Chem. Pharm. Bull. 30(8)2964—2970(1982)

Studies on the Antioxidants. XVI.[1] Synergistic Reaction between Butylated Hydroxyanisole and Butylated Hydroxytoluene in Hydrogen Donation to 2,2-Diphenyl-1-picrylhydrazyl

Tsutao Kurechi and Tetsuta Kato*

Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan

(Received January 9, 1982)

Hydrogen-donating processes of combined antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), were followed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as the hydrogen acceptor, and their relation to the synergism is discussed. The hydrogen donation to DPPH by BHA was almost complete in 10 min. DPPH and BHA were regenerated from this reaction mixture by addition of DPPH·H in the molar ratio of 2: 1, and BHA and 2,6-di-*tert*-butylquinone methide (QM) were formed by addition of BHT in the molar ratio of 1: 1. The phenoxy radical formed from BHA was lost upon further addition of DPPH. These results suggest the formation of a stable intermediate which accepts two hydrogens from BHT to regenerate BHA, and the possible reaction processes of BHA and BHT with DPPH are shown as Chart 1; BHA reacts with DPPH to form the stable intermediate *via* its phenoxy radical, and this intermediate reacts with BHT to regenerate BHA with the enhanced oxidation of BHT to QM *via* its phenoxy radical. These processes may be associated with the synergism between BHA and BHT in hydrogen donation to DPPH. The amount of loss of DPPH was in fair agreement with the sum of two hydrogens donated by BHT in the enhanced oxidation to QM and hydrogens donated by BHA. The rate of decrease in DPPH concentration by BHA was about 450 times that by BHT.

Keywords——antioxidants; butylated hydroxyanisole; butylated hydroxytoluene; 2,2-diphenyl-1-picrylhydrazyl; phenoxy radical; synergism

Synergism between butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in inhibition of lard and cumene oxidations[2,3] has been demonstrated. Several kinetic explanations have been suggested for the synergism between 4-methoxyphenol derivatives such as BHA and hindered phenols such as BHT,[3,4] but the behavior of antioxidants and their oxidation products in relation to the synergism has not been elucidated.

Hydrogen or electron donation of antioxidant to the free radicals formed during propagation reactions is known to be one of the most important reactions inhibiting the autoxidation of oils and fats.[5,6] Our previous experiments showed that a combination of BHA and BHT exhibited synergistic effects on the reduction of ferric ion[7] and on hydrogen donation to 2,2-diphenyl-1-picrylhydrazyl (DPPH),[8] and that in the latter case this effect might arise from the regeneration of BHA from the possible intermediate formed from BHA and DPPH, with enhanced loss of BHT.[1,8] This study was undertaken to characterize the reactions of BHA and BHT in hydrogen donation to DPPH. The processes related to the synergism are discussed.

Experimental

Materials——DPPH (Tokyo Kasei Kogyo Company, Ltd.) was recrystallized from benzene–ether mixture.[9] 2,2-Diphenyl-1-picrylhydrazine (DPPH·H) was the product of Tokyo Kasei Kogyo Company, Ltd. BHA and BHT, both supplied by Nikki-Universal Company, Ltd., were recrystallized from petroleum ether[10] and ethanol, respectively. 2,2′-Dihydroxy-5,5′-dimethoxy-3,3′-di-*tert*-butylbiphenyl (A-I) and 2′,3-di-*tert*-butyl-2-hydroxy-4′,5-dimethoxybiphenyl ether (A-II) were isolated by silica gel column chromatography from ultraviolet (UV)-irradiated BHA benzene solution.[10] 2,6-Di-*tert*-butylquinone methide (QM) was obtained by debromination[11] of 2,6-di-*tert*-butyl-4-bromo-4-methylcyclohexa-3,5-dienone, which was

itself prepared by bromination of BHT.[12]

