JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2002,** *50,* 2519−2523 **2519**

Characterization, Quantification, and Bioactivities of Anthocyanins in *Cornus* Species

NAVINDRA P. SEERAM,[†] ROBERT SCHUTZKI,[‡] AMITABH CHANDRA,[§] AND MURALEEDHARAN G. NAIR*,[†]

Bioactive Natural Products and Phytoceuticals, Department of Horticulture and National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan 48824, and Chemistry Services, Department of Analytical Services, Access Business Group, Ada, Michigan 49355

Cornus mas, *Cornus officinalis*, *Cornus controversa*, and *Cornus kousa* (Cornaceae) bear edible fruits that are consumed in parts of Europe and Asia. This study undertook the investigation of the presence and levels of anthocyanins in the fruits of these *Cornus* species by HPLC. The anthocyanins present in Cornelian cherries, *C. mas*, are delphinidin 3-*O*-β-galactopyranoside (**1**), cyanidin 3-*O*-β-galactopyranoside (**2**), and pelargonidin 3-*O*-β-galactopyranoside (**3**). *C. officinalis* contains only anthocyanins **1**−**3**, similar to *C. mas*, but in different proportions. However, *C. controversa* contains anthocyanins **1**−**3** among other anthocyanins, but Chinese dogwood, *C. kousa*, did not contain **1**−**3**. The contents of pure anthocyanins **1**, **2**, and **3** in 1 kg of fresh fruits of *C. mas*, *C. officinalis,* and *C. controversa* were 280, 1079, and 710 ppm; 11, 77, and 230 ppm; and 600, 1000, and 700 ppm, respectively. In cyclooxygenase (COX)-I and -II enzyme inhibitory assays, anthocyanins **1**−**3** (all 40 μM) showed activities of 9.2 and 11.7%; 7.6 and 12.4%; and 5.3 and 7.8%, respectively, compared to Naproxen (54.3 and 41.3%; 10 μM), ibuprofen (47.5 and 39.8%; 10 μM), Celebrex (46.2 and 66.3%; 1.67 ppm), and Vioxx (23.8 and 88.1%, 1.67 ppm). In the antioxidant assay, anthocyanins **1**−**3** (all 40 μM) showed activities of 70.2, 60.1, and 40.3%, respectively. At 10 μM concentration, commercial synthetic antioxidants *tert*-butylhydroquinone, butylated hydroxytoluene, butylated hydroxyanisole, and vitamin E gave 83.2, 79.7, 82.1, and 10.2% of antioxidant activity, respectively.

KEYWORDS: ***Cornus mas*; *Cornus officinalis*; *Cornus controversa*; *Cornus kousa*; dogwood; anthocyanins; delphinidin; cyanidin; pelargonidin; galactoside; antioxidant; cyclooxygenase**

INTRODUCTION

Anthocyanins are glycosides of anthocyanidins universally associated with attractive, colorful, and flavorful fruits. Recently, there has been a resurgence of interest in anthocyanins due to their potential health benefits as antioxidants and anti-inflammatory agents (*1*, *2*). The use of anthocyanin-containing foods as part of a diet may also be beneficial to human health (*3*). The recently publicized therapeutic benefits of anthocyanins, as well as the natural sources that contain them, have resulted in an increasing consumer demand for anthocyanin-containing products (*4*). This has resulted in the phytoceutical and botanical supplement industries investigating fruits that have a high content of anthocyanins for purposes of formulating new commercial products. Some of the most important candidates that supply beneficial anthocyanins are bilberries, elderberries, chokeberries, and tart cherries (*5*). However, there is an ongoing search for new and other high-yield sources of beneficial anthocyanins. In our continued phytoceutical investigation of cherries and berries for the improvement of health and the production of value-added food (*1*, *2*, *4*), we have turned our attention to fruits of *Cornus* species.

The genus *Cornus* (dogwoods) contains about 40 species including some used in preserves and sweets (*6*). These plants often assume a brilliant fall coloring and attractive flower and fruit colors and are widely grown for ornamental purposes (*7*). *Cornus mas* L. is known as the European and Asiatic Cornelian cherry, and its scarlet fruits were formerly fermented as a beverage and are now used in Turkey in the concoction of a kind of sherbet (*8*). Extracts from the fruits are also used in Europe for cosmetical purposes, replacing synthetic astringent substances, and are claimed to exert a favorable action on the human complexion (*9*). Two early studies revealed that berries of *C. mas* contain five anthocyanins, identified by paper chromatography, spectrophotometric, and peroxide oxidation analyses as delphinidin 3-galactoside, cyanidin 3-galactoside, cyanidin 3-rhamnosylgalactoside, pelargonidin 3-galactoside, and pelargonidin 3-rhamnosylgalactoside (*10*, *11*). However, a survey of the literature revealed that the anthocyanin content

* Author to whom correspondence should be addressed [telephone (517) 353-2915; fax (517) 432-2310; e-mail nairm@msu.edu].

