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Triterpenoids from the Fruits of *Cornus kousa* Burg. as Human Acyl-CoA: Cholesterol Acyltransferase Inhibitors

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Abstract The fruits of *Cornus kousa* Burg. were extracted with 80% aqueous methanol (MeOH) and the concentrated extract was partitioned with ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH), and H_2O. From the EtOAc fraction, 5 triterpenoids were isolated through repeated silica gel (SiO_2), octadecyl silica gel (ODS), and Sephadex LH-20 column chromatography (c.c.). These compounds were determined to be ursolic acid (**1**), corosolic acid (**2**), taraxasterol (**3**), betulinic acid (**4**), and betulinic aldehyde (**5**) on the basis of their spectroscopic data including electronic ionization mass spectrometry, ultraviolet spectroscopy, infrared spectroscopy, and nuclear magnetic resonance. This is the first reported isolation of these compounds from this plant. Also, compounds **1**, **3**, **4**, and **5** show a relatively high inhibitory activity against human acyl-CoA: cholesterol acyltransferase-1 (hACAT-1) with inhibition values of 52.8±0.7, 91.1±0.4, 93.0±0.7, and 96.2±0.2% at a concentration of 100 μM, respectively.

Keywords: *Cornus kousa,* ursolic acid, corosolic acid, taraxasterol, betulinic acid, betulinic aldehyde, acyl-CoA: cholesterol acyltransferase

Introduction

Cornus kousa Burg. (Cornaceae) is a climbing plant distributed in the mountains of South Korea, China, and Japan. It flowers from May to June and the seeds ripen from July to August. The fruits are red or pink and 2-3 cm in diameter. The *C. kousa* flower is colorful and attractive and the fruits are delicious (1). In Korean traditional medicine, the fruit of this plant has been used as a hemostatic agent and for the treatment of diarrhea (1). Additionally, immuno-regulatory properties for this fruit extract have been reported (2). Some chemical constituents of the leaves of *C. kousa* such as isoquercitrin, gallic acid, tannin, phenolics, and flavonoids have been reported (3). Also, our previous phytochemical research on the fruit of this plant demonstrated the presence of sterones, lignans, and flavonoids (4-6). Our ongoing work led to the isolation of 5 triterpenoids from the fruit of *C. kousa* Burg. Their chemical structures were determined using spectroscopic methods and their inhibitory activity against human acyl-CoA: cholesterol acyltransferases, hACAT-1, and hACAT-2, was evaluated.

Acyl-CoA: cholesterol acyltransferase (ACAT, E.C.2.3.1.26) catalyzes the acylation of cholesterol to cholesteryl esters with long chain fatty acids, making it a very attractive target for the treatment of hypercholesterolemia and atherosclerosis (7). ACAT is present as 2 isoforms in mammals (8,9), ACAT-1 and ACAT-2, which differ in their tissue distribution and membrane topology (10). However, most ACAT inhibitors are screened against rat liver microsomal ACAT and they are limited by low oral bioavailability and adrenal and/or hepatic toxicity in clinical trials (11,12). ACAT-1 plays a critical role in foam cell formation in macrophages, whereas ACAT-2 regulates the cholesterol absorption process in intestinal mucosal cells (13). These findings are consistent with the finding that atherosclerosis lesions are reduced in ACAT-1 mice, whereas ACAT-2 mice have limited cholesterol absorption in the intestine, and decreased cholesterol ester content in the liver and plasma lipoproteins (14). Therefore, ACAT inhibitors could be developed as effective anti-hypercholesterolemic or anti-atherogenic agents (15).

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Materials and Methods

Plant materials The fruits of *Cornus kousa* Burg. (Cornaceae) were collected from an experimental farm at Kyung Hee University in August, 2006. A voucher specimen (KHU060907) was reserved at the Laboratory of Natural Products Chemistry, Kyung Hee University, Yongin, Korea.

