Review:

# METABOLISM AND PHARMACOKINETICS OF PAEONIFLORIN, A BIOACTIVE COMPONENT FROM PEONY ROOTS

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## ABSTRACT

Paeoniflorin (PF, yield up to 5.8% of the dry weight) is the major and the most important component of peony roots. Recent studies demonstrate that PF improves the spatial and memory deficits in rodents and suggest possible utilization of this agent in the treatment of certain types of dementia. The understanding of the metabolism and pharmacokinetics of this agent may provide insights into the mechanism of its effects. Due to the poor absorption from intestine, PF is subjected to the metabolism to give three metabolites, by intestinal bacteria. Paeonimetabolin I (PM-I), the major intestinal bacterial metabolite of PF showed anticonvulsant action. And in the presence of thiol compounds, intestinal bacteria were found capable of transforming PF to more potent anticonvulsant thiopaeonimetabolin-I derivatives. The pharmacokinetics of PF and PM-I were investigated in rats by enzyme immunoassay (EIA), and were dose-dependent. PM-I was not detected in the rat plasma after intravenous (*i.v.*) administration of PF, and a significant difference in the plasma concentration of PM-I was observed between germ-free and conventional rats. After oral and *i.v.* administration of PM-I to rats, it was found at high concentrations in the plasma.

## INTRODUCTION

Paeoniae Radix, the dried roots of *Paeonia lactiflora* PALLAS (Paeoniaceae), is a potential crude drug traditionally used as a component of Japanese and Chinese prescriptions to treat abdominal pain and certain types of dementia.[1-4] Paeoniflorin (PF, **1**) is the major component in this Radix. The yield of PF (**1**) was found to be variable, and depends not only on plant species, but also on the stage of growth, the season of collection and the method used for processing. Phylogenetically, plant species with higher PF contents were mainly in the genus *Paeonia, P. lactiflora* and *P. veitchi* being the two with the highest contents.[5] The maximum yield of PF (**1**) from cultivated *P. lactiflora* amounts to 5.8% of plant dry weight.[5-7] Plants cultivated for one year and collected in spring or fall contain the highest percentage of PF (**1**) relative to that cultivated for three years. Wine-processed roots contain higher percentage of PF (**1**) than those in raw or roasted roots.[8] When determined by HPLC, the major components of *P. lactiflora* (higher content is in the core wood) were found to be PF (**1**), albiflorin (**2**) and pentagalloylglucose, while those of *P. vitchii* (higher content is in the cortex) were PF (**1**), pentagalloylglucose and oxypaeoniflorin (**3**) (Chart 1).[9] The content of PF (**1**) in herbal prescriptions (Kampo medicine in Japanese) containing peony roots was also determined by HPLC. After almost three decades of its discovery, PF (**1**) was synthesized by Corey and Wu,[10] and then by Hatakeyama *et al.*[11]

Animal experiments indicated diverse activities of peony roots and PF (**1**) as well. The crude extract of peony roots was reported to have improving effects on memory deficits.[3,4] Besides, anti-inflammatory[12] and immuno-modulating effects were also reported.[13] The water-soluble fraction of the methanol extract of peony roots significantly improved spatial cognition caused by M1-receptor antagonist scopolamine in rats.[14] Ohta *et al.* found that such a beneficial effect was attributed mainly to the aqueous extract of peony roots and the water-

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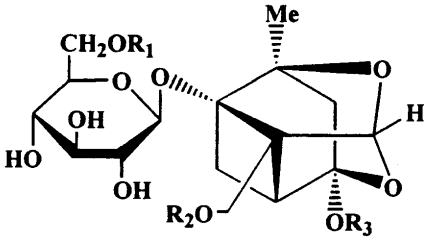
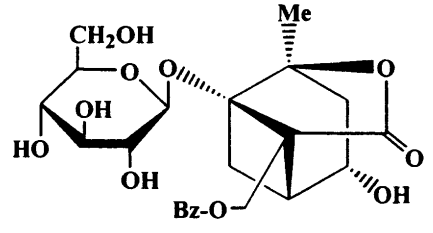
soluble glycosides such as PF (**1**) and related compounds.[15,16] PF (**1**) improved the spatial learning deficits produced by unilateral nucleus basalis magnocellularis (NBM)-lesion and learning impairment in aged (25 months) rats.[3] Since PF (**1**) did not induce any other pharmacological effects at the doses tested (0.1-1 mg/kg), the effects of this agent on cognitive functions were considered to be highly specific. In electrophysiological studies, Tabata *et al.*[17] found that PF (**1**, at a concentration of 0.1-1 $\mu$M) significantly reversed scopolamine- and pirenzepine-induced suppression of long-term potentiation (LTP) induction in the CA1 area in rat hippocampus. They suggested that this effect might be implicated in the ameliorative effect of PF (**1**) on spatial cognitive impairment caused by cholinergic dysfunction in rats. They also suggested that modulation, by PF (**1**), of adenosine A1 receptor-mediated inhibition of LTP in the rat hippocampal slice is implicated in its beneficial effect on learning and memory impairment in rodents.[18] PF (**1**) was found to show a very low acute toxicity ($LD_{50}$ 9.53 and 3.53 g/kg for *i.p.* and *i.v.*, respectively). These findings strongly suggested that PF (**1**) has beneficial effects in the treatment of dementia. Although albiflorin (**2**, 0.01-1 mg/kg, *p.o.*), which has a lactone ring in the pinane skeleton, exhibited anticonvulsant activity at smaller doses than PF (**1**), no effect on the maze performance disrupted by scopolamine was demonstrated. This observation suggested that a cage-like pinane skeleton including acetal and hemiketal structure as in PF (**1**), may play an important role in the ameliorating effect of these components.[16] Morevoer, the ameliorating effect of PF (**1**) was markedly enhanced by methylation, as in 3-*O*-methylpaeoniflorin (**4**) and 2,3,3,4,5-penta-*O*-methylpaeoniflorin (**5**) (Chart 1). However, deglucosylation, as in 6-deglucosyl-3-*O*-methylpaeoniflorin (**6**), significantly reduced the activity. And removal of both glucose and benzoyl moieties resulted in the loss of activity as seen in **7**.[19] These results suggested that, in addition to the cage-like pinane skeleton, the benzoyl and the glucosyl moieties are important structural elements of the paeoniflorin skeleton for its effects on spatial cognitive dysfunction in rats.

