-hexane to remove peroxides. *n*-Hexane and diisopropyl ether (IPE) were distilled in an all-glass still before use. L-3-phosphatidyl choline dipalmitoyl (PC) and L-3-phosphatidyl ethanolamine dipalmitoyl (PE) were purchased from Serdary Research Lab., Canada. TMAO and TOA were commercial products of analytical grade. γ-Toc was prepared from Toc mixture (Eisai Co.), which contains about 20% of edible oil. After saponification, unsaponifiable matter was column-chromatographed on silica gel, using a solvent system of *n*-hexane/ethyl ether. γ-Toc fractions were collected and then subjected to preparative high performance liquid chromatography (HPLC) for further purification. γ-Toc reducing



SCHEME 1. Pathway of the formation of the reducing dimers of γ-Toc.

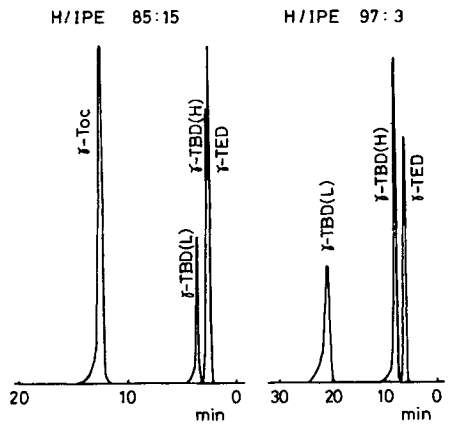


FIG. 1. HPLC of γ-Toc and its reducing dimers. Steel column (250 × 4 mm id) packed with LiChrosorb SI 60 (5μm) with *n*-hexane and diisopropyl ether as mobile phase (1 mL/min). A fluorescent detector with Ex 300 nm, Em 328 nm, and slit 10 nm.

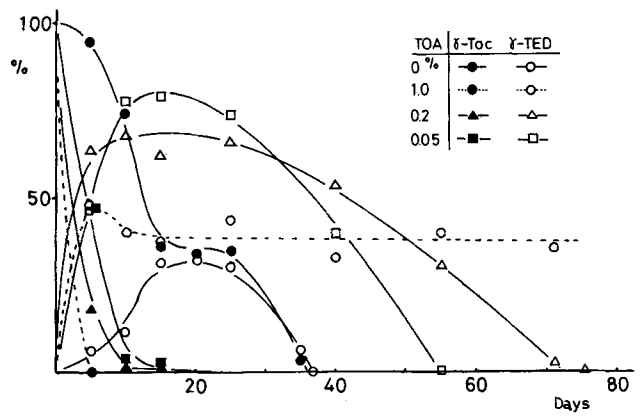


FIG. 2. Changes in the amounts of γ-Toc and γ-TED in the presence of tri-*n*-octylamine (TOA). The initial concentration of γ-Toc was 2.0%.

dimers were prepared by reacting γ-Toc dissolved in liquid paraffin with TMAO under N_2 at 180 C for 1 hr (21). After cooling, the reaction mixture was diluted ca. twice with *n*-hexane and applied on a silica gel column equilibrated with *n*-hexane. After liquid paraffin was washed away with *n*-hexane, the elution was continued with increasing amount of ethyl ether in *n*-hexane. The eluates were pooled into 2 fractions: one being γ-TED and γ-TBD(H); the other, γ-TBD(L). Each dimer was further purified by repeated preparative HPLC.

Autoxidation

One hundred and fifty mg of ML containing different concentrations of γ-Toc and amino compounds were placed in a vial with a flat bottom, 18 mm id, and autoxidized in the dark at 50 C. Samples in duplicate, taken out at varied time intervals, were dissolved in 5 mL of *n*-hexane and an aliquot was applied on HPLC for the determination of γ-Toc and its dimers.

HPLC

Preparative HPLC was done on silica gel (Merck LiChroprep SI 60) packed in a stainless steel column (8 × 500 mm) at 3 mL/min. The instrument used was a Hitachi liquid chromatograph Model 633A and the effluent was monitored with a Hitachi spectrophotometer at 295 nm. Mobile phases were *n*-hexane/IPE, 85:15 and 92:8, for the purification of γ-Toc and γ-TBD(L), respectively. The eluent *n*-hexane/IPE, 98:2 was used for separating γ-TED from γ-TBD(H).

