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Triterpenoids from the Flower of *Campsis grandiflora* K. Schum. as Human Acyl-CoA: Cholesterol Acyltransferase Inhibitors

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The flower of *Campsis grandiflora* K. Schum. was extracted with 80% aqueous MeOH, and the concentrated extract was partitioned with EtOAc, *n*-BuOH and H_2O. From the EtOAc fraction, seven triterpenoids were isolated through the repeated silica gel, ODS column chromatographies and preparative HPLC. From the result of physico-chemical data including NMR, MS and IR, the chemical structures of the compounds were determined as 3β-hydroxyolean-12-en-28-oic acid (oleanolic acid, **1**), 3β-hydroxyurs-12-en-28-oic acid (ursolic acid, **2**), 3β-hydroxyurs-12-en-28-al (ursolic aldehyde, **3**), 2α,3β-dihydroxyolean-12-en-28-oic acid (maslinic acid, **4**), 2α,3β-dihydroxyurs-12-en-28-oic acid (corosolic acid, **5**), 3β,23-dihydroxyurs-12-en-28-oic acid (23-hydroxyursolic acid, **6**) and 2α,3β,23-trihydroxyolean-12-en-28-oic acid (arjunolic acid, **7**). These teriterpenoids were isolated for the first time from this plant. Also, compounds **4**, **5**, **6**, and **7** revealed relatively high hACAT-1 inhibitory activity with the value of 46.2±1.1, 46.7±0.9, 41.5±1.3 and 60.8±1.1% at the concentration of 100 μg/mL, respectively.

Key words: *Campsis grandiflora*, Bignoniaceae, hACAT1 Inhibitory effect, Oleanolic acid, Ursolic acid

INTRODUCTION

Inhibition of Acyl-CoA: cholesterol acyltransferase (ACAT, E.C.2.3.1.26), which catalyzes the acylation of cholesterol to cholesteryl esters with long chain fatty acids, is a very attractive target for the treatment of hypercholesterolemia and atherosclerosis (Brown *et al.*, 1975). It was found to be present as two isoforms in mammals (Anderson *et al.*, 1998; Coses *et al.*, 1998), ACAT-1 and ACAT-2, with different tissue distribution and membrane topology (Joyce *et al.*, 2000). However, most ACAT inhibitors, which were screened by rat liver microsomal ACAT, have problems associated with low oral bioavailability and adrenal and/or hepatic toxicity in clinical trials (Dominick *et al.*, 1993; Matsuo *et al.*, 1996). ACAT-1 plays a critical role in foam cell formation in macrophages, whereas ACAT-2 is in charge of the cholesterol absorption process in intestinal mucosal cells (Rudel *et al.*, 2001). These findings were consistent with the following results that atherosclerosis lesions were reduced at ACAT-1 mice, whereas ACAT-1 mice have limited cholesterol absorption in the intestine, and decreased cholesterol ester content in the liver and plasma lipoproteins (Accad *et al.*, 2000; Yagyu *et al.*, 2000; Buhman *et al.*, 2000). Because ACAT inhibitors, most without selectivity for ACAT-1 versus ACAT-2, have not yet been identified that are effective in plasma cholesterol lowering in humans (Sliskovic *et al.*, 2002), the selective inhibitor of ACAT-1 or ACAT-2 may be effective for the development of a useful hypercholesterolemic or anti-atherogenic agent.

Campsis grandiflora K. Schum. (Bignoniaceae) is a climbing plant cultivated in the fields of Korea, China, and Japan. The flower of this plant has been used as a remedy for female disorders like uterine hemorrhage or menstrual irregularity and other several diseases due to extravasated blood in Korea and China, and as an ornamental plant in Japan (Soka, 1985). It is called 'Neung

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So Hwa' in Korean, 'Ryoshouka' or 'Noozenkazura' in Japanease and 'Ling Xiao Hua' in Chinese. The chemical constituents of genus *Campsis* have been studied by a number of researchers. For example, an iridoid glucoside and a phenylpropanoid glycoside have been isolated from *Campsis chinensis* (Imakura *et al.*, 1984, 1985). Also, analysis of the essential oil, phenolics and boron compounds from *C. grandiflora* (Ueyama *et al.*, 1989; Abdullahi *et al.*, 1986) has been carried out. However, the isolation of the chemical components from *C. grandiflora* has not been reported so far. So, in this paper the authors reported the isolation and identification of triterpenoids from the flower of *Campsis grandiflora* K. Schum, and inhibitory effects of the triterpenoids on hACAT-1 and hACAT-2.

