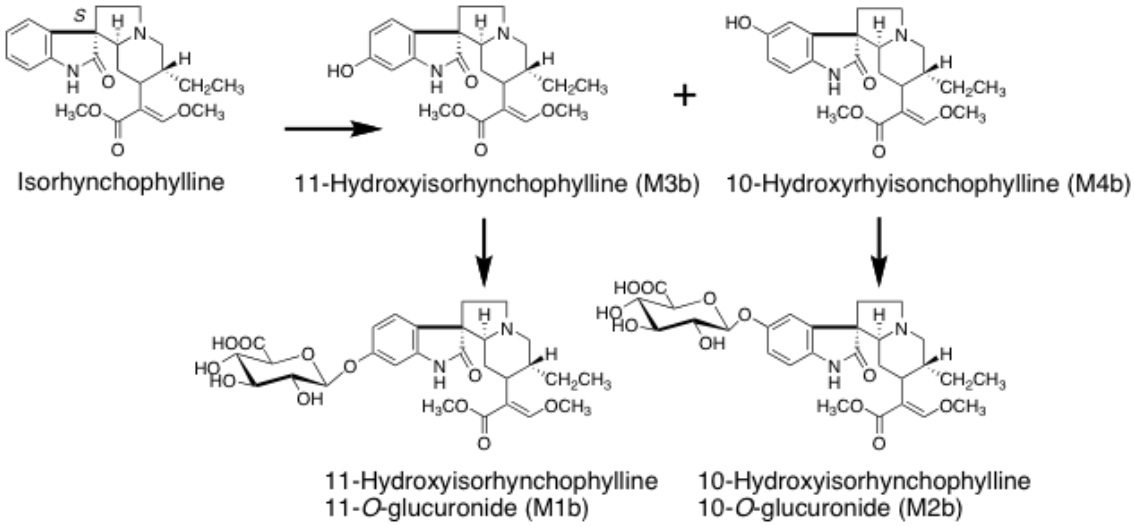
Isorhynchophylline



Metabolic processes of isorhynchophylline in rats

代謝実験

動物種　ラット

単一化合物 Isorhynchophylline 及び漢方処方釣藤散、抑肝散

11-Hydroxyisorhynchophylline 11-*O*-*β*-D-glucuronide (M1b)

White solid, mp 276-280 °C (uncorrected). $[\alpha]_D^{22}$ +12.3° (*c* = 0.42, MeOH). CD $\Delta\varepsilon$ (nm): -1.2 (285), -9.3 (255), +10.2 (233). HRFAB-MS *m/z*: 577.6083 ($[M+H]^+$, 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: 0.62 (1H, br m, H-14β), 0.90 (3H, t, H-18), 1.82 (2H, m, H-19), 2.10 (1H, br m, H-21α), 2.12 (1H, m, H-6α), 2.15 (1H, br m, H-14α), 2.22 (1H, br m, $J_{20,15}$ = 11.2 Hz, $J_{20,21\alpha}$ = 11.2 Hz, $J_{20,21\beta}$ = 3.2 Hz, H-20), 2.36 (1H, br m, $J_{15,14\beta}$ = 11.2 Hz, $J_{15,20}$ = 11.2 Hz, $J_{15,14\alpha}$ = 3.2 Hz, H-15), 2.46 (1H, m, H-6β), 2.57 (1H, m, H-5α), 2.65 (1H, m, H-3), 3.16 (1H, m, H-21β), 3.18 (1H, m, H-5β), 3.28 (1H, m, H-2′), 3.41 (1H, m, H-3′), 3.42 (1H, m, H-4′), 3.69 (1H, d, $J_{5',4'}$ = 7.6 Hz, H-5′), 3.70 (3H, s, H-23), 3.75 (3H, s, OC$\underline{H}_3$), 4.92 (1H, d, $J_{1',2'}$ = 7.6 Hz, H-1′), 6.89 (1H, d, $J_{12,10}$ = 1.2 Hz, H-12), 7.09 (1H, dd, $J_{10,9}$ = 8.0 Hz, $J_{10,12}$ = 1.2 Hz, H-10), 7.35 (1H, s, H-17), 7.43 (1H, d, $J_{9,10}$ = 8.0 Hz, H-9). ^{13}C-NMR (CD_3OD) δ: 11.8 (C-18), 24.3 (C-19), 28.8 (C-14), 35.7 (C-6), 37.3 (C-20),

