JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2008,** *56,* 10105–10114 **10105**

Identification of Hydroxycinnamoylquinic Acids of Arnica Flowers and Burdock Roots Using a Standardized LC-DAD-ESI/MS Profiling Method

LONG-ZE LIN* AND JAMES M. HARNLY

Food Composition and Methods Development Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, Building-161, BARC-East, U.S. Department of Agriculture, 103000 Baltimore Avenue, Beltsville, Maryland 20705

A screening method using LC-DAD-ESI/MS was developed for the identification of common hydroxycinnamoylquinic acids based on direct comparison with standards. A complete standard set for mono-, di-, and tricaffeoylquinic isomers was assembled from commercially available standards, positively identified compounds in common plants (artichokes, asparagus, coffee bean, honeysuckle flowers, sweet potato, and *Vernonia amygdalina* leaves) and chemically modified standards. Four C18 reversed phase columns were tested using the standardized profiling method (based on LC-DAD-ESI/MS) for 30 phenolic compounds, and their elution order and retention times were evaluated. Using only two columns under standardized LC condition and the collected phenolic compound database, it was possible to separate all of the hydroxycinnamoylquinic acid conjugates and to identify 28 and 18 hydroxycinnamoylquinic acids in arnica flowers (*Arnica montana* L.) and burdock roots (*Arctium lappa* L.), respectively. Of these, 22 are reported for the first time.

KEYWORDS: Arnica flowers; burdock roots; hydroxycinnamoylquinic acids; LC-DAD-ESI/MS; profiling method; elution order evaluation

INTRODUCTION

Hydroxycinnamoylquinic acids (**Figure 1**), especially chlorogenic acid [or 5-*O*-mono-(*E*)-caffeoylquinic acid] and other monocaffeoylquinic and dicaffeoylquinic acids, are widely found as the primary phenolic components in many food plants, such as fruits, vegetables, spices, and herbs. They demonstrate a variety of biological activities, such as radical-scavenging, antioxidant, anti-inflammatory, anti-HIV, and antihepatotoxic characteristics, inhibit mutagenesis and carcinogenesis, slow the aging process, and are considered to be beneficial to human health (*1*−*5*).

Chemically, each of the *E* (or *trans*)-hydroxycinnamic acids (more commonly known as hydroxycinnamic acids, such as caffeic acid) and (−)-quinic acid (1 *L*-OH using the IUPAC naming system) (*6*) (**Figure 1**) can form four (1-, 3-, 4-, and 5-)monocaffeoylquinic acids, six (1,3-, 1,4-, 1,5-, 3,4-, 3,5-, and 4,5-)dicaffeoylquinic acids, and four (1,3,4-, 1,3-,5-, 1,4,5-, and 3,4,5-)tricaffeoylquinic acids. Conceptually, the hydroxycinnamoylquinic acids can be divided into two groups (**Table 1**) that make discussion of elution order and retention times more systematic. Group I consists of those quinic acid conjugates that do not have a caffeoyl moiety at the 1-position. Thus, group I consists of the 3-, 4-, and 5-monocaffeoylquinic acids, the 3,4-, 3,5-, and 4,5-dicaffeoylquinic acids, and 3,4,5-tricaffeoylquinic acid. Group II consists of those quinic acids that have a caffeoyl moiety at the 1-position, such as 1-caffeoylquinic acid, the 1,3-, 1,4-, and 1,5-dicaffeoylquinic acids, and the 1,3,4-, 1,3,5-, and 1,4,5-tricaffeoylquinic acids.

Multiple hydroxycinnamoylquinic acid isomers usually coexist in plants, most notably in some plants from the family Compositae (or Asteraceae), making complete identification of the isomers difficult. Carefully targeted studies using liquid chromatography with tandem mass spectrometric detection (LC-MS^n) and appropriate standards or reference compounds have been used for the identification of many isomers (*7*−*17*). Technologically, positive identification based on MS^n fragmentation pattern analysis requires expensive instrumentation, a database for the fragmentation pattern of each isomer in a group, and sufficiently large analyte concentrations (sufficiently large peaks) to produce detectable ions after multiple collision processes ($n = 2$, 3, 4, or higher). However, even using MS^n, some of the isomers still cannot be positively identified (*7, 14*−*17*), which is similar to the limitation of tandem mass spectra for the assignment of some isomeric glycosylated flavonoids (*18, 26*).

Identification of the numerous hydroxycinnamoylquinic isomers is further complicated by the existence of their conjugation with glucose (or other sugars) and aliphatic acids. Glucosides of caffeoylquinic acids have been reported in chrysanthemum and several other plants (*7*). Methoxyoxalic acid, succinic acid, and 3-methyl-3-hydroxyglutaric acid conjugated hydroxycinnamoylquinic acids have been reported (*7, 19, 20*). Isomers

* Author to whom correspondence should be addressed [telephone (301) 504-9136; fax (301) 504-9136; e-mail longze.lin@ars.usda.gov].

10.1021/jf802412m
Published on Web 10/07/2008

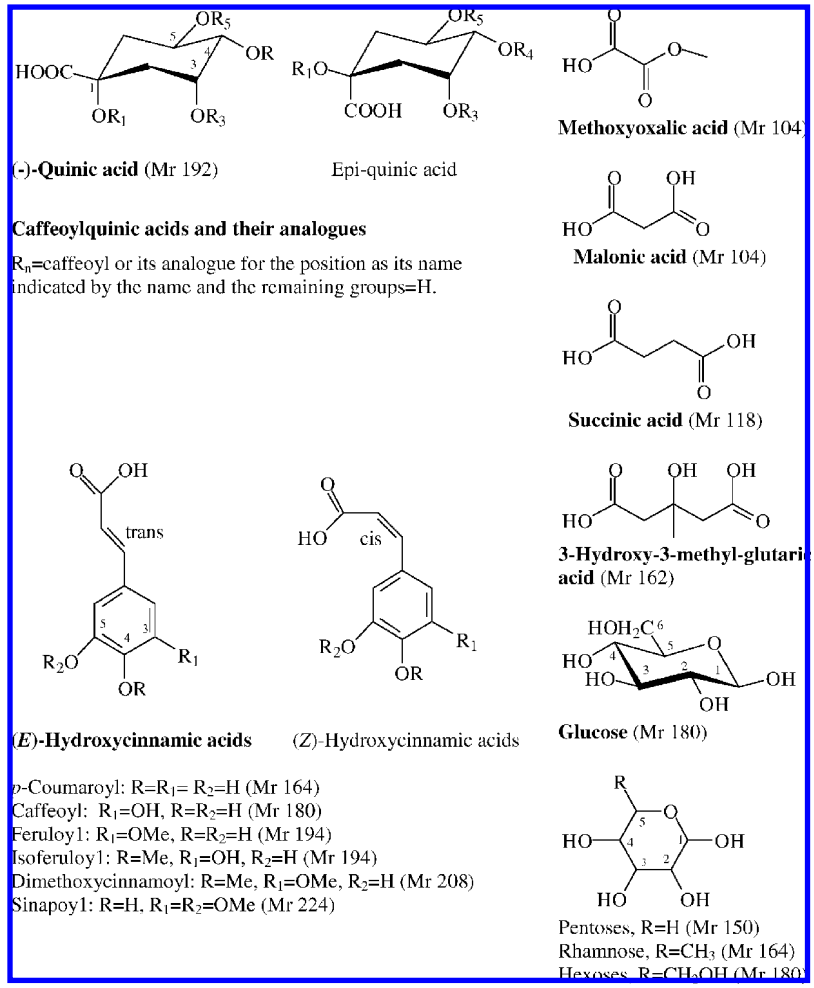

Figure 1. Structures of the hydroxycinnamoylquinic acids and their derivatives.

formed from (*Z*)-caffeic acid (also known as *cis*-caffeic acid) have also been found in some plants, even though they are much less common (*21, 22, 36*). The isomers formed from 1-*epi*-quinic acid (i.e., 1 *D*-OH) are very rare, but also reported (*7, 23*).

Two plant materials that contain the common caffeoylquinic acids and their novel derivatives are arnica flowers and burdock roots. Arnica flower (*Arnica montana* L.; Compositae) is a common herb that is used as a flavor component in food products such as beverages, desserts, candies, baked goods, and puddings. Dried arnica flower is available as a spice in the United States and commonly used as a diaphoretic, diuretic, and stimulant herb (*24*). Burdock root (*Arctium lappa* L.; Compositae), a vegetable in China and Japan, is used to make a general health tonic (*24*). The main phenolic components of these food plants are the hydroxycinnamoylquinic acids (*19, 20, 24, 25*).

A standardized profiling method based on LC separation with tandem diode array and MS detection (LC-DAD-ESI/MS) has been developed for the identification of phenolic compounds (*26*). This method acquires mass spectrometric data in the positive (PI) and negative ionization (NI) mode at low (70−100 V) and high (250 V) excitations energies. The NI low excitation energy provides the molecular ions, whereas the high-energy excitation provides fragments that indicate mono-, di-, or trihydroxycinnamoylquinic acids by the sequential loss of one or more hydroxycinnamoyls. Positive compound identification is obtained by comparing retention times and UV and MS data from sample peaks to those of known standards. This method has allowed the accumulation of a rich database for food phenolic compounds collected from the analysis of standards and routine profiling of plant foods and has also been used to identify them or to confirm their existence in new plant sources.

