Journal of Natural Products
Vol. 58, No. 12, pp. 1840-1847, December 1995

SYNTHESIS AND EVALUATION OF 3′,5′-DI-*TERT*-BUTYL-4′-HYDROXYFLAVONES AS POTENTIAL INHIBITORS OF LOW DENSITY LIPOPROTEIN (LDL) OXIDATION

GUY LEWIN,*

Laboratoire de Pharmacognosie, Faculté de Pharmacie, boulevard Becquerel, Hérouville Stain-Clair, 14032 Caen Cedex, France

YVES ROLLAND, SYLVIE PRIVAT, CHRISTINE BREUGNOT, ALBERT LENAERS, JEAN PAUL VILAINE,

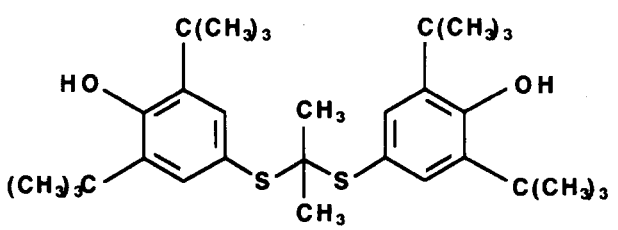
Institut de Recherches Servier, 11 rue des Moulineaux, 92150 Suresnes, France

JEAN-PIERRE BALTAZE, and JACQUES POISSON

Laboratoire de Chimie des Substances Thérapeutiques Naturelles, Centre d'Etudes Pharmaceutiques, Châtenay-Malabry 92296 Cedex, France

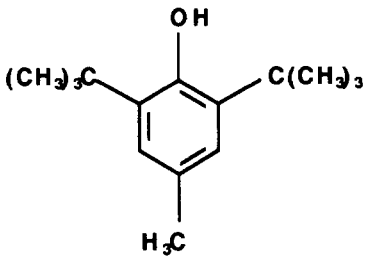
ABSTRACT.—The novel flavones **6–28**, which display structural analogies with the two well-known lipid peroxidation inhibitors, probucol [**1**] and butylated hydroxytoluene [**2**], were synthesized and studied in vitro for their ability to inhibit the copper sulfate or endothelial cell-induced lipid peroxidation of human low-density lipoprotein (LDL). Most of the flavones were active in the range of 0.1–1 μM.

Cholesterol delivery to cells is achieved in most tissues via the low-density lipoprotein (LDL) receptor-mediated pathway (1). During recent years, attention has focused on the importance of LDL oxidation in atherogenesis. The discovery that endothelial cells and smooth muscle cells are able to modify LDL in vitro was a major advance (2,3). Furthermore, the recent demonstration of the existence of oxidatively modified LDL in vivo suggested that they might have a role in the pathogenesis of atherosclerosis (4,5). Modification of LDL in the artery wall would appear to be a prerequisite to foam cell formation and to the development of fatty streaks, the earliest lesions in atherosclerosis. Recently, the protective effect of probucol [**1**], a hypocholesterolemic drug, against endothelial cell or copper-induced LDL oxidative modification was demonstrated by the group of Steinberg (6). Moreover, probucol was also reported to have a marked antiatherogenic action in animal models (7–9). This antiatherogenic action is supposed to be related to its antioxidant effect rather than to its relatively weak hypocholesterolemic potency (7), although a different mechanism cannot be completely excluded. On the other hand, flavonoids are known to display many antioxidant properties including scavenging free radicals and preventing lipid peroxidation (10–15). In particular, De Whalley *et al.* (16) pointed out that some flavonoids could inhibit the oxidative modification of LDL mediated by macrophages or cupric ions. The most active compounds in that study (fisetin, morin, quercetin, gossypetin, hypolaetin, and hypolaetin 8-glucoside) all possessed at least two hydroxyl groups, at C-7 and C-4′.