## Metabolism and Pharmacokinetics

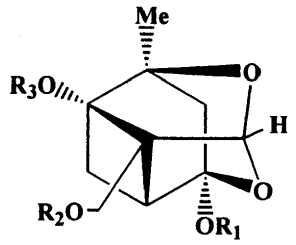
Since most of the therapeutic effects of peony roots are mainly explained by the pharmacological actions of PF (**1**), and most Kampo medicines are orally administered, the metabolic studies on PF




**1, Paeoniflorin (PF); $R_1$=H, $R_2$=Bz, $R_3$=H**
**3, Oxypaeoniflorin; $R_1$=H, $R_2$=*p*-OH-Bz, $R_3$=H**




**2, Albiflorin**




| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **4** | Me | Bz | Glc |
| **5** | Me | Bz | $Glc(OMe)_4$ |
| **6** | Me | Bz | H |
| **7** | Me | H | H |
| **8** | Me | H | Glc |
| **9** | Me | Bz | $Glc(OAc)_4$ |
| **10** | H | H | Glc |

Chart 1

(1) and related compounds by intestinal bacteria seem to provide insights into the mechanism of their therapeutic benefits. Hattori and co-workers have collaborated in developing PF (1) and related compounds through extensive studies on their metabolism and pharmacokinetics (either of individual compounds or Kampo formulas) in animals and humans. Following 24 hrs anaerobic incubation of PF (1) or 3 with a human fecal suspension, three metabolites, named paeonimetabolins I (PM-I, 11; the major metabolite), II (12) and III were obtained (Chart 2).[20] Fecal flora from different subjects showed potent metabolic ability but predominantly produced the 7*S*-diastereomer of PM-I. Most of the data showed that the fecal suspensions from aged subjects (over 40 years old) consumed PF (1) completely but produced relatively smaller amounts (less than 25%) of PM-I compared with those from younger subjects.

A number of bacterial strains isolated from human feces showed ability to convert PF (1) to paeonimetabolins I and II. Through 10 hrs incubation of PF (1) with *Lactobacillus brevis* or *Bacteroides fragilis*, PM-I (11) was obtained in a yield of *ca.* 20% of added PF (1).[21] This metabolite decreased in amount during prolonged incubation and disappeared almost completely in 16 hrs. PM-I was subsequently separated into 7*R*- and 7*S*-diastereomers in yields of 11% and 9.6%, respectively, by column chromatography on silica gel, and their structures were determined by various spectroscopic methods as well as X-ray analysis.[21] Similarly, paeonimetabolin II (12) could be separated into the 7*R*- and 7*S*-diastereomers. Formation

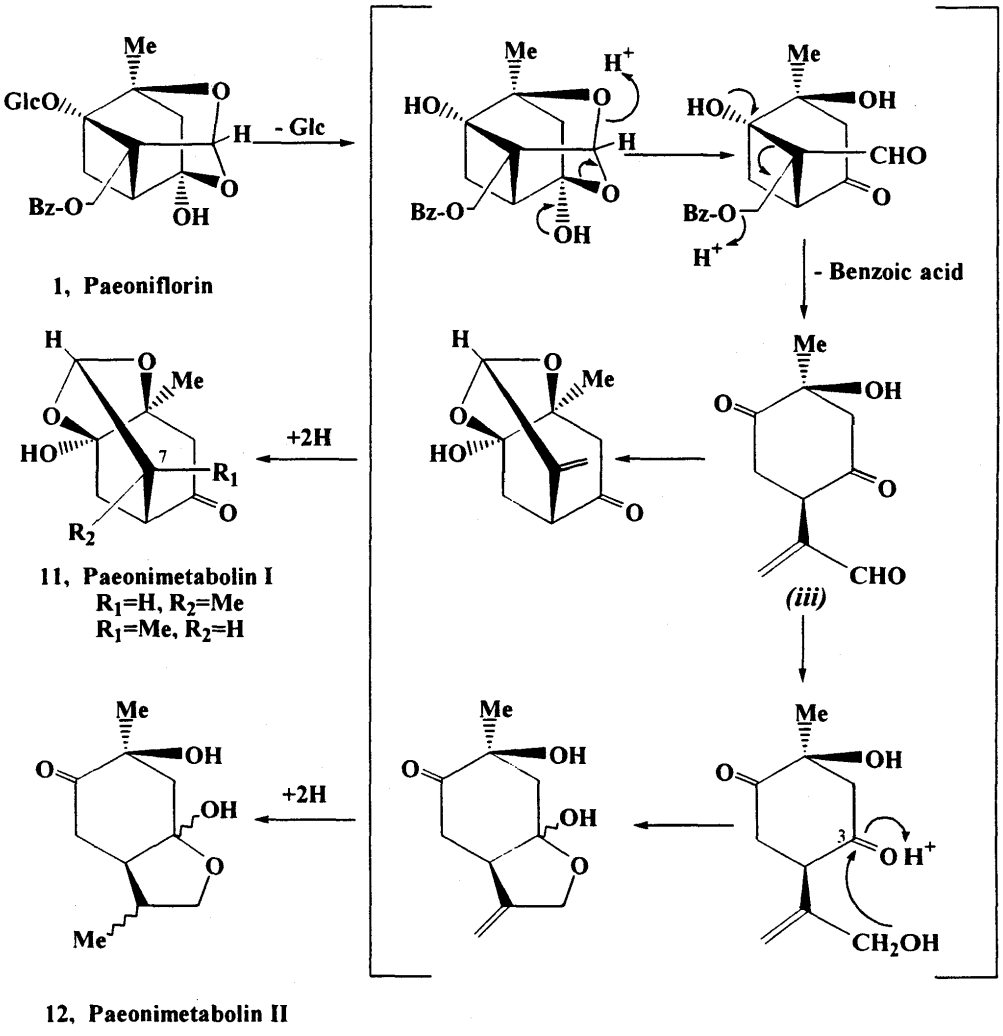



Chart 2. Possible metabolic pathway for the transformation of PF (1) by *Lactobacillus brevis*, a human intestinal bacterium.

