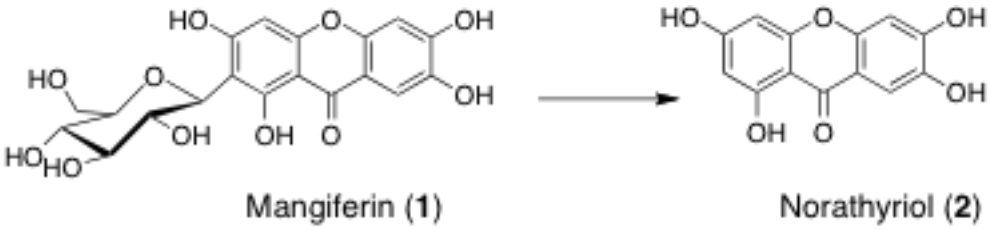
Mangiferin



Transformation of mangiferin (**1**) by human intestinal bactera

代謝実験

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Metabolism of mangiferin (1) by human intestinal flora
Mangiferin (**1**) was anaerobically incubated with a bacterial mixture from human feces, and a metabolite was isolated as a yellow powder, mp 300°C; UV λ_{max} at 236, 254, 312 and 360 nm. High resolution MS: the molecular formula $C_{13}H_{18}O_6$. IR, ^{1}H NMR, ^{13}C NMR and MS spectra were identical with those of authentic 1,3,6,7-tetrahydroxyxanthone (northyriol, **2**). [Hattori *et al*., *Phytochemistry,* **28**, 1289-1290 (1989)]

Metabolism of mangiferin (1) by a defined bacterium from human feces
A novel bacterium *Bacteroudes* species MANG was cultured with 0.5 mM mangiferin (**1**) in GAM broth under anaerobic conditions for 24 h. The metabolic product was extracted from 50 ml of the cultured broth with 50 ml of butanol 3 times. The extract was dried under vacuum, dissolved in 50% methanol, and then applied to preparative HPLC to purify the metabolite under the following conditions: Cosmosil 5C18-AR-II (20 × 250 mm) column (Nacalai Tesque, Kyoto, Japan); flow rate, 4 ml/min; detection, 260 nm; solvent system, 20―80% linear gradient of acetonitrile in 0.1% trifluoroacetic acid. [Sanugul *et al*., *Biol. Pharm. Bull.,* **28**, 1672-1678 (2005)]

Norathyriol (2)
The metabolite was obtained as yellow amorphous powder which showed 98% purity as a single peak on the HPLC chromatogram; UV λ_{max}^{MeOH} (ε) nm: 237 (24000), 254 (31000), 312 (15000), 362 (13000); EI-MS *m/z*: 260 $[M]^+$; ^{1}H NMR (500 MHz, CD_3OD) δ: 6.14 (1H, d, *J* = 2 Hz, 2-H), 6.28 (1H, d, *J* = 2 Hz, 4-H), 6.80 (1H, s, 5-H), 7.42 (1H, s, 8-H). [Sanugul *et al*., *Biol. Pharm. Bull.,* **28**, 1672-1678 (2005)]

