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Cytotoxic and ACAT-inhibitory Sesquiterpene Lactones from the Root of *Ixeris dentata* forma *albiflora*

Eun-Mi Ahn, Myun-Ho Bang[1], Myoung-Chong Song[1], Mi-Hyun Park[2], Hwa-Young Kim[2], Byoung-Mog Kwon[3], and Nam-In Baek[1]

Department of Herbal Food Science, Daegu Haany University, Gyeongsan, 712-715, Korea, [1]Graduate School of Biotechnology & Plant Metabolism Research Center, Kyung Hee University, Suwon 449-701, Korea, [2]Erom Life Co. Ltd., Seoul 135-825, Korea, and [3]Korea Research Institute of Bioscience and Biotechnology, Daejon 305-333, Korea

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Ixeris dentata forma *albiflora* was extracted with 80% aqueous MeOH, and the concentrated extract was partitioned with EtOAc, *n*-BuOH and H_2O. Eight sesquiterpenes were isolated through repeated silica gel and octadecyl silica gel (C_{18}, ODS) column chromatography of the EtOAc and *n*-BuOH fractions. Physicochemical analysis using NMR, MS and IR revealed the chemical structures of the sesquiterpenes, which were zaluzanin (**1**), 9*a*-hydroxyguaian-4(15),10(14),11(13)-triene-6,12-olide (**2**), 3β-*O*-β-D-glucopyranosyl-8β-hydroxyguaian-4(15),10(14)-diene-6,12-olide (**3**), 3-*O*-β-D-glucopyranosyl-8β-hydroxyguauan-10(14)-ene-6,12-olide (**4**), ixerin M (**5**), glucozaluzanin C (**6**), crepiside I (**7**), and ixerin D (**8**). This is the first time that these sesquiterpene lactones have been isolated from this plant. Compounds **1**, **2** and **7** revealed relatively high cytotoxicities on human colon carcinoma cell and lung adenocarcinoma cell, while compounds **5** and **7** showed acyl-CoA: cholesterol acyltransferase (ACAT) inhibitory activity.

Key words: *Ixeris dentata* forma *albiflora*, Sesquiterpene lactone, ACAT, HT29, A549.

INTRODUCTION

Ixeris dentata forma *albiflora* is an edible perennial herb, found all over Korea. *I. dentata* has been used as a traditional medicine for mithridatism, calculous and pneumonia (Soka, 1985). Despite these medical properties, the isolation of the chemical components of *I. dentate* forma *albiflora* has not been reported so far.

The chemical constituents of genus *Ixeris* have been studied by a number of researchers. Many classes of its secondary metabolites, including triterpenoids, sesquiterpene glycosides and flavonoids, have been investigated (Arai *et al.*, 1985; Kim and Lee, 1988; Seto *et al.*, 1986). Among them, many sesquiterpene lactones have exhibited a wide range of biological activities, such as antitumoral activity, cytotoxicity, phytotoxicity and antimicrobial activity (Geissman, 1973; Robles *et al.*, 1995).

In a previous study, we reported the Acyl-CoA: cholesterol acyltransferase (ACAT), diacylglycerol acyltransferase (DGAT) and farnesyl-protein transferase (FPTase) inhibitory activities of *I. dentata* forma *albiflora* and identified the active compounds as zaluzanin C (**1**), 9α-hydroxyguaian-4(15),10(14),11(13)-triene-6,12-olide (**2**), 3β-*O*-β-D-glucopyranosyl-8β-hydroxyguaian-4(15),10(14)-diene-6,12-olide (**3**), 3-*O*-β-D-glucopyranosyl-8β-hydroxyguauan-10(14)-ene-6,12-olide (**4**) (Bang *et al.*, 2004). In our continuing study of this plant, we isolated an additional four sesquiterpene lactones **5-8** for the first time. We evaluated the compounds for cytotoxic effects on lung adenocarcinoma A549 cells and colon carcinoma HT29 cells and for inhibitory activity on ACAT. Inhibition of Acyl-CoA, 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).

