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Comparative Study of the Alkyl and Peroxy Radical-scavenging Activity of 2-*t*-Butyl-4-methoxyphenol (BHA) and its Dimer, and their Theoretical Parameters

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Abstract. *Background: 2-t-Butyl-4-methoxyphenol (BHA) has considerable toxicity and undesirable potential tumor-promoting activities. To clarify the free radical mechanism of BHA-induced toxicity, the comparative radical-scavenging activity of BHA and its dimer (bis-BHA, 3,3'-ditert-butyl-5,5'-dimethoxy-1,1'-biphenyl-2,2'-diol) with or without 2-mercapto-1-methylimidazole (MMI) was studied using the induction period method. Materials and Methods: The induction period and propagation rate (Rp) were determined by differential scanning calorimetry (DSC) monitoring of polymerization of methyl methacrylate, initiated by the thermal decomposition of benzoyl peroxide (a source of the peroxy radical, PhCOO·) or 2,2'-azobisisobutyronitrile (a source of the alkyl radical, R·) under nearly anaerobic conditions. The anti-1,1'-diphenyl-2-picrylhydrazyl (DPPH) radical- and O_2^--scavenging activities were also investigated. Furthermore, theoretical parameters were calculated from the DEFT/B3LYP and HF/6-31G*//B3LYP levels. Results: For both PhCOO· and R· the inhibition rate constant (k_{inh}) for BHA and bis-BHA was almost identical, but a marked decrease in the Rp_{inh}/Rp_{con} was found for the former. The BHA/MMI mixture (1:1 molar ratio) oxidized by R· reduced the total radical-scavenging activity by approximately 20% . BHA showed lower anti-DPPH radical- and higher O_2^--scavenging activity. Conclusion: Upon PhCOO· or R· scavenging, BHA with a lower BDE, $IP_{koopman's}$, electronegativity, and electrophilicity value, but not bis-BHA with higher corresponding values, highly suppressed propagation. This may be due to the formation of highly reactive free-radical intermediates, which are potentially toxic.*

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Key Words: Butylmethoxyphenol, BHA, *bis*-BHA, radical-scavenging activity, theoretical parameters.

2-*t*-Butyl-4-methoxyphenol (BHA; compound **1**, Figure 1), a compound that exerts antioxidant activity due to its chain-breaking action during the autooxidation of lipids, is utilized for food preservation and suppression of the lipid peroxidation in biological materials. However, BHA has been found to exhibit tumor-promoting activity in some animal models. For example, BHA was found to be cytotoxic and carcinogenic in the mouse forestomach and urinary bladder (1), and in the forestomach and esophagus of rats, mice, hamsters and pigs (2, 3). BHA is toxic and carcinogenic, especially at higher concentrations. BHA was reported to be converted to *bis*-BHA (compound **5**, Figure 1), an *ortho* dimer of BHA, by rat intestine mucosa peroxidase (4). The toxicity of BHA can be reduced by dimerization of this compound *in vivo*. Therefore, we previously synthesized *bis*-BHA derived from BHA and investigated its antioxidant activity and cytotoxicity (5-9). As expected, *bis*-BHA did show less cytotoxicity than the original BHA (5-9).

A dominant metabolic pathway of BHA was reported to include its *O*-demethylation to 2-*tert*-butyl(1,4)hydroquinone (TBHQ) and subsequent peroxidation to a highly toxic 2-*tert*-butyl(1,4)paraquinone (TBQ) (10). It has been suggested that the induction of apoptosis in freshly isolated hepatocytes is caused by TBQ (11). However, the pharmacological actions of phenolic antioxidants are due mainly to their free radical-scavenging activity. Except for an *O*-demethylation mechanism of BHA, a free radical mechanism of BHA oxidation has been proposed (4, 12). In the peroxidation process, the manifestation of toxicity and induction of apoptosis by BHA is closely related to its antioxidant activity. However, the kinetics of the radical-scavenging activities of BHA remain unclear (5-9).