In this study, a collection of compounds composed of commercially available standards, and hydroxycinnamoylquinic acids found in common plants and foods, such as artichokes, asparagus, coffee beans, honeysuckle flowers, sweet potato, and *Vernonia amygdalina* leaves, and chemically modified standards were assembled. Using the profiling method, it was possible to identify 27 hydroxycinnamoylquinic acids in arnica flower and 18 in burdock root, 22 for the first time.

MATERIALS AND METHODS

Plant Materials. The dried arnica flowers (*A. montana* L.; plant origin, Germany) were obtained from Starwest Botanicals (Rancho Cordova, CA). Honeysuckle flowers were bought from Asia Natural Product, Inc. (San Francisco, CA). Dried nonroasted coffee beans (from Kenya) were purchased from Garry Burman Traders (Madison, WI). Fresh burdock roots, artichokes, and asparaguses were bought in local food stores in Maryland. The fresh food materials were cut into small pieces and freeze-dried. The dried materials were ground to a fine powder and passed through 20 mesh sieves prior to extraction.

Standards and Chemicals. Chlorogenic acid, caffeic acid, *p*-coumaric acid, sinapic acid, ferulic acid, and isoferulic acid were obtained from Sigma Chemical Co. (Saint Louis, MO). 1,3- and 1,5-dicaffeoylquinic acids were purchased from ChromaDex, Inc. (Santa Ana, CA). 3- and 4-mono- and 3,5-, 3,4-, and 4,5-dicaffeoylquinic acids were prepared or isolated in this laboratory (*26*).

Formic acid, sodium hydroxide, sodium bicarbonate, hydrochloric acid (37%), and HPLC grade solvents (acetonitrile, methanol) were

Table 1. Retention Times of Caffeoylquinic Acids and Some of Their Analogues in Tested Plants[a]

group and name of compound (peak no.)	t_R C1	C2	C3	C4
monocaffeoylquinic acid group II (M_r 354)				
1-caffeoylquinic acid (1)	6.2	10.2	5.1	7.0
monocaffeoylquinic acid group I (M_r 354)				
3-caffeoylquinic acid (2)	7.3	10.2	6.5	8.2
5-caffeoylquinc acid (3)	10.7	16.8	9.9	11.8
4-caffeoylquinic acid (4)	12.1	16.2	10.6	13.0
mono-*p*-coumaroylquinic acid group I (M_r 338)				
3-*p*-coumaroylquinic acid (5)	10.4	nd	9.7	nd
5-*p*-coumaroylquinc acid (7)	15.6	20.6	15.0	17.2
4-*p*-coumaroylquinic acid (8)	16.7	nd	15.3	17.9
monoferuloylquinic acid group I (M_r 368)				
3-feruloylquinic acid (6)	14.5	15.1	11.0	nd
5-feruloyllquinic acid (9)	18.0	23.9	17.1	19.5
4-feruloylquinic acid (10)	18.8	nd	17.8	nd
dicaffeoylquinic acid group II (M_r 516)				
1,3-dicaffeoylquinic acid (11)	17.9	25.5	16.7	18.5
1,4-dicaffeyolquinic acid (13)	29.9	40.8	28.8	31.9
1,5-dicaffeoylquinic acid (15)	31.7	42.7	30.7	33.0
dicaffeoylquinic acid group I (M_r 516)				
3,4-dicaffeoylquinic acid (12)	30.4	39.9	29.4	32.1
3,5-dicaffeoylquinic acid (14)	32.1	41.9	30.9	33.6
4,5-dicaffeoylquinic acid (16)	35.3	45.1	34.1	36.7
p-coumaroylcaffeoyl acid group I (M_r 500)				
3,4-*p*-coumaroylcaffeoylquinic acid (20)	37.2	46.9	34.1	37.9
3,4-*p*-coumaroylcaffeoylquinic acid (20)				
3-*p*-coumaroyl-5-caffeoyl-quinic acid (22)	38.5	48.3	36.0	39.9
3-caffeoyl-5-*p*-coumaroyl-quinic acid (23)	39.0	48.7	36.4	40.5
4-*p*-coumaroyl-5-caffeoylquinic acid (27)	42.2	50.3	39.3	43.3
4-caffeoyl-5-*p*-coumaroylquinic acid (28)	42.6	50.6	40.1	43.8
caffeoylferuloylquinic acid group I (M_r 530)				
3-feruloyl-4-caffeoylquinic acid (21)	38.4	46.5	35.5	38.8
3-caffeoyl-4-feruloylquinic acid (24)	39.7	46.8	36.7	39.9
3-feruloyl-5-caffeoyl-quinic acid (25)	40.7	48.4	38.4	41.5
3-caffeoyl-5-feruloyl-quinic acid (26)	41.3	48.6	39.0	42.2
4-feruloyl-5-caffeoylquinic acid (29)	43.5	49.9	40.9	43.9
4-caffeoyl-5-feruloylquinic acid (30)	44.1	50.1	41.6	44.5
tricaffeoylquinic acid group II (M_r 678)				
1,3,4-tricaffeoylquinic acid				
1,3,5-tricaffeoylquinic acid (17)	41.4	49.9	39.0	42.3
1,4,5-tricaffeoylquinic acid (18)	42.8	50.9	40.4	43.1
tricaffeoylquinic acid group I (M_r 678)				
3,4,5-tricaffeoylquinic acid (19)	48.8	53.8	47.4	49.3

[a] The isomeric caffeoylquinic acids of each group are listed in elution order; retention times were recorded with Waters Symmetry (C1), Waters SymmetryShield (C2), Agilent Zorbax Eclipse (C3), and Phenomenex Luna C18(2) (C4) columns.

purchased from VWR Scientific (Seattle, WA). HPLC grade water was prepared from distilled water by using Milli-Q (Millipore Laboratory, Bedford, MA).

Extraction Method. Dried ground material (100 mg of arnica flowers and 500 mg of burdock roots) was extracted with methanol/water (5.00 mL, 60:40, v/v) using an FS30 Ultrasonic sonicator (Fisher Scientific, Pittsburgh, PA) at 40 kHz and 100 W for 60 min at room temperature. The extract was filtered through a 0.45 μm nylon Acrodisk 13 filter (Gelman, Ann Arbor, MI). Volumes of 10 and 50 μL of the extracts were injected onto the analytical column for analysis, respectively.

Heated Extracts. The filtered extracts and standard solutions (1.0 mL) were heated in a capped glass tube with a heating block at 85 °C for 16 h to remove the methoxyoxaloyl group from the conjugates. Approximately 100 μL of each solution was taken from each tube after 1, 3, 6, 12, and 16 h of heating. After cooling at room temperature for 10 min, the solution was filtered as above before LC injection.

Caffeoyl Migration Reaction. Sodium bicarbonate powders (100 mg) were added to solutions of standards (1,3- or 1,5-dicaffoylquinic acid) or plant extract (0.5 mL). The solutions were shaken for 3 min to ensure the solution was saturated with sodium bicarbonate. The solutions were then filtered, heated in a covered glass tube at 85 °C for 10 min, cooled at room temperature for 10 min, adjusted to approximately pH 1 by adding of 1 drop of 10% HCl, and filtered again prior to LC analysis.

LC-DAD ESI/MS Conditions. The LC-DAD-ESI/MS consisted of an Agilent 1100 HPLC coupled to a diode array detector and mass spectrometer (MSD, SL mode) (Agilent Technologies, Palo Alto, CA). A 250 mm × 4.6 mm, 5 μm, Symmetry C18 column (Waters Corp., Milford, MA) with a 20 mm × 3.9 mm. i.d., 5 μm, Sentry guard column was used at a flow rate of 1.0 mL/min. The column oven temperature was set at 25 °C. The mobile phase consisted of a combination of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). The gradient was varied linearly from 10 to 26% B (v/v) in 40 min, to 65% B at 70 min, and finally to 100% B at 71 min and held at 100% B to 75 min. The DAD was set at 350, 310, 270, and 520 nm for real-time monitoring of the peak intensity, and full spectra (190−650 nm) were continuously recorded for plant component identification. Mass spectra were simultaneously acquired using electrospray ionization in the positive and negative ionization (PI and NI) modes at low and high fragmentation voltages (70 and 250 V) over the range of *m*/*z* 100−2000.

In this study, MS data were collected in both the total ion counting (TIC) and selective ion monitoring (SIM) modes. The SIM mode was used with NI at both low and high excitation energies (70 and 250 V) to detect the minor hydroxycinnamoylquinic acids more efficiently. NI ions were monitored at *m*/*z* 353 (monocaffeoylquinic acids), *m*/*z* 337 (*p*-coumaroylquinic acids), *m*/*z* 367 (feruloylquinic acids), *m*/*z* 515 (dicaffeoylquinic acids), *m*/*z* 677 (tricaffeoylquinic acids), and *m*/*z* 529 (caffeoylferuloylquinic acid). Detection in SIM mode allowed deconvolution of coeluting compounds (based on different molecular ions) and produced detection limits 10 times lower than those achievable in the TIC mode (*18, 26*).

RESULTS AND DISCUSSION

Hydroxycinnamoylquinic Acid Standards and Reference Compounds. Chlorogenic acid and 1,3- and 1,5-dicaffeoylquinic acids are available commercially. Two monomers, 3- and 4-caffeoylquinic acids, were prepared by isomerization of chlorogenic acid (300 mg in 10 mL of saturated sodium bicarbonate water solution at 90 °C for 30 min) (*27*) and isolated on a C18 column, as previously described (*26*).