1

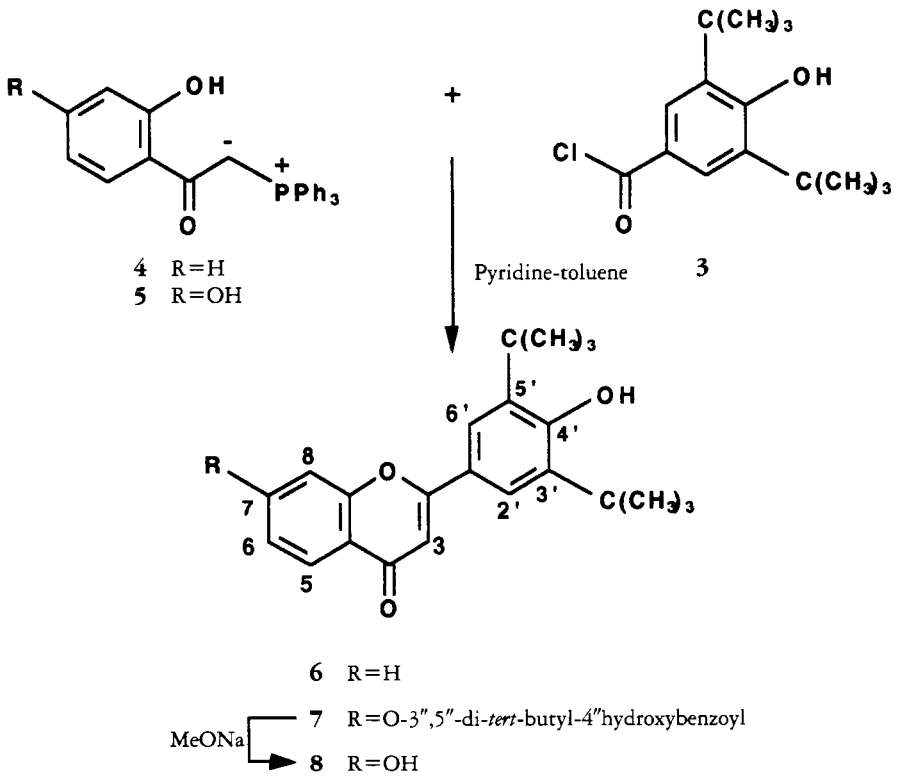
On this basis, we have synthesized novel hydroxylated flavones [**6–28**] which display structural analogies with probucol [**1**] or butylated hydroxytoluene (BHT) [**2**], a well-known lipid peroxidation inhibitor, and have investigated the effect of these compounds on copper and endothelial cell-induced LDL modification in vitro.



2

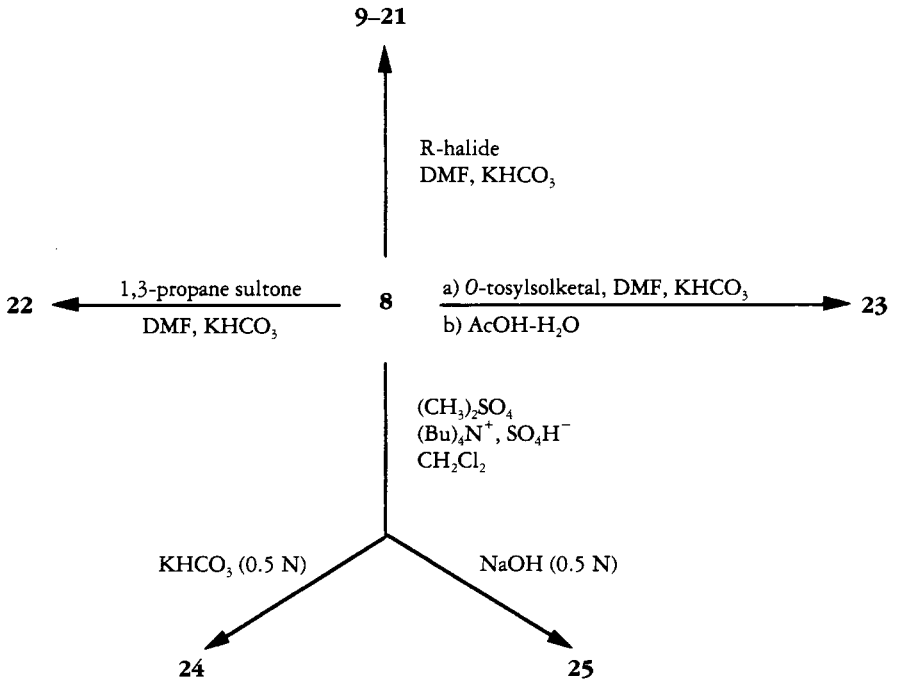
RESULTS AND DISCUSSION

Flavones were synthesized according to the method of Le Corre and Le Floc'h (17), by condensation of a phosphorane with 3,5-di-*tert*-butyl-4-hydroxybenzoyl chloride [**3**]. (2-Hydroxybenzoyl)-methylenetriphenyl-phosphorane [**4**] afforded in one step the flavone **6**, whereas (2,4-dihydroxybenzoyl)-methylenetriphenyl-phosphorane [**5**] (18) provided first the flavone ester [**7**] then, by methanolysis, 3′,5′-di-*tert*-butyl-7,4′-dihydroxyflavone [**8**] (Scheme 1).



SCHEME 1

Scheme 2 describes the preparation of most of the flavones from **8** through alkylation of the C-7 phenol group: compounds **9–21** were obtained with the appropriate halide, **22**, with 1,3-propane sultone, and **23** with 1,2-*O*-isopropylidene-3-*O*-tosylglycerol followed by hydrolysis of the acetonide. Phase-transfer catalysis alkylation of **8** with dimethyl sulfate afforded the flavone **24** with 0.5 N $KHCO_3$ and the dimethylated compound **25** with 0.5 N NaOH.



SCHEME 2

Finally, flavones **26** and **27** were prepared, respectively, from **9** by saponification with and without acidification and **28** through transesterification of **9**.

