JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Sclareol Exhibits Anti-inflammatory Activity in Both Lipopolysaccharide-Stimulated Macrophages and the λ-Carrageenan-Induced Paw Edema Model

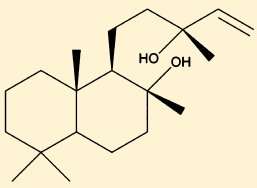
Guan-Jhong Huang,[†] Chun-Hsu Pan,[‡] and Chieh-Hsi Wu*[,‡]

[†]Institute of Chinese Pharmaceutical Sciences, China Medical University, Taichung 40402, Taiwan, Republic of China

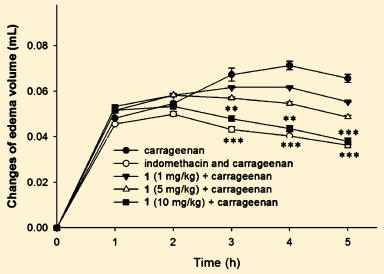
[‡]School of Pharmacy, China Medical University, Taichung 40402, Taiwan, Republic of China

Supporting Information

ABSTRACT: Sclareol (**1**) is a natural fragrance compound used widely in the cosmetic and food industries. Lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages and the λ-carrageenan-induced edema mouse paw model were applied to examine the anti-inflammatory potential of **1** and its possible molecular mechanisms. The experimental results obtained demonstrated that this compound inhibited cell growth, nitric oxide (NO) production, and the expression of the inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) proteins in LPS-stimulated macrophages. Compound **1** also reduced paw edema, the tissue content of NO, tumor necrosis factor-alpha (TNF-α), malondialdehyde (MDA), iNOS and COX-2 protein expression, and neutrophil infiltration within the tissues after λ-carrageenan stimulation. The present study suggests that the anti-inflammatory mechanisms of **1** might be related to a decrease of inflammatory cytokines and an increase of antioxidant enzyme activity.



1



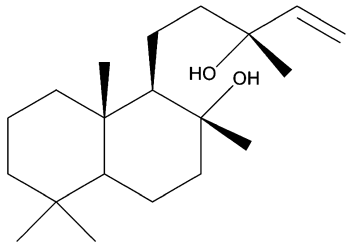
The acute inflammatory response is a series of local cellular and vascular responses that occur immediately following tissue damage, and this complex biological response is a protective mechanism by organisms to remove injurious stimuli, such as pathogens or irritants, or physical injury from the tissues and to initiate the healing process. However, chronic inflammation has been reported to involve the development of several disease conditions or disorders such as Alzheimer's disease,[1] asthma,[2] atherosclerosis,[3] autoimmune diseases,[4] cancer,[5] and rheumatoid arthritis,[6] which may lead to progressive destruction of the tissues, fibrosis, and necrosis.[7,8]

Numerous molecules have been reported to contribute to local tissue destruction during chronic inflammation.[9−11] Of these, inducible nitric oxide synthase (iNOS), a member of the NOS protein family, catalyzes the formation of nitric oxide (NO) from L-arginine.[12] NO can activate guanylate cyclase to induce smooth muscle relaxation under normal physiological conditions. High-output NO produced by activated macrophages via iNOS has been found to play a major role as an antimicrobial molecule.[13] However, high levels of NO have the opportunity to react with superoxide, resulting in peroxynitrite formation and cell toxicity, which have been found to play important roles in inflammation and carcinogenesis. The expression of COX-2 (cyclooxygenase 2) has also been reported to implicate prostaglandin biosynthesis in inflammation and pain, and selective inhibitors of COX-2 have been demonstrated clinically to provide effective anti-inflammatory action, with a marked reduction in gastrointestinal toxicity as compared to traditional NSAIDs (nonsteroidal anti-inflammatory drugs).[14] Similarly, TNF-α (tumor necrosis factor-alpha), an endotoxin-induced glycoprotein, is a critical modulator of the host immune response to infection, but its inappropriate or excessive production can be harmful. Use of an anti-TNF-α antibody and oral administration of soluble TNF receptors have been demonstrated to control the inflammatory condition.[11]

Compound **1** (labd-14-ene-8,13-diol) is a member of the labdane-type diterpenes and was isolated initially from the plant *Salvia sclarea* L. (Lamiaceae), of which the natural fragrance ingredients are applied widely in both the cosmetic and food industries. Several studies have demonstrated that **1** is a biologically active molecule with cytotoxic or cytostatic effects against human cancer cell lines.[15−18] Noori et al. noted that **1** modulates the immune response through affecting the cytokine pattern in the splenocytes of intratumorally injected mice.[19] Although this report suggested that **1** might have potential activity in the regulation of the inflammatory response, the possible mechanisms involved have remained unclear. The present study was carried out to examine whether **1** has potential effects against the inflammatory response in lipopolysaccharide (LPS)-stimulated macrophages and the λ-carrageenan-induced edema paw model and to clarify its possible molecular mechanisms, to help to further evaluate the clinical therapeutic potential of **1** in the treatment of inflammation.