[†] BNPP, Department of Horticulture and National Food Safety and Toxicology Center.

[‡] Department of Horticulture.

[§] Access Business Group.

10.1021/jf0115903 CCC: $22.00
Published on Web 03/09/2002

of *Cornus officinalis* and *Cornus controversa* has never been reported. *C. officinalis* is used in traditional medicine and is known for its tonic, analgesic, and diuretic activities (*12*). The stones of the fruits are also reported to have antioxidant properties (*13*). Tannins, galloylated glycosides, gallotannins, organic acids, and furan derivatives have been reported from fruits of this plant (*14*, *15*). *C. controversa* has recently been reported to contain phenolic compounds in its leaves (*16*). The fruits of *Cornus kousa* (Chinese dogwood) are attractive and edible and are fermented to wine in some parts of China where this plant is grown (*17*).

In addition to the comparison of anthocyanins in these four *Cornus* species, we describe the isolation, characterization, and quantification of anthocyanins by HPLC, LC-ES/MS, and NMR methods. This is the first report of the identification of anthocyanins from *C. mas* by NMR and LC-ES/MS spectral methods and of the cyclooxygenase inhibitory and antioxidant activities of the monogalactosides of delphinidin, cyanidin, and pelargonidin.

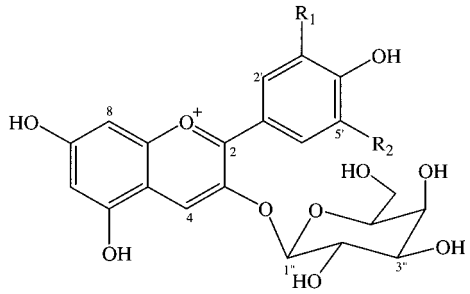
MATERIALS AND METHODS

General Experimental Procedures. All NMR spectra (^{1}H and ^{13}C) were recorded on a Varian VXR 500 MHz spectrometer. ^{13}C NMR spectra were recorded at 126 MHz. Chemical shifts were recorded in CD_3OD/DCl, and the values are in δ (parts per million) relative to CD_3OD at 3.31 for ^{1}H NMR and at 49.15 ppm for ^{13}C NMR. Coupling constants, *J*, are in hertz. All solvents were of ACS reagent grade and were purchased from Spectrum Chemical Co. Positive controls used in the antioxidant [*tert*-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and α-tocopherol] and COX inhibitory (ibuprofen and naproxen) bioassays were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Celebrex capsules and Vioxx tablets were physician's professional samples provided by Dr. Subash Gupta, Sparrow Pain Center, Sparrow Hospital, Lansing, MI. Standards of cyanidin 3-glucoside (kuromanin chloride), pelargonidin 3-glucoside (callistephin chloride), cyanidin chloride, and pelargonidin chloride were purchased from Indofine (Indofine Chemical Co., Somerville, NJ).

Fruits. Ripe fruits of *C. mas* L. were collected in mid October at the Northwest Michigan Horticulture Experimental Station, Sutton's Bay, MI. Samples of fruits of *C. officinalis* Sieb and Zucc., *C. controversa* Hemsl., and *C. kousa* Hance. were collected in mid September from trees on the campus of Michigan State University, East Lansing, MI. The locations of the trees are recorded in the Michigan State University Herbarium Plant Database. The fruits were stored in plastic ziplock bags at −20 °C prior to analyses.

Extraction of Anthocyanins for HPLC Analysis. Fresh fruits (25 g) were homogenized separately in 3 × 15 mL of methanol (1% HCl) for 5 min in a Kinematica CH-6010 (Roxdale, ON, Canada) homogenizer and centrifuged (model RC5C, Sorvall Instruments, Hoffman Estates, IL) at 10000*g* for 20 min at 4 °C. The extraction process was done three times to ensure that anthocyanins were exhasutively extracted. The supernatant from each fruit sample was quantitatively made up to 50 mL with methanol (1% HCl) and stored at −20 °C prior to analyses.

