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Radical-scavenging Activity of Natural Methoxyphenols *vs.* Synthetic Ones using the Induction Period Method

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Abstract: The radical-scavenging activities of the synthetic antioxidants 2-allyl-4-X-phenol (X=NO_2, Cl, Br, OCH_3, $COCH_3$, CH_3, *t*-$(CH_3)_3$, C_6H_5) and 2,4-dimethoxyphenol, and the natural antioxidants eugenol and isoeugenol, were investigated using differential scanning calorimetry (DSC) by measuring their anti-1,1-diphenyl-2-picrylhydrazyl (DPPH) radical activity and the induction period for polymerization of methyl methacrylate (MMA) initiated by thermal decomposition of 2,2'-azobisisobutyronitrile (AIBN) and benzoyl peroxide (BPO). 2-Allyl-4-methoxyphenol and 2,4-dimethoxy-phenol scavenged not only oxygen-centered radicals (PhCOO$^{\cdot}$) derived from BPO, but also carbon-centered radicals (R$^{\cdot}$) derived from the AIBN and DPPH radical much more efficiently, in comparison with eugenol and isoeugenol. 2-Allyl-4-methoxyphenol may be useful for its lower prooxidative activity

Keywords: Radical-scavenging activity; natural and synthetic methoxyphenols; DPPH; BPO, AIBN; induction method.

Introduction

Natural methoxyphenols such as eugenol (2-allyl-4-methoxyphenol) and isoeugenol (4-propenyl-2-methoxyphenol) are used in perfumes, soaps, detergents, air fresheners and cosmetics. Eugenol is also used as a component of zinc-oxide eugenol cement in dentistry, and has potent antioxidant and bactericidal activity [1]. It is known that exposure to light aggravates the inflammation reaction (hypersensitivity) triggered by eugenol [2]. With the aim of alleviating the side effects of eugenol and also to enhance its antioxidant activity, we previously synthesized various 2-methoxyphenol dimers derived from *ortho-ortho* coupling of the corresponding monomers and investigated the kinetics of their radical-scavenging activity [3]. The Claisen rearrangement of allyl aryl ethers provides a convenient route for obtaining allylphenols. Using this route we have synthesized allylphenols by selective conversion of allyl-*p*-X-phenylethers into 2-allyl-4-X-phenols and examined their ability for scavenging O_2^- (generated by the hypoxanthine–xanthine oxidase reaction) using ESR spectroscopy [4]. However, the kinetics of the radical scavenging activity remains unknown.

We have previously used DSC and induction methods to investigate the radical scavenging activity of phenolic antioxidants under nearly anaerobic conditions, and this method has proved to be reliable for evaluating the activity of these compounds [5]. In the present study, we investigated the radical-scavenging activity of synthetic antioxidants, 2-allyl-4-X-phenols (X=NO_2, Cl, Br, OCH_3, $COCH_3$, CH_3, *t*-$(CH_3)_3$, C_6H_5) and 2,4-dimethoxyphenol, and the natural methoxyphenols eugenol and isoeugenol, by determining the antiradical activity of these compounds against DPPH radicals and the induction period for polymerization of methyl methacrylate (MMA) initiated by thermal decomposition of 2,2'-azobisisobutyronitrile (AIBN) and benzoyl peroxide (BPO).

Results and Discussion

Anti-DPPH radical activity

The anti-DPPH radical activity is shown in Table 1. The activity assayed by optical methods increased in the order: 2-allyl-4-nitro- < 2-allyl-4-acetyl- < 2-allyl-4-chloro- < 2-allyl-4-bromo- < 2-allyl-4-*t*-butyl- < 2-allyl-4-phenyl- < 2-allyl-4-methylphenol < eugenol < isoeugenol < 2-allyl-4-methoxyphenol < 2,4-dimethoxyphenol. The synthetic compounds 2-allyl-4-methoxyphenol and 2,4-dimethoxyphenol showed greater activity than the natural compounds eugenol and isoeugenol. Correlations were found between the anti-DPPH radical activity and the bond dissociation enthalpy of the phenolic OH group (BDE)[4] or Hammett constants (σ_p) [6] in 2-allyl-4-X-phenols: -log EC_{20}=60.45-0.71BDE, n=7, R^2=0.74; -logEC_{20}=0.79-3.3 σ_p, n=7, R^2=0.82.

