The Inhibitory Effect of Prunioside A Acyl Derivatives on NO Production in RAW 264.7 Cell

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Prunioside A is a unique, highly oxidized monoterpene glycoside isolated from the methanol extract of *Spiraea prunifolia* var. *Simpliciflora*'s root. The ester derivatives were synthesized from the hydrolyzed compounds of prunioside A by β-glucosidase. The derivatives showed suppressive effects on the generation of nitric oxide in murine machrophage-like RAW 264.7 cells stimulated by lipopolysaccharide and interferon-γ.

Key Words : *Spiraea prunifolia*, Prunioside A, Nitric oxide, NO production, Inhibitory effects

Introduction

Nitric oxide (NO), a small molecule produced in mammalian cells, has been known to participate in diverse physiological processes including platelet inhibition, blood pressure homeostasis, neurotransmission, immune responses, and inflammation.[1,2] This molecule is produced by nitric oxide synthases (NOS) from L-arginine in certain cells. NO synthases can be classed into two types, constitutive nitric oxide synthase (cNOS) and inducible nitric oxide synthase (iNOS). The enzyme in macrophages is induced by cytokines such as lipopolysaccharide (LPS), interferon-γ (IFN-γ), interlukin-2 (IL-2), and tumor necrosis factor-α (TNF-α). NO synthesized by the inducible enzyme has showed cytotoxic effects on microbes, tumor cells, macrophages, and lymphocytes. However the increased NO production has been known to cause TNF-induced hypotension and various inflammatory diseases.[3-5] Therefore, many studies have been carried out to obtain a potential therapeutic agent from various natural substances which has the inhibitory effect on NO production by iNOS.

Recently, the methanol extract from *Spiraea prunifolia* var. *simpliciflora* root has been reported to suppress the generation of NO in polymyristic acetate stimulated RAW 264.7 cells.[6] *Spiraea prunifolia* var. *simpliciflora* (Rosaceae), a deciduous and latifoliate shrub, is found in the most parts of Korea and it's roots has been traditionally used for the treatment of fever and emetic conditions.[7,8] We have found the acetylated derivative of a secondary metabolite, prunioside A, from the roots of the plant showing the inhibitory effect on iNOS.[9,10] In this study we have synthesized the various ester derivatives of the hydrolyzed compounds, prepared from prunioside A by β-glucosidase, and analyzed their inhibitory effect on NO production in murine macrophage-like RAW 264.7 cells stimulated with INF-γ and lipopolysaccharide (LPS).

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Experimental Section

General experimental procedures. ESIMS data were obtained on a MicroMass Quatro LC with electro-spray ionization. NMR spectra were recorded in either acetone-d_6 or $CDCl_3$ using a JEOL Eclipse-500 MHz spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C), and chemical shifts were referenced relative to the corresponding residual solvent signals (acetone-d_6: δ 2.04/29.9, $CDCl_3$: δ 7.24/77.0). HPLC separations were performed on a Symmetry Prep C_{18} column (1.9 × 30 cm; 7-μm particle size; flow rate of 4 mL/min). Compounds were detected by measuring UV absorption at 210 and 254 nm on a Hewlett-Packard HP 8453 spectrophotometer.

Extraction and isolation of prunioside A (1). The roots of *S. prunifolia* were collected from Iksan City, Chonbuk Province, Korea in May 2002 and dried as previously described.[9] Prunioside A (232 mg) was isolated from the methanol extract of the air-dried roots (1 kg) of *S. prunifolia* according to the previous method.[10]

