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SYNTHESIS AND ANTIOXIDANT ACTIVITY OF NOVEL AMPHIPATHIC DERIVATIVES OF TEA POLYPHENOL

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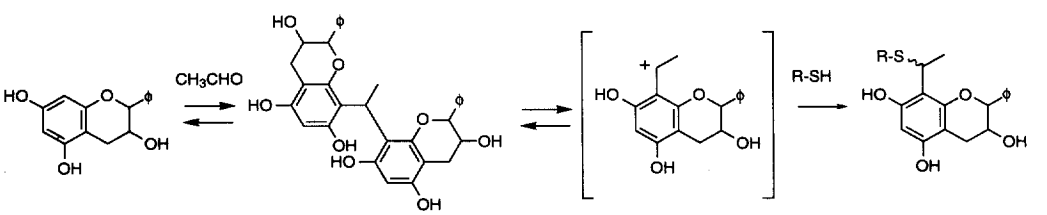
Abstract: Hydrophobic derivatives of a tea polyphenol have been synthesized. 6, 8-Bis(octylthiomethyl)-epigallocatechin 3-*O*-gallate, 6, 8-bis(octylthiomethyl)-4β-(2-hydroxyethylthio)epigallocatechin 3-*O*-gallate and epigallocatechin 3-*O*-[4-*O*-(*N*-octadecylcarbamoyl)gallate] showed strong inhibition activity against lipid peroxidation of liposome caused by both lipid-soluble and water-soluble radical generators.

Recent epidemiological and pharmacological studies on green tea and red wine suggested possible health benefits derived from plant polyphenols.[1] One of the most important biological activity is the radical scavenging effect which inhibit oxidation of plasma low density lipoproteins; hence, polyphenols is a potent protective agent against coronary heart disease and atherosclerosis. Epigallocatechin 3-*O*-gallate (**1**), the most active radical scavenger in tea polyphenols, inhibited lipid peroxidation in liposome lipid bilayer caused by water soluble radical initiator [2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH)] (Table 1). However, **1** was not effective against the peroxidation caused by lipophilic radical initiator [2,2'-azobis (2,4-dimethylvaleronitrile) (AMVN)], because **1** does not penetrate into the hydrophobic region of lipid bilayer. In order to design an amphipathic antioxidant having radical scavenging ability in both hydrophilic and hydrophobic environment, we have synthesized hydrophobic derivatives of **1** and compared their inhibition activity against lipid peroxidation.

Chemistry

It is known that flavan-3-ols and proanthocyanidins react with aldehyde to give unstable dimers, which are easily decomposed by addition of thiol compounds to yield sulfides (Scheme 1).[2] First, this reaction was



Scheme 1

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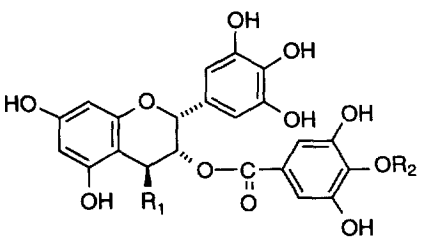
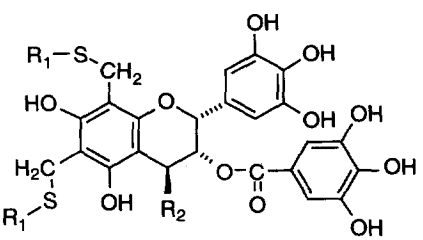
applied to the synthesis of hydrophobic derivatives of **1** having two substituents on the A ring. It was simple and one-pot procedure: *i.e.* to a solution of (-)-epigallocatechin-3-*O*-gallate (**1**) and excess of thiol compound in 10% acetic acid-ethanol was added 37% formaldehyde aqueous solution, and the mixture was heated at 80°C for 2–4 h. Successive separation by column chromatography on Sephadex LH-20 eluting with ethanol gave **3** (yield: 75 %),[3] **4** (60 %),[4] **5** (26 %),[5] **6** (60 %),[6] **7** (26 %),[7] and **8** (44 %).[8] Next, 4β-(2-hydroxyethylthio)epigallocatechin 3-*O*-gallate (**2**), which obtained by degradation of polymeric proanthocyanidins,[2] was treated with 1-octanethiol in a similar manner to yield derivatives having three substituents on the A and C rings [**9**[9] (15 %) and **10**[10] (38 %)]. Furthermore, a derivative **11** having a monoalkyl chain was obtained from **1** reacted with *n*-octadecylisocyanate in ethanol at room temperature (27%).[11] Distribution of these derivatives between *n*-octanol and water (partition coefficient values in Table 1) indicated that hydrophobicity of these derivatives was significantly higher than that of **1** and **2**.



