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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

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Phenolics from the American cranberry (*Vaccinium macrocarpon*) were fractionated into a series of proanthocyanidins and other flavonoid compounds by vacuum chromatography on a hydrophilic, porous polyvinylic gel permeation polymer. Antioxidant activity was not restricted to a particular class of components in the extract but was found in a wide range of the fractions. Significant chemopreventive activity, as indicated by an ornithine decarboxylase assay, was localized in one particular proanthocyanidin-rich fraction from the initial fractionation procedure. Further fractionation of the active anticarcinogenic fraction revealed the following components: seven flavonoids, mainly quercetin, myricetin, the corresponding 3-*O*-glycosides, (−)-epicatechin, (+)-catechin, and dimers of both gallocatechin and epigallocatechin types, and a series of oligomeric proanthocyanidins.

KEYWORDS: Anticarcinogenic activity; antioxidants; catechin; condensed tannins; cranberry; epicatechin; FRAP assay; ODC assay; proanthocyanidins; *Vaccinium macrocarpon* Ait.

INTRODUCTION

The American cranberry, *Vaccinium macrocarpon* Ait. (Ericaceae), with an attractive bright red appearance and distinctive flavor, is recognized as a concentrated source of dietary flavonoids, including anthocyanins (*1*), flavonol glycosides (*2*), and proanthocyanidins (condensed tannins) (*3*, *4*) as well as various phenolic acids (*5*, *6*).

The well-characterized anthocyanins of the American cranberry are mainly peonidin and cyanidin 3-galactosides and 3-arabinosides; this profile is distinct from the closely related European cranberry, *Vaccinium oxycoccus* L., which features peonidin 3-*O*-glucoside and cyanidin 3-*O*-glucoside as the main pigments (*6*). Flavonol glycosides previously reported from *V. macrocarpon* include quercetin, its 3-*O*-galactoside (hyperin), 3-*O*-arabinoside, and 3-*O*-rhamnoside (quercitrin); and myricetin 3-*O*-arabinoside and 3-*O*-digalactoside. Anthocyanidin glycosides previously reported are 3-*O*-galactoside and 3-*O*-arabinoside and peonidin 3-*O*-galactoside and 3-*O*-arabinoside (*2*, *7*). Minor quantities of carotenes were also identified (*8*). European cranberry and bilberry (*Vaccinium myrtillus* L.) have a relatively large percentage of myricetin derivatives relative to quercetin derivatives (*9*).

In comparison to simpler polyphenols, proanthocyanidins have not been as extensively studied in the past, in part because of their complex, multiunit nature. However, the recent development of improved analytical techniques (*10*−*12*) coupled with intense interest in the proanthocyanidins of the American cranberry as inhibitors of urinary tract infection has prompted the recent elucidation of some of these structures, including A-type trimers (*13*, *14*). *Vaccinium vitis-idaea* (lingonberry) similarly contains a variety of oligomeric proanthocyanidins with single links or with a doubly linked A-type unit; condensed tannins with A-1 units were characterized from this source (*15*). In the European cranberry, the ratio of procyanidin (with two B-ring hydroxyl groups, such as found in catechin or epicatechin) to prodelphinidin (with three B-ring hydroxyl groups, such as found in gallocatechin or epigallocatechin) is 78:22. In contrast, highbush blueberry (*Vaccinium corymbosum* L.) fruits do not contain prodelphinidin-type proanthocyanidins (*3*).

Flavonoid compounds, important in plant biochemistry and physiology as antioxidants, enzyme inhibitors or inducers, and precursors of toxic substances (*16*, *17*), are prominent constituents of a number of functional foods and have been cited frequently as bioactive agents for health maintenance. For example, quercetin markedly suppressed the effect of 12-*O*-

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tetradecanoyl phorbol-13-acetate (TPA) on skin tumor formation in mice initiated with dimethylbenzathracene (*18*), and potently inhibited epidermal lipoxygenase activity. Recent evidence indicates that ingested condensed tannins (proanthocyanidins) in many cases exert significant biological influence in metabolism, including antioxidant, anti-inflammatory, cancer chemopreventive, and other protective effects (*19*−*22*).

