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Coumaroyl Iridoids and a Depside from Cranberry (*Vaccinium macrocarpon*)

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Abstract

Cranberry (*Vaccinium macrocarpon* Ait., Ericaceae) juice has been used for urinary tract infections for approximately 50 years. Recent research suggests that this botanical blocks adherence of pathogenic *E. coli* to urinary tract cells, thus preventing infection. While current evidence indicates that proanthocyanidins are responsible for this activity, these compounds may not reach the urinary tract, thus further investigation is warranted. Fractionation of cranberry juice concentrate was guided by a recently published antiadherence assay, and the resulting fractions were phytochemically characterized. Two new coumaroyl iridoid glycosides, 10-*p*-*trans*- (**1**) and 10-*p*-*cis*-coumaroyl-1*S*-dihydromonotropein (**2**), and a depside, 2-*O*-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylmethylacetate (**3**) were isolated, and although these compounds did not have antiadherent activity in isolation, they might constitute a new group of marker compounds for this active fraction of cranberry.

Cranberry (*Vaccinium macrocarpon* Ait., Ericaceae) juice, generally in the form of cranberry juice cocktail consisting of a mixture of cranberry juice, water and sugar, is a popular botanical dietary supplement[1, 2] primarily used for the treatment and prevention of urinary tract infections, with documented human use for at least 47 years.[3] This use is supported by several clinical trials,[4–11] although some trials, notably for children with neuropathic bladder and catheterization, did not find cranberry to be effective.[12, 13] Cranberry juice was originally believed to be active due to its acidifying effect on urine, and/or the increased excretion of the cranberry urinary metabolite hippuric acid.[14, 15] However, subsequent research suggested that the bacteriostatic impact from acidification alone could not account for its demonstrated effects.[16, 17]

Inhibition of adherence of *Escherichia coli* to uroepithelial cells,[18–20] rather than direct bacteriostatic or bactericidal activity, has been proposed as the mechanism of action. Specifically, there is support for inhibition of the papG fimbrial attachment of uropathogenic strains of *E. coli* to human cells[21, 22] by cranberry's A-type proanthocyanidin compounds, but not by a B-type dimer or the (–)-epicatechin monomer.[18, 23] Proanthocyanidins, however, may not be assimilated in the gut, nor reach the urinary tract intact.[24] The putative active cranberry A-type proanthocyanidin oligomers, containing a second link (carbon-oxygen) between at least two of their epicatechin monomer units, are chemically similar to B-type proanthocyanidins, which contain only single, carbon-carbon links between units. Knowledge

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Supporting Information. HPLC chromatograms of the two lots of cranberry juice used in this research, including retention time for known cranberry compounds ideain chloride, resveratrol, and benzoic acid, are available free of charge via the Internet at http://pubs.acs.org.

about the metabolic route in humans of A-type proanthocyanidins is extremely sparse. With very limited recent exceptions for small amounts of dimers and possibly a trimer,[25] evidence currently indicates that B-type proanthocyanidins, especially trimers and larger, are degraded in the gut, and/or not assimilated in any quantity, and do not reach the urinary tract intact.[26–37] Whether this information can be applied to A-type proanthocyanidins is unknown, and should be verified before abandoning the search for other active cranberry constituents. Additionally, while urine after cranberry ingestion has been shown to be antiadherent,[16, 17, 19] and while proanthocyanidin metabolites[38] are possibly the active constituents, to date no researchers have elucidated any specific antiadherent cranberry compounds or metabolites thereof, proanthocyanidin or otherwise, found in urine after cranberry ingestion. It is also important to consider possible synergism between compounds, as seen for the antimutagenic activity of cranberry.[39] Relying on one class of isolated active compounds and ignoring interactions from the remaining constituents, while tempting for reasons of feasibility, can generally be misleading in terms of explaining efficacy of herbals. Therefore, one aim of the present study was to remove proanthocyanidins and bactericidal benzoic acid and then further characterize the *in vitro* antiadherent fraction of cranberry juice. In this small but important step in the complete understanding of the use of this botanical for urinary tract infection, we present data on two new compounds that, while not active in isolation, are potential new (phytochemical) marker compounds for the bioactive cranberry fraction.

An assay using biologically relevant cells, and of sufficiently high throughput such that it can efficiently guide the fractionation of cranberry, was developed in our laboratory and recently published.[40] We have used this assay in the bioactivity-guided fractionation of cranberry juice, and report here two new and one known compound (**1**–**3**) found in, and assisting in the identification of, the active fraction. Compounds **1** and **2** represent an isomeric pair of acylated dihydromonotropein iridoids and, together with previously reported congeners,[41] underline the significance of this class of phytochemicals for the characterization of cranberry preparations. The third compound is the depside, 2-*O*-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylmethylacetate (**3**), a methylated form[42] of another depside (**5**) so far only reported in *Papaver rhoeas*.[43]

Cranberry juice, obtained as a frozen concentrate, was thawed, partially neutralized with aqueous ammonia (28% NH_4OH), and fractionated over HP-20 resin. Two more levels of fractionation, guided by positive activity in the antiadhesion assay, resulted in an active fraction from which two new coumaroyl-iridoid glucosides (**1**, **2**) and a depside (**3**) were isolated using reverse-phase HPLC. These compounds were identified by spectroscopic analysis, and by comparison of their data with literature values of the similar compounds dihydromonotropein (**4**) and a *Papaver* depside (**5**).[41, 43]

