Human Acyl-CoA: Cholesterol Acyltransferase (hACAT) Inhibitory Activities of Triterpenoids from Roots of *Glycine max* (L.) Merr.

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Eight triterpenoids, six lanostanes **1-6**, one lupenane **7**, and one oleanane **8**, were isolated by bioactivity-guided fractionation of the ethylacetate extract from roots of *Glycine max* (L.) Merr. All isolated compounds were examined for their inhibitory activities against human ACAT-1 (hACAT-1) and human ACAT-2 (hACAT-2). Among them, three triterpenoids showed potent hACAT inhibitory activities. (24*R*)-ethylcholest-5-ene-3,7-diol (**1**) and 3β-hydroxylup-20(29)-en-28-oic acid (**7**) exhibited more potent inhibitory activity against hACAT-1 (**1**: IC_{50} = 25.0 ± 1.2 and **7**: IC_{50} = 11.5 ± 0.4 μM) than hACAT-2 (**1**: IC_{50} = 102.0 ± 5.4 and **7**: IC_{50} = 33.9 ± 3.7 μM), respectively. Interestingly, 5α,8α-epidioxy-24(*R*)-methylcholesta-6,22-diene-3β-ol (**4**) has proven to be a specific inhibitor against hACAT-1 (IC_{50} = 38.7 ± 0.8 μM) compared to hACAT-2 (IC_{50} > 200). In conclusion, this is the first study to demonstrate that triterpenoids of *G. max* have potent inhibitory activities against hACAT-1 and hACAT-2.

Key Words : *Glycine max*, Root, Human acyl-CoA: cholesterol acytransferase (hACAT), Triterpenoid, Atherosclerosis

Introduction

Acyl-CoA: cholesterol acyltransferase (ACAT, E.C. 2.3.1.26) is an allosteric enzyme that catalyzes the acylation of cholesterol to cholesteryl esters with long chain fatty acids, which is a very attractive target for the prevention and treatment of cardiovascular diseases and hypercholesterolemia.[1-3] In mammalian species, two isoforms such as ACAT-1 and ACAT-2 exhibited different tissue distribution and membrane.[4,5] In humans, ACAT-1 performs a critical role in the formation of macrophage foam cells, whereas ACAT-2 is in charge of the cholesterol absorption process in intestinal enterocytes.[6,7] Therefore, ACAT inhibitors are being investigated as potent therapeutic agents in the treatment of both atherosclerosis and hypercholesterolemia.[8] In our search for ACAT inhibitors from natural sources, we found that the EtOAc extract of *Glycine max* (L.) Merr. roots exhibited inhibitory activities against hACAT-1 and hACAT-2 with 82% and 59% inhibition at 100 μg/mL, respectively.

Soybeans (*G max*) are one of the most produced and commercialized commodities worldwide with not only high amounts of protein and oil but also several phytochemicals.[9-12] It is well established that isoflavones are responsible for the biological activities of soybean.[13] Even though researchers mainly have focused on secondary metabolites of soybean because of their potential medicinal values including antioxidant,[14] anticancer,[15] and antiatherosclerotic activities,[16] their application concerning ACAT inhibitor has so far not been reported. Recently, we were the first to report evaluation of low-density lipoprotein (LDL)-antioxidant pterocarpans concerning atherosclerosis from this species.[17] In this study, the isolation of phytochemicals from *G max*, bioactivity-guided fractionations, using hACAT inhibitory activity measurements led to the isolation of three potent hACAT inhibitors **1**, **4**, and **7** as well as five triterpenoids. Moreover here, we describe the isolation, structure characterization, and hACAT inhibitory activities.

