[Chem. Pharm. Bull. 31(5)1772—1776(1983)]

Studies on the Antioxidants. XX.[1] The Effect of Butylated Hydroxytoluene on *tert*-Butylhydroperoxide-Induced Oxidation of Butylated Hydroxyanisole

TSUTAO KURECHI and TETSUTA KATO*

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

(Received October 25, 1982)

Hydrogen donation from butylated hydroxyanisole (BHA) and/or butylated hydroxytoluene (BHT) to the peroxyl radical prepared by cobalt-catalyzed cleavage of *tert*-butylhydroperoxide was investigated, and the relation of this process to synergism in the antioxidative effect was discussed. BHA initially donated a hydrogen to the peroxyl radical to form its phenoxyl radical, and this radical reacted with either the peroxyl radical or another phenoxyl radical to form *o*-benzoquinone or the dimer. BHT reacted with the peroxyl radical to form its adduct. In the combination of BHA and BHT, BHA donated a hydrogen to the peroxyl radical initially, and its phenoxyl radical accepted hydrogen from BHT to regenerate BHA, with enhanced oxidation of BHT to quinone methide. This hydrogen acceptance of the phenoxyl radical of BHA from BHT may be closely correlated with the synergism in the antioxidative effect of the mixture of BHA and BHT.

Keywords——antioxidants; butylated hydroxyanisole; butylated hydroxytoluene; *tert*-butylhydroperoxide; peroxyl radical; phenoxyl radical; synergism

It has been demonstrated that peroxyl radicals of lipids may be the most important species in the propagation step of autoxidation, and antioxidants act as hydrogen donors to these radicals to inhibit the chain reaction.[2,3] In previous papers,[4–6] we investigated the hydrogen donating process of a mixture of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) to the stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and found that the synergistic effect might arise from the regeneration of BHA from its oxidized intermediates, with enhanced oxidation of BHT to 2,6-di-*tert*-butylquinone methide (QM).

Interaction between the peroxyl and the phenoxyl radical which was formed by the initial hydrogen donation may also participate in the antioxidative reaction, because the adducts of the peroxyl radical with BHT and tocopherol were obtained[7,8] and these reactions also eliminate the peroxyl radical to stop the propagation reaction. A kinetic explanation for the reaction of the peroxyl radical with a mixture of BHA and BHT, which showed synergism in the protection of lard from peroxidation,[9] was proposed,[10] but the details of the reaction process of the antioxidants are still obscure. This paper deals with the peroxyl radical-induced oxidation of BHA and BHT. The peroxyl radical prepared by cobalt-catalyzed cleavage of *tert*-butylhydroperoxide (BHPO) was used in this study, since we expected that it would react with antioxidants by analogy with peroxyl radicals of lipids. The oxidation process of the mixture of two antioxidants was compared with that of each antioxidant alone, and the relationship between the synergistic antioxidative effect and the reaction process is discussed.

Experimental

Materials——BHA and BHT, both supplied by Nikki-Universal Company, Ltd., were recrystallized from petroleum ether[11] and ethanol, respectively. BHA dimers, 2,2′-dihydroxy-5,5′-dimethoxy-3,3′-di-*tert*-butylbiphenyl (A-I) and 2′,3-di-*tert*-butyl-2-hydroxy-4′,5-dimethoxy biphenyl ether (A-II) were obtained by the methods described previously.[11] 3-*tert*-Butyl-5-methoxy-1,2-benzoquinone (A-III) was prepared by the method of Hewgill *et al.*[12] QM was synthesized from BHT according to the method of Bauer and Coppinger.[13] 2,6-Di-*tert*-butyl-4-*tert*-butylperoxy-4-methyl cyclohexa-2,5-dienone (T-adduct) was

prepared by the method of Campbell and Coppinger.[7] BHPO (70% in water) and cobalt naphthenate (6% Co^{3+}) were commercial products obtained from Nakarai Chemicals. Ltd. and Wako Pure Chemical Industries, Ltd., respectively. Wakogel B5F (Wako Pure Chemical Industries, Ltd.) and Silica-gel (100 mesh, Kanto Chemical Company, Ltd.) were used for thin layer chromatography (TLC) and column chromatography, respectively.

