Antioxidative and 5-Lipoxygenase Inhibiting Activities of Novel Bis(4-hydroxy-2,3,5-trimethylphenoxy)alkyl Derivatives

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Novel bis(4-hydroxy-2,3,5-trimethylphenoxy)alkyl derivatives were synthesized and evaluations were made of their inhibiting action on Fe^{3+}–ADP induced lipid peroxidation in rat liver microsome and reducing action on α,α-diphenyl-β-picrilhydrazyl (DPPH), a stable radical, in addition to their inhibiting action on 5-lipoxygenase (5-LO), an enzyme that synthesizes leukotrienes. We performed a structure–activity correlation study on these derivatives. A strong Fe^{3+}–ADP induced lipid peroxidation preventing activity was observed for the derivatives with an odd number of methylene groups including 1,3-bis(4-hydroxy-2,3,5-trimethylphenoxy)propane (3b) and 3a. No change in the DPPH reducing activity was found with change in the number of methylene groups. 5-LO inhibiting activity among the derivatives was the highest for 1,6-bis(4-hydroxy-2,3,5-trimethylphenoxy)hexane (3e). MM2 calculations were performed to find a stable steric structure for the derivatives, and 1,5-bis(4-hydroxy-2,3,5-trimethylphenoxy)pentane (3d) showed a strong activity in both antioxidative action and 5-LO inhibiting action.

Key words antioxidative action; 5-lipoxygenase; α,α-diphenyl-β-picrilhydrazyl; lipid peroxidation; trimethylhydroquinone; anti-allergic agent

When oxygen taken into the living body is utilized in the cells, active oxygen species are formed as by-products including superoxide anion radical ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), hydroxy radical ($\cdot OH$), and singlet oxygen ($^1O_2$) and free radicals of lipids such as alkoxy radical ($RO\cdot$) and peroxide radical ($ROO\cdot$). These active oxygen species and free radicals raise oxygen stress to produce lipid peroxide in the body, thereby causing inflammation or lesion on various organs. This triggers life-style related diseases as represented by cancer, ageing, arteriosclerosis, and allergy.[1)]

The involvement of leukotrienes (LTs) as a cause is indicated in the development of allergic diseases such as pollenosis and atopic dermatitis, the case numbers of which are now tending to increase now. LT is a chemical mediator that is produced by mast cells and eosinophils and is a 5-lipoxygenase (5-LO) metabolite of arachidonic acid.[2)] LTC_4, LTD_4, and LTE_4 are deeply involved in the aggravation of allergic symptoms with their activities like bronchial contraction.[3)] A number of effective 5-LO inhibiting drugs have so far been reported.[4—7)] There have also been reports that suggest an important role for the arachidonic acid cascade in the initiation and promotion processes of carcinogenesis[8)] and the suppression of prostate cancer by chemicals with 5-LO inhibiting activity.[9—11)] Since the arachidonic acid cascade is known to generate active oxygen through a free radical reaction, compounds with an inhibitory action on both 5-LO and the cascade are expected to be effective in treating allergic diseases and cancers.

Chemistry

We have synthesized various trimethylhydroquinone derivatives and reported their pharmacological activities. Among the derivatives synthesized, the compound shown in Fig. 1 was found to have a strong antioxidative activity.[12—14)] In this work, we synthesize compounds that have two trimethylhydroquinone moieties in their molecules by the method shown in Chart 1 in order to raise the antioxidative activity. Compounds **3a**—**i** were obtained by refluxing trimethylhydroquinone and a straight chain alkyldiol in toluene for 3—20 h at 130 °C in the presence of phosphomolybdic acid as the catalyst according to the method of Taniguchi.[15)] The reaction also gave compounds **4a**—**i**.

Results and Discussion

Symmetrical 4-hydroxy-2,3,5-trimethylphenoxyalkanes exhibited an interesting pharmacological activity. These compounds were suggested to have an antioxidative activity because of the presence of phenolic hydroxyl groups and side

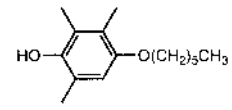
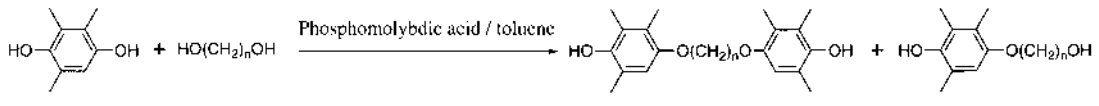


Fig. 1. Structure of 4-*O*-Hexyloxy-2,3,6-trimethylphenol



a ; n=2 **d** ; n=5 **g** ; n=8
b ; n=3 **e** ; n=6 **h** ; n=9
c ; n=4 **f** ; n=7 **i** ; n=10

Chart 1

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chains in their molecules. The side chain is important because it acts mutually with a lipid and appropriate hydrophobic is necessary. Action of these compounds on Fe^{3+}–ADP induced lipid peroxidation reaction was then examined using rat liver microsome.[16] Thus, the amount of lipid peroxide formed through induction with ADP/Fe^{3+} in rat liver microsome was determined as the amount of malondialdehyde using the reaction with 2-thiobarbituric acid (TBA), and the inhibition percentage was calculated against the control.[17] Compounds **3a**—**i** were examined using 2,6-di-*tert*-butyl-*p*-cresol (BHT), a widely used antioxidant, as the positive control. As shown in Fig. 2, the compounds with an odd number of methylene groups and **3a** exhibited a strong activity and the value of IC_{50} for **3b** that had the highest activity was 3.8×10^{-7} M. In contrast, **3c**, **3e**, **3g**, and **3i** were found to be less active than the positive control.