Instruments and reagents Optical rotations were measured on a JASCO P-1010 digital polarimeter (Jasco, Tokyo, Japan). Melting points were measured with a Fisher-John's melting point apparatus (Fisher Scientific, Waltham, MA, USA) and were not corrected. Electronic ionization mass spectrometry (EI-MS) data were recorded on a JEOL JMSAX 505-WA (Jeol, Tokyo, Japan) and infrared (IR) spectra were run on a Perkin Elmer One FT-IR spectrometer (Perkin Elmer, Waltham, MA, USA). Proton nuclear magnetic resonance (NMR, 400 MHz) and carbon NMR (100 MHz) spectra were taken on a Varian Unity Inova AS

400 FT-NMR spectrometer (Varian, Lake Forest, CA, USA). [1-14C] Oleoyl-CoA (56.0 mCi/mmol) was purchased from the Amersham Biosciences (Buckinghamshire, UK). KH_2PO_4, dithiothreitol, bovine serum albumin (BSA, fatty acid-free), and all reagent grade chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Radioactivity was measured with a scintillation cocktail (Lumac Co., Basle, Switzerland) in a liquid scintillation counter purchased from Wallac (1450 Micerobeta Trilux Wallac Oy; Turku, Finland).

Extraction and isolation of triterpenoids The dried and chopped fruits of *C. kousa* (10 kg) were extracted with 80% aqueous MeOH (10 L×3) 3 times at room temperature. The extracts were partitioned between EtOAc (2 L×3) and H_2O (2 L). The EtOAc extract (44 g) was applied to the silica gel column (10×60 cm) chromatography (c.c.) and eluted with *n*-hexane-EtOAc (4:12:1, 1.5 L of both) $CHCl_3$-MeOH (15:113:110:1, 1.5 L of each) monitoring by thin layer chromatography (TLC) to provide 17 fractions (CKFE1 to CKFE17) and to yield compound **1** [1.58 g, elution volume/total volume (Ve/Vt) 0.52-0.58 (CKFE-9), TLC (SiO_2 F_{254}) R_f 0.70 in $CHCl_3$-MeOH (7:1)]. Subfraction CKFE12 (1.76 g, Ve/Vt 0.70-0.76) was subjected to the SiO_2 c.c. (4×50 cm) and eluted with $CHCl_3$-MeOH (14:1) to produce 13 fractions (CKFE12-1 to CKFE12-13) and to isolate compound **2** [180 mg, Ve/Vt 0.30-0.45, TLC (SiO_2 F_{254}) R_f 0.60 in $CHCl_3$-MeOH (7:1)]. Subfraction CKFE6 (2.40 g, V_e/V_t 0.32-0.40) was subjected to the SiO_2 c.c. (4.5×50 cm) and eluted with *n*-hexane-EtOAc (7:1) to produce 12 fractions (CKFE6-1 to CKFE6-12) and to isolate compound **3** [22 mg, Ve/Vt 0.25-0.35, TLC (SiO_2 F_{254}) R_f 0.70 in *n*-hexane-EtOAc (1:1)]. Subfraction CKFE6-6 (478 mg Ve/Vt 0.50-0.65) was applied to the octadecyl silica gel (ODS) c.c. (3×20 cm) and eluted with MeOH-H_2O (10:1, 800 mL) to produce 5 fractions (CKFE6-6-1 to CKFE6-6-5) and ultimately compound **4** [35 mg, V_e/V_t 0.30-0.45, TLC (RP-18 F_{254}) R_f 0.55, MeOH-H_2O (5:1)]. Subfraction CKFE-6-6-3 (45 mg, V_e/V_t 0.46-0.65) was subjected to the Sephadex LH-20 (2×40 cm, 80% MeOH, 500 mL) to ultimately produce compound **5** [33 mg, V_e/V_t 0.50-0.90, TLC (RP-18 F_{254}) R_f 0.47, MeOH-H_2O (5:1)].