We have proposed a quantitative model rationalizing the antioxidant activity of phenolic antioxidants in the

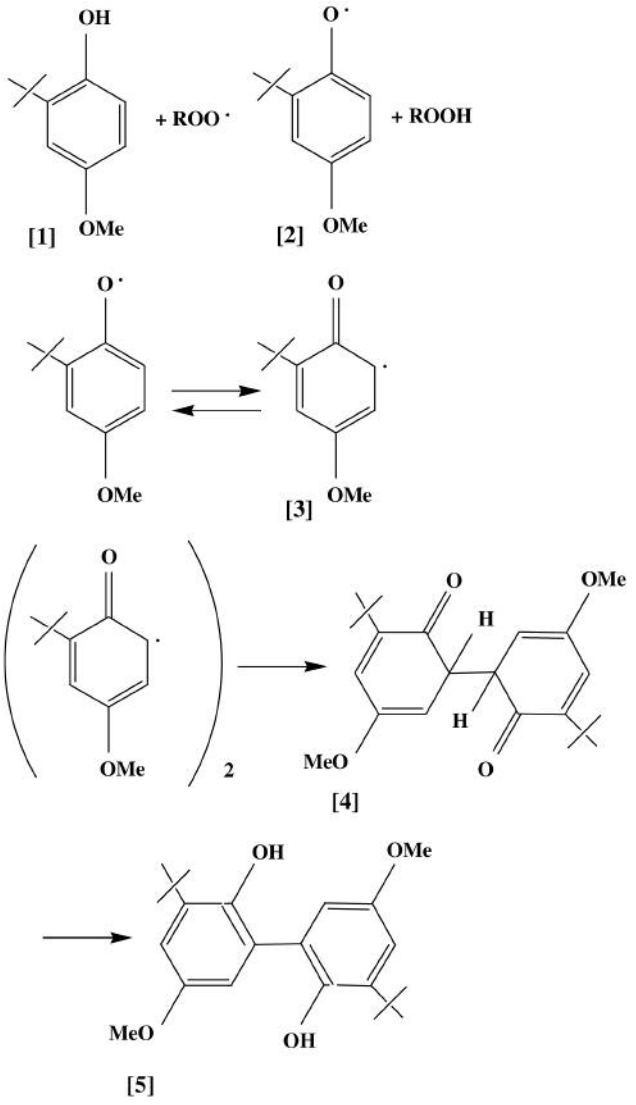

Figure 1. *Possible oxidation mechanism of 2-t-butyl-4-methoxyphenol (BHA, compound **1**).*

polymerization of methyl methacrylate (MMA) initiated by 2,2'-azo*bis*isobutyronitrile (AIBN) and benzoyl peroxide (BPO) using differential scanning calorimetry (DSC) under nearly anaerobic conditions, and this induction period method has proven to be reliable for evaluating the activity of these compounds because biological systems have a low oxygen tension (13, 14). Moreover, as cancer cells have an anaerobic metabolism (*i.e.* they do not utilize oxygen) (15), our biomimetic system under nearly anaerobic conditions may be a good model for evaluating the antioxidant activity of anticancer drugs. We previously reported that the well-known vitamin E-sparing action of vitamin C at high oxidation pressures was not observed under nearly anaerobic conditions, as judged by the induction period (13, 16). There are great discrepancies in the radical-scavenging activity of antioxidants such as vitamin C and vitamin E, and between aerobic and anaerobic conditions (17).

As antioxidants form an intricate antioxidant network together with co-antioxidants such as glutathione (GSH), an *in vivo* thiol, the aim of the present study was to determine whether the antioxidant activity of BHA and *bis*-BHA is influenced by the addition of a co-antioxidant, 2-mercapto-1-methylimidazole (MMI) using the induction period method. Moreover, their radical-scavenging activity for 1,1'-diphenyl-2-picrylhydrazyl (DPPH) and O_2^- was investigated. Furthermore, the theoretical parameters such as phenolic O-H bond dissociation enthalpy (BDE), ionization potential ($IP_{koopman's}$), chemical hardness (η), electronegativity (χ) and electrophilicity (ω) for BHA and *bis*-BHA were calculated from the DEFT/B3LYP and HF/6-31G*//B3LYP levels. On the basis of the results obtained, the possible mechanism of BHA and *bis*-BHA toxicity is discussed.

Materials and Methods

Materials. The following chemicals and detergents were obtained from the indicated companies: BHA (compound **1**), 2-methoxy-4-allyl phenol (EUG), MMI, MMA and DPPH (Tokyo Kasei Kogyo, Co., Ltd., Tokyo, Japan). *Bis*-BHA (compound **5**, Figure 1) was synthesized as previously (5, 6). AIBN and BPO (Wako Pure Chemical Industries Ltd. Japan) were recrystallized from methanol and chloroform/methanol, respectively.