Dietary mixtures of plant-derived proanthocyanidins and other flavonoids have been cited as most efficacious in terms of pharmacological value (*23*). Although differences in the level of pharmacological activity have been ascribed to individual polyphenols and to different classes of tannins, much of this activity was thought to result from generalized interactions of tannins with important molecules in test systems. Proanthocyanidins form complexes, both with metal ions and with macromolecules such as proteins and polysaccharides. These molecules also possess antioxidative and radical scavenging properties. More recently, however, a number of papers have appeared confirming that the interactions are more specific, in some cases involving binding to a specific receptor site (*24*).

The high antioxidant capacity of cranberries has been largely attributed to flavonoid content (*25*), which includes the proanthocyanidins previously noted as the natural components from cranberry juice that promote urinary tract health (*14*, *26*, *27*). Although a proanthocyanidin-containing fraction separated from cranberry fruits was earlier revealed to inhibit chemically induced carcinogenesis (*28*), only a preliminary identification of components was made.

In this study, we have chromatographically separated and further characterized the bioactive proanthocyanidin-rich fraction from cranberry, which has demonstrated chemopreventive efficacy as an inhibitor of TPA-induced ornithine decarboxylase (ODC) activity. The ODC enzyme is a key component that regulates cellular concentration of polyamines, and this assay has been shown to correlate with the ability of a substance to inhibit tumor promotion (*18*, *29*). The anticarcinogenic capacity of this isolated fraction was reconfirmed using an in vitro assay, and the antioxidant capacity and chemical composition of the whole extract and all fractions were assessed.

MATERIALS AND METHODS

General. Silica gel type G, 10−40 μm, $CaSO_4$ binder (Sigma S-6503; St. Louis, MO), cellulose powder (ICN Biochemicals, Cleveland, OH, no. 191499), and Toyopearl polymer HW-40C+HW-40F, 1:1 by weight (Tosohaas, Bioseparation Specialists, Montgomeryville, PA) were used for vacuum chromatography. Thin-layer chromatography (TLC) was carried out on precoated plates with silica gel (Merck 1.05554, DC-Alufolien, silica gel 60 with F_{254} fluorescent indicator, 0.2 mm, Sigma) and cellulose (Merck, 5577, DC-Plastikfolien cellulose, without fluorescent indicator, 0.1 mm). Unless otherwise indicated, all TLCs on silica gel were run in ethyl acetate/methanol/water (79:11:10). Spots were detected under UV light or visualized as below. Sephadex LH-20 (25−100 μm) was manufactured by Pharmacia (Sigma). All solvents were from Fisher Scientific (Pittsburgh, PA) and were of reagent grade or better.

The HPLC system consists of a Waters model 680 automated gradient controller, a Waters model 510 HPLC pump, a Waters Intelligent Sample Processor (WISP) model 712 autosampler, and a Waters series R-401 differential refractometer detector. A Hewlett-Packard 3390A reporting integrator was used to record the detector signal. An interaction model Ion-300 organic acid column (0.95 cm), measuring 300 × 7.8 mm i.d., was employed for the separation of compounds. The mobile phase was 0.001 N H_2SO_4 at room temperature. Retention times and peak areas were recorded for each component (flow rate = 1.2 mL/min and injection volume = 20 μL).

Mass spectra were determined by fast atom bombardment (FAB) on a Micromass ZAB-SE spectrometer and matrix-assisted laser desorption/ionization (MALDI) on a Micromass TofSpec spectrometer in the Mass Spectrometry Laboratory of the School of Chemical Sciences, University of Illinois, Urbana, IL.