39.3 (C-15), 50.4 (C-23), 55.6 (C-7), 56.5 (C-5), 57.5 (C-21), 63.3 (O<u>C</u>H$_3$), 71.8 (C-3), 72.3 (C-4′), 75.5 (C-2′), 78.3 (C-5′), 79.0 (C-3′), 99.8 (C-12), 100.6 (C-1′), 111.3 (C-10), 113.8 (C-16), 126.7 (C-9), 130.1 (C-8), 140.6 (C-13), 151.3 (C-11), 159.6 (C-17), 169.3 (C-22), 177.0 (C-6′), 182.6 (C-2). [Wang *et al*., *J. Pharmaceut. Sci*., **13**, 27-37 (2010)]

10-Hydroxyisorhynchophylline 10-*O*-β-D-glucuronide (M2b)

White solid, mp 273-278 °C (uncorrected). $[\alpha]_D^{22}$ +9.6° (*c* = 0.68, MeOH). CD $\Delta\varepsilon$ (nm): -2.1 (283), -7.2 (260), +9.1 (230). HRFAB-MS *m/z*: 577.6084 ($[M+H]^+$, 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: 0.51 (1H, br m, H-14β), 1.02 (3H, t, H-18), 1.79 (2H, m, H-19), 1.95 (1H, br m, H-21α), 2.02 (1H, m, H-6α), 2.19 (1H, br m, H-14α), 2.29 (1H, br m, $J_{20,15}$ = 11.2 Hz, $J_{20,21\alpha}$ = 11.2 Hz, $J_{20,21\beta}$ = 3.2 Hz, H-20), 2.35 (1H, br m, $J_{15,14\beta}$ = 11.2 Hz, $J_{15,20}$ = 11.2 Hz, $J_{15,14\alpha}$ = 3.2 Hz, H-15), 2.41 (1H, m, H-6β), 2.46 (1H, m, H-5α), 2.58 (1H, m, H-3), 3.11 (1H, m, H-21β), 3.20 (1H, m, H-5β), 3.27 (1H, m, H-2′), 3.40 (1H, m, H-3′), 3.46 (1H, m, H-4′), 3.65 (1H, d, $J_{5',4'}$ = 7.6 Hz, H-5′), 3.66 (3H, s, H-23), 3.70 (3H, s, OC<u>H</u>$_3$), 4.89 (1H, d, $J_{1',2'}$ = 7.6 Hz, H-1′), 6.72 (1H, d, $J_{12,11}$ = 7.6 Hz, H-12), 7.10 (1H, dd, $J_{11,9}$ = 1.2 Hz, $J_{11,12}$ = 7.6 Hz, H-11), 7.36 (1H, s, H-17), 7.46 (1H, d, $J_{9,11}$ = 1.2 Hz, H-9). ^{13}C-NMR (CD_3OD) δ: 11.6 (C-18), 24.1 (C-19), 28.9 (C-14), 35.6 (C-6), 37.5 (C-20), 39.1 (C-15), 50.3 (C-23), 55.7 (C-7), 56.0 (C-5), 57.3 (C-21), 63.3 (O<u>C</u>H$_3$), 71.3 (C-3), 71.8 (C-4′), 75.2 (C-2′), 78.5 (C-5′), 79.1 (C-3′), 100.4 (C-1′), 109.7 (C-12), 112.7 (C-16), 116.3 (C-9), 119.4 (C-11), 134.1 (C-13), 134.4 (C-8), 149.9 (C-10), 159.1 (C-17), 169.4 (C-22), 176.3 (C-6′), 182.2 (C-2). [Wang *et al*., *J. Pharmaceut. Sci*., **13**, 27-37 (2010)]

11-Hydroxyisorhynchophylline (M3b)

White solid from methanol, mp 231-234 °C (uncorrected). $[\alpha]_D^{20}$ +112.6° (*c* = 0.62, MeOH). CD $\Delta\varepsilon$ (nm): -1.7 (285), -6.3 (258), +7.6 (228). HRFAB-MS *m/z*: 401.4825 ($[M+H]^+$, 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: 0.61 (1H, br m, H-14β), 0.87 (3H, t, H-18), 1.80 (2H, m, H-19), 2.08 (1H, br m, H-21α), 2.10 (1H, br m, H-6α), 2.11 (1H, m, H-14α), 2.12 (1H, br m, $J_{20,15}$ = 11.2 Hz, $J_{20,21\alpha}$ = 11.2 Hz, $J_{20,21\beta}$ = 3.2 Hz, H-20), 2.32 (1H, br m, $J_{15,14\beta}$ = 11.2 Hz, $J_{15,20}$ = 11.2 Hz, $J_{15,14\alpha}$ = 3.2 Hz, H-15), 2.43 (1H, m, H-6β), 2.55 (1H, m, H-5α), 2.61 (1H, m, H-3), 3.11 (1H, m, H-21β), 3.17 (1H, m, H-5β), 3.68 (3H, s, H-23), 3.78 (3H, s, OC<u>H</u>$_3$),