Induction period (IP) and initial rate of polymerization (Rp). The induction periods (IP) and initial rate of polymerization in the presence (Rp_{inh}) or absence (Rp_{con}) of an antioxidant were determined by the method reported elsewhere (13, 14). The induction period (IP) was calculated from the difference between the IP of inhibitors and that of controls. The initial rates of polymerization in the absence (Rp_{con}) and presence (Rp_{inh}) of antioxidants, co-antioxidants and antioxidant/co-antioxidant mixtures were calculated from the slope of the plots during the initial linear phase of the conversion rate of MMA polymerization (tangent drawn at the early polymerization stage) as reported elsewhere (13, 14).

Measurement of stoichiometric factor (n). The relative *n* value can be calculated from the induction period in the presence of inhibitors:

$$n=R_i[IP]/[IH] \qquad \text{Equation (1)}$$

where R_i is the initiation rate, [IP] is the induction period in the presence of an inhibitor, [IH] is the concentration of inhibitors. The number of moles of peroxy or alkyl radicals trapped by the antioxidant is calculated with respect to 1 mole of inhibitor moiety unit. The R_i values for AIBN and BPO at 70˚C were 5.66×10^{-6} M s^{-1} and 2.28×10^{-6} M s^{-1}, respectively (13, 14).

Measurement of the inhibition rate constant (k_{inh}). When R_i is constant, *i.e.* when new chains are started at a constant rate, a steady-state treatment can be applied and the initial rate of polymerization of MMA is given by:

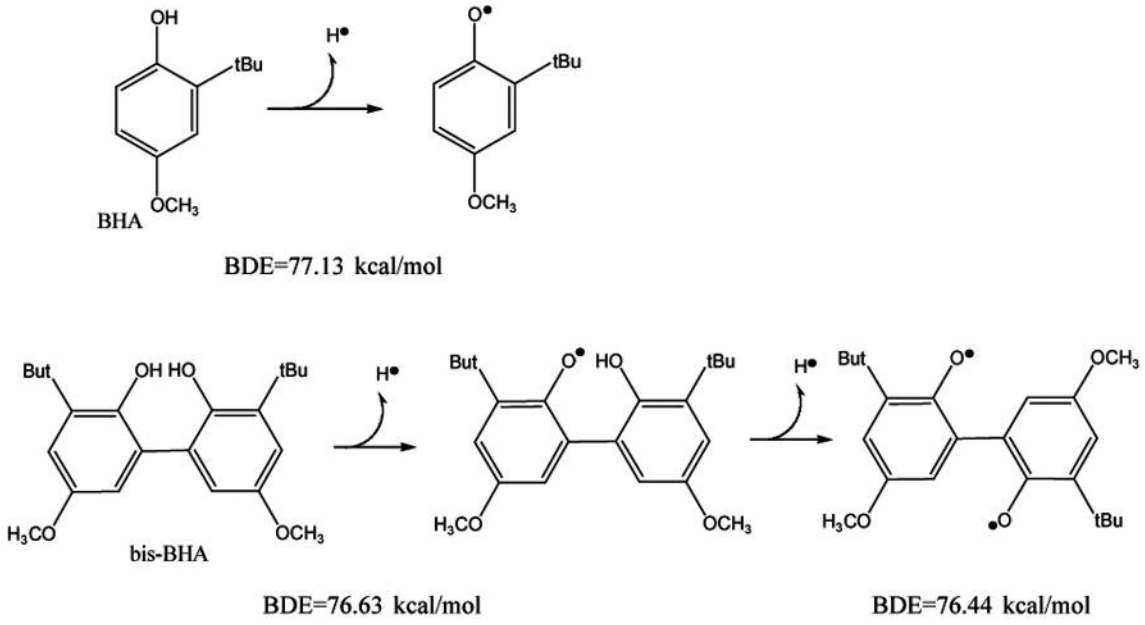

Figure 2. *Oxidation of BHA and bis-BHA, and their phenolic O-H bond dissociation enthalpy (BDE).*

$Rp_{con}=\{k_p\,[MMA]\,R_i^{1/2}\}/(2k_t)^{1/2}$ Equation (**2**)

where k_p and k_t are the rate constants for chain propagation and termination, respectively. The Rp_{inh} rates are determined by:

$Rp_{inh}=\{k_p\,[MMA]\,R_i\}/\{n\,k_{inh}\,[IH]\}$ Equation (**3**)

in which Rp_{inh} is the initial rate of inhibited polymerization, k_{inh} is the rate constant for scavenging (inhibition) of MMA radicals by an antioxidant. From Equation (**2**) and Equation (**3**), k_{inh}/k_p can be calculated:

$k_{inh}/k_p=[MMA]/\{[IP]\times[Rp_{inh}]\}$ Equation (**4**)

O_2^- scavenging activity. The values used here are taken from those reported elsewhere (5). Briefly, the superoxide anion (O_2^-) was produced by the hypoxanthine and xanthine oxidase reaction. Electron spin resonance (ESR) spectroscopy (JEOL JES RE1X, X-band, 100 kHz moduration frequency, Tokyo, Japan) was used for measuring radical intensity.

