molecules

Full Paper

Radical-scavenging Activity of the Reaction Products of Isoeugenol with Thiol, Thiophenol, Mercaptothiazoline or Mercaptomethylimidazole Using the Induction Period Method

Yoshinori Kadoma [1], **Wakako Kumada** [2], **Yuhta Asai** [2], **Yoshiaki Sugita** [2], **Ichiro Yokoe** [2] and **Seiichiro Fujisawa** [2,*]

[1] Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Kanda-surugadai, Chiyoda-ku, Tokyo 101-0062, Japan; E-mail: y-kadoma.fm@tmd.ac.jp

[2] Faculty of Pharmaceutical Sciences, Josai University, Saitama 3500295; Meikai Unversity School of Dentistry, Sakado, Saitama 3500283 Japan; E-mails: wakako-216@ezweb.ne.jp (Kumada); fqqrk256@yahoo.co.jp (Asai); yokoe@josai.ac.jp (Yokoe)

* Author to whom correspondence should be addressed; E-mail: fujisawa 33@nifty.com

Abstract: The reaction products in the presence of Lewis acid of isoeugenol (**1**) with ethanethiol, thiophenol, 2-mercaptothiazoline or 2-mercapto-1-methylimidazole (**ISO-S1** – **ISO-S-4**) were obtained. The radical-scavenging activity of these compounds was investigated using the induction period method for polymerization of methyl methacrylate (MMA) initiated by thermal decomposition of 2,2'-azobisisobutyronitrile (AIBN) and benzoyl peroxide (BPO) and monitored by differential scanning calorimetry (DSC). For BPO, the stoichiometric factor (number of free radicals trapped by one mole of antioxidant moiety, n) declined in the order isoeugenol (1.8) > **ISO-S-1** (1.6) > **ISO-S-2** (1.2) > **ISO-S-3** (0.9) > **ISO-S-4** (0.3), whereas for AIBN, their n values were about 1, except for **ISO-S-3** (0.6). The ratio of the rate constant of inhibition to that of propagation (k_{inh}/k_p) for BPO declined in the order **ISO-S-4** (56) > **ISO-S-3** (15) > **ISO-S-2** (11) > **ISO-S-1** (9) > isoeugenol (8). Similarly, for AIBN the k_{inh}/k_p of the reaction products (33-57) was greater than that of isoeugenol (31). The reaction products of isoeugenol with a SH group showed greater inhibition rate constants (k_{inh}) than the parent compound isoeugenol.

Introduction

Isoeugenol (4-propenyl-2-methoxyphenol, **1**) is used in perfumes, soaps, detergents, air fresheners and cosmetics. However, isoeugenol is also an important allergen [1]. Furthermore, isoeugenol has a moderate acute toxicity by dermal and oral routes. Although isoeugenol exhibited cytotoxic and the mentioned allergenic activity, it is a potent antioxidant [1]. It has been generally assumed that the antioxidant effects of phenolic antioxidants are responsible for their anti-inflammatory and chemopreventive effects. We previously synthesized a dimer of isoeugenol, dehydrodiisoeugenol, demonstrating that this compound, but not isoeugenol, displays potent cyclooxygenase-2 (COX-2) inhibition, an anti-inflammatory activity [2]. Also, we previously reported that the cytotoxicity of isoeugenol may be attributed to its induction of reactive oxygen species (ROS) and the reduction of gluthathione (GSH) levels, possibly due to its prooxidative activity [3]. Curcumin, an isoeugenol-related compound, was previously reported to form conjugates with GSH by separating mono- and diglutathionyl adducts of curcumin. This suggested that formation of curcumin-GSH adducts lead to inactivation of the parent curcumin's prooxidative activity [4]. These results led us a hypothesis: thioisoeugenols may reduce the cytotoxic and allergenic activity without decreasing their antioxidant activity. Thus, we synthesized **ISO-S-1**, a reaction product of isoeugenol (**1**) with ethanethiol; **ISO-S-2**, a reaction product of **1** with thiophenol; **ISO-S-3**, its reaction product with 2-mercaptothiazoline and **ISO-S-4**, a reaction product of **1** with 2-mercapto-1-methylimidazole (see Scheme 1).