Analytical HPLC Conditions for Analyses and Quantification. All samples (20 μL injection volume) were filtered (0.22 μm) and analyzed on an Xterra (Waters Corp.) RP-18 column, 250 × 4.6 mm i.d, 5 μm, at a column temperature of 35 °C. The mobile phase, 4% aqueous H_3PO_4/CH_3CN (9:1 v/v), was used under isocratic conditions at a flow rate of 0.75 mL/min. Anthocyanins were detected at 520 nm using a PDA detector (Waters Corp., Milford, MA). Quantification of anthocyanins was accomplished using the Millenium 2010 chromatography manager version 3.05.01 (Waters Corp.). Pure anthocyanins **1**, **2**, and **3**, 1 mg each, were weighed separately and dissolved in 1 mL of MeOH (1% HCl). Separate stock solutions were prepared for each anthocyanin, and samples were then prepared by serial dilution of the individual stock solutions to afford 0.50, 0.25, 0.10, 0.05, 0.025, and 0.0125 mg/mL concentrations, respectively. Each sample was injected in triplicate, and calibration curves were obtained by plotting the mean peak area percentages against concentration for each compound. Juice samples were analyzed in triplicate, and the mean peak area percentages of anthocyanins were used to determine the quantities of anthocyanins **1**−**3**.



1 R_1 = OH, R_2 = OH

2 R_1 = OH, R_2 = H

3 R_1 = H, R_2 = H

Figure 1. Anthocyanins **1**–**3** from *C. mas*.

LC-ES/MS Analyses. HPLC-ES/MS analyses were carried out on a MicroMass Quattro II LC-MS/MS system (Micromass, Division of Waters Corp., Beverly, MA), equipped with a Waters 2690 HPLC pump and a Waters 996 PDA detector (Waters Corp., Milford, MA). Data handling was carried out using MassLynx v. 3.4 software (Micromass). Conditions were as follows: column, HP ODS Hypersil HPLC column, 125 mm × 4.0 i.d., 5 μm (Agilent Technologies, Wilmington, DE); solvent A, 0.1% TFA/H_2O (v/v); solvent B, 50.4% H_2O/48.5% ACN/1.0% CH_3COOH/0.1% TFA (v/v/v/v); gradient, % B initial (20%), 26 min (60%), 30 min (20%), 35 min (20%); run time, 35 min; flow rate, 0.80 mL/min; injection volume, 10 μL (postcolumn split 10:1); column temperature, 30 °C; PDA range, 200−799 nm, 520 nm as detection wavelength. MS parameters were as follows: ionization mode, ES+; scan range, 200−1000 amu; scan rate, 1 scan/s; cone voltage, 20 eV. Peak identities were obtained by matching their molecular ions (M^+) obtained by ES/MS with the expected theoretical molecular weight from literature data (*5*). Reference standards of commercially available anthocyanins were used (independently and co-injected with test samples) for substantiating identities.

Purification of Anthocyanins 1—3. Anthocyanin mixture was isolated from *C. mas* berries according to a previously published method (*2*). The anthocyanin mixture (1.08 g) was dissolved in H_2O (2 mL) and fractionated by medium-pressure liquid chromatography (MPLC) on a C-18 column, 350 × 40 mm i.d., and eluted with a MeOH (0.01% TFA)/H_2O solvent system, under gradient conditions, starting with 30% MeOH to 100% MeOH (0.01% TFA). Five fractions, (I, 375 mL; II, 15 mL; III, 25 mL; IV, 30 mL; and V, 300 mL) were collected. HPLC analyses of these fractions revealed that fractions II (50 mg) (purple band), III (71 mg) (scarlet band), and IV (77 mg) (orange band) contained almost pure anthocyanins **1**, **2**, and **3**, respectively. The scarlet band, III (71 mg), was further chromatographed by MPLC on a C-18 column, 300 × 22 mm i.d., and eluted with a CH_3CN (0.01% TFA)/H_2O solvent system, under gradient conditions, starting with 10% CH_3CN to 100% CH_3CN (0.01% TFA). The pure, scarlet band collected (12 mL) was identified as cyanidin 3-galactoside (**2**) (11.8 mg). Similarly, the orange fraction IV (77 mg) yielded pelargonidin 3-galactoside (**3**) (6.2 mg). However, attempts to purify fraction II (50 mg) by the CH_3CN (0.01% TFA)/H_2O solvent system were not successful. A repeated MPLC, with a solvent system of CH_3CN/4% aqueous H_3PO_4 under gradient conditions starting with 10% CH_3CN to 100% CH_3CN, afforded pure delphinidin 3-galactoside (**1**) (3.4 mg). The purified anthocyanins **1**, **2**, and **3** are purple, scarlet, and orange amorphous powders, respectively (**Figure 1**).

