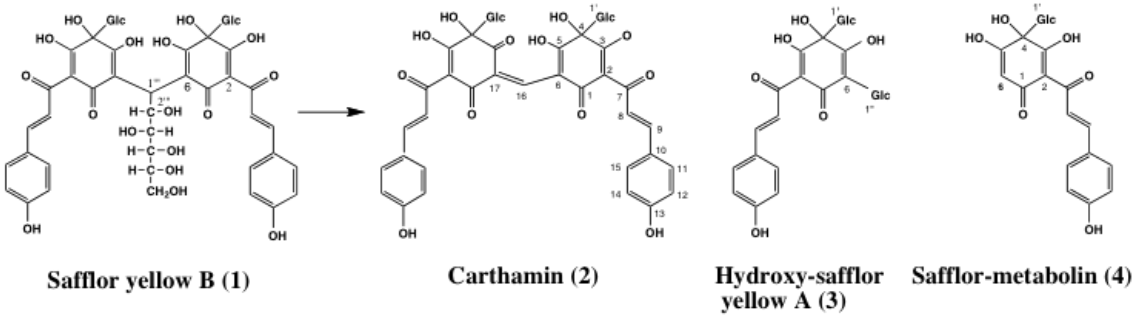
Safflor Yellow B



Transformation of safflor yellow B by a human intestinal microflora and *Peptostreptococcus anaerobius*

代謝実験

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Metabolism of safflor yellow B (1) by *Peptostreptococcus anaerobius*

A precultured bacterial suspension (100 ml) of *P. anaerobius* was added to GAM broth (900 ml) and cultured overnight at 37°C under anaerobic conditions. The bacterial culture was centrifuged at 7,800 x g for 10 min and the pellets were washed twice with saline and suspended in 0.1 M phosphate buffer (900 ml, pH 7.3). Safflor yellow B (900 mg, **1**) was added to the bacterial suspension and the mixture was anaerobically incubated for 4 d at 37°C. The mixture was then acidified to pH *ca.* 3 with 5% HCl and extracted with EtOAc (500 ml x 4). The EtOAc layer was washed with H_2O and evaporated *in vacuo* to give a residue. The residue (125 mg, 5%) was applied to a column of Sephadex LH-20 (25 x 2 cm), and elution with 50% MeOH afforded metabolites in mixture. The crude metabolites dissolved in water were passed through a column of cellulose powder (100 mg) and the column was eluted with 50% MeOH to give compound **2** (3.5 mg). The aqueous filtrate was subjected to preparative tlc with solvent system B to give compound **4** (R_f 0.35, 15 mg). The aqueous layer after EtOAc extraction was evaporated *in vacuo* to give a residue, which was applied to a column of Sephadex LH-20 (45 x 1.5 cm). Elution with 50% MeOH afforded crude

compound **3**, which was further purified by preparative tlc (solvent system A, R_f 0.33) to give a pure compound (37 mg).

Preparation of an intestinal bacterial mixture

Fresh feces obtained from a healthy subject were thoroughly suspended in 0.1 M phosphate buffer, pH 7.3, and filtered through layers of gauze to remove the sediments, the filtrate was kept under O_2-free CO_2 by bubbling for 3 min and used as an intestinal bacterial mixture.

Incubation of safflor yellow B (1) with an intestinal bacterial mixture

Safflor yellow B (120 mg, **1**) was incubated with an intestinal bacterial mixture (150 ml) for 7 d at 37°C in an anaerobic jar in which the air had been replaced by O_2-free C O_2. The incubation mixture was acidified with 5% HCl and extracted with EtOAc (50 ml x 4). Metabolites **3** and **4** were isolated and purified by preparative tlc (solvent system A).

Compound 2.

Reddish brown powder with metallic luster (1.5 mg, 0.16%). $[\alpha]^{25}_D$ –57.3° (*c* = 0.2, 50% Me_2CO). ir ν_{max} (KBr) cm^{-1}: 3400 (OH), 2900, 1620 (conj. C=O), 1600, 1500, 1400 (aromatic). uv λ_{max} (MeOH) nm (log ε): 280 (4.80), 380 (6.83), 520 (4.40). fabms *m/z* 909 $[M - 1]^-$. ^{1}H-nmr (DMSO-*d6*) δ_H : 3.07 (1H, m, H-4'), 3.20 (1H, t, *J* = 9 Hz, H-3'), 3.41 (1H, t, *J* = 9 Hz, H-2'), 3.51 (1H, m, H-5'), 3.53 (1H, m, H-6'), 3.77 (1H, m, H-6'), 3.88 (1H, d, *J* = 9 Hz, H-1'), 5.49 (1H, s, H-16), 6.87 (2H, d, *J* = 8.5 Hz, H-12 and H-14), 7.38 (1H, d, *J* = 16.5 Hz, H-9), 7.58 (2H, d, *J* = 8.5 Hz, H-11 and H-15), 7.65 (1H, d, *J* = 16.5 Hz, H-8), 9.37 (1H, s, 13-OH), 19.79 (1H, s, 3-OH). ^{13}C-nmr: see Table 2 in literature [Meselhy *et al*., *J. Nat. Prod*., **56**, 39-45 (1993)].

