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Article

Triterpenoids from Roots of *Glycine max* (L.) Merr.

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Metanolic root extract of *Glycine max* (L.) Merr. was chromatographed, affording three triterpenoids 1-3. The compound 3 was isolated from microorganism called *Fusarium sporotrichioides* but never been isolated from any plant sources. Compounds 1-3 showed cytotoxic activity against HT-59 human cancer cell line with IC_{50} values of 62.9, 20.0, and 44.2 μM, respectively. Compound 3 showed significant activities against Gram-positive bacterial such as *Bacillus cereus*, *Bacillus subtilis*, and *Staphylococcs aureus*.

Key words: *Glycine max (L.) Merr. roots, triterpenoid, cytotoxicity, antibacterial, HT-59 human cancer cell*

Numerous triterpenoids, which are widely distributed in plants, have been reported during the last decades due to their medicinal properties such as inhibition of HIV.[1-6] Recently, triterpenoids have become the focus of target compounds for human diseases including anticancer, antiviral, and LDL-antioxidant activities.[7-9]

Soybean (*Glycine max* (L.) Merr.) have been well-known to have health-protective properties against, among others, cancer,[10,11] coronary heart disease,[12-14] and osteoporosis.[15,16] The biologically active components in soybeans and soy products responsible for these beneficial effects are attributable to the groups of saponins and isoflavones namely genistein, daidzein, and glycitein.[17-19] Even though researchers have mainly focused on biological activities and secondary metabolites of soybeans due to their potential medicinal effects, only few studies have been performed on phytochemicals such as triterpenoids. We recently, for the first time, reported on the evaluation of LDL-antioxidant activities of soybean pterocarpans and isoflavones.[20] In addition, in search of new biological function of this species, we found that triterpenoids isolated from the methanolic extract from roots of *G. max* showed potent cytotoxic and antibacterial activities.

Here, we report on the isolation and structural elucidation of soyasapogenol B (**1**), (24*R*)-ethylcholestan-3β,7β-diol (**2**), and 12β-acetoxy-4,4-dimethyl-24-methylene-5α-cholesta-8, 14-dien-3β,11α-diol (3) from roots of *G. max* and also on the biological activities such as cytotoxic and antibacterial activities.

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Abbreviations: DEPT, distortionless enhancement by polarization transfer; HMBC, heteronuclear multiple-bond connectivity

Materials and Methods

Plant Material. The roots of *G. max* (Taekwangkong) were collected ten days after R8 at Moonsan, Jinju, Korea at the end of September 2003. The fresh roots of *G. max* were then dried.

Instruments. All purifications were monitored by TLC (E. Merck Co., Darmstadt, Germany), using commercially available glass-backed plates sprayed with 10% H_2SO_4 solution. Column chromatography was carried out using 230-400 mesh silica gel (Kieselgel 60, Merck, Germany). Melting points were measured on a Thomas Scientific capillary melting point apparatus (Electrothermal 9300, UK) and are uncorrected. IR spectra were recorded on a Bruker IFS66 (Bruker, karlsruhe, Germany) infrared Fourier transform spectrophotometer (KBr) and ^{1}H- and ^{13}C-NMR along with 2D-NMR data were obtained on a Bruker AM 500 (^{1}H-NMR at 500 MHz, ^{13}C-NMR at 125 MHz) spectrometer (Bruker, karlsruhe, Germany) in CD_3OD and pyridine-d_6. EIMS was obtained on a JEOL JMS-700 mass spectrometer (JEOL, Tokyo, Japan). Optical rotations were measured on a JASCO DIP-1000 polarimeter. All reagent-grade chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Extraction and isolation. The roots of *G. max* (2.4 kg) were cut into small pieces, air-dried, pulverized and extracted three times with methanol for 10 days at room temperature. The methanol extracts were combined, concentrated to dryness under reduced pressure to obtain a green gum (120 g) and dissolved in 1.2 L mixture of water and methanol (3 : 1), which was successively fractionated with *n*-hexane, EtOAc, and *n*-BuOH. The EtOAc extract (42.0 g) was subjected to chromatography over silica gel (450 g, 70-230 mesh), eluted initially with chloroform, followed by chloroform-acetone mixture of increasing polarity (50 : 11 : 2), yielding 15 fractions (Fr.1-Fr.15), which were differentiated based on the comparison of TLC profile. The fraction 9 (4.6 g) was eluted with *n*-

