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ARTICLE

Quantification of Flavoring Constituents in Cinnamon: High Variation of Coumarin in Cassia Bark from the German Retail Market and in Authentic Samples from Indonesia

Friederike Woehrlin,*,† Hildburg Fry,† Klaus Abraham,† and Angelika Preiss-Weigert†

†Federal Institute for Risk Assessment, Thielallee 88-92, 14195 Berlin, Germany

Coumarin is a flavoring which can cause hepatotoxicity in experimental animals and in a proportion of the human population. The tolerable daily intake (TDI) may be exceeded in consumers with high intake of cinnamon containing high levels of coumarin. The objective of this study was to determine these levels in cinnamon samples and to identify possible factors influencing them. A HPLC method to quantify coumarin and related constituents (cinnamaldehyde, cinnamic acid, cinnamyl alcohol, eugenol) in a single run was used. Results found in 47 cinnamon powder samples obtained from the German retail market confirmed high levels of coumarin in cassia cinnamon. A huge variation was observed in stick samples from two packages (range from below the limit of detection to about 10000 mg/kg). Cassia bark samples of five trees received directly from Indonesia were analyzed additionally. Interestingly, a high variation was observed in one of the trees, whereas no coumarin was detected in the samples of two other trees. In conclusion, coumarin levels in cassia cinnamon can vary widely even within a single tree.

KEYWORDS: Coumarin; cinnamaldehyde; eugenol; cinnamon; high-performance liquid chromatography (HPLC)

INTRODUCTION

Cinnamon is the second most important spice (next to black pepper) sold in the USA and European markets (*1*) and comprises two types: Commercial Ceylon cinnamon is the dried inner bark of the tree *Cinnamomum verum* J. S. Presl (syn. *C. zeylanicum*), belonging to the family Lauraceae; it is grown in Sri Lanka (the former Ceylon), the Seychelles, and Madagascar (*2*). Cassia or cassia cinnamon may have different origins, the important ones being the Chinese cassia (*Cinnamomum cassia Blume*, syn. *Cinnamomum aromaticum*), and Indonesian cassia (*Cinnamomum burmannii*) (*1*,*3*). There exists some confusion regarding the use of the terms cinnamon and cassia. In the UK, the term "cinnamon" applies only to *Cinnamomum verum* J. S. Presl, while "cassia" applies to *Cinnamomum cassia* J. S. Presl. In the USA and other countries, the term "cinnamon" applies to the bark of both types (*1*), as it is used in this article, comprising the types Ceylon cinnamon and cassia cinnamon.

Coumarin (1,2-benzopyrone) is a secondary plant ingredient with a pleasant flavor. It was isolated from the tonka bean (seed of *Dipteryx odorata,* also called *Coumarouna odorata*) in 1822 and, following its chemical synthesis in 1868, coumarin was used as a flavoring substance (*4*). In the 1950s, the use of coumarin as a food flavoring substance was prohibited following the discovery of its hepatotoxic properties in laboratory animals. Furthermore, coumarin proved to be carcinogenic (*5*), and until the 1990s, a genotoxic mechanism of action could not be excluded. After reviewing new experimental data in 2004, the European Food Safety Authority (EFSA) concluded that coumarin does not bind covalently to DNA, supporting a nongenotoxic mode of action for tumor induction. Thus, the derivation of a tolerable daily intake (TDI) was possible for the first time using animal data on hepatotoxicity (*6*). A value of 0.1 mg per kg body weight was derived. In 2006, the German Federal Institute for Risk Assessment (BfR) confirmed this value using human data from coumarin administration as a medicinal drug (*7*).

In 2006, high coumarin levels of up to 100 mg/kg were discovered in typical German Christmas cookies with high cinnamon content, indicating that coumarin exposure of heavy consumers of food spiced with cassia cinnamon may exceed the TDI (*8*). Other edible plants and fruits may also contain coumarin; however, the concentrations are much lower than those in cassia cinnamon (*3*, *9*). In 1988, a coumarin limit of 2 mg/kg for food in general (representing the limit of detection at that time) had been fixed in the European Community by Council Directive 88/388/EEC and was still valid in 2006. This Council Directive was replaced in 2008 by Regulation EC no. 1334/2008, defining higher maximum limits for cinnamon-containing foods.

In Germany, both types of cinnamon are available in dried form as sticks and powder (*10*). Regarding the latter, it is virtually impossible for consumers to distinguish between Ceylon and cassia cinnamon (*11*). However, the outer appearance of unground cassia sticks (hard and relatively thick layer of bark rolled

*To whom correspondence should be addressed. Phone: +49(0)-3084122355. Fax: +49(0)30184123457. E-mail: friederike.woehrlin@bfr.bund.de. Website: www.bfr.bund.de.

