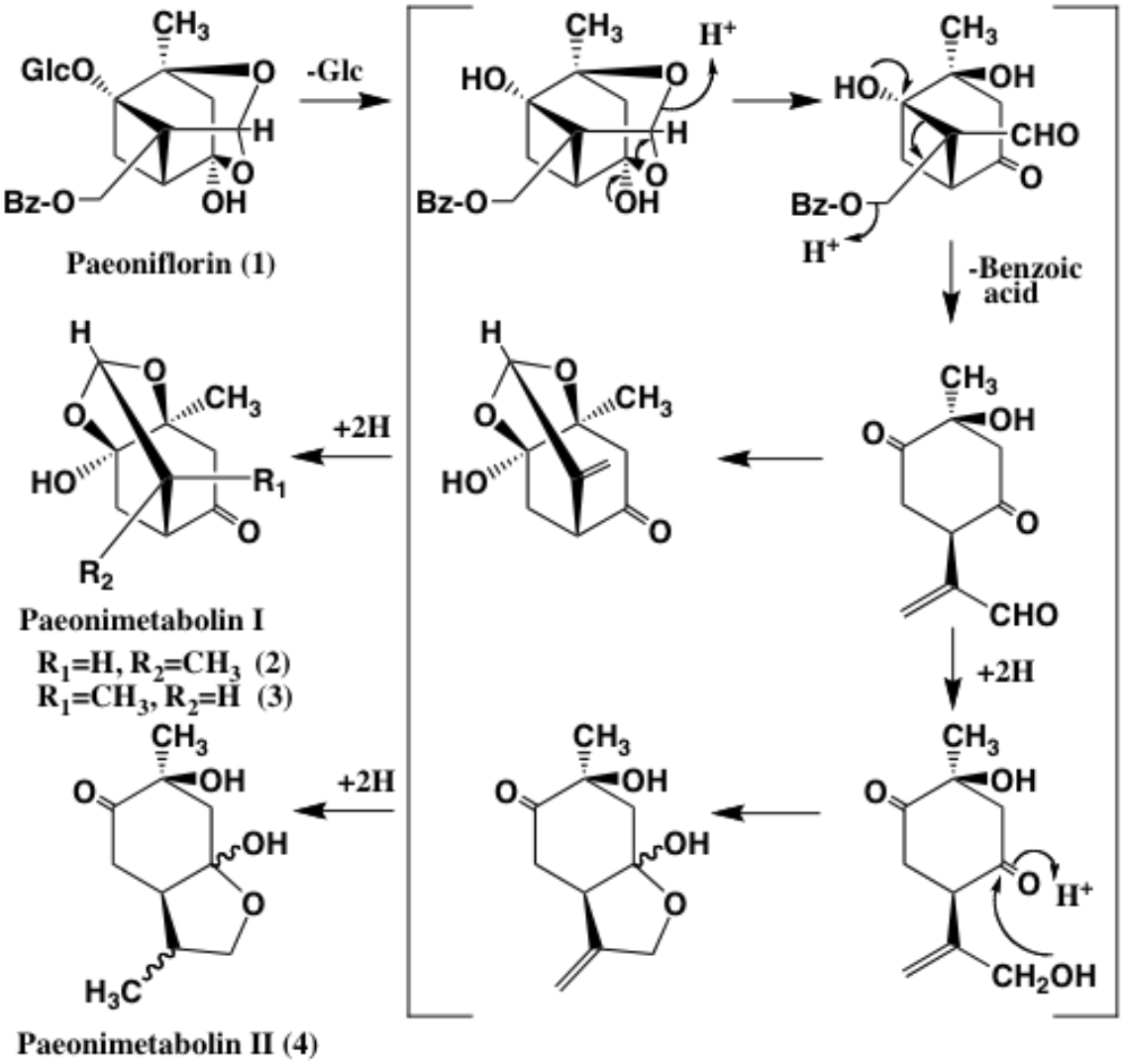
Paeoniflorin



Metabolic processes of paeoniflorin by human intestinal bacteria

代謝実験

腸内細菌代謝　ヒト腸内細菌フローラ、*Lactobacillus brevis*、*Bacteroides fragilis* ss. *Thetaotus*

動物代謝　ラット

単一化合物　paeoniflorin

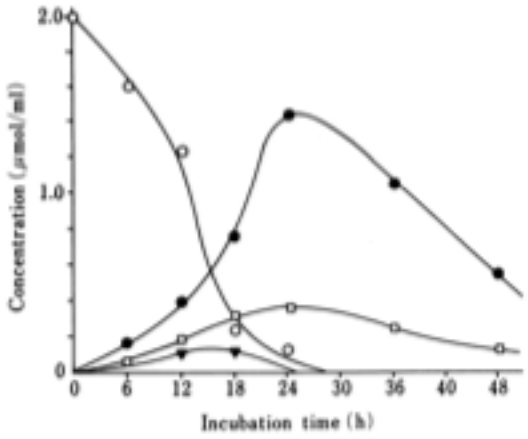


Fig. 1. Time course of metabolism of paeoniflorin by a human intestinal bacterial mixture

