生 薬 学 雑 誌
Shoyakugaku Zasshi
39(3), 223~227 (1985)

Evaluation of Crude Drugs by the Combination of Enfleurage and Chromatography (I) Paeonol in Moutan Cortex and Paeoniae Radix

Hitoshi Okugawa,[a] Masataka Moriyasu,*,[b] Atsushi Kato,[b]
Katsuhiko Matsumoto,[a] Akihiro Fujioka[a] and Yohei Hashimoto[b]

[a] *Institute for Oriental Medicine, Hyogo, Kitajonai, Amagasaki, Hyogo 660*
[b] *Kobe Women's College of Pharmacy, Motoyamakita-machi, Higashinada-ku, Kobe 658*

(Received February 27, 1985)

Moutan Cortex and Paeoniae Radix were examined for their content of paeonol by a combination of enfleurage method used in perfume industry and chromatographic technique. A very small amount of paeonol was efficiently detected by the present method without interference by coexisting foreign substances as encountered in conventional extraction methods. Addition of 4-methoxyacetophenone as an internal standard facilitated the quantitative determination of paeonol in these crude drugs. The presence of 0.025% by weight of paeonol was demonstrated by the present method in Paeoniae Radix, which was about 1/100 that in Moutan Cortex. It was also shown that the paeonol content was higher in portions near the cortex in both paeony root and Moutan Cortex.

Keywords——paeonol; Moutan Cortex; Paeoniae Radix; enfleurage method; HPLC analysis

Moutan Cortex (root bark of *Paeoniae moutan* Sims (Paeoniaceae)) and Paeoniae Radix (root of *Paeonia lactiflora* Pallas (Paeoniaceae)) are important crude drugs in Chinese traditional medicine. Paeonol is considered to be one of the effective components in Moutan Cortex,[1] but Paeoniae Radix has not been shown to contain paeonol. Although some authors quoted[2] that paeonol is contained in Paeoniae Radix, the literature cited therein[3] shows no evidence for the presence of paeonol. In the present investigation, we found that sublimating and volatile compounds were effectively detected by enfleurage chromatography, technique described in our previous communication,[4] by which volatile components are specifically absorbed into fat to be analyzed effectively by chromatography. This paper deals with microdetermination of paeonol in Paeoniae Radix and Moutan Cortex. Quantitative determination of paeonol was carried out with 4-methoxyacetophenone as an internal standard.

Experimental

Reagents—Paeonol (2-hydroxy-4-methoxyacetophenone) was purchased from Aldrich Chemical Co. Inc., 4-methoxyacetophenone, triolein, and tripalmitine were obtained from Wako Pure Chemicals Co. Ltd., lactose was purchased from Fujii Chemicals Co. Ltd., and filter papers (No. 5C, ashless) were supplied by Toyo Filter Paper Co. Ltd.

Plant materials—Eight commercial Paeoniae Radix and one commercial Moutan Cortex samples used in the present work were kindly offered by Tochimototenkaido Co., Ltd. These samples were powdered in porcelain mortar and dried in desiccator before use.

Apparatus—HPLC was conducted with LKB 2150 apparatus equipped with a variable wavelength UV detector (UVICORD SD). Satisfactory separation of paeonol from other components was achieved on a reverse phase column (Spherisorb ODS, 5 μm, 4.6 mm$\times$15 cm) with a solvent system, water-acetonitrile containing a small amount of acetic acid. Paeonol was also detected on normal phase chromatography using a silica gel column (Li-Chrosorb SI-100, 5 μm, 4.6 mm$\times$15 cm) with a solvent system hexane-ethyl acetate, but detection of paeonol on the silica gel column was often interferred by coexisting foreign substances, especially when the extract was prepared according to the conventional method.

Procedure—One gram of pulverized samples was placed in a small petri dish (4.5 cm in internal diameter and

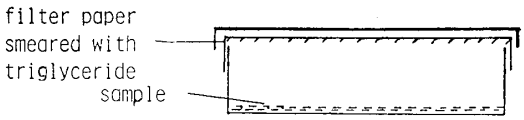

Fig. 1. The Container for Enfleurage Method

1.5 cm in height). For the quantitative analysis of paeonol by the present method, addition of an internal standard was essential. A certain amount of 4-methoxyacetophenone dissolved in methanol was added to the sample and then stirred well. About 0.2 g of fat (a mixture of triolein:tripalmitine, 10:1) was spread over a round filter paper (5.5 cm in diameter), which was placed on the top of the petri dish. The petri dish was closed (Fig. 1), and placed in a drying oven at 100°C for 2 hr. The filter paper was then extracted with 5 cm^3 of 50% aqueous acetonitrile in a glass tube for 20 min with ultrasonication. The extract was filtered through a small filter paper and the filtrate was used for HPLC analysis.