Scheme 1. Preparation of products ISO-S-1 – ISO-S-4.
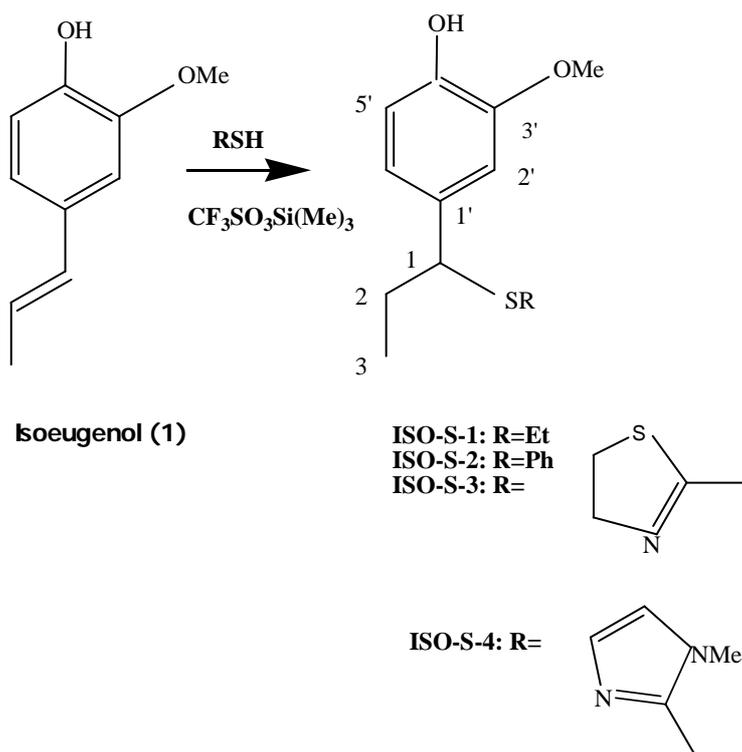
We have previously used DSC and the induction period method to investigate the radical scavenging activity of phenolic antioxidants under nearly anaerobic conditions, and this method has been proven to be reliable for evaluating the activity of these compounds [5,6].

In an extension of this work, we investigated here the radical-scavenging activity of **ISO-S-1**, **2**, **3** and **4**, and their parent compound, isoeugenol, by determining the induction period for polymerization of methyl methacrylate (MMA) initiated by thermal decomposition of 2,2'-azobisisobutyronitrile (AIBN, a R· radical) and benzoyl peroxide (BPO, a PhCOO· radical).

Results and Discussion

Radical scavenging activities determined by the induction period method

The time-exotherm and time-conversion curves for **ISO-S-1**, **2**, **3**, **4** and isoeugenol are shown in Figures 1 and 2, respectively.