Extraction and Fractionation of Plant Material. Freshly harvested fruits of *Vaccinium macrocarpon* Ait. var. Howes were obtained from Decas Cranberry Sales, Inc. (Wareham, MA). The Howes fruits were dry-harvested from Stuart Bog 3 in Carver, MA. Fruits were ground at room temperature in 70% aqueous acetone on arrival or frozen at −80 °C and extracted within 4 months of harvest. After filtration through cheesecloth, removal of acetone under vacuum, and removal of water by lyophilization, a strongly hygroscopic red-purple powder was obtained (2.3 kg of fruit yielded 202 g of lyophilized powder). A portion of the freeze-dried material (40 g) was dissolved in water, placed on top of the support, and fractionated by vacuum chromatography on Toyopearl (HW-40C+HW-40F, 1:1 by weight, 300 g, column diameter = 70 mm) using water as an eluant for fractions CR1−6, 50% methanol for fractions CR7−10, and 100% acetone for fractions CR11−13 (all fractions 1 L) (**Figure 1**). At this point, all colored materials were removed from the column. Analysis of the material eluted after fraction CR13 by TLC ensured that all compounds with TLC behaviors similar to that of fraction CR13 (R_f values and reactions with spray reagents such as vanillin−HCl, dichromate, and aluminum chloride) had been removed from the column. Fractions CR1−13 were concentrated under vacuum; water was removed by lyophilization. The fractionation was monitored by TLC on silica gel plates, using either vanillin−HCl reagent or dichromate solution, followed by heating at 100 °C in each instance, to determine the presence of proanthocyanidins, or methanolic aluminum chloride solution to determine the presence of simpler flavonoids.

Fraction CR13 from the Toyopearl fractionation, which demonstrated activity in the ODC assay below, was further fractionated on silica gel by vacuum chromatography (silica gel G with $CaSO_4$ binder, 25 g) to yield 16 subfractions (**Figure 1**). The column was washed with petroleum ether (100 mL) before use. Fraction CR13 (191 mg) was mixed with silica gel (~5 g, without solvent), placed on the column, and eluted. Solvents used were ethyl acetate with an increasing gradient of methanol and water (1, 100% EtOAc, 200 mL, 75.5 mg; 2, 5% MeOH in EtOAc, 200 mL, 19.4 mg; 3, 10% MeOH in EtOAc, 200 mL, 18.1 mg; 4, 15% MeOH in EtOAc, 150 mL, 6.0 mg; 5−7, EtOAc/MeOH/H_2O, 79:11:10; 200 mL, 29.5 mg total; 8, 40% MeOH in EtOAc, 200 mL, 12.8 mg; 9, MeOH/H_2O/EtOAc, 40:5:55, 50 mL, 4 mg; 10, MeOH/H_2O/EtOAc, 50:10:40, 60 mL, 12.1 mg; 11, MeOH/H_2O/EtOAc, 60:10:30, 75 mL, 3.1 mg; 12, MeOH/H_2O/EtOAc, 70:10:20, 125 mL, 2.4 mg; 13, MeOH/H_2O/EtOAc, 75:5:25, 125 mL, 2.2 mg; 14, 100% MeOH, 270 mL, 18.8 mg; 15, 16, MeOH/H_2O, 1:1, 400 mL, 50.6 mg).

On the basis of the results of silica gel TLC, subfractions 1−7 from the vacuum chromatography of fraction CR13 on silica gel above were combined (total = 148.5 mg). A portion of the combined subfractions (100 mg) was further fractionated on Sephadex LH-20 to produce another seven fractions. Fractions 1−6 were eluted with 95% EtOH, and fraction 7 was eluted with acetone (**Figure 1**). Each of these last seven fractions obtained was analyzed by TLC (cellulose) in two solvents: *n*-butanol/acetic acid/water (6:1:2) and acetic acid (15%). Thin-layer plates were visualized under UV light before and after spraying with methanolic aluminum chloride solution. Fraction 1 had two purple spots when viewed under UV light on cellulose TLC plates (R_f values 0.48 and 0.61, *n*-butanol/acetic acid/water, 0.46 and 0.63, 15% acetic acid; rutin R_f 0.61 and 0.63 under similar conditions). Fraction 2 had only one purple spot when viewed under UV light with R_f values 0.61 and 0.29, which changed to yellow when sprayed with methanolic aluminum chloride solution or when fumed with ammonium hydroxide. Fractions 3 and 4 had four purple spots under UV light on cellulose with R_f values of 0.48, 0.51, 0.66, and 0.72 in *n*-butanol/acetic acid/water (6:1:2) and an unresolved streak 0.3−0.63 in 15% HOAc. Fractions 5 and 6 had no observable spots when run in *n*-butanol/acetic acid/water (6:1:2) and one yellow spot in 15% acetic acid with and R_f identical to that of quercetin. When the TLC of fraction 7 was run with *n*-butanol/acetic acid/water (6:1:2), only two blue spots (at R_f 0.22 and 0.35) and three purple spots (at R_f 0.49, 0.61, and 0.66) were observed. On TLC with 15% HOAc, the purple spots streaked from 0.1 to 0.65.

