Synthesis and Anti Lipid-Peroxidation Activity of Hydroquinone Monoalkyl Ethers

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A series of hydroquinone monoalkyl ethers was synthesized and evaluated for anti lipid-peroxidation activity in rat liver microsomes. 4-Hexyloxy-2,3,6-trimethylphenol (9), having a low redox potential, as well as ascorbic acid exhibited the strongest anti lipid-peroxidation activity ($IC_{50}=4.2\times10^{-7}$ M). Structure–activity relationship studies demonstrated that the inhibitory effect of hydroquinone monoalkyl ethers on lipid peroxidation was increased by the acquisition of an optimum hydrophobicity and decreased by an insufficient or excessive hydrophobicity.

Keywords hydroquinone monoalkyl ether; 4-hexyloxy-2,3,6-trimethylphenol; antioxidant; lipid peroxidation

Active oxygen species and the free radicals derived from the biochemical utilization of O_2 or the prooxidant stimulation of O_2 metabolism participate in the pathogenesis of various kinds of animal injury models and human diseases.[1)] The pathogenesis is generally accepted to involve the chemical instability of polyunsaturated fatty acids, polynucleotides and some proteins under oxidative conditions.

Most polyunsaturated fatty acids, constituents of the cellular membrane, are susceptible to oxidation, and therefore protective agents against oxidative stimuli are expected to be beneficial in the clinical situation. Many candidate drugs with anti lipid-peroxidation activity have been proposed in the field of medicinal chemistry.[2–9)]

Our design concept is based on selection of an antioxidative moiety in a precursor form for targeting disease or injury. We reported the derivation of ascorbic acid to a form possessing a potent anti lipid-peroxidation activity.[3a)] The optimization of hydrophobicity was the most important feature to emerge from the structure–activity relationship study of 3-*O*-alkylascorbic acids.

In the present report, we describe the anti lipid-peroxidation activity of a series of hydroquinone monoalkyl ethers and discuss the structure–activity relationship, focusing on the hydrophobicity.

Results and Discussion

Chemical structures and anti lipid-peroxidative activity of the title hydroquinone monoalkyl ethers (1—13, types I—III) are listed in Table I. Compounds **1**—**12** (types I and II) were prepared by Taniguchi's method.[10)] Each hydroquinone and the corresponding alcohol were refluxed in the presence of phosphomolybdic acid to yield **1**—**12**. The regioisomer **13** (type III) of **9** was prepared *via* 2,3,6-trimethyl-4-pivaloyloxyphenol (**14**).[9)] The protected phenol was alkylated with 1-iodohexane, yielding 4-hexyloxy-2,3,5-trimethyl-1-pivaloyloxybenzene (**15**), which afforded **13** after hydrolysis (Chart 1).

We chemically evaluated the antioxidative profiles of the title compounds, as follows. The redox potentials of typical hydroquinones and ascorbic acid were measured by differential pulse voltammetry (Table II). Compound **9**, bearing a *bi*hindered phenol moiety (type II), had low potential, being equivalent to ascorbic acid. When the radical-scavenging activity was evaluated using the stable free radical, α,α-diphenyl-β-picrylhydrazyl (DPPH),[11)] compound **9** reduced the DPPH radical more quickly that did 2,6-di-*tert*-butyl-4-methylphenol (BHT) (Table II). The scavenging activity was almost equal to that of α-tocopherol (13.8 μM/min[11b)]).