In this study, it was observed that all the flavones bearing a 3′,5′-di-*tert*-butyl-4′-hydroxy substitution are able to protect LDL from oxidative stress (IC_{50} values in the 0.1–1 μM range). The presence of the OH-4′ group is crucial for this activity as demonstrated by the disappearance of the effect when the hydroxyl is methylated [**25**]. On the other hand, the OH-7 group appears to be of no importance because no significant difference was observed between **8** and its 7-deoxy compound, **6**. Finally, the weak activity of 5,7,4′-trihydroxyflavone (apigenin) and 7,4′-dihydroxyflavone (no protective effect up to 10^{-4} M) gave evidence of the role played by the 3′,5′-di-*tert*-butyl substituents.

When examining the structures of the active flavones, it is surprising to observe that the most lipophilic flavones are the least active compounds (**7**, **11**, **13**, and **24**). Interestingly, this is also true for vitamin E, which is the most lipophilic product among the reference compounds. Conversely, compounds that possess—or can generate—an amphiphilic structure (**8**, **12**, **18**, **19**, **22**, **23**, **26**, and **27**) are the most potent in this series and are even more potent than **1** and **2**. All these observations are in good accordance with the recent studies of Bowry and Stocker (19,20). These authors suggested that in an aqueous dispersion of lipid-bearing particles like LDL, a very lipophilic radical scavenger like vitamin E was not a very potent protector against peroxidation and could even favor radical propagation, at least in certain experimental

conditions. They also predicted that radical scavengers, which, due to their amphiphilic structure, are able to shuttle from the LDL particle to the external medium and *vice versa*, would be the most efficient compounds under these experimental conditions.

In summary, we have synthesized 23 novel flavones (see Table 1) displaying analogies with some natural flavonoids and with probucol **[1]** and BHT **[2]**, two known synthetic lipid peroxidation inhibitors. Testing of these new flavones has uncovered several new potent inhibitors of LDL oxidation This study also appears to confirm the interesting activities of amphiphilic flavones, and suggests that further analogues of this type may have even more potent inhibitory activity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were carried out at 200 MHz or 500 MHz on Bruker AC-200 or Bruker AMX-500 spectrometers in $CDCl_3$ or in DMSO-d_6 using TMS as internal reference. Microanalyses were performed on a Carlo Erba EA 1108 apparatus and mps were determined with a micro-Koffler instrument and are uncorrected.

FLAVONE **6**.—A solution of **3** (available by reaction of the carboxylic acid with $SOCl_2$) (0.616 g, 2.3 mmol) in toluene (10 ml) was added to a well-stirred solution of phosphorane **4** (0.792 g, 2 mmol) in toluene-pyridine (95:5) (30 ml) at 75°. The reaction mixture was stirred at 75° for 3 h, cooled to room temperature overnight, then filtered. The solution was poured into ice-H_2O (200 ml) and extracted with CH_2Cl_2 (3×100 ml). The combined extracts were dried (Na_2SO_4) and solvents were evaporated. Crystallization of the dried residue from MeOH afforded the crude desired compound which recrystallized from the same solvent to give **6** (0.225 g, 32%); mp 195–197°; ^{1}H nmr ($CDCl_3$) δ 1.50 (18H, s), 5.70 (1H, br s, exchanged with D_2O), 6.75 (1H, s), 7.40, 7.70 (2H, 2×td, J=8.5 and 1.9 Hz), 7.60 (1H, dd, J=8.5 and 1.9 Hz), 7.70 (2H, s), 8.25 (1H, dd, J=8.5 and 1.9 Hz); *anal.*, calcd for $C_{23}H_{26}O_3$, C 78.83, H 7.48; found C 78.67, H 7.39.

FLAVONE **7**.—A solution of **3** (24.6 g, 92 mmol) in toluene (140 ml) was added to a well-stirred solution of phosphorane **5** (16.5 g, 40 mmol) in dry pyridine (400 ml) at 75°. The same work-up as described above for **6** yielded pure **7** (8.8 g, 37%); mp 268–270°; ^{1}H nmr (DMSO-d_6) δ 1.50 (36H, s), 6.90 (1H, s), 7.40 (1H, dd, J=8.5 and 1.9 Hz, 1H), 7.70 (2H, br s, exchanged with D_2O), 7.75 (2H, s), 7.90 (1H, d, J=1.9 Hz), 8.00 (2H, s), 8.10 (1H, d, J=8.5 Hz); *anal.*, calcd for $C_{38}H_{46}O_6$, C 76.22, H 7.74; found C 75.97, H 7.66.

