JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Anti-inflammatory Effects of Phenolic Compounds Isolated from the Fruits of *Artocarpus heterophyllus*

SONG-CHWAN FANG,[†] CHIN-LIN HSU,[‡] AND GOW-CHIN YEN*,[‡]

Department of Food Nutrition, Chung Hwa University of Medical Technology, 89 Wenhwa First St., Tainan 71703, Taiwan, and Department of Food Science and Biotechnology, National Chung Hsing University, 250 Kuokuang Road, Taichung 40227, Taiwan

Artocarpus heterophyllus Lam is a large evergreen tree cultivated throughout Southeast Asia for its fruits. Its leaves and roots have been used for medicinal purposes. The aim of this work was to study the in vitro anti-inflammatory effects of phenolic compounds isolated from the ethyl acetate extracts of the fruits of *Artocarpus heterophyllus*. Three phenolic compounds were characterized as artocarpesin [5,7,2′,4′-tetrahydroxy-6-(3-methylbut-3-enyl) flavone] (**1**), norartocarpetin (5,7,2′,4′-tetrahydroxyflavone) (**2**), and oxyresveratrol [*trans*-2,4,3′,5′-tetrahydroxystilbene] (**3**) by spectroscopic methods and through comparison with data reported in the literatures. The anti-inflammatory effects of the isolated compounds (**1**−**3**) were evaluated by determining their inhibitory effects on the production of proinflammatory mediators in lipopolysaccharide (LPS)-activated RAW 264.7 murine macrophage cells. These three compounds exhibited potent anti-inflammatory activity. The results indicated that artocarpesin (**1**) suppressed the LPS-induced production of nitric oxide (NO) and prostaglandin E_2 (PGE_2) through the down-regulation of inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) protein expressions. Thus, artocarpesin (**1**) may provide a potential therapeutic approach for inflammation-associated disorders.

KEYWORDS: ***Artocarpus heterophyllus*; anti-inflammation; inducible nitric oxide synthase; cyclooxygenase 2**

INTRODUCTION

Macrophages play an important role in response to inflammation by nitric oxide (NO), superoxide anions, and cytokines (*1*). NO is synthesized from the amino acid arginine by nitric oxide synthase (NOS) and induces tissue injury at the inflammatory site (*2*). It is known to be an important mediator of acute and chronic inflammation (*3*). This nanomolar concentration of NO plays an important role as a vasodilator and neurotransmitter and in the immunological system as a defense against tumor cells, parasites, and bacteria (*4*). The large amount of NO produced in response to lipopolysaccharide (LPS) plays an important role in inflammatory conditions (*5*). Salerno et al. (*6*) indicated that enhanced gene expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) are associated with inflammatory responses. iNOS is the key enzyme that produced large amounts of NO in macrophages stimulated with LPS and proinflammatory cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor (TNF) (*7*). In addition, COX-2 is thought to be the predominant cyclooxygenase involved in the inflammatory responses (*8*). Therefore, reduction of excess NO generation as well as inhibition of iNOS and COX-2 protein expressions may be associated with the prevention and treatment of the oxidative stress-induced inflammatory diseases.

Artocarpus heterophyllus Lam is a large evergreen tree cultivated throughout Southeast Asia for its fruits. Its leaves and roots have been used for medicinal purposes, such as anemia, asthma, dermatosis, diarrhea, and cough as an expectorant (*9*–*11*). The root bark and the heartwood have been described as containing chemical compounds with antioxidant properties (*12*). *Artocarpus* species (*Moraceae*), a rich source of prenylated flavonoids and derivatives, have been investigated phytochemically and biologically (*13*–*15*). Wei et al. (*16*) indicated that artocarpanone from the roots of *A. heterophyllus* significantly inhibits the LPS-induced NO production and iNOS protein expression in RAW 264.7 cells. However, the literature regarding the anti-inflammatory activities of phenolic compounds isolated and identified from the fruits of *A. heterophyllus* remains unclear. The objective of this study was to investigate the anti-inflammatory effects of isolated bioactive compounds from the fruits of *A. heterophyllus* by modulation of iNOS and COX-2 expressions in LPS-stimulated RAW 264.7 cells.

MATERIALS AND METHODS

Materials. The fruits of *Artocarpus heterophyllus* were collected at Tainan Hsien, Taiwan, in August 2005. Lipopolysaccharide (LPS) and MTT dye [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bro-

* To whom correspondence should be addressed. Phone: 886-4-22879755. Fax: 886-4-22854378. E-mail: gcyen@nchu.edu.tw.
[†] Chung Hwa University of Medical Technology.
[‡] National Chung Hsing University.

