報 文

Synergism of Amines and Their Oxides with Tocopherols
in Inhibiting the Oxidation of LardYukihiro ISHIKAWA, Etsuji YUKI*, Hiromichi KATŌ**,
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The synergistic effects of several aliphatic amines and their oxides on the antioxidative activity of tocopherols in inhibiting the oxidation of lard were investigated and compared with those of trimethylamine oxide (TMAO). As for amines, the lower the volatility and the larger the molecular weight of amines become, the more effective they are, and tri-*n*-octylamine (TOA) showed the most excellent synergism with tocopherols. As for oxides, however, TMAO having the lowest molecular weight was found to be most effective. A distinct difference between TOA and TMAO was recognized in their synergistic mechanism.

1 Introduction

Although we have made much effort to clarify the synergistic mechanism between tocopherols and trimethylamine oxide (TMAO)^{1)~3)}, it was found that TMAO itself did not show any antioxidative activity but acted as an excellent synergist for phenolic antioxidants such as tocopherols and butylated hydroxyanisole¹⁾. In this case, it must be clarified whether TMAO itself or a substance derived from TMAO acts as a synergist for tocopherols.

Olcott *et al.*^{4)~6)} described on the synergistic effects of several amines with phenolic antioxidants, but did not discuss their synergistic mechanism. Among aliphatic amines tri-*n*-octylamine (TOA) showed synergism with phenolic antioxidants in inhibiting the oxidation of squalene, and TOA was more effective as an antioxidant and a synergist for ethoxyquin in the oxidation of menhaden oil at 70°C than it was at 50 and 60°C^{5)~6)}. It was suggested that the antioxidant and synergistic effects of TOA were due to a free-radical intermediate formed by the reaction of TOA with peroxides. This effective substance was isolated and determined to be dioctylhydroxylamine resulting from Cope elimination reaction

of tri-*n*-octylamine oxide (TOAO), and also obtained from the reaction of TOA with *t*-butyl hydroperoxide⁷⁾. On treatment at 70°C, synthesized TOAO formed radicals and showed an antioxidative activity, but no activity was observed in the case of TMAO⁷⁾.

The present paper, therefore, deals with the synergistic effects of several aliphatic amines and their oxides on the antioxidative activity of tocopherols compared with those of TMAO, and also discusses whether amines or their oxides take part in synergism with tocopherols.

2 Experimental

2.1 Tocopherols

Natural tocopherol mixture (M-Toc), a commercial product from Eisai Co. containing *ca.* 80% of tocopherols, was used; its composition was α -tocopherol 8.5%, γ -tocopherol 57.8%, and δ -tocopherol 33.7%. γ -Tocopherol (98.5%) was kindly provided by Eisai Co. and used without further purification.

2.2 Amines

Commercial products were used without further purification.

2.3 Oxides

TMAO is a commercial product of Tokyo Kasei

Co. and others were prepared from corresponding tertiary amines according to the method of Hoh *et al*⁹⁾, using 30% of hydrogen peroxide.

2.4 Antioxidative activity

The antioxidative activity of M-Toc was determined according to the weight gain method⁹⁾ and the Active Oxygen Method¹⁰⁾, and the reaction conditions are shown in Table-1 and 2, respectively.

2.5 GLC of amines

The conditions of GLC for the qualitative determination of amines were as follows: apparatus, Shimadzu GC-5A gas chromatograph equipped with FID; column, glass column (ϕ 3 mm $\times$ 2 m) packed with diglycerol+TEP+NaOH (15+5+2%) on Uniport KS, 60/80 mesh; column temperature, 80°C.

3 Results and Discussion

3.1 Synergism between M-Toc and amines

The synergistic effects of various aliphatic amines on the antioxidant activity of M-Toc were investigated by the weight gain method⁹⁾ using lard as a substrate, and the results are shown in Table-1.

All amines tested did not show any antioxidative activity and trimethylamine hydrochloride

Table-1 Synergism between tocopherols and amines.

