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Cassia Cinnamon as a Source of Coumarin in Cinnamon-Flavored Food and Food Supplements in the United States

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ABSTRACT: Coumarin as an additive or as a constituent of tonka beans or tonka extracts is banned from food in the United States due to its potentially adverse side effects. However, coumarin in food from other natural ingredients is not regulated. "True Cinnamon" refers to the dried inner bark of *Cinnamomum verum*. Other cinnamon species, *C. cassia*, *C. loureiroi*, and *C. burmannii*, commonly known as cassia, are also sold in the U.S. as cinnamon. In the present study, coumarin and other marker compounds were analyzed in authenticated cinnamon bark samples as well as locally bought cinnamon samples, cinnamon-flavored foods, and cinnamon-based food supplements using a validated UPLC-UV/MS method. The experimental results indicated that *C. verum* bark contained only traces of coumarin, whereas barks from all three cassia species, especially *C. loureiroi* and *C. burmannii*, contained substantial amounts of coumarin. These species could be potential sources of coumarin in cinnamon-flavored food in the U.S. Coumarin was detected in all locally bought cinnamon, cinnamon-flavored foods, and cinnamon food supplements. Their chemical profiles indicated that the cinnamon samples and the cinnamon in food supplements and flavored foods were probably Indonesian cassia, *C. burmannii*.

KEYWORDS: *cinnamon, coumarin, Cinnamomum verum, C. cassia, C. loureiroi, C. burmannii, cassia, cinnamaldehyde, UPLC-UV-MS*

INTRODUCTION

Cinnamon, one of the most important flavoring agents in the food and beverage industry, has been recognized for its flavoring and medicinal properties since antiquity. "True" cinnamon (Ceylon cinnamon) is the dried bark of *Cinnamomum verum* J. S. Presl (syn *C. zeylanicum*) (Lauraceae), a small evergreen tree native to Sri Lanka.[1] However, *C. cassia* (Nees & T. Nees) J. Presl (Syn. *C. aromaticum* Nees) (Chinese cassia), and *C. loureiroi* Nees (Saigon cassia), also can be sold under the label cinnamon in the United States.[2] The dried inner bark of *C. burmannii* (Nees & T. Nees) Blume (Indonesian cassia), which is listed in the "Generally Regarded as Safe" (GRAS) category under "Cassia, Padang" or "Batavia",[3] is also sold as cinnamon. (These species should not be confused with the *Cassia* genus, members of the family Fabaceae.) Trade data and recent studies indicate that *C. burmannii* or Indonesian cassia has replaced the more expensive true or Ceylon cinnamon (*C. verum*) in Europe, the United States, and Canada.[4−9] More than 90% of the "cinnamon" imported to the U.S. during the last five years was *C. burmannii*.[4]

Even though some clinical trials have shown that cinnamon had a modest antidiabetic activity, results remain inconclusive.[10,11] A majority of these trials have been carried out with ground *C. cassia* (*C. aromaticum*) bark or its aqueous extracts. There are a number of cinnamon-based dietary supplements in the market that claim to have glucose- and lipid-lowering properties.

Coumarins, a class of compounds that contains a 1,2-benzopyrone skeleton, are widespread in plants including many vegetables, spices, fruits, and medicinal plants.[12] Most of these compounds are not harmful to humans in the amounts present in edible plants. Coumarin (2*H*-chromen-2-one) (**1**), the simplest member of this class, as a pure compound or as a constituent of tonka beans had been used as a flavoring agent in food, alcoholic beverages and tobacco.[13] Evidence of the hepatotoxic effects of this compound in animal models led the U.S. Food and Drug Administration to ban coumarin as a food flavoring agent.[14] Development of tumors in animals which were exposed to coumarin for long periods of time indicated possible carcinogenicity of this compound.[15] In 1988, the Council of the European Communities set a maximum limit of 2 mg/kg for coumarin in many foods and beverages.[16] After it became evident that the carcinogenicity of coumarin has a nongenotoxic mechanism,[15] the European Food Safety Authority (EFSA) established a tolerable daily intake (TDI) of 0.1 mg/kg body weight on the basis of the nonobserved-adverse-effect level (NOAEL) for animals.[17]

Studies have shown differences in coumarin metabolism in primates and other animals. In contrast to the rodent model, the major metabolic pathway in primates does not result in

