Pharmacokinetic Study of Paeonimetabolin I, a Major Metabolite of Paeoniflorin from Paeony Roots

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Plasma concentrations of paeoniflorin (PF) and its major metabolite, paeonimetabolin I (PM-I), were estimated after oral administration of PF to rats at doses of 0.5 and 5 mg/kg. The maximal plasma concentrations ($C_{\max}$) of PF were 9.9 and 20.3, and those of PM-I were 16.5 and 101.7 ng/ml at each dose, respectively. The times to $C_{\max}$ ($t_{\max}$) of PF were 11.6 and 13.3, and those of PM-I were 60 and 80 min, respectively. The AUC_{0-180} of PM-I were 1873 and 12358, and those of PF were 300 and 1174 ng min/ml, respectively.

On the other hand, after intravenous administration of PM-I to rats at doses of 0.2 and 2 mg/kg (equal in molar ratio to 0.5 and 5 mg/kg PF), the plasma concentration of PM-I decreased rapidly and the plasma concentration–time curve profile of it fitted well with the two-compartment model at each dose, with terminal half lives ($t_{1/2}$) of 90.9 and 90.6 min. The V_{dss} values were 0.91 and 3.79 l/kg, the CL_{tot} values were 8.7 and 39.9 ml/min kg, and the AUC_{0-180} values were 5614.1 and 13176.0 ng min/ml, at each dose, respectively. The significant increase in V_{dss} and CL_{tot} with increasing doses suggested dose-dependent pharmacokinetics.

When PM-I was given orally at the same doses, the following parameters were shown: $C_{\max}$ of 102.2 and 285 ng/ml at $t_{\max}$ 6.2 and 7.5 min and AUCs of 4145.6 and 14182.1 ng min/ml, at each dose. The bioavailability (F) values were 0.8 and 1.07, respectively.

These findings indicated that the high percentage of PM-I transformed by intestinal bacteria was rapidly absorbed from the gastrointestinal tract, and a significantly high concentration of PM-I, rather than PF, was present in the plasma after oral administration of PF.

Key words pharmacokinetics; enzyme immunoassay; metabolism; paeonimetabolin I; paeoniflorin; paeony root

Paeoniflorin (PF; **1**) is a characteristic monoterpene glucoside (Fig. 1) present in paeony roots, which have been widely used in traditional Chinese medicine. Therapeutic effects of paeony roots have been explained by the pharmacological actions of PF (**1**).^{1,2} However, recent studies showed extremely poor absorption and low bioavailability of orally administered PF (**1**),^{3,4} suggesting the bacterial transformation of PF (**1**) in the gastrointestinal tract (GIT) and the role of metabolites in the therapeutic effects of PF (**1**). These were also supported by evidence that PF (**1**) is readily metabolized into a series of metabolites named paeonimetabolins I, II and III by human intestinal bacteria *in vitro*.^{5–7} Recently, we were able to determine the major metabolite, paeonimetabolin I (PM-I; **2**), in rat plasma, using the enzyme immunoassay (EIA) method,⁸ and PM-I (**2**) was found in comparatively higher concentrations than PF (**1**) after oral administration of PF (**1**).⁹ In view of the strong anti-

convulsant potency of PM-I (**2**),¹⁰ and the anti-cholinergic actions of PF (**1**) found only *in vivo*,¹¹ we suggested the contribution of PM-I (**2**) to the pharmacological actions of PF (**1**). This has stimulated us to investigate the plasma concentrations of PM-I (**2**) at low therapeutic doses, as well as the distribution and disposition of PM-I (**2**), as the first pharmacokinetic study of such a metabolite.

In this paper, we describe the plasma profiles and some pharmacokinetics of both PF (**1**) and PM-I (**2**) after oral doses of PF (**1**) in rats, and those of PM-I (**2**) after oral and intravenous doses of PM-I (**2**) in rats, to investigate the role of PM-I (**2**) in the therapeutic effect of PF (**1**).

