Prenylated and Benzylated Flavonoids from the Fruits of *Cudrania tricuspidata*

Xiang Hua Han,[†] Seong Su Hong,[†] Qinghao Jin,[†] Dayu Li,[†] Hyun-Kyu Kim,[‡] Jeongrai Lee,[†] Suk Hyung Kwon,[‡] Dongho Lee,[§] Chong-Kil Lee,[†] Myung Koo Lee,[†] and Bang Yeon Hwang*[,†]

College of Pharmacy, Chungbuk National University, Cheongju 361-763, Korea, Rexgene Biotech Co., Ltd., Ochang, Cheongwon, Chungbuk 363-883, Korea, and School of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Korea

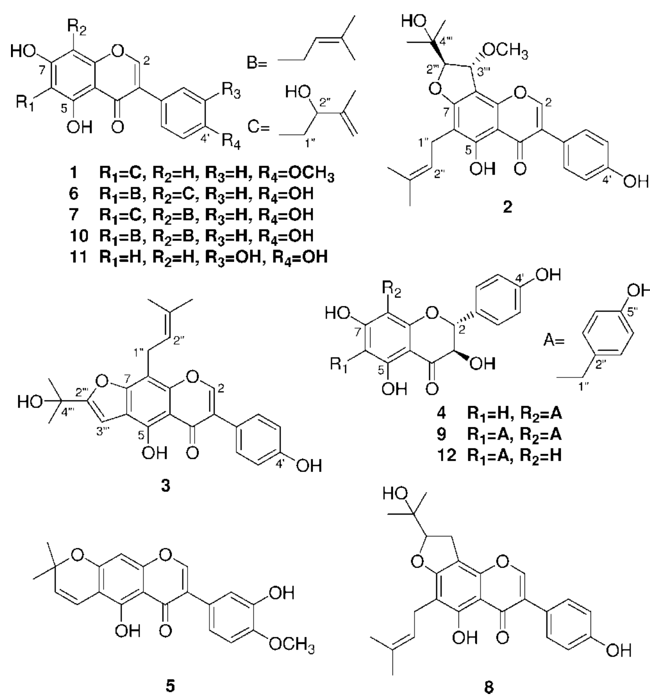
Received July 10, 2008

Three new prenylated isoflavones, 5,7-dihydroxy-6-(2′′-hydroxy-3′′-methylbut-3′′-enyl)-4′-methoxylisoflavone (**1**), 5,4′-dihydroxy-6-(3′′-methylbut-2′′-enyl)-2′′′-(4′′′-hydroxy-4′′′-methylethyl)-3′′′-methoxydihydrofurano-[4′′′,5′′′;7,8]isoflavone (**2**), and 5,4′-dihydroxy-8-(3′′-methylbut-2′′-enyl)-2′′′-(4′′′-hydroxy-4′′′-methylethyl)furano-[4′′′,5′′′;6,7]isoflavone (**3**), a benzylated dihydroflavonol, 5,7,4′-trihydroxy-8-*p*-hydroxybenzyldihydroflavonol (**4**), and eight known flavonoids (**5**−**12**) were isolated from the fruits of *Cudrania tricuspidata*. The structures of these compounds were determined on the basis of MS and ^{1}H and ^{13}C NMR spectroscopic data, including 2D NMR experiments. Compounds **2**, **3**, **6**, **7**, **8**, **10**, **11**, and **12** inhibited LPS-induced nitric oxide production, with IC_{50} values of 11.8−41.8 μM.

Cudrania tricuspidata (carr.) Bur. (Moraceae) is a small thorny tree native to East Asia and distributed mainly in the southern part of Korea. The cortex and root bark of this plant has been used for the treatment of gonorrhea, jaundice, hepatitis, neuritis, and inflammation.[1] Several prenylated xanthones and flavonoids,[2−6] isolated from the root of the genus *Cudrania*, were found to have cytotoxic,[7−10] antifungal,[11] antioxidant,[12] antiatherosclerotic,[13] anti-inflammatory,[13] and hepatoprotective[14,15] activities and display monoamine oxidase inhibitory effects.[16,17]

As part of our ongoing research program for the discovery of plant-derived inhibitors of nitric oxide (NO) production, we found that CH_2Cl_2 and EtOAc extracts of the fruits of *C. tricuspidata* inhibited NO production in RAW 264.7 cells. We isolated three new prenylated isoflavones and a new benzylated dihydroflavonol, together with eight known flavonoids, and the structures were determined on the basis of MS as well as ^{1}H NMR, ^{13}C NMR, and 2D NMR data. We describe herein the isolation, structure determination, and inhibitory effects of NO production in murine macrophage RAW 264.7 cells.