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Table 1. Overview of Bark Samples from Five Cassia Trees from Indonesia: Description of Segments (Photos of the Samples Are Available as Supporting Information)

tree	age	sampling segment	sample no.	no. of sticks	thickness of bark (mm)	length (cm)	total bark weight (g)
no. 1	approximately 12 years	stem low	1−1	2	3−4	47	573
		stem middle	1−2	2	3−4	41	374
		stem top	1−3	3	3	38/43	322
		branch low	1−4	2	2−4	41/44	287
		branch middle	1−5	2	2−3	40/43	333
		branch top	1−6	3	2−4	40/42	337
no. 2	approximately 2 years	not reported	2	29	0.25	23	176
no. 3	approximately 4 years	not reported	3	23	0.25−0.5	30	311
no. 4	approximately 8−12 years	stem low	4−1	5	1−2	40	486
		stem middle	4−2	5	2	40	516
		stem top	4−3	5	1−2	40	366
		branch	4−4	12	0.5−2	40−45	326
		branch	4−5	20	0.5−1	15−23	156
no. 5	approximately 15−20 years	stem low	5−1	3	5−6	40	1090
		stem middle	5−2	3	3−5	40	658
		stem top	5−3	3	2−3	40	459
		branch	5−4	8	1−2	40	334

up to a single stick) differs from that of Ceylon sticks (several soft and thin layers of bark rolled up to a comparatively compact cross-section looking more like a cigar) (*12*). In most cases, the type and origin of cinnamon is not labeled on the spice package in Germany.

According to literature data, coumarin concentrations are much higher in cassia than in Ceylon cinnamon. For example, levels were reported to be below the detection limit and up to 190 mg/kg in Ceylon cinnamon and between 700 and 12200 mg/kg in cassia cinnamon (*13*, *14*). Whereas coumarin levels in cassia powder were consistently reported to be at least 1500 mg/kg, recent measurements by the German control authorities revealed levels below 1000 mg/kg in some of the cassia sticks analyzed (*8*).

The objective of this study was to further elucidate the levels of coumarin, especially in cassia cinnamon, and to identify factors influencing these levels. For this purpose, samples from the German retail market, both sticks and powder, as well as cassia samples which were received from an Indonesian producer were analyzed. As it was considered to be helpful for the understanding of coumarin contents in cinnamon, other volatile constituents of cinnamon determining its smell and taste (*11*) were analyzed simultaneously. These are cinnamaldehyde, the major part of the essential oil determining the typical cinnamon flavor, as well as cinnamic acid, cinnamyl alcohol, and eugenol; the latter was reported to be a major compound in Ceylon cinnamon but not in cassia cinnamon (*2*, *11*, *15*, *16*). In addition, safrole, which is a genotoxic carcinogen detected in cinnamon leaf oil (*15*) and is a possible contaminant in powdered spices, was included.

Several methods have been established to determine coumarin in cinnamon such as thin layer chromatography (*11*, *17*, *18*), gas chromatography−mass spectrometry (*19*−*21*), liquid chromatography UV (*15*, *16*, *22*, *23*), and liquid chromatography DAD (*14*, *24*) as well as liquid chromatography−mass spectrometry (LC-MS) (*25*) and LC-MS/MS (*9*). For extraction of coumarin from cinnamon, magnetic stirring (*14*, *23*) or agitation on Vortex and sonication (*24*) as well as pressurized liquid extraction (*19*) are reported.

Moreover, it was the aim of this study to develop a fast and economic HPLC method for the simultaneous quantification of all above-mentioned compounds in a multitude of samples.

MATERIALS AND METHODS

Cinnamon Samples. Between the end of 2006 and the end of 2007, a total of 91 commercial cinnamon samples were purchased from the German retail market. Package sizes of 47 cinnamon powder samples were between 50 and 100 g. On the German market, ground cinnamon bark usually is cassia cinnamon (*10*), labeled as "Zimt" only, the German word for cinnamon. This applied to 40 packages; the other 7 ones were labeled as Ceylon cinnamon ("Ceylon-Zimt"). To prevent a possible loss of volatile compounds, all powdered samples were filled into a wide-necked volumetric flask and analyzed promptly. A sample of cassia cinnamon powder was split and stored at room temperature in two vessels for one year. Vessel 1 was open topped, vessel 2 was screw capped. The analytical results obtained one year later provide evidence of the stability of the samples for coumarin over one year (open topped vessel (91%); screw capped vessel (103%)).

Packages of cinnamon sticks (n = 44) contained between 5 and 15 sticks with a length of 6−10 cm. Their macroscopic appearance allowed a botanical differentiation, according to criteria like bark thickness and color (*26*), into cassia (n = 29) and Ceylon (n = 15) sticks. From each package, generally one single stick randomly chosen was analyzed; in addition, all sticks of two randomly chosen cassia packages ("A" and "B") were analyzed. The origin of cinnamon of package A was Indonesia, while the origin of cinnamon of package B was not specified.