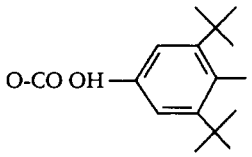
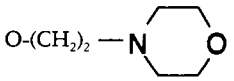
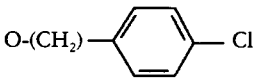
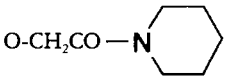
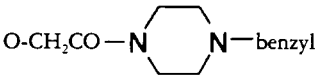
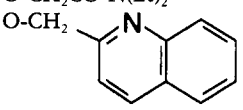
FLAVONE **8**.—Compound **7** (8.4 g, 14 mmol) was added to a 0.5 M solution of MeONa in MeOH (500 ml) at room temperature. The reaction mixture was stirred overnight, then poured into ice-H_2O (4 liters) and extracted with cyclohexane (3×500 ml). The aqueous layer was adjusted with 0.5 N HCl to pH 6, and extracted with CH_2Cl_2-MeOH (2:1) (4×500 ml). Standard work-up of the organic layer afforded by crystallization from MeOH afforded pure **8** (4.96 g, 96%); mp 298–300°; ^{1}H nmr (DMSO-d_6) δ 1.45 (18H, s, *tert*-butyl), 6.70 (1H, s, H-3), 6.95 (1H, dd, J=8.5 and 1.9 Hz, H-6), 7.00 (1H, d, J=1.9 Hz, H-8), 7.70 (1H, br s, OH-4′, exchanged with D_2O), 7.70 (2H, s, H-2′, H-6′), 7.85 (1H, d, J=8.5 Hz, H-5), 10.70 (1H, br s, OH-7, exchanged with D_2O); *anal.*, calcd for $C_{23}H_{26}O_4$, C 75.38, H 7.15; found C 75.35, H 7.30.

GENERAL PROCEDURE FOR THE PREPARATION OF FLAVONES **9**–**21**.—$KHCO_3$ was added to a solution of compound **8** (0.22 g, 0.6 mmol) in DMF (8 ml), and the mixture was stirred under N_2 at 120° for 5 min. The appropriate halide was added and the mixture was heated at the same temperature for 2–15 h. The reaction mixture was poured into H_2O (50 ml) and extracted with CH_2Cl_2 (4×30 ml). After the standard work-up, the dry residue was purified by two crystallizations. Some compounds crystallized only after first purifying the dry residue by flash chromatography (see below). All flavones were crystallized from MeOH except **18** (from Et_2O).

FLAVONE **9**.—Prepared by alkylation with ethyl chloroacetate (0.26 ml, 2.4 mmol) and $KHCO_3$ (0.072 g, 0.72 mmol) for 2 h. Yield (0.209 g, 77%); mp 172–174°; ^{1}H-nmr ($CDCl_3$) δ H-3, H-2′, H-6′, and di-*tert*-butyl protons, see flavone **8**; OH-4′ δ 5.65, H-5, H-6, H-8 δ 8.15, 7.05, and 6.95; additional signals at δ 1.35 (3H, t, J=7.5 Hz), 4.30 (2H, q, J=7.5 Hz), 4.75 (2H, s); *anal.*, calcd for $C_{27}H_{32}O_6$, C 71.66, H 7.13; found C 71.42, H 6.96.

FLAVONE **10**.—Prepared by alkylation with ethyl 2-bromoisobutyrate (1.1 ml, 7.5 mmol) and $KHCO_3$ (0.37 g, 3.7 mmol) for 15 h. The dry residue was first purified by flash chromatography [SiO_2, CH_2Cl_2-MeOH (98.5:1.5)]. Yield (0.181 g, 63%); mp 167–169°; ^{1}H nmr ($CDCl_3$) δ H-3, H-2′, H-6′, and di-*tert*-butyl protons, see flavone **8**; OH-4′ δ 5.65, H-5, H-6, H-8 δ 8.10, 6.90, and 6.90; additional signals at δ

TABLE 1. Structures and Biological Data of Flavones **6–28**.

Compound	C-7 substituent	C-4′ substituent	A:[a] IC_{50} (M)	B:[b] IC_{50} (M)
6	H	OH	3×10^{-6}	2.5×10^{-6}
7		OH	3×10^{-5}	7×10^{-5}
8	OH	OH	10^{-6}	2×10^{-6}
9	O-CH_2-COOEt	OH	3×10^{-6}	8×10^{-7}
10	O-$C(CH_3)_2$-COOEt	OH	3×10^{-6}	5×10^{-7}
11	O-$(CH_2)_5$-CH_3	OH	5×10^{-6}	5×10^{-5}
12		OH	8×10^{-7}	8×10^{-7}
13		OH	7×10^{-6}	
14		OH	3×10^{-6}	
15		OH	3×10^{-6}	
16	O-CH_2CO-$N(Et)_2$	OH	3×10^{-6}	
17		OH	3×10^{-6}	
18	O-$(CH_2)_2$-$N(Me)_2$	OH	7×10^{-7}	
19	O-$(CH_2)_3$-$N(Me)_2$	OH	7×10^{-7}	
20	O-$(CH_2)_4$-COOEt	OH	3×10^{-6}	
21	O-$(CH_2)_2$-OH	OH	3×10^{-6}	
22	O-$(CH_2)_3$-SO_3H	OH	5×10^{-7}	
23	O-CH_2CHOH-CH_2OH	OH	10^{-7}	
24	OMe	OH	10^{-6}	
25	OMe	OMe	$>10^{-4}$	
26	O-CH_2-COOH	OH	3×10^{-6}	5×10^{-7}
27	O-CH_2-COONa	OH	10^{-7}	
28	O-CH_2-COO$(CH_2)_4$-CH_3	OH	5×10^{-6}	
Probucol [**1**]			3×10^{-6}	4×10^{-6}
BHT [**2**]			3×10^{-6}	
Vitamin E			$>10^{-4}$	4×10^{-6}
Dihydroxyflavone			$>10^{-4}$	
Apigenin			$>10^{-4}$	

[a]A: Inhibition of copper-induced oxidation of human LDL.
[b]B: Inhibition of endothelial cell-induced oxidation of human LDL.