DPPH radical-scavenging activity. Radical-scavenging activities were determined using DPPH as a free radical. For each inhibitor, different concentrations were tested in ethanol. The decrease in absorbance was determined at 517 nm for 10 min at room temperature. Antiradical activity was calculated as the concentration (mole/l) of inhibitor necessary to reduce the initial DPPH radical concentration by 50% (IC_{50}).

Computation. The BDE was calculated as follows: First, the lowest and second lowest-energy conformers of both the phenol derivatives and their phenoxyl radical species were identified as candidates for geometry optimization using the conformer search procedure by MMFF (Merck molecular mechanics) force fields calculation. The tentative conformers were then optimized in geometry by *ab initio* molecular orbital calculation on a Hartree-Fock model with *ab initio* 6-31G* (HF//6-31G*) for the phenols and UHF//6-31G* level for the phenoxyl radicals *in vacuo* to afford the respective energetically minimized structures. The electronic energy was further preceded by single point calculation of density functional theory (DFT) using the B3LYP functional on the 6-31G* basis set. The BDE=Hr+Hh–Hp, where Hr is the enthalpy of the phenoxyl radical generated by H-abstraction, Hh is the enthalpy of the hydrogen radical and Hp is the enthalpy of the parent phenol (Figure 2).

The energy values of both the highest occupied molecular orbital (HOMO) and the lowest occupied molecular orbital (LUMO) energy of the fully optimized phenol derivatives were calculated on HF//6-31G* level basis set molecular orbital calculation. The absolute value of HOMO energy was adopted as an approximate ionization potential value ($IP_{koopman's}$) according to Koopman's theory. All of the molecular modeling and calculation were performed with Spartan 04 (Wavefunction Inc., Irvine, CA, USA). The η, χ and ω values were calculated using Equations **5-7**, respectively:

$\eta=(E_{LUMO}-E_{HOMO})/2$ Equation (**5**)
$\chi=-(E_{LUMO}+E_{HOMO})/2$ Equation (**6**)
$\omega=\chi^2/2\eta$ Equation (**7**)

where E_{LUMO} and E_{HOMO} are the energy levels for the frontier orbitals.

Results

Radical-scavenging activities determined by the induction period method. The IP- or the Rp_{inh}/Rp_{con}-antioxidant concentration curves for BHA and *bis*-BHA for the AIBN and BPO system

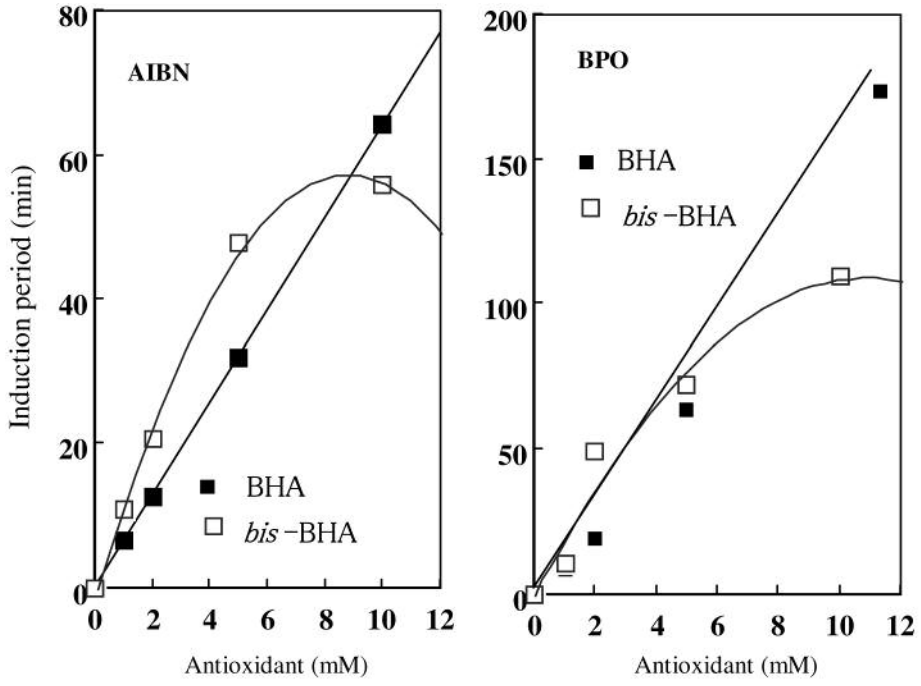

Figure 3. *Plots of the induction period vs. the concentration of BHA and bis-BHA for the AIBN and BPO systems. MMA, 9.4 mol/liter; AIBN (BPO), 100 mM; BHA (bis-BHA), 0-10 mM.*