To identify possible factors influencing the coumarin content in cassia cinnamon, bark samples directly delivered from an Indonesian producer (Pungut-RPT, District Kerinci, Provinci Jambi, Sumatra; naturally grown trees, no farm-grown trees) via a German spice manufacturer were analyzed. These samples with defined origin of the trees were harvested and delivered in October (tree no. 1, focus on different parts of the tree) and November 2007 (trees no. 2−5, focus on different age). From trees no. 1, no. 4, and no. 5 (age > 8 years), several segments were delivered. Trees no. 2 and no. 3 were younger (2 and 4 years, respectively); they had a thin bark and are normally not chosen for harvesting. Details of the samples are described in **Table 1**; photos are available as Supporting Information.

Preparation of Samples. Cinnamon powder was homogenized for 20 min using an overhead mixer and directly weighed into a thick-walled 30 mL test tube. Commercial cinnamon sticks were broken into small pieces, ground to fine powder in a mill (Micro beater mill MFC Culatti, IKA, Germany), and sieved (1 mm pore diameter). For the analysis of bark from trees no. 1−5 (**Table 1**), short pieces of bark (approximately 3 cm) were cut at one end of each stick of stem or branch of the respective sampling segment. In this way, a set of samples was obtained for each tree. Subsequently, the bark was prepared as described for the commercial sticks. Additionally, single bark sticks of tree no. 3 (five sticks randomly

Table 2. Laboratory Precision Based on Repeatability[a]

	mean level ± SD (mg/kg)	intraday repeatability[b] RSD (%)	interday repeatability[c] RSD (%)
		Coumarin	
sample 1	< LOQ		
sample 2	462 ± 8	1.76	2.7
sample 3	5060 ± 145	2.87	3.2
		Cinnamic Acid	
sample 1	110 ± 3	2.67	9.0
sample 2	105 ± 3	2.39	5.4
sample 3	463 ± 15	3.21	3.5
		Cinnamaldehyde	
sample 1	7410 ± 356	4.8	4.8
sample 2	831 ± 21	2.57	4.5
sample 3	23200 ± 821	3.54	4.5
		Eugenol	
sample 1	2100 ± 82	3.92	4.7
sample 2	< LOD		
sample 3	< LOD		
		Cinnamyl Alcohol	
sample 1	655 ± 19	2.92	8.4
sample 2	336 ± 10	2.98	12.2
sample 3	470 ± 18	3.89	5.0

[a] Ten-fold determination was carried out on three different samples on two different days amounts are mean values ± SD of $n = 10$. [b] Values refer to 10 replicates performed in one day. [c] Values refer to 10 replicates each performed on three different days.

chosen) and tree no. 4 (five sticks from each of the three defined segments of the stem: top, middle, low) were analyzed separately.

Extraction Procedures. Methanol (10 mL) was added to the homogenized, accurately weighed sample of powdered cinnamon (about 100 mg). During method development, sample amounts were tested in five different quantities in the range from 100 to 1000 mg. Sample amounts of 100 mg did not show higher variations than the other sample amounts, but prior sample homogenization by, e.g., overhead shaker is of pivotal importance.

After agitation on a Vortex for 30 s and sonication for 30 min at room temperature, the sample solution was centrifuged at 207*g* for 10 min at 10 °C. Subsequently, the supernatant was placed into a 20 mL volumetric flask. Extraction was repeated with 8 mL of methanol under agitation on a Vortex for 30 s. The sample solution was centrifuged again (1860*g*, 10 min at 10 °C) and the supernatant added into the volumetric flask, which subsequently was filled up to the calibration mark with methanol. After filtration of 500 μL of the sample solution using a centrifugal filter (VWR Centrifugal Filters, Nylon Membrane, 0.45 μm; 12000*g*; 1 min), 10 μL of the filtrate were injected into the chromatographic system. When high coumarin concentrations (> 5000 mg/kg) were observed, the sample extract was diluted with methanol in order to remain within the range of the calibration line. Every sample was prepared and analyzed in triplicate.

Chemicals and Solvents. Analytical standards of coumarin (≥99%), cinnamaldehyde (≥99%), cinnamic acid (≥99%), and safrole (≥97%) were purchased from Sigma-Aldrich (Taufkirchen, Germany). Analytical standards of cinnamyl alcohol (98%) and eugenol (≥99%) were purchased from Alfa Aesar (Karlsruhe, Germany) and Merck (Darmstadt, Germany), respectively. Acetonitrile (HPLC grade), methanol (HPLC grade), ethanol (HPLC grade), glacial acetic acid (pa 100%), and ammonium acetate (HPLC grade) were purchased from Merck (Darmstadt, Germany).