Analytical Methods——The absorbance was measured with a Hitachi 101 spectrophotometer or a Shimadzu UV-200S double-beam spectrophotometer. The concentrations of BHA, BHT and QM were determined by gas chromatography (GC) and high performance liquid chromatography (HPLC) according to the method described previously.[1] The concentrations of BHA dimers, A-I and A-II, were determined by gas chromatography using a Yanaco gas chromatograph equipped with a hydrogen flame ionization detector and a glass column (3 mm i.d. × 2 m) of Silicone OV-17 on Chromosorb WAW. The chromatograph was operated isothermally at 250°C (column temperature) and 270°C (injection temperature) with a nitrogen gas flow of 12.5 ml/min. The concentrations were determined by comparing the peak areas of samples with those of authentic standard solutions (1.0 mM).

Estimation of Decrease in DPPH Concentration——Solutions of DPPH and BHA and/or BHT were mixed and the mixtures were permitted to stand at 20°C in a thermostatically controlled water bath. The concentration of DPPH in the reaction mixture was calculated from the absorbance at 520 nm according to the equation reported by Boguth and Repges.[13]

Identification of the Phenoxy Radicals——A solution of BHA and/or BHT was added to a solution of DPPH in benzene. The mixture was poured into the sample cell, and a stream of nitrogen was passed through the solution to displace oxygen. The electron spin resonance (ESR) spectra were observed using a Varian E4 spectrometer with 100 kHz field modulation.

Results

BHA (0.1 mM) was treated with a 1.9-fold molar excess of DPPH with or without the addition of DPPH·H at 20°C in benzene. The time courses of decrease in DPPH (A) and BHA (B) concentrations are illustrated in Fig. 1. BHA concentration decreased rapidly owing to hydrogen donation to DPPH during about 10 min, and the reaction proceeded gradually thereafter. In the presence of DPPH·H (1.0 mM), the decreases in DPPH and BHA concentrations were retarded markedly. When DPPH·H was added to the reaction mixture of DPPH and BHA 10 min after the reaction, DPPH and BHA concentrations increased rapidly with increasing amount of DPPH·H added. The ratio of increased DPPH to increased BHA was 2 (1.89—2.00) under these conditions.

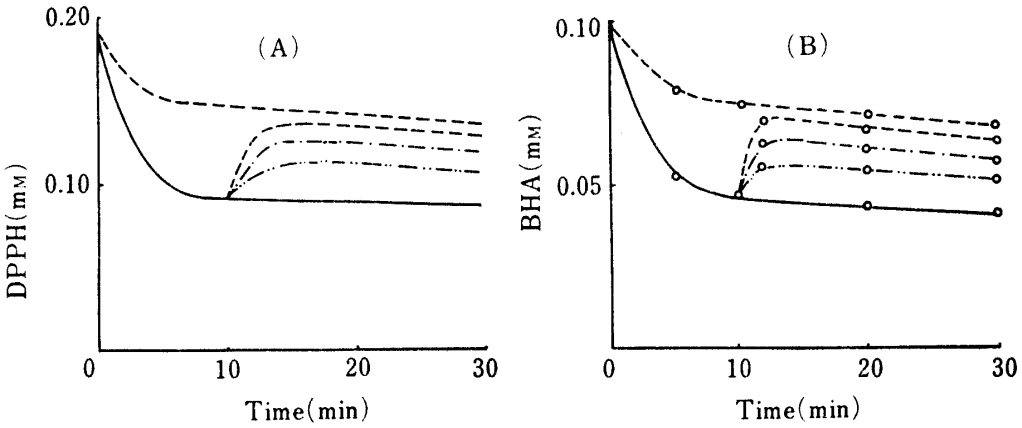

Fig. 1. Effect of DPPH·H on the Loss of DPPH and BHA in the Reaction between DPPH and BHA

DPPH·H was added to the reaction mixture of DPPH (0.19 mM) and BHA (0.10 mM) (————) at the start or after 10 min, and the concentration of BHA was determined by GLC. The concentrations of DPPH·H added were 0.25 mM (—··—··), 0.50 mM (—·—·—) and 1.00 mM (– – – –).

