Glycyrrhetic acid

Metabolic processes of glycyrrhetic acid by human intestinal bacteria

代謝実験
腸内細菌代謝 ヒト腸内細菌フローラ、ヒト腸内細菌株 Clostridium innocuum ES24-06, Ruminococcus sp. POI-3
単一化合物 glycyrrhetic acid

Fig. 1. Metabolic time course of 18β-glycyrrhetic acid by human intestinal flora

Tubes containing 18β-glycyrrhetic acid (1 mg), an intestinal bacterial mixture (1 ml) and GAM broth (9 ml) were incubated at 37° C in an anaerobic jar. The metabolites
were analyzed by TLC-densitometry. ○, 18β-glycyrrhetic acid; ●, 3-epi-18β-glycyrrhetic acid. [Hattori et al., Planta Med., 48, 38-42 (1983)]

**Preparation of a human intestinal bacterial mixture**

Fresh faeces were immediately transferred into a vinyl bag filled with CO₂ gas. The bag was then pressed by hand to uniformly mix the contents. The faeces thus obtained was suspended in five volumes of GAM broth or PGPY broth, then centrifuged at 16 x g for 1 min to eliminate the residue. The upper phase was used in all experiments as an intestinal bacterial mixture. [Hattori et al., Planta Med., 48, 38-42 (1983)]

**Incubation of 18β-glycyrrhetic acid with an intestinal bacterial mixture**

18β-Glycyrrhetic acid (272 mg) dissolved in EtOH (14 ml) and an intestinal bacterial mixture (200 ml) were added to GAM broth (4000 ml), which were then anaerobically incubated at 37°C for 48 hr. The culture medium was adjusted to pH 1 and extracted 4 times with AcOEt (2000 ml each). The AcOEt phase was washed with 2% NaCl and concentrated to a small volume in vacuo. The metabolic mixture obtained was applied to a silica gel column (2.4 × 44 cm). The column was first eluted with CHCl₃ (5:1) and fractions of 500 ml/flask were collected. The fourth fraction was evaporated to dryness in vacuo and the residue was further purified by repeated preparative TLC and crystallization from CHCl₃–petroleum ether. Yield 2 mg. mp > 300°C, UV λ_max (EtOH): 250 nm, MS: m/z 468 (M⁺, 29%), 453 (15%), 440 (15%), 422 (17%), 303 (73%), 262 (75%), 216 (12%), 135 (100%), IR ν_max (KBr): 3310 (COOH), 1726 (C=O) cm⁻¹. ¹H NMR (DMSO-d₆) δ: 0.79, 0.98, 1.03, 1.09, 1.12, 1.16, 1.38 (each 3H, each s, C-CH₃), 5.48 (1H, s, C = CH). This compound was identified to be 3-dehydro-18β-glycyrrhetic acid. The column was next eluted with CHCl₃-MeOH (100:1). Fractions of 10 ml/tube were collected, monitoring by silica gel TLC. Fr. 1-10 were pooled and evaporated to dryness in vacuo. The precipitate (ca. 25 mg) was purified by preparative TLC and by repeated crystallization from CHCl₃–benzene–MeOH to give pure 3-epi-18β-glycyrrhetic acid (3.3 mg). Fr. 11-39 contained two components, which were then separated to give 3-epi-18β-glycyrrhetic acid (ca. 1 mg) and 18β-glycyrrhetic acid (31 mg) by rechromatography. Fr. 40-49 were work up in a similar fashion. The precipitate (ca. 144 mg) was crystallized from

**Isolation of 3-epi-18α-glycyrrhetic acid**

Precultured *Clostridium innocuum* ES24-06 was inoculated into GAM broth (200 ml) and cultured for 5 h at 37 ºC. 18α-Glycyrrhetic acid (94 mg) in EtOH (20 ml) was added to the culture medium, which was further incubated for 10 h at 37 ºC. Then 1 N HCl (1000 ml) and NaCl (150 g) were added, and the medium was extracted three times with EtOAc (1.5 l). The EtOAc solution was concentrated to a volume of ca. 1000 ml, washed with a saturated NaCl solution (500 ml), dried over Na₂SO₄, and then evaporated to dryness *in vacuo* below 40 ºC. The residue was dissolved in a small volume of CHCl₃ and the solution was applied to a column of silica gel (36 x 2 cm). The column was washed with CHCl₃ (700 ml) and eluted with CHCl₃–MeOH (100:1). Fractions I—IV (100 ml each) were pooled, evaporated to dryness *in vacuo*, and washed with H₂O–EtOH. The precipitate (57.4 mg) was purified by preparative thin layer chromatography (Merck, Kieselgel 60 F254 S, 2 mm layer thickness) using solvent system F and the product was crystallized from n-PrOH-petroleum ether to give colorless prisms (5.1 mg). mp >300 ºC, Anal. Calcd for C₃₀H₄₆O₄: C, 76.55; H, 9.85. Found: C, 76.54; H, 9.63. ¹H-NMR (DMSO-d₆) δ: 0.65, 0.77, 0.84, 1.04, 1.13, 1.16, 1.35 (each 3H, each s, C-CH₃), 2.8 (1H, brs, CH-OH), 5.33 (1H, s, C = CH). UV λ_max: 244 nm. MS m/z: 470 (M⁺, 2%), 452 (4%), 437 (5%), 303 (100%), 262 (18%), 257 (15%), 175 (15%), 135 (60%). IR ν_max (KBr): 3480 (OH), 1709 (COOH), 1650 (conjugated C = O), 1615 (conjugated C = C) cm⁻¹. [Hattori et al., Chem. Pharm. Bull., 33, 210-217 (1985)]
Fig. 2. Time courses of metabolism of 3-dehydroglycyrrhetic acid by *Ruminococcus* sp. POI-3 (A) and *Clostridium innocuum* ES24-06 (B).

○, 3-dehydroglycyrrhetic acid; ●, glycyrrhetic acid; ▲, 3-**epi**-glycyrrhetic acid. [Hattori *et al.*, *Chem. Pharm. Bull.*, **33**, 210-217 (1985)]

**Reduction of 3-dehydroglycyrrhetic acid**

A human intestinal bacterium or a bacterial mixture was anaerobically cultured for 48 h at 37 °C in GAM broth (20 ml) containing 3-dehydroglycyrrhetic acid (1 mmol). A portion (10 ml) of the culture was acidified to pH 1 with HCl and extracted with ethyl acetate (EtOAc, 10 ml x 2) after adding NaCl (2g). The EtOAc solution was concentrated to a volume of 1 ml and an aliquot (12 µl) of it was spotted on a TLC plate, which was then developed with solvent system F. Metabolites glycyrrhetic acid and 3-**epi**-glycyrrhetic acid were quantitatively analyzed by TLC-densitometry. [Hattori *et al.*, *Chem. Pharm. Bull.*, **33**, 210-217 (1985)]

**参考文献**