Figure 1. Exothermic curves for the polymerization of MMA initiated by thermal decomposition of BPO in the presence of 0.01 mol% additives.
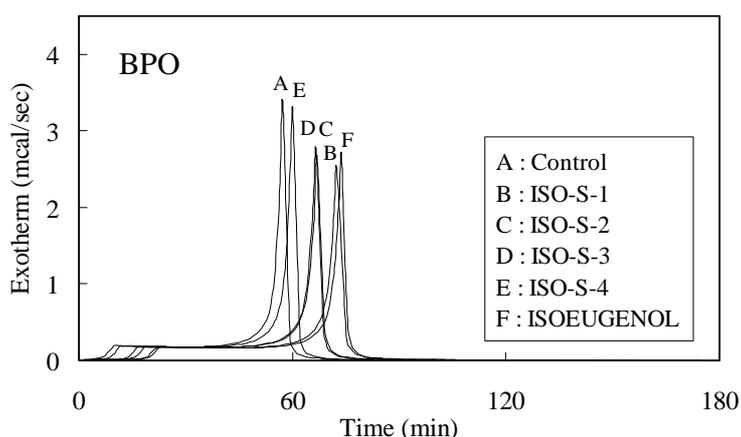
Figure 2. Time-conversion curves for the polymerization of MMA initiated by thermal decomposition of BPO in the presence of 0.01 mol% additives. Curves were obtained from findings shown in Figure 1.
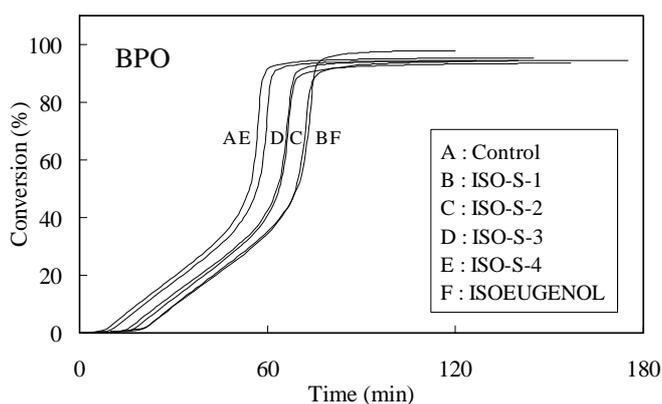
The *n* value was determined from each slope (Figure 3, see Equation 2). Results are shown in Table 1. For BPO, the *n* value declined in the order isoeugenol (1.8)> **ISO-S-1** (1.6) > **ISO-S-2** (1.2) > **ISO-S-3** (0.9) > **ISO-S-4** (0.3), whereas for AIBN, the *n* value of **ISO-1**, **2**, and **4** and isoeugenol were all about 1, while the value for **ISO-3** was 0.6. In general, the *n* value of phenolic compounds is close to 2 [7]. The *n* value for 2-methoxyphenol is less than 2, due to the strong internal hydrogen bond on the OH and the methoxy group [5c]. It is well known that the one-electron oxidation of phenolic compounds; the *n* of about 1 leads to dimmers and to polymers (lignin) [8]. In the present study, compounds with the *n* of about 1 may occur dimerization during induction period.

Figure 3. A typical plot of the induction period *vs* the concentration for **ISO-S-1**, **2**, **3**, and **4** in the polymerization of MMA initiated by thermal decomposition of AIBN (left panel) and BPO (right panel).
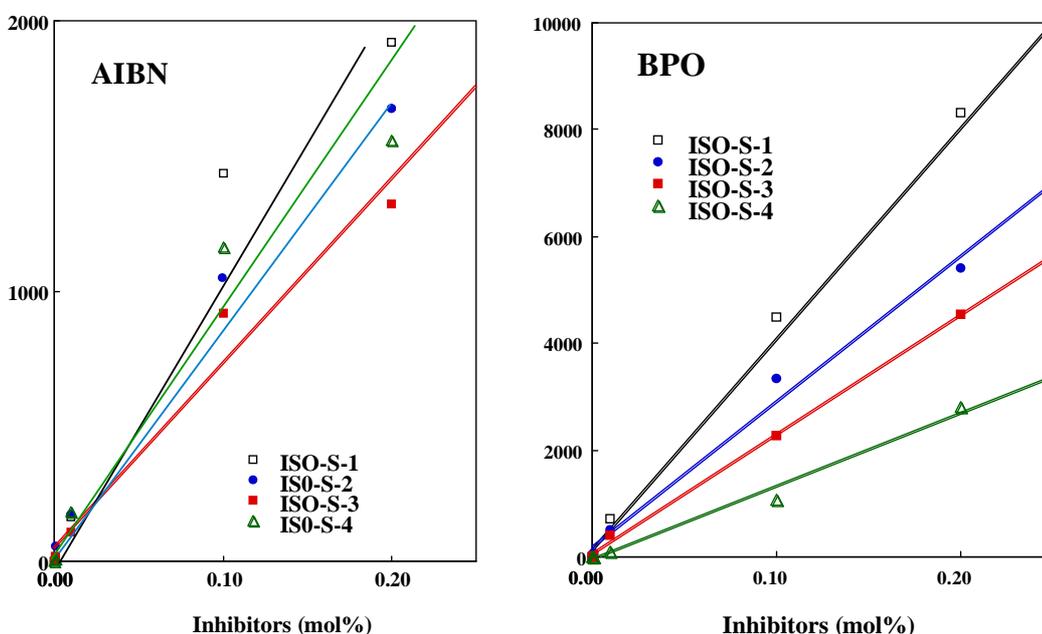
Table 1. Radical-scavenging activity of the reaction products of isoeugenol (**1**) with ethanethiol, thiophenol, 2-mercaptothiazoline or 2-mercapto-1-methylimidazole (ISO-S-1 – ISO-S-4) in the polymerization of methyl methacrylate initiated by AIBN and BPO.