Three dimers, 3,5-, 3,4-, and 4,5-dicaffeoylquinic acids, with a limited amount of 3- and 4-caffeoylquinic acids were extracted from honeysuckle flower, a Chinese traditional herb (**Figure 2A**) and isolated on a C18 column (*26*). The isolated standards were identified by comparing their proton nuclear magnetic resonance (^{1}H NMR) spectra with published data (*28, 29*).

Both 1,4-dicaffeoylquinic acid and 1-caffeoylquinic acid were formed by caffeoyl migration and partial decaffeoylation of 1,3-dicaffeoylquinc acid after 10 min of heating at 85 °C. The LC chromatogram is shown in **Figure 2C**. The latter compound was also reported to be formed by partial acid hydrolysis of 1,3-dicaffeoylquinic acid (*12*).

Artichokes (*Cynara scolymus* L.; Compositae) make an excellent reference because they contain all four monocaffeoylquinic acids and all six dicaffeoylquinic acids (**Figure 2D**) (*17*).

Nonroasted coffee beans are also an excellent reference material (**Figures 2B** and **3**). They contained over 30 hydroxycinnamoylquinic acids, including hetero-di-isomers such as caffeoylferuloylquinic acids and *p*-coumaroylcaffeoylquinic acids, which were positively identified by Clifford and coworkers using MS^n fragmentation patterns (*8*−*10*). Nonroasted coffee beans were analyzed (**Figures 2B** and **3**), and 26 hydroxycinnamoylquinic acids were detected. As shown in **Figure 2B**, 4- and 5-mono-*p*-coumaroyl- and feruloylquinic acids were detected in coffee extract, but their 3-isomers were

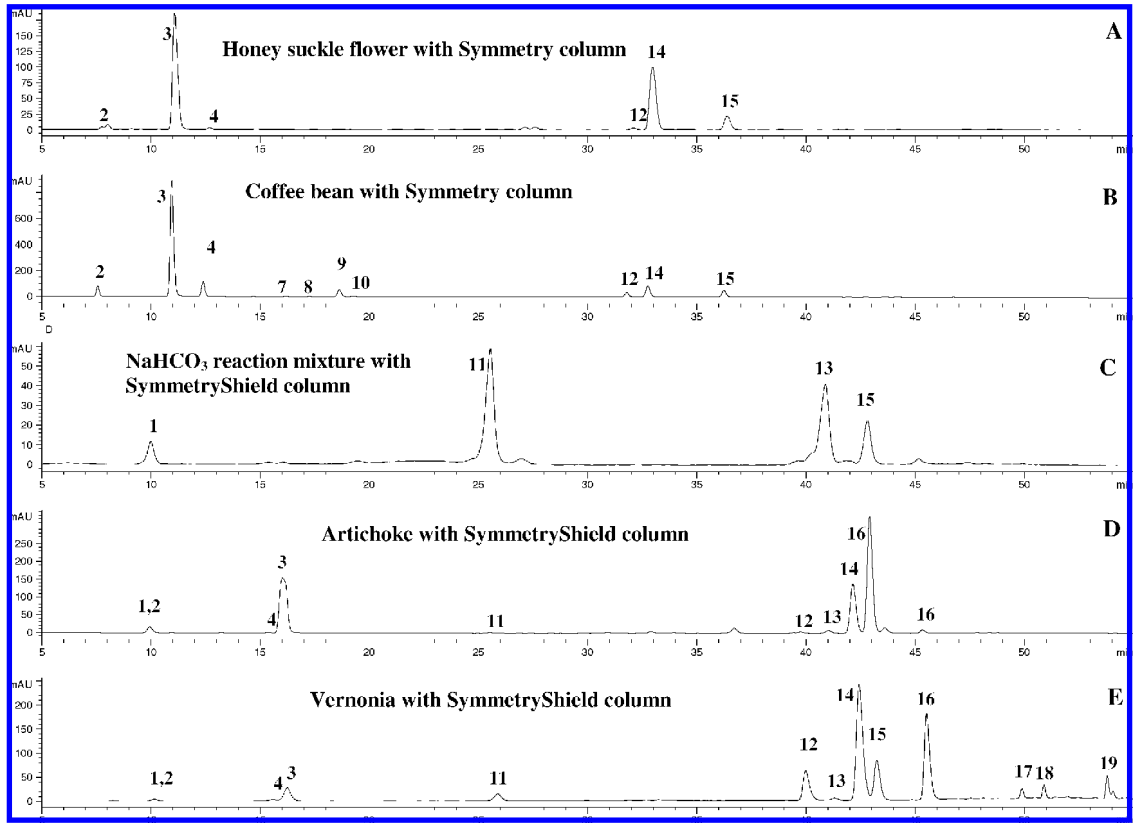

Figure 2. LC chromatograms (350 nm) of (**A**) honeysuckle flowers, (**B**) coffee bean, (**C**) the mixture of caffeoyl migration reaction of 1,3-dicaffeoylquinic acid with $NaHCO_3$, (**D**) the extracts of artichokes, and (**E**) *Vernonia amygdalina* leaf extract. Peaks assignments are listed in **Table 1**.

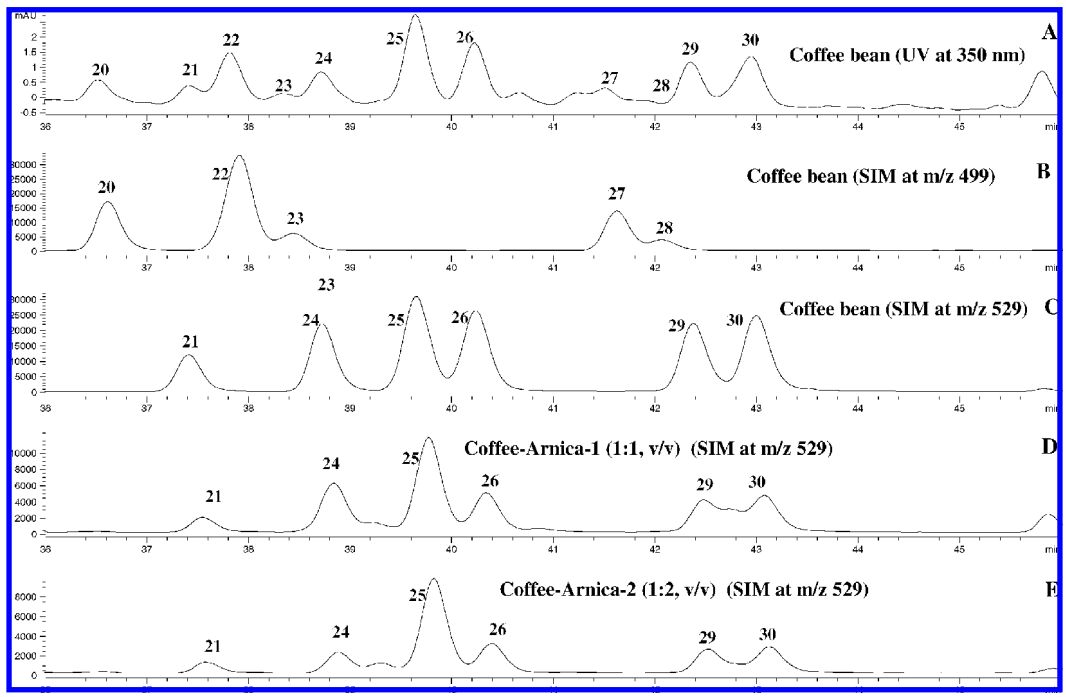

Figure 3. (**A**) Expanded LC (at 350 nm, Symmetry column) and (**B**) NI SIM chromatograms at *m/z* 499 and (**C**) *m/z* 529 of coffee bean extract and its (**D**) 1:1 and (**E**) 1:2 spiked samples with arnica flower extract. Peak assignments are listed in **Table 1**.

not clearly detected. However, they were found in some of the plant foods screened in this laboratory, such as asparagus (*Asparagus officinalis* L.; Liliaceae), in which 3-*p*-coumaroyl-, feruloyl- and caffeoylquinic acids all dominate their groups (**Figure 4**).

1,4,5-Tricaffeoylquinic acid in arnica flower and 3,4,5-tricaffeoylquinic acid in sweet potato leaves were isolated and identified using NMR (*5, 25*). A third isomer, 1,3,5-tricaffeoylquinic acid, has been isolated from the flowers of *Xanthium strumarium* L. (Compositae) (*31*), and its succinoyl derivative has also been isolated from burdock roots (*20*). Both were identified by NMR. The three tricaffeoylquinic acids have been identified in the leaves of *V. amygdalina* L. (Compositae), an African vegetable and herb previously analyzed (**Figure 2E**).