The time courses of formation of BHA dimers, A-I and A-II, in the reaction mixture of DPPH and BHA are shown in Fig. 2. When a mixture of DPPH (2.0 mM) and BHA (1.0 mM) was treated at 20°C in benzene, a small amount of the dimers was formed compared with the loss of BHA (10% of loss of BHA). The addition of a large excess of BHA (100 mM) at the

start of the reaction increased the concentrations of dimers rapidly, and the maximal concentrations were reached after 20 min. Almost the same amount of dimers was formed by addition of a large excess of BHA 10 min or 30 min after the reaction.

The formation of BHA dimers was compared under various conditions (Table I). When 1.0, 2.0 and 4.0 mM BHA were treated with 2.0 mM DPPH for 10 min, a small amount of BHA dimers was formed and DPPH was lost almost completely in the latter two combinations. Addition of excess BHA to those reaction mixtures (reaction I) increased the concentrations of the dimers to 0.74—0.83 mM at 20 min after excess BHA had been added, regardless of initial BHA concentration. When BHT and excess BHA were added to the mixtures of DPPH and BHA at the same time,

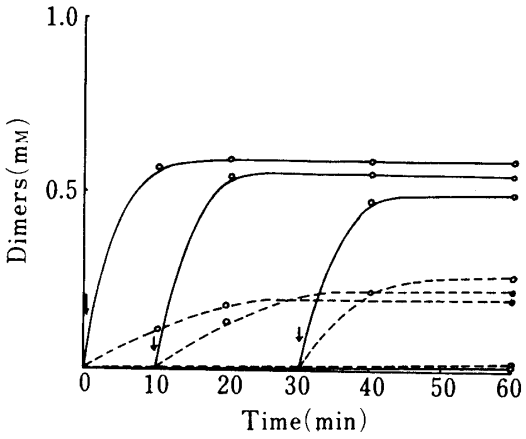

Fig. 2. Formation of BHA Dimers in the Reaction between DPPH and BHA

A mixture of DPPH (2 mM) and BHA (1 mM) was treated at 20°C and excess BHA (100 mM) was added to the reaction mixture at one of the times indicated by arrows. A-I; ———, A-II; – – – –.

Table I. Formation of BHA Dimers

Initial concentration (mM)		Pre-treating time (min)	Concentration after pretreatment (mM)				Concentration in the reaction miuture I (mM)		Concentration in the reaction miuture II (mM)	
DPPH	BHA		DPPH	BHA	A-I	A-II	A-I	A-II	A-I	A-II
2.00	1.00	0	2.00	1.00	0	0	0.63	0.18	0.04	0.06
		10	0.76	0.40	0.02	0.03	0.54	0.20	0.07	0.09
2.00	2.00	0	2.00	2.00	0	0	0.63	0.18	0.04	0.06
		10	0.04	0.93	0.02	0.06	0.52	0.27	0.09	0.12
2.00	4.00	0	2.00	4.00	0	0	0.63	0.18	0.04	0.06
		10	0	2.82	0.06	0.10	0.50	0.33	0.14	0.15

A solution of BHA and DPPH was treated at 20°C in benzene and excess BHA (100 mM) (reaction I) or BHA (100 mM) +BHT (10 mM) (reaction II) was added to the mixture at the start or after 10 min. The concentrations of BHA and its dimers were determined by GLC 20 min after addition of excess BHA or BHA+BHT.

Table II. Relationship between Recovery of BHA and Formation of Quinone Methide

Initial concentration (mM)		Pretreating time (min)	Concentration after pretreatment (mM)		BHA[a] recovered (mM) (B)	Quinone methide[a] formed (mM) (C)	(B–A)/C
DPPH	BHA		DPPH	BHA (A)			
1.00	1.00	10	0.10	0.54	0.96	0.47	0.89
		20	0.04	0.48	0.92	0.45	0.98
1.00	2.00	10	0	1.47	1.92	0.44	1.02
2.00	2.00	10	0.04	0.91	1.76	0.86	0.99
2.00	4.00	10	0	2.82	3.60	0.80	0.98

The mixtures (50 ml) of DPPH and BHA were treated for 10 or 20 min at 20°C, and 22 mg of BHT was added to the reaction mixture after pretreatment. Concentrations of BHA and quinone methide were determined by HPLC.

a) The concentrations were determined 20 min after the addition of BHT.

the concentrations of the dimers were scarcely increased (reaction II).