Raison and Standen reported that symmetrical diaminophenylalkanes have a schistosomicidal activity, and that there is a difference in the activity between the compounds with an odd number of methylene groups and those with an even number, as in our case.[18]

Molecular simulations (MM2 calculations) were then performed on **3a**—**i** to determine their steric structures. The results obtained for **3b**—**e** are shown in Fig. 3. Two benzene rings face each other in **3b** and **3d** which have an odd number of methylene groups, while they are located apart from each other and have the most stable state in **3c** and **3e** that have an even number of methylene groups.

The following mechanisms are generally possible for the action of antioxidant: 1) free radical capturing action, 2) singlet oxygen depleting action, and 3) metal chelating action. Vitamins C and E develop their antioxidant activity through the first mechanism while the second and third mechanisms work for carotenoids and flavonoids, respectively, when they develop the activity.

To check the above inference, evaluations were made of the antioxidative activity of the compounds by another method using α,α-diphenyl-β-picrilhydrazyl (DPPH), a stable radical, proposed by Blois.[19] This agent is often used in ESR measurements and is a stable crystalline radical. The color of ethanolic solution of DPPH is purple and it fades away when the radical is reduced. Figure 4 shows the reduction percentage 3 min after addition of inhibitor to the ethanolic solution for the compounds. No appreciable change in the activity with the length of side chain was observed for compounds **3a**—**i** which have two phenolic hydroxyl groups, in contrast to the change shown in Fig. 2, since the reducing activity against DPPH develops mainly through radical capturing action of the phenolic hydroxyl group.

This suggests that compounds **3a**—**i** develop their antioxidative activity by mechanisms 1) and 3). Compounds, **3b** and **3d** in which two benzene rings face each other would be highly active because of their strong chelation with Fe.

Separately, Fe^{3+}–ADP induced lipid peroxidation inhibiting activity was examined for 4-alkoxy-2,3,6-trimethylphenols (**4a**—**i**) having one trimethylhydroquinone moiety in their molecules. The compounds exhibited the activities shown in Fig. 5 where **4f**, **4g**, and **4h** exhibited the strongest activity with no remarkable difference among then, in contrast to that of **3** in Fig. 2. This would imply that the steric structure for **3a**—**i** changes with the length of side chain because they have two phenolic hydroxyl groups and among them the compounds with two benzene rings facing each other (**3a**, **3b**, **3d**, **3f**, **3h**) exhibit a high activity since their

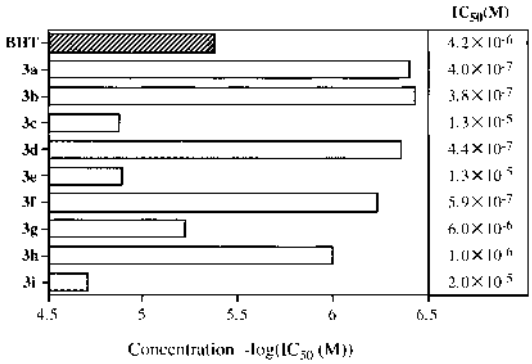

Fig. 2. Effect of **3a**—**i** and BHT on Fe^{3+}–ADP Induced NADPH Dependent Lipid Peroxidation in Rat Liver Microsomes

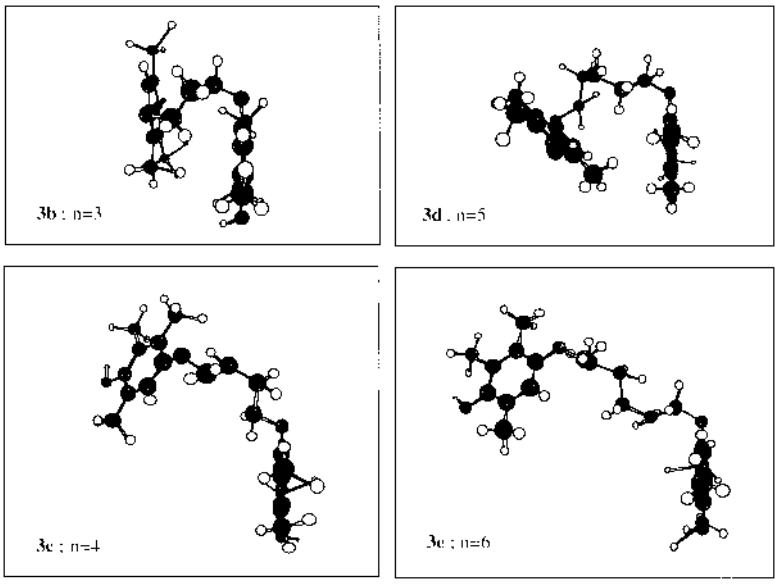

Fig. 3. Steric Structures of **3b**—**e** Obtained for Molecular Simulations (MM2 Caluculations)

