JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Triterpenoids Isolated from Apple Peels Have Potent Antiproliferative Activity and May Be Partially Responsible for Apple's Anticancer Activity

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Bioactivity-guided fractionation of apple peels was used to determine the chemical identity of bioactive constituents. Thirteen triterpenoids were isolated, and their chemical structures were identified. Antiproliferative activities of the triterpenoids against human HepG2 liver cancer cells, MCF-7 breast cancer cells, and Caco-2 colon cancer cells were evaluated. Most of the triterpenoids showed high potential anticancer activities against the three human cancer cell lines. Among the compounds isolated, 2α-hydroxyursolic acid, 2α-hydroxy-3β-{[(2*E*)-3-phenyl-1-oxo-2-propenyl]oxy}olean-12-en-28-oic acid, and 3β-*trans*-*p*-coumaroyloxy-2α-hydroxyolean-12-en-28-oic acid showed higher antiproliferative activity toward HepG2 cancer cells. Ursolic acid, 2α-hydroxyursolic acid, and 3β-*trans*-*p*-coumaroyloxy-2α-hydroxyolean-12-en-28-oic acid exhibited higher antiproliferative activity against MCF-7 cancer cells. All triterpenoids tested showed antiproliferative activity against Caco-2 cancer cells, especially 2α-hydroxyursolic acid, maslinic acid, 2α-hydroxy-3β-{[(2*E*)-3-phenyl-1-oxo-2-propenyl]oxy}olean-12-en-28-oic acid, and 3β-*trans*-*p*-coumaroyloxy-2α-hydroxyolean-12-en-28-oic acid, which displayed much higher antiproliferative activities. These results showed the triterpenoids isolated from apple peels have potent antiproliferative activity and may be partially responsible for the anticancer activities of whole apples.

KEYWORDS: Apple; apple peels; phytochemicals; phenolics; triterpenoids; breast cancer; antiproliferative activities; MCF-7 cells; cancer cells

INTRODUCTION

Epidemiologic studies have consistently shown that diets rich in fruits and vegetables have been associated with reduced risk of developing chronic diseases, such as cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataracts, and age-related functional declines (*1*−*3*). This suggests that a change in dietary behavior, such as increasing the consumption of fruits and vegetables, and related lifestyles is a practical strategy for significantly reducing the incidence of cancer.

Apples (*Malus pumila*) are a very significant part of the diet in humans and are rich in phenolic compounds (*4*). The total phenolic content ranges from 110 to 357 mg/100 g of fresh apple (*5*−*8*). Twenty-two percent of fruit phenolics consumed in the United States are from apples, which are the largest source. Apples also have a high content of flavonoids (*4*, *8*). In Finland, apples are the top contributors of flavonoids along with onions (*9*). Consumption of apples has been linked to the prevention of various chronic diseases (*4*). Apple intake can reduce lung cancer incidence (*9*−*10*), cardiovascular disease (*11*), symptoms of chronic obstructive pulmonary disease (*12*), and the risk of thrombotic stroke (*13*).

Phytochemicals have been suggested to be responsible for the health benefits of fruits and vegetables (*14*). Apple phytochemical extracts have been shown to have potent antioxidant activity (*5*, *8*) and antiproliferative activity against human cancer cells (*4*, *8*, *15*, *16*) and to prevent mammary cancers in rats in a dose-dependent manner (*17*).

Apple peels had exhibited more potent antioxidant activity and antiproliferative activity than apple flesh (*5*, *15*, *18*), suggesting that apple peels provided the major portion of bioactive phytochemicals. The high phenolic content, high antioxidant activity, and high antiproliferative activity of apple peels indicate that they may impart health benefits when consumed and should be regarded as a valuable source of natural antioxidants or bioactive compounds (*15*, *18*).

However, the bioactive compounds of apple peels that may be responsible for anticancer activity are not clear. In continuing efforts to seek bioactive components from fruits, vegetables, and other natural products (*19*−*21*), bioactivity-guided fractionation of apple peels was used to determine the identity of bioactive compounds that inhibit tumor cell growth. The

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10.1021/jf063563o CCC: $37.00
Published on Web 05/08/2007

objective of this research was to isolate and identify the bioactive compounds of apple peels with potent antiproliferative activity.