MATERIALS AND METHODS

Chemicals The goat antiserum to rabbit IgG and *N*-hydroxysuccinimide were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Bovine serum

Fig. 1. Chemical Structures of Paeoniflorin (**1**) and Paeonimetabolin I (**2**)

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albumin (BSA) was purchased from Sigma Chemicals (St. Louis, U.S.A.). Freund's complete adjuvant was a product of Difco Co. (Detroit, U.S.A.). β -Galactosidase (EC 3.2.1.23) from *Escherichia coli* was obtained from Boehringer Co. (Mannheim, Germany). 4-Methylumbelliferyl β -D-galactoside was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). PF (1) was isolated from the dried roots of *Paeonia albiflora* PALL.^{12,13} (7R)- and (7S)-PM-I (2) were prepared as reported previously,¹⁴ with some modifications. Buffer A was 20 mM phosphate buffered saline (pH 7.3) containing 0.1% BSA, 0.1% NaN₃ and 0.001% MgCl₂, and buffer C was 20 mM phosphate buffered saline (pH 7.3) containing 0.1% NaN₃ and 0.001% MgCl₂.

Animals Male Sprague-Dawley rats (7–8 weeks old, SLC Co., Hamamatsu, Japan) were used. Animals were fed standard laboratory chow with water *ad libitum* and fasted overnight before the experiments.

Administration of PF to Rats Under pentobarbital anesthesia, the left femoral artery of the animal was cannulated with SP10 polyethylene tubing (Natsume Seisakusho, Tokyo, Japan) which was filled with sodium heparin at a concentration of 100 unit/ml. After recovery from anesthesia, PF (1) dissolved in distilled water was given to rats by oral administration ($n=4$) at doses of 0.5 and 5 mg/kg. Arterial blood samples (300 μ l) were obtained at fixed time intervals after administration. Plasma samples were immediately separated by centrifugation, then used directly to measure the concentrations of PF (1) and PM-I (2) by their respective EIA methods, as described below.

Administration of PM-I to Rats Doses of 0.2 and 2 mg/kg PM-I (2) were dissolved in buffer A for oral and intravenous administration. For oral doses of PM-I (2), the same procedures were worked up as described above with PF (1), but for intravenous doses, both the femoral vein and artery were cannulated. After recovery from anesthesia, a drug was given through the venous cannula ($n=4$). Arterial blood samples (300 μ l) were obtained at fixed time intervals after administration. Plasma samples were treated as described above to measure the concentrations of PM-I (2) by the EIA method described below.

Determination of PF Concentrations of PF (1) in the rat plasma were determined according to the EIA method of Kanaoka *et al.*¹⁵

Determination of PM-I Concentrations of PM-I (2) in the rat plasma were determined according to the previously reported method.⁸ Antiserum and labeled antigen were prepared in the same manner. For the preparation of antiserum to PM-I (2), a mixture of (7R)- and (7S)-8-(2'-carboxyethylthio)paeonimetabolin-I (CEP) was coupled with BSA by the *N*-hydroxysuccinimide ester method to give a CEP immunogen. The immunogen was dissolved in saline and emulsified with Freund's complete adjuvant. The emulsion was subcutaneously injected into domestic albino female rabbits at multiple sites on the back. After several booster injections, blood was withdrawn and separated by centrifugation. The sera were stored at -80°C until use. For preparation of the labeled antigen, a mixture of (7R)- and (7S)-8-(carboxymethylthio)paeonimetabolin-I (CMP) was prepared, as with CEP,⁸ and coupled with β -galactosidase by the

N-hydroxysuccinimide ester method to give a CMP-labeled antigen. The antiserum and the labeled antigen were diluted with buffers A and C, respectively, to appropriate concentrations. A sample or a standard solution containing PM-I (2) (50 μ l) was added to 10000-fold diluted antiserum (50 μ l) and 1000-fold diluted labeled antigen (25 μ l). The mixture was kept for 2 h at room temperature, then 10-fold diluted goat anti-rabbit IgG (50 μ l) and 100-fold normal rabbit serum (20 μ l) were added, and the new mixture was incubated at 4°C overnight. After the incubation, 1 ml of buffer A was added and the solution was centrifuged at $3000 \times g$ for 25 min at 4°C . The supernatant was removed and the immunoprecipitate was washed with 1 ml of buffer A, followed by recentrifugation. The resulting immunoprecipitate was incubated with 0.1 mM 4-methylumbelliferyl β -D-galactoside (150 μ l) at 30°C for 30 min. Then, 3 ml of 0.1 M glycine-NaOH buffer (pH 10.3) was added to the reaction mixture, and the fluorescence intensity of 7-hydroxy-4-methylumbelliferon formed was measured spectrofluorometrically at wavelengths of 364 nm and 448 nm for excitation and emission, respectively.