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hepatotoxic metabolites.[13,15,18] Human clinical data indicated that a majority of people were less sensitive to coumarin than the rodent models used to investigate the toxic effects of this compound. However, a particular group of the population was found to be more susceptible to coumarin-induced hepatotoxicity.[19] Coumarin has been used in several countries for the treatment of edemas, renal cell carcinoma, and other tumors. After receiving reports of patients developing signs of hepatotoxicity with the use of coumarin, the registration of this drug was canceled in Australia[20,21] and France.[22] In clinical trials in the U.S.[23] and Ireland,[24] some of the patients receiving coumarin developed signs of drug-induced liver toxicity even at lower doses. After reviewing accumulated human data, the German Federal Institute for Risk Assessment (BfR) reaffirmed the TDI of 0.1 mg/kg of coumarin in 2007.[19]

Cinnamaldehyde is the major flavor constituent in all these *Cinnamomum* species, but the presence of a toxic compound, coumarin, in cassia cinnamon has raised safety concerns.[4−9] Studies carried out in Germany prior to 2008[5−8] revealed that the coumarin content in some of the cinnamon-flavored products and cinnamon capsules which contained cassia cinnamon as a substitute for true cinnamon exceeded the limits set by the Council of the European Communities in 1988. In some cases children who consume cinnamon-flavored food and people who take cinnamon capsules could exceed the TDI established by the EFSA. An Italian study also found that about 70% of the cinnamon-flavored foods analyzed had higher levels of coumarin than that set by the Council of the European Communities.[9] After deliberation on the regulatory limit of coumarin in food and the noncompliance in European countries in 2008, the European Parliament and the Council of the European increased the maximum level of coumarin in cinnamon-flavored traditional and/or seasonal baked goods to 50 mg/kg and that for other fine baked goods to 15 mg/kg.[25] The maximum coumarin limits for breakfast cereals and desserts were set at 20 and 5 mg/kg, respectively.

Even though coumarin as such or as a constituent of tonka beans or tonka extracts is listed under the substances generally prohibited from direct addition or use as human food in the U.S., coumarin content in cassia cinnamon-flavored food is not regulated as in Europe. The present study was initiated to determine the coumarin content in cinnamon and cinnamon-flavored food available in the U.S.

HPLC coupled with UV or mass spectrometry is currently the method of choice to determine coumarin in cinnamon.[7−9,26] Methods based on thin-layer chromatography[27,28] and gas chromatography−mass spectrometry[29] have also been reported in the past two decades. To determine coumarin and cinnamaldehyde in cinnamon samples, and compare the chemical profile of different *Cinnamomum* species, a fingerprinting UPLC-UV/MS method was developed and used to analyze samples of cinnamon powder or barks, cinnamon-flavored foods, and cinnamon-based dietary supplements. The developed method was used to characterize and quantitate the major compounds reported in cinnamon, viz., coumarin **1**, cinnamyl alcohol **2**, cinnamaldehyde **3**, cinnamic acid **4**, eugenol **5**, and cinnamyl acetate **6** (Figure 1). The method was also applied to determine these compounds in authenticated *C. verum*, *C. cassia*, *C. loureiroi*, and *C. burmannii* bark and cinnamon samples from Sri Lanka, China, Vietnam, and Indonesia. Cinnamon samples purchased from local grocery chains and ground cinnamon obtained from a leading coffee chain were also analyzed.

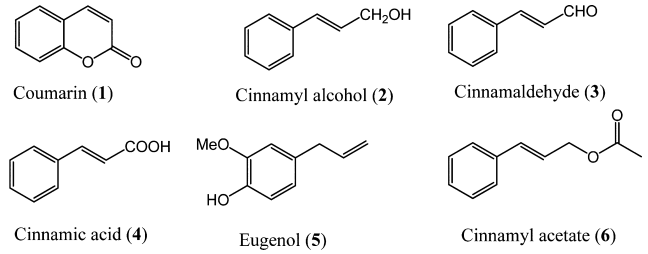

Figure 1. Structure of standard compounds **1**−**6**.