The deprotonated molecular ion of **1** at *m/z* 537.1629 (**1**) $[M-H]^-$ (high-resolution negative ion electrospray) corresponded to the molecular formula $C_{25}H_{30}O_{13}$ (calc. 537.1608). The IR spectra showed the characteristic absorption bands of hydroxy groups, free acid or ester groups, and alkenes at 3308, 1684, and 1138 cm^{-1}, respectively. UV absorptions at 215, 230 and 315 suggested that **1** had two independent conjugated systems, one of which was aromatic. In the ^1H NMR spectrum of **1** (Table 1), AM spin system resonances at δ_H 7.668 and 6.385 (each 1H, *d*, *J* = 15.9 Hz; H-3″, H-2″ resp.) revealed the presence of a *trans*-ethylene group conjugated with an aromatic ring, and a typical AA′XX′ spin system for 1,4-disubstituted aromatic ring protons at δ_H 7.487 and 6.804 (each 2H, *brddd*, *J* = 8.7, 2.9, 2.0 Hz; H-5″/H-9″, and H-6″/H-8″, resp.). The spectrum also revealed a six-carbon sugar moiety with all-axial ring protons at δ_H 4.725 (1H, *d*, *J* = 7.9 Hz; H-1′), 3.238 (1H, *dd*, *J* = 9.0, 7.9 Hz; H-2′), 3.329 (1H, overlapped with solvent signals; H-3′), 3.352 (1H, *t*, 9.0; H-4′), 3.336 (1H, overlapped with solvent signals; H-5′), 3.846 (1H, *dd*, *J* =11.8, 1.6 Hz; H-6a′), and 3.662 (1H, *dd*, *J* = 11.8, 5.2 Hz; H-6b′). In addition to the observed resonances of characteristic spin systems and a sugar moiety, an

olefinic proton was observed at δ_H 7.492 (1H, *d*, *J* = 1.4 Hz; H-3), a dioxymethine proton at δ_H 5.514 (1H, *d*, *J* = 5.3 Hz; H-1), methylene resonances at δ_H 4.256 and 4.182 (each 1H, *d*, *J* = 11.3 Hz; H-10), three proton resonances at δ_H 2.933 (1H, *ddddd*, *J* = 15.2, 9.2, 1.7, 1.4, 0.5 Hz; H-5), 2.319 (1H, *dd*, *J* = 9.2, 5.3 Hz; H-9), and 2.174 (1H, *m*; H-6a), and three overlapped multiplets in the range δ_H 1.831~1.653 accounting for the C-7 methylene protons and one of the two C-6 protons. The shielded H-6b at δ_H 1.704 overlapped with H-7, as revealed in the HMQC spectrum. The ^{13}C and DEPT NMR spectra of **1** (Table 2) included resonances for a *trans*-ethylene group at δ_C 114.89 (*d*, C-2″) and 147.03 (*d*, C-3″), as well as two resonances representing four carbons of a 1,4-disubstituted aromatic ring at δ_C 131.32 (*d*, C-5″ and 9″) and 116.85 (*d*, C-6″ and 8″), and six oxygenated carbon resonances belonging to the sugar substituent at δ_C 100.88 (*d*, C-1′), 74.62 (*d*, C-2′), 77.88 (*d*, C-3′), 71.22 (*d*, C-4′), 78.40 (*d*, C-5′), and 62.49 (*t*, C-6′). In addition, the spectra showed an acyl group and a carbonyl group at δ_C 169.23 (*s*, C-1″) and 170.74 (*s*, C-11), a pair of resonances for olefinic carbons at δ_C 153.28 (*d*, C-3) and 112.47 (*s*, C-4), three methine carbons at δ_C 96.14 (*d*, C-1), 35.21 (*d*, C-5), and 46.87 (*d*, C-9), three methylene carbons at δ_C 31.57 (*t*, C-6), 37.47 (*t*, C-7), and 70.88 (*t*, C-10), and an oxygenated quaternary carbon at δ_C 81.66 (*s*, C-8). These NMR data suggested that **1** was likely an iridoid glycoside, as they were similar to those of the known iridoid glucoside, 6,7-dihydromonotropein (**4**) recently isolated from cranberry by Jensen *et al*.[41]

The primary differences in the NMR data of **1** with **4**, in addition to the *trans*-coumaroyl signals in **1**, were that the protons at δ_H 3.56 (1H, *d*, *J* = 17.5 Hz; H-10b) and 3.62 (1H, *d*, *J* = 17.5 Hz; H-10a) in **4** were shifted toward lower field to δ_H 4.182 (1H, *d*, *J* = 11.3) and 4.256 (1H, *d*, *J* = 11.3 Hz) in **1**, and that the corresponding carbon at δ_C 68.2 (*t*, C-10) in **4** was also deshielded to δ_C 70.88 (*t*) in **1**. This evidence indicated that the coumaroyl group in compound **1** was connected to the C-10 hydroxyl function of the iridoid skeleton. Additionally, the shift in the geminal coupling constants for H-10a and H-10b from 17.5 Hz in **4** to 11.3 Hz in **1** suggested that the hydroxyl proton had been replaced by a bulky substituent.

In the HMBC spectrum, correlations of the C-10 methylene protons resonating at δ_H 4.182 and 4.256 with the quaternary carbon at δ_C 169.23 (C-1″) were observed. This quaternary carbon was also correlated with two other protons at δ_H 6.385 and 7.668 (H-2″ and H-3″, respectively). This evidence confirmed that the coumaroyl group must be attached at position 10. Two fragments of *m/z* 163.0344 and 373.1023 in the negative ion tandem mass spectrum supported this conclusion.