Calibration Curves for PF and PM-I The plasma samples were used directly without dilution. The calibration curves were prepared for PF (1) and PM-I (2) in the presence of plasma from normal rats and constructed with the linearized logit-log plot. The calibration curves ranged from 0.5–500 ng/tube for both PF (1) and PM-I (2).

Pharmacokinetic Analysis The individual plasma concentration data after intravenous administration were fitted to the following equation:

$$C_t = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

by nonlinear least squares regression analysis. The plasma total body clearance (CL_{tot}), the apparent volume of the central compartment (V_c), the distribution volume at the steady state (V_{dss}) and the elimination half-lives of α and β -phases ($t_{1/2\alpha}$ and $t_{1/2\beta}$) were calculated as follows:

$$CL_{\text{tot}} = \text{dose}/AUC \quad (2)$$

$$V_c = \text{dose}/(A + B) \quad (3)$$

$$V_{\text{dss}} = \text{dose} \cdot (A/\alpha^2 + B/\beta^2)/(A/\alpha + B/\beta) \quad (4)$$

$$t_{\alpha \text{ or } \beta} = 0.693/\alpha \text{ or } \beta \quad (5)$$

Pharmacokinetic analysis of the oral dosage of PM-I (2) was carried out using model-independent methods. The C_{max} and t_{max} were determined from the individual profile by inspection. The area under the plasma concentration curve from zero to 180 min (AUC_{0-180}) after intravenous or oral administration was calculated by the trapezoidal rule.

Systemic bioavailability (F) was calculated by the following equation:

$$F = AUC_{p.o.}/AUC_{i.v.} \quad (6)$$

The C_{max} and t_{max} of PF (1) and PM-I (2) after oral doses of PF (1) were determined from their individual plasma concentration–time curves by inspection, and AUC_{0-180} was calculated by the trapezoidal rule.

Pharmacokinetic parameters were represented as an estimated value $\pm$ S.E. Statistical analysis was performed by analysis of variance with the level of significance at 0.05.

RESULTS

Figures 2 and 3 show the plasma concentration-time curves of PF (1) and PM-I (2) after oral administration of PF (1) at doses of 0.5 and 5 mg/kg to rats. Table 1 summarizes the pharmacokinetic parameters of PF (1) and PM-I (2), respectively. The $C_{\max}$ values of PM-I (2) were 2–5 times greater than those of PF (1), and the AUC_{0-180} 6–10 times greater at each dose. The $t_{\max}$ values of PM-I (2) were delayed ($t_{\max}=60, 80$ min) from those of PF (1) ($t_{\max}=11.6, 13.3$ min) at each dose, respectively.

Figure 4 shows the plasma concentration-time curves of PM-I (2) after intravenous administration of PM-I (2) at doses of 0.2 and 2 mg/kg to rats, and the pharmacokinetic parameters were calculated and listed in Table 2. The plasma disappearance of PM-I (2) in rats was

Fig. 2. Plasma Concentration–Time Curves of Paeonimetabolin I (●) and Paeoniflorin (▲) after Oral Administration of Paeoniflorin at a Dose of 0.5 mg/kg to Rats

Each value represents the mean $\pm$ S.E. of 3 rats.

Table 1. Pharmacokinetic Parameters of Paeoniflorin (PF, 1) and Paeonimetabolin I (PM-I, 2) after 0.5 and 5 mg/kg Oral Administration of Paeoniflorin to Rats

Parameter	PF 0.5 mg/kg		PF 5 mg/kg	
	PF	PM-I	PF	PM-I
$C_{\max}$ (ng/ml)	9.9 $\pm$ 2.2	16.5 $\pm$ 2.64	20.3 $\pm$ 2.7	101.7 $\pm$ 26.4
$t_{\max}$ (min)	11.6 $\pm$ 1.7	60 $\pm$ 0.0	13.3 $\pm$ 1.7	80 $\pm$ 10.2
AUC_{0-180} (ng min/ml)	300 $\pm$ 79	1873 $\pm$ 176.8	1174 $\pm$ 287	12358 $\pm$ 3564

Each point represents the mean $\pm$ S.E. ($n=3$).

described by the biexponential curves. There was no large variability in the $t_{1/2}$ at the terminal phase at each dose (1.5 h). The V_{dss} and CL_{tot} significantly increased with increasing doses. The increase in the AUC was less than the

Fig. 3. Plasma Concentration–Time Curves of Paeonimetabolin I (●) and Paeoniflorin (▲) after Oral Administration of Paeoniflorin at a Dose of 5 mg/kg to Rats

Each value represents the mean $\pm$ S.E. of 3 rats.

