JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Identification of Triterpene Hydroxycinnamates with *in Vitro* Antitumor Activity from Whole Cranberry Fruit (*Vaccinium macrocarpon*)

BRIAN T. MURPHY,[†] SHAWNA L. MACKINNON,[‡] XIAOJUN YAN,[†,§] GERALD B. HAMMOND,[†] ABRAHAM J. VAISBERG,[||] AND CATHERINE C. NETO*,[†]

Department of Chemistry and Biochemistry, University of Massachusetts Dartmouth, North Dartmouth, Massachusetts 02747, Institute for Marine Biosciences, National Research Council of Canada, Halifax, NS, Canada, and Department of Microbiology, Universidad Peruana Cayetano Heredia, Lima, Peru

Bioactivity-guided fractionation of cranberry fruit was used to determine the identity of triterpenoid esters from *Vaccinium macrocarpon*, which inhibit tumor cell growth and may play a role in cancer prevention. In our previous study, a fraction from whole fruit exhibited tumor cell growth inhibition in vitro. The major components of this fraction were isolated by chromatographic separation of ethyl acetate extracts, purified by semipreparative HPLC, and identified by NMR as *cis*- (**1**) and *trans*- (**2**) isomers of 3-*O*-*p*-hydroxycinnamoyl ursolic acid. These triterpenoid esters have not been previously reported in *Vaccinium* fruit. Bioassay of the purified triterpene cinnamates in tumor cell lines in vitro showed slightly greater activity of compound **1** in most cell lines, with GI_{50} values of approximately 20 μM in MCF-7 breast, ME180 cervical and PC3 prostate tumor cell lines. Quercetin was slightly less active than **1,** while cyanidin-3-galactoside exhibited much lower cytotoxicity, with GI_{50} greater than 250 μM in all cell lines. Phenylboronic acid (**3**) was also isolated from the fruit but showed insignificant antitumor activity.

KEYWORDS: antitumor; cranberry; triterpenoids; hydroxycinnamic acid; cancer; cytotoxic; ursolic acid esters

INTRODUCTION

Cranberry (*Vaccinium macrocarpon* Ait. Ericaceae), a native fruit in North America, has attracted public interest as a functional food, due to its potential health benefits. Recently, cranberry fruit has been found to prevent bacterial adhesion in urinary tract infections (UTI) of *E. coli* and stomach ulcers (*1, 2*), contain significant antioxidant content (*3*), protect against lipoprotein oxidation (*4*), and exhibit in vitro anticancer activity (*5*). Many of these biological effects have been linked to the presence of a wide variety of phenolic compounds in the fruit, including flavonoids and various small phenolic acids. Cranberry contained the highest content of total phenolics per serving and by weight among 20 fruits analyzed in a study published in 2001 (*6*) and was ranked sixth in overall antioxidant quality. Ongoing studies in our laboratory have used bioassay-guided fractionation to determine the identity of compounds in cranberry with antioxidant and antitumor activity. We recently reported on the radical-scavenging activities of the various flavonol glycosides and anthocyanins in whole cranberry fruit and their considerable ability to protect against lipoprotein oxidation in vitro (*7*).

The anticancer properties of cranberry and the nature of the compounds that provide protection against tumor promotion and proliferation has not been fully investigated. Bomser and coworkers (*5*) reported in 1996 that extracts from berries of *Vaccinium* species were able to inhibit the induction of ornithine decarboxylase (ODC), an enzyme involved in tumor proliferation, and induce quinone reductase, an enzyme that can inactivate certain carcinogens. Proanthocyanidin-rich cranberry extracts containing other flavonoids were reported to inhibit ODC induction in epithelial cells in 2002 (*8*); recent studies also report on anti-angiogenic properties of the berries (*9*). Reports of two cranberry extracts that inhibited proliferation of MCF-7 and MDA-MB-435 breast cancer cells (*10*) did not identify the active constituents. Determination of the structure and activity of chemopreventive compounds in cranberry and medicinal plants (*11*) has been a primary goal of our ongoing research. Using this approach, we screened cranberry extracts for in vitro antitumor activity in nine cell lines and reported our initial findings in 2002 (*7*). A methanolic extract of whole berries prepared by solvent partitioning methods was observed

* Corresponding author. Tel: 508-910-6928. Fax: 508-999-9167. E-mail: cneto@umassd.edu.
[†] University of Massachusetts-Dartmouth.
[‡] Institute for Marine Biosciences, NRC Canada.
[||] Universidad Peruana Cayetano Heredia.
[§] Present address: Post Box 71, Ningbo University, Ningbo, P. R. China, 315211.