Compound	AIBN		BPO	
	Relative *n*	k_{inb}/k_p	Relative *n*	k_{inb}/k_p
ISO-S-1	0.92 ± 0.05	36.43 ± 1.78	1.61 ± 0.08	8.88 ± 0.44
ISO-S-2	0.96 ± 0.04	34.81 ± 1.71	1.19 ± 0.05	11.75 ± 0.58
ISO-S-3	0.59 ± 0.02	56.63 ± 2.27	0.91 ± 0.04	15.46 ± 0.76
ISO-S-4	1.01 ± 0.05	32.86 ± 1.48	0.25 ± 0.01	55.95 ± 2.79
Isoeugenol	1.11 ± 0.06	30.52 ± 1.36	1.75 ± 0.09	8.16 ± 0.42

n values calculated using Eqn. 2. k_{inb} and k_p are the rate constants of inhibition and propagation, respectively. The k_{inb}/k_p was calculated using Eqn. 5. MMA, 9.4 mol/L; compounds, 1mM; T=70°C; conversion: 90.3-95.3%; values, the mean ± SD for three different experiments.

The inhibition rate constant, k_{inh} plays a more important role for evaluating the activity, so we investigated this parameter. The relationships between propagation rate (Rp), the initial rate of polymerization and concentration of the ISO-S compounds are shown in Figure 4. Plots of the Rp_{inh}/Rp_{con} vs concentrations for both initiators decreased linearly as the concentration increased. In particular, **ISO-S-2** was strongly reduced in both initiators, followed by the great retardation of the growing MMA radicals. This was possibly due to the strong interaction between the oxidized products of **ISO-S-2** with a benzene ring and the growing MMA radicals. For AIBN and BPO, the k_{inh}/k_p was calculated using Equation 5.