Radical-scavenging activity was further evaluated using a radical chain lipid peroxidation model.[12)] The title

$$H_3C,\ CH_3,\ OH,\ HO,\ CH_3 \quad + COCl \longrightarrow \quad \mathbf{14}^{9)} \quad CH_3(CH_2)_5I \longrightarrow \quad \mathbf{15} \quad KOH \longrightarrow \quad \mathbf{13}$$

Chart 1

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Table I. Chemical and Physical Data and Inhibitory Effect on Lipid Peroxidation of Hydroquinone Monoalkyl Ethers

HO–C₆H₄–OR (type I, **1**—**6**); HO–(bihindered)–OR (type II, **7**—**12**); RO–(hindered)–OH (type III, **13**)

Compound	R	Yield (%)	mp (°C)	LO inhibition (%)[a]	
				10^{-5} M	10^{-6} M
Type I					
1	C_2H_5[10]	78	63—65	38	5
2	n-C_4H_9[10]	73	63—64	86	10
3	n-C_6H_{13}[10]	70	42.5—43	>99	9
4	n-C_8H_{17}	60	58.5—59	>99	10
5	n-$C_{10}H_{21}$	49	68.5—69	79	3
6	n-$C_{12}H_{25}$	55	77—78	73	7
Type II					
7	C_2H_5	70	87—88	—[b]	45
8	n-C_4H_9	73	65.5—66.5	—[b]	76
9	n-C_6H_{13}[15]	64	72.5—73	>99	>99
10	n-C_8H_{17}	54	70—71	>99	75
11	n-$C_{10}H_{21}$	42	76—77	>99	38
12	n-$C_{12}H_{25}$	55	81—83	64	31
Type III					
13	n-C_6H_{13}	—[c]	32—34	98	40

a) LO (lipid peroxidation) in rat liver microsomes. Malondialdehyde production of control, 19.3 ± 1.1 nM/mg of protein (mean ± S.D., $n=4$). *b*) Not tested. *c*) See Experimental.

Table II. Redox Potential and Reducing Activity towards DPPH of Compound **9**

Compound	Peak potential (mV)[a]	Reducing rate (μM/min)[b]
3	324	—[c]
9	164	15.8
BHT	—[c]	2.8
Ascorbic acid	137	(43.4)[11b]

a) The redox potentials were determined by differential pulse voltammetry. The reference electrode was Ag/AgCl. *b*) Reducing activity towards α,α-diphenyl-β-picrylhydrazyl (DPPH) of equimolar test compound. *c*) Not tested.

compounds inhibited the site-specific induction of linoleic acid peroxidation by Fe^{2+} (Table III). The lipid peroxidation was completely inhibited by triphenylphosphine pretreatment to reduce linoleic acid hydroperoxide (LOOH), and therefore it was concluded to have been induced by Fe^{2+}–LOOH reaction; the lipid hydroperoxide was present in linoleic acid. Lipid radicals derived from the Fenton-like reaction are promoters of radical chain reactions.[12] The anti lipid-peroxidative activity of **3**, **9**, **13**, and α-tocopherol was characteristic of chain-breaking antioxidants. These strong antioxidative potencies of **9** were chemically reflected by the low redox potential of the *bi*hindered phenol moiety.

The anti lipid-peroxidative activity of **1**—**13** and some known antioxidants was further evaluated in rat liver microsomes. The title compounds inhibited enzymatic lipid peroxidation in rat liver microsomes (Table I). The monohexyl ether of *bi*hindered phenol (**9**) was the most potent inhibitor in the series. The other hexyl ethers **3**

Table III. Inhibitory Effect of Hydroquinone Monoalkyl Ethers on Lipid Peroxidation by Fe^{2+} in TTAB Micelles

Compound	Concentration (μM)	Rate ($\Delta A_{234\,nm/min}$)[a]	Inhibition (%)
Control	—	1.053 ± 0.011	—
3	10	0.711 ± 0.058	33
	50	0.110 ± 0.020	90
9	10	0.033 ± 0.007	>97
13	10	0.262 ± 0.045	75
α-Tocopherol	10	0.025 ± 0.005	>97
Triphenylphosphine	80	>0.002	>99

a) The reaction mixture contained linoleic acid (5 mM) with $FeSO_4$ (20 μM) in tetradecyltrimethylammonium bromide (TTAB). The rate of diene conjugation was linear for approximately 3 min. The rate was determined from the initial velocity of the reaction (mean ± S.D., $n=5$).

