Synthesis and Structure–Activity Relationships of Phaffiaol and Related Antioxidants

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Total synthesis of a novel long chain alkyl phenol, phaffiaol (1), a potent antioxidant isolated from *Phaffia rhodozyma*, was achieved *via* three reaction steps starting from 3,5-dibromosalicylaldehyde (2). We also prepared several types of long chain alkyl phenol having different aromatic rings or different carbon length, and evaluated their antioxidative activity using the rabbit erythrocyte membrane ghost system. Amongst these compounds, the novel methoxy phenol derivatives (6c, d, 7) having a heptadecyl moiety at the *ortho*-position to the hydroxy group, were approximately 2 times more active than α-tocopherol. In addition, 4-methoxy-2-pentadecylphenol (10h), which has a 15-carbon length in the side chain moiety, showed the maximum activity.

Key words phaffiaol; long chain alkyl phenol; antioxidative activity; structure–activity relationship

A number of long chain alkyl phenols have been isolated from various plants[1] and microorganisms,[2] and many exhibit biological activity, such as 5-lipoxygenase inhibition,[1a] glycerol-3-phosphate dehydrogenase inhibition,[2a] thromboxane A_2 antagonism,[3] antitumor activity,[4] and antifungal activity.[5] We have previously reported the isolation, structure elucidation, and biological activity of the trisubstituted alkyl phenol, phaffiaol (**1**),[6] which was isolated from a red yeast *Phaffia rhodozyma*. Compound **1** has antioxidative activity equivalent to that of α-tocopherol, a well-known natural antioxidant. It has been considered recently that active oxygen species and free radicals are involved in the pathogenesis of certain clinical diseases, including cerebral ischemia,[7] atherosclerosis,[8] Parkinson's disease,[8a] inflammation,[9] diabetes,[10] and cancer.[11] Therefore, antioxidants have been proposed as effective therapeutic drugs for these diseases. For example, chemists at Sankyo reported that in the development of troglitazone, a market-launched antidiabetic, α-tocopherol served as a lead compound.[12]

As part of our study on antioxidants, we have prepared various kinds of long chain alkyl phenols and tested for antioxidative activity. In this paper, we describe a total synthesis of phaffiaol and its antioxidative activity. We also synthesized thirty-two long chain alkyl phenols having various aromatic rings or different carbon lengths in the alkyl side chain moiety, and evaluated their antioxidative activity. The structure–activity relationships (SAR) are discussed.

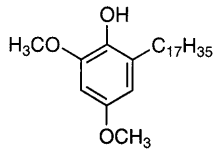


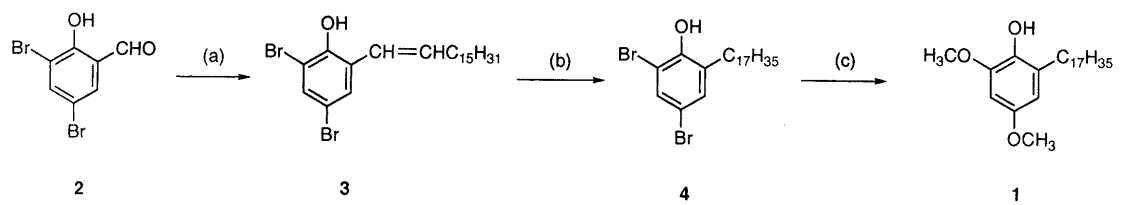
Fig. 1. Chemical Structure of Phaffiaol (**1**)

Chemistry

Many syntheses of long chain alkyl phenols have been described[4,13] and the first synthesis of 2,4-dimethoxy-6-heptadecylphenol (**1**) was carried out *via* the route shown in Chart 1.

The phosphonium salt was converted into the ylide using sodium methylsulfinylmethide as base, and then allowed to react with 3,5-dibromosalicylaldehyde (**2**).[14] The Wittig olefination of **2** at 50 °C afforded a *Z*/*E* mixture (approximately 1 : 1 ratio, δ 5.86 for *Z*-isomer and δ 6.26 for *E*-isomer in ^{1}H-NMR) of alkenyl phenol (**3**) in moderate yield, which was easily separated by chromatography on silica gel. Compound **3** was reduced by catalytic hydrogenation to give alkyl phenol **4**. Methoxylation of dibromide **4** with sodium methoxide, collidine, and cuprous iodide gave phaffiaol (**1**) in moderate yield.[15] The synthetic phaffiaol showed good agreement with the physico-chemical data and chromatographical behavior of the natural product, as shown in Table 1.

The synthetic procedures for the various substituted heptadecylphenol derivatives (**6a—o**, **7** and **8a—f**) are shown in Chart 2. Compounds **6a—o** were synthesized from the corresponding substituted benzaldehyde derivatives (**5a—o**) as described for the preparation of **4**. Compound **6a** was methoxylated to give **7** by the same procedure as that utilized for the preparation of **1**. Exposure of compounds **7** and **6b**, **c** to 2.2 eq of boron tribromide yielded trihydroxy derivatives (**8a—c**), respectively, and treatment of **6b** with 1.1 eq of boron tri-



reagents: (a) $C_{16}H_{33}PPh_3Br$/NaH/DMSO; (b) H_2/PtO_2/EtOAc; (c) NaOMe/collidine/CuI