Correspondence to: Nam-In Baek, Graduate School of Biotechnology, KyungHee University, Seochun-Dong 1, Kiheung-Gu, Suwon 449-701, Korea
Tel: 82-31-201-2661 Fax: 82-31-201-2157
E-mail: nibaek@khu.ac.kr

MATERIALS AND METHODS

General experimental procedures

Melting points were determined on a Fisher-John apparatus and uncorrected. Optical rotations were measured with a P-1020 polarimeter (JASCO, Japan). Infrared (IR) spectra were measured with a Perkin model 599B infrared spectrometer (Perkin-Elmer, Massachusetts, U.S.A.). Fast atom bombardment-mass spectroscopy (FAB-MS) analyses were recorded on a JEOL JMSAX 505-WA. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were measured with a Varian Unity Inova AS 400 FT-NMR spectrometer, the chemical shifts being represented as ppm with tetramethylsilane as an internal standard. Column chromatography was carried out using silica gel 60 (63-200 mm, Merck Co., Germany), ODS Lichroprep RP-18 (Merck Co.). Thin layer chromatography (TLC) was carried out using plates coated with silica gel 60 F_{254} (Merck Co.).

Plant materials

Whole *Ixeris dentata* forma *albiflora* plants were purchased from the farmer at Yangpyung, Kyunggido, Korea, in October 2000. A voucher specimen (KHU03052) was deposited at the Laboratory of Natural Products Chemistry, Kyung Hee University, Suwon, Korea.

Chemicals

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

Extraction and isolation

Ixeris dentata forma *albiflora* (27 kg) were extracted three times with 80% aqueous MeOH (360 L) at room temperature. The MeOH solution was filtered and evaporated to give a residue (348 g). The residue was partitioned with EtOAc (2 L × 3), *n*-BuOH (2 L × 3) and H_2O (2 L). The *n*-BuOH layer was concentrated and chromatographed on a silica gel column with $CHCl_3$-MeOH-H_2O (9:3:1→7:3:1→65:35:10) to yield ten fractions (IDR-B-1-10). Fraction (IDR-B-4, 3.9 g) was subjected to successive column chromatography on a silica gel (*n*-hexane-$CHCl_3$-MeOH =2:3:1) to give five subfractions (IDR-B-4-1 - IDR-B-4-5). Two of these subfractions (IDR-B-4-3, IDR-B-4-5) were subjected to successive column chromatography on a silica gel (*n*-hexane-$CHCl_3$-MeOH=2:3:1) to give compounds **5** (22.4 mg, R_f value on silica gel TLC in $CHCl_3$-MeOH-H_2O=7:3:1, 0.64), **6** (13.2 mg, R_f value on silica gel TLC in $CHCl_3$-MeOH-H_2O=7:3:1, 0.60) and **7** (51.9 mg, R_f value on silica gel TLC in $CHCl_3$-MeOH-H_2O=7:3:1, 0.62). IDR-B-5-8 (235 mg) was chromatographed on a ODS column, eluted with MeOH-H_2O (2:8) to obtain compound **8** (30 mg, R_f value on silica gel TLC in $CHCl_3$-MeOH=3:1, 0.45).

Ixerin M (5)

White powder ($CHCl_3$-MeOH); m.p. 202-205°C; IRν (KBr, cm^{-1}): 3370 (hydroxyl), 1760 (C=O); $[\alpha]_D$: -0.6° (*c*=1.5, MeOH); FAB-MS: *m/z* 547 $[M+Na]^+$; ^{1}H-NMR (400 MHz, pyridine-d_5): δ 6.33 (1H, d, *J*=3.0 Hz, H-13a), 5.96 (1H, br. s, H-15a), 5.52 (1H, br. s, H-15b), 5.38 (1H, d, *J*=3.0 Hz, H-13b), 5.21 (1H, br. s, H-14a), 5.06 (1H, m, H-8), 4.95 (1H, br. s, H-14b), 4.82 (1H, dd, *J*=6.3, 5.9 Hz, H-6), 4.72 (1H, d, *J*=4.3 Hz, H-β), 4.64 (1H, d, *J*=8.1 Hz, H-1'), 4.52 (1H, br. s, H-3), 3.69 (1H, dd, *J*=12.2, 4.8 Hz, H-6'a), 3.57-3.12 (4H, m, H-2', H-4', H-6'), 3.31 (1H, br. d, *J*=12.2 Hz, H-6'b), 3.14 (1H, dd, *J*=5.9, 1.8 Hz, H-7), 2.92 (1H, br. dd, *J*=5.3, 10.8 Hz, H-1), 2.74 (1H, dd, *J*=9.5, 6.3 Hz, H-5), 2.45 (1H, dd, *J*=13.9, 6.1 Hz, H-9a), 2.42 (1H, dd, *J*=13.9, 5.8 Hz, H-9b), 2.34 (1H, m, H-2a), 2.17 (1H, m, H-γ), 1.98 (1H, m, H-2b), 1.00 (3H, d, *J*=6.8 Hz, H-δ), 0.96 (3H, d, *J*=6.8 Hz, H-d'); ^{13}C-NMR (100 MHz, pyridine-d_5): δ 174.81 (C-α), 169.90 (C-12), 150.44 (C-4), 143.17 (C-10), 136.39 (C-11), 121.31 (C-13), 117.74 (C-14), 112.92 (C-15), 104.79 (C-1'), 80.22 (C-3), 78.48 (C-6), 78.31 (C-3'), 78.18 (C-5'), 77.05 (C-β), 75.76 (C-2'), 71.02 (C-4'), 68.71 (C-8), 63.50 (C-6'), 50.44 (C-5), 48.25 (C-7), 45.15 (C-1), 40.64 (C-9), 38.17 (C-2), 32.13 (C-γ), 19.16 (C-d), 17.47 (C-δ').