Preparation of Standards. Stock solutions (1 mg/mL) for all standards were prepared by dissolving individual standards in ethanol. For each compound, a standard mixture of individual concentrations was prepared and diluted with methanol to obtain adequate solutions. Calibration curves were generated by external standardization for each run of samples.

Instrumentation. The HPLC system used was an Agilent (Waldbronn, Germany) 1100 HPLC system (quaternary pump, degasser, and autosampler) with UV detector and a diode array detector. Best results of chromatographic separation were achieved on a reversed phase C18 column (Phenomenex Aqua 3 u; 125 Å; 150 mm × 2 mm; Phenomenex, Aschaffenburg, Germany). The column was thermostatted at 35 °C. HPLC analysis was performed using mobile phase A (buffer, 0.02 mol/L, pH 5.0; 0.98 g ammonium acetate and 412 μL acetic acid in water) and mobile phase B (acetonitrile) in a gradient program with a flow of 0.35 mL/min. The mobile phase composition started with 80% A, which was maintained for 50 s, followed by a linear decrease to 40% A within 7 min, holding for 6 min 10 s, and then returned to the initial condition in 6 min for the next run. Under the specified chromatographic conditions, the retention times for cinnamic acid, coumarin, cinnamyl alcohol, cinnamaldehyde, eugenol, and safrole were 5.4, 8.4, 10.4, 15.1, 17.4, and 19.0 min, respectively. For quantitative analysis, the wavelength representing the highest intensity for all compounds (274 nm) was chosen as a compromise.

Method Validation. The analytical method was validated in-house assessing repeatability, linearity, limit of detection (LOD), and limit of quantification (LOQ).

Because blank matrices were not available for all analytes, recovery studies were examined for coumarin only. Blank samples were fortified with three levels (100, 500, 1000 mg/kg) of coumarin ($n = 8$ each). Intraday and interday precision was determined by repeated analysis of three different cinnamon samples. Ten-fold determination was carried out for each sample on two different days. LOD and LOQ were calculated by means of the calibration curve method according to DIN 32 645 ($\alpha = 0.05$; $k = 3$) (*27*). For the calculations, samples below the limit of quantification were considered as 0.

Statistics. Statistical evaluations of correlations (Spearman rank correlation analysis) and differences between groups (Mann−Whitney U test) were performed using the SPSS software package (SPSS 12.0.1, version 4).

RESULTS

Method Validation Parameters. The calibration curve linearity was ascertained in the range of 0.25−25 ng/μL for coumarin, cinnamic acid, and cinnamyl alcohol, in the range of 0.1−10 ng/μL for eugenol and safrole, and in the range of 3−120 ng/mL for cinnamaldehyde ($r \geq 0.99$).

For fortified amounts of 100, 500, and 1000 mg/kg, coumarin contents were found to be 103.2 ± 3.7, 510.6 ± 24.9, and 1008 ± 22 mg/kg (mean value ± standard deviation) with recovery rates of 103%, 102%, and 101%. Data on the precision of the method, expressed as intra- and interday repeatability, is given in **Table 2**. LOD and LOQ were determined to be 30 and 80 mg/kg for coumarin, 130 and 360 mg/kg for cinnamaldehyde, 30 and 88 mg/kg for cinnamic acid, 45 and 125 mg/kg for eugenol, 30 and 80 mg/kg for cinnamyl alcohol, and 15 and 44 mg/kg for safrole.

Cinnamon Samples from German Retail Market. Results for coumarin in cassia powder and sticks as well as in Ceylon powder and sticks are shown in **Table 3**. Whereas coumarin concentrations in powder of cassia cinnamon varied between 1740 and 7670 mg/kg ($n = 40$), a much greater variation (from < LOD to 9900 mg/kg) was found in the 29 cassia sticks. Of the latter, more than half of the samples were found to contain less coumarin than the lowest powder sample (16 sticks below 1600 mg/kg, including 9 sticks below 400 mg/kg). Mean values of coumarin levels in cassia powder (4020 mg/kg) and sticks (3250 mg/kg) were not statistically different. The contents in Ceylon powder varied between < LOD and 297 mg/kg and in sticks between < LOD and 486 mg/kg.

