Cranberry Phytochemicals: Isolation, Structure Elucidation, and Their Antiproliferative and Antioxidant Activities

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Bioactivity-guided fractionation of cranberries was used to determine the chemical identity of bioactive constituents. Twenty compounds were isolated using gradient solvent fractionation, silica gel and ODS columns, and preparative RP-HPLC. Their chemical structures were identified using HR-MS, 1D and 2D NMR, and X-ray diffraction analysis. Antiproliferative activities of isolated compounds against HepG2 human liver cancer and MCF-7 human breast cancer cells were evaluated. Among the compounds isolated, ursolic acid, quercetin, and 3,5,7,3′,4′-pentahydroxyflavonol-3-O-β-D-glucopyranoside showed potent antiproliferative activities against HepG2 cell growth, with EC_{50} values of 87.4 ± 2.7, 40.9 ± 1.1, and 49.2 ± 4.9 μM, respectively. Ursolic acid, quercetin, and 3,5,7,3′,4′-pentahydroxyflavonol-3-O-β-D-glucopyranoside showed potent inhibitory activity toward the proliferation of MCF-7 cells, with EC_{50} values of 11.7 ± 0.1, 137.5 ± 2.6, and 23.9 ± 3.9 μM, respectively. Quercetin, 3,5,7,3′,4′-pentahydroxyflavonol-3-O-β-D-glucopyranoside, 3,5,7,3′,4′-pentahydroxyflavonol-3-O-β-D-galactopyranoside, and 3,5,7,3′,4′-pentahydroxyflavonol-3-O-α-L-arabinofuranoside showed potent antioxidant activities, with EC_{50} values of ≈10 μM. These results showed cranberry phytochemical extracts have potent antioxidant and antiproliferative activities.

INTRODUCTION

Cranberries (*Vaccinium macrocarpon*), a native fruit in North America, have attracted public attention due to their potential health benefits. Recently, cranberries have been found to be rich in phenolics, which exhibit potent antioxidant activity (*1, 2*), prevent bacterial adhesion to host cells in urinary tract infections of *Escherichia coli* and stomach ulcers (*3−5*), as well as the co-aggregation of many oral bacteria (*6, 7*), exhibit in vitro anticancer activity (*1, 8*), protect against lipoprotein oxidation (*9, 10*), and reduce cholesterol in vivo (*11*). Many of these bioactivities have been linked to the presence of a very wide variety of phytochemicals in cranberries. The attractive bright red appearance and distinctive flavor of the cranberry are due to its unique flavonoids (*1*), including anthocyanins (*12*), proanthocyanidins (*4*), and flavonol glycosides (*13, 14*). Some triterpenoids were also identified from cranberries, which have shown strong bioactivities against tumor cell proliferation (*15*).

In continuing efforts to seek bioactive components from fruits, vegetables, and other natural products, bioactivity-guided fractionation of cranberries was used to determine the identity of bioactive compounds that inhibit tumor cell growth and may play a role in cancer prevention and therapy. The objective of this research was to isolate and identify the bioactive compounds of cranberries with potent antiproliferative and antioxidant activities.

MATERIALS AND METHODS

Plant Material. Cranberries (*V. macrocarpon*) of Stevens cultivar were provided by the Cranberry Institute (Wareham, MA). They were harvested in October 2004 and were stored at −40 °C until use.

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 deuterated solvents, chloroform-*d*, methanol-*d*$_4$, dimethyl-*d*$_6$ sulfoxide, and acetone-*d*$_6$, pyridine-*d*$_5$, for NMR measurement were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Randomly methylated β-cyclodextrin (RMCD) for antioxidant activity assay was obtained from Cyclodextrin Technologies (High Springs, FL). 2,2′-Azobis(amidinopropane) was purchased from Wako Chemicals (Richmond, VA). Dichlorofluorescein diacetate was purchased from Sigma-Aldrich.

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).

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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. Mass spectra were obtained on an ESI-TOF II mass spectrometer (Micromass, Wythenshawe, U.K.) equipped with an orthogonal electrospray source (Z-spray) operated in positive ion mode. Optimal ESI conditions were as follows: capillary voltage, 3000 V; source temperature, 110 °C; and cone voltage, 55 V. The ESI gas was nitrogen. Data were acquired in continuum mode until acceptable averaged data were obtained.

Extraction, Isolation, and Purification Procedures of Bioactive Constituents from Cranberries. Fresh cranberries of Stevens cultivar (7.0 kg) were homogenized for 5 min with chilled 80% acetone (1:2, w/v) using a chilled Waring blender (*1*). 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 ≈90% of the filtrate had been evaporated. The residue was then resuspended in 4000 mL of water and extracted with the same volume of ethyl acetate and then extracted three times with water-saturated *n*-butanol.