Compound 1. ^{1}H and ^{13}C NMR spectra of pure anthocyanin **1** (delphinidin 3-*O*-β-galactopyranoside) were consistent with literature data (*7*). The LC-ES/MS gave the following major peaks at *m*/*z* (% intensity): 465 (M^+, 20), 304 (25), 303 (100, aglycon).

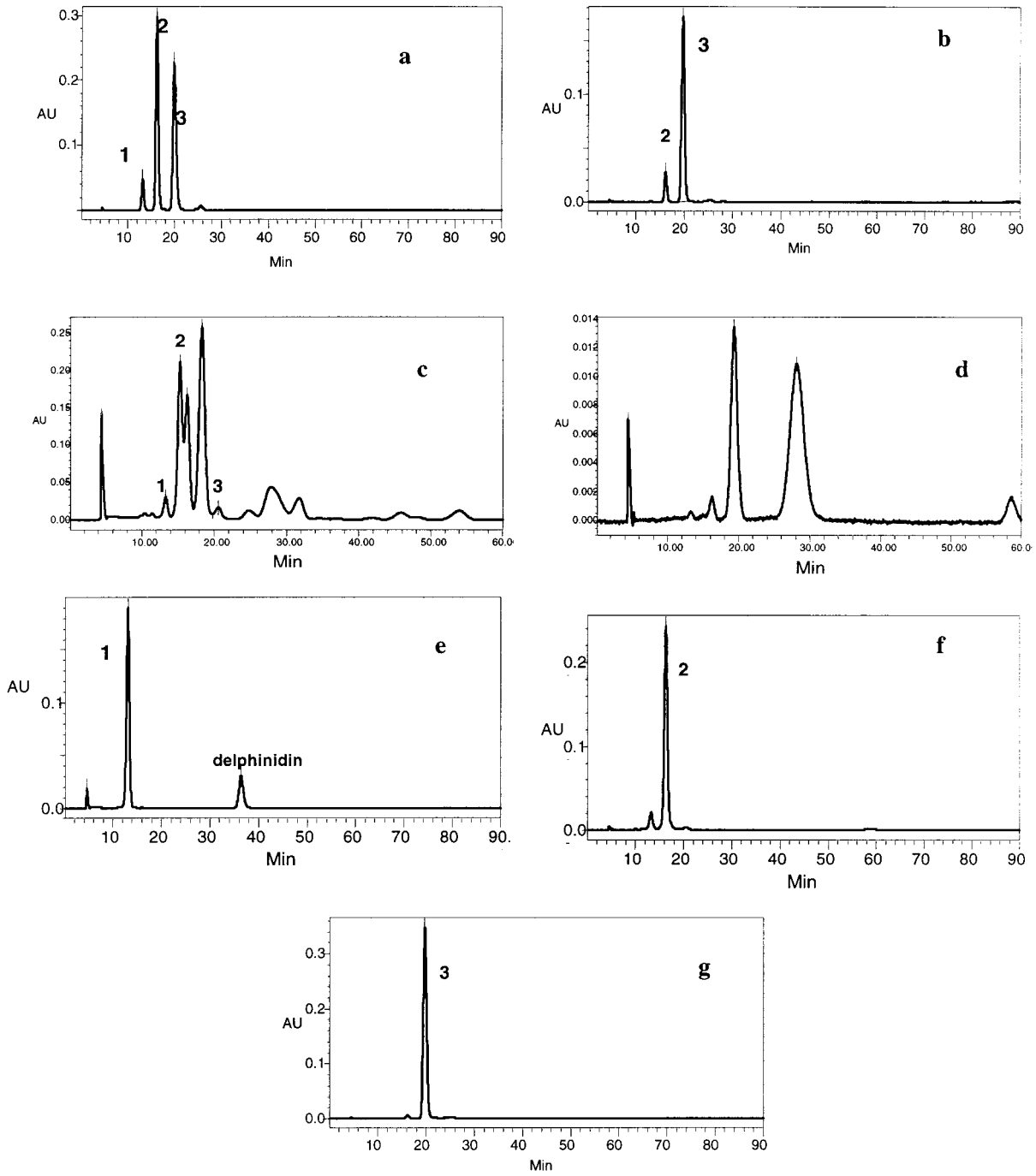


Figure 2. HPLC chromatograms of anthocyanins **1**–**3** in fresh fruits of *C. mas*, *C. officinalis*, *C. controversa*, and *C. kousa*: (a) anthocyanins **1**–**3** in *C. mas* at 13.2, 16.2, and 19.8 min, respectively; (b) anthocyanins **2** and **3** in *C. officinalis* at 16.1 and 19.7 min, respectively; (c) anthocyanins **1**–**3** in *C. controversa* at 13.3, 16.3, and 19.9 min, respectively; (d) unidentified anthocyanins in *C. kousa* at 18.2, 20.4, and 26.1 min, respectively; (e) pure anthocyanin **1** and its aglycon, delphinidin, from *C. mas* at 13.1 and 36.4 min, respectively; (f) pure anthocyanin **2** isolated from *C. mas* at 16.3 min; (g) pure anthocyanin **3** isolated from *C. mas* at 19.7 min.

Compound 2. ^{1}H NMR (CD_3OD/DCl) δ 8.98 (1H, s, H-4), 8.23 (1H, dd, J = 8.7, 2.5 Hz, H-6′), 8.04 (1H, d, J = 2.5 Hz, H-2′), 7.01 (1H, d, J = 8.7 Hz, H-5′), 6.92 (1H, d, J = 1.5 Hz, H-8), 6.67 (1H, d, J = 1.5 Hz, H-6), 5.43 (1H, d, J = 7.3 Hz, H-1″), 4.03 (1H, dd, J = 11.8, 6.2 Hz, H-6a″), 3.81 (1H, dd, J = 9.3, 9.0 Hz, H-3″), 3.71 (1H, dd, J = 9.0, 7.6 Hz, H-2″), 