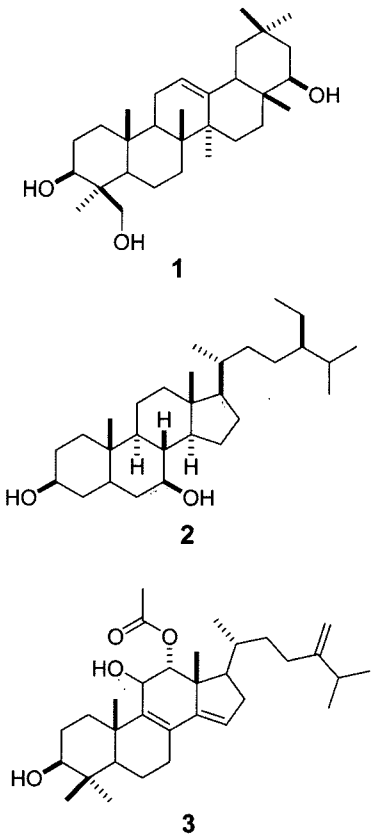

Fig. 1. Structures of isolated compounds 1-3 from roots of *G max*.

hexane:acetone mixtures of increasing polarity (30 : 15 : 1). Altogether, 110 subfractions were collected and combined to give ten major subfractions (72-81) based on the comparison of TLC profiles. The ten major subfractions were further purified in small chromatographic column containing silica gel, eluting with *n*-hexane:EtOAc (1 : 1) to afford the pure compound **1** (1.6 g). The fraction 14 (1.9 g) was subjected to a silica gel column chromatography (5.7 g, 230-400 mesh), eluted with a $CHCl_3$: acetone gradient (15 : 11 : 2) to give 55 subfractions. Subfractions 40-42 were concentrated and subjected to preparative TLC [$CHCl_3$: acetone (1 : 1)], yielding compound **2** (21 mg). Subfractions 45-54 were separately subjected to silica gel column chromatography with the same solvent as used for compound **2** and further purified by sephadex LH-20, eluting with MeOH to afford compound **3** (45 mg).

Compound **1**: white needles; mp 258-259°C; $[\alpha]_D^{20}$ +92.4 (*c* 1.00, CH_3OH); EIMS *m/z* 458; IR (KBr) ν_{max} 3440, 2250, 1360 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 0.74 (3H, s, H-28), 0.79 (1H, dd, *J* = 1.8, 12.3 Hz, H-6), 0.81 (3H, s, H-29), 0.85 (1H, m, H-19α), 0.86 (3H, s, H-25), 0.88 (3H, s, H-26), 0.90 (1H, m, H-1α), 0.91 (3H, s, H-30), 0.95 (1H, m, H-2α), 1.03 (3H, s, H-27), 1.10 (3H, s, H-23), 1.21 (1H, m, H-15α), 1.24 (1H, m, H-21α), 1.29 (2H, m, H-6), 1.33 (1H, m, H-21β), 1.35 (1H, m, H-7α), 1.46 (1H, m, H-7β), 1.51 (1H, m, H-9), 1.56 (1H, m, H-1β), 1.58 (1H, m, H-16α), 1.62 (1H, m, H-16β), 1.63 (1H, m, H-2β), 1.64 (1H, m, H-15β), 1.65 (1H, m, H-19β), 1.80 (2H, m, H-11), 1.96 (1H, d, *J* = 12.8 Hz, H-18), 3.25 (1H, dd, *J* = 3.9, 12.0 Hz, H-3), 3.28 (1H, d, *J* = 11.1 Hz, H-24α), 3.28 (1H, m, H-22), 4.03 (1H, d, *J* = 11.1 Hz, H-24β), and 5.16 (1H, t, *J* = 3.6 Hz, H-12). ^{13}C NMR (125 MHz, CD_3OD): see Table 1.