Results and Discussion

The methanolic extract of the dried roots of *G max* showed a strong inhibition upon hACAT. Solvent partition of the methanolic extract resulted in the localization of the active compounds in EtOAc-soluble fraction. Further bioactivity-guided fractionation of combined EtOAc fraction using hACAT inhibitory activity, yielded eight triterpenoids isolated by repeated column chromatography, Sephadex LH-20, and recrystallization. These compounds **1-8** were identified as (24*R*)-ethylcholest-5-ene-3,7-diol (**1**), (24*R*)-ethylcholest-5-en-3-ol-7-one (**2**), (24*R*)-ethylcholestane-3,7-diol (**3**), 5α,8α-epidioxy-24(*R*)-methylcholesta-6,22-diene-3β-ol (**4**), 24(*R*)-methylcholesta-5,7,22-tiene-3-ol (**5**), 17-acetoxy-4,4-dimethyl-24-methylene-5-cholesta-8,14-diene-3,11-diol (**6**), 3β-hydroxylup-20(29)-en-28-oic acid (**7**), and 12-oleanene-3β,22β,3,22,24-triol (**8**), respectively, by analyses of spectroscopic data and comparison of their physical data previously reported (Figure 1).[18-28] Among them, three compounds **4**, **7**, and **8** could be obtained in gram amount (**4**: 1.9 g, **7**: 1.1 g, and **8**: 2.1 g, respectively) as the dominant triterpenoids constituents. Moreover, five

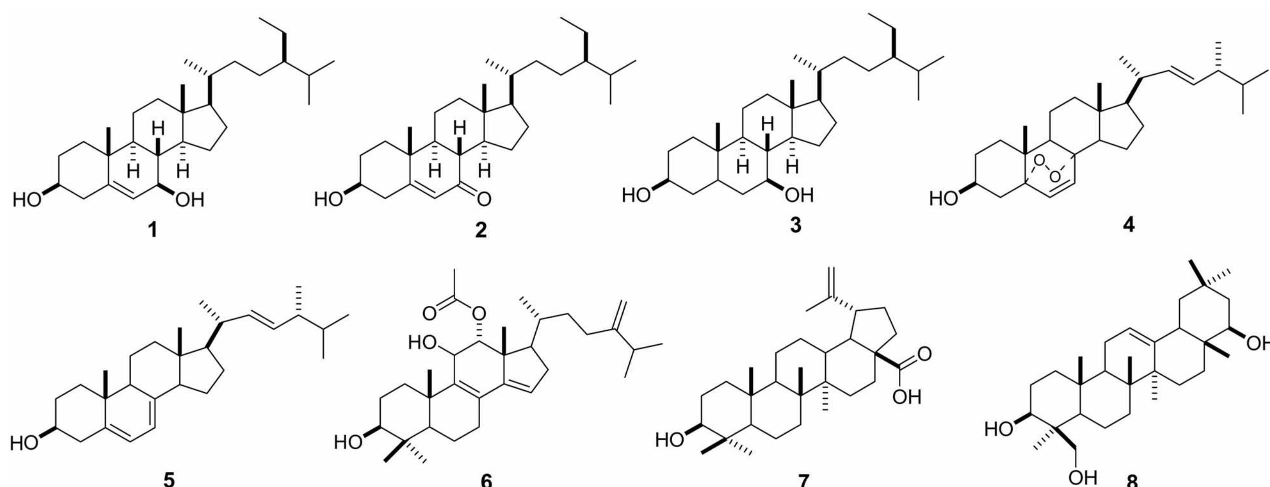

Figure 1. Chemical structures of isolated triterpenoids **1-8** from roots of *G. max*.

compounds **1**, **2**, **4**, **5**, and **7** were first isolated from this plant.