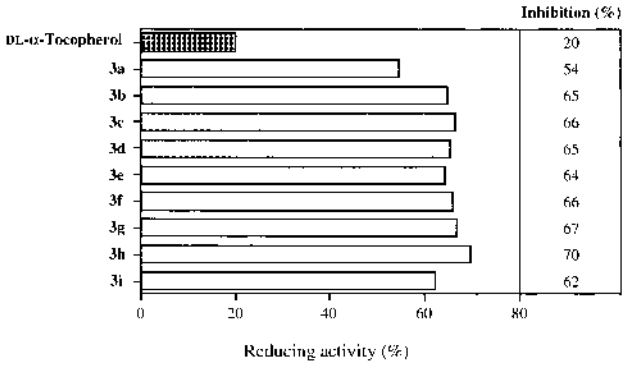

Fig. 4 α,α-Diphenyl-β-picrilhydrazyl (DPPH) Reducing Activity of **3a**—**i** and DL-α-Tocopherol

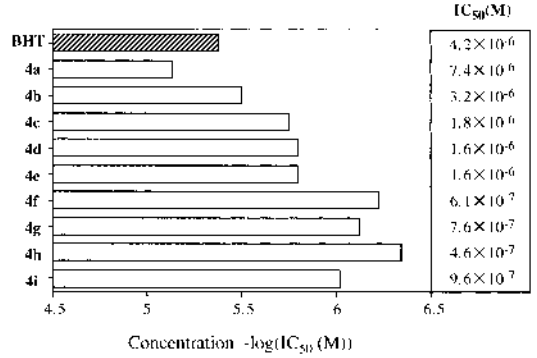

Fig. 5. Effect of **4a**—**i** and BHT on Fe^{3+}–ADP Induced NADPH Dependent Lipid Peroxidation in Rat Liver Microsomes

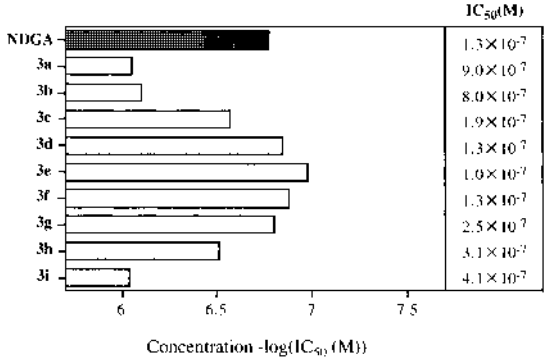

Fig. 6. Effect of **3a**—**i** and NDGA on RBL-1 Cell 5-Lipoxygenase Activity

chelation with Fe is strong.

From what has been mentioned so far, Fe^{3+} in liver microsome is considered to be localized to a certain extent, instead of being uniformly distributed over a wide area, since the compounds with a structure in which Fe^{3+} is enclosed by benzene rings had a strong activity.

5-LO inhibiting activity was then examined in the compounds.[20] As shown in Fig. 6, they exhibited an activity stronger than that of nordihydroguaiaretic acid (NDGA),[4] the positive control, with that of **3e** being the strongest. Bell *et al*. classified compounds that inhibit 5-lipoxygenase into three types, 1) substrate analogues, 2) compounds with radical scavenging activity, and 3) chelating agents of Fe, the active center metal of 5-LO.[21] Here, compounds **3a**—**i** are suggested to develop their activity as 2) and 3) because they have phenolic hydroxyl groups.

3d showed a strong activity in both antioxidative action and 5-LD inhibiting action. Novel 1,5-bis(4-hydroxy-2,3,5-trimethylphenoxy)-pentane (**3d**) is expected to be effective against various symptoms including tissue injury, cancer, and inflammation.

Experimental

NMR spectra were measured on a Varian Unity (^{1}H-NMR; 200 MHz, ^{13}C-NMR; 50 MHz) or Varian Gemini (^{1}H, 200 MHz; ^{13}C, 50 MHz) spectrometer in $CDCl_3$ or dimethyl sulfoxide (DMSO)-d_6 solution with tetramethylsilane (TMS) as an internal standard. IR spectra were measured on a JASCO FT/IR-5300 or JASCO FT/IR-410. Melting points were measured on a BÜCHI 510. MS spectra were measured on a JEOL JMS-AX 500. MM2 calculations were used on the iris of Silicon Graphics with biograf.

Synthesis of 1,2-Bis(4-hydroxy-2,3,5-trimethylphenoxy)ethane (3a) and 4-(2-Hydroxyethoxy)-2,3,6-trimethyphenol (4a) Trimethylhydroquinone (**1**) (5.00 g, 32.85 mmol) and ethyleneglycol (**2a**) (6.12 g, 98.57 mmol) were dissolved in 35 ml of toluene and the solution was refluxed at 130 °C for 20 h after addition of phosphomolybdic acid (1.00 g). The solvent was then removed from the reaction mixture by evaporation under reduced pressure and the residue was treated by silica gel chromatography ($CHCl_3$–AcOEt, 10 : 1). Compounds **3a** (1.03 g, 3.11 mmol, 10%) and **4a** (3.88 g, 19.78 mmol, 60%) were obtained by recrystallization from methanol and *n*-hexane–ethyl acetate, respectively.

