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Novel Flavonol Glycoside, 7-*O*-Methyl Mearnsitrin, from *Sageretia theezans* and Its Antioxidant Effect

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A novel flavonol glycoside, 7-*O*-methylmearnsitrin (7,4′-*O*-dimethylmyricetin 3-*O*-α-L-rhamnopyranoside), and myricetrin, kaempferol 3-*O*-α-L-rhamnopyranoside, europetin 3-*O*-α-L-rhamnoside, and 7-*O*-methyl quercetin 3-*O*-α-L-rhamnopyranoside were isolated from the leaves of *Sageretia theezans*, and their chemical structures were identified by spectroscopic analyses including two-dimensional NMR (HSQC, HMBC). Whereas myricetrin, europetin 3-*O*-α-L-rhamnoside, and 7-*O*-methylquercetin 3-*O*-α-L-rhamnopyranoside showed stronger activities than ascorbic acid and α-tocopherol, 7-*O*-methylmearnsitrin showed very weak antioxidant activities by ESR and LDL oxidation inhibition tests.

KEYWORDS: Flavonol glycosides; 7-*O*-methylmearnsitrin; *Sageretia theezans*; ESR; LDL oxidation

INTRODUCTION

Sageretia theezans is an evergreen tender shrub of the Rhamnaceae family native to Asia and warmer areas of North America. Recently it has been extensively grown in China and Japan for use as a bonsai. In Korea, it grows along the southern seashore areas, and traditionally the leaves have been used as tea materials. However, there have been only a few chemical composition studies of *Sageretia theezans* so far (*1*, *2*). In the course of our continuing study of natural antioxidants from Korean plant sources, we had found strong antioxidative activity in the aqueous extract of the leaves. Therefore, it was very interesting to us to isolate and identify the antioxidant compounds of the leaves of *S. theezans*. Furthermore, the antioxidant activities of the isolated compounds were examined by electron spin resonance (ESR) measurements and low-density lipoprotein (LDL) oxidation inhibition test.

MATERIALS AND METHODS

Plant Material. The leaves of *S. theezans* were collected at Kohung in Korea at the end of September 2001 and identified by Dr. Min-Sup Chung, Kyungpook National University; they were dried in the shade at room temperature.

General Apparatus and Chemicals. Ultraviolet (UV) and infrared (IR) spectra were recorded on UV-1601 PC (Shimadzu, Kyoto, Japan) and FT/IR-410 (Jasco, Tokyo, Japan) spectrometers, respectively. Optical rotation was measured with a P-1020 polarimeter (Jasco, Tokyo, Japan). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a JNM ECP-500 (JEOL, Tokyo, Japan; ^{1}H, 500 MHz; ^{13}C, 125 MHz). Chemical shifts for ^{1}H and ^{13}C NMR are recorded in parts per million (ppm) relative to solvent signals (methanol-d_4: δ_H 3.30 and δ_C 49.0) as internal standards. Fast atom bombardment mass (FAB-MS) spectra were measured with a JMS HX-110 (JEOL, Tokyo, Japan) using *m*-nitrobenzyl alcohol and glycerin as matrices. The ESR spectra were obtained with a JES-FR30 free radical monitor (JEOL, Akishima, Japan). Analytical TLC was performed on silica gel 60 F_{254} (E. Merck Co., Darmstadt, Germany), visualized under UV at 254 and 366 nm or sprayed with *p*-anisaldehyde solution. After hydrolysis of flavonol glycosides, TLC of sugars was performed in EtOAc/AcOH/MeOH/H_2O (65:20:15:15) and sprayed with 0.5% thymol in H_2SO_4/EtOH (5:95).

Solvents for NMR was purchased from Cambridge Isotope Laboratory Inc. (Andover, MA). Other solvents were spectro, HPLC, and special grade products of Wako Pure Chemical Industries Ltd. (Osaka, Japan) and Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). Diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid (DTPA) (Dojindo Laboratories Co., Kumamoto, Japan), 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) (Labotec Co., Tokyo, Japan), and hypoxanthine and xanthine oxidase (Sigma Chemical Co., St. Louis, MO) were used for ESR experiments. 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) for LDL oxidation was purchased from Wako Pure Chemical Industries Ltd.

