JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Anti-Inflammatory Effects of Resveratrol and Oligostilbenes from *Vitis thunbergii* var. *taiwaniana* against Lipopolysaccharide-Induced Arthritis

Kun-Teng Wang,[†,‡] Lih-Geeng Chen,[§] Sung-Hui Tseng,[∥,⊥] Jung-Shan Huang,[§] Ming-Shium Hsieh,[∥,⊥] and Ching-Chiung Wang*[,†,⊥]

[†]School of Pharmacy, College of Pharmacy, Taipei Medical University, Taipei, Taiwan

[‡]Section of Biologics & Advanced Therapeutic Product Analysis, Division of Research and Analysis, Food and Drug Administration, Department of Health, Executive Yuan, Taiwan

[§]Department of Microbiology, Immunology and Biopharmaceutical Sciences, College of Life Sciences, National Chiayi University, Chiayi, Taiwan

[∥]School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan

[⊥]Orthopedics Research Center, Taipei Medical University Hospital, Taipei, Taiwan

ABSTRACT: *Vitis thunbergii* Sieb. and Zucc. var. *taiwaniana* Lu is an endemic plant in Taiwan used as a dietary supplement for bone health. In this study, human chondrocytes were induced to produce COX-2, MMP-3, -13, and PGE_2 by LPS. An ^{18}F-FDG microPET imaging system was used to evaluate the anti-inflammatory arthritic effects in vivo. Six stilbenes, resveratrol (**1**), (+)-ε-viniferin (**2**), ampelopsin C (**3**), ampelopsin A (**4**), (−)-vitisin B (**5**), and (+)-vitisin A (**6**), were isolated from the stem part of *V. thunbergii*, which displayed the strongest PGE_2 inhibition. Among these compounds, **1** significantly decreased COX-2 activity, PGE_2, MMP-3, and -13 production in vitro, and ^{18}F-FDG uptake and serum PGE_2 in rabbits in vivo. Anti-inflammatory effects were enhanced through the combined usage of **1** and other oligostilbenes. Taken together, the synergistic effects of **1** and oligostilbenes resulted in stem part extracts with lower **1** content displaying the better anti-inflammatory arthritis effects.

KEYWORDS: *Vitis thunbergii* var. *taiwaniana*, ^{18}F-FDG microPET, inflammatory arthritis, oligostilbenes, resveratrol, synergistic effects

INTRODUCTION

Grape (*Vitis* species) is widely distributed across the world. Grape leaves and seeds have been commonly used in traditional Chinese medicine, and fruits are employed as dietary supplements. Recent studies have reported that *Vitis* species possess several pharmacological and clinical properties, including anti-inflammatory, anti-oxidative, anti-diabetic, and anti-carcinogenic properties.[1,2] In *Vitis* species, *Vitis thunbergii* Sieb. and Zucc. var. *taiwaniana* Lu (Vitaceae) is a wild grape native to Taiwan and widely used as folk medicine for treating hepatitis, jaundice, stomachache and so on. Aboriginal people of Taiwan used this plant to strengthen the body and to treat arthritis.[3]

In terms of chemical components, several oligostilbenes have been isolated from *Vitis thunbergii* var. *taiwaniana* and most were resveratrol derivatives. Resveratrol is a well-known anti-oxidative, anti-tumor, and anti-inflammatory component.[4,5] However, resveratrol derivatives in *Vitis* species also have several important pharmacological activities. For example, miyabenol A, viniferin, and vitisin A, the common resveratrol derivatives isolated from *Vitis* species, have been reported to have anti-inflammatory, anti-diabetogenic, and anti-allergic properties.[6] However, pharmacological effects of the combined usage of resveratrol and oligostilbenes in treating inflammatory arthritis have not been previously documented or discussed.

Nowadays, because of increasing life span, inflammatory arthritis is the most common disease in elderly people. Therapeutic strategies for treating arthritis are still analgesia and disease modification, but these have not been sufficiently effective. Etiologically, pro-inflammatory mediators played critical roles in the pathogenesis of inflammatory arthritis. Over-expressions of pro-inflammatory mediators caused the imbalance of synthesis and degradation of joint matrix components.[7] Prostaglandin E_2 (PGE_2) is a well known inflammatory mediator that is converted from arachidonic acid by cyclooxygenase-2 (COX-2). PGE_2 regulates nitric oxide production, cytokine release, and extracellular matrix (ECM) enzymes activities.[8] Overproduction of PGE_2 causes clinical symptoms in arthritis patients, including inflammation, joint pain, and cartilage damage. Matrix metalloproteinases (MMPs) are important mediators in the pathogenesis of inflammatory arthritis. MMPs consist of a zinc-dependent endopeptidase family and are important aggressive factors in damaging the ECM in articular cartilage. Articular cartilage can be degraded by MMPs, especially MMP-3 and MMP-13.[9] A recent study has reported that inflammatory arthritis levels are correlated with serum MMP-3 levels. Besides ECM degradation, MMP-3 also plays an important role as a proMMP activator, causing damage to articular cartilage.[10]

Received: December 8, 2010
Accepted: February 20, 2011
Revised: February 16, 2011
Published: March 10, 2011

dx.doi.org/10.1021/jf104718g | *J. Agric. Food Chem.* 2011, 59, 3649–3656

In the past decade, people have been seeking to discover a single element that would act as a "magic bullet" for treating malignant tumors, chronic hepatitis B infection, gout, etc.,[11] but therapeutic efficacy remains suboptimal. At the same time, approximately 30% of patients have looked for dietary supplements that contain more than one active component for treating their diseases. U.S. sales of dietary supplements are almost $20 billion annually.[12] *Harpagophytum procumbens* and *Zingiber officinale* are famous dietary supplements for arthritis patients. *Harpagophytum procumbens*, also called devil's claw, is an anti-inflammatory plants because of its selective COX-2 inhibitory effects that reduces symptoms of arthritis patients.[13] *Curcuma longa* and *Zingiber officinale* are well-known for their COX-2 inhibitory effects, blocking PGE_2 and leukotriene production, leading to relief of symptoms.[14] These finding suggest that plant extracts are good sources for developing dietary supplements for arthritis.

In this study, different parts of *V. thunbergii* var. *taiwaniana* were evaluated for anti-inflammatory effects in a LPS-induced HC model, and the chemical components were isolated. The anti-inflammatory arthritis effects of isolated-components were evaluated by in vitro assay, and then the most active components were observed by an in vivo ^{18}F-FDG microPET imaging system. In addition, we explored potential synergistic anti-inflammatory effects of components of *V. thunbergii* var. *taiwaniana* and discussed whether stem part extracts of *V. thunbergii* var. *taiwaniana* could be used as a dietary supplement for treating inflammatory arthritis.

MATERIALS AND METHODS

Chemicals. Dimethyl sulfoxide (DMSO), lipopolysaccharide (LPS), 3-(4.5-dimethylthiazol-2-yl) 2.5-diphenyltetrazolium bromide (MTT), arachidonic acid, and NS-398 (*N*-(2-cyclohexyloxy-4-nitrophenyl) methanesulfonamide) were purchased from Sigma Industries (St. Louis, MO, USA). Pronase and collagenase Type IV were purchased from Roche and Sigma, respectively. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), antibiotics, glutamine, and other cell culture materials were purchased from GIBCO BRL (Grand Island, NY, USA). Western blotting antibodies against COX-2 (clone C-20) anti-GAPDH (clone 6C5) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Acetonitrile and trifluoroacetic acid (TFA) used for HPLC analysis were of chromatographic grade and were purchased from J.T. Baker (Phillipsburg, NJ, USA). Sephadex LH-20 was purchased from Amersham Pharmacia Biotech AB (Uppsala, Sweden). LiChroprep RP-18 (40−63 μm) was purchased from Merck (Darmstadt, Germany).