○ , paeoniflorin; ●, paeonimetabolin I; □, paeonimetabolin II; ▼, paeonimetabolin III. [Hattori *et al*., *Chem. Pharm. Bull*., **33**, 3838-3846 (1985)]

Metabolism of 1 by *Lactobacillus brevis*

A precultured bacterial suspension (500 ml) of *L. brevis* was added to GAM broth (4.5 1) and cultivated for 12 h at 37 °C under anaerobic conditions. The culture was centrifuged at 7000 rpm for 10 min. The precipitates were washed with saline solution, centrifuged, and suspended in 0.1 M phosphate buffer (625 ml). The suspension was transferred into five tubes. Compound **1** (600 mg/10 ml in the same buffer) was then added portion wise into each tube and was anaerobically incubated for 4 h at 37 °C. The mixture was extracted three times with ethyl acetate (AcOEt, 200 ml each) and the organic layer was concentrated *in vacuo* to give an oily residue. The combined residues (0.3 g) were applied to a column of silica gel (40 g, 19 x 240 mm). The column was thoroughly washed with benzene and eluted with benzene–$CHCl_3$ (1:1). Fractions (50 ml each) were collected and monitored by silica gel TLC and ^{1}H-NMR spectroscopy. Fractions 1—5 afforded a colorless oil, (7*S*-paeonimetabolin I, **2**, 26 mg, 11%) and fractions 11—15 yielded a crystalline compound (23 mg, 9.6%), which gave pure crystals from hexane–$CHCl_3$ (9:1) (3, 11 mg) on recrystallization. Fractions 6—10 gave a mixture of **2** and **3** (21mg, 8.8%). [Shu *et al*., *Chem. Pharm. Bull*., **35**, 3726-3733 (1987)]

7*S*-Paeonimetabolin I (2)

The physical properties were reported in the literature: [Hattori *et al*., *Chem. Pharm. Bull*., **33**, 3838-3846 (1985)].

7*R*-Paeonimetabolin I (3)

Colorless prisms, mp 146-148 °C. High resolution MS: Found, 198.0853; Calcd for M^+ , $C_{10}H_{14}O_4$, 198.0892. IR ν_{max} cm^{-1}: 3420 (OH), 1705 (C=O). ^{1}H-NMR ($CDC1_3$, 400 MHz) δ: 0.90 (3H, d, *J*=7.3 Hz, 8-H_3), 1.29 (3H, s, 10-H_3), 2.07 (1H, dq, *J*=7.5, 7.3 Hz, 7-H), 2.15 and 2.35 (each 1H, dd, *J*= 13.4, 2.3 Hz; *J*= 13.4, 3.4 Hz, 5-H_2), 2.60 and 2.64 (2H, ABq, *J*= 17.7 Hz, 2-H_2), 2.65 (1H, m, 4-H), 5.14 (1H, brs, 9-H). MS *m/z*: 198 (M^+), 180 ($M^+ - H_2O$), 152, 124, 109, 98, 83, 69 (base peak), 55. ^{13}C-NMR: see Table I in the literature: [Shu *et al*., *Chem. Pharm. Bull*., **35**, 3726-3733 (1987)].

Metabolism of 1 by *Bacteroides fragilis* ss. *thetaotus*

Compound **1** (2.1 g) was incubated with *B. fragilis* ss. *thetaotus* under conditions similar to those described above. After extraction with AcOEt, the organic layer was evaporated *in vacuo* to give an oily residue (0.9 g). The residue was chromatographed on silica gel (80 g; column size, 19 x 350 mm). The column was washed with benzene and eluted with benzene–$CHCl_3$ (1:1). Fractions were collected (60 ml/flask). Fractions 32—41, 42–49 and 50—61 afforded **2** (colorless oil, 105 mg, 12.6%), a mixture of **2** and **3** (oil, 103 mg, 12.3%) and **3** (prisms, 52 mg, 6.2%), respectively. Another oily substance (8 mg, 0.9%) was obtained from a $CHC1_3$ eluate; this was identical with paeonimetabolin II (**4**). [Shu *et al*., *Chem. Pharm. Bull*., **35**, 3726-3733 (1987)]

Paeonimetaboline II (4)

