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Note: Bioactive Materials

Isoprenylated flavonoids from the root bark of *Morus alba* L. and their inhibition effect on NO production in LPS-induced RAW 264.7 cells

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Abstract The root bark of *Morus alba* L. were extracted with 80% aqueous MeOH, and the concentrated extract was partitioned with EtOAc, *n*-BuOH, and H_2O fractions. The repeated silica gel (SiO_2), octadecyl SiO_2 (ODS), and Sephadex LH-20 column chromatographies of the EtOAc fraction led to isolation of 12 phenolic compounds. The chemical structures of the compounds were determined as sanggenol Q (**1**), sanggenol A (**2**), sanggenol L (**3**), kuwanon T (**4**), cyclomorusin (**5**), sanggenon F (**6**), sanggenol O (**7**), sanggenon N (**8**), sanggenon G (**9**), mulberrofuran G (**10**), mulberrofuran C (**11**), and moracin E (**12**). All isolated compounds were evaluated for inhibit lipopolysaccharide-induced nitric oxide production in RAW 264.7 macrophages.

Keywords Anti-inflammatory · Isoprenylated flavonoid · *Morus alba* · Nitric oxide production · RAW 264.7 · Root bark

Introduction

The root bark of mulberry trees, known in Korea as Sang-Baek-Pi, is used for a variety of medicinal purposes in South Asian nations (Naoki et al. 2001; Ahn 2012). In previous research, we isolated isoprenylated flavonoids from root bark and evaluated them for neuroprotective and hepatoprotective activities (Jung et al. 2015). Under continued exploration for other pharmacological activities, the isolated isoprenylated flavonoids were evaluated for anti-inflammatory activity via assessment of the inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-induced RAW 264.7 cells.

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Materials and Methods

Isolation and identification of isoprenylated flavonoids

Previously, we isolated 12 isoprenylated flavonoids from the EtOAc fraction of the root bark using silica gel (SiO_2), octadecyl SiO_2 (ODS), and Sephadex LH-20 column chromatographies and were identified as sanggenol Q (**1**), sanggenol A (**2**), sanggenol L (**3**), kuwanon T (**4**), cyclomorusin (**5**), sanggenon F (**6**), sanggenol O (**7**), sanggenon N (**8**), sanggenon G (**9**), mulberrofuran G (**10**), mulberrofuran C (**11**), and moracin E (**12**) (Jung et al. 2015).

NO production assay

RAW 264.7 cells were maintained at 5×10^5 cells/mL in DMEM medium. Each cell type was supplemented with penicillin G (100 units/mL), streptomycin (100 mg/mL), L-glutamine (5 mM), and 10% heat-inactivated FBS followed by incubation under a humidified atmosphere containing 5% CO_2 and 95% air at 37 °C.

NO production was determined by measuring the amount of nitrite ion (NO_2^-) using a method based on the Griess reaction as previously described (Lee et al. 2004). RAW 264.7 cells were incubated in 96-well cell culture plates (2×10^4 cells/well) for 1 h. The cells were cultured with various concentrations of the test compounds (5, 10, 20, and 40 μM) for 12 h, and then stimulated with LPS (1 μg/mL) for additional 18 h. An aliquot of each cell culture supernatant (100 μL) was mixed with Griess reagent (100 μL) and incubated for 10 min at room temperature, and absorbance