6.85 (1H, d, $J_{12,10}$ = 1.2 Hz, H-12), 7.06 (1H, dd, $J_{10,9}$ = 8.0 Hz, $J_{10,12}$ = 1.2 Hz, H-10), 7.33 (1H, s, H-17), 7.45 (1H, d, $J_{9,10}$ = 8.0 Hz, H-9). ^{13}C-NMR (CD_3OD) δ: 11.7 (C-18), 24.2 (C-19), 28.9 (C-14), 35.5 (C-6), 37.3 (C-20), 39.2 (C-15), 50.3 (C-23), 55.7 (C-7), 56.3 (C-5), 57.3 (C-21), 63.5 (O$\underline{C}H_3$), 72.0 (C-3), 99.5 (C-12), 111.1 (C-10), 113.6 (C-16), 126.8 (C-9), 130.0 (C-8), 140.7 (C-13), 151.1 (C-11), 159.2 (C-17), 169.2 (C-22), 182.3 (C-2). [Wang *et al*., *J. Pharmaceut. Sci*., **13**, 27-37 (2010)]

10-Hydroxyisorhynchophylline (M4b)

White solid from methanol, mp 227-231 °C (uncorrected). $[\alpha]_D^{20}$ +103.8° (c = 0.76, MeOH). CD $\Delta\varepsilon$ (nm): -1.4 (284), -5.9 (257), +8.1 (228). HRFAB-MS *m/z*: 401.4826 ($[M+H]^+$, 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: 0.53 (1H, br m, H-14β), 0.97 (3H, t, H-18), 1.73 (2H, m, H-19), 1.91 (1H, br m, H-21α), 2.01 (1H, m, H-6α), 2.11 (1H, br m, H-14α), 2.19 (1H, br m, $J_{20,15}$ = 11.2 Hz, $J_{20,21\alpha}$ = 11.2 Hz, $J_{20,21\beta}$ = 3.2 Hz, H-20), 2.32 (1H, br m, $J_{15,14\beta}$ = 11.2 Hz, $J_{15,20}$ = 11.2 Hz, $J_{15,14\alpha}$ = 3.2 Hz, H-15), 2.37 (1H, m, H-6β), 2.50 (1H, m, H-5α), 2.56 (1H, m, H-3), 3.16 (1H, m, H-21β), 3.20 (1H, m, H-5β), 3.62 (3H, s, H-23), 3.75 (3H, s, OC$\underline{H}_3$), 6.82 (1H, d, $J_{12,11}$ = 7.6 Hz, H-12), 7.08 (1H, dd, $J_{11,9}$ = 1.2 Hz, $J_{11,12}$ = 7.6 Hz, H-11), 7.37 (1H, s, Hz, H-17), 7.40 (1H, d, $J_{9,11}$ = 1.2 Hz, H-9). ^{13}C-NMR (CD_3OD) δ: 11.7 (C-18), 24.2 (C-19), 28.7 (C-14), 35.6 (C-6), 37.9 (C-20), 39.1 (C-15), 50.1 (C-23), 55.8 (C-7), 56.6 (C-5), 57.1 (C-21), 63.3 (O$\underline{C}H_3$), 71.2 (C-3), 110.1 (C-12), 113.0 (C-16), 116.2 (C-9), 119.0 (C-11), 134.0 (C-13), 134.5 (C-8), 149.6 (C-10), 161.1 (C-17), 169.4 (C-22), 182.3 (C-2). [Wang *et al*., *J. Pharmaceut. Sci*., **13**, 27-37 (2010)]