3.64 (1H, m, H-5″), 3.62 (1H, dd, J = 11.8, 1.5 Hz, H-6b″), 3.34 (1H, dd, J = 9.6, 9.0 Hz, H-4″); ^{13}C NMR (CD_3OD) δ 170.51 (C-7), 168.87 (C-2), 159.26 (C-5), 155.79 (C-9), 154.11 (C-4′), 147.38 (C-3′), 145.74 (C-3), 133.32 (C-4), 128.41(C-6′), 121.30 (C-1′), 118.57 (C-2′), 117.63 (C-5′), 113.56 (C-10), 106.14 (C-6), 98.19 (C-1″), 95.34 (C-8), 77.94 (C-5″), 74.78 (C-3″), 71.64 (C-2″), 69.97 (C-4″), 62.74 (C-6″). The LC-ES/MS gave the following major peaks at *m*/*z* (% intensity): 449 (M^+, 20), 287 (70, aglycon), 256 (100).

Compound 3. ^{1}H NMR (CD_3OD/DCl) δ 9.04 (1H, s, H-4), 8.58 (2H, d, J = 9.0 Hz, H-2′, H-6′), 7.04 (2H, d, J = 9.0 Hz, H-3′, H-5′), 6.94 (1H, d, J = 2.0 Hz, H-8), 6.68 (1H, d, J = 2.0 Hz, H-6), 5.26 (1H, d, J = 7.9 Hz, H-1″), 3.99 (1H, dd, J = 12.5, 3.5 Hz, H-6a″), 3.97 (1H, dd, J = 9.0, 7.5 Hz, H-2″), 3.85 (1H, dd, J = 9.3, 9.2 Hz, H-3″), 3.78 (1H, dd, J = 12.5, 3.5 Hz, H-6b″), 3.71 (1H, m, H-5″), 3.35 (1H, dd, J = 12.5, 3.5 Hz, H-4″); ^{13}C NMR (CD_3OD) δ 170.67 (C-7), 166.54 (C-2), 159.32 (C-5), 157.80 (C-9), 153.12 (C-4′), 145.51 (C-3), 137.58 (C-4), 135.83 (C-3′, C-5′), 120.93 (C-1′), 117.94 (C-2′, C-6′), 113.62 (C-10), 104.37 (C-1″), 103.59 (C-6), 95.39 (C-8), 77.76 (C-5″), 74.99 (C-3″), 72.14 (C-2″), 70.10 (C-4″), 62.37 (C-6″). The LC-ES/MS gave the following major peaks at *m*/*z* (% intensity): 433 (M^+, 23), 271 (100, aglycon), 256 (35).

