Abstract. The dimers bis-EUG, bis-MMP, bis-BHA, bis-BMP and bis-DBP were synthesized from the monomers 4-allyl-2-methoxyphenol (EUG), 2-methoxy-4-methylphenol (MMP), 2-t-butyl-4-methoxyphenol (BHA), 2-t-butyl-4-methylphenol (BMP) and 2,4-di-t-butylphenol (DBP), respectively. The stoichiometric factors (n; number of free radicals trapped by one mole of phenolic moiety) of these compounds were determined by induction period methods with a kinetic approach in the 2′,2-azobisisobutyronitrile (AIBN) and benzoyl peroxide (BPO) systems at 70°C. The n values for bis-EUG, bis-MMP and bis-BHA were approximately two-fold greater than those for their monomers in both the AIBN and BPO systems, whereas the n values for bis-BMP and bis-DBP were identical to those of their monomers. bis-EUG, bis-MMP and bis-BHA, containing methoxy groups, were potent antioxidants. The n values (1.3-1.6) for EUG and MMP were considerably less than 2, as is commonly observed for the stoichiometric factors of phenolic compounds. The antiradical efficiencies against DPPH (diphenylpicrylhydrazyl) of the monomers and their dimers were also investigated, likewise indicating that bis-EUG, bis-MMP and bis-BHA were potent antioxidants. DBP and bis-DBP were less effective radical scavengers because of the steric factor of their bulky t-butyl groups. Phenolic activity is caused by the generation of radicals from phenols themselves. Additionally, phenols scavenge harmful radicals such as reactive oxygen species derived from biological systems. Thus, phenols exhibit both antioxidant and prooxidant activity.

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Key Words: 2-Methoxyphenols, butyl-substituted phenols, orthobisphenols.
methoxyphenols and 2-t-butyl-alkylphenols, and investigated the cytotoxicity and radical production/antioxidant activity of these compounds (2-5). However, the structure-activity relationship (SAR) models would be improved by knowledge of the radical-scavenging and cytotoxic mechanisms of five different phenolic monomers (EUG, MMP, BHA, BMP and DBP) and their dimers.

In the present study, we used a reliable and direct method previously developed (6, 7) to quantify the radical-scavenging activity of these monomers and their corresponding dimers against both alkyl radicals (R•) derived from AIBN and peroxy radicals (PhCOO•) derived from BPO. In addition, the antiradical efficiency of the monomers and dimers against DPPH radicals (DPPH•) was studied. Furthermore, the cytotoxic activities of the monomers and dimers against HGF (human gingival fibroblast) and HSG (human submandibular gland cancer cell line) cells were compared. The absolute 1H- and 13C-NMR assignments of the five prepared dimers are presented, and also the electrostatic charge, energy of the HOMO (high occupied molecular orbital) and bond-dissociation enthalpy of the phenolic O-H (¢H) for these compounds were obtained from PM3 calculations (8). A possible link between radicals and cytotoxicity is discussed.

Materials and Methods

Materials. The following chemicals and reagents were obtained from the indicated companies: EUG, MMP, BHA, BMP, DBP, 2,6-di-t-butyl-4-methylphenol (BHT), 2,4,6-tri-t-butylphenol (TBP), 2,6-di-t-butyl-4-methoxyphenol (DBMPH), MMA, AIBN, BPO and DPPH were purchased from Tokyo Kasei Chem. Co., Japan. MMA was purified by distillation. AIBN and BPO were recrystallized from methanol and chloroform/methanol, respectively.

Minimum essential Eagle medium (MEM) and minimum essential medium modification (MEM) were obtained from Sigma Chemical Co. (St. Louis, MO, USA); fetal bovine serum (FBS) and newborn calf serum (NBCS) were from JRH Biosciences (Lenexa, KS, USA); and Cell Titer 96 Aqueous One Solution of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was from Promega Co. (Madison, WI, USA).

