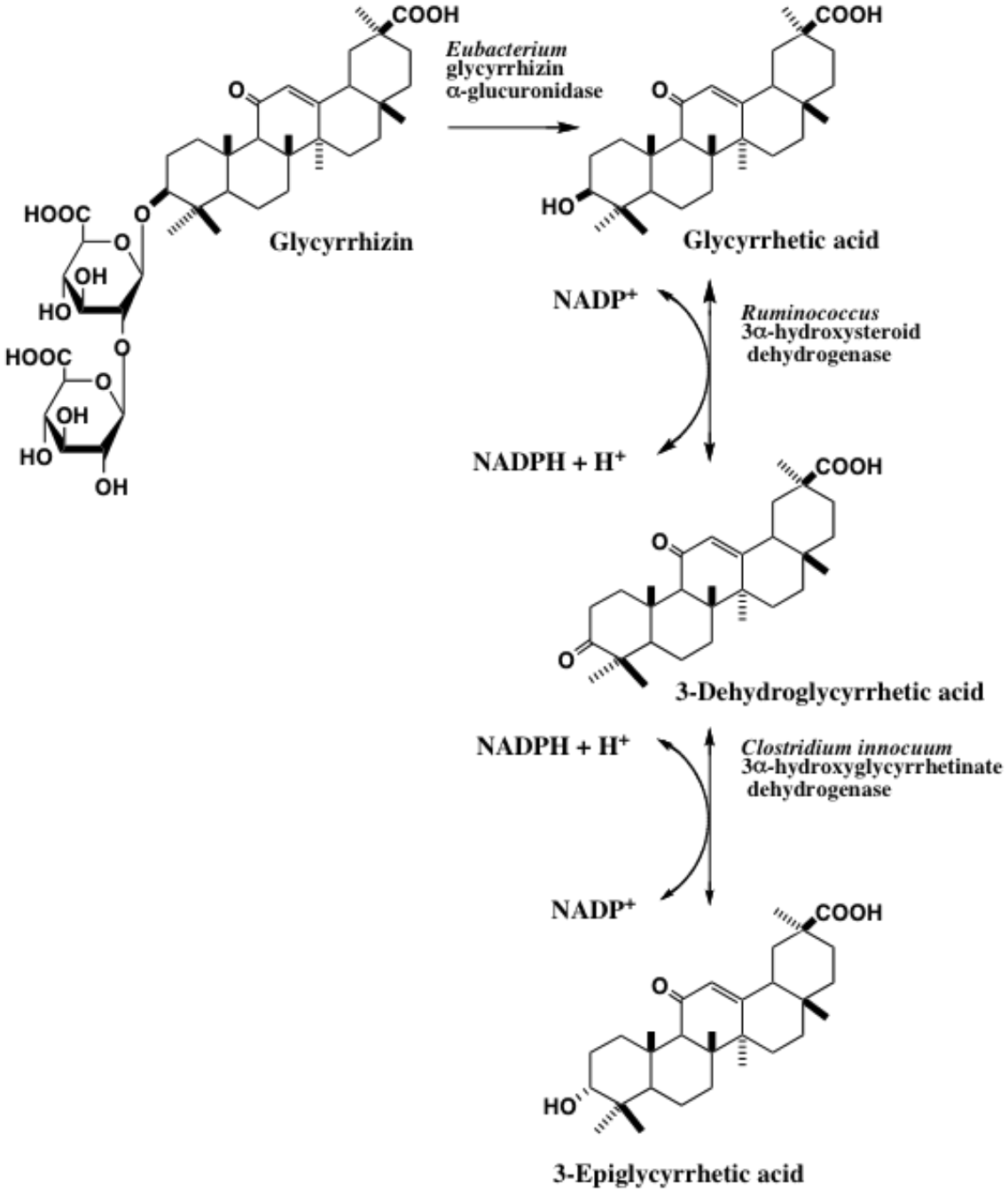
Glycyrrhizin



Metabolic processes of glycyrrhizin by human intestinal bacteria

代謝実験

腸内細菌代謝　ヒト腸内細菌フローラ、ヒト腸内細菌分離株 *Eubacterium, Clostridium innocum*

単一化合物　glycyrrhizin

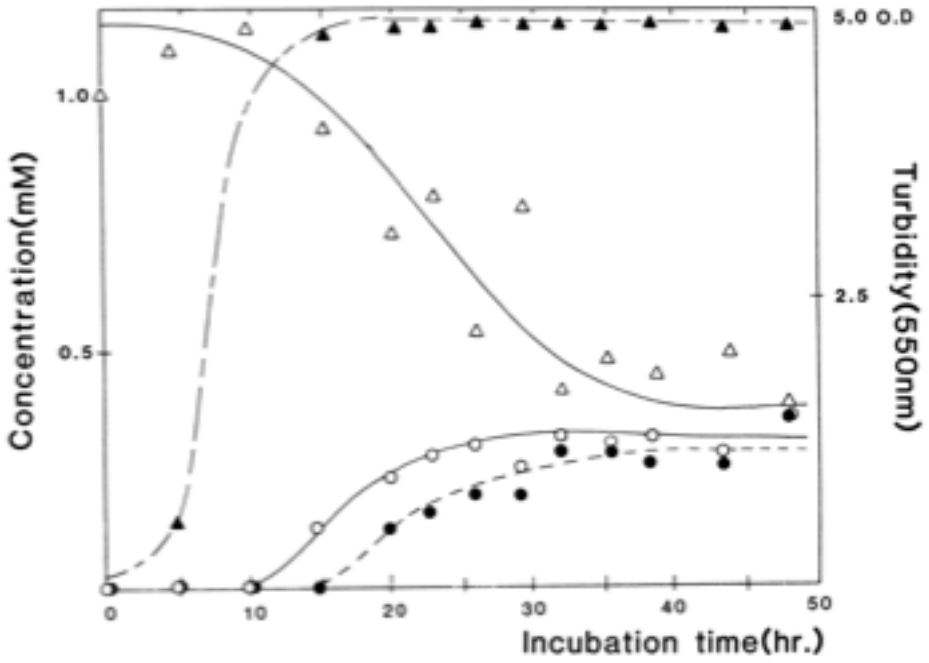


Fig. 1.　Metabolic time course of glycyrrhizin by human intestinal flora.

Tubes containing monoammonium glycyrrhizinate (1 mg), an intestinal bacterial mixture (0.1 ml) and GAM broth (0.9 ml) were incubated at 37° C in an anaerobic jar. The mixture was acidified and extracted with AcOEt (2 ml). Aliquots of the upper phase were chromatographed on TLC-plates in solvent systems A and B, and quantitatively analyzed by TLC-densitometry. △, glycyrrhizin; ○, 18-β-glycyrrhetic acid; ●, 3-epi-18β-glycyrrhetic acid; ▲, turbidity of the incubation mixture representing bacterial growth. [Hattori *et al*., *Planta Med.*, **48**, 38-42 (1983)]

Incubation of glycyrrhizin with an intestinal bacterial mixture

An intestinal bacterial mixture (40 ml) and GAM broth (310 ml) were mixed thoroughly and incubated at 37° C for 24 hr in an anaerobic jar, in which air had been replaced with CO_2 gas in the presence of activated steel wool. Glycyrrhizin (mono ammonium, 400 mg) was anaerobically incubated at 37°C for 24 hr in a similar fashion. The mixture were adjusted to pH *ca*. 1 with HC1, and extracted six times with $CHCl_3$ (200 ml each).