Enzymatic hydrolysis of prunioside A (1) with β-glucosidase. The enzymatic reaction was carrried out by incubating a solution of compound **1** (92 mg) in H_2O (10 mL) with β-glucosidase (46 mg) from yeast (G 8889, Sigma Chemical Co., St. Louis, MO) at 37 °C for 12 h. The reaction mixture was extracted with EtOAc (3 × 10 mL). The dried organic residue was applied to reversed-phase HPLC to afford 3-(4-Hydroxy-phenyl)-acrylic acid 1-[4-(2-hydroxy-

ethylidene)-5-oxo-tetrahydro-furan-2-yl]-2-methyl-allyl ester (compound **2**, 40 mg) (Scheme 1): ^{1}H NMR (500 MHz, acetone-d_6): δ 1.82 (3H, *s*), 2.75 (1H, *m*), 3.08 (1H, *m*), 4.58 (1H, *m*), 4.64 (1H, *m*), 4.91 (1H, *ddd*, J = 8.3, 5.5, 5.5), 5.04 (1H, *br s*), 5.10 (1H, *br s*), 5.40 (1H, *d*, J = 5.5), 6.32 (1H, *m*), 6.38 (1H, *d*, J = 16.0 Hz,), 6.89 (2H, *d*, J = 8.7 Hz), 7.56 (2H, *d*, J = 8.7 Hz), 7.62 (1H, *d*, J = 16.0 Hz); ^{13}C NMR: δ 18.5, 30.9, 58.1, 76.7, 77.4, 114.1, 114.4, 115.9, 124.3, 126.0, 130.3, 140.5, 143.0, 145.5, 160.0, 165.6, 168.9; ESI-MS: *m/z* 345 (100%, M + H$^+$).

Benzoic acid 4-[2-(2-methyl-1-{5-oxo-4-[2-(2-vinyl-but-2-enoyloxy)-ethylidene]-tetrahydro-furan-2-yl}-allyloxy-carbonyl)-vinyl]-phenyl ester (3). A solution of **2** (5 mg, 1.46×10^{-5} mol), 4-*N,N*-dimethylaminopyridine (0.5 mg), and benzoyl chloride (40 μL, 2.85×10^{-7}mol) in acetonitrile (2 mL) was stirred for 30 min at room temperature. The ester compound **3** (5.5 mg, yield 68%) was obtained by the separation procedure described above; ^{1}H NMR (500 MHz, acetone-d_6): δ 1.86 (3H, *s*), 2.82-2.89 (1H, *m*), 3.17 (1H, m), 5.02 (1H, *ddd*, J = 5.1, 5.1, 8.3), 5.07 (1H, *s*), 5.14 (1H, *s*), 5.36-5.49 (2H, *m*), 5.48 (1H, *d*, J = 4.6), 6.41 (1H, *m*), 6.63 (1H, *d*, J = 16.1), 7.33 (2H, *d*, J = 8.7), 7.46 (2H, *t*, J = 8.3), 7.59 (2H, *t*, J = 7.8), 7.71 (2H, *m*), 7.81 (2H, *d*, J = 8.7), 7.95 (2H, *d*, J = 8.3), 8.17 (2H, *d*, J = 7.3); ^{13}C NMR: δ 17.8, 24.9, 63, 80.8, 85.8.107.6, 118.1, 121.3, 126.6, 128.4, 128.5, 129.7, 130.1, 130.5, 130.6, 131.7, 132.8, 133.7, 135.6, 136.2, 143.3, 147.9, 152.3, 164, 165.5, 165.7, 167; ESI-MS: *m/z* 575 (100%, M + Na$^+$).

