Studies on the Preparation of Bioactive Lignans by Oxidative Coupling Reaction. I. Preparation and Lipid Peroxidation Inhibitory Effect of Benzofuran Lignans Related to Schizotenuins

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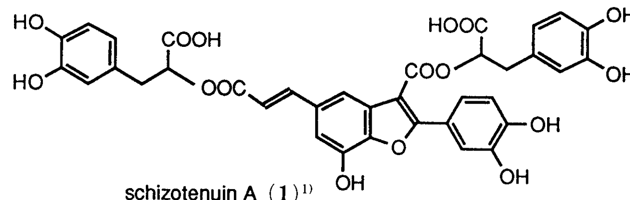
The parent benzofuran lignan 4 of schizotenuins 1—3 and related compounds were efficiently prepared by a judicious use of the oxidative coupling reaction, and were tested for their inhibitory effects on lipid peroxidation in rat brain homogenate and rat liver microsomes. Among twelve compounds tested in rat brain homogenate, compounds 13, 14 and 16 showed prominent inhibitory activity. Compounds 13 and 16 were then tested in rat liver microsomes, and their activity was found to be more potent than schizotenuin A (1) and much more potent than that of (±)-α-tocopherol.

Keywords lignan; oxidative coupling reaction; lipid peroxidation inhibitor; benzofuran

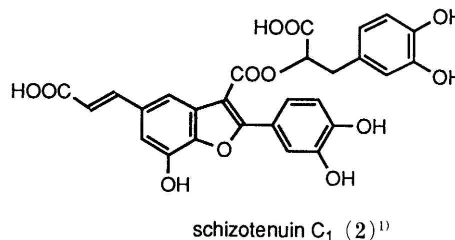
Schizotenuin A (**1**), schizotenuin C_1 (**2**) and schizotenuin C_2 (**3**), representing the oligomers of caffeic acid, are novel lignans having a benzofuran skeleton.[1] They have been isolated by Matsuta and his co-workers as the inhibitory constituents of 3α-hydroxy steroid dehydrogenase from the terrestrial part of *Schizonepeta tenuifolia* BRIQ. (Japanese name: Keigai), which has been used in traditional Chinese medicines, Bohu-tusyo-san, Keigai-rengyo-to and Zyumi-haidoku-to, as an anti-inflammatory Chinese crude drug. So far there have been many reports on the biologically active natural lignans which have a dihydrobenzofuran skeleton, *e.g.* hordatine A (antifungal activity),[2] a lignan isolated from *Zizyphus jujuba* MILL (PGI_2 inducer),[3] and magnesium lithospermate B (kallikrein activator and PGE_2 inducer).[4] However, those with a benzofuran skeleton were barely reported. Accordingly, we were interested in the biological activities of the benzofuran lignans related to schizotenuins **1**—**3**, and investigated establishing an efficient synthetic route to the parent benzofuran **4**[5] and testing its derivatives on their inhibitory effect against lipid peroxidation.

Synthesis For the synthesis of the compound **4** we conceived that the route based on biosynthesis would be the most convenient. The oxidative coupling reaction of caffeic acid (**5**, R = H) or its equivalent would afford the dihydrobenzofuran derivative **6**. Its dehydrogenation, followed by relevant conversion, would furnish our target compound **4**.

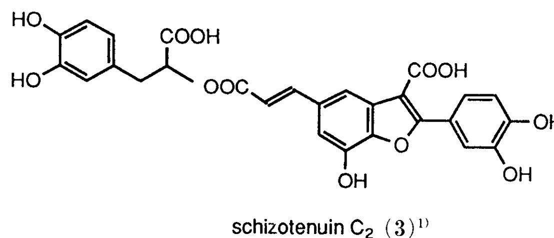
Whereas the one electron oxidation reaction of ferulic acid, a monomethyl ether of caffeic acid, was reported to give a dilactone compound,[6] the reactions of methyl caffeate[7] and methyl ferulate (**7**)[8] were known to afford corresponding dihydrobenzofuran compounds in 29% and 38% yields, respectively. Our investigation started from a re-examination of the oxidative coupling reaction of methyl ferulate (**7**), and we found that the same *trans*-dihydrobenzofuran **8** as reported was prepared in a yield of 50% on the treatment of **7** with silver oxide in



schizotenuin A (1)[1]



schizotenuin C_1 (2)[1]



schizotenuin C_2 (3)[1]