MATERIALS AND METHODS

Plant Material. Apples (*Malus pumila*) of the Red Delicious variety were purchased from Cornell Orchard (Cornell University, Ithaca, NY). They were harvested in October 2005.

Reagents. All chemicals used in the study, such as methanol, acetone, hexane, ethyl acetate, dichloromethane, and *n*-butanol, were of analytical grade and were purchased from Mallinckrodt Chemicals (Phillipsburg, NJ). All deuteriated solvents, chloroform-*d*, and pyridine-d_5 for NMR measurement were purchased from Sigma-Aldrich, Inc. (St. Louis, MO).

Chromatographic Materials. Silica gel for column chromatography, 230−400 mesh, and precoated silica gel 60 TLC plates were purchased from Merck KGaA (Darmstadt, Germany). Precoated Rp-18 TLC plates were obtained from Macherey-Nagel (Düren, Germany). Diaion HP-20 was purchased from Supelco, Inc. (Bellefonte, PA). Octadecylsilane (ODS) for open column chromatography was purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). The 100 × 7.8 mm i.d., 5 μm, XTera MS C_{18} semipreparative HPLC column was purchased from Waters Corp. (Milford, MA). The 250 × 22 mm i.d., 5 μm, Alltima C_{18} preparative HPLC column was purchased from Alltech Associates, Inc. (Deerfield, IL).

Instrumentation. HPLC analysis and purification were performed on a Millennium HPLC system composed of two 515 pumps with a 2480 UV−visible detector (Waters Corp.). All NMR spectra were obtained on a Varian INOVA 400 NMR spectrometer (Varian, Inc., Palo Alto, CA) operating at 400 MHz for proton and 100 MHz for carbon (*19*). Mass spectra were obtained on a Bruker Esquire 3D ion trap ESI mass spectrometer, and mass spectra conditions were as reported previously (*19*).

Extraction, Isolation, and Purification Procedures of Bioactive Constituents from Apple Peels. Fresh apples of the Red Delicious variety (105.0 kg) were peeled by an apple peeler as described previously (*15*, *18*). The apple peels (10.8 kg, 10.3% of whole apples as fresh weight) were homogenized for 5 min with chilled 80% acetone (1:2, w/v) using a chilled Waring blender as reported previously from our laboratory (*22*). Samples were then homogenized further using a Polytron homogenizer for an additional 3 min. The homogenates were filtered, and the filtrate was evaporated under vacuum at 45 °C until approximately 90% of the filtrate had been evaporated. The residue (1792.0 g, 16.6% of apple peels) was then resuspended in 4000 mL of water and extracted three times with the same volume of ethyl acetate and then extracted three times with water-saturated *n*-butanol. The ethyl acetate fraction (90.0 g, 5.0% yield) was further purified by silica gel chromatography (230−400 mesh, 430 × 75 mm) and eluted with a CH_2Cl_2/MeOH gradient elution (the ratios of CH_2Cl_2/MeOH were from 100:0 to 0:100). The CH_2Cl_2 eluant (1.0 g) was further subjected to silica gel column chromatography (200 × 25 mm) and eluted with hexane/ethyl acetate, and compound **13** (8.3 mg) was purified from the hexane/ethyl acetate (10:1) fraction. The fraction eluted with hexane/ethyl acetate (3:1, 205.0 mg) was purified on a semipreparative HPLC using the XTera C_{18} column eluted isocratically with 80% methanol in water at a flow rate of 2.5 mL/min, and compound **11** (10.5 mg, t_R = 9.0 min) was purified from this elution. Compound **1** (16.4 g) was obtained from CH_2Cl_2/MeOH (100:1) elution of the ethyl acetate fraction using the silica gel column. The CH_2Cl_2/MeOH (20:1) elution (5.75 g) of the ethyl acetate fraction was further isolated using the silica gel column (420 × 25 mm) eluted with CH_2Cl_2/MeOH. The CH_2Cl_2/MeOH (50:1) elution of the column was purified with a 250 × 22 mm, 5 μm, Alltima C_{18} preparative HPLC column using 83% methanol (containing 0.1% CF_3COOH, pH 2.0) as mobile phase. Compounds **6** (21.0 mg, t_R = 46.0 min), **2** (750.2 mg, t_R = 50.2 min), **10** (5.0 mg, t_R = 52.3 min), **9** (25.1 mg, t_R = 53.8 min), **5** (3.8 mg, t_R = 58.2 min), **4** (105.2 mg, t_R = 59.3 min), **8** (5.1 mg, t_R = 63.8 min), **7** (80.8 mg, t_R = 65.0 min), and **3** (158.2 mg, t_R = 71.5 min) were obtained from this fraction. The CH_2Cl_2/MeOH (20:1) elution (5.75 g) of the ethyl acetate fraction was purified by HPLC (250 × 22 mm, 5 μm, Alltima C_{18} preparative HPLC column), and compound **12** (15.2 mg, t_R = 22.8 min) was obtained using 80% methanol (containing 0.1% CF_3COOH, pH 2.0) as mobile phase.

