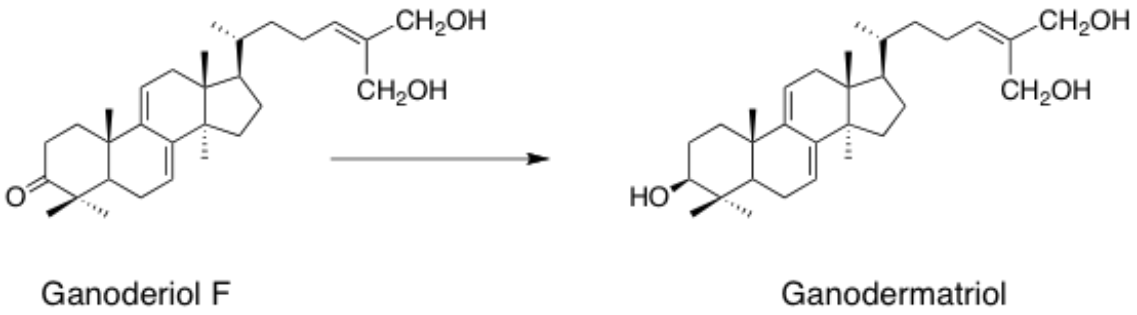
Ganoderiol F



Metabolism of ganoderiol F by human intestinal microflora

代謝実験
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Fresh feces (5 g each) from rats were suspended in 100 ml of GAM broth, and used for bacterial transformation of ganoderiol F (GF) *in vitro*; GF (2 mg) dissolved in 40 ml of MeOH and the above bacterial mixture (500 μl) were added to GAM broth (5 ml) and incubated at 37°C for 7 d under anaerobic conditions. Portions (200 μl) were removed at intervals and mixed with MeOH (400 μl). The mixture was centrifuged at 604 ×g for 15 min, and the upper layer was passed through a liquid chromatography disc followed by centrifugation at 6700 ×g for 5 min. Portions of supernatants were analyzed by HPLC/MS/MS.

Ganodermatriol

Retention time (t_R) = 6.5 min in the HPLC/MS/MS chromatogram. ^{1}H-NMR ($CDCl_3$) δ: 0.56 (3H, s, 18-H_3), 0.88 (6H, s, 29-H_3 and 30-H_3), 0.91 (3H, d, J = 6.2 Hz, 21-H_3), 0.98 (3H, s, 28-H_3), 1.01 (3H, s, 19-H_3), 3.24 (1H, dd, J = 4.8 and 10.6 Hz, 3-Ha), 4.21 (2H,

s, 26-H_2), 4.33 (2H, s, 27-H_2), 5.31 (1H, br d, J = 6.2 Hz, 11-H), 5.48 (1H, m, 7-H), 5.56 (1H, t, J = 7.5 Hz, 24-H). ^{13}C-NMR ($CDCl_3$) δ: 16.0, 16.6, 18.6, 23.0, 23.6, 24.7, 25.9, 28.1, 28.9, 28.9, 31.9, 36.4, 36.4, 36.9, 37.9, 38.1, 39.4, 49.8, 50.7, 51.2, 58.5, 65.5, 78.1, 116.5, 120.0, 127.7, 140.8, 143.0, 146.0.