Time Courses of Changes of BHA, BHT and QM Concentrations——One ml of benzene solution of BHA (20 mM) and/or BHT (20 mM), 5 ml of benzene solution of BHPO (4 or 40 mM) and 1 ml of benzene solution of cobalt naphthenate (1 mM Co^{3+}) were mixed and made up to 10 ml with benzene. The concentrations of BHA, BHT and QM in the reaction mixture were determined by gas liquid chromatography (GLC) at selected times. GLC was carried out under the conditions described in a previous paper.[4]

Estimation of Changes of Electron Spin (ESR) Spectra——The same mixtures as used above were subjected to ESR analysis. ESR spectra were measured with a Varian E-4 EPR spectrophotometer under the same operating conditions as described in a previous paper.[4]

Results and Discussion

BHA (2 mM) and/or BHT (2 mM) were treated with BHPO (2 or 20 mM) in the presence of Co^{3+}. Thin layer chromatograms of the products from these reaction mixtures are shown in Fig. 1. BHA dimers, A-I and A-II, were detected from the equimolar mixture of BHA and BHPO (A(a)). These compounds were scarcely detected in the reaction mixture with a large excess of BHPO, and an orange spot with a lower *Rf* value was detected (A(b)). This compound was separated by column chromatography and identified as A-III.[12] Formation of A-III may result from the coupling reaction between the phenoxyl radical of BHA and the *tert*-butylperoxyl radical, followed by the elimination of butyl alcohol.[14] These results suggest that BHA might initially donate hydrogen to the peroxyl radical to form the phenoxyl radical, and this phenoxyl radical can react with either the peroxyl radical or another phenoxyl radical. From the mixtures of BHT and BHPO, only one reaction product corresponding to T-adduct was detected, as described by Campbell and Coppinger[7] (B). When BHA (2 mM) and BHT (2 mM) were treated with BHPO (2 mM), neither of the reaction products of each antioxidant treated separately was detectable and QM was detected as a main reaction product (C(a)). When a large excess of BHPO (20 mM) was added, QM and A-III were detected as

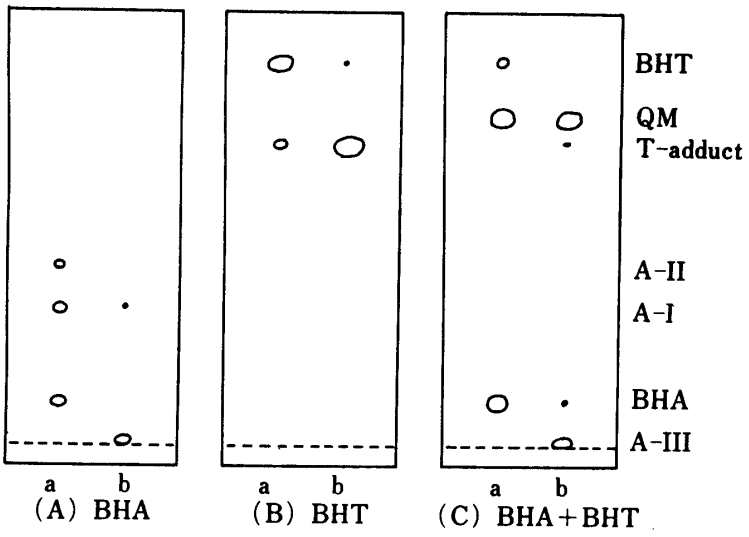

Fig. 1. Thin Layer Chromatogram of Reaction Products of BHA and/or BHT Treated with BHPO in the Presence of Co^{3+}

a: BHA (2 mM) and/or BHT (2 mM)+BHPO (2 mM)+Co^{3+} (0.1 mM)
b: BHA (2 mM) and/or BHT (2 mM)+BHPO (20 mM)+Co^{3+} (0.1 mM)
Solutions (a) and (b) were treated for 30 min at 20°C.
The chromatogram was developed with hexane–benzene (1: 1) mixture.

main products (C(b)).