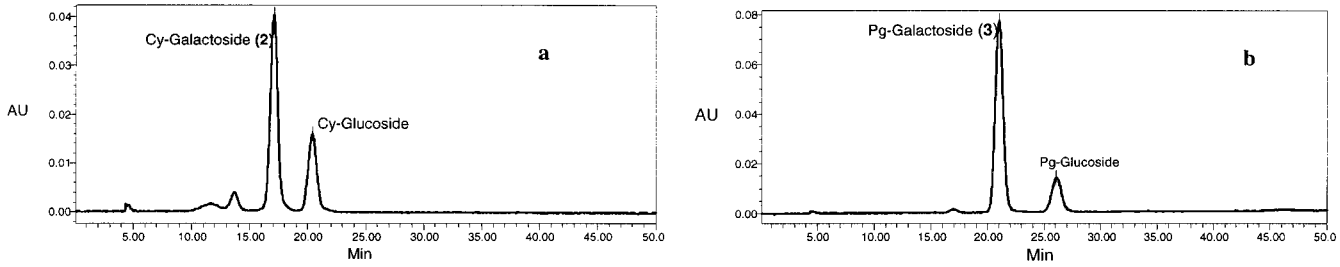


Figure 3. HPLC chromatograms of anthocyanin galactosides from *C. mas* co-injected with commercial standards of anthocyanin glucosides: (a) cyanidin 3-galactoside from *C. mas* and commercial cyanidin 3-glucoside at t_R = 16.3 and 20.4 min, respectively; (b) pelargonidin 3-galactoside from *C. mas* and commercial pelargonidin 3-glucoside at t_R = 19.9 and 26.1 min, respectively.

Cyclooxygenase (COX) Inhibitory Assay. A COX-I enzyme inhibitory assay was conducted with an enzyme preparation from ram seminal vesicles. COX-II activity was determined using a preparation from insect cell lysate. COX assays were measured at 37 °C and at pH 7.0 according to a published procedure (*1*, *2*).

Antioxidant Assay. Bioassays were conducted by analysis of model liposome oxidation using fluorescence spectroscopy according to the procedure reported previously (*1*, *2*). Peroxidation was initiated by the addition of $FeCl_2$ for positive controls (BHA, BHT, TBHQ, and α-tocopherol/vitamin E, all 10 μM, and test samples. Fluorescence was measured at 384 nm and monitored at 0, 1, 3, and every 3 min thereafter up to 21 min using a Turner model 450 digital fluorometer (Barnstead Thermolyne, Dubuque, IA). The decrease of relative fluorescence intensity with time indicated the rate of peroxidation, and these data are reported for 21 min after the initiation of peroxidation. Relative fluorescence (F_t/F_0) was calculated by dividing the fluorescence value at a given point (F_t) by that at t = 0 min (F_0).

RESULTS AND DISCUSSION

Pure anthocyanins **1**−**3** (**Figure 1**) were isolated from the enriched anthocyanin powder prepared from the fruits of *C. mas* according to previously published methods (*1*, *2*). Anthocyanin **1** was identified as delphinidin 3-*O*-β-galactoside by analyses of its NMR and LC/ES-MS spectral data. **Figure 2e** shows the HPLC chromatogram of delphinidin galactoside detected at 13.2 min and its aglycon, delphinidin, at 36.4 min. The LC-ES/MS spectrum of **1** gave a molecular ion at *m*/*z* 465 corresponding to delphinidin hexose, and the base peak at *m*/*z* 303 indicated the presence of a delphinidin aglycon. The NMR data of **1** were in agreement with the literature values for delphinidin 3-*O*-β-galactoside (*7*).

The structure of anthocyanin **2** was established as cyanidin 3-*O*-β-galactoside by examination of its NMR spectral data. The ^1H and ^{13}C NMR spectra were similar to those of delphinidin galactoside, **1**, except for differences in ring C, where cyanidin has one less OH than delphinidin. The LC-ES/MS of **2** gave a molecular cation at *m*/*z* 449 corresponding to cyanidin hexose, and the base peak at *m*/*z* 287 indicated the presence of cyanidin aglycon. Anthocyanin **2** corresponded to the peak at 16.2 min in the HPLC chromatogram (**Figure 2f**). Because the LC-ES/MS data had suggested the presence of a hexose sugar residue, a reference sample of cyanidin 3-*O*-β-glucoside was used in the HPLC analysis to rule out the possibility of a glucoside (**Figure 3a**). Similarly, anthocyanin **3** was identified as pelargonidin 3-*O*-β-galactoside by examination of its NMR spectral data. The MS peaks at *m*/*z* 433 and 271 were indicative of pelargonidin hexose and pelargonidin aglycon, respectively. Compound **3** corresponded to the peak observed at 19.8 min in the HPLC chromatogram (**Figure 2g**) and was distinct from the peak at t_R = 26.1 min for an authentic sample of pelargonidin 3-*O*-β-glucoside (**Figure 3b**).