Chart 1. Total Synthesis of Phaffiaol

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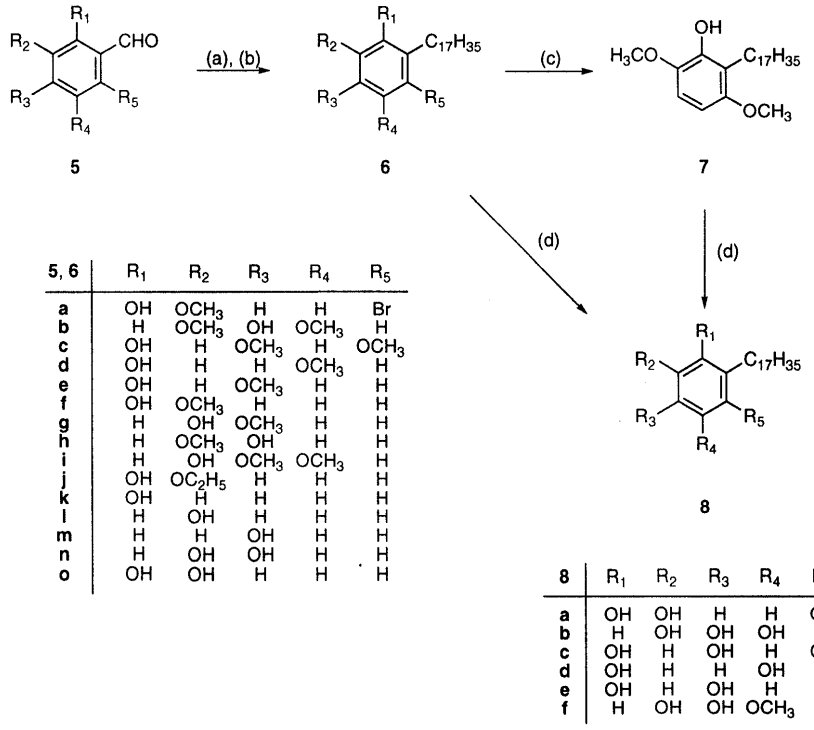
Table 1. Spectral Data for Phaffiaol

	Synthetic product	Natural product
^{1}H-NMR[a] (δ_H)	6.35 (1H, d, J=2.8 Hz), 6.28 (1H, d, J=2.8 Hz), 5.24 (1H, br s), 3.85 (3H, s), 3.75 (3H, s), 2.60 (2H, t, J=7.8 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (28H, m), 0.88 (3H, t, J=6.8 Hz)	6.35 (1H, d, J=2.5 Hz), 6.28 (1H, d, J=2.5 Hz), 5.24 (1H, br s), 3.85 (3H, s), 3.75 (3H, s), 2.60 (2H, t, J=7.8 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (28H, m), 0.87 (3H, t, J=7.0 Hz)
^{13}C-NMR[b] (δ_C)	152.7 (s), 146.7 (s), 137.5 (s), 128.7 (s), 105.8 (d), 96.6 (d), 56.0 (q), 55.8 (q), 31.9 (t), 30.1 (t), 29.9 (t), 29.7 (7C, each t), 29.6 (4C, each t), 29.4 (t), 22.7 (t), 14.1 (q)	152.7 (s), 146.7 (s), 137.4 (s), 128.8 (s), 105.9 (d), 96.6 (d), 56.0 (q), 55.8 (q), 31.9 (t), 30.0 (t), 29.9 (t), 29.7 (8C, each t), 29.6 (3C, each t), 29.4 (t), 22.7 (t), 14.1 (q)
UV λ_{max}^{EtOH} nm (ε)	289 (4000)	289 (3810)
IR ν_{max}^{KBr} cm^{-1}	3452, 2920, 2848, 1617, 1505	3480, 2915, 2845, 1610, 1500

a) 400 MHz in $CDCl_3$ (ppm). b) 100 MHz in $CDCl_3$ (ppm).



5, 6	R_1	R_2	R_3	R_4	R_5
a	OH	OCH_3	H	H	Br
b	H	OCH_3	OH	OCH_3	H
c	OH	H	OCH_3	H	OCH_3
d	OH	H	H	OCH_3	H
e	OH	H	OCH_3	H	H
f	OH	OCH_3	H	H	H
g	H	OH	OCH_3	H	H
h	H	OCH_3	OH	H	H
i	H	OH	OCH_3	OCH_3	H
j	OH	OC_2H_5	H	H	H
k	OH	H	H	H	H
l	H	OH	H	H	H
m	H	H	OH	H	H
n	H	OH	OH	H	H
o	OH	OH	H	H	H

8	R_1	R_2	R_3	R_4	R_5
a	OH	OH	H	H	OH
b	H	OH	OH	OH	H
c	OH	H	OH	H	OH
d	OH	H	H	OH	H
e	OH	H	OH	H	H
f	H	OH	OH	OCH_3	H

reagents: (a) $C_{16}H_{33}PPh_3Br$/NaH/DMSO; (b) H_2/PtO_2/EtOAc; (c) NaOMe/collidine/CuI; (d) BBr_3/CH_2Cl_2

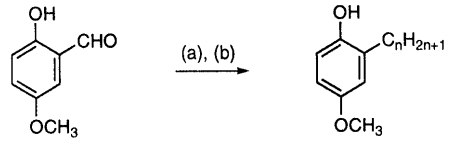
Chart 2. Preparation of Heptadecylphenol Derivatives

bromide afforded **8f**.[16]

In the synthesis of dihydroxy derivatives similar to compounds (**8d**, **e**), Itokawa and coworkers observed that the yields of Grignard reactions with β-resorcylaldehyde were very low.[4] An alternative way to the dihydroxy derivatives was demethylation of the corresponding dimethoxy derivatives.[13a] Actually, compounds **8d**, **e** were not obtained from gentisaldehyde or β-resorcylaldehyde by using the Wittig reaction described above, but were synthesized in a similar manner to **8f** using the corresponding methoxy phenols (**6d**, **e**).

Among the synthetic heptadecylphenols, mono and dihydroxy derivatives (**6k—o** and **8d**, **e**) possessing no methoxy groups or their congeners are known.[1b,c,4,13a] Some of them (**6l** and **6n**, **o**) were reported as natural products.[1b,c]

Synthetic routes to 2-substituted 4-methoxyphenols (**10a—k**) having a side chain of 7—18 methylene groups are illustrated in Chart 3. Wittig reaction of 5-methoxysalicylaldehyde (**9**) with ylides generated *in situ* from the corresponding alkyltriphenylphosphonium salt and methylsulfinyl carbanion–dimethyl sulfoxide, followed by catalytic hydrogenation, afforded compounds **10a—k**. As the alkyl length became



10	n
a	8
b	9
c	10
d	11
e	12
f	13
g	14
h	15
i	16
j	18
k	19

reagents: (a) $C_{n-1}H_{2n-1}PPh_3Br$/NaH/DMSO; (b) H_2/PtO_2/EtOAc

Chart 3. Preparation of 2-Alkyl-4-methoxyphenol Derivatives

longer, the yield decreased and compounds having an alkyl chain longer than 20 methylene groups were not obtained because of the low solubility of the ylides.