Time courses of loss of BHA and BHT and the formation of QM in the reaction mixtures of BHA and/or BHT with BHPO+Co^{3+} were followed by GLC (Fig. 2). When BHA (2 mM) was treated with an equimolar amount of BHPO (A), the concentration of BHA was gradually decreased and 0.58 mM BHA remained after 120 min. The loss of BHA was greatly depressed in the combination with BHT (2 mM), and 1.8 mM BHA remained after 120 min. In this combination, BHT was gradually oxidized to QM, which could not be detected in the absence of BHA. In the reaction mixture of combined antioxidants with a large excess of BHPO (20 mM) (B), the oxidation of BHT to QM was faster and was completed in 25 min. The loss of BHA was very gradual during this period and rapid thereafter. The formation of QM from BHT in the reaction mixture of BHA and BHT indicates that two-hydrogen donation of BHT may proceed preferentially, in contrast with the one-hydrogen-donating and one-adduct-forming process of BHT treated alone with BHPO. This two-hydrogen-donating process of BHT may participate in the suppression of the oxidation of BHA under these conditions.

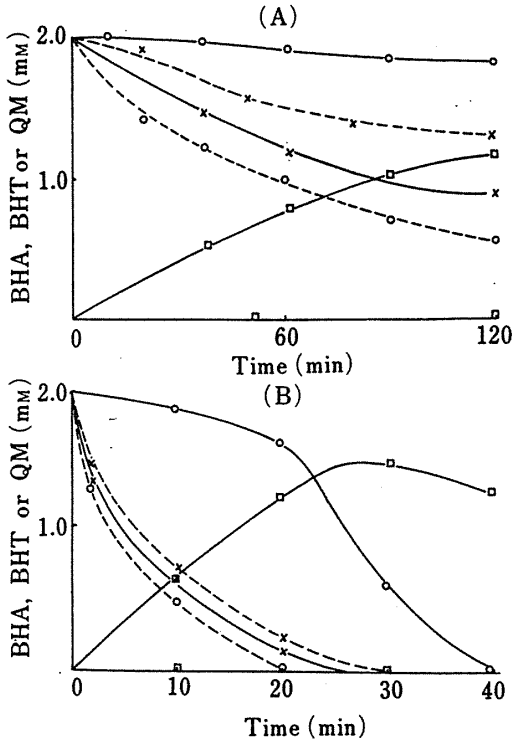

Fig. 2. Time Courses of Loss of BHA and BHT and Formation of QM in the Reaction Mixture of BHA and/or BHT with BHPO+Co^{3+}

BHA (2 mM) and BHT (2 mM) were treated with BHPO (2 mM (A) or 20 mM (B))+Co^{3+} (0.1 mM) at 20°C.
BHA+BHT mixture, ——; BHA or BHT alone, ----; BHA, ○; BHT, ×; and QM, □.

ESR spectra from the reaction mixtures of BHA and/or BHT with BHPO+Co^{3+} were measured under the same conditions as shown in Fig. 2(B). When BHA was treated with BHPO, the phenoxyl radical of BHA was detected at 2 min but not after 20 min. Detection and loss of the radical indicated that BHA initially donated one hydrogen to the *tert*-butylperoxyl radical to form the phenoxyl radical, and then coupled with the peroxyl radical or another phenoxyl radical to remove the phenoxyl radical. The phenoxyl radical of BHT could not be detected in the reaction mixture of BHT and BHPO, although the concentration of BHT decreased under these conditions. In the reaction mixture of BHA and BHT, only the phenoxyl radical of BHT was detected at 2 min, and only the phenoxyl radical of BHA was detected at 20 min. Furthermore, when BHT was added to the reaction mixture of BHA and BHPO at 5 min after the reaction, the phenoxyl radical of BHA was lost rapidly and only the phenoxyl radical of BHT was detected. The oxidation of BHT to the phenoxyl radical and further oxidation to QM in the combination with BHA may result from the rapid hydrogen donation from BHT to the phenoxyl radical of BHA formed from the initial hydrogen donationto the *tert*-butylperoxyl radical. Detection of the phenoxyl radical of BHA in the reaction mixture of the combined antioxidants at 20 min corresponded to the decrease in BHA concentration, as shown in Fig. 2.