Preparation of the *V. thunbergii* Extracts. Different parts of *V. thunbergii* (stem, branch, leaf, and root) were purchased from a herb store in Hualian, Taiwan, in December 2006 and identified by Chi-Luan Wen (Assistant Research Fellow in Taiwan Seed Improvement and Propagation Station). The voucher specimens (VT-001 to VT-004) were deposited in Department of Microbiology, Immunology and Biopharmaceutical Sciences, College of Life Sciences, National Chiayi University, Chiayi, Taiwan. Four parts of *V. thunbergii* were individually refluxed with methanol for 1 h, evaporated under vacuum using a rotatory evaporator, and freeze-dried to obtain the methanolic extract.

Human Chondrocytes Culture. Knee cartilage was obtained from patients undergoing total joint replacement surgery, and human chondrocytes (HC) were kindly obtained from Dr. Ming-Shium Hsieh's laboratory.[15] Informed consent was obtained in accordance with the guidelines set forth by the Taipei Medical University Hospital Institutional Review Board. Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 with DMEM medium and confirmed by Safranin O solutions (data not shown). Experiments were performed with third to fifth passage cells.

Isolation of Oligostilbenes from Stem and Root of *V. thunbergii* var. *taiwaniana*. The dried stems and roots of *V. thunbergii* var. *taiwaniana* were extracted with methanol under reflux for 1 h. The filtrate was concentrated in a rotary evaporator at 45 °C and freeze-dried to obtain the methanolic extract of stem and root.

A portion of the methanolic extract of the stem (16.85 g) dissolved in methanol was chromatographed through a Sephadex LH-20 column (2.5 cm i.d. × 51 cm) eluted with methanol (100 mL/fraction) to give fractions A_1 to X_1. Fraction F_1 (1.16 g) and fraction G_1 (835 mg) were rechromatographed through a LiChroprep RP-18 column (2.5 cm i.d. × 57 cm) eluted with 0.05% TFA−CH_3CN (75:25 and 70:30) to obtain resveratrol (**1**, 11 mg) and (+)-ε-viniferin (**2**, 352 mg), respectively. Fraction H_1 (717 mg) was rechromatographed through a LiChroprep RP-18 column (2.5 cm i.d. × 57 cm) eluted with 0.05% TFA−CH_3CN (70: 30) to obtain ampelopsin C (**3**, 110 mg).

A portion of the methanolic extract of the root (10 g) dissolved in methanol was chromatographed through a Sephadex LH-20 column (2.5 cm i.d. × 51 cm) eluted with methanol (100 mL/fraction) to give fractions A_2 to X_2. Fraction E_2−F_2 (1338 mg) was rechromatographed through a LiChroprep RP-18 column (2.5 cm i.d. × 52 cm) eluted with 0.05% TFA−CH_3CN (81:19) to obtain ampelopsin A (**4**, 110 mg). Fraction I_2 (413 mg) was purified with an ODS-80TS preparative HPLC column (20 mm i.d. × 300 mm, 10 μm, Tosoh, Tokyo, Japan) with 0.05% TFA−CH_3CN (65:35) at a flow rate of 10 mL/min and detected at 280 nm to obtain (−)-vitisin B (**5**, 28 mg). Fraction J_2−R_2 (2162 mg) was rechromatographed through a LiChroprep RP-18 column (2.5 cm i.d. × 52 cm) eluted with 0.05% TFA−CH_3CN (70:30) to obtain (+)-vitisin A (**6**, 208 mg). Structural determinations of these six oligostilbenes were estimated by ^{1}H- and ^{13}C NMR, including 2D-NMR techniques, and also by comparison of those data with authentic compounds in published papers.[3] The purities of resveratrol (**1**), (+)-ε-viniferin (**2**), ampelopsin C (**3**), ampelopsin A (**4**), (−)-vitisin B (**5**), and (+)-vitisin A (**6**) were determined by HPLC and were 98.4%, 95.6%, 94.8%, 96.2%, 91.5%, and 97.0%, respectively.

Resveratrol (**1**) ESI-MS *m*/*z*: 227 $[M - H]^-$. UV λ_{max} nm (log ε): 319 (4.55). ^{1}H NMR (500 MHz, acetone-d_6) δ (ppm): 7.40 (2H, d, J = 8.6 Hz, H-2, H-6), 7.00 (1H, d, J = 16.3 Hz, H-8), 6.87 (1H, d, J = 16.3 Hz, H-7), 6.83 (2H, d, J = 8.6 Hz, H-3, H-5), 6.53 (2H, d, J = 1.9 Hz, H-10, H-14), 6.26 (1H, t, J = 1.9 Hz, H-12). ^{13}C NMR (125 MHz, acetone-d_6) δ (ppm): 159.5 (C-11, C-13), 158.1 (C-4), 140.8 (C-9), 129.9 (C-1), 129.1 (C-8), 128.7 (C-2, C-6), 126.8 (C-7), 116.3 (C-3, C-5), 105.5 (C-10, C-14), 102.5 (C-12).

(+)-ε-Viniferin (**2**) ESI-MS *m*/*z*: 453 $[M - H]^-$. UV λ_{max} nm (log ε): 325 (4.47). ^{1}H NMR (500 MHz, acetone-d_6) δ (ppm): 7.19 (2H, d, J = 8.4 Hz, H-2′, H-6′), 7.16 (2H, d, J = 8.5 Hz, H-2, H-6), 6.90 (1H, d, J = 16.5 Hz, H-8), 6.83 (2H, d, J = 8.4 Hz, H-3′, H-5′), 6.72 (1H, brs, H-12), 6.73 (2H, d, J = 8.5 Hz, H-3, H-5), 6.70 (1H, d, J = 16.5 Hz, H-7), 6.32 (1H, brs, H-14), 6.23 (3H, brs, H-10′, H-12′, H-14′), 5.41 (1H, d, J = 5.4, H-7′), 4.47 (1H, d, J = 5.4, H-8′). ^{13}C NMR (125 MHz, acetone-d_6) δ (ppm): 162.4 (C-11), 159.8 (C-11′, C13′), 159.50 (C-4′), 159.45 (C-4), 158.1 (C-13), 147.4 (C-9′), 136.4 (C-9), 133.8 (C-1′), 130.1 (C-8), 129.8 (C-1), 128.7 (C-2, C-6), 127.9 (C-2′, C-6′), 123.4 (C-7), 119.8 (C-10), 116.2 (C-3, C-5), 116.0 (C-3′, C-5′), 106.9 (C-10′, C-14′), 104.1 (C-14), 101.9 (C-12′), 96.7 (C-12), 93.9 (C-7′), 57.1 (C-8′).

Ampelopsin C (**3**) ESI-MS *m*/*z*: 679 $[M - H]^-$. UV λ_{max} nm (log ε): 283 (4.14). ^{1}H NMR (500 MHz, acetone-d_6) δ (ppm): 7.25 (2H, *d*, J = 8.5 Hz, H-2b, H-6b), 7.17 (2H, *d*, J = 8.5 Hz, H-2a, H-6a), 7.00 (2H, *d*, J = 8.5 Hz, H-2c, H-6c), 6.79 (2H, *d*, J = 8.5 Hz, H-3b, H-5b), 6.71 (2H, *d*, J = 8.5 Hz, H-3c, H-5c), 6.67 (2H, *d*, J = 8.6 Hz, H-3a, H-5a), 6.33 (1H, *d*, J = 2.0 Hz, H-12b), 6.17 (2H, *d*, J=2.0 Hz, H-10c, H-14c), 6.16 (1H, *t*, J = 2.0 Hz, H-12c), 6.15 (1H, *d*, J = 2.0 Hz, H-14b), 6.14 (1H, *s*, H-12a),5.81 (1H, *d*, J = 11.7 Hz, H-7b), 5.25 (1H, *d*, J = 3.3 Hz, H-7a),