10.1021/jf034114g CCC: $25.00
Published on Web 05/01/2003

to have selective activity against some tumor cell lines. Further investigation of the composition of this extract has led to the isolation of two compounds, previously unreported in cranberry, that exhibit selective tumor cell growth inhibition in breast, prostate, lung, cervical, and leukemia cell lines. The identification of the *cis*- (**1**) and *trans*- (**2**) isomers of 3-*O*-*p*-hydroxycinnamoyl ursolic acid, the isolation of phenylboronic acid (**3**) in cranberry fruit, and results of our tumor cell growth inhibition bioassays, are discussed here.

MATERIALS AND METHODS

Plant Material. Cranberry fruit (*Vaccinium macrocarpon* cv. Stevens) were donated by Decas Cranberry. The berries were harvested in October, 2000 from cranberry bogs in Wareham, Massachusetts and kept frozen at −20 °C until use.

Reagents. All reagents were of analytical grade. CD_3OD, $CDCl_3$, quercetin dihydrate, and benzeneboronic acid (phenylboronic acid) were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Cyanidin-3-galactoside was isolated from whole cranberries, as described previously (*7*). High-performance liquid chromatography (HPLC) grade methanol and other solvents used in isolation were purchased from Fisher Scientific.

Instrumentation. HPLC analysis and purification were performed on a Waters Millenium HPLC system composed of two Waters 515 pumps with a Waters 996 photodiode array detector. Except where otherwise specified, the HPLC chromatograms were monitored at 250−600 nm. EI mass spectra for compounds **1** (MH^+ *m*/*z* 603.4027 Δ 3.7 ppm) and **2** (MH^+ *m*/*z* 603.4033 Δ 2.7 ppm) were obtained on a VG AutoSpec instrument at the Mass Spectrometry Facility at the University of Nebraska. HRMS analyses were obtained using fast atom bombardment (FAB) on a Kratos MS-50-TC-HM-TA mass spectrometer at the Department of Chemistry at the University of Montreal. Mass spectra for compound **3** were obtained using a Finnigan TSQ spectrometer. ^1H, ^{13}C, DEPT and 2-D NMR spectra for compounds **1** and **2** were measured on a Bruker DRX-500 spectrometer (Bruker, Canada Ltd.) at the Institute for Marine Biosciences. ^1H and ^{13}C NMR data used to identify **3** were obtained on a Bruker AC-300 MHz instrument at UMass-Dartmouth. Chemical shifts were reported relative to tetramethylsilane as an internal standard. $CDCl_3$ was the solvent for **1** and **2**; CD_3OD was the solvent for **3**.

Tumor Cell Growth Inhibition Bioassays. Cytotoxicity of compounds isolated as described below was evaluated in various tumor cell lines using published procedures (*12*). Cell lines tested included BALB/c3T3, H460, ME180, DU145, MCF-7, M-14, HT-29, PC3, and K562. Growth inhibition was evaluated at various concentrations of extract by spectrophotometrically quantifying the number of live cells remaining at the end of the incubation period. GI_{50} values were calculated as the concentration of extract required to inhibit tumor cell growth by 50%, relative to control.

Isolation of Triterpene Hydroxycinnamates from Whole Cranberry. An ethyl acetate extract of whole cranberries (1 kg) was prepared by macerating the berries in a blender containing 2% acetic acid in ethyl acetate (3 L) at high speed in four 15 min intervals. The supernatant was decanted, and dried by rotary evaporation and lyophilization to yield a light green solid (9.88 g). The ethyl acetate extract was loaded onto a silica gel column (40 × 5 cm) and eluted with methanol/chloroform/acetic acid (10:88:2 v/v). Several yellow and green bands eluted. The first yellow band (Fraction A, 793 mg) and a second light green band (Fraction B, 158 mg) were collected and evaporated to dryness.