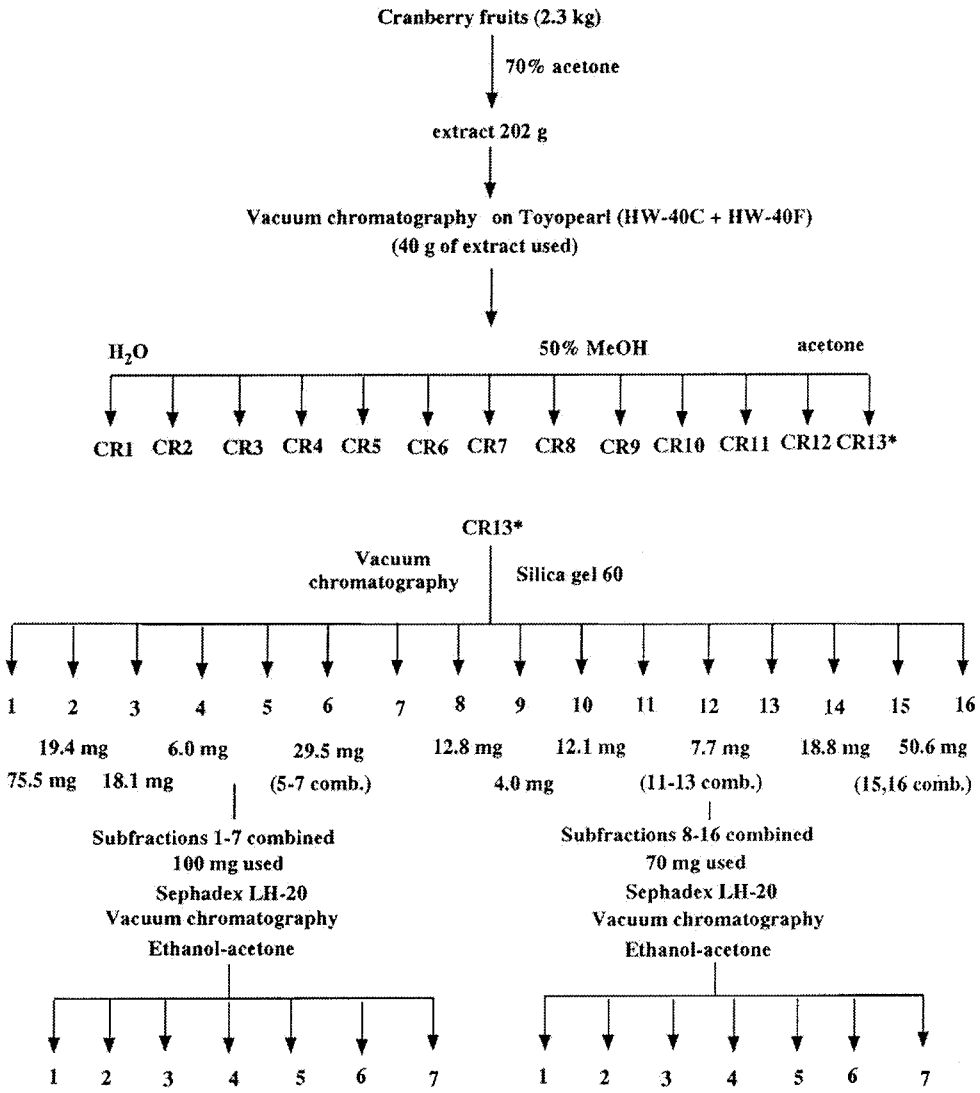

Figure 1. Flowchart showing the sequence of fractionation for cranberry fruit extract.

On the basis of a similar analysis of the silica gel TLC data, subfractions 8−16 from the vacuum chromatography of fraction CR13 on silica gel, above, were combined (106 mg of original sample, 70 mg used for fractionation). These were chromatographed on Sephadex LH-20 with ethanol and acetone as above to yield seven fractions (**Figure 1**), of which only fraction 4 possessed adequate material (30 mg) for further study.