Received: June 17, 2011
Published: January 17, 2012

dx.doi.org/10.1021/np200512a | J. Nat. Prod. 2012, 75, 54−59



1

RESULTS AND DISCUSSION

Effect of Sclareol (1) on Cell Viability of Macrophages. The growth regulation of **1** on macrophage cell viability was examined using an MTT assay (Figure S1, Supporting Information). The cells were pretreated with **1** at various concentrations (0, 1, 5, and 10 μg/mL) for 1 h and then co-incubated with 100 ng/mL of LPS for a further 24 h. The results indicated that 100 ng/mL of LPS did not change the cell viability of macrophages. Similarly, the cell viability of macrophages was not influenced even at the highest concentration (10 μg/mL) of **1** in the presence of LPS. However, several studies have reported that **1** can reduce markedly the cell viability of human leukemia cell lines (IC_{50} values from 6.0 to 24.2 μg/mL),[15,18] inhibit human breast cancer cells (IC_{50} values from 7.0 to 11.6 μg/mL),[17,18] and suppress human colon cancer cells (HCT116) (IC_{50} value 10.6 μg/mL).[18] In normal cells such as peripheral blood mononuclear cells (PBMC), **1** exhibited growth inhibition with an IC_{50} value of 10.7 μg/mL.[18]

Inhibitory Effect of Sclareol (1) on LPS-Stimulated NO, iNOS, and COX-2. Various concentrations (0, 1, 5, and 10 μg/mL) of **1** were used on macrophages to test whether this compound can reverse LPS-induced accumulation of NO (Figure 1).

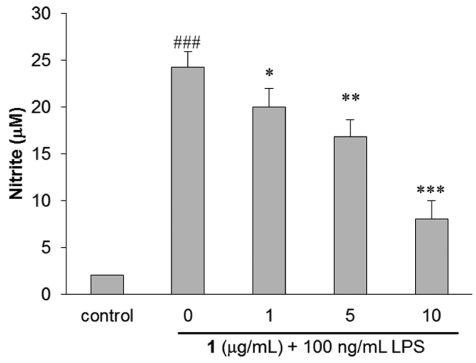


Figure 1. Sclareol (**1**) reduces LPS-induced NO production in macrophages. ###p < 0.001 compared with the control group (treated with normal saline only). *p < 0.05, **p < 0.01, and ***p < 0.001 compared to the group treated with LPS alone.

The results revealed that 100 ng/mL LPS can increase NO production as compared with a control group (p < 0.001), and this effect can be suppressed markedly in a dose-dependent manner by pretreatment of **1** (1, 5, and 10 μg/mL) as compared to those in a LPS-treated group only. Additionally, **1** (0, 1, 5, and 10 μg/mL) was also tested on macrophages to examine whether it can reduce protein expression of inflammation-associated molecules triggered by LPS (Figure 2). The results obtained suggested that 100 ng/mL of LPS can stimulate protein expression of iNOS and COX-2 significantly (p < 0.001), and pretreatment of **1** at concentrations of 5 and 10 μg/mL can

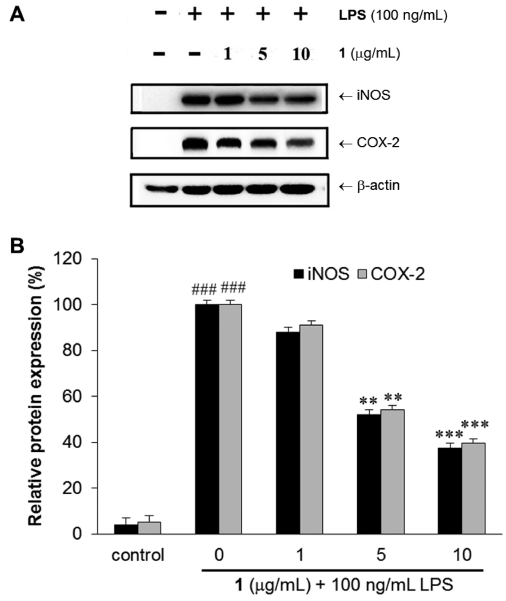


Figure 2. Sclareol (**1**) inhibits LPS-stimulated expression of inflammation-associated proteins in macrophages. (A) Western blot showing expression of iNOS and COX-2 proteins. The bar chart indicates the relative protein expression of iNOS and COX-2 in macrophages after treatment (B). ###p < 0.001 compared with the control group (treated with normal saline only). **p < 0.01 and ***p < 0.001 compared with the group treated by LPS alone.