The concentration range, mean value, and median of the other four analytes investigated are shown in **Table 3**. Mean levels of cinnamaldehyde were found to be higher in cassia powder (24100 mg/kg) and sticks (30800 mg/kg) than in Ceylon powder (11100 mg/kg) and sticks (16700 mg/kg). Eugenol was detectable in many samples of Ceylon cinnamon (60% of sticks and 43% of

Table 3. Contents of Coumarin, Cinnamic Acid, Cinnamaldehyde, Cinnamyl Alcohol, and Eugenol in Cinnamon (Powder and Sticks) Obtained from the German Retail Market[a]

	coumarin (mg/kg)	cinnamic acid (mg/kg)	cinnamaldehyde (mg/kg)	cinnamyl alcohol (mg/kg)	eugenol (mg/kg)
			Cassia Powder (n = 40)		
mean value	4020	849[c]	24100[b]	90[c]	143
median	3790	863	22400	27	<LOD
range	1740−7670	90−1270	12000−42600	< LOD−672	<LOD−1540
			Cassia Sticks (n = 29)		
mean value	3252	596	30800	257	295
median	1374	572	33000	276	<LOD
range	< LOD−9900	112−1320	8930−54300	< LOD−604	0−3650
			Ceylon Powder (n = 7)		
mean value	64	252	11100	334	183
median	< LOD	235	11500	260	<LOD
range	< LOD−297	88−436	2080−24800	<LOD−946	<LOD−509
			Ceylon Sticks (n = 15)		
mean value	185	231	16700	476	1210
median	160	208	17400	560	434
range	<LOD−486	62−522	3930−28200	<LOD−888	<LOD−8140

[a] For the calculation of mean values, contents < LOQ are considered as 0. Significant differences were found for the five compounds if results of all cassia and Ceylon cinnamon samples (sticks and powder) are compared ($p < 0.01$). [b] Results are significantly different in sticks of the same botanical species: $p < 0.05$. [c] Results are significantly different in sticks of the same botanical species: $p < 0.001$.

powder), as well as in samples of cassia cinnamon (28% of sticks and 20% of powder). An overall comparison of samples (sticks and powder) showed that contents differed significantly between cassia and Ceylon cinnamon ($p < 0.01$) for all four analytes. A comparison between sticks and powder of the same botanical species showed that mean concentrations of cinnamaldehyde, cinnamyl alcohol, and eugenol were higher in the sticks, whereas mean concentrations of cinnamic acid were higher in the powder samples. These differences were significant in the case of cassia samples for cinnamic acid, cinnamaldehyde, and cinnamyl alcohol only. Safrole was not detected in any of the samples examined.

Cinnamon Sticks of the Same Package. To further elucidate the prominent variation of coumarin contents among cassia stick samples, several cassia sticks were analyzed, taken from two packages obtained from different companies (package A and B) showing no visible differences. Within the 12 sticks analyzed from package A, contents of coumarin varied considerably (**Table 4**), i.e. a 33-fold difference between the lowest (300 mg/kg) and the highest (9900 mg/kg) coumarin content. An even greater difference of coumarin levels (83-fold) was found in a second analysis (package B, 11 sticks analyzed, **Table 4**). While the lowest coumarin content was 130 mg/kg, the highest concentration was found to be 10900 mg/kg.

The contents of the other compounds analyzed did not vary to such an extent. In packages A and B, cinnamic acid contents differed 12- and 7-fold, cinnamaldehyde 3- and 5-fold, and cinnamyl alcohol 3- and at least 6-fold, respectively. Eugenol was quantified only in five sticks from package A and two sticks from package B, all containing high coumarin contents (**Table 4**).

Authentic Bark Samples from Indonesian Trees. Results of bark samples of cassia cinnamon from Indonesia are compiled in **Table 5**. Coumarin could not be detected in any sampling segment of trees no. 1 and 5. The coumarin levels of samples from tree no. 4 (from five different segments) varied considerably between lower stem (4−1: 470 mg/kg), middle stem (4−2: 6180 mg/kg), top stem (4−3: < LOQ), and the two branches (4−4: 1060 mg/kg; 4−5: 2780 mg/kg). These contents were measured in the homogenized samples of the segments, which were prepared by mixing comparable parts (approximately 3 cm) of each stick belonging to the respective segment. To further elucidate the phenomenon of the high variation within this tree, all sticks originating from the three different stem segments (five sticks each) were analyzed one by one (**Table 5**, figures quoted in italic type). The results relating to middle stem (4−2−1 to 4−2−5; coumarin levels

Table 4. Contents of Analytes in Cassia Sticks from Package A and Package B Obtained from the German Market, Showing No Visible Differences; Sticks Are Listed in Increasing Order of Their Coumarin Content

stick no.	coumarin (mg/kg)	cinnamic acid (mg/kg)	cinnamaldehyde (mg/kg)	cinnamyl alcohol (mg/kg)	eugenol (mg/kg)
			Package A		
1	300	1320	39300	604	<LOD
2	552	572	34700	396	<LOD
3	565	1070	33000	394	<LOD
4	786	202	14600	242	<LOD
5	1067	962	44300	422	<LOD
6	6450	112	15500	492	<LOD
7	6740	359	20100	209	694
8	7410	242	50100	576	905
9	7470	397	29800	279	745
10	7730	298	17300	231	593
11	8200	454	46400	276	<LOD
12	9900	466	16900	291	1050
			Package B		
1	130	685	43900	451	<LOD
2	154	1010	60100	180	<LOD
3	231	948	47700	97	<LOD
4	420	1060	64700	141	<LOD
5	871	1280	56500	162	<LOD
6	4650	206	12500	263	175
7	6890	657	39100	329	<LOD
8	7450	188	14000	298	<LOD
9	9980	568	30300	407	<LOD
10	10400	252	32000	<LOD	758
11	10900	630	40400	245	<LOD