The coupling constants of $J_{1,2'}$ (7.9 Hz), $J_{2',3'}$ (9.0 Hz), $J_{3',4'}$ and $J_{4',5'}$ (9.0 Hz in CD_3OD and 8.6 Hz in D_2O) indicated that all of these protons are pyranose ring protons in an axial position, confirming that the sugar substituent was glucose. In the ROESY spectrum of **1**, the correlations between H-1 and H-1′ and one of two C-7 protons indicated that H-1 is in an α orientation. Both H-5 and H-9 correlated with H-6 (δ_H 2.174) and with one of the two C-7 protons (δ_H 1.840~1.726). Moreover, correlations of H-10 (δ_H 4.182 and 4.256) with H-9 (δ_H 2.319), H-5 (δ_H 2.933), and H-7 (δ_H 1.840~1.716, 2H) were observed. NOE experiments provided evidence that these protons were in β orientations, i.e., **1** had the same relative configuration as **4**. Consequently, compound **1** was deduced to be 10-*trans*-coumaroyl-6,7-dihydromonotropein.

Compound **2** was very similar to compound **1**. Comparing the ^{1}H, ^{13}C, and DEPT NMR spectra of **2** with those of **1**, the only difference was for the proton and carbon resonances within the coumaroyl moiety. In the ^{1}H NMR spectra, the H-2″ and H-3″ signals were shifted upfield from δ_H 6.385 and 7.668 in **1** to δ_H 5.858 and 6.914 in **2**, respectively. The coupling constant between H-2″ and H-3″ differed markedly from 15.9 Hz in **1** to 12.6 Hz in **2**. Comparing the ^{13}C NMR spectra, the chemical shift of C-2″ was shifted downfield from δ_C 114.89 in **1** to 116.89 in **2**, while the chemical shift of C-3″ moved to higher field from δ_C 147.03 in **1** to 144.32 in **2**. This evidence indicated that compound **2** had a *cis*-coumaroyl substituent, as

opposed to the *trans*-coumaroyl seen in **1**. Therefore, compound **2** was determined to be 10-*cis*-coumaroyl-6,7-dihydromonotropein.

Additional iridoid compounds are likely to be present in cranberry. A ^{1}HNMR analysis of the parent fraction, from which the two iridoids **1** and **2** were isolated, reveals a total of four pairs of doublets between δ_H 4.6 and 4.1 with the typical coupling of C-10 acylated geminal protons (J = 11.1 to 11.4 Hz). While **1** and **2** account for one pair of doublets each, two pairs remain, for a possibility of at least two more compounds belonging to the same series of iridoids. At least one compound is highly likely to be a 10-*O*-substituted iridoid glycoside: a doublet can be clearly seen downfield from the H-10 resonances of both **1** and **2**, at δ_H 4.329 (J = 11.4 Hz); resonances for the second C-10 proton for this third iridoid are likely to be found at around 4.2, overlapping with the resonances of **1** and **2** (likely a J = 11.4 Hz doublet at 4.205). While the low available sample mass limited analysis in the present study, further analysis of this fraction with regard to the presence of additional iridoid glycosides is clearly warranted (Figure 1).

Compound **3** was isolated in a yield of 1.7 mg (13 ppm, dry weight) as an amorphous, colorless solid. The high-resolution negative ion electrospray mass spectrum of compound **3** revealed a deprotonated molecule at *m/z*: 333.0606 $[M-H]^-$, corresponding to a molecular formula of $C_{16}H_{14}O_8$ (calc. 333.0611). The negative ion tandem mass spectrum of compound **3** contained a fragment ion *m/z* 301, which corresponded to a loss of methanol and indicated the presence of a methoxy group in **3**. The IR spectrum showed the characteristic absorption bands corresponding to a hydroxy group, acyl or carboxy groups, and alkenes at 3362, 1684, and 1138 cm^{-1}, respectively. In the ^{1}H NMR (Table 3) and ^{1}H-^{1}H COSY spectra of **3**, only two aromatic spin systems were observed at lower field. One was a 1,2,4-trisubstituted ABX spin system with resonances at δ_H 7.529 (1H, *dd*, J = 8.0, 2.0 Hz; H-6′), 7.517 (1H, *dd*, J = 2.0, 0.4 Hz; H-2′), and 6.863 (1H, *dd*, J = 8.0, 0.4 Hz; H-5′), respectively. The other was a 1,2,3,5-tetrasubstituted AX spin system with resonances at δ_H 6.251 (1H, *d*, J = 2.3 Hz; H-3) and 6.135 (1H, *d*, J = 2.3 Hz; H-5). In addition to the above resonances, a methoxy and a similarly shifted methylene resonance were observed at δ_H 3.550 (3H, *s*; OMe) and 3.479 (2H, *brs*; H-7a, H-7b), respectively. The ^{13}C and DEPT NMR resonances could be ascribed to a 1,2,3,5-tetrasubstituted aromatic ring containing resonances at δ_C 107.14 (*s*, C-1), 158.63 (*s*, C-2), 101.02 (*d*, C-3), 158.42 (*s*, C-4), 102.12 (*d*, C-5), and 152.40 (*s*, C-6), and to a 1,2,4-trisubstituted aromatic ring with carbons resonating at δ_C 121.65 (*s*, C-1′), 117.81 (*d*, C-2′), 146.45 (*s*, C-3′), 152.51 (*s*, C-4′), 116.09 (*d*, C-5′), and 124.37 (*d*, C-6′), respectively. In addition, there were two carbonyl resonances at δ_C 174.34 (*s*, C-8) and 166.29 (*s*, C-7′), a methylene at δ_C 29.89 (*t*, C-7), and one methoxy resonance at δ_C 52.33. The HMBC spectrum of **3** exhibited correlations between the respective protons and carbons, as illustrated in Figure 2. Compared to what is typical of a free carboxylic acid,[43] C-7′ in **3** was shifted to a higher field, indicating that this is the site of esterification, which, by a process of elimination, must have occurred between the two rings of this depside. However, the C-7′ shift supplied only indirect evidence; additional support for this ester configuration was provided by MS data. The positive ion electrospray tandem mass spectrum contained the dominant fragment ion of *m/z* 137.0239 ($C_7H_5O_3$) corresponding to the cleavage of the ester bond (see Figure 2). This ion further fragmented to lose CO, which further supported the assignment of the ester group. The NMR data were consistent with a similar compound recently discovered in *Papaver rhoeas* petals,[43] with the exception of the 8.2 Hz coupling constant reported for H-2′. It is our understanding that this is a typographical error on the part of Hillenbrand *et al.*,[43] because the presence of three *ortho* coupling constants is inconsistent with their tri- and *para*-substituted ring, and, additionally, a matching 2.2 Hz meta coupling constant (H-2′ with H-6′) is missing from their data. Compound **3** was thus deduced to be 2-*O*-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylmethylacetate (**3**).[42]