Bioactivity Assays. *Ferric Reducing/Antioxidant Power (FRAP) Assay*. The FRAP assay, which evaluates antioxidants on the basis of their activity as reductants in a redox-linked colorimetric method and has been demonstrated as a direct indicator of "total antioxidant power" for both simple and heterogeneous solutions of antioxidants (*30*), was used to gauge the antioxidant capacity of cranberry extracts. Reduction of a ferric tripyridyltriazine (Fe^{III}-TPTZ) complex (FRAP reagent) results in the formation of the ferrous complex, which has an intense blue color. This reaction was monitored by measuring the change in absorption at 593 nm.

The assay was adapted from that of Benzie and Strain (*30*) for cranberry samples by using a 273 mM acetate buffer to dissolve $FeCl_3 \cdot 6H_2O$ just prior to assay. This acetate buffer/$FeCl_3$ solution was mixed with a 0.001 M solution of 2,4,6-tripyridyl-*s*-triazine (TPTZ) at a final ratio of 11:1, to prepare the FRAP reagent. The reaction of the FRAP reagent with an $FeSO_4$ standard is linear; therefore, by measuring nine 100 μL aliquots of the 2.0 mM standard solution, interspersed with the unknown cranberry extract samples, a good calibration curve was obtained. For the spectrophotometer blank, 100 μL of dimethyl sulfoxide (DMSO) was added to 3 mL of FRAP reagent. Each cranberry extract (from fractions 1−13 after vacuum chromatography) was dissolved in DMSO at a 0.5 mg L^{-1} target concentration. At 60 s intervals, 100 μL aliquots of either an $FeSO_4$ standard or a cranberry extract were added to the FRAP reagent and vortexed, and the absorbance at 593 nm was read with a Shimadzu UV-1601 UV−vis spectrophotometer. FRAP values were obtained for triplicates of unknown samples, and catechin and quercetin standards were used as additional controls.

Ornithine Decarboxylase (ODC) Assay. Mouse epidermal cells, line 308, were grown at 37 °C in humidified incubators containing 5% CO_2 in air. Minimal essential medium-spinner modification (S-MEM) supplemented with 5% dialyzed fetal bovine serum, nonessential amino acids (1×), Ca^{2+} (0.05 mM), and antimycotic-antibiotic (1%) was used as the growth medium and was replaced three times per week.

Ninety percent confluent cells were washed with Ca^{2+}- and Mg^{2+}-free Dulbecco's phosphate-buffered saline (PBS), refed with growth medium, allowed to grow for an additional 24 h, and then plated at 2 × 10^5 cells/mL/well in 24-well plates. Plates were placed in an incubator (37 °C, 5% CO_2) for 18 h, after which time 5 μL of sample (in DMSO) and 20 μL of TPA solution (final concentration of 200 nM, dissolved in 2.5% DMSO) were added to each well. Additional wells did not receive any fruit extract, only an equivalent amount of DMSO (0.6%) and TPA, and served as the experimental control. Cells were incubated for an additional 6 h, washed twice with cold Ca^{2+}- and Mg^{2+}-free PBS, and then immediately placed in a −80 °C freezer until the ODC assay was performed, usually within 3 days.

ODC activity was determined by measuring the release of $^{14}CO_2$ from L-[1-^{14}C]ornithine essentially according to the procedure of Lichti and Gottesman (*31*), as described previously (*32*). The protein content of each of the 24 wells used for the ODC assay was determined

following the addition of chloramine T (50 μL, 8 mg/mL) to destroy dithiothreitol (30 min) and NaOH (50 μL, 5.7 N) to solubilize protein (*33*). TPA-induced ODC activity was expressed as counts per minute (cpm) of $^{14}CO_2$ released/mg of protein/h. The amount of fraction required to inhibit ODC activity by 50% (IC_{50}) was determined graphically from quadruplicate measurements.

Identification of Free Sugars Using HPLC. When a portion of the 70% acetone extract (40 g) was treated with methanol (1 L) and filtered, a light pink material remained behind. A portion of this methanol-insoluble material (100 mg) was taken up in H_2O and analyzed by high-performance liquid chromatography (HPLC).

RESULTS AND DISCUSSION

For the isolation and characterization of phytochemicals and for identification of bioactive compounds, extraction with organic solvents is usually necessary. Aqueous acetone (70%) is an especially effective solvent for phenolic materials, especially for proanthocyanidins (*34*). In this instance, extracts had pronounced antioxidant effects as well as chemopreventive effects in the ODC assay below. Previous studies suggested that the latter activity is associated with fractions rich in proanthocyanidins (*11*, *28*).