Fresh fruits of *C. tricuspidata* were extracted with MeOH and successively fractionated with CH_2Cl_2, EtOAc, and H_2O. Repeated column chromatography (CC) and semipreparative HPLC of the CH_2Cl_2- and EtOAc-soluble extracts resulted in the isolation of three new prenylated isoflavones (**1**−**3**) and a benzylated dihydroflavonol (**4**), together with eight known compounds (**5**−**12**). The eight known compounds were identified as 5,3′-dihydroxy-4′-methoxy-2′′,2′′-dimethylpyrano-(5′′,6′′;6,7)-isoflavone (**5**),[18] erysenegalensein E (**6**),[19] isoerysenegalensein E (**7**),[20] senegalensin (**8**),[21,22] gericudranin D (**9**),[23] 5,7,4′-trihydroxy-6,8-diprenylisoflavone (**10**),[24] orobol (**11**),[25] and gericudranin E (**12**)[23] by comparison of spectroscopic data with reported values in the literature.



Compound **1** was obtained as pale yellow needles. The molecular formula was determined to be $C_{21}H_{20}O_6$ by HRFABMS. The ^{1}H and ^{13}C NMR spectra of compound **1** were similar to those of laburnetin,[26] except for an additional signal arising from an OCH_3 group. The ^{1}H NMR spectrum had a sharp singlet at δ_H 7.83 and a downfield signal at δ_H 13.25, confirming the presence of an intramolecular hydrogen-bonded group at the C-5 position of an isoflavone skeleton. An aromatic proton signal at δ_H 6.46 (1H, s, H-8), an OCH_3 signal at δ_H 3.84 (3H, s), and two sets of doublet signals for the AA′BB′ spin system at δ_H 6.97 and 7.44 (each 2H, d, J = 8.5 Hz) were assigned to a *p*-disubstituted benzene ring. The presence of a 2-hydroxy-3-methyl-3-butenyl group was deduced from the ^{1}H NMR signals of two benzylic proton signals at δ_H 2.92 and 3.17 (each 1H, dd, J = 7.8 and 14.9 Hz), an oxymethine proton signal at δ_H 4.41 (1H, br d, J = 7.8 Hz), two olefinic *gem*-proton signals at δ_H 4.88 and 4.99 (each 1H, br s), and a methyl proton signal at δ_H 1.86 (3H, s). The ^{13}C NMR and DEPT spectra of compound **1** showed 21 carbon signals including an isoflavone moiety with a methoxy and a 2-hydroxy-3-methyl-3-butenyl group. In the HMBC experiment, the OCH_3 at δ_H 3.84 (3H, s) was correlated with C-4′ at δ_C 159.7, which suggested that the OCH_3 group is located at C-4′. HMBC correlations of the benzylic proton signals (δ_H 2.92 and 3.17) with C-5 (δ_C 160.3), C-6 (δ_C 109.2), and C-7 (δ_C 163.1) indicated that the 2-hydroxy-3-methyl-3-butenyl group was attached to C-6 of the A-ring. Complete assignment of the ^{1}H, ^{13}C NMR chemical shifts was obtained using ^{1}H−^{1}H COSY, HMQC, DEPT, and HMBC NMR correlations (see Experimental

* Corresponding author. Tel: +82-43-261-2814. Fax: +82-43-268-2732. E-mail: byhwang@chungbuk.ac.kr.
† Chungbuk National University.
‡ Rexgene Biotech Co., Ltd.
§ Korea University.

10.1021/np800418j CCC: $40.75 © 2009 American Chemical Society and American Society of Pharmacognosy
Published on Web 12/29/2008

Section). Thus, compound **1** was identified as 5,7-dihydroxy-6-(2″-hydroxy-3″-methylbut-3″-enyl)-4′-methoxylisoflavone.

