JOURNAL OF NATURAL PRODUCTS

ARTICLE

pubs.acs.org/jnp

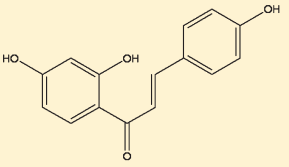
Comparative Evaluation of Two Structurally Related Flavonoids, Isoliquiritigenin and Liquiritigenin, for Their Oral Infection Therapeutic Potential

Mark Feldman, Juliana Santos, and Daniel Grenier*

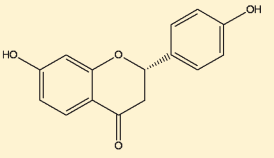
Groupe de Recherche en Écologie Buccale, Faculté de Médecine Dentaire, Université Laval, 2420 Rue de la Terrasse, Québec City, QC, Canada, G1V 0A6

Supporting Information

ABSTRACT: Isoliquiritigenin (**1**) and liquiritigenin (**2**) are structurally related flavonoids found in a variety of plants. The purpose of this study was to perform a comparative analysis of biological properties of these compounds in regard to their therapeutic potential for oral infections. Compound **1** demonstrated significant antibacterial activity against three major periodontopathogens, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Prevotella intermedia*. In contrast, **2** exerted less pronounced effects on the above bacterial species. Neither compound was effective against cariogenic bacteria (*Streptococcus mutans* and *Streptococcus sobrinus*). Furthermore, **1** exhibited a stronger inhibitory activity than **2** toward *P. gingivalis* collagenase and human matrix metalloproteinase 9. Finally, the capacity of **1** to attenuate the inflammatory response of macrophages induced by *Aggregatibacter actinomycetemcomitans* lipopolysaccharide (LPS) was much higher when compared to **2**. The activation of transcriptional factors nuclear factor-κB (NF-κB) p65 and activator protein-1 (AP-1) associated with the LPS-induced inflammatory response in macrophages was inhibited strongly by **1**, but less affected by **2**.



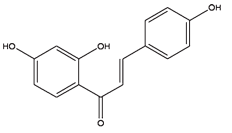
Isoliquiritigenin (1)



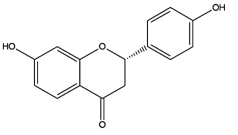
Liquiritigenin (2)

Flavonoids comprise a large group of phenolic secondary metabolites that are widespread throughout the plant kingdom.[1] A wide range of flavonoids has been shown to exhibit various biological properties, including antibacterial, antiprotozoal, anti-inflammatory, antioxidant, estrogenic, and vascular activities.[2,3] Isoliquiritigenin (**1**) and liquiritigenin (**2**) are flavonoids contained in edible plants and some oriental medicines. Although both compounds are found in numerous plants, most studies have investigated **1** and **2** as constituents of licorice.[4,5] Biosynthetically and structurally, **1** is the precursor and an isomer of **2**.[6] During the early stages of the biosynthesis of these flavonoids, chalcone isomerase (CHI) catalyzes the intramolecular cyclization of chalcone **1** into flavanone **2**.[7] The major therapeutic potential of **1** and **2** has been attributed to their anticarcinogenic and anti-inflammatory activities. For example, both compounds isolated from *Platymiscium floribundum* were effective against five human cancer cell lines in vitro.[8] Compound **1** has been reported as a promising chemopreventive agent against colon carcinogenesis[9] as well as an effective heme oxygenase-1 inducer capable of inhibiting macrophage-mediated inflammation.[10] Flavonoids **1** and **2** were shown to inhibit airway eosinophilic inflammation, which is a major feature of allergic asthma.[6] In addition, **2** showed hepatoprotective effects against acetaminophen-induced acute liver injuries.[11]

Although both flavonoids **1** and **2** have been the topic of numerous studies in the cancer and inflammation research fields, there are few reports in the literature regarding their antimicrobial activity. It has been documented recently that **1** and **2** possess similar antibacterial activity against *Mycobacterium tuberculosis*.[12] In other studies, **1** was found to inhibit growth of *Mycobacterium bovis* at 50 μg/mL,[13] while a concentration of 250 μg/mL was required to inhibit the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus hemolyticus*.[14]



Isoliquiritigenin (1)



Liquiritigenin (2)

Periodontal diseases and dental caries are oral infections that affect a large proportion of the population throughout the world. Mutans streptococci such as *Streptococcus mutans* and *Streptococcus sobrinus* are considered the principal etiological agents of dental caries formation, through their aciduric, acidogenic, and adhesion properties.[15,16] Dietary sucrose is essential for the accumulation of these bacteria on teeth and for the formation of carious lesions in enamel.[17] Periodontitis represents one of the most common chronic bacterial infections in man. This disease is associated with an

