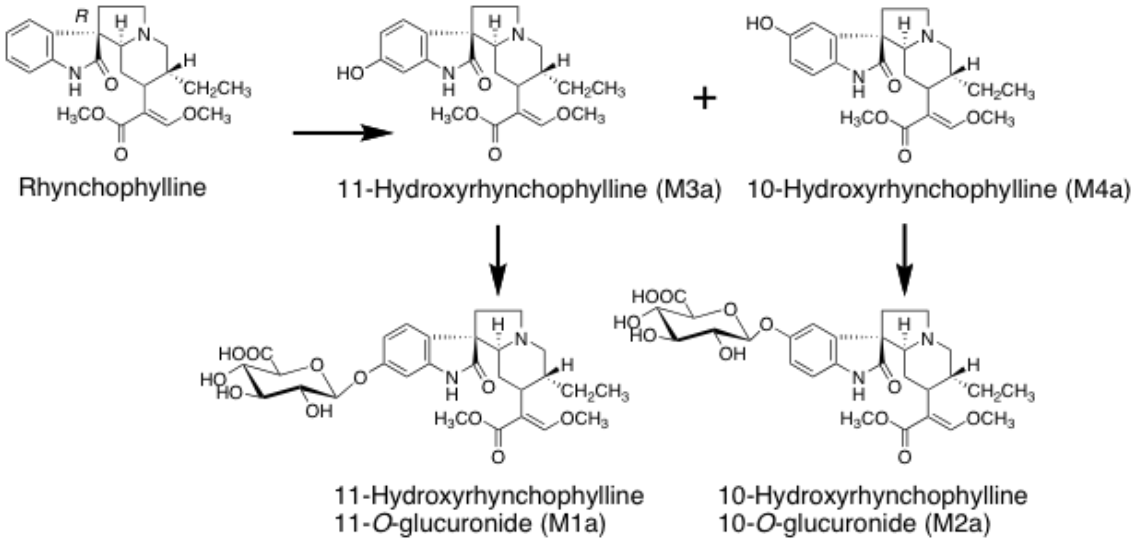
Rhynchophylline



Metabolic processes of rhynchophylline in rats

代謝実験

動物種　ラット

単一化合物 Rhynchophylline 及び漢方処方釣藤散、抑肝散

11-Hydroxyrhynchophylline 11-*O*-*β*-D-Glucuronide (M1a)

White solid, mp 285-290°C (uncorrected). $[\alpha]_D^{22}$ -8.3° (*c* = 0.56, MeOH). CD $\Delta\varepsilon$ (nm): +8.7 (288), -6.4 (259), -7.0 (222). HRFAB-MS *m/z*: 577.6081 ($[M+H]^+$, Calcd for $[C_{28}H_{36}N_2O_{11}+H]$: 577.6087). ESI-MS *m/z*: 577 ($[M+H]^+$). ^{1}H-NMR (CD_3OD) δ: 0.89 (3H, t, H-18), 1.16 (1H, br m, H-14β), 1.80 (2H, m, H-19), 2.08 (1H, br m, H-21α), 2.10 (1H, m, H-6α), 2.21 (1H, br m, H-20), 2.30 (1H, br m, H-15), 2.36 (1H, br m, H-14α), 2.38 (1H, m, H-3), 2.52 (1H, m, H-6β), 2.54 (1H, m, H-5α), 3.21 (1H, m, H-21β), 3.25 (1H, m, H-5β), 3.30 (1H, m, H-2′), 3.42 (1H, m, H-3′), 3.46 (1H, m, H-4′), 3.68 (3H, s, H-23), 3.70 (1H, d, $J_{5',4'}$ = 7.6 Hz, H-5′), 3.78 (3H, s, O$\underline{C}H_3$), 4.98 (1H, d, $J_{1',2'}$ = 7.6 Hz, H-1′), 6.92 (1H, d, $J_{12,10}$ = 1.2 Hz, H-12), 7.12 (1H, dd, $J_{10,9}$ = 8.0 Hz, $J_{10,12}$ = 1.2 Hz, H-10), 7.20 (1H, d, $J_{9,10}$ = 8.0 Hz, H-9), 7.35 (1H, s, H-17). ^{13}C-NMR (CD_3OD) δ: 11.9 (C-18), 24.3 (C-19), 28.5 (C-14), 35.4 (C-6), 40.0 (C-20), 40.9 (C-15), 50.2 (C-23), 55.0 (C-7), 57.2 (C-5), 57.2 (C-21), 63.2 (O$\underline{C}H_3$), 71.7 (C-4′), 74.5 (C-3), 75.2 (C-2′), 78.2 (C-5′), 78.8 (C-3′), 100.2 (C-12), 100.8 (C-1′), 111.3 (C-10), 113.7

