Phenolic Compounds from the Roots of *Lindera fruticosa*

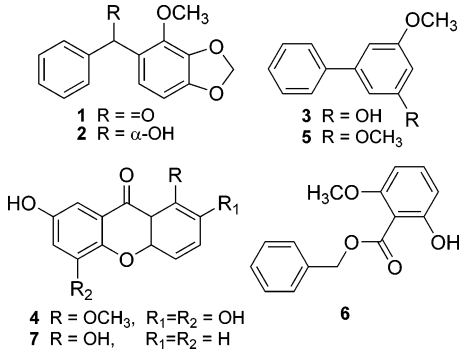
Myoung-Chong Song,[†] Fikru Nigussie,[‡] Tae-Sook Jeong,[§] Chang-Yong Lee,[⊥] Fekadu Regassa,[∥] Takele Markos,[∥] and Nam-In Baek*,[†]

Graduate School of Biotechnology and Plant Metabolism Research Center, Kyung Hee University, Suwon 449-701, Korea, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-333, Korea, Department of Food Science and Technology, Cornell University, Geneva, New York 14456, and Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit 34, Ethiopia

Received February 1, 2006

The phenolic compounds isolated from the roots of *Lindera fruticosa* included four new compounds, 2-methoxy-3,4-methylenedioxybenzophenone (**1**), (*S*)-2-methoxy-3,4-methylenedioxybenzhydryl alcohol (**2**), 3-hydroxy-5-methoxybiphenyl (**3**), and 1-methoxy-2,5,7-trihydroxyxanthone (**4**). Three previously identified phenolics were also identified, namely, 3,5-dimethoxybiphenyl (**5**), benzyl 2-hydroxy-6-methoxybenzoate (**6**), and 1,7-dihydroxyxanthone (**7**). These compounds were evaluated for their inhibitory effects on human acyl-CoA:cholesterol acyltransferase activity and on the in vitro oxidation of low-density lipoprotein.

Lindera fruticosa Hemsley is a shrub that grows in China, Nepal, India, and Ethiopia. In Nepal, the fruit of this plant is used for the a treatment of gastric disease,[1] and in Ethiopia, the root is a traditional anti-inflammatory medicine. However, no phytochemical research has been carried out on *L. fruticosa* to date. This paper describes the isolation of four new (**1**−**4**) and three known (**5**−**7**) phenolic compounds from *L. fruticosa.* To determine whether these compounds might be effective in the development of hypercholesterolemic or antiatherogenic agents, their potential for inhibiting human acyl-CoA:cholesterol acyltransferase (hACAT) and low-density lipoprotein (LDL) oxidation was evaluated.



Compound **1** showed characteristic phenyl (2937, 1470 cm^{-1}), ketone (1731 cm^{-1}), and ether (1281, 1071 cm^{-1}) absorption bands in the IR spectrum. A molecular formula of $C_{15}H_{12}O_4$ was determined by HREIMS ($[M]^+$, *m*/*z* 256.0734). The ^1H NMR spectrum of **1** indicated the presence of a monosubstituted benzene ring [δ 7.76 (2H, br dd, J = 8.0, 1.6 Hz), 7.51 (1H, dddd, J = 8.0, 8.0, 1.6, 1.6 Hz), 7.41 (2H, br dd, J = 8.0, 8.0 Hz)], a 1,2,3,4-tetrasubstituted benzene ring [δ 6.93 (1H, d, J = 8.0 Hz), 6.57 (1H, d, J = 8.0 Hz)], a methylenedioxy (δ 5.59, 2H, s), and an aromatic methoxy group (δ 3.81, 3H, s). The ^{13}C NMR spectrum indicated the presence of a characteristic nonchelated ketone at δ 195.0 (C-7), five quaternary sp^2 carbons at δ 151.2 (C-4), 141.8 (C-2), 138.3 (C-1′), 136.8 (C-3), and 126.1 (C-1), seven methine