Glucozaluzanin C (6)

White powder ($CHCl_3$-MeOH); m.p. 104-107°C; IRν (KBr, cm^{-1}): 3374 (hydroxyl), 1750 (C=O); $[\alpha]_D$: -12.6° (*c*=0.9, MeOH); FAB-MS: *m/z* 431 $[M+Na]^+$; ^{1}H-NMR (400 MHz, pyridine-d_5): δ 6.24 (1H, d, *J*=3.2 Hz, H-13a), 5.47 (1H, d, *J*=3.2 Hz, H-13b), 5.22 (1H, br. s, H-15a), 5.07 (1H, br. s, H-15b), 5.01 (1H, br. s, H-14a), 4.85 (1H, br. s, H-14b), 4.59 (1H, dd, *J*=6.4, 6.4 Hz, H-3), 4.56 (1H, d, *J*=8.0 Hz, H-1'), 3.85 (1H, dd, *J*=10.1, 9.2 Hz, H-6), 3.75 (1H, br. d, *J*=11.8 Hz, H-6a'), 3.67 (1H, dd, *J*=11.8, 4.8 Hz, H-6b'), 3.15-3.35 (4H, overlapping, H-2', 3', 4', 5'), 3.14 (1H, m, H-7), 3.10 (1H, ddd, *J*=8.4, 8.8, 8.8 Hz, H-1), 2.91(1H, m, H-5), 2.57 (1H, m, H-8a), 2.52 (1H, m, H-8b), 2.30 (1H, m, H-9a), 2.27 (1H, m, H-9b), 2.04 (1H, m, H-2a), 2.03 (1H, m, H-2b); ^{13}C-NMR (100 MHz, pyridine-d_5): δ 170.32 (C-12), 150.51 (C-4), 148.83 (C-10), 140.90 (C-11),120.19 (C-13), 113.12 (C-14), 111.95 (C-15), 103.43 (C-1'), 83.84 (C-6), 80.86 (C-3), 78.46 (C-3'), 78.12 (C-5'), 75.57 (C-2'), 71.20 (C-4'), 62.89 (C-6), 50.19 (C-5), 45.84 (C-7), 44.76 (C-1), 38.19 (C-2), 34.57 (C-9), 30.59 (C-8).

Crepiside I (7)

Amorphous powder ($CHCl_3$-MeOH); m.p. 224-225°C; IRν (KBr, cm^{-1}): 3450 (hydroxy), 1745 (C=O); $[\alpha]_D$: -22.6° (*c*=