MATERIALS AND METHODS

Plant materials

The *Campsis grandiflora* K. Schum. was imported from China in March, 2003 and identified by Prof. Dae-Keun Kim, Woosuk University, Jeonju. A voucher specimen (KHU03061) was reserved at the Laboratory of Natural Products Chemistry, KyungHee University, Suwon, Korea.

Instrumentation

Melting points were determined on a Fisher-John apparatus and uncorrected. Optical rotations were measured on a JASCO P-1010 digital polarimeter. EI-MS were recorded on a JEOL JMSAX 505-WA. IR spectra were run on a Perkin Elmer Spectrum One FT-IR spectrometer. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were taken on a Varian Unity Inova AS 400 FT-NMR spectrometer. HPLC was performed on a Shimadzu LC-10AT.

Chemicals

[1-^{14}C]oleoyl-CoA (56.0 mCi/mmol) was purchased from the Amersham Biosciences Korea Ltd. KH_2PO_4, dithiothreitol, bovine serum albumin (fatty acid free) were purchased from the Sigma-Aldrich Korea Ltd. All the reagent grade chemicals were purchased from the Sigma-Aldrich Korea Ltd.

Isolation of triterpenoids from the flower of *Campsis grandiflora* K. Schum.

The dried and powdered flower of *C. grandiflora* (6 kg) were extracted three times at room temperature with 80% aqueous MeOH (45 L×3). The extract was partitioned with water (4 L), EtOAc (4 L×3) and *n*-BuOH (4 L×3), successively. The EtOAc extract (114 g) was applied to the silica gel (70-230 mesh, Merck) column (9×20 cm) chromatography (c.c.) and eluted with *n*-hexane : $CHCl_3$: MeOH (30 : 10 : 1) monitoring by thin layer chromatography (TLC) to produce fifteen fractions (CGE1 to CGE15). CGE4 (14.7 g) was subjected to the silica gel c.c. (7×14 cm) eluted with *n*-hexane : $CHCl_3$: MeOH (20 : 50 : 1 → 10 : 10 : 1) to afford ten fractions (CGE4-1 to CGE4-10).

Two triterpenoids from CGE4-6 (884 mg) were isolated by high performance liquid chromatography (HPLC). As a stationary phase the COSMOSIL 5C_{18} Waters column (10×250 mm) was used and MeOH : H_2O (30 : 1) was used as the mobile phase. The flow rate was 2.0 mL/min and the detector was used with the refractive index detector (RID-10A, Shimadzu, Japan). Two peaks were detected at 14.81 and 15.45 min. Through repeat collecting each peak, compounds **1** (6 mg) and **2** (10 mg) were obtained. CGE4-4 (4.48 g) containing remarkable triterpenoids was applied to the silica gel c.c. (6×13 cm) eluted with *n*-hexane : EtOAc (6 : 1) to give thirteen fractions (CGE4-4-1 to CGE4-4-13) and CGE4-4-1 (350 mg) was subjected to the silica gel c.c. (3×12 cm) eluted with *n*-hexane : EtOAc (10 : 1) to afford seven fractions (CGE4-4-1-1 to CGE4-4-1-7). The fourth fraction (CGE4-4-1-4) was purified by ODS (Octadecyl silica gel, Merck) c.c. (3.8 ×6 cm) using MeOH : H_2O (25 : 1) as eluents to ultimately produce compound **3** (20 mg). CGE7 (26 g) was chromatographed on the silica gel column (7×14 cm) with $CHCl_3$: EtOH (35 : 1 → 25 : 1 → 15 : 1) as eluting solution to yield eighteen fractions (CGE7-1 to CGE7-18). CGE7-6 (1.2 g) was applied to the ODS c.c. (4.5×10 cm) eluted with MeOH : H_2O (2 : 1) to ultimately produce compounds **4** (27 mg) and **5** (32 mg). CGE7-7 (1.0 g) was subjected to the silica gel c.c. (4.5×9 cm) eluted with MeOH : H_2O (5 : 1) to afford eight fractions (CGE7-7-1 to CGE7-7-8). CGE7-7-7 (468 mg) was applied to the silica gel c.c. (3.8×9 cm) eluted with MeOH : H_2O (3 : 1) to give five fractions (CGE7-7-7-1 to CGE7-7-7-5) and CGE7-7-7-3 (195 mg) was purified by the ODS c.c. (3.8×9 cm) using MeOH : H_2O (2 : 1) as eluent to yield compound **6** (10 mg). CGE7-13 (668 mg) was subjected to the silica gel c.c. (4.5×13 cm) eluting with $CHCl_3$: EtOH (8 : 1) to afford nine fractions (CGE7-13-1 to CGE7-13-9). CGE7-13-7 (253 mg) including a triterpenoid was purified by the ODS c.c. (4.5×7 cm) using MeOH : H_2O (1 : 5 → 2 : 1 → 5 : 1) as eluent to yield compound **7** (33 mg).