Chart 1

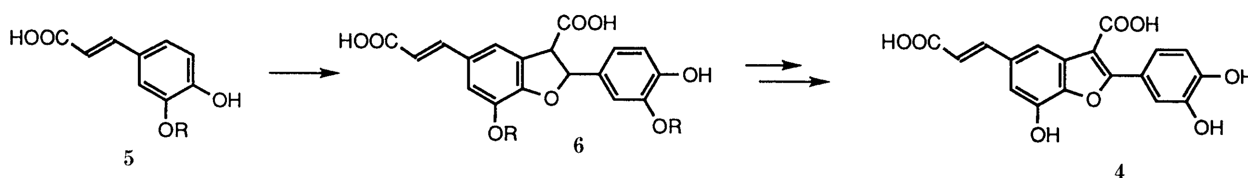


Chart 2

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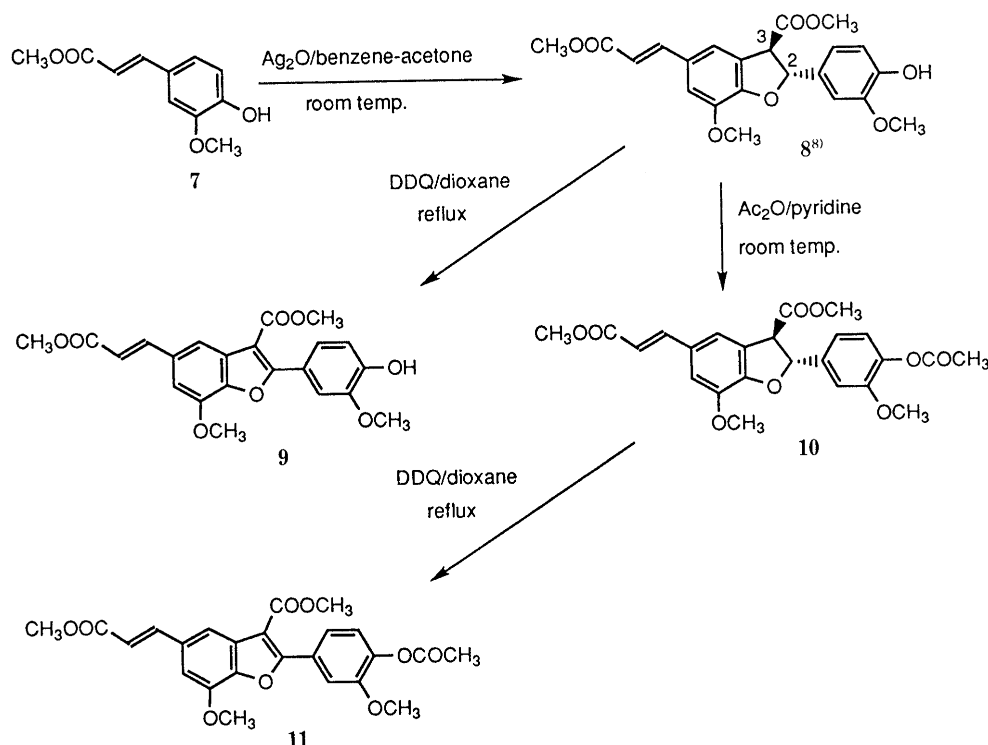

Chart 3

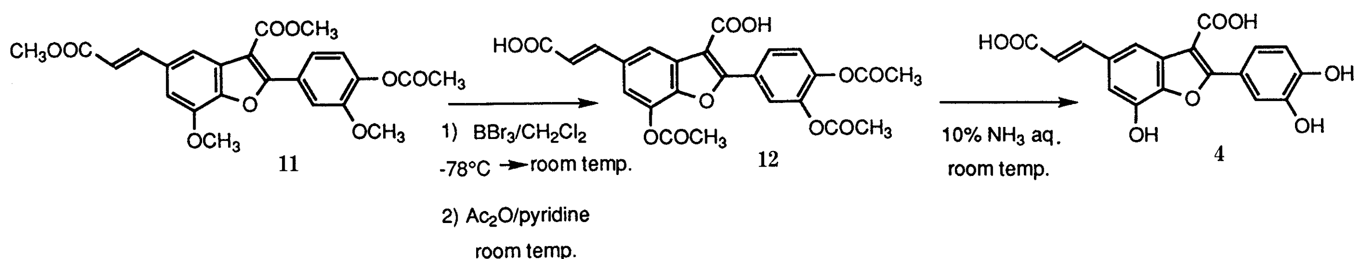

Chart 4

benzene–acetone.

The dihydrobenzofuran **8** was then dehydrogenated by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dry dioxane to afford the desired benzofuran **9**, but only in a low yield of 20%. The reason for the low yield might be due to the susceptibility of the phenolic hydroxyl group to oxidation. Therefore, **8** was converted into its acetate **10**, which was treated with DDQ to give benzofuran acetate **11** in 76% yield. In the ^{1}H-NMR spectrum of **11**, the signals due to H-2 and H-3 present in the educt **10** had disappeared.

The remaining task for the synthesis of benzofuran **4** was then the removal of four methyl protecting groups. To this end, we investigated the reaction of **11** with boron tribromide in CH_2Cl_2. The purification of the product, after acetylation, by chromatography on silica gel afforded triacetate **12** in 65% yield. Finally, **12** was converted to the desired benzofuran **4** in quantitative yield by hydrolysis with 10% ammonia solution. The ^{1}H-NMR, ^{13}C-NMR spectra and elemental analysis of **4** were compatible with the benzofuran structure.