10.1021/jf800444g CCC: $40.75
Published on Web 05/24/2008

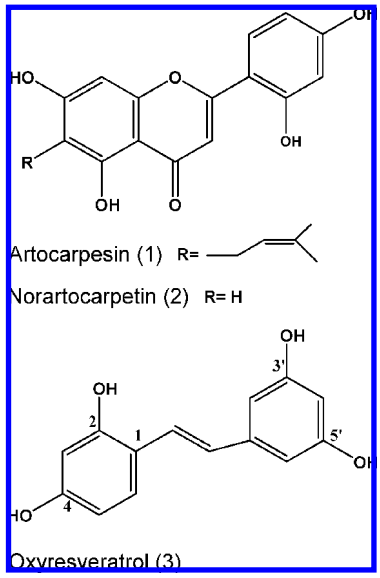

Figure 1. Structures of artocarpesin (**1**), norartocarpetin (**2**), and oxyresveratrol (**3**) isolated from the ethyl acetate extracts of the fruits of *A. Heterophyllus*.

mide] were purchased from the Sigma Chemical Co. (St. Louis, MO). Dimethylsulfoxide (DMSO) was purchased from the Merck Co. (Darmstadt, Germany). Dulbecco's modified Eagle's medium, fetal bovine serum, L-glutamine, and the antibiotic mixture (penicillin−streptomycin) were purchased from the Invitrogen Co. (Carlsbad, CA). Anti-COX-2 and anti-iNOS antibody were purchased from ABcam (Cambridge, MA). Anti-β-actin antibody was purchased from Cell Signaling Technology (Beverly, MA). Antirabbit or antimouse secondary horseradish peroxidase antibodies were purchased from Bethyl Laboratories (Montgomery, TX). Protein molecular mass markers were obtained from Pharmacia Biotech (Saclay, France). Polyvinyldifluoride (PVDF) membranes for Western blotting were obtained from Millipore (Bedford, MA). All other chemicals were reagent grade.

Extraction and Isolation of Phenolic Compounds from the Fruits of *Artocarpus heterophyllus*. The dried fruit pulps (22 kg) were extracted twice with 20 L of methanol at room temperature for 8 days. The crude methanolic extracts were concentrated in vacuo, and the resulting aqueous suspension was partitioned with *n*-hexane, EtOAc, and *n*-BuOH to afford dried *n*-hexane (71.3 g), EtOAc (32.6 g), and *n*-BuOH (69.5 g) extracts. The ethyl acetate soluble material was subjected to chromatography over a silica gel column (70−230 mesh, 1.5 kg, Merck) using a step gradient, eluted with *n*-C_6H_{14}−EtOAc as the solvent system (9:1, 5:1, 2:1, 1:1, 0:1, v/v, each solvent system containing 2.2 L) and EtOAc−MeOH (20:1, v/v, 1.5 L), to afford fractions A−D by TLC profile. Fraction A contained a mixture of aliphatic compounds, fraction D contained an intractable mixture of phloroglucinols, and only fractions B and C were examined in detail. Fraction B (*n*-C_6H_{14}−EtOAc, 5:1−2:1, 5.3 g) was rechromatographed on a silica gel column (70−230 mesh, 150 g, Merck) and eluted with *n*-C_6H_{14}−EtOAc as the solvent system (5:1, 4:1, 1:1, 0:1, v/v, each solvent system containing 800 mL) to yield 18 fractions. The fractions (*n*-C_6H_{14}−EtOAc, 4:1−2:1, fractions 8−10) were rechromatographed on a silica gel column (230−400 mesh, 52.5 g, Merck) and eluted with C_6H_6−EtOAc (4:1, v/v) to afford six fractions. The fraction (fraction 3) was purified by preparative TLC developing with $CHCl_3$−MeOH (10:1, v/v) to obtain compound **1** (15.2 mg). The fractions (*n*-C_6H_{14}-EtOAc, 1:1, fractions 13−14) were applied to preparative TLC with $CHCl_3$−MeOH (10:1, v/v) to obtain compound **2** (18.5 mg). Fraction C (*n*-C_6H_{14}−EtOAc, 1:1, 3.6 g) was rechromatographed on a silica gel column (230−400 mesh, 350 g, Merck) and eluted with CH_2Cl_2−EtOAc as the solvent system (10:1, 8: 1, 5:1, 3:1, 2:1, 1:1, 0:1, v/v, each solvent system containing 800 mL) to yield 13 fractions. The fractions (CH_2Cl_2−EtOAc, 3:1−2:1, fractions 5−6) were recrystallized from MeOH−EtOAc (1:8) to obtain compound **3** (11.6 mg).

Spectrometry. UV spectra were obtained on a Thermo model α UV−vis spectrophotometer (Thermo Spectronic, Cambridge, England). ^1H (400 MHz) and ^{13}C NMR (100 MHz) experiments were performed with a Varian Unity 400 NMR spectrophotometer (Varian, CA). MS spectra were obtained on a JMS HX-100 mass spectrometer (JEOL Ltd., Tokyo, Japan).

Cell Culture. RAW 264.7 cell line (BCRC 60001) was obtained from the Bioresource Collection and Research Center (BCRC, Food Industry Research and Development Institute, Hsinchu, Taiwan). Cells were cultured in Dulbecco's modified Eagle's medium, 10% fetal bovine serum, 2 mM L-glutamine, and 100 U/mL penicillin−streptomycin. These cells were cultured at 37 °C in a humidified 5% CO_2 incubator.