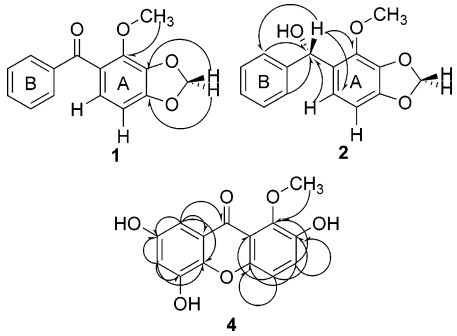


Figure 1. ^1H and ^{13}C long-range correlations observed in the HMBC spectra of compounds **1**, **2**, and **4**. The arrows indicate the long-range correlations between proton and carbon signals in the HMBC spectrum.

sp^2 carbons at δ 132.6 (C-4′), 124.3 (C-6), 102.7 (C-5), 129.5 (C-2′,6′), and 128.0 (C-3′,5′), a methylenedioxy sp^3 carbon at δ 101.5, and a methoxy signal at δ 60.0. The structure of compound **1** deduced from the data above was very similar to that of secuiphenone A, a 4-methoxy-2,3-methylenedioxybenzophenone.[2] The small discrepancies were derived from a difference in the location of the methylenedioxy and the methoxy on the benzene ring. Determination of the final structure of **1**, including the location of the functional group, was accomplished by a gradient heteronuclear multiple bonding connectivity (gHMBC) NMR experiment (Figure 1). The methoxy hydrogen signal (δ 3.81) correlated with the oxygenated olefinic quaternary carbon C-2 (δ 141.8), and the methylenedioxy proton signal (δ 5.59) correlated with the oxygenated olefinic quaternary carbons C-3 (δ 136.8) and C-4 (δ 148.6). Thus, the new compound **1** was identified as 2-methoxy-3,4-methylenedioxybenzophenone.

Compound **2** showed absorptions characteristic of phenolic alcohol (3427 cm^{-1}), phenyl (2924, 1468 cm^{-1}), and ether (1258, 1065 cm^{-1}) groups in the IR spectrum. A molecular formula of $C_{15}H_{14}O_4$ was determined by HREIMS ($[M]^+$, *m*/*z* 258.0887). The ^1H and ^{13}C NMR spectra revealed compound **2** to be similar to compound **1**, with the exception of a singlet oxygenated methine proton at δ 5.91 (1H, s, H-7), a carbon at δ 72.5 (C-7), and the absence of any characteristic nonchelated ketone carbonyl. In the gHMBC spectrum, the C-7 (δ 72.5) signal showed cross-peaks with H-2′/6′ (δ 7.34) and H-6 (δ 6.73), and the H-7 (δ 5.91) proton signal showed cross-peaks with C-2 (δ 140.9), C-6 (δ 120.7), and C-2′/6′ (δ 128.0) by J_3 correlation. Therefore, rather than a ketone, a hydroxyl group was present between the A and B rings. The

* To whom correspondence should be addressed. Tel: 82-31-201-2661. Fax: 82-31-201-2157. E-mail: nibaek@khu.ac.kr.
[†] Kyung Hee University.
[‡] Seoul National University.
[§] Korea Research Institute of Bioscience and Biotechnology.
[⊥] Cornell University.
[∥] Addis Ababa University.

10.1021/np060048b CCC: $33.50
Published on Web 04/28/2006

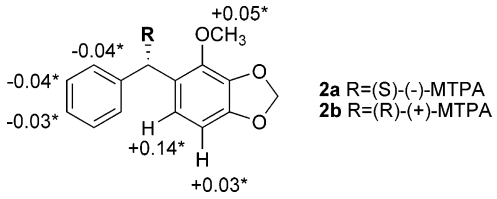


Figure 2. NMR chemical shift differences for the (*S*)-(−)-MTPA ester of **2** (**2a**) and the (*R*)-(+)-MTPA ester of **2** (**2b**) in ppm at 400 MHz. *$\Delta\delta^{SR}$: Defined as δ_H in **2a** − δ_H in **2b**.