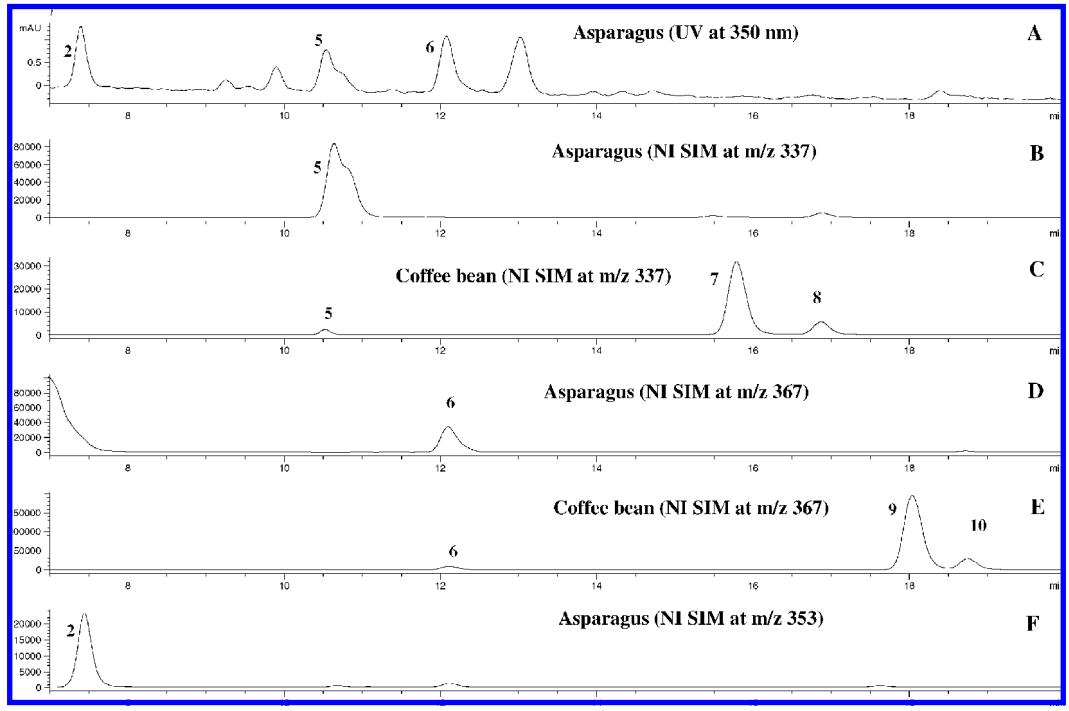

Figure 4. (**A**) Expanded LC at 350 nm (Symmetry column) and (**B**) NI SIM chromatograms of asparagus extract at *m/z* 337, (**D**) *m/z* 367, and (**F**) *m/z* 353) and (**C**) coffee bean extract at *m/z* 337 and (**E**) *m/z* 367. Peak assignments are listed in **Table 1**.

However, the existence of 1,3,4- or 1,4,5-tricaffeoylquinic acid in herbal chrysanthemum has not been identified by a systematic tandem MS study (*7*).

Elution Order of Common Hydroxycinnamoylquinic Acids. The 30 standards described above and listed in **Table 1** were analyzed using the standardized LC-DAD-ESI/MS profiling method. Their retention times and UV and mass data were recorded for four different columns. Hydroxycinnamoylquinic acids in new plant materials, such as arnica flowers and burdock roots, were positively identified by matching peak retention times and UV and MS spectra to the standards. The identification of the peaks was also further confirmed by spiking the extract with the standards as shown in **Figure 3D**,**E**. This is part of the normal procedure used for positive identification with the standardized profiling method (*26*).

Chromatographic separation of the 13 caffeoylquinic acid isomers and 17 other hydroxycinnamoylquinic acids was optimized for four reversed phase C18 columns (Symmetry, SymmetryShield, Luna, and Zorbax Eclipse XDB-C18) (all C18, 5 μm, 250 mm $\times$ 4.6 mm i.d.) with the standardized LC condition. The results in **Table 1** show that the Symmetry column could separate all of the monocaffeoylquinic acids and the isomers of each group, but could not separate the six dicaffeoylquinic acids. The SymmetryShield column could separate the six dicaffeoylquinic acids, but could not separate some of the monocaffeoylquinic acids. Use of the Symmetry and SymmetryShield columns together allowed the four mono- and six dicaffeoylquinic acids to be detected and recognized (**Figure 2B**,**C**).

Some general patterns can be seen in the elution order of the compounds in **Table 1**. The isomers of the monocaffeoylquinic acids of group I elute in the order of the 3-, 5-, and 4-isomer on three of the four columns. This pattern also holds for the other monohydroxycinnamoylquinic acids, such as mono-*p*-coumaroylquinic and monoferuleoylquinic acids in group I. For the dicaffeoylquinic acids of group II, the 1,3-isomer eluted first, then the 1,4-isomer, and finally the 1,5-isomer. For the dicaffeoylquinic acids of group I, the 3,4-isomer eluted first, then the 3,5-isomer, and finally the 4,5-isomer. The elution order for all six dicaffeoylquinic acids on the SymmetryShield column was 1,3-, 3,4-, 1,4-, 3,5-, 1,5-, and 4,5-isomer.

This elution order also appears to be valid for the di-isomer formed from two different phenolic acids. As shown in **Table 1**, the 3,4-mixed isomers always eluted before the 3,5-mixed isomers and were followed by the 4,5-mixed isomers. This order was maintained regardless the identity of the phenolic acids. In each pair, for example, 3-feruloyl-5-caffeoylquinic acid and 3-caffeoyl-5-feruloylquinic acid, the elution order was made by referring to the elution orders for them or their analogues, recorded on Phenonemex Luna or Kromasli C18 columns (*8*−*10*, *13*). It is interesting to note that when the equatorial positions (4 for the 3,4-isomer and 5 for the 3,5- and 4,5-isomers) of quinic acid were connected with the relatively more polar phenolic acid (caffeoyl), elution occurred earlier. Thus, 3-feruloyl-5-caffeoylquinic acid eluted earlier than 3-caffeoyl-5-feruloylquinic acid.

The elution order observed in this study for the di-isomers suggested that two isomers identified in *Artemisia annua* using MS^2 and MS^3 could be incorrect. 4-Feruloyl-5-caffeoylquinic acid elutes earlier than the two 3,5-isomers and 3-caffeoyl-4-feruloylquinic acid elutes later than the two 3,5-isomers on the Agilent Zorbax-C18 column (*16*), which was different from the elution order obtained in this study (**Table 1**).

The grouping of the hydroxycinnamoylquinic acids proposed in the introduction also reflects the plant taxonomy. In general, some plants from the family Compositae (or Asteraceae), such as arnica flower (*19, 25*), burdock root, artichoke (*17*), chrysanthemum flower (*7*), and others (*21*−*23*), contain caffeoylquinic acids from both groups I and II as their main phenolic components. The plants from most other taxonomic families, such as coffee beans (*Coffea arabica* L. or *C. robusta* L.; Rubiaceae) (*8*−*10*), *Hemerocallis* spp. (Liliaceae) (*11*), honeysuckle flowers (*Lonicera japonica* L.), trumpet honeysuckle flowers (*Lonicera sempervirens* Blanche Sandman; Capri-

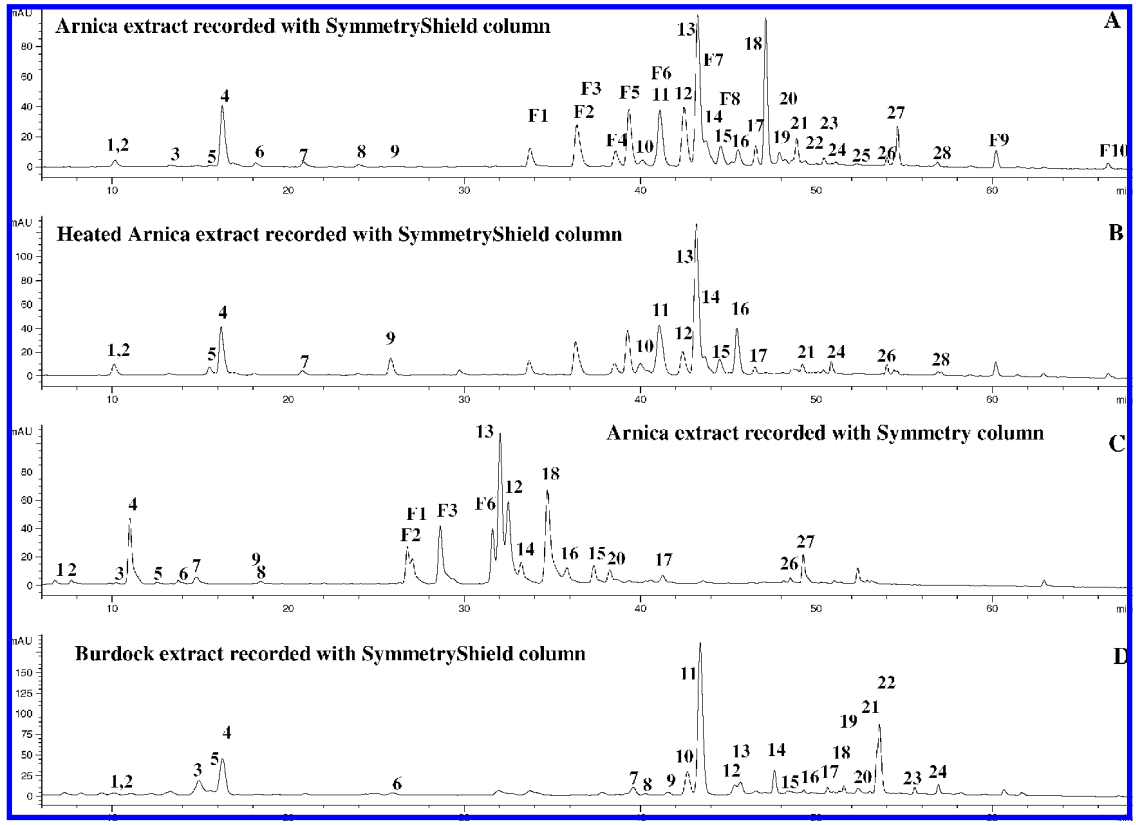

Figure 5. LC chromatograms of arnica flower extract (**A**, **C**), (**B**) heated extract, and (**D**) burdock roots. Chromatograms **A**, **B**, and **D** were recorded on a SymmetryShield column, whereas chromatogram **C** was recorded on a Symmetry column. Peak assignments are given in **Tables 2** and **3**, respectively. Peaks F1−F10 are for the flavonoinds of arnica, but the identifications are omitted.

foliaceae) (*26, 30, 32*), sweet potato leaves (*Ipomoea batatas* L.; Dioscoreaceae) (*5*), potatoes (*Solanum tuberosum* L.; Salonaceae), elder flowers (*Sambucus canadensis* L., Ericaceae), Fuji apples (*Malus domesestica* Borkh. cv. Fuji; Rosaceae) (*26*), and green tea (*Camellia sinensis* L., Theaceae) (*33*), contain mainly the isomers in group I. There are some reported exceptions. For example, fennel and its seeds (*Foeniculum vulgare* Mill; Apiaceae) contain both group I and II isomers as their main phenolic components (*34, 35*).