Results and Discussion

Alkyl phenols obtained in this study were evaluated for antioxidative activity using *tert*-butylhydroperoxide-initiated lipid peroxidation of rabbit erythrocyte ghost membrane.[17] Table 6 summarizes the 50% inhibitory concentration (IC_{50}) for each compound against lipid peroxidation. α-Tocopherol (Toc) was used as a reference compound and its IC_{50} value (30.50 μM) agreed with the reported value.[18]

Different substitution patterns on the phenol ring influenced antioxidative activity, however, all compounds tested, except **6m**, showed potent activity. Synthetic phaffiaol (**1**) and the natural material showed equal potency. Among the synthesized heptadecylphenols, several (*ex.* **6b**—**d**) exhibited higher activity than Toc or phaffiaol.

Initially, we examined the effect of the number and regiochemistry of hydroxy groups. Activity with respect to the substitution pattern of monohydroxy compounds having no methoxy group was *ortho* (**6k**)>*meta* (**6l**)>*para* (**6m**). Dihydroxy compounds (**6n**, **o**, **8d**, **e**) exhibited relatively high activity and tended to show increased activity compared to the corresponding methoxy analogues (**6d**—**h**). At this stage, we anticipated that more hydroxy groups would lead to better activity. Contrary to our expectation, the activity of the trihydroxy compounds (**8a**—**c**) was not strong and was in fact inferior to that of the corresponding methoxy analogues (**7**, **6b**, **c**, **8f**). These results showed that increasing the number of hydroxy groups did not necessarily increase the activity. Laccol (**6o**), isolated from poison ivy, poison oak and lac tree,[1b,c] has proven to be strongest among the compounds synthesized and its potency was at least 4 times greater than Toc. However, since laccol is known to cause irritation and inflammation,[1b] it is not appropriate as a lead compound.

We then investigated substituents on the phenol ring. Conversion to bromo substituents resulted in significant loss of activity. In contrast, the methoxylation of bromides led to a remarkable increase in the activity (**4** *vs.* **1**, **6a** *vs.* **7**). These observations suggested that electron-withdrawing substituents reduced activity. Replacement of the methoxy group in **6f** with an ethoxy moiety enhanced the activity (**6j**). The size of the alkoxy substituents seemed to influence the activity.

The effect of introduction of a methoxy group was examined. In the case of non methoxy-substituted analogues, incorporation of a methoxy group to the inferior compounds (**6l**, **m**) resulted in a significant increase in activity (**6g**, **h**). In contrast, incorporation of the function to the superior compounds (**6k**, **n**) did not remarkably improve the activity (**6d**—**f**, **8f**). The addition of another methoxy group to the mono methoxy-substituted analogues (**6d**—**h**) also enhanced the activity (**6b**, **c**, **i**, **7** except **1**). Comparison of **1** and its desmethoxy analogues (**6d**, **f**, **k**) revealed that the relative activity profile was 4-methoxy (**6d**)≥non-methoxy (**6k**)>2,4-dimethoxy (**1**)>2-methoxy (**6f**) and in this series a methoxy substituent at the 2-position was not suitable for improving activity.

Among the novel synthetic derivatives possessing methoxy groups, compounds (**6c**, **d**, **7**) were approximately twice as active as Toc but less active compared to laccol. However, they had no significant toxicity.[19]

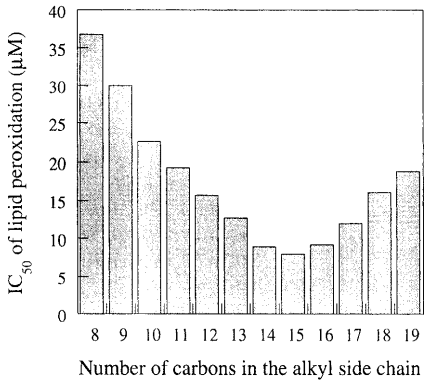


Fig. 2. Antioxidative Activity of 2-Alkyl-4-methoxyphenols

Finally, we focused our attention on the effect of the side chain length, while the aromatic ring contribution was fixed to the 2-alkyl-4-methoxyphenols (**10a**—**k**) because of the potent activity, no severe toxicity, and the novel simple structure of compound **6d**. As shown in Fig. 2, the length of the side chain affected activity. As the alkyl group became longer, the activity tended to increase and optimal antioxidative activity was obtained with compound **10h** having a 15-carbon length. Compound **10h** showed about 4 times greater activity than Toc and similar activity to laccol. Moreover, **10h** did not show significant toxicity,[19] therefore this compound appears to be a potential promising antioxidant *in vivo*.

Phaffiaol, isolated from a yeast, has 17-carbon atoms in the side chain moiety, while activity reaches a maximum at the 15-carbon length in this antioxidative activity system using the rabbit erythrocyte ghost membrane. These results imply that the difference in optimum carbon length may be attributable to the composition of the membrane in each species.[20]

Kinetic studies on tocopherol model compounds have suggested that alkyl functions at *ortho*-positions increase the antioxidant activity.[21] In this study, most of the *ortho*-alkyl phenols showed significant activity and these results may be related to these earlier findings. Many efforts to understand the antioxidant mechanism have focused on the localization of α-tocopherol in membranes. Recently, it has became evident that the hydrophobic phytyl side chain is embedded in the membrane and the hydroxy group is at or near the membrane surface.[22] In the alkyl phenols described in this report, it is reasonable to expect that the long alkyl chain went into the interior of the membrane and the phenolic hydrogen located at or near the membrane surface. The position of the hydroxy group at the membrane surface played an important role in the enhancement of activity. In addition, incorporation of substituents on the phenol ring influenced potency, since the substituents might affect steric hindrance near the hydroxy group, mobility in the membrane, or the stability of the hydroxy radical.