Compound **2** showed a molecular ion peak at *m/z* 453.1914 [M + H]$^+$ (HRFABMS), corresponding to a molecular formula of $C_{26}H_{28}O_7$. The ^{1}H NMR spectrum had a sharp singlet at δ_H 8.37 and a downfield signal at δ_H 13.87 that were characteristic of a 5-hydroxyisoflavone skeleton. Two sets of doublet signals at δ_H 7.63 (2H, d, J = 8.5 Hz) and 7.06 (2H, d, J = 8.5 Hz) were assigned to a *p*-disubstituted benzene, as found in the B-ring. A group of signals at δ_H 5.43 (1H, d, J = 7.2 Hz), 3.52 and 3.45 (each 1H, dd, J = 7.2 and 14.1 Hz), 1.93 (3H, s), and 1.81 (3H, s) suggested the presence of a 3-methylbut-3-enyl (prenyl) group in the molecule. Furthermore, two singlet methyl signals (δ_H 1.51 and 1.28), two oxygenated methine signals at δ_H 5.46 (1H, d, J = 2.1 Hz) and 4.71 (1H, d, J = 2.1 Hz), and an OCH_3 signal (δ_H 3.61) indicated a dihydrofuran ring with a 2-(1-hydroxy-1-methylethyl)-3-methoxy substituted group. The HMBC correlations particularly from the OCH_3 signal at δ_H 3.61 to C-3‴ (δ_C 79.8) supported the attachment of the OCH_3 group at C-3‴, and correlations from H-2‴ (δ_H 4.71) to C-7 (δ_C 166.8) and C-3‴ (δ_C 79.8) as well as correlations from the H-3‴ (δ_H 5.46) to C-7 (δ_H 166.8) and C-8 (δ_C 104.9) indicated the location of a 2-(1-hydroxy-1-methylethyl)-3-methoxy-2,3-dihydrofuran ring at the C-7 and C-8 positions of the A-ring. The position of the prenyl group was determined as C-6 according to the HMBC correlation of H-1″ (δ_H 3.52 and 3.45) to C-5 (δ_C 162.4), C-6 (δ_C 108.0), and C-7 (δ_C 166.8). All of the NMR assignments were obtained using ^{1}H−^{1}H COSY, HMQC, DEPT, and HMBC correlations. The relative configuration of the two oxygenated methine protons on the dihydrofuran ring was determined to be *trans* by a comparison of the coupling constant between H-2‴ and H-3‴ (J = 2.1 Hz).[27] The strong NOE correlations observed between H-3‴ (δ_H 5.46) and the two methyl protons at δ_H 1.51 and 1.28 in the NOESY spectrum further supported the *trans* relationship. Thus, compound **2** was identified as 5,4′-dihydroxy-6-(3″-methylbut-2″-enyl)-2‴-(4‴-hydroxy-4‴-methylethyl)-3‴-methoxydihydrofurano-[4‴,5‴;7,8]isoflavone.

Compound **3** was obtained as a yellow powder. The molecular formula of **3** was determined to be $C_{25}H_{24}O_6$ by HRFABMS. The ^{1}H NMR signals at δ_H 8.01 and 13.40 were also characteristic of a 5-hydroxyisoflavone. The ^{1}H and ^{13}C NMR data of **3** were similar to those of **2**, except for the presence of signals for the 1-hydroxy-1-methylethylfuran ring at δ_H 6.81 (1H, s, H-3‴), 1.70 (3H, s, CH_3-6‴), and 1.26 (3H, s, CH_3-5‴); δ_C 162.9 (C-2‴), 98.4 (C-3‴), 69.2 (C-4‴), 29.7 (C-6‴), and 25.8 (C-5‴) instead of the 2-(1-hydroxy-1-methylethyl)-3-methoxy-2,3-dihydrofuran ring present in **2**. The location of a 1-hydroxy-1-methylethylfuran ring was determined to be at the C-6 and C-7 positions of the A-ring on the basis of HMBC correlations between H-3‴ (δ_H 6.81) and C-6 (δ_C 113.4) and C-7 (δ_C 157.4). HMBC correlations of the benzylic proton signal (δ_H 3.69) with C-7 (δ_C 157.4), C-8 (δ_C 104.0), and C-8a (δ_C 151.1) indicated that the prenyl group was attached to C-8 of the A-ring. Therefore, compound **3** was identified as 5,4′-dihydroxy-8-(3″-methylbut-2″-enyl)-2‴-(4‴-hydroxy-4‴-methylethyl)furano-[4‴,5‴;6,7]isoflavone.