Although a large number of reports have been issued on the biological activities of *G. max*, there is no report on the component of soybean which inhibits hACAT. The isolated triterpenoids **1-8** from *G. max* roots have been evaluated for the first time for their inhibitory activities on hACAT-1 and hACAT-2 for the development of useful antiatherogenic and hyperchole-sterolemic substances. The rate of incorporation of [1-^{14}C] oleoyl-CoA into cholesteryl ester was determined using the expressed hACAT-1 and hACAT-2 from Hi5 cells and oleic acid anilide was used as positive control.[25] As shown in Table 1, three triterpenoids **1**, **4**, and **7** showed potent hACAT inhibitory activities, whereas the other compounds exhibited a very weak or no inhibitory activities. The compounds **1**, **4**, and **7** were detected to inhibit hACAT-1 and hACAT-2 activities in a dose-dependent manner with significant hACAT inhibitory effects observed at 10-100 μM (Figure 2).

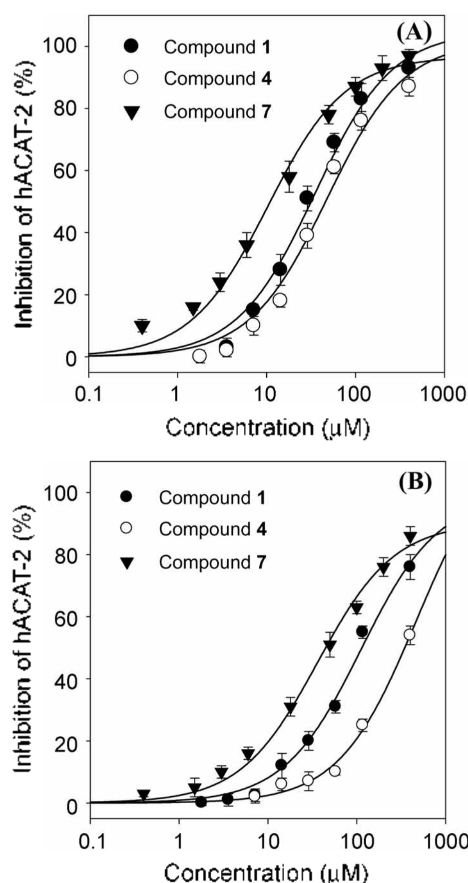

Figure 2. Effect of triterpenoids 1, 4, and 7 on hACAT-1 (A) and hACAT-2 (B) inhibitory activities.

(24*R*)-Ethylcholest-5-ene-3,7-diol (**1**) of lanostane-type triterpenoid inhibited hACAT-1 with IC_{50} values of 25.0 ± 1.2 μM as well as hACAT-2 with IC_{50} values of 102.0 ± 5.4 μM (Table 1). Among other lanostane-type triterpenoids, 5α,8α-epidioxy-24(*R*)-methylcholesta-6,22-diene-3β-ol (**4**) exhibited relatively high hACAT-1 inhibitory activity with IC_{50} value of 38.7 ± 0.8 μM, although inhibition of the hACAT-2 activity was barely detected (> 200 μM). Allyl alcohol group in B-ring of lanostane skeleton may play a role as a key functionality for inhibitory activity on hACAT. Because compound **1** has allyl alcohol in B-ring apparently

Table 1. Human ACAT inhibitory activities of triterpenoids **1-8**

Compound	IC_{50} (μM) values[a]	
	hACAT-1	hACAT-2
1	25.0 ± 1.2	102.0 ± 5.4
2	NI[c]	NI
3	NI	NI
4	38.7 ± 0.8	>200
5	23%[b]	NI
6	7%	4%
7	11.5 ± 0.4	33.9 ± 3.7
8	37%	9%

[a]*In vitro* ACAT inhibitory activity was measured using expressed hACAT-1, and hACAT-2. Data are shown as mean values of three independent experiments performed in triplicate (Mean ± S.D., *n* = 3). [b]Percentage at 25 μM. [c]NI: no inhibition.

and compound **4** has 1,2-dioxinyl group that could be transformed to allyl alcohol functionality in aqueous condition easily. In lupenane-type triterpenoids, 3β-hydroxylup-20(29)-en-28-oic acid (**7**) showed the most potent inhibitory activities against both hACAT-1 and hACAT-2 with IC_{50} values of 11.5 ± 0.4 and 33.9 ± 3.7 μM, respectively, which was in accordance with our previous report.[30] This result suggested that lanostane-type triterpenoids **1** and **4** and lupenane-type triterpenoids **7** in soybean could be expected as hACAT inhibitors.

