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Research Letter

Practical Synthesis and Biological Evaluation of Bergenin Analogs

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Here, we describe the practical synthesis and biological properties of bergenin and its structural analogs. Synthetic bergenin compounds were prepared by acylation of bergenin. These compounds were then evaluated for suppression of lipopolysaccharide-induced nitric oxide (NO) generation in cultured cells and anti-narcotic effects on morphine-dependent mice. We found that bergenin derivatives showed potent anti-inflammatory activity (suppression of NO generation) at concentrations ranging from 20 to 30 μM *in vitro*, and bergenin derivatives (10–20 mg/kg) exhibited significant anti-narcotic effects on morphine dependence in mice. These results suggest the potential utility of bergenin and its analogs as anti-narcotic agents and the design of more potent anti-inflammatory compounds.

Key words: bergenin, methylation, acylation reaction, NO generation, neurotoxicity

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Natural polyphenolic products are often used as intermediates for industrial products and phytochemical applications including food sciences, as well as for traditional drugs (1). These compounds act as scavengers for free radicals that are correlated with the progression of various pathological conditions. Isocoumarin derivatives norbergenin **1** and bergenin **2** have been isolated from the roots of *Bergenia crassifolia* (2,3), bark of *Corylopsis spicata* (4), heartwood of *Shorea leprosula* (5), and roots of *Caesalpinia digyna* (6) and have evoked a great deal of interest because of their biological properties and characteristic fused ring molecular architecture (Figure 1) (7,8).

Bergenin moieties possess important pharmacological properties including analgesic (9), anti-arthritis (10), anti-arrhythmic, antioxidative antimicrobial, hepatoprotective, anti-inflammatory, and anticancer effects (11–15). They are also used to enhance wound healing and improve symptoms of Alzheimer's disease and senescence.

Bergenin-containing herbs were traditionally used for cardiac arrhythmias, and a number of current comparative pharmacological investigations of bergenin and its derivatives have found good antioxidant activity with low side effects and little toxicity.

A recent report (16–18) described the simple synthesis of bergenin derivatives and their biological properties. The Fukuyama and other groups have researched the synthesis of synthetic bergenin moieties and their antioxidant and neuroprotective activities (19). Our medicinal research program is focused on the synthesis of biologically active bergenin derivatives, and we report here the simple derivatization of bergenin by an acylation reaction. The biological activities of bergenin and its structural analogs in suppression of lipopolysaccharide (LPS)-induced nitric oxide (NO) generation *in vitro* suggest their possible usefulness as novel antioxidant and anti-inflammatory lead compounds.

Experimental Section

General procedure

Reactions requiring anhydrous conditions were performed with standard precautions for rigorous exclusion of air and moisture. Thin layer chromatography was performed on precoated silica gel G and GP uniplates from Analtech and visualized with 254-nm UV light. Flash chromatography was carried out on Merck silica gel 60 [particle size 230–400 μm, pore size 60 Å]. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX 250 (Bruker, Germany) at 250 and 63 MHz, respectively. The chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane, and *J*-values are in Hz. Infrared (IR) spectra were obtained on an JASCO FT/IR-430 spectrometer (USA). Mass spectra were recorded with an Applied Biosystems (USA) 4700 proteomics analyzer spectrometer. When necessary, chemicals were purified as previously described (20).

General procedure for the preparation of compounds 3–6 by condensation of bergenin 2 and various acid chlorides

A stirred solution of bergenin **2** (1 g, 3.05 mmol) in pyridine (10 mL) was added dropwise to appropriate acid chlorides or ethyl chloroformate (16.0 mmol) at 0 °C, and the reaction mixture was warmed to room temperature and then stirred for 16 h. The reaction was quenched with slow addition of cold water (20 mL) and extracted with ethyl acetate (20 mL). The organic phase was separated and washed with saturated aqueous $NaHCO_3$ solution (20 mL) and brine (20 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to provide crude product, which was purified by flash silica chromatography to obtain pure bergenin derivatives **3–6**.