Radical scavenging activities determined by the induction period method

The time-exotherm and time-conversion curves for 2-allyl-4-methoxyphenol, 2,4-dimethoxyphenol, eugenol and isoeugenol, having potent DPPH radical-scavenging activity, are shown in Figures 1 and 2.

Table 1. Radical-scavenging activity (EC) of methoxyphenols and their descriptors (phenolic O-H bond dissociation enthalpy, BDE; Hammett-constants, σ.

No.	Compound	2-Allyl-4-X-phenol	EC_{20} μM	BDE kcal/mol[a]	σ_p^b
1	2-Allyl-4-chlorophenol	X=Cl	2,850	84.46	0.23
2	2-Allyl-4-phenylphenol	Ph	50	84.64	-0.01
3	2-Allyl-4-methoxyphenol	MeO	5	81.99	-0.27
4	2-Alyl-4-acetylphenol	COMe	10,000	86.52	0.66
5	2-Allyl-4-nitrophenol	NO_2	>10,000	89.32	0.78
6	2-Allyl-4-*t*-butylphenol	*t*-Bu	142	84.35	-0.20
7	2-Allyl-4-methylphenol	Me	40	84.05	-0.17
8	2-Allyl-4-bromophenol	Br	1,950	85.93	0.23
9	2,4-Dimethoxyphenol		4	80.28	
10	4-Allyl-2-methoxyphenol (Eugenol)		14	79.25	
11	4-Propenyl-2-methoxyphenol (Isoeugenol)		13		

EC_{20} is 20% inhibitive concentration of anti-DPPH-radical activity, respectively. [a]ref. [4]; [b]ref. [6], the hammett constant for 2-allylphenol (X=H) as 0.00.

Figure 1. Exotherm curves for the polymerization of MMA with AIBN in the presence of 0.1 mol% of additives. C: control, E: eugenol, I: isoeugenol, D: 2,4-dimethoxyphenol, A: 2-allyl-4-methoxyphenol.
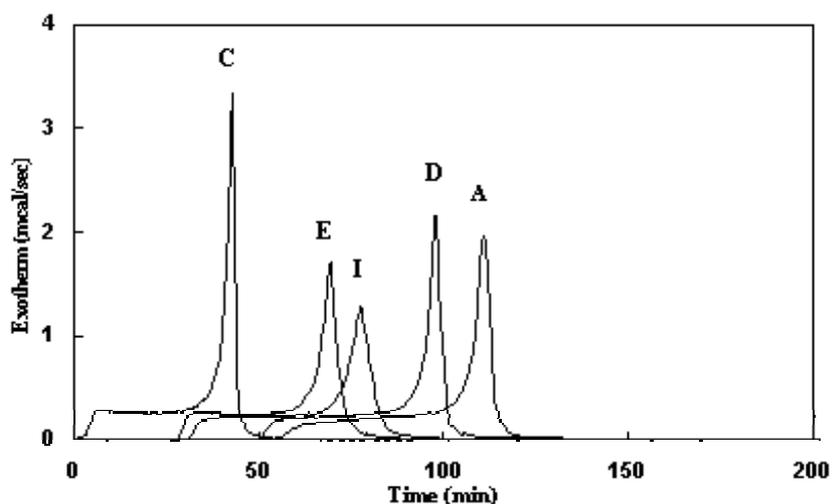
Figure 2. Time-conversion curves for the polymerization of MMA with AIBN in the presence of 0.1 mol% of additives. Curves were obtained from findings shown in Figure 1. For the compound name abbreviations see Figure 1.
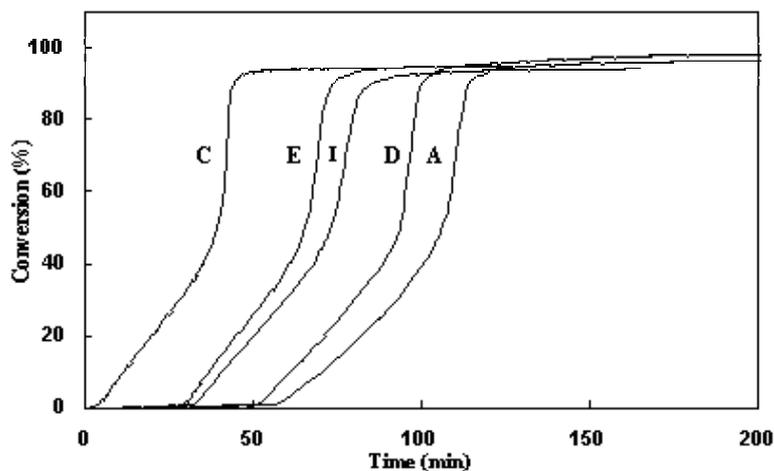
The *n* value (number of moles of radicals trapped by methoxyphenol calculated with respect to one mole of inhibitor moiety for methoxyphenol) was determined from each slope (Figure 3; see Equation 2).