3a: White powder, mp 192—193 °C. IR (neat) cm^{-1}: 3355, 2934, 1595, 1132. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 2.04 (3H, s), 2.07 (3H, s), 2.13 (3H, s), 4.12 (2H, s), 6.60 (1H, s), 7.60 (1H, s). ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 11.8 (q), 12.6 (q), 16.8 (q), 68.0 (t), 112.7 (d), 121.9 (s), 122.9 (s), 124.7 (s), 146.8 (s), 149.4 (s). HR-EI-MS: 330.1857 (M^+) (Calcd for $C_{20}H_{26}O_4$: 330.1831).

4a: White powder, mp 124—126 °C. IR (neat) cm^{-1}: 3374, 2920, 1588, 1123. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 2.16 (3H, s), 2.18 (3H, s), 2.22 (3H, s), 3.98 (4H, m), 4.35 (1H, s), 6.55 (1H, s). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 11.9 (q), 12.7 (q), 16.9 (q), 60.0 (t), 70.9 (t), 112.6 (d), 122.0 (s), 123.0 (s), 124.8 (s), 146.8 (s), 149.8 (s). HR-EI-MS: 196.1074 (M^+) (Calcd for $C_{11}H_{16}O_3$: 196.1099).

Synthesis of 1,3-Bis(4-hydroxy-2,3,5-trimethylphenoxy)propane (3b) and 4-(3-Hydroxypropyloxy)-2,3,6-trimethyphenol (4b) Trimethyhydroquinone (**1**) (5.00 g, 32.85 mmol) and 1,3-propanediol (**2b**) (7.50 g, 98.57 mmol) were dissolved in 35 ml of toluene and the solution was refluxed at 130 °C for 5 h after addition of phosphomolybdic acid (1.00 g). The solvent was then removed from the reaction mixture by evaporation under reduced pressure and the residue was treated by silica gel chromatography ($CHCl_3$–AcOEt, 10 : 1). Compounds **3b** (1.08 g, 3.14 mmol, 10%) and **4b** (4.61 g, 21.94 mmol, 67%) were obtained by recrystallization from methanol and *n*-hexane–ethyl acetate, respectively.

3b; White powder, mp 124 °C. IR (neat) cm^{-1}: 3437, 2917, 1595, 1120. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 2.02 (3H, s), 2.06 (3H, s), 2.11 (3H, s), 4.00 (2H, t, J=6.2 Hz), 6.54 (1H, s), 7.58 (1H, s). ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 11.8 (q), 12.7 (q), 16.8 (q), 29.4 (t), 65.3 (t), 112.0 (d), 121.9 (s), 122.6 (s), 124.7 (s), 146.6 (s), 149.5 (s). HR-EI-MS: 344.1975 (M^+) (Calcd for $C_{21}H_{28}O_4$: 344.1987).

4b: White powder, mp 90—91 °C. IR (neat) cm^{-1}: 3283, 2949, 1589, 1123. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 2.04 (2H, tt, J=5.5, 5.5 Hz), 2.13 (3H, s), 2.17 (3H, s), 2.22 (3H, s), 3.89 (2H, t, J=5.9 Hz), 4.04 (2H, t, J=5.7 Hz), 4.39 (1H, s), 6.55 (1H, s). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 12.7 (q), 12.9 (q), 16.9 (q), 32.9 (t), 61.9 (t), 68.3 (t), 113.0 (d), 120.9 (s), 124.3 (s), 124.8 (s), 147.0 (s), 151.1 (s). HR-EI-MS: 210.1240 (M^+) (Calcd for $C_{12}H_{18}O_3$: 210.1256).

Synthesis of 1,4-Bis(4-hydroxy-2,3,5-trimethylphenoxy)butane (3c) and 4-(4-Hydroxybutoxy)-2,3,6-trimethylphenol (4c) Trimethylhydroquinone (**1**) (1.00 g, 6.57 mmol) and 1,4-butanediol (**2c**) (1.78 g, 19.75 mmol) were dissolved in 25 ml of toluene and the solution was refluxed at 130 °C for 3 h after addition of phosphomolybdic acid (0.25 g). The solvent was then removed from the reaction mixture by evaporation under reduced pressure and the residue was treated by silica gel chromatography ($CHCl_3$–AcOEt, 10 : 1). Compounds **3c** (0.38 g, 1.05 mmol, 16%) and **4c** (0.16 g, 0.71 mmol, 43%) were obtained by recrystallization from methanol and *n*-hexane–ethyl acetate, respectively.

3c: White powder, mp 188—189 °C. IR (neat) cm^{-1}: 3362, 2924, 1593,

1117. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 1.81—1.87 (2H, m), 2.02 (3H, s), 2.06 (3H, s), 2.12 (3H, s), 3.86—3.92 (2H, m), 6.53 (1H, s), 7.56 (1H, s). ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 11.8 (q), 12.7 (q), 16.9 (q), 26.0 (t), 68.1 (t), 112.0 (d), 121.8 (s), 122.5 (s), 124.7 (s), 146.5 (s), 149.6 (s). HR-EI-MS: 358.2157 (M^+) (Calcd for $C_{22}H_{30}O_4$: 358.2114).