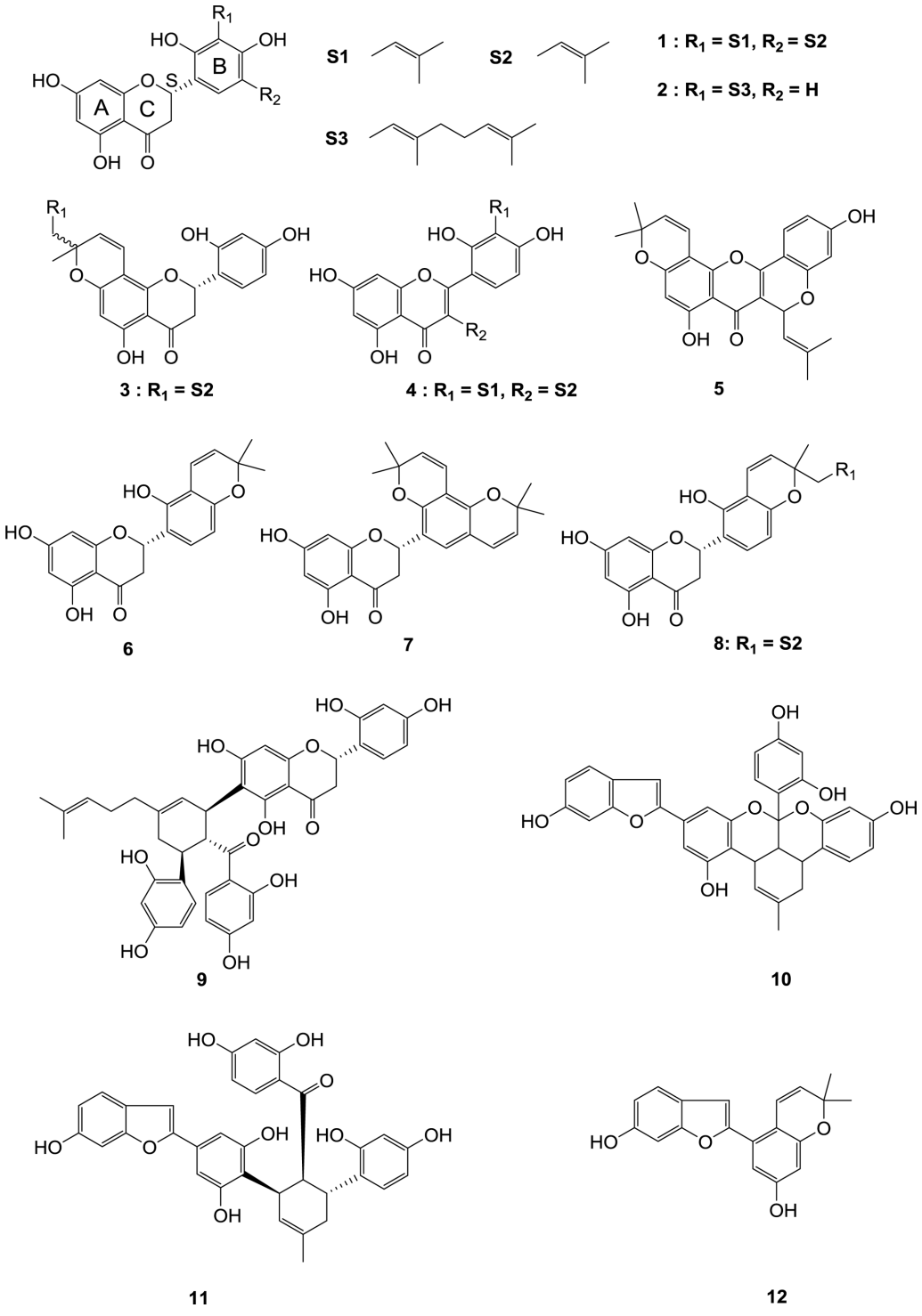

Fig. 1 Chemical structures of compounds **1-12** isolated from the root bark of *Morus alba* L.

was measured at 550 nm. The NO_2^- concentration was determined by comparison of the standard curve using the known concentration of sodium nitrite ($NaNO_2$) (Chun et al. 2012). Butein (10 μM) was used as a positive control (Lee et al. 2004).

Results and Discussion

All isolated compounds **1-12** (Fig. 1). were evaluated for inhibit LPS-induced nitric oxide production in RAW 264.7 macrophages.