(C-16), 124.2 (C-9), 129.8 (C-8), 141.0 (C-13), 151.4 (C-11), 160.1 (C-17), 169.2 (C-22), 176.8 (C-6′), 182.9 (C-2). [Wang *et al*., *Biol. Pharm. Bull.* **33**, 669-676 (2010)]

10-Hydroxyrhynchophylline 10-*O*-*β*-D-Glucuronide (M2a)

White solid, mp 283-286°C (uncorrected). $[\alpha]_D^{22}$ -5.6° (*c* = 0.72, MeOH). CD $\Delta\varepsilon$ (nm): +6.9 (292), -3.2 (262), -5.0 (220). HRFAB-MS *m/z*: 577.6082 ($[M+H]^+$, Calcd for $[C_{28}H_{36}N_2O_{11}+H]$: 577.6087). ESI-MS *m/z*: 577.3 or 577.4 ($[M+H]^+$). ^{1}H-NMR (CD_3OD) δ: 1.00 (3H, t, H-18), 1.20 (1H, br m, H-14β), 1.78 (2H, m, H-19), 1.90 (1H, br m, H-21α), 2.02 (1H, m, H-6α), 2.20 (1H, br m, H-20), 2.28 (1H, br m, H-15), 2.30 (1H, br m, H-14α), 2.36 (1H, m, H-3), 2.40 (1H, m, H-6β), 2.44 (1H, m, H-5α), 3.15 (1H, m, H-21β), 3.28 (1H, m, H-5β), 3.31 (1H, m, H-2′), 3.44 (1H, m, H-3′), 3.50 (1H, m, H-4′), 3.62 (3H, s, H-23), 3.66 (1H, d, $J_{5',4'}$ = 7.6 Hz, H-5′), 3.73 (3H, s, O$\underline{C}H_3$), 4.96 (1H, d, $J_{1',2'}$ = 7.6 Hz, H-1′), 6.78 (1H, d, $J_{12,11}$ = 7.6 Hz, H-12), 7.12 (1H, dd, $J_{11,9}$ = 1.2 Hz, $J_{11,12}$ = 7.6 Hz, H-11), 7.22 (1H, d, $J_{9,11}$ = 1.2 Hz, H-9), 7.34 (1H, s, H-17). ^{13}C-NMR (CD_3OD) δ: 11.7 (C-18), 24.2 (C-19), 28.3 (C-14), 35.2 (C-6), 40.2 (C-20), 40.3 (C-15), 50.1 (C-23), 55.3 (C-7), 57.1 (C-5), 57.1 (C-21), 63.2 (O$\underline{C}H_3$), 71.7 (C-4′), 74.5 (C-3), 75.0 (C-2′), 78.4 (C-5′), 78.8 (C-3′), 100.6 (C-1′), 110.2 (C-12), 112.1 (C-16), 114.2 (C-9), 119.6 (C-11), 133.9 (C-8), 135.0 (C-13), 150.1 (C-10), 160.9 (C-17), 169.1 (C-22), 176.2 (C-6′), 182.8 (C-2). [Wang *et al*., *Biol. Pharm. Bull.* **33**, 669-676 (2010)]

11-Hydroxyrhynchophylline (M3a)

White solid from methanol, mp 239-241°C (uncorrected). $[\alpha]_D^{20}$ -120.0° (*c* = 0.78, MeOH). CD $\Delta\varepsilon$ (nm): +12.0 (289), -4.8 (260), -9.0 (221). HRFAB-MS *m/z*: 401.4822 ($[M+H]^+$, Calcd for $[C_{22}H_{28}N_2O_5+H]$: 401.4827). ESI-MS *m/z*: 401 ($[M+H]^+$). ^{1}H-NMR (CD_3OD) δ: 0.88 (3H, t, H-18), 1.16 (1H, br m, H-14β), 1.80 (2H, m, H-19), 2.08 (1H, br m, H-21α), 2.10 (1H, m, H-6α), 2.20 (1H, br m, H-20), 2.27 (1H, br m, H-15), 2.28 (1H, br m, H-14α), 2.39 (1H, m, H-3), 2.51 (1H, m, H-6β), 2.52 (1H, m, H-5α), 3.20 (1H, m, H-21β), 3.25 (1H, m, H-5β), 3.68 (3H, s, H-23), 3.80 (3H, s, O$\underline{C}H_3$), 6.88 (1H, d, $J_{12,10}$ = 1.2 Hz, H-12), 7.08 (1H, dd, $J_{10,9}$ = 8.0 Hz, $J_{10,12}$ = 1.2 Hz, H-10), 7.24 (1H, d, $J_{9,10}$ = 8.0 Hz, H-9), 7.37 (1H, s, H-17). ^{13}C-NMR (CD_3OD) δ: 11.6 (C-18), 24.1 (C-19), 28.3 (C-14), 35.3 (C-6), 40.1 (C-20), 40.5 (C-15), 50.2 (C-23), 55.2 (C-7), 57.1 (C-5),