Table 5. Analysis of Bark Samples Obtained Directly from Indonesia[a]

tree	samples no.	coumarin (mg/kg)	cinnamic acid (mg/kg)	cinnamaldehyde (mg/kg)	cinnamyl alcohol (mg/kg)	eugenol (mg/kg)
no. 1	1−1	<LOQ	568	95400	622	<LOD
	1−2	<LOQ	694	92400	393	<LOD
	1−3	<LOQ	649	87900	421	<LOD
	1−4	<LOQ	744	99200	448	<LOD
	1−5	<LOQ	670	96900	403	<LOD
	1−6	<LOQ	799	94100	421	<LOD
no. 2	2	3120	260	17600	765	217
no. 3	3	4250	173	25400	1610	335
	3−1	*4810*	*136*	*41300*	*1660*	*259*
	3−2	*6660*	*105*	*45500*	*1490*	*221*
	3−3	*2240*	*134*	*29600*	*881*	*<LOD*
	3−4	*2890*	*130*	*24300*	*1540*	*<LOD*
	3−5	*2690*	*185*	*29200*	*1260*	*<LOD*
no. 4	4−1	470	579	69405	288	<LOD
	4−1−1	*<LOQ*	*1010*	*52000*	*279*	*<LOD*
	4−1−2	*<LOQ*	*990*	*55000*	*285*	*<LOD*
	4−1−3	*<LOQ*	*1000*	*54000*	*294*	*<LOD*
	4−1−4	*<LOQ*	*1050*	*64300*	*383*	*<LOD*
	4−1−5	*5705*	*285*	*52800*	*729*	*793*
	4−2	6180	128	77700	751	806
	4−2−1	*5550*	*202*	*48600*	*1140*	*765*
	4−2−2	*5980*	*231*	*46400*	*734*	*747*
	4−2−3	*6760*	*307*	*45800*	*1060*	*918*
	4−2−4	*7380*	*235*	*41300*	*1080*	*796*
	4−2−5	*5850*	*316*	*44200*	*1080*	*860*
	4−3	<LOQ	728	75818	394	<LOD
	4−3−1	*<LOQ*	*933*	*45300*	*422*	*<LOD*
	4−3−2	*<LOD*	*781*	*30800*	*228*	*<LOD*
	4−3−3	*<LOQ*	*734*	*49000*	*617*	*<LOD*
	4−3−4	*<LOQ*	*1041*	*41200*	*315*	*<LOD*
	4−3−5	*<LOQ*	*760*	*47200*	*845*	*<LOD*
	4−4	2780	179	65400	1210	445
	4−5	1060	578	71800	356	<LOD
no. 5	5−1	<LOD	999	66600	325	<LOD
	5−2	<LOD	820	60300	470	<LOD
	5−3	<LOD	621	52300	444	<LOD
	5−4	<LOD	538	40500	496	<LOD

[a] The figures written in nonitalicized type are average values obtained from analysis (one per segment) of homogenized bark pieces of all sticks of the respective segment or tree. Additional analyses (figures quoted in italic type) were performed for single bark sticks of tree no. 3 (5 sticks randomly chosen) and tree no. 4 (5 pieces from each of the tree stem segments). Nomenclature of samples is given in **Table 1**.

5550−7380 mg/kg) and top stem (4−3−1 to 4−3−5; coumarin levels below LOQ) correspond to the data obtained from analyses of homogenized bark pieces. For the low stem samples (4−1−1 to 4−1−5), coumarin levels were found to be below the LOQ in four single sticks and 5710 mg/kg in the fifth one, which explains the relatively low level of 470 mg/kg obtained for the analysis of the homogenized bark pieces of this segment. Samples of the young trees no. 2 (age 2 years) and no. 3 (age 4 years) revealed relatively high coumarin levels of 3120 and 4250 mg/kg, respectively; additional analyses of five single sticks from tree no. 3 showed levels between 2240 and 6660 mg/kg (italic figures in **Table 5**).

Regarding cinnamaldehyde, especially in comparison to cassia samples from the German retail market, relatively high concentrations (between 40000 and 100000 mg/kg) were found in the bark of trees no. 1, no. 4, and no. 5 (**Table 3**), while they were distinctly lower in the younger trees no. 2 and no. 3 (17600 and 25400 mg/kg, respectively).