The ethyl acetate fraction (59.0 g) was further purified by silica gel chromatography (230−400 mesh, 300 × 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 (11.4 g) was further subjected to silica gel column chromatography (250 × 50 mm) and eluted with hexane/ethyl acetate (10:1), and compound **14** (100.3 mg) was purified from this fraction. The fraction eluted with CH_2Cl_2/MeOH (100:1, 100.0 mg) was purified by silica gel column (380 × 10 mm) with hexane/ethyl acetate, and compound **6** (5.6 mg) was obtained from hexane/ethyl acetate (20:1) elution. The fraction (350.0 mg) eluted with CH_2Cl_2/MeOH (100:1) was further applied to a silica gel column (200 × 20 mm) using hexane/ethyl acetate (100:0 to 0:100), and compound **19** (40.7 mg) was obtained from hexane/ethyl acetate (10:1) elution. The fraction (1001.3 mg) eluted with CH_2Cl_2/MeOH (100:3) was further separated by a silica gel column (210 × 25 mm) using hexane/ethyl acetate, and compound **10** (12.1 mg) was isolated from the hexane/ethyl acetate (20:1) eluant, compound **3** (142.0 mg) was isolated from hexane/ethyl acetate (10:1) eluant, and compound **5** (20.0 mg) was purified from hexane/ethyl acetate (5:1) eluant, respectively. The CH_2Cl_2/MeOH (10:1) eluant (1.4 g) of the cranberry ethyl acetate fraction was chromatographed on a silica gel column (235 × 25 mm) using hexane/acetone (10:1) as solvent. Then the subfraction was further purified on semipreparative HPLC using the XTera C_{18} column, which was eluted isocratically with 79% methanol in water at a flow rate of 2.5 mL/min. Three triterpenoids, compounds **11** (55.1 mg), **12** (3.2 mg), and **13** (3.1 mg), were obtained at retention times of 13.5, 32.5, and 35.0 min, respectively. The later CH_2Cl_2/MeOH (10:1) eluant (13.0 g) of cranberry ethyl acetate fraction was separated by a silica gel column (300 × 50 mm) with a CH_2Cl_2/MeOH solvent system, yielding compounds **15** (1.2 mg) and **20** (14.0 mg) from CH_2Cl_2/MeOH 50:1 and 5:1 fractions, respectively. The CH_2Cl_2/MeOH (5:1) elution (4.73 g) of the cranberry ethyl acetate fraction was applied to a silica gel column (450 × 35 mm) using CH_2Cl_2/MeOH as eluant, and compound **18** (2.2 mg) was obtained from the CH_2Cl_2/MeOH (10:1) fraction. The CH_2Cl_2/MeOH (4:1) eluant (2.75 g) of the cranberry ethyl acetate fraction was separated by a silica gel column (360 × 34 mm) using CH_2Cl_2/MeOH (8:1) as eluant, and two flavonoid glycosides, **16** (10.0 mg) and **17** (15.0 mg), were obtained.

The butanol fraction (204.0 g) of cranberry extracts was subjected to a Diaion HP-20 column (550 × 55 mm) and eluted with 3000 mL of water, 30% methanol (v/v), 50% methanol (v/v), and methanol, respectively. The water eluant (68.95 g, of which 25.10 g was taken for further isolation) was applied to a silica gel column (300 × 50 mm) and eluted with CH_2Cl_2/MeOH, and compound **9** (268.0 mg) was obtained from CH_2Cl_2/MeOH (5:1) elution. The 30% methanol eluate (16.50 g) was further purified by silica gel column (230−400 mesh, 235 × 75 mm) and eluted with CH_2Cl_2/MeOH with a gradually increased ratio of methanol. Compound **1** (605.2 mg) was isolated from CH_2Cl_2/MeOH (10:1) elution, compound **2** (12.1 mg) was from the

CH_2Cl_2/MeOH (5:1) fraction, and compound **4** (15.1 mg) was obtained from the CH_2Cl_2/MeOH (4:1) fraction, respectively. The methanol elution (5.25 g) of the butanol fraction was subjected to silica gel column (200 × 75 mm) and eluted with CH_2Cl_2/MeOH/H_2O. The fraction eluted with CH_2Cl_2/MeOH/H_2O (80:20:1.5) was purified by a silica gel column (300 × 25 mm) and eluted with CH_2Cl_2/MeOH with a gradually increased ratio of methanol. Compound **7** (37.3 mg) was obtained from the CH_2Cl_2/MeOH elution (20:1, v/v) as a colorless crystalline solid from methylene dichloride. Compound **8** (40.5 mg) was obtained from CH_2Cl_2/MeOH (10:1) elution.