HPLC Analysis of Cranberry Isolates and Fractions. HPLC analysis was conducted on 20-μL injections of sample on a 150 × 3.9 mm i.d. Nova-Pak C18 RP (Waters) column, using solvent A (2% aqueous acetic acid) and solvent B (methanol with 2% acetic acid). A program of isocratic elution with 100% A from 0 to 15 min, a linear gradient to 100% B from 15 to 45 min, followed by an isocratic elution with 100% B from 45 to 60 min, at a flow rate of 0.8 mL/min, was used. Photodiode array (PDA) detection from 250 to 600 nm was used to monitor the effluent.

Table 1. Cytotoxicity of Ursolic Acid *p*-Hydroxycinnamate Esters[a] from Cranberry Fruit[b] in Tumor Cell Lines

cell line	GI_{50} (μM)[c] (**1**)	(**2**)
BALB/3T3 (murine fibroblasts)	21.6	75
H460 (human large cell lung carcinoma)	27.1	84
ME180 (human epidermoid cervical carcinoma)	23.3	42
DU145 (human prostate metastatic carcinoma)	28.4	25–100
MCF-7 (human breast adenocarcinoma)	18.8	25–100
M-14 (human melanoma)	46.4	25–100
HT-29 (human colon adenocarcinoma)	32.9	40
PC3 (human prostate adenocarcinoma)	24.3	25–100
K562 (human chronic myelogenous leukemia)	28.9	25–100

[a] Ursolic acid was previously isolated by our group from Polylepis racemosa (*11*) and gave GI_{50} values ranging from 42 to 117 μg/mL in these cell lines (93–230 μM), using the same bioassay methods. [b] Cytotoxicity of cranberry extracts were previously reported by us (*7*) with GI_{50} values on the order of 500 μg/mL for crude and 16–250 μg/mL for the methanolic fraction containing the triterpene esters. [c] GI_{50} = concentration required to inhibit tumor cell growth by 50% relative to control.

Purification of *cis*- and *trans*-3-*O*-*p*-Hydroxycinnamoyl Ursolic Acid. Compounds **1** and **2** were purified from Fraction A by first obtaining the methanol soluble solids (70 mg) by extracting the fraction with 200 mL of methanol. The resultant methanol soluble solid was subjected to semipreparative HPLC on a 300 × 7.8 mm i.d. Nova-Pak HR C18 (Waters) column, which was eluted isocratically with 90% solvent C (85% methanol in 2% acetic acid) and 10% solvent B at a flow rate of 4.0 mL/min. The two triterpene hydroxycinnamates **1** and **2** were obtained in a yield of 20 mg each at retention times of 28.0 min, (λ_{max} = 308.9 nm) and 34.5 min, (λ_{max} = 312.4 nm), respectively, and identified by NMR and MS.

Identification of Triterpene Hydroxycinnamates and Phenylboronic Acid in Original Cytotoxic Methanolic Extract. As previously reported (*7*), a methanolic extract of cranberry fruit exhibited selective cytotoxicity in various tumor cell lines (see footnote to **Table 1**). The major components were separated by semipreparative HPLC for identification. A portion (59 mg) of the original methanolic extract was separated using a 300 × 7.8 mm i.d. Nova-Pak HR C18 column (Waters), which was eluted isocratically, at a flow rate of 2.0 mL/min, with 90% solvent D (90% methanol in 2% acetic acid) and 10% solvent B. Two major peaks eluting at 2.2 min (34 mg) and 7.4 min (6.1 mg) were collected. The material eluting at 2.2 min was further purified, using the same semipreparative column as before, but this time eluting it isocratically with 90% solvent E (40% methanol in 2% acetic acid) and 10% solvent B, to yield compound **3** (4 mg, 10.6 min, λ_{max} = 273.4 nm), which was identified by NMR and MS. HPLC analysis, using the program described above, was performed on the material eluting at 7.4 min and on the methanol-soluble solid from Fraction A containing the triterpene hydroxycinnamates. The major peaks in both extracts were identified as compounds **1** (46.1 min, λ_{max} = 308.9 nm) and **2** (47.1 min, λ_{max} = 312.4 nm). Thus, the triterpene hydroxycinnamates isolated from Fraction A were also present in the previously reported cytotoxic methanolic extract. The other major component of that methanolic extract, compound **3**, was also present in Fraction B from the ethyl acetate extract.