	R_1	R_2
1:	H	H
2:	SCH_2CH_2OH	H
11:	H	CO-NH-$C_{18}H_{37}$



	R_1	R_2
3:	C_8H_{17}	H
4:	$CH_2C_6H_5$	H
5:	$CH_2CH{=}CH_2$	H
6:	$CH_2CO_2CH_3$	H
7:	$C_{16}H_{33}$	H
8:	$C_{18}H_{37}$	H
9:	C_8H_{17}	SC_8H_{17}
10:	C_8H_{17}	SCH_2CH_2OH

Figure 1. Chemical structure of the derivatives

Inhibition of lipid peroxidation.

Inhibition activities (IC_{50} value) against lipid peroxidation of egg-phosphatidylcholine (PC) liposome[12] were evaluated by measuring the concentration of thiobarbituric acid reactive substance (Table 1).[13] Compared to **1**, pentagalloylglucose and *n*-octyl gallate, compounds **3**, **9**, **10** and **11** strongly inhibited lipid peroxidation caused by AMVN. In particular, **9** and **11**, which have three C_8-alkyl chains and a C_{18}-alkyl chain, respectively, showed the strongest inhibition activity. On the other hand, the activities of **4, 5** and **6** were similar to that of **1**. These results apparently suggested that the derivatives having long alkyl chains penetrated into the hydrophobic region of lipid bilayer and scavenged radicals.

Compounds **7** and **8** having C_{16} and C_{18} alkyl chains, respectively, strongly aggregated and precipitated the lipids; hence, the evaluation IC_{50} values was impossible. This aggregation was probably

caused by, in addition to penetration of two alkyl chains into bilayer, adsorption of the epigallocatechin-3-*O*-gallate moiety on the surface of phosphatidylcholine bilayers, and formation of an interbilayer bridge between apposing two bilayers by these large molecules (Fig. 1).[14] The possibility of the adsorption of **1** on lipid bilayer was supported by following observation: 1) on ultrafiltration of an aqueous solution of **1**, the permeability of **1** through membrane filter was reduced in the presence of liposome; 2) the ^{1}H-NMR signals of **1** in deuterium oxide were significantly broadened by addition of liposome; and 3) electron microscopic observation showed morphological change (adhesion and fusion) of liposome in the presence of high concentration of **1**. In addition, despite its high hydrophobicity, compound **4** having π-electron rich benzyl groups showed strong inhibition activity against AAPH and relatively low activity against AMVN, indicating that this compound was located on the surface of the bilayer. This fact suggested that electrostatic interaction between π-electron of aromatic rings and -$N(CH_3)_3$ groups on the phosphatidylcholine headgroups is of importance for the adsorption.[14] Since polyphenols are multiple hydrogen-bond donor, hydrogen bonding with hydrogen-bond-accepting groups on bilayer probably increase the overall strength of the adsorption. From these results, it was presumed that the epigallocatechin 3-*O*-gallate moiety of the derivatives, such as **3** and **10**, was electrostatically adsorbed on the ionic surface of bilayer, and the alkyl chains were anchored into the hydrophobic region (Fig. 1). The most hydrophobic derivative **9** having three C_8 alkyl chains showed the highest inhibition activity against AMVN and the lowest activity against AAPH. This derivative was supposed to be present in the hydrophobic region. Compound **11**, which have a C_{18}-long chain alkyl group on its galloyl group, did not aggregated lipids and showed high inhibition activity against lipid peroxidation caused by both water-soluble and lipid-soluble radical generators. Masking of a phenolic hydroxy group of galloyl residue by carbamoyl ester may weaken the interaction with membrane surface, and the molecule may penetrate deeper into hydrophobic region.