Received: February 21, 2011
Published: August 25, 2011

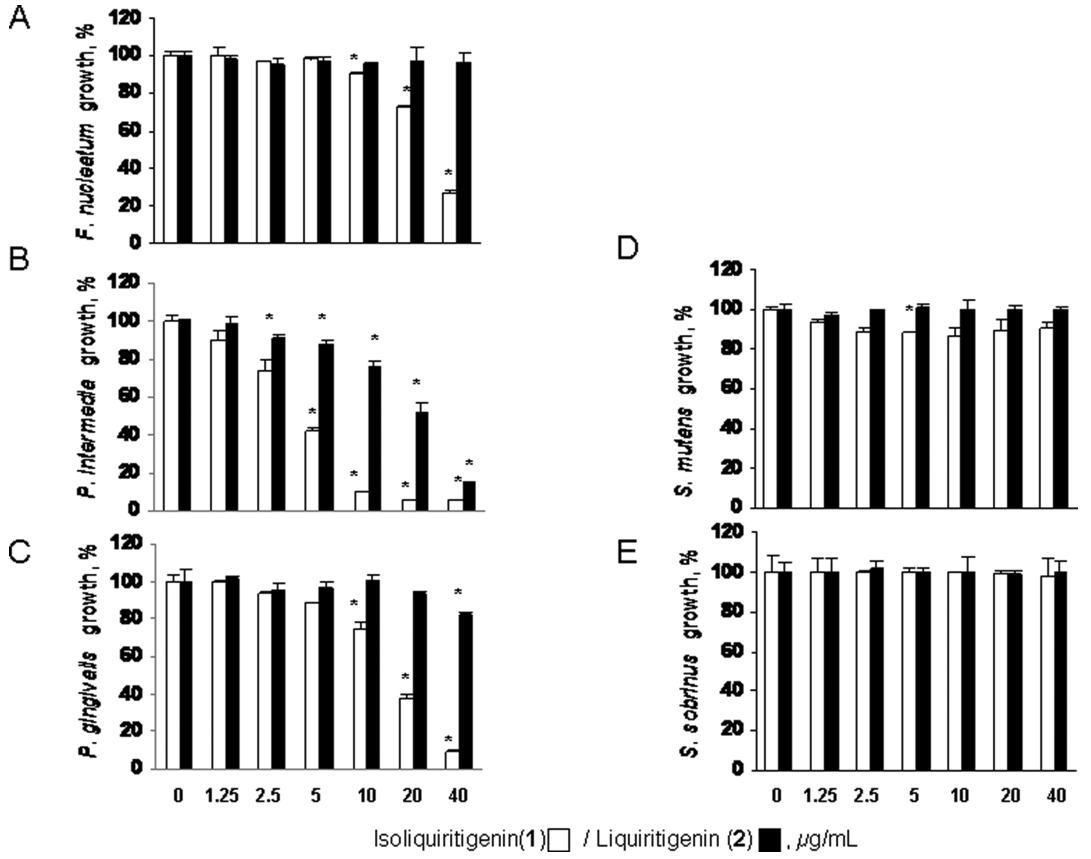


Figure 1. Antibacterial activity of isoliquiritigenin (**1**) or liquiritigenin (**2**) on *F. nucleatum* (A), *P. intermedia* (B), *P. gingivalis* (C), *S. mutans* (D), and *S. sobrinus* (E). A value of 100% was given to growth obtained in the absence of compounds. The data are means ± SD of triplicate assays for two independent experiments. $^*p < 0.01$ compared to control with no **1** or **2**.

inflammatory response leading to gingival tissue destruction and progressive loss of alveolar bone around the teeth. A limited number of bacterial species, known as periodontopathogens, contribute to the formation of periodontal lesions, including *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, and *Treponema denticola*.[18,19] Although the periodontopathogens found in the supra- and subgingival biofilms play an important role in the initiation of periodontitis, they are not sufficient for disease progression. There is now a consensus that the host inflammatory response induced by periodontopathogens is responsible for most of the tissue breakdown leading to the clinical signs of periodontitis. Due to the immune defense of the host against bacteria, there is an accumulation of certain inflammatory mediators, including interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), and prostaglandins, causing a chronic inflammation associated with the production of tissue-degrading enzymes such as matrix metalloproteinases (MMPs).[20] It was demonstrated that such excessive production of inflammatory mediators and MMPs is induced by virulence factors, including lipopolysaccharide (LPS), fimbriae, and proteinases, produced by periodontopathogens.[21−25]

This study was undertaken to compare flavonoids **1** and **2** in regard to their therapeutic potential for dental caries and periodontitis. Specifically, (a) their antibacterial activity against five major oral pathogens, (b) their antiprotease activity on MMP-9 and *P. gingivalis* collagenase, and (c) their anti-inflammatory activity using a macrophage model stimulated with LPS were evaluated.

