JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2004,** *52,* 2213−2217 **2213**

Cyanidin and Malvidin from *Oryza sativa* cv. Heugjinjubyeo Mediate Cytotoxicity against Human Monocytic Leukemia Cells by Arrest of G_2/M Phase and Induction of Apoptosis

JIN WON HYUN† AND HA SOOK CHUNG*,§

Department of Biochemistry, College of Medicine, Cheju National University, Jeju 690-756, Korea, and Department of Foods and Nutrition, College of Natural Sciences, Duksung Women's University, Seoul 132-714, Korea

Oryza sativa cv. Heugjinjubyeo (Gramineae), anthocyanin-pigmented rice, having dark purple grains, is known broadly as enriched rice with an improved taste. Two bioactive compounds were isolated from the 0.5% HCl−ethyl alcohol soluble fraction of the aleurone layer of *O. sativa* cv. Heugjinjubyeo through an activity-monitored fractionation and isolation method. From spectral analysis, the cytotoxic components were the anthocyanidins cyanidin (**1**) and malvidin (**2**). The 50% growth inhibitory concentrations (IC_{50}) of cyanidin and malvidin on U937, human monocytic leukemia cells, were 60 and 40 μg/mL, respectively. These compounds showed cytotoxicity through the arrest of the G_2/M phase of cell cycle and induction of apoptosis.

KEYWORDS: ***Oryza sativa*** **cv. Heugjinjubyeo; Gramineae; anthocyanidin; cyanidin; malvidin; cytotoxicity; phytonutrients; human monocytic leukemia cells; cell cycle arrest; apoptosis**

INTRODUCTION

Within the past decade, the so-called health food industry has experienced tremendous growth such that these products are commonplace in most community pharmacies and markets in Korea. These products have been identified in the industry as nutraceuticals. Plant natural products have had, and continue to have, an important role as nutritious and medicinal agents, as purified isolates and extractives (*1*).

With growing concerns for national health and the expanding markets of health food, research in the area of the industrial use of phytochemicals from diverse crops became more universal. Extraction and industrial use of new constituents from crops have been accomplished for added-value agricultural products. Development of high-quality varieties containing increased levels of bioactive constituents could increase the nutritional value of the harvest grain.

In general, cancer cells escape growth control and thus prohibit a normal functioning of the organ in which these cells are located. Dietary polyphenols such as anthocyanins and anthocyanidins, and their aglycons, which are the major sources of red, violet, and blue pigmentation, are suggested to be involved in the inhibitory influence on colon cancer development *in vitro* and *in vivo*, respectively (*2*, *3*) and antioxidant and anti-inflammatory (*4*) and estrogenic activities (*5*). Recently, it has been reported that cyanidin and delphinidin, major anthocyanidins of natural plant sources, inhibit the growth of human tumor cells *in vitro* in the human vulva carcinoma cell line A431 (*6*), where cyanidin strongly inhibited the epidermal growth factor receptor by shutting off the downstream signaling cascades (*7*).

Rice (*Oryza sativa* L.) is the staple food in many Asian countries. Cultivation of pigmented rice through genetic engineering in the early 1970s began a surge in world production of various kinds of rice grains (*8*). The anthocyanin-pigmented rices (*Oryza sativa* cvs. Heugjinjubyeo, Heugnambyeo, Jakwangdo, Sanghaehyeolla, Hongmi, Suwon #405, Suwon #415, Suwon 420, Suwon 425, and Kilimheugmi) having dark red, dark purple, dark blue, red brown, black purple, and dark red-purple grains are widely known as enriched rice for taste and health improvements in Korea. They are also widely used as food colorants in bread, ice creams, and liquor (*9*, *10*).

As a part of our studies on the characterization of cytotoxic components from plant sources (*11*−*14*), *O. sativa* cv. Heugjinjubyeo, which has a dark purple color by modification of genomes in the aleurone layer of rice grains, was selected for study, because the 0.5% HCl−EtOH extract of the aleurone layer of grain was found to exhibit significant cytotoxic activity against U937, human monocytic leukemia cells.