Analytical HPLC was done on a silica gel (Merck LiChrosorb SI 60, 5μm) column (4 × 250 mm) at 1 mL/min. The effluent was monitored with a Hitachi fluorescence spectrophotometer Model 650-10S with Ex 300 nm, Em 328 nm, and a slit of 10 nm. Mobile phases were as follows: *n*-hexane/IPE, 85:15, for determining γ-Toc and γ-TBD(L); and *n*-hexane/IPE, 97:3, for determining γ-TED and γ-TBD(H). The amount of each substance was calculated from the height of each peak as compared to the height of a known amount of each standard. Figure 1 shows a typical chromatogram of HPLC for the analyses of γ-Toc and its reducing dimers.

RESULTS AND DISCUSSION

Effect of TOA on Dimerization of γ-Toc

The amount of γ-TBD formed was larger than that of γ-TED during the autoxidation of ML containing γ-Toc (4.0%) and TOA (2.0%) (2). Because higher concentrations of γ-Toc cannot be used in practice, we attempted to determine whether TOA influenced dimerization of lower concentrations of γ-Toc. Figure 2 shows the changes in the amounts of γ-Toc and γ-TED at 2.0% γ-Toc. The presence of 0.2% and 0.05% TOA led to the formation of a large amount of γ-TED until ca. 20 days. Then γ-TED decreased as the autoxidation of ML proceeded. The amount of γ-TED formed at 1.0% TOA was lesser than that in the former cases. However, it was maintained almost at constant values within the period tested. The amount of γ-TBD formed at 1.0% TOA was larger than that at 0.2% and 0.05% TOA although no data were shown.

As Table I shows, the additive effect of TOA, especially at the 0.2% and 0.05% levels, on dimerization of 0.1% and 0.5% γ-Toc was characterized by the preferential formation of γ-TED. However, the higher concentration of TOA was found to promote the formation of γ-TBD as in the case of TMAO.

Effect of TMAO on Dimerization of γ-Toc

As Figure 3 shows, a large amount of γ-TBD was formed in the presence of TMAO. These data are quite different from those described above. The amount of γ-TED formed was slightly influenced by the respective concentrations of γ-Toc and TMAO. However, the amount of γ-TED was always less than that of γ-TBD. Similarly, in the case of δ-Toc, δ-TBD was preferentially formed at the higher levels of TMAO (19). Therefore, TMAO was found to stimulate the preferential formation of biphenyl dimers of Toc.

Effect of Phospholipid on Dimerization of γ-Toc

During the thermal oxidation of trilinolein, the addition of a small amount of soybean lecithin or sheep brain cephalin greatly decreased γ-Toc consumption (22). The amount of γ-TED formed increased slightly and that of γ-TBD did not. Soybean lecithin was also reported greatly to inhibit the thermal oxidation of various Toc in lard (23). Then the effects of PC and PE on oxidation of γ-Toc were investigated in the ML autoxidation system.

As shown in Figures 4 and 5, the additive effect of PC was almost the same as that of PE. The addition of PC or PE to 0.5% γ-Toc resulted in an increase in the amount of γ-TED formed, whereas they seemed not to affect the formation of γ-TBD. In the case of 0.1% γ-Toc, the additive effects of PC and PE were hardly observed although the amount of γ-TED was increased in appearance due to rapid oxidation of γ-Toc.

TABLE I

Changes in the Amounts of γ-Toc and its Reducing Dimers During the Autoxidation of Methyl Linoleate Containing TOA