RESULTS AND DISCUSSION

The present study showed clearly that both **1** and **2** exhibited significant antibacterial activities against Gram-negative periodontal bacteria, while having no effect on Gram-positive streptococci (Figure 1). More specifically, **1** demonstrated a dose-dependent antibacterial activity against all tested periodontopathogens. The most pronounced antibacterial effect was observed for *P. intermedia* (Figure 1B). At 5 μg/mL, **1** reduced bacterial growth by 58%, while 10 μg/mL represented the minimal inhibitory concentration (MIC). Moreover, it was found that **1** at 20 μg/mL kills *P. intermedia*, as no bacteria could be recovered on an agar plate from the inoculated broth culture (data not shown). *P. gingivalis* was more resistant than *P. intermedia* to **1** with an MIC of 40 μg/mL (Figure 1C). At this concentration, the inoculated bacteria reminded viable, indicating that it was not bactericidal. *F. nucleatum* was found to be the most resistant to **1**. This compound at 40 μg/mL was able to reduce *F. nucleatum* growth by only approximately 70% (Figure 1A). In contrast to the significant antibacterial activity of chalcone **1**, flavanone **2** was effective only on *P. intermedia*, which was the least resistant bacterium to **1**. At a concentration of 40 μg/mL, **2** inhibited the growth of *P. intermedia* by 85% (Figure 1B). Considering that oral pathogens persist in the oral cavity in the presence of saliva and serum exudate, the above growth studies were also carried out in the presence of 1% fetal bovine serum or with 10% of unstimulated whole saliva, and similar results were obtained (data not shown).

Table 1. Effect of Isoliquiritigenin (1) and Liquiritigenin (2) on Enzymatic Activity of *P. gingivalis* Collagenase and MMP-9[a]

compound	concentration (μg/mL)	relative activity (%) *P. gingivalis* collagenase	relative activity (%) MMP-9
none		100 ± 2	100 ± 2
isoliquiritigenin (**1**)	5	82 ± 5	97 ± 1
	25	78 ± 2[b]	91 ± 1[b]
	100	48 ± 2[b]	67 ± 1[b]
liquiritigenin (**2**)	5	83 ± 2[b]	95 ± 1
	25	82 ± 3[b]	95 ± 2
	100	79 ± 4[b]	87 ± 2[b]

[a] A value of 100% was given to the activity obtained in the absence of the tested compounds. Means ± SD of triplicate assays are presented. [b] $p < 0.01$ for all inhibition values when compared to respective level of activity in the absence of the tested compounds (Student's *t* test).

The marked difference regarding the antibacterial activity of **1** on Gram-negative and Gram-positive bacteria could be attributed to the distinct bacterial cell wall structures. Gram-positive bacteria have a thick peptidoglycan layer (50−60 nm) as part of the cell membrane, and this structure is known to help bacteria overcome physical stresses.[26] This peptidoglycan layer is also believed to reduce the penetration of small molecules. On the other hand, Gram-negative bacteria have a thin layer of peptidoglycan between the outer and inner cell membranes. Moreover, the porins present in the outer membrane of Gram-negative bacteria act as channels for low molecular weight substances to enter the cells.[27] The obvious difference in antibacterial activity between **1** and **2** may be explained by their distinct chemical structures. It is proposed that the chalcone skeleton may be a key factor for the difference in the activity of the tested compounds against bacteria. Chalcones are well-known antibacterial agents, and they exert their activity through cell membrane disruption.[28] Moreover, it was proposed that **1**, which does not bear an oxygen-containing ring, represents the most potent inhibitor of fatty acid biosynthesis of *M. bovis* among flavonoids having this ring.[13]

The capacity of **1** and **2** to inhibit the collagenase activity of *P. gingivalis* was investigated. Chalcone **1** was found to be a potent inhibitor of *P. gingivalis* collagenase, showing a dose-dependent effect. At the highest concentration tested (100 μg/mL), **1** reduced collagenase activity by 52% compared to the control (Table 1). In contrast, flavanone **2** at the above concentration decreased *P. gingivalis* collagenase activity by only 21% (Table 1). Considering the key roles that *P. gingivalis* proteases may play in nutrient acquisition and the tissue destructive process of periodontitis, inhibitors of these enzymes are considered potential therapeutic agents. Thus, chalcone **1**, in affecting both the growth of periodontopathogens and an important virulence factor of *P. gingivalis*, may be of high interest. To further investigate the effects of **1** and **2** on tissue-degrading enzymes, they were tested on MMP-9 activity. As reported in Table 1, a marked inhibition of MMP-9 activity was obtained only at the highest concentration (100 μg/mL) used. As for the collagenase activity of *P. gingivalis*, **1** was more effective than **2** in inhibiting MMP-9 activity.