In this study, we report the isolation and identification of two bioactive compounds, cyanidin (**1**) and malvidin (**2**), and show that these compounds mediate the cytotoxicity through the arrest of the G_2/M phase of the cell cycle and by induction of apoptosis.

* Address correspondence to this author at the Department of Foods and Nutrition, Duksung Women's University, Ssangmoon-dong, Dobong-ku, Seoul 132-714, Korea (telephone +82-2-901-8593; fax +82-2-901-8442; e-mail hasook@duksung.ac.kr).

† Cheju National University.

§ Duksung Women's University.

10.1021/jf030370h CCC: $27.50
Published on Web 03/25/2004

MATERIALS AND METHODS

Plant Material. The fully ground aleurone layer of *O. sativa* cv. Heugjinjubyeo was supplied by the National Crop Experiment Station, Rural Development Administration (RDA), Suwon, Gyeonggi-do, Korea, in February 2002. A voucher specimen has been deposited at the RDA.

Reagents. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), propidium iodide, and RNase were purchased from the Sigma Chemical Co. (St. Louis, MO).

Extraction, Fractionation, and Isolation of Anthocyanidins. The dried and ground aleurone layer of *O. sativa* cv. Heugjinjubyeo (1.0 kg) was extracted with acetone, and the residue was extracted with ethyl alcohol (EtOH) containing 0.5% HCl three times at room temperature overnight. The combined extracts were concentrated under reduced pressure to give a dark purple extract. The dried 0.5% HCl in EtOH soluble fraction (10.4 g) was chromatographed over a vacuum silica gel column (4.0 × 6.0 cm) using a chloroform ($CHCl_3$)−EtOH gradient to afford six fractions. Fraction 4 (2.8 g) was measured as an active portion, with a 50% growth inhibitory concentration (IC_{50}) value of 70.6 μg/mL on U937, human monocytic leukemic cells, and eluted with further chromatography on an open silica gel column (2.0 × 30 cm) using $CHCl_3$−EtOH (94:6, v/v); subfractions 32−37 (760.2 mg) were rechromatographed on a Sephadex LH-20 column (1.0 × 110 cm) by elution with EtOH in order to yield compounds **1** (48.1 mg) and **2** (26.3 mg). The chromatographic technique needed to be accomplished in a short time, because these compounds are sensitive to exposure in air. The results of column chromatographic separations can be conveniently monitored by applying aliquots of the fractions to silica gel and cellulose thin-layer chromatographic (TLC) plates and developing in a mixture of *n*-butyl alcohol (*n*-BuOH)−acetic acid (HOAc)−H_2O (4:1:5, v/v, top layer) and HCl−formic acid (HCOOH)−H_2O (2:5:3, v/v). The fractions were bioassayed before additional chromatographic fractionation, and then fractions with the desired activity were applied for the isolation of bioactive compounds. The elution on condensation resulted as a solid material and was further purified by recrystallization with highly purified methyl alcohol (MeOH) to give the pure compounds **1** and **2**.

Instrumental Analysis. Melting points (mp) were determined using a Mitamura-Riken melting point apparatus and are uncorrected. Electron impact mass spectrometry (EI-MS) spectra were obtained on a Hewelett Packard model 5985B gas chromatography (GC)−MS system. The ultraviolet (UV)−visible and infrared (IR) spectra were recorded on Hitachi 3100 UV−vis and JASCO Fourier transform (FT)-IR-5300 spectrophotometers, respectively. A Bruker AMX500 spectrometer was used to record nuclear magnetic resonance (NMR) spectra (500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR) with tetramethylsilane (TMS), and one drop of CD_3COOD-d_4 in DMSO-d_6 as an internal standard and NMR solvents, respectively. The correlation spectroscopy (COSY) and heteronuclear multiple bond correlation (HMBC) experiments were conducted by two-dimensional NMR spectroscophic method. TLC analysis was performed on silica gel (Kieselgel 60 F_{254}) and cellulose plates (0.25 mm layer thickness; Merck, Darmstadt, Germany), with compounds visualized after developing in a mixture of *n*-BuOH−HOAc−H_2O and HCl−HCOOH−H_2O by natural purple color. Silica gel (Merck 60 A, 230−400 mesh ASTM) and Sephadex LH-20 (25−100 μm; Pharmacia Fine Chemicals, Piscataway, NJ) were used for column chromatography.