absolute configuration at C-7 between the phenyl groups was clarified by applying the modified Mosher method to the α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) esters of compound **2.** Thus, compound **2** was esterified with (*R*)-(−)-MTPA chloride and (*S*)-(+)-MTPA chloride in the presence of pyridine, affording the (*S*)-(−)-MTPA ester (**2a**) (in a 82.9% yield) and the (*R*)-(+)-MTPA ester (**2b**) (in a 86.8% yield), as colorless crystals, respectively. In the ^{1}H NMR spectrum of MTPA esters, $\Delta\delta$ (ppm) = (*S*)-(−)-MTPA − (*R*)-(+)-MTPA was calculated for each proton around the C-7 MTPA ester group. The proton signals assigned for H-2′/6′, H-3′/5′, and H-4′ in the (*S*)-(−)-MTPA derivative (**2a**) were observed at a higher field compared to those of the (*R*)-(+)-MTPA derivative (**2b**), while the proton signals due to H-5, H-6, and the methoxy of (*S*)-(−)-MTPA (**2a**) were observed at a lower field compared to that in the (*R*)-(+)-MTPA (**2b**). The $\Delta\delta$ values and their signs are shown in Figure 2 and showed unequivocally that the absolute stereochemistry of the asymmetric carbon at C-7 is *S*. Thus, the new compound **2** was identified as (*S*)-2-methoxy-3,4-methylenedioxybenzhydryl alcohol, a reduced form of compound **1**.

Compound **3** showed hydroxy (3610 cm^{-1}), phenyl (3010, 1610, 1430 cm^{-1}), and ether (1225, 1210 cm^{-1}) bands in the IR spectrum. A molecular formula of $C_{13}H_{12}O_2$ was determined by HREIMS ([M]$^+$, 200.0834). The ^{1}H NMR spectrum revealed the signals for a monosubstituted benzene ring [δ 7.53 (2H, br dd, J = 8.0, 1.6 Hz), 7.40 (2H, dddd, J = 8.0, 8.0, 1.6, 1.6 Hz), 7.33 (1H, br dd, J = 8.0, 8.0 Hz)], a 1,3,5-trisubstituted benzene ring [δ 6.71 (1H, dd, J = 1.6, 1.6 Hz), 6.65 (1H, dd, J = 1.6, 1.6 Hz), 6.40 (1H, dd, J = 1.6, 1.6 Hz)], and a methoxy group (δ 3.82, 3H, s). The ^{13}C NMR spectroscopic data indicated four quaternary sp^2 carbons at δ 161.0 (C-5), 156.8 (C-3), 143.6 (C-1′), and 140.7 (C-1), eight methine sp^2 carbons at δ 128.6 (C-2′,6′), 127.5 (C-4′), 127.0 (C-3′,5′), 106.8 (C-2), 105.6 (C-6), and 100.3 (C-4), and a methoxy signal at δ 55.4. On the basis of this evidence, the new compound **3** was determined to be 3-hydroxy-5-methoxybiphenyl. Although obtained as a natural product for the first time in the present investigation, **3** has been synthesized from 1,3-cyclohexanedione previously.[3]