Time treated (days)	Concn. of TOA (%)	γ-Toc 0.1%					γ-Toc 0.5%				
		γ-Toc	γ-TED	γ-TBD(H)	γ-TBD(L)	Total	γ-Toc	γ-TED	γ-TBD(H)	γ-TBD(L)	Total
1	1.0	12.5	12.5	14.9	18.6	86.5	69.1	16.5	6.4	7.5	99.5
2	1.0	12.3	41.0	16.0	20.5	89.8	59.1	20.7	7.9	8.2	95.9
	0.2	56.0	38.9	3.2	5.2	103.3	85.4	12.7	trace	1.6	99.7
	0.05	79.1	16.8	trace	trace	95.9	96.5	3.5	trace	trace	100.0
5	1.0	9.5	41.4	15.3	21.4	87.6	33.8	36.3	13.5	16.4	100.0
	0.2	36.2	53.9	4.9	7.2	102.2	70.0	26.0	2.1	2.5	100.6
	0.05	69.3	24.8	trace	2.4	96.5	83.4	17.4	0.8	1.1	102.7
10	1.0	11.8	39.2	14.4	19.0	84.4	22.3	40.1	15.5	17.8	95.7
	0.2	37.4	49.9	3.6	5.7	96.6	58.3	37.5	3.3	4.3	103.4
	0.05	54.9	37.7	1.2	2.0	95.8	71.2	24.9	0.6	1.5	98.2
20	1.0	10.3	38.6	14.3	18.6	81.8	27.3	36.5	14.0	17.2	95.0
	0.2	27.7	50.5	3.3	5.0	86.5	59.4	32.7	2.7	3.7	98.5
	0.05	23.2	54.2	1.4	2.4	81.2	39.0	52.9	2.1	3.4	97.4
36	1.0	5.9	34.7	13.9	18.4	72.9	36.0	30.7	11.7	13.9	92.3
	0.2	10.1	46.0	1.6	5.2	62.9	47.0	42.7	2.8	5.6	98.1
	0.05	2.7	11.9	trace	trace	14.6	15.5	71.7	2.2	2.8	92.2
50	1.0	6.0	33.6	11.6	16.8	68.0	29.3	32.7	12.6	15.6	90.2
	0.2	2.3	13.8	trace	1.4	17.5	31.9	48.6	4.3	5.5	90.3
	0.05	—	—	—	—	—	3.6	26.2	0.5	1.1	31.4
58	0.2	2.1	10.5	0.0	0.0	12.6	—	—	—	—	—
	0.05	—	—	—	—	—	trace	0.3	0.0	0.0	0.3
70	1.0	7.1	33.0	13.9	14.0	68.0	29.0	31.8	12.2	14.7	87.7
	0.2	—	—	—	—	—	17.7	60.0	4.6	6.1	88.4

The amount of each compound was expressed in its weight proportion (%) to the initial weight of γ-Toc.

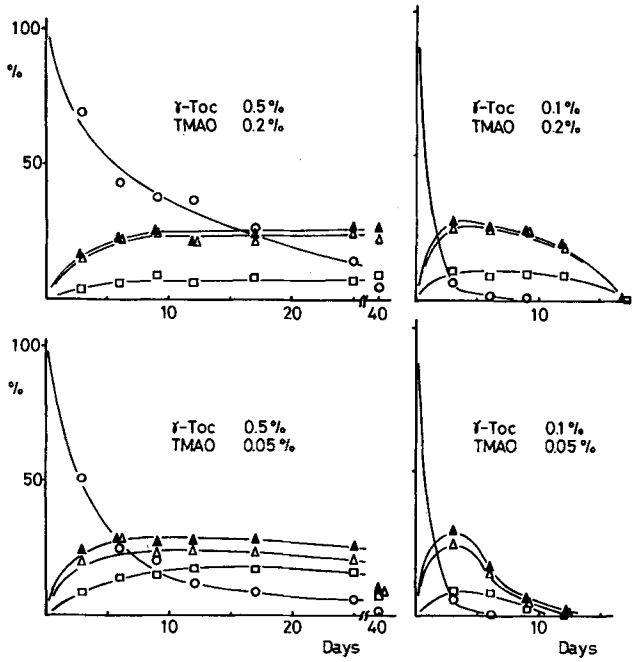


FIG. 3. Changes in the amounts of γ-Toc and its reducing dimers in the presence of trimethylamine oxide (TMAO). γ-Toc (○), γ-TED (□), γ-TBD(H) (△), and γ-TBD(L) (▲).