Table 1. ^{13}C-NMR of compounds 1-3 at 125 MHz (ppm, m)[a]

Position	Compounds		
	1	2	3
1	40.2 (t)	33.8 (t)	34.4 (t)
2	27.3 (t)	27.5 (t)	27.4 (t)
3	81.7 (d)	71.1 (d)	86.9 (d)
4	43.8 (s)	38.1 (t)	39.2 (s)
5	57.7 (d)	32.4 (d)	51.7 (d)
6	20.2 (t)	36.6 (t)	18.5 (t)
7	35.0 (t)	68.8 (d)	24.9 (t)
8	41.2 (s)	46.1 (d)	126.1 (s)
9	49.6 (d)	54.4 (d)	139.5 (s)
10	39.0 (s)	36.5 (s)	37.7 (s)
11	25.2 (t)	21.6 (t)	68.9 (d)
12	124.0 (d)	42.9 (t)	79.3 (d)
13	145.7 (s)	42.8 (s)	47.4 (s)
14	44.0 (s)	56.5 (d)	148.0 (s)
15	30.3 (t)	23.5 (t)	121.0 (d)
16	28.8 (t)	26.4 (t)	35.4 (t)
17	38.3 (t)	56.6 (d)	49.9 (d)
18	47.9 (d)	13.8 (q)	16.5 (q)
19	47.9 (t)	12.3 (q)	22.2 (q)
20	32.0 (s)	27.5 (d)	33.9 (d)
21	42.7 (t)	19.0 (q)	18.1 (q)
22	77.4 (d)	34.7 (t)	35.1 (t)
23	23.6 (q)	24.8 (t)	31.0 (t)
24	65.7 (t)	19.3 (d)	156.9 (s)
25	17.0 (q)	33.9 (d)	34.2 (d)
26	18.0 (q)	19.0 (q)	21.8 (q)
27	26.0 (q)	19.3 (q)	21.9 (q)
28	20.8 (q)	23.5 (d)	106.4 (t)
29	33.0 (q)	12.2 (q)	16.2 (q)
30	29.5 (q)		28.2 (q)
1'			171.6 (s)
2'			20.4 (q)

[a]The chemical shifts of compounds **1** and **3** were determined in CD_3OD and compound **2** was measured in pyridine-d_6.

Compound **2**: white crystals; mp 185-186°C; $[\alpha]_D^{20}$ −28.1 (*c* 0.80, CH_3OH); EIMS *m/z* 432; IR (KBr) ν_{max} 3450, 1760 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_6) δ1.85 (1H, m, H-9), 1.99 (3H, s, H-18), 2.00 (3H, d, *J* = 2.7 Hz, H-26), 2.02 (3H, s, H-19), 2.03 (3H, s, H-29), 2.03 (3H, d, *J* = 2.7 Hz, H-27), 2.10 (1H, m, H-8), 2.13 (3H, d, *J* = 6.5 Hz, H-21), 2.19 (2H, m, H-15), 2.20 (1H, m, H-17), 2.22 (1H, m, H-6α), 2.27 (1H, m, H-14), 2.27 (1H, m, H-4α), 2.35 (1H, m, H-12α), 2.37 (1H, m, H-28α), 2.38 (2H, m, H-23), 2.39 (2H, m, H-16), 2.40 (1H, m, H-11α), 2.41 (1H, m, H-20), 2.43 (1H, m, H-28β), 2.48 (1H, m, H-24), 2.52 (2H, m, H-22), 2.55 (2H, m, H-1), 2.61 (1H,

m, H-25), 2.65 (1H, m, H-11β), 2.85 (1H, m, H-6β), 2.85 (1H, m, H-2α), 3.12 (1H, m, H-4β), 3.20 (1H, m, H-2β), 3.40 (1H, m, H-12β), 4.14 (1H, m, H-5), 4.83 (1H, m, H-7), and 5.05 (1H, m, H-3). ^{13}C NMR (125 MHz, pyridine-d_6): see Table 1.