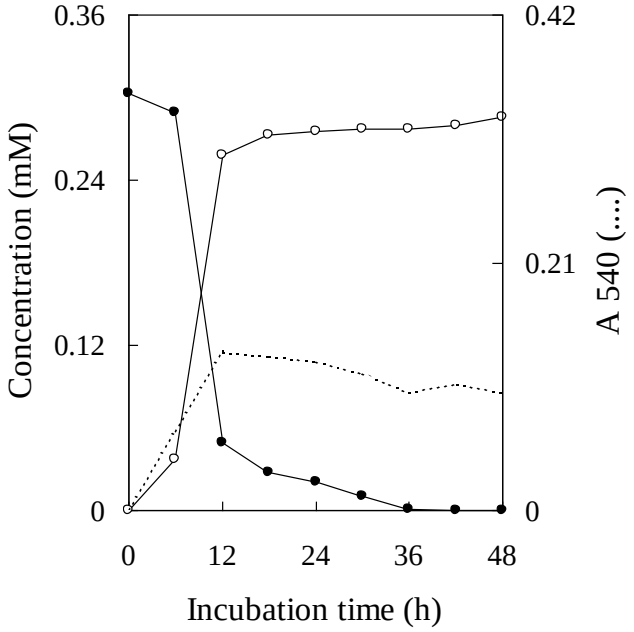

Fig. 1. Time course of conversion of mangiferin (**1**) (●) to norathyriol (**2**) (○) by *Bacteroides* sp. MANG in PYF broth

Bacterial growth (····) was monitored by measuring absorbance at 540 nm. [Sanugul *et al*., *Biol. Pharm. Bull.,* **28**, 1672-1678 (2005)]

Qualitative assays of mangiferin (1) and norathyriol (2) by TLC and HPLC

The butanol extract was separated by TLC (Silica gel RP-18 F254 S, Merck, Darmstadt, Germany) using $CH_3OH–H_2O–CH_3COOH$ (5:5:0.2) as a mixed solvent, and then mangiferin (**1**) and norathyriol (**2**) were visualized under UV light (Spectroline CM-10, Spectronics Corporation, NY, USA). The reversed phase HPLC (Shimadzu Co., Japan) conditions were as follows: recorder, C-R6A Chromatopac; pump, LC-6A; system controller, SCL-6B; monitor, SPD-6A; injector, SIL-9A; column, YMC-Pack ODS-AP AP-302 (4.6×150 mm) (YMC Co., Kyoto, Japan); flow rate, 1 ml/min; detection, 260 nm; solvent system, 10―40% acetonitrile linear gradient in 0.1% trifluoroacetic acid. The retention time of mangiferin (**1**) and norathyriol (**2**) were 7.5 and 14.5 min, respectively. [Sanugul *et al*., *Biol. Pharm. Bull.,* **28**, 1672-1678 (2005); Sanugul *et al*., *Biol. Pharm. Bull.,* **28**, 2035-2039 (2005)]

Bacterial suspension (100 μl) precultured in GAM broth was cultivated in 5 ml of PYF broth containing 0.4 mM of mangiferin (**1**) at 37°C under anaerobic conditions. Two 100 μl portions were removed every 6 h. Bacterial growth was measured in one portion at 540 nm (absorbance) and mangiferin (**1**) and norathyriol (**2**) were quantified by HPLC. The acidified butanol extract (5 μl) of a 100 μl portion was dried in a Speed Vac SC 110 (Savant Instruments, NY, USA) and then dissolved in 100 μl of 50% methanol for HPLC application. [Sanugul *et al*., *Biol. Pharm. Bull.,* **28**, 1672-1678 (2005)]

