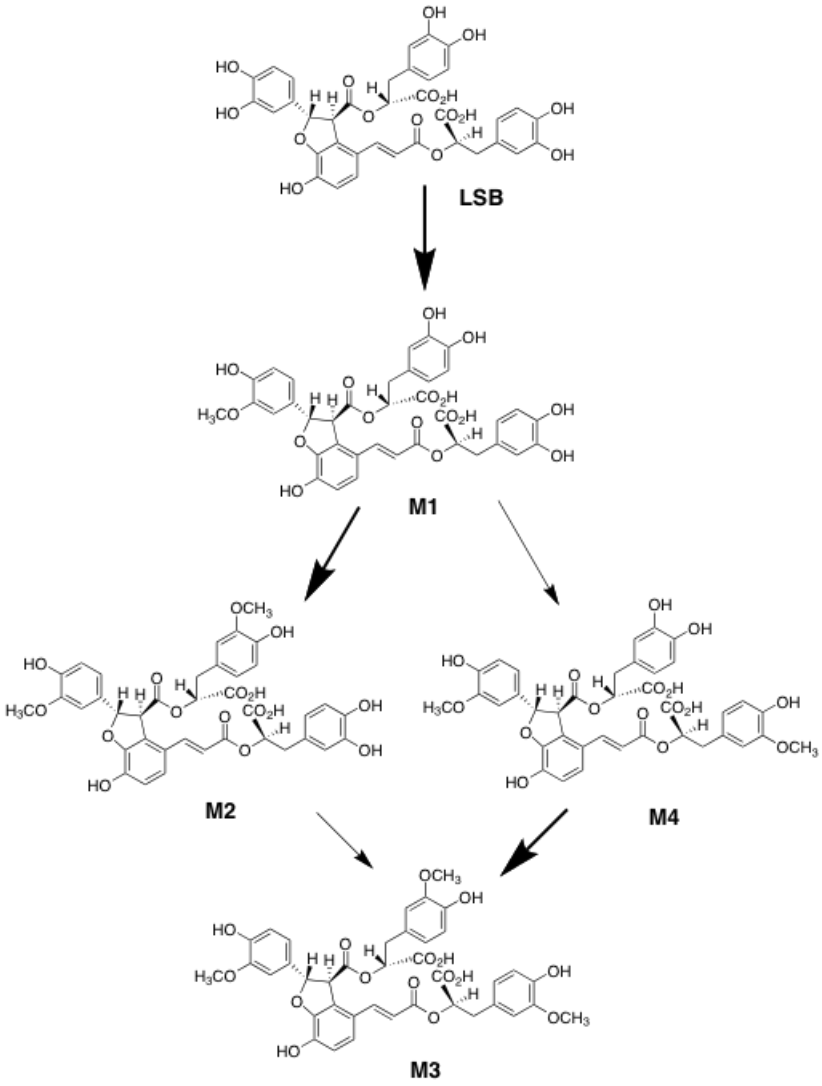
Lithospermic acid B



Metabolic processes of lithospermic acid B in rats

代謝実験

代謝動物　ラット

単一化合物　lithospermic acid B

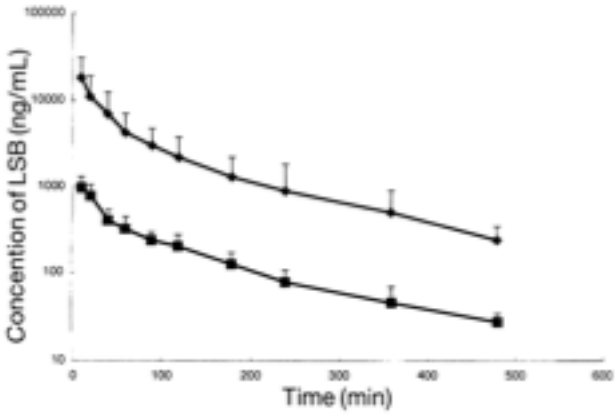


Fig. 1 Plasma concentration-time curves of lithospermic acid B (LSB) after intravenous administration of its magnesium salt (MLB) at doses of 4 (■) and 20 mg/kg (+) to rats.