MATERIALS AND METHODS

Chemicals, Plant Material, and Samples. The standard compounds coumarin **1**, cinnamyl alcohol **2**, cinnamaldehyde **3**, cinnamic acid **4**, eugenol **5**, and cinnamyl acetate **6** were purchased from Sigma (St. Louis, MO, USA), The identity and purity of the standards were confirmed by chromatographic methods and spectroscopic data (NMR and HR-ESI-MS). Acetonitrile, methanol, water, and formic acid were HPLC grade, and purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Authentic samples of *Cinnamomum verum* J. Presl (Syn. *C. zeylancium* Blume) (S-1), *C. burmannii* (Nees & T. Nees) Blume (S-18), and *C. loureiroi* Nees (S-26) were obtained from Savory Spice Shop, Denver, CO, and authenticated by macroscopic and microscopic methods. An authentic sample of *C. cassia* (Nees & T. Nees) J. Presl (Syn. *C. aromaticum* Nees) (S-28) was purchased in China and authenticated by macroscopic and microscopic methods. The identity, species, and sample type/source of all the analyzed samples are listed in Table 1. Commercial and authenticated samples are deposited at the National Center for Natural Products Research (NCNPR), University of Mississippi, MS, USA.

Instrumentation and Chromatographic Conditions for UPLC-UV/MS Analysis. All analyses were performed on a Waters Acquity UPLC system (Waters Corp., Milford, MA) that included a binary solvent manager, sampler manager, heated column compartment, photodiode array (PDA) detector, and single quadrupole detector (SQD). The instrument was controlled by Waters Empower 2 software. The column used was a 100 mm × 2.1 mm i.d., 1.7 μm, Acquity UPLC BEH shield RP18 column also from Waters. The column and sample temperatures were maintained at 40 and 25 °C, respectively. The eluent consisted of water with 0.05% formic acid (A) and methanol/acetonitrile (90:10, v/v) with 0.05% formic acid (B). Analysis was performed using the following linear gradient elution at a flow rate of 0.23 mL/min: 37% B to 67% B in 5.5 min, and increasing B to 100% B in next 1.5 min. The analysis was followed by a 1.5-min washing procedure with 100% B and re-equilibration period of 3.5 min. All solutions were filtered through 0.20-μm membrane filters and the injection volume was 2 μL. The total run time for an analysis was 7 min. The PDA detection wavelength was 280 nm.

Mass spectrometer conditions were optimized to obtain maximal sensitivity. The source temperature and the desolvation gas temperature were maintained at 150 and 350 °C, respectively. The probe voltage (capillary voltage), cone voltage, and extractor voltage were fixed at 3.0 kV, 35 V, and 2.0 V, respectively. Nitrogen was used as the desolvation gas (650 L/h) and drying gas (25 L/h). Analyte identity was confirmed in selected ion recording (SIR) mode. Signals at m/z 146.9 $[M + H]^+$, 116.9 $[M + H - H_2O]^+$, 132.9 $[M + H]^+$, 130.9 $[M + H - H_2O]^+$, 163.9 $[M]^+$, and 116.9 $[M + H -$ acetic acid$]^+$ were used to detect ions of coumarin, cinnamyl alcohol, cinnamaldehyde, cinnamic acid, eugenol, and cinnamyl acetate, respectively. Mass spectra were obtained at a dwell time of 0.1 s in SIR and 500 Da/s scan rate.

Sample Preparation. The samples analyzed in this work were received in multiple forms including barks, powders, capsules, snacks, jams, bread, rolls, bun, swirl, bar, and pastries. To perform the determinations on these different matrices, an extraction protocol specific for each type of sample was developed.

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Table 1. Content of Coumarin 1, Cinnamyl Alcohol 2, Cinnamaldehyde 3, Cinnamic Acid 4, Eugenol 5, and Cinnamyl Acetate 6 in *Cinnamomum* Species and Cinnamon Commercial Samples (g/kg)[a]