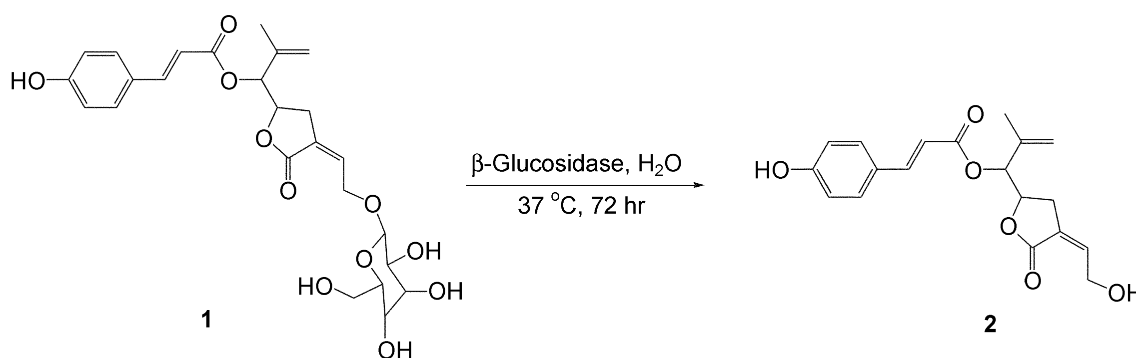
Cyclopentanecarboxylic acid 4-(2-{1-[4-(2-cyclopentane-carbony-loxyethylidene)-5-oxo-tetrahydro-furan-2-yl]-2-methylallyloxy-carbonyl}-vinyl)-phenyl ester (4). A solution of **2** (5 mg, 1.46×10^{-5} mol), 4-*N,N*-dimethylamino-pyridine (0.5 mg), and cyclopentanecarbonyl chloride (40 μL, 3.03×10^{-7} mol) in acetonitrile (2 mL) was allowed for the esterification at room temperature for 30 min. The HPLC separation followed by the solvent extraction gave 5.6 mg of compound **4** (yield 72%); ^{1}H NMR (500 MHz, acetone-d_6): δ 1.51-1.98 (18H, *m*), 1.83 (3H, *s*), 2.84 (1H, *m*), 3.06 (1H, *m*), 4.98 (1H, *ddd*, J = 4.6, 4.6, 8.7), 5.09-5.16 (2H, *m*), 5.05 (1H, *s*), 5.11 (1H, *s*), 5.45 (1H, *d*, J = 4.6), 6.23 (1H, *m*), 6.60 (1H, *d*, J =16.0), 7.20 (2H, *d*, J = 8.7), 7.71 (1H, *d*, J = 16.1), 7.77 (2H, *d*, 8.7); ^{13}C NMR: δ 17.8, 22.8, 23.1, 24.9, 25.4, 25.4, 40.8, 41.3, 63.7, 80.8, 85.8, 107.6, 118.1, 121.3, 126.6, 131.7, 135.6, 136.2, 143.3, 147.9, 152.3, 165.5, 165.7, 173, 176; ESI-MS: *m/z* 559 (100%, M + Na$^+$).

3-Methyl-but-2-enoic acid 4-[2-(2-methyl-1-{4-[2-(3-methyl-but-2-enoyloxy)-ethylidene]-5-oxo-tetrahydro-furan-2-yl}-allyloxycarbonyl)-vinyl]-phenyl ester (5). To a solution of **2** (5 mg, 1.46×10^{-5} mol), 4-*N,N*-dimethylaminopyridine (0.5 mg) in acetonitrile (2 mL) was added dimethylacryloyl chloride (40 μL, 3.38×10^{-7} mol). The reaction mixture was stirred for 30 min at room temperature. The dried organic residue obtained from the solvent extraction with EtOAc and H_2O was subjected to reversed-phase HPLC to afford 5.6 mg (yield 76%) of compound **5**; ^{1}H NMR (500 MHz, acetone-d_6): δ 1.73 (3H, *s*), 1.84 (3H, *s*), 2.00 (3H, *s*), 2.20 (3H, *s*), 2.78-2.84 (1H, *m*), 3.00 (2H, *s*), 3.12 (1H, *m*), 4.78 (1H, *s*), 4.83 (1H, *s*), 4.96 (1H, *ddd*, J = 4.6, 4.6, 8.7), 5.06 (1H, *s*), 5.11 (1H, *s*), 5.16 (2H, *m*), 5.44 (1H, *d*, J = 4.6), 5.93 (1H, *s*), 6.23 (1H, *m*), 6.57 (1H, *d*, J = 16.1), 7.19 (2H, *d*, J = 8.7), 7.68 (1H, *d*, J = 16.1), 7.75 (2H, *d*, 8.3); ^{13}C NMR: δ 17.8, 18.7, 19.1, 24.7, 24.7, 24.9, 63.5, 80.8, 85.8, 107.6, 113.1, 115.6, 118.1, 121.3, 126.6, 131.7, 135.6, 136.2, 143.3, 147.6, 147.9, 151.9, 152.3, 165.5, 165.7, 168, 171; ESI-MS: *m/z* 531 (100%, M + Na$^+$).