In this context, a noticeable correlation between eugenol and coumarin levels should be mentioned. Samples with a low coumarin level (< LOD to about 3000 mg/kg) did not have detectable eugenol levels, contrary to those with higher coumarin levels. The correlation between coumarin and eugenol was highly significant. The same is true for coumarin and cinnamyl alcohol, whereas coumarin correlated negatively with cinnamic acid and cinnamaldehyde. These correlations were not observed among the 29 samples of cassia sticks from the German retail market (**Table 3**).

DISCUSSION

Analytical Method. For analysis of coumarin contents of a large number of samples over a wide concentration range, the use of a fast and low-cost HPLC method with UV detection was preferred. It was our intention to quantify simultaneously prevalent cinnamon compounds (cinnamaldehyde, cinnamic acid, cinnamyl alcohol, eugenol) and safrole as a possible contaminant.

In comparison to the use of other solvents and extracting procedures, extracting cinnamon samples twice using methanol yielded the best results for all relevant compounds. This technique is similar to those described in previous studies (*16*, *24*), except we used an additional extracting step. The overall effort of analysis, including small sample amounts, uncomplicated sample treatment,

and fast HPLC determination, makes the presented method suitable for a rapid screening and a high throughput of samples.

Because there are inherent limitations to specificity of a HPLC-UV method, i.e. that it is impossible to exclude interferences of related minor compounds, a more specific GC-MS method was used in parallel for some samples to confirm HPLC results. The latter were found to compare favorably with those of GC-MS, nor did the use of a UV/vis DAD detector give evidence of considerable interferences in the quantification.

In accordance with results obtained by Sproll et al., the advantage of HPLC-UV methods over LC-MS/MS (*14*) in regard to lower cost for instrumentation, simple sample preparation and adequate LODs and LOQs should be emphasized.

Method Validation. As no blank matrices for the analysis of the other volatile constituents were available, recovery has been established for coumarin only. The average recovery rates ranging from 101 to 103% were satisfactory. Low % RSD values indicate the high precision and reliability of the method.

Coumarin in Cassia Cinnamon. Samples of cassia powder from the German retail market revealed coumarin levels between 1740 and 7670 mg/kg. In contrast, the range of concentrations in cassia sticks was considerably greater (< LOD and 9900 mg/kg) than expected on the basis of the usual botanical variation of plant constituents. Even in cassia sticks from the same package, supposed to originate from the same harvest, levels between 300 and 9900 mg/kg (package A) and between 130 and 10900 mg/kg (package B) were found. On the basis of analytical results from bark samples directly delivered from Indonesia, it can be assumed that major parts of bark may not contain detectable amounts of coumarin. It was even observed that the bark from the same segment of a single tree (tree no. 4, samples 4−1−1 to 4−1−5) may contain very different levels of coumarin (ranging between undetectable and high contents). Neither the age of trees nor the specific sampling segment on a tree seemed to be determining factors for the coumarin level in cassia cinnamon of our samples from Indonesia. Because commercially available cinnamon powder is produced by grinding bark deriving from several trees, the smaller variation range of coumarin in the powder samples from the German retail market is plausible.

In general, data on coumarin levels in cassia cinnamon obtained in this study confirms the results of earlier investigations (*8*, *13*, *14*, *16*, *19*). Analyses performed by the control laboratories of the Federal States in Germany in 2006 (n = 170, mostly based on cinnamon ground to powder) revealed mean coumarin levels of 2680 mg/kg (minimum < LOD, median = 2920 mg/kg, maximum = 8790 mg/kg) (*8*). Miller et al. (*13*) also observed a large range of coumarin concentrations from 700 to 12200 mg/kg in 11 samples of cassia cinnamon (sticks or powder not specified) from grocery stores in the USA and Korea. He et al. (*16*) analyzed bark samples of *Cinnamomum cassia*. They reported coumarin levels from below the LOD to 870 mg/kg (mean value 337 mg/kg) based on 15 samples obtained directly from trees of different regions in China, whereas much higher levels between 130 and 12200 mg/kg (mean 3520 mg/kg) were found in 15 samples from the retail market in Hong Kong. Lv et al. investigated 15 samples of Cassia cinnamon all originating from different regions in China (sticks or powder not specified). Coumarin contents varied between 330 mg/kg to 6500 mg/kg (*19*). It can be taken from these findings that interpretation of published data of coumarin analyses often appears difficult, as many factors may have influenced the results (e.g., botanical species, bark/powder, origin of market samples). Because this is the first time a sampling scheme with defined origin, age, and sampling segment was used to analyze bark of cassia cinnamon for coumarin levels, no comparable analytical data are available.