Since tissue destruction in periodontitis is modulated mainly by the inflammatory response induced by periodontopathogens, agents exhibiting a dual mode of action including antibacterial and anti-inflammatory activities represent promising therapeutics for controlling periodontitis.[29] In the present study, the ability of flavonoids **1** and **2** to attenuate the *A. actinomycetemcomitans* LPS-induced inflammatory response in a macrophage model was evaluated. As shown in Figure 2, secretion of TNF-α, IL-1β, IL-6, IL-8, and CCL5 was increased significantly when macrophages were stimulated with *A. actinomycetemcomitans* LPS compared to untreated cells. More specifically, the secretion of TNF-α, IL-1β, IL-6, IL-8, and CCL5 was increased by 22-, 96-, 33-, 22-, and 9-fold, respectively. IL-1β and TNF-α are critical factors for periodontitis progression, and local inhibition of these two mediators in periodontal tissues was found to significantly reduce the inflammatory response and bone loss in ligature-induced periodontitis in monkeys.[30] IL-6, IL-8, and CCL5 are believed to be also involved in the initiation and amplification of the inflammatory process. In this study, **1** was shown to reduce dose-dependently the LPS-induced secretion of the above inflammatory mediators (Figure 2). More specifically, the elevated production of TNF-α was markedly decreased by 87% by 5 μg/mL chalcone **1** (Figure 2E), while the LPS-induced secretion of CCL5, IL-6, and IL-1β was totally blocked by **1** at this concentration (Figure 2A, B, and D). In spite of the marked effect on the above cytokines, **1** at 5 μg/mL reduced IL-8 secretion by only 34% (Figure 2C). At the lowest concentration of **1** tested (0.2 μg/mL), a significant inhibition of cytokine secretion by macrophages was obtained only for IL-6 and IL-1β (Figure 2B, D). In contrast to its chalcone isomer, **2** exhibited a much weaker anti-inflammatory activity. The LPS-induced production of TNF-α, IL-1β, IL-6, IL-8, and CCL5 was poorly altered by low concentrations of **2**, while the highest concentration (5 μg/mL) decreased the above cytokine secretion by 17%, 34%, 23%, 11%, and 24%, respectively (Figure 2). None of the treatments of macrophages with either LPS, **1**, or **2** were associated with a loss of cell viability (data not shown).

Periodontal tissue destruction has been associated with the presence of high levels of MMPs, including MMP-9 in gingival crevicular fluid.[31] As a matter of fact, MMP-9 has been suggested as a useful marker for assessing the clinical severity of periodontal disease.[32] In the macrophage model used, LPS increased by 2-fold the secretion of MMP-9 compared to the nontreated control (Figure 3). Chalcone **1** demonstrated a marked capacity to inhibit MMP-9 secretion by LPS-treated macrophages. At a concentration of 5 μg/mL, **1** reduced MMP-9 secretion by 89% (Figure 3). Flavonone **2** was much less effective in inhibiting MMP-9 secretion. At a concentration of 5 μg/mL, **2** decreased MMP-9 secretion by 24% (Figure 3).

It is well documented that LPS induces the production of inflammatory mediators in mammalian cells via recognition and activation of toll-like receptor 4 (TLR 4) and subsequent initiation of a series of nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) associated intracellular signaling events.[33,34] The present results showed that *A. actinomycetemcomitans* LPS increased NF-κB p65 and AP-1 activation in macrophages by 83% and 70%, respectively (Figure S1, Supporting Information). Pretreatment of macrophages with 5 μg/mL of **1** prior to the stimulation with LPS totally neutralized the LPS-induced activation of NF-κB p65 and AP-1 (Figure S1, Supporting Information). This is in agreement with a previous study reporting that **1** exerts its anti-inflammatory and anticarcinogenic properties by blocking the NF-κB p65 and AP-1 activation pathways.[35] In contrast, compound **2** exhibited a less pronounced inhibitory