When the treatment of **11** with boron tribromide was performed under a milder condition (stirring of the reaction mixture at 0 °C for a shorter time), a monomethyl ether **13** (2%) and, after acetylation, two products **14** (50%) and **15** (21%) were obtained (see Experimental for details of the separation procedure). The ^{1}H-NMR spectrum of **13** exhibited signals due to three methoxy group protons (δ 3.77, 3.91, 4.01), of which the assignment was possible by analysis of the ^{1}H–^{13}C-NMR correlation spectroscopy *via* long-range couplings (COLOC). Namely, the former two originated from the carbomethoxy groups and the last signal was from a phenolic methoxy group. Since a correlation between signals of the phenolic methoxy protons (δ 4.01) and the aromatic carbon of C-7 position (δ 144.8) was observed, the remaining methoxy group was confirmed to be located at the C-7 position. In the ^{1}H-NMR spectrum of **14**, where the signals due to two phenolic methoxy groups disappeared, the presence of signals due to three acetyl (δ 2.33, 2.34, 2.43) and two carbomethoxy groups (δ 3.82, 3.96) were indicated. The acetate **14** was converted to a dimethyl ester **16** by hydrolysis with 25% ammonia in dioxane in quantitative yield. The hydrolysis of the acetate **15** in the same way afforded a monomethyl ester **17**. The ^{1}H-NMR spectrum of **17** exhibited only one signal due to a carbomethoxy

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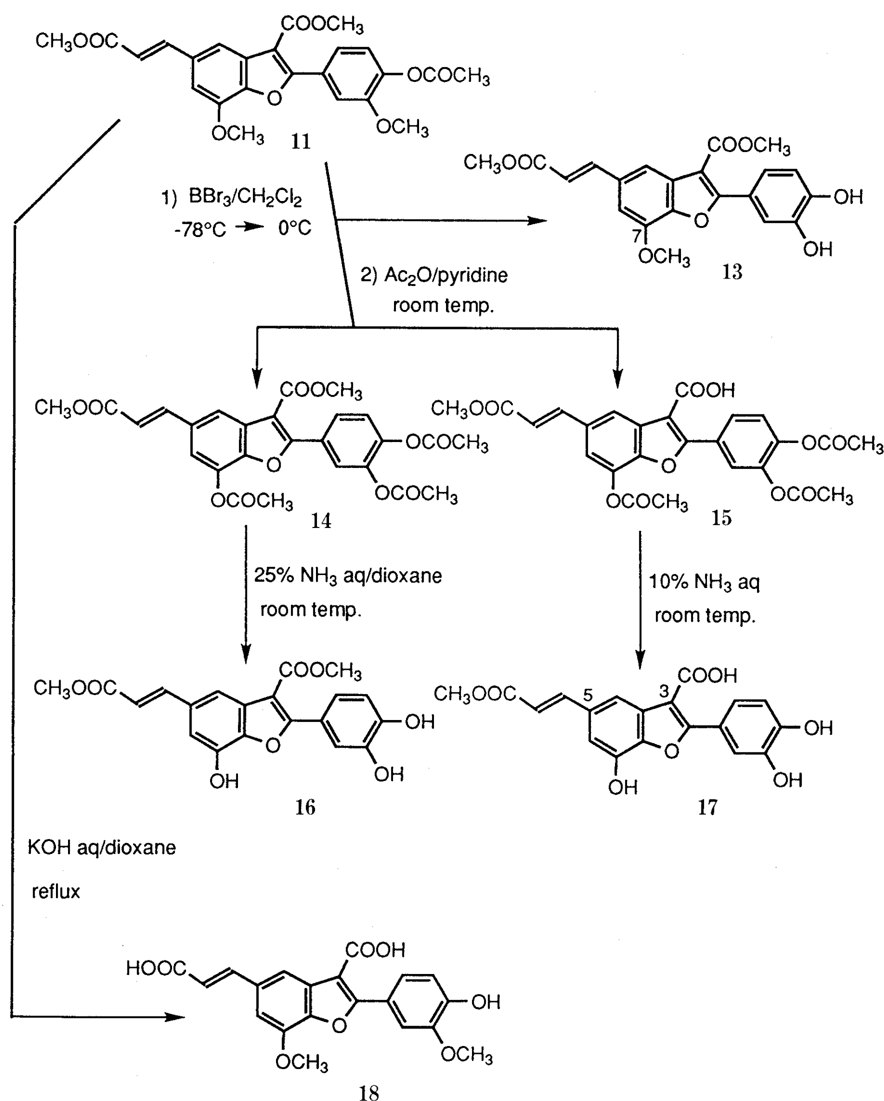