Bis-EUG (3,3'-dimethoxy-5,5'-di-2-propenyl-1,1'-biphenyl-2,2' diol), bis-BHA (3,3'-di-t-butyl-5,5'-dimethoxy-1,1'-biphenyl-2,2' diol), bis-MMP (3,3'-dimethoxy-5,5'-dimethyl-1,1'-biphenyl-2,2' diol), bis-BMP (3,3'-di-t-butyl-5,5'-dimethyl-1,1'-biphenyl-2,2' diol) and bis-DBP (3,3',5,5'-tetra-t-butyl-1,1'-biphenyl-2,2'-diol) were synthesized using the CuCl (OH) TMEDA complex, as reported previously (1, 2, 4). The chemical structures of the monophenols and dimers are shown in Figure 1.

NMR measurement. All 1D and 2D NMR spectra were obtained on a JEOL JNM-Alpha500 spectrometer at room temperature, and chemical shifts are expressed as ‰ value down from an internal TMS standard in CDCl3. Assignments were performed by a combination of spectral analytical methods, such as 1H (500MHz), homodecoupled spectra, NOE difference spectra, 13C (125MHz), 13C-DEPT, and field gradient mode of 2D spectra such as HMQC, HMBC and NOESY. Absolute assignments of the prepared dimers are as follows.

Bis-Eug: H-7 4H, d, J=6.7Hz, H-7, 3.92 (6H, s, 3-OCH3), 5.06 (2H, m, H-9b), 5.10 (4H, m, H-9a), 5.98 (2H, s, 2-OH). Bis-MMP: H-4 2H, d, J=1.5Hz, H-4, 6.72 (2H, d, J=1.8Hz, H-6). Bis-BHA: H-4 2H, d, J=1.8Hz, H-6, 6.77 (2H, d, J=1.8Hz, H-6). Bis-BMP: H-4 2H, d, J=1.8Hz, H-6. Bis-DBP: H-4 2H, d, J=1.8Hz, H-6.
Chemical shifts. δC 21.17 (5-CH₃), 56.06 (3-OCH₃), 111.32 (C4), 123.44 (C6), 124.35 (C1), 129.59 (C5), 140.35 (C2), 147.05 (C3).

Bis-BMP: δH 1.42 (18H, s, 3-C(CH₃)₃), 2.30 (6H, s, 5-CH₃), 5.15 (2H, s, OH), 6.88 (2H, d, J=1.8Hz, H-6), 7.14 (2H, d, J=1.8Hz, H-4). δC 20.79 (5-CH₃), 29.62 (3-C(C₃H₈)₃), 34.89 (3-C(CH₃)₃), 122.55 (C1), 128.43 (C4), 128.74 (C6), 129.55 (C5), 136.88 (C3), 149.83 (C2).

Bis-BHA: δH 1.43 (18H, s, 3-C(CH₃)₃), 3.77 (6H, s, 5-OCH₃), 5.01 (2H, s, OH). 6.63 (2H, d, J=3.1Hz, H-6), 6.96 (2H, d, J=3.1Hz, H-4). δC 29.49 (3-C(CH₃)₃), 35.17 (3-C(CH₃)₃), 55.73 (5-OCH₃), 111.76 (C6), 115.27 (C4), 123.18 (C1), 138.92 (C3), 145.88 (C2), 153.22 (C5).

Bis-DBP: δH 1.32 (18H, s, 5-C(CH₃)₃), 1.45 (18H, s, 3-C(CH₃)₃), 5.20 (2H, s, OH), 7.11 (2H, d, J=2.4Hz, H-6), 7.39 (2H, d, J=2.4Hz, H-4). δC 29.69 (3-C(CH₃)₃), 31.64 (5-C(CH₃)₃), 34.47 (5-C(CH₃)₃), 35.21 (3-C(CH₃)₃), 122.34 (C1), 124.84 (C4), 125.28 (C6), 136.26 (C3), 143.00 (C5), 149.78 (C2).