Table 1. Inhibition activity against lipid peroxidation of egg-PC liposome caused by water-soluble (AAPH) and lipid-soluble (AMVN) radical initiators, and the partition coefficient (P) between *n*-octanol and water.[a]

	IC_{50} (μM)		P
	AAPH	AMVN	(27°C)
1	20.8±3	>500	8
2	20.5±7	>500	12
3	7.9±0.5	60.0±11.3	10^4
4	9.6±0.9	432.0±91.8	4×10^3
5	11.6±1.1	>500	480
6	12.0±1.8	>500	9
9	135.1±39.3	19.4±3.1	8.9×10^4
10	8.8±0.4	60.8±22.3	1.6×10^3
11	24.6±4.9	22.1±9.3	1.3×10^3
pentagalloylglucose	10.0±0.4	>500	92
n-octyl gallate	18.6±2.4	188.3±38.1	2.5×10^4
α-tocopherol	40.3±10.2	5.9±0.9	-

a Partition coefficient values between *n*-octanol and water were evaluated by comparison of HPLC peak area.

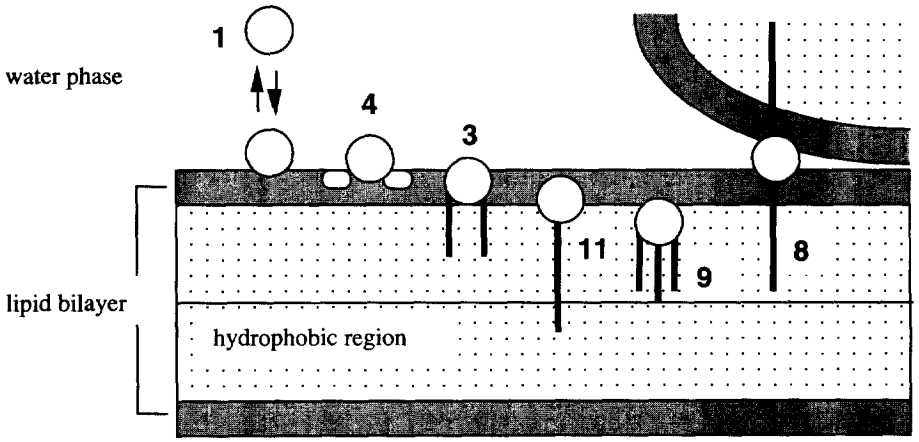


Fig. 1 Plausible interactions of **1**, **3**, **4**, **8** and **11** with lipid bilayer

Conclusion.

The compounds **3**, **10** and **11** are the first tea polyphenol derivatives with strong inhibition activity against lipid peroxidation in liposome lipid bilayer caused by both lipid-soluble and water-soluble radical generators. The relation between the structure and the activity suggested that, in addition to hydrophobicity, the interaction of the polyphenol moiety with surface of the lipid bilayer affected penetration of the derivatives into the hydrophobic region of the bilayer.