It should be noted that it is rare to find depsides in organisms that are not lichens,[44] although there is evidence that ionizing radiation, such as that used to preserve foods, can break down quercetin into various depsides.[45] Whether **3** is in fact a genuine cranberry secondary metabolite, or from some other biological source, related or unrelated to cranberries, deserves further investigation. Considering published evidence,[42] however, the methyl ester group in **3** appears unlikely to be due to artifact formation in the presence of methanol/acid.

Experimental Section

General Experimental Procedures

Optical rotations were obtained with a Perkin-Elmer 241 polarimeter (Perkin-Elmer, Inc., MA). ^{1}H and ^{13}C NMR were measured on Bruker Avance 360 and Avance 500 instruments (Bruker, Zürich, Switzerland). Chemical shifts (δ) were expressed in ppm with reference to the deuterated MeOH signals (^{1}H: 3.305, ^{13}C: 49.000 ppm) or D_2O (4.8000 ppm). The digital resolution was always better than 0.1 Hz equivalent to 0.0002 ppm (e.g., 32K real datapoints, 8 ppm spectral width for ^{1}H NMR) in the ^{1}H and 1.2 Hz equivalent to 0.008 ppm (32K real data points, 250 ppm spectral width) in the ^{13}C domain. HR-ESI MS data were recorded on a Micromass (Manchester, UK) Q-TOF-2 system. Infrared spectra were recorded from a thin film on a germanium ATR unit (JASCO FT/IR-410, JASCO Inc., MD). Thin-layer chromatography was performed on pre-coated TLC plates (250 μm thickness, KGF Si gel 60 and KGF RP-18 Si gel 60, EM Science, Germany, or 200 μm ALUGRAM® SIL G/UV_{254}, Macherey-Nagel, Germany) with compounds visualized by spraying the dried plates with 5% H_2SO_4 in EtOH or 2% anisaldehyde and 5% H_2SO_4 in EtOH, followed by heating. Semipreparative HPLC was carried out on a Waters Delta 600 system with a Waters 996 photodiode array detector, Waters 717 plus autosampler, and Millennium32 Chromatography Manager (Waters Co., MA) on a GROM-Sil 120 ODS-4 HE (Watrex-International, Inc., CA) semipreparative column (5 μm, 300 × 20 mm) with a flow rate of 6 mL/min. Diaion® HP-20 (Supelco Co., PA) and lipophilic Sephadex LH-20 (25–100 μm, Sigma Chemical Co., MO) were used for column chromatography. A High Speed Countercurrent Chromatography system (Pharma-Tech CCC-1000, Pharma-Tech, MD) was also used for fractionation. All solvents were HPLC grade (Fisher Scientific Co., Hanover Park, IL).

Biological Activity

Fractionation was guided by an assay that measured inhibition of adherence of uropathogenic *E. coli* to human uroepithelial cells; for experimental details see our recent report.[40] In brief, p-fimbriated *E. coli* were grown on CFA agar to promote fimbrial growth. Human T-24 cells were grown to confluence in 96-well microplates. Substances to be tested were briefly incubated with *E. coli*, and then poured over the T-24 cells, and incubated at 35 °C for one hour. Unadhered bacteria were carefully rinsed away, and new media was added to wells. Adherent bacteria were allowed to multiply for four to six hours, and detected with a microplate reader. Differences in initial quantities of adhered bacteria, compared with solvent controls, were calculated using a standard curve.

Botanical Source

Cranberry juice concentrate was obtained from Ocean Spray, Inc., in two separate lots (lot numbers T073101 and T111802). Because juice does not contain recognizable genetic material or cellular matter, botanical identification was not possible. An HPLC chromatogram for each lot, with peak matching for several cranberry metabolites, is available; see Supporting Information.

Fractionation and Isolation

Briefly, fractionation of cranberry juice consisted of four levels: initially following the general guidelines of Kandil *et al.*,[46] the first column was HP-20 reversed phase resin. HP-20 active fractions were secondarily separated with high-speed countercurrent chromatography (HSCCC, conditions see below), followed, third, by a 4-meter Sephadex LH-20 column, and the final fourth-level fractionation was accomplished on a semipreparative HPLC reverse-phase column (Figure 3). Bacterial antiadherence activity-guided fractionation was performed through the first three levels. The overall method was specifically designed to eliminate benzoic acid early in the fractionation scheme; this method primarily follows bioactivity, and differs from more traditional schemes developed for the targeted isolation of polyphenolic compounds.