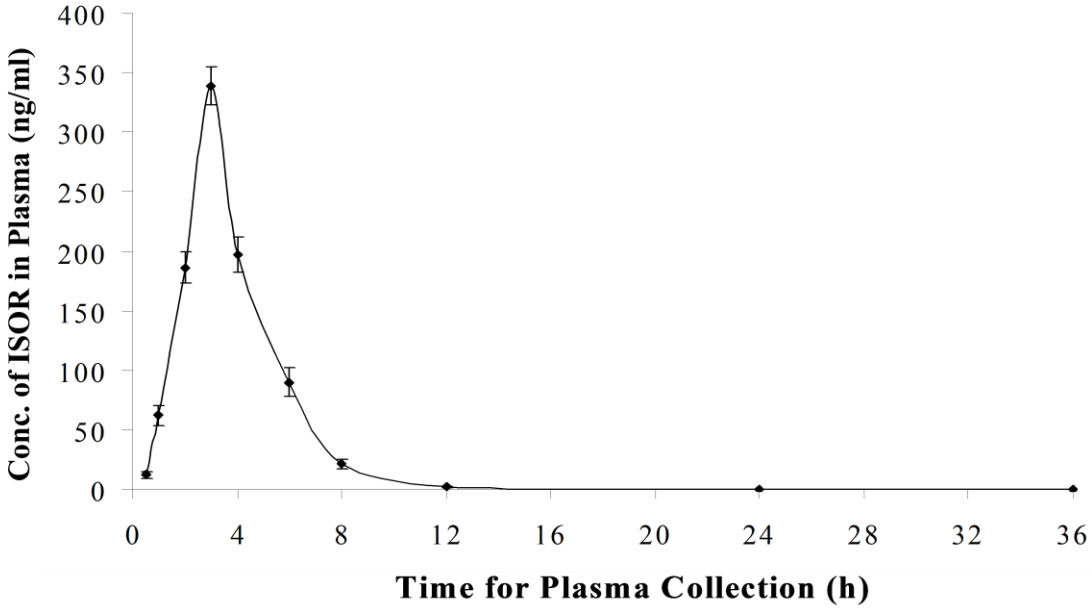


Fig. 1. Time course of isorhynchophylline (ISOR) in rat plasma after oral administration at 37.5 mg/kg,

ISOR concentrations in plasma were quantified by LC-MS (EIC) monitored at *m/z* 385 ± 0.5 in the positive ion mode ($[M+H]^+$). Data is shown as mean ± SD, n = 3. [Wang *et al*., *J. Pharmaceut. Sci*., **13**, 27-37 (2010)]