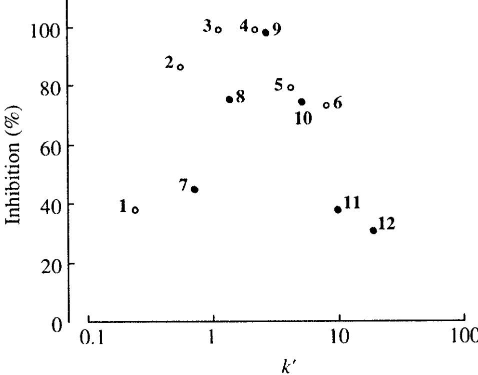

Fig. 1. Relationship between the Hydrophobicity (k') of Hydroquinone Monoalkyl Ethers and Their Antioxidant Activity against Lipid Peroxidation in Rat Liver Microsomes

Activity of compounds **1**—**6** at 10^{-5} M (○), compounds **7**—**12** at 10^{-6} M (●). Hydrophobicity (k') was determined by HPLC analysis. HPLC analysis was performed with a Cosmosil C8 column; the mobile phase consisted of H_2O/MeOH (20/80).

and **13** were less effective than **9**, in accordance with the strength of their antioxidative activities against Fe^{2+}-induced LOOH-dependent lipid peroxidation.

The relationship between the hydrophobicity (k') of **1**—**12** and the anti lipid-peroxidative activity is presented in Fig. 1. Stronger inhibitors (**3**, **4**, **9**), which were selected among each series of phenolic compounds (**1**—**6**, type I) and *bi*hidered phenolic compounds (**7**—**12**), type II), were located in the same hydrophobicity range. We reported a similar parabolic relationship of antioxidative activity and hydrophobicity of 3-*O*-alkylascorbic acids elsewhere.[3a] Interestingly, the most potent inhibitors among the series of 3-*O*-alkylascorbic acids bearing a long alkyl group exhibited almost the same hydrophobicity in the present study. In both cases, the antioxidative activity was more effectively elicited by optimizing hydrophobicity.

We propose that the anti lipid-peroxidative activity depends on the hydrophobicity of compounds bearing an antioxidative moiety. Insufficient or excessive hydrophobicity is unacceptable, resulting in loss of the activity. This our hypothesis is supported by other studies.[13] The phytyl group of tocopherol is believed to be an important moiety for its biological activity.[14] However, the anti lipid-peroxidation activity of tocopherol homologs differing in the lengths of their alkyl side chains decreased with

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TABLE IV. Inhibitory Effect of Compound **9** and Known Antioxidants on Lipid Peroxidation in Rat Microsomes

Compound	Concentration (μM)	Inhibition (%)
9	10	>99
	1.0	>99
	0.3	24
Hydroquinone	10	38
BHT	10	>99
	1.0	14
BHA	1.0	35
α-Tocopherol	100	11
	10	<1

Malondialdehyde production of control, 20.5 ± 0.5 nM/mg of proteins (mean ± S.D., $n=5$).

increasing chain length.[13] Biological antioxidative activity may be lost when the solubility of the antioxidants in the lipid membrane becomes insufficient.

In a comparative study, **9** inhibited lipid peroxidation more strongly than did known antioxidants (Table IV). We are planning it for further pharmacological evaluations. Recently, anti-mutagenesis and anti-carcinogenic activity of **9** were reported.[15] The results of further studies on **9** (HX-1171) as an inhibitor of lipid peroxidation will be reported in due course.

Experimental

^{1}H-NMR spectra were recorded on a JEOL FX-90Q spectrometer (90 MHz), using Me_4Si as an internal standard. All elemental analyses were found to be within ±0.4% of the calculated values. Melting points were obtained on a micro melting point apparatus (Yanagimoto) without correction. Column chromatography was carried out on a Kieselgel 60 column (70—230 mesh, Merck).