Measurement of Inhibition Activity on Tumor Cell Proliferation. Antiproliferative activities of all fractions and pure compounds isolated from cranberries were measured by the MTS assay (MTS-based cell titer 96 non-radioactivity cell proliferation assay) (Promega, Madison, WI) described previously (*1, 16*). 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, and 5% fetal bovine serum (Gibco Life Technologies, Grand Island, NY). HepG2 cells were maintained at 37 °C in 5% CO_2 in an incubator. A total of 2.5×10^4 HepG2 cells in growth media 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, 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. At least three replications for each sample were used to determine the cell proliferation.

MCF-7 human breast cancer cells (ATCC, Rockville, MD) were maintained at 37 °C with 5% CO_2 in minimum essential medium alpha medium (MEM) containing 10 mM Hepes, 10 µg/mL insulin, 50 units/mL penicillin, 50 µg/mL streptomycin, 100 µg/mL gentamicin, and 10% fetal bovine serum (Gibco Life Technologies). MCF-7 cells in growth media 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 the MCF-7 cell line of all fractions and pure compounds isolated from cranberry extracts were measured using the same procedures described above.

Measurement of Antioxidant Activity Using Peroxyl Radical Scavenging Capacity (PSC) Assay. Antioxidant activities of extracts and pure compounds were determined according to the PSC assay described previously (*17*). Just prior to use in the reaction, 107 µL of 2.48 mM dichlorofluorescein diacetate was hydrolyzed to dichlorofluorescein with 893 µL of 1.0 mM KOH for 5 min in a vial to remove the diacetate moiety and then diluted with 7 mL of 75 mM phosphate buffer (pH 7.4). 2,2′-Azobis(amidinopropane) (200 mM) was prepared fresh in the buffer and was kept at 4 °C between runs. In an assay, 100 µL of pure compounds or extracts was diluted in 75 mM phosphate buffer (pH 7.4) and then transferred into reaction cells on a 96-well plate, and 100 µL of dichlorofluorescein was added. The 96-well plate was loaded into the Fluoroskan Ascent fluorescence spectrophotometer (Thermo Labsystems, Franklin, MA), and the solution in each cell was mixed by shaking at 1200 rpm for 20 s. The reaction was then initiated by adding 50 µL of 2,2′-azobis(amidinopropane) from the autodispenser on the equipment. Each set of dilutions for a replicate and control was analyzed three times in adjacent columns. The reaction was carried out at 37 °C, and fluorescence was monitored at 485 nm excitation and 538 nm emission with the fluorescence spectrophotometer. The buffer was used for control reactions. Data were acquired with Ascent software, version 2.6 (Thermo Labsystems). The areas under the average fluorescence−reaction time kinetic curve (AUC) for both control and samples (up to 36 min) were integrated and used as the basis for calculating antioxidant activity according to the equation

$$PSC\ unit = 1 - (SA/CA)$$

where SA is AUC for sample or standard dilution and CA is AUC for the control reaction using only buffer. Compounds inhibiting the