HPLC separations of the anthocyanins in all *Cornus* spp. studied were obtained by isocratic conditions utilizing 4% aqueous H_3PO_4/CH_3CN as the mobile phase. The HPLC profile of fresh fruits of *C. mas* (**Figure 2a**) showed peaks at retention times (13.2, 16.2, and 19.8 min) corresponding to anthocyanins **1**, **2**, and **3**, respectively. The anthocyanins in *C. officinalis* (**Figure 2b**) were identical to those in *C. mas* but differed in their concentrations. Only a trace amount of anthocyanin **1** was detected in fresh fruits of *C. officinalis*. Therefore, it was quantified in the enriched anthocyanin powder prepared from the fruits. Fresh fruits of *C. controversa* contain anthocyanins **1**−**3** as well as a number of unidentified peaks, at 520 nm in its HPLC chromatogram, which were not quantified for purposes of this study (**Figure 2c**). The yields of pure anthocyanins **1**, **2**, and **3** in 10 g of fresh berries of *C. mas*, *C. officinalis*, and *C. controversa* were 2.8, 10.79, and 7.1 mg; 0.11, 0.77, and 2.3 mg; and 6.0, 10.0, and 0.21 mg, respectively. HPLC chromatogram revealed that Chinese dogwood, *C. kousa*, did not contain anthocyanins **1**−**3** (**Figure 2d**). However, *C. kousa* fruits have previously been reported to contain delphinidin 3-glucoside, cyanidin 3-glucoside, and pelargonidin 3-glucoside (*17*).

The cyclooxygenase enzyme inhibitory activities of *C. mas* anthocyanins were determined using COX-I and COX-II isozymes, which are used to discover potential anti-inflammatory compounds for phytoceutical and pharmaceutical applications. The assay is based on the ability of the enzymes to convert arachidonic acid to prostaglandins, which evoke the physiological response of inflammation. Anthocyanins **1**−**3** were assayed at 40 μM concentrations. Ibuprofen and naproxen showed 47.5 and 54.3% of COX-I and 39.8 and 41.3% of COX-II inhibitory activities, respectively, at 10 μM concentrations. Celebrex and Vioxx showed 46.2 and 23.8% and 66.3 and 88.1% COX- I and COX-II inhibition, respectively, at 1.67 ppm concentrations. Anthocyanins **1**, **2**, and **3** displayed 9.2, 7.6, and 5.3% COX-I and 11.7, 12.4, and 7.8% COX-II activities, respectively.

Anthocyanins **1**−**3** were tested at 40 μM concentrations for antioxidant activity by using an iron-catalyzed liposomal model and fluorescence spectroscopy to monitor the inhibition of lipid peroxidation as described before (*1*, *2*). At a test concentration of 40 μM, the *Cornus* spp. anthocyanins **1**, **2**, and **3** showed antioxidant activities of 70.2, 60.1, and 40.3%, respectively. The commercial antioxidants TBHQ (83.2%), BHT (79.7%), BHA (82.1%), and vitamin E (10.2%) were tested at 10 μM concentrations (**Figure 4**).

Although anthocyanins in the *Cornus* spp. did not show appreciable cyclooxygenase inhibitory activities, it was reported that the aglycon of cyanidin glycosides, cyanidin, acted as a strong COX-I and COX-II inhibitor (*1*, *2*). Similarly, aglycons of anthocyanins are shown to possess strong antioxidant activities (*1*, *2*). *Cornus* spp. also contain several other bioactive compounds and are currently being investigated in our laboratory. The antioxidant and anti-inflammatory activities of the

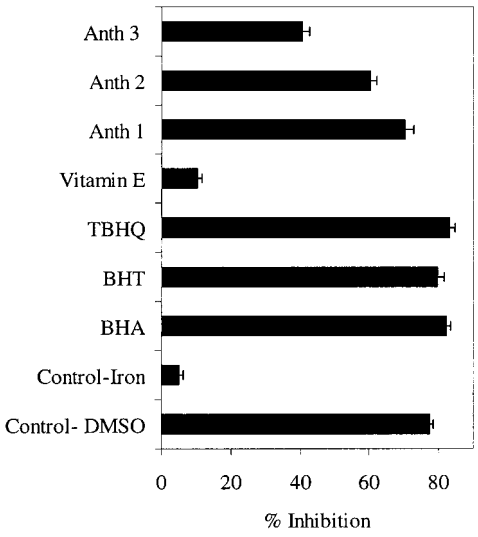


Figure 4. Antioxidant activities of anthocyanins **1**–**3** from *Cornus* spp. assayed in a liposomal model system. Samples were tested at 40 μM. Commercial antioxidants TBHQ, BHT, BHA, and vitamin E were assayed at 10 μM.

anthocyanins from these fruits suggest that their consumption would be beneficial to human health.