down-regulate the expression of these LPS-stimulated proteins as compared to LPS alone-treated group (p < 0.01 and p < 0.001). It has been reported that **1** can increase the expression of interferon-gamma (INF-γ) and decrease interleukin-4 (IL-4) in the splenocytes isolated from intratumorally injected mice.[19] Chan and Riches demonstrated that co-incubation of LPS and INF-γ can induce both TNF-α and IL-1β production, which up-regulates protein expression of iNOS via activation of the ERK and JNK MAPK pathways in RAW 264.7γNO(−) cells, which do not produce NO with IFN-γ stimulation alone.[20] Roach et al. also reported that co-treatment of LPS and INF-γ will increase both transcriptional and translational levels of iNOS in the macrophages.[21] Moreover, the up-regulatory effect of INF-γ on the expression of iNOS has also been identified in aortic smooth muscle cells, epithelial cells, and the saphenous vein endothelium.[22−24] In human foreskin fibroblasts, IFN-γ has been reported to suppress COX-2 promoter activity.[25] On the other hand, IL-4 has been shown to down-regulate IFN-γ-inducible iNOS transcription in the RAW264.7 murine macrophage cell line.[26] Cui et al. also noted that IL-4 has the capacity to inhibit COX-2 mRNA transcription in human non-small-cell lung cancer and follicular dendritic cells.[27,28] In contrast to the regulation of IFN-γ, Guo et al. reported that IL-4 plays a critical role in mRNA stabilization of iNOS in primary human airway epithelial cells stimulated by IFN-γ.[29] To date, it is still unknown as to whether **1** can modulate the expression of INF-γ and IL-4 in the macrophages. In the present study, the experimental data obtained suggested that **1** can decrease markedly LPS-stimulated iNOS expression and then reduce NO production in the macrophages.

Inhibitory Effect of Sclareol (1) on λ-Carrageenan-Induced Mouse Paw Edema. The carrageenan-induced mouse paw edema model was used to evaluate the in vivo anti-inflammatory effect of **1** (Figure 3). The results showed that

dx.doi.org/10.1021/np200512a | J. Nat. Prod. 2012, 75, 54−59

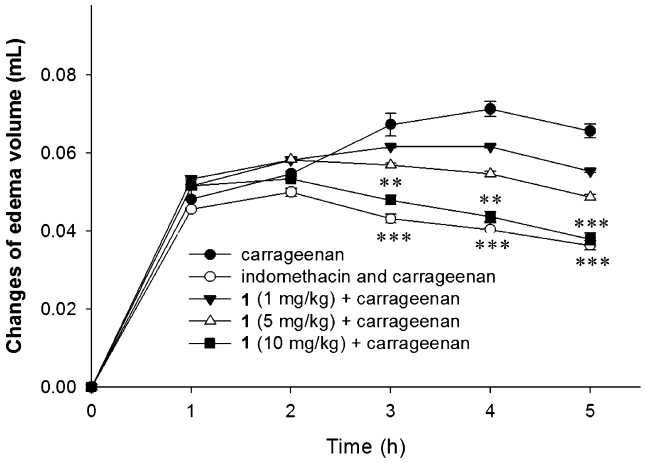


Figure 3. Sclareol (**1**) reduces mice hind paw edema induced by λ-carrageenan (1%). $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with the carrageenan-treated group. A dose of 10 mg/kg was used of indomethacin.

injection of carrageenan will stimulate local inflammation and then induce edema of the paw tissues. Indomethacin, a common clinical NSAID, was used as a positive control to indicate that pretreatment of 10 mg/kg of indomethacin can reduce paw edema after a 3 h stimulation by carrageenan ($p < 0.001$). Pretreatment of 10 mg/kg of **1** attenuated markedly paw edema after 3 h of carrageenan stimulation, in a similar manner to the positive control group ($p < 0.01$).

Inhibitory Effect of Sclareol (1) on NO, TNF-α, and MDA in a Carrageenan-Induced Edema Paw Model. The NO levels increased significantly in the edema serum 5 h after injection with carrageenan ($p < 0.001$), which could be reversed by **1** at concentrations greater than 1 mg/kg ($p < 0.01$) (Figure 4A). Likewise, both the TNF-α and MDA levels increased significantly in the edema paw 5 h after carrageenan injection ($p < 0.001$), and this effect was decreased significantly by treatment with **1** as well as 10 mg/kg of indomethacin (Figure 4B and C). The carrageenan test is highly sensitive to nonsteroidal anti-inflammatory drugs and has long been accepted as a useful phlogistic tool for investigating drug therapies.[30] In the present study, statistical analysis revealed that 10 mg/kg of indomethacin and 10 mg/kg of **1** inhibited significantly the development of edema 3 h after treatment ($p < 0.001$ or $p < 0.01$) (Figure 3). The L-arginine–NO pathway has been proposed to play an important role in the carrageenan-induced inflammatory response[31] and the expression of the inducible isoform of NO synthase as an important mediator of inflammation.[32] The results obtained confirmed that the carrageenan-induced paw edema model led to the production of NO, in which the level of NO decreased significantly by treatment with 1, 5, and 10 mg/kg of **1** (Figure 4A). These results suggest that the anti-inflammatory mechanism of **1** may be through the L-arginine–NO pathway, because this diterpenoid inhibited significantly NO production. TNF-α is also a mediator of carrageenan-induced inflammatory incapacitation and is able to induce the further release of kinins and leukotrienes, which is suggested to have an important role in the maintenance of a long-lasting nociceptive response.[33] In the present study, the results showed that **1** decreased the levels of serum TNF-α after carrageenan injection by treatment with 5 and 10 mg/kg of **1** (Figure 4B).