Compound 3.

Yellow amorphous powder (37 mg, 4.1%). $[\alpha]^{25}_D$ –54.3° (*c* = 0.1, MeOH). ir ν_{max} (KBr) cm^{-1}: 3400, 2950, 1640 (conj. C=O), 1610, 1520, 1450. uv λ_{max} (MeOH) nm (log ε): 280 (4.63), 400 (4.43). fabms *m/z* 611 $[M - 1]^-$. ^{1}H-nmr (DMSO-*d6*) δ_H: 2.91 (1H, m,

H-4'), 2.95 (1H, m, H-5'), 3.05 (1H, m, H-5"), 3.08 (1H, m, H-4"), 3.10 (1H, m, H-3'), 3.13 (1H, m, H-3"), 3.31 (1H, m, H-2'), 3.38 (1H, m, H-6'), 3.40 (1H, m, H-6"), 3.60 (1H, m, H-6"), 3.61 (1H, m, H-6'), 3.64 (1H, d, *J* = 10 Hz, H-1'), 4.01 (1H, s, 2"-OH), 4.14 (1H, m, H-2"), 4.14 (1H, s, 3"-OH), 4.21 (1H, d, *J* = 10 Hz, H-1"), 4.44 (1H, s, 6"-OH), 4.64 (1H, s, 4-OH), 4.69 (2H, s, 2'-OH and H-4"), 4.81 (1H, s, 4'-OH), 4.88 (2H, s, 3' and 6'-OH), 6.77 (2H, d, *J* = 9 Hz, H-12 and H-14), 7.28 (1H, d, *J* =16.5 Hz, H-9), 7.4 (2H, d, *J* = 9 Hz, H-11 and H-15), 7.42 (1H, d, *J* = 16.5 Hz, H-8), 8.29 (1H, s, 13-OH), 9.75 (1H, s, 5-OH), 18.61 (1H, s, 3-OH). ^{13}C-nmr: see Table 2 in literature [Meselhy *et al*., *J. Nat. Prod*., **56**, 39-45 (1993)].

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Compound 4.

Yellow amorphous powder (15 mg, 1.6 %). $[\alpha]^{25}_{D}$ −61.4° (*c* = 0.1, MeOH). ir ν_{max} (KBr) cm^{-1}: 3400, 2900, 1620, 1600, 1520, 1420, 1380. uv λ_{max} (MeOH) nm (log ε): 275 (4.49), 400 (4.36). fabms *m/z* 449 $[M-1]^-$. ^{1}H-nmr (DMSO-*d6*) δ_H : 2.97 (1H, t, *J* = 8.5 Hz, H-5'), 3.07 (1H, m, H-4'), 3.33 (1H, m, H-2'), 3.47 (1H, m, H-6'), 3.58 (1H, m, H-6'), 3.60 (1H, d, *J* = 10 Hz, H-1'), 4.65 (1H, s, 6'-OH), 4.65 (1H, s, H-6), 6.80 (2H, d, *J* = 9 Hz, H-11 and H-15), 7.31 (1H, d, *J* = 16.5 Hz, H-9), 7.43 (2H, d, *J* = 9 Hz, H-12 and H-14), 7.45 (1H, d, *J* = 16.5 Hz, H-8), 9.82 (1H, s, 5-OH), 18. 51 (1H, s, 3-OH). ^{13}C-nmr: see Table 2 in literature [Meselhy *et al*., *J. Nat. Prod*., **56**, 39-45 (1993)].

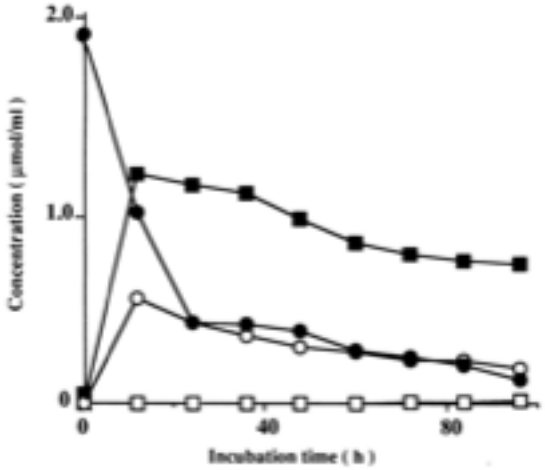

Fig. 1. Metabolic time course of safflor yellow B by *Peptostreptococcus anaerobius*.

（□）, carthamin (**2**);（■）, hydroxysafflor yellow A (**3**);（○）, safflor-metabolin;（●）, safflor yellow B (**1**)

Safflor yellow B (50 mg, **1**) was incubated with a precultured bacterial suspension of *Peptostreptococcus anaerobius* (100 ml) under anaerobic conditions for 4 d. An aliquot (10 ml) was taken out at 12 h intervals and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH (0.5 ml) and analyzed by tlc-densitometry. [Meselhy *et al*., *J. Nat. Prod*., **56**, 39-45 (1993)]

参考文献

1) Meselhy M. R., Kadota S., Hattori M. and Namba T.: Metabolism of safflor yellow B by human intestinal bacteria. *J. Nat. Prod*., **56**, 39-45 (1993).