General patterns were also observed in the concentration levels (peak intensity) of the caffeoylquinic acid isomers in the plants analyzed in this study. If all three monocaffeoylqunic acids of group I were detected in the extract, the 5-isomer was always more abundant than the 4- or 3-isomer. Similarly, the order of abundance for the group II isomers was 1,5- > 1,4- > 1,3-isomer. For the group I isomers, the order was 3,5- > 4,5- > 3,4-isomer. Thus, chlorogenic acid and 3,5- and 1,5-dicaffeoylquinic acid are the most abundant, generally. Compared to coexisting mono- and dicaffeoylquinic acids, the tricaffeoylquinic acids are always the least abundant. There are some exceptions; for example, in asparagus, the 3-isomer was most abundant.

Hydroxycinnamoylquinic Acids in Arnica Flowers. Two columns [SymmetryShield and Symmetry (**Figures 5A−C**)] were used to separate all phenolic compounds (including 10 flavonoids) of arnica flower extract and its heating extract (**Figure 5B**). **Table 2** lists the analytical data (t_R, λ_{max}, deprotonated molecules $[M - H]^-$, and major diagnostic fragments) obtained with the SymmetryShield column. Twenty-eight peaks shown in **Figure 5A**,**C** and **Table 2** display typical caffeic acid UV absorption (λ_{max} 240, 300sh, 326 nm), suggesting that they are caffeic acid conjugates. Peak 7 ($[M - H]^-$ at *m*/*z* 179 and an ion at *m*/*z*135 by loss of CO_2) was identified as caffeic acid by comparison to a standard. Peak 3 ($[M - H]^-$ at *m*/*z* 341 and two major fragments at *m*/*z* 179 and 135) was tentatively identified as a caffeic acid hexoside and is most likely caffeic acid-4-glucoside instead of the isomeric 3-glucoside, or one of the five possible caffeoylglucoses (*7*).

Seventeen of the chromatographic peaks in **Figure 5** are recognizable as hydroxycinnamoylquinic acids (**Table 2**). They contained one or more hydroxycinnamoyls; the type and number can be directly deduced from the mass spectrometric data. On the basis of retention times, deprotonated masses, and fragment masses that matched those for the standards (**Table 1**), peaks 1, 2, 4, and 5 were easily identified as 1-, 3-, 4-, and 5-monocaffeoylquinic acids (*m*/*z* 353, 191, and 173 for quinic acid and its daughter ion by loss of H_2O, 179 and 135 for caffeic acid and its daughter ion by loss of COO, and 161 for caffeoyl), respectively. Similarly, peaks 9, 10, 11, 12, 13, and 16 were identified as 1,3-, 3,4-, 1,4-, 3,5-, 1,5-, and 4,5-dicaffeoylquinic acids (*m*/*z* 515, 353 by loss of caffeoyl and other ions for monocaffeoylquinic acids), and peaks 24 and 26 were identified as 1,4,5-, and 3,4,5-tricaffeoylquinic acids (*m*/*z* 677, and the diagnostic ions shown in **Figure 6**), respectively.

Similarly, peak 8 [molecular ion at *m*/*z* 367, 193 (ferulic acid ion), 191 (quinic acid), 149 (ferulic acid ion − COO)] was identified as 5-feruloylquinic acid, peak 6 [*m*/*z* 397, 223 (sinapic acid ion), 191, 179 (sinapic acid ion − COO)] was identified as 5-sinapoylquinic acid, and peak 21A was identified as 3-feruloyl-5-caffeoylquinic acid. The positive identification of peak 21A was based on the enhanced ratios of 3-feruloyl-5-caffeoylquinic acid to its isomers in coffee bean extract when arnica extract was spiked. As shown in **Figure 3C−E**, after arnica extract was added to coffee extract (1:1 v/v), peak 25 (3-feruloyl-5-caffeoylquinic acid) was significantly increased

Table 2. Peak Assignments of the Extracts of Arnica Flower[a]

peak	t_R, C2 (min)	$[M - H]^-$ (*m*/*z*)	diagnostic NI ions (*m*/*z*)	UV λ_{max} (nm)	identification
1	10.1	353	191, 179, 173, 135	240, 300sh, 328	1-caffeoylquinic acid[b,c]
2	10.1	353	191, 179, 173, 135	240, 300sh, 328	3-caffeoylquinic acid[c]
3	13.4	341	179, 135	240, 300sh, 328	caffeic acid 4-glucoside
4	16.3	353	191, 179, 173, 135	240, 300sh, 328	4-caffeoylquinic acid[b,c]
5	16.9	353	191, 179	240, 300sh, 328	chlorogenic acid[b,c]
6	18.1	397	223, 191, 179	240, 300sh, 326	5-sinapoylquinic acid
7	20.9	179	179, 135	240, 322	caffeic acid[b,c]
8	23.7	367	193, 191, 149	240, 324	5-feruloylquinic acid[b]
9	25.5	515	353, 191, 179, 135	nd[d]	1,3-dicaffeoylquinic acid[b,c]
10	39.0	515	353, 191, 173, 161, 135	240, 300sh, 328	3,4-dicaffeoylquinic acid[b,c]
11	40.8	515	353, 191, 173, 161, 135	240, 300sh, 328	1,4-dicaffeoylquinic acid[b,c]
12	41.9	515	353, 191, 179, 161, 135	240, 300sh, 330	3,5-dicaffeoylquinic acid[b,c]
13	42.7	515	353, 191, 179	240, 300sh, 330	1,5-dicaffeoylquinic acid[b,c]
14	43.7	559	397, 223, 191, 179	240, 300sh, 328	3-sinapoyl-5-caffeoylquinic acid
15	44.6	559	397, 223, 191, 179	240, 300sh, 328	4-sinapoyl-5-caffeoylquinic acid
16	45.5	515	353, 179	240, 300sh, 328	4,5-dicaffeoylquinic acid[b,c]
17	46.5	645	601, 397, 223, 191, 179	240, 300sh, 328	3-sinapoyl-5-caffeoyl-1-methoxyoxaloylquinic acid
18	47.1	601	557, 395, 353, 233, 191 179	240, 300sh, 328	1,5-dicaffeoyl-3-methoxyoxaloylquinic acid
19	47.9	645	601, 397, 223, 191, 179	240, 300sh, 328	4-sinapoyl-5-caffeoyl-1-methoxyoxaloylquinic acid
20	48.2	601	557, 395, 353, 233, 191, 179	240, 300sh, 328	3,5-dicaffeoyl-1-methoxyoxaloylquinic acid[b]
21A	48.7	529	367, 193, 191, 179	240, 300sh, 328	3-feruloyl-5-caffeoylquinic acid
21B	48.9	601	557, 395, 353, 233, 191, 179	240, 300sh, 328	4,5-dicaffeoyl-1-methoxyoxaloylquinic acid
22	49.3	645	601, 397, 223, 191, 179	240, 300sh, 328	3-sinapoyl-5-caffeoyl-4-methoxyoxaloylquinic acid
23	50.4	687	643, 395, 353, 191, 179, 161	240, 300sh, 328	1,5-dicaffeoyl-3,4-dimethoxyoxaloylquinic acid
24	50.9	677	515, 497, 353, 191, 179	240, 300sh, 328	1,4,5-tricaffeoylquinic acid[b]
25	51.1	615	515, 353, 191, 179	240, 300sh, 328	dicaffeoylsuccinylquinic acid
26	54.0	677	515, 353,	240, 300sh, 328	3,4,5-tricaffeoylquinic acid
27	54.6	763	719, 601, 557, 395, 179	240, 300sh, 328	1,4,5-tricaffeoyl-3-methoxyoxaloylquinic acid

[a] Recorded with a SymmetryShield column; detection limitation as 0.003% for arnica. [b] Previously reported. [c] Identified with standards or reference compounds. [d] nd, not determined or unidentified.