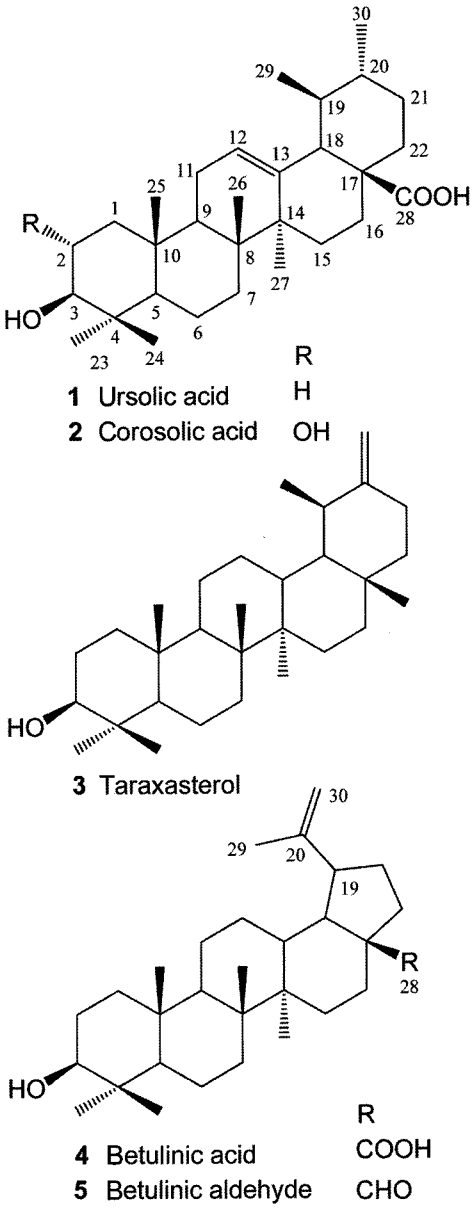

Fig. 1. Chemical structures of triterpenoids isolated from the fruits of *C. kousa*.

Compound **1**, white powder (in $CHCl_3$); m.p. 287; $[\alpha]^{25}_D$=+70.0° (*c*=0.7, $CHCl_3$); EI-MS *m/z*: 456 $[M]^+$; IR_υ ($CHCl_3$, 1/cm) 3,360, 1,732, 1,648; ^{1}H-NMR (400 MHz, C_5D_5N, δ_H) 5.48 (1H, br s, H-12), 3.45 (1H, dd, *J*=9.6, 5.4 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, C_5D_5N, δ_C) see Table 1.

Compound **2**, white powder (in $CHCl_3$); m.p. 253; $[\alpha]^{23}_D$=+42.2° (*c*=0.1, $CHCl_3$); EI-MS *m/z*: 472 $[M]^+$; IR_υ ($CHCl_3$, 1/cm) 3,404, 1,911, 1,700, 1,685; ^{1}H-NMR (400 MHz, C_5D_5N, δ_H) 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, C_5D_5N, δ_C) see Table 1.

Compound **3**, white powder (in $CHCl_3$); m.p. 225°C; $[\alpha]^{20}_D$=+52.2° (*c*=0.1, $CHCl_3$); EI-MS *m/z*: 426 $[M]^+$; IR_υ ($CHCl_3$, 1/cm) 3,410, 2,927, 1,659; ^{1}H-NMR (400 MHz, $CDCl_3$, δ_H) 4.65 (1H, br s, H-30a), 4.54 (1H, br s, H-30b), 3.16 (1H, dd, *J*=12.6, 4.8 Hz, H-3), 2.35 (1H, d, *J*=11.2 Hz, H-19), 1.03 (3H, d, *J*=6.4, H-29), 1.00 (3H, s, H-26), 0.98 (3H, s, H-23), 0.94 (3H, s, H-27), 0.85 (3H, s, H-25), 0.83 (3H, s, H-28), 0.78 (3H, s, H-24); ^{13}C-NMR (100 MHz, $CDCl_3$, δ_C) see Table 1.