Compound **4** showed a [M + H]$^+$ peak at *m/z* 395.1136 (HRFABMS), corresponding to the molecular formula $C_{22}H_{18}O_7$. The ^{1}H and ^{13}C NMR spectroscopic data of **4** were similar to those reported for gericudranin E.[23] The ^{1}H NMR spectrum of **4** showed two sets of proton signals at δ_H 7.57 (2H, d, J = 8.4 Hz) and 7.05 (2H, d, J = 8.4 Hz), typical of an AA′BB′ system of a *p*-substituted ring B, and two AB systems at δ_H 5.23 (1H, d, J = 11.5 Hz) and 4.75 (1H, d, J = 11.5 Hz) characteristic of *trans* diaxial H-2 and H-3 protons of a dihydroflavonol, and a proton signal at δ_H 6.26 (1H, s) assignable to H-6 or H-8 of ring A. The spectrum also showed the presence of a *p*-substituted benzyl group at δ_H 7.21 (2H, d, J = 8.3 Hz), 6.80 (2H, d, J = 8.3 Hz), 3.91 (1H, d, J = 14.0 Hz), and 3.84 (1H, d, J = 14.0 Hz). The structure was also supported by the ^{13}C NMR, DEPT, and HMQC spectra. The position of a *p*-substituted benzyl group was confirmed by the HMBC correlations. The benzylic methylene protons at δ_H 3.91 and 3.84 showed long-range correlations with C-7 (δ_C 165.3), C-8 (δ_C 109.1), and C-8a (δ_C 160.8). The absolute configuration of **4** was determined by measurement of the CD spectrum, which showed a positive Cotton effect at 326 nm and a negative Cotton effect at 294 nm, which are characteristic of 2*R*, 3*R* absolute stereochemistry.[28] Therefore, compound **4** was identified as 5,7,4′-trihydroxy-8-*p*-hydroxybenzyldihydroflavonol.

Table 1. Inhibition of NO Production by Compounds **1**−**12**[a]

compound	IC$_{50}$ (μM)	compound	IC$_{50}$ (μM)
1	>50	**8**	13.1 ± 0.12
2	11.8 ± 0.28	**9**	>50
3	12.1 ± 0.21	**10**	19.2 ± 0.21
4	>50	**11**	18.7 ± 0.14
5	>50	**12**	41.8 ± 0.27
6	18.4 ± 0.17	AG[b]	28.0 ± 0.25
7	12.7 ± 0.16		

[a] Data are presented as a mean ± SD from three separate experiments. [b] Aminoguanidine was used as the positive control.

All of the isolates (**1**−**12**) were evaluated for their inhibitory effects of LPS-induced NO production in RAW 264.7 cells. Compounds **2**, **3**, **6**, **7**, **8**, **10**, **11**, and **12** significantly inhibited LPS-induced NO production with IC$_{50}$ values of 11.8−41.8 μM (Table 1). Cell viability, as measured by the CCK assay, indicated that none of the compounds had significant cytotoxicity at concentrations that could inhibit NO production (data not shown). These results indicate that the fruits of *C. tricuspidata* may be useful for the treatment of inflammatory diseases caused by excessive production of NO.

Experimental Section

General Experimental Procedures. Melting points were measured on Büchi model B-540 without correction. Optical rotations were determined with a JASCO DIP-1000 polarimeter. CD spectra were recorded on a JASCO J-715 spectrometer. UV and IR spectra were obtained on JASCO UV-550 and Perkin-Elmer model LE599 spectrometers, respectively. NMR spectra were recorded on a Bruker DRX 500 MHz NMR spectrometer using $CDCl_3$ and acetone-d_6 as solvents. High-resolution fast atom bombardment (HRFAB) and electron impact (EI) mass spectra were obtained on JMS 700 (JEOL, Tokyo, Japan) and VG Autospec Ultima (Micromass, Manchester, UK) mass spectrometers, respectively. Semipreparative HPLC was performed using a Waters HPLC system equipped with two Waters 515 pumps, a 2996 photodiode array detector, and Millenium 32 software using a YMC J'sphere ODS-H80 column (4 μm, 150 × 20 mm, i.d., flow rate 6.5 mL/min). Silica gel (70−230 mesh, Merck, Germany) and Sephadex LH-20 (25−100 μM, Amersham Biosciences, Sweden) were used for open column chromatography (CC). Thin-layer chromatography (TLC) was performed on a precoated silica gel 60 F$_{254}$ (0.25 mm, Merck, Germany). All other chemicals and reagents were analytical grade. Fetal bovine serum, media, and supplement materials for cell culture were purchased from Gibco BRL (Grand Island, NY).