3β-Hydroxyolean-12-en-28-oic acid (oleanolic acid, 1) White powder ($CHCl_3$); m.p. 296-298°C; $[\alpha]_D$ = +70.0° (*c*=0.4, $CHCl_3$); EI/MS *m/z*: 456, 438, 248, 207, 203, 189; IR_ν ($CHCl_3$, cm^{-1}) 3420, 2930, 1680; ^{1}H-NMR (400 MHz, pyridine-d_5, δ) 5.48 (1H, br. s, H-12), 3.32 (1H, dd, *J*=14.0, 4.0 Hz, H-3), 1.30 (3H, s, H-23), 1.26 (3H, s, H-27), 1.04 (3H, s, H-24), 1.04 (3H, s, H-30), 1.02 (3H, s, H-25), 0.97 (3H, s, H-26), 0.91 (3H, s, H-29), ^{13}C-NMR (100 MHz, pyridine-d_5, δ) see Table I.

Table I. ^{13}C-NMR chemical shifts of triterpenoids from the flower of *Campsis grandiflora*

Carbon No.	1	2	3	4	5	6	7
1	38.5	38.6	38.7	47.3	47.5	38.4	47.2
2	27.7	28.2	27.3	68.0	68.1	27.2	68.3
3	77.5	77.6	79.0	83.2	83.2	72.8	77.7
4	39.3	39.0	38.8	39.4	39.4	42.4	43.2
5	55.3	55.3	55.2	55.4	55.4	48.0	47.7
6	18.4	18.4	18.4	18.4	18.4	18.1	18.1
7	32.7*	33.1	32.0	32.8	33.0	30.6	32.4
8	39.5	39.5	39.9	39.3	39.5	39.5	39.4
9	47.6	47.6	47.6	47.7	47.6	47.5	47.5
10	36.9	37.0	37.0	38.0	37.9	36.6	38.0
11	23.3**	23.3	23.3	23.5	23.3	23.2	23.3
12	122.0	125.0	126.1	121.7	124.8	125.0	121.8
13	144.2	138.6	137.7	144.4	138.8	138.7	144.3
14	41.7	42.0	42.2	41.7	42.0	42.0	41.7
15	27.9	29.2	28.2	27.8	28.2	28.2	27.9
16	23.4**	24.5	23.4	23.3	24.5	24.5	23.5
17	46.0	47.6	50.2	46.2	47.6	47.5	46.2
18	41.5	53.0	52.6	41.5	53.1	53.1	41.5
19	46.2	39.0*	39.0	46.0	39.0	39.0	46.0
20	31.7	38.9*	38.9	30.5	38.9	38.9	30.5
21	33.8	30.5	30.2	33.8	30.7	37.0	33.8
22	32.8*	36.8	33.2	32.7	37.0	32.8	32.8
23	28.3	28.4	29.8	28.9	28.9	67.3	66.0
24	16.1	16.1	15.7*	17.1	17.1	12.7	13.9
25	15.1	15.3	15.6*	16.4	16.5	15.7	16.9
26	17.0	17.0	17.3	17.2	17.3	17.1	17.1
27	25.7	23.5	23.3	25.7	23.4	23.4	25.7
28	179.5	179.2	207.3	179.0	179.8	178.7	180.9
29	34.4	17.1	16.7	32.8	17.1	17.1	32.8
30	23.3**	21.0	21.2	23.3	21.0	20.9	23.3