Measurement of Inhibition Activity on Tumor Cell Proliferation. Antiproliferative activities against HepG2 human liver cancer cells (*23*−*24*), MCF-7 human breast cancer cells (*16*, *25*), and Caco-2 human colon cancer cells (*5*, *23*) of the pure compounds isolated from apple peels were measured by the MTS assay as described previously.

HepG2 cells [American Type Culture Collection, (ATCC), Rockville, MD] were maintained in Williams medium E (WME), containing 10 mM Hepes, 5 μg/mL insulin, 2 μg/mL glucagon, 0.05 μg/mL hydrocortisone, 5% fetal bovine serum (Gibco, Life Technologies, Grand Island, NY), 50 units/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL gentamicin (*26*, *27*). HepG2 cells were maintained at 37 °C in 5% CO_2. A total of 2.5 × 10^4 HepG2 cells in growth medium were placed in each well of a 96-well flat-bottom plate. After 4 h of incubation at 37 °C in 5% CO_2, the growth medium was replaced by media containing different concentrations of fruit extracts and purified compounds. Control cultures received the extraction solution minus the fruit extracts or purified compounds, and blank wells contained 100 μL of growth medium with no cells. After 96 h of incubation, cell proliferation was determined by colorimetric MTS assay. Cell proliferation (percent) was determined at 96 h from the MTS absorbance (490 nm) reading for each concentration compared to the control (*23*). At least three replications for each sample were used to determine the cell proliferation.

MCF-7 human breast cancer cells (ATCC) were maintained at 37 °C with 5% CO_2 in minimum essential medium (MEM) α medium containing 10 mM Hepes, 10 μg/mL insulin, 10% fetal bovine serum (Gibco, Life Technologies), 50 units/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL gentamicin (*16*, *25*). MCF-7 cells in growth medium were placed in each well of a 96-well flat-bottom plate at a concentration of 2.5 × 10^4 cells/well. Antiproliferative activities to MCF-7 cell line of all pure compounds were measured using the same procedures described above.

Caco-2 human colon cancer cells (ATCC) were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco Life Technologies) supplemented with 5% fetal bovine serum (Gibco Life Technologies), 10 mM Hepes, 50 units/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL gentamicin and were maintained at 37 °C with 5% CO_2 (*23*, *25*). Caco-2 cells in growth medium were placed in each well of a 96-well flat-bottom plate at a concentration of 2.5 × 10^4 cells/well. Antiproliferative activities to Caco-2 cell line of all pure compounds were measured using the same procedures described above.

RESULTS AND DISCUSSION

Structure Identification of the Purified Compounds. The chemical structures of compounds **1**−**13** are shown in **Figure 1**.