Experimental Section

General Experimental Procedures. 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 $CDCl_3$, pyridine-d_6, DMSO-d_6, and CD_3OD. Optical rotation values were measured by a Perkin-Elmer 343 polarimeter and $[\alpha]_D$-values are given in units of 10^{-1} deg cm^{2}g^{-1}. A scintillation counter was used with 1450 Microbeta Trilux, Qallac Oy (Turku, Finland) for hACAT inhibitory activity. The [1-^{14}C] oleoyl-CoA (56.0 μCi/mmol) solution was purchased from the Amersham Biosciences Korea Ltd. KH_2PO_4, dithiothreitol, bovine serum albumin (fatty acid free) were purchased from the Sigma-Aldrich. All the reagent grade chemicals were purchased from Sigma (Sigma Chemical Co, St. Louis, MO, USA).

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

Extraction and Isolation. The dried roots of *G. max* (2.0 kg) were chopped and extracted three times (6 L × 3) with methanol for 10 days at room temperature. The concentrated methanolic extract (98.0 g) was suspended in H_2O and successively partitioned with *n*-hexane (1.0 L), EtOAc (1.5 L), and *n*-BuOH (1.2 L), which yielded a *n*-hexane (10.4 g), EtOAc (29.8 g), and *n*-BuOH (12.6 g) extracts. The EtOAc extract showed potent inhibitory activities against hACAT-1 and hACAT-2 with 82% and 59% inhibition at 100 μg/mL, respectively, which was chromatographed on a silica gel (6 × 60 cm, 230-400 mesh, 680 g), eluting a step gradient of $CHCl_3$-acetone (25:1 → 1:2) to give twelve fractions (F1-F12), based on the comparison of TLC profiles. The active fraction F4 (6.1 g) was submitted to a flash silica gel column chromatography (5.0 × 60 cm, 580 g, 230-400 mesh), eluted with a step gradient of *n*-hexane-EtOAc (20:1 → 1:1). Altogether, 65 subfractions were collected. Among them, subfractions 24-35 (1.7 g) were concentrated and separated by a silica gel column (3.0 × 50 cm, 190 g, 230-400 mesh) eluting with *n*-hexane-EtOAc gradient (25:1 → 6:1) to yield compounds **2** (89 mg) and **5** (74 mg). Subfractions 49-61 (1.2 g) were subjected to silica gel column chromatography (3.0 × 50 cm, 150 g, 230-400 mesh) with *n*-hexane-acetone (15:1 → 4:1) and then purified by a second silica gel column (2.5 × 50 cm, 110 g, 230-400 mesh) using a gradient of *n*-hexane-acetone (16:1 → 4:1) to obtain compounds **1** (29 mg) and **3** (23 mg). The active fractions F8-9 (5.9 g) were applied to silica gel column chromatography (5.0 × 60 cm, 520 g, 230-400 mesh) by eluting initially with $CHCl_3$, then with $CHCl_3$-acetone mixture of increasing polarity (25:1 → 1:1), resulting in 78 subfractions. Subfractions 13-29 (1.9 g) were evaporated and further purified in small chromatographic column containing silica gel, eluting with $CHCl_3$-acetone (20:1 → 8:1) to yield the pure compound **4** (1.4 g) as a white powder. Also, subfractions 38-43 (1.5 g) were pooled and rechromatographed on silica gel (3.5 × 50 cm, 180 g, 230-400 mesh) with $CHCl_3$-acetone (16:1 → 3:1) to give compound **8** (2.1 g). Subfractions 55-65 (1.3 g) were evaporated and recrystallized from $CHCl_3$-acetone mixture to give compound **7** (1.1 g). Subfraction 70-75 (675 mg)