Chart 5

Table I. ^{1}H-NMR Chemical Shifts of Benzofuran Lignans in $CDCl_3$

H	8	9	10	11	14	15
2	4.35 (1H, d, 8)		4.35 (1H, d, 8)			
3	6.11 (1H, d, 8)		6.19 (1H, d, 8)			
4		7.78 (1H, br s)		7.79 (1H, d, 1)	8.07 (1H, d, 1)	8.13 (1H, d, 1)
6	6.87—9.93 (3H, m)	7.00 (1H, br s)	7.19 (1H, br s)	7.03 (1H, d, 1)	7.33 (1H, d, 1)	7.33 (1H, d, 1)
2′	7.03 (1H, br s)	7.76 (1H, d, 2)	6.97—7.04 (4H, m)	7.84 (1H, d, 2)	7.94 (1H, d, 2)	7.92 (1H, d, 2)
5′	7.19 (1H, br s)	7.02 (1H, d, 8)		7.15 (1H, d, 8)	7.34 (1H, d, 9)	7.36 (1H, d, 9)
6′		7.66 (1H, dd, 2, 8)		7.67 (1H, dd, 2, 8)	7.93 (1H, dd, 2, 9)	7.97 (1H, dd, 2, 9)
=CHCOO	6.32 (1H, d, 16)	6.46 (1H, d, 16)	6.33 (1H, d, 16)	6.47 (1H, d, 16)	6.46 (1H, d, 16)	6.48 (1H, d, 16)
ArCH=	7.65 (1H, d, 16)	7.81 (d, 16)	7.64 (1H, d, 16)	7.81 (1H, d, 16)	7.78 (1H, d, 16)	7.81 (1H, d, 16)
OH	5.70 (1H, br s)	5.99 (1H, br s)				
$OCOCH_3$			2.31 (3H, s)	2.35 (3H, s)	2.33, 2.34, 2.43 (each 3H, s)	2.34 (6H, s), 2.44 (3H, s)
OCH_3 / $COOCH_3$	3.81, 3.84, 3.87, 3.92 (each 3H, s)	3.84, 3.96, 3.99, 4.05 (each 3H, s)	3.80, 3.82, 3.85, 3.93 (each 3H, s)	3.84, 3.94, 3.96, 4.05 (each 3H, s)	3.82, 3.96 (each 3H, s)	3.82 (3H, s)

group, and the COLOC spectrum revealed a correlation between the signals of the methyl protons (δ 3.76) in this group and the carbonyl carbon of methyl (E)-propenoate chain (δ 166.7), indicating that the carbomethoxy group in this moiety remained unhydrolyzed in **17** and, hence, in **15** as well. In addition, the treatment of **11** with 95% KOH in dioxane afforded the dimethyl ether **18** in 81% yield.

Inhibitory Effects on Lipid Peroxidation Biogenerated free radicals and active oxygen are scavenged by

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Table II. ^{1}H-NMR Chemical Shifts of Benzofuran Lignans in DMSO-d_6

H	4	12	13	16	17	18
2						
3						
4	7.67 (1H, br s)	8.13 (1H, d, 1)	7.69 (1H, s)	7.68 (1H, d, 1)	7.71 (1H, s)	7.76 (1H, d, 1)
6	7.12 (1H, br s)	7.74 (1H, d, 1)	7.35 (1H, s)	7.13 (1H, d, 1)	7.15 (1H, s)	7.40 (1H, d, 1)
2′	7.55 (1H, d, 2)	7.91 (1H, d, 2)	7.53 (1H, d, 2)	7.55 (1H, d, 2)	7.57 (1H, s)	7.72 (1H, d, 2)
5′	6.91 (1H, d, 8)	7.48 (1H, d, 8)	6.91 (1H, d, 8)	6.91 (1H, d, 8)	6.92 (1H, d, 8)	6.93 (1H, d, 8)
6′	7.47 (1H, dd, 2, 8)	7.93 (1H, dd, 2, 8)	7.46 (1H, dd, 2, 8)	7.47 (1H, dd, 2, 8)	7.50 (1H, d, 8)	7.50 (1H, dd, 2, 8)
=CHCOO	6.36 (1H, d, 16)	6.55 (1H, d, 16)	6.67 (1H, d, 16)	6.48 (1H, d, 16)	6.45 (1H, d, 16)	6.59 (1H, d, 16)
ArCH=	7.66 (1H, d, 16)	7.74 (1H, d, 16)	7.73 (1H, d, 16)	7.72 (1H, d, 16)	7.74 (1H, d, 16)	7.70 (1H, s)
OH	9,33, 9.66, 10.52 (each 1H, br s)		9.54 (2H, br s)	9.38, 9.69, 10.56 (each 1H, br s)	9.34, 9.66, 10.54 (each 1H, br s)	9.77 (1H, s)
$OCOCH_3$		2.33 (6H, s), 2.43 (3H, s)				
OCH_3			4.01 (3H, s)			3.85, 4.03 (each 3H, s)
3-$COOCH_3$			3.91 (3H, s)	3.75, 3.92 (each 3H, s)	3.76 (3H, s)	
=$CHCOOC\underline{H}_3$			3.77 (1H, s)			
COOH	12.70 (2H, br s)	12.95 (1H, br s)			13.01 (1H, br s)	12.76 (2H, br s)