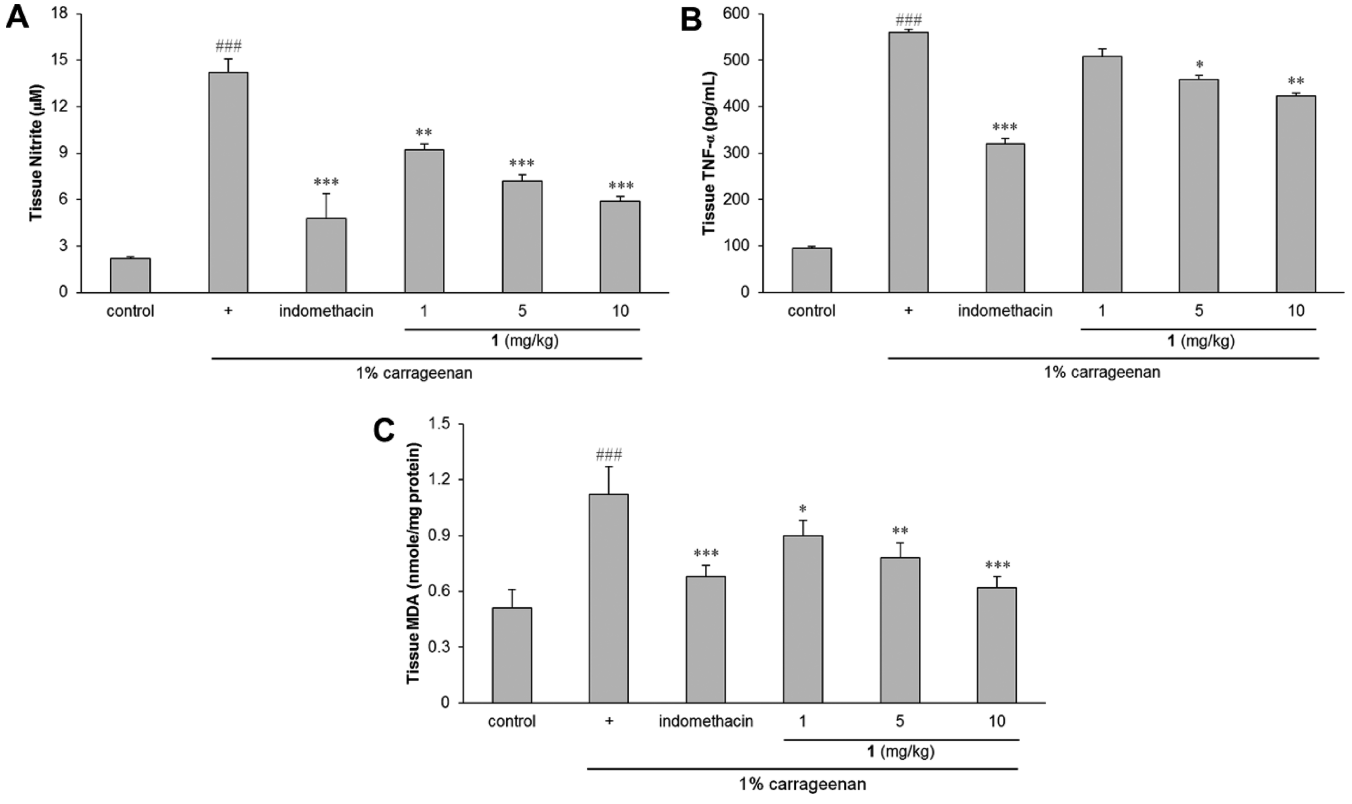


Figure 4. Sclareol (**1**) inhibits tissue content of (A) NO, (B) TNF-α, and (C) MDA in the carrageenan-induced paw edema model. $^{###}p < 0.001$ compared with the control group (treated with normal saline only). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with the carrageenan group. A dose of 10 mg/kg of indomethacin was used.