Cell Culture. U937 (human monocytic leukemia cells) were cultured at 37 °C in 5% CO_2 in RPMI 1640 medium containing 2 mM glutamine, 10% heat-inactivated fetal calf serum, penicillin (100 units/mL), and streptomycin (100 μg/mL).

Cell Viability Assay. The effect of plant fractions on the viability of U937 cells was determined by an MTT assay (*15*, *16*). Cells at the exponential phase were collected and transferred into each well (~10^4−10^5 cells in 180 μL/well). The cells were incubated for 96 h in the presence of various amounts of fractions and pure anthocyanidins (0−200 μg/mL) in a total reaction volume of 200 μL; 50 μL of 2 mg/mL MTT solution was then added to each well (0.1 mg/well). After 4 h of incubation, the plates were centrifuged at 800*g* for 5 min and supernatants were aspirated. The formazan crystals in each well were dissolved in 150 μL of dimethyl sulfoxide, and the A_{540} was read on a scanning multiwell spectrophotometer (Molecular Devices Co., Sunnyvale, CA). The value for IC_{50} was determined at the concentration that inhibited cell growth by 50% using the MTT assay. Data are expressed as means ± standard error (SE).

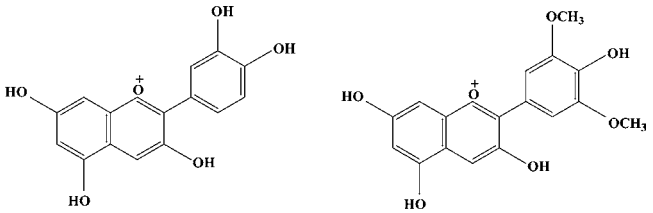

Figure 1. Structures of cyanidin (**1**) and malvidin (**2**) isolated from the aleurone layer of *O. sativa* cv. Heugjinjubyeo.

Flow Cytometry. Cells were harvested at the times indicated and fixed in 1 mL of 70% EtOH (2 × 10^5 cells/mL). The cells in each of these EtOH solutions were then washed twice with phosphate-buffered saline (PBS) and incubated in the dark in 1 mL of PBS containing 100 μg of propidium iodide (Sigma Chemical Co.) for 30 min at 37 °C. Flow cytometric analysis was performed with a FACSCalibur flow cytometer (Becton Dickson, Mountain View, CA). The effect on the cell cycle was assessed by determining changes in the percentages of cells at the different phases of cell cycle as assessed by histograms generated by a computer program, Cell Quest and Mod-Fit (*17*). The effect on apoptosis was determined by the increase in the proportion of sub-G_1 hypo-diploid cells (*18*). Data are expressed as means ± standard error (SE).

DNA Fragmentation on Agarose Gel. DNA fragmentation assay was performed by electophoresis on 1.8% agarose gel. Briefly, cells (2 × 10^5/mL) were incubated with cyanidin or malvidin for 48 h and harvested. DNA was extracted using a genomic DNA extraction kit (Wako Junyaku, Tokyo, Japan). Aliquots of the DNA (4 μg) dissolved in 5 μL of a loading buffer were electrophoresed in a 1.8% agarose gel containing 0.1 μg/mL of ethidium bromide.

RESULTS AND DISCUSSION

Isolation and Identification of Anthocyanidins. The 0.5% HCl−EtOH extract, which showed moderate cytotoxicity with an IC_{50} value of 83 μg/mL on U937 human monocytic leukemia cells, was fractionated, and the acetone extract and 0.5% HCl−EtOH soluble fractions showed IC_{50} values of 300 and 68 μg/mL, respectively. The 0.5% HCl−EtOH fraction was chromatographed using vacuum liquid chromatography to obtain six subfractions visualizing TLC patterns. Of these, subfraction 4, which possessed cytotoxic activity with an IC_{50} value of 70.6 μg/mL on U937 cells, was chosen for further column chromatography to isolate two active pure compounds. Complete identification of isolated compounds made use of a variety of physical and chemical methods, which includes MS spectrometry, UV−vis, IR, and ^1H and ^{13}C NMR spectroscopy. The structures of these compounds (**Figure 1**) were identified by comparing spectra with published data (*19*, *20*). The isolated cytotoxic compounds were determined as cyanidin (**1**) and malvidin (**2**), and detailed data are described as follows.