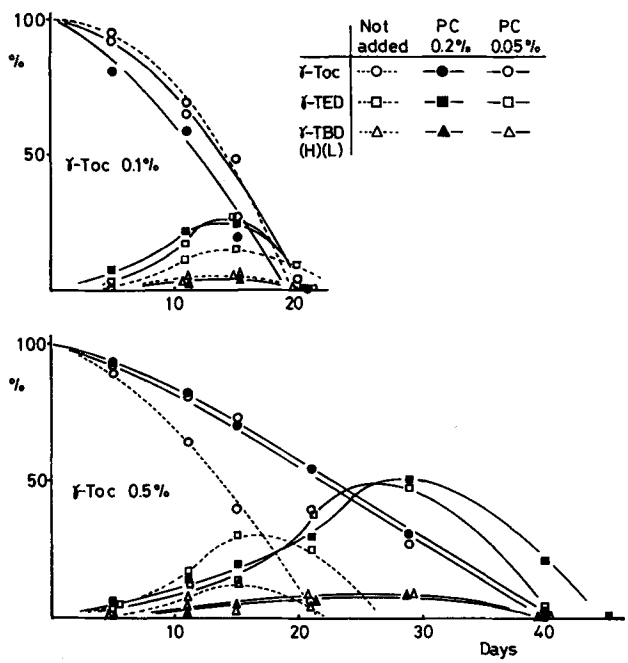


FIG. 4. Changes in the amounts of γ-Toc and its reducing dimers in the presence of phosphatidyl choline (PC).

Effect of Temperature on the Formation of γ-Toc Dimers

As described above, both the amounts and the ratios of γ-Toc reducing dimers which were formed, were changed not only with the concentration of γ-Toc, but also with the concentrations and the kinds of amino compounds. In order to clarify whether TMAO, a potent prooxidant for ML (24), forms γ-TBD preferentially due to the acceleration of ML autoxidation, the changes in the amounts of γ-Toc and its dimers were investigated during the autoxidation of ML with 0.1% and 0.5% γ-Toc at various temperatures.

As Table II shows, the amount of γ-TED formed was

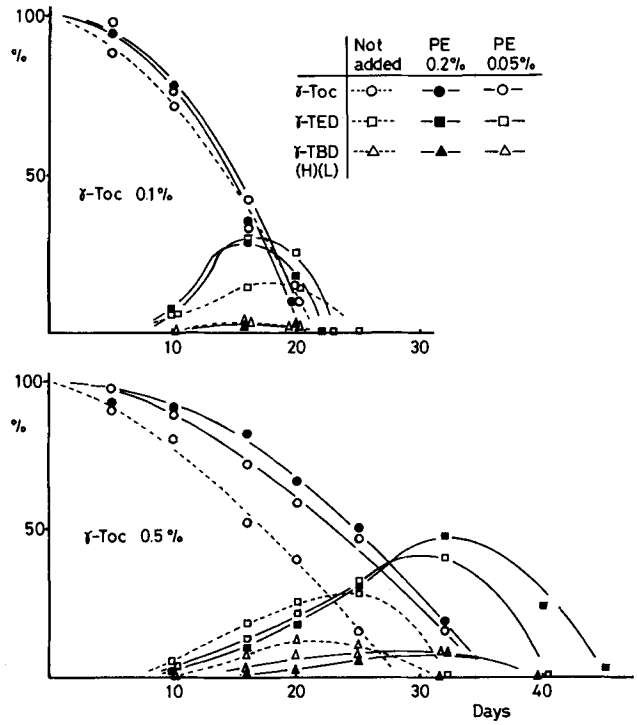


FIG. 5. Changes in the amounts of γ-Toc and its reducing dimers in the presence of phosphatidyl ethanolamine (PE).

larger at lower temperatures. These facts indicate that higher temperature leads γ-TED to decompose immediately after its formation. The higher the initial concentration of γ-Toc was, the larger was the amount of γ-TBD formed. In general, however, the amount of γ-TBD was smaller than that of γ-TED in every temperature tested. These results show that autoxidation of ML with γ-Toc tends to form γ-TED, rather than γ-TBD. This means that TMAO does not form γ-TBD preferentially according to its potent prooxidant effect (24). Therefore, it should be pointed out that the preferential formation of γ-TBD when TMAO is used as a synergist may be due to the interaction between TMAO and oxidation products formed from γ-Toc.

As PC and PE could slightly inhibit the autoxidation of ML, γ-TED may accumulate. TOA at the 0.2% and 0.05% levels seems to contribute effectively to the formation of a larger amount of γ-TED.