3-(4-Yryloxy-phenyl)-acrylic acid 1-[4-(2-butyryloxy-ethylidene)-5-oxo-tetrahydro-furan-2-yl]-2-methyl-allyl ester (6). A solution of **2** (5 mg, 1.46×10^{-5} mol), 4-*N,N*-dimethylamino-pyridine (0.5 mg), and butyryl chloride (4 μL, 3.75×10^{-7} mol) in acetonitrile (2 mL) was stirred at room temperature for 30 min. The solvent was evaporated under N_2. The resulting residue was purified as described above to afford 5.1 mg (yield 73%) of compound **6**; ^{1}H NMR (500 MHz, acetone-d_6): δ 0.88 (3H, *t*, J = 7.4), 1.00 (3H, *t*, J = 7.3), 1.56 (2H, *m*), 1.73 (2H, *m*), 1.84 (3H, *s*), 2.20 (2H, *t*, J = 7.3), 2.57 (2H, *t*, J = 7.4), 2.80 (1H, *m*), 3.15 (1H, *m*), 4.98 (1H, *ddd*, J = 5.0, 5.0, 8.3), 5.05-5.15 (2H, *m*), 5.05 (1H, *s*), 5.11 (1H, *s*), 5.45 (1H, *d*, J = 4.6), 6.23 (1H, *m*), 6.59 (1H, *d*, J = 16.1), 7.20 (2H, *d*, J = 8.7), 7.70 (1H, *d*, J = 16.0), 7.75 (2H, *d*, 8.7); ^{13}C NMR: δ 13.4, 13.7, 17.8, 18.2, 18.2, 24.9, 35.4, 35.9, 63.4, 80.8, 85.8, 107.6,, 118.1, 121.3, 126.6, 131.7, 135.6, 136.2, 143.3, 147.9, 152.3, 165.5, 165.7, 169, 172; ESI-MS: *m/z* 507 (100%, M + Na$^+$).

Benzoic acid 4-(2-{1-[4-(2-hydroxy-ethylidene)-5-oxo-tetrahydro-furan-2-yl]-2-methyl-allyloxycarbonyl}-vinyl)-phenyl ester (7). Compound **2** (5 mg, 1.46×10^{-5} mol) was stirred with 4-*N,N*-dimethylaminopyridine (0.5 mg) and benzoyl chloride (2 μL, 1.42×10^{-7} mol) in acetonitrile (2 mL) for 15 min at room temperature. Compound **7** (4.3 mg, yield 66%) was obtaind by the separation procedures described above; ^{1}H NMR (500 MHz, acetone-d_6): δ 1.84 (3H, *s*), 2.76-2.83 (1H, *m*), 3.09 (1H, *m*), 4.61 (2H, *m*), 4.92 (1H, *ddd*, J = 5.5, 5.5, 8.3), 5.06 (1H, *s*), 5.12 (1H, *s*), 5.43 (1H, *d*, J = 5.1), 6.32 (1H, *m*), 6.60 (1H, *d*, J = 16.1), 7.37 (2H, *d*, J = 8.7), 7.59 (2H, *t*, J = 8.3), 7.72 (2H, *t*, J = 8.3), 7.82 (2H, *d*, J = 8.7), 8.17 (2H, *d*, J = 7.4); ^{13}C NMR: δ 17.8, 24.9, 59.7, 80.8, 85.8, 107.6, 118.1, 121.3, 126.6, 128.5, 130.1, 130.6, 131.7, 133.7, 135.6, 136.2, 143.3, 147.9, 152.3, 164, 165.5, 165.7; ESI-MS: *m/z* 471 (100%, M + Na$^+$).