Table III. ^{13}C-NMR Chemical Shifts of Benzofuran Lignans in DMSO-d_6

C	4	13	17
2	160.7	161.1	160.8
3	107.8	106.7	107.8
3a	129.7	128.6	129.8
4	113.7	115.8	114.1
5	131.1	131.2	130.9
6	109.9	106.1	110.0
7	142.7	144.8	142.7
7a	143.1	143.1	143.3
1′	120.0	119.4	120.0
2′	116.6	116.3	116.6
3′	144.9	144.9	144.9
4′	148.1	148.4	148.1
5′	115.4	115.4	115.4
6′	121.6	121.5	121.6
=$\underline{C}HCOO$	118.0	117.1	116.6
ArCH=	144.8	145.1	145.4
7-OCH_3		56.1	
3′-OCH_3			
3-$COO\underline{C}H_3$		51.7	
=$CHCOO\underline{C}H_3$		51.4	51.5
3-COO	167.6	163.5	164.8
=$CH\underline{C}OO$	164.8	166.8	166.7

Table IV. Inhibitory Effects of Benzofuran Lignans on Lipid Peroxidation in Rat Brain Homogenate[a]

Compound	Inhibition (%)[b]			IC_{50} (10^{-6} M)[c]
	10^{-4} M	10^{-5} M	10^{-6} M	
4	97	50	4	—
8	65	16	—	—
9	30	—	—	—
10	50	15	—	—
11	68	25	—	—
12	77	33	—	—
13	97	100	32	1.20 (1.15—1.26)
14	95	89	61	0.70 (0.65—0.79)
15	93	59	13	—
16	97	100	56	0.77 (0.73—0.79)
17	96	95	25	—
18	6	—	—	—
Idebenone	93	27	—	23.7 (20.5—27.0)

a) MDA productions of control were 250—300 nmol/g wet tissue. *b*) The inhibition % values were the average of three to four experiments. *c*) IC_{50} values and its 95% confidence limits were calculated by probit analysis by using 4 determinations of 5 different concentrations for each compound.

Table V. Inhibitory Effects of Benzofuran Lignans on Lipid Peroxidation in Rat Liver Microsomes[a]

Compound	IC_{50} (10^{-6} M)[b]
13	3.66 (3.48—3.83)
16	4.49 (4.31—4.70)
Schizotenuin A (**1**)	36.26 (33.54—39.76)
(±)-α-Tocopherol	976 (880—1149)

a) MDA productions of control were 20—28 nmol/g protein. *b*) IC_{50} values and its 95% confidence limits were calculated by probit analysis by using 4 determinations of 3—5 different concentrations (geometric ratio = 1.4) for each compound.

antioxidants and superoxide dismutase. However, when the defense function of living body is weakened by pathologic change, lipid peroxidation in tissue might proceed without homeostatic control. On the other hand, with arteriosclerosis, damage to the intima of the aorta provoked by lipid peroxides could plausibly be the initial event in the pathogenesis of human atheroma.[9] It has also been reported[10] that probucol, an antihyperlipemic agent, can inhibit atherogenesis by limiting oxidative low density lipoprotein (LDL) modification, and other compounds with antioxidant properties might behave similarly. Furthermore, in the search for an antiarteriosclerotic drug, the determination of lipid peroxidation inhibiting activity for rat liver microsomes has been shown to be capable of being a standard of antioxidation activity for an *in vitro* test.[11] Firstly, we examined all of the synthetic lignans for their inhibitory activity of lipid peroxidation in rat brain homogenate, and for those compounds which possessed significant activity, the test has been carried out in rat liver microsomes. The results are summarized in Tables IV and V.

Among the twelve compounds tested in rat brain homogenate, **4**, **12**—**17** showed inhibitory activities more potent than idebenone, and especially, inhibition by **13**,

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14 and 16 was the highest. Therefore, 13 and 16 were tested in rat liver microsomes, and the lipid peroxidation inhibiting activity was found to be more potent than that of schizotenuin A (1)[12] and much more potent than that of (±)-α-tocopherol.

In summary, the synthesis of the benzofuran lignan 4 and its analogs has been achieved efficiency using an oxidative coupling method. Several compounds were found to exhibit prominent lipid peroxidation activities in rat brain homogenate and also in rat liver microsomes. The easy synthetic accessibility to the benzofuran lignans described in this paper, we believe, should promote investigations directed toward the developement of new drugs from these lignans. Further biological activities of the synthetic lignans are now under examination.

Experimental

All melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were measured with a Nicolet 60 SX spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were obtained at 300 and 75 MHz, respectively, using a Brucker AM 300 instrument. Chemical shift values were expressed as ppm downfield from tetramethylsilane as an internal standard.