RESULTS AND DISCUSSION

Tumor Cell Growth Inhibition Bioassays. Compounds **1** and **2** were evaluated for ability to inhibit tumor cell growth in nine cell lines **(Table 1)**. The *cis*-hydroxycinnamate isomer (**1**) was more effective than the trans isomer (**2**) in inhibiting tumor growth in all cell lines tested, with the greatest effectiveness observed in MCF-7 breast tumor cells, where 50% growth inhibition occurred at concentrations of less than 20 μM. Cervical (ME180) and prostate (PC3) tumor growth was

inhibited at similar concentrations. In our assay, quercetin, a known inhibitor of tumor growth (*13*, *14*) and a major constituent in cranberry, was less active than **1** in most of the tumor cell lines tested, with GI_{50} ranging from 37.5 μM in MCF-7 (breast) to 62 μM in HT-29 (colon) cells. Cyanidin-3-galactoside, one of the most powerful antioxidants in cranberry fruit (*7*), was not a significant tumor growth inhibitor; GI_{50} values in all cell lines were greater than 250 μM. This suggests that tumor growth inhibition is not necessarily correlated with antioxidant activity. However, the hydroxycinnamates of ursolic acid inhibited tumor growth at lower concentrations than did ursolic acid alone in our assays. Previous studies by our group (*11*) using the same assay protocol and cell lines found ursolic acid isolated from *Polylepis racemosa* cytotoxic at higher concentrations than those reported here for the hydroxycinnamate esters (see footnote to **Table 1**). Although the presence of hydroxycinnamate esters of triterpenoids has not been previously reported in cranberry or other *Vaccinium* species, ursolic acid and 19α-hydroxyursolic (pomolic) acid have been isolated from *Vaccinium corymbosum* L. (highbush blueberry) and found to be cytotoxic against HL-60 leukemia cells in vitro (*15*). The cytotoxicity of ursolic acid has been reported in P-388 and L-1210 lymphocytic leukemia cells and A-549 human lung carcinoma (*16*), as was its inhibitory effect on B16 mouse melanoma proliferation (*17*) and ability to inhibit growth of implanted sarcoma-180 tumors in mice (*18*).

Ursolic acid and other common plant triterpenoids are well-known antiinflammatories (*19*), and it is therefore possible that their antitumor activity may be linked to antiinflammatory properties. We are presently investigating the antiinflammatory activity and mechanism of anticancer action of **1** and **2**.