Values are expressed as means ± SE (n = 5). [Zhang *et al*., *Planta Med*., **70**, 138-142 (2004)]

Blood sampling

Magnesium lithospermate B (MLB) dissolved in saline was injected in a tail vein at 4 or 20 mg/kg. The aqueous solution of MLB was orally given by gastric intubation at 20 or 100 mg/kg. Blood samples were collected from a tail vein (the vein opposite to the injected side in the case of intravenous injection) through heparinized microcapillaries at 5, 10, 20, 40 min, 1, 1.5, 2,3, 4, 6, 8, 10, and 24 h after administration, and centrifuged at 1000 g for 15 min to obtain plasma, which was stored at -30 °C until analysis. [Zhang *et al*., *Planta Med*., **70**, 138-142 (2004)]

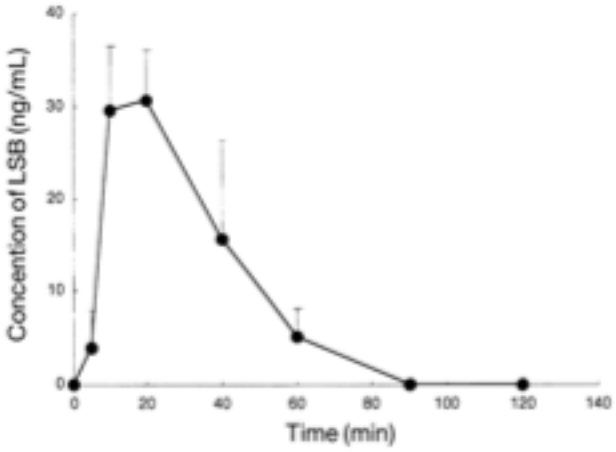


Fig. 2　Plasma concentration-time curve of lithospermic acid B (LSB) after oral administration of magnesium lithospermate (MLB) 100 mg/kg (●).

Values are expressed as means ± SE (n = 5). [Zhang *et al*., *Planta Med*., **70**, 138-142 (2004)]

Tab|e 1.　Pharmacokinetic parameters of lithospermic acid B (LSB) after intravenous administration of magnesium lithospermic acid B (MLB) to rats

Parameter	Dose	
	4 mg/kg	20 mg/kg
A μg/ml)	1.50 ± 0.25	19.5 ± 7.41
α (min^{-1})	0.063 ± 0.001	0.035 ± 0.007
$t_{1/2\alpha}$ (min)	12.3 ± 2.14	22.7 ± 4.29
B (μg/mL)	0.34 ± 0.05	2.74 ±1.41
β (min^{-1})	0.0076 ±0.0004	0.0062 ±0.0009
$t_{1/2\beta}$ (min)	128 ± 4.68	176 ± 30.4
AUC (μg･min/mL)	87.8 ± 10.9	1130 ± 329
Vss (L/kg)	7.60 ± 1.03	3.61 ± 1.16*
MRT (min)	137 ± 3.71	141 ± 23.4
CL_{tot} (mL/min/kg)	55.5 ± 7.1	23.5 ± 6.0*

MRT: mean residence time. Values are given as means ± SE of five rats. [Zhang *et al*., *Planta Med*., **70**, 138-142 (2004)]

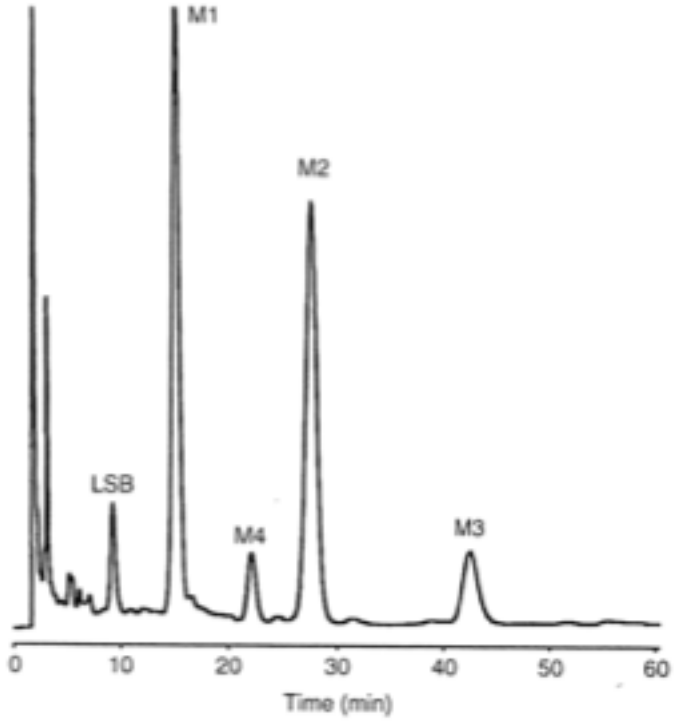


Fig. 3. HPLC chromatogram of bile after oral administration of magnesium lithospermate B (MLB).

Bile was collected from rats during 0 to 2 h after oral administration of magnesium lithospermate B (MLB) at a dose of 100 mg/kg. The identified metabolites are labeled Ml through M4 in the order of peak size, and the parent compound is labeled as LSB.

Bile and urine collection

Under light anesthesia with diethyl ether, bile fistulas in 3 or 4 male rats were cannulated with PE-5 polyethylene tubing for collection of bile. The bile was collected into successive vials on ice in 2 ml of 0.1 % H_3PO_4 at 2-h intervals for 6 h after dosing and in 4 ml of 0.1 % H_3PO_4 at 4-h intervals for up to 30 h thereafter. Urine was also drawn into a cooled vial over 30 h through a silicone tube (4 mm, i.d.) fixed to the penis.