57.1 (C-21), 63.0 (O<u>C</u>H_3), 74.6 (C-3), 99.7 (C-12), 111.2 (C-10), 113.1 (C-16), 124.5 (C-9), 129.6 (C-8), 141.6 (C-13), 151.3 (C-11), 159.4 (C-17), 169.0 (C-22), 182.7 (C-2). [Wang *et al*., *Biol. Pharm. Bull.* **33**, 669-676 (2010)]

10-Hydroxyrhynchophylline (M4a)

White solid from methanol, mp 233-237°C (uncorrected). $[\alpha]_D^{20}$ -115.2° (*c* = 0.91, MeOH). CD $\Delta\varepsilon$ (nm): +9.6 (290), -6.2 (261), -7.20 (222). HRFAB-MS *m/z*: 401.4823 ($[M+H]^+$, Calcd for $[C_{22}H_{28}N_2O_5+H]$: 401.4827. ESI-MS *m/z*: 401 ($[M+H]^+$). ^{1}H-NMR (CD_3OD) δ: 0.95 (3H, t, H-18), 1.18 (1H, br m, H-14β), 1.70 (2H, m, H-19), 1.89 (1H, br m, H-21α), 2.00 (1H, m, H-6α), 2.12 (1H, br m, H-20), 2.25 (1H, br m, H-15), 2.33 (1H, br m, H-14α), 2.35 (1H, m, H-3), 2.39 (1H, m, H-6β), 2.45 (1H, m, H-5α), 3.15 (1H, m, H-21β), 3.28 (1H, m, H-5β), 3.60 (3H, s, H-23), 3.72 (3H, s, OC<u>H</u>$_3$), 6.86 (1H, d, $J_{12,11}$ = 7.6 Hz, H-12), 7.07 (1H, dd, $J_{11,9}$ = 1.2 Hz, $J_{11,12}$ = 7.6 Hz, H-11), 7.18 (1H, d, $J_{9,11}$ = 1.2 Hz, H-9), 7.33 (1H, s, Hz, H-17). ^{13}C-NMR (CD_3OD) δ: 11.6 (C-18), 24.1 (C-19), 28.2 (C-14), 35.1 (C-6), 39.7 (C-20), 40.4 (C-15), 50.0 (C-23), 55.1 (C-7), 57.3 (C-5), 57.3 (C-21), 63.1 (O<u>C</u>H_3), 74.4 (C-3), 110.4 (C-12), 112.5 (C-16), 114.0 (C-9), 119.5 (C-11), 134.0 (C-8), 134.7 (C-13), 149.7 (C-10), 161.7 (C-17), 169.2 (C-22), 182.9 (C-2). [Wang *et al*., *Biol. Pharm. Bull.* **33**, 669-676 (2010)]