Cell Viability Assay. The MTT assay was performed according to the method of Mosmann (*17*). RAW 264.7 cells were plated into 96-well microtiter plates at a density of 1×10^4 cells/well. After 24 h, the culture medium was replaced by 200 μL serial dilutions (0−100 μM) of artocarpesin (**1**), norartocarpetin (**2**), and oxyresveratrol (**3**), and the cells were incubated for 24 h. The final concentration of solvent was less than 0.1% in the cell culture medium. The culture medium was removed and replaced by 90 μL of fresh culture medium. Sterile filtered MTT solution (10 μL, 5 mg/mL) in phosphate buffered saline (PBS, pH=7.4) was added to each well, reaching a final concentration of 0.5 mg MTT/mL. After 5 h, unreacted dye was removed, and the insoluble formazan crystals were dissolved in 200 μL/well DMSO and measured spectrophotometrically in a FLUOstar galaxy spectrophotometer (BMG Labtechnologies Ltd., Offenburg, Germany) at 570 nm. The relative cell viability (%) related to control wells containing cell culture medium without samples was calculated by $A_{570\ nm}$[sample]/$A_{570\ nm}$[control] × 100.

Measurement of Nitric Oxide/Nitrite. Nitrite levels in the cultured media, which reflect intracellular nitric oxide synthase activity, were determined by Griess reaction. The cells were incubated with 0−50 μM of artocarpesin (**1**), norartocarpetin (**2**), and oxyresveratrol (**3**) in the absence or presence of LPS (1 μg/mL) for 24 h. Briefly, cells were dispensed into 96-well plates, and 100 μL of each supernatant was mixed with the same volume of Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride, and 5% phosphoric acid) and incubated at room temperature for 10 min. By using sodium nitrite to generate a standard curve (*18*), the concentration of nitrite was measured by OD reading at 550 nm.

Measurement of Prostaglandin E_2 (PGE_2). The cells were incubated with 0−25 μM of artocarpesin (**1**) in the absence or presence of LPS (1 μg/mL) for 24 h. PGE_2 was determined using a prostaglandin E_2 Express EIA Kit (Cayman Chemical Company, Ann Arbor, MI). The concentration of PGE_2 was photometrically determined using a microplate reader (Awareness Technology, Palm City, FL) at 405 nm.

Determination of ROS Production. The intracellular ROS scavenging activity of the sample was measured using the oxidant-sensitive fluorescent probe DCFH-DA. DCFH converted from DCFH-DA by deacetylase within the cells is oxidized by a variety of intracellular ROS to DCF, a highly fluorescent compound. The cells were incubated with 0−10 μM of artocarpesin (**1**) in the absence or presence of LPS (1 μg/mL) for 1 h. Briefly, cells were harvested by trypsin−EDTA (TE) solution (0.05% trypsin and 0.02% EDTA in PBS) and wash with PBS twice. The cells were stained with 20 μM of DCFH-DA for 15 min at room temperature and subjected to determination of intracellular ROS production by using a FACScan flow cytometric (Becton Dickinson Immunocytometry Systems, San Jose, CA). Approximately 1×10^4 counts were made for each sample. The ROS production (%) was calculated by CELL Quest software.

Western Blot Analysis. The cells were incubated with 0−25 μM of artocarpesin (**1**) in the absence or presence of LPS (1 μg/mL) for 3 and 6 h. After stimulation, cells were collected and lysed in ice-cold lysis buffer [20 mM tris-HCl (pH 7.4), 2 mM EDTA, 500 μM sodium orthovanadate, 1% Triton X-100, 0.1% SDS, 10 mM NaF, 10 μg/mL leupeptin, and 1 mM PMSF]. The COX-2 and iNOS proteins were assessed in RAW 264.7 cells. The protein concentration of extracts was estimated with the Bio-Rad DC protein assay (Bio-Rad Laboratories, Hercules, CA) using bovine serum albumin as the standard. The total proteins (50−60 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 12% poly-