*, ** : assignments may be interchangeable.

3β-Hydroxyurs-12-en-28-oic acid (ursolic acid, 2)

White powder ($CHCl_3$); m.p. 287-288°C; $[\alpha]_D$ = +70.0° (*c*=0.7, $CHCl_3$); EI/MS *m/z*: 456, 438, 248, 207, 203, 189, 175, 133; IR_ν ($CHCl_3$, cm^{-1}) 3360, 2900, 1695; ^{1}H-NMR (400 MHz, pyridine-d_5, δ) 5.49 (1H, br. s, H-12), 3.45 (1H, dd, *J*=9.6, 6.0 Hz, H-3), 1.24 (3H, s, H-23), 1.23 (3H, s, H-27), 1.04 (3H, s, H-26), 1.02 (3H, s, H-24), 1.01 (3H, d, *J*=6.4 Hz, H-30), 0.96 (3H, d, *J*=6.4 Hz, H-29), 0.89 (3H, s, H-25), ^{13}C-NMR (100 MHz, pyridine-d_5, δ) see Table I.

3*b*-Hydroxyurs-12-en-28-al (ursolic aldehyde, 3)

White powder ($CHCl_3$); m.p. 217-219°C; $[\alpha]_D$ = +62.4° (*c*=0.9, $CHCl_3$); EI/MS *m/z*: 440, 422, 232, 207, 203, 189, 133; IR_ν ($CHCl_3$, cm^{-1}) 3368, 2915, 2890, 1715; ^{1}H-NMR (400 MHz, $CDCl_3$, δ) 9.30 (1H, s, H-28), 5.29 (1H, dd, *J*=3.6, 3.6 Hz, H-12), 3.19 (1H, dd, *J*=10.8, 4.8 Hz, H-3), 1.06 (3H, s, H-27), 0.96 (3H, s, H-23), 0.97 (3H, d, *J*=6.4 Hz, H-30), 0.90 (3H, s, H-24), 0.85 (3H, d, *J*=6.4 Hz, H-29), 0.75 (3H, s, H-25), 0.74 (3H, s, H-26), ^{13}C-NMR (100 MHz, $CDCl_3$, δ) see Table I.

2α,3β-Dihydroxyolean-12-en-28-oic acid (maslinic acid, 4)

White powder ($CHCl_3$-MeOH); m.p. 241-245°C; $[\alpha]_D$ = +49.0° (*c*=0.5, pyridine); EI/MS *m/z*: 472, 426, 408, 248, 223, 203; IR_ν ($CHCl_3$, cm^{-1}) 3400, 2910, 1675; ^{1}H-NMR (400 MHz, $CDCl_3$, δ) 5.46 (1H, br. s, H-12), 4.09 (1H, ddd, *J*=11.4, 9.0, 4.4 Hz, H-2), 3.39 (1H, d, *J*= 9.0 Hz, H-3), 1.26 (3H, s, H-23), 1.25 (3H, s, H-27), 1.07 (3H, s, H-24), 1.01 (3H, s, H-30), 0.99 (3H, s, H-25), 0.98 (3H, s, H-26), 0.93 (3H, s, H-29), ^{13}C-NMR (100 MHz, $CDCl_3$, δ) see Table I.