Compound **4**, amorphous powder (in MeOH); m.p. 282°C; $[\alpha]^{20}_D$=+12.4° (*c*=0.15, $CHCl_3$); IR_υ (KBr, 1/cm) 3,413, 1,725, 1,653; EI-MS *m/z*: 456 $[M]^+$, 438, 411, 248, 207, 203, 189; ^{1}H-NMR (400 MHz, C_5D_5N, δ_H) 4.92 (1H, br s, H-30a), 4.77 (1H, br s, H-30b), 3.57 (1H, dd, *J*=11.2, 5.0 Hz, H-3), 2.98 (1H, ddd, *J*=11.2, 10.8, 4.9 Hz, H-19), 1.80 (3H, s, H-29), 1.23 (3H, s, H-27), 1.07 (3H, s, H-23), 1.05 (3H, s, H-25), 1.02 (3H, s, H-26), 0.82 (3H, s, H-24); ^{13}C-NMR (100 MHz, C_5D_5N, δ_C) see Table 1.

Table 1. ^{13}C-NMR data of compounds 1, 2, 4, 5 (in C_5D_5N) and 3 (in $CDCl_3$) from the fruits of *C. kousa*

Carbon No.	Compound **1**	Compound **2**	Compound **3**[1)]	Compound **4**[1)]	Compound **5**[1)]
1	38.6	47.5	38.0	38.7	39.3
2	28.2	68.1	27.5	27.4	27.3
3	78.1	83.2	78.9	80.3	78.0
4	39.0	39.4	38.7	38.8	39.6
5	55.8	55.4	55.3	57.4	55.8
6	18.4	18.4	18.3	18.3	18.8
7	33.1	33.0	34.3	34.3	34.7
8	41.7	39.5	42.1	42.0	41.1
9	47.6	47.6	50.4	51.5	50.7
10	37.0	37.9	37.2	37.2	37.5
11	23.3	23.3	21.1	20.8	21.0
12	125.5	124.8	24.8	25.5	25.9
13	139.2	138.8	38.8	38.4	39.0
14	42.0	42.0	43.0	43.6	42.8
15	29.2	28.2	27.4	30.5	28.7
16	24.5	24.5	40.0	32.1	29.1
17	47.6	49.2	36.3	58.1	59.4
18	53.6	53.1	48.3	48.5	48.0
19	39.0	39.0	37.7	50.4	48.3
20	38.9	38.9	150.8	151.3	150.1
21	30.5	30.7	24.0	29.7	29.4
22	36.8	37.0	40.8	37.0	33.3
23	28.4	28.9	28.0	27.9	28.4
24	16.7	17.3	15.4	16.1	16.2
25	15.8	17.1	16.0	17.2	16.5
26	17.5	17.1	16.1	16.9	16.6
27	23.5	23.4	14.6	14.9	14.4
28	179.9	179.8	25.1	180.1	206.4
29	17.1	16.5	19.3	19.4	19.1
30	21.0	21.0	109.1	110.5	110.5

[1)]Assignments are based on 2D-NMR (COSY, HSQC, HMBC) experiments.

Compound **5**, colorless amorphous powder (in MeOH); m.p. 193°C; $[\alpha]^{20}_D$=+19.5° (*c*=0.10, $CHCl_3$); IR_υ (KBr, 1/cm) 3,413, 2,850, 2,760, 1,720, 1,651; EI-MS *m/z*: 440 $[M]^+$, 232, 220, 207, 189; ^{1}H-NMR (400 MHz, C_5D_5N, δ_H) 9.80 (1H, s, H-28), 4.89 (1H, br s, H-30a), 4.74 (1H, br s, H-30b), 3.45 (1H, dd, *J*=11.0, 4.8 Hz, H-3), 3.06 (1H, ddd, *J*=11.0, 10.8, 4.9 Hz, H-19), 1.71 (3H, s, H-29), 1.21 (3H, s, H-27), 1.01 (3H, s, H-23), 0.95 (3H, s, H-25), 0.91 (3H, s, H-26), 0.80 (3H, s, H-24); ^{13}C-NMR (100 MHz, C_5D_5N, δ_C) see Table 1.