From the results, the reaction process of BHA and/or BHT with BHPO+Co^{3+} may be as follows (Chart 1). In the case of BHA alone, BHA donates one hydrogen to the *tert*-butylperoxyl radical to form the phenoxyl radical, and this radical reacts with either the peroxyl radical or another phenoxyl radical (Reactions (1), (2), (3), and (4)). BHT reacts with the

peroxyl radical to form T-adduct (Reaction (5)). In the combination of BHA and BHT, BHA rapidly donates one hydrogen to the peroxyl radical initially (Reaction (1)). Two hydrogens are transferred from BHT to the phenoxyl radicals of BHA formed in the reaction (1), and then BHA is regenerated, with enhanced oxidation of BHT to QM (Reactions (6) and (7)). This reaction process is consistent with the findings that the oxidation of BHA was depressed

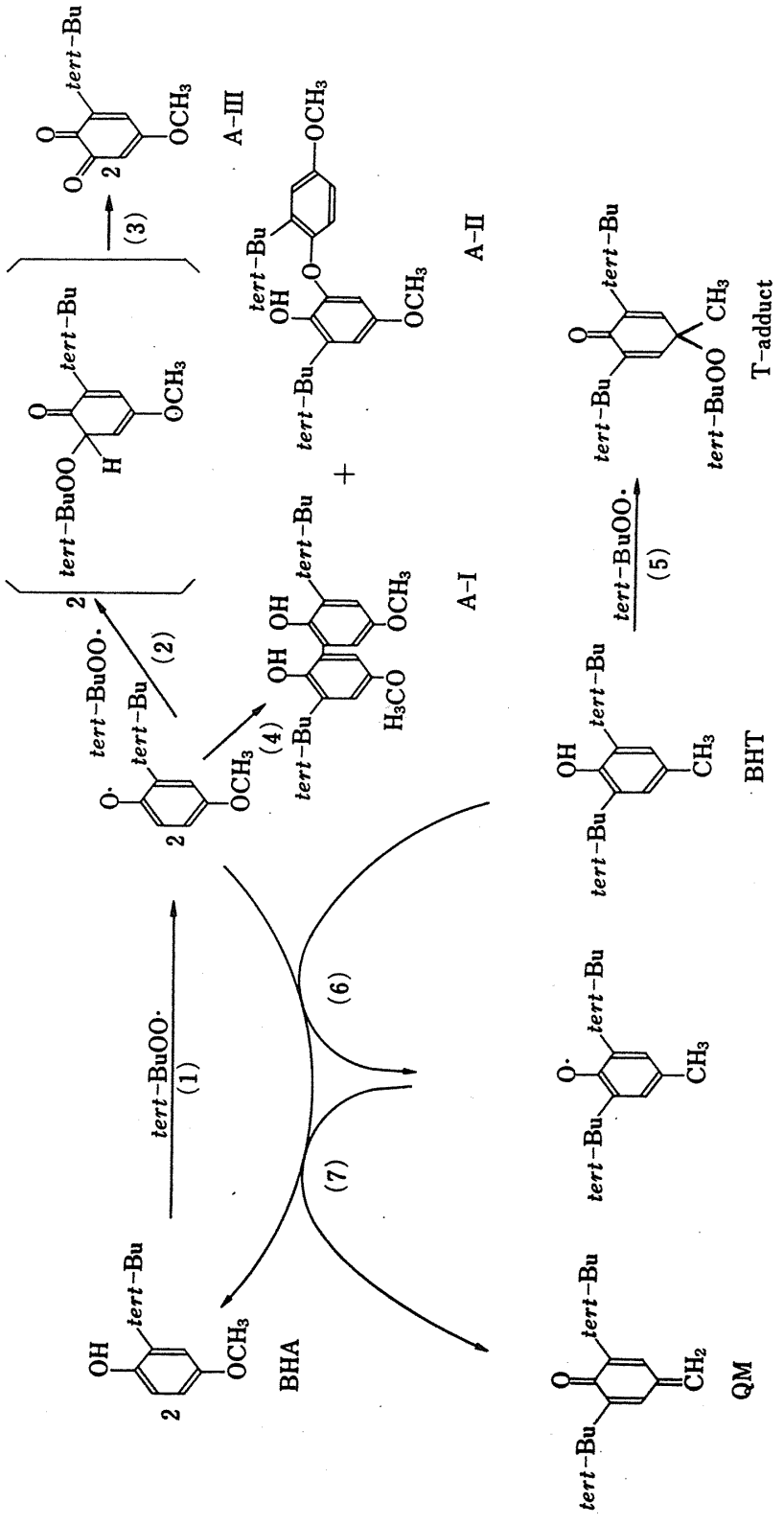

Chart 1. Reaction of BHA and/or BHT with *tert*-Butylperoxyl Radical

in the combination with BHT and proceeded rapidly after the disapperance of BHT.