2α,3β-Dihydroxyurs-12-en-28-oic acid (corosolic acid, 5)

White powder ($CHCl_3$-MeOH); m.p. 253-254°C; $[\alpha]_D$ = +44.2° (*c*=0.4, pyridine); EI/MS *m/z*: 472, 454, 426, 409, 248, 219, 203, 189, 133; IR_ν ($CHCl_3$, cm^{-1}) 3404, 2915, 1685; ^{1}H-NMR (400 MHz, pyridine-d_5, δ) 5.45 (1H, br. s, H-12), 4.09 (1H, ddd, *J*=9.6, 9.6, 3.2 Hz, H-2), 3.40 (1H, d, *J*= 9.6 Hz, H-3), 1.27 (3H, s, H-23), 1.19 (3H, s, H-27), 1.07 (3H, s, H-24), 1.02 (3H, s, H-25), 0.99 (3H, d, *J*= 6.0 Hz, H-29), 0.97 (3H, s, H-26), 0.93 (3H, d, *J*=6.0 Hz, H-30), ^{13}C-NMR (100 MHz, pyridine-d_5, δ) see Table I.

3β,23-Dihydroxyurs-12-en-28-oic acid (23-hydrxyursolic acid, 6)

White powder ($CHCl_3$-MeOH); m.p. 283-287°C; $[\alpha]_D$ = +64.0° (*c*=0.3, MeOH); EI/MS *m/z*: 472, 454, 446, 441, 248, 223, 203, 175, 133; IR_ν ($CHCl_3$, cm^{-1}) 3450, 2944, 1702; ^{1}H-NMR (400 MHz, pyridine-d_5, δ) 5.48 (1H, br. s, H-12), 4.22 (1H, dd, *J*=9.6, 6.0 Hz, H-3), 4.18 (1H, d, *J*=10.4 Hz, H-23a), 3.72 (1H, d, *J*=10.4 Hz, H-23b), 1.17 (3H, s, H-27), 1.06 (3H, s, H-26), 1.04 (3H, s, H-24), 0.98 (3H, d, *J*= 6.4 Hz, H-29), 0.96 (3H, s, H-25), 0.92 (3H, d, *J*=6.4 Hz, H-30), ^{13}C-NMR (100 MHz, pyridine-d_5, δ) see Table I.

2α,3β,23-Trihydroxyolean-12-en-28-oic acid (arjunolic acid, 7)

White powder ($CHCl_3$-MeOH); m.p. 330-332°C; $[\alpha]_D$ = +58.1° (*c*=0.4, pyridine); EI/MS *m/z*: 488, 470, 452, 442, 424, 248, 240, 222, 203, 191; IR_ν ($CHCl_3$, cm^{-1}) 3450, 2910, 1690; ^{1}H-NMR (400 MHz, pyridine-d_5, δ) 5.45 (1H, br. s, H-12), 4.21 (1H, m, H-2), 4.18 (1H x 2, d, *J*=10.4 Hz, H-3, 23a), 3.70 (1H, d, *J*=10.4 Hz, H-23b), 1.19 (3H, s, H-

27), 1.06 (3H, s, H-25), 1.05 (3H, s, H-24), 1.02 (3H, s, H-26), 0.98 (3H, s, H-30), 0.90 (3H, s, H-29), ^{13}C-NMR (100 MHz, pyridine-d_5, δ) see Table I.