Figure 4. A typical plot of the Rp_{inh}/Rp_{con} *vs* the concentration for **ISO-S-1**, **2**, **3**, and **4**.
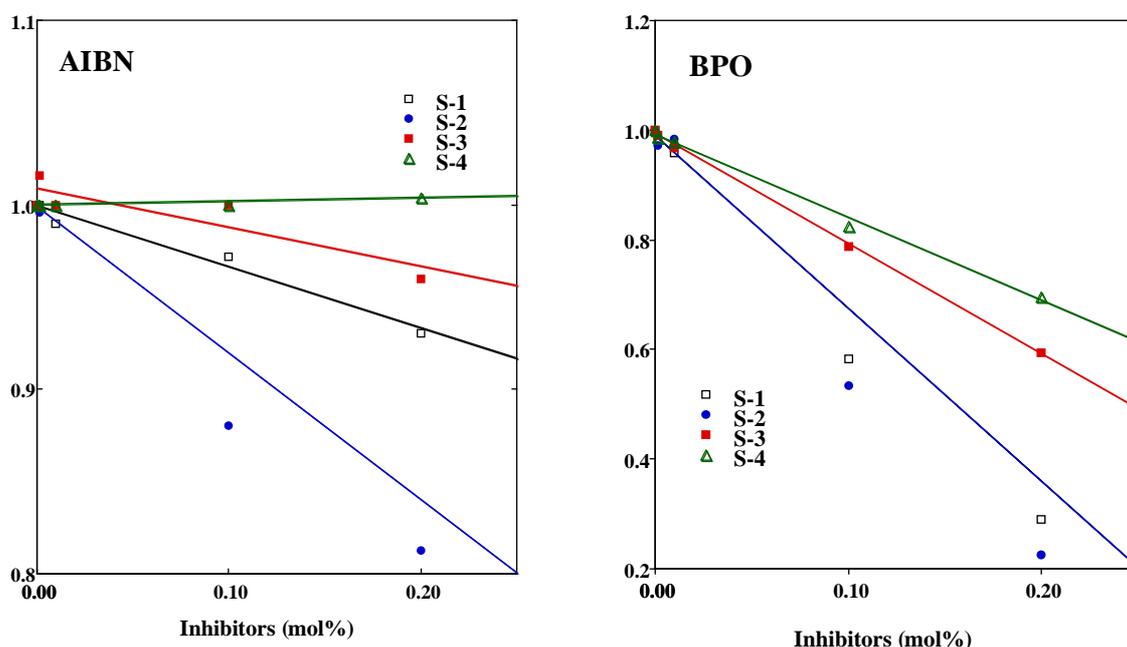
Results are shown also in Table 1. For AIBN, the k_{inh}/k_p value for isoeugenol (31) was the smallest. The k_{inh}/k_p value of **ISO-S-3** (57) was the greatest, but was about only twice as large as that of other reaction products. For BPO, the k_{inh}/k_p declined in the order **ISO-S-4** (56) > **ISO-S-3** (15) > **ISO-S-2** (12) > **ISO-S-1** (9) > isoeugenol (8). The k_{inh} of **ISO-S-4** was estimated as 4.4 x 10^4 $mol^{-1}s^{-1}$, assuming that the k_p value for MMA is 797 $mol^{-1}s^{-1}$ [5c]. Antioxidants having a *n* value of about 1 or less than 1 may undergo dimerization during the induction period. In contrast, the *n* of isoeugenol for BPO was about 2, suggesting the formation of vinyl quinone methide derived from a two-electron oxidation of this compound [9]. The radical-scavenging activity of **ISO**-**S-1**, **2**, **3**, and **4** against PhCOO·, an oxygen-centered radical derived from BPO, was clearly different from that against R·, a carbon-centered radical derived from AIBN. These compounds preferred an oxygen-centered radical rather than a carbon-centered radical.

Conclusions

ISO-**S-1**, **2**, **3**, and **4** were obtained by reaction of isoeugenol with compounds having a SH group Their radical-scavenging activity using two methods under nearly anaerobic conditions (*n* and k_{inh})

was determined using the induction period method. The reaction products showed the greater k_{inh}/k_p than that of isoeugenol.

Experimental

General

The chemicals were purchased from Tokyo Kasei Kogyo Ltd. (Tokyo Japan) . NMR spectra were measured in CDCl3 solutions at ambient temperature on a JEOL, JNM-A500 spectrometer (JEOL, Tokyo, Japan). The chemical shifts δ are given in ppm related to tetramethylsilane, TMS as internal standard. Mass spectra were measured on **aJMS/700 (JEOL).**

General procedure for the synthesis of **ISO-S-1, 2, 3**, *and* **4**: r*eaction of isoeugenol* (**1**) *with thiols in the presence of Lewis acid*