Compound **1** was obtained as a white powder. The ESI-MS of compound **1** showed the $[M - H]^-$ ion at *m*/*z* 455, and its molecular formula was inferred as $C_{30}H_{48}O_3$ on the basis of the analyses of ^1H and ^{13}C NMR and DEPT spectra. In the ^1H NMR spectra, there were seven methyl groups in the molecule, showing characteristic peaks at the high field. One carbonyl signal appeared at 180.2 ppm in the ^{13}C NMR spectra. The signals at 139.6 and 126.0 ppm were two olefinic carbons, which implied there was a carbon−carbon double bond in the molecule. On the basis of the analyses of spectra, compound **1** was identified as ursolic acid, which was the same as a previously reported compound isolated from cranberries (*19*). Compound **1** was one of the major triterpenoids isolated from apple peels and was present in the largest quantity.

Compound **2** was obtained as a white amorphous powder. The molecular formula was inferred as $C_{30}H_{48}O_4$ according to its MS, ^1H and ^{13}C NMR, and DEPT spectra. In the lower field of the ^1H NMR, it had an olefinic proton signal at 5.48 ppm (H, br d). It showed the characteristic signals of triterpenoids in the higher field. Compared to the ^{13}C NMR data of compound

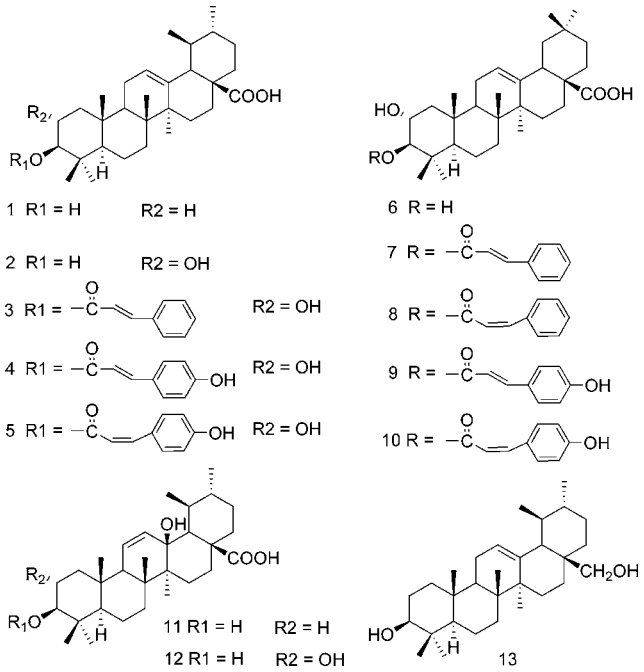

Figure 1. Chemical structures of triterpenoids isolated from apple peels.

1, they were almost the same except that of the A-ring. Compound **2** had one more hydroxyl group than compound **1** as revealed by its molecular weight. The additional hydroxyl group was assigned to connect at C-2 as judged from the chemical shifts of carbon signals and HMBC. From the above analysis, compound **2** was identified as 2α-hydroxyursolic acid, which was the same as the compound isolated from *Melaleuca leucadendron* (*28*).

The molecular formula of compound **3** was drawn as $C_{39}H_{54}O_5$ from HR-MS and ^{13}C NMR. In the lowest field of the ^1H NMR, there were three characteristic group signals of monosubstituted benzene, which were at 8.14 (2H, br d, J = 8.7 Hz), 7.55 (2H, br t, J = 8.7 Hz), and 7.18 ppm (H, br t, J = 8.7 Hz). It also had a pair of *trans*-conjugated olefinic protons, which were at 8.01 (H, d, J = 15.9 Hz) and 6.67 ppm (H, d, J = 15.9 Hz). In the ^{13}C NMR, there were 12 sp^2 hybrid carbons at the lower field, which included two carbonyl carbons. A *trans*-cinnamic acid unit could be drawn from its ^1H and ^{13}C NMR and DEPT spectra. The carbon signals of compound **3** were very similar to those of compound **2** at the triterpenoid skeleton. Compared to those of compound **2**, the signal at C-3 of compound **3** had a 1.1 ppm shift to the lower field, whereas C-2 shifted to the higher field by about 2.2 ppm, suggesting a linkage of the cinnamic acid group at C-3, which was confirmed by HMBC. Combined with the above analysis, compound **3** was identified as 2α-hydroxy-3β-{[(2*E*)-3-phenyl-1-oxo-2-propenyl]oxy}urs-12-en-28-oic acid, or 3β-*trans*-cinnamoyloxy-2α-hydroxyurs-12-en-28-oic acid. Compound **3** was a new compound that has not been reported previously in the literature.