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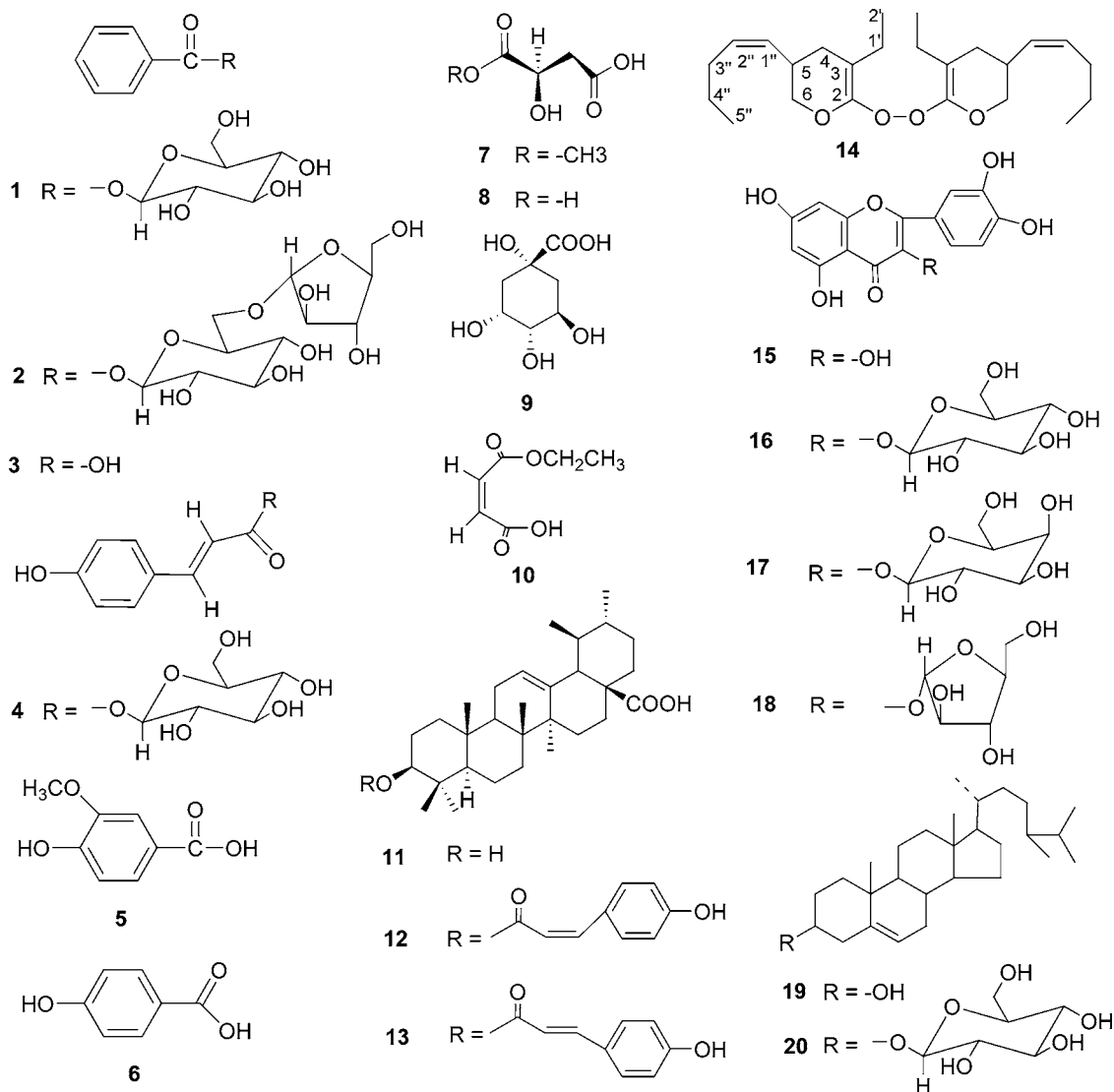*J. Agric. Food Chem.,* Vol. 54, No. 19, 2006   **7071**

Figure 1. Chemical structures of the compounds isolated from cranberries.

oxidation of dichlorofluorescein produced smaller SA and higher PSC units. The median effective concentration (EC$_{50}$) was defined as the dose required to cause a 50% inhibition (PSC unit 0.5) for each pure compound or extract. Results were expressed as micromoles of vitamin C equivalents per micromole of pure compound or gram of sample extract ± standard deviation (SD) for triplicate analyses.

Solubility of lipophilic compounds was ensured by dissolving them in 12% RMCD prepared in 50% acetone in water. The fluorescent dye was prepared by hydrolyzing 11 μL of 2.48 mM dichlorofluorescein diacetate with 898 μL of 1.0 mM KOH and then diluted with 7 mL of 75 mM phosphate buffer (pH 7.4). The reaction mixture of the lipo-PSC assay contained 100 μL of appropriate dilutions of pure compounds in 12% RMCD, 100 μL of dichlorofluorescein, and 50 μL of 200 mM 2,2′-azobis(amidinopropane). Control reactions used 12% RMCD. The reaction conditions, data acquisition, and processing were as described above for the hydro-PSC assay. The AUC was calculated up to 40 min as described previously.

RESULTS AND DISCUSSION

Structure Identification of the Purified Compounds. The chemical structures of compounds **1**−**20** are shown in **Figure 1**.

Compound **1**, colorless cubic crystals from methanol, was one of the main constituents of cranberries. The molecular formula was inferred as C$_{13}$H$_{16}$O$_7$ according to its MS, ^{1}H and ^{13}C NMR, and DEPT spectra. In the lowest field of ^{1}H NMR, it showed monosubstituted benzene characteristic signals at 8.04

(2H, dt, *J* = 8.7, 1.5 Hz), 7.70 (H, td, *J* = 7.5, 1.5 Hz), and 7.56 ppm (2H, td, *J* = 7.5, 1.5 Hz), respectively. There was an anomeric proton signal at 5.61 ppm (H, d, *J* = 7.5 Hz), which showed that there was a sugar unit in the molecule. In the ^{13}C NMR, there were 13 carbon signals. In the lowest field, there was a carbonyl carbon (164.8 ppm). From 60 to 100 ppm, there were 6 carbon signals altogether. From the carbon signals in ^{13}C NMR, DEPT, and the signals of ^{1}H NMR, the sugar was identified as a glucose unit. The β-configuration of glucose was drawn from the coupling constant of the anomeric proton (*J* = 7.5 Hz). From the above analysis, compound **1** was identified as benzoic acid β-D-glucopyranoside. The X-ray diffraction analysis showed the configuration of compound **1** (*18*).