To a solution of **1** (0.5 g, 3 mmol) and ethanethiol (0.76 g, 12 mmol) in dry chloroform (20 mL) trimethylsilyl trifloromethanesulfonate (0.5 mL) was added and the reaction mixture was stirred at room temperature for 0.5 h. After washing with 5% sodium carbonate solution the mixture was evaporated and purified by silicagel chromatography to give an oily product (**ISO-S-1**) in 66.0% yield. MS; m/z 165 (100%), 226 (M$^+$, $C_{12}H_{18}O_2S$); ^{1}H-NMR (CDCl$_3$) δ (ppm): 6.86 (1H, d, *J*=1.8 Hz, C2'-H), 6.83 (1H, d, *J*=8.0 Hz, C5'-H), 6.75 (1H, dd, *J*=8.0, 1.8 Hz, C6'-H), 3.90 (3H, s, C3'-OC$\underline{H}$3), 3.65 (1H, dd, *J*=8.7, 6.1 Hz, C$_1$-H), 2.3-2.4 (2H, m, SC$\underline{H}_2$-CH$_3$), 1.78-1.92 (2H, m, C$_1$-C$\underline{H}_2$CH$_3$), 1.15 (3H, t, *J*=7.3 Hz, S-CH$_2$C$\underline{H}_3$), 0.90 (3H, t, *J*=7.3 Hz, C$_1$-CH$_2$C$\underline{H}_3$).

Reaction of **1** *with thiophenol*

According to the general procedure, the reaction of isoeugenol with thiophenol for 1.5 h gave an oily product (**ISO-S-2**) in 75.0% yield. MS; m/z 165 (100%), 274 (M$^+$, $C_{16}H_{18}O_2S$); ^{1}H-NMR (CDCl$_3$) δ: 7.18-7.26 (5H, m, S-$\underline{Ph}$), 6.80 (1H, d, *J*=8.0 Hz, C$_{5''}$-H), 6.74 (1H, d, *J*=2.1 Hz, C$_{2'}$-H), 6.72 (1H, dd, *J*=8.0, 2.1 Hz, C6'-H), 3.97 (1H, dd, *J*=8.9, 6.1 Hz, C$_1$-C$\underline{H}$-), 3.83 (3H, s, C3'-OC$\underline{H}_3$), 1.80-1.98 (2H, m, C$_1$-C$\underline{H}_2$CH$_3$), 0.90 (3H, t, J=7.3Hz, C$_1$-CH$_2$C$\underline{H}_3$).

Reaction of **1** *with 2-mercaptothiazoline*

According to the general procedure, the reaction of isoeugenol with 2-mercaptothiazoline for 24 h gave an oily product (**ISO-S-3**) in 36.5% yield. MS; m/z 165 (100%), 283 (M$^+$, $C_{13}H_{17}O_2NS_2$); ^{1}H-NMR (CDCl$_3$) δ: 6.96 (1H, d, *J*=1.7 Hz,C2'-H), 6.89 (1H, d, *J*=8.2 Hz, C$_5$'-H), 6.86 (1H, dd, *J*=8.2, 1.7 Hz, C$_6$'-H), 6.10 (1H, m, C$_1$-C$\underline{H}$-), 3.88 (3H, s, C$_3$'-OC$\underline{H}_3$), 3.6-3.9 (2H, m, -S-C$\underline{H}_2$CH$_2$-N), 3.05-3.24 (2H, m, -S-CH$_2$C$\underline{H}_2$-N=), 1.88-2.12 (2H, m, C$_1$-C$\underline{H}_2$CH$_3$), 1.01(3H, t, *J*=7.3Hz, C$_1$-CH$_2$C$\underline{H}_3$).

Reaction of **1** *with 2-mercapto-1-methylimidazole*

According to the general procedure, the reaction of eugenol with 2-mercapto-1-methylimidazole for 24 h gave a crystalline product (**ISO-S-4**); mp 137-138°C (hexane-AcOEt) in 48.2% yield. MS; m/z 165 (100%), 246 (M$^+$, $C_{14}H_{18}O_2N_2S$); ^{1}H-NMR (CDCl$_3$) δ: 6.96 (1H, d, J=1.7Hz, $C_{2'}$-H), 6.87-6.91 (2H, m, C_5', 6'-H), 6.64 (2H, s, imidazole-C$\underline{H}$=C$\underline{H}$-), 5.94 (1H, t, J=7.9Hz, C_1-C$\underline{H}$-), 3.87 (3H, s, $C_{3'}$-OC$\underline{H}$3), 3.62 (3H, s, N-$\underline{Me}$), 2.08-2.19 (2H, m, C_1-C$\underline{H}_2$CH$_3$), 0.95 (3H, t, J=7.9Hz, C_1-CH$_2$C$\underline{H}_3$).