4.45 (1H, *d*, *J* = 11.7 Hz, H-8b), 4.23 (1H, *d*, *J* = 9.5 Hz, H-7c), 3.73 (1H, *dd*, *J* = 11.7, 9.5 Hz, H-8c), 3.63 (1H, *d*, *J* = 11.7 Hz, H-8a). ^{13}C NMR (125 MHz, acetone-d_6) δ (ppm): 159.5 (C-11c), 159.2 (C-13c), 158.5 (C-4b), 156.7 (C-11b, C-13b), 155.8 (C-4a), 155.7 (C-4c), 154.6 (C-11a, C-13a), 146.8 (C-9c), 144.1 (C-9a), 141.7 (C-9b), 133.3 (C-1a), 132.8 (C-1c), 130.8 (C-1b), 130.5 (C-2a, C-6a), 130.2 (C-2b, C-6b), 130.0 (C-2c, C-6c), 124.7 (C-10b),121.0 (C-14a), 116.0 (C-3b, C-5b, C-10a), 115.8 (C-3c, C-5c), 115.4 (C-3a, C-5a), 107.3 (C-10c, C-14c), 105.9 (C-14b), 101.6 (C-12c), 101.5 (C-12b), 96.6 (C-12a), 90.6 (C-7b), 62.1 (C-8c), 57.4 (C-7c), 52.5 (C-8a), 48.8 (C-8b), 37.5 (C-7a).

Ampelopsin A (**4**) $[a]_D^{25} = -216.7°$ (*c* 0.09, MeOH). ESI-MS *m*/*z*: 469 $[M - H]^-$. ^{1}H NMR (500 MHz, acetone-d_6) δ (ppm): 7.09 (2H, d, *J* = 8.6 Hz, H-2a, 6a), 6.88 (2H, d, *J* = 8.2 Hz, H-2b, 6b), 6.76 (2H, d, *J* = 8.4 Hz, H-3a, 5a), 6.62 (2H, d, *J* = 8.6 Hz, H-3b, 5b), 6.60 (1H, d, *J* = 2.2 Hz, H-14b), 6.41 (1H, d, *J* = 2.2 Hz, H-12a), 6.21 (1H, brs, H-14a), 6.14 (1H, d, *J* = 2.2 Hz, H-12b), 5.74 (1H, d, *J* = 11.4 Hz, H-7a), 5.43 (1H, d, *J* = 4.8 Hz, H-7b), 5.39 (1H, d, *J* = 4.8 Hz, H-8b), 4.14 (1H, d, *J* = 11.4 Hz, H-8a). ^{13}C NMR (125 MHz, acetone-d_6) δ ppm): 160.2 (C-13a), 158.9 (C-11b), 158.8 (C-13b), 158.4 (C-4b), 157.2 (C-4a), 156.0 (C-11a), 143.1 (C-9a), 140.4 (C-9b), 132.7 (C-1a), 130.9 (C1b), 129.9 (C-2a, C-6a), 128.73 (C-2b, C-6b), 118.9 (C-10a), 118.3 (C-10b), 115.9 (C-3a, C-5a), 115.4 (C-3b, C-5b), 110.4 (C-14b), 105.4 (C-14a), 101.4 (C-12a), 97.0 (C-12b), 88.4 (C-7a), 71.1 (C-8b), 49.6 (C-8a), 43.9 (C-7b).

(−)-Vitisin B (**5**) $[a]_D^{25} = -171.04°$ (*c* 0.6, MeOH). ESI-MS *m*/*z*: 905 $[M - H]^-$. UV λ_{max} nm (log ε): 288 (4.32), 323 (4.50). ^{1}H NMR (500 MHz, acetone-d_6) δ (ppm): 7.26 (2H, d, *J* = 8.6 Hz, H-2a, H-6a), 7.20 (2H, d, *J* = 8.5 Hz, H-2d, H-6d), 7.14 (1H, d, *J* = 8.3 Hz, H-6b), 6.90 (2H, d, *J* = 8.6 Hz, H-3a, H-5a), 6.83 (2H, d, *J* = 8.6 Hz, H-3d, H-5d), 6.76 (1H, d, *J* = 16.8 Hz, H-8b), 6.70−6.62 (5H, m, H-5b, H-2b, H-14b, H-2c, H-6c), 6.60 (1H, d, *J* = 16.8 Hz, H-7b), 6.58 (2H, d, *J* = 8.6 Hz, H-3c, H-5c), 6.31 (2H, brs, H-12c), 6.24−6.20 (5H, m, H-12b, H-10d, H-14d, H-12d, H-14c), 6.19 (1H, d, *J* = 2.1 Hz, H-12a), 6.12 (2H, d, *J* = 2.2 Hz, H-10a, H-14a), 5.53 (1H, d, *J* = 4.7 Hz, H-7c), 5.41 (2H, m, H-7d, H-7a), 4.53 (1H, d, *J* = 4.5 Hz, H-8a), 4.46 (1H, d, *J* = 5.4 Hz, H-8d), 4.32 (1H, d, *J* = 4.7 Hz, H-8c). ^{13}C NMR (125 MHz, acetone-d_6) δ (ppm): 162.4 (C-11b), 162.3 (C-11c), 160.2 (C-13c), 160.0 (C-4b), 159.8 (C-11a, C-13a), 159.4 (C-11d, C-13d), 158.1 (C-13b), 157.9 (C-4d), 157.7 (C-4a), 147.3 (C-4c), 142.1 (C-9a), 136.3 (C-9d), 134.3 (C-9c), 134.2 (C-9b), 133.8 (C-1a), 132.5(C-1d), 132.3 (C-1b, C-1c), 131.7 (C-3b), 130.3 (C-8b), 127.9 (C-2c, C-6c), 127.8 (C-2d, C-6d), 127.5 (C-2a, C-6a), 126.4 (C-6b), 125.6 (C-2b), 123.9 (C-7b), 119.8 (C-10b), 119.7 (C-10c), 116.3 (C-3a, C-5a), 116.1 (C-3d, C-5d), 115.8 (C-3c, C-5c), 110.5 (C-5b), 107.0 (C-10d, C-14d), 106.9 (C-14c), 106.8 (C-10a, C-14a), 104.4 (C-14b), 102.4 (C-12a), 102.1 (C-12d), 96.8 (C-12b), 96.4 (C-12c), 94.0 (C-7d), 93.8 (C-7a), 91.1 (C-7c), 57.0 (C-8d), 56.8 (C-8a), 52.1 (C-8c).