Compounds **4** and **5** were a pair of *cis* and *trans* isomers, which had a close retention time on the semipreparative C_{18} HPLC column. The molecular weights of compounds **4** and **5** were 618, and the molecular formula of $C_{39}H_{54}O_6$ was inferred from analysis of ^1H and ^{13}C NMR and DEPT spectra. In the ^1H NMR of compound **4**, the signals at 7.91 (2H, d, J = 8.2 Hz) and 7.14 ppm (2H, d, J = 8.4 Hz) showed that there was a 1,4-disubstituted benzene fragment in the molecule. The signals at 8.01 (H, d, J = 15.6 Hz) and 6.68 ppm (H, d, J = 15.6 Hz) were characteristic signals of *trans*-olefinic protons. When the ^{13}C NMR was compared to that of compound **2**, there were nine additional carbon signals, which were all olefinic protons and appeared at lower fields. Except for the signals of the A-ring, the carbon signals of the skeleton of compounds **2** and **4** were almost the same. The additional nine olefinic carbons were identified as a *trans*-*p*-coumaroyloxy group. The signal at C-3 of compound **4** was shifted downfield from 84.2 ppm in compound **2** to 85.2 ppm. Therefore, the *trans*-*p*-coumaroyloxy group was linked at C-3. On the basis of the above analysis, compound **4** was identified as 3β-*trans*-*p*-coumaroyloxy-2α-hydroxyurs-12-en-28-oic acid as reported previously (*28*).

The ^1H NMR of compound **5** was similar to that of compound **4** and also had a *p*-coumaroyloxy unit in the molecule. The difference between these two compounds was the configuration of the carbon−carbon double bond of cinnamic acid. The *cis*-*p*-coumaroyloxy configuration was drawn from the coupling constant of 12.6 Hz for the carbon−carbon double bond in **5** with olefinic signals at 6.09 and 6.93 ppm. Therefore, compound **5** was identified as 3β-*cis*-*p*-coumaroyloxy-2α-hydroxyurs-12-en-28-oic acid, the same as reported previously (*28*).

Compound **6** was obtained as a white powder. The negative ESI-MS of compound **6** gave the $[M - H]^-$ ion at *m*/*z* 471, and the molecular formula of $C_{30}H_{48}O_4$ was inferred from analysis of ^1H and ^{13}C NMR and DEPT spectra. In the ^1H NMR spectra, there were seven methyl groups in the molecule, showing characteristic peaks at the high field. One acid carbonyl signal appeared at 180.5 ppm in the ^{13}C NMR spectra. The signals at 145.2 and 122.8 ppm were two olefinic carbons, which implied there was a carbon−carbon double bond in the molecule. On the basis of the analyses of spectra and the reported data (*29*), compound **6** was identified as maslinic acid, which was an oleane-type triterpenoid.