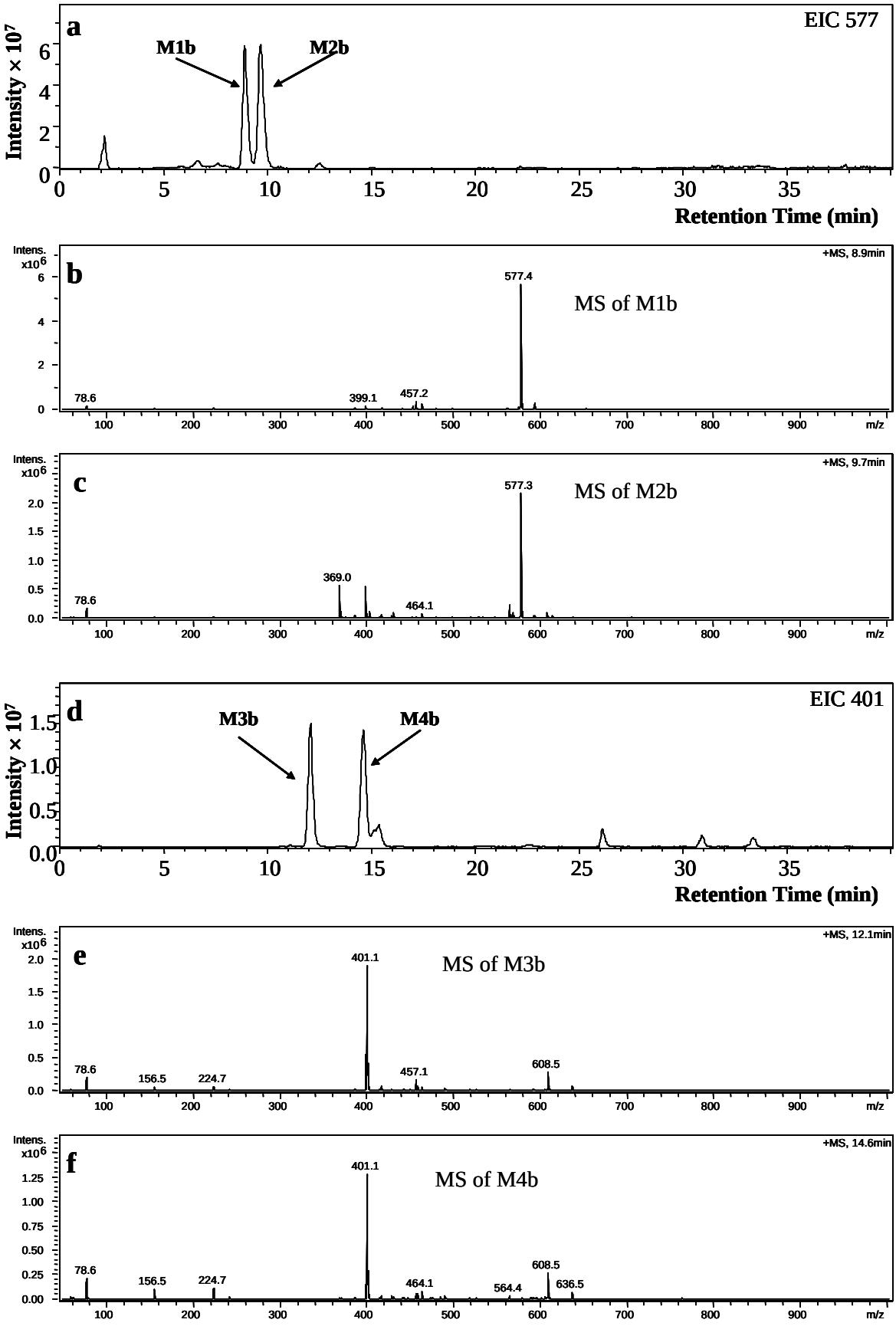

a
EIC 577
M1b
M2b
Intensity × 10^7
Retention Time (min)
b
+MS, 8.9min
577.4
MS of M1b
78.6
399.1
457.2
c
+MS, 9.7min
577.3
MS of M2b
369.0
78.6
464.1
d
EIC 401
M3b
M4b
Intensity × 10^7
Retention Time (min)
e
+MS, 12.1min
401.1
MS of M3b
78.6
156.5
224.7
457.1
608.5
f
+MS, 14.6min
401.1
MS of M4b
78.6
156.5
224.7
464.1
564.4
608.5
636.5
m/z

Fig. 2. LC-MS (extracted ion chromatogram: EIC) elution profiles monitored at *m/z* 577 ± 0.5 (**M1b** and **M2b**) of a bile sample collected 2 to 3 h after oral isorhynchophylline (ISOR) administration and at *m/z* 401 ± 0.5 (**M3b** and **M4b**) of that digested with *β*-glucuronidase. LC-MS: solvent B (0.01% v/v acetic acid in CH_3CN) from 10 to 25% in solvent A (0.01% v/v acetic acid) in 40 min, from 25 to 65% in 10 min, then to 100% in 10 min. a, EIC of **M1b** and **M2b** at *m/z* 577; b, MS of **M1b**; c, MS of **M2b**; d, EIC of **M3b** and **M4b** at *m/z* 401; e, MS of **M3b**; f, MS of **M4b**. [Wang *et al*., *J. Pharmaceut. Sci*., **13**, 27-37 (2010)]

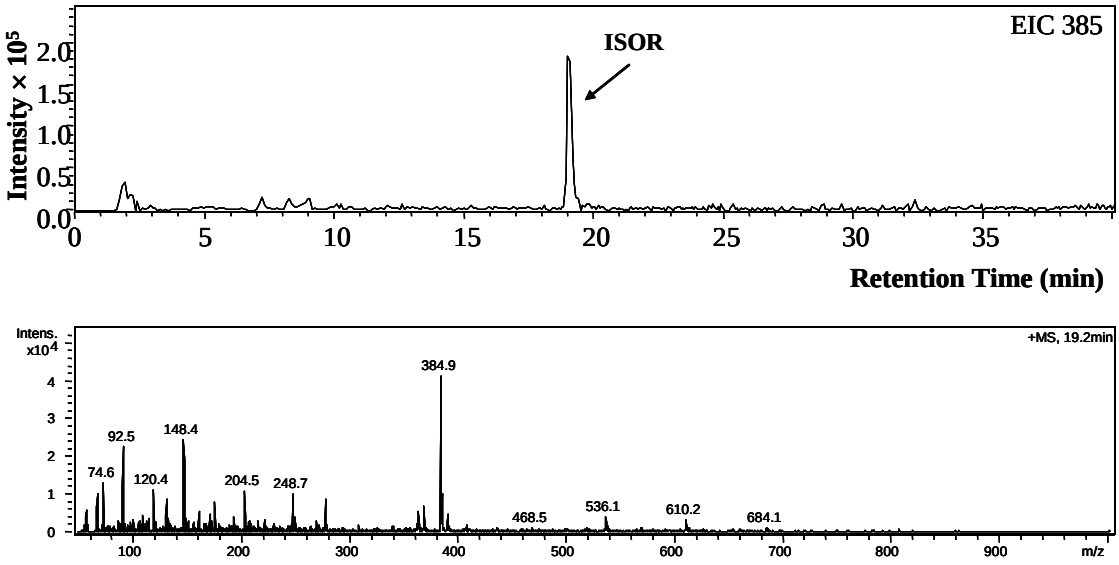


Fig. 3. LC-MS (EIC) elution profiles of rat brain sample (upper) collected at 3 h after oral isorhynchophylline (ISOR) administration at 37.5 mg/kg and MS spectrum (lower) monitored at *m/z* 385 ± 0.5 (ISOR). LC-MS: solvent B (0.01% v/v acetic acid in CH_3CN) from 10 to 30% in solvent A (0.01% v/v acetic acid) in 40 min, then to 100% in 20 min. [Wang *et al*., *J. Pharmaceut. Sci*., **13**, 27-37 (2010)]