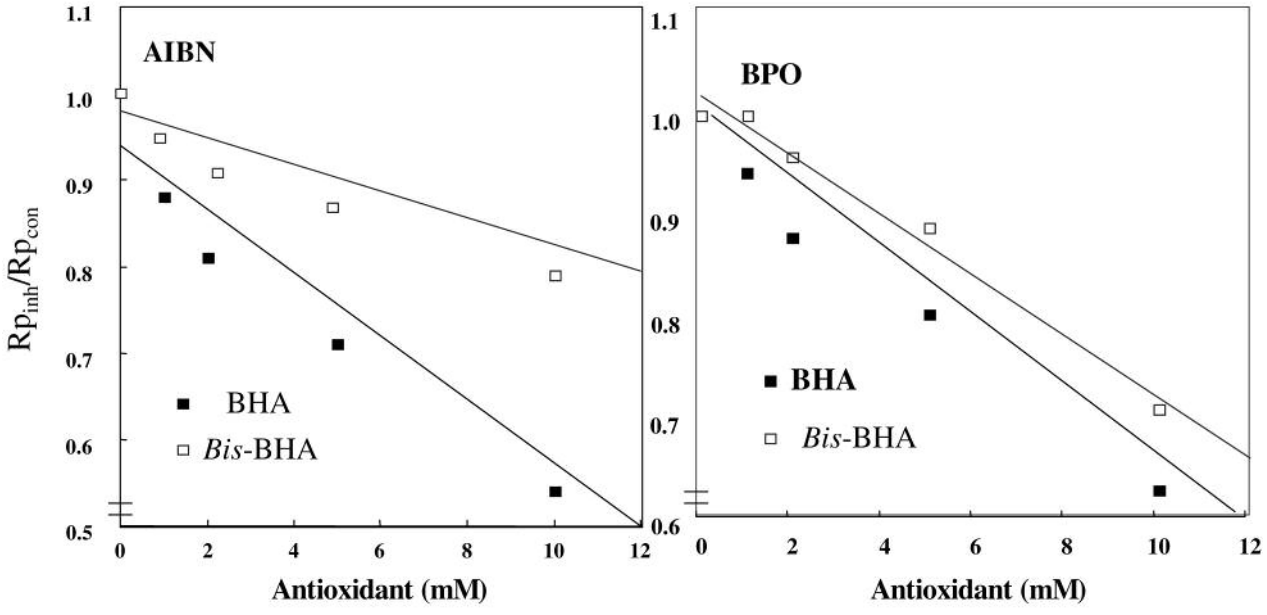

Figure 4. *Plots of the Rp_{inh}/Rp_{con} vs. the concentration of BHA and bis-BHA for the AIBN and BPO systems. MMA, 9.4 mol/liter; AIBN (BPO), 100 mM; BHA (bis-BHA), 0-10 mM.*

are shown in Figures 3, and 4, respectively. The IP-concentration curves of BHA for both systems were linear, whereas the corresponding curves of *bis*-BHA were parabolic, but were probably linear up to a concentration of 5 mM. Thus, the *n* value was determined from each linear slope (Figure 3) (see Equation **1**). The IP-concentration curve of eugenol for both the AIBN and BPO system was linear (data not shown). The respective values of their *n* and Rp_{inh}/Rp_{con} are shown in Table I.

Table I. *Radical-scavenging activity of methoxyphenols 2-t-butyl-4-methoxyphenol (BHA), bis-BHA and eugenol (EUG) using the induction period, DPPH scavenging and O_2^- scavenging method.*

	Induction period method						DPPH	O_2^-
	AIBN			BPO			EC_{50}	IC_{50}
Compound	relative *n*	Rp_{inh}/Rp_{con}	k_{inh}/k_p	relative *n*	Rp_{inh}/Rp_{con}	k_{inh}/k_p	mM	mM
BHA	1.98	0.76	2.69	1.68	0.71	2.31	0.053	14.2
bis-BHA	3.19	0.86	1.45	1.38	0.89	2.33	0.013	90.4
EUG	1.18	0.97	4.39	1.42	0.88	2.18	0.062	2.30

The methods are described in the text. Values were the mean of three independent experiments. Errors <5%. ments. AIBN, 2,2'-azo*bis* isobutyronitrile; BPO, benzoyl peroxide; DPPH, 1,1'-diphenyl-2-picrylhydrazyl. Errors <5%.