Cyanidin (1): dark purple amorphous powder from MeOH; mp 194−195 °C; UV (MeOH + HCl) λ_{max} (log ϵ) 535 (4.09), 335 (3.28), 280 (4.11) nm; IR (KBr) ν_{max} 3440 (OH), 1686 (C═C) cm^{-1}; EI-MS (70 eV), *m*/*z* (relative intensity %) 305 $[M + 1]^+$ (1.8); ^1H NMR and ^{13}C NMR data were consistent with those in the literature (*19*), described in **Table 1**.

Malvidin (2): dark purple amorphous powder from MeOH; mp 207−210 °C; UV (MeOH + HCl) λ_{max} (log ϵ) 536 (4.12), 333 (3.17), 282 (4.03) nm; IR (KBr) ν_{max} 3375 (OH), 1702 (C═C) cm^{-1}; EI-MS (70 eV), *m*/*z* (relative intensity %) 349

Table 1. ^{1}H, ^{13}C, ^{1}H–^{1}H COSY, and ^{1}H–^{13}C HMBC NMR Data for Isolated Compounds[a]

	δ^1H[a] (mult, *J* in Hz)		^{13}C[b]			
position	**1**	**2**	**1**	**2**	^{1}H–^{1}H COSY	^{1}H–^{13}C HMBC
2			156.2	157.0		
3			116.1	116.7		
4	8.86 (s)	8.76 (s)	97.7	96.9		C-5, C-9
5			152.8	152.5		
6	6.84 (d, $J = 2.2$)	6.87 (d, $J = 2.1$)	95.3	95.4	H-8	C-8, C-10
7			152.3	151.9		
8	6.94 (d, $J = 2.2$)	6.92 (d, $J = 2.1$)	96.2	96.8	H-6	C-6
9			111.7	119.4		
10			115.6	116.2		
1′			119.3	119.7		
2′	7.35 (d, $J = 2.1$)	7.52 (s)	107.9	109.1	H-6′	C-2, C-6′
3′			145.8	154.2		
4′			137.0	128.9		
5′	7.50 (d, $J = 7.6$)		102.2	154.2	H-6′	C-1′
6′	7.27 (dd, $J = 2.1, 7.6$)	7.52 (s)	99.6	109.7	H-2′, H-5′	C-4′
OH	12.23	11.59				
	12.08	10.35				
	10.91	10.15				
	10.54					
	9.54					
4′-OCH_3		3.93		56.1		C-4′
6′-OCH_3		3.95		59.1		C-6′

[a] TMS was used as the internal standard; chemical shifts are shown in the δ scale with *J* values in parentheses; measured at 500 MHz in DMSO-d_6 (with one drop of CD_3COOD-d_4). [b] Measured at 125 MHz in DMSO-d_6 (with one drop of CD_3COOD-d_4).

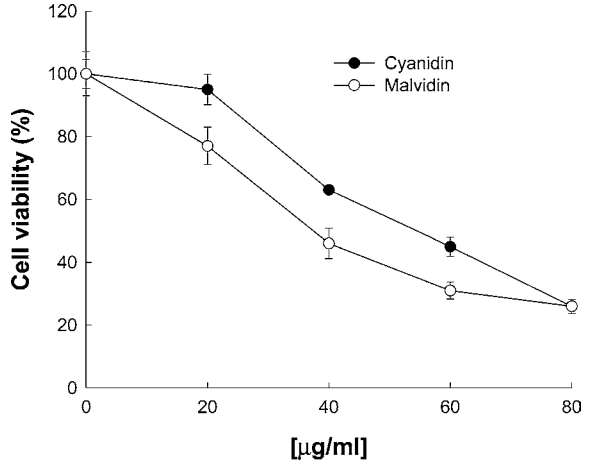

Figure 2. Cytotoxic activity of cyanidin and malvidin at dose-dependent pattern. U937 cells were incubated for 96 h in the presence of various amounts of cyanidin or malvidin, and then cell viability was detected using the MTT test; values are expressed as means ± standard error (SE).