(+)-Vitisin A (**6**) $[a]_D^{25} = +363.5°$ (*c* 0.8, MeOH). ESI-MS *m*/*z*: 905 $[M - H]^-$. UV λ_{max} nm (log ε): 286 (4.37), 331 (4.50). ^{1}H NMR (500 MHz, acetone-d_6) δ (ppm): 7.19 (2H, d, *J* = 8.5 Hz, H-2a, 6a), 7.14 (2H, d, *J* = 8.5 Hz, H-2c, H-6c), 7.02 (2H, d, *J* = 8.3 Hz, H-2d, H-6d), 6.87 (1H, dd, *J* = 8.4, 2.2 Hz, H-6b), 6.81 (2H, d, *J* = 8.5 Hz, H-3a, 5a), 6.77 (2H, d, *J* = 8.5 Hz, H-3c, H-5c), 6.68 (1H, d, *J* = 8.4 Hz, H-5b), 6.65 (2H, d, *J* = 8.3 Hz, H-3d, H-5d), 6.50 (1H, d, *J* = 2.2 Hz, H-14b), 6.38 (2H, brs, H-7b, H-8b), 6.25 (1H, d, *J* = 2.2 Hz, H-12b), 6.23 (1H, d, *J* = 2.2 Hz, H-14c), 6.21 (1H, d, *J* = 2.2 Hz, H-12a), 6.16 (2H, d, *J* = 2.2 Hz, 10a, 14a), 6.08 (1H, d, *J* = 1.8 Hz, H-2b), 6.07 (1H, d, *J* = 2.2 Hz, H-12d), 6.04 (1H, d, *J* = 1.8 Hz, H-14d), 6.01 (1H, d, *J* = 2.2 Hz, H-12c), 5.87 (1H, d, *J* = 11.6 Hz, H-7c), 5.47 (1H, d, *J* = 3.3 Hz, H-8d), 5.38 (1H, d, J=3.3 Hz, H-7d), 5.35 (1H, d, J=5.3 Hz, H-7a), 4.40 (1H, d, *J* = 5.3 Hz, H-8a), 4.23 (1H, d, *J* = 11.6 Hz, H-8c). ^{13}C NMR (125 MHz, acetone-d_6) δ (ppm): 162.5 (C-11b), 160.3 (C-13d), 159.7 (C-11a, C-13a), 159.4 (C-13b), 158.7 (C-11d), 158.4 (C-4c), 158.1 (C-4a), 157.8 (C-11c), 156.9 (C-13c), 155.9 (C-4d), 155.1 (C-4b), 147.2 (C-9a), 142.3 (C-9c), 141.2 (C-9d), 136.5 (C-9b), 135.3 (C-1d), 133.9 (C-1a), 132.7 (C-3b), 132.5 (C-2b), 131.1 (C-1c), 131.0 (C-8b),130.1 (C-2c, C-6c), 128.9 (C-1b), 128.8 (C-2d, C-6d), 127.9 (C-2a, C-6a), 123.6 (C-6b), 122.6 (C-7b), 120.3 (C-10c), 120.0 (C-10d), 119.1 (C-10b), 116.0 (C-3a, C-5a), 115.9 (C-3c, C-5c), 115.4 (C-3d, C-5d, C-5b), 110.0 (C-14d), 106.8 (C-10a, C-14a), 104.9 (C-14c), 104.3 (C-14b), 102.0 (C-12a), 100.7 (C12c), 96.5 (C-12b), 96.0 (C-12d), 93.8 (C-7a), 88.4 (C-7c), 57.1 (C-8a), 49.4 (C-8c), 41.3 (C-8d), 40.7 (C-7d).

HPLC analysis of stem parts of *V. thunbergii* var. *taiwaniana* was as follows: reversed-phase LiChrospher 100 RP-18e column (4 mm × 250 mm, 5 μm, Merck); column temperature, 40 °C; mobile phase, 0.05% TFA-acetonitrile (0 min, 95:5; 50 min, 55:45; 60 min, 55:45; 61 min, 95:5; 70 min, 95:5) (v/v); flow rate, 1.0 mL/min, monitored at 280 nm. Ten microliter portions were injected into the column. The retention times of **1** to **6** were 30.66 min, 39.37 min, 36.34 min, 24.66 min, 46.84 min, and 41.56 min, respectively.

Cytotoxicity, PGE_2, and MMPs Inhibitory Assays. HC (1×10^5 cells/mL) were seeded on a 96-well plate for 1 day. We co-treated the test samples and LPS (1 μg/mL) or IL-1β (5 ng/mL) for 18 h. In the PGE_2 synergistically inhibitory assay, the experimental design was modified from Murakami's study.[16,17] The synergistic effect was defined as the experiment data (%) > calculation data (%), where experiment data (%) = inhibitory effect of compounds A in combination with B, and calculation data (%) = inhibitory effect of compound A plus B. HC were cotreated with LPS (1 μg/mL), **1** (10 μM), and other oligostilbenes (25 μM) for 18 h to evaluate the PGE_2 inhibitory effects. HC cell viability was examined by the MTT assay, and the cell culture supernatant was collected. The PGE_2, MMP-3 and -13 concentrations were detected by commercial assay kits (Assay Designs, Michigan, USA).

COX-2 Protein Expression Assay. Equal amounts of protein were prepared and separated on 10% nonreducing SDS−polyacrylamide gels. Gels were applied to immobilon-P PVDF membranes (Millipore, Bedford, MA, USA), and non-specific binding was blocked with 1% bovine serum albumin at 37 °C for 1 h. Blots were incubated overnight at 4 °C with anti-COX-2 and anti-α-GAPDH antibodies. Membranes were washed with PBST and incubated for 1 h at 37 °C with alkaline phosphatase-conjugated secondary antibodies. Protein expression was visualized by staining with the 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium substrate solution (BCIP/NBT).

COX-2 Activity Assay. The assay procedure was modified from that in previous studies.[18] HC was pre-treated with LPS (1 μg/mL) for 18 h in a 10-cm dish. After overnight incubation, HC was washed with PBS and seeded on a 24-well plate, with 1×10^5 cells/mL. We co-treated the test samples and arachidonic acid (100 μM) for 30 min. The cell culture supernatant was collected for PGE_2 detection.

Animals. New Zealand White rabbits (1.5−2.5 kg) were used in the in vivo experiment. They were maintained at 21 ± 2 °C with food and water ad libitum and kept on a 12-h light/12-h dark cycle. All rabbits used in this experiment were cared for according to the Ethical Regulations on Animal Research of Taipei Medical University (Approval No.: LAC-97-0005).

Table 1. Cytotoxicity and PGE_2 Inhibitory Effects of Different Parts of *V. thunbergii*

		PGE_2 inhibition[a] (%)	
parts	cell viability[a] (%)	LPS-induced	IL-1β-induced
stem	97.06 ± 2.71	71.29 ± 7.08	22.50 ± 6.20
branch	98.23 ± 1.60	67.86 ± 4.19	4.90 ± 4.10
leaf	80.66 ± 1.84	54.25 ± 4.25	1.02 ± 1.77
root	73.57 ± 1.95	24.06 ± 9.85	3.50 ± 5.53
indomethacin[b]	98.59 ± 1.35	93.47 ± 0.61	94.14 ± 1.11

[a] Concentrations of all test samples were 100 μg/mL. [b] Concentration of indomethacin was 10 μM.