Compound **3**: white crystals; mp 181°C; $[\alpha]_D^{20}$ +32.7 (*c* 0.40, CH_3OH); EIMS *m/z* 498; IR (KBr) ν_{max} 3460, 1712 cm^{-1}; UV λ_{max} nm 248 (CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 0.80 (3H, s, H-29), 0.83 (3H, d, *J* = 6.5 Hz, H-21), 0.93 (3H, d, *J* = 3.5 Hz, H-26), 0.94 (3H, d, *J* = 3.5 Hz, H-27), 0.97 (3H, s, H-30), 0.99 (3H, s, H-18), 1.08 (1H, s, H-5), 1.18 (3H, s, H-19), 1.49 (1H, m, H-22α), 1.68 (1H, m, H-20), 1.70 (2H, m, H-6), 1.75 (1H, m, H-7α), 1.86 (1H, m, H-17), 1.90 (3H, s, H-2'), 1.90 (1H, m, H-1α), 1.98 (1H, m, H-16α), 2.01 (1H, m, H-22β), 2.02 (1H, m, H-1β), 2.05 (1H, m, H-23α), 2.09 (1H, m, H-23β), 2.10 (1H, m, H-7β), 2.15 (1H, m, H-25), 2.20 (2H, m, H-2), 2.30 (1H, m, H-16β), 3.88 (1H, m, H-3), 4.13 (1H, d, *J* = 1.1 Hz, H-11), 4.57 (1H, s, H-28α), 4.64 (1H, s, H-28β), 4.96 (1H, d, *J* = 1.1 Hz, H-12), and 5.47 (1H, s, H-15). ^{13}C NMR (125 MHz, CD_3OD): see Table 1.

Cytotoxic activity (Sulforhodamine B assay). Human cancer cell line, HT-59 (colon) was cultivated in humidified incubators (37°C, 5% CO_2) and the cells were grown in RPMI 1640 to assess the growth inhibition by a colorimetric assay, which estimates cell number indirectly by staining total cellular protein with the dye Sulforhodamine B (SRB).[21] In brief, cells were fixed by layering 100 μ*l* ice-cold 15% trichloroacetic acid (TCA) on top of the growth medium. They were then incubated at 4°C for 1 h. The plates were then washed five times with cold water, the excess water drained off, and the plates were air-dried. SRB stain (100 μ*l*; 0.4 in 1% acetic acid) was added to each well and left in contact with the cells for 1 h. Subsequently, the cells were washed with 1% acetic acid and rinsed four times. The plates were dried, and 1 m*l* of 10 mM Tris base was added to each well to dissolve the dye. The plates were shaken gently for 20 min on a gyratory shaker, and the absorbance (OD) of each well was read on a spectrophotometer at 540 nm. Cell survival was measured as the percentage absorbance compared to the control.

Antibacterial assay. The antibacterial activity was evaluated *in vitro* by the paper-disk diffusion method against eight bacterial strains,[22] selected as representative classes of Gram positive and Gram negative. The used microorganisms were *Bacillus subtilis* (ATCC 9372), *Bacillus cereus* (ATCC 27348), *Staphylococcus aureus* (ATCC 13301), *Pseudomonas aeruginosa* (ATCC 21636), *Staphylococcus epidermidis* (ATCC 12228), *Salmonella enteritidis* (ATCC 13076), *Salmonella typhimurium* (ATCC 14028), and *Escherichia coli* (ATCC 15489). Aliquots of the samples were dissolved in DMSO, and a 20 μL portion of each solution was placed on an 8 mm Whatman paper disk to give a concentration of 5 μg/disk. The disk was then placed on an agar plate seeded with the microorganism and, after incubating for 12 h, the zone of inhibition was measured.