$[M + 1]^+$ (2.8); ^{1}H NMR and ^{13}C NMR data were consistent with those in the literature (*20*), described in **Table 1**.

Cytotoxicity, Arrest of G_2/M Phase of Cell Cycle, and Induction of Apoptosis. Cyanidin and malvidin showed cytotoxic activity in a dose-dependent pattern (**Figure 2**). The IC_{50} values of these two anthocyanidins for U937 cells were 60 and 40 μg/mL, respectively (**Table 2**). The glucosides of cyanidin and malvidin did not have efficacy on U937 cells because the cell viability of these glucosides on U937 was not affected at the maximum dose (80 μg/mL) tested, suggesting that the cytotoxic activity is due to the aglycon moiety. To determine the mechanisms of U937 cell inhibition by these cytotoxic components, change of cell cycle was assessed using flow cytometry. As shown in **Figure 3**, the population of the G_2/M phase increased 15% in cyanidin-treated cells and 20% in malvidin-treated cells, compared to the untreated cells. This suggests that cyanidin and malvidin showed arrest at the G_2/M phase of the cell cycle. It is reported that anthocyanins of Concord grape juice inhibit cell growth of RBA cells, rat adenocarcinoma cells, through accumulation of G_1 phase of the cell cycle (*21*).

Table 2. Values of IC_{50} of Anthocyanidins Isolated from 0.5% HCl–EtOH Soluble Fraction against U937 Human Monocytic Leukemia Cells

compound	yield (%)	IC_{50}[a] (μg/mL)
cyanidin	4.81×10^{-5}	60 ± 0.6
malvidin	2.63×10^{-5}	40 ± 2.1
cisplatin[b]		1.5 ± 0.01

[a] Fifty percent inhibitory concentration (IC_{50}) was measured by MTT assay after 96 h of incubation and values are expressed as means ± standard error (SE). [b] Positive control.

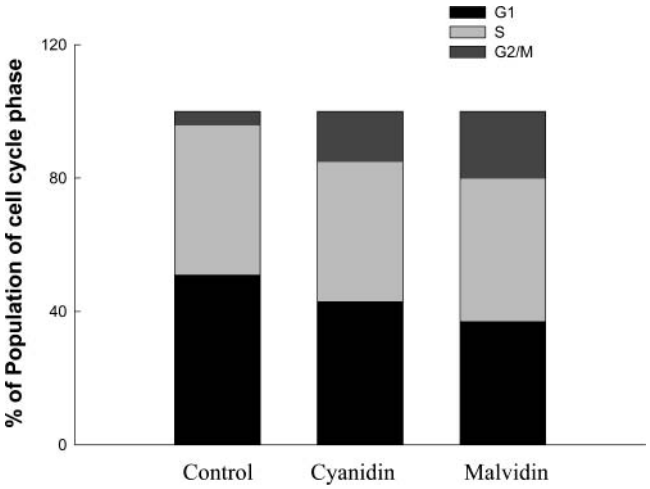

Figure 3. Accumulation of G_2/M phase of cell cycle in cyanidin- or malvidin-treated U937 cells. The cyanidin- or malvidin-treated cells were harvested, stained with propidium iodide, and subjected to flow cytometric analysis for cell distributions at each phase of the cell cycle.