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References and Notes

1. Ramarathnan, N.; Osawa, T.; Ochi, H.; Kawakishi, S. *Trends in Food Sci. Tech.*, **1995**, 75–82; Guo, Q.; Zhao, B.; Li, M.; Shen, S.; Xin, W. *Biochim. Biophys. Acta*, **1996**, *1304*, 210–222; Jankun, J.; Selman, S.H.; Swiercz, R.; Skrzypczak-Jankun, E. *Nature*, **1997**, *387*, 561.
2. Tanaka, T.; Takahashi, R.; Kouno, I.; Nonaka, G. *J. Chem. Soc. Perkin Trans. 1*, **1994**, 3013–3022.
3. **3**: $[\alpha]_D$ -95.8° (*c*=0.9, acetone); Negative FABMS *m/z*: 773 $(M-H)^-$; Found: C, 60.86; H, 6.83. $C_{40}H_{54}O_{11}S_2 \cdot 3/4\ H_2O$ requires C, 69.93; H, 7.09. ^1H NMR (300 MHz, acetone-d_6) δ: 0.86 (6H, m, CH_3), 1.26 (20H, m, CH_2), 1.55 (4H, m, -S-CH_2-CH_2-), 2.49 (4H, m, -S-CH_2-), 2.98 (1H, dd, *J* =17, 2 Hz, H-4a), 3.16 (1H, dd, *J* =17, 5 Hz, H-4b), 3.85, 3.88, 3.93, 4.04 (each 1H, d, *J* =13 Hz, benzyl), 5.10 (1H, s, H-2), 5.61 (1H, m, H-3), 6.68 (2H, s, H-2', 6') 7.01 (2H, s, galloyl-2, 6).
4. **4**: $[\alpha]_D$ -76.4° (*c*=0.8, acetone); Negative FABMS *m/z*: 729 $(M-H)^-$; Found: C, 61.17; H, 4.99.

$C_{38}H_{34}O_{11}S_2 \cdot 3/4\ H_2O$ requires C, 61.32; H, 4.81. ^{1}H NMR (300 MHz, acetone-d_6) δ: 2.99 (1H, dd, J =17, 2 Hz, H-4a), 3.16 (1H, dd, J =17, 5 Hz, H-4b), 3.78, 3.91 (each 2H, s, -S-CH_2-), 3.75, 3.80, 3.84, 4.05 (each 1H, d, J =13 Hz, benzyl), 5.13 (1H, s, H-2), 5.61 (1H, m, H-3), 6.70 (2H, s, H-2, 6), 7.03 (2H, s, galloyl-2, 6), 7.1-7.3 (10H, m, Ar-H).