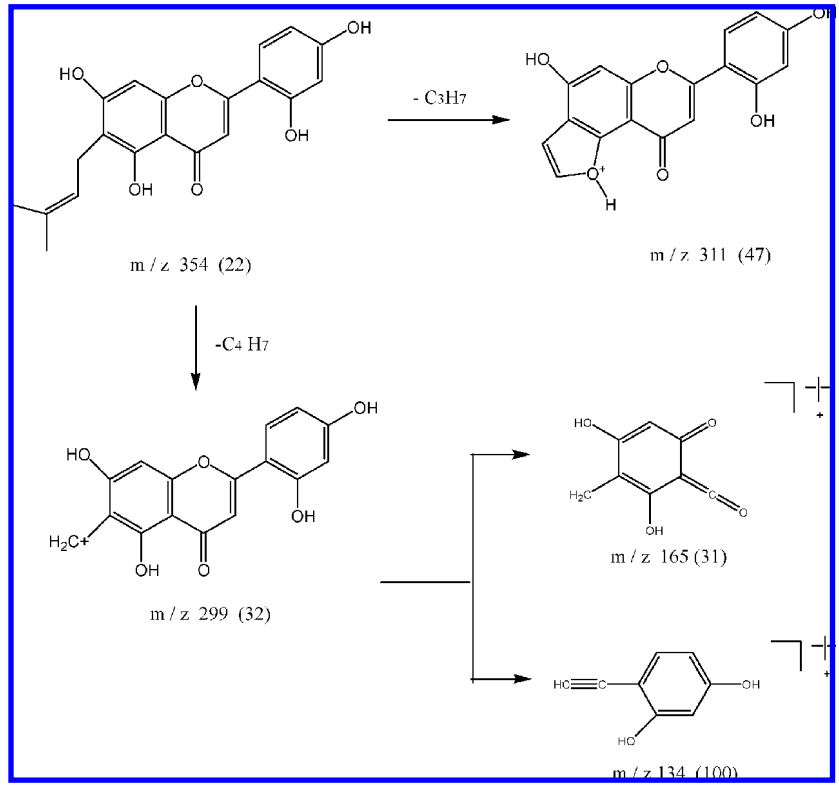

Figure 2. Mass spectral fragmentation of artocarpesin (**1**).

acrylamide gel. The proteins in the gel were transferred to a PVDF membrane. The membrane was blocked with 5% skim milk in PBST (0.05% v/v Tween-20 in PBS, pH 7.2) for 1 h. Membranes were incubated with primary antibody (1:5000) at 4 °C overnight and then with secondary antibody (1:5000) for 1 h. Membranes were washed 3 times in PBST for 10 min between each step. The signal was detected using the Amersham ECL system (Amersham-Pharmacia Biotech, Arlington Heights, IL). Relative protein expression was quantified densitometrically using the LabWorks 4.5 software and calculated according to the β-Actin reference bands.

Statistical Analysis. Each experiment was performed in triplicate. The results are expressed as mean ± SD. Statistical analysis was performed using SAS software. Analysis of variance was performed using ANOVA procedures. Significant differences ($p < 0.05$) between the means were determined by Duncan's multiple range tests.

RESULTS AND DISCUSSION

Isolation and Identification of Phenolic Compounds from *Artocarpus heterophyllus*. The EtOAc-soluble fraction of the fruits of *A. heterophyllus* led to the isolation of two flavones (compounds **1** and **2**) and a stilbene (compound **3**). The structures of these three compounds (**Figure 1**) were identified by comparing their physical and spectroscopic data (UV, ^{1}H NMR, ^{13}C NMR, and EI-MS) with those of reported values. Compound **1**, artocarpesin, was a brown amorphous powder. The UV spectrum exhibited maxima at 251, 270, and 352 nm, resembled the spectra of flavanone derivatives (*19*), and showed a bathochromic shift in the presence of aluminum chloride, sodium acetate, and sodium methoxide, respectively. The ^{1}H NMR spectrum of compound **1** showed the signals due to a prenyl group, ABX type protons, and two aromatic protons. The ^{13}C NMR spectrum of compound **1** showed 20 resonance signals; the EI-MS of compound **1** showed the base molecular ion at *m*/*z* 354 and the fragment ion at *m*/*z* 203, 177, and 165, suggesting that a prenyl group was located in the A ring. From the above results, compound **1** was proposed for the structure of artocarpesin. The chemical shifts of this compound were matched to reported values (*20*). The fragment pathway of the main ions of compound **1** is shown in **Figure 2**.

Compound **2**, norartocarpetin, was a yellow amorphous powder. The UV spectrum resembled that of compound **1**. The ^{1}H NMR spectrum of compound **2** revealed the presence of ABX type protons, two weakly meta-coupled protons, and an aromatic proton. The EI-MS of compound **2** showed the base molecular ion at *m*/*z* 286 and the fragment ion at *m*/*z* 153 and 134. From the above results, compound **2** was proposed for the structure of norartocarpetin. The chemical shifts of this compound were matched to reported values (*21*).

Compound **3**, oxyresveratrol, was a yellow amorphous powder. The ^{1}H NMR spectrum of compound **3** revealed the presence of proton signals for ABX type protons, two *trans*-olefinic protons, and a 1,3,5-trisubstituted symmetrical aromatic proton. The ^{13}C NMR spectrum, four oxygenated aromatic carbons (159.5 × 2, 159.2, 157.3), and 10 aromatic carbons (102.2−142.2) were shown. The EI-MS of compound **3** showed the base molecular ion at *m*/*z* 244 and the fragment ion at *m*/*z* 110 ($C_6H_6O_2$, moiety). From the above results, compound **3** was proposed for the structure of oxyresveratrol. The chemical shift of this compound was matched to reported values (*22*).

Effects of Artocarpesin (1), Norartocarpetin (2), and Oxyresveratrol (3) on NO Production. NO is synthesized from the amino acid arginine by nitric oxide synthase (NOS). Under pathological conditions, NO production is increased by the inducible NOS (iNOS) and, subsequently, brings about cytotoxicity and tissue damage (*23*). **Figure 3** shows the effects of artocarpesin (**1**), norartocarpetin (**2**), and oxyresveratrol (**3**) on nitrite production stimulated with LPS in RAW 264.7 cells. The NO production, measured as nitrite, was increased remarkably up to 49.6 ± 5.3 nmol/mL when 1 μg/mL of LPS was added to RAW 264.7 cells, compared to 0.7 ± 0.0 nmol/mL of the control without LPS. The inhibition of NO production in RAW 264.7 cells occurred in a dose-dependent manner when cells were exposed to artocarpesin (**1**), norartocarpetin (**2**), and oxyres-