In conclusion, we have prepared a series of long chain alkyl phenols, evaluated their antioxidative activity, and examined the SAR. In this study, it was found that structural requirements for strong activity are the *ortho*-location of the alkyl side chain relative to the hydroxy group, incorporation

Table 2. Physical Data for Heptadecylphenols

Compound No.	Yield[a] (%)	mp (°C)	Formula	Analysis (%) Calcd C	Calcd H	Found C	Found H
6a	51	47.4—48.2	$C_{24}H_{41}BrO_2$	65.29	9.36	65.30	9.61
6b	61	56.8—57.3	$C_{25}H_{44}O_3$	76.48	11.30	76.40	11.61
6c	8	74.9—76.9	$C_{25}H_{44}O_3$	76.48	11.30	76.58	11.43
6d	27	76.5—77.2	$C_{24}H_{42}O_2$	79.50	11.68	79.27	12.04
6e	29	63.2—64.1	$C_{24}H_{42}O_2$	79.50	11.68	79.21	11.97
6f	24	52.1—52.7	$C_{24}H_{42}O_2$	79.50	11.68	79.47	11.98
6g	22	61.1—61.7	$C_{24}H_{42}O_2$	79.50	11.68	79.46	11.86
6h	50	59.5—60.2	$C_{24}H_{42}O_2$	79.50	11.68	79.47	11.94
6i	48	58.5—59.9	$C_{25}H_{44}O_3$	76.48	11.30	76.31	11.71
6j	6	61.0—61.9	$C_{25}H_{44}O_2$	79.73	11.78	79.76	12.17
6k	24	60.5—61.4	$C_{23}H_{40}O$	83.07	12.12	83.05	12.49
6l	17	60.4—60.8	$C_{23}H_{40}O$	83.07	12.12	83.02	12.44
6m	42	79.3—79.8	$C_{23}H_{40}O$	83.07	12.12	83.11	12.47
6n	12	95.5—97.5	$C_{23}H_{40}O_2$	79.25	11.57	79.12	11.71
6o	8	63.1—64.2	$C_{23}H_{40}O_2$	79.25	11.57	79.27	11.89
7	11	45.9—47.1	$C_{25}H_{44}O_3$	76.48	11.30	76.49	11.64
8a	99	104.2—105.2	$C_{23}H_{40}O_3$	75.78	11.06	76.01	11.28
8b	86	94.5—95.3	$C_{23}H_{40}O_3$	75.78	11.06	75.79	11.24
8c	69	119.1—120.6	$C_{23}H_{40}O_3$	75.78	11.06	75.89	11.31
8d	73	111.9—113.1	$C_{23}H_{40}O_2$	79.25	11.57	79.35	11.95
8e	69	90.6—91.4	$C_{23}H_{40}O_2$	79.25	11.57	78.85	11.96
8f	88	78.0—79.0	$C_{24}H_{42}O_3$	76.14	11.18	75.87	11.36

a) Total yields obtained from the Wittig reaction and hydrogenation on **6a**—**o**.

of a methoxy group, and the appropriate carbon length for the side chain. 4-Methoxy-2-pentadecylphenol (**10h**) (IC_{50}, 8.00 μM) exhibited about 4 times more potent activity than α-tocopherol and also showed no severe toxicity. These results indicated that **10h** was a promising potential antioxidant *in vitro*.

Experimental

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Ultraviolet (UV) spectra were recorded on a HITACHI U-3210 spectrophotometer. Infrared (IR) spectra were recorded on a JASCO FT/IR spectrometer VALOR III. ^{1}H- and ^{13}C-NMR spectra were obtained at 400 MHz (^{1}H-NMR) and 100 MHz (^{13}C-NMR) using a Brucker DPX400. Chemical shifts are given on the δ (ppm) scale downfield from tetramethylsilane as an internal standard. The electron impact mass (EI-MS) spectra were taken on a JEOL JMS-AX500. Elemental analysis was carried out with a Perkin-Elmer CHNS/O Analyzer 2400 Series II. Melting points (mp) were measured with a Yanaco micro melting point apparatus MP-500D and are uncorrected. Column chromatography was carried out using Micro Sphere Gel D75-60A (Asahi Glass Co.). TLC experiments were performed on silica gel plates 60 F_{254} 0.25 mm (E. Merck).

2,4-Dibromo-6-(1-heptadecenyl)phenol (3) A suspension of sodium hydride (60% oil dispersion, 5.0 g, 125 mmol), washed with *n*-hexane, in freshly distilled dimethyl sulfoxide (DMSO) (62.5 ml) was stirred for 2 h at 60 °C under an argon atmosphere. Hexadecyltriphenylphosphonium bromide (35.3 g, 62 mmol) in DMSO (26 ml) was then added to the solution at room temperature, and the mixture was stirred at the same temperature for 30 min. 3,5-Dibromosalicylaldehyde (**2**) (8.7 g, 31 mmol) in DMSO (8.7 ml) was then added dropwise to the solution and the mixture was stirred at 50 °C overnight. The reaction mixture was poured into diluted HCl and extracted with EtOAc. The extract was washed with water and brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was purified by column chromatography (*n*-hexane–EtOAc, 50 : 1) to give *E*-**3** (*Rf* 0.50: *n*-hexane–EtOAc, 50 : 1, 2.54 g) and *Z*-**3** (*Rf* 0.35: *n*-hexane–EtOAc, 50 : 1, 2.65 g) as colorless needles in 57% yield (based on the recovery of **2**). *E*-**3**: mp 67.1—67.9 °C (from MeOH). EI-MS *m/z*: 488 (M^+, 81), 278 (100), 210 (37), 131 (57). IR ν_{max}^{KBr} cm^{-1}: 3409, 2917, 2851, 1583, 1560, 967. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.88 (3H, t, J=6.8 Hz), 1.2—1.4 (24H, m), 1.5—1.6 (2H, m), 2.2—2.3 (2H, m), 5.60 (1H, br s), 6.26 (1H, dt, J=6.9, 15.9 Hz), 6.56 (1H, dt, J=1.8, 15.9 Hz), 7.43 (1H, d, J=2.3 Hz), 7.44 (1H, d, J=2.3 Hz). *Anal.* Calcd for $C_{23}H_{36}Br_2O$: C, 56.57; H, 7.43. Found: C, 56.71; H, 7.65. *Z*-**3**: mp 43.4—44.6 °C (from MeOH). EI-MS *m/z*: 488 (M^+, 68), 278 (100), 210 (30), 131 (51). IR ν_{max}^{KBr} cm^{-1}: 3425, 2918, 2851, 1583, 1559, 717. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.88 (3H, t, J=6.8 Hz), 1.2—1.4 (24H, m), 1.4—1.5 (2H, m), 2.1—2.2 (2H, m), 5.53 (1H, br s), 5.86 (1H, dt, J=5.7, 11.5 Hz), 6.36 (1H, dt, J=1.7, 11.5 Hz), 7.24 (1H, d, J=2.3 Hz), 7.50 (1H, d, J=2.3 Hz). *Anal.* Calcd for $C_{23}H_{36}Br_2O$: C, 56.57; H, 7.43. Found: C, 56.79; H, 7.66.