DSC measurements

The induction period (IP) and initial rate of polymerization in the presence (Rp$_{inh}$) or absence (Rp$_{con}$) of **ISO-S-1, 2, 3, 4** and isoeugenol (**1**) (Figure 2) were determined by the previously reported method [6]. In brief, the experimental resin consisted of MMA and AIBN (or BPO) with or without additives. AIBN (or BPO) were added at 1.0 mol%, and the additives were used at 0, 0.001, 0.01, 0.02 and 0.1 mol%. Approximately 10 µL of the experimental resin (MMA: 9.12-9.96 mg) 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 the thermal changes induced by polymerization were recorded for the appropriate periods. The heat due to polymerization of MMA was 13.0 kcal/mole in this experiment. The conversion of all samples, as calculated from the DSC thermograms, was 91-96%. Polymerization curves were derived from DSC thermograms using the integrated heat evoked by the polymerization of MMA. Polymerization curves break when an inhibitor is consumed (Figure 2). These breaks are sharp and provide a reliable measure of the IP of the inhibitor. The presence of oxygen retards polymerization because oxygen reacts with MMA radicals activated by the initiator and then subsequently produces a non-radical product. Thus, polymerization of the control was slightly inhibited, even though the reaction was carried out in a sealed DSC pan, because the pan contained a small amount of oxygen; approximately 8.128 x 10^{-8} mole/L since it had been sealed in air. Tangents were drawn to polymerization curves at an early stage in the run. The IP of test compounds was determined from the length of time between the zero point on the abscissa and the point of intersection of tangents drawn to the early stage of polymerization. The IP was calculated from the difference between the induction period of specimens and that of controls. The initial rates of polymerization in the absence (Rp$_{con}$) and presence (Rp$_{inh}$) of natural and synthetic antioxidants were calculated from the slope of the plots of the first linear line of the conversion rate of MMA polymerization (tangent drawn at the early polymerization stage).

Rate of initiation

The induction period method was used to determine the rate of initiation (R$_i$) due to the thermal decomposition of AIBN or BPO according to Eqn. (1):

$$R_i = n \, [IH]_0/[IP] \tag{1}$$

where [IH]$_0$ is the concentration of the inhibitor at time zero and [IP] is the induction period. 2,6-di-*tert*-butyl-4-methoxyphenol (DTBM) was used to determine R$_i$, since its stoichiometric factor, *n*, is

known to be 2.00 [7]. In the case of [MMA] = 9.4 M and [AIBN or BPO] = 0.1 M at 70°C, the induction period method using DTBM gave the rate of initiation, R_i, at 70°C. The R_i values of AIBN and BPO were 5.66×10^{-6} Ms^{-1} and 2.28×10^{-6} Ms^{-1}, respectively.

Measurement of stoichiometric factor (n)

The relative *n* value in Eqn. (2) can be calculated from the induction period in the presence of inhibitors:

$$n = R_i[IP]/[IH] \tag{2}$$

where [IP] is the induction period in the presence of an inhibitor. The number of moles of peroxy radicals trapped by the antioxidant is calculated with respect to 1 mole of inhibitor moiety unit.

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 Eqn. (3) [6]:

$$Rp_{con} = \{k_p [MMA] R_i^{1/2}\}/(2k_t)^{1/2} \tag{3}$$

where MMA represents methyl methacrylate and k_p and k_t are the rate constants for chain propagation and termination, respectively.