Table 2. ^{13}C NMR of compounds **1**, **2**, **4**, **5**, and **7** at 125 MHz (ppm, m)[a]

Position	Compound				
	1	**2**	**4**	**5**	**7**
1	37.9 (t)	38.9 (t)	34.7 (t)	38.4 (t)	38.6 (t)
2	31.8 (t)	34.3 (t)	30.1 (t)	32.0 (t)	27.5 (t)
3	65.8 (d)	73.7 (d)	66.4 (d)	70.5 (d)	77.2 (d)
4	39.6 (t)	40.0 (t)	37.0 (t)	40.8 (t)	38.9 (s)
5	146.6 (s)	168.8 (s)	82.2 (s)	141.3 (s)	55.3 (d)
6	124.3 (d)	126.7 (d)	130.7(d)	119.6 (d)	18.3 (t)
7	65.7 (d)	200.8 (s)	135.4 (d)	116.3 (d)	34.3 (t)
8	37.4 (d)	37.5 (d)	79.4 (s)	140.0 (s)	40.6 (s)
9	49.8 (d)	54.0 (d)	51.2 (d)	46.3 (d)	50.3 (d)
10	37.8 (s)	38.4 (s)	37.0 (s)	37.1 (s)	38.0 (s)
11	21.1 (t)	21.4 (t)	23.4 (t)	21.1 (t)	20.8 (t)
12	42.4 (t)	42.9 (t)	39.4 (t)	28.3 (t)	25.5 (t)
13	42.5 (s)	46.2 (s)	44.6 (s)	42.9 (s)	38.6 (d)
14	56.2 (d)	56.4 (d)	51.7 (d)	55.8 (d)	42.4 (s)
15	24.7 (t)	24.5 (t)	20.6 (t)	22.2 (t)	29.3 (t)
16	28.7 (t)	28.6 (t)	28.6(t)	38.4 (t)	32.1 (t)
17	56.1 (d)	56.3 (d)	56.1 (d)	54.6 (d)	55.8 (s)
18	12.0 (q)	12.4 (q)	12.9 (q)	12.1 (q)	47.0 (d)
19	18.6 (q)	19.9 (q)	18.2 (q)	16.3 (q)	48.9 (d)
20	36.5 (d)	36.5 (d)	39.7 (d)	40.4 (d)	150.7 (s)
21	19.2 (q)	19.1 (q)	20.9 (q)	19.7 (q)	30.5 (t)
22	34.3 (t)	34.6 (t)	132.3 (d)	135.6 (d)	36.7 (t)
23	26.4 (t)	26.5 (t)	135.2 (d)	132.0 (d)	28.5 (q)
24	49.8 (d)	51.6 (d)	42.8 (d)	42.9 (d)	16.1 (q)
25	28.7 (t)	29.6 (d)	33.1 (d)	33.1 (d)	16.1 (q)
26	20.2 (q)	20.2 (q)	20.0 (q)	20.0 (q)	16.3 (q)
27	19.4 (q)	19.4 (q)	19.6 (q)	23.0 (q)	14.7 (q)
28	23.5 (t)	23.5 (t)	17.6 (q)	17.6 (q)	177.6 (s)
29	12.4 (q)	12.4 (q)			109.9 (t)
30					19.3 (q)

[a]The chemical shifts of compounds **1**, **2**, **4**, and **5** were determined in $CDCl_3$, and compound **7** was measured in DMSO-d_6.

were concentrated and submitted to a silica gel column chromatography (2.5 × 50 cm. 130 g. 230-400 mesh) eluting with $CHCl_3$-acetone (10:1 → 1:1) and rechromatographed on a Sephadex LH-20 (1.5 × 50 cm) for elution with methanol in order to yield compound **6** (39 mg). The spectroscopic data of five isolated triterpenoids were described as follows and the other compounds were described as previously reported.[18]