Identification of (1) and (2) as *cis*- and *trans*- 3-*O*-*p*-Hydroxycinnamoyl Ursolic Acid. High-resolution FABMS experiments determined the molecular formula of both **1** and **2** to be $C_{39}H_{54}O_5$. Inspection of the downfield region of the proton NMR spectra indicated the presence of compounds containing a 4-hydroxycinnamic acid moiety. Compound **1** was shown to have a characteristic cis configuration, as it displayed chemical shifts at δ 7.65 (d, 8.48 Hz, H-2″, 6″), 6.84 (d, 12.7 Hz, H-3′), 6.81 (d, 8.49 Hz, H-3″, 5″), and 5.85 ppm (d, 12.7 Hz, H-2′). Further evidence suggesting the presence of this moiety was the agreement of ^{1}H shifts and coupling constants with other reported triterpene hydroxycinnamates (*20*). The coupling constants were crucial to distinguishing between the cis and trans isomers. A survey of the literature showed compound **1** to be identical to that of a triterpenoid that was earlier reported from the plant *Mimusops elengi* (*21*). The λ_{max} value of **1** (308.9 nm) closely matched the previously reported value for *cis*-3-*O*-*p*-hydroxycinnamoyl ursolic acid **(Figure 1)**. Identification of the triterpenoid moiety came by evaluating one- and two-dimensional NMR spectra as well as comparison of chemical shifts with published ^{13}C data on ursolates (*22*, *23*). Our assignments of ^{13}C signals for the triterpenoid portion of **1**, shown in **Table 2**, agree closely with those published on ursolic acid by Sang et al. (*22*). The shift at 29.75 ppm is assigned to the C23 methyl group on ring A. Its spatial proximity to the ester oxygen at C3 (80.88 ppm) results in a downfield shift, also confirming the position of the ester at C3. The position of the double bond, characteristic of ursolic acid, was confirmed by the correlation spectroscopy and heteronuclear multiple-bond correlation data, which showed correlation of the proton on C12 (125.5 ppm) with those on C11 (23.25 ppm). The characteristic C28 carboxylic acid group of ursolic acid leads to a shift of 47.88 ppm for the quaternary C17. To further confirm the identification of **1,** the EI mass spectrum gave rise to signals at *m*/*z* 483, 438, 248, 203, 189, 147, and 133, corresponding with earlier reported data (*21*).

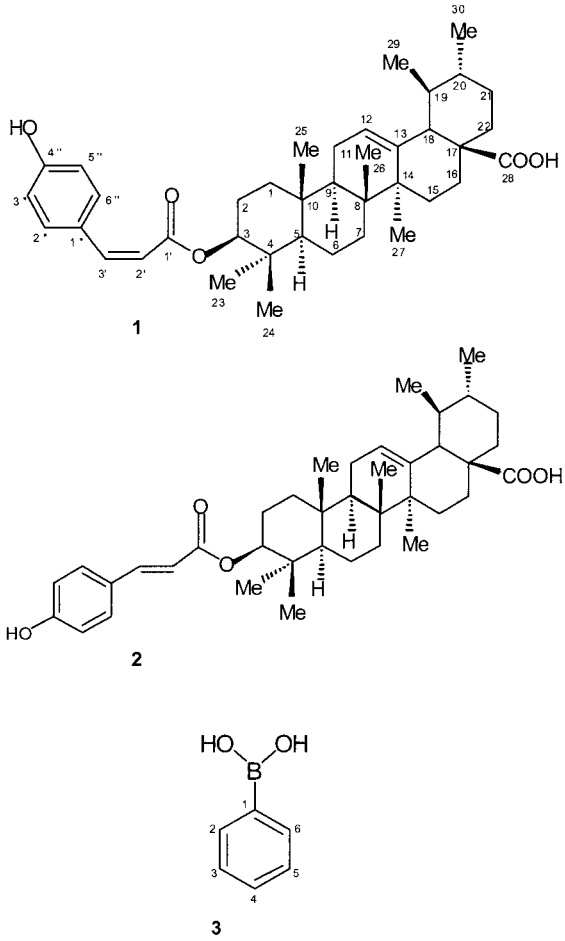

Figure 1. Structures of isolates from selected extracts of cranberry fruit.

Compound **2** had a λ_{max} of 312.4 nm, was originally identified in the wood from *Tripetaleia paniculata* (*24*), and was later reported from *Mimusops elengi* (*21*) as *trans*-3-*O*-*p*-hydroxycinnamoyl ursolic acid. The trans configuration of the 4-hydroxycinnamic acid moiety was confirmed using published ^{1}H data on *trans*-hydroxycinnamates (*20*). Proton signals appeared at δ 7.61 (d, 15.9 Hz, H-3′), 7.44 (d, 8.05 Hz, H-2″, 6″), 6.84 (d, 8.55 Hz, H-3″, 5″), and 6.31 ppm (d, 15.9 Hz, H-2′), to confirm the presence of this moiety. The ^{13}C shifts of **2** were nearly identical to those of **1** (**Table 2**). The signal for equivalent carbons at C-2″ and C-6″ in the *cis*-*p*-hydroxycinnamate moiety (132.32 ppm) appeared slightly downfield from the signals for the corresponding carbons of the trans isomer (129.87 ppm). This is likely due to its spatial proximity to C1′ (166.41 ppm) in the cis isomer. The assignments for the *cis*- and *trans*-*p*-hydroxycinnamic acid moiety correspond with previously published data on similar triterpene esters (*20*). The EI mass spectrum gave rise to signals at *m*/*z* 556, 483, 438, 248, 203, 189, 147, and 133.