Table 1. Main pharmacokinetic parameters for ISOR in rats

Parameter	Units	Intravenous administration	Oral administration
		15.0 mg/kg	37.5 mg/kg
k	min^{-1}	$(8.318 \pm 0.23)\times10^{-3}$	
$t_{1/2}$	min	83.31 ± 2.6	
V	l/kg	$(1.062 \pm 0.0092)\times10^{-1}$	
CL	$l\cdot kg^{-1}\cdot min^{-1}$	$(8.834 \pm 0.21)\times10^{-4}$	
t_{max}	min		180.0 ± 0.56
C_{max}	ng/ml		338.8 ± 1.9
$AUC_{0-\infty}$	$\mu g\cdot ml^{-1}\cdot min$	$(16.98 \pm 0.31)\times10^{3}$	177.4 ± 2.9

Data is shown as mean ± SD, n = 3. [Wang *et al*., *J. Pharmaceut. Sci*., **13**, 27-37 (2010)]

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

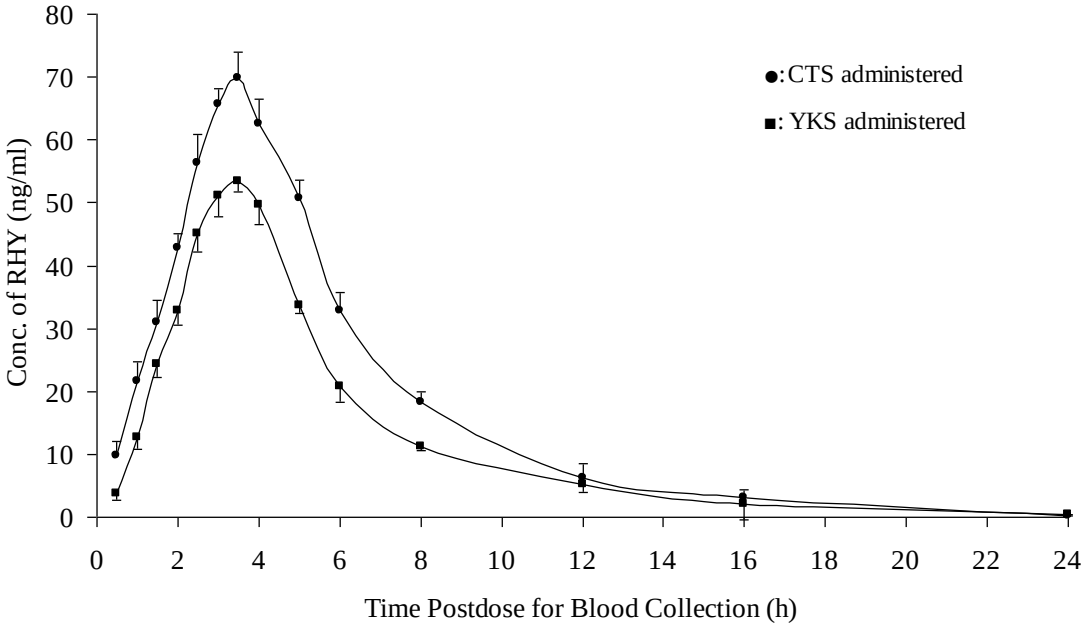


Fig. 4　Time course of the plasma concentration of rhynchophylline after oral administration of Chotosan (CTS, 釣藤散) and Yokukansan (YKS, 抑肝散)

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)]