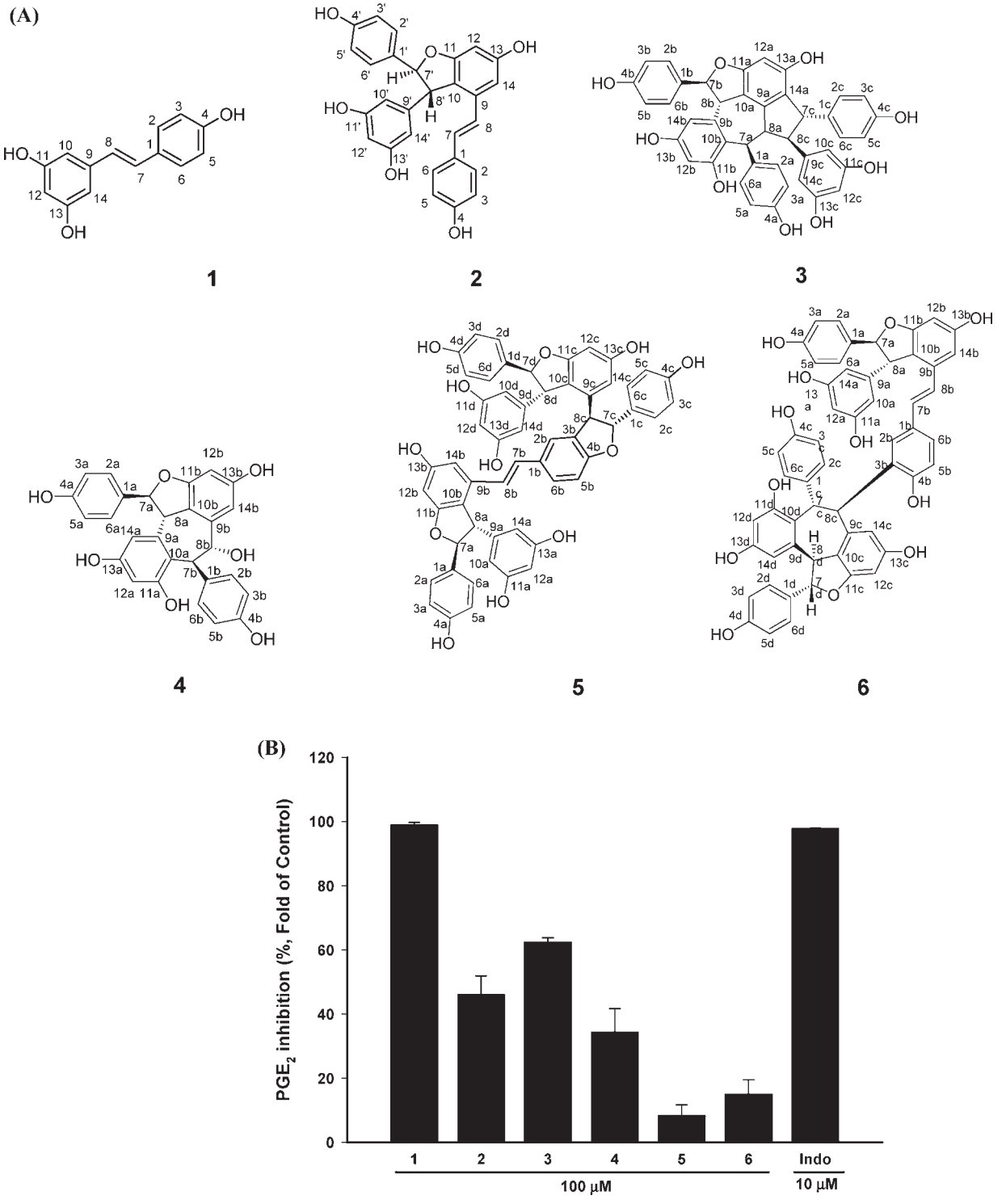

Figure 1. Chemical structure (A) and inhibitory percentage of 6 oligostilbenes isolated from the stem part of *V. thunbergii* in LPS-induced HC PGE_2 expression (B). The 6 oligostilbenes were resveratrol (**1**), (+)-ε-viniferin (**2**), ampelopsin C (**3**), ampelopsin A (**4**), (−)-vitisin B (**5**), and (+)-vitisin A (**6**). The significance between the blank and control group was statistically calculated by the one-way ANOVA test.

Acute Inflammatory Arthritis Induced by LPS. LPS (10 ng in 100 μL of phosphate buffered saline (PBS)) was used to induce acute arthritis in rabbits. Experimental procedures were established in our previous study.[19] On day 0, LPS was injected into the rabbits' right knee joint. The experimental group received oral administration of **1** (10 mg/kg) on day 0, 2, 4, and 6. The control group received the vehicle. Serum was collected from the ear veins on day 0, 2, 4, and 6. ^{18}F-FDG PET was performed on day 1 (16 h after LPS injection) and day 7. Anti-arthritis activity of **1** was assessed with 2-^{18}F-fluoro-2-deoxy-D-glucose positron emission tomography (^{18}F-FDG PET) and simultaneous assessment of serum PGE_2 concentrations

^{18}F-FDG PET Examination. A Concorde microPET R4 scanner (Concorde Microsystems, Knoxville, TN, USA) was used in the ^{18}F-FDG-PET analysis. Rabbits received an averaged 38.0 MBq of FDG by

intravenous bolus injection into one of the ear veins. A 1200-s emission PET scan was done after FDG administration for 2 h. We reconstructed the PET images through Fourier rebinning and ordered-subset expectation maximization (OSEM) using default corrections for radioactive decay, dead time, and attenuation provided by the vendor. To evaluate the arthritis level, standard uptake value (*SUV*), the most popular quantitation parameter of PET, was used and defined as follows:

$$SUV = \frac{ROI(\mu Ci/mL)}{dose(\mu Ci)/[BW(g)/\delta(g/mL)]}$$

where *ROI* indicates the region of interest (maximum radioactivity concentration in the *ROI* is usually used). Dose indicates the total dose injected. *BW* and δ are the rabbit's body weight and average body density, respectively. Generally, δ was set to 1.[20] We used ASIPro VM analysis software to draw the *ROIs* and to determine the *SUVs*.

Statistical Analysis. Data are presented as the mean and SD. Data were analyzed with the Student's *t*-test and the one-way ANOVA test using SPSS software.

RESULTS

PGE_2 Inhibitory Effects of Different Parts of *V. thunbergii* var. *taiwaniana*. Different parts of *V. thunbergii* (stem, branch, leaf, and root) were extracted with methanol. The cytotoxicity and PGE_2 concentrations were measured in a LPS and IL-1β-induced HC model. As Table 1 showed, the stem part displayed the better PGE_2 inhibitory effects than the other parts in both LPS and IL-1β-induced HC without any cytotoxicity. Thus, the active chondroprotective components were isolated from the stem part of *V. thunbergii*.

PGE_2 Inhibitory Effects of the 6 Oligostilbenes in LPS-Induced HC. Six oligostilbenes isolated from the stem part of *V. thunbergii* did not show any cytotoxicity in HC at 100 μM (data not shown). Among the 6 oligostilbenes, **1** and **3** showed better PGE_2 inhibitory effects than the others (Figure 1C), while the IC_{50} values were 73.32 $\pm$ 1.74 and 15.52 $\pm$ 2.69 μM, respectively.

Inhibitory Effects of 1 on COX-2 Enzyme Activity. The COX-2 protein expression and enzyme activity of **1** were evaluated in LPS-induced HC. Results showed that **1** did not inhibit the protein expression of COX-2 in LPS-induced HC (Figure 2A), but significantly inhibited COX-2 activity in a dose-dependent manner, while the IC_{50} value was 27.01 $\pm$ 1.11 μM (Figure 2B). NS-398, the COX-2-selective inhibitor, was used as a positive control and displayed strong inhibitory effects on COX-2 enzyme activity.

Inhibitory Effects of 1 on MMP-3 and MMP-13 Expressions. MMP-3 and MMP-13 played an important role in the degradation and turnover of articular cartilage. First, the MMP-3 and -13 levels in HC could be activated by LPS-induction. As shown in Figure 2B, **1** (25−100 μM) inhibited MMP-3 expressions in a dose-dependent down-regulating manner in LPS-induced HC and showed a more than 40% inhibitory rate at 100 μM. Moreover, **1** also significantly inhibited the MMP-13 expressions in a dose-dependent manner, while the IC_{50} value was 38.42 $\pm$ 0.46 μM.

Inhibitory Effects of 1 on LPS-Induced Rabbit Acute Inflammatory Arthritis. In the microPET imaging system, the brighter areas in transverse and coronal views of the knee joint indicate the accumulation of ^{18}F-FDG, showing the more severe inflammatory arthritis status. As shown in Figure 3A, the micro-PET image of the knee joint of the sham group displayed a weak signal, suggesting non-inflammatory status. After injecting the LPS into the right joint, the brighter areas displayed on the microPET image indicated the acute inflammatory arthritis of the control group. Administration of **1** (10 mg/kg) resulted in displaying a less bright area than the control group in the transverse and coronal views of the knee joint. The level of