The main oxidation product from the reaction of BHA and BHT with DPPH was QM.[1] The amount of QM formed from the reaction of BHT with the mixture of DPPH and BHA was compared with the amount of BHA recovered (Table II). When BHT (2 mM) was added to the reaction mixture of DPPH and BHA, BHA was recovered to the extent of 88—96%, and 0.44—0.47 mM and 0.80—0.86 mM QM were formed from the reaction mixtures containing 1.0 mM and 2.0 mM DPPH, respectively. The ratio of BHA regenerated (B–A) to QM formed (C) was 1 (0.98—1.02) to 1 in the reaction mixture in which DPPH was lost during pretreatment.

ESR spectra of the reaction mixtures of BHA and/or BHT with DPPH are shown in Fig. 3. When BHA (4 mM) and DPPH (1 mM) were treated for 2 min (A), BHA phenoxy radical signals, a ($H_{3,5}$)=0.82 G, (0.90 G), a (H_6)=6.1 G (6.2 G), a (H_{-OCH_3})=1.6 G (1.7 G),[14]

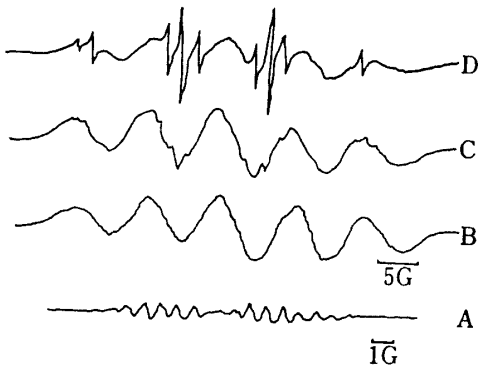

Fig. 3. ESR Spectra of Reaction Mixtures of BHA and/or BHT with DPPH

A; DPPH (1 mM)+BHA (4 mM), B; DPPH (1 mM) +BHA (1 mM), C; DPPH (1 mM)+BHT (1 mM), D; DPPH (1 mM)+BHA (0.01 mM)+BHT (1 mM). Reaction time: A and B; 2 min, C and D; 10 min.

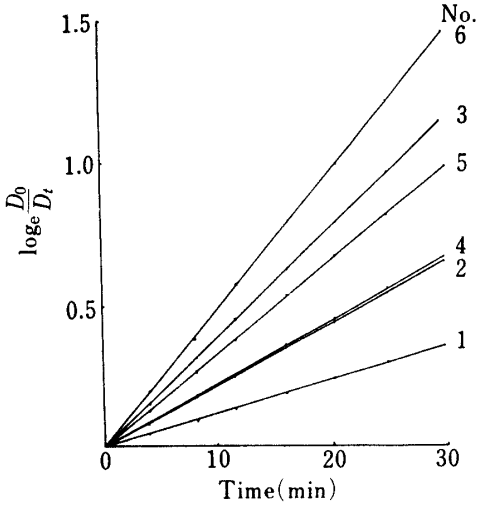

Fig. 4. Reaction Kinetics for BHA and BHT with DPPH

Reaction conditions were as indicated in Table III.

were detected. Only the signals of DPPH were detected in the reaction mixture of BHA and DPPH in equimolar ratio (B). On further addition of DPPH (4 mM) to the reaction mixture of BHA and DPPH (4 mM+1 mM), the signals of BHA phenoxy radical were lost rapidly, and the signals of DPPH were detected. In the reaction mixture of BHT and DPPH, the signals of BHT phenoxy radical, a ($H_{3,5}$)=1.7 G (1.8 G), a (H_{-CH_3})=11.0 G (10.7 G),[15] were detected accompanied by those of DPPH (C). Addition of BHA to this mixture increased the amount of BHT phenoxy radical (D), but the signals of BHA phenoxy radical were not detected.