Cells and cell culture. Two different cell types were used in this study: HGF, a primary culture of human gingival fibroblasts and HSG, a human epidermoid carcinoma cell line derived from a human submandibular gland. HGF cells were obtained by a method similar to that previously reported (1). Ethical clearance for the study was obtained from the local ethics committee.

Induction period. The induction period was determined by the method reported previously (6, 7). In brief, the experimental resin consisted of MMA and AIBN (or BPO) with or without additives. The concentration of the initiators (AIBN or BPO) was 0.1 M and of the additives was 2 mM. About 10 μl (MMA: 9.12-9.96 mg) of the experimental resin was loaded into an aluminum sample container and sealed by applying pressure. The container was placed in a differential scanning calorimeter (model DSC 3100; MAC Science Co., Tokyo, Japan) kept at 70°C and thermal changes induced by polymerization were recorded for the appropriate periods. The heat due to the polymerization of MMA was 13.0 kcal/mole in this experiment. This was in agreement with the cited value (9).

The conversion of all samples, as calculated from the DSC thermogram, was 91.0-96.5%. Exothermic curves and polymerization curves for controls and test compounds were derived from DSC thermograms using integrated heat evoked by the polymerization of MMA (Figure 2, top). Polymerization curves break when the phenol inhibitor is consumed (Figure 2, bottom). These breaks are very sharp and provide a reliable measure of the induction period of the inhibitor. The presence of oxygen retards polymerization because oxygen, a biradical, reacts with the initiator. Thus, polymerization in the control was slightly inhibited, even though the reaction was carried out in a sealed DSC pan, since it contained a small amount.
of oxygen having been sealed in air. Tangents were drawn to polymerization curves at an early stage in the run. The induction period (T ind) of the test compounds was determined from the length of time between the zero point on the abscissa and the point of the intersection of the tangents drawn to the early stage of polymerization. T ind was calculated from the difference between the induction period of specimens and that of controls.

Measurement of the induction rate (R i). The induction period method was used to determine Ri due to thermal decomposition, using Eq. (I):

\[ n = \frac{R_i T_{\text{ind}}}{[\text{II}]_0} \]

where [I]_0 is the concentration of the inhibitor at time zero and T ind is the induction period. DTBM was used to determine R i, since its stoichiometric factor n, is known to be 2.00 (10, 11). In the case of [MMA] = 9.4 mole/l and [AIBN or BPO] = 0.1 mole/l at 70°C, the induction period method using DTBM gave Ri for AIBN = 5.66 x 10⁻⁶ mole/(l·s) and for BPO = 2.28 x 10⁻⁶ mole/(l·s).

Stoichiometric factors (n). The relative n values in Eq. (II) can be calculated from the induction periods in the presence of an inhibitor:

\[ n = \frac{R_i T_{\text{ind}}}{[\text{II}]_0} \]

where T ind is the induction period in the presence of an inhibitor [II]. The number of moles of peroxy radicals trapped by one mole of the relevant phenol was calculated with respect to one phenolic moiety unit.

Anti-DPPH radical activity of inhibitors. Radical-scavenging activities were determined with DPPH as a free radical. For each inhibitor, various concentrations were tested in ethanol. The decrease in absorbance was determined at 517 nm for 10 minutes at room temperature. Antiradical activity was defined as the amount of inhibitor necessary to decrease the initial DPPH radical concentration by 50% (EC50% (mole/l)) (12).

Anti-DPPH radical activity of inhibitors. Radical-scavenging activities were determined with DPPH as a free radical. For each inhibitor, various concentrations were tested in ethanol. The decrease in absorbance was determined at 517 nm for 10 minutes at room temperature. Antiradical activity was defined as the amount of inhibitor necessary to decrease the initial DPPH radical concentration by 50% (EC50% (mole/l)) (12).