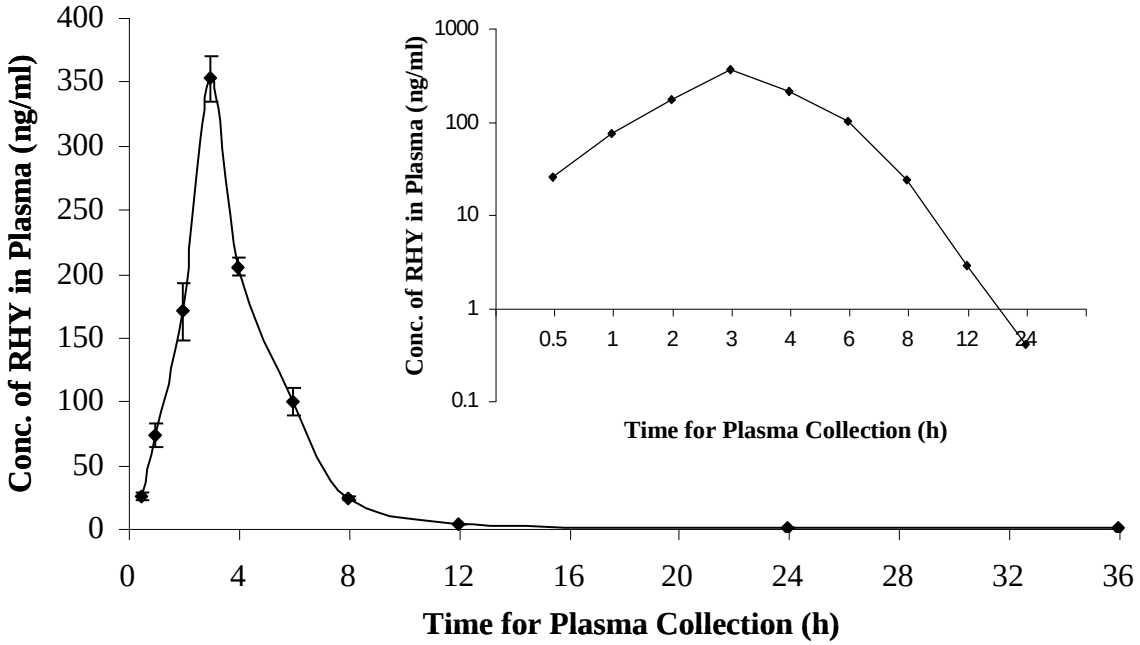


Fig. 1. Time course of rhynchophylline (RHY) in rat plasma after oral administration.

After oral administration of 37.5 mg/kg, RHY concentrations in plasma were quantified by LC-MS (EIC) monitored at *m/z* 385 ± 0.5 in the positive ion mode. [Wang *et al*., *Biol. Pharm. Bull.* **33**, 669-676 (2010)]

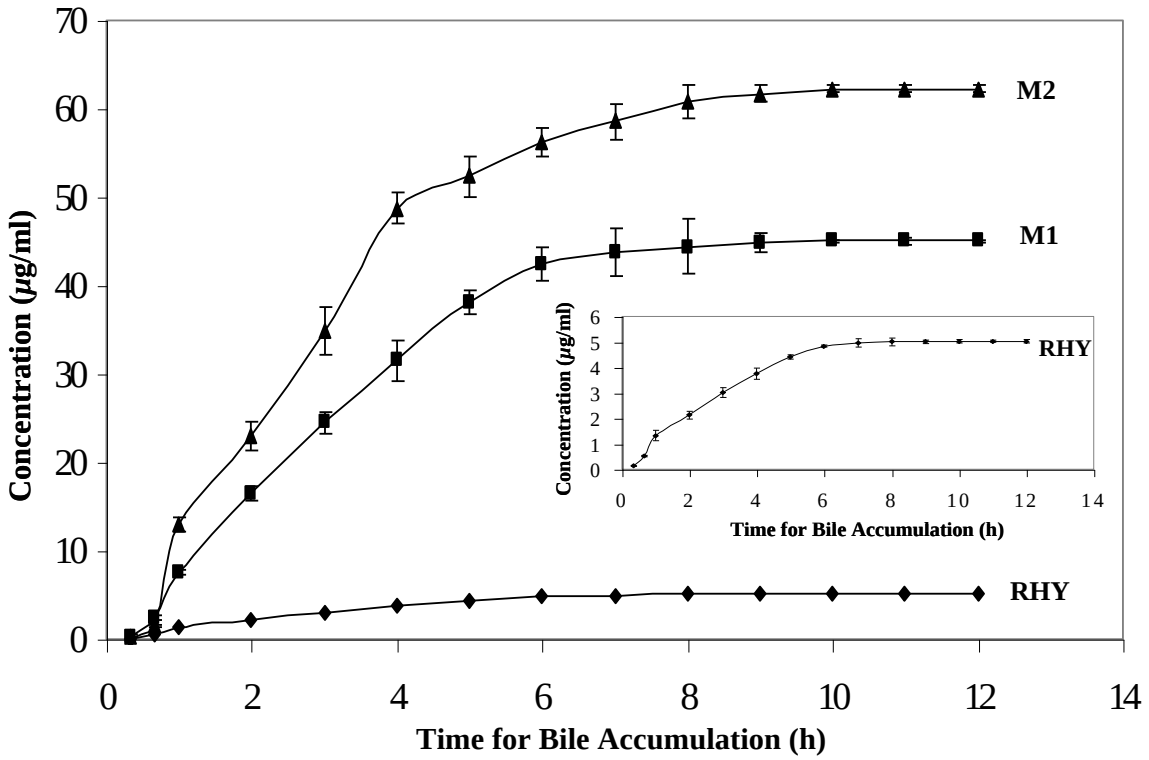


Fig. 2. Cumulative curves of rhynchophylline (RHY), 11-hydroxy rhynchophylline 11-*O*-glucuronide (M1) and 10-hydroxyrhynchophylline 10-*O*-glucuronide (M2) in rat bile after oral RHY administration.