4c: White powder, mp 70—71 °C. IR (neat) cm^{-1}: 3395, 2944, 1557, 1119. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.68—1.98 (4H, m), 1.78 (2H, tt, J=5.5, 5.5 Hz), 2.13 (3H, s), 2.16 (3H, s), 2.20 (3H, s), 3.68 (2H, t, J=5.9 Hz), 3.86 (2H, t, J=6.2 Hz), 4.76 (1H, s), 6.51 (1H, s). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 11.9 (q), 12.2 (q), 16.2 (q), 26.2 (t), 29.7 (t), 62.9 (t), 69.2 (t), 113.0 (d), 120.9 (s), 124.3 (s), 124.8 (s), 147.0 (s), 151.1 (s). HR-EI-MS: 224.1404 (M^+) (Calcd for $C_{13}H_{20}O_3$: 224.1412).

Synthesis of 1,5-Bis(4-hydroxy-2,3,5-trimethylphenoxy)pentane (3d) and 4-(5-Hydroxypentyloxy)-2,3,6-trimethylphenol (4d) Trimethylhydroquinone (**1**) (5.00 g, 32.85 mmol) and 1,5-pentanediol (**2d**) (10.27 g, 98.57 mmol) were dissolved in 35 ml of toluene and the solution was refluxed at 130 °C for 6 h after addition of phosphomolybdic acid (1.00 g). The solvent was then removed from the reaction mixture by evaporation under reduced pressure and the residue was treated by silica gel chromatography ($CHCl_3$–AcOEt, 10 : 1). Compounds **3d** (0.97 g, 2.62 mmol, 8%) and **4d** (5.33 g, 22.38 mmol, 68%) were obtained by recrystallization from *n*-hexane and *n*-hexane–ethyl acetate, respectively.

3d: White powder, mp 124—125 °C. IR (neat) cm^{-1}: 3335, 2940, 1593, 1121. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 1.57—1.77 (3H, m), 2.02 (3H, s), 2.06 (3H, s), 2.12 (3H, s), 3.84 (2H, t, J=6.2 Hz), 6.52 (1H, s), 7.56 (1H, s). ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 11.8 (q), 12.7 (q), 16.9 (q), 22.5 (t), 28.8 (t), 68.3 (t), 112.0 (d), 121.8 (s), 122.5 (s), 124.7 (s), 146.5 (s), 149.6 (s). HR-EI-MS: 372.2286 (M^+) (Calcd for $C_{23}H_{32}O_4$: 372.2300).

4d: White powder, mp 79—81 °C. IR (neat) cm^{-1}: 3277, 2940, 1588, 1124. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.52—1.66 (4H, m), 1.78 (2H, tt, J=6.7, 6.7 Hz), 2.13 (3H, s), 2.16 (3H, s), 2.20 (3H, s), 3.68 (2H, t, J=5.9 Hz), 3.86 (2H, t, J=6.2 Hz), 4.76 (1H, s), 6.51 (1H, s). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 11.9 (q), 12.2 (q), 16.2 (q), 22.4 (t), 29.3 (t), 32.3 (t), 62.7 (t), 69.1 (t), 112.4 (d), 120.4 (s), 123.8 (s), 124.2 (s), 145.9 (s), 150.6 (s). HR-EI-MS: 238.1567 (M^+) (Calcd for $C_{14}H_{22}O_3$: 238.1569).

Synthesis of 1,6-Bis(4-hydroxy-2,3,5-trimethylphenoxy)hexane (3e) and 4-(6-Hydroxyhexyloxy)-2,3,6-trimethyphenol (4e) Trimethylhydroquinone (**1**) (1.00 g, 6.57 mmol) and 1,6-hexanediol (**2e**) (2.33 g, 19.72 mmol) were dissolved in 10 ml of toluene and the solution was refluxed at 130 °C for 3 h after addition of phosphomolybdic acid (0.40 g). The solvent was then removed from the reaction mixture by evaporation under reduced pressure and the residue was treated by silica gel chromatography ($CHCl_3$–AcOEt, 10 : 1). Compounds **3e** (0.20 g, 0.52 mmol, 8%) and **4e** (1.00 g, 3.99 mmol, 61%) were obtained by recrystallization from *n*-hexane and *n*-hexane–ethyl acetate, respectively.

3e: White powder, mp 175—177 °C. IR (neat) cm^{-1}: 3295, 2915, 1591, 1119. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 1.48—1.51 (2H, m), 1.67—1.74 (2H, m), 2.01 (3H, s), 2.06 (3H, s), 2.12 (3H, s), 3.82 (2H, t, J=6.3 Hz), 6.51 (1H, s), 7.56 (1H, s). ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 11.8 (q), 12.7 (q), 16.8 (q), 29.0 (t), 68.3 (t), 112.0 (d), 121.8 (s), 122.5 (s), 124.7 (s), 146.5 (s), 149.6 (s). HR-EI-MS: 386.2432 (M^+) (Calcd for $C_{24}H_{34}O_4$: 386.2457).