Plant Material. Fruits of *C. tricuspidata* were collected from the herb garden at Chungbuk National University, Cheongju, Korea, in October 2005. The plant material was identified by Emeritus Professor Kyong Soon Lee. A voucher specimen of this plant was deposited at the Herbarium of the College of Pharmacy, Chungbuk National University, Korea (CBNU 05021).

Extraction and Isolation. The fresh fruits of *C. tricuspidata* (4.1 kg) were sliced and extracted with MeOH (3 × 8 L) at room temperature. The solvent was evaporated *in vacuo* to afford a MeOH extract (D01, 120 g), which was then suspended in H_2O (1.5 L). The aqueous solution was partitioned with CH_2Cl_2 (3 × 1.5 L) and EtOAc (3 × 1.5 L), sequentially, to afford dried CH_2Cl_2- (D02, 41.7 g), EtOAc- (D03, 13.5 g), and H_2O-soluble (D04, 62.5 g) residues. Bioassay indicated that the CH_2Cl_2- and EtOAc-soluble extracts showed significant inhibitory effects on LPS-induced NO production with IC$_{50}$ values of 17.2 and 28.6 μg/mL, respectively. Accordingly, the CH_2Cl_2-soluble

fraction was separated on a silica gel column eluted with CH_2Cl_2/MeOH (1:0 to 1:1, then pure MeOH) to yield five fractions (CT-C1−CT-C5). Fraction CT-C4 was further purified by silica gel CC eluted with hexane/acetone (10:1 to 1:1, then pure acetone) to give four subfractions (CT-C41−CT-C44). Fraction CT-42 was separated by semipreparative HPLC using CH_3CN/H_2O (65:35, v/v) to yield compounds **1** (6.5 mg) and **5** (3.6 mg). Faction CT-C43 was passed over a silica gel column and eluted with a gradient of hexane/CH_2Cl_2 (2:1 to 0:1) and CH_2Cl_2/MeOH (1:0 to 0:1) to afford four subfractions (CT-C431−CT-C434). Fraction CT-431 was purified by semipreparative HPLC using CH_3CN/H_2O (60:40, v/v) to yield compounds **2** (5.9 mg) and **8** (6.8 mg). Fraction CT-432 was further purified by semipreparative HPLC using CH_3CN/H_2O (65:35, v/v) to yield compounds **6** (5.9 mg) and **7** (6.8 mg). Fraction CT-C44 was further separated over silica gel eluted with CH_2Cl_2/acetone (3:1 to 1:1) to give compound **3** (5.3 mg). Fraction CT-C5 was purified by silica gel CC eluted with CH_2Cl_2/EtOAc (5:1 to 0:1) to give three subfractions (CT-C51−CT-C53). Fraction CT-C52 was further separated over Sephadex LH-20 eluted with MeOH to afford four subfractions (CT-C521−CT-C524). Fraction CT-C522 was purified by semipreparative HPLC using CH_3CN/H_2O (65:35, v/v) to furnish compound **4** (3.5 mg). Fraction CT-C523 was further purified by semipreparative HPLC using CH_3CN/H_2O (60:40, v/v) to yield compound **9** (3.9 mg).

The EtOAc fraction was separated on a silica gel column eluted with CH_2Cl_2/MeOH (1:0 to 1:1, then pure MeOH) to yield six fractions (CT-E1−CT-E6). Fraction CT-E2 was passed through Sephadex LH-20 eluted with MeOH and purified by semipreparative HPLC using CH_3CN/H_2O (55:45, v/v) to afford compound **10** (1.9 mg). Fraction CT-E4 was separated by Sephadex LH-20 eluted with MeOH and further purified by semipreparative HPLC using CH_3CN/H_2O (50:50, v/v) to yield compounds **11** (6.5 mg) and **12** (5.0 mg).