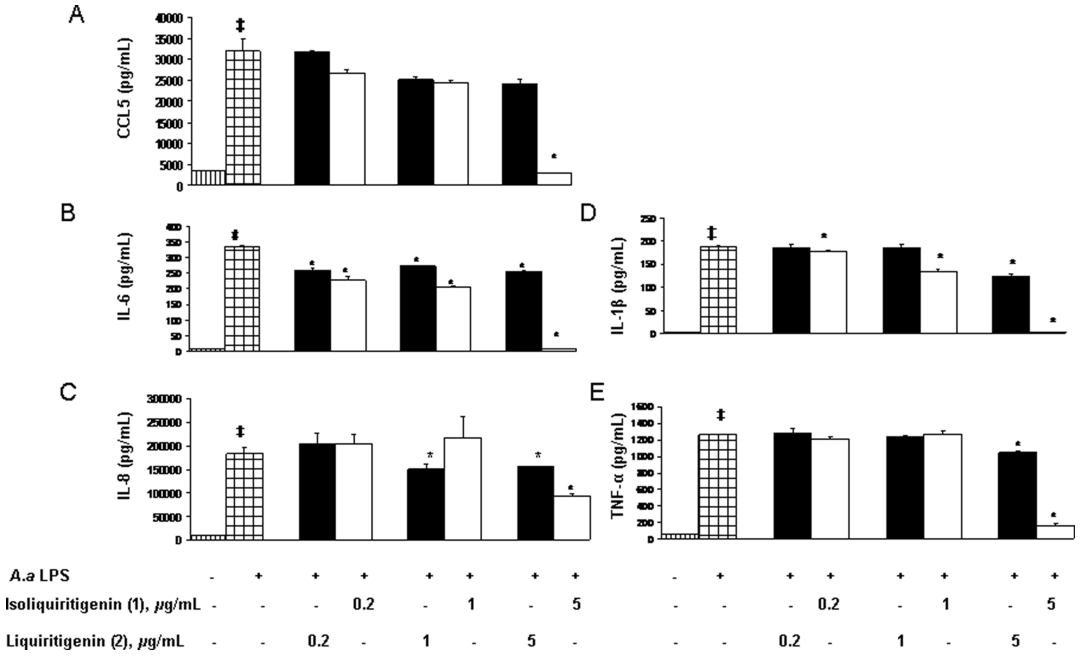


Figure 2. Effect of treating macrophages with isoliquiritigenin (**1**) or liquiritigenin (**2**) on the secretion of CCL-5 (A), IL-6 (B), IL-8 (C), IL-1β (D), and TNF-α (E) induced by *A. actinomycetemcomitans* ATCC 29522 LPS. The data are means ± SD of triplicate assays for two independent experiments. $^{\dagger}p < 0.01$ compared to control with no LPS and with no **1** or **2**. $^{*}p < 0.01$ compared to an LPS control with no **1** or **2**.

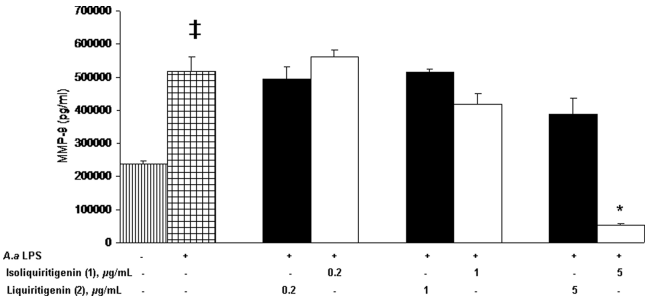


Figure 3. Effect of treating macrophages with isoliquiritigenin (**1**) or liquiritigenin (**2**) on the secretion of MMP-9 induced by *A. actinomycetemcomitans* LPS. The data are means ± SD of triplicate assays for two independent experiments. $^{\dagger}p < 0.01$ compared to control with no LPS and with no **1** or **2**. $^{*}p < 0.01$ compared to an LPS control with no **1** or **2**.

effect on activation of both transcriptional factors. At a concentration of 5 μg/mL, **2** was able to reduce LPS activation of NF-κB p65 and AP-1 by 39% and 20%, respectively (Figure S1, Supporting Information).

The difference in the anti-inflammatory properties of **1** and **2** may be attributed to their distinct chemical structures. Compound **1** is known as a potent suppressor of LPS-induced TLR4 dimerization that is correlated strongly with inhibition of NF-κB p65 and AP-1 activation and subsequent reduction of cytokine production.[36] Phytochemicals with an α,β-unsaturated carbonyl group conferring Michael addition have been shown to disrupt dimerization of TLR4.[37] These molecules are highly reactive with biological nucleophiles such as the thiol group of cysteines found in extracellular and cytoplasmic domains of TLR4.[38] Therefore, since **1** has a structural motif of an α,β-unsaturated carbonyl group, it has been suggested that TLR4 receptor cysteine residues are the potential targets for this chalcone.[36] In contrast to **1**, its isomer **2** does not possess this type of functionality and, therefore, probably interacts with another sensor on mammalian cells. Indeed, Kim et al.[39] have hypothesized that **2** is a selective agonist at the estrogen receptor-β (ERβ) and may inhibit the activation of NF-κB and AP-1.