After initial extraction in aqueous acetone and trituration of the freeze-dried solid with methanol, a portion of this extract did not dissolve in methanol. Analysis by HPLC and comparison to standard materials indicated that a large portion of methanol-insoluble material was composed of sugars, especially glucose, fructose, and sucrose, but primarily polysaccharides (possibly pectins) as determined by comparison with known standards. Carbohydrates were readily separated from other constituents of lyophilized cranberry extracts by vacuum chromatography on Toyopearl resin.

Additional vacuum chromatography on a polyvinylic gel permeation polymer (Toyopearl HW-40C+HW-40F, 1:1 by weight) separated a series of phenolic compounds from cranberries (**Figure 1**). Use of water, methanol, and acetone gradients with this support is especially effective for fractionation of phenolic materials (*35*−*37*). Fractionation of simple flavonoids (such as flavonols and flavonol glycosides) from mixtures containing predominantly proanthocyanidins is readily accomplished by this method.

As indicated by the FRAP assay, antioxidant activity was noted in scattered fractions after Toyopearl separation of fractions 1−13, but the highest antioxidant activity was noted in the latter two fractions, which approached the activity of the catechin standard (**Figure 2**). However, of the 13 fractions initially collected after vacuum chromatography of the cranberries extracted in 70% aqueous acetone, only extract from fraction 13 exhibited antipromotor activity, as indicated by 98% inhibition of ODC at a concentration of 20 μg/mL in the ODC assay. Whereas crude, unfractionated cranberry extract exhibited 48% ODC activity, none of the other 12 fractions from vacuum chromatography exhibited significant activity at 20 μg/mL. In the present study, because the ODC activity of these fruits was largely concentrated in one fraction (fraction 13), the results suggest that activity is not a generalized response of proanthocyanidins or flavonoids, as mixtures of these compounds also were found in fractions 6−13.

When tested in the ODC assay with the ME-308 cell line, the IC_{50} value for cranberry fraction 13 (CR13) was 5.67 μg/mL, which was indicative of significant activity against the promotion stage of chemically induced carcinogenesis (**Figure 3**). ODC activity of DMSO-treated controls and TPA+DMSO-treated controls were 248 and 3720 nmol/mg of protein/h, respectively. The amounts of CR13 extract needed to provide

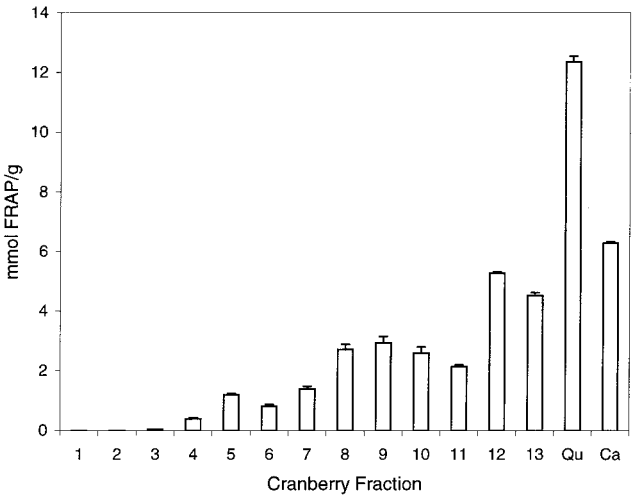

Figure 2. Antioxidant activity of cranberry fractions CR1–13 as indicated by a FRAP assay. Quercetin (Qu) and catechin (Ca) standards were included for comparison. Values are the average of quadruplicate measurements.