Isolation and Identification of Flavonol Glycosides. The dried and ground leaves (300 g) were defatted with $CHCl_3$ and extracted with 60% (v/v) aqueous acetone (3 L × 2) for 12 h at room temperature. After evaporation of the solvents, the aqueous slurry (~500 mL) was extracted with diethyl ether (1 L × 3). The ether extract (5.02 g) was loaded on a silica gel column and eluted with mixtures of hexane/ethyl acetate (9:1, 7:3, and 5:5), ethyl acetate, methanolic ethyl acetate (20%, v/v), and methanol. The ethyl acetate fraction (620 mg) was rechromatographed by an ODS-MPLC system using a 300 × 25 mm i.d. column at a flow rate of 3 mL/min with H_2O/MeOH (75:25, 50:50, 25:75, and MeOH), and six fractions were collected on the basis of TLC analysis. The second fraction (210 mg) was purified by preparative HPLC with a 250 × 20 mm i.d. Develosil ODS-5 column and a MeOH/H_2O (7:3) solvent eluted at 4 mL/min, with detection at

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254 nm to give compounds **1** (5.4 mg), **2** (2.8 mg), **3** (12.6 mg), **4** (23.7 mg), and **5** (16.9 mg).

*Myricetin myricitrin 3-O-α-L-rhamnopyranoside (**1**):* $[\alpha]_D$ −138.6° (MeOH; *c* 0.5); IR, ν (KBr) cm^{-1} 3747, 3362, 2356, 1652, 1612, 1512, 1200; UV, λ_{max} nm 354, 257 (MeOH), 392, 322, 269 (NaOMe), 415, 311, 270 ($AlCl_3$), 403, 310, 273 ($AlCl_3$ + HCl), 356, 264 (NaOAc), 374, 300, 259 (NaOAc + H_3BO_3); ^1H NMR (CD_3OD), δ 0.95 (3H, d, 6.4 Hz, H-6″), 3.33 (1H, t, 9.2 Hz, H-4″), 3.51 (1H, dq, 9.2, 6.4 Hz, H-5″), 3.78 (1H, dd, 9.2, 3.2 Hz, H-3″, 4.21 (1H, dd, 3.2, 1.8 Hz, H-2″), 5.30 (1H, d, 1.8 Hz, H-1″), 6.19 (1H, d, 2.3 Hz, H-6), 6.35 (1H, d, 2.3 Hz, H-8), 6.94 (2H, s, H-2′, 6′); ^{13}C NMR (CD_3OD), δ 17.5 (C-6″), 71.7 (C-2″), 71.9 (C-5″), 72.0 (C-3″), 73.2 (C-4″), 94.6 (C-8), 99.7 (C-6), 103.5 (C-1″), 105.6 (C-10), 109.4 (C-2′,6′), 121.7 (C-1′), 136.1 (C-3), 137.7 (C-4′), 146.7 (C-3′,5′), 158.4 (C-9), 159.2 (C-2), 163.1 (C-5), 164.0 (C-7), 159.3 (C-2), 179.5 (C-4); FAB-MS (positive), *m*/*z* 465 $[M + H]^+$.

*Kaempferol 3-O-α-L-rhamnopyranoside afzelin (**2**):* $[\alpha]_D$ −73.5° (MeOH; *c* 0.5); IR, ν (KBr) cm^{-1} 3747, 3370, 2361, 1651, 1607, 1176; UV, λ_{max} nm 342, 265 (MeOH), 390, 325, 272 (NaOMe), 393, 345, 303, 273 ($AlCl_3$), 397, 343, 301, 274 ($AlCl_3$ + HCl), 343, 265 (NaOAc), 342, 264 (NaOAc + H_3BO_3); ^1H NMR (CD_3OD), δ 0.92 (3H, d, 5.5 Hz, H-6″), 3.33 (2H, m, H-4″, H-5″), 3.71 (1H, dd, 9.2, 3.2 Hz, H-3″), 4.22 (1H, dd, 3.2, 1.8 Hz, H-2″), 5.38 (1H, d, 1.8 Hz, H-1″), 6.20 (1H, d, 2.3 Hz, H-6), 6.38 (1H, d, 2.3 Hz, H-8), 6.93 (2H, d, 8.7 Hz, H-3′, 5′), 7.77 (1H, d, 8.7 Hz, H-2′, 6′); ^{13}C NMR (CD_3OD) δ 17.7 (C-6″), 71.9 (C-2″), 72.0 (C-5″), 72.2 (C-3″), 73.2 (C-4″), 94.8 (C-8), 99.9 (C-6), 103.5 (C-1″), 106.0 (C-10), 116.6 (C-3′, 5′), 122.7 (C-1′), 131.9 (C-2′, 6′), 121.7 (C-1′), 136.2 (C-3), 158.6 (C-9), 159.3 (C-2), 159.3 (C-4′), 161.6 (C-5), 163.3 (C-7), 179.7 (C-4). FAB-MS (positive), *m*/*z* 433 $[M + H]^+$.