In order to compare the enfleurage extraction technique with usual extraction method, extracts of crude drug samples were prepared with several solvents, methanol, ethanol, and ether and the extracts were subjected to HPLC.

Results and Discussion

Comparison of enfleurage and usual extraction method

Figures 2 and 3 compare HPLC chromatograms of conventional extracts from Moutan Cortex and Paeoniae Radix with those of enfleurage extracts. As shown in these chromatograms, the detection of paeonol in extracts by HPLC was seriously affected by coextracted substances. Thus, in conventional solvent extracts from Paeoniae Radix in which the content of paeonol was very low the presence of paeonol was often not clearly shown. In contrast, the enfleurage method proved to be much superior and demonstrated the presence of paeonol in Paeoniae Radix and Moutan Cortex without being affected by coexisting substances. The present results suggest that volatile components and presumably sublimating components are both specifically absorbed by fat.

Effect of temperature

To determine the optimum temperature for the enfleurage method, samples were subjected to enfleurage in drying oven kept at various temperatures for 2 hr, and the content of paeonol in each extract was measured by HPLC, the results being shown in Fig. 4. The peak height of paeonol increased gradually as the enfleurage temperature increased and reached maximum at 100°C. Further increase in temperature brought about slight decrease in the peak height, and the samples colored a little brown. Therefore, in the present experiment the enfleurage step was carried out at 100°C.

Effect of time

To determine the optimum time for the enfleurage process, enfleurage was conducted at 100°C for a

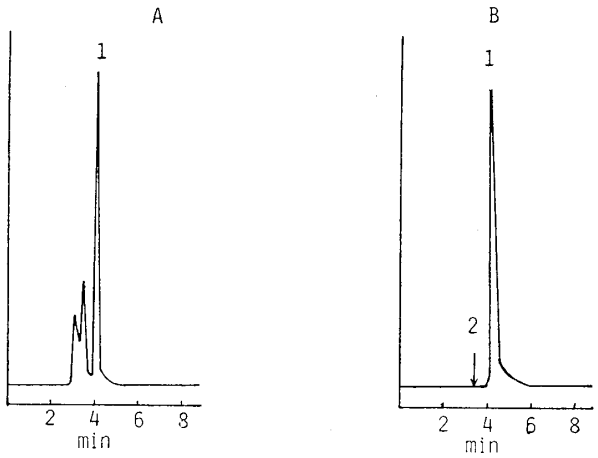

Fig. 2. Comparison of Enfleurage and Extraction Method for Moutan Cortex by HPLC Analysis

A: extracted with ethanol, B: enfleurage method. 1: paeonol, 2: 4-methoxyacetophenone (internal standard). HPLC conditions: Column: Spherisorb ODS, 5 μm (4.6 mm$\times$ 15 cm); Eluent: acetonitrile–water–acetic acid=100:10:0.2, 1.2 cm^3/min; Detection: UV 280 nm.

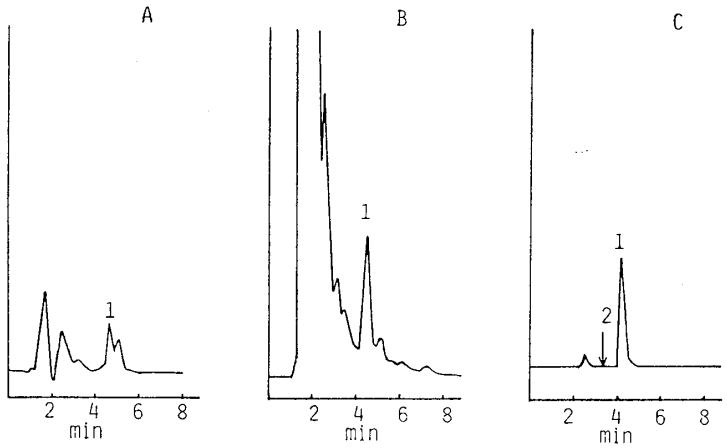

Fig. 3. Comparison of Enfleurage and Extraction Method for Paeoniae Radix by HPLC Analyses
A: extracted with ether, B: extracted with methanol, C: enfleurage method. 1: paeonol, 2: 4-methoxyacetophenone. For HPLC conditions, see Fig. 2.

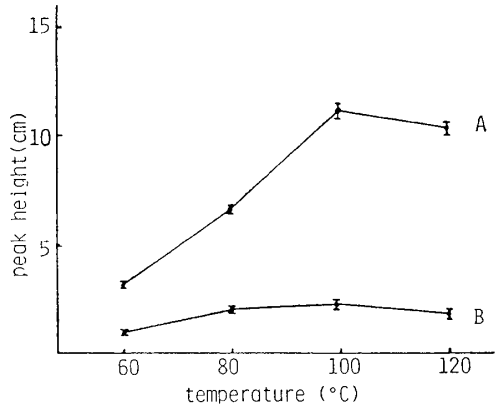

Fig. 4.