of these metabolites from **1** and **3** was initially depends on membrane-bound $\beta$-glucosidase (commercially available $\beta$-glucosidase from almonds or that of animal liver origin hardly hydrolyzed **1** as well as **2**[22]) to liberate an aglycone which underwent reversible conversion of the hemiketal-acetal system to ketone, hydroxyl and aldehyde groups. A free hydroxyl at $C_3$ is an important prerequisite (similar reaction by emulsin, intestinal or soil bacteria was not achieved when the hydroxyl group, $C_3$-OH, was methylated) for this conversion which seems to be essential for the subsequent steps. Then, cleavage of the four-membered ring ($C_4$-$C_5$-$C_6$-$C_7$) may be responsible for the formation of a new double bond at $C_7$ and $C_8$ and liberation of a benzoyl (or a *p*-hydroxybenzoyl) residue. This key intermediate may again cyclize to form a hemiketal-acetal system at $C_1$, $C_6$ and $C_9$ for stereochemical reasons. Non-stereospecific reduction of the terminal double bond may lead to a mixture of the epimeric 7*R*- and 7*S*- PM-I (**11**).

It is noteworthy that the 7*S*-isomer of PM-I showed a more potent anticonvulsant effect ($ED_{50}$: 41.3 mg *i.v.*) than PF (**1**, $ED_{50}$: >100 mg) in EI mice, a model of hereditary epilepsy. When the metabolite was given intraduodenally or intraventricularly to rats, the convulsions induced by pentylenetetrazol were greatly inhibited, and the inhibitory effect of PF (**1**) on the carbachol-induced contraction of rat isolated proximal colon was found only *in vivo*.[23,24] These findings emphasize the role of intestinal flora in the metabolic activation of PF (**1**). Consequently, compounds of this unique skeleton have been the targets of considerable synthetic activity, and synthesis of 7*R*- and 7*S*-PM-I (**11**) was effectively achieved.[25,26]

Since no conversion from **11** to **12** could be demonstrated during anaerobic incubation with either *L. brevis* or *B. fragilis*, the formation of **12** proceeds via an aldehyde intermediate (*iii*) and its reduced product. The alcohol might cyclize to form a hemiketal at the $C_3$ position, followed by non stereospecific reduction of the terminal double bond to yield the 7*R* and 7*S* isomers of paeonimetabolin II (**12**) (Chart 2).[20] Similar to the case of PF (**1**), albiflorin (**2**) was converted to paeonilactones A (**13**) and B (**14**) (Chart 3).[27]

Akao *et al.*[28] reported that in the presence of 3-mercaptopropionic acid, 2-mercaptoethanol and thiobenzoic acid (hydroxy, carboxy and amino

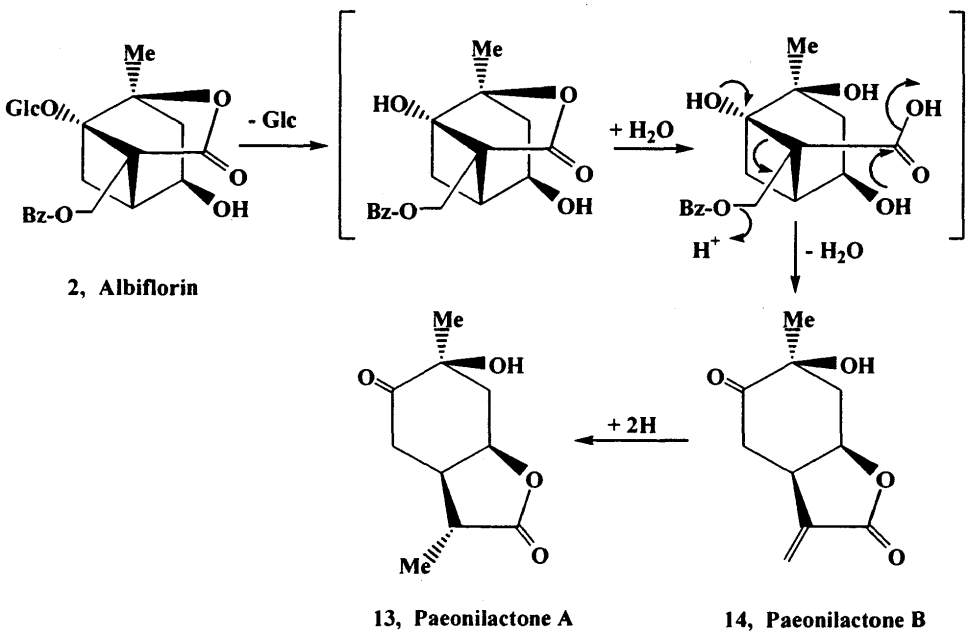



Chart 3. Possible metabolic pathway for the transformation of albiflorin (**2**) by *Lactobacillus brevis*, a human intestinal bacterium

groups are less reactive), the key intermediate (*iii*), reacts in the preference with a mercapto group of the compounds by Michael addition, followed by the formation of a hemiketal-acetal system and, almost equal amounts of the 7*R*- and 7*S*-isomers of **15**, **16** and **17** could also be obtained. These adduct possessed similar structures in which the sulfhydryl compounds are covalently bound at the $C_8$ position of PM-I (**11**) through a thioether linkage (Chart 4). It is interesting to mention that during the course of preparing these compounds, new paeonilactone-A adducts (**33**－**37**) were obtained, but only in the presence of aromatic thiols. Related adducts of paeonilactone-A were not obtained when aliphatic thiols were used.[29] Formation of these adducts from PF (**1**) was presumed to follow the same pathway as in Chart 4 to the key intermediate *iii*. And this intermediate may be converted to *v* through *iv*, possibly by redox reaction followed by lactonization. Michael addition of aromatic thiols on the double bond conjugated with a carbonyl group of *v* leads to the formation of **33**－**37**.