2,4-Dibromo-6-heptadecylphenol (4) A mixture of alkenyl phenol **3** (*E*/*Z*=1/1) (4.0 g, 82 mmol) and EtOAc (50 ml) was treated with platinum (IV) oxide hydrate catalyst (200 mg) and stirred for 1 h under a hydrogen atmosphere. Removal of the catalyst and concentration gave a powder. The residue was purified by column chromatography (*n*-hexane–EtOAc, 50 : 1) to give **4** (3.90 g) as colorless needles in 97% yield, mp 65.4—66.2 °C (from MeOH). EI-MS *m/z*: 490 (M^+, 100), 410 (13), 265 (48), 185 (35), 57 (75). IR ν_{max}^{KBr} cm^{-1}: 3408, 2916, 2849, 1591, 1568. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz), 1.2—1.4 (28H, m), 1.5—1.6 (2H, m), 2.61 (2H, t, J=7.8 Hz), 5.49 (1H, br s), 7.18 (1H, d, J=2.2 Hz), 7.43 (1H, d, J=2.2 Hz). *Anal.* Calcd for $C_{23}H_{38}Br_2O$: C, 56.34; H, 7.81. Found: C, 56.20; H, 7.98.

Compounds **6a**—**o** and **10a**—**k** were prepared as described above. The physical data for these compounds **6a**—**o** and **10a**—**k** are summarized in Tables 2—5.

2,4-Dimethoxy-6-heptadecylphenol (1) Freshly cut sodium (0.84 g, 36.5 mmol) was added to dry MeOH (14.6 ml) under an argon atmosphere at room temperature. When dissolution was complete, the warm solution was diluted with distilled 2,4,6-collidine (6.1 ml), and vacuum-dried cuprous iodide (1.17 g, 6.1 mmol), dibromide **4** (3.0 g, 6.1 mmol) and additional collidine (20 ml) was added to the solution. The mixture was refluxed under an argon atmosphere for 16 h, and then filtered. The filtrate was poured into diluted HCl and extracted with EtOAc. The extract was washed with water and brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was purified by column chromatography (*n*-hexane–EtOAc, 95 : 5). This product was recrystallized from MeOH to give **1** (1.61 g) as colorless needles in 67% yield, mp 77.7—79.1 °C. ^{1}H-NMR, ^{13}C-NMR, IR and UV spectral data are given in Table 1. EI-MS *m/z*: 392 (M^+, 100), 168 (61), 153 (12), 139 (14). *Anal.* Calcd for $C_{25}H_{44}O_3$: C, 76.48; H, 11.30. Found: C, 76.49; H, 11.58.

Compound **7** was prepared as described above. The physical data for this compound are summarized in Tables 2 and 3.

1,2,4-Trihydroxy-3-heptadecylphenol (8a) To a solution of 2,5-dimethoxy-6-heptadecylphenol (**7**) (1.54 g, 3.92 mmol) in CH_2Cl_2 (24 ml) was