sample	NCNPR code	species	sample type/source	3	1	2	4	5	6
S-1	3997	*C. verum*	authentic sample	16.8	0.017	0.096	0.072	DUL	2.01
S-2	3974	*C. verum*	barks/Sri Lanka, commercial source	8.79	0.007	0.049	0.044	0.243	1.17
S-3	3975	*C. verum*	barks/Sri Lanka, commercial source	12.1	0.016	0.145	0.043	0.961	0.983
S-4	3976	*C. verum*	barks/Sri Lanka, commercial source	4.72	0.09	0.070	0.061	0.219	0.374
S-5	3977	*C. verum*	barks/Sri Lanka, commercial source	6.37	0.020	0.040	0.052	0.131	0.174
S-6	3978	*C. verum*	barks/Sri Lanka, commercial source	14.2	0.016	0.116	0.092	0.385	0.683
S-7	3979	*C. verum*	barks/Sri Lanka, commercial source	11.1	0.025	DUL	0.046	0.568	0.255
S-8	3980	*C. verum*	barks/Sri Lanka, commercial source	7.80	0.009	DUL	0.079	0.150	0.249
S-9	3981	*C. verum*	barks/Sri Lanka, commercial source	9.09	0.019	0.289	0.061	0.168	2.38
S-10	3982	*C. verum*	barks/Sri Lanka, commercial source	3.12	0.005	0.340	0.044	0.227	4.24
S-11	3983	*C. verum*	barks/Sri Lanka, commercial source	10.0	0.011	0.419	0.112	0.382	0.771
S-12	3984	*C. verum*	barks/Sri Lanka, commercial source	18.1	0.007	0.452	0.052	1.15	2.73
S-13	3985	*C. verum*	barks/Sri Lanka, commercial source	6.60	0.020	0.211	0.054	0.225	1.26
S-14	3986	*C. verum*	barks/Sri Lanka, commercial source	9.67	0.012	0.096	0.056	0.358	1.02
S-15	3987	*C. verum*	barks/Sri Lanka, commercial source	9.53	0.013	0.265	0.038	0.213	1.83
S-16	4895	*C. verum*	barks/Sri Lanka, commercial source	22.1	0.019	ND	0.219	0.526	0.567
S-17	7545	*C. verum*	barks/U.S., commercial source	21.0	0.013	ND	0.122	ND	0.677
S-18	3995	*C. burmannii*	authentic sample	46.3	2.14	DUL	0.578	ND	0.871
S-19	4881	*C. burmannii*	barks/U.S., commercial source	52.2	6.19	DUL	1.22	0.013	0.185
S-20	4887	*C. burmannii*	barks/U.S., commercial source	32.6	3.99	ND	0.283	DUL	1.01
S-21	4892	*C. burmannii*	barks/U.S., commercial source	22.4	2.37	ND	0.240	ND	1.01
S-22	4896	*C. burmannii*	barks/U.S., commercial source	50.9	9.30	ND	1.31	ND	1.14
S-23	4897	*C. burmannii*	barks/U.S., commercial source	63.8	4.03	ND	0.993	ND	0.163
S-24	4898	*C. burmannii*	barks/U.S., commercial source	12.4	5.01	ND	0.176	ND	0.314
S-25	5605	*C. burmannii*	barks/U.S., commercial source	34.5	7.31	ND	0.540	0.050	0.576
S-26	3996	*C. loureiroi*	authentic sample	55.8	6.97	0.121	1.11	0.019	0.166
S-27	5229	*C. loureiroi*	barks/Vietnam, commercial source	76.1	1.06	0.031	0.622	ND	0.564
S-28	5227	*C. cassia*	authentic sample	18.7	0.310	0.045	0.605	ND	0.035
S-29	5226	*C. cassia*	barks/China, commercial source	15.4	0.145	ND	0.192	ND	0.030
S-30	4893	*C. cassia*	barks/U.S., commercial source	17.4	0.085	ND	0.369	ND	0.247
S-31	4899	*C. cassia*	barks/U.S., commercial source	22.3	0.262	ND	0.924	ND	DUL
S-32	4882	*Cinnamomum spp.*	barks/U.S., commercial source	53.6	5.79	0.048	1.16	DUL	ND
S-33	4883	*Cinnamomum spp.*	powder/U.S., commercial source	33.1	4.44	ND	0.909	ND	ND
S-34	4884	*Cinnamomum spp.*	powder/U.S., commercial source	52.2	6.19	ND	1.61	DUL	ND
S-35	4885	*Cinnamomum spp.*	powder/U.S., commercial source	20.9	3.19	ND	0.715	0.016	0.028
S-36	4886	*Cinnamomum spp.*	powder/U.S., commercial source	31.9	3.25	0.044	0.645	0.007	0.052
S-37	4888	*Cinnamomum spp.*	powder/U.S., commercial source	32.0	3.25	ND	0.613	0.005	0.025
S-38	4921	*Cinnamomum spp.*	powder/U.S., commercial source	8.33	2.06	ND	0.904	ND	0.041
S-39	9248	*Cinnamomum spp.*	barks/U.S., commercial source	31.9	3.32	DUL	0.640	0.007	0.034
S-40	9249	*Cinnamomum spp.*	barks/U.S., commercial source	41.7	2.00	DUL	0.420	DUL	0.975
S-41	9250	*Cinnamomum spp.*	barks/U.S., commercial source	32.6	3.47	0.023	0.736	ND	0.024
S-42	7546	*Cinnamomum spp.*	barks/U.S., commercial source	26.7	3.93	ND	0.869	ND	ND
S-43	7547	*Cinnamomum spp.*	barks/U.S., commercial source	28.0	4.05	ND	0.887	ND	ND
S-44	7548	*Cinnamomum spp.*	barks/U.S., commercial source	21.0	3.80	ND	0.889	ND	0.027

[a]DUL = Detected under limits of quantitation; ND = not detected.