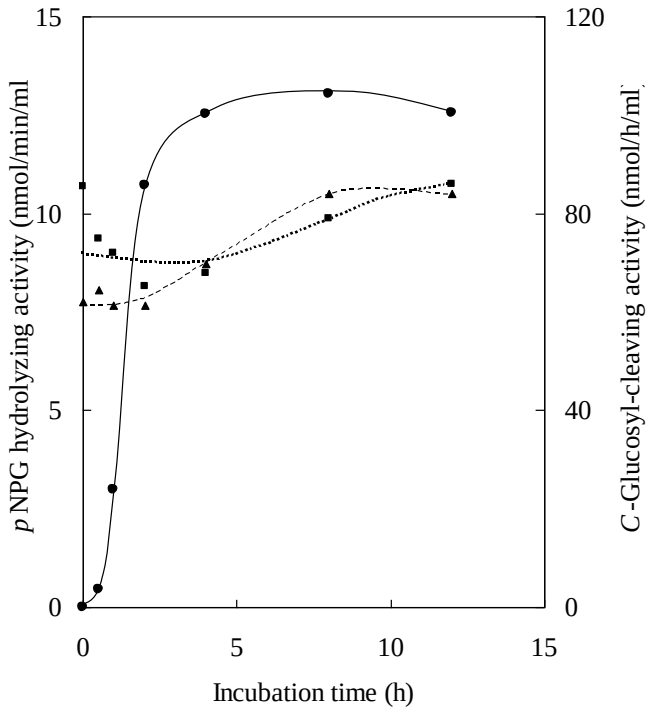

Fig. 2. Time course of *C*-glucosyl-cleavage activity of *Bacteroides*. sp. MANG induced by 0.2 mM mangiferin (**1**) at 37°C

C-glucosyl-cleavage activity (●) compared with *p*-nitrophenyl-α-D-glucose (*p*-NP-α-G) (▲) and *p*-nitrophenyl-β-D-glucose *(p*-NP-β-G) (■) hydrolyzing activities under the same conditions. [Sanugul *et al*., *Biol. Pharm. Bull.,* **28**, 1672-1678 (2005)]

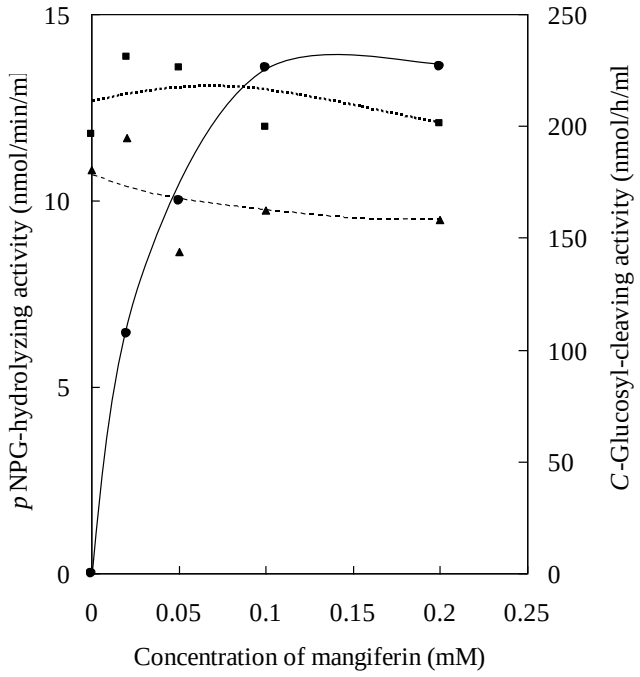

Fig. 3. Effect of mangiferin (**1**) concentrations on the stimulation of *C*-glucosyl-cleaving activity of *Bacteroides* sp. MANG at 37°C for 4 h

C-glucosyl-cleaving activity (●) was determined together with *p*NP-α-G- (▲) and *p*NP-β-G- (■) hydrolyzing activities [Sanugul *et al*., *Biol. Pharm. Bull.,* **28**, 1672-1678 (2005)]

1) Hattori M., Shu Y. Z., Tomimori T., Kobashi K. and Namba T.: A bacterial cleavage of the *C*-glucosyl bond of mangiferin and bergenin. *Phytochemistry*, **28**, 1289-1290 (1989).
2) Sanugul K., Akao T., Li Y., Kakiuchi N., Nakamura N. and Hattori M.: Isolation of a human intestinal bacterium that transforms mangiferin to norathyriol and inducibility of the enzyme that cleaves a *C*-glucosyl bond. *Biol. Pharm. Bull.,* **28**, 1672-1678 (2005).
3) Sanugul K., Akao T., Nakamura N., and Hattori M.: Two proteins, Mn^{2+}, and low molecular cofactor are required for *C*-glucosyl-cleavage of mangiferin. *Biol. Pharm. Bull.,* **28**, 2035-2039 (2005).