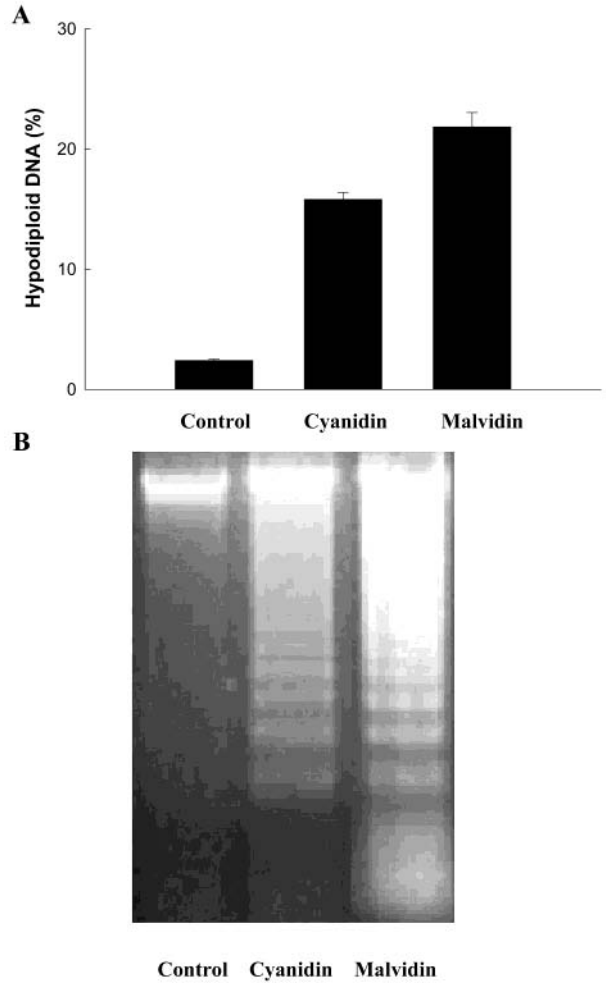

Figure 4. Apoptotic patterns of cyanidin- or malvidin-teated U937 cells. U937 cells cultured with cyanidin (60 μM) or malvidin (40 μM) were examined for apoptosis. (**A**) For the measurement of sub G_1 hypo-diploid cells, the cyanidin- or malvidin-treated cells were harvested at the indicated times and fixed in 1 mL of 70% EtOH for 30 min. After washing with PBS, the cells were treated with 1 mL of PBS containing 100 μg of propidium iodide and 100 μg of RNase A for 30 min at 37 °C. Flow cytometric analysis was performed with a FACSCalibur flow cytometer. (**B**) Gel electrophoresis for the detection of DNA fragmentation after incubation with cyanidin or malvidin for 48 h was performed. Genomic DNA was extracted using a genomic DNA extraction kit; DNA (4 μg) was electrophoresed on a 1.8% agarose gel containing 0.1 μg/mL ethidium bromide and visualized under ultraviolet light.

Many cytotoxic agents and/or DNA-damaging agents accumulate the cell cycle to ensure the cells repair the damaged DNA and then induce apoptotic cell death in the case of irreparable cells (*22*−*26*). Apoptosis is a common mechanism that regulates cell death physiological processes such as embryogenesis and normal tissue and organ involution. Apoptosis also can be induced in cells by the imposition of external stresses such as bacterial toxins, heat shock, radiation, and oxidative stress (*27*), and failure of apoptosis is considered to contribute to the development of human cancer (*28*). Because it has recently been suggested that cancer chemotherapeutics exert part of their pharmacological effects by triggering apoptotic cell death, the induction of apoptosis in tumor cells has become a candidate for cancer treatment (*29*). Also, from the result of flow cytometry as shown in **Figure 4A**, it was found that the sub G_1 hypo-diploid population increased in cyanidin- or malvidin-treated U937 cells, which are known to be apoptotic cells (*18*). Also, DNA fragmentation, which is another characteristic of apoptosis (*18*, *22*), was detected in cyanidin- or malvidin-treated U937 cells (**Figure 4B**). There are also some reports that anthocyanins induce apotosis on various cancer cell lines (*30*−*32*).

From these results, we suggest that cyanidin and malvidin showed cytotoxicity through the arrest of G_2/M phase of the cell cycle and induction of apoptosis. It is believed that bioactive anthocyanidins isolated from *O. sativa* cv. Heugjinjubyeo can supply beneficial effects on not only taste but also health and have been shown to inhibit the growth of human monocytic leukemia cells.