First Level Fractionation—Two lots of cranberry juice concentrate, 31.8 kg total including H_2O content (approximately 6.4 kg dry weight, processed in two runs, approximately 12 kg and 19 kg, respectively), were adjusted on ice from pH 2.4 to approximately pH 5.5 with 28% ammonia (Fisher), and were fractionated over 2.25 kg Dianon® HP-20 (Supelco/Sigma) that had been conditioned with deionized H_2O. Juice was allowed to flow through the column, which retained all colored anthocyanins. Effluent of this first fraction contained 95% of the mass (dry weight). The subsequent mobile phase sequence consisted of 100% deionized H_2O (10 and 14 L, first and second run, respectively), followed by 20% MeOH in deionized H_2O (8 and 9 L, resp.), then 50% MeOH (11 and 19 L, resp.), and finally 100% MeOH (11 and 13 L, resp.). Transitions in MeOH concentration were accomplished with a short gradient of approximately one liter total volume. One-liter fractions were collected throughout and tested for antiadherent activity. Antiadherent fraction yield, from 100% MeOH fractions, was 34.6 g.

Second Level Fractionation—Because the above active HP-20 first level fractions contained large quantities of bactericidal benzoic acid, which interfered with antiadherence assessment, HSCCC separation on a Pharma-Tech CCC-1000 system was next used. 12.9 g of the active fraction from level one was separated using a solvent system of $CHCl_3$-MeOH-H_2O 10:7:5, flow rate of 1.5 mL/min, UV detection (T2H mode, upper phase mobile). The resulting fractions were recombined per TLC into 7 fractions. The first of these 7 fractions, collected in 10 runs averaging 62.5 mL solvent each (total 625 mL), with a yield of 5.05 g dry weight, contained antiadherent activity but no benzoic acid.

Third Level Fractionation—The first HSCCC fraction (5.05 g, K_D<0.2), containing all visible anthocyanins but no benzoic acid, was then fractionated over a four-meter Sephadex LH-20 column (300 mm × 25 mm ID pre-column; main column 4 m × 10 mm ID, 108 g total LH-20; isocratic 100% MeOH mobile phase) in six injections of approximately 800 mg each (300 fractions at 8 mL each collected for each injection). Fractions obtained were recombined into 16 fractions per TLC.

Fourth Level Fractionation—Fractions 6, 8–12, 14, and 16 from level three were further separated on a semiprep HPLC reversed phase column (Grom, SIL-120 ODS-4 HE, 300 × 20 mm, 5 um) at 5 mL/min, with a mobile phase consisting of a mixture of MeOH (solvent A) and H_2O containing 0.1% TFA (solvent B). Compounds **1** and **2** were isolated using a gradient of 50:50 to 76:24 A:B over 35 min. Retention time for **1** was 23.6 min (6.8 mg), and retention time for **2** was 28.4 min (1.9 mg). For the isolation of compound **3**, an isocratic method (55:45 A:B) was used. Retention time for **3** was 23.3 min (1.7 mg).

10-*p*-*trans*-coumaroyl-1*S*-dihydromonotropein (1):colorless, amorphous solid; $[\alpha]^{25}_D$ −44.4 (*c* 0.20, MeOH); UV(MeOH/H_2O) λ_{max} 215, 230, 310; IR (Ge ATR) ν_{max} 3308, 1684

br, 1634, 1604, 1515, 1169 and 1074 $cm^{-1;}$ ^{1}H NMR and ^{13}C NMR see Tables 1 and 2; HR-ESIMS m/z 537.1629 (calcd for $[C_{25}H_{30}O_{13}\text{-}H]^{-}$, 537.1608)

10-*p-cis*-coumaroyl-1*S*-dihydromonotropein (2):colorless, amorphous solid; $[\alpha]^{25}_{D}$ +37.8 (*c* 0.10, MeOH); UV(MeOH/H_2O) λ_{max} 215, 231, 314; IR (Ge ATR) ν_{max} 3308, 1695, 1684, 1558, 1507, 1457, 1188, 1160, and 1071 $cm^{-1;}$ ^{1}H NMR and ^{13}C NMR see Tables 1 and 2; HR-ESIMS m/z 537.1649 (calcd for $[C_{25}H_{30}O_{13}\text{-}H]^{-}$, 537.1608)

2-O-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylmethylacetate (3):colorless, amorphous solid; UV(MeOH/H_2O) λ_{max} 215, 230, 315; IR (Ge ATR) ν_{max} 3308, 1717, 1700, 1684, 1653, 1560, 1295, 1191, and 1138 $cm^{-1;}$ ^{1}H NMR and ^{13}C NMR see Tables 1 and 2; HR-ESIMS m/z 333.0606 (calcd for $[C_{16}H_{14}O_{8}\text{-}H]^{-}$, 333.0611).

Supporting Information

Refer to Web version on PubMed Central for supplementary material.