Epimeric mixture consisting of **4a** (33%) and **4b** (67%). The following assignments of 'H-NMR signals ($CDC1_3$, 400 MHz) were made on the basis of the peak intensities of paired signals. **4a**: δ 1.12 (3H, d, *J*= 6.4 Hz, 8-H_3), 1.36 (3H, s, 10-H_3), 2.02 (1H, ddq, *J*=14.5, 8.0, 6.5 Hz, 7-H), 2.25 and 2.29 (2H, ABq, *J*= 12.8 Hz, 2-H_2), *ca*. 2.27 (1H, m, overlapped, 4-H), 2.41 and 2.85 (each 1H, d and dd, *J*= 14.5 Hz; *J*= 14.5, 7.4 Hz, 5-H_2), 3.65 and 4.05 (each 1H, dd, *J*= 16.8, 7.8 Hz; *J*= 16.8, 8.0 Hz, 9-H_2). **4b**: δ 1.13 (3H, d, *J*= 6.4 Hz, 8-H_3), 1.33 (3H, s, 10-H_3), 2.15 (1H, m, 7-H), 2.32 and 2.38 (2H, ABq, *J*=

13.2 Hz, 2-H_2), *ca*. 2.38 (1H, m, overlapped, 4-H), 2.37 and 2.72 (each 1H, d and dd, *J*= 14.0 Hz; *J*= 14.0, 7.0 Hz, 5-H_2), 3.67 and 4.08 (each 1H, dd, *J*= 16.8, 9.2 Hz; *J*= 16.8, 8.7 Hz, 9-H_2). [Shu *et al*., *Chem. Pharm. Bull*., **35**, 3726-3733 (1987)]

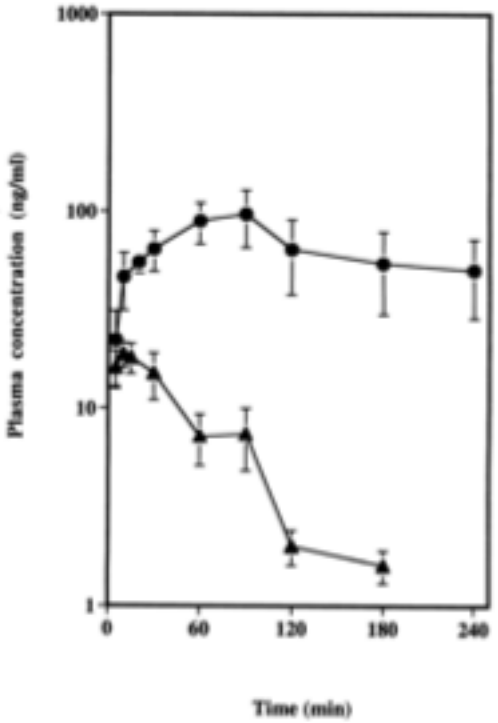


Fig. 2. Plasma concentration–time curves of paeonimetabolin I (●) and paeoniflorin (▲) after oral administration of paeoniflorin at a dose of 5 mg/kg to rats.
Each value represents the mean ± S.E. of 3 rats. [Heikal *et al*., *Biol. Pharm. Bull*., **20**, 517-521 (1997)]

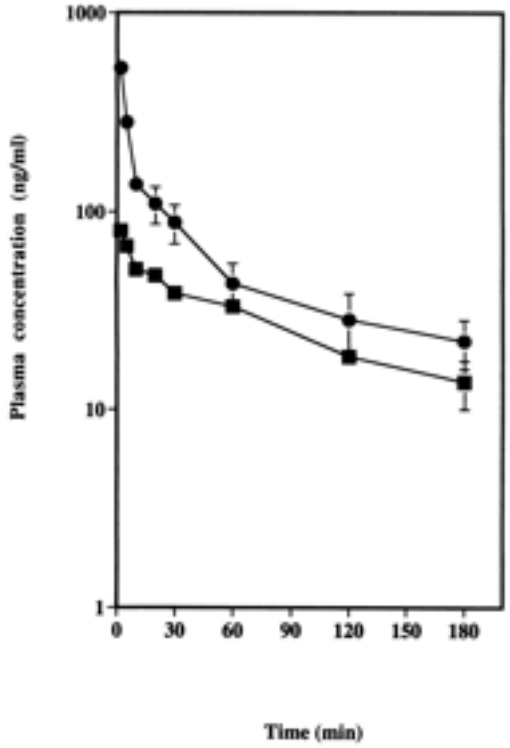


Fig. 3. Plasma concentration–time curves of paeonimetabolin I after intravenous administration of paeonimetabolin I at doses of 0.2 (■) and 2 (●) mg/kg to rats. Each value represents the mean ± S.E. of 4 rats. [Heikal *et al*., *Biol. Pharm. Bull*., **20**, 517-521 (1997)]