0.31, MeOH); FAB-MS: *m/z* 581 $[M+Na]^+$; ^{1}H-NMR (400 MHz, CD_3OD): δ 7.23 (2H, d, *J*=6.4 Hz, H-2", 6"), 7.04 (2H, d, *J*=6.4 Hz, H-3", 5"), 6.51 (1H, d, *J*=3.4 Hz, H-13a), 5.82 (1H, br. s, H-15b), 5.63 (1H, d, *J*=3.4 Hz, H-13b), 5.46 (1H, br. s, H-15a), 5.12 (3H, br. s, H-14a), 4.48 (1H, m, H-8), 4.81 (1H, br. s, H-14b), 4.64 (1H, d, *J*=8.2 Hz, H-1'), 4.43 (1H, dd, *J*=10.4, 9.2 Hz, H-6), 3.71 (1H, dd, *J*=12.1, 5.2 Hz, H-6a), 3.66 (2H, s, H-β), 3.21 (1H, dd, *J*=12.1, 3.3 Hz, H-6b), 3.11~3.61 (4H, overlapping, H-2,3,4,5), 3.07 (1H, dd, *J*=9.2, 6.0 Hz, H-7), 2.91 (1H, br. dd, *J*=16.6, 8.4 Hz, H-1), 2.72 (1H, dd, *J*=9.2, 9.2 Hz, H-5), 2.58 (1H, dd, *J*=14.2, 5.8 Hz, H-9a), 2.40 (1H, dd, *J*=14.2, 5.6 Hz, H-9b), 2.31(1H, m, H-2b), 2.29 (1H, m, 2a); ^{13}C-NMR (100 MHz, CD_3OD): δ 171.64 (C-α), 170.47 (C-12), 157.62 (C-4"), 148.20 (C-4), 144.52 (C-10), 136.93 (C-11), 130.10 (C-2", 6"), 125.80 (C-1"), 121.96 (C-13), 118.63 (C-3", 5"), 117.11 (C-14), 112.77 (C-15), 104.52 (C-1'), 80.79 (C-3), 78.37 (C-3'), 78.04 (C-6), 75.25 (C-2'), 74.82 (C-5'), 71.47 (C-4'), 67.82 (C-8), 64.90 (C-6'), 50.84 (C-5), 48.11 (C-7), 45.06 (C-1), 43.55 (C-9), 40.55 (C-β), 37.43 (C-2).

Ixerin D (8)

White powder ($CHCl_3$-MeOH); m.p. 229-233°C; IRν (KBr, cm^{-1}): 3450 (hydroxy), 1773 (C=O); $[\alpha]_D$: -32.2° (*c*=0.5, MeOH); FAB-MS: *m/z* 449 $[M+Na]^+$; ^{1}H-NMR (400 MHz, CD_3OD): δ 5.42 (1H, br. s, H-15a), 5.67 (1H, br. s, H-15b), 5.33 (1H, d, *J*=3.6 Hz, H-13a), 6.16 (1H, d, *J*=3.6 Hz, H-13b), 4.82 (1H, br. s, H-3), 5.05 (1H, d, *J*=8.2 Hz, H-1'), 3.27 (1H, m, H-7), 2.98 (1H, dd, *J*=9.4, 9.4 Hz, H-5), 1.32 (3H, s, H-14); ^{13}C-NMR (100 MHz, CD_3OD): δ 171.01 (C-12), 150.28 (C-4),143.19 (C-11), 119.52 (C-13), 112.91 (C-15), 102.06 (C-1'), 80.83 (C-6), 78.96 (C-3), 77.98 (C-3'), 77.76 (C-5'), 75.05 (C-2'), 73.24 (C-10), 71.72 (C-4'), 62.27 (C-6'), 50.75 (C-1), 51.94 (C-5), 44.84 (C-7), 35.79 (C-2), 35.53 (C-9), 30.81 (C-14), 24.96 (C-8).

Assay of cytotoxic activity

The human A549 lung adenocarcinoma cells and HT29 human colon carcinoma cells were purchased from the Korea Cell Line Bank (KCLB). The cells were maintained in RPMI 1640 (Gibco, N.Y, U.S.A.), supplemented with 10% heat inactivated FBS (JRH Bio Science, Lenexa, U.S.A.) and antibiotics (penicillin 100 U/mL and streptomycin 100 μg/m, Sigma, St. Louis, MO, U.S.A.). The cells were harvested by trypsinization (0.25% trypsin) and plated at a concentration of 2.0-2.5×10^4 cells/well in RPMI 1640 supplemented with 10% FBS in 96-well tissue culture plates and allowed to attach for 24 h. Various concentrations of test samples were added. After 3 days, in a humidified 5% CO_2 incubator at 37°C, cell proliferation reagent MTS (Promega Co., U.S.A.) was added to each well. Cytotoxic activity was determined using the absorbance value of each well at 492 nm (Chan *et al.*, 2001). The 50% inhibitory concentration (IC_{50}) values for cell growth were expressed as the dose resulting in 50% reduction of tumor cell growth.

ACAT activity assay

Rat liver microsomes were used as the source of the enzyme. The activity of ACAT was measured according to the method of Brecher and Chan (1980), with slight modifications (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 BSA (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 left for 2 mins to allow phase separation under gravity. 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 scintillation cocktail (Lipoluma, Lumac Co.) using a liquid scintillation counter (1450 Microbeta Trilux Wallac Oy, Turku, Finland). Background values (200-250 cpm) were obtained by preparing heat inactivated microsomes or normal insect cell lysate microsomes. ACAT reaction samples gave values of around 8000 cpm. The ACAT activity was expressed as a defined unit of pmol of cholesteryl oleate/min/mg protein.