Assay for cytotoxicity. HSG or HGF cells were inoculated into 96-well plates at a density of 4 x 10³ cells/well in 0.1 ml of MEM or α-MEM supplemented with 10% FBS and cultured at 37°C for 2 days. The medium was replaced by serum-free medium 1 hour before the assay. Phenol compounds (10⁻⁵ to 10⁻⁴ M) dissolved in DMSO were added to the cells, giving a final concentration of 10⁻⁷ to 10⁻³ M. The final DMSO concentration was 1%. After incubation for 2 days, viability was estimated by the MTT method (1). In brief, 25 μl of Cell Titer 96 Aqueous One solution of MTT was added to each well. After incubation for 3 hours at 37°C, the absorbance at 492 nm was determined with a microplate reader (Biochromatic; Labsystem, Helsinki, Finland). The viability was defined as the ratio (percent) of the absorbance in the experimental well to that of the control well, which contained 1% DMSO (no test compound). A dose-response curve of viability (%) was plotted to define the concentration of eugenol-related compounds that reduced MTT-formazan production. HSG cells were maintained as monolayer cultures at 37°C in MEM medium supplemented with 10% FBS, in a humidified 5% CO₂ atmosphere (13). The fifty % cytotoxic concentration (CC50) was determined from dose-response curves.

Computational details. Theoretical calculations, i.e. charges, electrostatic charges, HOMO density and ΔH, were performed with the PM3 semiempirical MO method as implemented in the MOPAC program on a Tektronix CAChe worksystem (8, 14).

Results

Stoichiometric factors (n) in the AIBN and BPO systems. Typical time-exothermic curves and time-conversion curves are shown in Figure 2. Relative n values of monomers and dimers were determined, and the results are shown in Table I. In the AIBN system, the n value for EUG and MMP was 1.3-1.4, whereas that for BHA, BMP and DBP was approximately 2. The n values for dimers, except for bis-DBP, were 2.0-3.5. In the BPO system, the n value for EUG (1.3) was also the smallest, followed by MMP (1.6). BHA, BMP, bis-EUG and bis-BHA showed n values of approximately 2, whereas the monomer and dimer of DBP showed an n value less than 2. The n of bis-MMP (2.8) was the largest in the BPO system. In both systems, the n values of EUG and MMP were less than 2. Based on the ratio of dimer/monomer, bis-EUG, bis-MMP and bis-BHA were potent radical scavengers.

Antiradical efficiency for DPPH radicals. The antiradical efficiency of each inhibitor is shown in Table II as EC50 values (the concentration causing 50% reduction of DPPH). The EC50 value assayed by optical methods increased in the following order: bis-BHA < bis-EUG < bis-MMP < bis-BMP < BHA < EUG < MMP < BHT < BMP < DBP = bis-DBP. Except for bis-DBP, the antiradical efficiency for dimers was markedly higher than that for monomers. BHT, DBP, bis-BMP and bis-DBP, with t-butyl substituted groups, showed the lowest antiradical efficiency, with DBP and bis-DBP having the lowest activity among the compounds tested. This suggests that the lower scavenging activity for DPPH• of phenolic compounds with bulky 2,6-di-t-butyl substituent groups is due to steric factors. The number of reduced DPPH• for MMP, EUG and BHA was 0.5-1, suggesting dimerization of these compounds. Dimerization of EUG has previously been reported (12).

Cytotoxicity. CC50 values (concentrations causing 50% cell death) for monomers and dimers against HSG and HGF cells are shown in Table III. Among the compounds tested, MMP had the lowest cytotoxicity against both HSG and HGF cells, whereas DBP (in addition to the positive controls BHT and TBP) was classified into the most cytotoxic group. The sensitivity index (SI) for EUG, MMP and bis-MMP was about 5-10, whereas that for other compounds was about 1 (Table III). HSG cells were particularly sensitive to 2-methoxy-4-alkylphenols such as EUG and MMP. The cytotoxicity of EUG and BHA was reduced by dimerization, whereas the cytotoxicity of MMP and BMP was enhanced. The high cytotoxicity of DBP was not altered by dimerization.