Barks/Powders/Capsules. For barks, an adequate amount of plant material was pulverized with a mortar and pestle. For capsules, five samples were weighted, opened, and the contents were emptied, then mixed and triturated in a mortar and pestle.

Dry plant samples (0.5 g) or an adequate amount of powdered capsule contents (0.5 g) were weighed and sonicated in 2.5 mL of methanol for 30 min followed by centrifugation for 10 min at 3000 rpm. The supernatant was transferred to a 10-mL volumetric flask. The procedure was repeated three times and respective supernatants were combined. The final volume was adjusted to 10 mL with methanol and mixed thoroughly.

Prior to injection, an adequate volume (ca. 2 mL) was passed through a 0.2-μm nylon membrane filter. The first 1 mL was discarded and the remaining volume was collected in a LC sample vial.

Snack Foods/Toothpaste. An adequate amount of snack food (20 g) was weighed and crushed. After mixing, samples were ground with a mortar and pestle.

Powdered samples or tooth paste (3.0 g) were weighed and sonicated in 4.0 mL of methanol for 30 min followed by centrifugation for 10 min at 3000 rpm. The supernatant was transferred to a 10-mL volumetric flask. The procedure was repeated three times but using 2.0 mL of methanol. The respective supernatants were combined, and the final volume was adjusted to 10 mL with methanol. The extracts were then processed as stated previously for barks.

Bread, Rolls, Bun, Swirl, Bar, and Pastries. Portions of each bread sample were selected from different parts of a loaf. For rolls, bun, swirl, bar, and pastries samples, a piece of bread roll was cut from each type. An adequate amount (50−100 g) was taken for sample

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homogenizing. The selected samples were cut into less than 0.5 cm cubes and mixed in a zip bag.

Homogenized samples of bread or rolls (3.0 g) were weighed and sonicated in 4.0 mL of methanol for 30 min followed by centrifugation for 10 min at 3000 rpm. The supernatant was transferred to a 10-mL volumetric flask. The procedure was repeated three times but using 2.0 mL of methanol. The respective supernatants were combined, and the final volume was adjusted to 10 mL with methanol. The extracts were then processed as stated previously for barks.

Preparation of Standard Solution. Individual stock solutions of the standard compounds were prepared at a concentration of 2.0 mg/mL in methanol. Calibration curves were prepared using seven different concentration levels.

Validation Procedure. The developed UPLC method was validated in terms of precision, accuracy, and linearity according to International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines.[30] The precision of the assay method was determined using three independent test solutions on three consecutive days. Recovery experiments were conducted using concentrations of the standards 20 μg/mL. The limit of detection (LOD) and limit of quantitation (LOQ) were estimated by injecting the dilute solutions of the standards with known concentration.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions. In a preliminary phase, different sub-2-μm UPLC columns were tested in order to optimize the condition for separation. The different columns tested were Acquity UPLC BEH C18 (50 mm × 2.1 mm i.d., 1.7 μm), BEH Shield RP18 (50 mm × 2.1 mm i.d., 1.7 μm), BEH C18 (100 mm × 2.1 mm i.d., 1.7 μm), and BEH Shield RP18 (100 mm × 2.1 mm i.d., 1.7 μm). The best separation and peak shapes were achieved using a 100 mm × 2.1 mm BEH Shield RP18 column. Different gradient systems, which included acetonitrile/water, methanol/water, and methanol/acetonitrile/water, were evaluated for the best separation of the standard compounds. Optimal chromatographic conditions were observed with methanol/acetonitrile (90:10, v/v) with 0.05% formic acid and water containing 0.05% formic acid as mobile phase. A mixture of methanol and acetonitrile was preferred as the mobile phase because it was able to enhance the separation. Formic acid was used as a modifier because it improved the peak shapes and separation, as well as increased the sensitivity of mass spectrometer in positive ions mode.

UPLC Method Validation. The UPLC method was proposed as a suitable method for quantitative determination and routine analysis of coumarin, cinnamyl alcohol, cinnamaldehyde, cinnamic acid, eugenol, and cinnamyl acetate in *Cinnamomum* plant samples and cinnamon-flavored products. The UPLC method was validated for precision, accuracy, linearity, limits of detection, and limits of quantitation.