5,7-Dihydroxy-6-(2′′-hydroxy-3′′-methylbut-3′′-enyl)-4′-methoxy-lisoflavone (1): pale yellow needles; mp 208−210 °C; $[\alpha]^{25}_{D}$ +4.44 (*c* 0.19, MeOH); UV (MeOH) λ_{max} nm (log ε) 265.4 (4.5); IR ν_{max} (dried film) 3433, 2081, 1639, 1247, 582 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 13.25 (1H, s, 5-OH), 7.83 (1H, s, H-2), 7.44 (2H, d, J = 8.7 Hz, H-2′, 6′), 6.97 (2H, d, J = 8.7 Hz, H-3′, 5′), 6.46 (1H, s, H-8), 4.99 (1H, s, Ha-4′′), 4.88 (1H, s, Hb-4′′), 4.41 (1H, d, J = 7.8 Hz, H-2′′), 3.84 (3H, s, OCH_3-4′), 3.17 (1H, dd, J = 7.8, 14.9 Hz, Ha-1′′), 2.92 (1H, dd, J = 7.8, 14.9 Hz, Hb-1′′), 1.86 (3H, s, CH_3-5′′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 180.9 (C-4), 163.1 (C-7), 160.3 (C-5), 159.7 (C-4′), 156.7 (C-8a), 152.6 (C-2), 136.6 (C-3′′), 130.2 (C-2′, 6′), 123.4 (C-1′), 123.3 (C-3), 114.1 (C-3′, 5′), 110.5 (C-4′′), 109.2 (C-6), 105.6 (C-4a), 95.2 (C-8), 77.5 (C-2′′), 55.4 (-OCH_3), 28.2 (C-1′′), 18.6 (C-5′′); EIMS *m/z* 368 [M]$^+$; HRFABMS *m/z* 369.1333 [M + H]$^+$ (calcd for $C_{21}H_{21}O_6$, 369.1338).

5,4′-Dihydroxy-6-(3′′-methylbut-2′′-enyl)-2′′′-(4′′′-hydroxy-4′′′-methylethyl)-3′′′-methoxydihydrofurano-[4′′′,5′′′;7,8]isoflavone (2): yellow powder; mp 156−158 °C; $[\alpha]^{25}_{D}$ +1.38 (*c* 0.12, MeOH); UV (MeOH) λ_{max} nm (log ε) 217.0 (4.6), 265.4 (4.4); IR ν_{max} (dried film) 3460, 1647 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 13.87 (1H, s, 5-OH), 8.37 (1H, s, H-2), 7.63 (2H, d, J = 8.5 Hz, H-2′, 6′), 7.06 (2H, d, J = 8.5 Hz, H-3′, 5′), 5.46 (1H, d, J = 2.1 Hz, H-3′′′), 5.43 (1H, t, J = 7.2 Hz, H-2′′), 4.71 (1H, d, J = 2.1 Hz, H-2′′′), 4.12 (1H, s, OH-4′′′), 3.61 (3H, s, OCH_3-3′′′), 3.52 (1H, dd, J = 7.2, 14.2 Hz, Ha-1′′), 3.45 (1H, dd, J = 7.2, 14.2 Hz, Hb-1′′), 1.93 (3H, s, CH_3-4′′), 1.81 (3H, s, CH_3-5′′), 1.51 (3H, s, 5′′′-Me), 1.28 (3H, s, 6′′′-Me); ^{13}C NMR (acetone-d_6, 125 MHz) δ 181.8 (C-4), 166.8 (C-7), 162.4 (C-5), 158.5 (C-4′), 153.8 (C-2), 153.2 (C-8a), 132.3 (C-3′′), 131.3 (C-2′, 6′), 124.3 (C-3), 123.1 (C-1′), 122.4 (C-2′′), 116.0 (C-3′, 5′), 108.0 (C-6), 106.4 (C-4a), 104.9 (C-8), 97.3 (C-2′′′), 79.8 (C-3′′′), 71.0 (C-4′′′), 56.3 (-OCH_3), 27.1 (C-6′′′), 25.8 (C-4′′), 24.4 (C-5′′′), 22.2 (C-1′′), 17.9 (C-5′′); EIMS *m/z* 452 [M]$^+$; HRFABMS *m/z* 453.1914 [M + H]$^+$ (calcd for $C_{26}H_{29}O_7$, 453.1914).