1.25 (3H, t, J=7.5 Hz), 1.70 (6H, s), 4.30 (2H, q, J=7.5 Hz); *anal.*, calcd for $C_{29}H_{36}O_6$, C 72.48, H 7.55; found C 72.55, H 7.56.

FLAVONE **11**.—Prepared by alkylation with 1-bromohexane (0.84 ml, 6 mmol) and $KHCO_3$ (0.3 g, 3 mmol) for 2 h. Yield (0.2 g, 75%); mp 133–135°; ^{1}H nmr ($CDCl_3$) δ H-3, H-2′, H-6′, and di-*tert*-butyl

protons, see flavone **8**; OH-4′ δ 5.65, δ H-5, H-6, H-8 δ 8.10, 6.95, 6.95; additional signals at δ 0.90 (3H, t, J=7.5 Hz), 1.30–1.60 (6H, m), 1.85 (2H, m), 4.10 (2H, t, J=7.3 Hz); *anal.*, calcd for $C_{29}H_{38}O_4$, C 77.30, H 8.50; found C 77.14, H 8.45.

FLAVONE **12**.—Prepared by alkylation with 4-(2-chloroethyl) morpholine hydrochloride (0.14 g, 0.75 mmol) and $KHCO_3$ (0.54 g, 5.4 mmol) for 4 h. Yield (0.161 g, 56%); mp 175–176°; ^{1}H nmr ($CDCl_3$) δ H-3, H-2′, H-6′, and di-*tert*-butyl protons, see flavone **8**; OH-4′ δ 5.65, H-5, H-6, H-8 δ 8.10, 7.00, 7.00; additional signals at δ 2.60 (4H, m), 2.90 (2H, t, J=7.0 Hz), 3.85 (4H, m), 4.25 (2H, t, J=7.0 Hz, 2H); *anal.*, calcd for $C_{29}H_{37}NO_5$, C 72.62, H 7.78, N 2.92; found C 72.74, H 7.75, N 3.02.

FLAVONE **13**.—Prepared by alkylation with 4-chlorobenzyl chloride (0.193 g, 1.2 mmol) and $KHCO_3$ (0.066 g, 0.66 mmol) for 2 h. Yield (0.194 g, 66%); mp 190–191°; ^{1}H nmr ($CDCl_3$) δ H-3, H-2′, H-6′, and di-*tert*-butyl protons, see flavone **8**; OH-4′ δ 5.65, H-5, H-6, H-8 δ 8.15, 7.05, 7.05; additional signals at δ 5.15 (2H, s), 7.40 (4H, s); *anal.*, calcd for $C_{30}H_{31}O_4Cl$, C 73.38, H 6.36; found C 73.14, H 6.33.

FLAVONE **14**.—Preparation of 1-chloroacetylpiperidine: chloroacetyl chloride (0.92 ml, 11.5 mmol) was added to a solution of piperidine (2 ml, 20 mmol) in CH_2Cl_2 (10 ml) at 0°. The reaction mixture was stirred for 3 h at room temperature. The solution was extracted with 0.2 N aqueous HCl, washed with H_2O, dried over Na_2SO_4, and the solvent was evaporated to yield quantitatively the attempted compound as a colorless oil. Flavone **14** was prepared by alkylation with 1-chloroacetylpiperidine (0.125 g, 0.78 mmol) and $KHCO_3$ (0.3 g, 3 mmol) for 3 h. Yield (0.18 g, 61%); mp 215–216°; ^{1}H nmr ($CDCl_3$) δ H-3, H-2′, H-6′, and di-*tert*-butyl protons, see flavone **8**; OH-4′ δ 5.65, H-5, H-6, H-8 δ 8.15, 7.05, 7.05; additional signals at δ 1.65 (6H, m), 3.55 (4H, m), 4.80 (2H, s); *anal.*, calcd for $C_{30}H_{37}NO_5$, C 73.29, H 7.59, N 2.85; found C 72.83, H 7.59, N 2.80.

FLAVONE **15**.—Preparation of 1-benzyl-4-chloroacetylpiperazine: chloroacetyl chloride (1 ml, 12.5 mmol) was added to a solution of 1-benzylpiperazine (2 ml, 11.5 mmol) in CH_2Cl_2 (10 ml) at 0°. The reaction mixture was stirred for 3 h at room temperature. The solution was washed with 0.5 N aqueous NaOH, then with H_2O. Standard work-up afforded quantitatively the desired product as a colorless oil. Flavone **15** was prepared by alkylation with 1-benzyl-4-chloroacetylpiperazine (0.196 g, 0.78 mmol) and $KHCO_3$ (0.3 g, 3 mmol) for 3 h. Yield (0.227 g, 65%); mp 167–169°; ^{1}H nmr ($CDCl_3$) δ H-3, H-2′, H-6′, di-*tert*-butyl protons, see flavone **8**; OH-4′ δ 5.65, H-5, H-6, H-8 δ 8.15, 7.00, and 7.00: additional signals at δ 2.50 (4H, m), 3.55 (2H, s), 3.65 (4H, m), 4.80 (2H, s), 7.30 (5H, m); *anal.*, calcd for $C_{36}H_{42}N_2O_5$, C 74.20, H 7.26, N 4.81; found C 73.96, H 7.12, N 4.81.