ACAT activity assay

Microsomal fractions of Hi5 cells containing baculovirally expressed hACAT-1 or hACAT-2 and rat liver microsomes were used as sources of the enzyme. The activities of the hACAT-1 and hACAT-2 were measured according to the method of Brecher and Chan (Brecher *et al.*, 1980), with slight modification (Jeong *et al.*, 1995; Lee *et al.*, 2001). The reaction mixture, containing 4 μL of microsomes (8 mg/mL protein), 20 μL of 0.5 M potassium phosphate buffer (pH 7.4) with 10 mM dithiothreitol, 15 μL of bovine serum albumin (fatty acid free, 40 mg/mL), 2 μL of cholesterol in acetone (20 μg/mL, added last), 41 μL of water, and 10 μL of test sample in a total volume of 92 μL, was preincubated for 20 min at 37°C with brief vortexing and sonication. The reaction was initiated by the addition of 8 μL of [1-^{14}C] oleoyl-CoA solution (0.05 μCi, final conc. 10 μM). After 25 min of incubation at 37°C, the reaction was stopped by the addition of 1 mL of isopropanol-heptane (4 : 1; v/v). A mixture of 0.6 mL of heptane and 0.4 mL of 0.1 M potassium phosphate buffer (pH 7.4) with 2 mM dithiothreitol was then added to the terminated reaction mixture. The above solution was mixed and allowed to phase separation under gravity for 2 min. Cholesterol oleate was recovered in the upper heptane phase (total volume 0.9-1.0 mL). The radioactivity in 100 μL of the upper phase was measured in a liquid scintillation viral with 3 mL of scintillation cocktail (Lipoluma, Lumac Co.) using a liquid scintillation counter (1450 Microbeta 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, while 8000 cpm of ACAT reaction. The ACAT activity was expressed as a defined unit, cholesteryl oleate pmol/min/mg protein.

RESULTS AND DISCUSSION

When the methanol extract of *C. grandiflora* was developed on the silica gel TLC, the spots showed pink colorization by spaying 10% H_2SO_4 solution and heating, which indicated the presence of triterpenoids in the extracts. The methanol extract was fractionated into EtOAc, *n*-BuOH and H_2O layers through solvent fractionation. The repeated silica gel, ODS column chromatographies, and prep HPLC of EtOAc layer supplied seven triterpenoids, compounds **1-7**.

Compound **1**, white powder, showed the absorbance bands due to the hydroxyl (3420 cm^{-1}) and carboxyl (1680 cm^{-1}) in the IR spectrum ($CHCl_3$) and molecular ion peak (M^+) at *m/z* 456 in the EI/MS spectrum. In the ^{1}H-NMR spectrum, an olefinic methine (δ_H 5.48) and an oxygenated methine (δ_H 3.32) were observed. Also in the high magnet field region, several methine and methylene signals and seven singlet methyl signals (δ_H 1.30, 1.26, 1.04, 1.04, 1.02, 0.97, 0.91) were observed. In the ^{13}C-NMR spectrum, thirty signals consisting of one carboxyl (δ_C 179.5), one olefinic quaternary (δ_C 144.2) and one olefinic methine (δ_C 122.0), one oxygenated methine (δ_C 77.5), six quaternary, three methine and ten methylene and seven methyl (δ_C 28.8, 23.7, 21.4, 17.5, 17.5, 16.6, 15.7) signals were observed. These led to the conclusion for compound **1** to be a pentacyclic triterpenoid of oleanane type with one carboxyl, one double bond, one hydroxyl and seven singlet methyls. Compound **1** was finally identified as 3β-hydroxyolean-12-en-28-oic acid (oleanolic acid) through the comparison of several physical and spectral data with those of literature (Shim *et al.*, 2002).

Compound **2**, white powder, showed the absorbance bands due to the hydroxyl (3360 cm^{-1}) and carboxyl (1695 cm^{-1}) in the IR spectrum ($CHCl_3$) and molecular ion peak (M^+) at *m/z* 456 in the EI/MS spectrum. In the ^{1}H-NMR spectrum, an olefinic methine (δ_H 5.49) and an oxygenated methine (δ_H 3.45) were observed. Also in the high magnet field region, five singlet (δ_H 1.24, 1.23, 1.04, 1.02, 0.89) and two doublet (δ_H 1.01, 0.96) methyl signals were observed. In the ^{13}C-NMR spectrum, thirty signals consisting of one carboxyl (δ_C 179.2), one olefinic quaternary (δ_C 138.6) one olefinic methine (δ_C 125.0), one oxygenated methine (δ_C 77.6), five quaternary, five methine, nine methylene, and seven methyl (δ_C 28.3, 25.7, 23.3, 17.0, 17.5, 16.1, 15.1) signals were observed. These led to the conclusion for compound **2** to be a pentacyclic teriterpenoid of ursane type with one carboxyl, one double bond, one hydroxy, five tertiary and two secondary methyls. Compound **2** was finally identified as 3β-hydroxyurs-12-en-28-oic acid (ursolic acid) through the comparison of several physical and spectral data with those of literature (Houghton *et al.*, 1986).