Methyl (*E*)-3-[(2*R,3*R**)-2,3-Dihydro-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-3-methoxycarbonylbenzofuran-5-yl]propenoate (8)** Methyl ferulate (7) (12.0 g, 0.058 mol) was dissolved in benzene–acetone (300 ml, 2:1) and stirred with silver oxide (8.1 g, 0.035 mol) under nitrogen atmosphere at room temperature for 19 h. The suspension was filtered, the filtrate was evaporated to dryness, and the residue was chromatographed on silica gel (*n*-hexane–AcOEt, 5:2) giving 8 (6.0 g, 50%) as colorless needles, mp 149–151 °C (lit.[8]) mp 151—152 °C). IR (KBr): 3392 (OH), 1741, 1730 (C=O) cm^{-1}.

Methyl (*E*)-3-[2-(4-Hydroxy-3-methoxyphenyl)-7-methoxy-3-methoxycarbonylbenzofuran-5-yl]propenoate (9) To a solution of 8 (4.0 g, 9.7 mmol) in dry dioxane (50 ml) was added a solution of DDQ (2.9 g, 0.013 mol) in dry dioxane (40 ml), and the mixture was refluxed for 7 h. After cooling, the precipitate formed was filtered and it was washed with CH_2Cl_2. The filtrate and washing were combined and evaporated to dryness. The residue was purified by chromatography on silica gel (*n*-hexane–AcOEt, 5:3) and recrystallization from AcOEt, giving 9 (0.8 g, 20%) as colorless needles, mp 190—191 °C. IR (KBr): 3346 (OH), 1709, 1691 (C=O) cm^{-1}. *Anal.* Calcd for $C_{22}H_{20}O_8$: C, 64.07; H, 4.90. Found: C, 63.89; H, 4.88.

Methyl (*E*)-3-[(2*R,3*R**)-2-(4-Acetoxy-3-methoxyphenyl)-2,3-dihydro-7-methoxy-3-methoxycarbonylbenzofuran-5-yl]propenoate (10)** A solution of 8 (57.3 g, 0.14 mol) in dry pyridine (165 ml) and acetic anhydride (138 ml, 1.5 mol) was stirred at room temperature for 24 h. The reaction mixture was poured into 6 M HCl–ice water, and the precipitate was collected by filtration, washed with water, and dried. Recrystallization from MeOH gave 10 (62.2 g, 99%) as colorless needles, mp 122—124 °C. IR (KBr): 1766, 1736, 1695 (C=O) cm^{-1}. *Anal.* Calcd for $C_{24}H_{24}O_9$: C, 63.14; H, 5.31. Found: C, 63.14; H, 5.32.

Methyl (*E*)-3-[2-(4-Acetoxy-3-methoxyphenyl)-7-methoxy-3-methoxycarbonylbenzofuran-5-yl]propenoate (11) To a solution of 10 (64.5 g, 0.14 mol) in dry dioxane (800 ml) was added a solution of DDQ (37.6 g, 0.17 mol) in dry dioxane (400 ml). After having been refluxed for 18 h, to the reaction mixture was added an additional DDQ (26.5 g, 0.12 mol) dissolved in dry dioxane (300 ml), and then the mixture was refluxed for 24 h. The reaction mixture was cooled to room temperature and the precipitate formed was filtered, and was washed with benzene. The filtrate and the washing were combined, and evaporated to dryness. The residue was chromatographed on silica gel (CH_2Cl_2–benzene, 2:1 then CH_2Cl_2–benzene–MeOH, 2:1:0.1). The first eluate was recrystallized from MeOH to give unreacted 10 (9.4 g). The second eluate was recrystallized from acetone to give 11 (48.6 g, 76%) as colorless needles, mp 156—157 °C. IR (KBr): 1760, 1717 (C=O) cm^{-1}. *Anal.* Calcd for $C_{24}H_{22}O_9$: C, 63.42; H, 4.89. Found: C, 63.42; H, 4.91.

(*E*)-3-[7-Acetoxy-3-carboxy-2-(3,4-diacetoxyphenyl)benzofuran-5-yl]propenoic Acid (12) To a solution of 11 (10.0 g, 0.022 mol) in dry CH_2Cl_2 (500 ml) was added dropwise at −78 °C a solution of boron tribromide (37 ml, 0.40 mol) in dry CH_2Cl_2 (200 ml). The mixture was stirred at −78 °C for 3 h and then at room temperature for 18 h. After the excess reagent was decomposed by the addition of MeOH, water was added. The precipitate formed was collected by filtration and dissolved in AcOEt. The solution was washed with sat. NaCl sol., dried over $MgSO_4$ and evaporated to dryness. The residue was dissolved in dry pyridine (150 ml) and acetic anhydride (170 ml, 1.8 mol), and the mixture was stirred at room temperature for 18 h. The reaction mixture was poured into 6 M HCl–ice water and extracted with AcOEt. The organic layer was washed with sat. NaCl sol., dried over $MgSO_4$, and evaporated to dryness. The residue was purified by chromatography on silica gel (CH_2Cl_2–AcOEt, 9:1 then 9:2) and recrystallization from AcOEt, giving 12 (6.9 g, 65%) as pale yellow needles, mp 242—245 °C. IR (KBr): 1775, 1690 (C=O) cm^{-1}. *Anal.* Calcd for $C_{24}H_{18}O_{11}$: C, 59.75; H, 3.77. Found: C, 59.47; H, 3.73.