Rat bile was collected from 10 rats after oral RHY administration at a dose of 60 mg/kg over 8 h within 1 day, lyophilized and then applied to an LH-20 column (2.5 × 30 cm). The methanol eluate was analyzed by HPLC. [Wang *et al*., *Biol. Pharm. Bull.* **33**, 669-676 (2010)]

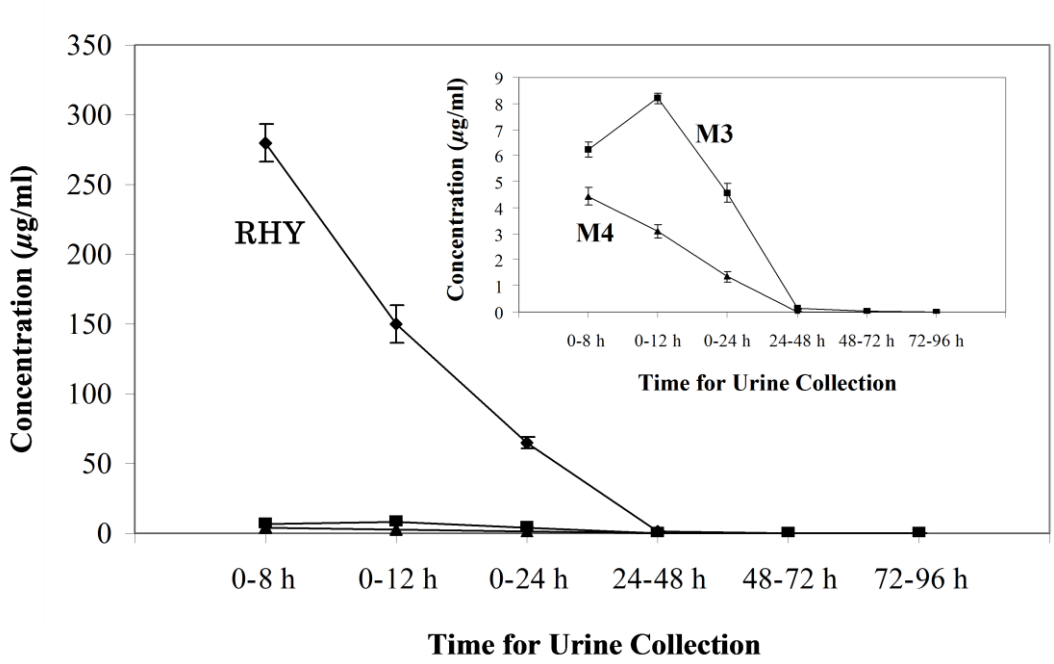


Fig. 3. Excretion curves of rhynchophylline (RHY), 11-hydroxy rhynchophylline (M3) and 10-hydroxyrhynchophylline (M2) into rat urine after oral RHY administration (n = 3). [Wang *et al*., *Biol. Pharm. Bull.* **33**, 669-676 (2010)]

Main pharmacokinetic parameters for RHY in rats.

Parameter	Units	Administration 15 mg/kg i.v.	Administration 37.5 mg/kg p.o.
k	min^{-1}	$(8.521 \pm 0.19)\times10^{-3}$	
$t_{1/2}$	min	81.33 ± 1.8	
V	l/kg	$(1.009 \pm 0.0069)\times10^{-1}$	
CL	$l\cdot kg^{-1}\cdot min^{-1}$	$(8.597 \pm 0.13)\times10^{-4}$	
t_{max}	min		180.0 ± 0.82
C_{max}	ng/ml		351.5 ± 1.2
$AUC_{0-\infty}$	$\mu g\cdot ml^{-1}\cdot min$	$(17.44 \pm 0.27)\times10^{3}$	191.3 ± 4.2

[Wang *et al*., *Biol. Pharm. Bull.* **33**, 669-676 (2010)]