Formation of **16** could be regarded as an analogue of paeoniflorigenone (**38**) in which the benzoyl moiety is replaced by a thiobenzoyl group and the formation of this adduct in the presence of thiobenzoic acid suggests a similar pathway for biosynthesis of **5** in paeonies.[30-32] For better understanding the metabolism and pharmacokinetics of PF (**1**) and its intestinal bacterial metabolite, PM-I, enzyme immunoassay methods for the determination of PF (**1**) and PM-I were established.[33,34] Kanaoka *et al.*[33] used the *N*-hydroxysuccinimide ester method to couple a hemisuccinyl PF derivative with β-galactosidase (to give a labeled antigen) and a hemiglutaryl PF derivative with BSA (to give an immunogen) (Chart 5). The anti-PF antisera were elicited in rabbits by immunization with the immunogen, and were used for the determination of PF. A satisfactory standard curve for the enzyme immunoassay (EIA) of PF was obtained in the range of 1-400 ng/ml. The anti-PF antisera reacted with oxypaeoniflorin (**3**) (0.31%) and albiflorin (**2**) (0.22%) (Table 1).

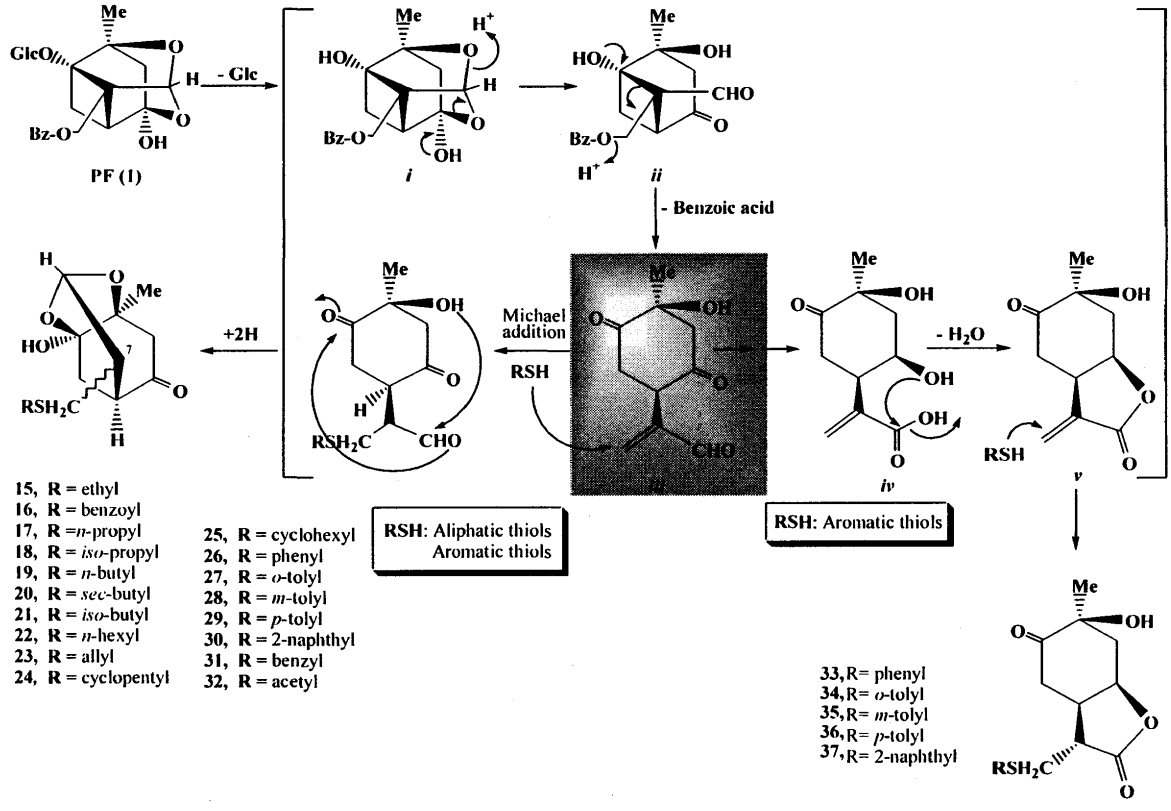
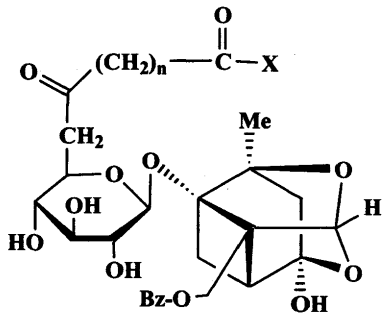



Chart 4. Possible pathway for the formation of thiopaeonnimetabolin-I derivatives and thiopaeonilactone-A derivatives after incubation of PF (**1**) and thiol compound with *Lactobacillus brevis*.

Table 1. Specificity of Anti-PF-BSA Antiserum

| Compound | Cross reaction (%) |
|---|---|
| Paeoniflorin (**1**) | 100 |
| Albiflorin (**2**) | 0.22 |
| Oxypaeoniflorin (**3**) | 0.31 |
| Paeoniflorigenone (**38**) | 0.10 |
| Desbenzoylpaeoniflorin (**10**) | 0.03 |
| Paeoniflorin tetraacetate | 0.07 |
| Paeoniflorin pentaacetate | 0.01 |



PF hemiglutarate-BSA conjugate:
X=BSA....NH, n=3
PF hemisuccinate-β-Gal conjugate:
X=β-Gal...NH, n=2

Chart 5. BSA-and $\beta$-Gal conjugates of PF(**1**)

Hattori and co-workers developed the EIA method of Kanaoka to be used for the determination of PM-I.[34] The study was accomplished by preparing the carboxymethylthio, carboxyethylthio and carboxypentylthio PM-I derivatives, as haptens possessing short, medium and long spacers to prepare the BSA-conjugate and labeled antigen (Chart 6).