Compound **4** showed absorbances of phenolic alcohol (3306 cm^{-1}), phenyl (2922, 1487 cm^{-1}), and ketone (1734 cm^{-1}) in the IR spectrum. A molecular formula of $C_{14}H_{10}O_6$ was determined by HREIMS ([M]$^+$, 274.0473). The ^{1}H NMR spectrum indicated the proton signals for a 1,2,3,4-tetrasubstituted benzene ring at δ 7.30 (1H, d, J = 8.8 Hz) and 6.86 (1H, d, J = 8.8 Hz), a 1,2,3,5-tetrasubstituted benzene ring at δ 8.00 (1H, br s) and 7.57 (1H, br s), and an aromatic methoxy at δ 3.85 (3H, s). The ^{13}C NMR spectrum indicated a characteristic ketone at δ 182.7 (C-9), eight quaternary sp^2 carbons at δ 156.0 (C-5a), 155.1 (C-2), 150.3 (C-7), 149.8 (C-5), 146.6 (C-4a), 140.8 (C-1), 126.5 (C-8a), and 109.8 (C-1a), four methine sp^2 carbons at δ 120.5 (C-3), 120.1 (C-6), 109.3 (C-8), and 108.6 (C-4), and a methoxy carbon at δ 57.4. The tetraoxygenated xanthone character of compound **4** was inferred from the above data. In the gHMBC NMR spectrum (Figure 1), the methoxy hydrogen signal (δ 3.85) showed a cross-peak with the oxygenated olefinic quaternary carbon C-1 (δ 155.1). The H-3 (δ 7.30) proton signal exhibited cross-peaks with C-1 (δ 155.1) and C-4a (δ 146.5) by J_3 correlation, as well as with C-2 (δ 140.8) by J_2 correlation. The H-4 signal (δ 6.86) showed cross-peaks with C-2 (δ 140.8) and C-1a (δ 109.8) by J_3 correlation and C-4a (δ 146.5) by J_2 correlation. Therefore, in the A ring, the hydroxyl group could be placed at C-2 and the methoxy group at C-1. The H-6 signal (δ 7.54) showed a cross-peak with C-5a (δ 156.0) by J_3 correlation and with C-5 (δ 149.8) and C-7 (δ 150.4) by J_2 correlation. In turn, the H-8 signal (δ 8.00) indicated cross-peaks with C-5a (δ 156.0) and C-9 (δ 182.7) by J_3 correlation and with C-8a (δ 126.5) and C-7 (δ 150.4) by J_2 correlation. Therefore, the other two hydroxyl groups were assigned at C-5 and C-7, repectively. Thus, compound **4** was established as a 1-methoxy-2,5,7-trihydroxyxanthone.

The three known compounds **5**−**7** were identified as 3,5-dimethoxybiphenyl, benzyl 2-hydroxy-6-methoxybenzoate, and 1,7-dihydroxyxanthone, respectively, through the comparison of several spectroscopic data with those of the literature.[4−6]

The phenolic compounds isolated from *L. fruticosa* were tested for inhibition of hACAT-1 and LDL oxidation activity. It has been suggested that oxidation of LDL cholesterol is an important step in the formation of atherosclerotic lesions.[7] Evidence to support this hypothesis is based in part on observational studies that demonstrate associations between oxidized LDL cholesterol and both the presence of atherosclerotic lesions[8] and the progression of carotid artery atherosclerosis.[9] Acyl-CoA:cholesterol acyltransferase (ACAT, E.C. 2.3.1.26) is an allosteric enzyme[10] that plays an important role in the esterification of cholesterol, facilitating intracellular storage[11] and intercellular circulation via incorporation into apolipoprotein-B (apo-B)-containing lipoproteins.[12] Additionally, many in vitro and in vivo experiments have demonstrated that ACAT plays a critical role in the development of foam cells, a prominent feature of atherosclerotic lesions.[13]

Naturally occurring hACAT-1 and LDL oxidation inhibitors have been rarely reported thus far. The activity of hACAT-1 was inhibited by compounds **1**, **5**, and **6** (0.40 mM) by 54 ± 0.7%, 43 ± 1.3%, and 46 ± 0.6%, respectively. However, these compounds exhibited much weaker inhibitory activity than oleic acid anilide (the positive control), with 57 ± 2.1% (0.3 μM) inhibition. Compounds **3**−**5** demonstrated LDL antioxidant activity with IC_{50} values of 15.5, 25.5, and 48.1 μM, respectively. These values were also somewhat lower than the positive control, probucol, which had an IC_{50} value of 4.3 μM.

Experimental Section

General Experimental Procedures. For instrumental and general methods, see a previous paper.[14]

Plant Material. The roots of *L. fruticosa* were collected from a rural forest in Addis Ababa Province, Ethiopia, by one of the authors (F.N.). They were identified by Dr. Dae-Keun Kim, Woosuk University, Jeonju. A voucher specimen (KHU02031) was deposited in the Laboratory of Natural Products Chemistry, Kyung Hee University, Suwon, Republic of Korea.