The rats were allowed to recover from anesthesia before receiving a 4-mg/kg intravenous dose of MLB in saline or a 100 mg/kg oral dose of MLB in distilled water. During the period of bile and urine collection, rats were kept in restraining cages with free access to water. All bile and urine samples were stored at -30°C until analysis. [Zhang *et al*., *Drug Met. Disp*. **32**, 752-757 (2004)]

Isolation of biliary metabolites

Bile from 16 rats with biliary fistulization for 10 h after bolus intravenous injection at a dose of 25 mg/kg was combined, diluted with water to 200 ml, and applied to a Diaion HP-20 (porous polymer resin, aromatic type absorbents based on crosslinked polystyrenic matrix) column. After washing with 800 ml of water, the column was eluted with MeOH–H_2O (700 ml with 1:4 and 1000 ml with 3:2, respectively). Further repeated column chromatography using Wakogel 100C18, which was eluted with 0.1% TFA–MeOH, afforded Ml (80 mg), M2 (40 mg), M3 (11 mg), and M4 (2 mg) as free acid forms. The purity of each metabolite was 97, 98, 90, and 90%, respectively, based on HPLC analyses. [Zhang *et al*., *Drug Met. Disp*. **32**, 752-757 (2004)]

M1

A pale yellow powder, $[\alpha]_D^{25}$ +62.5° (*c* = 0.2, MeOH). UV (MeOH) λ_{max} (log ε): 287.5 (4.16), 309 (4.11) nm. IR (KBr) ν_{max}: 3421, 1720, 1612, 1529, 1469, 1265, 1180 cm^{-1}. FAB-MS (positive ion mode) *m/z*: 755 $[M+Na]^+$, 733 $[M+H]^+$. ^{1}HNMR and ^{13}C NMR: see reference [Zhang *et al*., *Drug Met. Disp*. **32**, 752-757 (2004)]

M2

A pale yellow powder, $[\alpha]_D^{25}$ +68.3° (*c* = 0.1, MeOH). UV (MeOH) λ_{max} (log ε): 287 (4.15), 309.5 (4.10) nm. IR (KBr) ν_{max}: 3421, 1720, 1608, 1516, 1469, 1273, 1173, 1030 cm^{-1}. FAB-MS (positive ion mode) *m/z*: 769 $[M+Na]^+$, 747 $[M+H]^+$. ^{1}HNMR and ^{13}C NMR: see reference [Zhang *et al*., *Drug Met. Disp*. **32**, 752-757 (2004)]

M3. A pale yellow powder, $[\alpha]_D^{26}$ +73.0° (*c* = 0.1, MeOH). UV (MeOH) λ_{max} (log ε: 287-5 (4.09), 309 (4.15) nm. IR (KBr) ν_{max}: 3444, 1720, 1628, 1516, 1469, 1273, 1180, 1034 cm^{-1}. FAB-MS (positive ion mode) *m/z*: 783 $[M+Na]^+$.

^{1}HNMR and ^{13}C NMR: see reference [Zhang *et al*., *Drug Met. Disp*. **32**, 752-757 (2004)]

M4. A pale yellow powder, $[\alpha]_D^{26}$ +62.5° (*c* = 0.05, MeOH). UV (MeOH) λ_{max} (log ε): 287.5 (3.89), 309 (3.97) nm. IR (KBr) ν_{max} 3425, 1720, 1608, 1520, 1469, 1273, 1034 cm^{-1}. FAB-MS (positive ion mode) *m/z*: 769 $[M+Na]^+$. ^{1}HNMR: see reference. [Zhang *et al*., *Drug Met. Disp*. **32**, 752-757 (2004)]

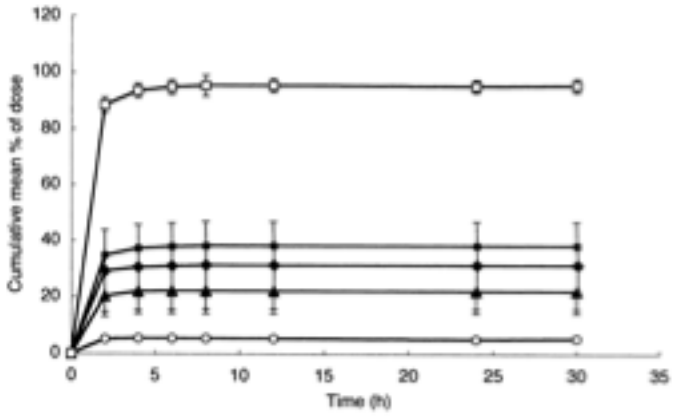


Fig. 4. Cumulative biliary excretion of metabolites as a function of time after intravenous injection of magnesium lithospermate (MLB) (4 mg/kg).