Identification of Phenylboronic Acid (3). The identity of phenylboronic acid **3 (Figure 1)**, was confirmed by comparison of ^{1}H and ^{13}C NMR spectra in CD_3OD with a purchased standard, as well as confirmation of distinct fragment peaks in the mass spectrum. The aromatic region of the ^{1}H spectrum provided the information necessary for elucidation of **3**; chemical shifts were observed at δ 8.01 (d, J = 7.2 Hz, H-2,

Table 2. ^{13}C NMR Data for Compounds **1** and **2** in $CDCl_3$

	δ_C (ppm)	
assignment	(**1**)	(**2**)
1	37.71	37.91
2	27.92	27.93
3	80.88	80.76
4	38.24	38.23
5	55.30	55.27
6	18.14	18.15
7	32.78	32.81
8	39.44	39.45
9	47.38	47.40
10	36.88	36.88
11	23.25	23.26
12	125.73	125.75
13	137.89	137.88
14	41.87	41.91
15	28.08	28.11
16	24.05	24.06
17	47.88	47.90
18	52.52	52.54
19	38.98	38.98
20	38.78	38.78
21	30.55	30.55
22	36.66	36.66
23	29.75	29.69
24	17.00	20.50[a]
25	15.52	15.54[a]
26	16.71	16.80[a]
27	23.55	23.65
28	182.68	182.61
29	17.02	17.00
30	21.17	21.17
1′	166.41	167.19
2′	117.84	116.33
3′	143.08	143.88
1′	127.59	127.40
2″, 6″	132.32	129.87
3″, 5″	114.90	115.78
4″	156.45	157.38

[a] Some discrepancies exist in the literature (*21*, *22*) for these carbon shift assignments.

6), 7.56 (t, H-4), and 7.45 (t, H-3, 5). A characteristic "roofing effect" was observed among peaks, due to coupling between adjacent protons H-2 with H-3 and H-3 with H-4. The ^{13}C spectrum showed three characteristic peaks at δ 133.8 (C-2, 6), 130.9 (C-1), and 129.5 (C-3, 4, 5). The chemical shifts and peak intensity patterns displayed by **3** agreed with that of the phenylboronic acid standard and with those found in the Spectral Data Base System (SDBS) (*25*). The mass spectrum of **3** ($C_6H_7O_2B$) gave a molecular ion [M] at *m*/*z* 122, loss of a hydroxyl group at *m*/*z* 105, and cleavage of the H_2O_2B moiety at *m*/*z* 77 as the predominant peaks. We have found no previous reports of phenylboronic acid isolated from plant sources. However, boron is an important micronutrient in cranberry production, as fertilizers containing calcium borate are often applied to cultivated bogs to increase fruit set (*26*). Boron has been reported to play a role in calcium metabolism in humans (*27*) and is also believed to play a role in phenolic production in plants (*28*, *29*). Further studies of the effect of boron on the composition of phenolics in cranberry are underway. Phenylboronic acid did not inhibit tumor cell growth at concentrations below 250 μM in our assays.

The discovery of triterpene cinnamates in whole cranberries adds to our overall knowledge of the chemistry of *Vaccinium* fruit. While both isomers of 3-*O*-*p*-hydroxycinnamoyl ursolic acid were present in whole berries, HPLC analysis did not detect significant amounts in aqueous extracts, suggesting that a quantitative comparison study of the triterpene content in cranberry products including juice would be worthwhile. Further antitumor studies investigating juice versus whole fruit would also be useful toward determination of whether any additional benefits may be derived from consumption of whole fruit. It is possible that the triterpenoids and their phenolic esters found in the fruit of cranberry and other *Vaccinium* species may contribute to the anticancer properties of these fruits; however, the bioavailability of these compounds must be further investigated in vivo. Our findings here, together with previous studies by our group and others cited herein, indicate that cranberry fruit has the potential to offer a broad range of health benefits due to the diverse nature of its chemistry. Future studies will be directed toward discerning the mechanisms by which cranberry components offer protection against cancers and other diseases related to oxidative and inflammatory processes.