Specificity of the method was established through determination of peak purity in samples with PDA detection at UV 280 nm and MS detection with an ESI interface. The specificity of the UPLC method was determined by injecting individual standard samples. No interference was observed for any of the components. The purity of the principal and other chromatographic peaks was found to be satisfactory.

The precision and stability of the assay method was evaluated by carrying out three independent assays on three different days. Multiple assays and injections showed that the results are highly reproducible and showed low standard error. The RSD of assay results obtained in interday and intraday study was within 5.3%. The inter- and intraday assays confirmed the good precision of the method.

The recoverability of the method was determined for the related substance by spiking samples with a known amount of each standard. The assay method was assessed from three replicate samples at the concentration of the standards 20 μg/mL. The average recoveries of the analytes in sample S-1 were 97.0%, 96.2%, 126.8%, 100.5%, 100.5%, and 98.1% for standard compounds coumarin, cinnamyl alcohol, cinnamaldehyde, cinnamic acid, eugenol, and cinnamyl acetate, respectively.

The LOD and LOQ were estimated by injecting the dilute solutions of the standards with known concentration. The LOQs were 0.2 μg/mL for compounds **1**, **3**, and **4**, 1.0 μg/mL for compounds **2** and **6**, and 0.5 μg/mL for compound **5**. The LODs and LOQs were defined, respectively, as signal-to-noise ratio equal to 3 and 10.

Linear calibration plots for the related substance were obtained over the calibration range at seven concentration levels. The linear dynamic range for coumarin, cinnamaldehyde, and cinnamic acid was 0.2−200 μg/mL, for eugenol was 0.5−200 μg/mL, and for cinnamyl alcohol and cinnamyl acetate was 1.0−200 μg/mL for UPLC-UV analysis. The results showed good linear correlation ($r^2 > 0.999$).

Cinnamon Plant Samples and Commercial Products (Powders and Barks). The major flavor constituents identified in each sample and their concentrations are given in Table 1. The LC-UV chromatograms of reference samples showed (Figure 2) that cinnamaldehyde (**3**) is a dominant component in the species *C. verum* (S-1), *C. burmannii* (S-18), *C. loureiroi* (S-26), and *C. cassia* (S-28), and its concentration was determined to be 16.8, 46.3, 55.8, and 18.7 g/kg, respectively, whereas the coumarin contents of these samples were 0.017, 2.15, 6.97, and 0.31 g/kg, respectively. Cinnamyl alcohol, cinnamaldehyde, cinnamic acid, and cinnamyl acetate were detected in all samples. Eugenol was found mainly in *C. verum* and *C. loureiroi*, but not detected in samples of *C. cassia* or *C. burmannii*.

Comparison of reference compounds in the voucher specimen of *C. verum* (S-1) with the cinnamon purchased in Sri Lanka (S-2 to S-16) or sold in the U.S. as *C. verum* (S-17) indicated similar profiles where percentages of flavor components varied broadly. Cinnamaldehyde and coumarin contents of these samples varied from 3.1 (S-10) to 22 g/kg (S-16), and 0.005 (S-10) to 0.025 g/kg (S-7), respectively. Samples sold as Indonesian cinnamon or *C. burmannii* samples (S-19 to S-24) and Vietnamese cinnamon *C. loureiroi* (S-26) had higher contents of both cinnamaldehyde and coumarin which varied from 12.5 (S-24) to 76.1 (S-27), and 1.06 (S-27) to 9.30 g/kg (S-22) respectively. Cinnamaldehyde and coumarin levels in the sample bought in China (S-29) or sold as Chinese cinnamon *C. cassia* (S-30 and S-31) varied from 15.4 (S-29) to 23.3 g/kg (S-31) and 0.085 (S-30) to 0.261 g/kg (S-31), respectively. In summary, the results shown in Table 1 suggest that true cinnamon *C. verum* contains only traces of coumarin, whereas Indonesian cinnamon *C. burmannii* contained substantial amounts. Replicates of *C. verum* and *C. burmannii* are numbered 17 and 8, respectively. Among them, the median value for the ratio of cinnaamaldehyde to coumarin for *C. verum* and *C. burmannii*, respectively, is 805 and 8.3. The remarkable difference of the ratio of cinnamaldehyde versus coumarin between *C. verum* and *C. burmannii* would be useful to differentiate the two species.