FLAVONE **16**.—Prepared by alkylation with 2-chloro-*N*,*N*-diethylacetamide (0.11 ml, 0.78 mmol) and $KHCO_3$ (0.3 g, 3 mmol) for 3 h. Yield (0.208 g, 72%); mp 180–182°; ^{1}H nmr ($CDCl_3$) δ H-3, H-2′, H-6′, and di-*tert*-butyl protons, see flavone **8**; OH-4′ δ 5.65, H-5, H-6, H-8 δ 8.15, 7.00, 7.00; additional signals at δ 1.20 (3H, t, J=7.5 Hz), 1.30 (3H, t, J=7.5 Hz), 3.45 (4H, m), 4.80 (2H, s); *anal.*, calcd for $C_{29}H_{37}NO_5$, C 72.62, H 7.78, N 2.92; found C 72.15, H 7.65, N 2.93.

FLAVONE **17**.—Prepared by alkylation with 2-(chloromethyl)quinoline hydrochloride (0.167 g, 0.78 mmol) and $KHCO_3$ (0.3 g, 3 mmol) for 3 h. Yield (0.176 g, 58%); mp 213–215°; ^{1}H nmr ($CDCl_3$) δ H-3, H-2′, H-6′, and di-*tert*-butyl protons, see flavone **8**; OH-4′ δ 5.65; H-5, H-6, H-8 δ 8.10, 7.15, 7.15; additional signals at δ 5.50 (2H, s), 7.60 (1H, dd, J=8.2 and 6.8 Hz, 1H), 7.70–7.80 (2H, m), 7.85 (1H, dd, J=8.2 and 1.6 Hz, 1H), 8.00–8.20 (1H, m), 8.20 (1H, d, J=8.3 Hz); *anal.*, calcd for $C_{33}H_{33}NO_4$, C 78.08, H 6.55, N 2.76; found C 77.86, H 6.55, N 2.91.

FLAVONE **18**.—Prepared by alkylation with 2-dimethylaminoethyl chloride hydrochloride (0.108 g, 0.75 mmol) and $KHCO_3$ (0.54 g, 54 mmol) for 4 h. The dry residue was purified by flash chromatography [alumina, CH_2Cl_2-MeOH (99:1)] then crystallization from Et_2O. Yield (0.11 g, 42%); mp 136–137°; ^{1}H nmr ($CDCl_3$) δ H-3, H-2′, H-6′, and di-*tert*-butyl protons, see flavone **8**; OH-4′ δ 5.65, H-5, H-6, H-8 δ 8.15, 7.00, 7.00; additional signals at δ 2.40 (6H, s), 2.70 (2H, t, J=7.3 Hz), 4.20 (2H, t, J=7.3 Hz); *anal.*, calcd for $C_{27}H_{35}NO_4$, C 74.11, H 8.06, N 3.20; found C 74.20, H 8.34, N 3.43.

FLAVONE **19**.—Prepared by alkylation with 3-dimethylaminopropyl chloride hydrochloride (0.118 g, 0.75 mmol) and $KHCO_3$ (0.54 g, 54 mmol) for 4 h. Yield (0.16 g, 59%); mp 183–185°; ^{1}H nmr ($CDCl_3$) δ H-3, H-2′, H-6′, and di-*tert*-butyl protons, see flavone **8**; OH-4′ δ 5.65, H-5, H-6, H-8 δ 8.10, 6.95, 6.95; additional signals at δ 2.05 (2H, quint., J=7.5 Hz), 2.30 (6H, s), 2.50 (2H, t, J=7.5 Hz), 4.15 (2H, t, J=7.5 Hz); *anal.*, calcd for $C_{28}H_{37}NO_4$, C 74.47, H 8.26, N 3.10; found C 74.01, H 8.31, N 3.14.

FLAVONE **20**.—Prepared by alkylation with ethyl bromovalerate (0.13 g, 0.78 mmol) and $KHCO_3$ (0.18 g, 1.8 mmol) for 3 h. Yield (0.19 g, 64%); mp 131–132°; ^{1}H nmr ($CDCl_3$) δ H-3, H-2′, H-6′, and di-*tert*-butyl protons, see flavone **8**; OH-4′ δ 5.65, H-5, H-6, H-8 δ 8.15, 6.95, 6.95; additional signals at

δ 1.30 (3H, t, J=7.4 Hz), 1.90 (4H, m), 2.40 (2H, t, J=7.5 Hz), 4.15 (4H, m); *anal.*, calcd for $C_{30}H_{38}O_6$, C 72.85, H 7.74; found C 73.22, H 8.02.