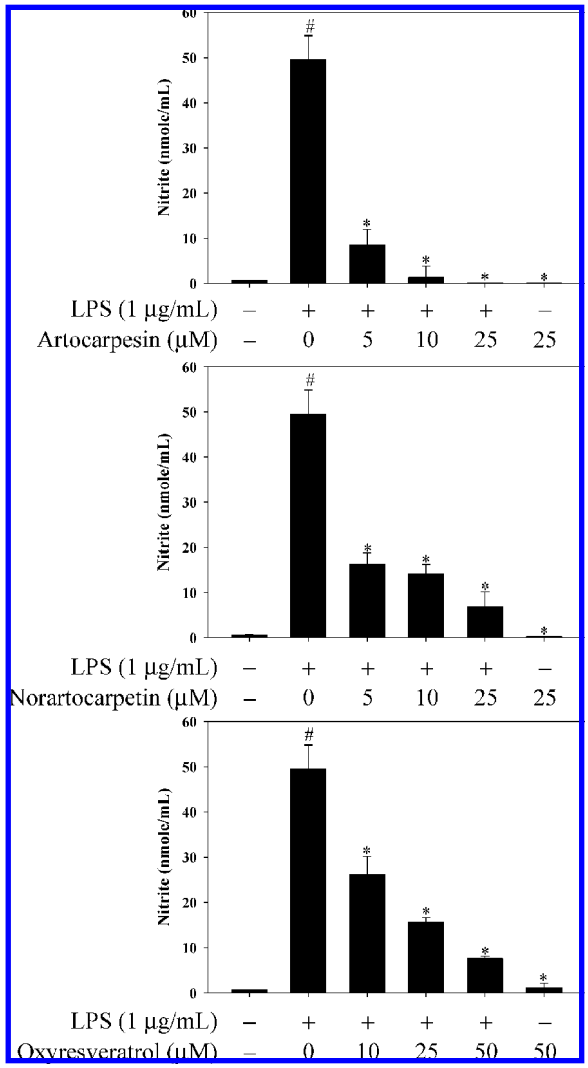

Figure 3. Effects of artocarpesin (**1**), norartocarpetin (**2**), and oxyresveratrol (**3**) on LPS-induced nitrite production in RAW 264.7 cells. The cells were incubated with 0−50 μM of compounds in the absence or presence of LPS (1 μg/mL) for 24 h. (#) $p < 0.05$ indicates significant differences from the control group. (*) $p < 0.05$ indicates significant differences from the LPS treated group.

veratrol (**3**) at the concentrations of 0−50 μM. However, artocarpesin (**1**) had the highest inhibition on NO production among the test compounds (**1**−**3**). Billack (*1*) indicated that macrophages play an important role in the response to inflammation by NO, superoxide anions, and cytokines. These functions of NO are beneficial in maintaining proper physiological homeostasis; however, excess NO acts as a toxic radical and causes many diseases such as mutagenesis, cancer, atherosclerosis, cell apoptosis, and necrosis (*24, 25*). An examination of the cell viability of test compounds in RAW 264.7 cells by the MTT assay indicated that even 50 μM of test compounds did not affect the viability of the RAW 264.7 cells (data not shown). Thus, the inhibitory effects were not attributable to cytotoxic effects. In the present study, artocarpesin (**1**) showed the greatest inhibition of NO production in LPS-stimulated RAW 264.7 cells. Artocarpesin isolated from *Artocarpus incisus*, *Artocarpus elasticus*, and *Cudrania tricuspidata* was reported to have potent tyrosinase inhibitory activity and growth inhibition of cancer cells (*26–28*). However, the literature regarding the anti-inflammatory activities of artocarpesin from the fruits of *A. heterophyllus* remains unclear. Therefore, artocarpesin (**1**) was selected as the sample in the following study.

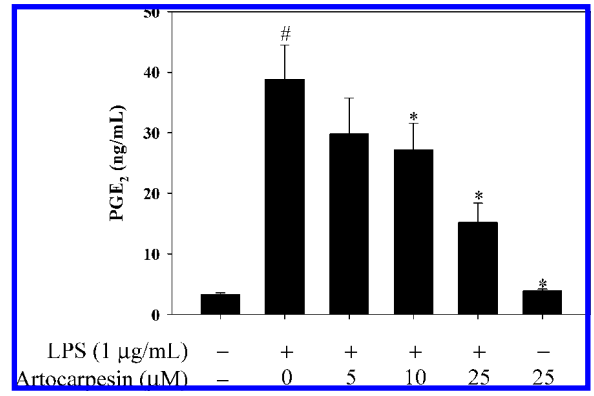

Figure 4. Effect of artocarpesin (**1**) on LPS-induced PGE_2 production in RAW 264.7 cells. The cells were incubated with 0−25 μM of artocarpesin (**1**) in the absence or presence of LPS (1 μg/mL) for 24 h. (#) $p < 0.05$ indicates significant differences from the control group. (*) $p < 0.05$ indicates significant differences from the LPS treated group.