The kinetic data on loss of DPPH in the presence of BHA and BHT are shown in Fig. 4.

Tabll III. Rate Constants for the Bimolecular Reactions of BHA and BHT with DPPH

No.	Initial concentration (mM)			$\frac{1}{t}\log_e\frac{D_0}{D_t}$ [a]	k_1 ($M^{-1}s^{-1}$) [b]	k_2 ($M^{-1}s^{-1}$) [b]
	DPPH	BHA	BHT			
1	0.100	1.00	0	0.0116	0.193	
2	0.100	1.00	0.0020	0.0220		86.7
3	0.100	1.00	0.0050	0.0372		85.3
4	0.0996	2.00	0	0.0223	0.187	
5	0.0996	2.00	0.0020	0.0327		86.7
6	0.0996	2.00	0.0050	0.0481		86.0

a) D_0 and D_t indicate the concentrations of DPPH at reaction times of 0 and t min, respectively.
b) k_1 and k_2 are the rate constants for the bimolecular reactions of BHT and BHA with DPPH in benzene at 20°C, respectively.

and Table III. Loss of DPPH in the presence of BHT was enhanced by addition of BHA in a concentration-dependent manner. Plots of the reaction time (min) *vs.* $\log_e D_0/D_t$ gave a straight line, and indicated that the rate of decrease in DPPH concentration caused by BHA and BHT may be pseudo-first order, because the regeneration of BHA by BHT may proceed rapidly and completely under reacton conditions such that BHT$\gg$DPPH$\gg$BHA. The rate constants of decrease in DPPH concentration produced by BHT and BHA, k_1 and k_2, were calculated by means of the following equation.

$$-\frac{dD}{dt} = k_1TD + k_2AD \quad (1)$$

In this equation, D, A and T are the concentrations of DPPH, BHA and BHT, respectively. The rate of loss of DPPH caused by BHA was about 450 times that by BHT, as shown in Table III.

Discussion

The results in the previous paper showed that the intermediate formed from the interaction of BHA and DPPH enhanced the oxidation of BHT and might play an important role in the synergism.[1,8] In the present paper, the formation process and reactivity of the intermediate were further investigated by following the behavior of these antioxidants and their oxidation products. The decrease in DPPH and BHA concentrations in the mixture was retarded in the presence of DPPH·H, and DPPH and BHA were regenerated rapidly by addition of DPPH ·H to their mixture 10 min after the reaction. These observations suggested a mode of this retardation of loss of DPPH alternative to that proposed previously, in which the retardation was explained in terms of the following reversible equation;[9,16,17]

$$\text{DPPH} + \text{phenol} \rightleftarrows \text{DPPH}\cdot\text{H} + \text{phenoxy radical} \quad (2)$$

A species differing from the phenoxy radical may be formed from the reaction of BHA and DPPH, because it was relatively stable and the molar ratio of regenerated DPPH and BHA upon addition of DPPH·H was 2: 1. This assumption was confirmed by measuring the ESR spectra in the reaction mixture of BHA and DPPH; the phenoxy radical was not detected in the reaction mixture of BHA and DPPH (1: 1) and the radical formed by their interaction (BHA: DPPH=4: 1) was lost upon further addition of DPPH. These observations indicate the formation of non radical species from the interaction of the phenoxy radical and DPPH. The reversible reaction may involve hydrogen donation from DPPH·H to this species to regenerate BHA and DPPH.

The dimers of BHA, A-I and A-II, formed from coupling of the BHA phenoxy radical,[10,18] were scarcely detected in BHA and DPPH (1: 2) mixture. When excess BHA was added to the reaction mixture, the concentrations of BHA dimers increased rapidly even in the reaction mixtures in which DPPH was almost lost, and in these cases, the amount of BHA transformed into dimers was larger than that lost in pretreatment. These results indicate that BHA may react with the non radical species formed from the reaction of BHA and DPPH to form dimers *via* its phenoxy radical, namely the species may act as a hydrogen acceptor from BHA.