*7-O-Methylmyricitrin europetin 3-O-α-L-rhamnopyranoside (**3**):* $[\alpha]_D$ −152.3° (MeOH; *c* 0.5); IR, ν (KBr) cm^{-1} 3362, 2360, 1657, 1596, 1498, 1345, 1212, 1162, 1038, 964; UV, λ_{max} nm 355, 256 (MeOH), 408, 250, (NaOMe), 393, 269 ($AlCl_3$), 403, 308, 273 ($AlCl_3$ + HCl), 355, 301, 257 (NaOAc), 376, 258 (NaOAc + H_3BO_3); ^1H NMR (CD_3-OD), δ 0.97 (3H, d, 6.2 Hz, H-6″), 3.35 (1H, t, 9.8 Hz, H-4″), 3.53 (1H, dq, 9.8, 6.2 Hz, H-5″), 3.80 (1H, dd, 9.8, 3.3 Hz, H-3″), 3.86 (3H, s, 7-OMe), 4.23 (1H, dd, 3.3, 1.7 Hz, H-2″), 5.34 (1H, d, 1.7 Hz, H-1″), 6.31 (1H, d, 1.9 Hz, H-6), 6.51 (1H, d, 1.9 Hz, H-8), 6.97 (2H, s, H-2′, 6′); ^{13}C NMR (CD_3OD), δ 17.7 (C-6″), 56.5 (7-OMe), 71.9 (C-2″), 72.0 (C-5″), 72.1 (C-3″), 73.3 (C-4″), 93.1 (C-8), 98.9 (C-6), 103.6 (C-1″), 106.7 (C-10), 109.6 (C-2′, 6′), 121.8 (C-1′), 136.5 (C-3), 138.0 (C-4′), 146.8 (C-3′, 5′), 158.3 (C-9), 159.7 (C-2), 162.9 (C-5), 167.2 (C-7), 179.7 (C-4); FAB-MS (positive), *m*/*z* 479 $[M + H]^+$.

*7,4′-O-Dimethylmyricetin 3-O-α-L-rhamnopyranoside 7-O-methylmearnsitrin (**4**):* $[\alpha]_D$ −86.4° (MeOH; *c* 0.5); IR, ν (KBr) cm^{-1} 3415, 2938, 2357, 1659, 1598, 1503, 1445, 1372, 1214, 116, 1059, 964, 806, 719; UV, λ_{max} nm 339, 264 (MeOH), 348, 258 (NaOMe), 341, 304, 273 ($AlCl_3$), 394, 339, 274 ($AlCl_3$ + HCl), 338, 264 (NaOAc), 337, 264 (NaOAc + H_3BO_3); spectroscopic data of ^1H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) are shown in **Table 1**; FAB-MS (positive), *m*/*z* 493 $[M + H]^+$; HRFAB-MS (positive), *m*/*z* 493.1318 $[M + H]^+$, $C_{23}H_{25}O_{12}$ requires 493.1346.