LITERATURE CITED

(1) Kinghorn, A. D., Balandrin, M. F., Eds. In *Human Medicinal Agents from Plants*; ACS Symposium Series 534; American Chemical Society: Washington, DC, 1993.
(2) Briviba, K.; Abrahamse, S. L.; Pool-Zobel, B. L.; Rechkemmer, G. Neurotensin- and EGF-induced metabolic activation of colon carcinoma cells is diminished by dietary flavonoid cyanidin but not its glycosides. *Nutr. Cancer* **2001**, *41*, 172−179.
(3) Kang, S. Y.; Seeram, N. P.; Nair, M. G.; Bourquin, L. D. Tart cherry anthocyanins inhibit tumor development in APC^{Min} mice and reduce proliferation of human colon cancer cells. *Cancer Lett.* **2003**, *194*, 13−19.
(4) Wang, H.; Nair, M. G.; Strasburg, G. M.; Chang, Y.-C.; Booren, A. M.; Gray, J. L.; DeWitt, D. L. Antioxidant and anti-inflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. *J. Nat. Prod.* **1999**, *62*, 294−296.
(5) Elmar, S.; Helga, S. Estrogenic activity of naturally occurring anthocyanidins. *Nutr. Cancer* **2001**, *41*, 145−149.
(6) Meiers, S.; Kemeny, M.; Weyand, U.; Gastpar, R.; von Angerer, E.; Marko, D. The anthocyanidins cyanidin and delphinidin are potent inhibitors of the epidermal growth-factor receptor. *J. Agric. Food Chem.* **2001**, *49*, 958−962.
(7) Wang, J.; Mazza, G. Effects of anthocyanins and other phenolic compounds on the production of tumor necrosis factor α in LPS/IFN-γ-activated RAW 264.7 macrophages. *J. Agric. Food Chem.* **2002**, *50*, 4183−4189.
(8) Chung, H. S.; Woo, W. S. A quinolone alkaloid with antioxidant activity from the aleurone layer of anthocyanin-pigmented rice. *J. Nat. Prod.* **2001**, *64*, 1579−1580.
(9) Lee, H. J.; Oh, S. K.; Choi, H. C.; Kim, S. U. Identification of anthocyanins from pigmented rice seed. *Agric. Chem. Biotechnol.* **1988**, *41*, 257−262.
(10) Yoshinaga, K.; Yakahashi, K.; Yoshizawa, K. Liquor with pigments of red rice. *J. Brew. Soc. Jpn.* **1986**, *81*, 337−342.
(11) Yang, Y. M.; Hyun, J. W.; Sung, M. S.; Chung, H. S.; Kim, B. K.; Paik, W. H.; Kang, S. S.; Park, J. G. The cytotoxicity of psoralidin from *Psoralea corylifolia*. *Planta Med.* **1996**, *62*, 353−354.
(12) Hyun, J. W.; Yang, Y. M.; Sung, M. S.; Chung, H. S.; Paik, W. H.; Kang, S. S.; Park, J. G. The cytotoxic activity of sterol derivatives from *Pulsatilla chinensis* Regal. *J. Kor. Cancer Assoc.* **1996**, *28*, 145−150.
(13) Chung, H. S. Cytotoxicity from some Korean edible plants. *Kor. J. Soc. Food Sci.* **1999**, *15*, 108−113.
(14) Chung, H. S. Guaianolide sesquiterpene lactone from *Ixeris sonchifolia* Hance with cytotoxicity in cultured human stomach and colon cancer cell lines. *Food Sci. Biotechnol.* **2001**, *10*, 433−436.
(15) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Cancer Res.* **1987**, *47*, 936−942.

(16) Liu, Y.; Peterson, D. A.; Kimura, H.; Schubert, D. Mechanism of cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction. *J. Neurochem*. **1997**, *69*, 581−593.

(17) Wang, X. W.; Zhan, Q.; Coursen, J. D.; Khan, M. A.; Kontny, H. U.; Yu, L.; Hollander, M. C.; Oconnor, P. M.; Fornace, A. J.; Harris, C. C. GADD45 induction of a G2/M cell cycle checkpoint. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 3706−3711.

(18) Nicoletti, I.; Migliorati, G.; Pagliacci, M. C.; Grignani, F.; Riccardi, C. A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. *J. Immunol. Methods* **1991**, *139*, 271−279.