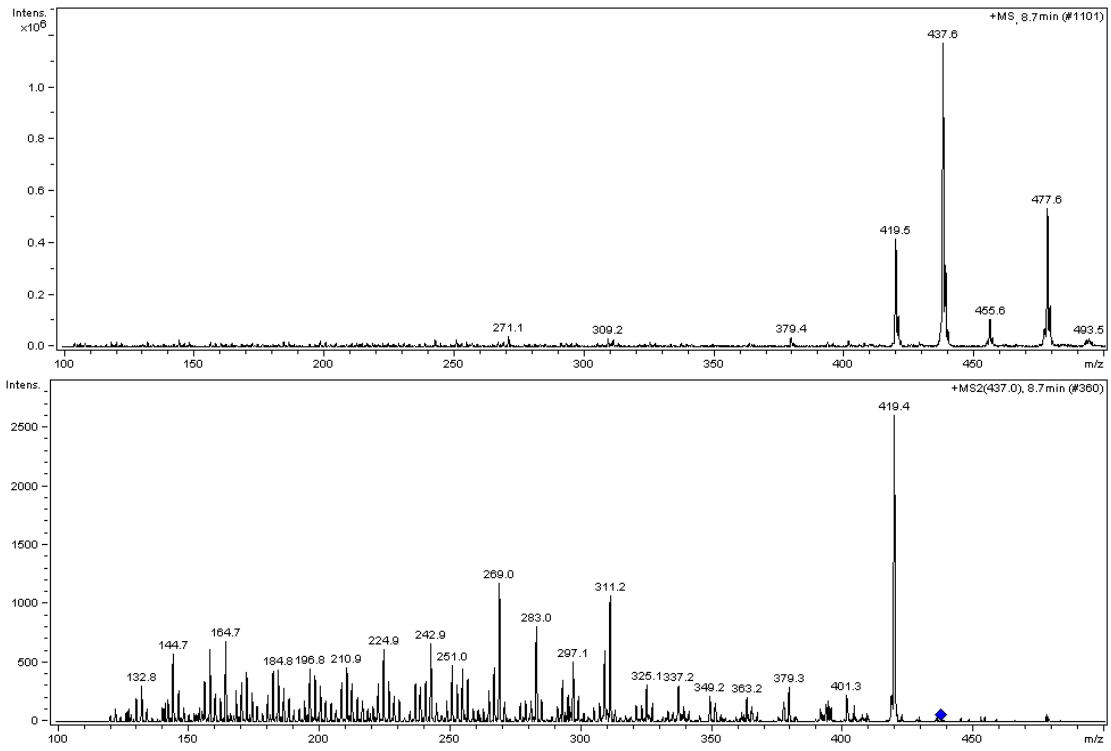

Fig. 1 MS and MS/MS spectra of ganoderiol F

Liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) data were acquired using Esquire Control software and analyzed using software from Bruker Daltonik. Samples were applied to a column of TSK gel ODS-80 Ts (particle size, 5 μm; 4.6 9 150 mm; Tosoh Co., Tokyo, Japan) attached to an HPLC/ESI ion trap mass spectrometer (ESI-Ion Trap MS), and the column was eluted with a gradient of 0.5% (v/v) AcOH in water and CH_3CN (20–80%, v/v) at a flow rate of 1.0 ml/min at 30°C. The standard positive ion mode was selected and the following conditions were used: second MS scan, *m/z* 437; scan resolution, 13,000 *m/z* per scan; nebulizer pressure, 70.0 psi; dry gas, 12 l/min; dry temperature, 365°C.