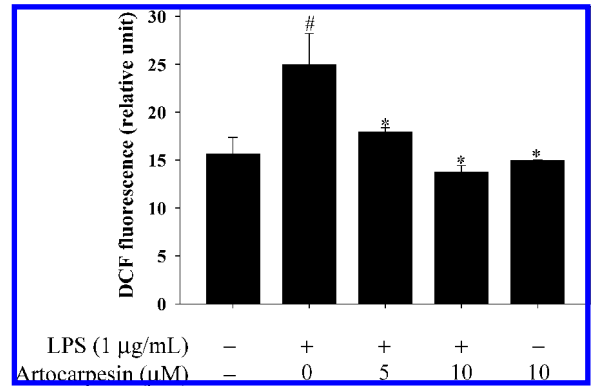

Figure 5. Effect of artocarpesin (**1**) on LPS-induced ROS production in RAW 264.7 cells. The cells were incubated with 0−10 μM of artocarpesin (**1**) in the absence or presence of LPS (1 μg/mL) for 1 h. (#) $p < 0.05$ indicates significant differences from the control group. (*) $p < 0.05$ indicates significant differences from the LPS treated group.

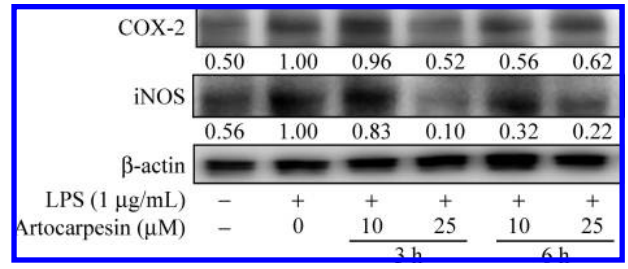

Figure 6. Effect of artocarpesin (**1**) on LPS-induced COX-2 and iNOS protein expression in RAW 264.7 cells. The cells were incubated with 0−25 μM of artocarpesin (**1**) in the absence or presence of LPS (1 μg/mL) for 3 and 6 h.

Effect of Artocarpesin (1) on PGE_2 Production. PGE_2 is produced by macrophages and contributes to physiologic responses such as vasodilation, pain, and fever (*29*). The effect of artocarpesin (**1**) on PGE_2 production stimulated with LPS in RAW 264.7 cells is shown in **Figure 4**. Artocarpesin (**1**) showed marked inhibitory action toward PGE_2 production in the LPS-stimulated RAW 264.7 cells. Harris et al. (*30*) indicated that phenolic compounds (such as luteolin) have been found to inhibit inflammation-associated PGE_2 formation in the LPS-stimulated RAW 264.7 cells. In this study, the phenolic compounds isolated from the fruits of *Artocarpus heterophyllus* such as artocarpesin (**1**) could suppress PGE_2 production in the LPS-stimulated RAW 264.7 cells.

Effect of Artocarpesin (1) on Reactive Oxygen Species (ROS) Production. There is considerable evidence that ROS induce oxidative damage in biomolecules causing atherosclerosis, hypertension, diabetes, and cancer (*31*). In the present study, LPS was used as a model compound for the generation of ROS. **Figure 5** shows the effect of artocarpesin (**1**) on ROS production in RAW 264.7 cells stimulated with LPS. ROS determination was measured by using the fluorescent probe DCFH-DA using a flow cytometer. Treatment of cells with artocarpesin (**1**) at the concentrations of 5 and 10 μM significantly inhibited LPS to induce ROS generation. Our data indicated that the NO and PGE_2 production in LPS-stimulated RAW 264.7 cells were inhibited by the removal of ROS. Ginn-Pease and Whisler (*32*) indicated that *N*-acetyl-L-cystein inhibits inflammatory gene expression and NO production through the removal of ROS.

Effect of Artocarpesin (1) on Protein Expression of COX-2 and iNOS. COX appears to have an important role in the conversion of arachidonic acid to prostaglandin (PGE_2) and is a rate-limiting enzyme in the biosynthesis of prostaglandins (*33*). Posadas et al. (*34*) indicated that the pro-inflammatory mediators such as NO and PGE_2 are generated by COX-2 and iNOS. The effect of artocarpesin (**1**) on COX-2 and iNOS protein expression in RAW 264.7 cells was examined by Western blot analysis. **Figure 6** shows the effect of artocarpesin (**1**) on COX-2 and iNOS protein expression in RAW 264.7 cells. LPS at 1 μg/mL induced a significant increase in COX-2 and iNOS protein expression, as compared to the control without LPS and artocarpesin (**1**). When artocarpesin (**1**) in the range 0−25 μM was added to the medium simultaneously with LPS (1 μg/mL), the results showed that the inhibitory effect of artocarpesin (**1**) on COX-2 and iNOS protein expression was in a dose-dependent manner. These results indicated that the inhibitory effect of artocarpesin (**1**) on COX-2 and iNOS protein expression might be responsible for suppression of NO production when stimulated with LPS. There is considerable evidence suggested that COX-2 is upregulated in several pathological conditions including cancer and autoimmune disease (*35*). These results indicated that artocarpesin (**1**) inhibits PGE_2 production through the suppression of COX-2 and iNOS expression at protein levels in LPS-stimulated RAW 264.7 cells.