Table 3. IR, MS and ^{1}H-NMR[a] Data for Heptadecylphenols

Compound No.	IR ν_{max}^{KBr} cm^{-1}	EI-MS *m/z*	^{1}H-NMR (δ_H)
6a	3450, 2916, 2851, 1605, 1585, 1558	440 (M^+, 100), 215 (37)	7.03 (1H, d, *J*=8.6 Hz), 6.59 (1H, d, *J*=8.6 Hz), 5.75 (1H, br s), 3.86 (3H, s), 2.77 (2H, t, *J*=7.9 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (28H, m), 0.88 (3H, t, *J*=6.9 Hz)
6b	3505, 2918, 2848, 1612, 1522	392 (M^+, 100), 167 (93)	6.39 (2H, s), 5.33 (1H, br s), 3.87 (6H, s), 2.52 (2H, t, *J*=7.8 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (28H, m), 0.88 (3H, t, *J*=6.8 Hz)
6c	3410, 2919, 2851, 1622, 1512	392 (M^+, 40), 167 (100)	6.08 (1H, d, *J*=2.3 Hz), 6.04 (1H, d, *J*=2.3 Hz), 4.68 (1H, br s), 3.77 (3H, s), 3.76 (3H, s), 2.52 (2H, t, *J*=7.6 Hz), 1.4—1.5 (2H, m), 1.2—1.4 (28H, m), 0.88 (3H, t, *J*=6.8 Hz)
6d	3340, 2918, 2850, 1600, 1508	362 (M^+, 85), 137 (100)	6.6—6.7 (2H, m), 6.61 (1H, dd, *J*=2.8, 8.7 Hz), 4.35 (1H, br s), 3.75 (3H, s), 2.56 (2H, t, *J*=7.8 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (28H, m), 0.88 (3H, t, *J*=6.9 Hz)
6e	3389, 2923, 2849, 1619, 1523	362 (M^+, 18), 137 (100)	6.99 (1H, d, *J*=8.3 Hz), 6.43 (1H, dd, *J*=2.5, 8.3 Hz), 6.37 (1H, d, *J*=2.5 Hz), 4.67 (1H, br s), 3.76 (3H, s), 2.52 (2H, t, *J*=7.7 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (28H, m), 0.88 (3H, t, *J*=6.8 Hz)
6f	3448, 2917, 2849, 1593, 1480	362 (M^+, 100), 137 (87)	6.7—6.8 (3H, m), 5.65 (1H, br s), 3.87 (3H, s), 2.62 (2H, t, *J*=7.8 Hz), 1.6—1.7 (2H, m), 1.2—1.4 (28H, m), 0.88 (3H, t, *J*=6.8 Hz)
6g	3510, 2920, 2850, 1591, 1518	362 (M^+, 98), 137 (100)	6.7—6.8 (2H, m), 6.64 (1H, dd, *J*=2.0, 8.1 Hz), 5.53 (1H, br s), 3.86 (3H, s), 2.50 (2H, t, *J*=7.7 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (28H, m), 0.88 (3H, t, *J*=6.9 Hz)
6h	3506, 2919, 2852, 1603, 1522	362 (M^+, 74), 137 (100)	6.82 (1H, d, *J*=7.8 Hz), 6.7—6.8 (2H, m), 5.43 (1H, br s), 3.87 (3H, s), 2.52 (2H, t, *J*=7.7 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (28H, m), 0.88 (3H, t, *J*=6.6 Hz)
6i	3408, 2918, 2850, 1595, 1510	392 (M^+, 66), 167 (100)	6.43 (1H, d, *J*=1.8 Hz), 6.28 (1H, d, *J*=1.8 Hz), 5.68 (1H, br s), 3.87 (3H, s), 3.85 (1H, s), 2.50 (2H, t, *J*=7.8 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (28H, m), 0.88 (3H, t, *J*=6.9 Hz)
6j	3467, 2919, 2850, 1614, 1473	376 (M^+, 100), 151 (94)	6.7—6.8 (3H, m), 5.71 (1H, br s), 4.09 (2H, q, *J*=7.0 Hz), 2.62 (2H, t, *J*=7.8 Hz), 1.6—1.7 (2H, m), 1.43 (3H, t, *J*=7.0 Hz) 1.2—1.4 (28H, m), 0.88 (3H, t, *J*=6.8 Hz)
6k	3319, 2920, 2849, 1591, 1506	332 (M^+, 84), 107 (100)	7.11 (1H, dd, *J*=1.4, 7.6 Hz), 7.07 (1H, ddd, *J*=1.4, 7.6, 7.6 Hz), 6.86 (1H, ddd, *J*=1.4, 7.6, 7.6 Hz), 6.75 (1H, dd, *J*=1.4, 7.6 Hz), 4.62 (1H, br s), 2.59 (2H, t, *J*=7.8 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (28H, m), 0.88 (3H, t, *J*=6.8 Hz)
6l	3361, 2917, 2849, 1587, 1500	332 (M^+, 31), 107 (100)	7.13 (1H, dd, *J*=7.6, 7.6 Hz), 6.7—6.8 (1H, m), 6.6—6.7 (2H, m), 4.62 (1H, br s), 2.55 (2H, t, *J*=7.7 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (28H, m), 0.88 (3H, t, *J*=6.9 Hz)
6m	3412, 2920, 2849, 1616, 1517	332 (M^+, 40), 107 (100)	7.04 (2H, dd, *J*=2.0, 6.5 Hz), 6.74 (2H, dd, *J*=2.0, 6.5 Hz), 4.56 (1H, br s), 2.52 (2H, t, *J*=7.7 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (28H, m), 0.88 (3H, t, *J*=6.9 Hz)
6n	3460, 3345, 2919, 2851, 1607, 1530	348 (M^+, 100), 123 (72)	8.58 (2H, br), 6.60 (1H, d, *J*=8.0 Hz), 6.53 (1H, d, *J*=2.0 Hz), 6.39 (1H, dd, *J*=2.0, 8.0 Hz), 2.37 (2H, t, *J*=7.8 Hz), 1.4—1.5 (2H, m), 1.2—1.3 (28H, m), 0.85 (3H, t, *J*=6.8 Hz)
6o	3387, 2921, 2849, 1596, 1478	348 (M^+, 100), 123 (82)	6.6—6.7 (3H, m), 5.12 (1H, br s), 5.06 (1H, br s), 2.60 (2H, t, *J*=7.8 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (28H, m), 0.88 (3H, t, *J*=6.8 Hz)
7	3434, 2917, 2850, 1599, 1491	392 (M^+, 100), 167 (56)	6.63 (1H, d, *J*=8.8 Hz), 6.32 (1H, d, *J*=8.8 Hz), 5.67 (1H, br s), 3.84 (3H, s), 3.76 (3H, s), 2.64 (2H, t, *J*=7.7 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (28H, m), 0.88 (3H, t, *J*=6.7 Hz)
8a	3492, 3292, 2920, 2848, 1488, 1468	364 (M^+, 100), 139 (93)	8.35 (1H, br), 8.28 (1H, br), 7.77 (1H, br), 6.37 (1H, d, *J*=8.5 Hz), 6.06 (1H, d, *J*=8.5 Hz), 2.46 (2H, t, *J*=7.7 Hz), 1.4—1.5 (2H, m), 1.2—1.3 (28H, m), 0.85 (3H, t, *J*=6.8 Hz)
8b	3372, 2916, 2851, 1617, 1536	364 (M^+, 43), 139 (100)	8.33 (3H, br), 6.06 (2H, s), 2.29 (2H, t, *J*=7.5 Hz), 1.5—1.6 (2H, m), 1.2—1.3 (28H, m), 0.85 (3H, t, *J*=6.8 Hz)
8c	3308, 2919, 2849, 1617, 1522	364 (M^+, 21), 139 (100)	8.67 (2H, br s), 8.64 (1H, br s), 5.74 (2H, s), 2.33 (2H, t, *J*=7.3 Hz), 1.3—1.4 (2H, m), 1.2—1.3 (28H, m), 0.85 (3H, t, *J*=6.7 Hz)
8d	3280, 2919, 2849, 1617, 1459	348 (M^+, 53), 123 (100)	8.45 (1H, br), 8.38 (1H, br), 6.54 (1H, d, *J*=8.7 Hz), 6.43 (1H, d, *J*=3.0 Hz), 6.36 (1H, dd, *J*=3.0, 8.7 Hz), 2.40 (2H, t, *J*=7.7 Hz), 1.4—1.5 (2H, m), 1.2—1.3 (28H, m), 0.85 (3H, t, *J*=6.8 Hz)
8e	3382, 3314, 2918, 2849, 1607, 1526	348 (M^+, 38), 123 (100)	8.92 (1H, br), 8.87 (1H, br), 6.74 (1H, d, *J*=8.0 Hz), 6.24 (1H, d, *J*=2.4 Hz), 6.10 (1H, dd, *J*=2.4, 8.0 Hz), 2.36 (2H, t, *J*=7.8 Hz), 1.4—1.5 (2H, m), 1.2—1.3 (28H, m), 0.85 (3H, t, *J*=6.8 Hz)
8f	3471, 3289, 2918, 2849, 1608, 1519	378 (M^+, 59), 153 (100)	6.43 (1H, d, *J*=1.7 Hz), 6.29 (1H, d, *J*=1.7 Hz), 5.21 (1H, br s), 5.19 (1H, br s), 3.86 (3H, s), 2.48 (2H, t, *J*=7.7 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (28H, m), 0.88 (3H, t, *J*=6.8 Hz)