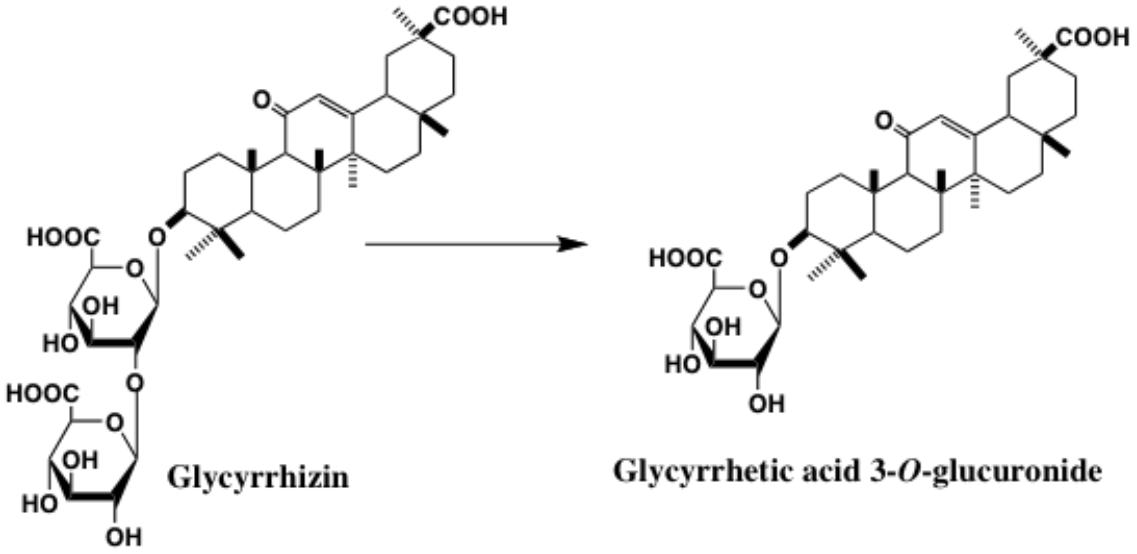
The $CHCl_3$ phase was washed with water, then concentrated to *ca*. 20 ml *in vacuo*. The mixture was applied to a column of silica gel (2.4 x 44 cm). The column was washed with $CHCl_3$, and eluted with $CHCl_3$–MeOH (100:1). Fractions of 3.6 ml/tube were collected. Fr. 102-112 (metabolite I) and fr. 125-136 (metabolite II) were separately pooled and evaporated to dryness *in vacuo*. The metabolites I (*ca*. 150 mg) and II (*ca*. 43 mg) were then purified by repeated crystallization from $CHCl_3$-petroleum ether. [Hattori *et al*., *Planta Med.*, **48**, 38-42 (1983)]

Metabolite I (18β-glycyrrhetic acid)

Colorless prisms, yield 45 mg, mp. 294-296° C, $C_{30}H_{46}O_4$ (Anal. Calcd.: C, 76.55; H, 9.85, Found: C, 76.48; H, 9. 93), UV λ_{max} (EtOH): 250 nm, IR (KBr) 3430 (OH), 1700 C=O), 1661 (conjugated C=O), MS: *m/z* 470 (M^+, 10 %), 303 (93 %), 262 (82 %), 216 (17 %), 175 (52%), 135 (100%), $[\alpha]_D^{25}$= + 153.8 (*c*, 1.3 in $CHCl_3$–MeOH (19:1), NMR (DMSO-d_6): 0.70, 0.77, 0.92, 1.04, 1.05, 1.11, 1.37 (each 3H, each s, C-CH_3), 3.04 (1H, m, CH-OH), 5.44 (1H, s, C =CH) ppm. [Hattori *et al*., *Planta Med.*, **48**, 38-42 (1983)]

Metabolite II (3-epi-18β-glycyrrhetic acid)

Yield 10 mg, colorless prisms, mp > 300°C, $C_{30}H_{46}O_4$ (Anal. Calcd: C, 76.55; H, 9.85, Found: C, 76.77; H, 9.83), UV λ_{max} (EtOH): 250 nm, ε = 13600, IR (KBr): 3500 (OH), 1717 (C = O), 1641 (conjugated C = O), 1615 (conjugated C = C) cm^{-1}, MS: *m/z* 470 (M^+, 25 %), 303 (96 %), 262 (84 %), 216 (16%), 175 (76%), 135 (100%), $[\alpha]_D^{24}$ = +146.9 *(c*, 1.3 in $CHCl_3$–MeOH (19:1), NMR (DMSO-d_6): 0.80 (6H, s, 2 x C-CH_3), 0.88 (3H, s, C-CH_3), 1.07 (6H, s, 2 x C-CH_3), 1.14, 1.40 (each 3H, each s, C-CH_3), 3.21 (1H, b. s, CH-OH), 5.46 (1H, s, C = CH) ppm. [Hattori *et al*., *Planta Med.*, **48**, 38-42 (1983)]



Conversion of glycyrrhizin to glycyrrhetic acid 3-*O*-glucuronide by animal livers

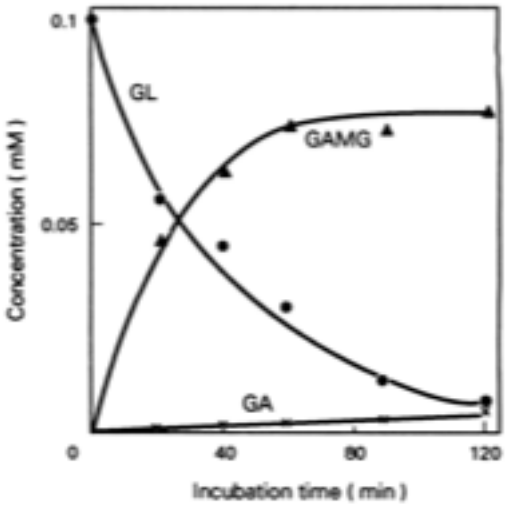


Fig. 2. Time course of glycyrrhizin-hydrolysis with rat liver lysosomes.