For the AIBN system, the *n* value declined in the order *bis*-BHA (3.2) >BHA (2.0) > eugenol (1.2). In general, the *n* for fully oxidized hindered monophenols such as 2,6-*tert*-butyl-4-methoxyphenol is 2. The fully oxidized *n* of *bis*-BHA, compound **5** for the AIBN system is 4 (assuming two-electron oxidation, and consequently the formation of compound **4**; Figures 1 and 2). That of BHA (compound **1**) was 2. In contrast, the n of EUG, a less hindered phenol, was about 1, suggesting the formation of a dimer (8). On the other hand, for the BPO system, the *n* value for all antioxidants was less than 2, with a range of 1.4-1.7.

The inhibition rate constant, k_{inh} plays a more important role in the evaluation of activity. Thus, we investigated the k_{inh}/k_p value. Plots of the ratio of the propagation rate with an inhibitor (Rp_{inh}) to that without an inhibitor (Rp_{con}), the Rp_{inh}/Rp_{con} *vs*. antioxidant concentrations for both initiators decreased linearly as the concentration increased (Figure 4). In particular, the Rp_{inh}/Rp_{con} value for BHA was markedly reduced in the both initiator systems. This was possibly due to the strong interaction between the oxidized products of BHA and the growing number of MMA radicals. For the AIBN and BPO system, the kinh/kp was calculated using Equation **4**. Results are shown also in Table I. For the AIBN system, the k_{inh}/k_p value declined in the order EUG (4.4) > BHA (2.7) > *bis*-BHA (1.5), whereas for BPO the values (2.2-2.3) were almost identical. For both systems, the kinh/kp for BHA was similar to that for *bis*-BHA.

Table II. *Effects of 2-mercapto-1-methylimidazole (MMI) on the induction period (IP) and propagation rate (Rp) of methoxyphenol antioxidants BHA, bis-BHA and eugenol (EUG) in the BPO- and AIBN-MMA system.*

		IP (min)				
Initiator	System+	Observed (A)	Calculated (B)	B-A	A/B	Rp_{inh}/Rp_{con}
BPO	BHA	16.73				0.91
BPO	BHA+ MMI	15.55	17.78	+2.23	0.87	0.91
BPO	*bis*-BHA	20.59				0.96
BPO	*bis*-BHA + MMI	19.60	21.64	+2.04	0.91	0.95
BPO[a]	EUG	16.83				0.86
BPO[a]	EUG + MMI	19.88	17.93	–1.95	1.10	0.82
BPO	MMI	1.05				0.97
AIBN	BHA	5.11				0.95
AIBN	BHA+MMI	4.61	5.95	+1.34	0.77	0.97
AIBN	*bis*-BHA	8.94				0.98
AIBN	*bis*-BHA + MMI	9.81	9.78	–0.03	1.00	0.98
AIBN	EUG	2.62				0.98
AIBN	EUG+ MMI	3.42	3.46	+0.02	0.99	0.98
AIBN	MMI	0.84				1.01

The IP value of control for BPO and AIBN was 7.43 min and 3.79 min, respectively. $IP_{observed} = IP_{exptl} - IP_{control}$. The Rp_{con} value for the AIBN and BPO system was 2.01×10^{-3} M s^{-1} and 1.37×10^{-3} M s^{-1}, respectively. Calculated IP, the simple sum of IP value (antioxidant + MMI). Values were the mean of three different experiments. Computational errors <5%. [a]Obtained from the literature (14); +Chemicals used at 1 mM each.

Effects of MMI on the induction period. The IP values of antioxidants, BHA, *bis*-BHA and EUG, with or without MMI, a co-antioxidant, for the BPO and AIBN system are shown respectively in Table II. The polymerization was carried out at an antioxidant to co-antioxidant molar ratio of 1:1. The observed IP (A), calculated IP (B), B-A, the ratio of A to B (A/B) and the ratio of Rp_{inh} to Rp_{con} (Rp_{inh}/Rp_{con}) for the BPO and AIBN systems are shown in Table II. The reaction of antioxidants with MMI was well characterized by the A/B and Rp_{inh}/Rp_{con} values. For the BPO system, the A/B of the BHA and of the *bis*-BHA/MMI mixture was 0.9. For the AIBN system, the A/B of the BHA/MMI mixture was 0.8, whereas that for the *bis*-BHA and for the EUG mixture was 1.0. In contrast, the A/B of the EUG/MMI mixture for the BPO system was 1.1. In other words, upon $R^{\cdot}$ scavenging, BHA reduced the total antioxidant activity by approximately 20% in the presence of MMI. Upon $PhCOO^{\cdot}$

scavenging, BHA and *bis*-BHA decreased the antioxidant activity by approximately 10% , whereas EUG increased it by approximately 10% . Upon $R^{\cdot}$ scavenging, the A/B of the *bis*-BHA and of the EUG/MMI mixture was 1.0, showing no changes in the activity