Compound **1**: White powder. mp 179-181 °C. $[\alpha]_D^{20}$ +32.7° (*c* 0.44. CH_3OH); IR (KBr): 3460. 1712 cm^{-1}; ^1H NMR (500 MHz. $CDCl_3$) δ 0.62 (3H. s. H-18), 0.63 (3H, s. H-19). 0.75 (3H. s. H-29). 0.76 (3H. d, *J* = 6.8 Hz. H-21). 0.85 (3H. d. *J* = 6.4 Hz. H-27), 0.87 (3H, d, *J* = 6.4 Hz, H-26). 0.93 (1H. m, H-24), 1.09 (1H. m. H-17), 1.10 (1H. m. H-14). 1.11 (1H. m, H-1β), 1.39 (1H, m, H-8), 1.40 (1H. m. H-9), 1.78 (1H. m. H-1α), 1.95 (2H. m. H-4), 2.17 (2H. m. H-15). 2.27 (2H, m, H-12). 2.36 (1H. m. H-28α). 2.37 (2H. m, H-23). 2.39 (2H. m, H-16). 2.41 (1H. m, H-20), 2.38 (1H. m, H-11α). 2.43 (1H. m. H-28β). 2.50 (2H. m. H-22). 2.56 (1H, m, H-11β), 2.60 (1H, m. H-25), 2.83 (1H. m. H-2α). 3.19 (1H. m. H-2β). 3.52 (1H, m. H-3), 3.78 (1H. s, H-7). and 5.54 (1H, d, *J* = 5.1 Hz, H-6). ^{13}C NMR (125 MHz. $CDCl_3$): see Table 2.

Compound **2**: Whiter powder; mp 140-142 °C. $[\alpha]_D^{20}$ −3.9° (*c* 0.62. $CHCl_3$); IR (KBr): 3400. 1640 cm^{-1}; ^1H NMR (500 MHz. $CDCl_3$) δ 0.61 (3H, s. H-18). 0.75 (3H, d. *J* = 6.7 Hz, H-26). 0.76 (3H. d. *J* = 7.0 Hz, H-27), 0.77 (3H, t. *J* = 9.3 Hz. H-29), 0.83-0.89 (1H. m. H-24), 0.83-0.86 (1H. m. H-9), 0.85 (3H. d. *J* = 6.4 Hz. H-21). 0.89-0.93 (1H. m, H-14). 0.94 (3H. s. H-19). 0.91-1.01 (1H, m, H-15α). 0.95-1.05 (1H, m. H-1α), 0.99-1.08 (1H, m. H-17), 1.06-1.12 (2H, m. H-23). 1.06-1.12 (1H, m. H-12α), 1.10 (2H, m, H-22), 1.15-1.21 (2H. m, H-28). 1.16-1.19 (1H. m, H-16α), 1.18 (1H, m. H-25). 1.24-1.30 (1H, m. H-20), 1.37-1.41 (1H, m, H-8). 1.37-1.44 (2H, m. H-11), 1.48-1.53 (1H. m. H-15β). 1.75-1.81 (1H, m, H-1β). 1.75-1.79 (2H. m, H-2), 1.77 (1H, m, H-16β). 1.88-1.95 (1H, m, H-12β). 2.17 (1H. ddd. *J* = 11.4, 4.9. 2.0 Hz. H-4α), 2.21 (1H, ddd. *J* = 13.1. 4.9. 2.0 Hz. H-4β). 3.41-3.45 (1H. m. H-3). 5.28 (1H. dd. *J* = 2.6. 5.2 Hz. H-6). ^{13}C NMR (125 MHz. $CDCl_3$): see Table 2.