Penta-O-acetyl bergenin (3)

White solid, mp 206–207 °C; IR ν_{max} (KBr) 3024, 2950, 1755, 1748, 1732, 1609, 1486, 1455, 1428, 1372, 1328, 1196, 1091, 1032, 962, 892/cm; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.77 (s, 1H), 5.48 (t, J = 9.48 Hz, 1H), 5.13 (t, J = 9.64 Hz, 1 H), 4.82 (d, J = 10.74 Hz, 1H), 4.40–4.29 (m, 2H), 4.14 (dd, J = 12.64, J = 3.79 Hz, 1H), 3.91 (s, 3H), 3.85–3.79 (m, 1H), 2.34 (d, J = 2.53 Hz, 6H), 2.11–2.07 (m, 9H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 170.5, 170.0, 169.7, 168.3, 167.7, 161.7, 150.2, 144.4, 141.5, 129.7, 124.1, 118.8, 77.7, 77.2, 76.8, 76.7, 73.0, 72.3, 68.5, 62.0, 61.6, 20.8, 20.7; MALDI-TOF-MS calcd. for $C_{24}H_{26}O_{14}$ 561.1215 $[M+Na]^+$, found: 561.3566.

Penta-O-caproyl bergenin (4)

Light yellow liquid, IR ν_{max} (KBr) 2956, 2891, 1747, 1609, 1484, 1459, 1425, 1378, 1327, 1217, 1165, 1095, 1016, 964, 885/cm; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.75 (s, 1H) 5.50 (t, J = 9.47, 1H) 5.17(t, J = 9.55 Hz, 1H), 4.84 (d, J = 10.58 Hz, 1H), 4.23–4.41 (m, 2H), 4.07–4.18 (m, 1 H), 3.88 (s, 3H), 3.82–3.78 (m, 1H), 2.60 (t, J = 7.42 Hz, 4H), 2.42–2.23 (m, 6H), 1.82–1.55 (m, 10H), 1.43–1.28 (m, 20H), 0.95–0.85 (m, 15H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 173.3, 172.7, 172.2, 171.2, 170.6, 161.7, 150.1, 144.4, 141.6, 129.6, 124.0, 118.8, 77.4, 76.9, 73.0, 71.9, 67.9, 61.5, 34.0, 34.0, 33.9, 33.8, 31.2, 31.2, 24.5, 24.4, 24.4, 22.4, 22.3; MALDI-TOF-MS calcd. for $C_{44}H_{66}O_{14}$ 841.4345 $[M+Na]^+$, found: 841. 5308.

Penta-O-ethoxycarbonyl bergenin (5)

White solid, mp 69–72 °C; IR ν_{max} (KBr) 2985, 2912, 1762, 1612, 1488, 1467, 1430, 1394, 1371, 1335, 1281, 1126, 1094, 1008, 876, 758/cm; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.85 (s, 1H), 5.38 (t, J = 9.48 Hz, 1H), 5.34–4.97 (m, 2H), 4.47–4.16 (m, 13H), 3.98 (s, 3H), 3.93 (t, J = 3.16 Hz, 1H), 1.42–1.28 (m, 15H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 161.2, 154.7, 154.2, 153.9, 152.4, 151.9, 150.1, 144.4, 141.5, 129.4, 123.5, 118.4, 77.7, 77.2, 76.7, 76.4, 76.0, 75.8, 72.3, 72.1, 65.7, 65.1, 64.9, 64.8, 64.4, 61.8, 14.1, 14.0; MALDI-TOF-MS calcd. for $C_{29}H_{36}O_{19}$ 711.1743 $[M+Na]^+$, found: 711.4653.