*7-O-Methylquercetin 3-O-α-L-rhamnopyranoside (**5**):* $[\alpha]_D$ −119.8° (MeOH; *c* 0.5); IR, ν (KBr) cm^{-1} 3747, 3337, 2361, 1653, 1596, 1209; UV, λ_{max} nm 349, 256 (MeOH), 395, 264 (NaOMe), 396, 273 ($AlCl_3$), 400, 362, 271 ($AlCl_3$ + HCl), 349, 256 (NaOAc), 369, 260 (NaOAc + H_3BO_3); ^1H NMR (CD_3OD), δ 0.94 (3H, d, 6.2 Hz, H-6″), 3.33 (1H, t, 9.5 Hz, H-4″), 3.43 (1H, dq, 9.5, 6.2 Hz, H-5″), 3.74 (1H, dd, 9.5, 3.2 Hz, H-3″), 3.88 (3H, s, 7-OMe), 4.21 (1H, dd, 3.2, 1.7 Hz, H-2″), 5.36 (1H, d, 1.7 Hz, H-1″), 6.33 (1H, d, 2.3 Hz, H-6), 6.57 (1H, d, 2.3 Hz, H-8), 6.90 (1H, d, 8.4 Hz, H− 5′), 7.32 (1H, dd, 8.4, 1.9 Hz, H-6′), 7.35 (1H, d, 1.9 Hz, H-2′); ^{13}C NMR (CD_3OD), δ 17.7 (C-6′), 56.5 (7-OMe), 71.9 (C-2″), 72.0 (C-5″), 72.1 (C-3″), 73.3 (C-4″), 93.1 (C-8), 99.0 (C-6), 103.6 (C-1″), 106.8 (C-10), 116.4 (C-5′), 117.0 (C-2′), 122.8 (C-1′), 122.9 (C-6′), 136.4 (C-3), 146.5 (C-3′), 150.1 (C-4′), 158.4 (C-2), 159.7 (C-9), 163.0 (C-5), 167.3 (C-7), 179.8 (C-4); FAB-MS (positive), *m*/*z* 463 $[M + H]^+$.

Antioxidant Activity Effects. Antioxidant activities of isolated flavonol glycosides were assessed through radical scavenging activities by the ESR measurement and LDL oxidation inhibition methods.

Table 1. ^1H and ^{13}C NMR Spectroscopic Data for 7-*O*-Methylmearnsitrin Isolated from the Leaves of *S. theezans*

position	δ_H (multiplicity, *J*)	δ_C	HMBC correlation
2		159.48	
3		136.98	
4		179.80	
5		162.96	
6	6.32 (1H, d, 2.3)	99.06	C-5, C-7, C-8, C-10
7		167.36	
8	6.54 (1H, d, 2.3)	93.17	C-7, C-9, C-6, C-10
9		158.45	
10		106.85	
1′		126.90	
2′	6.90 (2H, s)	109.89	C-1′, C-3′, C-2, C-4′
3′		151.90	
4′		139.49	
5′		151.90	
6′	6.90 (2H, s)	109.89	C-1′, C-4′, C-5′, C-2
1″	5.33 (1H, d, 1.4)	103.62	C-3
2″	4.24 (1H, dd, 3.4, 1.4)	71.89	
3″	3.75 (dd, 9.2, 3.4)	72.07	
4″	3.30–3.31 (1H, m)	73.21	
5″	3.34–3.36 (1H, m)	72.05	
6″	0.95 (3H, d, 5.5)	17.71	
7-OCH_3	3.87 (3H, s)	56.49	C-7
4′-OCH_3	3.88 (3H, s)	60.93	C-4′

ESR Measurement. •OH and $O_2^{\bullet-}$ scavenging activities were measured by using a modified procedure of the spin-trapping techniques of Noda et al. (*3*). For the •OH scavenging test, into a glass test tube were added 20 μL of 0.90 M DMPO, 37.5 μL of 40 mM iron(II) sulfate, 37.5 μL of 1 mM DTPA, 30 μL of sample solution, and 75 μL of 1 mM hydrogen peroxide in that order and mixed. Measurement of the ESR spectrum was started at 30 s after the addition of hydrogen peroxide. In the case of the $O_2^{\bullet-}$ scavenging test, into a glass test tube were added 30 μL of 4.49 M DMPO, 50 μL of 5 mM hypoxanthine, 20 μL of 9.625 mM DTPA, 50 μL of sample solution, and 50 μL of 0.4 unit/mL xanthine oxidase in that order and were immediately mixed. Reagents were dissolved in 100 mM phosphate buffer (pH 7.4) prepared using deionized water. Samples were dissolved in ethanol and diluted with 100 mM phosphate buffer (pH 7.4) to test the concentration. Measurement of the ESR spectrum was started at 30 s after the addition of xanthine oxidase. Antioxidant activity were exhibited as median radical scavenging concentration (IC_{50}) values of •OH and $O_2^{\bullet-}$. From the intensity ratio of sample radical signal to manganese reference signal in the observed spectra, each radical scavenging activity of four different test concentrations (0.1, 0.5, 1, and 2 mg/mL) were calculated, and the IC_{50} value was obtained by the least-squares method. The measurement conditions were as follows: microwave power, 4 mW; center field, 335.500 mT ($O_2^{\bullet-}$, 335.600 mT); sweep width, ±5 mT; sweep time, 1 min; modulate width, 0.32 mT ($O_2^{\bullet-}$, 79 μT).