Effects of TOA and TMAO on the Interconversion of γ-Toc Dimers

During the autoxidation of ML with γ-Toc, γ-TED, γ-TBD(H), and γ-TBD(L) were always formed. The ratios of each dimer varied greatly with different conditions. If a difference in the ratio develops due to an interconversion of the γ-Toc reducing dimers themselves, TMAO and TOA must function as promoters in the formation of γ-TBD and γ-TED, respectively. Table III shows the effects of TMAO an TOA on the behaviors of 0.1% γ-TED dissolved in ML. γ-Toc and γ-TBD(L) were formed from γ-TED, but γ-TBD(H) could not be detected. The amount of γ-TBD(L) formed was very small. TMAO and TOA were found not to be involved in the conversion of γ-TED to γ-TBD by comparison with the results of a control experiment. Therefore, it may be concluded that the amounts of γ-TED and γ-TBD formed from γ-Toc in ML autoxidation system depend on the extent to which either of the two dimerization processes occur after the oxidation of γ-Toc. The formation of a small amount of γ-Toc and γ-TBD(L) suggests that after the cleavage of the ether linkage in γ-TED: (a) γ-Toc is regen-

TABLE II

Effect of Temperature on the Formation of γ-Toc Reducing Dimers

Temp. (C)	Initial concn. of γ-Toc (%)	Time treated (days)	γ-Toc	γ-TED	γ-TBD(H)	γ-TBD(L)	Total
70	0.5	2	62.5	7.1	3.7	5.1	78.4
		3	23.4	13.0	4.1	6.1	46.6
		4	2.6	4.6	0.8	1.2	9.2
	0.1	1	89.2	1.3	trace	trace	90.5
		2	51.9	4.0	0.0	0.0	55.9
60	0.5	7	47.2	20.3	5.0	6.8	79.3
		9	20.0	23.4	4.5	6.6	54.5
	0.1	4	83.5	4.1	trace	trace	87.6
		7	18.1	12.2	1.0	2.0	33.3
40	0.5	18	85.8	4.8	1.0	2.2	93.8
		30	78.4	9.2	1.9	3.7	93.2
		50	46.5	28.0	4.1	6.6	85.2
	0.1	18	78.9	5.5	trace	1.8	86.2
		30	67.2	11.4	trace	2.4	81.0
		50	23.2	26.2	trace	3.2	52.6

The amount of each compound was expressed in its weight proportion (%) to the initial weight of γ-Toc.

TABLE III

Behavior of γ-TED during the Autoxidation of Methyl Linoleate

Time treated (days)		None	TOA 0.05%	TOA 0.2%	TMAO 0.05%	TMAO 0.2%
2	γ-TED	90.7	58.8	64.9	41.8	49.2
	γ-Toc	2.0	5.4	4.6	2.2	3.9
	γ-TBD(L)	0.0	0.0	0.6	1.4	1.1
4	TED	75.5	55.5	64.2	44.4	40.1
	Toc	5.3	5.4	4.8	2.5	3.8
	TBD(L)	0.7	1.9	1.1	1.1	1.1
7	TED	51.4	41.4	50.2	41.4	23.2
	Toc	8.2	4.7	4.6	2.5	2.7
	TBD(L)	0.7	0.3	0.0	0.7	0.7
10	TED	31.8	34.5	53.2	43.8	16.6
	Toc	6.7	4.2	4.5	3.1	2.0
	TBD(L)	0.0	0.3	0.0	0.4	1.4
13	TED	0.0	14.1	49.3	36.7	0.0
	Toc	trace	2.4	3.8	2.8	0.0
	TBD(L)	0.0	trace	0.0	trace	0.0
20	TED	–	–	50.0	7.0	–
	Toc	–	–	4.3	1.1	–
30	TED	–	–	22.1	–	–
	Toc	–	–	2.5	–	–

The amount of each compound was expressed in its weight proportion (%) to the initial weight of γ-TED. γ-TBD(H) could not be detected.

erated by way of phenoxy radical and the following oxidation of γ-Toc leads to the formation of γ-TBD(L); and/or (b) γ-TBD(L) itself is formed from the phenyl radical.

Tables IV and V show the behaviors of 0.1% γ-TBD(H) and 0.1% γ-TBD(L), respectively, dissolved in ML. γ-TBD (H) and (L) can be formed from their respective isomers. Atropisomerization of γ-TBD(H) and (L) required a considerably higher temperature when each of them was heated in liquid paraffin under N_2 (21). However, it is found that they can easily atropisomerize with each other in autoxidizing ML even at comparatively low temperatures. Conversion of γ-TBD(H) into (L) occurred more easily than that of γ-TBD(L) into (H).