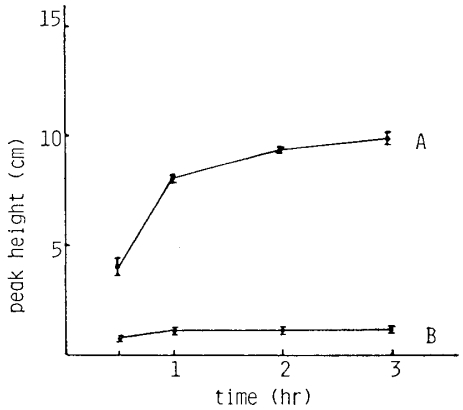

Fig. 5.

Fig. 4. Dependence of Peak Height of Paeonol on Temperature in Enfleurage Process
A: Moutan Cortex (China), 2.5 μl, B: Paeoniae Radix (Japan, No. 1 in TABLE 1), 20 μl. $n=5$, $\bar{\bullet}$: mean$\pm$S.E.

Fig. 5. Dependence of Peak Height of Paeonol on Heating Time in Enfleurage Process
A: Moutan Cortex (China), 2.5 μl, B: Paeoniae Radix (Japan, No. 1 in TABLE 1), 20 μl. $n=5$, $\bar{\bullet}$: mean$\pm$S.E.

various period of time (0.5–3.0 hr). Each sample was then analyzed by HPLC. As shown in Fig. 5, the peak height grew gradually with the increase in the enfleurage time. The increase in the peak height, however, was not so great after an hour. Consequently, 2 hr was considered to be suitable.

Addition of internal standard

By the method described above, it was demonstrated that even a very small amount of paeonol could be specifically detected. The quantity of paeonol was, however, not determined by this method because of the poor reproducibility even when the experimental conditions such as temperature and time were strictly controlled. In order to overcome this difficulty internal standard substance was introduced into the system. 4-Methoxyacetophenone was chosen as an internal standard as its structure resembled that of paeonol. Separation of paeonol from the internal standard in HPLC was shown to be quite easy and not interferred by coexisting substances. First, a certain amount of the internal standard dissolved in methanol was added to 1.0 g of lactose, and then various amounts of paeonol dissolved in methanol were added to it. Each sample was mixed well and subjected to enfleurage-HPLC. The ratios of the peak height of paeonol to that of the internal standard were calculated for each sample, and plotted (Fig. 6) against the paeonol content. A linear calibration graph was obtained, which showed that quantitative analysis was possible by the incorporation of an internal standard. To show that this internal

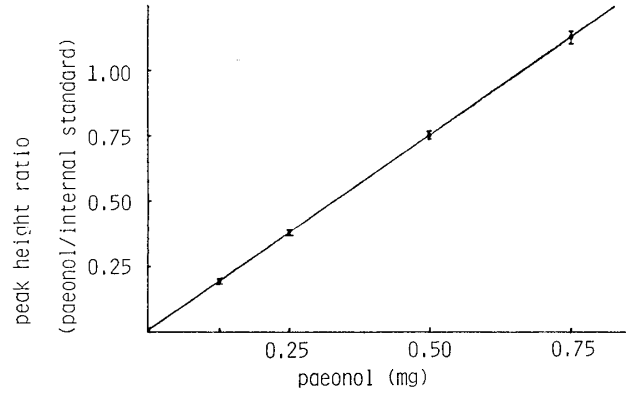

Fig. 6. Calibration Graph of Paeonol Using 4-Methoxyacetophenone as an Internal Standard
$y=1.503x+0.016$, $r=0.999$, $n=5$, ⊕: mean±S.E.

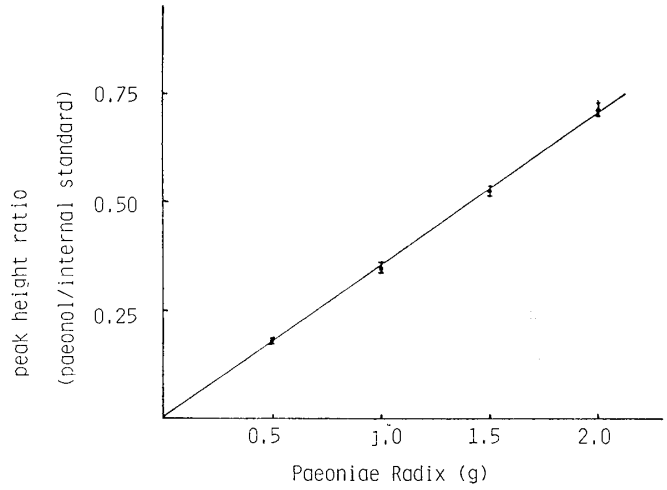

Fig. 7. Calibration Graph of Paeonol in Paeoniae Radix Sample
$y=1.504x-0.005$, $r=0.999$, $n=5$, ⊕: mean±S.E.