Two antisera, named **1CEP** and **2CPP**, were prepared by immunizing rabbits with the corresponding BSA-conjugates of CEP and CPP. The sensitivity was higher in heterologus combinations, using the labeled antigens linked with a shorter spacer than that used for antiserum production than the homologous combinations. The cross reactivities of **2CPP** against various PM-I related compounds were higher than those of **1CEP** (Table 2).

In order to clarify the site(s) of their formation, absorption and excretion, Hattori *et al.*[35-41] carried out intensive pharmacokinetic studies of PF (**1**) and its major metabolite, the mixture, 7*R*- and 7*S* PM-I (**11**) in rats. After *p.o.* and *i.v.* administration of PF (**1**), the plasma concentrations of PF (**1**) and PM-I were monitored by the respective EIA methods. Takeda *et al.*[35] found that orally administered PF (**1**) was not metabolized in the gut wall, liver and lung, and concluded that the unabsorbed fraction of

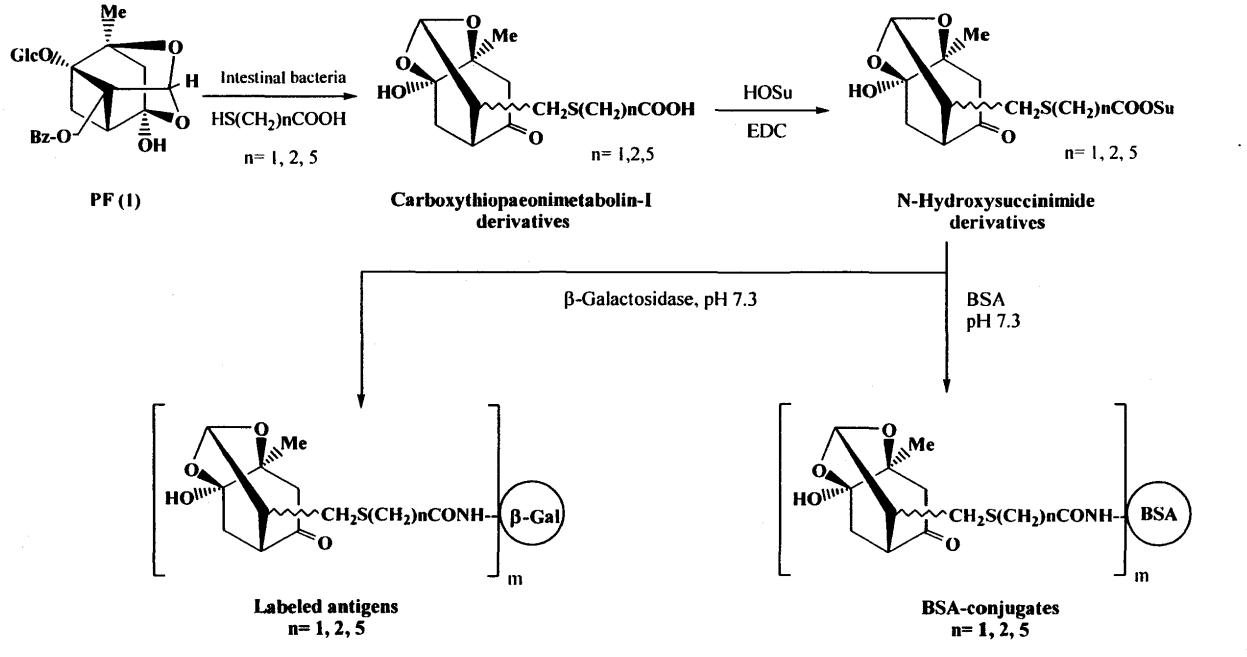
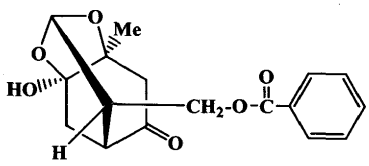



Chart 6. BSA-and $\beta$-Gal-conjugates of paeonimetabolin-I

Table 2. Specificity of Anti-PM-I Antisera

| Compound | Antisera Cross reaction (%) 1CEP | 2CPP |
|---|---|---|
| (7*R*)-, (7*S*)-PM-I (**11**) | 100 | 100 |
| (7*S*)-PM-I | 120 | 120 |
| (7*R*)-PM-I | 110 | 90 |
| Paeoniflorin (**1**) | 0.02 | 0.3 |
| Paeoniflorigenone (**38**) | 0.03 | 0.5 |
| Benzoylpaeoniflorin | 0.01 | 1.0 |
| Oxypaeoniflorin (**3**) | 0.02 | 0.7 |
| Paeoniflorin pentaacetate | 0.02 | 0.02 |

**1CEP**, Carboxyethylthiopaeonimetabolin-I-BSA
**2CPP**, carboxypentylthiopaeonimetabolin-I-BSA




**38, Paeoniflorigenone**

PF (**1**) was degraded by the intestinal flora to paeonimetabolins I-III because of its poor absorption from the intestine and the extremely low bioavailability. A trace amount of PF (**1**) ($C_{max}$= 95 ng/ml) was immediately absorbed from the GIT after *p.o.* administration of PF (**1**) at a dose of 20 mg/kg, and rapidly eliminated within a short period of time ($T_{max}$= 30 min, disappearance after 240 min), and a high concentration of PM-I (**11**) ($C_{max}$ = 400 ng/ml) was absorbed later and retained in the plasma for a longer period ($T_{max}$= 140±24.7 min, disappearance after 480 min).[36] The plasma concentration of PF (**1**) after its *i.v.* administration at a dose of 0.5, 2.0 and 5.0 mg/kg declined rapidly with mean terminal half-lives of 11.0, 9.9 and 12.6 min, respectively, and plasma concentration time curve obtained was best described by a two-compartment model at each dose.[37-39] Plasma concentration of PM-I (**11**) was higher than that of PF (**1**) at each dose, and reached the $C_{max}$ after the time when most of the PF (**1**) was eliminated (Table 3). PM-I (**11**) was retained in the plasma at higher concentration for a relatively longer time than PF. The plasma concentration profiles after intraportal administration of PF (**1**) was very close to those after *i.v.* administration, suggesting a negligible hepatic extraction ration of PF (**1**) [supported by the fact that PF (**1**) is not degraded after incubation with a rat liver homogenate].[35]