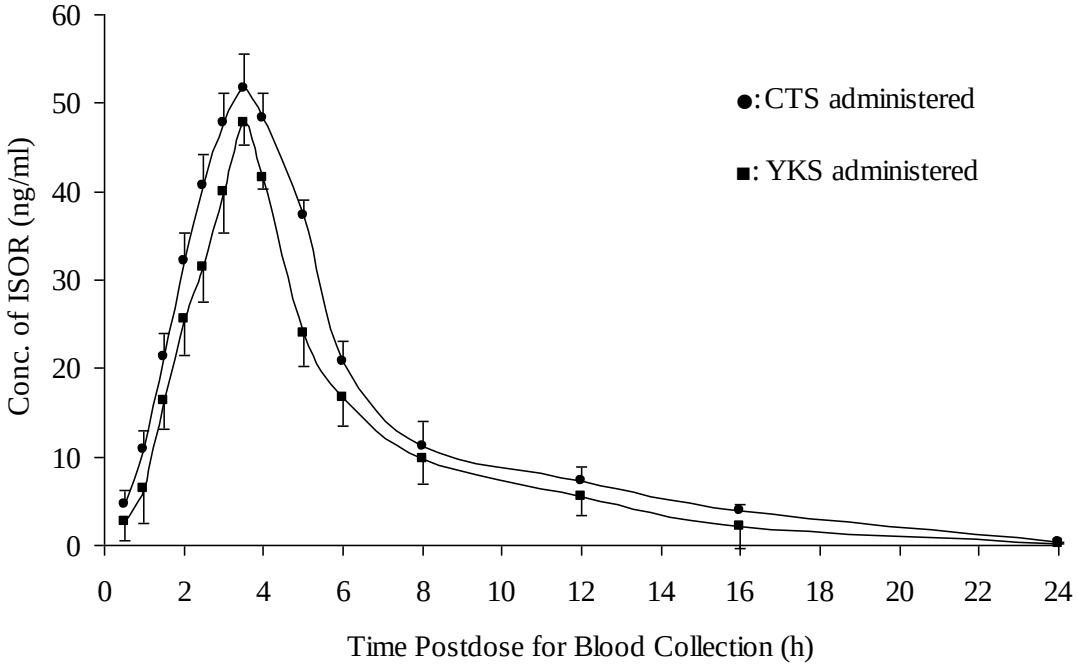


Fig. 5　Time course of the plasma concentration of isorhynchophylline after oral administration of Chotosan (CTS, 釣藤散) and Yokkansan (YKS, 抑肝散)

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)]

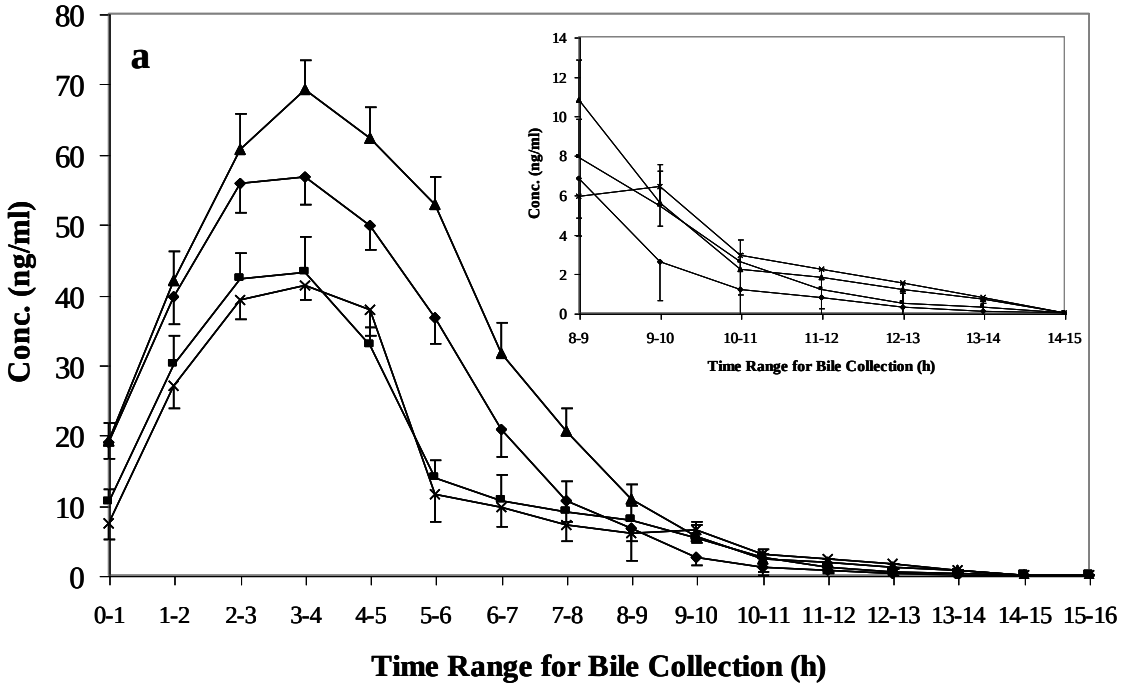


Fig. 6 The metabolites **M1a**, **M1b**, **M2a**, and **M2b** excreted into bile in 16 h after oral administration of Chotosan (CTS).