Each point represents the mean ± S.E. for four rats. (◆), M1; (■), M2; (▲), M3; (○), M4; (□), total metabolites. [Zhang *et al*., *Drug Met. Disp*. **32**, 752-757 (2004)]

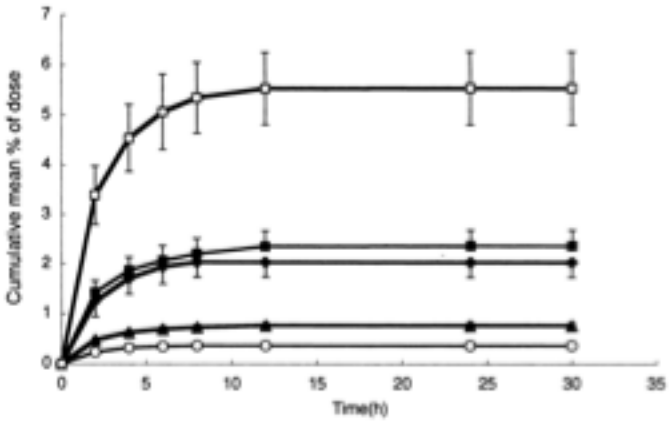


Fig. 5. Cumulative biliary excretion of metabolites as a function of time after oral administration of magnesium lithospermate (MLB) (100 mg/kg).

Each point represents the mean ± S.E. for three rats. (◆), M1; (■), M2; (▲), M3; (○), M4; (□), total metabolites. [Zhang *et al*., *Drug Met. Disp*. **32**, 752-757 (2004)]

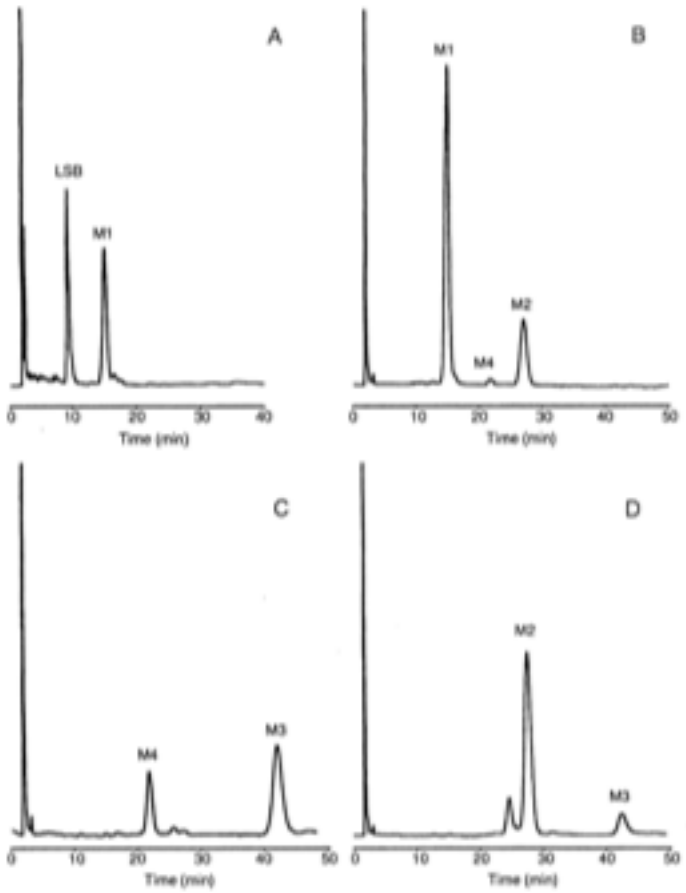


Fig. 6. HPLC chromatogram of rat cytosol incubation products.

MLB (A), Ml (B), M4 (C), and M2 (D) at 0.25 mM were respectively added as substrates to theincubation mixtures containing rat cytosol and *S*-adenosyl-L-methionine. After incubation for 10 min, each methanol extract of the reaction mixtures was subjected to HPLC. [Zhang *et al*., *Drug Met. Disp*. **32**, 752-757 (2004)]

参考文献

1) Zhang Y., Akao T., Nakamura N., Duan C. L., Hattori M., Yang X. W., and Liu J. X.: Extremly low bioavailability of magnesium lithospermate B, an active component from *Salvia miltiorrhiza*, in rat. *Planta Med*., **70**, 138-142 (2004).

2) Zhang Y., Akao T., Nakamura N., Hattori M., Yang X. W., Duan C. L., and Liu J. X.: Magnesium lithospermate B is excreted rapidly into rat bile mostly as methylated metabolites, which are potent antioxidants. *Drug Met. Disp*. **32**, 752-757 (2004).