(*E*)-3-[3-Carboxy-2-(3,4-dihydroxyphenyl)-7-hydroxybenzofuran-5-yl]propenoic Acid (4) The acetate 12 (1.5 g, 3.1 mmol) was dissolved in 10% ammonia solution (45 ml) and stirred at room temperature for 1 h. After the addition of AcOEt (50 ml), the mixture was acidified with 6 M HCl and extracted with AcOEt. The organic layer was washed with water and filtered. The filtrate was concentrated and CH_2Cl_2 was added. The precipitate was collected by filtration and dried, giving 4 (1.1 g, 100%) as whitish brown powder, mp >300 °C. IR (KBr): 3426 (OH), 1717, 1703 (C=O) cm^{-1}. *Anal.* Calcd for $C_{18}H_{12}O_8$: C, 60.67; H, 3.40. Found: C, 60.26; H, 3.47.

Methyl (*E*)-3-[2-(3,4-Dihydroxyphenyl)-7-methoxy-3-methoxycarbonylbenzofuran-5-yl]propenoate (13), Methyl (*E*)-3-[7-Acetoxy-2-(3,4-diacetoxyphenyl)-3-methoxycarbonylbenzofuran-5-yl]propenoate (14), Methyl (*E*)-3-[7-Acetoxy-3-carboxy-2-(3,4-diacetoxyphenyl)benzofuran-5-yl]propenoate (15) To a solution of 11 (4.0 g, 8.8 mmol) in dry CH_2Cl_2 (200 ml) was added dropwise at −78 °C a solution of boron tribromide (12 ml, 0.13 mol) in dry CH_2Cl_2 (100 ml). The mixture was stirred at −78 °C for 1 h and at 0 °C for 1 h. The excess reagent was decomposed by the addition of MeOH, water was added, and the precipitate formed was filtered. The organic layer of the filtrate was washed with sat. NaCl sol., dried over $MgSO_4$ and evaporated to dryness. The residue was recrystallized from MeOH, giving 13 (0.05 g, 2%) as pale yellow scales, mp 252—254 °C. The precipitate was dissolved in AcOEt. The solution was washed with sat. NaCl sol., dried over $MgSO_4$ and evaporated to dryness. The residue was dissolved in dry pyridine (88 ml) and acetic anhydride (75 ml, 0.79 mol), and the mixture was stirred at room temperature for 24 h. The reaction mixture was poured into 6 M HCl–ice water and extracted with AcOEt. The organic layer was washed with sat. NaCl sol., dried over $MgSO_4$, and evaporated to leave a residue, which was chromatographed on silica gel. The CH_2Cl_2–AcOEt (9:1) eluate was recrystallized from MeOH, giving 14 (2.25 g, 50%) as pale yellow needles, mp 172—174 °C, and the CH_2Cl_2–AcOEt–AcOH (9:1:0.5) eluate was recrystallized from MeOH, giving 15 (0.91 g, 21%) as pale yellow needles, mp 202—205 °C. 13: IR (KBr): 3513, 3278(OH), 1711, 1683(C=O) cm^{-1}. *Anal.* Calcd for $C_{21}H_{18}O_8$: C, 63.31; H, 4.56. Found: C, 63.26; H, 4.59. 14: IR (KBr): 1775, 1717(C=O) cm^{-1}. *Anal.* Calcd for $C_{26}H_{22}O_{11}$: C, 61.17; H, 4.35. Found: C, 61.10; H, 4.34. 15: IR (KBr): 1767, 1717(C=O) cm^{-1}. *Anal.* Calcd for $C_{25}H_{20}O_{11}$: C, 60.48; H, 4.07. Found: C, 60.48; H, 4.06.

Methyl (*E*)-3-[2-(3,4-Dihydroxyphenyl)-7-hydroxy-3-methoxycarbonylbenzofuran-5-yl]propenoate (16) To a solution of 14 (1.0 g, 2.0 mmol) in dioxane (40 ml) was added 25% ammonia solution (10 ml) and the mixture was stirred at room temperature for 4 h. AcOEt and water was added, and the mixture was acidified with 6 M HCl and extracted with AcOEt. The organic layer was washed with water and filtered. The filtrate was concentrated and CH_2Cl_2 was added. The precipitate collected by filtration was dried, giving 16 (0.75 g, 100%) as pale yellow powder, mp 269—271 °C. IR (KBr): 3520, 3503, 3381, 3286 (OH), 1702, 1668 (C=O) cm^{-1}. *Anal.* Calcd for $C_{20}H_{16}O_8$: 62.49; H, 4.20. Found: C, 62.19; H, 4.21.