釣藤散および抑肝散に含まれる rhynchophylline, isorhynchophylline 体内動態

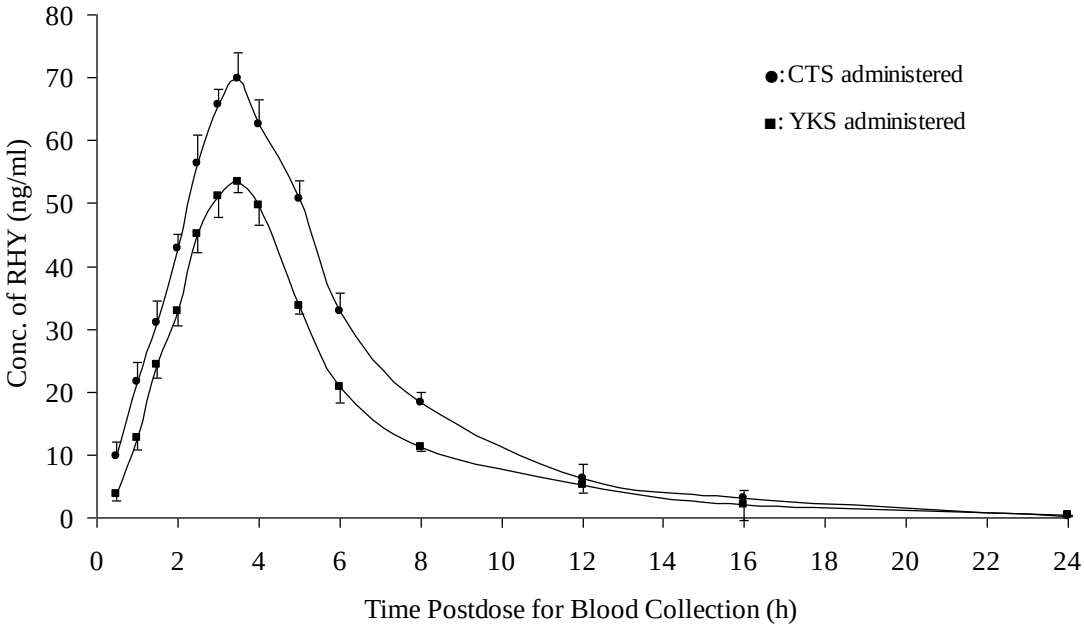


Fig. 4　Time course of the plasma concentration of rhynchophylline after oral administration of Chotosan (CTS, 釣藤散) and Yokukansan (YKS, 抑肝散)

CTS and YKS extracts were orally administered at doses of 0.29 and 0.31 g, respectively, to rats. [Wang et al., *J. Trad. Med*., **27**, 15-29 (2010)]

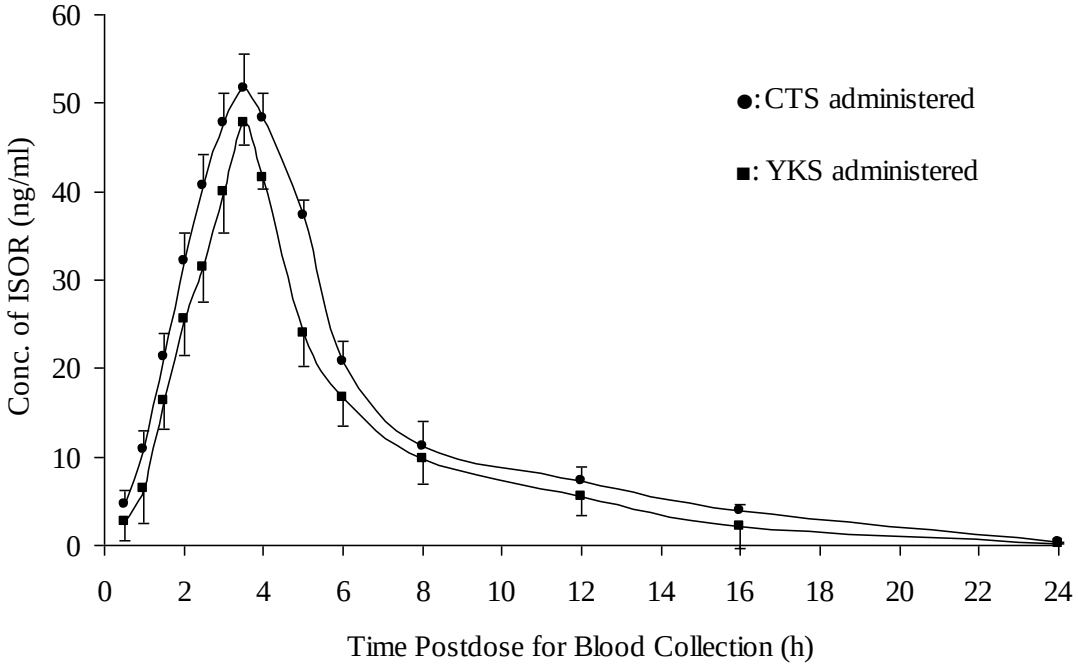


Fig. 5　Time course of the plasma concentration of isorhynchophylline after oral administration of Chotosan (CTS, 釣藤散) and Yokkansan (YKS, 抑肝散).