RESULTS AND DISCUSSION

MeOH extracts from the *I. dentata* forma *albiflora* inhibited the growth of A549 and the activity of ACAT by 82% and 89% respectively, at the concentration of 1 mg/mL. The principal components manifesting the activity were therefore isolated. The TLC of the EtOAc and *n*–BuOH fractions obtained from the MeOH extracts of the plant demonstrated the presence of some sesquiterpenes, and finally eight compounds were isolated by silica gel and ODS column chromatographies. Out of these compounds, the structure determination and ACAT, DGAT and FPTase inhibitory activities of compounds **1-4** have been described in a previous paper (Bang *et al.*, 2004). Compounds **5-8** were identified as ixerin M (**5**), glucozaluzanin C (**6**), crepiside I (**7**), and ixerin D (**8**) through the interpretation

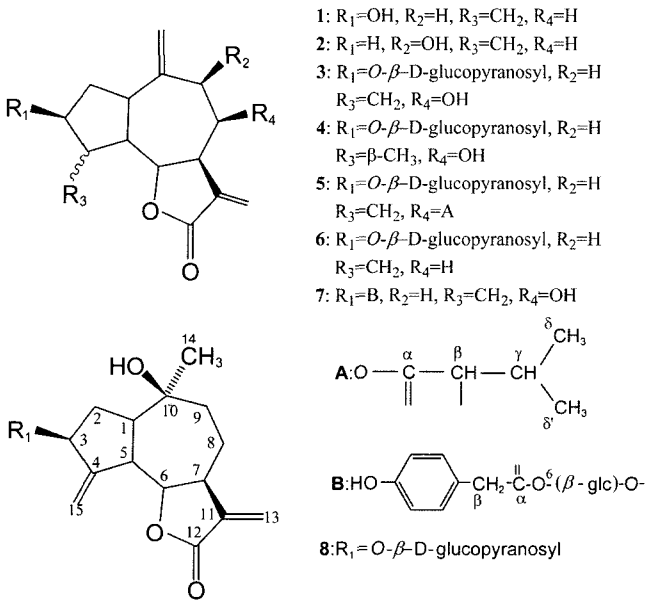

Fig. 1. Chemical structures of sesquiterpene lactones isolated from the roots of *Ixeris dentata* forma *albiflora*

of spectral data including 2D-NMR as well as by comparing with spectral data from the literature (Hidehisa *et al.*, 1984; Mamoru *et al.*, 1986; Miyase *et al.*, 1985; Miyase and Fukushima, 1984; Warashina *et al.*, 1990).

Compounds **5-8** have similar NMR spectral patterns. Compound **5** was obtained as a white powder. The IR spectrum showed the presence of a hydroxyl group (3370 cm^{-1}) and γ-lactone (1760 cm^{-1}). The ^{1}H-NMR spectrum showed doublets at δ 6.33 (1H, d, *J*=3.0 Hz, H-13a) and 5.38 (1H, d, *J*=3.0 Hz, H-13b), which were characteristic of exocyclic α-methylene-γ-lactone. Furthermore, two exomethylene groups were observed at δ 5.21 (1H, br.s H-14a), 4.95 (1H, br.s H-14b) and 5.96 (1H, br.s, H-15a), 5.52 (1H, br.s H-15b). The ^{13}C-NMR spectrum showed the presence of a γ-lactone carbonyl signal at δ 78.48 (C-6) and 169.90 (C-12), oxygen-bearing carbons were observed at δ 80.22 (C-3) and 68.71 (C-8), two methylene signals at δ 38.17 (C-2), 40.64 (C-9) and three methine signals at 45.51 (C-1), 50.44 (C-5) and 48.25 (C-7). These signals suggested the characteristics of a guaianolide-type sesquiterpene lactone. In the ^{1}H-NMR spectrum, an anomeric proton at δ_H 4.64 (1H, d, *J*=8.1 Hz, H-1') and a sugar moiety were also observed. The presence of isopentanoyl groups were confirmed from the ^{1}H-NMR signals [(δ_H 4.72 (1H, d, *J*=4.3 Hz, H-b), 2.17 (1H, m, H-g), 1.00 (3H,d, *J* =6.8 Hz, H-d) and 0.96 (3H, d, *J* =6.8 Hz, H-d¢). From these data, the structure of this compound was confirmed by direct comparison (^{1}H- and ^{13}C-NMR) with the literature (Warashina *et al.*, 1990). Therefore compound **5** was assumed to be ixerin M, which has been isolated from *Ixeris debilis*.