The $k_p/(2k_t)^{1/2}$ rate of polymerization of MMA (9.4 M) by AIBN (1 mol%) and BPO (1 mol%) at 70°C was a constant value, 9.86×10^{-2} $M^{-1/2}$ $s^{-1/2}$. The Rp_{inh} rates are determined by Eqn. (4):

$$Rp_{inh} = \{k_p [MMA] R_i\} /\{n\, k_{inh} [IH]\} \tag{4}$$

in which Rp_{inh} is the initial rate of inhibited polymerization, [MMA], *n*, [IH] and k_p are defined above, and k_{inh} is the rate constant for scavenging (inhibiting) of MMA radicals by an antioxidant. From Eqn. (2) and Eqn. (4), k_{inh}/k_p can be calculated (Eqn. 5):

$$k_{inh}/k_p = [MMA]/\{[IP] \times [Rp_{inh}]\} \tag{5}$$

References

1. (a) Van Joost, T. H.; Siolz, E.; Van Der Hoek, J. C. S. Simultaneous allergy to perfume ingredients. *Contact Dermatitis* **1985**, *12*, 115-116; (b) White, I. R.; Johansen, J. D.; Gimenez Arnau, E.; Lepoittevin, J.-P.; Rastogi, S.; Bruze, M.; Andersen, K. E.; Frosch, P. J.; Goossens, A; Menne, T. Isoeugenol is an important contact allergen: can it be safely replaced with isoeugenol acetate? *Contact Dermatitis* **1999**, *41*, 272-275.
2. Murakami, Y.; Shoji, M.; Hirata, A.; Tanaka,S.; Yokoe, I.; Fujisawa, S. Dehydrodiisoeugenol, an isoeugenol dimer, inhibits lipopolysaccharide-stimulated nuclear factor kappa B activation and cyclooxygenase-2 expression in macrophages. *Arch. Biochem. Biophys.* **2005**, *434*, 326-332.

3. Atsumi, T.; Fujisawa, S.; Tonosaki, K. A comparative study of the antioxidant/prooxidant activities of eugenol and isoeugenol with various concentrations and oxidation conditions. *Toxicol. In Vitro* **2005**, *19*, 1025-1033.

4. Awashi, S.; Pandya, U.; Singhal, S .S.; Lin, J. T. Thiviyanathan, V.; Seifert, w. E.; Jr. Awashi, Y. C.; Ansari, G. A. Curcumin-glutathione interactions and the role of human glutathione S-transferase P1-1. *Chem. Boil. Interact*. **2000**, *128*, 19-38.

5. (a) Fujisawa, S.; Ishihara, M.; Kadoma, Y. Kinetic evaluation of the reactivity of flavonoids as radical scavengers. *SAR QSAR Environ. Res.* **2002**, *13,* 617-627; (b) Fujisawa, S.; Kadoma, Y.; Yokoe, I. Radical-scavenging activity of butylated hydroxytoluene (BHT) and its metabolites. *Chem. Phys. Lipid* **2004**, *130*, 189-195; (c) Fujisawa, S.; Kadoma, Y. Comparative study of the alkyl and peroxy radical scavenging activities of polyphenols. *Chemosphere* **2006**, *62*, 71-79.

6. (a) Kadoma, Y.; Atsumi, T.; Okada, N.; Ishihara, M.; Yokoe, I., Fujisawa, S. Radical-scavenging activity of natural methoxyphenols versus synthetic ones using the induction period method. *Molecules* **2007**, *12*, 130-138; (b) Kadoma, Y.; Fujisawa, S. Radical-scavenging activity of estrogen and estrogen-like compounds using the induction period method. *Int. J. Mol. Sci.* **2007**, *8*, 295-303.

7. Burton, G. W.; Ingold, K. U. Autooxidation of biological molecules.1. The Antioxidant activity of vitamin E and related chain-breaking phenolic antioxidant In Vivo. *J. Am. Chem. Soc.* **1981**, *103*, 6472-6477.

8. Taylor, W. L.; Battersby, A.R., Eds. *Oxidative coupling of phenols*; Marcel Dekker: New York, **1967**.

9. Zanarotti, A. Synthesis and reactivity of vinyl quinone methides. *J. Org. Chem.* **1985**, *50*, 941-945.

Sample Availability: Available from the authors.