ACAT activity assay Microsomal fractions of Hi5 cells containing baculovirally expressed hACAT-1 and hACAT-2 were used as enzyme sources. The activities of hACAT-1 and hACAT-2 were measured according to the method of Brecher and Chan (16), with slight modifications (17). The reaction mixture contained 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 BSA (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. This mixture 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 isopropanolheptane (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 undergo 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 vial with 3 mL of scintillation cocktail using a liquid scintillation counter. Background values were obtained by preparing heat-inactivated microsomes or normal insect cell lysate microsomes, with a usual background count of 200-250 cpm and a count of 8,000 cpm for the ACAT reaction. The hACAT activity was expressed as a defined unit of cholesteryl oleate pmol/min/mg protein. Oleic acid analide was used as a positive control.

Table 2. hACAT-1 and hACAT-2 inhibitory activities of the triterpenoids from the fruits of *C. kousa*[1)]

Compounds	Concentration (μM)	hACAT-1 (%)	hACAT-2 (%)
1	100	52.8±0.7	54.1±0.3
2	100	17.5±6.0	13.7±4.6
3	100	91.1±0.4	41.5±1.5
4	100	93.0±0.7	81.9±0.8
5	100	95.2±0.2	52.2±2.4
Oleic acid anilide[2)]	100 nM	49	60

[1)]Data are presented as the mean±SD of 3 replications.
[2)]Used as a positive control.

Results and Discussion

Isolation and identification of active compounds from the fruits of *C. kousa* When the methanol extracts of *C. kousa* were developed on silica gel TLC, the spots showed pink and purple colorization upon spraying of 10% H_2SO_4 solution and heating, which indicates the presence of triterpenoids in the extracts. The methanol extracts were fractionated into EtOAc and H_2O layers through solvent fractionation. Repeated silica gel, ODS, and Sephadex LH-20 column chromatographies for EtOAc fractions resulted in 5 triterpenoids, compounds **1-5**.

Compound **1**, a white powder, showed absorbance bands due to hydroxyl (3,430/cm), carboxyl (1,790/cm), and olefine (1,694/cm) groups in the IR spectrum ($CHCl_3$) and a molecular ion peak $[M]^+$ at *m/z* 456 in the EI-MS spectrum. The ^{1}H-NMR spectrum (400 MHz, C_5D_5N) demonstrated an olefine methine (δ_H 5.48, br s) and an oxygenated methine (δ_H 3.45, dd, *J*=9.6, 5.4 Hz), and the chemical shift and coupling constants of the latter were in accordance with those of a 3β-OH substitution pattern. Additionally, in the high magnet field, 5 singlet methyl signals [δ_H 1.24 (H-23), 1.23 (H-27), 1.04 (H-26), 1.02 (H-24), 0.89 (H-25)] and 2 doublet methyl signals [δ_H 1.01 (*J*=6.4 Hz, H-30), 0.96 (*J*=6.4 Hz, H-29)] were observed. These indicate that compound **1** could be an ursane type triterpenoid. The ^{13}C-NMR spectrum (100 MHz, C_5D_5N) confirmed the presence of a carboxyl group (δ_C 179.9), the sp^2 hybridized carbons C-12 (δ_C 125.5), and C-13 (δ_C 139.2), indicating that compound **1** is an urs-12-en-oic acid derivative. Also, 1 oxygenated methine (δ_C 78.1) and 7 methyl (δ_C 28.4, 21.0, 18.4, 17.1, 17.5, 16.7, 15.8) signals were observed. These led to the conclusion that compound **1** was a pentacyclic teriterpenoid of ursane type with 5 singlet and 2 doublet methyls. Compound **1** was finally identified as 3β-hydroxyurs-12-en-28-oic acid (ursolic acid) by comparison of its spectral data with those in the literature (18).