Fig. 4. Plasma Concentration–Time Curves of Paeonimetabolin I after Intravenous Administration of Paeonimetabolin I at Doses of 0.2 (■) and 2 (●) mg/kg to rats

Each value represents the mean $\pm$ S.E. of 4 rats.

Table 2. Pharmacokinetic Parameters of Paeonimetabolin I (PM-I, 2) after 0.2 and 2 mg/kg Intravenous Administration of Paeonimetabolin I to Rats

Parameter	Dose (mg/kg)	
	0.2	2
A (ng/ml)	48 ± 0.006	584 ± 0.087
α (min^{-1})	0.22 ± 0.059	0.23 ± 0.04
B (ng/ml)	51 ± 0.001	109 ± 0.03
β (min^{-1})	0.008 ± 0.001	0.01 ± 0.004
$t_{1/2\alpha}$ (min^{-1})	3.69 ± 1.19	3.32 ± 0.65
$t_{1/2\beta}$ (min^{-1})	90.6 ± 13.4	90.9 ± 28.5
V_c (l/kg)	0.5 ± 0.029	0.76 ± 0.1
V_{dss} (l/kg)	0.91 ± 0.19	3.79 ± 1.0
CL_{tot} (ml/min kg)	8.7 ± 1.0	39.9 ± 5.0
AUC_{0-180} (ng min/ml)	5614.1 ± 436.6	13176.0 ± 2067.5

Each value represents mean $\pm$ S.E. ($n=3, 4$).

Fig. 5. Plasma Concentration-Time Curves of Paeonimetabolin I after Oral Administration of Paeonimetabolin I at Doses of 0.2 (■) and 2 (●) mg/kg to Rats

Each value represents the mean $\pm$ S.E. of 4 rats.

value proportional to the increase of administered dose.

Figure 5 shows the plasma concentration-time curves of PM-I (2) after oral administration of PM-I (2) at doses of 0.2 and 2 mg/kg to rats. PM-I (2) was absorbed rapidly and reached maxima of 102.2 and 285 ng/ml within 6.2 and 7.5 min at each dose, respectively, followed by an elimination phase with terminal half lives of 240.6 and 229.5 min, respectively. The bioavailability (F) calculated from the AUC s after the intravenous and oral administration were 0.8 and 1.07, at each dose, respectively.

DISCUSSION

It is important to evaluate the significance of PF (1) and the role of its metabolites in the pharmacological

Table 3. Pharmacokinetic Parameters of Paeonimetabolin I (PM-I, 2) after 0.2 and 2 mg/kg Oral Administration of Paeonimetabolin I to Rats

Parameter	Dose (mg/kg)	
	0.2	2
C_{max} (ng/ml)	102 ± 17.0	285 ± 41.7
t_{max} (min)	6.2 ± 1.3	7.5 ± 1.4
$t_{1/2}$ (min)	240.6 ± 137	229.5 ± 58.5
AUC_{0-180} (ng min/ml)	4145.6 ± 973.7	14182.1 ± 1642
F	0.8 ± 0.15	1.07 ± 0.07

Each value represents mean $\pm$ S.E. ($n=3, 4$).

actions of paeony roots to understand the therapeutic efficiency of paeony roots in traditional Chinese medicine. The transformation of PF (1) to other metabolites was concluded by *in vitro* intestinal floral experiments,⁵⁻⁷ and recently by an *in vivo* experiment,^{3,4} where the AUC of PF (1) was greater in germ free rats than in conventional rats.¹⁶ PF (1) was reported to be metabolized to a major metabolite, PM-I (2),⁵⁻⁷ which showed stronger anti-convulsant activity than PF (1), in rats given pentylen-tetrazole, and in El mice, a model animal of heredity epilepsy.¹⁰ On the other hand, the recent findings that PF (1) significantly improved aging-induced and nucleus basalis magnocellularis lesion-induced learning and spatial working memory deficits, at quite low dosages,¹⁷⁻²¹ seem not to coincide with the extremely low bioavailability of PF (1),^{3,4} and it may be difficult to ascribe the pharmacological actions of PF (1) without considering the role of its metabolite.