LITERATURE CITED

(1) Foo, L. Y.; Howell, A. B.; Vorsa, N. The structure of cranberry proanthocyanidins, which inhibit adherence of uropathogenic P-fimbricated *Escherichia coli* in vitro. *Phytochemistry* **2000**, *54*, 173−181.

(2) Burger, O.; Ofek, I.; Tabak, M.; Weiss, E. I.; Sharon, N.; Neeman, I. A high molecular mass constituent of cranberry juice inhibits *Helicobacter pylori* adhesion to human gastric mucus. *FEMS Immunol. Med. Microbiol.* **2000**, *29*, 295−301.

(3) Wang, S. Y.; Jiao, H. Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. *J. Agric. Food Chem.* **2000**, *48*, 5677−84.

(4) Wilson, T.; Porcari, J.; Harbin, D. Cranberry extract inhibits low-density lipoprotein oxidation. *Pharmacol. Lett.* **1998**, *62*, 381−386.

(5) Bomser, J.; Madhavi, D. L.; Singletary, K.; Smith, M. A. *In vitro* anticancer activity of fruit extracts from *Vaccinium* species. *Planta Med.* **1996**, *62*, 212−6.

(6) Vinson, J. A.; Su, X.; Zubik, L.; Bose, P. Phenol antioxidant quantity and quality in foods: Fruits. *J. Agric. Food Chem.* **2001**, *49*, 5315−5321.

(7) Yan, X.; Murphy, B. T.; Hammond, G. B.; Vinson, J. A.; Neto, C. C. Antioxidant activities and antitumor screening of extracts from cranberry fruit (*Vaccinium macrocarpon*). *J. Agric. Food Chem.* **2002**, *50*, 5844−5849.

(8) Kandil, F. E.; Smith, M. A. L.; Rogers, R. B.; Pepin, M.-F.; Song, L. L.; Pezzuto, J. M.; Seigler, D. S. Composition of a chemopreventive proanthocyanidin-rich fraction from cranberry fruits responsible for the inhibition of 12-*O*-tetradecanoyl phorbol-13-acetate (TPA)-induced ornithine decarboxylase (ODC) activity. *J. Agric. Food Chem.* **2002**, *50*, 1063−1069.

(9) Roy, S.; Khanna, S.; Alessio, H. M.; Vider, J.; Bagchi, D.; Bagchi, M.; Sen, C. K. Anti-angiogenic property of edible berries. *Free Rad. Res.* **2002**, *36*, 1023−1031.

(10) Guthrie, N. Effect of cranberry juice and products on human breast cancer cell growth. *Proc. Exp. Biol. Conf.*, April 14−18, 2000; San Diego, CA, 2000; Abstract #531.13.

(11) Neto, C. C.; Vaisberg, A. J.; Zhou, B.-N.; Kingston, D. G. I.; Hammond, G. B. Cytotoxic triterpene acids from the Peruvian medicinal plant *Polylepis racemosa*. *Planta Med.* **2000**, *66*, 483−484.

(12) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J.; Bokesch, H.; Kenney, S.; Boyd, M. New colorimetric cytotoxicity assay for anticancer drug screening. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.

(13) Kawaii, S.; Tomono, Y.; Katase, E.; Ogawa, K.; Yano, M. Antiproliferative activity of flavonoids on several cancer cell lines. *Biosci., Biotechnol., Biochem.* **1999**, *63*, 896−899.

(14) Manthey, J. A.; Guthrie, N. Antiproliferative activities of citrus flavonoids against six human cancer cell lines. *J. Agric. Food Chem.* **2002**, *50*, 5837−5843.