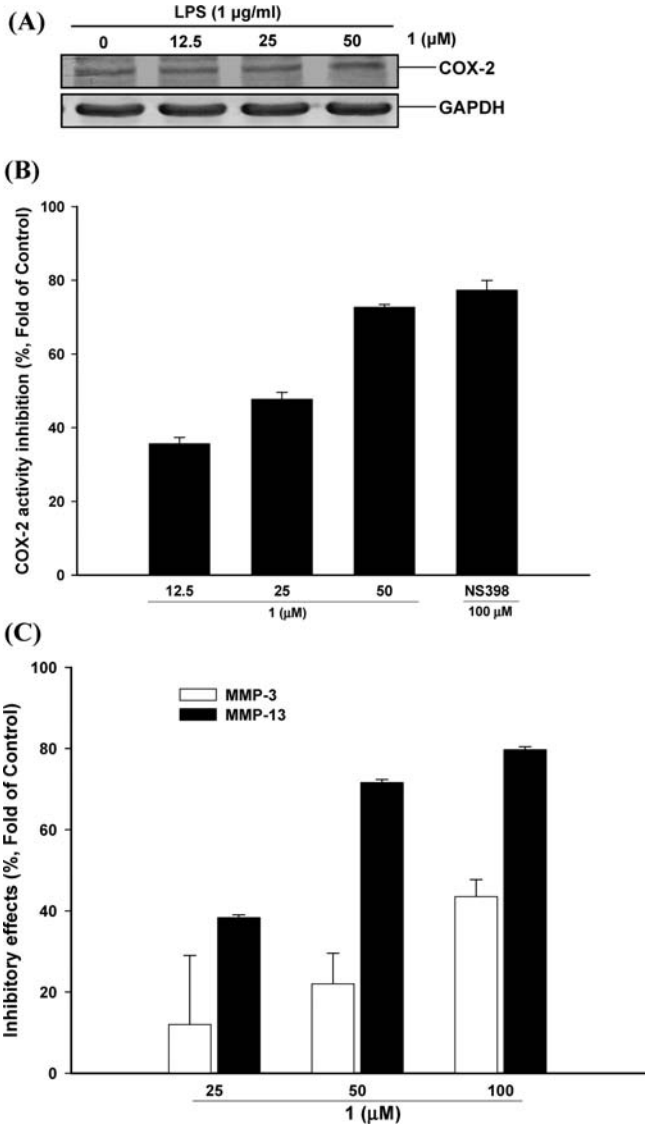

Figure 2. Inhibitory effects of **1** on COX-2 protein expression (A), enzyme activity (B), and MMP-3 and MMP-13 expression (C). The significance between the blank and control group was statistically calculated by the one-way ANOVA test.

Table 2. Analysis of 1 on LPS-Induced Rabbit Knee Arthritis in *SUV*

	variations of the maximal *SUV*	
group	day 1	day 7
control	0.59 $\pm$ 0.07	2.11 $\pm$ 0.18[a]
1	1.11 $\pm$ 0.57	1.68 $\pm$ 0.03[a]

[a] Day 7 of control compared with Day 1, $p < 0.005$. [b] Day 7 of **1** compared with Day 7 of control, $p < 0.05$.

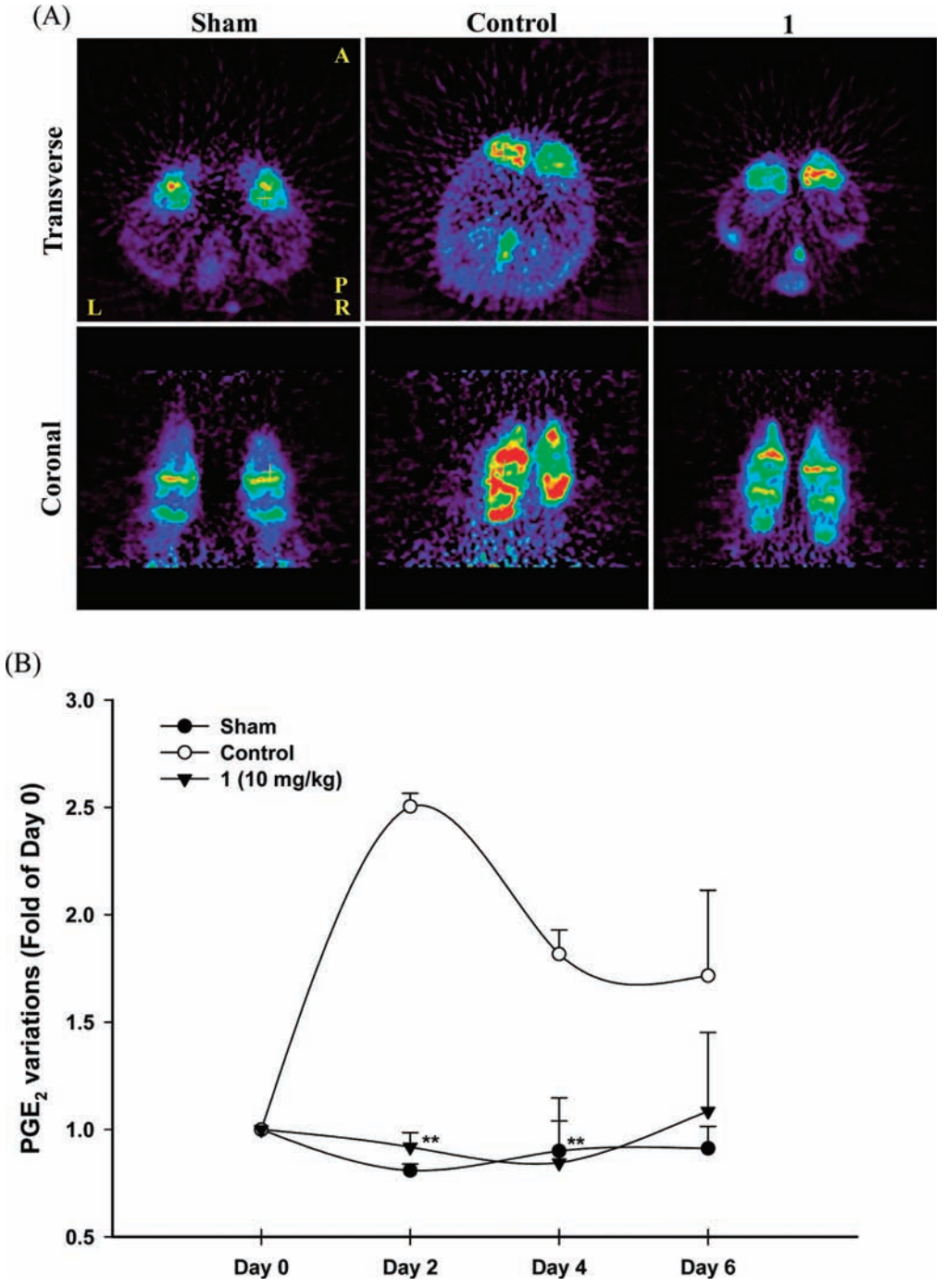

Figure 3. Transverse and coronal orthogonal views of a typical PET image volume of a pair of rabbit knees on day 7 (A) and inhibitory effects of **1** on serum PGE_2 of LPS-induced rabbits (B). Note that the anterior (A, head)—posterior (P, tail) and right (R)—left (L) labels are all reversed with respect to the actual imaging orientations. Sham, no LPS induction; control, injection of LPS in right knee; treatment, oral administration of **1** (10 mg/kg) before LPS injection. **: $p < 0.005$, compared with the control group.

inflammatory arthritis was performed by *SUV*, the most popular quantitation parameter of microPET. The *SUV* of the control group on day 7 was significantly higher than that on day 1. After treating with **1**, *SUV* was significantly lower than that of the control group, meaning the potential anti-inflammatory arthritis ability (Table 2). In addition, the serum PGE_2 levels were also evaluated. The control group displayed the higher serum PGE_2 levels than the sham group on days 2, 4, and 6. The **1**-treated group displayed a down-regulating trend of serum PGE_2, while it showed significantly inhibitory effects on days 2 and 6 (Figure 3B).

Synergistic Effects of 1 and Other Oligostilbenes on PGE_2 Inhibition. First, the PGE_2 inhibitory effects of lower dosages of **1** (10 μM) and other oligostilbenes (25 μM) were about 47% and 7 to 11%, respectively. The synergistically anti-inflammatory arthritic effects of **1** combined with other oligostilbenes were significantly better than **1** or oligostilbenes alone (Table 3). Moreover, the synergistic effect of **5** was stronger than the others.