Table 3. Peak Assignments of the Extracts of Burdock Root (SymmetryShield Column)[a]

peak	t_R, C2 (min)	$[M - H]^-$ (*m*/*z*)	diagnostic ions (*m*/*z*)	UV λ_{max} (nm)	identification
1	10.1	353	191, 179, 173, 135	240, 300sh, 328	1-caffeoylquinic acid[b]
2	10.1	353	191, 179, 173, 135	240, 300sh, 328	3-caffeoylquinic acid[b]
3	14.9	415 or 325	316	nd[d]	
4	16.3	353	191, 179, 173, 135	240, 300sh, 328	4-caffeoylquinic acid[b]
5	16.9	353	191, 179	240, 300sh, 328	chlorogenic acid[b,c]
6	25.5	515	353, 191, 179, 135	nd	1,3-dicaffeoylquinic acid[b]
7	39.6	631	353, 191, 179	240, 300sh, 328	caffeoylquinic acid glycoside
8	40.0	515	353, 191, 173, 161, 135	240, 300sh, 328	3,4-dicaffeoylquinic acid[b]
9	40.8	515	353, 191, 173, 161, 135	240, 300sh, 328	1,4-dicaffeoylquinic acid[b]
10	41.9	515	353, 191, 179, 161, 135	240, 300sh, 330	3,5-dicaffeoylquinic acid[b]
11	42.7	515	353, 191, 179	240, 300sh, 330	1,5-dicaffeoylquinic acid[b,c]
12	45.5	515	353, 191, 179, 173, 135	240, 300sh, 328	4,5-dicaffeoylquinic acid[b]
13	45.4	631	353, 191, 179, 173, 135	240, 300sh, 328	caffeoylquinic acid glycoside
14	47.6	615	497, 453, 353, 191	240, 300sh, 328	1,5-dicaffeoyl-3-succinoylquinic acid[c]
15	49.3	615	497, 453, 353, 191	240, 300sh, 328	1,5-dicaffeoyl-4-succinoylquinic acid[c]
16	49.9	677	515, 497, 353, 335, 191, 179, 161	240, 300sh, 328	1,3,5-tricaffeoylquinic acid
17	50.6	793	631, 469,191	240, 300sh, 328	caffeoylquinic acid glycoside
18	51.3	715	553, 391, 291, 191	nd	1,5-dicaffeoyl-3,4-disuccinoylquinic acid[c]
19	51.6	793	631, 469, 191	240, 300sh, 328	caffeoylquinic acid glycoside
20	53.4	793	631, 469, 191	240, 300sh, 328	caffeoylquinic acid glycoside
21	53.6	777	615, 453, 191, 179	240, 300sh, 328	1,3,5-dicaffeoyl-4-succinoylquinic acid[c]
22	54.0	677	515, 353	240, 300sh, 328	3,4,5-tricaffeoylquinic acid
23	55.6	477	179	240, 300sh, 328	nd
24	56.9	535	179, 161, 135	240, 300sh, 328	nd

[a] The phenolic component detection limitation was 0.0002% for burdock roots. [b] Identified with standards or reference compounds. [c] Previously reported. [d] nd, not determined or unidentified.

(**Figure 3D**) compared to that in **Figure 3C** (no arnica extract was added) and was much greater in **Figure 3E** with arnica extract to coffee ratio of 2.

Minor peaks 14 and 15 have $[M - H]^-$ at *m*/*z* 559 and fragments at *m*/*z* 397 (loss of one caffeoyl), 353 (loss of sinapoyl), 223 (sinapic acid ion), 191(quinic acid ion), and 179 (caffeic acid ion or sinapic acid ion − COO) to suggest that they were sinapoylcaffeoylquinic acids. The former might be 3-sinapoyl-5-caffeoylsinapoylquinic acid, and the latter might be 4-sinapoyl-5-caffeoylquinic acids formed in the same biogenetic way as 3-feruloyl-5-caffeoylquinic acid detected in this plant. Unfortunately, the positions of the two hydroxycinnamoyls could not be completely assigned because reference compounds for the isomers of each pair were not available for a direct comparison.

As shown in **Figure 6A−C**, the NI mass spectra for three tricaffeoylquinic acids showed $[M - H]^-$ at *m*/*z* 677, and diagnostic fragments at *m*/*z* 515 (loss of the first caffeoyl), *m*/*z*

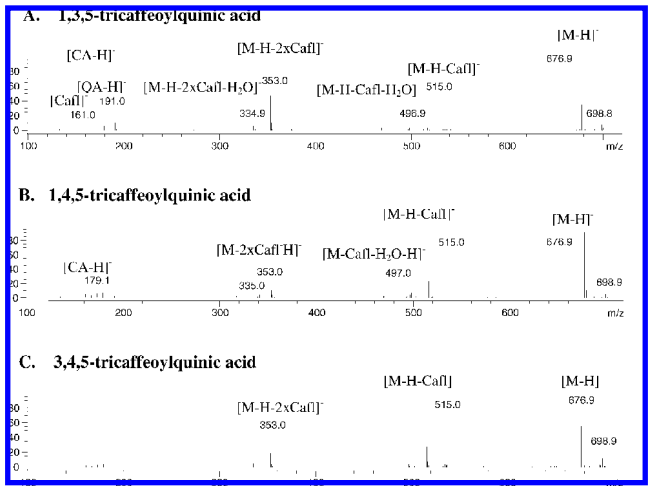

Figure 6. Mass spectra of (**A**) 1,3,5-, (**B**) 1,4,5-, and (**C**) 3,4,5-tricaffeoylquinic acids obtained from NI 250 V MS chromatogram. Cafl, caffeoyl; QA, quinic acid; CA, caffeic acid.

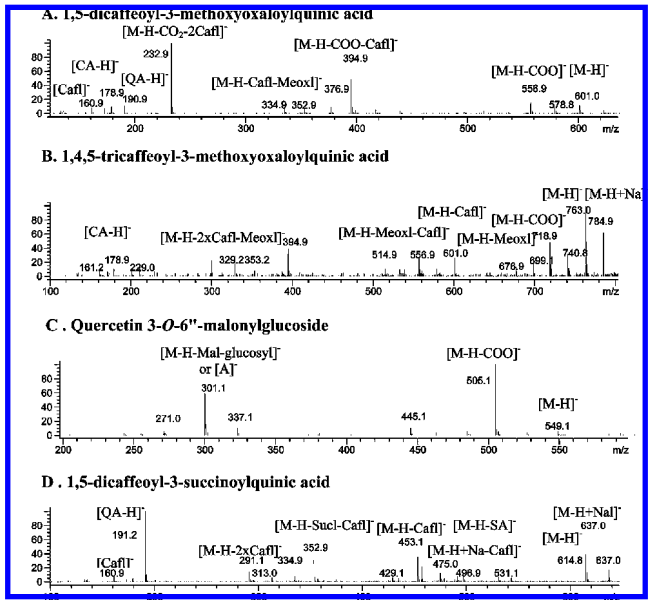

Figure 7. Mass spectra of (**A**) 1,5-dicaffeoyl-3-methoxyoxaloylquinic acid, (**B**) 1,4,5-tricaffeyol-3-methoxyoxaloylquinic acid, (**C**) quercetin 3-*O*-6-malonylglucoside in pear skin (*36*), and (**D**) 1,5-dicaffeoyl-3-succinoylquinic acid obtained from NI 250 V MS chromatogram. Meoxl, methothoxyoxaloyl; SA, succinic acid; Sucl, succinoyl; Mal, malonyl.

353 (loss of the second caffeoyl), *m/z* 191 (loss of the third caffeoyl to give quinic acid ion), *m/z* 179 (caffeic acid ion), *m/z* 161 (caffeoyl), and *m/z* 135 (the fragment of caffeic acid ion by loss of COO), which indicated that they were tricaffeoylquinic acids. These mass spectra also show the differences between the isomers, primarily in terms of the relative intensities of fragment ions, such as 3,4,5-dicaffeoylquinic acid with 1,3,5- and 1,4,5-tricaffeoylquinic acids,. It might be possible to use the relative intensities to distinguish between the isomers, although it is not required in this case because their positive identification was made by direct comparison (spiking) with reference compounds.

There were nine aliphatic acid-containing hydroxycinnamoylquinic acids detected in arnica. Peak 25 was identified as a dicaffeoylsuccinylquinic acid ([M − H]$^-$ at 615) by direct comparison of its mass data with those shown in **Figure 7D** for the positively identified 1,5-dicaffeoyl-3-succinoloylquinic acid in burdock roots (*20*). The eight remaining peaks were considered to be methoxyoxaloyl-(or malonyl-)hydroxycinnamoylquinic acid derivatives with one (plus 86 amu to the [M − H]$^-$) or two methoxyoxaloyls (plus 176 amu).

1-Methoxyoxaloyl-3,5-dicaffeoylquinic acid has been isolated from arnica flower and identified by proton and carbon NMR (in DMSO-d_6) analysis and showed the fragments of *m/z* 557 ([M − COOH]$^-$ or [M − H − COO]$^-$), and 395 ([M − caffeoyl − COOH]$^-$) as its major negative ion mass fragments (*19*).This means that this compound easily loses COO (44 amu) instead of COOMe (59 amu) (**Figure 1**). The fragment of [M − H − COO]$^-$ was frequently found as the major negative ionization (NI) mass fragment for many flavonoid malonylglycosides, for example, quercetin 3-*O*-malonylglucoside (**Figure 7C**), because they have a COOH in their malonyl group.