4e: White powder, mp 77—78 °C. IR (neat) cm^{-1}: 3387, 2938, 1591, 1119. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.41—1.81 (8H, m), 2.14 (3H, s), 2.16 (3H, s), 2.21 (3H, s), 3.65 (2H, t, J=6.4 Hz), 3.87 (2H, t, J=6.3 Hz), 4.51 (1H, s), 6.51 (1H, s). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 11.9 (q), 12.2 (q), 16.2 (q), 25.5 (t), 26.0 (t), 29.5 (t), 32.7 (t), 62.9 (t), 69.2 (t), 112.4 (d), 120.2 (s), 123.6 (s), 124.2 (s), 145.9 (s), 150.7 (s). HR-EI-MS: 252.1698 (M^+) (Calcd for $C_{15}H_{24}O_3$: 252.1726).

Synthesis of 1,7-Bis(4-hydroxy-2,3,5-trimethylphenoxy)heptane (3f) and 4-(7-Hydroxyheptyloxy)-2,3,6-trimethylphenol (4f) Trimethylhydroquinone (**1**) (1.00 g, 6.67 mmol) and 1,7-heptanediol (**2f**) (2.61 g, 13.14 mmol) were dissolved in 10 ml of toluene and the solution was refluxed at 130 °C for 4 h after addition of phosphomolybdic acid (0.25 g). The solvent was removed from the reaction mixture by evaporation under reduced pressure and the residue was treated by silica gel chromatography (CHC_3–AcOEt, 10 : 1). Compounds **3**f (0.22 g, 0.55 mmol, 8%) and **4f** (1.43 g, 5.38 mmol. 82%) were obtained by recrystallization from *n*-hexane and *n*-hexane–ethyl acetate, respectively.

3f: White powder, mp 110—112 °C. IR (neat) cm^{-1}: 3293, 2936, 1593, 1123. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 1.40—1.48 (4H, m), 1.65—1.72 (2H, m), 2.02 (3H, s), 2.06 (3H, s), 2.12 (3H, s), 3.82 (2H, t, J=6.3 Hz), 6.51 (1H, s), 7.55 (1H, s). ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 11.8 (q), 12.7 (q), 16.8 (q), 25.6 (t), 28.4 (t), 28.9 (t), 68.3 (t), 112.0 (d), 121.8 (s), 122.5 (s), 124.7 (s), 146.5 (s), 149.6 (s). HR-EI-MS: 400.2627 (M^+) (Calcd for $C_{25}H_{36}O_4$: 400.2613).

4f: White powder, mp 73—75 °C. IR (neat) cm^{-1}: 3347, 2934, 1590, 1119. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.33–1.79 (10H, m), 2.13 (3H, s), 2.16 (3H, s), 2.20 (3H, s), 3.63 (2H, t, J=6.4 Hz), 3.86 (2H, t, J=6.4 Hz), 4.62 (1H, s), 6.51 (1H, s). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 11.9 (q), 12.2 (q), 16.2 (q), 25.6 (t), 26.1 (t), 29.1 (t), 29.5 (t), 32.6 (t), 62.9 (t), 69.2 (t), 112.3 (d), 120.3 (s), 123.7 (s), 124.2 (s), 145.9 (s), 150.7 (s). HR-EI-MS: 266.1890 (M^+) (Calcd for $C_{16}H_{26}O_3$: 266.1882).

Synthesis of 1,8-Bis(4-hydroxy-2,3,5-trimethylphenoxy)octane (3g) and 4-(8-Hydroxyoctyloxy)-2,3,6-trimethylphenol (4g) Trimethylhydroquinone (**1**) (5.00 g, 32.85 mmol) and 1,8-octanediol (**2g**) (14.41 g, 98.56 mmol) were dissolved in 35 ml of toluene and the solution was refluxed at 130 °C for 3 h after addition of phosphomolybdic acid (1.00 g). The solvent was removed from the reaction mixture by evaporation under reduced pressure and the residue was treated by silica gel chromatography ($CHCl_3$–AcOEt, 10 : 1). Compounds **3g** (1.09 g, 2.63 mmol, 8%) and **4g** (6.57 g, 23.47 mmol, 72%) were obtained by recrystallization from n-hexane and *n*-hexane–ethyl acetate, respectively.

3g: White powder, mp 136—137 °C. IR (neat) cm^{-1}: 3358, 2913, 1595, 1121. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 1.36—1.42 (4H, m), 1.64—1.68 (2H, m), 2.01 (3H, s), 2.06 (3H, s), 2.12 (3H, s), 3.81 (2H, t, J=6.4 Hz), 6.51 (1H, s), 7.55 (1H, s). ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 11.8 (q), 12.7 (q), 16.9 (q), 25.6 (t), 28.7 (t), 29.0 (t), 68.3 (t), 112.0 (d), 121.8 (s), 122.5 (s), 124.7 (s), 146.5 (s), 149.6 (s). HR-EI-MS: 414.2783 (M^+) (Calcd for $C_{26}H_{38}O_4$: 414.2770).