Coumarin in Ceylon Cinnamon. In general, coumarin mean levels in Ceylon cinnamon are by far lower than those in cassia cinnamon as shown in many other investigations (*2*, *8*, *9*, *13*−*16*, *22*). Yet coumarin contents of Ceylon cinnamon may exceed the range of traces: out of the 22 samples (sticks and labeled powder) analyzed, 13 showed detectable levels of coumarin, with a maximum level of 486 mg/kg. These results are consistent with those of Miller et al. (coumarin levels from < LOD to 190 mg/kg) (*13*) and Lungarini et al. (from 3 to 736 mg/kg) (*24*). In some investigations performed earlier (*17*, *22*), no coumarin was detected in samples of Ceylon cinnamon, probably due to a relatively poor LOD or a limited number of samples. Considering that coumarin levels in Ceylon sticks in some cases were found up to several hundred mg per kg and in cassia sticks in some cases below the LOD, it is not possible to infer that the presence of coumarin can be used to differentiate between the two types. But on the basis of our findings of coumarin in cinnamon powder, it is plausible that coumarin contents higher than approximately 1700 mg/kg refer to cassia cinnamon (**Table 3**).

Cinnamaldehyde. Generally, the concentration of cinnamaldehyde determines the flavor quality of cinnamon, low levels being known to represent material of inferior quality (*11*, *13*). Many other authors also report levels averagely lower in Ceylon cinnamon than in cassia cinnamon and levels on average lower in powder samples than in stick samples from the same botanical species (*2*, *13*, *22*, *28*). Remarkably, relatively low levels of cinnamaldehyde in the bark of trees nos. 2 and 3 (two and four years of age, respectively, which normally are too young for being harvested) were found compared to bark samples from the older trees (nos. 1, 4, and 5) from Indonesia. The latter had levels of cinnamaldehyde at least 2-fold higher than the younger ones, thus indicating that levels of cinnamaldehyde increase with increasing age of the tree. Such a phenomenon was not observed for any of the other analytes.

Powder and Stick Samples. In samples of the same botanical species obtained from German retailers, less cinnamic acid and more cinnamaldehyde and cinnamyl alcohol were found on average in the stick samples than in the powder samples (**Table 3**). Differences between powder and sticks were significant in the case of cassia only. Lower amounts of cinnamaldehyde and cinnamyl alcohol are probably due to evaporation over time in ground cinnamon, depending on storage conditions. Considering furthermore that cinnamaldehyde is known to undergo oxidation to cinnamic acid when exposed to air (*2*, *29*), this may be an explanation to levels of the latter increasing over time. A loss of cinnamaldehyde and an increase in cinnamic acid was also observed by other authors in methanolic extracts of cinnamon when allowed to stand at room temperature (*22*). A similar observation of an increase of cinnamic acid (+21%) and a decrease of cinnamaldehyde (−57%) and cinnamyl alcohol (−31%) was made after storage of cassia cinnamon powder at room temperature in an open-top vessel for one year. Poole et al. assumed that the concentration of cinnamic acid is an indicator of age and/or storage conditions (*2*).

Eugenol. Whereas eugenol was reported to be absent in cassia cinnamon or present in trace amounts only (*30*−*32*), it was detected though in the range of 190−1650 mg/kg in nine samples of Ceylon cinnamon (*13*). Actually, even though not detectable in all samples, our analyses revealed relatively high mean levels of eugenol in Ceylon cinnamon. As also reported by He et al. (*16*), eugenol was detectable in some of the cassia samples. Therefore, the detection of eugenol is not suitable to reliably distinguish individual samples of Ceylon cinnamon from those of cassia cinnamon. Furthermore, we observed a surprising correlation between coumarin and eugenol in samples of the same packages A and B, as well as in the samples received directly from

Indonesia. Because such correlations were not observed in the cassia samples from the German retail market, it may be taken as a phenomenon observable in samples from Indonesia only (supposing the sticks of package B also originate from Indonesia, as did those of package A), thus indicating a relation to *Cinnamomum burmannii*.

A contamination with safrole deriving from cinnamon leaves was not detected in any sample of cinnamon powder, as reported by Lv et al. (*19*).

In summary, coumarin levels in cassia cinnamon were found to be relatively high in powder samples, thus confirming the results of other authors. These levels are average values of the many sticks ground to powder. In contrast, coumarin levels can vary widely between single bark sticks, even within the sticks of a package and also within bark samples originating from the same tree. Single sticks may contain coumarin levels as low as those of Ceylon cinnamon. In Germany, it was recommended that heavy consumers of this spice use Ceylon cinnamon. Because of its limited availability on the world market, the use of cassia cinnamon with low coumarin levels may be an alternative. Therefore, further research is necessary to identify factors influencing the coumarin levels in cassia cinnamon and to possibly allow the harvesting of cassia cinnamon with low coumarin levels in the future.

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Supporting Information Available: Photos of cinnamon bark samples directly delivered by an Indonesian producer (trees 1−5). Analytical results are shown in **Table 5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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