Penta-O-benzoyl bergenin (6)

White solid, mp 145–147 °C; IR ν_{max} (KBr) 3064, 3021, 2949, 1731, 1601, 1584, 1485, 1451, 1427, 1370, 1330, 1314, 1279, 1065, 1025, 992, 851/cm; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.20 (d, J = 7.58 Hz, 3H), 7.95 (d, J = 7.90 Hz, 5H), 7.81 (d, J = 7.27 Hz, 2H), 7.68–7.23 (m, 16 H), 6.05 (t, J = 9.64 Hz, 1H), 5.72 (brs, 1H), 5.16 (d, J = 9.16 Hz, 1H), 4.73 (t, J = 10.11 Hz, 1H), 3.97–4.05 (m, 2 H) 3.93 (s, 3 H), 3.48 (d, J = 10.16 Hz, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 165.6, 165.0, 161.7, 144.6, 134.2, 133.5, 133.4, 133.1, 130.4, 130.0, 129.8, 129.7, 129.5, 129.0, 128.9, 128.6, 128.4, 77.7, 77.2, 76.7, 76.6, 72.6, 68.8, 61.9; MALDI-TOF-MS calcd. for $C_{49}H_{36}O_{14}$ 871.1997 $[M+Na]^+$, found: 871.5660.

BV-2 microglia culture

The murine BV-2 microglia cell line was maintained in DMEM supplemented with 10% fetal bovine serum and penicillin/streptomycin at 37 °C in a humidified incubator under 5% CO_2. For all experiments, cells were plated at a density of 1×10^5 cells/mL in 24-well plates and then treated with 100 ng/mL LPS alone or with various concentrations of compounds for 24 h at 37 °C.

Nitric oxide assay

The Griess reaction was used to perform nitrite assays. Cells were incubated with LPS (100 ng/mL) and various concentrations of bergenin derivatives for 24 h at 37 °C. The culture media were then mixed with an equal volume of reagent (one part 0.1% *N*-1-naphthylethylenediamine dihydrochloride, one part 1% sulfanilamide in 5% phosphoric acid) in 96-well plates. The absorbance was determined at 540 nm using a microplate reader. Data are reported as the mean ± the SD of three observations.

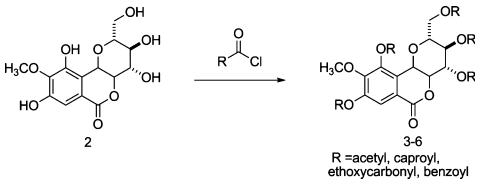
Animals and drug administration

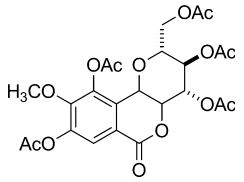
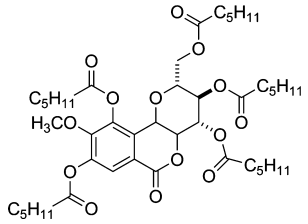
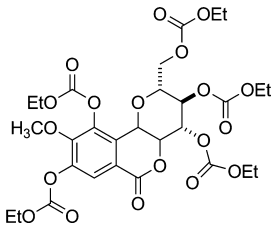
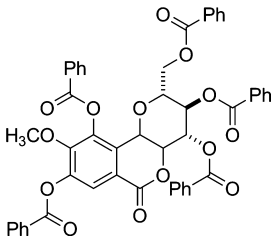
C57BL/6 mice were obtained from Daehan Biolink (Eumsung, Korea). Mice were housed with a 12-h light/dark cycle and maintained at 24 ± 3 °C. All animal procedures were performed in accordance with the Institutional Animal Care and Use Committee of Ewha Womans University. The mice (male, 20 ± 2 g) were randomly divided into each group (n = 10) and were given saline, morphine, or both morphine and bergenin derivatives. The morphine chloride (10 mg/kg/day, Myungmun Pharm., Seoul) was dissolved in saline, and bergenin derivatives (10 or 20 mg/kg/day) were dissolved in 10% cremophor solution containing 2% dimethyl sulfoxide. Morphine was administered daily for 7 days intraperitoneally (i.p.), and bergenin derivatives were administered orally 30 min prior to injection of morphine. Naloxone hydrochloride (5 mg/kg, i.p.) was injected 6 h after the final morphine injection for induction of morphine withdrawal syndrome in mice.