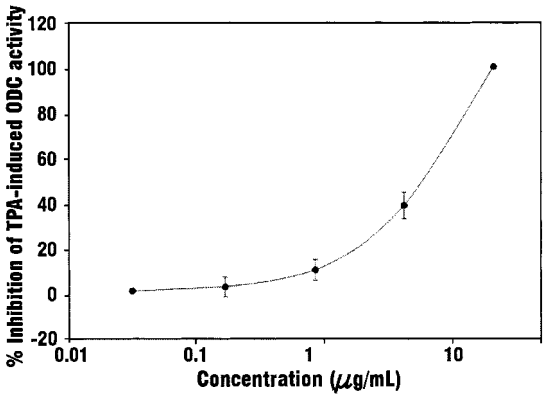

Figure 3. Percent inhibition of ODC activity induced by small concentrations of fraction CR13 from original extraction of *V. macrocarpon* (cranberry) fruits. IC_{50}, the concentration of extract required to inhibit ODC activity by 50%, was reached at 5.67 μg/mL, indicative of significant inhibitory activity. Values are the mean of quadruplicate determinations; bars indicate standard error.

significant inhibition in the ODC assay were not associated with cytotoxicity. Induction of ODC correlates with the potency of tumor promoters in the mouse skin model, and ODC activity assays are therefore useful for the development of chemopreventive agents capable of inhibiting carcinogenesis and tumor growth. In mammalian cells, ODC is a key enzyme in the biosynthesis of polyamines and the only route to production of putrescine, which is then further converted by the action of other enzymes into the polyamines spermidine and spermine. ODC activity and the resulting polyamines are demonstrated as essential for the process of cellular proliferation. In accordance with this essential role, ODC activity is greatly and rapidly induced in response to growth-promoting stimuli such as growth factors, hormones, and tumor promoters (*29*). In vivo carcinogenesis studies will need to be performed to further confirm anti-tumor promotion activity and to identify individual components and mixtures responsible for activity.

In an effort to determine the major constituents of this bioactive fraction, fraction 13 (CR13) was further fractionated by silica gel vacuum chromatography (**Figure 1**). TLC R_f values and reactions with spray reagents indicated that the fractions

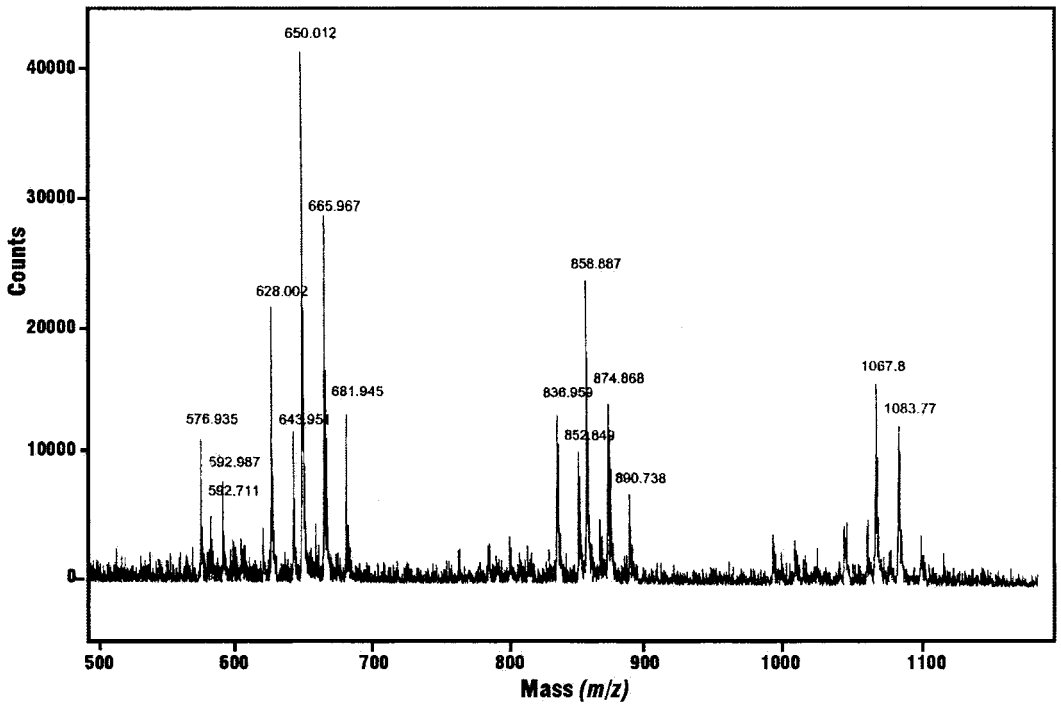

Figure 4. MALDI mass spectrum of fraction 4 from fractionation of CR13 (subfractions 8–16).

consisted largely of flavonol glycosides (*1*−*7*) and a mixture of flavonol derivatives and proanthocyanidins (*8*−*16*).