Extraction and Isolation. The dried, powdered roots (1 kg) were extracted with 80% aqueous methanol (20 L × 3) and concentrated in vacuo. The extracts were partitioned with H_2O (2 L), EtOAc (2 L × 3), and *n*-BuOH (2 L × 3). The concentrated EtOAc fraction (LFE, 14 g) was subjected to silica gel column chromatography (150 g, ϕ 5 × 12 cm) and eluted with a gradient of $CHCl_3$−MeOH (10:1 → 7:1, 1 L of each), resulting in 12 fractions (LFE1−LFE12). Fraction LFE2 [1.0 g, V_e/V_t (elution volume/total volume) 0.05−0.10] was subjected to ODS column chromatography (100 g, ϕ 3 × 6 cm) and eluted with H_2O−MeOH (5:4, 1.8 L), yielding compound **1** [112 mg, V_e/V_t 0.28−0.44; TLC (RP-18 F_{254S}) R_f 0.4, MeOH−H_2O, 3:1] and 3,5-dimethoxybiphenyl (**5**)[4] [32 mg, V_e/V_t 0.44−0.53; TLC (RP-18 F_{254S}) R_f 0.3, MeOH−H_2O, 3:1]. Fraction LFE2-7 (290 mg, V_e/V_t 0.11−0.14) was separated by ODS column chromatography (100 g, ϕ 4 × 6 cm) and eluted with MeOH−H_2O (3:1, 1.6 L), yielding benzyl 2-hydroxy-6-methoxybenzoate (**6**)[5] [41 mg, V_e/V_t 0.13−0.16; TLC (RP-18 F_{254S}) R_f 0.4, MeOH−H_2O, 5:1]. Fraction LFE3 (1.4 g, V_e/V_t 0.10−0.15) was separated by ODS column chromatography (150 g, ϕ 4 × 6 cm) and eluted with MeOH−H_2O (1:1 → 2:1, 1 L of each), resulting in 11

Table 1. Inhibitory Activity of Compounds **1**−**7** on hACAT-1 and LDL Oxidation[a]

	1	2	3	4	5	6	7	positive control[b]
hACAT-inhibition (%)[c]	54 ± 0.7	31 ± 2.8	30 ± 2.2	7.0 ± 0.7	43 ± 1.3	46 ± 0.6	31 ± 0.4	57 ± 2.1
LDL antioxidation (IC_{50}, μM)	524	192	15.5	25.5	48.1	478	386	5.4

[a] The data are presented as mean ± standard deviation of three replicates. [b] Probucol and oleic acid anilide were used as inhibitors on LDL oxidation and on hACAT-1, respectively. [c] The treatment concentration of compounds isolated from *Lindera fruticosa* was 0.4 mM, and that of oleic acid anilide was 0.3 μM.

fractions (LFE3-1−LFE3-11). Fraction LFE3-7 (36 mg, V_e/V_t 0.51−0.53) was subjected to silica gel column chromatography (100 g, ϕ 3 × 7 cm) and eluted with *n*-hexane−EtOAc (3:1, 2 L), yielding compound **2** [23 mg, V_e/V_t 0.13−0.16; TLC (Keiselgel 60 F_{254}) R_f 0.7, *n*-hexane−EtOAc, 1:1]. LFE3-8 (162 mg, V_e/V_t 0.54−0.62) was separated by ODS column chromatography (70 g, ϕ 3 × 5 cm) and eluted with MeOH−H_2O (4:1, 2.5 L), yielding compounds **3** [14 mg, V_e/V_t 0.06−0.20; TLC (RP-18 F_{254S}) R_f 0.5, MeOH−H_2O, 3:1] and **4** [16 mg, V_e/V_t 0.30−0.40; TLC (RP-18 F_{254S}) R_f 0.45, MeOH−H_2O, 3:1]. Fraction LFE3-9 (122 mg, V_e/V_t 0.63−0.69) was separated by ODS column chromatography (70 g, ϕ 3 × 5 cm) and eluted with MeOH−H_2O (8:1, 1 L), yielding 1,7-dihydroxyxanthone (**7**)[6] [23 mg, V_e/V_t 0.40−0.50; TLC (RP-18 F_{254S}) R_f 0.35, MeOH−H_2O, 4:1].