11-Hydroxyrhynchophylline 11-*O*-glucuronide (**M1a**, ■), 11-hydroxyisorhynchophylline 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)]

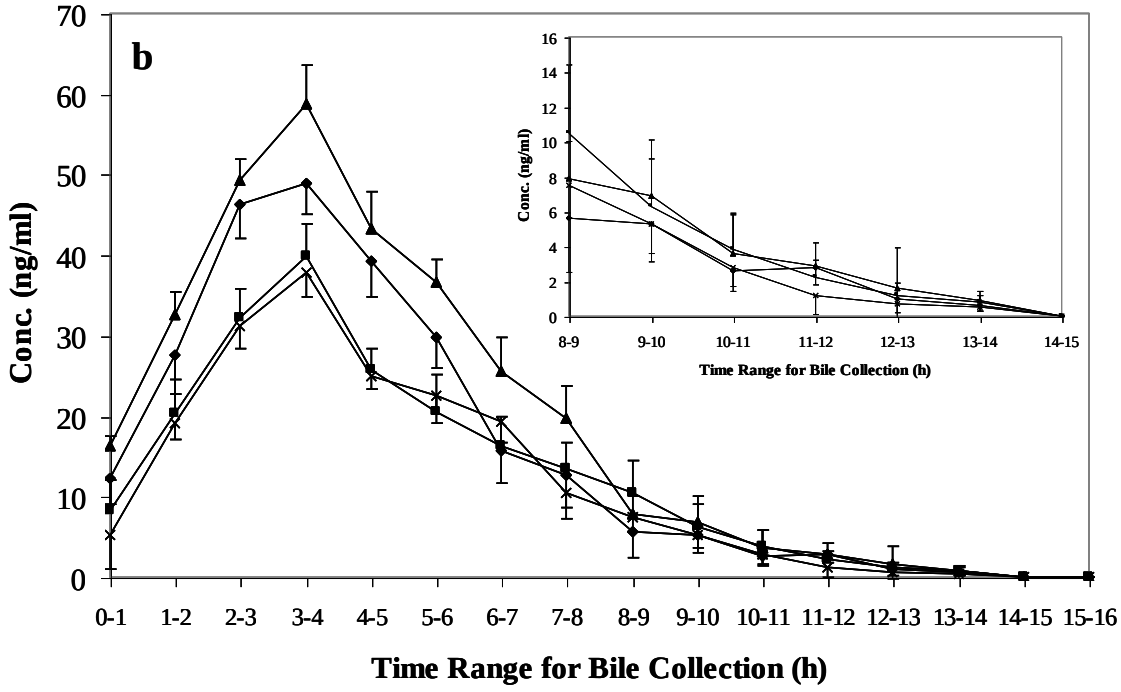


Fig. 7 The metabolites **M1a**, **M1b**, **M2a**, and **M2b** excreted into bile in 16 h after oral administration of Yokukansan (YKS).

11-Hydroxyrhynchophylline 11-*O*-glucuronide (**M1a**, ■), 11-hydroxyisorhynchophylline 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)]

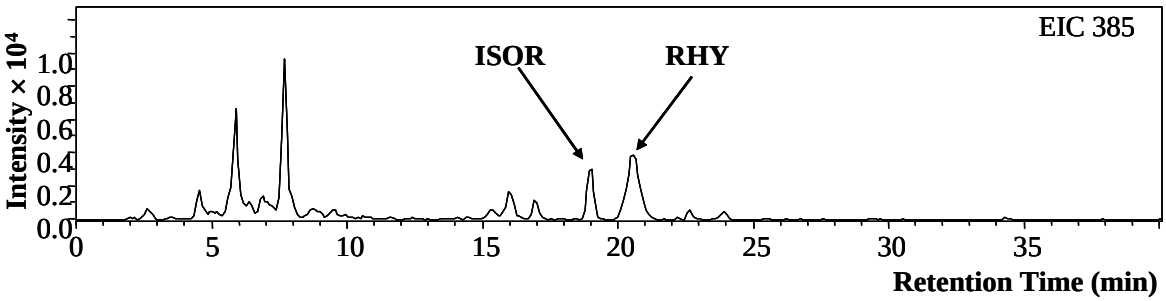


Fig. 8. LC-MS (EIC) elution profiles of a rat brain sample isolated at 3.5 h after oral administration of CTS, monitored at *m/z* 385 ± 0.5. [Wang *et al*., *J. Trad. Med*., **27**, 15-29 (2010)]

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 isorhynchophylline in rats detected by LC-MS. *J. Pharmaceut. Sci*., **13**, 27-37 (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).