Measurement of morphine withdrawal syndrome

Morphine withdrawal syndrome was induced by injection of naloxone (5 mg/kg), a competitive antagonist with high opioid receptor affinity. Immediately after the naloxone injection, mice were placed into individual observation cylinders (24 cm in diameter and 50 cm high), and the frequency of jumps of each mouse was observed for 30 min. The data were expressed as mean ± SE. Statistical differences were analyzed by Student's t-test.

Table 1: Synthesis of bergenin derivatives **3–6**



Product	Compound	Formula	Yield[a] (%)
3		$C_{24}H_{26}O_{14}$	71
4		$C_{44}H_{66}O_{14}$	59
5		$C_{29}H_{36}O_{19}$	69
6		$C_{49}H_{36}O_{14}$	95

[a]Isolated pure yield.

Results and Discussion

Chemistry

A series of bergenin derivatives was prepared from naturally extracted bergenin 2-(4-methoxy-2-[(1*S*,2*R*,3*S*,4*S*,5*R*)-3,4,5,6-tetrahydro-3,4,5-trihydroxy-6-(hydroxymethyl)-2*H*-pyran-2-yl]-α-resorcylic acid δ-lactone), which was treated with several acyl chlorides or ethyl chloroformate (acetyl chloride, caproyl chloride, ethyl chloroformate, and benzoyl chloride) in the presence of two equivalents of base in dichloromethane to provide bergenin analogs **3–6** at high yields (Table 1). Even though bergenin analogs **3–6** were modified by acyl groups on hydroxyl groups of the bergenin skeleton, their structural composition remained as a six-membered ring, aromatic ring, glucopyranose ring, and annellated δ-lactone ring. In addition, these compounds contain three stereogenic centers and secondary or primary alcohol groups on the tetrahydropyran ring (21).

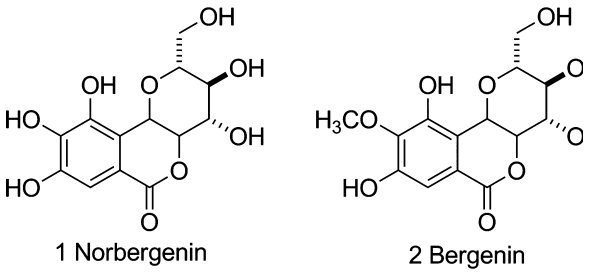


Figure 1: Structures of norbergenin **1** and bergenin **2**.

This simple one-step condensation reaction produced novel derivatives **3–6** as listed in Table 1. All physical and spectral data for synthetically prepared compound **3** were identical to those reported (19), and all new compounds **4–6** have given satisfactory analytical and spectral data.

Biology

Nitrite was used as a measure of NO production. The *in vitro* suppression of LPS-induced NO generation by the prepared bergenin derivatives was evaluated as previously described (22). Bergenin and most of the bergenin **3–6** derivatives inhibited nitrite accumulation in LPS-stimulated microglia BV-2 cells at a concentration of 20–30 μM (Figure 2). In addition, bergenin derivatives acted well as anti-narcotics for morphine dependence. Interestingly, compounds **3–6** showed more favorable anti-narcotic activity compared with bergenin. These compounds seemed to be enhancing bioavailability by increasing lipophilicity in the *in vitro* experiment in cells.