5. **5**: $[\alpha]_D^{14}$ -133.7° (c=0.8 acetone); Negative FABMS m/z: 629 $(M-H)^-$; Found: C, 55.20; H, 4.81. $C_{30}H_{30}O_{11}S_2 \cdot H_2O$ requires C, 55.55; H, 4.97. ^{1}H NMR (300 MHz, acetone-d_6) δ: 2.94-3.24 (6H, m, H-4a,b, and allyl-1), 3.86, 3.99 (each 1H, d, J =13 Hz, benzyl), 3.87 (2H, s, benzyl), 4.96, 5.02 (each 1H, br d, J =10 Hz, allyl-3), 5.12, 5.16 (each 1H, br d, J =17 Hz, allyl-3), 5.12 (1H, s, H-2), 5.61 (1H, m, H-3), 5.85 (2H, m, allyl-2), 6.69 (2H, s, H-2', 6'), 7.01 (2H, s, galloyl-2, 6).
6. **6**: $[\alpha]_D^{18}$ -109.5° (c=0.8 acetone); Negative FABMS m/z: 693 $(M-H)^-$; Found: C, 51.64; H, 4.69. $C_{30}H_{30}O_{15}S_2 \cdot 1/4\ H_2O$ requires C, 51.53; H, 4.40. ^{1}H NMR (300 MHz, acetone-d_6) δ: 2.99 (1H, dd, J =17, 2 Hz, H-4a), 3.15 (1H, dd, J =17, 4 Hz, H-4b), 3.34, 3.41 (each 1H, d, J =16 Hz, -S-CH_2-), 3.63, 3.70 (each 3H, s, OMe), 3.94, 4.10 (each 1H, d, J =13 Hz, benzyl), 3.98 (2H, s, benzyl), 5.14 (1H, s, H-2), 5.61 (1H, m, H-3), 6.69 (2H, s, H-2', 6'), 7.02 (2H, s, galloyl-2, 6).
7. **7**: $[\alpha]_D^{18}$ -74.3° (c=0.8 acetone); Negative FABMS m/z: 997 $(M-H)^-$; Found: C, 66.36; H, 8.57. $C_{56}H_{86}O_{11}S_2 \cdot 3/4\ H_2O$ requires C, 66.4; H, 8.71. ^{1}H NMR (300 MHz, acetone-d_6) δ: 0.88 (6H, t, J =7 Hz, CH_3), 1.28 (52H, m, CH_2), 1.56 (4H, m, -S-CH_2-C$\underline{H}_2$-), 2.49 (4H, m, -S-CH_2-), 2.99 (1H, dd, J =17, 3 Hz, H-4a), 3.15 (1H, dd, J =17, 5 Hz, H-4b), 3.86, 3.88, 3.93, 4.04 (each 1H, d, J =13 Hz, benzyl), 5.10 (1H, s, H-2), 5.61 (1H, m, H-3), 6.68 (2H, s, H-2', 6'), 7.01 (2H, s, galloyl-2, 6).
8. **8**: $[\alpha]_D^{18}$ -59.6° (c=0.8 acetone); Negative FABMS m/z: 1053 $(M-H)^-$; Found: C, 67.12; H, 9.05. $C_{60}H_{94}O_{11}S_2 \cdot H_2O$ requires C, 67.13; H, 9.01. ^{1}H NMR (300 MHz, acetone-d_6) δ: 0.88 (6H, t, J =7 Hz, CH_3), 1.29 (60H, m, CH_2), 1.56 (4H, m, -S-CH_2-C$\underline{H}_2$-), 2.49 (4H, m, -S-CH_2-), 2.98 (1H, dd, J =17, 2 Hz, H-4a), 3.15 (1H, dd, J =17, 5 Hz, H-4b), 3.86, 3.87, 3.93, 4.04 (each 1H, d, J =13 Hz, benzyl), 5.10 (1H, s, H-2), 5.61 (1H, m, H-3), 6.68 (2H, s, H-2', 6'), 7.01 (2H, s, galloyl-2, 6).
9. **9**: $[\alpha]_D^{18}$ -84.2° (c=0.8 acetone); Negative FABMS m/z: 917 $(M-H)^-$; Found: C, 60.68; H, 7.28. $C_{48}H_{70}O_{11}S_3 \cdot 3/2\ H_2O$ requires C, 60.92; H, 7.78. ^{1}H NMR (300 MHz, acetone-d_6) δ: 0.88 (9H, m, CH_3), 1.26 (30H, m, CH_2), 1.56 (4H, m, -S-CH_2-C$\underline{H}_2$-), 1.81 (2H, m, C-4-S-CH_2-C$\underline{H}_2$-), 2.47 (4H, m, -S-CH_2-), 2.95, 3.08 (each 1H, dt, J =13, 7 Hz, C-4-S-C$\underline{H}_2$-), 3.84, 3.85, 3.92, 4.05 (each 1H, d, J =13 Hz, benzyl), 4.27 (1H, d, J =2 Hz, H-4), 5.43 (1H, m, H-3), 5.48(1H, s, H-2), 6.71 (2H, s, H-2', 6'), 6.98 (2H, s, galloyl-2, 6).
10. **10**: $[\alpha]_D^{18}$ -100.1° (c=0.8 acetone); Negative FABMS m/z: 849 $(M-H)^-$; Found: C, 58.50; H, 6.66. $C_{42}H_{58}O_{12}S_3 \cdot 1/2\ H_2O$ requires C, 58.65; H, 6.91. ^{1}H NMR (300 MHz, acetone-d_6) δ: 0.86 (6H, m, CH_3), 1.26 (20H, m, CH_2), 1.55 (4H, m, -S-CH_2-C$\underline{H}_2$-), 2.48 (4H, m, -S-CH_2-), 2.89 (1H, dt, J =14, 3 Hz, -C$\underline{H}_2$-CH_2OH), 3.32 (1H, m, -C$\underline{H}_2$-CH_2OH), 3.80, 3.83, 3.91, 4.01 (each 1H, d, J =13 Hz, benzyl), 3.85, 4.24 (each 1H, m, J =14 Hz, -C$\underline{H}_2$OH), 4.30 (1H, d, J =2 Hz, H-4), 5.39 (1H, s, H-2), 5.40 (1H, m, H-3), 6.71 (2H, s, H-2', 6'), 6.98 (2H, s, galloyl-2, 6).
11. **11**: $[\alpha]_D^{15}$ -110.3° (c=0.8 acetone); Positive FABMS m/z: 754 $(M+H)^+$; Found: C, 63.63; H, 7.27; N, 1.90. $C_{41}H_{55}O_{12}N \cdot H_2O$ requires C, 63.80; H, 7.44; N, 1.81. ^{1}H NMR (300 MHz, acetone-d_6) δ: 0.88 (3H, t, J =6 Hz, CH_3), 1.29 (32H, m, CH_2), 1.54 (2H, m, -S-CH_2-C$\underline{H}_2$-), 2.48 (4H, m, -S-CH_2-), 2.89 (1H, dt, J =14, 3 Hz, -C$\underline{H}_2$-CH_2OH), 3.32 (1H, m, -C$\underline{H}_2$-N), 2.91 (1H, dd, J =16, 2 Hz, H-4), 3.60 (1H, dd, J =16, 4 Hz, H-4), 5.08 (1H, s, H-2), 5.60 (1H, m, H-3), 6.03, 6.06 (each 1H, d, J = 2 Hz, H-6 and H-