Descriptors in the SAR model. Computational chemistry finds its most important applications in the support of experimental efforts. ¹H-NMR signals due to phenolic OH
for bis-MMP and bis-EUG were shifted to a lower field than those for bis-BHA, bis-BMP and bis-DBP, suggesting that the OH groups of 2-t-butyl-substituted phenols are much more shielding than those of 2-methoxyphenols. Electrostatic charge, the energy of the HOMO, \( \Delta H \) and shape/size (projection into the YZ plane, YZshadow) were calculated as descriptors for the SAR models. The charge and electrostatic charge distributions of the less hindered phenols EUG, MMP, BHA, BMP and DBP are shown in Table IV. The minimum charge \( Q_{\text{min}} \) in the molecule for all compounds appeared at 2-OH with an almost similar value. In the 3-OH position for 2-methoxyphenols, the \( Q_{\text{min}} \) of EUG is greater than that of MMP, suggesting that enzymatic demethylation of the methoxy group may be favored in EUG compared to MMP. Ortho-quinone formation, due to the demethylation of methoxyphenols, is well known to induce cytotoxic activity (15).

The energy of the highest HOMO for the phenolic compounds tested exists at either the C5 or the C2 position (Table V). In monomers, the energy of the highest HOMO for EUG, MMP and BHA exists at the C2 position, whereas that for BMP and DBP exists at the C5 position. In dimers, the energy of the highest HOMO for bis-EUG, bis-BHA, bis-BMP and bis-DBP exist at the C2 position, whereas that for bis-MMP exists at the C5 position. The \( \Delta H \) (approx. 79 kcal/mol) for EUG and MMP was significantly less than that (82-87 kcal/mol) for the other phenolic compounds, suggesting that EUG and MMP are oxidizable and consequently have lower \( n \) values and may undergo dimerization.

Next, we calculated the shape/size (YZshadow) of the dimer molecules. The YZshadow for bis-EUG is illustrated in Figure 3. Calculations using PM3 performed on the two aromatic ring planes of bis-EUG, an ortho-bisphenol, predict the counter aromatic plane to be 90° twisted, suggesting that the two phenolic OH functional groups of bis-EUG do not form an OH-OH hydrogen bond. The YZshadow for other dimers was similar to that for bis-EUG. This finding suggests that bulky DPPH+ radicals could be quite efficiently scavenged by dimers, since OH-OH bonding does not occur.

### Discussion

In the present study, we investigated whether the synthetic phenolic dimers we designed could act as potent radical scavengers. The compounds (Figure 1) showed quite wide variation in their reactivity toward radicals derived from AIBN or BPO and in their cytotoxic activity toward HSG and HGF cells. This suggests that substituents in phenols play a crucial role in their radical-scavenging activity and cytotoxicity. Dimerization of EUG, BHA and MMP enhanced the radical-scavenging activity for both R• and PhCOO• radicals. The DPPH• (cyanoisopropyl) radical is classified as an alkyl radical (R•), and the radical-scavenging behavior of these compounds

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>EC(_{50}) (mM)</th>
<th>Stoichiometric Value</th>
<th>Number of reduced DPPH radicals*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP</td>
<td>0.091</td>
<td>1.85</td>
<td>0.54</td>
</tr>
<tr>
<td>EUG</td>
<td>0.065</td>
<td>1.29</td>
<td>0.77</td>
</tr>
<tr>
<td>BHA</td>
<td>0.052</td>
<td>1.04</td>
<td>0.96</td>
</tr>
<tr>
<td>BMP</td>
<td>1.01</td>
<td>20.01</td>
<td>0.05</td>
</tr>
<tr>
<td>DBP</td>
<td>&gt;1</td>
<td>&gt;1</td>
<td>&gt;1</td>
</tr>
<tr>
<td>BHT</td>
<td>0.76</td>
<td>14.3</td>
<td>0.07</td>
</tr>
<tr>
<td>bis-MMP</td>
<td>0.024</td>
<td>0.48</td>
<td>2.08</td>
</tr>
<tr>
<td>bis-EUG</td>
<td>0.015</td>
<td>0.3</td>
<td>3.33</td>
</tr>
<tr>
<td>bis-BHA</td>
<td>0.012</td>
<td>0.24</td>
<td>4.16</td>
</tr>
<tr>
<td>bis-BMP</td>
<td>0.047</td>
<td>0.94</td>
<td>1.06</td>
</tr>
<tr>
<td>bis-DBP</td>
<td>1&gt;</td>
<td>&gt;1</td>
<td>0.0&gt;</td>
</tr>
</tbody>
</table>