Compound **2**, a white amorphous power, was obtained from the *n*-butanol fraction of cranberries. High-resolution TOF-MS showed the ion [M + Na]$^+$ at 439.1149, corresponding to its molecular formula C$_{18}$H$_{24}$O$_{11}$. In the lower field of the ^{1}H NMR, it also showed monosubstituted signals, which were at 8.12 (2H, dt, *J* = 8.2, 1.2 Hz), 7.45 (H, td, *J* = 7.2, 1.2 Hz), and 7.30 ppm (2H, td, *J* = 7.5, 1.2 Hz), respectively. There were two anomeric proton signals at 6.59 (H, d, *J* = 7.8 Hz) and 5.69 ppm (H, d, *J* = 2.1 Hz), respectively. In the ^{13}C NMR and DEPT spectra, there were 18 carbon signals, which consisted of 2 quaternary carbons, 14 methines, and 2 methylenes. There

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were two anomeric carbon signals at 110.6 and 97.0 ppm, respectively, which showed there were two sugar units in the molecule. When the ^{13}C NMR spectrum of compound **2** was compared to that of compound **1**, the former had an additional pentose unit in the molecule, and all other carbon signals were the same. From the proton and carbon signals of the additional sugar unit, it was identified as an L-arabinose. The carbon signal at C-6 of glucose was obviously shifted to lower field CH_2, 68.4 ppm. In the HMBC spectra, the anomeric proton signal of L-arabinose at 5.69 ppm (H, d, $J = 2.1$ Hz) had long-range correlations with the carbon signal at C-6 of glucose, which showed that the arabinose was linked at the C-6 of glucose. Combined with the above analysis, compound **2** was identified as benzoic acid α-L-arabinopyranosyl (1→6)-β-D-glucopyrano-side. Compound **2** is a new compound, which has not been reported in the literature.

Compound **3** was a colorless cubic crystal from methanol. It was identified as benzoic acid according to its MS and ^{1}H and ^{13}C NMR spectra. Benzoic acid is a very common food additive, used as an antimicrobial agent in foods. Here we showed benzoic acid was one of the main constituents of cran-berry extracts, which may be responsible for the antimicrobial activity of cranberries.

The ESI-MS of compound **4** showed the ion $[M - H]^-$ at 325, and its molecular formula was inferred as $C_{15}H_{18}O_8$ on the basis of the analyses of ^{1}H and ^{13}C NMR and DEPT spectra. The proton signals at low field at 7.58 (2H, br d, $J = 8.7$ Hz) and 6.80 ppm (2H, br d, $J = 8.7$ Hz) were characteristic 1,4-disubstituted benzene protons. The pair of proton signals at 7.65 (H, d, $J = 15.9$ Hz) and 6.40 ppm (H, d, $J = 15.9$ Hz) were *trans*-olefinic protons, which were conjugated with a carbonyl group. There was an anomeric proton signal at 5.46 ppm (H, d, $J = 8.4$ Hz). There were 15 carbon signals in the ^{13}C NMR spectra, which consisted of 1 carbonyl carbon, 8 olefinic carbons, 1 anomeric carbon, and 5 oxygenated carbons. The sugar unit was determined to be D-glucose when compared to that of compound **1**. The coupling constant of the anomeric proton ($J_{H1-H2} = 8.4$ Hz) indicated it had a β-configuration. On the basis of the above analysis, compound **4** was identified as 1-[3-(4-hydroxyphenyl)-2-propenoate]-β-D-glucopyranoside, which was consistent with literature reports (*19*).

The ESI-MS of compound **5** gave the $[M - H]^-$ ion at *m/z* 167, and its molecular formula of $C_8H_8O_4$ was inferred from its ^{1}H and ^{13}C NMR spectra. In the ^{1}H NMR spectrum, there was a low-field ABX spin system at 7.46 (H, d, $J = 7.9$ Hz), 6.84 (H, dd, $J = 7.9, 1.5$ Hz), and 7.44 ppm (H, d, $J = 1.5$ Hz). Therefore, compound **5** was a 1,3,4-trisubstituted benzene derivative. In the high field of the ^{1}H NMR, there was an oxygenated methyl group at 3.80 ppm (3H, s), and it had eight carbon signals in the ^{13}C NMR. At lowest field, there was a carbonyl carbon signal (167.3 ppm). According to the above analysis, compound **5** was determined to be 3-methoxy-4-hydroxybenzoic acid. Compound **6** was determined to be *p*-hydroxybenzoic acid according to its MS, ^{1}H and ^{13}C NMR, and DEPT spectra.