Results and Discussions

Repeated silica gel chromatography and recyclization of methanol extracts from roots of *G. max* yielded soyasapogenol B (**1**), (24*R*)-ethylcholestan-3β,7β-diol (**2**), and 12β-acetoxy-4,4-dimethyl-24-methylene-5α-cholesta-8,14-dien-3β,11α-diol (**3**). Compound **1** was obtained as white needles. IR spectrum revealed absorption bands due to hydroxyl (3440 cm^{-1}) group. The ^{1}H-NMR data showed the evidence for seven methyl protons [δ 0.74, 0.81, 0.86, 0.88, 0.91, 1.03, and 1.10 (each 3H, s)], five methine proton [δ 3.25 (H-3), 0.79 (H-6), 1.51 (H-9), 1.96 (H-18), and 3.28 (H-22)], one olefinic methine proton δ 5.16 (1H, t, *J* = 3.6 Hz, H-12), and ten methylenes protons. The complex overlapping proton signals in the 0.5-2.1 ppm region of the proton spectrum was analyzed using ^{1}H-^{1}H COSY. Figure 2 showed important HMBC correlation and the positioning of the substituents on the ring system. All data mentioned above indicate that the structure of compound **1** is soysapogenol B.[23] Compound **2** was obtained as white crystals and in the EIMS, the molecular ion peak showed at *m/z* 432. The IR spectrum showed the presence of hydroxyl (3450 cm^{-1}). The ^{1}H-NMR data showed the evidence for six methyl protons [δ 1.99 (s, H-18), 2.02 (s, H-19), 2.13 (d, *J* = 6.5 Hz, H-21), 2.00 (d, *J* = 2.7 Hz, H-26), 2.03 (d, *J* = 2.7 Hz, H-27), and 2.03 (s, H-29)], ten methine protons [δ 5.05 (H-3), 4.14 (H-5), 4.83 (H-7), 2.10 (H-8), 1.85 (H-9), 2.27 (H-14), 2.20 (H-17), 2.41 (H-20), 2.48 (H-24), and 2.61 (H-25)], and eleven methylene protons [δ 2.55 (H-1), 2.85 (H-2α), 3.20 (H-2β), 2.27 (H-4α), 3.12 (H-4β), 2.22 (H-6α), 2.85 (H-6β), 2.40 (H-11α), 2.65 (H-11β), 2.35 (H-12α), 3.40 (H-12β), 2.19 (H-15), 2.39 (H-16), 2.52 (H-22), 2.38 (H-23), 2.37 (H-28α), 2.43 (H-28β)]. In the ^{1}H-^{1}H COSY spectrum, the signal of the methine proton attached to carbon bearing a hydroxyl group at δ 5.05 (1H, m, H-3) was coupled with two methylene proton signals at δ 2.55 (2H, m, H-1) and at δ 2.85 (1H, m, H-2α) and 3.20 (1H, m, H-2β). However, in the HMBC correlations, these methylene signal at δ (71.1, C-3), and the methylene carbon signal at δ (33.8, C-1) was correlated with the methyl proton signal at δ 2.02 (3H, s, H-19) (Fig. 2). All data mentioned above indicate that the structure of compound **2** is identified as (24*R*)-ethylcholestan-3β,7β-diol. This compound was first reported from this plant. Compound **3** was obtained as white crystals with a molecular ion peak at *m/z* 498. The IR spectrum showed the presence of hydroxyl (3460 cm^{-1}) and carbonyl (1712 cm^{-1}). The ^{1}H-NMR data showed the evidence for eight methyl protons [δ 0.99 (H-18), 1.18 (H-19), 0.83 (d, *J* = 6.5 Hz, H-21), 0.93 (d, *J* = 3.5 Hz, H-26), 0.94 (d, *J* = 3.5 Hz, H-27), 0.80 (H-29), 0.97 (H-30), and 1.90 (H-2')], eight methine protons [δ 3.88 (H-3), 1.08 (H-5), 4.13 (H-11), 4.96 (H-12), 5.47 (H-15), 1.86 (H-17), 1.68 (H-20), and 2.15 (H-25)], and eight methylene protons [δ 1.90 (H-1α), 2.02 (H-1β), 2.20 (H-2), 1.70 (H-6), 1.75 (H-7α), 2.10 (H-7β),1.98 (H-16α), 2.30 (H-16β), 1.49 (H-22α), 2.01 (H-22β), 2.05 (H-23α), 2.09 (H-23β), 4.57 (H-28α), and 4.64 (H-28β)]. In the