Figure 3. Plot of the induction period *vs* the concentration for methoxyphenols. I: isoeugenol, E: eugenol, A: 2-allyl-4-methoxyphenol, D: 2,4-dimethoxyphenol.
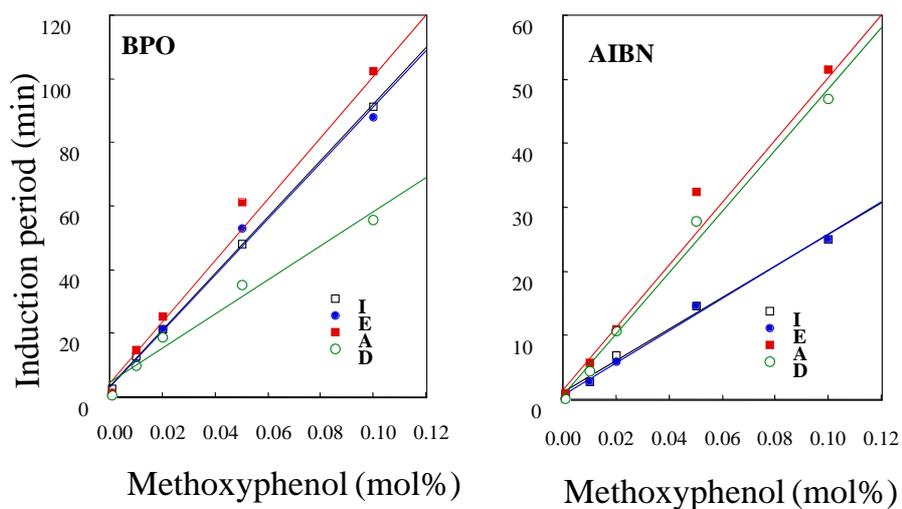

For BPO, the *n* value for 2-allyl-4-methoxyphenol was approximately 2, whereas that of 2,4-dimethoxyphenol was approximately 1. The *n* values for eugenol and isoeugenol were less than 2. In

contrast, for AIBN, the *n* values for 2-allyl-4-methoxyphenol and 2,4-dimethoxyphenol were approximately 2, whereas that for eugenol and isoeugenol was approximately 1.

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 between the OH and the methoxy group [3b]. In the present study, the *n* values for eugenol, isoeugenol and 2,4-dimethoxyphenol were less than 2, whereas that for 2-allyl-4-methoxyphenol was 2 for both inhibitors. On the basis of the *n* value, the intermolecular OH---π-hydrogen bridge at the allyl C=C double bond for 2-allyl-4-methoxyphenol appeared to be considerably weaker than the OCH_3---OH bond for 2-methoxyphenol. In allyl-4-X-phenols, the phenolic hydrogen is more easily abstracted than the hydrogen of the allyl group at room temperature, as judged using the PM 3 semiempirical method [4]. 2-Allyl-4-methoxyphenol produces a phenoxyl radical after oxidation [4]. The less hindered phenols eugenol and isoeugenol underwent dimerization when the *n* value was less than 2. We have previously reported the mechanism of dimerization for eugenol and isoeugenol [3a]. In contrast, since the fully oxidized 2-allyl-4-methoxyphenol had an *n* value of 2, it could produce a quinonemethide, but not a dimer, during the induction period.

The relationships between propagation rate (Rp), the initial rate of polymerization, and concentration for the methoxyphenols are shown in Figure 4. Except for eugenol in BPO, plots of the Rp_{inh}/Rp_{con} vs concentrations for both initiators decreased linearly as the concentration increased. In particular, 2-allyl-4-methoxyphenols were strongly reduced in both initiators, suggesting retardation of the growing MMA radicals. This was possibly due to the strong interaction between the oxidized products of 2-allyl-4-methoxyphenol and the growing MMA radicals. However, despite the alteration of the induction period and the Rp for BPO and AIBN, the ratio of the rate constant for inhibition and that for propagation, k_{inh}/k_p, calculated using Equation 5, showed similar values of 2.7-3.3.