Table 3. Synergistic PGE_2 Inhibitory Effects of Oligostilbenes in LPS-Induced HC[a]

samples	inhibition (%, calculation data[b])	inhibition (%, experiment data[c])
1		47.10 ± 5.24
2		11.11 ± 9.72
3		2.57 ± 4.40
4		7.93 ± 6.64
5		11.48 ± 3.20
6		0.00 ± 1.04
1 + 2	57.21 ± 14.9	93.16 ± 4.98[d]
1 + 3	49.67 ± 9.64	72.98 ± 4.61[d]
1 + 4	55.03 ± 11.9	88.88 ± 0.50[d]
1 + 5	58.58 ± 8.44	95.77 ± 0.57[d]
1 + 6	47.10 ± 6.26	62.49 ± 7.40[d]

[a] Concentration of **1** was 10 μM. Concentrations of other oligostilbenes (**2**−**6**) were 25 μM. [b] PGE_2 inhibitory percentage of the summarization of **1** and other oligostilbenes. [c] PGE_2 inhibitory percentage of co-treatments of **1** and other oligostilbenes. [d] $p < 0.005$, experiment data compared with the calculation data.

We suggest that the anti-inflammatory arthritis effects can be enhanced through combined usage.

DISCUSSION

The prevalence of overall arthritis has grown since the past decade because of the world's aging population. In 2003, about 46.4 million people in the U.S. had suffered arthritis, and that number will exceed 67 million by 2030.[21] The trend in managements of inflammatory arthritis is to reduce pain and physical limitations. However, side effects are a big problem when we use modern medicines to treat inflammatory arthritis.[22] Increasingly, dietary supplements are widely used to treat inflammatory arthritis. According to the 2007 National Health Interview Survey (NHIS) in the U.S., the use of natural products (17.7%) is an important part of alternative medicine. Specifically, the frequency of adults using alternative medicine to treat arthritis, including joint pain or stiffness, reached 8.7%, indicating that treating arthritis with natural products is popular in the U.S.[23]

Vitis species are rich in phytophenol and have been used for centuries as therapeutic agents for treating inflammatory diseases, with its NO and PGE_2 inhibitory properties.[4,24] Phytophenols are classified as single-ring phenolic acids, bisphenols, including stilbenes, tricyclic phenols, and subclasses. In this study, six polyphenol-type oligostilbenes were isolated from the stem part of *Vitis thunbergii* var. *taiwaniana*, including **1** (resveratrol monomer), **2** and **4** (resveratrol dimer), **3** (resveratrol trimer), and **5** and **6** (resveratrol tetramer). Of the six oligostilbenes, **1**, the oligostilbene monomer, displayed the strongest anti-inflammatory arthritis properties the in vitro and in vivo models. In the pathogenesis of arthritis, PGE_2 and MMP-3 are involved in the formation of intra-articular inflammation and cartilage damage, leading to inflammatory arthritis.[25] Among the 6 isolated oligostilbenes, only **1** displayed significant MMP-3 and -13 inhibitory effects and COX-2 activity inhibition, resulting in the down-regulation of PGE_2 expression, which ameliorated joint degradation resulting from inflammation (Figure 2).

At present, extensive research on the structure activity relationship (SAR) of natural oligostilbenes derivatives has been reported. Evidence has shown that no regular rules were observed between the cytotoxicity and molecular structure of resveratrol derivatives.[26] Moreover, in Ha's study, the relationship of different resveratrol derivatives on anti-oxidative abilities was discussed. Results were the same as our previous finding that resveratrol displayed the strongest bioactivity,[27] while oligostilbenes with complex structures showed weaker bioactivity than stilbenes. In this study, stem part extracts of *V. thunbergii* displayed the strongest PGE_2 inhibitory effects (Table 1). However, in Yang's study, amounts of **1** in the leaf epidermis and grape skins ranged from 571.0 to 38.0 μg/100 g fresh samples and even less in other parts.[28] However, **1** and **3** were the minor and **2**, **4**, and **5** were the major components in the stem part of *V. thunbergii* (data not shown), which displayed the strongest PGE_2 inhibitory effects among the four parts (Table 1). As for the PGE_2 inhibitory effects, single treatment with the lower dosage of **1** (10 μM) displayed approximately 50% PGE_2 inhibition, but in higher doses of other oligostilbenes (25 μM), the inhibitory effects were not prominent. Once we co-treated with **1** and other oligostilbenes, the PGE_2 inhibitory effects were stronger than the single treatment. Moreover, the major component, **5**, showed the strongest enhancing effects in the six oligostilbenes from *V. thunbergii* (Table 3). We suggested that the synergistic inhibitory mechanisms of **1** could relate to the anti-oxidation of the co-treated compound. According to the results of our other project, the anti-oxidative effects of **5** was strongest in the six oligostilbenes by the $ABTS^{\cdot+}$ radical scavenging assay. The anti-oxidative compound, **5**, could more prevent the oxidation of **1**. Therefore, the synergistic effect of **5** was stronger than the others. This is the first report that indicates that **1** in combination with other oligostilbenes could synergistically inhibit inflammatory arthritis.

Resveratrol (**1**) is a well-known natural anti-tumor product, and toxicity is an important side effect. In Cottart's study, the administration of **1** (over 5 g/70 kg) as a single dose, approximately 1/40 and 1/200, would cause nephrotoxicity.[29] In the LPS-induced rabbit acute inflammatory arthritis model, rabbits were only administrated with **1** at 10 mg/kg, which resulted in displaying inhibition effects. The effective dose (about 0.7 g/70 kg) of **1** was 7 times less than the approximate toxicity dosage (5 g/70 kg). Combined usage of a low dose **1** and other oligostilbenes would increase its anti-inflammatory efficacy, while the toxicity induced by **1** could be avoided. The results indicate that the extract of *V. thunbergii* (mixture components) has value as a medical food and can be used to develop nutritional supplements.

In conclusion, we found that **1** was a potential anti-inflammatory arthritis component in the stem part of *V. thunbergii* through both in vitro and in vivo models. The possible mechanism was through decreasing PGE_2, MMP-3, and MMP-13 expressions. Moreover, it was the first time that other oligostilbenes have been shown to possess synergistic anti-inflammatory arthritis effects when taken with **1**. Besides the well-known grape seed, we suggest that the stem part of *V. thunbergii* is also a fine source for developing anti-inflammatory arthritis dietary supplements.

AUTHOR INFORMATION

Corresponding Author

*School of Pharmacy, College of Pharmacy, Taipei Medical University, 250 Wu-Hsing Street, Taipei 110, Taiwan. Tel: 886-2-27361661 ext. 6161. Fax: 886-2-27329368. E-mail: crystal@tmu.edu.tw.

■ ACKNOWLEDGMENT

We thank the Council of Agriculture, Executive Yuan, Taiwan (Grant no. 96AS-1.2.1-ST-a1-21 and 97AS-1.2.1-ST-a1-27) for financially supporting this study. The microPET technology was supported by the Molecular and Genetic Imaging Core/National Research Program for Genomic Medicine at National Yang-Ming University.

■ ABBREVIATIONS USED

COX-2, cyclooxygenase-2; ECM, extracellular matrix; ^{18}F-FDG PET, 2-^{18}F-fluoro-2-deoxy-D-glucose positron emission tomography; HC, human chondrocytes; HPLC, high-performance liquid chromatography; LPS, lipopolysaccharide; MMPs, matrix metalloproteinases; PBS, phosphate buffered saline; PGE_2, prostaglandin E_2; *SUV*, standard uptake value.

■ REFERENCES

(1) Cheng, M.; Gao, H. Q.; Xu, L.; Li, B. Y.; Zhang, H.; Li, X. H. Cardioprotective effects of grape seed proanthocyanidins extracts in streptozocin induced diabetic rats. *J. Cardiovasc. Pharmacol.* **2007**, *50* (5), 503–509.

(2) Caillet, S.; Salmieri, S.; Lacroix, M. Evaluation of free radical-scavenging properties of commercial grape phenol extracts by a fast colorimetric method. *Food Chem.* **2006**, *95* (1), 1–8.