2-Methoxy-3,4-methylenedioxybenzophenone (1): colorless oil; IR (CaF_2 window in $CHCl_3$) ν_{max} 2937, 2841, 1731, 1658, 1470, 1281, 1071 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.76 (2H, br dd, J = 8.0, 1.6 Hz, H-2′, 6′), 7.51 (1H, dddd, J = 8.0, 8.0, 1.6, 1.6 Hz, H-4′), 7.41 (2H, br dd, J = 8.0, 8.0 Hz, H-3′, 5′), 6.93 (1H, d, J = 8.0 Hz, H-6), 6.57 (1H, d, J = 8.0 Hz, H-5), 5.59 (2H, br s, −OCH_2O−), 3.81 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 195.0 (C, C-7), 151.2 (C, C-4), 141.8 (C, C-2), 138.3 (C, C-1′), 136.8 (C, C-3), 132.6 (CH, C-4′), 129.5 (CH, C-2′, 6′), 128.0 (CH, C-3′, 5′), 126.1 (C, C-1), 124.3 (CH, C-6), 102.7 (CH, C-5), 101.5 (CH_2, −OCH_2O−), 60.0 (CH_3, OCH_3); EIMS *m*/*z* 256 $[M]^+$ (40), 240 (32), 180 (9), 150 (100), 92 (100), 57 (35); HREIMS *m*/*z* 256.0734 (calcd for $C_{15}H_{12}O_4$, 256.0736).

(*S*)-2-Methoxy-3,4-methylenedioxybenzhydryl alcohol (2): fine, yellow, amorphous powder (EtOAc); $[\alpha]^{26}_D$ −13.1 (*c* 0.26, $CHCl_3$); IR (CaF_2 window in $CHCl_3$) ν_{max} 3427, 2924, 2854, 1728, 1574, 1468, 1258, 1065 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) δ 7.34 (2H, dd, J = 8.4, 2.0 Hz, H-2′, 6′), 7.30 (2H, dd, J = 8.4, 8.4 Hz, H-3′, 5′), 7.23 (1H, tt, J = 8.4, 2.0 Hz, H-4′), 6.73 (1H, d, J = 8.0 Hz, H-6), 6.49 (1H, d, J = 8.0 Hz, H-5), 5.91 (3H, br s, −OCH_2O− and H-7), 3.81 (3H, s, OCH_3); ^{13}C NMR (pyridine-d_5, 100 MHz) δ 148.9 (C, C-4), 140.9 (C, C-2), 143.6 (C, C-1′), 136.5 (C, C-3), 128.6 (C, C-1), 128.0 (CH, C-2′, 6′), 127.0 (CH, C-4′), 126.2 (CH, C-3′, 5′), 120.7 (CH, C-6), 102.5 (CH, C-5), 101.0 (CH_2, −OCH_2O−), 72.5 (CH, C-7), 59.5 (CH_3, OCH_3); EIMS *m*/*z* 258 $[M]^+$ (40), 239 (78), 200 (13), 178 (97), 164 (32), 152 (13), 148 (64), 105 (100), 96 (38), 72 (80), 59 (100); *m*/*z* 258.0887 (calcd for $C_{15}H_{14}O_4$, 258.0892).

Esterification of Compound 2 with (*R*)-(−)-MTPA Chloride.[15] The esterification of compounds **2** (2.5 mg × 2) with (*R*)-(−)-MTPA chloride (24 μL) and (*S*)-(+)-MTPA chloride (24 μL) yielded the (*S*)-(−)-MTPA ester **2a** (3.9 mg) and the (*R*)-(+)-MTPA ester **2b** (4.0 mg) as colorless crystals, respectively.