ACKNOWLEDGMENT

The COX-II enzyme was supplied by Dr. Dave Dewitt, Department of Biochemistry, Michigan State University.

LITERATURE CITED

(1) Wang, H.; Nair, M. G.; Strasburg, G. M.; Chang, Y.-C.; Booren, A. M.; Gray, I. J.; Dewitt, D. L. Antioxidant and anti-inflammatory activities of anthocyanins and their aglycone, cyanidin, from tart cherries. *J. Nat. Prod.* **1999**, *62*, 294−296.

(2) Seeram, N. P.; Momin, R. A.; Bourquin, L. D.; Nair, M. G. Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries. *Phytomedicine* **2001**, *8*, 362−369.

(3) Kalt, W.; Dufour, D. Health functionality of blueberries. *Hortic. Technol.* **1997**, *7*, 216−221.

(4) Seeram, N. P.; Bourquin, L. D.; Nair, M. G. Degradation products of cyanidin glycosides from tart cherries and their bioactivities. *J. Agric. Food Chem.* **2001**, *49*, 4924−4929.

(5) Chandra, A.; Rana, J.; Li Y. Separation, identification, quantification and method validation of anthocyanins in botanical supplement raw materials by HPLC and HPLC-MS. *J. Agric. Food Chem.* **2001**, *49*, 3515−3521.

(6) Bailey, L. H. In *Manual of Cultivated Plants*; Macmillan: New York, 1977; p 755.

(7) Slimestad, R.; Andersen, O. M. Cyanidin-3-(2-glucosylgalactoside) and other anthocyanins from fruits of *Cornus suecica*. *Phytochemistry* **1998**, *49*, 2163−2166.

(8) Millspaugh, C. F. In *American Medicinal Plants*; Dover Publications: New York, 1974; p 282.

(9) Polinicencu, C.; Popescu, H.; Nistor, C. Vegetal extracts for cosmetic use: 1. Extracts from fruits of *Cornus mas*. Preparation and characterization. *Clujul Med.* **1980**, *53*, 160−163.

(10) Du, C.-T.; Francis, F. J. New Anthocyanin from *Cornus mas*. *HortScience* **1973**, *8*, 29−30.

(11) Du, C.-T.; Francis, F. J. Anthocyanins from *Cornus mas*. *Phytochemistry* **1973**, *12*, 2487−2489.

(12) Kim, D.-K.; Kwak, J. H. A Furan derivative from *Cornus officinalis*. *Arch. Pharmacal Res.* **1998**, *21*, 787−789.

(13) Shang, S.; Liu, Y.; Xiao, X.; Sun, Z.; Zhang, J.; Tian, S.; Jiang, X. Antioxidant properties of extracts from the stone of *Cornus officinalis*. *Linchan Huaxue Yu Gonye* **1990**, *10*, 217−225.

(14) Okuda, T.; Hatano, T.; Ogawa, N.; Kira, R.; Matsuda, M. Cornusiin A, a dimeric ellagitannin forming four tautomers, and accompanying new tannins in *Cornus officinalis*. *Chem. Pharm Bull.* **1984**, *32*, 4662−4665.

(15) Lee, S.-H.; Tanaka, T.; Nonaka, G.; Nishioka, I. Tannins and related compounds. Part 86. Sedoheptulose digallate from *Cornus officinalis*. *Phytochemistry* **1989**, *28*, 3469−3472.

(16) Lee, D.; Kang, S.-J.; Lee, S.-H.; Ro, J.; Lee, K.; Kinghorn A. D. Phenolic compounds from the leaves of *Cornus controversa*. *Phytochemistry* **2000**, *53*, 405−407.

(17) Du, C.-T.; Wang, P. L.; Francis, F. J. Anthocyanins of Cornaceae, *Cornus kousa* and *Cornus florida*. *HortScience* **1974**, *9*, 243−244.

Received for review December 4, 2001. Revised manuscript received February 6, 2002. Accepted February 7, 2002. Funding for this project was partially provided by Agricultural Experiment Station and the Center for Plant Products and Technologies (CPPT) at Michigan State University. The NMR data were obtained on instrumentation that was purchased in part with funds from NIH Grant 1-S10-RR04750, NSF Grant CHE-88-00770, and NSF Grant CHE-92-13241.

JF0115903