The previous study showed that QM was the main oxidation product from the reaction of BHA and BHT with DPPH. Enhanced oxidation of BHT to QM proceeded even when BHT was added to a reaction mixture of BHA and DPPH in which DPPH was lost almost completely (Table II). Non radical species formed from BHA and DPPH, which acts as a hydrogen acceptor from DPPH·H and BHA, may represent an intermediate enhancing the oxidation of BHT to QM with the regeneration of BHA. The hydrogen accepting ability of the intermediate was estimated by comparing the amount of QM formed with the amount of BHA regenerated from the reaction mixture of BHA and DPPH by addition of BHT. Since QM and BHA were formed in the molar ratio of 1: 1 from the reaction mixture, the

intermediate may accept two hydrogens from BHT to regenerate BHA. This property of the intermediate was consistent with the observed regeneration of DPPH and BHA in the molar ratio of 2: 1 on addition of DPPH·H.

Thus, the reaction of BHA and BHT with DPPH may be shown as follows (Chart 1). BHA reacts with DPPH to form the stable intermediate *via* its phenoxy radical (Reactions 1 and 2). These oxidized species react with BHT to regenerate BHA with the enhanced oxidation of BHT to QM *via* its phenoxy radical (Reactions 3 and 4). From the kinetic results, Ivanova *et al.*[3] suggested the rapid reaction of BHA phenoxy radical with BHT to regenerate BHA and to form BHT phenoxy radical. Our data indicate the rapid reaction of BHA phenoxy radical with DPPH to form the intermediate which abstracts hydrogens from BHT. The result that only BHT phenoxy radical was detected under our reaction conditions (Fig. 3.D) may be explained by both reaction processes, and the molar ratio of BHA, BHT and DPPH in the reaction mixture may be the deciding factor in determining which of the reaction processes actually operates.

The processes in Chart 1 indicate that the amount of loss of DPPH may be equal to the

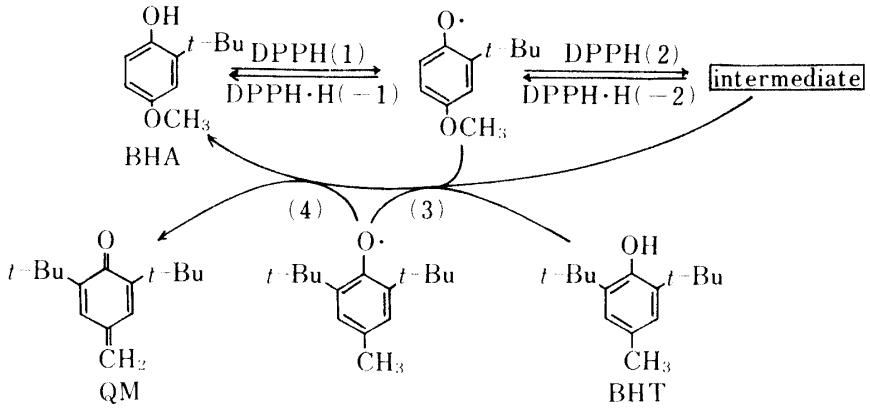

Chart 1

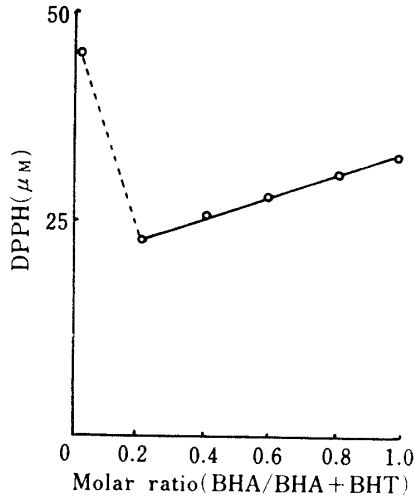

Fig. 5. DPPH Concentration in Mixtures of the Combined Antioxidants

Mixtures of DPPH (44 μM) and combined antioxidants (total concentration: 12.5 μM) were treated for 60 min. Residual concentrations of DPPH (○) were compared with the expected values (—).