The Rp_{inh}/Rp_{con} value of BHA with or without MMI was smaller than that of the corresponding *bis*-BHA value for both BPO and AIBN systems, suggesting marked interaction between their oxidized products and the growing number of MMA radicals. Similarly, a marked decrease in the Rp_{inh}/Rp_{con} value was found in EUG with MMI for the BPO system.

Anti-DPPH radical and O_2^- scavenging activity. The anti-DPPH radical and O_2^--scavenging activity were investigated, and the results are also shown in Table I. The O_2^--scavenging activity ($1/IC_{50}$) declined in the order EUG > BHA > *bis*-BHA, whereas the anti-DPPH radical activity (1/EC50) declined in the order *bis*-BHA > BHA > EUG. The number of reduced DPPH radicals (the number of DPPH moles reduced by one mole of inhibitor) was calculated from the IC_{50} value, and this indicated that the number declined in the order *bis*-BHA (3.9) > BHA (0.9) > EUG (0.8). Bis-BHA scavenged about 4 DPPH radicals, whereas BHA and EUG scavenged about 1 radical. The radical-scavenging activity of *bis*-BHA for DPPH radical, a nitrogen-centered radical was the highest among the three compounds. Bis-BHA preferentially favored more $R^{\cdot}$ derived from AIBN or DPPH radical than $PhCOO^{\cdot}$ (an oxygen-centered radical) derived from BPO or reactive oxygen species (ROS) such as O_2^-.

Theoretical parameters. The parameters are shown in Figure 2 and Table III. The BDE (kcal/mol) declined in the order EUG (84.00) > BHA (77.13) > *bis*-BHA (76.63, first oxidation value) (Figure 2). In contrast, the BDE of *bis*-BHA for two electron oxidation (153.07 kcal/mol) was the highest among the antioxidants. The $IP_{koopman's}$ declined in the order *bis*-BHA > EUG > BHA. The χ and ω values for *bis*-BHA were greater than those for BHA or EUG, whereas the η value for *bis*-BHA was the smallest.

Table III. *HOMO, LUMO, chemical hardness, electronegativity and electrophilicity for BHA, bis-BHA and eugenol (EUG) using the HF//6-31G* and the HF/6-31G*//B3LYP method.*

	HF//6-31G*			HF/6-31G*//B3LYP		
	BHA	*bis*-BHA	EUG	BHA	*bis*-BHA	EUG
LUMO orbital energy (eV)	3.914	3.369	4.088	0.203	–0.246	0.332
LUMO eigenvalue	0.144	0.124	0.151	0.007	–0.009	0.012
HOMO eigenvalue	–0.286	–0.289	–0.291	–0.193	–0.198	–0.200
HOMO orbital energy (eV)	–7.770	–7.867	–7.898	–5.260	–5.382	–5.375
Chemical hardness (η)	5.842	5.618	5.993	2.732	2.568	2.854
Electronegativity (χ)	1.928	2.249	1.905	2.285	2.814	2.522
Electrophilicity (ω)	0.318	0.450	0.303	0.956	1.542	1.114
$IP_{koopmans'}$ (eV)	7.770	7.867	7.898	5.260	5.382	5.375