Cyclopentanecarboxylic acid 4-(2-{1-[4-(2-hydroxy-ethylidene)-5-oxo-tetrahydro-furan-2-yl]-2-methyl-allyloxy-carbonyl}-vinyl)-phenyl ester (8). Compound **8** (4.6 mg, yield 72%) was prepared by allowing the reaction for 15 min from compound and cyclopentanecarbonyl chloride (20 μL, 1.51×10^{-7} mol) using the acylation procedure described above; ^{1}H NMR (500 MHz, acetone-d_6): δ 1.62-2.00 (9H, *m*), 1.84 (3H, *s*), 2.78-2.83 (1H, *m*), 3.08 (1H, *m*), 4.61 (2H, *m*), 4.91 (1H, *ddd*, J = 5.1, 5.1, 8.7), 5.05 (1H, *s*), 5.11 (1H, *s*), 5.41 (1H, *d*, J = 5.1), 6.32 (1H, *m*), 6.55 (1H, *d*, J = 16.1), 7.18 (2H, *d*, J = 8.7), 7.67 (1H, *d*, J = 16.1), 7.74 (2H, *d*, 8.7); ^{13}C NMR: δ 17.8, 22.8, 24.9, 25.4, 40.8, 59.7, 80.8, 85.8, 107.6, 118.1, 121.3, 126.6, 131.7, 135.6, 136.2, 143.3, 147.9, 152.3, 165.5, 165.7, 173; ESI-MS: *m/z* 463 (100%, M + Na$^+$).



Scheme 1

3-Methyl-but-2-enoic acid 4-(2-{1-[4-(2-hydroxy-ethylidene)-5-oxo-tetrahydro-furan-2-yl]-2-methyl-allyloxycarbonyl}-vinyl)-phenyl ester (9). Dimethylacryloyl chloride (20 μL, 1.69×10^{-7} mol) was used to synthesize compound **9** (4.7 mg, yield 76%) from compound **2**; ^{1}H NMR (500 MHz, acetone-d_6): δ 1.83 (3H, *s*), 2.00 (3H, *s*), 2.19 (3H, *s*), 2.75-2.81 (1H, *m*), 3.06 (1H, *m*), 4.61 (2H, *m*), 4.92 (1H, *ddd*, *J* = 5.5, 5.5, 8.3), 5.05 (1H, *s*), 5.11 (1H, *s*), 5.16 (2H, *m*), 5.42 (1H, *d*, *J* = 5.1), 5.92 (1H, *br*, *s*), 6.31 (1H, *m*), 6.56 (1H, *d*, *J* = 16.0), 7.19 (2H, *d*, *J* = 8.8), 7.68 (1H, *d*, *J* = 16.1), 7.75 (2H, *d*, 8.7); ^{13}C NMR: δ 17.8, 18.7, 24.7, 24.9, 59.7, 80.8, 85.8, 107.6, 113.1, 118.1, 121.3, 126.6, 131.7, 135.6, 136.2, 143.3, 147.9, 151.9, 152.3, 165.5, 165.7, 168; ESI-MS: *m/z* 449 (100%, M − Na$^+$).

3-(4-Butyryloxy-phenyl)-acrylic acid 1-[4-(2-hydroxy-ethylidene)-5-oxo-tetrahydro-furan-2-yl]-2-methyl-allyl ester (10). Compound **10** (4.6 mg, yield 77%) was obtained from butyryl chloride (20 μL, 1.88×10^{-7} mol) and compound **2**; ^{1}H NMR (500 MHz, acetone-d_6): δ 1.01 (3H, *t*, *J* = 7.4), 1.75 (2H, *m*), 1.84 (3H, *s*), 2.58 (2H, *t*, *J* = 7.4), 2.80 (1H, *m*), 3.08 (1H, *m*), 4.60 (2H, *m*), 4.93 (1H, *ddd*, *J* = 5.5, 5.5, 8.3), 5.05 (1H, *s*), 5.11 (1H, *s*), 5.42 (1H, *d*, *J* = 5.1), 6.32 (1H, *m*), 6.58 (1H, *d*, *J* = 16.1), 7.20 (2H, *d*, *J* = 8.7), 7.71 (1H, *d*, *J* = 16.1), 7.76 (2H, *d*, 8.7); ^{13}C NMR: δ 13.4, 17.8, 18.2, 24.9, 35.4, 59.7, 80.8, 85.8, 107.6, 118.1, 121.3, 126.6, 131.7, 135.6, 136.2, 143.3, 147.9, 152.3, 165.5, 165.7, 169; ESI-MS: *m/z* 437 (100%, M + Na$^+$).