4g: White powder, mp 70—71 °C. IR (neat) cm^{-1}: 3385, 2934, 1581, 1119. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.28–1.83 (12H, m), 2.14 (3H, s), 2.16 (3H, s), 2.21 (3H, s), 3.63 (2H, t, J=6.9 Hz), 3.86 (2H, t, J=6.7 Hz), 6.51 (1H, s). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 11.9 (q), 12.2 (q), 16.2 (q), 25.6 (t), 26.1 (t), 29.3 (t), 29.3 (t), 29.3 (t), 29.5 (t), 32.7 (t), 63.0 (t), 69.3 (t), 112.4 (d), 120.2 (s), 123.6 (s), 124.2 (s), 145.9 (s), 150.7 (s). HR-EI-MS: 280.2048 (M^+) (Calcd for $C_{17}H_{28}O_3$: 280.2038).

Synthesis of 1,9-Bis(4-hydroxy-2,3,5-trimethylphenoxy)nonane (3h) and 4-(9-Hydroxynonyloxy)-2,3,6-trimethylphenol (4h) Trimethylhydroquinone (**1**) (1.00 g, 6.57 mmol) and 1,9-nonanediol (**2h**) (3.16 g, 19.70 mmol) were dissolved in 10 ml of toluene and the solution was refluxed at 130 °C for 19 h after addition of phosphomolybdic acid (0.50 g). The solvent was removed from the reaction mixture by evaporation under reduced pressure and the residue was treated by silica gel chromatography ($CHCl_3$–AcOEt, 10 : 1). Compounds **3h** (0.18 g, 0.42 mmol, 6%) and **4h** (0.81 g, 2.75 mmol, 42%) were obtained by recrystallization from *n*-hexane and *n*-hexane–ethyl acetate, respectively.

3h: White powder, mp 103—104 °C. IR (neat) cm^{-1}: 3355, 2919, 1595, 1128. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 1.32—1.41 (6H, m), 1.64—1.68 (2H, m), 2.02 (3H, s), 2.06 (3H, s), 2.12 (3H, s), 3.81 (2H, t, J =6.3 Hz), 6.51 (1H, s), 7.55 (1H, s). ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 11.8 (q), 12.6 (q), 16.8 (q), 25.6 (t), 28.6 (t), 29.0 (t), 68.3 (t), 112.0 (d), 121.8 (s), 122.5 (s), 124.6 (s), 146.5 (s), 149.6 (s). HR-EI-MS: 428.2932 (M^+) (Calcd for $C_{27}H_{40}O_4$: 428.2926).

4h: White powder, mp 72—74 °C. IR (neat) cm^{-1}: 3341, 2932, 1593, 1121. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.21—1.79 (14H, m), 2.14 (3H, s), 2.16 (3H, s), 2.21 (3H, s), 3.62 (2H, t, J=6.5 Hz), 3.86 (2H, t, J=6.4 Hz), 4.61 (1H, s), 6.51 (1H, s). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 11.9 (q), 12.2 (q), 16.2 (q), 25.7 (t), 26.1 (t), 29.3 (t), 29.3 (t), 29.5 (t), 29.5 (t), 32.7 (t), 63.0 (t), 69.3 (t), 112.4 (d), 120.3 (s), 123.7 (s), 124.2 (s), 145.9 (s), 150.7 (s). HR-EI-MS: 294.2202 (M^+) (Calcd for $C_{18}H_{30}O_3$: 294.2195).

Synthesis of 1,10-Bis(4-hydroxy-2,3,5-trimethylphenoxy)decane (3i) and 4-(10-Hydroxydecanyloxy)-2,3,6-trimethylphenol (4i) Trimethylhydroquinone (**1**) (5.00 g, 32.85 mmol) and 1,10-decanediol (**2i**) (17.18 g, 98.56 mmol) were dissolved in 35 ml of toluene and the solution was refluxed at 130 °C for 4 h after addition of phosphomolybdic acid (1.00 g). The solvent was removed from the reaction mixture by evaporation under reduced pressure and the residue was treated by silica gel chromatography ($CHCl_3$–AcOEt, 10 : 1). Compounds **3i** (0.96 g, 2.18 mmol, 7%) and **4i** (7.99 g, 25.93 mmol, 79%) were obtained by recrystallization from *n*-hexane and *n*-hexane–ethyl acetate, respectively.

3i: White powder, mp 111—113 °C. IR (neat) cm^{-1}: 3331, 2932, 1616, 1125. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 1.30—1.40 (6H, m), 1.63—1.67 (2H, m), 2.01 (3H, s), 2.06 (3H, s), 2.11 (3H, s), 3.80 (2H, t, J=6.3 Hz), 6.51 (1H, s), 7.55 (1H, s). ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 11.8 (q), 12.6 (q), 16.8 (q), 25.6 (t), 28.7 (t), 28.9 (t), 29.0 (t), 68.3 (t), 112.0 (d), 121.8 (s), 122.4 (s), 124.6 (s), 146.5 (s), 149.6 (s). HR-EI-MS: 442.3087 (M^+) (Calcd for $C_{28}H_{42}O_4$: 442.3082).