Since the *AUC* value, from time zero to infinity, by the intraperitoneal route (24.5±2.91 μg/min/ml) is not less than that after intraportal administration (19.1±2.60 mg/min/ml), PF (**1**) seems not to be metabolized in gut wall.[35] The oral bioavailability of PF (**1**) was very low (3-4%) when calculated by *AUCs* after *p.o.* or *i.v.* administration (agreed with that reported in rabbits[41]) and extremely low fecal excretion (0.07-0.22%) is observed. This low bioavailability might be induced by the first-pass effects in the gut wall and liver or by the bacterial degradation of PF (**1**), which has the opportunity to occur due to poor intestinal absorption.[35,38] The plasma concentration of PF (**1**) after usual oral dosing was very low ($C_{max}$= 9.8, 30.7 and 101.5 ng/ml at 0.5, 2.0 and 5.0 mg/kg, respectively) when compared with that after *i.v.* dos-

Table 3. Pharmacokinetic Parameters of Paeoniflorin (**1**) and Paeonimetabolin I (**11**) after Oral Administration of Paeoniflorin (0.5 and 5 mg/kg) to Rats

| Parameter | PF 0.5 mg/kg | | PF 5 mg/kg | |
|---|---|---|---|---|
| | PF (**1**) | PM-I (**11**) | PF (**1**) | PM-I (**11**) |
| $C_{max}$ (ng/ml) | 9.9±2.2 | 163.5±2.64 | 20.3±2.7 | 101.7±26.4 |
| $T_{max}$ (min) | 11.6±1.7 | 60±0.0 | 13.3±1.7 | 80±10.2 |
| $AUC_{0\text{-}180}$ (ng.min/ml) | 300±79 | 1873±176.8 | 1174±287 | 12358±3564 |

Each value represents mean±S.E (n= 3).

ing.[38] Following *i.v.* administration of PF (**1**) to rats, as much as 50% of the dose was excreted in urine and 0.22% was excreted in the feces within 72 hrs, while 1.0 and 0.08% was, respectively, excreted after *p.o.* administration within 48 hrs.[37] Cumulative biliary excretion after *i.v.* or *p.o.* administration was 6.9 and 1.3% of the dose (0.5 mg/ kg) within 24 hrs, respectively, and suggesting an enterohepatic cycling may occur.[38] In germ-free rats, PF (**1**) was rapidly absorbed as well as in conventional rats, but the plasma concentration was kept at the nearly constant level around 25 ng/ml for 240 min after *p.o.* administration, and a significant difference of plasma concentrations between germ-free and conventional rats was observed (Table 4).[41]

When the kinetics of PM-I (**11**) were independently investigated, appreciable increase in the plasma concentration of PM-I was observed after *p.o.* or *i.v.* administration of PM-I (0.2 and 2 mg/kg) (Table 5), which then decreased and followed by a long elimination half lives (240.6 and 229.5 min, respectively) and the curves were well fitted to the two-compartment model at each dose.[40,41]

The disposition of PF and its major metabolite, PM-I in rats was investigated, after *p.o.* administration of traditional Chinese prescriptions containing paeony root. Shakuyaku-kanzo-to (SK), a prescription contained equal amounts of paeony root and licorice root, while Toki-shakuyaku-san (TS) contained peony root mixed with other 5 herbal drugs

Table 4. Pharmacokinetic Parameters of Paeoniflorin (**1**) in Germ-free and Conventional Rats after 2 mg/kg Oral Administration of PF (**1**).

| Rat | n | $C_{max}$ (ng/ml) | $T_{max}$ (min) | $AUC_{0\text{-}240}$ (ng.min/ml) | $t_{1/2}$ (min) |
|---|---|---|---|---|---|
| Conventional | 6 | 32.7±04.1 | 10±01.8 | 2799.7±276.6 | 40.2±6.1 |
| Germ-free | 3 | 42.3±11.5 | 130±60.8 | 5528±1461.8* | |

- : not calculated; Each value represents mean±S.E.; *$p$<0.05 : significantly different from conventional rat

Table 5. Pharmacokinetic Parameters of Paeonimetabolin I (**11**) 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 |
| $AUC_{0\text{-}180}$ (ng.min/ml) | 4145.6±973.7 | 14182.1±1642 |
| $F$ | 0.8±0.15 | 1.07±0.07 |

Each value represents mean±S.E (n= 3, 4).

Table 6. Pharmacokinetic Parameters of PF (**1**) and PM-I (**11**) after Oral Administration of TS and SK at Doses of 100 and 500 mg to Rats.

| Prescription | | $C_{max}$ (ng/ml) | $t_{max}$ (min) | $t_{1/2}$ (min) | $AUC_{0\text{-}24h}$ (ng·min/ml) |
|---|---|---|---|---|---|
| TS (100 mg) | PF (**1**) | 146.3 | 60 | 140.3 | 14305 |
| | PM-I (**11**) | 184.0 | 120 | 426.7 | 98497 |
| (500 mg) | PF (**1**) | 165.1 | 45 | 970.0 | 19385 |
| | PM-I (**11**) | 400.3 | 180 | 569.1 | 182188 |
| SK (100 mg) | PF (**1**) | 128.5 | 5 | 921.2 | 48857 |
| | PM-I (**11**) | 141.7 | 360 | 508.7 | 102136 |
| (500 mg) | PF (**1**) | 153.5 | 5 | 69.1 | 32518 |
| | PM-I (**11**) | 726.5 | 480 | 325.8 | 469305 |