dx.doi.org/10.1021/np200512a | J. Nat. Prod. 2012, 75, 54–59

Inhibitory Effect of Sclareol (1) on iNOS and COX-2 in the Carrageenan-Induced Edema Paw Model. The experimental results obtained showed that **1** (10 mg/kg) can inhibit ($p < 0.001$) both iNOS and COX-2 protein expression in the edema paw as compared to the carrageenan-treated alone group (Figure 5). The experiments showed an average of 69.6%

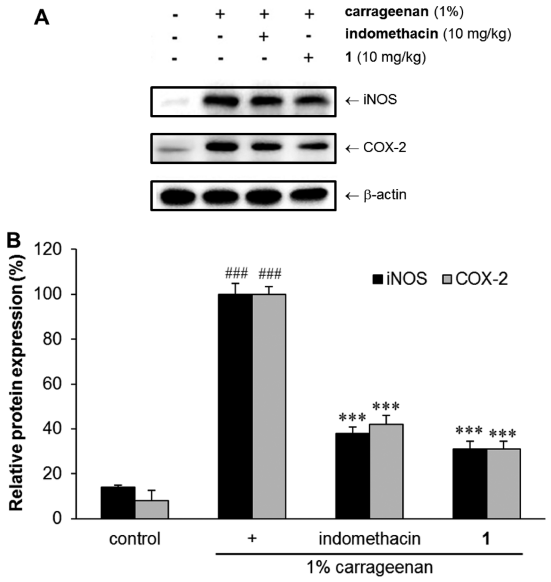


Figure 5. Sclareol (**1**) reduces expression of inflammation-associated proteins in the edema paw induced by carrageenan (1%). (A) Western blot showing protein expression of iNOS and COX-2. The bar chart indicates the relative protein expression of iNOS and COX-2 within the paw tissue after treatment (B). ###$p < 0.001$ compared with the control group (normal saline treated only). ***$p < 0.001$ compared with the carrageenan group. A dose of 10 mg/kg of indomethacin was used.

and 70.3% down-regulation of the iNOS and COX-2 proteins, respectively, after treatment with **1** at 10 mg/kg compared with the carrageenan-treated group (Figure 5B). In addition, the protein expression showed an average of 61.5% and 58.1% down-regulation of the iNOS and COX-2 proteins after treatment with indomethacin at 10 mg/kg compared with the carrageenan-treated group (Figure 5B). The potency of **1** (10 mg/kg) on down-regulating the expression of iNOS and COX-2 proteins was similar to that of indomethacin (10 mg/kg).

Inhibitory Effect of Sclareol (1) on Neutrophil Infiltration in the Carrageenan-Induced Edema Paw Model. In Figure S2 (Supporting Information), neutrophils increased with carrageenan treatment ($p < 0.001$). Indomethacin and **1** (10 mg/kg) decreased significantly neutrophil numbers as compared to the carrageenan-treated group ($p < 0.001$). The carrageenan-induced inflammatory response has been linked to neutrophil infiltration and the production of neutrophil-derived free radicals as well as the release of other neutrophil-derived mediators.[33] It has been demonstrated that free radicals and NO will be released when administrated with carrageenan, and increasing free radicals might attack plasma membranes and result in the accumulation of MDA. The present study demonstrated that 10 mg/kg of **1** decreased markedly neutrophil infiltration and the accumulation of MDA in the edema paw after carrageenan treatment (Figures 4C and S2, Supporting Information).

In Vivo Regulatory Effect of Sclareol (1) on the Activity of Antioxidative Enzymes. At 5 h after the intrapaw injection of carrageenan, liver tissues were analyzed for biochemical parameters such as CAT, SOD, and GPx activities (Table S1, Supporting Information). CAT, SOD, and GPx activities in liver tissue were decreased significantly by carrageenan administration, but increased after being treated with 5 mg/kg of **1** ($p > 0.05$ or $p < 0.01$) and 10 mg/kg of indomethacin ($p < 0.01$). Glutathione (GSH) plays an important role against carrageenan-induced local inflammation,[34] and endogenous GSH can reduce MDA production. In the present study, increases of CAT, SOD, and GPx activities were found in the group with treatment with **1** (Figure 4C and Table S1, Supporting Information). Therefore, the suppression of MDA production is probably due to increases of CAT, SOD, and GPx activities. It has also been demonstrated that sclareol (**1**) possesses antioxidant activity in vitro,[35] with such an effect being a possible explanation for the reduced MDA found in hepatic tissues.

EXPERIMENTAL SECTION

Chemicals. Sclareol (**1**), lipopolysaccharide from *Escherichia coli* (serotype 0127:B8), λ-carrageenan, indomethacin, and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). The purity of **1** used in the present study was >95%. TNF-α was purchased from Biosource International, Inc. (Camarillo, CA, USA). Anti-iNOS, anti-COX-2, anti-β-actin antibody (Santa Cruz Biotechnology, CA, USA) and a protein assay kit (Bio-Rad Lab, Watford, UK) were obtained as indicated. Polyvinylidene fluoride (PVDF) membrane (Immobilon-P) was obtained from Millipore Corporation (Bedford, MA, USA).