Figure 4. Plot of the Rp_{inh}/Rp_{con} *vs* the concentration for methoxyphenols. For the compound name abbreviations see Figure 3.
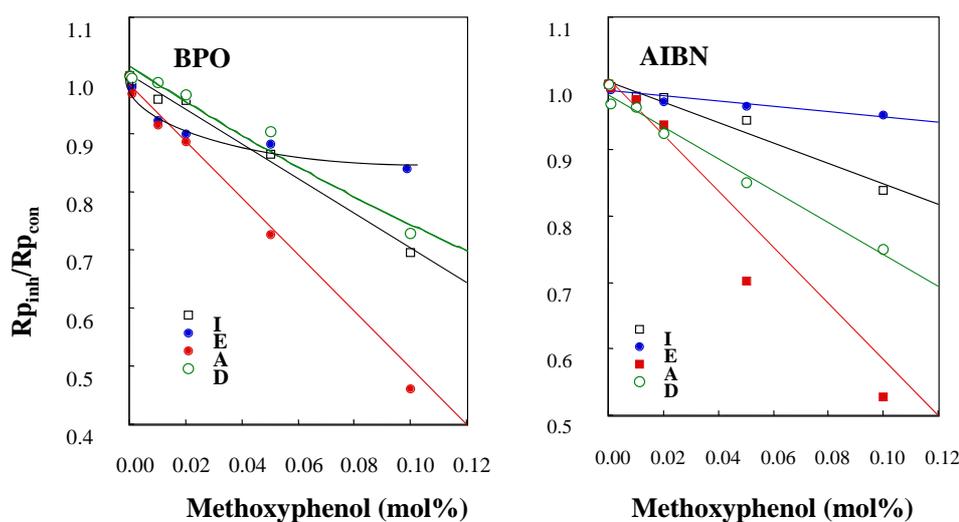
BPO is widely used in dentistry as an initiator for polymerization of MMA. Prosthetic devices such as dentures are made of resins cured by the BPO-MMA system. BPO is also used as an additive in cosmetics and pharmaceuticals, especially those related to the treatment of acne. However, the

hyperplastic and skin-irritant effects of BPO are well known [8]. In general, the kinetics of polymerization of styrene and methacrylates have been investigated in AIBN, but not in BPO, because use of BPO results in inductive decomposition. However, the k_{inh}/k_p obtained experimentally for methoxyphenols did not differ significantly between the initiator systems used in the present study and consequently BPO was selected as an initiator, because it is already used widely as an additive in cosmetics and also dentistry. 2-Allyl-4-methoxyphenol and 2,4-dimethoxyphenol efficiently scavenged PhCOO⁻ radicals derived from decomposition of BPO. In addition, these compounds efficiently scavenge O_2^- as well as eugenol [4]. Naturally occurring eugenol-related compounds such as ferulic acid inhibit superoxide anion radicals produced by interaction of the tumor promotor BPO with murine peritoneal macrophages *in vitro* [8]. In addition to scavenging oxygen-centered radicals, 2-allyl-4-methoxyphenol and 2,4-dimethoxyphenol efficiently scavenge much more R⁻ radicals derived from AIBN, and also DPPH radicals, in comparison with eugenol and isoeugenol.

Conclusions

2-Allyl-4-methoxyphenol and 2,4-dimethoxyphenol have much more potent radical-scavenging activity against DPPH, oxygen-centered and carbon-centered radicals than eugenol and isoeugenol. Among the eugenol isomers, 2-allyl-4-methoxyphenol may be useful for its lower prooxidative activity in the feeding and health aspects.

Experimental procedures

Anti-DPPH radical activity

Radical-scavenging activities were determined with DPPH as a free radical [9]. For each compound, various concentrations were tested in ethanol. The decrease in absorbance was determined at 517 nm for 10 min at room temperature. Antiradical activity was defined as the amount of inhibitor (phenolic compound) necessary to decrease the initial DPPH radical concentration by 20% (EC_{20}).

DSC measurements

The induction period (IP) and initial rate of polymerization in the presence (Rp_{inh}) or absence (Rp_{con}) of a methoxyphenol antioxidant were determined by the previously reported method [5]. 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, 0.05 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 in this experiment was 13.0 kcal/mole. The conversion of all samples, as calculated from 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 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 Equation 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-*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 Equation 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 Equation 3 [5]:

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

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} $M^{-1/2}$ $s^{-1/2}$ (13). The Rp_{inh} rates are determined by Equation 4:

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

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

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

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Sample Availability: Available from the authors.