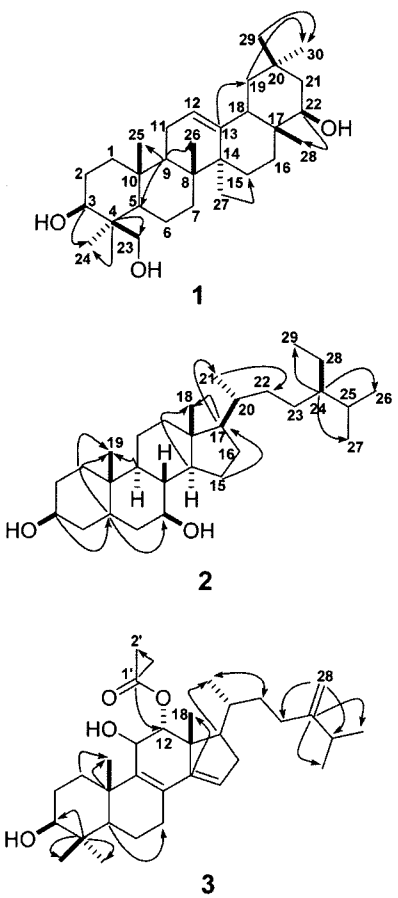

Fig. 2. Important HMBC correlations in compounds 1-3.

^{1}H-^{1}H COSY spectrum, the signal of the methine proton attached to the carbon bearing a hydroxyl group at δ 3.88 (1H, m, H-3) was coupled with two methylene proton signals at δ 1.90 (1H, m, H-1α) and 2.02 (1H, m, H-1β) and at δ 2.20 (2H, m, H-2). Most all of the methine and methylene proton signals overlapped, like other sterols: therefore, it was ambiguous and difficult to distinguish the ^{1}H-^{1}H COSY. However, in the HMBC correlations, these three methylene signal at δ (86.9, C-3), and the methylene carbon signal at δ (34.4, C-1) were correlated with the methyl proton signal at δ 1.18 (3H, s, H-19). The methyl proton signal at δ 1.18 (3H, s, H-19) was long-range correlated to the methylene carbon signal at δ (51.7, C-5), to which the proton signals at δ 1.70 (2H, m, H-6α) were correlated. In addition, in the HMBC spectrum, the following long-range couplings appeared: the methine proton δ 4.96 (H-12) with C-1' and the methyl proton δ 1.90 (H-2') with C-1'. These facts allowed assignment of acetoxy group to site at C12 on the C-ring (Fig. 2). Analysis of HMBC spectra allowed the unequivocal assignment of all carbons. Figure 2 shows important HMBC correlation and the positioning of the substituents on the ring system. Consequently, compound **3** was identified as 12β-acetoxy-4,4-dimethyl-24-methylene-5α-cholesta-8,14-dien-3β,11α-diol.[24, 25] This is the first report on the isolation of compound **3** from natural plant.

Isolated compounds **1-3** were examined for their *in vitro* cytotoxic activities against human cancer cell line including HT-59 (cancer) by the sulphorhodamine B (SRB). Compounds **1-3** showed cytotoxic activity against HT-29 human cancer cell line with the IC_{50} values of 62.9, 20.0, and 44.2 μM, respectively (Table 2). These compounds showed dose-dependent activities against HT-59 human cancer cell (Fig. 3).

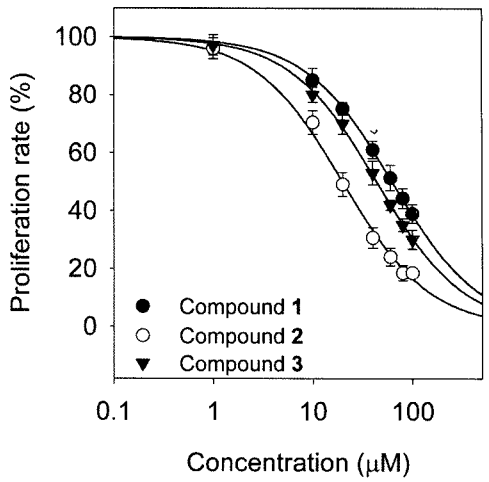

Fig. 3. Effects of compounds 1-3 on the proliferation of HT-59 cancer cell.

Table 2. *In vitro* cytotoxicity of compounds 1-3 from roots of *G. max* on human cell line HT-29[a]

Compounds	IC_{50} (μM)
1	62.9 ± 1.99
2	20.0 ± 1.25
3	44.2 ± 0.94

[a]Results are expressed as IC_{50} values (μM). Data are shown as mean ± S.D. of triplicate experiments.