In conclusion, artocarpesin (**1**) isolated from the fruits of *Artocarpus heterophyllus* is able to inhibit LPS-induced NO, PGE_2, and ROS production in RAW 264.7 cells. The down regulation of COX-2 and iNOS proteins may be responsible for the inhibition of NO production in LPS-stimulated RAW 264.7 cells. The fruits of *Artocarpus heterophyllus* may provide a beneficial effect for inflammatory-mediated diseases.

ABBREVIATIONS USED

COX-2, cyclooxygenase 2; DMSO, dimethylsulfoxide; IFN-γ, interferon-γ; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; NO, nitric oxide; NOS, nitric oxide synthase; PGE_2, prostaglandin E_2; PVDF, polyvinyldifluoride; TNF, tumor necrosis factor.

LITERATURE CITED

(1) Billack, B. Macrophage activation: role of toll-like receptors, nitric oxide, and nuclear factor kappa B. *Am. J. Pharm. Educ.* **2006**, *70*, 102.

(2) Nathan, C. Nitric oxide as a secretory product of mammalian cells. *FASEB J.* **1992**, *6*, 3051–3064.

(3) Wang, H. Q.; Smart, R. C. Overexpression of protein kinase C-alpha in the epidermis of transgenic mice results in striking alterations in phorbol ester-induced inflammation and COX-2, MIP-2 and TNF-alpha expression but not tumor promotion. *J. Cell Sci.* **1999**, *112*, 3497–3506.

(4) Nakagawa, T.; Yokozawa, T. Direct scavenging of nitric oxide and superoxide by green tea. *Food Chem. Toxicol.* **2002**, *40*, 1745–1750.

(5) Stoclet, J. C.; Muller, B.; Andriantsitohaina, R.; Kleschyov, A. Overproduction of nitric oxide in pathophysiology of blood vessels. *Biochemistry* **1998**, *37*, 826–832.

(6) Salerno, L.; Sorrenti, V.; Di Giacomo, C.; Romeo, G.; Siracusa, M. A. Progress in the development of selective nitric oxide synthase (NOS) inhibitors. *Curr. Pharm. Des.* **2002**, *8*, 177–200.

(7) Komatsu, W.; Ishihara, K.; Murata, M.; Saito, H.; Shinohara, K. Docosahexaenoic acid suppresses nitric oxide production and inducible nitric oxide synthase expression in interferon-γ plus lipopolysaccharide stimulated murine macrophages by inhibiting the oxidative stress. *Free Radical Biol. Med.* **2003**, *34*, 1006–1016.

(8) Griswold, D. E.; Adams, J. L. Constitutive cyclooxygenase (COX-1) and inducible cyclooxygenase (COX-2): rationale for selective inhibition and progress to date. *Med. Res. Rev.* **1996**, *16*, 181–206.

(9) Fernando, M. R.; Wickramasinghe, N.; Thabrew, M. I.; Ariyananda, P. L.; Karunanayake, E. H. Effect of *Artocarpus heterophyllus* and *Asteracanthus longifolia* on glucose tolerance in normal human subjects and in maturity-onset diabetic patients. *J. Ethnopharmacol.* **1991**, *31*, 277–282.

(10) Lin, C. N.; Lu, C. M.; Lin, H. C.; Fang, S. C.; Shieh, B. J.; Hsu, M. F.; Wang, J. P.; Ko, F. N.; Teng, C. M. Novel antiplatelet constituent from Formosan Moraceous plants. *J. Nat. Prod.* **1996**, *59*, 834–838.

(11) Maia, J. G. S.; Andrade, E. H. A.; Zoghbi, M. G. B. Aroma volatiles from two fruit varieties of Jackfruit (*Artocarpus heterophyllus* Lam). *Food Chem.* **2004**, *85*, 195–197.

(12) Ko, F. N.; Cheng, Z. C.; Lin, C. N.; Teng, C. M. Scavenger and antioxidant properties of prenylflavones isolated from *Artocarpus heterophyllus*. *Free Radical Bio. Med.* **1998**, *25*, 160–168.

(13) Hano, Y.; Aida, M.; Nomura, T. Two new natural diels-alder type adducts from the root bark of *Artocarpus heterophyllus*. *J. Nat. Prod.* **1990**, *53*, 391–395.

(14) Kijjoa, A.; Cidade, H. M.; Pinto, M. M. M.; Gonzalez, M. J. T. G.; Anantachoke, C.; Gedris, T. E.; Herz, W. Prenylflavonoids from *Artocarpus elasticus*. *Phytochemistry* **1996**, *43*, 691–694.

(15) Ko, H. H.; Lu, Y. H.; Yang, S. Z.; Won, S. J.; Lin, C. N. Cytotoxic prenylflavonoids from *Artocarpus elasticus*. *J. Nat. Prod.* **2005**, *68*, 1692–1695.

(16) Wei, B. L.; Weng, J. R.; Chiu, P. H.; Hung, C. F.; Wang, J. P.; Lin, C. N. Anti-inflammatory flavonoids from *Artocarpus heterophyllus* and *Artocarpus communis*. *J. Agric. Food Chem.* **2005**, *53*, 3867–3871.