Compound **7** was colorless, long-needle crystals isolated from the *n*-butanol fraction of cranberry extracts. The molecular formula of $C_5H_8O_5$ was inferred from its MS and ^{1}H and ^{13}C NMR. There were four groups of signals in the proton NMR spectrum, including a pair of geminal protons at 2.62 (H, dd, $J = 15.9, 5.1$ Hz) and 2.48 (H, dd, $J = 15.9, 7.2$ Hz) ppm, an oxygenated methine proton signal at 4.33 (H, dd, $J = 7.2, 5.1$ Hz) ppm, and an oxygenated methyl signal at 3.62 ppm (3H, s). Two carbonyl carbons, an oxygenated methine, an

Table 1. Antiproliferative Activities (EC_{50}) and Cytotoxicity (CC_{50}) of Compounds Isolated from Cranberries against HepG2 Human Liver Cancer Cells and MCF-7 Human Breast Cancer Cells

	HepG2 cells		MCF-7 cells	
compd	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
1	>500	>500	>500	>500
2	>625	>625	>625	>625
3	>2500	>2500	>2500	>2500
4	>625	>625	>625	>625
5	>2500	>2500	>2500	>2500
6	>1000	>1000	>1000	>1000
7	>1000	>1000	>1000	>1000
8	>1000	>1000	>1000	>1000
9	>500	>500	>500	>500
10	671.78 ± 6.06	>1000	>1000	>1000
11	87.35 ± 2.74	>200	14.35 ± 1.77	18.25 ± 0.64
14	>500	>500	227.86 ± 4.57	385.13 ± 45.38
15	40.90 ± 1.12	>200	137.46 ± 2.55	>200
16	49.22 ± 4.90	>500	23.90 ± 3.86	>500
17	>500	>500	>500	>500
19	>1000	>1000	>1000	>1000
20	>625	>625	>625	>625

oxygenated methyl, and a methylene were shown in the ^{13}C NMR spectra. It was determined to be 2(*R*)-hydroxybutanedioic acid 1-methyl ester. This structure was reconfirmed by the X-ray diffraction analysis showing that C-2 had the *R*-configuration (*20*).

The ^{1}H and ^{13}C NMR spectra of compound **8** were very similar to those of compound **7**, except for lacking an oxygen-ated methyl signal, and it was determined to be 2(*R*)-hydroxy-butanedioic acid.

Compound **9** was obtained as colorless cubic crystals from the *n*-butanol fraction of cranberry extracts, and it was one of the main constituents of cranberries. The molecular formula was inferred as $C_7H_{12}O_6$ according to its TOF-MS and ^{1}H and ^{13}C NMR spectra. There was a carbonyl carbon signal at 175.7 ppm and four oxygenated carbon signals at 75.6 (quaternary carbon), 74.5 (CH), 69.0 (CH), and 66.8 ppm (CH), respectively. In the high field of the ^{13}C NMR, there were two saturated methylene signals at 40.5 and 37.5 ppm. Compound **9** was identified to be quinic acid on the basis of analysis of the above spectra. The stereochemistry of compound **9** was determined by X-ray diffraction analysis and identified as (1*R*,3*R*,4*R*,5*R*)-1,3,4,5-tetrahydroxycyclohexanecarboxylic acid, which was the same as reported previously (*21*). Compound **10** was identified as maleic acid monoethyl ester by comparison of its ^{1}H and ^{13}C NMR with the authentic compound.

Compounds **11−13** were derivatives of triterpenoids. The ESI-MS of compound **11** gave the ion $[M + H]^+$ at 457, and the molecular formula of $C_{30}H_{48}O_3$ was inferred from analysis of ^{1}H, ^{13}C NMR, and DEPT spectra. In the ^{1}H NMR spectrum, there were seven methyl groups in the molecule, showing characteristic peaks at high field. One acid carbonyl signal appeared at 181.5 ppm in the ^{13}C NMR spectra. The signals at 139.9 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 **11** was identified as ursolic acid, which was the same as reported previously (*15*). Compound **11** had potent antiproliferative activity against the HepG2 human liver cancer cell line and the MCF-7 human breast cancer cell line (**Table 1**).