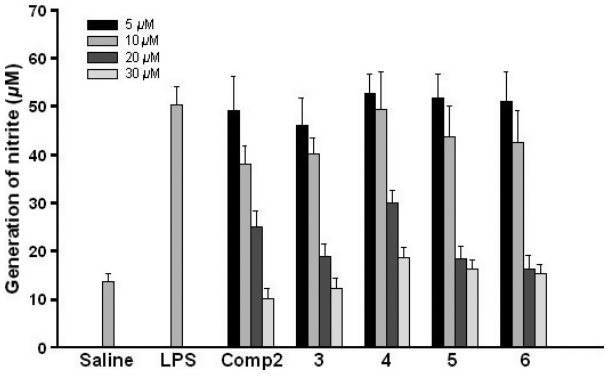


Figure 2: Effect of bergenin and bergenin derivatives **3–6** on nitrite production in lipopolysaccharide (LPS)-stimulated BV-2 microglia cells. Cells were treated with 100 ng/mL LPS. Various concentrations of bergenin compounds (5–30 μM) were added for 24 h at 37 °C. Values indicate nitrite production from culture supernatants of LPS-treated cells with or without bergenin compounds. Data represent the mean ± SD of three observations.

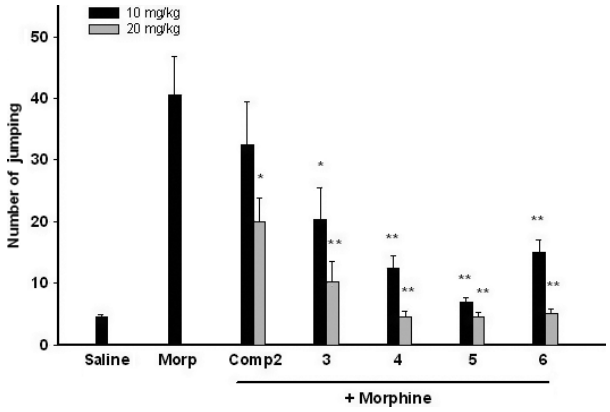


Figure 3: Effects of bergenin derivatives on naloxone-induced jumping behavior in morphine-dependent mice. Bergenin and its derivatives (10, 20 mg/kg, p.o.) were administered 30 min before morphine injection (10 mg/kg, i.p., n = 10) for 7 days. On the 7th day, naloxone (5 mg/kg, i.p.) was injected 6 h after final drug administration. The number of jumps in a 30-min interval was counted after naloxone injection. Statistical differences were analyzed by Student's t-test. *$p < 0.05$, **$p < 0.01$ in comparison with morphine-only group.

Anti-narcotic properties of bergenin and its derivatives

Mice received morphine (10 mg/kg/day, i.p.) for 7 days to develop a dependence on morphine. The effects of bergenin derivatives (10, 20 mg/kg/day, p.o.) were examined on naloxone-induced jumping behavior in morphine-dependent mice. The frequency of jumping, which is an indicator of morphine withdrawal symptom, was decreased by treatment with bergenin **2** and bergenin derivatives **3–6** (Figure 2). Interestingly, each of the bergenin derivatives inhibited the number of jumps in morphine-dependent mice after administration of 10 and 20 mg/kg, respectively: **2** (19%, 50%), **3** (50%, 75%), **4** (69%, 88%), **5** (83%, 89%), and **6** (63%, 88%) (Figure 3).

Compounds **4**, **5**, and **6** displayed especially strong attenuation at 20 mg/kg oral administration. By employing the naloxone-induced jumping test, we found that compound **5** displayed the most effective anti-narcotic activity among bergenin derivatives.

Conclusions

Here, we demonstrated the simple and practical synthesis and evaluation of bergenin derivatives. We expect that this method will prove to be useful for the practical preparation of new derivatives and modifications of bergenin. Biological studies showed that bergenin compounds **3–6** possessed enhanced activities for suppression of LPS-induced NO generation and anti-narcotic effects compared with bergenin. Our data are potentially useful for the design of more potent anti-inflammatory and anti-narcotic compounds.

Acknowledgments

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