CTS and YKS extracts were orally administered at doses of 0.29 and 0.31 g, respectively, to rats. [Wang et al., *J. Trad. Med*., **27**, 15-29 (2010)]

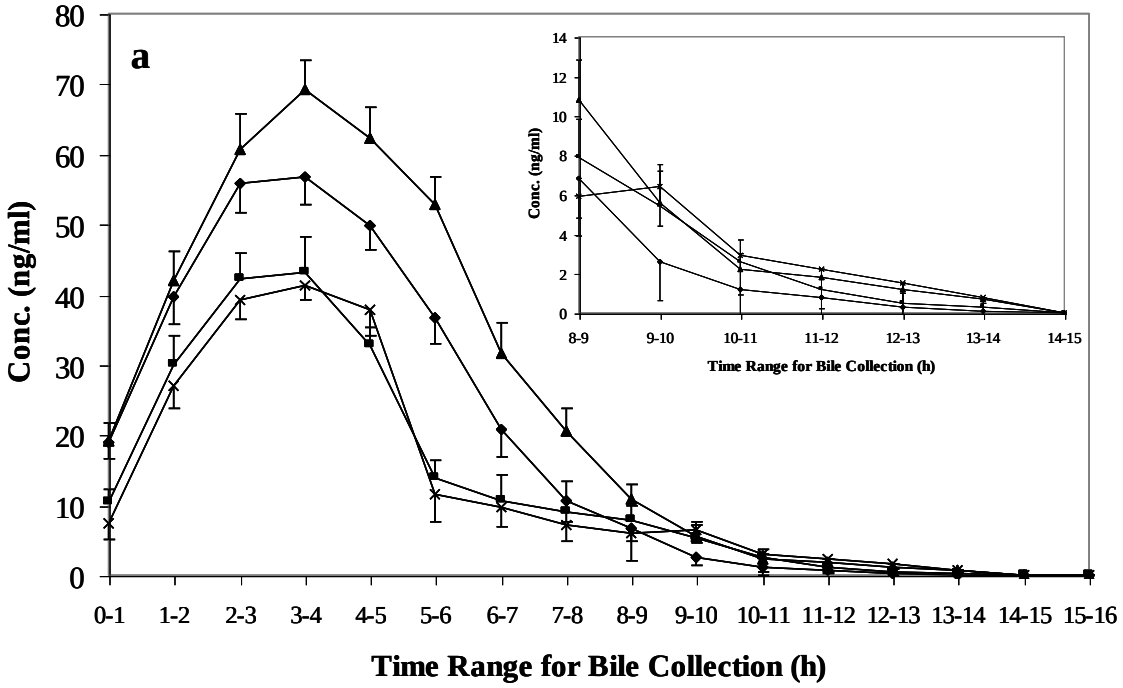


Fig. 6 The metabolites **M1a**, **M1b**, **M2a**, and **M2b** excreted into bile in 16 h after oral administration of Chotosan (CTS).

11-Hydroxyrhynchophylline 11-*O*-glucuronide (**M1a**, ■), 11-hydroxyisorhyncho-phylline 11-*O*-glucuronide (**M1b**, ▲), 10-hydroxyrhynchophylline 10-*O*-glucuronide (**M2a**, ◆) and 10-hydroxyisorhynchophylline 10-*O*-glucuronide (**M2b**, ×) [Wang et al., *J. Trad. Med*., **27**, 15-29 (2010)]