FLAVONE **21**.—Prepared by alkylation with 2-chloroethanol (0.2 ml, 3 mmol) and $KHCO_3$ (1 g, 10 mmol) for 6 h. The reaction mixture was poured into H_2O and extracted with EtOAc. After standard work-up, the dry residue was purified by flash chromatography [alumina, CH_2Cl_2-MeOH (99:1)] then crystallization from MeOH. Yield (0.123 g, 50%); mp 230–231°; ^{1}H nmr ($CDCl_3$) δ H-3, H-2′, H-6′, and di-*tert*-butyl protons, see flavone **8**; OH-4′ δ 5.65, H-5, H-6, H-8 δ 8.15, 7.00, 7.00; additional signals at δ 2.30 (1H, br t, exchanged with D_2O), 4.05 (2H, q, J=7.5 Hz), 4.25 (2H, t, J=7.5 Hz); *anal.*, calcd for $C_{25}H_{30}O_5$, C 73.15, H 7.37; found C 72.91, H 7.29.

FLAVONE **22**.—$KHCO_3$ (0.18 g, 1.8 mmol) was added to a solution of compound **8** (0.22 g, 0.6 mmol) in DMF (8 ml), and the mixture was stirred under N_2 at 120° for 5 min. 1,3-Propane sultone (0.092 g, 0.75 mmol) was added and the mixture was heated at the same temperature for 3 h. The reaction mixture was poured into H_2O (100 ml), then 0.2 N aqueous HCl added until pH 4. The solution was extracted with *n*-BuOH (3×40 ml) and the organic layer evaporated to dryness. Crystallization of the dry residue from EtOAc afforded pure **22** (0.22 g, 75%); mp 290–292°; ^{1}H nmr (DMSO-d_6) δ H-3, H-2′, H-6′, OH-4′, and di-*tert*-butyl protons, see flavone **8**; H-5, H-6, H-8 δ 7.90, 7.05, 7.25; additional signals at δ 2.10 (2H, quint., J=7.6 Hz), 2.70 (2H, t, J=7.6 Hz), 4.25 (2H, t, J=7.6 Hz); *anal.*, calcd for $C_{26}H_{32}O_7S$, C 63.91, H 6.60, S 6.56; found C 63.71, H 6.77, S 6.71.

FLAVONE **23**.—$KHCO_3$ (0.3 g, 3 mmol) was added to a solution of compound **8** (0.366 g, 1 mmol) in DMF (10 ml), and the mixture was stirred under N_2 at 120° for 5 min. 1,2-*O*-Isopropylidene-3-*O*-tosylglycerol (available from solketal by tosylation) (0.343 g, 1.2 mmol) was added and the mixture was heated at same temperature for 3 h. The reaction mixture was poured into H_2O (100 ml), extracted with *n*-BuOH (3×50 ml), then the organic layer was evaporated to dryness. The dry residue was refluxed in the mixture AcOH-H_2O (4:1) (15 ml) for 40 min under N_2. The reaction mixture was evaporated, and the residue was purified by flash chromatography [alumina, CH_2Cl_2-MeOH (96:4)] then crystallization from EtOAc to yield pure **23** (0.07 g, 16%); mp 190–191°; ^{1}H nmr (DMSO-d_6) δ H-3, H-2′, H-6′, OH-4′, and di-*tert*-butyl protons, see flavone **8**; H-5, H-6, H-8 δ 7.95, 7.05, 7.30; additional signals at δ 3.50 (2H, t, J=7.5 Hz), 3.85 (1H, m), 4.05 (1H, dd, J=10.0 and 7.0 Hz), 4.20 (1H, dd, J=10.0 and 7.0 Hz), 4.80 (1H, t, J=7.5 Hz, exchanged with D_2O), 5.10 (1H, d, J=7.5 Hz, exchanged with D_2O); *anal.*, calcd for $C_{26}H_{32}O_6$, C 70.89, H 7.32; found C 70.54, H 7.28.

FLAVONE **24**.—A solution of compound **8** (0.183 g, 0.5 mmol) in 0.5 N aqueous $KHCO_3$ solution (15 ml) was stirred well for 15 h at room temperature in the presence of CH_2Cl_2 (20 ml), dimethyl sulfate (0.5 ml, 5 mmol), and tetrabutylammonium hydrogen sulfate (0.02 g) as phase-transfer catalyst. The aqueous layer was added with 0.5 N aqueous HCl until pH 5, then extracted with CH_2Cl_2 (2×20 ml). The combined organic layers were washed with H_2O, dried over Na_2SO_4 then evaporated to dryness. The dry residue was purified by flash chromatography [SiO_2, CH_2Cl_2-MeOH (99:1)] then crystallized from MeOH to yield pure **24** (0.095 g, 50%); mp 195–197°; ^{1}H nmr ($CDCl_3$) δ H-3, H-2′, H-6′, and di-*tert*-butyl protons, see flavone **8**; OH-4′ δ 5.65, H-5, H-6, H-8 δ 8.15, 7.00, 6.95; additional signal at δ 3.95 (3H, s); *anal.*, calcd for $C_{24}H_{28}O_4$, C 75.76, H 7.42; found C 75.54, H 7.31.