5,4′-Dihydroxy-8-(3′′-methylbut-2′′-enyl)-2′′′-(4′′′-hydroxy-4′′′-methylethyl)-furano-[4′′′,5′′′;6,7]isoflavone (3): yellow powder; mp 160−162 °C; UV (MeOH) λ_{max} nm (log ε) 268.9 (4.6); IR ν_{max} (dried film) 3435, 1631, 604 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 13.40 (1H, s, 5-OH), 8.01 (1H, s, H-2), 7.44 (2H, d, J = 7.3 Hz, H-2′, 6′), 6.91 (2H, d, J = 7.3 Hz, H-3′, 5′), 6.81 (1H, s, H-3′′′), 5.33 (1H, t, J = 7.1 Hz, H-2′′), 3.69 (2H, d, J = 7.1 Hz, H-1′′), 1.87 (3H, s, 5′′-Me), 1.70 (3H, s, 4′′-Me), 1.70 (3H, s, 6′′′-Me), 1.26 (3H, s, 5′′′-Me); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 182.9 (C-4), 162.9 (C-2′′′), 157.4 (C-7), 153.4 (C-2, C-5), 151.1 (C-8a), 132.8 (C-3′′), 130.4 (C-4′), 123.3 (C-2′, 6′), 122.4 (C-3, C-1′), 121.1 (C-2′′), 115.6 (C-3′, 5′), 113.4 (C-6), 106.8 (C-4a), 104.0 (C-8), 98.4 (C-3′′′), 69.2 (C-4′′′), 29.7 (C-6′′′), 28.6 (C-4′′), 25.8 (C-5′′′), 22.2 (C-1′′), 17.9 (C-5′′); HRFABMS *m/z* 421.1653 [M + H]$^+$ (calcd for $C_{25}H_{25}O_6$, 421.1651).

5,7,4′-Trihydroxy-8-*p*-hydroxybenzyldihydroflavonol (4): yellow powder; mp 126−128 °C; $[\alpha]^{25}_{D}$ +30.81 (*c* 0.03, MeOH); UV (MeOH) λ_{max} nm (log ε) 296.2 (4.3); CD (c 0.01, MeOH) $[\theta]_{260}$ +4905, $[\theta]_{294}$ −9734, $[\theta]_{326}$ +4822; IR ν_{max} (dried film) 3439, 1635, 609 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 11.81 (1H, s, 5-OH), 7.57 (2H, d, J = 8.4 Hz, H-2′, 6′), 7.21 (2H, d, J = 8.3 Hz, H-3′′, 7′′), 7.05 (2H, d, J = 8.4 Hz, H-3′, 5′), 6.80 (2H, d, J = 8.3 Hz, H-4′′, 6′′), 6.26 (1H, s, H-6), 5.23 (1H, d, J = 11.5 Hz, H-2), 4.75 (1H, d, J = 11.5 Hz, H-3), 3.91 (1H, d, J = 14.0 Hz, Ha-1′′), 3.84 (1H, d, J = 14.0 Hz, Hb-1′′); ^{13}C NMR (acetone-d_6, 125 MHz) δ 198.3 (C-4), 165.3 (C-7), 162.6 (C-5), 160.8 (C-8a), 158.5 (C-4′), 156.0 (C-5′′), 132.7 (C-2′′), 130.1 (C-3′′, 7′′), 129.7 (C-2′, 6′), 129.1 (C-1′), 115.6 (C-3′, 5′), 115.3 (C-4′′, 6′′), 109.1 (C-8), 101.3 (C-4a), 96.5 (C-6), 84.0 (C-2), 73.0 (C-3), 27.5 (C-1′′); EIMS *m/z* 394 [M]$^+$; HRFABMS *m/z* 395.1136 [M + H]$^+$ (calcd for $C_{22}H_{19}O_7$: 395.1131).

Determination of NO Production and Cell Viability. The level of nitric oxide production was determined by measuring the amount of nitrite in the cell culture supernatant as previously described.[29] The viability of the cells remaining after the Griess assay was determined using a CCK-8 assay as previously described.[29]

Acknowledgment. This work was supported by a grant from the Korea Research Foundation Grant funded by the Korea Government (MOCIE) (Chungbuk QOL grant). The authors wish to thank the Korea Basic Science Institute for the NMR spectroscopic measurements.