Like the reported mass major fragments for 1-methoxyoxaloyl-3,5-dicaffeoylquinic acid (*19*), peaks 18, 20, and 21B had the characteristic fragments of [M − H − COO]$^-$ at *m/z* 557 and [M − H − COO − caffeoyl]$^-$ at *m/z* 395, [M − H − COO − 2 × caffeoyl]$^-$ at *m/z* 233, [M − H + Na − COO]$^-$ at *m/z* 579, [M − H]$^-$ at *m/z* 601, and [M − H + Na]$^-$ at *m/z* 623, as well as other ions at *m/z* 353, 191, 179, and 161 (**Figure 7A**), suggesting that they were methoxyoxaloyl-dicaffeoylquinic acids.

As shown in **Figure 7B**, peak 27 showed its molecular ion at *m/z* 763, [M − H+Na]$^-$ at *m/z* 785, fragments of [M − H − COO]$^-$ at *m/z* 719, [M − H − caffoeyl] $^-$ at *m/z* 601, [M − H − COO − caffeoyl]$^-$ at *m/z* 557, [M − H − COO − 2 × caffeoyl]$^-$ at *m/z* 395, [M − H + Na − COO]$^-$ ions at *m/z* 741, [M − H + Na − methoxyoxalic acid] at *m/z* 699, and other ions at *m/z* 515, 353, 179, and 161, suggesting that this peak was a tricaffoeyl-methoxyoxaloylquinic acid. Peak 23 showed its [M − H]$^-$ at *m/z* 687, [M − H − COO]$^-$ at *m/z* 643, and other diagnostic fragments at *m/z* 353, 191, and 179, suggesting a dicaffeoyldimethoxyoxaloylquinic acid.

Similarly, peaks 17, 19, and 22 have their [M − H]$^-$ at *m/z* 645, [M − H − COO]$^-$ at *m/z* 601, and other diagnostic fragments at *m/z* 397, 353, 223, 191, and 179 and were identified as methoxyoxaloylcaffeoylsinapoylquinic acids.

Among the eight detected derivatives in the extract, 1-methoxyoxaloyl-3,5-dicaffeoylquinic acid, one other monomethoxaloyldicaffoeylquinic acid (M_r 602), and one dimethoxaloyldicaffeoylquinic acids (M_r 688) were reported in this plant previously, but the two latter ones were not identified structurally (*19*).

To find the corresponding parent compound for each methoxyoxaloyl-hydroxycinnamoylquinic acid, samples were heated in a carefully controlled manner. Arnica flowers, artichokes, and five dicaffeoylquinic acid standards in an aqueous methanol solution were placed on a heating block at 85 °C. Aliquots were taken at 1, 2, 4, and 6 h and analyzed by LC-MS. The changes in the peak areas (UV absorption) were carefully monitored. Increases in the peak areas of the arnica flower caffeoylquinic acids were due to both caffeoyl migration and loss of the methoxyoxaloyl by hydrolysis of the methoxyoxaloylcaffeoylquinc acids. Using the artichoke extract and each standard as references, the contribution of migration to the increase in peak area was determined. This permitted estimation of the increase in areas due only to loss of methoxyoxaloyl and identification of four of the methoxyoxaloyl derivatives. It was determined that migration of the caffeoyls contributed much less to the increase in peak area than the loss of methoxyoxaloyl groups upon heating for 1 and 2 h. This time, heating of the arnica flower extract resulted in a loss in area for peak 18 that was accompanied by a proportional increase in area for peak

13 (1,5-dicaffeoylquinic acid). This led to the identification of peak 18 as 1,5-dicaffeoyl-3-methoxyoxaloylquinic acid. Similarly, peaks 20, 21B, and 27 were identified as 3,5- and 4,5-dicaffeoyl-1-methoxyoxaloylquinic acids and 1,4,5-dicaffeoyl-3-methoxyoxaloylquinic acid, respectively (**Figure 5A**).

This procedure could not positively indicate the parent compounds for the remaining four minor peaks (23, 17, 19, and 22) because the small changes could not be clearly observed. However, they could still be provisionally identified on the basis of the systematic consideration of the information from LC-MS (such as elution order), biogenetic pathways, and the relative content in the plant. Peak 23 was most likely 1,5-caffeoyl-3,4-dimethoxyoxaloylquinic acid because 1,5-dicaffeoylquinic acid (peak 13) and 1,5-dicaffeoyl-3-methoxyoxaloylquinic acid (peak 18) dominate all of the dicaffeoylquinic acid isomers and their monomethoxyoxaloyl derivatives in this flower. Peaks 17, 19, and 22 were most likely the methoxyoxaloyl derivatives of the two detected caffeoylsinapoylquinic acids (peaks 14 and 15) with their methoxyoxaloyl substituents at the 1-position, and the remaining might be 3-sinapoyl-5-caffeoyl-4-methoxyoxaloylquinic acid because the parent compound dominated this group, and the assignment was made according to the elution order at this LC condition.

Hydroxycinnamoylquinic Acids in Burdock Roots. Twenty-four hydroxycinnamoylquinic acids were detected in burdock roots (**Figure 5D**; **Table 3**). Twelve of them are common caffeoylquinic acids, that is, four monocaffeoylchlorogenic acids (peaks 1, 2, 4, and 5), six dicaffeoylquinic acids (peaks 6 and 8−12), and 1,3,5- and 3,4,5-tricaffeoylquinic acids (peaks 16 and 22). They were identified by direct comparison with standards in the database (**Table 1**) and in arnica flower (**Table 2**). The mass spectrum of 1,3,5-tricaffeoylquinic acid (peak 16) is shown in **Figure 6A**. The demonstrated fragments strongly supported this identification.

1,3,5-Tricaffeoyl-4-succinoylquinic acid (M_r 778), 1,5-dicaffoeyl-3,4-disuccinoylquinic acid (M_r 716), 1,5-dicaffeoyl-3-succinoylquinic acid, and 1,5-dicaffeoyl-4-succinoylquinic acid (M_r 616) were isolated from this plant and identified by NMR (*20*). Thus, it is easy to assign peaks 14 and 15 as diccafeoylsuccinoylquinic acids on the basis of their $[M - H]^-$ at *m/z* 615 and $[M - H + Na]^-$ at *m/z* 637, diagnostic fragments at *m/z* 497 (loss of succinic acid), 475 (loss of succinic acid from $[M - H + Na]^-$ ion), 353 (loss of one caffeoyl and succinoyl), 291 (loss of two caffeoyl), 191 (loss of two caffeoyls and one succinoyl), and 161 (caffeoyl) (shown in **Figure 7D**). According to their elution order mentioned above, peaks 14 and 15 were assigned further as 1,5-dicaffeoyl-3-succinylquinic acid and 1,5-dicaffeoyl-4-succinylquinic acid, respectively.

Similarly, peak 21 was assigned as 1,3,5-tricaffeoyl-4-succinoylquinic acid on the basis of its mass data: $[M - H]^-$ at *m/z* 777, 615 (loss of one caffeoyl), 453 (loss of two caffeoyls), 291 (loss of three caffeoyls), 191 (quinic acid ion produced by loss of three caffeoyls and one succinoyl), and 179 (caffeic acid). Peak 18 was assigned as 1,5-dicaffeoyl-3,4-succinoylquinic acid on the basis of its mass data $[M - H]^-$ at *m/z* 715, 553 (loss of one caffeoyl), 453 (loss of one succinoyl), 291 (loss of two caffeoyls and one succinoyl), 191 (quinic acid ion produced by loss of two caffeoyls and two succinoyl), and 179 (caffeic acid).

Peaks 7 and 13 (t_R 39.6 and 45.4 min) had their $[M - H]^-$ at *m/z* 631 and fragments at *m/z* 353, 191, and 179. Their mass spectra suggested that they might contain a pentose (plus 132) and rhamnose (or *p*-coumaroyl) (plus 146) or a glycosyl consisting of both the sugars (plus 278), because their molecular weights cannot be matched by addition of any combination of the reported aliphatic acids, such as methoxyoxalic, succinic, and 3-hydroxy-3-methoxyglutaric acids (*7*). The other three peaks [peaks 17, 19, 20, molecular ions at *m/z* 793, fragments at *m/z* 631 (loss of 162), 469, and 191] were the analogues of peaks 7 and 13 and contained one more caffeoyl (plus 162). All of them were the minor phenolics of burdock roots, and their structures were not identified in this study. Peaks 3 (t_R 14.9 min), 23 (t_R 55.6 min), and 24 (t_R 56.9 min) were phenolic acid derivatives, too, but they were not identified. 1,3,5- and 3,4,5-tricaffeoylquinic acids and most of the remaining ones have not been previously reported (*20*).

This study has demonstrated that the complex assortment of mono-, di-, and trihydroxycinnamoylquinic acids be separated and identified using two reversed phase columns, diode array, and ESI/MS detection. The key to the success of this approach was the use of standards or plant materials containing positively identified reference compounds. This approach significantly reduces the instrumental complexity and cost (no systematic tandem MS study required) necessary to achieve accurate identification.

LITERATURE CITED

(1) Kurata, R.; Adachi, M.; Yamakawa, O.; Yoshimoto, M. Growth suppression of human cancer cells by polyphenolics from sweetpotato (*Ipomoea batatas* L.) leaves. *J. Agric. Food Chem.* **2007**, *55*, 185–190.

(2) Tamura, H.; Akioka, T.; Ueno, K.; Chujyo, T.; Okazaki, K.; King, P. J.; Robinson, W. E., Jr. Anti-human immunodeficiency virus activity of 3,4,5-tricaffeoylquinic acid in cultured cells of lettuce leaves. *Mol. Nutr. Food Res.* **2006**, *50*, 396–400.