Cell Culture. The RAW264.7 murine macrophage cell line (BCRC No. 60001) was purchased from the Bioresources Collection and Research Center (BCRC) of the Food Industry Research and Development Institute (Hsinchu, Taiwan). Cells were cultured in culture dishes containing Dulbecco's modified Eagle medium (DMEM; Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich) in a CO_2 incubator (5% CO_2 in air) at 37 °C and subcultured every 3 days at a dilution of 1:5 using 0.05% trypsin−0.02% EDTA in Dulbecco's phosphate-buffered saline (DPBS) without Ca^{2+} and Mg^{2+} ions.

Mouse Model of Carrageenan-Induced Paw Edema. Twenty-four male ICR mice were obtained from BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan). The animals were housed in Plexiglas cages with free access to food and water and maintained at a constant temperature of 22 ± 1 °C and relative humidity of 55 ± 5% with a photocycle of 12 h light/dark. The experimental procedures were performed according to the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals*. In addition, all tests were conducted under the guidelines of the International Association for the Study of Pain.[36] After a 2-week adaptation period, the mice (about 18−25 g) were assigned randomly to four groups ($n = 6$) for further experiments. The control group received normal saline, and the other three groups included carrageenan, carrageenan and indomethacin (a positive control), and carrageenan and **1** administered groups. The carrageenan-induced hind paw edema model was used for determination of anti-inflammatory activity.[37] Animals were treated with normal saline, indomethacin, or **1** (1, 5, and 10 mg/kg) with intraperitoneal injection, 30 min prior to injection of 1% carrageenan (50 μL) in the plantar side of right hind paws of the mice. The paw volume was measured immediately after carrageenan injection and at 1, 2, 3, 4, and 5 h intervals after the administration of the edematogenic agent using a Plethysmometer (model 7159; Ugo Basile, Varese, Italy). The degree of swelling induced was evaluated by the ratio A/B, where A is the volume of the right hind paw after carrageenan treatment and B is the volume of the right hind paw before carrageenan treatment. Finally, the animals were sacrificed, and all of the right hind paws were dissected and stored at −80 °C. Also, blood was withdrawn and kept at −80 °C.

dx.doi.org/10.1021/np200512a | J. Nat. Prod. 2012, 75, 54−59

MTT Cell Viability Assay. Macrophages (2×10^5) were cultured in a 96-well plate containing DMEM supplemented with 10% FBS for 1 day to become nearly confluent. Then, the cells were pretreated with several concentrations (0, 1, 5, and 10 μg/mL) of **1** for 1 h and then co-stimulated with 100 ng/mL of LPS for 24 h. Next, the cells were washed twice with DPBS and incubated with 100 μL of 0.5 mg/mL MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) for 2 h at 37 °C, the medium was discarded, and 100 μL of dimethyl sulfoxide (DMSO) was added. After 30 min incubation, absorbances at 570 nm were read using a microplate reader.

Measurement of Nitric Oxide/Nitrite. NO production was indirectly assessed by measuring the nitrite levels in the culture medium and serum as determined according to a previous study.[37] The cells were preincubated with **1** (0, 1, 5, and 10 μg/mL) for 1 h and then co-treated with 100 ng/mL LPS at 37 °C for 24 h. Subsequently, 100 μL of each collected culture medium was mixed with the same volume of Griess reagent (1% sulfanilamide, 0.1% naphthyl ethylenediamine dihydrochloride, and 5% phosphoric acid) and incubated at room temperature for 10 min. The absorbance of the mixture was measured at 540 nm with a Micro-Reader (Molecular Devices, Sunnyvale, CA, USA). Homogenized tissue samples were diluted four times with distilled water and deproteinized by adding 1/20 volume of zinc sulfate (300 mg/mL) to a final concentration of 15 mg/mL. After centrifugation at 10000*g* for 5 min at room temperature, 100 μL of supernatant was added into a microtiter plate, followed by 100 μL of Griess reagent. After 10 min of color development at room temperature, the absorbance was measured at 540 nm with a Micro-Reader. Using sodium nitrite to generate a standard curve, the concentration of nitrite was measured by absorbance at 540 nm.