(19) Johansen, O. P.; Andersen, O. M.; Nerdal, W.; Aksnes, D. W. Cyanidin 3-[6-(*p*-coumaroyl)-2-(xylosyl)-glucoside]-5-glucoside and other anthocyanins from fruits of *Sambucus canadensis*. *Phytochemistry* **1991**, *30*, 4137−4141.

(20) Degenhardt, A.; Hofmann, S.; Knapp, H.; Winterhalter, P. Preparative isolation of anthocyanins by high-speed countercurrent chromatography and application of the color activity concept to red wine. *J. Agric. Food Chem*. **2000**, *48*, 5812−5818.

(21) Singletary, K. W.; Stansbury, M. J.; Giusti, M.; van Breemen, R. B.; Wallig, M.; Rimando, A. Inhibition of rat mammary tumorigenesis by Concord grape juice constituents. *J. Agric. Food Chem.* **2003**, *51*, 7280−7286.

(22) Purohit, A.; Hejaz, H. A. M.; Walden, L.; MacCarthy-Morrogh, L.; Packham, G.; Potter, B. V. L.; Reed, M. J. The effect of 2-methoxyoestrone-3-*O*-sulphamate on the growth of breast cancer cells and induced mammary tumors. *Int. J. Cancer* **2000**, *85*, 584−589.

(23) Kessel, D.; Luo, Y. Cells in cryptophycin-induced cell-cycle arrest are susceptible to apoptosis. *Cancer Lett*. **2000**, *151*, 25−29.

(24) Fujimoto, K.; Hosotani, R.; Doi, R.; Wada, M.; Lee, J. U.; Koshiba, T.; Miyamoto, Y.; Tsuji, S.; Nakajima, S.; Imamura, M. Induction of cell-cycle arrest and apoptosis by a novel retinobenzoic-acid derivative, TAC-101, in human pancreatic-cancer cells. *Int. J. Cancer* **1999**, *81*, 637−644.

(25) Johnson, P. A.; Clements, P.; Hudson, K.; Caldecott, K. W. A mitotic spindle requirement for DNA damage-induced apoptosis in Chinese hamster ovary cells. *Cancer Res*. **1999**, *59*, 2696−2700.

(26) Orren, D. K.; Petersen, L. N.; Bohr, V. A. Persistent DNA damage inhibits S-phase and G2 progression, and results in apoptosis. *Mol. Biol. Cell* **1997**, *8*, 1129−1142.

(27) McConkey, D. L.; Orrenius, J. Signal transduction pathways in apoptosis. *Stem Cell* **1996**, *14*, 619−631.

(28) Que, F. G.; Gores, G. Cell death by apoptosis: basic concepts and disease relevance for the gastroenterologist. *Gastroenterology* **1996**, *110*, 1238−1246.

(29) Ahmad, N.; Feyes, D. K.; Nieminen, A. L.; Agarwal, R.; Mukhtar, H. Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. *J. Natl. Cancer Inst*. **1997**, *89*, 1881−1886.

(30) Katsube, N.; Iwashita, K.; Tsushida, T.; Yamaki, K.; Kobori, M. Induction of apoptosis in cancer cells by bilberry (*Vaccinium myrtillus*) and the anthocyanins. *J. Agric. Food Chem.* **2003**, *51*, 68−75.

(31) Hou, D. X. Potential mechanisms of cancer chemoprevention by anthocyanins. *Curr. Mol. Med.* **2003**, *3*, 149−159.

(32) Katsuzaki, H.; Hibasami, H.; Ohwaki, S.; Ishikawa, K.; Imai, K.; Date, K.; Kimura, Y.; Komiya, T. Cyanidin 3-*O*-*β*-D-glucoside isolated from skin of black glycine max and other anthocyanins isolated from skin of red grape induce apoptosis in human lymphoid leukemia Molt 4B cells. *Oncol. Rep*. **2003**, *10*, 297−300.

Received for review May 16, 2003. Revised manuscript received February 3, 2004. Accepted February 3, 2004. This study was supported by Specific Research-promoting Joint Projects, RDA, Korea.

JF030370H