According to the above results, it is proposed that targeting host cell receptors activated by pathogens is a potential strategy for designing new anti-inflammatory agents. Further research should focus on the structural modification of the chalcone unit by incorporation of functional groups (hydroxy, methyl, halogen), without disruption of the α,β-unsaturated carbonyl moiety in order to enhance the bioactivity of compound **1**. Altogether, the present findings have demonstrated distinct antibacterial, antiprotease, and anti-inflammatory activities between structurally related flavonoids. Among the two isomers tested, chalcone **1** offers the greater potential as a therapeutic lead compound for infectious and inflammatory diseases.

EXPERIMENTAL SECTION

Tested Compounds. Isoliquiritigenin (**1**) and liquiritigenin (**2**) were purchased from Chromadex (Irvine, CA). Both compounds tested in the present study were extracted from licorice (*Glycyrrhiza glabra*) at ≥98% purity as determined by HLPC, according to the manufacturer's protocol.

Determination of Minimal Inhibitory and Minimal Bactericidal Concentrations. The protocol used to determine the MICs and minimal bactericidal concentrations (MBCs) of *P. gingivalis* ATCC 33277, *F. nucleatum* ATCC 25586, *P. intermedia* ATCC 25611, *S. mutans* ATCC 25175, and *S. sobrinus* ATCC 27352 was as described previously.[40] Using this microdilution assay, MIC values (μg/mL) of **1** and **2** for each bacterial species were determined as the lowest concentration at which no growth occurred. MBC values of **1** and **2** were determined as the lowest concentration at which no colony formation occurred when aliquots of wells showing no visible bacterial

growth were spread on culture plates and incubated for five days under appropriate conditions.

Determination of *P. gingivalis* Collagenase Activity. The culture supernatant of a 2-day-old culture of *P. gingivalis* ATCC 33277 was harvested. Compounds **1** and **2** (3 μL) were added to a mixture containing 7.5 μL of *P. gingivalis* supernatant, 15 μL of fluorescein-conjugated DQ type I collagen (Molecular Probes) at 100 μg/mL, and 124.5 μL of TCNB buffer to obtain final concentrations of 0, 5, 25, and 100 μg/mL. Incubation was carried out for 4 h in the dark at room temperature, and fluorescence was measured using a fluorometer, as described above.

Determination of MMP-9 Activity. Human recombinant MMP-9 (Calbiochem, San Diego, CA) was obtained in the active form and diluted to a final concentration of 100 ng/mL in TCNB buffer (50 mM Tris·HCl, 10 mM $CaCl_2$, 150 mM NaCl, and 0.05% Brij35, pH 7.5). MMP-9 (75 μL) was mixed with fluorogenic substrate (15 μL; 1 mg/mL fluorescein-conjugated DQ gelatin, obtained from Molecular Probes, Eugene, OR), TCNB buffer (57 μL), and **1** or **2** (3 μL) at final concentrations of 0, 5, 25, and 100 μg/mL. The assay mixtures were incubated in the dark for 4 h at 37 °C. The fluorescence was then measured using a fluorometer (VersaFluor Fluorometer System, Bio-Rad Laboratories, Hercules, CA) with excitation and emission wavelengths set at 490 and 520 nm, respectively.

Preparation of Monocyte-Derived Macrophages and Treatments. The protocol used to prepare and stimulate macrophages was as described previously.[40] Briefly, U937 monocytes (ATCC CRL-1593.2) were differentiated into adherent macrophage-like cells by incubation in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) (RPMI-FBS) and 100 μg/mL of penicillin−streptomycin and containing 10 ng/mL of phorbol myristic acid (PMA) (Sigma Chemical Co., St. Louis, MO) for 48 h at 37 °C in a 5% CO_2 atmosphere. Following the PMA treatment, adherent macrophages were suspended in RPMI-1% FBS medium and seeded in a six-well plate at 37 °C in a 5% CO_2 atmosphere for 2 h prior to treatment. Various concentrations of **1** or **2** (0, 0.2, 1, and 5 μg/mL) were added to macrophages, which were then incubated at 37 °C in a 5% CO_2 atmosphere for 2 h prior to adding the *A. actinomycetemcomitans* LPS at a final concentration of 1 μg/mL. After 24 h, the culture medium supernatants were removed and stored at −20 °C until used. Cells incubated with no LPS and with no **1** or **2** as well as cells treated with LPS in the absence of **1** or **2** were used as controls. An MTT (3-[4,5-diethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay (Roche Diagnostics, Mannheim, Germany) was used to show that treatment of macrophages with LPS, **1**, or **2** did not result in loss of cell viability.