Western Blot Analysis. The stimulated macrophages were washed with PBS and lysed in an ice-cold lysis buffer [10% glycerol, 1% Triton X-100, 1 mM sodium orthovanadate, 1 mM EGTA, 10 mM sodium fluoride, 1 mM sodium pyrophosphate, 20 mM Tris buffer (pH 7.9), 100 mM β-glycerophosphate, 137 mM sodium chloride, 5 mM EDTA, and a protease inhibitor cocktail tablet (Roche, Indianapolis, IN, USA)] on ice for 1 h, followed by centrifugation at 12000*g* for 30 min at 4 °C. Soft tissues were removed from individual mice paws and homogenized in a solution containing 10 mM CHAPS, 1 mM phenylmethylsulfonyl fluoride (PMSF), 5 μg/mL, aprotinin, 1 μM pepstatin, and 10 μM leupeptin. The homogenates were centrifuged at 12000*g* for 20 min, and the supernatant was collected for Western blot analysis. Protein concentration was measured by the Bio-Rad protein assay kit with bovine serum albumin as a standard. About 30 μg of protein from the supernatants was then separated on 10% sodium dodecylsulfate−polyacrylamide gel (SDS-PAGE) and transferred to PVDF membranes. After transfer, the membrane was blocked for 2 h at room temperature with 5% skim milk in TBST buffer (20 mM Tris, 500 mM NaCl, pH 7.5, and 0.1% Tween 20). The membranes were then incubated with mouse monoclonal anti-iNOS or anti-COX-2 antibody in 5% skim milk in TBST buffer for 2 h at room temperature. The membranes were washed three times with TBST at room temperature and incubated with a 1:2000 dilution of anti-mouse IgG secondary antibody conjugated to horseradish peroxidase (Sigma-Aldrich) in 2.5% skim milk in TBST for 1 h at room temperature. The membranes were washed three times, and the immunoreactive proteins were detected by enhanced chemiluminescence (ECL) using Hyperfilm ECL reagent (Amersham Biosciences, Inc., Amersham, UK). The results of Western blot analysis were quantified by measuring the relative intensity compared to the control using Kodak molecular imaging software ver.4.0.5 (Eastman Kodak Company, Rochester, NY, USA) and represented in the relative intensities. The results for iNOS and COX-2 were normalized to the band density of internal control (β-actin), and the relative protein expression was calculated according to the value of the LPS-treated control group as 100%.

Malondialdehyde Assay. Malondialdehyde from carrageenan-induced edema paws was evaluated using the thiobarbituric acid reacting substance (TRARS) method.[37] Briefly, MDA reacted with thiobarbituric acid in the acidic high temperature and formed a red complex, TBARS. The absorbance of TBARS was determined at 532 nm.

Measurement of Serum TNF-α. Serum levels of TNF-α were determined using a commercially available ELISA kit (Biosource International, Inc., Camarillo, CA, USA), according to the manufacturer's instructions. The concentration of serum TNF-α was presented as pg/mL and determined according to the regression equation of the standard curve.

Statistical Analysis. Data are expressed as means ± standard errors of the means. Statistical evaluation was carried out by one-way analysis of variance (ANOVA, Scheffe's post hoc test). A value of $p < 0.05$ was regarded as being statistically significant.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and results regarding the following studies: (a) Effect of sclareol (**1**) on cell viability of LPS-stimulated macrophages, (b) effect of sclareol (**1**) on neutrophil infiltration in the carrageenan-induced edema paw model, and (c) regulation of sclareol (**1**) on the enzyme activities of CAT, SOD, and GPx in the mouse liver. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +886-4-22053366#5101. Fax: +886-4-22073709. E-mail: chhswu@mail.cmu.edu.tw.

■ ACKNOWLEDGMENTS

Financial support was obtained from the National Science Council (NSC 97-2313-B-039-001-MY3), China Medical University (CMU) (CMU99-S-29, CCM-P99-RD-042, and CMU99-COL-10), and the Taiwan Department of Heath Clinical Trial and Research Center of Excellence (DOH100-TD-B-111-004).