On the basis of kinetic experiments on the inhibition of cumene oxidation by BHA and BHT, Ivanova *et al.*[10] suggested that rapid one-hydrogen transfer proceeded from BHT to the phenoxyl radical of BHA. A similar result was reported for the combination of BHT and 4-methoxyphenol.[15] Our observations suggest that the phenoxyl radical of BHA may accept hydrogen from BHT to regenerate BHA, and two-step oxidation of BHT to QM was enhanced in this process. The coupling between the phenoxyl radical of BHT and the peroxyl radical was not observed in the combination with BHA, although this reaction proceeded quantitatively in the case of BHT alone.

Synergistic inhibitory effects of the mixture of BHA and BHT on the oxidation of lard and cumene,[9,10] hydrogen donation to DPPH[6] and electron donation to ferric ion[16] were described previously. The synergistic effect in this combination on hydrogen donation to DPPH may result from the rapid hydrogen donation from BHT to the stable intermediate formed from the interaction of BHA and DPPH.[4] The hydrogen donating process to the *tert*-butylperoxyl radical was similar to that to DPPH, except that no stable intermediate was formed in the case of BHPO, in which only the phenoxyl radical of BHA might act as a species accelerating two-hydrogen donation of BHT. Peroxyl radicals of lipids may be the most important species in the propagation step of autoxidation, and we used the *tert*-butylperoxyl radical as a model compound of peroxyl radicals of lipids. The regeneration of BHA from its phenoxyl radical by BHT described in this paper may play an important role in the occurrence of the synergistic antioxidative effect in this combination.

References

1) Part XIX: T. Kurechi and A. Kunugi, *J. Am. Oil Chem. Soc.*, "submitted."
2) J.L. Bolland and P.T. Have, *Trans. Faraday Soc.*, **43**, 201 (1947).
3) L.A. Witting "Free Radicals in Biology," Vol. IV, ed. by W.A. Pryor, Academic Press, Inc., New York, 1980, chapter 9.
4) T. Kurechi and T. Kato, *Chem. Pharm. Bull.*, **30**, 2964 (1982).
5) T. Kurechi and T. Kato, *Chem. Pharm. Bull.*, **29**, 3012 (1981).
6) T. Kurechi, K. Kikugawa, and T. Kato, *Chem. Pharm. Bull.*, **28**, 2089 (1980).
7) T.W. Campbell and G.M. Coppinger, *J. Am. Chem. Soc.*, **74**, 1469 (1952).
8) H.W. Gardner, K. Eskins, G.M. Grams, and G.E. Inglett, *Lipids*, **7**, 324 (1972).
9) W.M. Gearhart and B.N. Stuckey, *J. Am. Oil Chem. Soc.*, **32**, 386 (1955).
10) R.A. Ivanova, N.S. Pimenova, E.I. Kozlov, and V.F. Tsepalov, *Kinet. Katal.*, **20**, 1423 (1979).
11) T. Kurechi, *Eisei Kagaku*, **13**, 191 (1967).
12) F.R. Hewgill, B.R. Kennedy, and D. Kilpin, *J. Chem. Soc.*, **1965**, 2904.
13) R.H. Bauer and G.M. Coppinger, *Tetrahedeon*, **19**, 1202 (1963).
14) E.C. Horswill and K.U. Ingold, *Can. J. Chem.*, **44**, 263 (1966).
15) L.R. Mahoney and M.A. DaRooge, *J. Am. Chem. Soc.*, **89**, 5619 (1967).
16) T. Krechi, K. Kikugawa, T. Kato, and T. Numasato, *Chem. Pharm. Bull.*, **28**, 2228 (1980).