Both the TLC (R_f values and reactions with spray reagents) and positive ion FAB mass spectra of subfractions 1−7 (from vacuum chromatography of CR13 on silica gel) indicated that these fractions consisted mainly of flavonols and flavonol glycosides. Mass spectra had peaks that corresponded to quercetin $[M + 1]^+$ *m*/*z* 303.4; myricetin $[M + 1]^+$ *m*/*z* 319.3; quercetin monoglycoside $[M + 1]^+$ *m*/*z* 465.0; myricetin monoglycoside $[M + 1]^+$ *m*/*z* 481.0; quercetin monoglycoside $[M + Na]^+$ *m*/*z* 487.4; myricetin monoglycoside $[M + Na]^+$ *m*/*z* 495.3; quercetin diglycoside $[M + Na]^+$ *m*/*z* 650.4; myricetin diglycoside $[M + Na]^+$ *m*/*z* 666.3; gallocatechin/epigallocatechin $[M + Na]^+$ *m*/*z* 329.2; and catechin/epicatechin *p*-hydroxybenzoate $[M + Na]^+$ *m*/*z* 433.2. These compounds correspond in mass spectra and TLC R_f values and reactions with spray reagents to flavonoids previously reported by other workers (*2*, *7*), although the sugars of the glycosides in this study were not established.

Fractions 8−16 from vacuum chromatography of CR13 on silica gel were combined. TLC analysis confirms that a number of vanillin−HCl positive compounds, presumably proanthocyanidins, are present in these fractions. After further vacuum chromatography on Sephadex LH-20, seven fractions were recovered. The TLC of the major fraction indicated the presence of proanthocyanidins. However, this fraction also contained other flavonoids. An FAB positive ion mass spectrum of this fraction (**Figure 4**) had peaks corresponding to flavonol glycosides: rutin $[M + Na]^+$ *m*/*z* 632.9; quercetin diglycoside $[M + Na]^+$ *m*/*z* 650.0; quercetin diglycoside $[M + K]^+$ *m*/*z* 666.0; myricetin diglycoside $[M + Na]^+$ *m*/*z* 666.0; and myricetin diglycoside $[M + K]^+$ *m*/*z* 681.9. Although TLC followed by development with vanillin−HCl indicated the presence of proanthocyanidins, only peaks corresponding to an A-type dimer (based on catechin/epicatechin) $[M + 1]^+$ *m*/*z* 576.9 were observed (**Figure 4**). The complexity of the mixtures of proanthocyanidins in these fractions suggests that additional fractionation is warranted.

On the basis of these and published data (*3*, *13*, *14*), it can be seen that the proanthocyanidins of cranberry contain compounds that are based on (+)-catechin/(−)-epicatechin and gallocatechin/epigallocatechin monomers. In most other examples studied, gallate esters are attached at position 3 of the flavonoid units (*37*).

Previously, we prepared a continuous suspension culture of *Vaccinium pahalae* Ait. and extracted, fractionated, and subfractionated it to obtain a series of proanthocyanidins of increasing molecular weights (*11*, *38*). In the present study, partial characterization of the proanthocyanidins of the active cranberry fraction was facilitated by comparison of R_f values and mass spectral properties of the subfractions containing proanthocyanidins with those previously isolated from these cell cultures.

Myricetin and gallocatechin/epigallocatechin derivatives, with triply substituted B-rings, are typical components of cranberry fruits. These compounds are inherently less stable than quercetin derivatives and proanthocyanidins derived from (+)-catechin and (−)-epicatechin, which may account for a greater sensitivity of cranberry fruit bioactivity to physical environmental stress such as heating and length of storage time. In previous work with ODC assays, we noted loss of activity in bioactive cranberry subfractions after storage. A knowledge of the chemistry of the bioactive principles is desirable in order to understand why differences in activity or stability may occur, especially after prolonged storage. This knowledge has special significance for nutraceutical components that will be delivered or administered to humans.

ABBREVIATIONS USED

FRAP, ferric reducing, antioxidant power; ODC, ornithine decarboxylase; TLC, thin-layer chromatography; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate.

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