In the course of our studies on the metabolism of PF (1), we developed a sensitive EIA method to measure the concentration of PM-I (2) in the plasma.⁸ After the oral administration of 20 mg/kg PF (1) to rats, we found a higher concentration of PM-I (2) in the plasma compared to that of PF (1),⁹ using their respective EIA methods. No PM-I (2) was detected in the plasma after the intravenous administration of PF (1),⁹ suggesting that PM-I (2) is formed exclusively in the GIT. To clarify further the role of PM-I (2) in the therapeutic effects of PF (1), we compared their plasma concentration-time curves (Figs. 2, 3) after the oral administration of PF (1) at low therapeutic doses of 0.5 and 5 mg/kg to rats. Plasma concentrations of PM-I (2) were higher than those of PF (1) at each dose, and reached the C_{max} after the time when most of the PF (1) was eliminated. PM-I (2) was retained in the plasma at high concentrations for a relatively longer time than PF (1) (Table 1). The pharmacokinetic parameters of PF (1) support the low plasma concentrations and the short elimination half life of PF (1), as reported previously by Takeda *et al.*³ Thus, these results prompted us to investigate the kinetics of PM-I (2) independently.

Figure 4 shows the plasma concentration-time curves of PM-I (2) after its intravenous administration at doses of 0.2 and 2 mg/kg to rats, the doses being equivalent to those of PF (1) in a molar ratio for comparison (PM-I, 2 mg=10.5 mmol; PF, 5 mg=10.4 mmol). There was a rapid decrease in the plasma concentrations, followed by a flat slope of elimination. The curves were well fitted

to the two-compartmental model, at each dose, and the concentrations of PM-I (2) slowly decreased because of long elimination half-lives (Table 3). The V_c and V_{dss} values, derived from Eqs. 3 and 4 after the intravenous dosing of PM-I (2), were relatively large at each dose, suggesting that PM-I (2) may reside more in the intracellular space than in the extracellular space²²; however, further study on the concentrations of PM-I (2) in the tissues is required. A significant increase in both V_{dss} and CL_{tot} values with increasing plasma concentrations was observed in a range of doses administered, while $t_{1/2}$ values at the terminal phase were almost the same at each dose (1.5 h) (Table 2). The variability in the CL_{tot} values explains the non-proportional increase in AUC values to the increase of the administered doses,²² and indicates dose-dependent kinetics of PM-I (2). Comparing these results with those of the intravenous plasma profiles of PF (1), reported by Takeda *et al.*,³ higher concentrations of PF (1) in the plasma, with relatively smaller V_{dss} and V_c values, indicate less intracellular distribution due to its higher hydrophilic character than that of PM-I (2). Thus, we suggest that the rapid decrease of PM-I (2) from the plasma is a result of redistribution into the tissues, since it showed a long elimination half-life.

Figure 5 shows the plasma concentration–time curves of PM-I (2) after oral doses of 0.2 and 2 mg/kg PM-I (2) to rats. PM-I (2) was rapidly absorbed from the GIT with t_{max} values of 6.2 and 7.5 min, respectively. The plasma concentrations after oral doses were relatively high, then decreased slowly with elimination half-lives of 240.6 and 229.5 min at each dose, respectively, suggesting that the absorption process is faster than elimination. The increase in the AUC values was not proportional to the administered dose, similar to that observed after intravenous doses, suggesting that the disposition kinetics of PM-I (2) after oral and intravenous administrations of PM-I (2) to rats are the same (Figs. 4 and 5). The F values, obtained from AUC s after intravenous and oral dosing, were considerably high (0.8 and 1.07), suggesting almost complete absorption and negligible metabolism in the liver.

Comparing these results with those of plasma concentrations of PF (1) after its oral administration, reported by Takeda *et al.*,³ PM-I (2) showed shorter absorption times (t_{max} , 6.2 and 7.5 min) and higher C_{max} (102 and 285 ng/ml) values with larger concentrations in the plasma, suggesting a greater extent of absorption of PM-I (2) from the intestinal membrane than that of PF (1). Comparing the AUC s of PM-I (2) after oral administrations of PF (1) and PM-I (2) to rats, a large amount of PM-I (2) was shown to be absorbed from the GIT after it was transformed from PF (1) by intestinal bacteria.

The high F value, as a result of negligible hepatic metabolism, will cause increases in both the volumes of distribution and CL_{tot} as the dose increases,²³ and this coincided with our observation described above. Further studies on the tissue distribution and excretion of PM-I (2) are in progress.