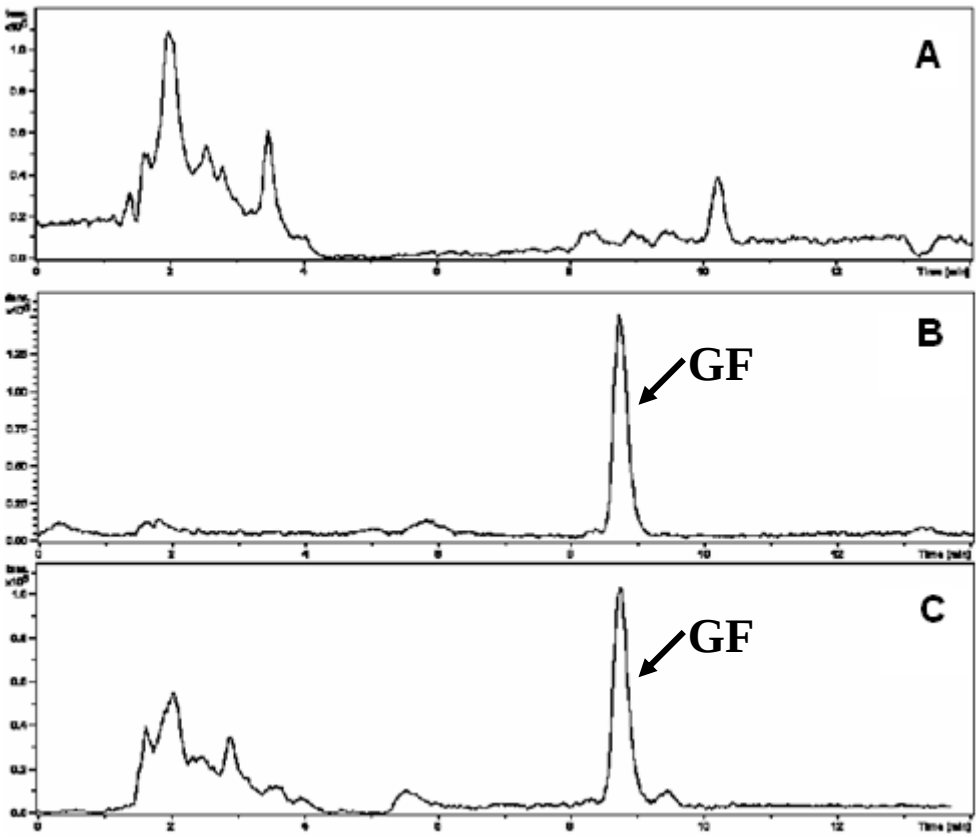

Fig. 2 MS/MS chromatograms of ganoderiol F in plasma

A, blank plasma; B, GF; C, plasma sample after oral administration of ganoderiol F (GF).

GF (25 mg/ml in DMSO) was administered orally at doses of 20 and 50 mg/kg to rats that were deprived of food, but given free access to water for 18 h. GF (2.5 mg/ml) was administered to rats *via* the femoral vein at a dose of 0.5 mg/kg for i.v. experiments. The rats were killed and whole blood samples were collected 0.5, 1, 2, 4, 8, 12, 24, 36, and 48 h after oral administration and at 5, 10, 15, 30, 45, 60, 120, 240, and 480 min after i.v. administration. The plasma was separated by centrifugation at 6049g for 15 min at 4°C and stored at -20°C. Plasma samples were thawed at room temperature and slowly applied to a solid-phase extraction cartridge (Waters Co., Milford, MA, USA) that had been washed with MeOH (3 ml) and equilibrated with water (6 ml). After sample absorption, the cartridge was washed with water (4 ml) and then GF was eluted with MeOH (3 ml). The eluate was evaporated under a stream of nitrogen at 35°C to yield a residue, which was dissolved in MeOH (100 μl). After centrifugation at 6,7009g

for 5 min, a 20-ll portion of the supernatant was analyzed by LC/MS/MS.

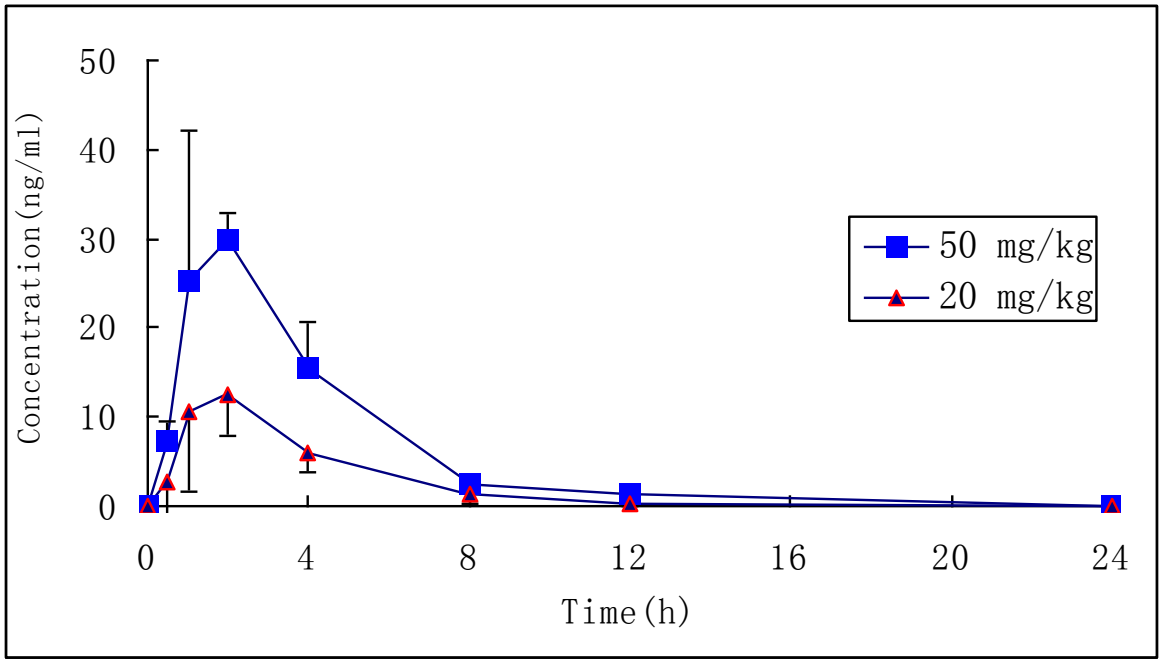

Fig. 3 Mean plasma concentration-time curves of GF (n=4) after oral administration at dose of 20 and 50 mg/kg in rats