TABLE I. Content of Paeonol in Various Paeoniae Radix

No.	Crude drug samples	Paeonol content (mg/g) mean±S.E. ($n=5$)
1	Japan (Nagano)	0.230±0.009
2*	Japan (Nara)	0.006±0.001
3	Korea	0.140±0.002
4*	China (Bai-Shao-Yao)	trace
5	China (Chi-Shao-Yao)	0.240±0.004
6	China (Chi-Shao-Yao)	0.234±0.002
7	Japan (Hokkaido)	0.044±0.005
8	North Korea	trace

*scalded.

TABLE II. Distribution of Paeonol in Different Tissues of Paeniae Radix

Section	Paeonol content (mg/g)
A	0.062
B	0.016
C	0.031

$n=5$

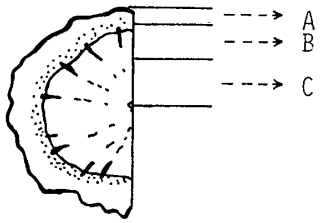

Fig. 8. Transverse Section of Paeoniae Radix

standard method is applicable to the practical assay of paeonol in crude drug samples, the following experiments were carried out: A certain amount of the internal standard was mixed into various amounts of powdered Paeoniae Radix (0.5–2.0 g) and then the mixture was subjected to enfleurage. As shown in Fig. 7, the ratios of the peak height of paeonol in Paeoniae Radix to that of the internal standard added produced, when plotted against the amount of Paeoniae Radix, a linear calibration graph, demonstrating that the internal standard method is applicable to the quantitative determination of paeonol in crude drugs.

Contents of paeonol in Moutan Cortex and Paeoniae Radix

By using the calibration graph shown in Fig. 6, Moutan Cortex and Paeoniae Radix were assayed for their paeonol content. The content of paeonol in Moutan Cortex (Chinese occurrence) was found to be 1.6% by weight, which was close to the value reported by other authors.[5] The content of paeonol in various Paeoniae Radix samples are given in TABLE I. Paeonol was found to be contained in all the samples tested.

Then, Paeoniae Radix (No. 6 in TABLE I) was divided into three parts as shown in Fig. 8, and the content of paeonol in each part was determined. TABLE II shows that a larger portion of paeonol in Paeoniae Radix exists near the cortex as in the case of root of *Paeonia moutan* SIMS.[6]

The present study clearly demonstrated that volatile and sublimating components can be selectively assayed by the present enfleurage—chromatography technique with very high sensitivity. This technique accordingly provides a very effective in microdetermination method which enables detection of such compounds even when their content is very low, with no interference by coexisting substances as encountered in the usual extraction procedures.

Acknowledgement: The authors wish to express their gratitude to Dr. Usha Shome, National Botanical Research Institute, Lucknow, India, who kindly corrected the English of this paper.

References and Notes

1) a) N. Nagai, *Yakugaku Zasshi*, **7**, 283 (1887); **8**, 495 (1888); b) M. Harada, Y. Yamashita, *ibid.*, **89**, 1205 (1969); c) M. Harada, A. Yamashita, M. Aburada, *ibid.*, **92**, 750 (1972).
2) a) Jiang Su New Medical College (ed.), "Dictionary of Chinese Crude Drugs," "Zhong-yao-dai-ci-dian" (中薬大辞典), Shanghai Scientific Technological Publisher, Shanghai, 1977, p. 707; b) "Abstracts of Chinese Traditional Medicine," "Zhong-yao-yan-jui-wen-xian-zhai-yao" (中薬研究文献摘要), Scientific Publisher, Beijing, 1975, p. 255.
3) L. Chi-shau, C. Lee, W. Mu-chou, L. Cheng, *Acta Pharm. Sinica, Beijing* (薬学学報), **4**, 17 (1956).
4) Y. Hashimoto, K. Kawanishi, H. Tomita, M. Moriyasu, Y. Uhara, A. Kato, *Anal. Lett.*, **16**, 317 (1983).
5) a) M. Konoshima, Y. Kano, K. Otsuka, J. Ihara, *Shoyakugaku Zasshi*, **27**, 124 (1973); b) Y. Akada, S. Kawano, M. Yamagishi, Y. Tanase, *Yakugaku Zasshi*, **100**, 212 (1980).
6) T. Tani, T. Katsuki, M. Kubo, S. Arichi, I. Kitagawa, *Shoyakugaku Zasshi*, **34**, 292 (1980).