Table 3. Antibacterial activities of compounds 1-3 and ampicillin at 5 μg/disc[a]

Compounds	Inhibition zone (mm)		
	B. cereus	*B. subtilis*	*S. aureus*
1	NI	NI	NI
2	NI	NI	NI
3	16	14	11
Ampicillin	21	22	23

[a]Values indicate the diameter of inhibition zone of the disc (8 mm). All assays were carried out on nutrient agar. NI = no inhibition

We also, in search of investigating new biological function of these compounds, isolated compounds **1-3**, which were examined for antibacterial activity. The antibacterial activities of compounds **1-3** along with commercially available antibiotics such as ampicillin were evaluated by comparing the inhibition zone diameters determined by the paper disc diffusion bioassay (Table 3). At 5 mg/disc, compound **3** exhibited strong activity against Gram positive bacteria such as *B. cereus* (16 mm), *B. subtilis* (14 mm), and *S. aureus* (11

mm) in comparison with ampicillin as a positive control.

In conclusions, three triterpenoids **1-3** were isolated from *G. max* and their structures were identified by spectroscopic methods. Isolated compounds **1-3** showed cytotoxic activities against HT-59 human cancer cell line with IC_{50} values of 62.9, 20.0, and 44.2 μM, respectively. Compound **3** showed significant activities against Gram positive bacterial such as *B. cereus*, *B. subtilis*, and *S. aureus*.