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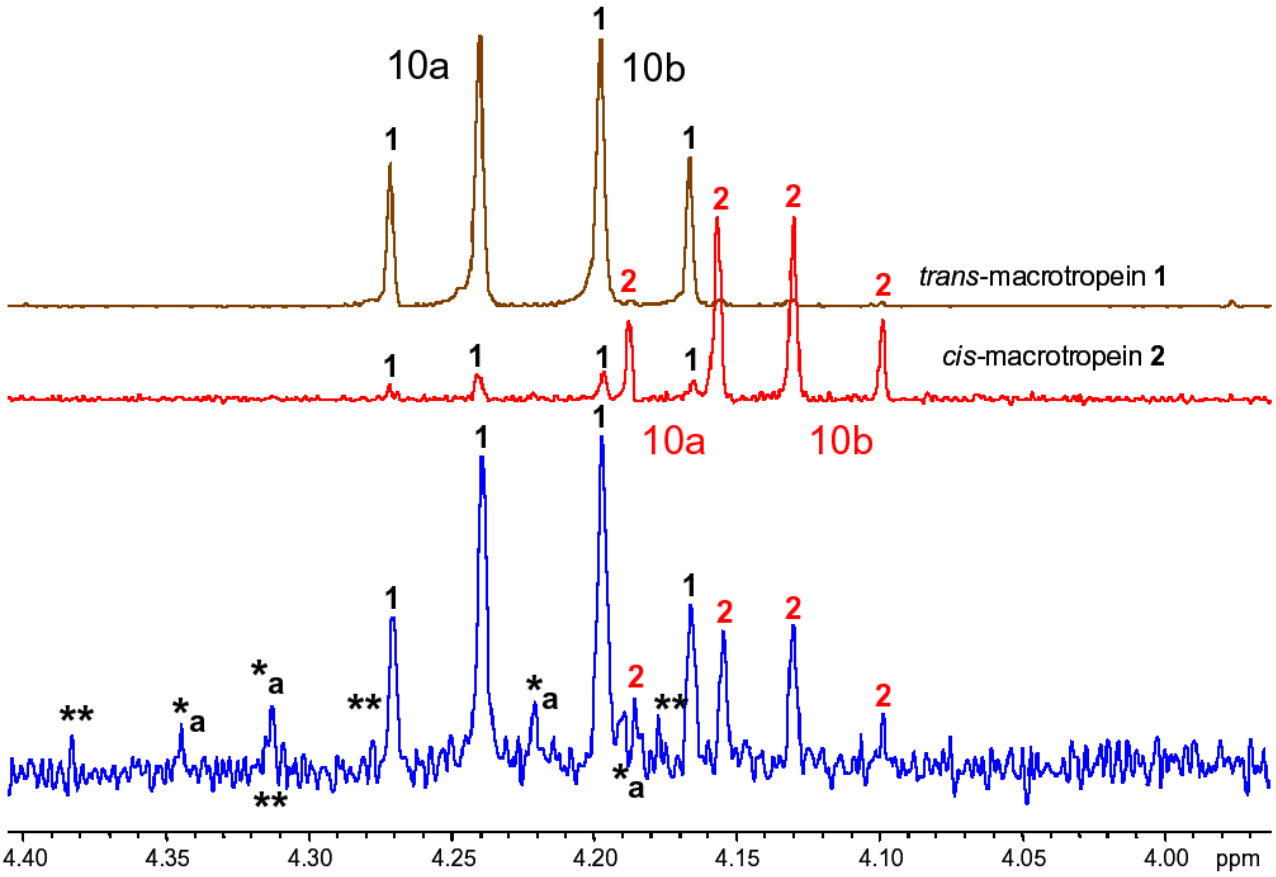

Figure 1.

Additional resonances (*/**) in the ^{1}H NMR spectrum of the parent fraction, from which **1** and **2** were isolated, revealed the presence of structurally related minor iridoids, indicated by the presence of pairs of doublets with the typical ~11 Hz geminal coupling of acylated C-10 oxymethylene resonances. *E.g.*, the signals at δ 4.329 and 4.205 (*a) likely belong to a third major monotropein-type iridoid. This indicates that these iridoids are distinct phytochemical markers of the antiadherence active fraction of *V. macrocarpon.*

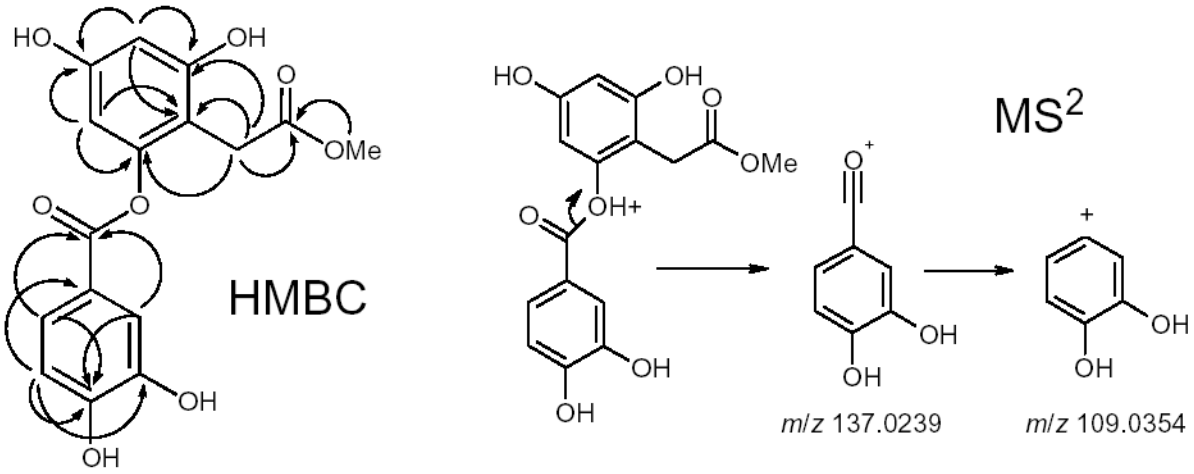

Figure 2.
HR-MS fragmentation pattern and HMBC correlations of 2-*O*-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylmethylacetate (**3**).