From these observations, we concluded that PM-I (2)

was found at higher concentrations in the plasma than PF (1), after either oral administration of PF (1) or PM-I (2) to rats. (Tables 1 and 3). Intravenous and oral administration of PM-I (2) suggested considerable intracellular distribution and dose-dependent kinetics of PM-I (2). Furthermore, it is important to study the therapeutic effects of paeony roots in view of the pharmacological actions of both PF (1) and PM-I (2), since we can expect a considerable role of PM-I (2) in the pharmacological actions from the present kinetic studies.

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REFERENCES

- 1) Takagi K., Harada M., *Yakugaku Zasshi*, **89**, 879–886 (1969).
- 2) Takagi K., Harada M., *Yakugaku Zasshi*, **89**, 887–892 (1969).
- 3) Takeda S., Isono T., Wakui Y., Matsuzaki Y., Sasaki H., Amagaya S., Maruno M., *J. Pharm. Pharmacol.*, **47**, 1036–1040 (1995).
- 4) Takeda S., Isono T., Wakui Y., Mizuhara Y., Amagaya S., Maruno M., Hattori M., *J. Pharm. Pharmacol.*, **49**, 35–39 (1997).
- 5) Hattori M., Shu Y. Z., Shimizu M., Hayashi T., Morita N., Kobashi K., Xu G., Namba T., *Chem. Pharm. Bull.*, **33**, 3838–3846 (1985).
- 6) Shu Y. Z., Hattori M., Akao T., Kobashi K., Kagi K., Fukuyama K., Tsukihara T., Namba T., *Chem. Pharm. Bull.*, **35**, 3726–3733 (1987).
- 7) Shu Y. Z., Hattori M., Akao T., Kobashi K., Namba T., *J. Med. Pharm. Soc., Wakan-Yaku*, **4**, 82–87 (1987).
- 8) Hattori M., Yang X., Shu Y., Heikal O. A., Miyashiro H., Kanaoka M., Akao T., Kobashi K., Namba T., *J. Trad. Med.*, **13**, 73–80 (1996).
- 9) Heikal O. A., Miyashiro H., Akao T., Hattori M., *J. Trad. Med.*, in press (1997).
- 10) Shu Y., Hattori M., Namba T., Mibu K., Akao T., Kobashi K., *J. Pharmacobio-Dyn.*, **10**, s-58 (1987).
- 11) Kobayashi M., Ueda C., Aoki S., Tajima K., Tanaka N., Yamahara J., *Yakugaku Zasshi*, **110**, 964–968 (1990).
- 12) Kaneda M., Iitaka Y., Shibata S., *Tetrahedron*, **28**, 4309–4317 (1972).
- 13) Shibata S., Nakahara M., *Chem. Pharm. Bull.*, **11**, 372–378 (1963).
- 14) Akao T., Shu Y., Matsuda Y., Hattori M., Namba T., Kobashi K., *Chem. Pharm. Bull.*, **36**, 3043–3048 (1988).
- 15) Kanaoka M., Yano S., Kato H., Nakanishi K., Yoshizaki, M., *Chem. Pharm. Bull.*, **32**, 1461–1466 (1984).
- 16) Takeda S., Wakui Y., Mizuhara Y., Ishihara K., Amagaya S., Maruno M., Hattori M., *J. Trad. Med.*, in press (1997).
- 17) Ohata H., Matsumoto K., Watanabe H., Shimidzu M., *Jpn. J. Pharmacol.*, **62**, 199–202 (1993).
- 18) Ohata H., Matsumoto K., Shimidzu M., Watanabe H., *Pharmacol. Biochem. Behav.*, **49**, 213–217 (1994).
- 19) Ohata H., Ni J., Matsumoto K., Watanabe H., Shimidzu M., *Pharmacol. Biochem. Behav.*, **45**, 719–723 (1993).
- 20) Ohata H., Nishi K., Matsumoto K., Shimidzu M., Watanabe H., *Phytomedicine*, **1**, 117–121 (1994).
- 21) Nishi K., Matsumoto K., Ohata H., Shimidzu M., Watanabe H., *J. Trad. Med.*, **11**, 118–122 (1994).
- 22) Tozer R., "Clinical Pharmacokinetics, Concept and Application," William & Wilkins Co., Baltimore, 1995, pp. 18–50.
- 23) Gilman A., "Goodman and Gilman's The Pharmacological Basis of Therapeutics," Pergamon Press, New York, 1990, pp. 20–31.