8), 6.62 (2H, s, H-2', 6'), 7.02 (2H, s, galloyl-2, 6). The location of carbamoyl group was determined by analysis of the ^{13}C NMR spectrum, which showed upfield shift of the galloyl C-4 ($\Delta\delta$ -6.0) signal and low field shift of the galloyl C-1 ($\Delta\delta$ 6.6) and C-3, 5 ($\Delta\delta$ 5.6) signals compared to those of **1**. Presence of a minor isomer which have the carbamoyl group at 3-*O*-position of the galloyl group was presumed by observation of *meta*-coupled aromatic proton signals at δ 7.28 and 7.32.

12. Preparation of liposome was as follows: egg-phosphatidylcholine (sigma, 47 mg) was dissolved in chloroform, and the solvent was removed by evaporation to obtain a thin film on a flask wall. Degassed water (13 ml) was added and liposomes of the lipids were prepared by vortex mixing, followed by probe-type sonication at a power of 50W for 10 min at 0°C under a stream of nitrogen. Formation of small unilamellar liposome was confirmed by electron microscopic observation ($\times 10^5$).
13. The thiobarbitulic acid reactive substance was quantified by the method of Buege, J.A.; Aust, S.D. *Methods Enzymol.* **1978**, *52*, 302-310, and Yokozawa, T.; Dong, E.; Liu, Z.-W.; Oura, H.; Nishioka, I. Natural Medicines **1996**, *50*, 243-246. The reaction mixture was composed of liposome solution (0.65 ml), sample (DMSO solution 25 µl), AMVN (8 mM DMSO solution, 25 µl) and water (150 µl) [or AAPH (0.21 M aqueous solution, 150 µl) and DMSO (25 µl)]. The mixture was incubated at 37°C for 20 min in a capped tube, then 4 ml of stop solution, consisting of 0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25N HCl, was added to each tube, and all the tubes were heated at 100°C for 15 min. After a cooling period of 10 min in ice water, centrifugation was carried out at 3,000 rpm for 10 min, then determination of the supernatant was done spectrophotometrically at 535 nm. The concentration of TBA-reactive substance generated in the mixture was calculated using an absorption coefficient of 1.56×10^5 $M^{-1}cm^{-1}]^{-1}$.
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