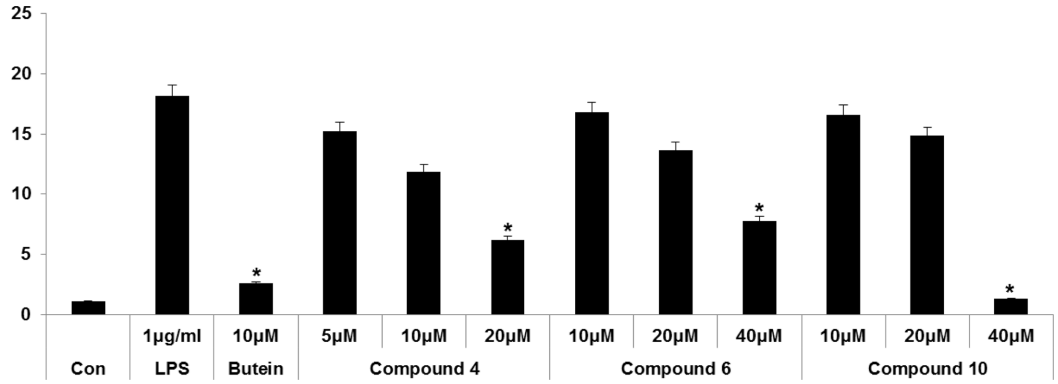

Fig. 2 Anti-inflammatory effects of compounds **4**, **6**, and **10** on NO production in LPS-induced RAW 264.7 cells. Each bar represents the mean ± S.D. of three independent experiments. *p <0.05, compared to the group treated with LPS. Butein (10 ìM) was used as a positive control

LPSs, in the form of lipoglycans and endotoxins, are found in the outer membrane of Gram-negative bacteria. The activation of LPS produces a variety of inflammatory mediators, including NO, interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), IL-6, and prostaglandins via inducible cyclooxygenase (COX-2) (Chun et al. 2012). Over-activation of NO results in many biological responses to tissue and neuronal injury, fever, and septic shock (Lee et al. 2004). Therefore, in this study, active compounds capable of blocking NO production in LPS-stimulated RAW 264.7 macrophage cells were identified. Compounds **4**, **6**, and **10** exhibited an inhibitory effect on NO production in LPS-induced RAW 264.7 cells in a dose-dependent manner (Fig. **2**). The IC_{50} values of compounds **4**, **6**, and **10** were 12.85 ± 1.84, 32.35 ± 5.25, and 24.03 ± 4.96 μM, respectively. Yang et al. (2011) also reported that isoprenylated flavonoids including kuwanon T (**4**) and sanggenon F (**6**) showed similar inhibitory effects on NO production in LPS-stimulated RAW 264.7 cells, the IC_{50} values of which were 10.0 and 19.0 μM, respectively. However, the IC_{50} values of moracin D (18.0 μM) and kuwanon E (14.9 μM) were very different from those of similar compounds **2** and **12** (greater than 40 μM). Therefore, the anti-inflammatory mechanism of extracts of Sang-Baek-Pi and its isopropylated flavonoids should be explored in further studies through the analysis of nuclear factor-κB (NF-κB), LPS-stimulated THP-1 monocytes, and inhibition of the iNOS pathway.

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References

Ahn DK (2012) Sinnongbonchogyung. Euiseungdang, Seoul

Chun JM, Cheon MS, Park M, Lee AY, Moon BC, Ji Y, Kim HK (2012) Inhibitory effects of an ethyl acetate fraction from *Cephalonoplos segetum* on inflammatory mediators from lipopolysaccharide-induced RAW 264.7 macrophages. J Korean Soc Appl Biol Chem 55: 41–46

Jung JW, Ko WM, Park JH, Seo KH, Oh EJ, Lee DY, Lee DS, Kim YC, Lim DW, Han D, Baek NI (2015) Isoprenylated flavonoids from the root bark of *Morus alba* and their hepatoprotective and neuroprotective activities. Arch Pharm Res 38: 2066–2075

Lee SH, Seo GS, Sohn DH (2004) Inhibition of lipopolysaccharide-induced expression of inducible nitric oxide synthase by butein in RAW 264.7 cells. Biochem Biophys Res Commun 323: 125–132

Naoki A, Toru Y, Kayo Y, Kyoto I, Haruhisa K, Yukihiko K, Atsushi K, Robert JN, Lee HS, Ryu KS (2001) Polyhydroxylated alkaloids isolated from mulberry trees (*Morus alba* L.) and silkworms (*Bombyx mori* L.). J Agric Food Chem 49: 4208–4213

Yang ZG, Matsuzaki K, Takamatsu S, Kitanaka (2011) Inhibitory effects of constituents from *Morus alba* var. *multicaulis* on differentiation of 3T3-L1 cells and nitric oxide production in RAW264.7 cells. Molecules 16: 6010–6022