(3) Chen, L. G.; Wang, C. C. Preparative separation of oligostilbenes from *Vitis thunbergii* var. *taiwaniana* by centrifugal partition chromatography followed by Sephadex LH-20 column chromatography. *Sep. Purif. Technol.* **2009**, *66*, 65–70.

(4) Quilez, A. M.; Saenz, M. T.; Garcia, M. D.; de la Puerta, R. Phytochemical analysis and anti-allergic study of *Agave intermixta* Trel. and *Cissus sicyoides* L. *J. Pharm. Pharmacol.* **2004**, *56* (9), 1185–1189.

(5) Kim, S.; Min, S. Y.; Lee, S. K.; Cho, W. J. Comparative molecular field analysis study of stilbene derivatives active against A549 lung carcinoma. *Chem. Pharm. Bull. (Tokyo)* **2003**, *51* (5), 516–521.

(6) Matsuda, H.; Asao, Y.; Nakamura, S.; Hamao, M.; Sugimoto, S.; Hongo, M.; Pongpiriyadacha, Y.; Yoshikawa, M. Antidiabetogenic constituents from the Thai traditional medicine *Cotylelobium melanoxylon*. *Chem. Pharm. Bull. (Tokyo)* **2009**, *57* (5), 487–494.

(7) Brama, P. A.; TeKoppele, J. M.; Beekman, B.; van El, B.; Barneveld, A.; van Weeren, P. R. Influence of development and joint pathology on stromelysin enzyme activity in equine synovial fluid. *Ann. Rheum. Dis* **2000**, *59* (2), 155–157.

(8) Kunisch, E.; Jansen, A.; Kojima, F.; Loffler, I.; Kapoor, M.; Kawai, S.; Rubio, I.; Crofford, L. J.; Kinne, R. W. Prostaglandin E_2 differentially modulates proinflammatory/prodestructive effects of TNF-alpha on synovial fibroblasts *via* specific E prostanoid receptors/cAMP. *J. Immunol.* **2009**, *183* (2), 1328–1336.

(9) Lohmander, L. S.; Roos, H.; Dahlberg, L.; Hoerrner, L. A.; Lark, M. W. Temporal patterns of stromelysin-1, tissue inhibitor, and proteoglycan fragments in human knee joint fluid after injury to the cruciate ligament or meniscus. *J. Orthop. Res.* **1994**, *12* (1), 21–28.

(10) Nagase, H.; Visse, R.; Murphy, G. Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc. Res.* **2006**, *69* (3), 562–573.

(11) Lau, G. K. K. A new magic bullet for chronic hepatitis B infection: Is this the end of the story? *Gastroenterology* **2009**, *136* (5), 1830–1832.

(12) Gregory, P. J.; Sperry, M.; Wilson, A. F. Dietary supplements for osteoarthritis. *Am. Fam. Physician* **2008**, *77* (2), 177–184.

(13) Wegener, T.; Lupke, N. P. Treatment of patients with arthrosis of hip or knee with an aqueous extract of devil's claw (*Harpagophytum procumbens* DC.). *Phytother. Res.* **2003**, *17* (10), 1165–1172.

(14) Deodhar, S. D.; Sethi, R.; Srimal, R. C. Preliminary study on antirheumatic activity of curcumin (diferuloyl methane). *Indian J. Med. Res.* **1980**, *71*, 632–634.

(15) Hsieh, M. S.; Ho, H. C.; Chou, D. T.; Pan, S.; Liang, Y. C.; Hsieh, T. Y.; Lan, J. L.; Tsai, S. H. Expression of matrix metalloproteinase-9 (gelatinase B) in gouty arthritis and stimulation of MMP-9 by urate crystals in macrophages. *J. Cell. Biochem.* **2003**, *89* (4), 791–799.

(16) Murakami, A.; Takahashi, D.; Koshimizu., K.; Ohigashi, H. Synergistic suppression of superoxide and nitric oxide generation from inflammatory cells by combined food factors. *Mutat. Res.* **2003**, *523*−*524*, 151–161.

(17) McCormick, D. L.; Wilson, A. M. Combination chemoprevention of rat mammary carcinogenesis by indomethacin and butylated hydroxytoluene. *Cancer Res.* **1986**, *46* (8), 3907–3911.

(18) Caughey, G. E.; Cleland, L. G.; Penglis, P. S.; Gamble, J. R.; James, M. J. Roles of cyclooxygenase (COX)-1 and COX-2 in prostanoid production by human endothelial cells: selective up-regulation of prostacyclin synthesis by COX-2. *J. Immunol.* **2001**, *167* (5), 2831–2838.

(19) Hsieh, M. S.; Wang, K. T.; Tseng, S. H.; Lee, C. J.; Chen, C. H.; Wang, C. C. Using ^{18}F-FDG microPET imaging to measure the inhibitory effects of *Clematis chinensis* Osbeck on the pro-inflammatory and degradative mediators associated with inflammatory arthritis. *J. Ethnopharmacol.* **2010**10.1016/j.jep.2010.06.042.

(20) Keyes, J. W., Jr. SUV: standard uptake or silly useless value? *J. Nucl. Med.* **1995**, *36* (10), 1836–1839.

(21) Hootman, J. M.; Helmick, C. G. Projections of US prevalence of arthritis and associated activity limitations. *Arthritis. Rheum.* **2006**, *54* (1), 226–229.

(22) Callahan, L. F.; Wiley-Exley, E. K.; Mielenz, T. J.; Brady, T. J.; Xiao, C.; Currey, S. S.; Sleath, B. L.; Sloane, P. D.; DeVellis, R. F.; Sniezek, J. Use of complementary and alternative medicine among patients with arthritis. *Prev. Chronic Dis.* **2009**, *6* (2), A44.

(23) Barnes, P. M.; Bloom, B.; Nahin, R. L. Complementary and alternative medicine use among adults and children: United States, *2007*. *Natl. Health. Stat. Rep.* **2008**, *12*, 1–23.

(24) Sakurai, T.; Kitadate, K.; Nishioka, H.; Fujii, H.; Kizaki, T.; Kondoh, Y.; Izawa, T.; Ishida, H.; Radak, Z.; Ohno, H. Oligomerized grape seed polyphenols attenuate inflammatory changes due to anti-oxidative properties in coculture of adipocytes and macrophages. *J. Nutr. Biochem.* **2010**, *21* (1), 47–54.

(25) Nerusu, K. C.; Warner, R. L.; Bhagavathula, N.; McClintock, S. D.; Johnson, K. J.; Varani, J. Matrix metalloproteinase-3 (stromelysin-1) in acute inflammatory tissue injury. *Exp. Mol. Pathol.* **2007**, *83* (2), 169–176.

(26) Muhtadi; Hakim, E. H.; Juliawaty, L. D.; Syah, Y. M.; Achmad, S. A.; Latip, J.; Ghisalberti, E. L. Cytotoxic resveratrol oligomers from the tree bark of *Dipterocarpus hasseltii*. *Fitoterapia* **2006**, *77* (7−8), 550–555.

(27) Ha do, T.; Kim, H.; Thuong, P. T.; Ngoc, T. M.; Lee, I.; Hung, N. D.; Bae, K. Antioxidant and lipoxygenase inhibitory activity of oligostilbenes from the leaf and stem of *Vitis amurensis*. *J. Ethnopharmacol.* **2009**, *125* (2), 304–309.

(28) Yang, J.; Martinson, T. E.; Liu, R. H. Phytochemical profiles and antioxidant activities of wine grapes. *Food Chem.* **2009**, *116* (1), 332–339.

(29) Cottart, C. H.; Nivet-Antoine, V.; Laguillier-Morizot, C.; Beaudeux, J. L. Resveratrol bioavailability and toxicity in humans. *Mol. Nutr. Food Res.* **2010**, *54* (1), 7–16.