The supernatant (0.57 mg of protein) of the sonicated lysosomes was incubated with 0.1 mM glycyrrhizin in 0.5 mL of 0.1 M acetate buffer (pH 5.6). Metabolites were analysed at the indicated period of time. [Akao *et al.*, *Biochem. Pharmacol.*, **40**, 291-296 (1990)]

Animals and livers.

Wistar strains of male and female rats at 4-14 weeks of age and ddy strains of

male mice at 4-6 weeks of age were used. One bovine and three porcine livers were purchased from Nippon Ham Co. (Osaka, Japan). Human liver tissues were obtained from non-involved part of the resected specimen of the metastatic liver tumors. [Akao *et al.*, *Biochem. Pharmacol*., **40**, 291-296 (1990)]

Preparation of subcellular fractions and lysosomes

Fresh livers obtained from rat, mouse and human were used for preparation without storage, and livers from cattle and porcine after storage at -20° for a few months. Liver homogenates in 0.25 M sucrose were separated into nuclear, mitochondrial, lysosomal, microsomal and soluble fractions. Hepatic lysosomes were prepared by centrifuging liver homogenates in 0.15 MKC1 at 5000 g for 10 min and then the supernatant at 10,000g for 20 min. Lysosomes suspended in 50 mM potassium phosphate buffer (pH 7.0) were sonicated and then centrifuged at 100,000 g for 90 min to obtain a clear supernatant. [Akao *et al.*, *Biochem. Pharmacol*., **40**, 291-296 (1990)]

Isolation of glycyrrhetic acid 3-*O*-glucuronide produced from glycyrrhizin by rat liver lysosomes.

The supernatant (230 mg of protein) of the sonicated lysosomes from four male rats (14 weeks old) was incubated with 26 μmol of glycyrrhizin in 100 mL of 0.1 M acetate buffer (pH 5.6). After incubation at 37° for 1 hr, the reaction was stopped by the addition of 1 M HC1. It was extracted twice with an equal volume of ethyl acetate. After evaporating
the ethyl acetate phase, glycyrrhetic acid 3-*O*-glucuronide was isolated as powder by preparative TLC, though glycyrrhizin was also detected on the plate. [Akao *et al.*, *Biochem. Pharmacol*., **40**, 291-296 (1990)]

Glycyrrhetic acid 3-*O*-glucuronide.

FABMS *m/z* (negative ion): 691(M + 2Na – 1)$^-$, 668(M + Na – 1)$^-$, 651(668 – OH)$^-$, 623(668 – COOH)$^-$, 469(aglycone – l)$^-$, 451(469 – H_2O)$^-$. ^{1}HNMR (270 MHz, CD_3OD): 60.82, 0.87, 1.08, 1.12, 1.14, 1.15, 1.43 (each 3H, s, Me), 4.37(1H, d, J = 7.3 Hz, H-l'), 5.67(1H, s, H-12). ^{13}CNMR (100 MHz, pyridine-d_5): aglycone moiety, δ 39.6(C-1), 26.9(C-2), 88.8(C-3), 40.0(C-4), 55.4(C-5), 17.7(C-6), 33.0(C-7), 43.5(C-8), 62.2(C-9), 37.3(C-10), 199.3(C-11), 128.7(C-12), 169.5(C-13), 45.6(C-14), 26.6(C-15),

26.7(C-16), 32.2(C-17), 48.7(C-18), 41.8(C-19), 44.1(C-20), 31.7(C-21), 38.5(C-22), 28.3(C-23), 16.8(C-24), 17.0(C-25), 18.9(C-26), 23.7(C-27), 28.76(C-28), 28.81(C-29), 179.1(C-30); sugar moiety, 107.0(C-1'), 78.2(C-2'), 75.5(C-3'), 73.5(C-4'), 73.4(C-5'), 150.4(C-6'). [Akao *et al.*, *Biochem. Pharmacol*., **40**, 291-296 (1990)]

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