LDL Oxidation Inhibition Method. LDL was isolated from human plasma taken from fasting nomolipidemic healthy females by sequential density ultracentrifugation between densities of 1.006 and 1.063 g/mL using an Optima TLX ultracentrifuger (Beckman Instruments, Palo Alto, CA) at 44000 rpm (*4*). The LDL preparation was dialyzed with a phosphate-buffered saline (PBS) containing 0.01% ethylenediaminetetraacetic acid (EDTA). EDTA was removed by extensive dialysis with PBS without EDTA for 48 h at 4 °C. The protein content was determined according to the method of Bradford (*5*). LDL (0.1 mg of protein/mL) oxidation was carried out in a water bath at 37 °C for 2 h. One hundred microliters of LDL was added to 100 μL of 10 μM $CuSO_4$ or 5 mM AAPH in the absence or presence of tested flavonoids dissolved in dimethyl sulfoxide (DMSO). After incubation, 1 mM EDTA and 10 μM butylated hydroxytoluene (BHT) were added. LDL oxidation inhibition activity was determined by measuring the amount of thiobarbituric acid reactive substances (TBARS) (*6*) and determination of free malondialdehyde (MDA) contents (*7*) using HPLC on a 250 × 4.6 mm i.d. 5U, Econosphere NH_2 column with a CH_3CN/30 mM tris buffer, pH 7.4 (1:9, v/v) mobile phase eluted at 0.8 mL/min

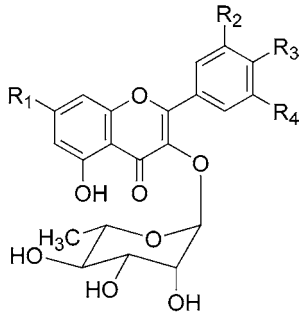
and detection at 267 nm. Standard MDA was prepared by the hydrolysis of 1,1,3,3-tetraethoxypropane with 1 N HCl.

RESULTS AND DISCUSSION

Identification of Flavonol Glycosides. The five compounds were isolated and purified through silica gel, ODS column, and preparative HPLC from the 60% aqueous acetone extract of *S. theezans* leaves. Among them, compound **4**, which was obtained in the largest amount, was found to be a new compound.