FLAVONE **25**.—Compound **25** was prepared from **8** (0.5 mmol) as described above for **24**, except with 0.5 N NaOH aqueous solution instead of $KHCO_3$ and dimethyl sulfate (2.5 ml instead of 0.5 ml). The dry residue was purified by tlc [SiO_2, CH_2Cl_2-MeOH (99:1)] then crystallized from MeOH to yield pure **25** (0.097 g, 49%); mp 164–166°; ^{1}H nmr ($CDCl_3$) δ H-3, H-2′, H-6′, and di-*tert*-butyl protons, see flavone **8**; H-5, H-6, H-8 δ 8.15, 7.00, 6.95; additional signals at δ 3.75 (3H, s), 3.95 (3H, s); *anal.*, calcd for $C_{25}H_{30}O_4$, C 76.11, H 7.67; found C 75.94, H 7.43.

FLAVONES **26** AND **27**.—A solution of flavone **9** (0.452 g, 1 mmol) in THF-1 N aqueous NaOH (1:3) (80 ml) (15 ml) was kept at room temperature for 5 h. The reaction mixture was poured into ice-H_2O (100 ml), with 5 N aqueous HCl added until pH 2, then extracted with CH_2Cl_2 (3×50 ml). Evaporation to dryness of the organic layer afforded pure **26** as an amorphous compound (0.41 g, 96%); ^{1}H nmr (DMSO-d_6) δ H-3, H-2′, H-6′, OH-4′, and di-*tert*-butyl protons, see flavone **8**; H-5, H-6, H-8 δ 7.90, 7.05, 7.25; additional signal at δ 4.90 (2H, s); *anal.*, calcd for $C_{25}H_{28}O_6$, C 70.74, H 6.65; found C 70.28, H 6.67. A solution of **26** (0.212 g, 0.5 mmol) in THF (30 ml) was added to aqueous $NaHCO_3$ solution (0.042 g, 0.5 mmol in 20 ml). THF was evaporated, then the aqueous solution was lyophilized and afforded quantitatively **27** as an amorphous powder (0.22 g). ^{1}H nmr (DMSO-d_6) δ H-3, H-2′, H-6′, OH-4′, and di-*tert*-butyl protons, see flavone **8**; H-5, H-6, H-8 δ 7.90, 7.00, 7.00; additional signal at δ 4.50 (2H, s); *anal.*, calcd for $C_{25}H_{27}O_6Na$, C 67.25, H 6.10; found C 67.17, H 6.44.

FLAVONE **28**.—A solution of flavone **9** (0.181 g, 0.4 mmol) in *n*-pentanol (20 ml) was heated at 125° in the presence of *p*-toluenesulfonic acid monohydrate (0.02 g, 0.1 mmol) for 4 h. The reaction mixture was poured into H_2O (100 ml) and extracted with CH_2Cl_2 (3×50 ml). Standard work-up of the organic layer afforded by crystallization from MeOH pure **28** (0.145 g, 73%); mp 175–177°; ^{1}H nmr ($CDCl_3$) δ H-3, H-2′, H-6′, and di-*tert*-butyl protons, see flavone **8**; OH-4′ δ 5.65, H-5, H-6, H-8 δ 8.15, 7.00, 6.95; additional signals at δ 0.90 (3H, t, J=7.3 Hz), 1.30 (4H, m), 1.70 (2H, m), 4.25 (2H, t, J=7.4 Hz), 4.80 (2H, s); *anal.*, calcd for $C_{30}H_{38}O_6$, C 72.85, H 7.74; found C 72.42, H 7.85.

LDL OXIDATIVE MODIFICATION ASSAYS.—Low Density Lipoprotein (LDL) was prepared from fresh normal human plasma according to Havel *et al.* (21), and oxidation was induced by copper ions or endothelial cells as described previously (22,23). Flavones to be tested were dissolved in EtOH or in DMSO and added at various concentrations simultaneously with the purified LDL in both experiments. The extent of lipid peroxidation was assessed by measuring the thiobarbituric acid-reactive substances calculated as nanomoles of equivalent malondialdehyde per milligram of LDL protein. Results are expressed as inhibitory concentrations 50 (IC_{50} values) calculated from the measurement of oxidized LDL with the flavones in the medium at various concentrations, in comparison with the extent of oxidation in the absence of drug (100% of oxidation).

ACKNOWLEDGMENTS

We thank J.P. Volland, Institut de Recherches Servier, and staff for microanalytical and spectral data, M. Wierzbicki for 1,2-*O*-isopropylidene-3-*O*-tosylglycerol, D. Steinberg, La Jolla, CA, for the gift of the endothelial cell strain, F. Mahlberg for assays on apigenin and 7,4′-dihydroxyflavone, and B. Saint Paul (Hôpital du Chesnay) for supplying human plasma.

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Received 11 May 1995