Compound **2** was obtained as a white powder. The IR spectrum was similar to that of compound **1,** including a hydroxyl group at 3,404/cm, a carboxyl group at 1,700/cm, and a double bond at 1,685/cm. The EI-MS spectrum showed a molecular ion peak at *m/z* 472. The NMR spectrum of compound **2** was similar to that of ursolic acid (**1**) with the exception of 2 oxygenated methine protons at δ_H 4.09 (1H, ddd, *J*=9.6, 9.6, 3.2 Hz, H-2) and δ_H 3.40 (1H, d, *J*=9.6 Hz, H-3), suggesting an α-OH at the C-2 position and a β-OH at the C-3 position. The ^{1}H- and ^{13}C-NMR data of compound **2** were assigned according to data from the literature (19). Thus, compound **2** was determined to be 2α, 3β-dihydroxyurs-12-en-28-oic acid (corosolic acid).

Compound **3** was obtained as a white powder. The IR spectrum indicated absorption for the hydroxyl group (3,410/cm) and the exo-methylene (1,659/cm). The EI-MS spectrum showed a molecular ion peak at *m/z* 426. In the ^{1}H-NMR spectrum (400 MHz, $CDCl_3$), 2 broad singlet proton signals at δ_H 4.65 (H-30a) and 4.54 (H-30b) due to exomethylene protons, and a doublet methine proton signal at δ_H 3.16 (*J*=12.6, 4.8 Hz) due to an oxygenated methine proton with 3β-OH substitution pattern were observed. In the high magnet field, 6 singlet methyl signals [δ_H 1.00 (H-26), 0.98 (H-23), 0.94 (H-26), 0.85 (H-25), 0.83 (H-28), 0.78 (H-24)], and 1 doublet methyl signal [δ_H 1.03 (*J*=6.4 Hz, H-29)] were observed, indicating that compound **3** was an ursane triterpenoid. The ^{13}C-NMR spectrum (100 MHz, $CDCl_3$) exhibited the presence of 30 carbon signals, consisting of 7 methyl signals (δ_C 25.1, 19.3, 18.3, 16.1, 16.0, 15.4, and 14.6), 2 olefine carbon signals (δ_C 109.3 and 150.8), and 1 oxygenated methine carbon signal (δ_C 78.9). The multiplicity of each carbon was determined using a DEPT experiment. The position of the exomethylene and the doublet methyl groups were determined using HSQC and HMBC experiments. The exomethylene proton signals at δ_H 4.65 and 4.54 (H-30) showed correlations with a singlet carbon signal at δ_C 150.8 (C-20) in the HMBC spectrum. Furthermore, the HSQC spectrum exhibited a cross peak between a doublet methyl proton signal at δ_H 1.03 (H-29) and a doublet carbon signal at δ_C 19.3, therefore the latter could be identified as C-29. Based on the cross peak in the HSQC, the doublet of doublet methine proton signal at δ_H 2.35 corresponds with the proton on C-19 (δ_C 37.7). Also, H-29 showed cross peaks with C-19 and a quaternary C-20 in the HMBC spectrum. As a result, compound **3** was determined to be 3β-hydroxy-urs-20(30)-ene (taraxasterol) (20).