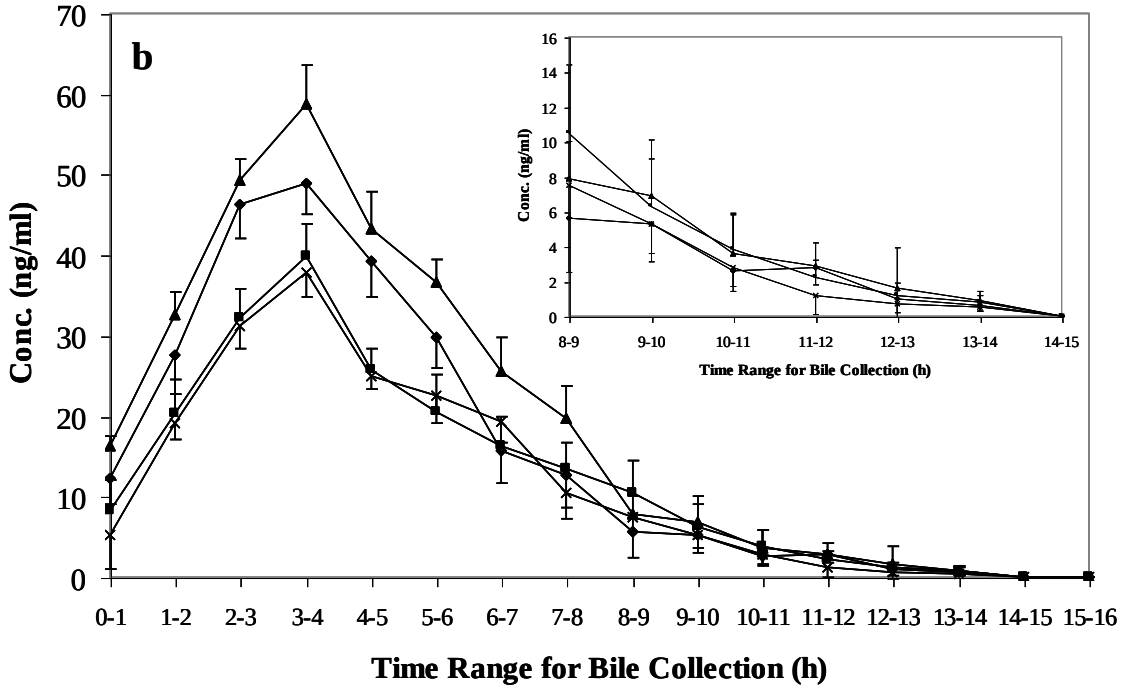


Fig. 7　The metabolites **M1a**, **M1b**, **M2a**, and **M2b** excreted into bile in 16 h after oral administration of Yokukansan (YKS).

11-Hydroxyrhynchophylline 11-*O*-glucuronide (**M1a**, ■), 11-hydroxyisorhyncho-phylline 11-*O*-glucuronide (**M1b**, ▲), 10-hydroxyrhynchophylline 10-*O*-glucuronide (**M2a**, ◆) and 10-hydroxyisorhynchophylline 10-*O*-glucuronide (**M2b**, ×). [Wang et al., *J. Trad. Med*., **27**, 15-29 (2010)]

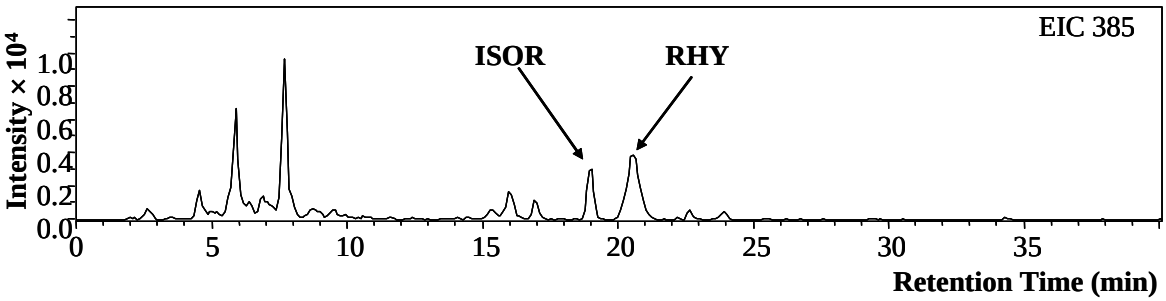


Fig. 8. LC-MS (EIC) elution profiles of a rat brain sample isolated at 3.5 h after oral administration of CTS, monitored at *m/z* 385 ± 0.5. [Wang et al., *J. Trad. Med*., **27**, 15-29 (2010)]

Table 1. Amounts of rhynchophylline (RHY) and isorhynchophylline (ISOR) detected in rat brain after oral administration of RHY, ISOR, and prescriptions Chotosan (CTS), and Yokukansan (YKS)

Dose (mg/kg)			Concentration (ng/g)	
			RHY	ISOR
RHY		37.5	0.13 ± 0.01	ND
ISOR		37.5	ND	0.10 ± 0.01
CTS	RHY	24.8	1.29 ± 0.02	
	ISOR	16.6		0.40 ± 0.03
YKS	RHY	23.9	1.02 ± 0.05	
	ISOR	16.2		0.38 ± 0.02

Data is shown as mean ± SD (n = 3), rat brain in dry weight, ND: not detected. [Wang et al., *J. Trad. Med*., **27**, 15-29 (2010)]

参考文献

1) Wang W., Ma C. M., and Hattori M.: Metabolism and pharmacokinetics of rhynchophylline in rats. *Biol. Pharm. Bull.* **33**, 669-676 (2010).

2) Wang W., Ma C. M., and Hattori M.: Simultaneous determination of rhynchophylline, isorhynchophylline, and their eight metabolites in rats. *J. Trad. Med*., **27**, 15-29 (2010).