(17) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55–63.

(18) Dirsch, V. M.; Stuppner, H.; Vollmar, A. M. The griess assay: suitable for a bio-guided fractionation of anti-inflammatory plant extracts. *Planta Med.* **1998**, *64*, 423–426.

(19) Mabry, T. J.; Markham, K. R.; Thomas, M. B. *The Systematic Identification of Flavonoids*; Springer-Verlag: New York, 1970.

(20) Monache, G. D.; Scurria, R.; Vitali, A.; Botta, B.; Monacelli, B.; Pasqua, G.; Palocci, C.; Cernia, E. Two isoflavones and a flavone from the fruits of *Maclura pomifera*. *Phytochemistry* **1994**, *37*, 893–898.

(21) Lin, C. N.; Lu, C. M.; Huang, P. L. Flavonoids from *Artocarpus heterophyllus*. *Phytochemistry* **1995**, *39*, 1447–1451.

(22) Tripetch, K.; Kotomi, S.; Ryoji, K.; Kazuo, Y.; Mohamed, S. K.; Mahmoud, H. M. Stilbene and 2-arylbenzofuran glucosides from the rhizomes of *Schoenocaulon officinale*. *Chem. Pharm. Bull.* **2002**, *50*, 863–865.

(23) Kim, H. K.; Cheon, B. S.; Kim, Y. H.; Kim, S. Y.; Kim, H. P. Effects of naturally occurring flavonoids on nitric oxide production in the macrophage cell line RAW 264.7 and their structure-activity relationships. *Biochem. Pharmacol.* **1999**, *58*, 759–765.

(24) Tamir, S.; Tannenbaum, S. R. The role of nitric oxide (NO) in the carcinogenic process. *Biochim. Biophys. Acta* **1996**, *1288*, F31–F36.

(25) Darley-Usmar, V. M.; Hogg, N.; O'Leary, V. J.; Wilson, M. T.; Moncada, S. The simultaneous generation of superoxide and nitric oxide can initiate lipid peroxidation in human low density lipoprotein. *Free Radical Res. Commun.* **1992**, *17*, 9–20.

(26) Shimizu, K.; Kondo, R.; Sakai, K.; Lee, S. H.; Sato, H. The inhibitory components from *Artocarpus incisus* on melanin biosynthesis. *Planta Med.* **1998**, *64*, 408–412.

(27) Cidade, H. M.; Nacimento, M. S.; Pinto, M. M.; Kijjoa, A.; Silva, A. M.; Herz, W. Artelastocarpin and carpelastofuran, two new flavones, and cytotoxicities of prenyl flavonoids from *Artocarpus elasticus* against three cancer cell lines. *Planta Med.* **2001**, *67*, 867–870.

(28) Zou, Y. S.; Hou, A. J.; Zhu, G. F.; Chen, Y. F.; Sun, H. D.; Zhao, Q. S. Cytotoxic isoprenylated xanthones from *Cudrania tricuspidata*. *Bioorg. Med. Chem.* **2004**, *12*, 1947–1953.

(29) Astiz, M.; Saha, D.; Lustbader, D.; Lin, R.; Rackow, E. Monocyte response to bacterial toxins, expression of cell surface receptors, and release of anti-inflammatory cytokines during sepsis. *J. Lab. Clin. Med.* **1996**, *128*, 594–600.

(30) Harris, G. K.; Qian, Y.; Leonard, S. S.; Sbarra, D. C.; Shi, X. Luteolin and chrysin differentially inhibit cyclooxygenase-2 expression and scavenge reactive oxygen species but similarly inhibit prostaglandin-E2 formation in RAW 264.7 cells. *J. Nutr.* **2006**, *136*, 1517–1521.

(31) Pong, K. Oxidative stress in neurodegenerative diseases: therapeutic implications for superoxide dismutase mimetics. *Expert Opin. Biol. Ther.* **2003**, *3*, 127–139.

(32) Ginn-Pease, M. E.; Whisler, R. L. Redox signals and NF-κB activation in T cells. *Free Radical Biol. Med.* **1998**, *25*, 346–361.

(33) Smith, W. L.; Garavito, R. M.; DeWitt, D. L. Prostaglandin endoperoxide H synthases (cyclooxygenase)-1 and -2. *J. Biol. Chem.* **1996**, *271*, 33157–33160.

(34) Posadas, I.; Terencio, M. C.; Guillén, I.; Ferrándiz, M. L.; Coloma, J.; Payá, M.; Alcaraz, M. J. Co-regulation between cyclooxygenase-2 and inducible nitric oxide synthase expression in the time-course of murine inflammation. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2000**, *361*, 98–106.

(35) Chun, K. S.; Surh, Y. J. Signal transduction pathways regulating cyclooxygnase-2 expression: potential molecular targets for chemoprevention. *Biochem. Pharmacol.* **2004**, *68*, 1089–1100.

Received for review February 13, 2008. Revised manuscript received March 30, 2008. Accepted April 8, 2008. This research work was partially supported by the National Science Council, the Republic of China, under grant NSC95-2321-B005-002.

JF800444G