Determination of Cytokine and MMP-9 Production. Commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN) were used to quantify IL-1β, TNF-α, IL-6, IL-8, CCL5, and MMP-9 in the samples, according to the manufacturer's protocols. The absorbance at 450 nm was read in a microplate reader with the wavelength correction set at 550 nm. The sensitivities of the commercial ELISA kits were 31.2 pg/mL for IL-8 and MMP-9, 15.6 pg/mL for TNF-α and CCL5, 9.3 pg/mL for IL-6, and 3.9 pg/mL for IL-1β.

Analysis of NF-κB p65 and AP-1 Activation. Macrophages were incubated in the presence of **1** or **2** (5 μg/mL) for 1 h and then stimulated with LPS (1 μg/mL) for an additional 1 h at 37 °C in a 5% CO_2 atmosphere. Macrophages without pretreatment with **1** or **2** served as controls. Whole-cell extracts were prepared according to the manufacturer's protocol (Nuclear Extract kits; Active Motif, Carlsbad, CA), adjusted to a protein concentration of 1 mg/mL, and stored at −80 °C until use. The extracts were used to measure NF-κB p65 and AP-1 activation using commercial kits (Trans-AM; Active Motif).

Statistical Analysis. All experiments were carried out in triplicates and the means ± standard deviations were calculated. The statistical analysis was performed using Student *t* test with a level of significance of $p < 0.01$.

■ ASSOCIATED CONTENT

Supporting Information. Figure reporting the effect of treating macrophages with isoliquiritigenin (**1**) or liquiritigenin (**2**) on the activation of nuclear factor-kappa B (NF-kB) p65 and activator protein-1 (AP-1) induced by *A. actinomycetemcomitans* LPS. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*Tel: (418) 656-7341. Fax: (418) 656-2861. E-mail: Daniel.Grenier@greb.ulaval.ca.

■ ACKNOWLEDGMENT

This study was supported by the Ministère du Développement Économique, de l'Innovation et de l'Exportation du Québec. We would like to thank Shinichi Tanabe for technical assistance.

■ REFERENCES

(1) Koes, R. E.; Quattrocchio, F; Mol, J. N. M. *Bioessays* **1994**, *16*, 123–132.
(2) Crozier, A.; Jaganath, I. B.; Clifford, M. N. *Nat. Prod. Rep.* **2009**, *26*, 1001–1043.
(3) Saleem, M.; Nazir, M.; Ali, M. S.; Hussain, H.; Lee, Y. S.; Riaz, N.; Jabbar, A. *Nat. Prod. Rep.* **2009**, *27*, 238–254.
(4) Lee, Y. S.; Kim, S. H.; Jung, S. H.; Kim, J. K.; Pan, C. H.; Lim, S. S. *Biol. Pharm. Bull.* **2010**, *33*, 917–921.
(5) Shibano, M.; Ozaki, K.; Watanabe, H.; Tabata, A.; Taniguchi, M.; Baba, K. *Planta Med.* **2010**, *76*, 729–733.
(6) Jayaprakasam, B.; Doddaga, S.; Wang, R.; Holmes, D.; Goldfarb, J.; Li, X. M. *J. Agric. Food. Chem.* **2009**, *57*, 820–825.
(7) Liu, C. J.; Dixon, R. A. *Plant Cell* **2001**, *13*, 2643–2658.
(8) Falcao, M. J.; Pouliquem, Y. B.; Lima, M. A.; Gramosa, N. V.; Costa-Lotufo, L. V.; Militao, G. C.; Pessoa, C.; de Moraes, M. O.; Silveira, E. R. *J. Nat. Prod.* **2005**, *68*, 423–426.
(9) Takahashi, T.; Takasuka, N.; Iigo, M.; Baba, M.; Nishino, H.; Tsuda, H.; Okuyama, T. *Cancer Sci.* **2004**, *95*, 448–453.
(10) Lee, S. H.; Kim, J. Y.; Seo, G. S.; Kim, Y. C.; Sohn, D. H. *Inflammat. Res.* **2009**, *58*, 257–262.
(11) Kim, Y. W.; Ki, S. H.; Lee, J. R.; Lee, S. J.; Kim, C. W.; Kim, S. C.; Kim, S. G. *Chem. Biol. Interact.* **2006**, *161*, 125–138.
(12) Chokchaisiri, R.; Suaisom, C.; Sriphota, S.; Chindaduang, A.; Chuprajob, T.; Suksamrarn, A. *Chem. Pharm. Bull.* **2009**, *57*, 428–432.
(13) Brown, A. K.; Papaemmanouil, A.; Bhowruth, V.; Bhatt, A.; Dover, L. G.; Besra, G. S. *Microbiology* **2007**, *153*, 3314–3322.
(14) de Barros Machado, T.; Leal, I. C.; Kuster, R. M.; Amaral, A. C.; Kokis, V.; de Silva, M. G.; dos Santos, K. R. *Phytother. Res.* **2005**, *19*, 519–525.
(15) Kuramitsu, H. K.; Smorawinska, M.; Yamashita, Y. In *Cariology for the Nineties*; Bowen, W. H.; Tabak, L. A., Eds.; University of Rochester Press: Rochester, NY, 1994; pp 301−308.
(16) Liljemark, W. F.; Bloomquist, C. *Crit. Rev. Oral Biol. Med.* **1996**, *7*, 180–198.
(17) Rolla, G. *Scand. J. Dent. Res.* **1989**, *97*, 115–119.
(18) Feng, Z.; Weinberg, A. *Periodontol. 2000* **2006**, *40*, 50–76.
(19) Nishihara, T.; Koseki, T. *Periodontol. 2000* **2004**, *36*, 14–26.
(20) Kinane, D. F.; Lappin, D. F. *Acta Odontol. Scand.* **2001**, *59*, 154–160.
(21) Bainbridge, B. W.; Darveau, R. P. *Acta Odontol. Scand.* **2001**, *59*, 131–138.
(22) Giacaman, R. A.; Asrani, A. C.; Ross, K. F.; Herzberg, M. C. *Microbiology* **2009**, *155*, 3238–3246.