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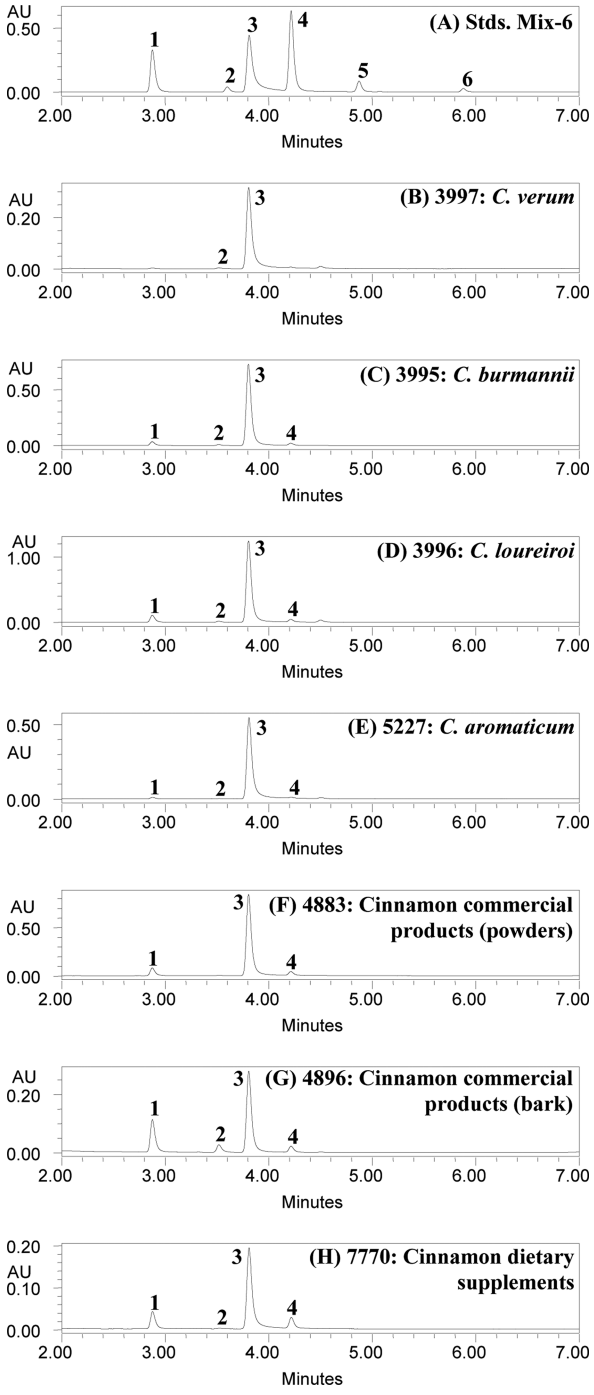

Figure 2. Typical UPLC-UV (280 nm) chromatograms of (A) mixture of coumarin **1**, cinnamyl alcohol **2**, cinnamaldehyde **3**, cinnamic acid **4**, eugenol **5**, and cinnamyl acetate **6**, and methanolic extracts of (B) *C. verum*; (C) *C. burmannii*; (D) *C. loureiroi*; (E) *C. cassia*; (F) cinnamon commercial products (powders); (G) cinnamon commercial products (barks), and (H) cinnamon dietary supplements.

Cinnamon-Flavored Products and Dietary Supplements. As shown in Table 2, 21 food products, which included different type of cinnamon-flavored foods, such as snacks, bread, rolls, bun, swirl, bar, and pastries, were analyzed. Except for cinnamaldehyde which is essential for cinnamon flavor, coumarin was detected in all cinnamon-flavored food products (S-45 to S-63). The coumarin content in these foods varied from 0.05 to 2.4 mg per serving.

Two dietary supplements (S-64 and S-65) that contained powders of cinnamon bark were also analyzed. Both of them contained high amounts of coumarin which amounted to 2.5 and 3.9 mg per serving.