Compounds **12** and **13** 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 **12** and **13** were 602, and the molecular formula of $C_{39}H_{54}O_5$ was

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inferred from analysis of ^1H, ^{13}NMR, and DEPT spectra. In the ^1H NMR of compound **12**, the signals at 7.60 (2H, d, $J = 8.2$ Hz) and 6.83 ppm (2H, d, $J = 8.4$ Hz) showed that there was a 1,4-disubstituted benzene fragment in the molecule. The signals at 6.86 (H, d, $J = 12.6$ Hz) and 5.76 ppm (H, d, $J = 12.6$ Hz) were characteristic signals of *cis*-olefinic protons. When the ^{13}C NMR spectrum was compared to that of compound **11**, 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 **11** and **12** were almost the same. The additional nine olefinic carbons were identified as *p*-hydroxycinnamic acid. The signal at C-3 of compound **12** was shifted downfield from 78.5 ppm in compound **11** to 82.4 ppm. Therefore, the *p*-hydroxycinnamic acid was linked at C-3. On the basis of the above analysis, compound **12** was identified as *cis*-3-*O*-*p*-hydroxycinnamoylursolic acid.

The ^1H NMR spectrum of compound **13** was similar to that of compound **12** and also had a *p*-hydroxycinnamic acid unit in the molecule. The difference between these two compounds was the configuration of the carbon−carbon double bond of cinnamic acid. The *trans*-*p*-hydroxycinnamic acid configuration was drawn from the coupling constant of 16.0 Hz for the carbon−carbon double bond in **12** with olefinic signals at 7.46 and 6.32 ppm. Therefore, compound **13** was identified as *trans*-3-*O*-*p*-hydroxycinnamoylursolic acid, the same as reported previously (*15*).

Compound **14** was obtained as a pale yellow liquid from the ethyl acetate fraction of cranberry extracts with lower polarity. The HR TOF-MS spectrum showed the ion $[M + Na]^+$ at 413.2510, and the molecular formula of $C_{24}H_{38}O_4$ was derived, combined with analyses of NMR spectra. There were 12 carbon signals in the ^{13}C NMR spectra, which indicated that compound **14** had symmetric units and each unit had 12 carbon atoms. For one 12-carbon unit, there were 2 quaternary carbons, 3 methines, 5 methylenes, and 2 methyl groups in the DEPT spectra. From $^1H-^1H$ COSY and HMBC spectra, the fragments of ethyl, 1-pentenyl, and 2-hydroxy-2,3-dehydropyran were present in the molecule. In the HMBC spectrum, the methyl signals (0.88 ppm, 3H, t, $J = 6.6$ Hz) in the ethyl group had correlations with the olefinic carbon (132.4, C-3), and therefore the ethyl was linked at C-3 of the pyran. The olefinic proton in the 1-pentenyl (7.45 ppm, H, m) unit had correlations with the C-5 (38.7 ppm) carbon of the pyran, and the other olefinic proton in the 1-pentenyl (7.65 ppm, H, m) had correlations with the C-5 (38.7 ppm) and C-6 (67.9 ppm) carbons of pyran. On the basis of the above analysis and its HR-MS spectrum, **14** was identified as 5-ethyl-6-[3-ethyl-5-((*Z*)-pent-1-enyl)-5,6-dihydro-4*H*-pyran-2-ylperoxy]-3-[(*Z*)-pent-1-enyl]-3, 4-dihydro-2*H*-pyran. Compound **14** is a new compound that has not been reported in the literature and is named vaccinperoxypyran.

Compounds **15**−**18** were flavonoids with different sugar moieties. Compound **15** was obtained as a pale yellow powder. It was identified as quercetin by its ^1H and ^{13}C NMR spectra. Compound **16** was obtained as a pale yellow powder. The TOF-MS spectrum showed the ion $[M + Na]^+$ at 487, and the molecular formula of $C_{21}H_{20}O_{12}$ was inferred from ^1H, ^{13}C NMR, and DEPT. In the ^1H NMR spectrum, an ABX spin-coupling system was ascribed to the three protons of the B-ring of a flavone, with signals at 7.67 (H, dd, $J = 8.4, 2.4$ Hz), 7.51 (H, d, $J = 2.4$ Hz), and 6.81 ppm (H, d, $J = 8.4$ Hz), respectively. The signals at 6.40 (H, d, $J = 2.4$ Hz) and 6.20 ppm (H, d, $J = 2.4$ Hz) were meta-substituted protons of the A-ring. The signal at 12.64 ppm (H, s) was the characteristic

Table 2. Antioxidant Activity of Selected Compounds Isolated from Cranberries (Mean ± SD)

compd	EC_{50} (μM)	PSC value (μmol of vitamin C equiv/μmol of compound)
15	8.21 ± 0.81	1.09 ± 0.05
16	10.07 ± 0.24	0.88 ± 0.07
17	10.34 ± 1.14	0.86 ± 0.08
18	7.39 ± 0.17	1.21 ± 0.02

signal of the hydroxyl at C-5 of a flavone. Compared with the NMR data of compound **15**, the aglycone of compound **16** was quercetin. There was an anomeric proton at 5.46 ppm (H, d, $J = 7.5$ Hz). The signals in the ^{13}C NMR showed 21 carbons in the molecule. The sugar unit was linked at C-3 according to its ^1H, ^{13}C, and literature data. Compound **16** was identified as 3,5,7,3′,4′-pentahydroxyflavonol-3-*O*-β-D-glucopyranoside, which was identical to the reported data in the literature (*22*).