DPPH (2,2’-diphenyl-1-picrylhydrazyl), 0.1 mM. The antiradical activity was determined as the amount of inhibitor necessary to decrease the initial DPPH concentration by 50% (EC\(_{50}\), mol/l). The stoichiometry was obtained by multiplying the EC\(_{50}\) of each inhibitor by two which gives the theoretical efficient concentration of each inhibitor needed to reduce 100% of DPPH radicals. *The number of DPPH moles reduced by one mole of inhibitor. The EC\(_{50}\) was determined from dose- response curves. Values were the mean for three separate measurements. Standard errors<10%.

<table>
<thead>
<tr>
<th>Compound</th>
<th>AIBN</th>
<th>BPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUG</td>
<td>1.30</td>
<td>2.58</td>
</tr>
<tr>
<td>MMP</td>
<td>1.41</td>
<td>2.56</td>
</tr>
<tr>
<td>BHA</td>
<td>2.14</td>
<td>3.52</td>
</tr>
<tr>
<td>BMP</td>
<td>1.98</td>
<td>1.98</td>
</tr>
<tr>
<td>DBP</td>
<td>1.92</td>
<td>1.70</td>
</tr>
</tbody>
</table>

Results are the means of 3 determination. Standard errors<10%; MMA, 9.4 M; AIBN (or BPO), 0.1 M; 70° C; 2,6-di-t-butyl-4-methoxyphenol with \( n=2.00 \) was used as a standard. The measurement of \( n \) values is described in the text.
The CC50 was determined from dose-response curves depicted by 8 independent experiments. Standard errors were <15%.

### Table IV. Charge (upper) and electrostatic charge (lower) distribution of methoxy and t-butyl phenols.*

<table>
<thead>
<tr>
<th>Position</th>
<th>EUG</th>
<th>MMP</th>
<th>BHA</th>
<th>DBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>-0.13</td>
<td>-0.13</td>
<td>-0.20</td>
<td>-0.17</td>
</tr>
<tr>
<td>C2</td>
<td>-0.28</td>
<td>-0.26</td>
<td>-0.26</td>
<td>-0.15</td>
</tr>
<tr>
<td>C3</td>
<td>0.09</td>
<td>0.09</td>
<td>0.10</td>
<td>0.07</td>
</tr>
<tr>
<td>C4</td>
<td>0.30</td>
<td>0.31</td>
<td>0.31</td>
<td>0.28</td>
</tr>
<tr>
<td>C5</td>
<td>-0.04</td>
<td>-0.03</td>
<td>-0.09</td>
<td>-0.06</td>
</tr>
<tr>
<td>C6</td>
<td>0.18</td>
<td>0.03</td>
<td>-0.18</td>
<td>-0.24</td>
</tr>
<tr>
<td>C7</td>
<td>-0.28</td>
<td>-0.22</td>
<td>-0.23</td>
<td>-0.23</td>
</tr>
<tr>
<td>C8</td>
<td>-0.48</td>
<td>-0.49</td>
<td>-0.46</td>
<td>-0.45</td>
</tr>
<tr>
<td>C9</td>
<td>-0.21</td>
<td>-0.22</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>C10</td>
<td>-0.48</td>
<td>-0.39</td>
<td>0.09</td>
<td>0.16</td>
</tr>
<tr>
<td>3-Me</td>
<td>0.05</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-C</td>
<td>0.32</td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-OMe</td>
<td>-0.02</td>
<td>-0.06</td>
<td>-0.06</td>
<td>-0.19</td>
</tr>
<tr>
<td>5-Me</td>
<td>0.17</td>
<td>0.08</td>
<td>0.09</td>
<td>-0.36</td>
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<tr>
<td>5-Q</td>
<td>0.14</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-OH</td>
<td>-0.10</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-C</td>
<td>-0.17</td>
<td></td>
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<td></td>
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<tr>
<td>5-C</td>
<td>-0.23</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*Values were calculated by semiempirical molecular orbital calculation (PM3)