(3) Robards, K.; Prenzler, P. D.; Tucker, G.; Swatsitang, P.; Glover, W. Phenolic compounds and their role in oxidative processes in fruits. *Food Chem.* **1999**, *66*, 401–436.

(4) Manach, C.; Scalbert, A.; Morand, C.; Remesy, C.; Jimenez, C. Bioavailability and bioefficacy of polyphenols in humans. *Am. J. Clin. Nutr.* **2004**, *79*, 727–747.

(5) Yoshimoto, M.; Yahara, S.; Okuno, S.; Islam, M. S.; Ishiguro, K.; Yamakawa, O. Antimutagenicity of mono-, di-, and tricaffeoylquinic acid derivatives isolated from sweetpotato (*Ipomoea batatas* L.) leaf. *Biosci., Biotechnol., Biochem.* **2002**, *66*, 2336–2341.

(6) IUPAC. Nomenclature of cyclitols. *Biochem. J.* **1976**, *153*, 23−31.

(7) Clifford, M. N.; Wu, W.; Kirkpatrick, J.; Kuhnert, N. Profiling the chlorogenic acids and other caffeic acid derivatives of herbal chrysanthemum by LC-MS^n. *J. Agric. Food Chem.* **2007**, *55*, 929–936.

(8) Clifford, M. N.; Johston, K. L.; Knight, S.; Kuhnert, N. Hierachical scheme for LC-MS^n identification of chlorogenic acids. *J. Agric. Food Chem.* **2003**, *51*, 2900–2911.

(9) Clifford, M. N.; Marks, S.; Knight, S.; Kuhnert, N. Characterization by LC-MS(*n*) of four new classes of *p*-coumaric acid-containing diacyl chlorogenic acids in green coffee beans. *J. Agric. Food Chem.* **2006**, *54*, 4095–4101.

(10) Clifford, M. N.; Knight, S.; Surucu, B.; Kuhnert, N. Characterization by LC-MS^n of four new classes of chlorogenic acids in green coffee beans: dimethoxycinnamoylquinic acids, diferuloylquinic acids, caffeoyl-dimethoxycinnamoylquinic acids, and feruloyl-dimethoxycinnamoylquinic acids. *J. Agric. Food Chem.* **2006**, *54*, 1957–1969.

(11) Clifford, M. N.; Wu, W.; Kuhnert, N. The chorogenic acids of *Hemenrollis*. *Food Chem.* **2006**, *95*, 574–578.

(12) Clifford, M. N.; Knight, S.; Kuhnert, N. Discriminating between the six isomers of dicaffeoylquinic acids by LC-MS(*n*). *J. Agric. Food Chem.* **2005**, *53*, 3821–3832.

(13) Clifford, M. N.; Zheng, W.; Kuhnert, N. Profiling the chlorogenic acids of aster by HPLC-MS(*n*). *Phytochem. Anal.* **2006**, *17*, 384–393.

(14) Gobbo-Neto, L.; Lopes, N. P. Online identification of chlorogenic acids, sesquiterpene lactones, and flavonoids in the Brazilian arnica *Lychnophora ericoides* Mart. (Asteraceae) leaves by HPLC-DAD-MS and HPLC-DAD-MS/MS and a validated HPLC-DAD method for their simultaneous analysis. *J. Agric. Food Chem.* **2008**, *56*, 1193–1204.

(15) Lai, J. P.; Lim, Y. H.; Su, J.; Shen, H. M.; Ong, C. N. Identification and characterization of major flavonoids and caffeoylquinic acids in three Compositae plants by LC/DAD-APCI/MS. *J. Chromatogr., B* **2007**, *848*, 215–225.

(16) Han, J.; Ye, M.; Qiao, X.; Xu, M.; Wang, B.-R.; Gua, D.-R. Characterization of phenolic compounds in the Chinese herbal drug Artemisia annua by liquid chromatography coupled to electrospray ionization mass spectrometry. *J. Pharm. Biomed. Anal.* **2008**, *47*, 516–525.

(17) Schütz, K.; Kammerer, D.; Carle, R.; Schieber, A. Identification and quantification of caffeoylquinic acids and flavonoids from artichoke (*Cynara scolymus* L.) heads, juice, and pomace by HPLC-DAD-ESI/MS*n*. *J. Agric. Food Chem.* **2004**, *52*, 4090–4096.

(18) Cuyckens, F.; Claeys, M. Mass spectrometry in the structural analysis of flavonoids. *J. Mass Spectrom.* **2004**, *39*, 1–15.

(19) Spitaler, R.; Schlorhaufer, P. D.; Ellmerer, E. P.; Merfort, I.; Bortenschlager, S.; Stuppner, H.; Zidorn, C. Altitudinal variation of secondary metabolite profiles in flowering heads of *Arnica montana* cv. ARBO. *Phytochemistry* **2006**, *67*, 409–417.

(20) Maruta, Y.; Jun Kawabata, J.; Ryoya Niké, R. Antioxidative caffeoylquinic acid derivatives in the roots of burdock (*Arctium lappa* L.). *J. Agric. Food Chem.* **1995**, *43*, 2592–2595.

(21) Whitaker, B. D.; Stommel, J. R. Distribution of hydroxycinnamic acid conjugates in fruit of commercial eggplant (*Solanum melongena* L.) cultivars. *J. Agric. Food Chem.* **2003**, *51*, 3448–3454.

(22) Clifford, M. N.; Kirkpatrick, J.; Kuhnert, N.; Roozendaal, H.; Rodrigues Salgado, P. R. LC-MS^n analysis of the cis isomers of chlorogenic acids. *Food Chem.* **2008**, *106*, 379–385.

(23) Kim, H. J.; Lee, Y. S. Identification of new dicaffeoylquinic acids from Chrysanthemum morifolium and their antioxidant activities. *Planta Med.* **2005**, *71*, 871–876.

(24) Leung, A. Y.; Foster, S. Arnica; artichoke; burdock; coffee; fennel In *Encyclopedia of Common Natural Ingredients Used in Food, Drugs and Cosmetics*, 2nd ed.; Leung, A. Y., Foster, S. , Eds.; Wiley: New York, 1996; pp 40−42, 42−44, 107−108, 187−190, 240−243, respectively.

(25) Merfort, I. Caffeoylquinic acids from flower of *Arnica montana* and *Arnica chamissonis*. *Phytochemistry* **1992**, *31*, 22111–2113.

(26) Lin, L.-Z.; Harnly, J. A screening method for the identification of glycosylated flavonoids and other phenolic compounds using a standard analytical approach for all plant materials. *J. Agric. Food Chem.* **2007**, *55*, 1084–1096.

(27) Nagels, L.; Herrmann, K.; Brucker, J. D.; Potter, H. D. High-performance liquid chromatographyic separation of naturally occurrence esters of phenolic acids. *J. Chromatogr., A* **1980**, *187*, 181–187.

(28) Nakatani, N.; Kayano, S.-I.; Kikuzaki, H.; Sumino, K.; Katagiri, K.; Mitani, T. Identification, quantitative determination, and antioxidative activities of chlorogenic acid isomers in prune (*Prunus domestica* L.). *J. Agric. Food Chem.* **2000**, *48*, 5512–5516.

(29) Islam, M. S.; Yoshimoto, M.; Yahara, S.; Okuno, S.; Ishiguro, K.; Yamakawa, S. Identification and characterization of foliar polyphenolic compound in sweet potapo (*Ipomoea batatas* L.) genotypes. *J. Agric. Food Chem.* **2002**, *50*, 3718–3722.

(30) Peng, L. Y.; Mei, S. X.; Jiang, B.; Zhou, H.; Sun, H. D. Constituents from *Lonicera japonica*. *Fitoterapia* **2000**, *71*, 713–715.

(31) Agata, I.; Goto, S.; Hatano, T.; Nishibe, S.; Okuda, T. 1,3,5-Tricaffeoylquinic acid from *Xanthium strumarium*. *Phytochemistry* **1993**, *33*, 508–509.

(32) Wang, Z.; Clifford, M. N. Comparison of the profiles of chlorogenic acids and their derivatives from three Chinese traditional herbs by LC-MS^n. *Yao Xue Xue Bao* **2008**, *43*, 185–190.

(33) Lin, L.-Z.; Chen, P.; Harnly, J. M. New phenolic components and chromatographic profiles of green and fermented teas *J. Agric. Food Chem.* **2008**, *56*, 8130–8140.

(34) Krizman, M.; Baricevic, D.; Prosek, M. Determination of phenolic compounds in fennel by HPLC and HPLC-MS using a monolithic reversed-phase column. *J. Pharm. Biomed. Anal.* **2007**, *43*, 481–485.

(35) Parejo, I.; Jauregui, O.; Sánchez-Rabaneda, F.; Viladomat, F.; Bastida, J.; Codina, C. Separation and characterization of phenolic compounds in fennel (*Foeniculum vulgare*) using liquid chromatography−negative electrospray ionization tandem mass spectrometry. *J. Agric. Food Chem.* **2004**, *52*, 3679–3687.

(36) Lin, L.-Z.; Harnly, J. M. New phenolic components and chromatographic profiles of pear skins (*Pyrus* spp.) *J. Agric. Food Chem.* **2008**, in press.

Received for review April 30, 2008. Revised manuscript received August 21, 2008. Accepted August 22, 2008. This research is supported by the Agricultural Research Service of the U.S. Department of Agriculture and an Interagency Agreement with the Office of Dietary Supplements of the National Institutes of Health.

JF802412M