Compound **17** had the same aglycone, quercetin, as compound **16**. There were 21 carbons in the molecule. The difference between them was the sugar unit. The sugar was galactose in compound **17**. Therefore, **17** was identified as 3,5,7,3′,4′-pentahydroxyflavonol-3-*O*-β-D-galactopyranoside (hyperin) (*23*).

Compound **18** also had the same aglycone, quercetin, as compound **16**. The TOF-MS gave the ion $[M + Na]^+$ at 457 corresponding to the molecular formula of $C_{20}H_{18}O_{11}$. There were 20 carbon signals in the ^{13}C NMR, which showed the sugar of compound **18** was pentose instead of hexose. By comparison with the literature, compound **18** was identified as 3,5,7,3′,4′-pentahydroxyflavonol-3-*O*-α-L-arabinofuranoside.

Compounds **19** and **20** were plant sterols, which exist widely in many plants and were identified as β-sitosterol and β-sitosterol-3-*O*-β-D-glucoside, respectively, according to their TOF-MS and ^1H and ^{13}C NMR.

Antiproliferative Activities of the Pure Compounds Isolated from Cranberries. All compounds were evaluated for antiproliferative activities against HepG2 human liver cancer cells and MCF-7 human breast cancer cells. The antiproliferative activities and cytotoxicities are summarized in **Table 1**. Among the pure compounds isolated from cranberry extracts, compounds **11**, **15**, and **16** showed potent antiproliferative activities against HepG2 cell growth in a dose-dependent manner, with EC_{50} values of 87.35 ± 2.74, 40.90 ± 1.12, and 49.22 ± 4.90 μM, respectively, whereas they did not display any toxicity at the concentrations applied in the experiments. Compounds **11**, **15**, and **16** showed potent inhibitory activities against the proliferation of MCF-7 cells, with EC_{50} values of 11.70 ± 0.06, 137.46 ± 2.55, and 23.90 ± 3.86 μM, respectively. It is interesting to note that ursolic acid, compound **11**, showed potent antiproliferative activities against the HepG2 cell line and espccially the MCF-7 cell lines. However, it displayed strong cytotoxicity to MCF-7 cells, not to HepG2 cells. The discrepancy of ursolic acid against MCF-7 cells and HepG2 cells and the mechanism of action are worth further investigation.

Antioxidant Activities of the Pure Compounds Isolated from Cranberries. The antioxidant activities of the 20 pure compounds were evaluated using the PSC assay (*17*). Four flavonoids (compounds **15**−**18**) showed potent antioxidant activities (**Table 2**). Compound **18** had the highest antioxidant activity with an EC_{50} of 7.39 ± 0.17 μM, followed by compound **15** (EC_{50} = 8.21 ± 0.81 μM), compound **16** (EC_{50} = 10.07 ± 0.24 μM), and compound **17** (EC_{50} = 10.34 ± 1.14 μM). The antioxidant activities of the four flavonoids were comparable to the activity of vitamin C with PSC value ranging from 0.86 to 1.21 μmol of vitamin C equiv/μmol.

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Whole cranberry phytochemical extracts have been shown to have potent antioxidant and antiproliferative activities (*1*, *9*). Among the compounds isolated, ursolic acid, quercetin, and 3,5,7,3′,4′-pentahydroxyflavonol-3-*O*-β-D-glucopyranoside showed potent antiproliferative activities against the growth of human HepG2 liver cancer cells and human MCF-7 breast cancer cells. Quercetin, 3,5,7,3′,4′-pentahydroxyflavonol-3-*O*-β-D-glucopyranoside, 3,5,7,3′,4′-pentahydroxyflavonol-3-*O*-β-D-galactopyranoside, and 3,5,7,3′,4′-pentahydroxyflavonol-3-*O*-α-L-arabinofuranoside, isolated from cranberry extracts, showed potent antioxidant activities. This further confirmed our hypothesis that the additive and synergistic effects of phytochemicals in whole fruits and vegetables are responsible for their potent antioxidant and anticancer activities and that the health benefits of fruits and vegetables are attributable to the complex mixtures of phytochemicals present in whole foods (*1*, *24−26*).

ACKNOWLEDGMENT

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