Reagent	Induction period (d)	
	without Toc	with Toc
None	1	27
Trimethylamine oxide	1	94
Trimethylamine aq. soln.	1	27
Trimethylamine HCl salt	< 1	4
Triethylamine	1	31
Tri- <i>n</i> -butylamine	1	67
Tri- <i>n</i> -octylamine	1	>150
Diethylamine	2	37
Di- <i>n</i> -butylamine	2	49
Ethylamine	2	38
<i>n</i> -Propylamine	2	43
<i>n</i> -Butylamine	2	43
Stearylamine	1	53

Eight hundred and fifty mg of lard containing M-Toc (0.04%) and each reagent (2×10^{-3} M) was placed in a Petri dish (I. D., 45 mm) and kept in the dark at 60°C. Induction period was defined as the days required to increase the weight of lard by 0.5%.

acted as a potent prooxidant. Some primary, secondary, and tertiary amines had the synergistic effects with M-Toc, and the lower the volatility becomes, the more effective they are, especially TOA. Although trimethylamine having high volatility did not show synergism with M-Toc, its oxide, TMAO, was found to be one of the most effective compounds tested owing to its very low volatility.

3.2 Comparison between the effects of amines and their oxides

From the facts that tertiary amines like TOA and oxides like TMAO show excellent synergism with M-Toc in inhibiting the oxidation of lard, differences between tertiary amines and their oxides in the synergistic effect with M-Toc, were investigated. The results are shown in Table-2.

Table-2 Comparison of synergistic effects of amines and their oxides on the antioxidative activity of tocopherols.

Reagent	Induction period (h)	
	without Toc	with Toc
None	2.5	38.5
Trimethylamine	2.5	39.5
Triethylamine	2.5	39.5
Tri- <i>n</i> -butylamine	3.0	47.0
Tri- <i>n</i> -octylamine	4.0	125.0
Trimethylamine oxide	3.0	98.0
Triethylamine oxide	6.0	48.0
Tri- <i>n</i> -butylamine oxide	16.0	57.5
Tri- <i>n</i> -octylamine oxide	22.5	56.0

Lard containing M-Toc (0.04%) and each reagent (2×10^{-3} M) was treated under the AOM conditions. Induction period was defined as the hour required to reach POV 100.

In the absence of M-Toc, amines did not show any antioxidant activity regardless of their volatility. However, their oxides having very low volatility showed some antioxidative activity, and the activity was found to be closely related to the size of alkyl groups in molecule; *i.e.* TOAO had the largest activity.

The synergistic effects of amines with M-Toc increased with the increase of their molecular weight. Then TOA showed the most excellent synergistic effect with M-Toc. On the contrary, the synergistic effects of oxides were not always

related mutually with their size of molecular weight, and TMAO having the smallest alkyl group had the most remarkable effect.

A mixture of TMAO and some substances in liquid paraffin was kept in a sealed vessel at 50°C. After standing in the dark for 2 days, a head space gas of the mixture was tested with a litmus paper on its alkalinity. The results are shown in **Table-3**.

Table-3 Alkalinity of head space gas of reaction mixtures containing trimethylamine oxide.

	Alkalinity (Litmus)
TMAO	—
TMAO + methyl linoleate	+
TMAO + AIBN*	++
TMAO + γ -Toc	±
TMAO + γ -Toc + AIBN*	++
AIBN* + γ -Toc	—
AIBN*	—

*: Azo-bis-isobutyronitrile

The analysis of the gas by GLC gave only a peak corresponding to trimethylamine, and the retention times of amines were as follows: GLC (t_R), trimethylamine, 0.6 min; dimethylamine, 1.2 min; monomethylamine, 2.2 min. Azo-bis-isobutyronitrile is an initiator of radical chain reaction and it was found that trimethylamine was generated by reacting TMAO with radicals formed from oxidized methyl linoleate and with antioxidants such as γ -tocopherol.

Synergism between tocopherols and TOA was thought to be related to the degradation of one of three larger alkyl groups of tertiary amines by Cope elimination reaction and the resulting formation of dioctylhydroxylamine^{6)~7)}. However, the facts that only trimethylamine generated from the reaction mixture containing TMAO as

previously stated, indicate that there is a difference between TMAO and TOA in the mode of their synergistic effects with tocopherols. As trimethylamine had no synergistic effect with M-Toc due to its large volatility, it is concluded that TMAO does not show synergism with tocopherols after being degraded to trimethylamine but TMAO itself serves as a potent, synergist for tocopherols.

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