Table 7. Anticonvulsant Potency and Neurotoxicity of **16** (7*S*) and **22** (7*S*)

| Parameter | PF 0.5 mg/kg | | PF 5 mg/kg | |
|---|---|---|---|---|
| | PF (**1**) | PM-I (**11**) | PF (**1**) | PM-I (**11**) |
| $C_{max}$ (ng/ml) | 9.9±2.2 | 163.5±2.64 | 20.3±2.7 | 101.7±26.4 |
| $T_{max}$ (min) | 11.6±1.7 | 60±0.0 | 13.3±1.7 | 80±10.2 |
| $AUC_{0\text{-}180}$ (ng.min/ml) | 300±79 | 1873±176.8 | 1174±287 | 12358±3564 |

Each value represents mean±S.E (n= 3).

but no licorice root.[42] It was clear that the plasma concentrations and the *AUC* values of PM-I were higher than those of PF (Table 6). Another important finding was that the effects of the two formulas on the disposition of PM-I were significantly different. The maximal plasma concentration of PM-I appeared at 360-480 min in the case of SK and at 120-180 min in TS. The decline of the PF level was very slow when SK was given but very fast when TS was given. The $C_{max}$ of PF in the plasma was at 5 min when SK was given, while the shoulder peak at 5 min when TS was given. These difference can be explained by the fact that SK affects the gastric emptying rate, the peristaltic movement of the small intestine which delayed the time for PF to reach the large intestine where most of PF was metabolized to PM-I by intestinal bacteria.

## Application of Bacterial Transformation to the Development of New Drugs

Although efficient chemical processes are available for the synthesis of PF (**1**)[10,11] and its major metabolite, PM-I,[25,26] chemical reactions carried out by intestinal bacteria offer a promising method for developing these compounds. As mentioned earlier, the 7*S*-isomer of PM-I, showed anticonvulsant activity in EI mice, an animal model of heredity epilepsy. Attempts have been made to improve the anticonvulsant activity of PM-I by using intestinal bacterial reactions. In the presence of various thiol compounds (RSH, where R = aliphatic and aromatic groups), *Lactobacillus brevis*, a human intestinal bacterium, showed high ability to transform PF (**1**) into seventeen thiopaeonimetabolin-I derivatives (**16**−**32**) (with preference for the 7*S*-isomers (Chart 4).[43,44] When the anticonvulsant activity of these derivatives was investigated in mice using the maximal subcutanous pentylenetetrazol seizure test, 13 compounds (**16**−**22, 24, 26**−**30**) showed dose-dependent prolongation of latencies of clonic and tonic convulsions in a concentration range of 0.125-0.5 mmol/kg. It was apparent that introduction of an R-thio residue at C-8 of PM-I effectively enhanced its anticonvulsant activity.

For the most potent anticonvulsant adducts (**16** and **22**), a marked stereospecificity was noticed, where the 7*S*-isomers displayed anticonvulsant activity while the diasteroisomers (7*R*-ones) showed skeletal muscle relaxation effect.[44] When the $ED_{50}$ values of the 7*S*-isomers of **16** and **22** were compared with that of valproic acid (VPA), **16** (7*S*) and **22** (7*S*) were approximately 11.7 and 8.8-times as active as VPA, respectively (Table 7). Furthermore, the higher protective indices (4.0 and 5.0, repectively) for **16** (7*S*) and **22** (7*S*), when compared with that of VPA (PI=1.8), suggested that the maximal anticonvulsant effect of these compounds was achieved at non-toxic doses.

## Conclusion

Paeoniflorin (PF, **1**), the major monoterpene glycoside in peony roots, is a promising compound that demonstrates neuropharmacological effects in rodents, beside its activity as muscle relaxant, antispasmodic, and antiinflammatory agent. It is noteworthy to mention that methylation of PF (**1**) enhanced its antiamnesic action, possibly by increasing its lipophilicity and bioavilability. But, because of its poor absorption from the small intestine, metabolism of PF (**1**) by intestinal bacteria gave three metabolites, paeonimetabolin I-III. PM-I (**11**), its major intestinal bacterial metabolite, was detected in rat plasma at higher concentrations than that of PF after oral administration of PF (**1**) or

herbal prescriptions containing peony root. When compared with PF, PM-I showed a potent anticonvulsant activity. Moreover, transformation of PF (**1**) by intestinal bacteria in the presence of various thiol compounds was conveniently exploited for the preparation of more potent anticonvulsant thiopaeonimetabolin-I derivatives, which are otherwise inaccessible.

Although it is too early to draw a conclusion, the above findings suggested possible utilization of PF (**1**) in the treatment of certain types of dementia and other CNS disorders.