4i: White powder, mp 58—61 °C. IR (neat) cm^{-1}: 3389, 2928, 1587, 1117. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.23—1.82 (16H, m), 2.14 (3H, s), 2.17 (3H, s), 2.21 (3H, s), 3.63 (2H, t, J=6.5 Hz), 3.86 (2H, t, J=6.3 Hz), 4.43 (1H, s), 6.52 (1H, s). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 11.9 (q), 12.2 (q), 16.2 (q), 25.7 (t), 26.1 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.5 (t), 29.6 (t), 32.7 (t), 63.0 (t), 69.3 (t), 112.4 (d), 120.1 (s), 123.6 (s), 124.3 (s), 145.9 (s), 150.8 (s). HR-EI-MS: 308.2354 (M^+) (Calcd for $C_{19}H_{32}O_3$: 308.2357).

Pharmacology Materials Male Wistar rats were obtained from Japan SLC Inc. RBL-1 cells were obtained from Dainippon Pharmaceutical Co., Ltd. 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) was obtained from Sigma Chemical Co. Adenosine 5′-diphosphate disodium salt (ADP), nicotinamide adenine dinucleotide phosphate reduced form (β-NADPH), and sodium dodecyl sulfate (SDS) were purchased from Wako Pure Chemical Industries, Ltd. BHT, TBA, D,L-α-tocopherol, L-ascorbic acid, NDGA, and arachidonic acid were purchased from Nacalai Tesque, Inc.

Measurement of Fe^{3+}–ADP Induced NADPH Dependent Lipid Peroxidation in Rat Liver Microsome.[12] The modified method of Kiso *et al*.[16] was used. Microsomes were prepared from male Wistar rats weighing about 200 g. The rat liver was homogenized in cold 0.25 M sucrose and the homogenate was centrifuged at 4 °C (8000×***g***, 30 min). The supernatant fraction was then collected and ultracentrifuged at 4 °C (105000×***g***, 30 min). The pellet obtained was resuspended in 83.5 mM KCl 37.2 mM Tris–HCl buffer (pH 7.4) and stocked at −20 °C until use. Protein concentration was determined by the method of Lowry *et al*.[22] The assay system (1 ml) consisted of 83.5 mM KCl 37.2 mM Tris–HCl buffer (pH 7.4), the test compound in 1% DMSO, 0.2 mM NADPH, 1 mM ADP, 1 mg protein/ml rat liver microsomes and 10 μM $FeCl_3$. The reaction mixture was incubated at 37 °C for 20 min, and then cooled on ice to terminate the reaction. Lipid peroxide was measured by the method of Ohkawa *et al*.[17] Thus, 8.1% SDS (0.2 ml), 20% AcOH (1.5 ml) and 0.8% TBA (1.5 ml) were added to the reaction mixture. The mixture was then boiled at 100 °C for 20 min and the reaction was stopped by cooling on ice. Thereafter, *n*-BuOH–pyridine (15 : 1, 4 ml) was added and mixed vigorously. After centrifugation (780×***g***, 10 min), the organic layer was separated, and the absorbance was measured at 532 nm. The amount of TBA-positive material was expressed as the corresponding amount of malondialdehyde.

Measurement of DPPH Reducing Activity[19] The modified method of Nihro *et al*.[23] was used. The assay system (2 ml) consisted of 0.1 mM DPPH in ethanol and the test compound in 1% DMSO. The reaction mixture was left at room temperature for 3 min, and the absorbance was measured at 517 nm.

Measurement of RBL-1 Cell 5-Lipoxygenase Activity[24] The modified method of Blackham *et al*.[20] was used. RBL-1 cells were grown in Roswell Park Memorial Institute (RPMI)-1640 medium containing 10% heat-inactivated newborn calf serum, 100 units/ml penicillin and 0.1 mg/ml streptomycin. Cells were cultured at 37 °C in 5% CO_2/air. Cells in the growth phase (5×10^5—10^6 cells/ml) were collected by centrifugation (100×***g***, 5 min) and suspended at 3×10^7 cells/ml in 50 mM phosphate buffer (0.25 M sucrose, 1 mM EDTA, 2 mM glutathione, pH 7.4), then stored at −80 °C until use. The assay system (0.5 ml) consisted of 50 mM phosphate buffer (0.25 M sucrose, 1 mM EDTA, 2 mM glutathione, pH 7.4), the test compound in 1% dimethyl sulfoxide (DMSO), 2 mM $CaCl_2$, 0.2 mg/ml arachidonic acid (10 mg/ml MeOH soln; 10 μl) and 10^7 cells/ml RBL-1 cells of homogenate. The reaction mixture was incubated at 37 °C for 3 min, and then 0.5 ml of MeOH was added to terminate the reaction. The mixture was centrifuged (2000×***g***, 15 min), 5-HETE in the supernatant was analyzed by HPLC (column; COSMOSIL 5C18-MS Waters 4.6×150 mm (Nacalai Tesque, Inc.), mobile phase; CH_3CN : 0.1% AcOH aq. (6 : 4), flow rate; 1 ml/min, temperature; r.t., absorbance; 235 nm).

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