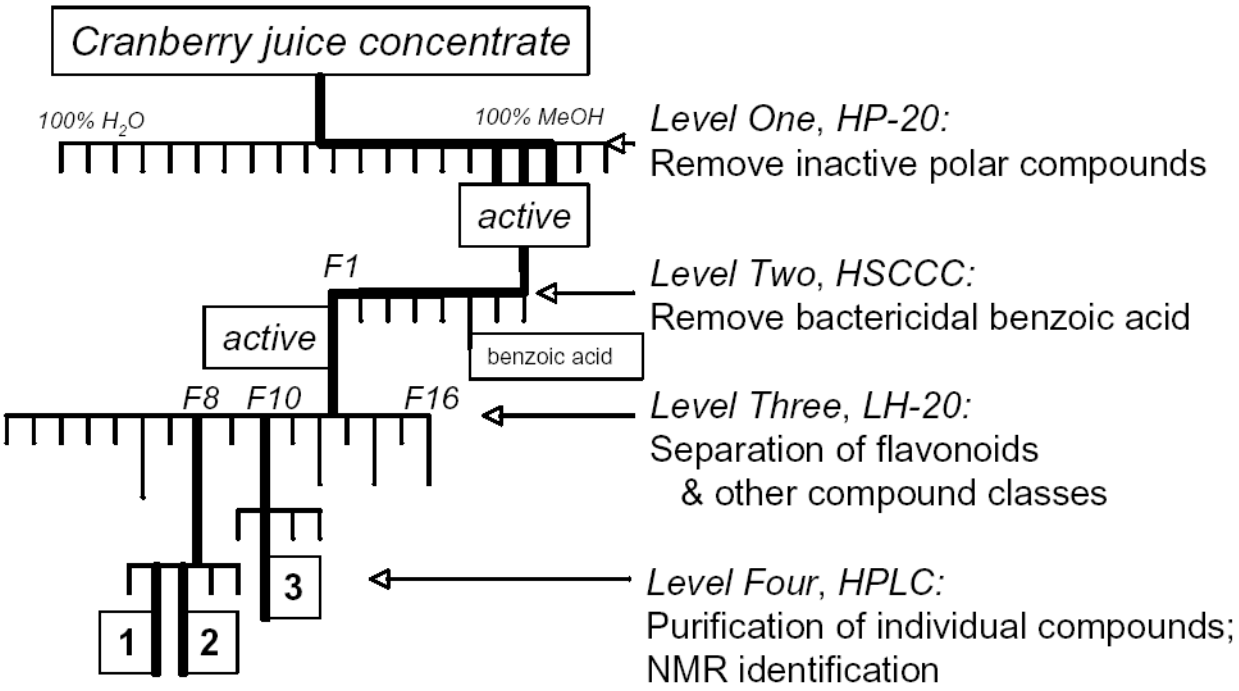

Figure 3.
Bioactivity-guided fractionation of cranberry juice, leading to isolation of three compounds (**1**–**3**). Bold lines follow activity through the first three levels of fractionation. Longer lines from level three indicate further separation on HPLC.

Table 1

^{1}H NMR [δ in ppm (mult, *J* in Hz)] data of compounds **1**, **2** and **4** in CD_3OD[b] and D_2O[a,c]

pos.	1[b]	1[c]	2[b]	2[c]	4[d]
1	5.514 (*d*, 5.3)	5.543 (*d*, 3.9)	5.466 (*d*, 5.4)	5.496 (*d*, 4.0)	5.54 (*d*, 3.6)
3	7.492 (*d*, 1.4)	7.526 (*br s*)	7.463 (*d*, 1.4)	7.527 (*br s*)	7.55 (*br s*)
5	2.933 (*ddddd*, 15.2, 9.2, 1.7, 1.4, 0.5)	2.915 (*m*)	2.840 (*ddddd*, 15.2, 9.4, 2.0, 1.4, 0.5)	2.828 (*m*)	2.95 (*m*)
6	Ha: 1.704 (*m*)	1.672 (*m*)	Ha: 1.630 (*m*)	1.674 (*m*)	2.08 – 1.63 (*m* w/ H7)
	Hb: 2.174 (*m*)	2.091 (*m*)	Hb: 2.113 (*m*)	2.073 (*m*)	
7	1.840~1.716 (2H, *m*)	1.831~1.710 (2H, *m*)	1.749~1.645 (2H, *m*)	1.794~1.670 (2H, *m*)	2.08 – 1.63 (*m* w/ H6)
9	2.319 (*dd*, 9.2, 5.3)	2.353 (*dd* 9.3, 4.0)	2.224 (*dd*, 9.4, 5.4)	2.270 (*dd*, 9.2, 4.2)	2.34 (*dd*, 9.3, 3.6)
10	4.182 (*d*, 11.3)	4.159 (*d*, 11.1)	4.114 (*d*, 11.2)	4.194 (*d*, 11.2)	3.56 (*d*, 17.5)
	4.256 (*d*, 11.3)	4.199 (*d*, 11.1)	4.176 (*d*, 11.2)	4.230 (*d*, 11.2)	3.62 (*d*, 17.5)
1′	4.725 (*d*, 7.9)	4.797 (overlapped[e])	4.711 (*d*, 7.9)	4.799 (overlapped[e])	4.82 (*d*, 8.4)
2′	3.238(*dd*, 9.0, 7.9)	3.285 (*dd*, 9.1, 8.6)	3.243 (*dd*, 9.0, 7.9)	3.291 (*dd*, 9.2, 8.1)	3.29 (*t*, 9.1, 8.1)
3′	3.329 (overlapped[e])	3.487 (*t*, 9.1)	3.327 (overlapped[e])	3.509 (*dd*, 9.5, 9.2)	3.51 (*t*, 9.1)
4′	3.352 (*t*, 9.0)	3.389 (*t*, 8.6)	3.349 (*t*, 9.0)	3.388 (*t*, 9.5)	3.41 (*t*, 9.1, 9.1)
5′	3.336 (overlapped[e])	3.436~3.406 (*m*)	3.330 (overlapped[e])	3.500~3.465 (*m*)	3.54–3.48 (*m*)
6′	3.662 (*dd*, 11.8, 5.2)	3.638 (*dd*, 12.4, 5.2)	3.667 (*dd*, 11.8, 5.1)	3.708 (*dd*, 12.4, 5.5)	3.74 (*dd*, 12.3, 5.7)
	3.846 (*dd*, 11.8, 1.6)	3.788 (*dd*, 12.4, 2.1)	3.860 (*dd*, 11.8, 1.4)	3.897 (*dd*, 12.4, 2.2)	3.92 (*br d*, 12.3)
2″	6.385 (*d*, 15.9)	6.276 (*d*, 16.0)	5.858 (*d*, 12.6)	5.992 (*d*, 12.4)	-
3″	7.668 (*d*, 15.9)	7.570 (*d*, 16.0)	6.914 (*d*, 12.6)	7.143 (*d*, 12.4)	-
5″, 9″	7.487 (2H, *br ddd*, 8.7, 2.9, 2.0)	7.445 (*d*, 8.6)	7.616 (2H, *br ddd*, 8.7, 3.0, 2.1)	7.471 (*d*, 8.9)	-
6″, 8″	6.804 (2H, *br ddd*, 8.7, 2.9, 2.0)	6.875 (*d*, 8.6)	6.750 (2H, *br ddd*, 8.7, 3.0, 2.1)	6.903 (*d*, 8.9)	-

