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Note

Determination of cinnamaldehyde, coumarin and cinnamyl alcohol in cinnamon and cassia by high-performance liquid chromatography

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In Europe and Australia cinnamon is defined as the dried inner bark of the coppiced tree *Cinnamomum zeylanicum* Blume (Lauraceae) and is the sole source of cinnamon oil; cassia is defined as the dried bark of *C. cassia* Blume[1]. The substitution of cassia for cinnamon is obvious when the products are not powdered but the powdered spices are not readily differentiated. Various techniques have been used to distinguish between the two products: differences in fluorescence[2], mucilage ash values[3], thin-layer chromatography (TLC) to detect eugenol in cinnamon[4], one- and two-dimensional TLC patterns[5], differences in sedimentation[6], TLC to differentiate *Cinnamomum* species[7] and TLC to detect coumarin in cassia[8]. Cinnamon and cassia may be distinguished by the presence of coumarin (2*H*-1-benzopyran-2-one) in the latter and the presence of coumarin in cinnamon is indicative of the addition of other *Cinnamomum* species. The major aromatic compound present in the *Cinnamomum* species is cinnamaldehyde (3-phenyl-2-propenal) together with smaller quantities of cinnamyl alcohol, cinnamyl acetate, eugenol and 2-methoxy-cinnamaldehyde. Cinnamaldehyde has been determined in cinnamon by colorimetry[9], polarography[10], fluorimetry[11], gas chromatography[11] and high-performance liquid chromatography (HPLC)[11] using a silica column with a mobile phase of heptane and chloroform. Cinnamaldehyde and eugenol have been determined in cinnamon and cassia oils by HPLC using a Corasil column and a mobile phase of ethyl acetate in cyclohexane[12]. This note describes the separation and determination of coumarin, cinnamyl alcohol and cinnamaldehyde in cinnamon and cassia powder using an aqueous mobile phase with a C_8 stationary phase and vanillin as an internal standard.

EXPERIMENTAL

Chromatography

The apparatus used consisted of an Altex Model 321 liquid chromatograph with a Rheodyne 7125 sample injector fitted with a 10-μl loop, and an Erma Model ERC-7210 variable-wavelength detector set at 275 nm and 0.08 absorbance units. A LiChrosorb RP-8 reversed-phase column, 250 × 7 mm O.D., 5-μm particle size with a Brownlee RP-8 5-μm, 3-cm guard column, was used with a flow-rate of 1 ml/min. The mobile phase was a mixture of 600 ml of filtered de-ionised water with 120 ml

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of methanol, 200 ml of acetonitrile and 80 ml of tetrahydrofuran. All organic solvents were of HPLC grade. TLC was carried out using 10 × 5 cm silica plates, Merck 5719, with a mobile phase of dichloromethane. Coumarin was detected by spraying with 1 *N* potassium hydroxide and examination under long-wavelength UV light[13].

Reagents

Cinnamaldehyde and cinnamyl alcohol were both purum grade (Fluka), cinnamic acid and vanillin were Unilab grade (Ajax Chemicals, Sydney, Australia), cinnamyl acetate, coumarin, eugenol, methyl cinnamate and safrole were from Tokyo Kasei and 2-methoxycinnamaldehyde was from Aldrich. Methanol for extraction was AR grade, May and Baker.

A standard solution was prepared to contain 200 mg cinnamaldehyde, 30 mg coumarin and 25 mg cinnamyl alcohol in 100 ml of methanol. A volume of 5 ml of this solution with 5 ml internal standard solution were diluted to 100 ml with methanol to give a dilute standard solution. The internal standard solution consisted of 0.1% (w/v) vanillin in methanol.

Procedure

Inject 10 μl of the dilute standard solution and determine the ratios of the peak areas of cinnamaldehyde, cinnamyl alcohol and coumarin to vanillin. Weigh accurately about 0.5 g of powdered sample and add about 80 ml of methanol. Boil gently for 15 min, cool and add 5 ml of internal standard solution and dilute to 100 ml with methanol. Allow to stand briefly, filter the supernatant liquid through a 0.45-μm filter and inject 10 μl of the filtrate. From the peak area ratios of cinnamaldehyde, cinnamyl alcohol and coumarin to vanillin, calculate the percentage of cinnamaldehyde, cinnamyl alcohol and coumarin in the sample.

RESULTS AND DISCUSSION

Cinnamon[14] and cassia[15] contain a large number of compounds including terpenes and aromatic aldehydes and esters. The major component in both spices is cinnamaldehyde, together with cinnamyl acetate, cinnamyl alcohol and eugenol. In addition cinnamon is reported to contain safrole[14,16], absent from cassia, and 2-methoxycinnamaldehyde[8,17], although this latter compound was earlier reported to be present in cassia alone[7,18]. All these compounds have UV absorption maxima in the range 250–290 nm; a detector wavelength of 275 nm was chosen as this is one of the UV maxima of coumarin.

Methanol readily dissolves the compounds of interest and has been used to extract cinnamon and cassia for analysis by TLC[8]; it was therefore selected as the solvent for extraction. Preliminary experiments showed that an aqueous phase containing methanol, acetonitrile and tetrahydrofuran was required to give a separation of the compounds of interest. The most effective separation, *i.e.* minor components eluted before the major component, was obtained with the mobile phase composition listed under *Chromatography*. Vanillin was found to be a suitable internal standard. Methanolic solutions of vanillin have been reported[19] to produce additional peaks on standing, possibly due to acetal or hemi-acetal formation but this was not found in the present work. All standard solutions were kept at 4°C in the dark when not

in use. Methanolic extracts of cinnamon, when allowed to stand at room temperature (20–28°C) for 4 weeks, showed a loss of cinnamaldehyde, an increase in cinnamic acid and the production of an additional peak, with a retention time of 19.3 min, which was identified by co-chromatography as methyl cinnamate. Cinnamon extracts were usually analysed within 1–2 h of preparation.