Compounds **7** and **8** were a pair of *cis* and *trans* isomers. The HR-MSI-MS showed the $[M - H]^-$ ions at *m*/*z* 601.3967 and 601.3970, respectively, and their molecular formula was inferred as $C_{39}H_{54}O_5$. In the lowest field of ^1H NMR of compound **7**, there were three characteristic group signals of monosubstituted benzene, which were at 8.15 (2H, br d, J = 8.7 Hz), 7.57 (2H, br t, J = 8.7 Hz), and 7.17 ppm (H, br t, J = 8.7 Hz). It also had a pair of *trans*-conjugated olefinic protons, which were at 8.02 (H, d, J = 15.9 Hz) and 6.69 ppm (H, d, J = 15.9 Hz). In the ^{13}C NMR, there were 12 sp^2-hybrid carbons at the lower field, which included 2 carbonyl carbons. A *trans*-cinnamoyloxy unit could be drawn from its ^1H and ^{13}C NMR and DEPT spectra. The carbon signals of compound **7** were very similar to those of compound **6** at the triterpenoid skeleton. Compared to those of compound **6**, the signal at C-3 of compound **3** had a 0.7 ppm shift to the lower field (shifting from 84.7 ppm in **6** to 85.4 ppm in **7**), whereas C-2 shifted to the higher field by about 2.2 ppm (shifting from 68.9 ppm in **6** to 66.7 ppm in **7**). From the correlations in HMBC and the chemical shifts, the cinnamic acid group was linked at C-3. Combined with the above analysis, compound **7** was identified as 2α-hydroxy-3β-{[(2*E*)-3-phenyl-1-oxo-2-propenyl]oxy}-olean-12-en-28-oic acid, or 3β-*trans*-cinnamoyloxy-2α-hydroxyolean-12-en-28-oic acid. Accordingly, compound **8** was identified as 2α-hydroxy-3β-{[(2*Z*)-3-phenyl-1-oxo-2-propenyl]oxy}olean-12-en-28-oic acid. Compounds **7** and **8** are new compounds that have not been reported in the literature previously.

Compounds **9** and **10** were another pair of *cis* and *trans* isomers. There were identified as 3β-*trans*-*p*-coumaroyloxy-2α-hydroxyolean-12-en-28-oic acid and 3β-*cis*-*p*-coumaroyloxy-2α-hydroxyolean-12-en-28-oic acid according to the reported literature (*30*, *31*).

Table 1. Antiproliferative Activities (EC_{50}) and Cytotoxicity (CC_{50}) of the Triterpenoids Isolated from Apple Peels against HepG2 Human Liver Cancer Cells, MCF-7 Human Breast Cancer Cells, and Caco-2 Human Colon Cancer Cells

	HepG2 cells			MCF-7 cells			Caco-2 cells		
compd	EC_{50} (μM)	CC_{50} (μM)	SI[a]	EC_{50} (μM)	CC_{50} (μM)	SI	EC_{50} (μM)	CC_{50} (μM)	SI
1	87.4 ± 2.7	>219.3	>2.5	14.4 ± 1.8	18.2 ± 0.6	1.3	34.4 ± 1.9	157.5 ± 8.2	4.6
2	10.6 ± 1.4	32.3 ± 6.3	3.0	4.7 ± 1.7	61.2 ± 5.7	13.0	12.9 ± 0.6	28.8 ± 2.1	2.2
3	30.0 ± 2.9	>166.1	>5.5	22.4 ± 1.8	>166.1	>7.4	49.2 ± 2.0	>166.1	>3.4
4	34.8 ± 1.8	>161.8	>4.6	54.9 ± 4.9	>161.8	>2.9	32.3 ± 1.4	>161.8	>5.0
6	69.1 ± 2.0	229.2 ± 14.5	3.3	136.0 ± 2.3	351.9 ± 23.2	2.6	15.4 ± 0.4	133.6 ± 2.0	8.7
7	20.6 ± 1.3	>166.1	>8.1	29.2 ± 3.3	118.4 ± 5.3	4.0	8.9 ± 0.1	112.6 ± 12.7	12.6
9	17.9 ± 2.6	>161.8	>9.0	20.9 ± 2.3	67.5 ± 1.5	3.2	14.2 ± 1.9	55.5 ± 6.3	3.9
11	>211.9	>211.9		>105.9	>105.9		59.2 ± 5.1	>105.9	>1.8
12	>204.9	>204.9		52.0 ± 2.7	>204.9	>3.9	37.8 ± 2.7	>204.9	>5.4

[a] Selective index = CC_{50}/EC_{50}.

The molecular formula of compound **11** was inferred as $C_{30}H_{48}O_4$ on the basis of analyses of ESI-MS and ^1H and ^{13}C NMR spectra, suggesting it had seven unsaturated degrees in the molecule. The ^1H NMR spectrum contained the signals for seven skeletal methyl groups, of which five were singlets and two were doublets. These data, coupled with the presence of 30 carbon atom signals in its ^{13}C NMR spectrum, suggested that **11** was an ursane-type triterpenoid. It was identified as 3β,13β-dihydroxyurs-11-en-28-oic acid (*32*).