In the previous paper (20), a possible mechanism of synergism between Toc and TMAO in the inhibition of the autoxidation of ML was proposed as follows. The reducing dimers of Toc were consumed by offering H• to peroxy radical of ML to give radicals of the dimers and hydroperoxide. An active intermediate of TMAO, (TMAO-H)•, offers H• to radicals of the dimers, and thus the dimers are regenerated. In this experiment with TMAO, therefore, it was assumed that after γ-TBD (a radical type which has no steric hindrance by the OH groups) atropisomerized, the original γ-TBD was regenerated. However, the atropisomerization occurred in the presence of TOA and also in the absence of TMAO and TOA. It is believed that the synergistic effects of TMAO (20) and TOA (2) on the antioxidative activities of Toc differ from each other. Thus further investigations must be done on other possible mechanisms of the conversion.

TABLE IV

Behavior of γ-TBD(H) during the Autoxidation of Methyl Linoleate

Time treated (days)		None	TOA 0.05%	TOA 0.2%	TMAO 0.05%	TMAO 0.2%
2	γ-TBD(H)	83.0	45.7	49.6	38.7	45.1
	γ-TBD(L)	12.6	13.5	32.8	33.6	35.4
4	(H)	82.0	30.3	52.7	38.0	40.7
	(L)	15.1	19.7	29.7	31.0	33.3
7	(H)	50.3	24.2	43.0	8.1	16.1
	(L)	24.0	20.6	30.7	9.9	18.1
10	(H)	18.9	12.1	19.7	3.0	0.0
	(L)	19.2	11.0	18.1	5.2	0.0

The amount of each compound was expressed in its weight proportion (%) to the initial weight of γ-TBD(H). γ-Toc and γ-TED could not be detected.

TABLE V

Behavior of γ-TBD(L) during the Autoxidation of Methyl Linoleate

Time treated (days)		None	TOA 0.05%	TOA 0.2%	TMAO 0.05%	TMAO 0.2%
2	γ-TBD(L)	91.8	60.0	46.3	26.9	38.5
	γ-TBD(H)	4.9	15.0	16.3	13.2	16.3
4	(L)	77.9	57.6	44.2	21.7	40.4
	(H)	8.6	13.0	17.5	15.0	17.5
7	(L)	63.0	34.2	38.8	25.5	26.1
	(H)	15.1	13.5	16.2	10.8	15.1
10	(L)	3.8	12.0	26.6	0.0	0.0
	(H)	2.6	7.9	14.2	0.0	0.0

The amount of each compound was expressed in its weight proportion (%) to the initial weight of γ-TBD(L). γ-Toc and γ-TED could not be detected.

As shown in Tables IV and V, the formations of γ-Toc and γ-TED from 0.1% γ-TBD could not be detected in any ML autoxidation system tested at 50 C. However, during the autoxidation of ML containing 4.0% γ-Toc and 2.0% TOA at 50 C (2), the amount of γ-TED increased with decreasing amount of γ-TBD in a later stage of the reaction, whereas γ-Toc had been already consumed at the time. Therefore, γ-TED must be formed from γ-TBD because γ-Toc did not exist. In order to resolve this conflict, investigations will be required at higher concentrations of γ-TBD in ML. Furthermore, tests will be done to determine whether even small amounts of γ-Toc and γ-TED could be formed from γ-TBD at the higher temperatures that cause the atropisomerization of γ-TBD.

As the conversion of γ-TED into γ-TBD and vice versa can be neglected in any event, TMAO was found to be closely involved by its interaction with oxidation products formed from γ-Toc for the preferential formation of γ-TBD. It is preferable for effective synergists to contribute to the formation of the reducing dimers of biphenyl ether type and to synergize with the dimers. TMAO is not an ideal synergist. However, the ternary system of γ-Toc, TMAO, and some phospholipids show a marked inhibition of the oxidative decomposition of γ-Toc dissolved in ML (unpublished data). Therefore, clarifying the mechanism of the preferential formation of each reducing dimer of Toc will lead to develop safer and more effective synergists for Toc.

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