a) ^{1}H-NMR spectra were taken in $CDCl_3$ except for compounds **6n** and **8a**—**e** in DMSO-d_6 (ppm).

Table 4. Physical Data for 2-Alkyl-4-methoxyphenols

Compound No.	Yield[a] (%)	mp (°C)	Formula	Analysis (%) Calcd C	Calcd H	Found C	Found H
10a	46	30.7—32.2	$C_{15}H_{24}O_2$	76.23	10.23	76.15	10.60
10b	25	40.6—41.5	$C_{16}H_{26}O_2$	76.75	10.47	76.61	10.75
10c	40	45.6—47.2	$C_{17}H_{28}O_2$	77.22	10.67	77.37	10.83
10d	12	51.8—53.5	$C_{18}H_{30}O_2$	77.65	10.86	77.83	11.27
10e	45	55.4—57.2	$C_{19}H_{32}O_2$	78.03	11.03	77.87	11.37
10f	40	59.5—61.1	$C_{20}H_{34}O_2$	78.38	11.18	78.26	11.64
10g	26	65.1—65.8	$C_{21}H_{36}O_2$	78.70	11.32	78.92	11.64
10h	11	70.6—71.0	$C_{22}H_{38}O_2$	78.99	11.45	79.05	11.65
10i	23	71.2—72.0	$C_{23}H_{40}O_2$	79.25	11.57	79.11	11.88
10j	6	75.4—76.6	$C_{25}H_{44}O_2$	79.73	11.78	79.53	12.05
10k	3	79.9—81.3	$C_{26}H_{46}O_2$	79.94	11.87	79.56	11.98

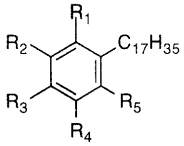
a) Total yields obtained from the Wittig reaction and hydrogenation.

Table 5. IR, MS and ^{1}H-NMR[a] Data for 2-Alkyl-4-methoxyphenols

Compound No.	IR ν_{max}^{KBr} cm^{-1}	EI-MS *m/z*	^{1}H-NMR (δ_H)
10a	3327, 2923, 2853, 1601, 1509	236 (M^+, 100), 137 (85)	6.6—6.7 (2H, m), 6.62 (1H, dd, *J*=2.9, 8.6 Hz), 4.37 (1H, br s), 3.75 (3H, s), 2.56 (2H, t, *J*=7.8 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (10H, m), 0.88 (3H, t, *J*=6.9 Hz)
10b	3331, 2922, 2853, 1600, 1509	250 (M^+, 100), 137 (91)	6.6—6.7 (2H, m), 6.62 (1H, dd, *J*=2.9, 8.7 Hz), 4.37 (1H, br s), 3.75 (3H, s), 2.56 (2H, t, *J*=7.8 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (12H, m), 0.88 (3H, t, *J*= 6.9 Hz)
10c	3326, 2922, 2852, 1600, 1509	264 (M^+, 100), 137 (94)	6.6—6.7 (2H, m), 6.62 (1H, dd, *J*=3.0, 8.6 Hz), 4.38 (1H, br s), 3.75 (3H, s), 2.56 (2H, t, *J*=7.8 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (14H, m), 0.88 (3H, t, *J*=6.9 Hz)
10d	3337, 2922, 2852, 1601, 1509	278 (M^+, 100), 137 (72)	6.6—6.7 (2H, m), 6.61 (1H, dd, *J*=2.9, 8.7 Hz), 4.39 (1H, br), 3.75 (3H, s), 2.56 (2H, t, *J*=7.8 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (16H, m), 0.88 (3H, t, *J*=6.8 Hz)
10e	3337, 2921, 2852, 1601, 1509	292 (M^+, 92), 137 (100)	6.6—6.7 (2H, m), 6.61 (1H, dd, *J*=2.8, 8.9 Hz), 4.37 (1H, br s), 3.75 (3H, s), 2.56 (2H, t, *J*=7.8 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (18H, m), 0.88 (3H, t, *J*=6.8 Hz)
10f	3337, 2921, 2851, 1600, 1509	306 (M^+, 57), 137 (100)	6.6—6.7 (2H, m), 6.61 (1H, dd, *J*=2.9, 8.7 Hz), 4.39 (1H, br), 3.75 (3H, s), 2.56 (2H, t, *J*=7.8 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (20H, m), 0.88 (3H, t, *J*=6.9 Hz)
10g	3338, 2921, 2851, 1600, 1509	320 (M^+, 80), 137 (100)	6.6—6.7 (2H, m), 6.62 (1H, dd, *J*=2.9, 8.7 Hz), 4.36 (1H, br s), 3.75 (3H, s), 2.56 (2H, t, *J*=7.8 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (22H, m), 0.88 (3H, t, *J*=6.8 Hz)
10h	3339, 2920, 2851, 1600, 1509	334 (M^+, 100), 137 (70)	6.6—6.7 (2H, m), 6.62 (1H, dd, *J*=2.8, 8.7 Hz), 4.36 (1H, br), 3.75 (3H, s), 2.56 (2H, t, *J*=7.7 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (24H, m), 0.88 (3H, t, *J*=6.8 Hz)
10i	3339, 2920, 2851, 1600, 1508	348 (M^+, 80), 137 (100)	6.6—6.7 (2H, m), 6.62 (1H, dd, *J*=2.8, 8.8 Hz), 4.35 (1H, br), 3.75 (3H, s), 2.56 (2H, t, *J*=7.8 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (26H, m), 0.88 (3H, t, *J*=6.8 Hz)
10j	3349, 2920, 2851, 1600, 1508	376 (M^+, 100), 137 (91)	6.6—6.7 (2H, m), 6.62 (1H, dd, *J*=2.8, 8.8 Hz), 4.35 (1H, br), 3.75 (3H, s), 2.56 (2H, t, *J*=7.8 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (30H, m), 0.88 (3H, t, *J*=6.9 Hz)
10k	3341, 2920, 2850, 1600, 1508	390 (M^+, 78), 137 (100)	6.6—6.7 (2H, m), 6.62 (1H, dd, *J*=2.9, 8.9 Hz), 4.32 (1H, br), 3.75 (3H, s), 2.56 (2H, t, *J*=7.8 Hz), 1.5—1.6 (2H, m), 1.2—1.4 (32H, m), 0.88 (3H, t, *J*=6.9 Hz)