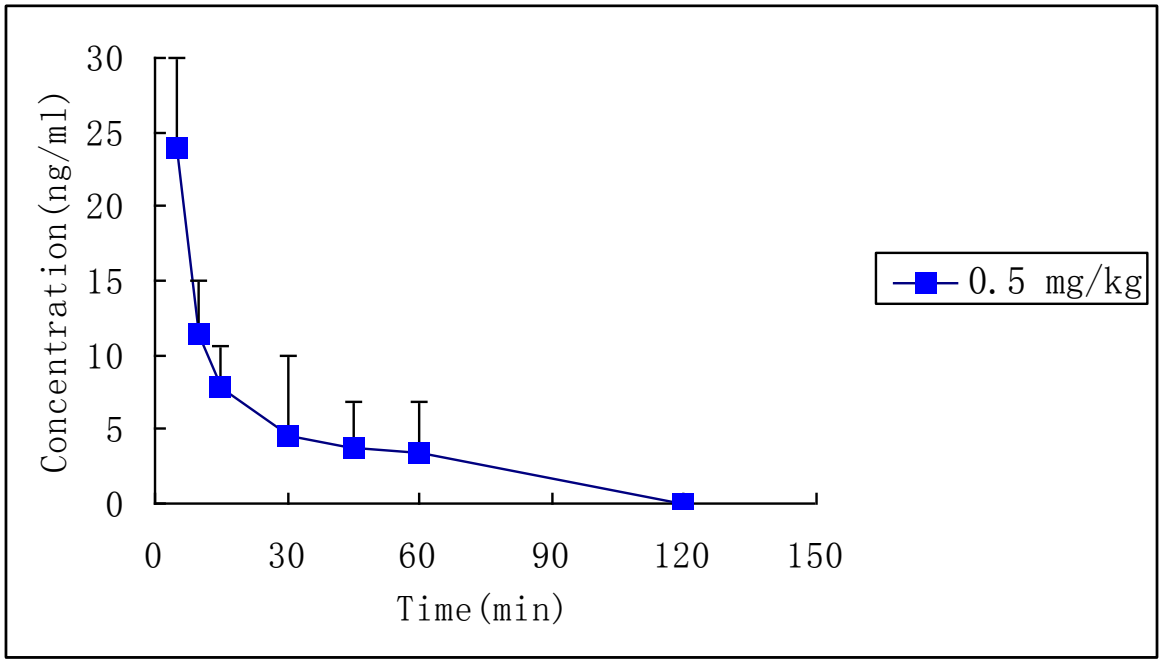

Fig. 4 Mean plasma concentration-time curves of GF (n=4) after intravenous administration at dose of 0.5 mg/kg in rats

Table 1. Pharmacokinetic parameters of ganoderiol F after intravenous and oral administration in rats (n=4).

Pharmacokinetic parameters	**Units**	**Values**		
		0.5 mg/kg (i.v.)	**20 mg/kg (p.o.)**	**50 mg/kg (p.o.)**
$T_{1/2\alpha}$	**h**	**0.04±0.0036**	**0.24±0.02**	**0.31±0.027**
$T_{1/2\beta}$	**h**	**0.58±0.187**	**2.39±0.569**	**1.91±0.535**
T_{max}	**h**		**0.88±0.025**	**0.96±0.03**
C_{max}	**ng/ml**		**11.1±4.12**	**28.5±3.48**
$AUC_{0\text{-}lim}$	**(ng/ml)h**	**11.17±3.02**	**49.4±7.92**	**111.6±5.905**
MRT	**h**	**0.44±0.073**	**3.41±0.669**	**3.19±0.483**

参考論文

1) Zhang　Q., Zuo F. Nakamura N., Ma C. M., and Hattori M.: Metabolism and pharmacokinetics in rats of ganoderiol F, a highly cytotoxic and antitumor triterpene from *Ganoderma lucidum*. *J. Nat. Med*., **63**, 304–310 (2009).