(*S*)-(−)-MTPA ester 2a: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.30 (2H, dd, J = 8.4, 2.0 Hz, H-2′, 6′), 7.26 (2H, dd, J = 8.4, 8.4 Hz, H-3′, 5′), 7.19 (1H, tt, J = 8.4, 2.0 Hz, H-4′), 7.18−7.28 (phenyl groups of MTPA overlapped with H-2′−H-6′), 6.86 (1H, d, J = 8.0 Hz, H-6), 6.52 (1H, d, J = 8.0 Hz, H-5), 5.90 (1H, d, J = 1.2 Hz, −OCH_2O−), 5.87 (1H, d, J = 1.2 Hz, −OCH_2O−), 5.53 (1H, s, H-7), 3.86 (3H, s, OCH_3).

(*R*)-(+)-MTPA ester 2b: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.34 (2H, dd, J = 8.4, 2.0 Hz, H-2′, 6′), 7.30 (2H, dd, J = 8.4, 8.4 Hz, H-3′, 5′), 7.22 (1H, tt, J = 8.4, 2.0 Hz, H-4′), 7.20−7.31 (phenyl groups of MTPA overlapped with H-2′−H-6′), 6.73 (1H, d, J = 8.0 Hz, H-6), 6.49 (1H, d, J = 8.0 Hz, H-5), 5.91 (1H, d, J = 1.2 Hz, −OCH_2O−), 5.90 (1H, d, J = 1.2 Hz, −OCH_2O−), 5.90 (1H, s, H-7), 3.81 (3H, s, OCH_3).

3-Hydroxy-5-methoxybiphenyl (3):[3] colorless oil; IR (CaF_2 window in $CHCl_3$) ν_{max} 3610, 3010, 2960, 2840, 1610 (s), 1590, 1500, 1430, 1360, 1225, 1210 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.53 (2H, br dd, J = 8.0, 1.6 Hz, H-2′, 6′), 7.40 (2H, dddd, J = 8.0, 8.0, 1.6, 1.6 Hz, H-3′, 5′), 7.33 (1H, br dd, J = 8.0, 8.0 Hz, H-4′), 6.71 (1H, dd, J = 1.6, 1.6 Hz, H-6), 6.65 (1H, dd, J = 1.6, 1.6 Hz, H-2), 6.40 (1H, dd, J = 1.6, 1.6 Hz, H-4), 3.82 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.0 (C, C-5), 156.8 (C, C-3), 143.6 (C, C-1′), 140.7 (C, C-1), 128.6 (CH, C-2′, 6′), 127.5 (CH, C-4′), 127.0 (CH, C-3′, 5′), 106.8 (CH, C-2), 105.6 (CH, C-6), 100.3 (CH, C-4), 55.4 (CH_3, OCH_3); EIMS *m*/*z* 200 $[M]^+$ (100), 170 (100), 128 (91), 115 (46), 102 (20), 77 (34), 51 (15), 18 (8); HREIMS *m*/*z* 200.0834 (calcd for $C_{13}H_{12}O_2$, 200.0837).

1-Methoxy-2,5,7-trihydroxyxanthone (4): fine, yellow, amorphous powder (MeOH); IR (CaF_2 window in MeOH) ν_{max} 3306, 2922, 2853, 1734, 1637, 1605, 1581, 1487 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) δ 8.00 (1H, br s, H-8), 7.57 (1H, br s, H-6), 7.30 (1H, d, J = 8.8 Hz, H-3), 6.86 (1H, d, J = 8.8 Hz, H-4), 3.85 (1H, s, OCH_3); ^{13}C NMR (pyridine-d_5, 100 MHz) δ 182.7 (C, C-9), 156.0 (C, C-5a), 155.1 (C, C-2), 150.3 (C, C-7), 149.8 (C, C-5), 146.6 (C, C-4a), 140.8 (C, C-1), 126.5 (C, C-8a), 120.5 (CH, C-3), 120.1 (CH, C-6), 109.8 (C, C-1a), 109.3 (CH, C-8), 108.6 (CH, C-4), 57.4 (CH_3, OCH_3); EIMS *m*/*z* 274 $[M]^+$ (10), 230 (43), 215 (11), 200 (12), 187 (15), 172 (5), 149 (12), 135 (17), 115 (10), 97 (12), 84 (100), 56 (100), 18 (83); HREIMS *m*/*z* 274.0473 (calcd for $C_{14}H_{10}O_6$, 274. 0477).