a) ^{1}H-NMR spectra were taken in $CDCl_3$ (ppm).

Table 6. Antioxidative Activity for Heptadecylphenols



Compound No.	R_1	R_2	R_3	R_4	R_5	IC_{50} (μM)
1	OH	OCH_3	H	OCH_3	H	26.99
4	OH	Br	H	Br	H	92.42
6a	OH	OCH_3	H	H	Br	50.00
6b	H	OCH_3	OH	OCH_3	H	19.93
6c	OH	H	OCH_3	H	OCH_3	13.09
6d	OH	H	H	OCH_3	H	13.56
6e	OH	H	OCH_3	H	H	29.33
6f	OH	OCH_3	H	H	H	41.78
6g	H	OH	OCH_3	H	H	25.42
6h	H	OCH_3	OH	H	H	38.13
6i	H	OH	OCH_3	OCH_3	H	31.77
6j	OH	OC_2H_5	H	H	H	33.15
6k	OH	H	H	H	H	15.17
6l	H	OH	H	H	H	105.17
6m	H	H	OH	H	H	>300
6n	H	OH	OH	H	H	16.58
6o	OH	OH	H	H	H	6.50
7	OH	OCH_3	H	H	OCH_3	12.25
8a	OH	OH	H	H	OH	13.80
8b	H	OH	OH	OH	H	21.33
8c	OH	H	OH	H	OH	52.59
8d	OH	H	H	OH	H	12.66
8e	OH	H	OH	H	H	21.18
8f	H	OH	OH	OCH_3	H	11.91

added dropwise a solution of 1 M boron tribromide in CH_2Cl_2 (8.63 ml, 8.63 mmol) under an argon atmosphere at −20 °C. The mixture was then stirred at room temperature for 3 h. The reaction mixture was poured into ice water and extracted with EtOAc. The extract was washed with water and brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was purified by column chromatography (*n*-hexane–EtOAc, 2 : 1) to give **8a** (1.42 g) as colorless needles in 99% yield. Physical data are given in Tables 2 and 3.

Compounds **8b—f** were prepared as described above. In the synthesis of **8f**, 1.1 eq of 1 M boron tribromide solution was used. The physical data for these compounds are summarized in Tables 2 and 3.

Antioxidative Activity Antioxidative activity was examined by using the *tert*-butylhydroperoxide-initiated lipid peroxidation of rabbit erythrocyte ghost membrane.[17] Rabbit blood (100 ml) obtained from Japan Biotest Institute Co., Ltd. was diluted with 100 ml of isotonic buffer solution (10 mM phosphate/152 mM sodium chloride, pH 7.4). After centrifugation (1500 ***g***, 20 min, 4 °C), the red blood cells were collected and washed three times with 100 ml of the isotonic buffer solution. Washed red blood cells were diluted with 100 ml of hypotonic buffer solution (10 mM phosphate, pH 7.4) and centrifuged (20000 ***g***, 40 min, 4 °C). Erythrocyte membrane ghosts were collected and washed six times with 100 ml of the hypotonic buffer solution. The precipitate was diluted with the hypotonic buffer solution to give a suspension (1—2.5 mg of protein/ml) and used for the antioxidative assay. Synthetic compounds were dissolved in DMSO and the sample solution (100 μl) was mixed with the ghost suspension (850 μl) and 24 mM *tert*-butylhydroperoxide solution (50 μl). After incubation at 37 °C for 30 min, 1 ml of 2.0 M trichloroacetic acid/1.7 M HCl solution and 2 ml of 0.67% thiobarbituric acid (TBA) solution (including a pellet of NaOH per liter) were added to quench the reaction. The quantity of TBA reactive substances was determined at 535 nm using a UV spectrophotometer. As a positive control, DL-α-tocopherol was used and the values without test compounds were taken as 100% lipid peroxidation.

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