General Procedure for the Alkylation of the Hydroquinones[10] (Hydroquinone Monoalkyl Ethers; 1—12 except 13) 4-Hexyloxy-2,3,6-trimethylphenol (9) Trimethylhydroquinone (3.0 g, 19.7 mmol), phosphomolybdic acid (0.7 g) and 1-hexanol (30 ml, 238 mmol) were stirred under reflux for 6 h. After cooling of the mixture, EtOAc (100 ml) and H_2O (100 ml) were added and the mixture was vigorously shaken. The organic layer was separated, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was subjected to column chromatography on SiO_2 and eluted with EtOAc–*n*-hexane (1 : 10) to give 2.6 g of **9** (54%), mp 72.5—73 °C (recrystallized from *n*-hexane). NMR ($CDCl_3$) δ: 0.97 (3H, t), 1.36—1.97 (8H, m), 2.14 (3H, s), 2.17 (3H, s), 2.21 (3H, s), 3.87 (2H, t), 4.24 (1H, s), 6.51 (1H, s). Elemental analyses of compounds **4**—**12** (except **9**) were as follows.

Compound **4**: *Anal.* Calcd for $C_{14}H_{22}O_2$: C, 75.63; H, 9.97. Found: C, 75.88, H, 9.78. **5**: *Anal.* Calcd for $C_{16}H_{26}O_2$: C, 76.75, H, 10.47. Found: C, 76.76, H, 10.44. **6**: *Anal.* Calcd for $C_{18}H_{30}O_2$: C, 77.65, H, 10.86. Found: C, 77.86, H, 10.92. **7**: *Anal* Calcd for $C_{11}H_{16}O_2$: C, 73.3, H, 8.95. Found: C, 73.43, H, 8.74. **8**: *Anal.* Calcd for $C_{13}H_{20}O_2$: C, 74.96, H, 9.68. Found: C, 74.88, H, 9.74. **10**: *Anal.* Calcd for $C_{17}H_{28}O_2$: C, 77.22, H, 10.67. Found: C, 77.33, H, 10.56. **11**: *Anal.* Calcd for $C_{19}H_{32}O_2$: C, 78.03, H, 10.94. Found: C, 78.1, H, 10.89. **12**: *Anal.* Calcd for $C_{21}H_{36}O_2$: C, 78.7, H, 11.32. Found: C, 78.65, H, 11.43.

4-Hexyloxy-2,3,5-trimethylphenol (13) Pivaloyl chloride (2.8 g, 23.2 mmol) in CH_2Cl_2 (20 ml) was added dropwise to a mixture of trimethylhydroquinone (3.5 g, 23.0 mmol), CH_2Cl_2 (25 ml), and pyridine (6 ml). The mixture was left to stand at room temperature overnight, then AcOH (4.25 ml) and H_2O (20 ml) were added. The organic layer was separated, washed with H_2O, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was subjected to column chromatography on SiO_2 with EtOAc–benzene (1 : 8) to give 2.4 g of 2,3,6-trimethyl-4-pivaloyloxyphenol (**14**).[9] A solution of **14** (2.4 g, 10.0 mmol) in methylethylketone (70 ml) was treated with 1-iodohexane (21.2 g, 100.0 mmol) and K_2CO_3 (6.9 g, 20.0 mmol), and the mixture was stirred for 8 h under reflux. After cooling, the mixture was evaporated *in vacuo*. To the residue, EtOAc (150 ml) and H_2O (100 ml) were added, and the mixture was vigorously shaken. The organic layer was separated, washed with H_2O, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was subjected to column chromatography on SiO_2 with EtOAc–*n*-hexane (1 : 15) to give 1.5 g of 4-hexyloxy-2,3,5-trimethyl-1-pivaloyloxybenzene (**15**). A mixture of **15** (1.5 g, 4.0 mmol), KOH (0.45 g), and MeOH (5 ml) was stirred for 6 h at room temperature, and then EtOAc (150 ml) and H_2O (100 ml) were added, and the mixture was shaken. The organic layer was separated, washed with H_2O, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was subjected to column chromatography on SiO_2 with EtOAc–*n*-hexane (1 : 10) to give 0.7 g (13% from the starting material) of **13**, mp 32—34 °C (recrystallized from hexane): *Anal.* Calcd for $C_{15}H_{24}O_2$: C, 76.23, H, 10.23. Found: C, 76.26, H, 10.46. NMR ($CDCl_3$) δ: 0.91 (3H, t), 1.23—1.79 (8H, m), 2.05 (3H, s), 2.12 (3H, s), 2.18 (3H, s), 3.66 (2H, t), 4.53 (1H, s), 6.45 (1H, s).