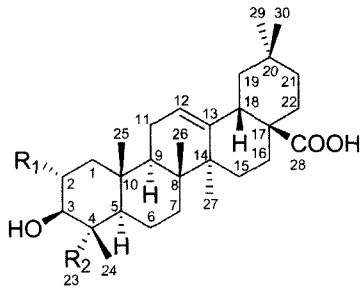
The NMR spectra of compound **3** were almost identical with those of ursolic acid (**2**) with the exception of signal of C-28 atom. Carbon-28, which was a carboxyl group (δ_C 179.2) in the compound **2**, might be transformed to an aldehyde group {(δ_C 207.3), (δ_H 9.30, 1H, s)} by the reduction. Thus, compound **3** was determined to be 3β-hydroxyurs-12-en-28-al (ursolic aldehyde). Because no ^{13}C-NMR data of compound **3** have been reported so far, the ^{13}C-NMR data were assigned on the basis of the ^{1}H- and ^{13}C-NMR data of ursolic acid (**2**).

The NMR spectra of compound **4** were nearly same as those of oleanolic acid (**1**) with the exception of the resonances of C-2 atom. C-2, which was a methylene in oleanolic acid, was modified as an oxygenated methine

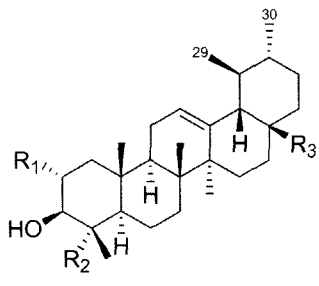
by the oxidation. This fact was proved that not only H-2 (δ_H 4.09) signal showed the correlation with H-3 (δ_H 3.39) and H-1 (δ_H 2.24 and δ_H 1.30) signals in the ^{1}H-^{1}H COSY spectrum but also C-2 (δ_C 68.0) signal with H-3 and H-1 signals in the gHMBC spectrum. Also as the result of observing the coupling constant (J=11.4, 9.0, 4.4 Hz) of H-2, the configuration of hydroxyl at C-2 was determined to be α-bond. The ^{1}H- and ^{13}C-NMR data of compound **4** were assigned on the basis of those of the literature (Kuang *et al.*, 1989). Consequently, compound **4** was determined to be 2α,3β-dihydroxyolean-12-en-28-oic acid (maslinic acid).

NMR spectra of compound **5** were similar to those of ursolic acid (**2**) with the exception of the resonances of C-2 atom. The stereochemistry of a hydroxy group at C-2 {(δ_C 68.1), (δ_H 4.09, 1H, ddd, J=9.6, 9.6, 3.2 Hz)} of compound **5** was determined to be α on the basis of the same procedure as that of compound **4**. The ^{1}H- and ^{13}C-NMR data of compound **5** were assigned by comparing the data with those of literature (Kuang *et al.*, 1989). Thus compound **5** was determined to be 2α,3β-dihydroxyurs-12-en-28-oic acid (corosolic acid).

Chemical shifts of compound **6** were almost identical with those of ursolic acid (**2**) with the exception of signal of C-23. By the oxidation of the methyl group, C-23 was altered to an oxygenated methylene (δ_C 67.3, δ_H 4.18 and 3.72) and this fact was confirmed in the gHMBC spectrum, in which C-23 showed the correlation with H-3 (δ_H 4.22) and H-24 (δ_H 1.04). The ^{1}H- and ^{13}C-NMR data of compound **6** were assigned on the basis of those of the literature (Srivastava *et al.*, 1989). Therefore compound **6** was determined to be 3β,23-dihydroxyurs-12-en-28-oic acid (23-hydroxyursolic aicd).