ACAT Activity Assay. The inhibitory activity on ACAT was determined using a previous method as described by Brecher and Chan,[16] with slight modifications.[17]

LDL Oxidation Assay. The inhibitory activity on LDL oxidation was determined using a previous method as described in the literature,[18] with slight modifications.[17]

Acknowledgment. This study was supported by the SRC program of MOST/KOSEF (R11-2000-081) through the Plant Metabolism Research Center, Kyung Hee University, and a grant from the BioGreen 21 Program, Rural Development Administration, Republic of Korea.

References and Notes

(1) Manadhar, N. P. *Fitoterapia* **1994**, *65*, 7−13.
(2) Yang, X. D.; Xu, L. Z.; Yang, S. L. *Chin. Chem. Lett.* **2003**, *14*, 930−931.
(3) Kotnis, A. S. *Tetrahedron Lett.* **1991**, *32*, 3441−3444
(4) Burkhardt, G.; Schild, W.; Becker, H.; Grubert, M. *Phytochemistry* **1992**, *31*, 543−548.
(5) Kodpinid, M.; Sadavongvivid, C.; Thebtaranonth, C.; Thebtaranonth, Y. *Phytochemistry* **1984**, *23*, 199−200.
(6) Yang, X. D.; Xu, L. Z.; Yang, S. L. *Phytochemistry* **2001**, *58*, 1245−1249.
(7) Diaz, M. N.; Frei, B.; Vita, J. A.; Keaney, J. F., Jr. *N. Engl. J. Med.* **1997**, *337*, 408−416.
(8) Regnstrom, J.; Nilsson, J.; Tornvall, P.; Landou, C.; Hamsten, A. *Lancet* **1992**, *339*, 1183−1186.
(9) Salonen, J. T.; Yla-Herttuala, S.; Yamamoto, R.; Butler, S.; Korpela, H.; Salonen, R.; Nyyssonen, K.; Palinski, W.; Witztum, J. L. *Lancet* **1992**, *339*, 883−887.
(10) Chang, T. Y.; Chang, C. Y.; Cheng, D. *Annu. Rev. Biochem.* **1997**, *66*, 613−638.
(11) Chang, C. C.; Huh, H. Y.; Cadigan, K. M.; Chang, T. Y. *J. Biol. Chem.* **1993**, *268*, 20747−20755.
(12) Suckling, K. E.; Stange, E. F. *J. Lipid Res.* **1985**, *26*, 647−671.
(13) Accad, M.; Smith, S. J.; Newland, D. L.; Sanan, D. A.; King, L. E. Jr.; Linton, M. F.; Fazio, S.; Farese, R. V., Jr. *J. Clin. Invest.* **2000**, *105*, 711−719.
(14) Kim, D. H.; Han, K. M.; Chung, I. S.; Kim, D. K.; Kim, S. H.; Kwon, B. M.; Jeong, T. S.; Park, M. H.; Baek, N. I. *Arch. Pharm. Res.* **2005**, *28*, 550−556.
(15) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *24*, 512−519.
(16) Brecher, P.; Chan, C. T. *Biochim. Biophys. Acta* **1980**, *617*, 458−471.
(17) Kim, Y. K.; Tomoda, H.; Nishida, H.; Sunazuka, T.; Obata, R.; Omura, S. *J. Antibiot.* **1994**, *47*, 154−162.
(18) (a) Havel, R. J.; Eder, H. A.; Bragdon, J. H. *J. Clin. Invest.* **1955**, *34*, 1345−1353. (b) Buege, J. A.; Aust, S. D. *Methods Enzymol.* **1978**, *52*, 302−310. (c) Ahn, B. T.; Lee, S. K.; Lee, S. B.; Lee, E. S.; Kim, J. G.; Bok, S. H.; Jeong, T. S. *J. Nat. Prod.* **2001**, *64*, 1562−1564.

NP060048B