References and Notes

(1) Jung, B. S.; Shin, M. K. *Encyclopedia of Illustrated Korean Natural Drugs*; Young Lim Sa: Seoul, 1990; pp 544−545.
(2) Zou, Y. S.; Hou, A. J.; Zhu, G. F. *Chem. Biodiversity* **2005**, *2*, 131–138.
(3) Hano, Y.; Matsumoto, Y.; Shinohara, K.; Sun, J. Y.; Nomura, T. *Planta Med.* **1991**, *57*, 172–175.
(4) Hano, Y.; Matsumoto, Y.; Sun, J. Y.; Nomura, T. *Planta Med.* **1990**, *56*, 478–481.
(5) Hano, Y.; Matsumoto, Y.; Sun, J. Y.; Nomura, T. *Planta Med.* **1990**, *56*, 399–402.
(6) Fujimoto, T.; Hano, Y.; Nomura, T. *Planta Med.* **1984**, *50*, 218–221.
(7) Lee, I. K.; Kim, C. J.; Song, K. S.; Kim, H. M.; Koshino, H.; Uramoto, M.; Yoo, I. D. *Phytochemistry* **1996**, *41*, 213–216.
(8) Zou, Y. S.; Hou, A. J.; Zhu, G. F.; Chen, Y. F.; Sun, H. D.; Zhao, Q. S. *Bioorg. Med. Chem.* **2004**, *12*, 1947–1953.
(9) Lee, B. W.; Gal, S. W.; Park, K. M.; Park, K. H. *J. Nat. Prod.* **2005**, *68*, 456–458.
(10) Sun, N. J.; Chang, C. J.; Cassady, J. M. *Phytochemistry* **1988**, *27*, 951–952.
(11) Wang, Y. H.; Hou, A. J.; Zhu, G. F.; Chen, D. F.; Sun, H. D. *Planta Med.* **2005**, *71*, 273–274.
(12) Lee, B. W.; Lee, J. H.; Lee, S. T.; Lee, H. S.; Lee, W. S.; Jeong, T. S.; Park, K. H. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5548–5552.
(13) Park, K. H.; Park, Y. D.; Han, J. M.; Im, K. R.; Lee, B. W.; Jeong, I. Y.; Jeong, T. S.; Lee, W. S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5580–5583.
(14) An, R. B.; Sohn, D. H.; Kim, Y. C. *Biol. Pharm. Bull.* **2006**, *29*, 838–840.
(15) Tian, Y. H.; Kim, H. C.; Cui, J. M.; Kim, Y. C. *Arch. Pharm. Res.* **2005**, *28*, 44–48.
(16) Hwang, J. H.; Hong, S. S.; Han, X. H.; Hwang, J. S.; Lee, D.; Lee, H.; Yun, Y. P.; Kim, Y.; Ro, J. S.; Hwang, B. Y. *J. Nat. Prod.* **2007**, *70*, 1207–1209.
(17) Han, X. H.; Hong, S. S.; Hwang, J. S.; Jeong, S. H.; Hwang, J. H.; Lee, M. H.; Lee, M. K.; Lee, D.; Ro, J. S.; Hwang, B. Y. *Arch. Pharm. Res.* **2005**, *28*, 1324–1327.
(18) Olivares, E. M.; Lwande, W.; Monache, F. D.; Bettolo, G. B. M. *Phytochemistry* **1982**, *21*, 1763–1765.
(19) Wandji, J.; Fomum, Z. T.; Tillequin, F.; Seguin, E.; Koch, M. *Phytochemistry* **1994**, *35*, 245–248.
(20) El-Masry, S.; Amer, M. E.; Abdel-Kader, M. S.; Zaatout, H. H. *Phytochemistry* **2002**, *60*, 783–787.
(21) Wandji, J.; Nkengfack, A. E.; Fomum, Z. T.; Ubillas, R.; Killday, K. B.; Tempesta, M. S. *J. Nat. Prod.* **1990**, *53*, 1425–1429.
(22) Tanaka, H.; Doi, M.; Etoh, H.; Watanabe, N.; Shimizu, H.; Hirata, M.; Ahmad, M.; Qurashi, I.; Khan, M. R. *J. Nat. Prod.* **2001**, *64*, 1336–1440.

(23) Lee, I. K.; Kim, C. J.; Song, K. S.; Kim, H. M.; Yoo, I. D.; Koshino, H.; Esumi, Y.; Uramoto, M. *J. Nat. Prod.* **1995**, *58*, 1614–1617.
(24) Shirataki, Y.; Manaka, A.; Komatsu, M. *Phytochemistry* **1982**, *21*, 2959–2963.
(25) El-Sohly, H. N.; Joshi, A.; Li, X. C.; Ross, S. A. *Phytochemistry* **1999**, *52*, 141–145.
(26) Sato, H.; Tahara, S.; Ingham, J. L.; Dziedzic, S. Z. *Phytochemistry* **1995**, *39*, 673–676.
(27) Takashima, J.; Ohsaki, A. *J. Nat. Prod.* **2002**, *65*, 1843–1847.
(28) Islam, M. T.; Tahara, S. *Phytochemistry* **2000**, *54*, 901–907.
(29) Hong, S. S.; Lee, S. A.; Han, X. H.; Hwang, J. S.; Lee, C.; Lee, D.; Hong, J. T.; Kim, Y.; Lee, H.; Hwang, B. Y. *J. Nat. Prod.* **2008**, *71*, 1055–1058.

NP800418J