Methyl (*E*)-3-[3-Carboxy-2-(3,4-dihydroxyphenyl)-7-hydroxybenzofuran-5-yl]propenoate (17) The acetate 15 (0.6 g, 1.2 mmol) was dissolved in 10% ammonia solution (50 ml) and the mixture was stirred at room temperature for 1 h. After the addition of AcOEt, the mixture was acidified with 6 M HCl and extracted with AcOEt. After filtration, the filtrate was concentrated and CH_2Cl_2 was added. The precipitate was collected by filtration and dried, giving 17 (0.45 g, 100%) as whitish brown powder, mp 266—269 °C. IR (KBr): 3432, 3272, 3187 (OH), 1703, 1680 (C=O) cm^{-1}. *Anal.* Calcd for $C_{19}H_{14}O_8$: C, 61.62; H, 3.82. Found:

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C, 61.33; H, 3.85.

(*E*)-3-[3-Carboxy-2-(4-hydroxy-3-methoxyphenyl)-7-methoxybenzofuran-5-yl]propenoic Acid (18) To a solution of **11** (1.0 g, 2.2 mmol) in dioxane (75 ml) was added a solution of KOH (0.7 g) in water (4 ml), and the mixture was refluxed for 1.5 h. The reaction mixture was concentrated and acidified with 6 M HCl. The precipitate was collected by filtration, washed with water, and dried. Recrystallization from acetone gave **18** (0.69 g, 81%) as pale yellow prisms, mp 274—275 °C. IR (KBr): 3421(OH), 1706, 1686(C=O) cm^{-1}. *Anal.* Calcd for $C_{20}H_{16}O_8$: 62.50; H, 4.20. Found: C, 62.22; H, 4.21.

Evaluation of the Inhibitory Effects on Lipid Peroxidation in Rat Brain Homogenate Lipid peroxides produced in the brain homogenate were determined according to the method of Suno *et al.*[13] and Kubo *et al.*[14] with some modifications. Male Wistar rats, weighing about 200—250 g, were purchased from S.L.C., Inc., and were fed allowing free access to food and water. Whole brain, except the cerebellum, was homogenized in a glass-Teflon homogenizer with four-fold volume of phosphate buffered saline (50 mM, pH 7.4), followed by centrifugation at 2800 rpm for 10 min. The resulting supernatant was stored at −40 °C until used.

The stock brain homogenate (0.1 ml) was mixed with the solution, prepared by adding a solution of the test sample in dimethyl sulfoxide (DMSO) (10 μl) to the same buffer (phosphate buffered saline (PBS)) as above (0.9 ml), and the mixture was incubated at 37 °C for 30 min. As a control, the mixture of DMSO (10 μl) and PBS (0.9 ml) was processed in the same way. The reaction was stopped by adding 35% $HClO_4$ (0.2 ml), and the mixture was centrifuged at 2800 rpm for 10 min. The resulting supernatant (1.0 ml portion) was heated with thiobarbituric acid solution (0.5 ml) (5 g/l in 50% acetic acid) at 100 °C for 15 min and, after cooling, the absorbance was measured at 532 nm. The level of lipid peroxides in the supernatant is expressed by reducing to the concentration of malon-dialdehyde (MDA) by using 1,1,3,3-tetraethoxypropane as the standard.

$$\text{Inhibitory activity on lipid peroxidation}(\%) = [1-[\text{MDA(sample)}-\text{MDA(0 min)}] / [\text{MDA(control)}-\text{MDA(0 min)}]] \times 100$$

The concentration causing 50% inhibition (IC_{50} value) was calculated.[14]

Evaluation of Inhibitory Effects on Fe^{2+}-Ascorbic Acid-Induced Lipid Peroxidation in Rat Liver Microsomes The same breed of animal as above was used. Lipid peroxides produced in the liver microsomes were determined according to the method of Thiele *et al.*[15] with some modification. The liver microsomes was suspended in phosphate buffer (50 mM, pH 7.0) and adjusted to 5 mg protein/ml. The amount of protein was determined according to the method of Lowry.[16] The stock liver microsomes prepared above (0.1 ml) was added to the mixture of a solution of the sample in DMSO (10 μl) and 0.9 M KCl solution (0.1 ml) diluted with the same phosphate buffer as above (0.59 ml). As a control, plain DMSO (10 μl) was used, and the mixture was preincubated at 37 °C for 10 min. Subsequently, 0.5 mM $FeSO_4 \cdot 7H_2O$ solution (0.1 ml) and 50 mM ascorbic acid solution (0.1 ml) were added and the mixture was incubated at 37 °C for 10 min. The reaction was stopped by the addition of 10% trichloroacetic acid solution (1 ml), followed by centrifugation at 3000 rpm for 15 min. The resulting supernatant (0.5 ml portion) was heated with 0.8% thiobarbituric acid solution (2 ml, dissolved in 20% acetic acid buffer, pH 3.5) at 100 °C for 15 min and, after cooling, the absorbance was measured at 532 nm. The inhibitory activity on lipid peroxidation was calculated as above.

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