Macrophage cell line culture. The murine macrophage cell line RAW264.7 was purchased from the American Tissue Culture Collection (Rockville, MD). The cells were maintained in complete RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum, 1% *L*-glutamine, 1% nonessential amino acids, 1% antibiotic/antimycotic (100 U/mL of penicillin, 25 μg/mL of amphotericin D, and 100 mg/mL of streptomycin), 1.5% sodium bicarbonate, and 1% minimal essential vitamins at 37 °C in a humidified 5% CO_2 atmosphere

Measurement of nitrite concentration. Experiments were undertaken on cells grown in the presence of various concentrations of test compounds dissolved in DMSO with LPS (1 μg/mL) for 18 hr. The final concentration of DMSO in culture media was 0.1%. Supernatants in cultured macrophages were collected and mixed with an equal volume of the Griess reagent (1% sulfanilamide, 0.1% *N*-(1-naphthyl)-ethylenediamine dihydrochloride in 2.5% phosphoric acid solution) and incubated for 10 min at room temperature. Nitrite concentration was determined by measuring the absorbance at 540 nm using an ELISA plate reader. The level of nitrite reflects nitric oxide synthesis. Sodium nitrite was used as a standard. The cell-free medium contained 5-8 μM of nitrite, and this value was determined in each experiment and subtracted from the value obtained with cells.

Results and Discussion

NO, which is produced in a large amount by cytokines such as LPS and IFN-γ in macrphages and several immune cells, can be deleterious to the host and has been implicated in the pathogenesis of various inflammatory diseases.[4,5] Prunioside A, a unique terpene glycoside, has been recently identified from the methanol extract of *S. pruniofolia*'s roots showing a suppressive effect on NO production in murine macrophage-like RAW 264.7 cells stimulated with IFN-γ plus LPS. Though the terpene glycoside did not show any inhibitory effect on NO production in IFN-γ and LPS-stimulated macrophage-like RAW 264.7 cells, its acetylated derivatives showed the inhibitory effect on the NO generation.[9,10]

The various ester derivatives were synthesized from the hydrolyzed prunioside A with β-glucosidase (Scheme 1) and their inhibitory effect on NO production (IC_{50}) of RAW 264.7 cells were determined (Table 1). Figure 1 showes the biological activities of ester derivatives (compounds **3-10**) on NO production in the RAW 264.7 cells. All of them exhibited a clear suppressive effect on NO production in a dose-dependent manner in a range from 1 μg/mL to 50 μg/mL. Particularly, it is noticeable that compound **10** displays the most apparent inhibitory effect among the compounds examined (Fig. 1). RT-PCR (Reverse transcriptase-polymerase chain reaction) was conducted to determine whether the inhibition of NO production by compound **10** was owing to the interfering with the transcription of iNOS. The inhibitory effect on NO production by the compound **10** in IFN-γ and LPS-stimulated macrophage-like RAW 264.7 cells appeared to be correlated with the suppression of iNOS mRNA (data not shown).