Compound **6** was obtained as a white powder as well. The IR spectrum also showed the presence of a hydroxyl group (3374 cm^{-1}) and γ-lactone (1750 cm^{-1}). The ^{1}H-NMR spectrum showed doublets at δ 6.24 (1H, d, *J*=3.2 Hz, H-13a) and 5.47 (1H, d, *J*=3.2 Hz, H-13b), which were characteristic of exocyclic α-methylene-g-lactone. The ^{1}H- and ^{13}C-NMR spectra were similar to those of compound **5**, except for the oxygenated methine signal and 2-hydroxy-3-methylbutanoyl signals. In the ^{13}C-NMR spectrum, a methylene signal was observed at δ 30.59 (C-8). From these results compound **6** was determined to be glucozaluzanin C, which has been isolated from *Ixeris dentata* and *Ainsliaea acerifolia* (Mamoru *et al.*, 1986, Miyase and Fukushima, 1984).

Compound **7** was obtained as an amorphous powder. The IR spectrum of **7** also indicated the presence of a hydroxyl group (3450 cm^{-1}) and g-lactone (1745 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra were similar to those of compound **5**. The ^{1}H-NMR spectrum showed a *para*-substituted signal δ_H 7.23 (2H, d, *J*=6.4 Hz, H-2", 6"), 7.04 (2H, d, *J*=6.4 Hz, H-3", 5"), which were correlated with carbon-13 signals at δ_C 130.10 (C-2", 6") and 118.63 (C-3", 5") respectively, and a singlet methylene signal 3.66 (2H, s, H-β). The ^{13}C-NMR spectrum showed *para*-substituted oxygen-bearing carbon δ_C 157.62 (C-4"), carbon-bearing δ_C 125.80 (C-1"), four methine carbon signals [δ 130.10 (C-2", 6"), 118.63 (C-3", 5")], methylene and carbonyl signals δ_C 171.64 (C-α) and δ_C 40.55 (C-β) respectively. From these data the structure of compound **7** was determined to be crepiside I, which has been isolated from *Crepis japonica* (Miyase *et al.*, 1985).

Compound **8** was obtained as a white powder. The IR spectrum again showed the presence of a hydroxyl group (3450 cm^{-1}) and γ-lactone (1773 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra were similar to those of compound **6**, except for the methyl signal [δ_H 1.32 (3H, s, H-14), δ_C 30.81 (C-14)] and an oxygen-bearing carbon δ_C 73.24 (C-10). Thus compound **8** was identified as an ixerin D, which has been isolated from *I. tamagawaensis* and *I, dentata Nakai* (Hidehisa *et al.*, 1984, Mamoru *et al.*, 1896).

In order to assess the potential of compounds **1-8** as useful anti-cancer, hypercholesterolemic and anti-atherogenic agents, the compounds were tested for cytotoxicity on a cultured human colon carcinoma cell line (HT-29) and lung adenocarcinoma cell line (A549), and for inhibitory activity on ACAT. Compounds **1**, **2**, and **7** exhibited relatively mild cytotoxicity (**1**: A549, IC_{50}: 0.26 mM; HT-29, IC_{50}: 0.19 mM; **2**: A549, IC_{50}: 1.63 mM; HT-29, IC_{50}: 0.25 mM; **7**: HT-29, IC_{50}: 6.75 mM), with IC_{50} values many orders of magnitude higher than the positive control, mithramycin (A549, IC_{50}: 0.06 μM; HT-29, IC_{50}: 0.07 μM). These results suggest that the attachment of a glucose to the isolated guaianolide sesquiterpene led to the decrease of the cytotoxicity on some cancer cells.

Compounds **5** and **7** showed ACAT inhibitory activity with values of 46.4±1.1% and 66.5±0.9% respectively, at 100 μg/mL. The positive control, oleic acid anilide, inhibited ACAT by 45.1±0.9% at 0.1 μg/mL. The other compounds showed little cytotoxic activity against the tumor cell lines and low ACAT inhibitory activity. Although the sesquiterpene lactones isolated form *Ixeris dentata* forma *albiflora* had lower inhibitory activity than oleic acid anilide, naturally occurring ACAT inhibitors have rarely been reported. This finding therefore may lead to further study to facilitate the development of safer hypercholesterolemic, anti-atherogenic and anti-cancer agents.

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