Compound **4** was obtained as an amorphous powder. The EI-MS spectrum showed a molecular ion peak at *m/z* 456. The IR spectrum suggested that compound **4** contained hydroxyl groups (3,413/cm), 1 carboxyl group (1,725/cm), and 1 terminal double bond (1,653/cm). The ^{1}H-NMR spectrum showed 5 singlet methyl signals [δ_H 1.23 (H-27), 1.07 (H-23), 1.05 (H-25), 1.02 (H-26), 0.82 (H-24)], an isopropenyl group [δ_H 4.92 (H-29a), 4.77 (H-29b), 1.80 (H-30)], an oxygenated methine proton [δ_H 3.57 (dd, *J*=11.2, 5.0 Hz, H_β-3)], and a typical lupane 19β-proton signal at δ_H 2.98 (ddd, *J*=11.2, 10.8, 4.9 Hz). All these data indicate that compound **4** is a lupane triterpenoid. Its ^{13}C-NMR spectrum (Table 1) showed 30 carbon signals, which were assigned as 6 methyls, 10 methylenes, 5 methines, 5 quaternary carbons, 1 oxygenated methine, 2 olefins, and 1 carboxyl carbon using DEPT experiments. The 2 sp^2 carbons observed at δ_C 151.3 and 110.5 in the ^{13}C-NMR spectrum confirmed the $\Delta^{20,29}$-functionality of a lupane skeleton. Thus, compound **4** was identified as the lupane-triterpenoid, 3β-hydroxylup-20(30)-en-28-oic acid (betulinic acid) through comparison with reported spectral data (21).

Compound **5** was obtained as a colorless amorphous

powder in methanol. The EI-MS spectrum showed a molecular ion peak at *m/z* 440. The IR spectrum suggested that it contained a hydroxyl group (3,413/cm), aldehyde group (2,850 and 2,760/cm), and exomethylene group (1,651/cm). The NMR spectra of compound **5** was almost identical to that of betulinic acid (**4**), with the exception of an aldehyde signal [δ_C 206.4, δ_H 9.80 (s)]. Carbon-28, which was a carboxyl group (δ_C 180.1) in compound **4**, may have been transformed to an aldehyde group through a reduction reaction. The aldehyde group was determined to be located at C-28 using an HMBC experiment in which the aldehyde proton signal at δ_H 9.80 showed ^2J and ^3J correlations with C-17 (δ_C 59.4) and C-18 (δ_C 48.0), respectively. Thus, compound **5** was identified as hydroxylup-20(30)-en-28-al (betulinic aldehyde) (22). This is the first report of extraction of all 5 of these compounds from the fruit of *C. kousa*.

Inhibitory activity against human ACAT For the development of a useful hypercholesterolemic or anti-atherogenic agent, the ACAT inhibitory activity of compounds **1-5** was evaluated (Table 2). Oleic acid anilide was used as the positive control. In this experiment, compounds **1** and **3-5** showed significant inhibitory activity towards hACAT-1 of 52.8± 0.7, 91.1±0.4, 93.0±0.7, and 95.2±0.2%, respectively, and towards hACAT-2 of 54.1± 0.3, 41.5±1.5, 81.9±0.8, and 52.2±2.4%, respectively, at a concentration of 100 μM. The positive control, oleic acid anilide, showed inhibitory activity towards hACAT-1 and hACAT-2 of 49.0 and 60.0%, respectively, at a concentration of 100 nM. Although the triterpenoids isolated form *C. kousa* showed a lower inhibitory activity than oleic acid anilide, naturally occurring hACAT inhibitors have rarely been reported. In general, lupane triterpenoids exhibit a slightly higher inhibitory activity on ACAT than ursane triterpenoids. Compound **2**, a ursane triterpenoic acid with *trans*-1,2-diol at C-2 and C-3, barely inhibited ACAT activity. The exomethylene group at C-30 in the ursane triterpenoid might be a key factor decreasing the inhibitory activity of this compound towards hACAT-1, while the carboxylic acid at C-28 in the lupane triterpenoid might be responsible for the increased inhibitory activity of this compound towards hACAT-2. In conclusion, the findings of this study suggest that the methanolic extract of *C. kousa*, as well as its isolated compounds, may prove useful in the treatment of hypercholesterolemia and atherosclerosis.

Acknowledgments

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