In addition to the synthesis of various ester derivatives

Table 1. Inhibitory effect of prunioside A derivatives on NO production

RO— ... —OR'

The ester derivatives of **2**

Entry	R	R'	Product	Yield (%)	IC_{50} Inhibition
1	—C(=O)—C$_6$H$_5$	—C(=O)—C$_6$H$_5$	**3**	68	5.0 μg/mL
2	—C(=O)—C$_5$H$_9$ (cyclopentyl)	—C(=O)—C$_5$H$_9$ (cyclopentyl)	**4**	72	6.0 μg/mL
3	—C(=O)—CH=C(CH$_3$)$_2$	—C(=O)—CH=C(CH$_3$)$_2$	**5**	76	7.5 μg/mL
4	—C(=O)—CH$_2$CH$_2$CH$_3$	—C(=O)—CH$_2$CH$_2$CH$_3$	**6**	73	6.0 μg/mL
5	—C(=O)—C$_6$H$_5$	H	**7**	66	4.0 μg/mL
6	—C(=O)—C$_5$H$_9$ (cyclopentyl)	H	**9**	72	5.5 μg/mL
7	—C(=O)—CH=C(CH$_3$)$_2$	H	**9**	76	5.0 μg/mL
8	—C(=O)—CH$_2$CH$_2$CH$_3$	H	**10**	77	1.0 μg/mL

from the β-glucosidase treated prunioside A, the present study is the first report that the ester derivatives of prunioside A may be the potential therapeutic agents for some inflammatory diseases.

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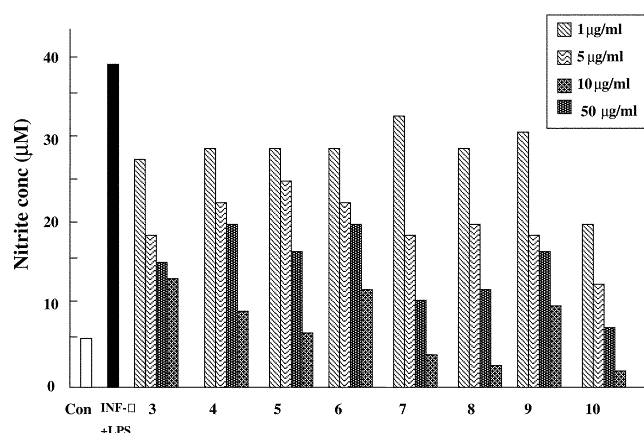

Figure 1. Effects of compounds **3-10** on NO production in LPS-stimulated RAW 264.7 cells. Cells were stimulated for 24 h with 1 μg/mL of LPS in the presence or absence of test compounds at indicated concentrations.

References

1. Natha, C. *FASEB J.* **1992**, *6*, 3051.
2. Moncad, S.; Palmer, R. M. J.; Higgs, E. A. *Pharmacol. Rev.* **1991**, *43*, 109.
3. Kilbourn, R. G.; Gross, S. S.; Jubran, A.; Adams, J.; Griffith, O. W.; Levi, R. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 3629.
4. Lalenti, A.; Ianaro, A.; Moncada, S.; DiRosa, M. *Eur. J. Pharmacol.* **1992**, *211*, 177.
5. McCartney-Francis, N.; Allen, J. B.; Mizel, D. E.; Albina, J. E.; Xie, Q. W.; Nathan, C. F.; Wahl, S. M. *J. Exp. Med.* **1993**, *178*, 749.
6. So, H. S.; Park, R.; Oh, H. M.; Pae, H. O.; Lee, J. H.; Chai, K. Y.; Chung, S. Y.; Chung, H. T. *J. Ethnopharmacol.* **1999**, *68*, 209.
7. Ro, J. S. *Kor. J. Pharmacol.* **1982**, *13*, 39.
8. Woo, M. H.; Lee, E. H.; Chung, S. O.; Kim, C. W. *Kor. J. Pharmacol.* **1996**, *27*, 389.
9. Oh, H.; Oh, G. S.; Seo, W. G.; Pae, H. O.; Chai, K. Y.; Kwon, T. O.; Lee, Y. H.; Chung, H. T.; Lee, H. S. *J. Nat. Prod.* **2001**, *64*, 942.
10. Oh, H.; Shin, H.; Oh, G. S.; Pae, H. O.; Chai, K. Y.; Chung, H. T.; Lee, H. S. *Phytochem.* **2003**, *64*, 1113.