Determination of the Reducing Activity towards a Stable Radical, DPPH[11] The test compound, in *N*,*N*-dimethylformamide (DMF) (40 μl), was added to a solution of a stable radical, DPPH, in EtOH (4 ml, 1×10^{-4} M), and the decrease of absorption of DPPH was continuously monitored at 517 nm.

Determination on Redox Potentials of the Hydroquinone Derivatives Voltammetric analysis was conducted in 0.01 M phosphate buffer (pH 7.4), which was degassed with argon before use. An Ag/AgCl reference electrode, a glassy carbon working electrode (3 mm i.d.), and a Pt counter electrode (0.05 mm i.d. × 2 cm) were used (Yanagimoto). The redox potentials were determined by using a Yanagimoto electrochemical analyzer to obtain the peak potential in the voltammograms by using the differential pulse voltametry mode. The experimental conditions were: initial E (mV) = 100, final E (mV) = 500, V (mV/s) = 4, pulse amplitude (mV) = 50, pulse width (ms) = 60, pulse ratio (ms) = 1000. Test compound solutions in MeOH were added to the buffer (final concentration 0.05 mM, final concentration of MeOH 5%). Electrochemical reversibility was assessed by analyzing the reversed differential pulse voltammograms (initial E (mV) = 500, final E (mV) = −100).

Fe^{2+}-Induced LOOH-Dependent Lipid Peroxidation in TTAB Micelles[12] Linoleic acid micellar solutions were prepared as follows. The interior base of the apparatus was filmed with 25 μmol of linoleic acid (and test compound) by evaporation of the solvent. Five ml of TTAB (50 mM) solution was added to the film, followed by vortexing and sonication. The pH was adjusted to 7.0 by adding 5% acetic acid and 5% $NaHCO_3$. Reactions were initiated by the addition of $FeSO_4$. Diene conjugation during lipid peroxidation was continuously monitored at 234 nm. The rate of diene conjugation was linear for approximately 3 min, after which a decrease in rate was observed. All rates were determined from the initial velocity of the reaction. The lipid hydroperoxide content of linoleic acid, determined iodometrically, was 70 μM in the reaction mixture.

Fe^{3+}-ADP-Induced NADPH-Dependent Lipid Peroxidation in Rat Liver Microsomes Rat liveer microsomal fraction was prepared in the usual way, and stocked at −25 °C until use. The assay system (1 ml) consisted of 83.5 mM KCl and 37.2 mM Tris–HCl buffer (pH 7.4), the test compound in DMF (10 μl), 1 mM ADP, 10 μM $FeCl_3$, the microsomal fraction (2.0 mg of protein), and 20 μM NADPH. The reaction mixtures were incubated at 37 °C for 20 min and then cooled on ice to terminate the reaction. The lipid peroxidates generated were measured by the method of Ohkawa *et al.*[16] In brief, 8.1% sodium dodecyl sulfate (0.2 ml) and 20% AcOH containing 0.27 M HCl adjusted with NaOH to pH 3.5 (1.5 ml) were added to the reaction mixture. The mixture was then heated at 95 °C for 20 min and the reaction was stopped by cooling on ice. The *n*-BuOH–pyridine (15 : 1, 5.0 ml) was added and vigorous mixing was performed. After centrifugation at 800 × ***g*** for 10 min, the organic layer was separated and the absorbance was measured at 532 nm.

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