sum of two hydrogens donated by BHT in the enhanced oxidation to QM and hydrogens donated by BHA. These expected values were in fair agreement with the experimental values (Fig. 5).[8]

The rate of decrease in DPPH concentration by BHA and BHT was pseudo-first order according to equation (1) under our reaction conditions, BHT≫DPPH≫BHA. The results indicate that the concentration of BHA in the reaction mixture was constant during the period of the reaction, because the regeneration of BHA by BHT with its oxidation to QM proceeded rapidly. The rate constant of decrease in DPPH concentration by BHT was similar to that by other 2,6-di-*tert*-butyl phenols,[9,17] and the rate by BHA was about 450 times that by BHT. It is apparant that the rate of hydrogen donation to DPPH was increased considerably by rapid regeneration of BHA by BHT, in other words, by the catalytic action of BHA in the synergistic

reaction.

From the kinetic results, Ivanova *et al.*[3] suggested that the synergistic effect of BHA and BHT on inhibition of cumene oxidation arose as a result of regeneration of BHA by means of its phenoxy radical reaction with BHT. In this paper, we suggest the formation of the stable intermediate which abstracts two hydrogens from BHT to regenerate BHA, on the basis of the behavior of the antioxidants and their oxidation products. This intermediate may play an important role in hydrogen donation to DPPH, and these reaction processes may help to cast light on the general mechanisms of synergism in the inhibition of lipid oxidation.

References and Notes

1) Part XV: T. Kurechi and T. Kato, *Chem. Pharm. Bull.*, **29**, 3012 (1981).
2) W.M. Gearhart and B.N. Stukey, *J. Am. Oil Chemists' Soc.*, **32**, 386 (1955).
3) R.A. Ivanova, N.S. Pimenova, E.I. Kozlov, and V.F. Tsepalov, *Kinet. Katal.*, **20**, 1423 (1979).
4) L.R. Mahoney and M.A. DaRooge, *J. Am. Chem. Soc.*, **89**, 5619 (1967).
5) J.L. Bolland and P.T. Have, *Trans. Faraday Soc.*, **43**, 201 (1947).
6) J.R. Shelton and D.N. Vincent, *J. Appl. Pol. Sci.*, **85**, 2433 (1963).
7) T. Kurechi, K. Kikugawa, T. Kato, and T. Numasato, *Chem. Pharm. Bull.*, **28**, 2228 (1980).
8) T. Kurechi, K. Kikugawa, and T. Kato, *Chem. Pharm. Bull.*, **28**, 2089 (1980).
9) P.B. Ayscough and K.E. Russell, *Can. J. Chem.*, **43**, 3039 (1965).
10) T. Kurechi, *Eisei Kagaku*, **13**, 191 (1967).
11) G.M. Coppinger and T.W. Champbell, *J. Am. Chem. Soc.*, **75**, 734 (1953).
12) R.H. Bauer and G.M. Coppinger, *Tetrahedron*, **19**, 1201 (1963).
13) W. Boguth and R. Repges, *Internat. Z. Vit. Forschung*, **39**, 289 (1969).
14) W.G.B. Huysmans and W.A. Waters, *J. Chem. Soc. (B)*, **1966**, 1047.
15) J.K. Becconsall, S. Clough, and D. Scott, *Proc. Chem. Soc. London* **1959**, 308.
16) J.S. Hogg, D.H. Lohman, and K.E. Russell, *Can J. Chem.* ,**39**, 1588 (1961).
17) P.B. Ayscough and K.E. Russell, *Can. J. Chem.*, **45**, 3019 (1967).
18) J. Baltes and F. Volbert, *Fette, Seifen, Anstrichmittel.* **57**, 660 (1955).