Compound **4** had the molecular formula of $C_{23}H_{25}O_{12}$ determined by HR FAB-MS. The ^{13}C NMR spectrum showed 21 carbon signals. Distortionless enhancement by polarization transfer (DEPT) spectra indicated that this compound is composed of 3 primary, 8 tertiary, and 10 quaternary carbons. The ^1H NMR signals showed a typical flavonol α-L-rhamnopyranoside pattern having δ 0.95 [3H, d; H-6″(Me)], 3.33−3.36 (2H, m; H-4″ and H-5″), 3.75 (1H, dd; H-3), 4.24 (1H, dd; H-2″), and 5.33 (1H, d; H-1″). All signals of the sugar moiety were assigned on the basis of the $^1H-^1H$ correlation spectroscopy (COSY), heteronuclear multiple bond correlation spectroscopy (HMBC) spectra, and reported data (*8*). Furthermore, two methoxyl signals at δ 3.87 (7-OMe) and 3.88 (4′-OMe) and four aromatic protons at δ 6.90 (H-2′,6′), 6.32 (H-6), and 6.54 (H-8) were observed in the ^1H NMR spectrum. The signal for two protons at δ 6.90, which was observed as a singlet, correlated with a carbon signal at δ 109.9 on heteronuclear single-quantum correlation (HSQC) spectrum. This indicates that compound **4** has a symmetrical tetrasubstituted benzene ring, and, combined with the HMBC spectrum, it is suggestive of a 3′,4′,5′-trioxygenated B-ring (*9*). The δ 6.32 and 6.54 signals having the coupling constant values of 2.3 Hz revealed that they are meta coupled at the A ring. The downfield shift of C-3′ and C-5′ compared to myricetin (**1**) indicates the presence of a methoxy group at the C-4′ position (*10*, *11*). The cross-peaks of C-7 (167.4)/OMe (3.87) and C-4′ (139.5)/OMe (3.88) in the HMBC spectrum demonstrated that these methoxyl groups are linked to C-7 and C-4′, respectively. Furthermore, the anomeric proton of rhamnose correlated with carbon at C-3 (137.0). Therefore, together with the spectroscopic data of known compounds such as mearnsitrin (*12*), compound **4** was identified as 7-*O*-methylmearnsitrin (7-*O*-methylmearnsetin 3-*O*-α-L-rhamnopyranoside). The aglycon of this compound, 7-*O*-methylmearnsetin, and its galactoside were first isolated from *Rhus lancea* (*13*). However, its 3-*O*-rhamnoside, 7-*O*-methylmearnsitrin, is isolated from plants for the first time here, although compound **4** was obtained by the methylation of myricitrin (*14*). As there have been only a few reports (*15*, *16*) on mearnsetin so far, it might be a rare flavonoid contained in the plant.

Compound **1** showed nearly the same ^1H NMR pattern as **4**, that is, chemical shift values of a sugar moiety and a singlet of two protons at δ 6.94 (H-2′,6′) with a pair of doublets at δ 6.19 (1.8 Hz; H-6) and 6.35 (2.3 Hz; H-8). Accordingly, it was identified as myricetin 3-*O*-α-L-rhamnopyranoside. Compound **2** was identified as kaempferol 3-*O*-α-L-rhamnopyranoside, because a pair of doublet signals for two protons, δ 6.92 (8.7 Hz, H-3′,5′) and 7.77 (8.7 Hz, H-2′,6′), revealed that this compound corresponds to an analogue of compound **1**, which possesses a 1,4-disubstituted B-ring. NMR data of compound **3** were almost the same as that of compound **1**, except for a methoxyl group at δ 3.85. The methoxyl group was correlated with C-7 in the HMBC spectrum, so it was identified as 7-*O*-methylmyricetin 3-*O*-α-L-rhamnopyranoside. 7-*O*-Methylmyricetin (europetin) was first isolated from *Plumbago europea* (*17*).



1 $R_1=R_2=R_3=R_4=OH$
2 $R_1=R_3=OH$, $R_2=R_4=H$
3 $R_1=OCH_3$, $R_2=R_3=R_4=OH$
4 $R_1=R_3=OCH_3$, $R_2=R_4=OH$
5 $R_1=OCH_3$, $R_2=R_3=OH$, $R_4=H$

Figure 1. Chemical structures of flavonol glycosides isolated from *S. theezans*.

Table 2. Reactive Oxygen Radical Scavenging Activities of Flavonol Glycosides Isolated from *S. theezans*

compound	IC_{50} value[a] (mM)	
	•OH	$O_2^{\bullet-}$
1	0.697	0.034
2	0.838	0.250
3	0.906	0.027
4	1.698	19.179
5	0.954	0.231
ascorbic acid	0.938	0.108

[a] Median radical scavenging concentration (IC_{50}) value.

Compound **5**, having a series of three aromatic protons, δ 7.35 (br s; H-2′), 6.90 (d; H-5′), and 7.32 (br d; H-6′), and a methoxyl signal at δ 3.75 (7-OMe), was identified as 7-*O*-methylquercetin 3-*O*-α-L-rhamnopyranoside. Their chemical shift values on the ^{13}C NMR spectrum were assigned by HSQC and HMBC spectra and were confirmed by comparison with literature data (*8*). After hydrolysis of the five glycosides, the residue were identified as L-rhamnose by TLC with a colored yellow spot of R_f 0.81. Their chemical structures are shown in **Figure 1**.