in the AIBN system appeared to be similar to that in the DPPH system. The phenolic compounds (BHT, BMP, DBP and dimers) with high hydrophobicity showed high cytotoxic activity, but in terms of cytotoxicity EUG, MMP and bis-BMP are of particular interest because their SIs were significantly greater than those of the other compounds. 2-Methoxy-4-alkyl phenols such as EUG and MMP have n values less than 2 and may dimerize via quinone methide intermediates (QMIs) (16). 2-Methoxy-4-alkyl phenols have previously been reported to form QMIs, and their toxicity was dependent on the life-time of the QMI. EUG, with a longer QMI life-time, was shown to be much more toxic than MMP, with a shorter life-time (17), because of the extent of injury caused by QMI-reactive nucleophiles. In the present study, the n value (2.8) of bis-MMP against peroxy radicals (PhCOO•), which are considered to be a biomimetic model for peroxy radicals (ROO•) derived from unsaturated lipids, was the highest, suggesting that bis-MMP preferentially undergoes oxidation in biological systems. We previously demonstrated that bis-MMP, but not bis-EUG or bis-HA, enhances the cytotoxicity of visible light irradiation. (3). Also, the PM3 calculations indicated that the energy of the HOMO of the carbon in the ring at the para position (C5) is most highly enhanced in bis-MMP, which adds considerable support to the conjecture that bis-MMP can yield a highly reactive substance, CH₃⁺, via oxidation (Figure 4). Bis-EUG and bis-HA were less cytotoxic and less potent antioxidants. We recently reported that bis-EUG, but not EUG, shows potent anti-inflammatory activity (19). SAR models using PM3 calculations are useful to predict the biological activity of anticancer and anti-inflammatory drugs (8, 18).

### Acknowledgements

This work was supported in part by a Grant-in-Aid from the Japanese Ministry of Education, Science, Sports and Culture.
Table V. Energy of the highest HOMO (density) and bond-dissociation enthalpy of Ph O-H ($\Delta H$ kcal/mol)*

<table>
<thead>
<tr>
<th></th>
<th>A. Monomer</th>
<th></th>
<th>B. Dimer</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Position</td>
<td>EUG</td>
<td>MMP</td>
<td>BMP</td>
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*calculated values by PM3 method. Bold is energy of the highest HOMO. Positions see numbering of chemical structures (Figure 1).

Figure 3. $YZ_{\text{shadow}}$ for bis-EUG calculated by the PM3 method.

Figure 4. Potential reaction pathways for the oxidation of bis-MMP. For the reaction of bis-MMP (1) with oxygen, the heat of reaction is exothermic by 24.7 kcal/mol. Also, the reaction of compound 2 with oxygen is exothermic by 24.2 kcal/mol and compound 3 would be produced. The final product (compound 5) with CH$_3$OH would be obtained via the self-reaction of the quinone compound (4).
References


17 Thompson DC, Pera K, Krol ES and Bolton JL: o-Methoxy-4-alkyl phenols that form quinone methides of intermediate reactivity are the most toxic in rat liver slices. Chem Res Toxicol 8: 323-327, 1995.


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