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A FLUOROMETRIC ASSAY OF *trans*-CINNAMALDEHYDE IN CINNAMON

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In China (1), cinnamon is often used as both a medicine and a condiment. The principal component of this drug is *trans*-cinnamaldehyde. Accordingly, the evaluation of this drug is usually performed by determination of its *trans*-cinnamaldehyde content. For this purpose, several methods have been reported. They are bisulfite (2), tlc (3), gc (3,4), hplc (5), paper chromatography (6), and nmr spectrometric methods (7). However, no fluorometric method has been found.

It is well known that fluorometry is more specific than absorptiometry. It is frequently used to determine a biogenic substance present in a complex mixture. Owing to its high specificity, fluorometry is frequently characterized by simplicity of sample preparation and methodology that commends it for routine use. For these advantages, the authors developed the following fluorometric method of analysis for determining the *trans*-cinnamaldehyde content of cinnamon and compared it to both gc and hplc methods of analysis.

EXPERIMENTAL

MATERIALS AND REAGENTS.—All chemicals used were reagent grade obtained from commercial sources. Isonicotinylhydrazine (INH) from Asia Chemical Co. (Japan) and *trans*-cinnamaldehyde from Tokyo Chemical Industry Co. (Tokyo, Japan) were used without further purification.

The reagent for the fluorescence reaction was freshly prepared by mixing 1 ml of 120 mM methanolic INH, 1 ml of 60 mM methanolic $AlCl_3$, and 1 ml of MeOH.

Two kinds of cinnamon, common Kuei-pi (*Cinnammoum zeylanium* Nees.) and Ching Hua Yu-kuei (*Cinnammoum cassia* Blume.), were purchased from Taiwan markets and were ground to a fine powder just before being used.

PREPARATION OF THE SAMPLE SOLUTION.—One-half gram of the powder of common Kuei-pi (250 mg, for Ching Hua Yu-kuei) was accurately weighed and extracted in a boiling H_2O bath for 10 min with eight 5-ml portions of H_2O in a glass-stoppered test-tube (1.5×15 cm). The supernatant was separated with a centrifuge and transferred to a 100-ml separator with a dropper. The H_2O extract was extracted with five 5-ml portions of petroleum ether (bp, 50-90°). The petroleum ether extract was then dried with a sufficient amount of anhydrous Na_2SO_4 and diluted to 50 ml. The solution obtained was used as a sample solution for gc and hplc. A tenfold diluted solution was used for the fluorometric determination.

RECOMMENDED PROCEDURE FOR FLUOROMETRIC DETERMINATION[1] OF *trans*-CINNAMALDEHYDE CONTENT OF CINNAMON.—The reagent (3 ml) was added to 0.1 ml of the sample solution in a test tube. The mixture was then warmed at 50° for 10 min. After cooling in running H_2O for 3 min, the fluorescence intensity was immediately measured with excitation and emission wavelengths at 399 nm and 510 nm, respectively, against a blank. A calibration curve was prepared by the same procedure as above.

GC DETERMINATION[2] OF *trans*-CINNAMALDEHYDE CONTENT OF CINNAMON.—The conditions for gc were as follows: column: 10% PEG-20M on Shimalite in a stainless steel tube (2 m×0.4 cm); injection port temperature: 215°; column temperature: 120°; carrier gas: N_2, 60 ml/min.

HPLC DETERMINATION[3] OF *trans*-CINNAMALDEHYDE CONTENT OF CINNAMON.—The sample solution was chromatographed on a LiChrosorb Si 100 column (5 μm, 250 mm×4.6 mm, internal diameter) at 30° and detected at 254 nm. The mobile phase used was a mixture of $CHCl_3$ and *n*-heptane (12:88, v/v) at a flow rate of 2.0 ml/min.

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[1]A Shimadzu RF-520 dual beam difference spectrofluorophotometer (Tokyo, Japan) was used.

[2]A Shimadzu GC-5A gas chromatograph equipped with a flame ionization detector (Tokyo, Japan) was used.

[3]A Hewlett Packard 1084B high-performance liquid chromatograph equipped with a Hewlett Packard 79850B LC terminal and a variable wave-length detector (USA) was used.

RESULTS AND DISCUSSION

The sample solution used in the recommended procedure was prepared by an extraction method described by Wu *et al.* (4). However, there was no investigation on the extraction procedure in that paper. Thus, the extraction procedure was reinvestigated with 500 mg of common Kuei-pi as a sample. The investigation was performed with the procedure described in the Experimental section except that extraction times were changed. The results show that the sample powder and, successively, the H_2O extract should be extracted eight times with 5-ml portions of H_2O and five times with 5-ml portions of petroleum ether, respectively.

Horikawa *et al.* (8) described a fluorometric method for the determination of Δ^4-3-ketosteroids by using INH and an aluminum salt as the reagents, and also indicated that this fluorogenic reaction was highly specific for certain α,β-unsaturated carbonyl compound such as *trans*-cinnamaldehyde and citral. In order to search for a specific and simple method of determining the *trans*-cinnamaldehyde content of cinnamon, this reaction was studied.

By changing the reaction time or temperature of the recommended procedure, the optimal reaction time and temperature were found to be 10 min and 50°, respectively.

With this procedure, a calibration curve, which was linear up to 8 nmole/ml of petroleum ether extract, was obtained and was used for the determination of the cinnamaldehyde content of cinnamon.

Due to their high efficiency and selectivity, gc and hplc were chosen as the standard methods for the evaluation of the present method.

trans-Cinnamaldehyde content of cinnamon had been determined by gc with a column packed with 1.5% SE-30 on Chromosorb W (4). With this column, a chromatogram with an asymmetric peak of *trans*-cinnamaldehyde had been obtained. To improve this drawback, a relatively high polar column, PEG-20M, was used. With this column and the optimal conditions indicated in the Experimental section, a straight calibration curve through the origin was obtained.

An hplc method was also developed for this evaluation. After investigation of LiChrosorb RP-18, LiChrosorb RP-8, and LiChrosorb Si 100, LiChrosorb Si 100 was chosen for the separation of *trans*-cinnamaldehyde. The chromatogram shows good separation. With the conditions indicated in the Experimental section, a straight calibration curve through the origin was obtained.

By using these three methods, the *trans*-cinnamaldehyde contents of common Kuei-pi and Ching Hua Yu-kuei were determined and are shown in Table 1. The results indicate that the fluorometric method is in good agreement with the results obtained using established chromatographic procedures. In addition, the fluorometric method is simpler and easier to perform; hence, it is recommended for the deter-

TABLE 1. The *trans*-Cinnamaldehyde Contents Determined with Different Methods

Method	*trans*-Cinnamaldehyde content of common Kuei-pi (% w/w)[b]	*trans*-Cinnamaldehyde content of Ching Hua Yu-kuei (% w/w)[b]
fluorometry[a]	5.41±0.11	9.11±0.06
gc[a]	5.60±0.29	8.88±0.58
hplc[a]	4.49±0.08	7.89±0.13

[a]The procedure was described in Experimental section.
[b]Mean±SD of six determinations.

mination of *trans*-cinnamaldehyde content of cinnamon.

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