Antioxidant Effects by ESR and LDL Oxidation Inhibition. We examined the antioxidant activities of the five flavonoids isolated by •OH and $O_2^{\bullet-}$ spin-trapping methods using ESR. Each scavenging activity was measured at four different test concentrations and expressed as IC_{50} values (**Table 2**). Myricitrin (**1**) and 7-*O*-methylmyricitrin (**3**) showed lower IC_{50} values for •OH and $O_2^{\bullet-}$ than ascorbic acid. Kaempferol 3-*O*-α-L-rhamnopyranoside (**2**) and 7-*O*-methylquercetin 3-*O*-α-L-rhamnopyranoside (**5**) showed weaker activities, but comparable to that of ascorbic acid. 7-*O*-Methylmearnsitrin (**4**) showed significantly higher values, meaning very weak radical scavenging activities. Flavonoids having *ortho*-dihydroxy and trihydroxyl groups in the B-ring can act as strong radical scavengers through formation of a hydrogen bond with the semiquinone radical of the B-ring (*18*). Donald et al. (*19*) reported that myricetin reacts 6 times more quickly than quercetin to the galvinoxyl radical and concluded that flavonoid reactivity depends highly on the substitution pattern of OH groups in the B-ring. Our results also confirm the report (*20*) that the $O_2^{\bullet-}$ scavenging rate constants of pyrogallol substituents in the B-ring exceed those of *ortho*-dihydroxy substituents. However, it is supposed that the effect of both iron chelation (*21*, *22*) and xanthine oxidase inhibition (*23*) of each flavonol

Table 3. LDL Oxidation Inhibition Effects by TBA Method and Free MDA Contents of Flavonol Glycosides (10 μM) Isolated from *S. theezans*

compound	inhibition rate (%) AAPH	inhibition rate (%) $CuSO_4$	MDA contents (nM/mg of protein)
1	78.1 ± 2.3[a]	68.8 ± 4.9	4.4 ± 0.7
2	51.0 ± 10.1	54.8 ± 3.2	14.4 ± 1.2
3	74.6 ± 17.4	73.5 ± 2.3	5.5 ± 0.4
4	34.0 ± 13.4	–3.7 ± 7.4	43.1 ± 6.9
5	69.5 ± 8.1	68.2 ± 3.5	6.0 ± 0.6
α-tocopherol	41.4 ± 1.6	61.5 ± 4.7	7.9 ± 1.1

[a] Mean ± SD of triplicate assays.

glycoside might affect the radical scavenging activities of them in this experiment.

Oxidation of human LDL induced by reactive oxygen radicals plays a significant role in the pathogenesis of atherosclerosis and coronary heart disease (*24*). Because flavonoids had been shown to be effective in the inhibition of LDL oxidation (*25*), many studies have been reported on the protecting effects of LDL oxidation. These effects may arise from the reactive oxygen radical scavenging and transition metal ion chelating activity (*26*). The inhibition effects of the five flavonol glycosides on LDL oxidation induced by copper ions or AAPH using the TBA method are exhibited in **Table 3**. The pyrogallol substiuents in the B-ring of compounds **1** and **3** also showed slightly stronger inhibition effects of LDL oxidation induced by copper ion and AAPH than the *ortho*-dihydroxy-substituted flavonoid, compound **5**. However, compound **4** was inactive to oxidation induced by copper ion, and it showed moderate LDL oxidation inhibition activity by AAPH, which represents similar results to biochanin, a 4′-methylated isoflavone (*27*). The free MDA contents and LDL oxidation inhibition effects of the five flavonol glycosides by HPLC are shown in **Table 3**. Compounds **1** and **3**, having trihydroxy structures in the B-ring, also exhibited lower free MDA contents and slightly stronger inhibition activities than **5**, having a dihydroxy structure. Although compound **4** showed very weak antioxidant activities by ESR and LDL oxidation inhibition test, Matsuda et al. (*28*) reported that methylation of the 4′-hydroxyl group of flavonol enhanced nitric oxide radical production inhibition activity. Therefore, it is proposed that an advanced biological activity study of compound **4**, a novel compound having the same 4′-methylated structure, might be needed. Besides both scavenging reactive oxygen radicals and chelating iron and copper, which can catalyze LDL oxidation, flavonoids can convert the α-tocopheryl radical into α-tocopherol, the main endogenous antioxidant in LDL (*29*).

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