Compound **4**: Amorphous white powder. mp 178-179 °C. $[\alpha]_D^{20}$ −25.4° (*c* 1.00. $CHCl_3$); IR (KBr): 3400, 1459 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.10 (3H, d, *J* = 11.0 Hz. H-21). 0.79 (3H. d. *J* = 7.6 Hz, H-27), 0.81 (3H, d, *J* = 7.6 Hz. H-26). 0.82 (3H. s. H-18). 0.88 (3H. s. H-19). 0.91 (3H. d. *J* = 11.4 Hz. H-28). 1.22 (1H. m. H-17). 1.22 (1H. m. H-11α). 1.24 (1H. m. H-12α). 1.38 (1H, m. H-15α). 1.40 (1H. m. H-16α). 1.47 (1H, m, H-25). 1.48 (1H, s, H-9), 1.50 (1H. m, H-11β), 1.53 (1H, m. H-2α). 1.55 (1H. m. H-14). 1.59 (1H. m. H-15β). 1.67 (1H, m, H-1α). 1.75 (1H, m, H-16β), 1.84 (1H. m, H-2β). 1.85 (1H, m, H-24). 1.90 (1H, m, H-4β). 1.94 (1H, m, H-1β). 1.95 (1H. m. H-12β). 2.03 (1H, m, H-20). 2.09 (1H. m. H-4α). 3.96 (1H, m, H-3), 5.13 (1H. dd. *J* = 15.2, 7.7 Hz. H-23). 5.23 (1H. dd. *J* =15.2, 7.7 Hz. H-22). 6.23 (1H. d. *J* = 8.6 Hz. H-7), and 6.49 (1H. d. *J* = 8.6 Hz. H-6). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 2.

Compound **5**: White powder. mp 165-167 °C. $[\alpha]_D^{20}$ −130° (*c* 0.80, $CHCl_3$); IR (KBr): 3450, 1565 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.65 (3H. s. H-18). 0.84 (3H, d. *J* = 7.6 Hz. H-26). 0.86 (3H. d, *J* = 7.6 Hz. H-27). 0.93 (3H. d. *J* = 11.4 Hz, H-28). 0.96 (3H. s. H-19), 1.05 (3H, d. *J* = 11.0 Hz, H-21). 1.15 (2H. m, H-12). 1.26 (1H, m, H-14). 1.27 (1H. m. H-1β). 1.46 (2H, m, H-15). 1.47 (2H. m, H-11). 1.48 (1H. m, H-25). 1.64 (2H. m, H-2). 1.66 (1H. m, H-16α), 1.66 (1H, m. H-1α), 1.75 (1H, s. H-9), 1.77 (1H, m. H-17). 1.78 (1H, m. H-20), 1.79 (1H. m. H-24), 1.99 (1H. m. H-16β), 2.29 (1H. m, H-4β). 2.46 (1H, m. H-4α). 3.65 (1H, m. H-3). 5.21 (2H. m. H-22 and H-23). 5.40 (1H. m. H-7). and 5.59 (1H, dd, *J* = 4.0, 9.3 Hz. H-6). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 2.

Compound **7**: White needles, mp 307-309 °C. $[\alpha]_D^{20}$ +7.6° (*c* 1.00. pyridine); IR (KBr): 3450, 1689 cm^{-1}; ^1H NMR (500 MHz. CD_3OD) δ 0.54 (1H, s, H-5). 0.56 (3H, s. H-24), 0.68 (3H. s, H-25). 0.78 (3H. s. H-23). 0.78 (3H, s, H-26), 0.84 (3H, s. H-27). 0.99 (1H, m. H-12α). 1.01 (2H, m. H-15). 1.15 (1H. m. H-11α), 1.15 (1H, m, H-9), 1.21 (1H, m, H-6α). 1.22 (2H. m, H-7), 1.29 (1H, m. H-11β), 1.26 (1H, m. H-16α), 1.35 (2H, m, H-2). 1.37 (1H. m. H-6β), 1.42 (1H. m. H-18). 1.48 (1H, m, H-1β), 1.51 (1H, m. H-1α), 1.56 (1H, m, H-12β). 1.56 (3H. s, H-30), 1.70 (2H. m. H-22). 1.71 (2H, m, H-21). 2.13 (1H. m. H-16β). 2.14 (1H. m, H-13). 2.86 (1H. m. H-19). 2.88 (1H, m. H-3). 4.47 (1H, d. *J* = 2.1 Hz, H-29α), and 4.60 (1H. d, *J* = 2.1 Hz, H-29β). ^{13}C NMR (125 MHz, CD_3OD): see Table 2.