The separation of reference compounds is shown in Fig. 1A. Typical retention times were (in min): cinnamic acid, 4.0–4.1; vanillin, 6.2–6.4; coumarin, 8.3–8.5; cinnamyl alcohol, 9.3–9.5; cinnamaldehyde, 11.1–11.4; eugenol, 15.5–16.1 and cinnamyl acetate, 22.5–24. The detector response for cinnamaldehyde, cinnamyl alcohol and coumarin was linear up to concentrations of 15, 2 and 3 mg/100 ml respectively, corresponding to 3% cinnamaldehyde, 0.4% cinnamyl alcohol and 0.6% coumarin in the sample. Cinnamaldehyde (10 mg), cinnamyl alcohol (1 mg) and coumarin (1.5 mg) were added to a sample of powdered cinnamon containing 0.65% cinnamaldehyde and no cinnamyl alcohol or coumarin. The means of duplicate recoveries were 95%, 110% and 103% respectively.

Chromatograms of genuine cinnamon extracts, Fig. 1B and C, showed coumarin to be absent; cinnamon was reported to contain less than 0.0008% (8 ppm) coumarin[8]. The chromatograms also showed the presence of low concentrations of cinnamyl alcohol and the presence of a peak m, identified by co-chromatography as 2-methoxycinnamaldehyde, retention time 14.0–14.4 min. These peaks, together with eugenol and cinnamyl acetate, were present in the stronger extract, Fig. 1C. The chromatogram of genuine cassia, Fig. 1D, showed the presence of coumarin and the absence of 2-methoxycinnamaldehyde. All chromatograms showed cinnamaldehyde as a major peak. Safrole, retention time 31 min, was not detected in any of the samples examined.

Samples of cinnamon, cassia, *C. burmanni* Blume and retail samples of powdered cinnamon were examined by HPLC; the results are shown in Table I. The mean cinnamaldehyde concentration found in genuine cinnamon was 1.99% and in

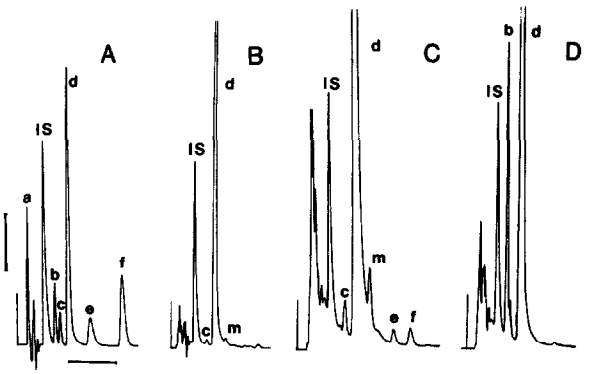

Fig. 1. HPLC chromatograms of (A) reference compounds; (B) genuine cinnamon (0.5 g/100 ml); (C) genuine cinnamon (2.5 g/100 ml); (D) genuine cassia (0.5 g/100 ml). Peaks: a = cinnamic acid, b = coumarin, c = cinnamyl alcohol, d = cinnamaldehyde, e = eugenol, f = cinnamyl acetate, m = 2-methoxy-cinnamaldehyde, IS = internal standard (vanillin). Vertical bar = 0.01 a.u.; horizontal bar = 10 min. For chromatographic conditions see text.

TABLE I

HPLC ANALYSIS OF CINNAMON AND CASSIA SAMPLES

N.D. = Not detected. Detection limits: coumarin, 0.01%; cinnamyl alcohol, 0.02%.

Sample	*Cinnamaldehyde (%)*	*Cinnamyl alcohol (%)*	*Coumarin (%)*
Cinnamon, quills (*C. zeylanicum*)			
9 Samples, mean	1.99	0.043	N.D.
Range	1.49–3.20	N.D.–0.083	
Cassia, bark (*C. cassia*)			
6 Samples, mean	2.56	N.D.	0.45
Range	0.76–3.37		0.14–0.70
C. burmanni, bark	0.054	0.046	0.042
Cinnamon powder retail			
1	1.10	0.031	N.D.
2	3.37	0.035	N.D.
3	0.91	ND	ND
4	1.02	0.050	ND
5	0.66	0.024	ND
6	2.57	ND	0.41
7	1.69	ND	0.20
8	3.02	ND	0.60
9	1.52	ND	0.33
10	2.39	ND	0.031
11	2.12	ND	0.046
12	1.81	ND	0.050

cassia, 2.56%. Tsai and Chen reported[11] cinnamaldehyde concentrations of 4.49% and 7.89% for cinnamon and cassia respectively, when determined by HPLC. *C. zeylanicum* is reported to contain 1–2% volatile oil[1] which contains 60–80% cinnamaldehyde[17], equivalent to 0.6–1.6% cinnamaldehyde in cinnamon. Cinnamon (Ceylon Zimt) was reported to contain 1.3–1.8% cinnamaldehyde and cassia (Chinesischer Zimt) 1.3–2.8% cinnamaldehyde[20]. The sample of *C. burmanni* contained coumarin and cinnamyl alcohol but very little cinnamaldehyde. The retail samples 1–5 appeared to be genuine cinnamon, lacking coumarin, but samples 6–9 were identified as cassia. The presence of coumarin in these samples was confirmed by TLC as described above under *Chromatography*. The remaining samples, 10–12, contained only traces of coumarin and did not appear to be either cinnamon or cassia. They may be *C. burmanni* or *C. loureirii* Nees, both of which are reported to contain coumarin[7,8].

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