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## REFERENCES

1. Ching Su New Medical College, "Dictionary of Chinese Materia Medica," Zhongguo Shanghai Renmin Chubanshe, Shanghai, p. 706-709, 1977.
2. Harada, M. (1969) *Yakugaku Zasshi* **89**, 899.
3. Ohta, H., Matsumoto, K., Watanabe, H. and Shimizu, M. (1993) *Jpn. J. Pharmacol.* **62**, 345.
4. Ohta, H., Matsumoto, K., Shimizu, M. and Watanabe, H. (1994) *Pharmacol. Biochem. Behav.* **49**, 213.
5. He, L. Y. (1983) *Chin. Pharm. Bull.* **18**, 230.
6. Ho, L. Y., Feng, R. Z. and Xiao P. G. (1980) *Acta Pharm. Sin.* **15**, 429.
7. Shimizu, M., Hashimoto, T., Iskikawa, S., Kurosaki, F., Morita, N. (1979) *Yakugaku Zasshi* **99**, 432.
8. Li, L. and Jingle, W. (1995) *Zhongguo Yaoxue Zazhi* **30**, 539.
9. Ikeda, N., Fukuda, T., Jyo, H., Shimada, Y., Murakami, N., Saka, M., and Yoshikawa, M. (1996) *Yakugaku Zasshi* **116**, 138.
10. Corey, E. J. and Wu, Y.-J. (1993) *J. Amer. Chem. Soc.* **115**, 8871.
11. Hatakeyama, S., Kawamura, M. and Takano, S. (1994) *J. Amer. Chem. Soc.* **116**, 4081.
12. Takagi, K. and Harada, M. (1969) *Yakugaku Zasshi* **89**, 887.
13. Zhidong, G., Aiwu, Z., Bin, W., Yuxian, S., Changhai, D., Anping, Z., Wei, W. and Shuyun, X. (1995) *Zhongguo Yaolixue Tongbao* **11**, 303.
14. Sugiyama, K., Muteki, T., and Kano, T. (1996) *Neurosci. Lett*, **216**, 147.
15. Nishi, K., Matsumoto, K., Ohta, H., Shimizu, M., and Watanabe, H. (1994) *J. Trad. Med.* **11**, 118.
16. Akazawa, K., Matsumoto, K., Shimizu, M., Ichiki, H., Hattori, M. and Watanabe, H. (1996) *J. Trad. Med.* **13**, 243.
17. Tabata, T., Matsumoto, K., and Watanabe, H. (2000) *Jpn. J. Pharmacol.* **83**, 25.
18. Tabata, T., Matsumoto, K., Murakami, Y., and Watanabe, H. (2001) *Biol. Pharm. Bull,* **24**, 496.
19. Abdel-Hafez, A. A., Meselhy, M. R., Nakamura, N., Hattori, M., Watanabe, H., El-Gendy, M. A., Mahfouz, N. M. and Mohamed, T. A. (1998) *Biol. Pharm. Bull.* **21**, 1174.
20. Hattori, M., Shu, Y-Z., Shimizu, M., Hayashi, K., Xu, G-J. and Namba, T. (1985) *Chem. Pharm. Bull.* **33**, 3838.
21. Shu, Y-Z, Hattori, M., Akao, T., Kobashi, K., Kagei, K., Fukuyama, K., Tsukihara, T. and Namba, T. (1987) *Chem. Pharm. Bull.* **35**, 3726.
22. Kobashi, K., Akao, T. and Hattori, M. (1992) *Bifidobacteria Microflora* **11**, 9.
23. Kobayashi, M., Ueda, C., Akao, S., Tajima, K., Tanaka, N. and Yamahara, J (1990) *Yakugaku Zasshi* **110**, 964.
24. Shu, Y-Z., Hattori, M., Namba, T., Mibu, K., Akao, T. and Kobashi, K. (1987) *J. Pharmacobio-Dyn.* **10,** s-58.
25. Kadota, S., Takeshita, M., Makino, K. and Kikuchi, T. (1989) *Chem. Pharm. Bull.* **37**, 843.
26. Hatakeyama, H., Kawamura, M., Shimanuki, E. and Takano, S. (1992) *Tetrahedron Lett.* **33**, 333.
27. Shu, Y-Z, Hattori, M., Akao, T., Kobashi, K. and Namba, T. (1987) *J. Med. Pharm. Soc. Wakan-Yaku* **4**, 82.
28. Akao, T., Shu, Y-Z., Matsuda, Y., Hattori, M., Namba, T. and Kobashi, K. (1988) *Chem. Pharm. Bull.* **36**, 3043.
29. Abdel-Hafez, A. A., Meselhy, M. R., Nakamura, N., Hattori, M., El-Gendy, M., Mahfouz, N. M., and Mohamed, T. A. (2001) *Chem. Pharm. Bull.*, **49**, 918.

30. Shimizu, M., Hayashi, T., Morita, N., Kimura, I., Kimura, M., Kiuchi, F., Noguchi, H., Iitaka, Y. and Sankawa, U. (1981) *Tetrahedron Lett.* 3069.
31. Shimizu, M., Hayashi, T., Morita, N., Kiuchi, F., Noguchi, H., Iitaka, Y. and Sankawa, U. (1983) *Chem. Pharm. Bull.* **31**, 577.
32. Hayashi, T., Kurosawa, S., Shimizu, M. and Morita, N. (1985) *Shoyakugaku Zasshi* **39**, 214.
33. Kanaoka, M., Yano, S., Kato, H., Nakanishi, K. and Yoshizaki, M. (1984) *Chem. Pharm. Bull.* **32**, 1461.
34. Hattori, M., Yang, X-W., Shu, Y-Z., Heikal, O. H., Miyashiro, H., Kato, H., Kanaoka, M., Akao, T., Kobayashi, K. and Namba, T. (1996) *J. Trad. Med.* **13**, 73.
35. Takeda, S., Isono, T., Wakui, Y., Mizuhara, Y., Amagaya, S., Maruno, M. and Hattori, M. (1997) *J. Pharm. Pharmacol.* **49**, 35.
36. Heikal, O. A., Miyashiro, H., Akao, T. and Hattori, M. (1997) *J. Trad. Med.* **14**, 15.
37. Heikal, O. A., Akao, T., Takeda, S. and Hattori, M. (1997) *Biol. Pharm. Bull.* **20**, 517.
38. Takeda, S., Isono, T., Wakui, Y., Matsuzaki, Y., Sasaki, H., Sakae, A. and Maruno, M. (1995) *J. Pharm. Pharmacol.* **47**, 1036.
39. Ishida, S., Sakiya, Y. and Ichikawa, T. (1990) *Xenobiot. Metabol. Dispos.* **5**, 771.
40. Xu, S., Chen, C. and Chen, G. (1990) *Eur. J. Pharmacol.* **183**, 2390.
41. Takeda, S., Waku, Y., Mizuhara, Y., Ishihara, K., Amagaya, S., Maruno, M. and Hattori, M. (1996) *J. Trad. Medicines* **13**, 248.
42. Meselhy, M. R., Heikal, O. A., Akao, T., Khojimatov, O., Hattori, M., Ono, H. and Sadakane, C. (1998) *Natural Medicines* **52**, 265.
43. Abdel-Hafez, A. A., Meselhy, M. R., Nakamura, N., Hattori, M., Watanabe, H., Mohamed, T. A., Mahfouz, N. M. and El-Gendy, M. A. (1998) *Chem. Pharm. Bull.* **46**, 1486.
44. Abdel-Hafez, A. A., Meselhy, M. R., Nakamura, N., Hattori, M., Watanabe, H., El-Gendy, M. A., Mahfouz, N. M. and Mohamed, T. A. (1998) *Biol. Pharm. Bull.* **22**, 491