Discussion

Our results demonstrated that although BHA was an efficient radical scavenger, its oxidized products might cause adverse effects in biological systems (assuming reaction products of compound **3** with $R^{\cdot}$, $ROO^{\cdot}$ or proteins with nucleophilic groups, and compound **4**; Figure 1). Through peroxidative oxidation, BHA was previously repoted to form *bis*-BHA due to an *orth-ortho* coupling reaction of two BHA molecules (Figure 1) (4). A similar reaction mechanism was previously shown to occur in the peroxidative oxidation of phenols, and less hindered phenols such as eugenol, isoeugenol, 2-methoxy-4-methyl phenol (8). In general, a dimer is less toxic than the parent monomers (8). On the other hand, antioxidants alone do not act as radical scavengers *in vivo* but act in a network of non-enzymatic co-antioxidants such as GSH, ascorbate and vitamin E. Therefore, in the present study, we examined the radical-scavenging activity of BHA and *bis*-BHA with MMI. MMI was used as a representative for compounds with thio groups, because GSH had only limited solubility in MMA. Upon both $PhCOO^{\cdot}$ and $R^{\cdot}$ scavenging, the BHA/MMI mixture greatly reduced the total antioxidant activity, possibly due to the lower BDE and $IP_{koopman's}$ values of BHA. BHA may possess antioxidant/pro-oxidant activity. In addition, BHA may preferentially produce intermediates derived from oxidation. It has been reported that BHA prevents damage to lipid membranes by terminating the free radical chain reaction (18), but interferes with membrane integrity and the function of membrane-bound proteins (19). The cytotoxicity for BHA/butylated hydroxytoluene (BHT) mixtures is known to be greater than that of BHA or BHT alone and might be caused by reactive intermediates (20). These findings suggest a potential role for phenoxy radicals in the activation of xenobiotic chemicals to toxic metabolites (12).

On the other hand, an increase in total antioxidant activity was found in EUG/MMI mixtures. This finding for eugenol with conjugate groups may be due to intermediates formed by peroxidation with MMI (14). Although the cytotoxicity of eugenol was lower than that of BHA, this compound was previously reported to lack potent anti-inflammatory activity (21). This may be related to the interference of the EUG oxidized product with biological systems, estimated from the marked decrease in the Rp_{inh}/Rp_{con} for EUG/MMI mixtures.

The protective effect of low BHA concentration on the biological systems was presumably attributed to its ability to induce phase II detoxifying enzymes such as glutathione

S-transferases and quinone reductase (22). BHA is known to activate mitogen-activated protein kinase (MAPK) and to induce phase II/III drug metabolizing enzymes/transporter in mouse liver (23). BHA regulates the antioxidant responsive element (ARE)-mediated gene expression *via* nuclear-factor-like 2 (Nrf2) coupled with the extracellular signal-regulated kinase (ERK) and c-jun *N*-terminal kinase (JNK) signaling pathways (24). We previously investigated whether bioactive BHA possesses any anti-inflammatory activity. Fimbria-induced expression of the interleukin-1β and neutrophil chemoattractant KC genes in RAW264.7 murine macrophages was strongly inhibited by *bis*-BHA, but not by BHA; moreover *bis*-BHA significantly inhibited fimbria-stimulated phosphorylation-dependent degradation of the alpha inhibitor of nuclear factor-kappaB and the transcriptional activity of this factor in the cells (25). In addition, we previously reported that the fimbria-stimulated AP-1 activation of RAW 264.7 murine macrophages was markedly inhibited by *bis*-BHA, but not by BHA, and also that *bis*-BHA significantly inhibited fimbria-induced COX-2 gene expression, which is closely involved in inflammation and carcinogenesis (26). These findings suggest a marked difference between BHA and *bis*-BHA in the manifestation of their biological activities. Therefore, we carried out the biological analysis using theoretical parameters. Indeed, at the semiempirical PM3 level the cytotoxicity of 2-methoxyphenols is known to be related to η; as η increases, the cytotoxicity also increases (27). Their COX-2 inhibition is known to be related to χ; as χ increases, the inhibitory activity also increases (27). The η and χ and ω were previously reported to be possible criteria for determining the toxic natures of chemicals (28). In the present study, the χ and ω values for *bis*-BHA having lower cytotoxicity and potent COX-2 inhibition were clearly higher than those of BHA or EUG, which show higher cytotoxicity and no COX-2 inhibition. In contrast, the η value for *bis*-BHA was lower than that for BHA or EUG. Vanillin, a methoxyphenol showing potent COX-2 inhibition, had higher χ and ω values, and a smaller η value compared with the corresponding values for isoeugenol and eugenol, which show no COX-2 inhibition, while the BDE for vanillin was higher than that for eugenol and isoeugenol (29). Thus, these parameters are possible descriptors that have a direct relationship with toxicity and anti-inflammatory activities.

It has been shown that the cytotoxicity of phenols is due to the radical-mediated toxicity (30). The preset results suggested that the cytotoxicity of BHA may be induced by the reactions of radicals with this compound. The IP, k_{inh} and Rp_{inh}/Rp_{con} values of butylmethoxyphenols derived from the induction period method provided considerable insight into a complex manifestation of phenol-induced toxicity and will provide valuable guidance for future studies.

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