The developed UPLC-UV/MS method for quantitative and qualitative analysis of coumarin, cinnamyl alcohol, cinnamaldehyde, cinnamic acid, eugenol, and cinnamyl acetate was found to achieve shorter retention times and better resolution than that observed with conventional HPLC. The results were consistent with previous results[5−9] that showed true cinnamon *C. verum* contains only traces of coumarin, whereas Indonesian cinnamon *C. burmannii* contained substantial amounts. The observed high variations of volatile flavoring agents within a species may be due to the maturity of the bark and clonal differences, as well as duration and conditions of storage. Even though it was difficult to confirm the identity of a sample based solely on its flavor profile, a sample could generally be assigned to *C. verum*, *C cassia*, or *C. burmannii*−*C. loureiroi* group based on its cinnamaldehyde and coumarin content. All the cinnamon samples bought from local groceries (S-32 to S-41) and those collected from three different stores of a leading coffee chain (S-42 to S-44) contained more than 20 g/kg of cinnamaldehyde and 1.9 g/kg of coumarin. This indicates that these samples are either *C. burmannii* or *C. loureiroi.* Because around 90% of the "cinnamon" imported annually to the U.S. during the last five years originated from Indonesia and was the least expensive of the four major varieties, these samples are most probably *C. burmannii.*[4] As shown in Table 2, in addition to cinnamaldehyde, coumarin was detected in all cinnamon-flavored food products (S-45 to S-63). The coumarin content in these foods varied from 0.05 to 2.4 mg per serving. The origin of the cinnamon in these products was not stated in these product labels. The high coumarin to cinnamaldehyde ratios indicated that the cinnamon used to flavor these foods also was probably *C. burmannii.* These ratios were higher than that observed for authentic *C. burmannii* samples. A higher loss of more volatile cinnamaldehyde during the food processing compared to coumarin may account for this difference. The dietary supplements analyzed in this study (S-64 and S-65) contained high amounts of coumarin found as 2.5 and 3.9 mg per serving. These supplements usually suggest to use 1−2 servings per day which means 1−2 g powders of cinnamon bark is consumed.

New research has generated a vast amount of new information on human toxicity of coumarin since the U.S. banned it in food in 1954 based on animal data. Discovery of a nongenotoxic mechanism for the carcinogenicity of coumarin led the EFSA to establish a TDI of 0.1 mg/kg body weight for this compound. Differential metabolism of coumarin in rodents and human indicated that it is less toxic to humans. However, idiosyncratic toxicity observed for coumarin in human clinical trials showed that a subpopulation was sensitive to this compound.[19] Ingesting substantial amounts of coumarin on a daily basis may pose a health risk to individuals who are more sensitive to this compound. When coumarin was banned in food in the U.S., Tonka beans was considered to be its major source.[14,31] Now it is known that this compound is also present in small amounts in some vegetables and herbs and spices. Cinnamon is one of the most popular flavors in the U.S. As found in this study, coumarin was present, sometimes in substantial amounts, in cinnamon-based food supplements and cinnamon-flavored foods.

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Table 2. Content of Coumarin 1 and Cinnamaldehyde 3 in Cinnamon-Flavored Food and Dietary Supplements Samples (mg per Serving)[a]

sample	sample type/source	1 (mg/g)	3 (mg/g)	serving size (g)	average weight of per capsule content	coumarin per serving (mg)
S-45	cinnamon and apple sauce/local store	0.005	0.013	128	n/a	0.64
S-46	cinnamon pecan/local store	0.016	0.116	30	n/a	0.48
S-47	ice cream topper/local store	0.003	0.020	16	n/a	0.05
S-48	breakfast cereals/local store	0.004	0.004	28	n/a	0.11
S-49	breakfast cereals/local store	0.012	0.094	31	n/a	0.37
S-50	breakfast cereals/local store	0.044	0.181	30	n/a	1.3
S-51	breakfast cereals/local store	0.040	0.317	30	n/a	1.2
S-52	instant oatmeal/local store	0.056	0.150	43	n/a	2.4
S-53	bread/local store	0.020	0.104	47	n/a	0.93
S-54	bread/local store	0.029	0.041	38	n/a	1.1
S-55	muffin and quick bread mix/local store	0.020	0.122	36	n/a	0.73
S-56	bun/local store	0.003	0.011	65	n/a	0.18
S-57	roll/local store	0.024	0.059	85	n/a	2.1
S-58	cracker/local store	0.009	0.034	31	n/a	0.28
S-59	swirl/local store	0.006	0.0003	104	n/a	0.59
S-60	granola bar/local store	0.038	0.117	37	n/a	1.4
S-61	toaster pastries/local store	0.003	0.058	52	n/a	0.15
S-62	graham snack stick/local store	0.013	0.050	28	n/a	0.37
S-63	rice snack/local store	0.003	0.016	30	n/a	0.10
S-64	dietary supplement/commercial source	2.45	7.92	2 capsules	515.4 mg	2.5
S-65	dietary supplement/commercial source	3.61	2.26	2 capsules	537.9 mg	3.9

[a]n/a = not applicable.

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Notes

The authors declare no competing financial interests.

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