Compound **12** was obtained as a white amorphous powder. The molecular formula was inferred as $C_{30}H_{48}O_5$ according to its MS, ^1H and ^{13}C NMR, and DEPT spectra. In the lower field of ^1H NMR, it had two olefinic protons, which resonated at 5.65 (dd, J = 10.4, 3.0 Hz) and 6.03 ppm (br d, J = 10.4 Hz). It showed the characteristic signals of triterpenoids in the higher field. The ^{13}C NMR data of this compound were almost the same as those of compound **11** except that of the A-ring. On the basis of its molecular weight, compound **12** had one more hydroxyl group than compound **11**. The additional hydroxyl group was assigned at the C-2 position on the basis of the chemical shifts of carbon signals and HMBC. The α-orientation of the hydroxyl group at C-2 was drawn from its NOE spectra and the literature. From the above analysis, compound **12** was identified as 2α,3β,13β-trihydroxyurs-11-en-28-oic acid. To our knowledge, compound **12** is a new compound that has not been reported in the literature previously.

Compound **13** was an ursolic alcohol, which was a hydroxyl methyl group instead of a carbonyl acid at C-17. It was identified as 3β,28-dihydroxy-12-ursene as reported previously (*33*).

Antiproliferative Activities. All triterpenoids isolated were evaluated for antiproliferative activities against HepG2 human liver cancer cells, MCF-7 human breast cancer cells, and Caco-2 human colon cancer cells. The antiproliferative activities and cytotoxicities are summarized in **Table 1**. Most of the triterpenoids showed potent antiproliferative activities against all three cancer cell lines. Among the pure triterpenoids isolated from apple peels, compounds **2**, **7**, and **9** showed the highest antiproliferative activities toward HepG2 cancer cells, with EC_{50} values of 10.56 ± 1.44, 20.58 ± 1.32, and 17.94 ± 2.56 μM, respectively. Compound **2** also showed strong cytotoxicity against HepG2 cancer cells (CC_{50} = 32.34 ± 6.30 μM), whereas compounds **1**, **3**, **4**, **7**, and **9** did not show any cytotoxicity, even at the highest concentrations tested. Compounds **2**, **3**, **7**, and **9** exhibited high antiproliferative activity against MCF-7 cancer cells, with low EC_{50} and high selectivity. All triterpenoids tested here showed antiproliferative activity against Caco-2 human colon cancer cells, with EC_{50} values of <60 μM; compounds **2**, **6**, **7**, and **9** had especially low EC_{50} values of 12.96 ± 0.61, 15.38 ± 0.43, 8.89 ± 0.14, and 14.19 ± 1.96 μM, respectively. These results show the triterpenoids isolated from apple peels have potent antiproliferative activity and may be partially responsible for their anticancer activities.

Apple phytochemical extracts have been shown to have potent antiproliferative activity against HepG2 human liver cancer cells and Caco-2 human colon cancer cells in dose-dependent manners (*5*, *8*). Recently we reported apple phytochemical extracts inhibited mammary cancer in vivo in a rat model in a dose-dependent manner (*17*). We proposed that the additive and synergistic interactions of phytochemicals in fruits and vegetables are responsible for their health benefits (*14*, *34*). Here we reported triterpenoids isolated from apple peels had potent antiproliferative activity against HepG2 human liver cancer cells, Caco-2 human colon cancer cells, and MCF-7 human breast cancer cells. Those triterpenoids may be partially responsible for apple's anticancer activity along with other bioactive compounds. The mechanisms of action of those compounds in cancer prevention are worthy of further investigation.

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Received for review December 8, 2006. Revised manuscript received March 26, 2007. Accepted March 27, 2007. This work was supported in part by No. 06A127 from the American Institute for Cancer Research and the Ngan Foundation.

JF063563O