(23) Hajishengallis, G.; Martin, M.; Schifferle, R. E.; Genco, R. J. *Infect. Immun.* **2002**, *70*, 6658–6664.
(24) Hajishengallis, G.; Sojar, H.; Genco, R. J.; DeNardin, E. *Immunol. Invest.* **2004**, *33*, 157–172.
(25) Muthukuru, M.; Jotwani, R.; Cutler, C. W. *Infect. Immun.* **2005**, *73*, 687–694.
(26) Isobe, K. *J. Surf. Sci. Soc. Jpn.* **2001**, *22*, 652–662.
(27) Madigan, M. T.; Martinko, J. M.; Dunlap, P. V.; Clark, D. P. In *Brock Biology of Microorganisms*, 12th ed.; Brock, T. D., Ed.; Pearson Benjamin Cummings: San Francisco, 2009; p 1061.
(28) Nielsen, S. F.; Larsen, M.; Boesen, T.; Schonning, K.; Kromann, H. *J. Med. Chem.* **2005**, *48*, 2667–2677.
(29) Kornman, K. S. *Clin. Infect. Dis.* **1999**, *28*, 520–526.
(30) Assuma, R.; Oates, T.; Cochran, D.; Amar, S.; Graves, D. T. *J. Immunol.* **1998**, *160*, 403–409.
(31) Teng, Y. T.; Sodek, J.; McCulloch, C. A. *J. Periodont. Res.* **1992**, *27*, 544–552.
(32) Smith, P. C.; Munoz, V. C.; Collados, L.; Oyarzun, A. D. *J. Periodont. Res.* **2004**, *39*, 87–92.
(33) Liu, W.; Ouyang, X.; Yang, J.; Liu, J.; Li, Q.; Gu, Y.; Fukata, M.; Lin, T.; He, J. C.; Abreu, M.; Unkeless, J. C.; Mayer, L.; Xiong, H. *J. Biol. Chem.* **2009**, *284*, 24006–24016.
(34) Mandrekar, P.; Szabo, G. *J. Hepatol.* **2009**, *50*, 1258–1266.
(35) Kumar, S.; Sharma, A.; Madan, B.; Singhal, V.; Ghosh, B. *Biochem. Pharmacol.* **2007**, *73*, 1602–1612.
(36) Park, S. J.; Youn, H. S. *Phytochemistry* **2010**, *71*, 1736–1740.
(37) Ahn, S. I.; Lee, J. K.; Youn, H. S. *Mol. Cells* **2009**, *27*, 211–215.
(38) Na, H. K.; Surh, Y. J. *Mol. Carcinogen.* **2006**, *45*, 368–380.
(39) Kim, Y. W.; Zhao, R. J.; Park, S. J.; Lee, J. R.; Cho, I. J.; Yang, C. H.; Kim, S. G.; Kim, S. C. *Br. J. Pharmacol.* **2008**, *154*, 165–173.
(40) Feldman, M.; Tanabe, S.; Epifano, F.; Genovese, S.; Curini, M.; Grenier, D. *J. Nat. Prod.* **2011**, *74*, 26–31.