■ REFERENCES

(1) Weninger, S. C.; Yankner, B. A. *Nat. Med.* **2001**, *7*, 527−528.
(2) Bousquet, J.; Jeffery, P. K.; Busse, W. W.; Johnson, M.; Vignola, A. M. *Am. J. Resp. Crit. Care Med.* **2000**, *161*, 1720−1745.
(3) Libby, P. *Nature* **2002**, *420*, 868−874.
(4) Flavell, R. A. *Curr. Top. Microbiol. Immunol.* **2002**, *266*, 1−9.
(5) Rajput, S.; Wilber, A. *Front. Biosci. (Schol. Ed.)* **2010**, *2*, 176−183.
(6) Christodoulou, C.; Choy, E. H. *Clin. Exp. Med.* **2006**, *6*, 13−19.
(7) Liu, H.; Pope, R. M. *Rheum. Dis. Clin. North Am.* **2004**, *30*, 19−39.
(8) Wynn, T. A.; Barron, L. *Sem. Liver Dis.* **2010**, *30*, 245−257.
(9) Suschek, C. V.; Schnorr, O.; Kolb-Bachofen, V. *Curr. Mol. Med.* **2004**, *4*, 763−775.
(10) Crofford, L. J. *J. Rheumatol. Suppl.* **1997**, *49*, 15−19.
(11) Bradley, J. R. *J. Pathol.* **2008**, *214*, 149−160.
(12) Knowles, R. G.; Moncada, S. *Biochem. J.* **1994**, *298* (Part 2), 249−258.
(13) James, S. L. *Microbiol. Rev.* **1995**, *59*, 533−547.
(14) Antoniou, K.; Malamas, M.; Drosos, A. A. *Exp. Opin. Pharmacother.* **2007**, *8*, 1719−1732.
(15) Dimas, K.; Kokkinopoulos, D.; Demetzos, C.; Vaos, B.; Marselos, M.; Malamas, M.; Tzavaras, T. *Leuk. Res.* **1999**, *23*, 217−234.
(16) Dimas, K.; Hatziantoniou, S.; Tseleni, S.; Khan, H.; Georgopoulos, A.; Alevizopoulos, K.; Wyche, J. H.; Pantazis, P.; Demetzos, C. *Apoptosis* **2007**, *12*, 685−694.
(17) Dimas, K.; Papadaki, M.; Tsimplouli, C.; Hatziantoniou, S.; Alevizopoulos, K.; Pantazis, P.; Demetzos, C. *Biomed. Pharmacother.* **2006**, *60*, 127−133.
(18) Hatziantoniou, S.; Dimas, K.; Georgopoulos, A.; Sotiriadou, N.; Demetzos, C. *Pharmacol. Res.* **2006**, *53*, 80−87.

(19) Noori, S.; Hassan, Z. M.; Mohammadi, M.; Habibi, Z.; Sohrabi, N.; Bayanolhagh, S. *Cell. Immunol.* **2010**, *263*, 148−153.
(20) Chan, E. D.; Riches, D. W. *Am. J. Physiol. Cell Physiol.* **2001**, *280*, C441−C450.
(21) Roach, T. I.; Barton, C. H.; Chatterjee, D.; Liew, F. Y.; Blackwell, J. M. *Immunology* **1995**, *85*, 106−113.
(22) Teng, X.; Zhang, H.; Snead, C.; Catravas, J. D. *Am. J. Physiol. Cell Physiol.* **2002**, *282*, C144−C152.
(23) Arany, I.; Brysk, M. M.; Brysk, H.; Tyring, S. K. *Cancer Lett.* **1996**, *110*, 93−96.
(24) Stefano, G. B.; Salzet, M.; Magazine, H. I.; Bilfinger, T. V. *J. Cardiovasc. Pharm.* **1998**, *31*, 813−820.
(25) Deng, W. G.; Montero, A. J.; Wu, K. K. *Arterioscler. Thromb. Vasc. Biol.* **2007**, *27*, 1752−1759.
(26) Coccia, E. M.; Stellacci, E.; Marziali, G.; Weiss, G.; Battistini, A. *Int. Immunol.* **2000**, *12*, 977−985.
(27) Cui, X.; Yang, S. C.; Sharma, S.; Heuze-Vourc'h, N.; Dubinett, S. M. *Biochem. Biophys. Res. Commun.* **2006**, *343*, 995−1001.
(28) Cho, W.; Kim, Y.; Jeoung, D. I.; Kim, Y. M.; Choe, J. *Mol. Immunol.* **2011**, *48*, 966−972.
(29) Guo, F. H.; Uetani, K.; Haque, S. J.; Williams, B. R.; Dweik, R. A.; Thunnissen, F. B.; Calhoun, W.; Erzurum, S. C. *J. Clin. Invest.* **1997**, *100*, 829−838.
(30) Spector, W. G.; Willoughby, D. A. *Bacteriol. Rev.* **1963**, *27*, 117−154.
(31) Cuzzocrea, S.; Zingarelli, B.; Calapai, G.; Nava, F.; Caputi, A. P. *Life Sci.* **1997**, *60*, 215−220.
(32) Pan, M. H.; Lai, C. S.; Dushenkov, S.; Ho, C. T. *J. Agric. Food Chem.* **2009**, *57*, 4467−4477.
(33) Dawson, J.; Sedgwick, A. D.; Edwards, J. C.; Lees, P. *Int. J. Tissue React.* **1991**, *13*, 171−185.
(34) Chaturvedi, P. *Evid.-Based Complementary Altern. Med.* **2008**, *5*, 55−59.
(35) Kolak, U.; Hacibekiröglu, I.; Öztürk, M.; Özgökçe, F.; Topçu, G.; Ulubelen, A. *Turk. J. Chem.* **2009**, *33*, 813−823.
(36) Zimmermann, M. *Pain* **1983**, *16*, 109−110.
(37) Huang, G. J.; Deng, J. S.; Huang, S. S.; Chang, C. I.; Chang, T. N.; Shie, P. H.; Kuo, Y. H. *J. Agric. Food Chem.* **2011**, *59*, 11211−11218.