Inhibitory Activity against hACAT. The microsomal fractions of Hi5 cells containing baculovirally expressed hACAT-1 and hACAT-2 were used as sources of the enzyme.[29] The inhibitory activities of hACAT-1 and hACAT-2 were determined as previously described with some slight modifications.[18,31] Briefly. the reaction mixture. which contained 4 μL of microsomes (8 mg/mL protein) and 20 μL of KH_2PO_4 buffer (0.5 M. pH 7.4) with 10 mM dithiothreitol. 15 μL of bovine serum albumin (BSA, fatty acid free, 40 mg/mL). 2 μL of cholesterol in acetone (20 μg/mL), 41 μL of water, and 10 μL of test compound in a total volume of 92 μL. The tube containing the reaction mixture was incubated for 20 min in a shaking water bath set at 37 °C. After adding 8 μL of [1-^{14}C] oleoyl-CoA solution (56.0 μCi/*m*mol. final con. 10 μM). the tube was vortexed and then placed back into the water bath for 25 min at 37 °C. To stop the reaction mixture. 1.0 mL of isopropanol-heptane (4:1; v/v) solution was added to the tube. A solution mixture of 0.6 mL heptane and 0.4 mL potassium phosphate buffer (0.1 M, pH 7.4) with 2 mM dithiothreitol was added to the terminated reaction mixture. After centrifugation for 2 min. cholesterol oleate was recovered in the upper phase (total volume 0.9-1.0 mL). The radioactivity in 100 μL of the upper layer was measured in a 3 mL liquid scintillation vial with 3 mL of scintillation cocktail (Lipoluma. Lumac Co.) using a liquid scintillation counter (1450 Micerobeta Trilux Wallac Oy. Turku. Finland). Background values were obtained by preparing heat inactivated microsomes or normal insect cell lysate microsomes. usually background value was 200-250 cpm. at 8000 cpm of the ACAT reaction. ACAT activity was expressed as a

defined unit, cholesteryl oleate pmol/min/mg protein. The hACAT inhibitory activities of the isolated compounds were confirmed by the positive control with oleic acid anilide, which inhibited hACAT-1 and hACAT-2 with IC_{50} values of 0.14 and 0.17 μM, respectively.[29]

In conclusion, we reported eight triterpenoids **1-8** isolated from roots of *G. max* and examined their hACAT inhibitory activities. Especially, three triterpenoids **1**, **4**, and **7** exhibited high inhibitory activities against hACAT-1 (**1**: 25.0 ± 1.2, **4**: 38.7 ± 0.8, and **7**: 11.5 ± 0.4 μM, respectively) and hACAT-2 (**1**: 102.0 ± 5.4, **4**: > 200, and **7**: 33.9 ± 3.7 μM, respectively). This is nevertheless the first report to demonstrate that triterpenoids of *G. max* showed potent inhibitory activities against hACAT-1 and hACAT-2. Further studies on hACAT inhibitory activity for the treatment of hypercholesterolemia and atherosclerosis are under investigation.

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Supporting Information Available: ^{1}H, ^{13}C, and 2D NMR of compounds **1-8**; EIMS of compounds **3**, **4**, and **7**; X-ray crystal structure analysis of compound **5**. This material is available *via* the internet at *http://www.kcsnet.or.kr*.

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