	R_1	R_2
1 (oleanolic acid)	H	CH_3
4 (maslinic acid)	OH	CH_3
7 (arjunolic acid)	OH	CH_2OH



	R_1	R_2	R_3
2 (ursolic acid)	H	CH_3	COOH
3 (ursolic aldehyde)	H	CH_3	CHO
5 (corosolic acid)	OH	CH_3	COOH
6 (23-hydroxyursolic acid)	H	CH_2OH	COOH

Fig. 1. Chemical structures of triterpenoids isolated from the flower of *Campsis grandiflora* K. Schum

Table II. hACAT-1 and hACAT-2 inhibitory activities of the triterpenoids from the flower of *Campsis grandiflora* K. Schum

Compounds	hACAT-1 (100 μg/mL)*	hACAT-2 (100 μg/mL)*
1	22.6 ± 1.0 %	10.2 ± 0.4 %
2	23.1 ± 0.6 %	16.7 ± 0.6 %
3	21.8 ± 0.7 %	13.0 ± 0.5 %
4	46.2 ± 1.1 %	17.3 ± 1.1 %
5	46.7 ± 0.9 %	3.0 ± 0.2 %
6	41.5 ± 1.3 %	22.2 ± 0.4 %
7	60.8 ± 1.1 %	5.3 ± 0.3 %
Positive control (oleic acid anilide at 0.3 mM)	45.1 ± 0.9 %	50.5 ± 1.6 %

The data are presented as mean ± standard deviation of three replications. *The value denotes the concentration of each compound.

The NMR data of compound **7** were almost same as those of oleanolic acid (**1**) with the exclusion of some signals of C-2 and C-23. By the oxidation of C-2 and C-23, they were converted into the oxygenated methine (δ_C 68.3, δ_H 4.21) and methylene (δ_C 66.0, δ_H 4.18, 3.70), repectively. Each position of the oxidized carbons was verified in the gHMBC spectrum exhibiting the correlation between C-2 and H-3 (δ_H 4.18) and H-24 (δ_H 1.05), and between C-23 and H-3 and H-24, respectively. The ^{1}H- and ^{13}C-NMR data of compound **7** were assigned on the basis of those of the literature (Takemoto *et al.*, 1984). Consequently, compound **7** was determined to be 2α,3β,23-trihydroxyolean-12-en-28-oic acid (arjunolic acid). It is valuable not only that compounds **1-7** were isolated for the first time from this plant but also it is very rare occasion for both oleanane and ursane triterpenoids to be observed in the same plant.

For the development of a useful hypercholesterolemic or anti-atherogenic agent, the compounds **1-7** were examined for ACAT inhibitory activity (Table I). Oleic acid anilide was used as the positive control (Roth *et al.*, 1992; Kim *et al.*, 1994). As the result of this experiment, compounds **4-7** exhibited relatively high hACAT-1 inhibitory activity although all compounds hardly inhibit hACAT-2 activity. Compounds **4**, **5**, **6**, and **7** showed hACAT-1 inhibitory activity with the value of 46.2±1.1, 46.7±0.9, 41.5±

1.3, and 60.8±1.1%, respectively, at the concentration of 100 μg/mL. And the positive control, oleic acid anilide, showed the inhibitory activity with the value of 45.1±0.9% at the concentration of 0.3 mM. Although the triterpenoids isolated form *Campsis grandiflora* showed the lower inhibitory activity than oleic acid anilide, thus far, however, naturally occurring hACAT1 inhibitors have been rarely reported. Especially, the authors could infer the relationship between the chemical structures and the hACAT1 inhibitory activity of the pentacyclic triterpenoids. Compounds **1-3**, which have only one hydroxyl group in the position of C-3 of A-ring, showed relatively low activities, whereas compounds **4-6**, which have two hydroxyl groups at the A-ring, exhibited nearly two times as high activities as compounds **1-3**. Compound **7**, which has three hydroxyl groups at C-2, C-3, and C-23 in the A-ring, revealed the highest hACAT-1 inhibitory activity. In conclusion, it is clear that pentacyclic triterpenoids of more hydroxyl group at the A-ring exhibited higher hACAT-1 inhibitory activity than those of less hydroxyl group without relating to the skeleton type.

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