Table 1. Pharmacokinetic parameters of paeoniflorin and paeonimetabolin I (PM-I) 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-1	PF	PM-1
C_{max} (ng/ml)	9.9±2.2	16.5±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 point represents the mean ± S. E. (n=3). [Heikal *et al*., *Biol. Pharm. Bull*., **20**, 517-521 (1997)]

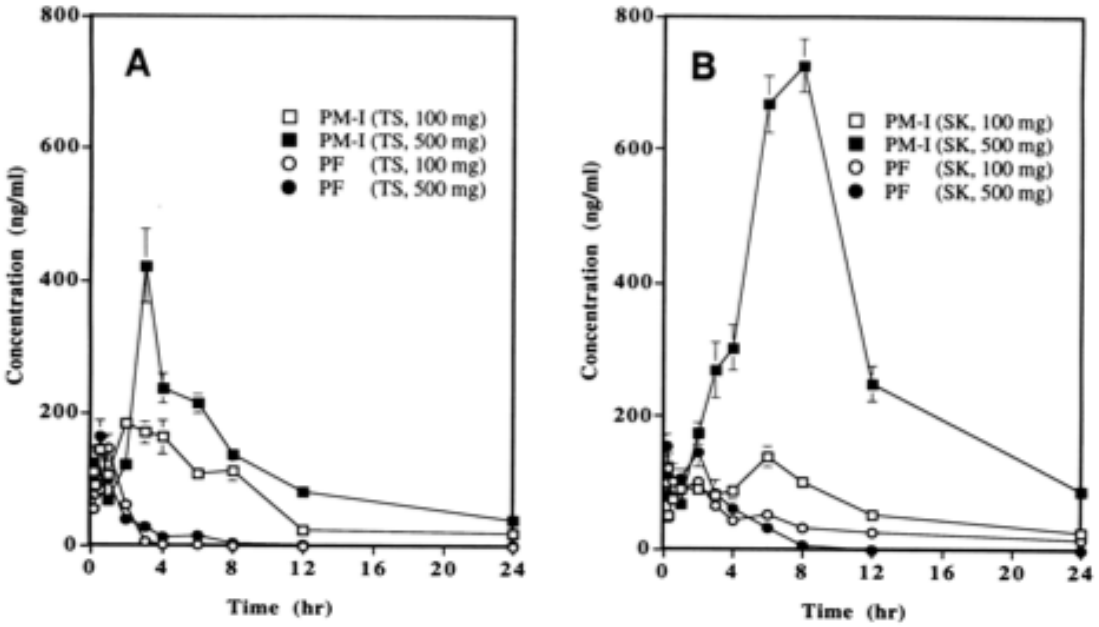


Fig. 4. Time cources of plasma levels of paeoniflorin (PF) and paeonimetabolin I (PM-I) in rats after oral administration of 100 and 500 mg prescriptions: Toki-Shakuyaku-San (TS) (A) and Shakuyaku-Kanzo-To (SK) (B).

TS, 当帰芍薬散; SK, 芍薬甘草湯; PF, paeoniflorin; PM-I, paeonimetabolin I.

Each point represents the mean ± S.E. of 4 rats. [Meselhy *et al*., *Natural Med.,* **52**, 265-268 (1998)]

Male Crj:CD Sprague-Dawley rats (7 weeks old, weighing *ca*. 220 g) obtained from Charles River (Japan) were fed standard laboratory chow. Forty four animals were fasted overnight with free access to water prior to drug administration. Prescriptions TS and SK were dissolved in distilled water, and doses of 100 and 500 mg/10 ml were orally given to the rats by gastric intubation. Four rats were randomly selected at time intervals, anesthetized, and all the blood was collected from the lower vena cava with a heparinized syringe. Blood samples were centrifuged at 1000 x *g* for 10 min, and the plasma was separated and kept at -20 °C until analysis. [Meselhy *et al*., *Natural Med.,* **52**, 265-268 (1998)]

Table 2. Pharmacokinetic parameters of PF and PM-I after *p.o.* 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	146.3	60	140.3	14305
	PM-I	184.0	120	426.7	98497
(500 mg)	PF	165.1	45	970.0	19385
	PM-I	400.3	180	569.1	182188
SK (100 mg)	PF	128.5	5	921.2	48857
	PM-I	141.7	360	508.7	102136
(500 mg)	PF	153.5	5	69.1	32518
	PM-I	726.5	480	325.8	469305

TS, 当帰芍薬散; SK, 芍薬甘草湯; PF, paeoniflorin; PM-I, paeonimetabolin I. [Meselhy *et al*., *Natural Med.,* **52**, 265-268 (1998)]

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