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References

1. EL-Mekkawy, S., Meselhy, M. R., Nakamura, N., Tezuka, Y., Hattori, M., Kakiuchi, N., Shimotohno, K., Kawahata, T. and Otake, T. (1998) Anti-HIV-1 and anti-HIV-protease substances from *Ganoderma lucidum*. *Phytochemistry* **49**, 1651-1657.
2. Honda, T., Rounds, B. V., Bore, L., Finlay, H. J., Favaloro, F. G., Jr., Suh, N., Wang, Y., Sporn, M. B. and Gribble, G. W. (2000) Synthetic oleanane and ursane triterpenoids with modified rings A and C: a series of highly active inhibitors of nitric oxide production in mouse macrophages. *J. Med. Chem.* **43**, 4233-4246.
3. Lee, S. M., Park, J. G., Lee, Y. H., Lee, C. G., Min, B. S., Kim, J. H. and Lee, H. K. (2004) Anti-complementary activity of triterpenoids from fruits of *Zizyphus jujube*. *Biol. Pharm. Bull.* **27**, 1883-1886.
4. Leu, Y. L., Kuo, S. M., Hwang, T. L. and Chiu, S. T. (2004) The inhibition of superoxide anion generation by neutrophils from *Viscum articulactum*. *Chem. Pharm. Bull.* **52**, 858-860.
5. Yim, T. K., Wu, W. K., Pak, W. F. and Ko, K. M. (2001) Hepatoprotective action of an oleanolic acid-enriched extract of *Ligustrum lucidum* fruits is mediated through an enhancement on hepatic glutathione regeneration capacity in mice. *Phytother. Res.* **15**, 589-592.
6. Kim, K. A., Lee, J. S., Park, H. J., Kim, J. W., Kim, C. J., Shim, I. S., Kim, N. J. and Han, S. M. (2004) Inhibition of cytochrome P450 activities by oleanolic acid and ursolic acid in human liver microsomes. *Life Sci.* **74**, 2769-2779.
7. Hsu, H. Y., Yang, J. J. and Lin, C. C. (1997) Effects of oleanolic acid ursolic acid on inhibiting tumor growth and enhancing the recovery of hematopoietic system postirradiation in mice. *Cancer Lett.* **111**, 7-13.
8. Choi, C. Y., You, H. J. and Jeong, H. G. (2001) Nitric oxide and tumor necrosis factor-alpha production by oleanolic acid via nuclear factor-kappaB activation in macrophages. *Biochem. Biophys. Res. Commun.* **288**, 49-55.
9. Andrikopoulos, N. K., Kaliora, A. C., Assimopoulou, A. N. and Papageorgiou, V. P. (2002) Inhibitory activity of minor polyphenolic and nonpolyphenolic constituents of olive oil against in vitro low-density lipoprotein oxidation. *J. Med. Food* 5, 1-7.
10. Pollard, M. and Wolter, W. (2000) Prevention of spontaneous prostaterelated cancer in Lobund-Wistar rats by a soy protein isolate/isoflavone diet. *Prostate* **45**, 101-105.
11. Wu, A. H., Ziegler, R. G., Nomura, A. M. Y., West, D. W., Kolonel, L. N., Ross, P. L. H. and Pike, M. C. (1998) Soy intake and risk of breast cancer in Asians and Asian Americans. *Am. J. Clin. Nutr.* **68**, 1437S-1443S.
12. Anthony, M. S., Clarkson, T. B., Hughes, C. L., Morgan, T. M. and Burke, G. L. (1996) Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal Rhesus monkeys. *J. Nutr.* **126**, 43-50.
13. Merz-Demlow, B. E., Duncan, A. M., Wangen, K. E., Xu, X., Carr, T. P., Phipps, W. R. and Kurzer, M. S. (2000) Soy isoflavones improve plasma lipids in normcholesterolemic permenopausal women. *Am. J. Clin. Nutr.* **71**, 1462-1469.
14. Wilcox, J. N. and Blumenthal, B. F. (1995) Thrombotic mechanisms in atherosclerosis: potential impact of soy proteins. *J. Nutr.* **125**, 631S-638S.
15. Arjmandi, B. H., Getlinger, M. J., Goyal, N. V., Alekel, L., Hasler, C. M., Juma, S., Drum, M. L., Hollis, B. W. and Kukreja, S. C. (1998) Role of soy protein with normal or reduced isoflavone content in reversing bone loss induced by ovarian hormone deficiency in rats. *Am. J. Clin. Nutr.* **68**, 1358S-1363S.
16. Arjmandi, B. H., Alekel, L., Hollis, B. W., Amin, D., Stacewicz-Sapuntzakis, M., Guo, P. and Kukreja, S. C. (1996) Dietary soybean protein prevents bone loss in an ovariectomized rat model of osteroporosis. *J. Nutr.* **126**, 161-167.
17. Wu, Q., Wang, M., Sciarappa, W. J. and Simon, J. E. (2004) LC/UV/ESI-MS analysis of isoflavones in edamame and tofu soybeans. *J. Agric. Food Chem.* **52**, 2763-2769.
18. Rostagno, M. A., Palma, M. and Barroso, C. G. (2004) Pressurized liquid extraction of isoflavones from soybeans. *Anal. Chim. Acta* **522**, 169-177.
19. Dwyer, J. T, Goldin, B. R., Saul, N., Gualtieri, L., Barakat, S. and Adlercreutz, H. (1994) Tofu and soy drinks contain phytoestrogens. *J. Am. Diet. Assoc.* **94**, 739-743.
20. Lee, J. H., Lee, B. W., Kim, J. H., Jeong T.-S., Kim, M. J., Lee, W. S. and Park, K. H. (2006) LDL-antioxidant pterocarpans from roots of *Glycine max* (L.) Merr. *J. Agric. Food Chem.* **54**, 2057-2063.
21. Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D. and Warren, J. (1990) New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.* **82**, 1107-1112.
22. Piddok, L. J. V. (1990) Techinques used for the determination of antibacterial resistance and sensitivity in bacteria. *J. Appl. Bacteriol.* **68**, 307-318.
23. Kitagawa, I., Yoshikawa, M., Wang, H. K., Saito, M., Tosirisuk, V., Fujiwara, T. and Tomita, K. I. (1982) Revised structures of soyasapogenols A, B, and E, oleanene-sapogenols from soybean. *Chem. Pharm. Bull.* **30**, 2294-2297.
24. Yagen, B., Horn, P., Joffe, A. Z. and Cox, R. H. (1980) Iso-

lation and structural elucidation of a novel sterol metabolite of *Fusarium sporotrichioides J. C. S. Perkin I.* **921**, 2914-2917.

25. Singh, S. B., Zink, D. L., Dombrowski, A. W., Polishook, J. D., Ondeyka, J. G., Hirshfield, J., Felock, P. and Hazuda, D. J. (2003) Integracides: tetracyclic triterpenoid inhibitors of HIV-1 integrase produced by *Fusarium* Sp. *Bioorg. Med. Chem.* **11**, 1577-1582.