(15) Wang, M.; Li, J.; Shao, Y.; Huang, T.-C.; Huang, M.-T.; Chin, C.-K.; Rosen, R. T.; Ho, C.-T. Antioxidative and cytotoxic components of highbush blueberry (*Vaccinium corymbosum* L.) In *Phytochemicals and Phytopharmaceuticals*; AOCS Press: Champaign, IL, 2000; 271−277.
(16) Lee, K.-H.; Lin, Y.-M.; Wu, T.-S.; Zhang, D.-C.; Yamagishi, T.; Hayashi, T.; Hall, I. H.; Chang, J.-J.; Wu, R.-Y.; Yang, T.-H. The cytotoxic principles of *Prunella vulgaris*, *Psychotria serpens*, and *Hyptis capitata*: Ursolic acid and related derivatives. *Planta Med.* **1988**, *54*, 308−310.
(17) Es-saady, D.; Simon, A.; Ollier, M.; Maurizis, J. C.; Chulia, A. J.; Delage, C. Inhibitory effect of ursolic acid on B16 proliferation through cell cycle arrest. *Cancer Lett.* **1996**, *106*, 193−197.
(18) Hsu, H.-Y.; Yang, J.-J.; Lin, C.-C. Effects of oleanolic acid and ursolic acid on inhibiting tumor growth and enhancing the recovery of hematopoietic system postirradiation in mice. *Cancer Lett.* **1997**, *111*, 7−13.
(19) Safayhi. H.; Sailer, E.-R. Antiinflammatory actions of pentacyclic triterpenes. *Planta Med.* **1997**, *63*, 487−493.
(20) McLean, S.; Reynolds, W. F.; Yang, J.-P.; Jacobs, H.; Jean-Pierre, L. L. Total assignment of the ^{1}H and ^{13}C shifts for a mixture of cis and trans-p-hydroxycinnamoyl esters of taraxerol with the aid of high-resolution, ^{13}C-detected, ^{13}C-^{1}H shift correlation spectra. *Magn. Reson. Chem.* **1994**, *32*, 422−428.
(21) Jahan, N.; Malik, A.; Afza, N.; Choudhary, M. I.; Shahzad-ul-Hassan, S. Triterpenes from *Mimusops elengi*. *Z. Naturforsch. B: Chem. Sci.* **2000**, *556*, 1206−1210.
(22) Sang, S.; Lapsley, K.; Rosen, R. T.; Ho, C.-T. New prenylated benzoic acid and other constituents from almond hulls (*Prunus amygdalus* Batsch). *J. Agric. Food Chem.* **2002**, *50*, 607−609.
(23) Seo, S.; Tomita, Y.; Tori, K. Carbon-13 NMR spectra of urs-12-enes and application to structural assignments of components of *Isodon japonicus* hara tissue cultures. *Tetrahedron Lett.* **1975**, *1*, 7−10.
(24) Yasue, M.; Sakakibara, J.; Ina, H. Studies on the constituents of *Tripetaleia paniculata* Sieb. et Zucc. III. On the constituents of the wood. *Yakugaku Zasshi* **1973**, *93*, 687−697.
(25) SDBSWeb. http://www.aist.go.jp/RIODB/SDBS/(access date: 7/2001).
(26) Demoranville, C. J.; Deubert, K. H. Effect of commercial calcium−boron and manganese−zinc formulations on fruit set of cranberries. *J. Hortic. Sci.* **1987**, *62*, 163−169.
(27) Nielsen, F. H. The emergence of boron as nutritionally important throughout the life cycle. *Nutrition* **2000**, *16*, 512−514.
(28) Lewis, D. H. Are there inter-relations between the metabolic role of boron, synthesis of phenolic phytoalexins and the germination of pollen? *New Phytol.* **1980**, *84*, 261−270.
(29) Blevins, D. G.; Lukaszewski, K. M. Boron in plant structure and function. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1988**, *49*, 481−500.

Received for review February 4, 2003. Revised manuscript received March 31, 2003. Accepted April 1, 2003.

JF034114G