[a] Some proton data were deduced from HMQC.

[b] Data were recorded on 360 MHz in CD_3OD. The signals of CD_3OD at δ 3.305 ppm were used as reference.

[c] Data were recorded on 500 MHz in D_2O. The signal of D_2O at δ 4.800 ppm was used as reference.

[d] Data were recorded on 300 MHz in D_2O; from Jensen, *et al*. 2002.[41]

[e] Signals overlapped with solvent signals.

Table 2

^{13}C NMR data [δ in ppm, mult] of compounds **1**, **2** and **4** in CD_3OH[a] and D_2O[b]

pos.	1[a]	2[b]	4[c]
1	96.14 *d*	95.05 *d*	95.6 *d*
3	153.28 *d*	152.50 *d*	153.4 *d*
4	112.47 *s*	111.57 *s*	112.4 *s*
5	35.21 *d*	32.40 *d*	32.5 *d*
6	31.57 *t*	29.67 *t*	30.1 *t*
7	37.47 *t*	35.61 *t*	35.7 *t*
8	81.66 *s*	80.37 *s*	82.8 *s*
9	46.87 *d*	45.66 *d*	45.7 *d*
10	70.88 *t*	69.55 *t*	68.2 *t*
11	170.74 *s*	171.25 *s*	172 *s*
1′	100.88 *d*	98.88 *d*	99.4 *d*
2′	74.62 *d*	72.72 *d*	73.3 *d*
3′	77.88 *d*	75.72 *d*	76.3 *d*
4′	71.22 *d*	70.14 *d*	70.1 *d*
5′	78.40 *d*	76.38 *d*	77.0 *d*
6′	62.49 *t*	60.78 *d*	61.3 *d*
1″	169.23 *s*	169.22 *s*	-
2″	114.89 *d*	116.89 *d*	-
3″	147.03 *d*	144.32 *d*	-
4″	127.13 *s*	127.31 *s*	-
5″, 9″	131.32 *d*	131.51 *d*	-
6″, 8″	116.85 *d*	115.34 *d*	-
7″	161.39 *s*	156.83 *s*	-

[a] 90 MHz in CD_3OD.

[b] 90 MHz in D_2O.

[c] 75 MHz in D_2O; from Jensen, *et al.* 2002.[41]

Table 3

NMR data of compounds **3** and **5.**

pos.	$\delta_H(3)^a$	$\delta_H(5)^b$	$\delta_C(3)^a$	$\delta_C(5)^b$
1	-		107.14 *s*	107.7
2	-		158.63 *s*	158.5
3	6.251 (*d*, 2.3)	6.18 (*d* 2.2)	101.02 *d*	101.2
4	-		158.42 *s*	158.6
5	6.135 (*d*, 2.3)	6.27 (*d* 2.2)	102.12 *d*	102.1
6	-		152.40 *s*	152.4
7	3.479 (*brs*)	3.47 *s* (2H)	29.89 *t*	30.3
8	-		174.34 *s*	176.1
1′	-		121.65 *s*	121.8
2′	7.517 (*dd* 2.0, 0.4)	7.55 (*d* 8.2)	117.81 *d*	117.9
3′	-		146.45 *s*	146.4
4′	-		152.51 *s*	152.5
5′	6.863 (*dd* 8.0, 0.4)	6.86 (*d* 8.2)	116.09 *d*	116.1
6′	7.529 (*dd*, 8.0, 2.0)	7.56 (*dd*, 8.2, 2.2)	124.37 *d*	124.5
7′	-		166.33 *s*	166.4
MeO	3.550 *s*	-	52.33 *q*	-

[a] 360 MHz for ^1H and 90 MHz for ^{13}C in CD_3OD

[b] 500 MHz for ^1H and 125 MHz for ^{13}C in CD_3OD, from Hillenbrand *et al.*[42]