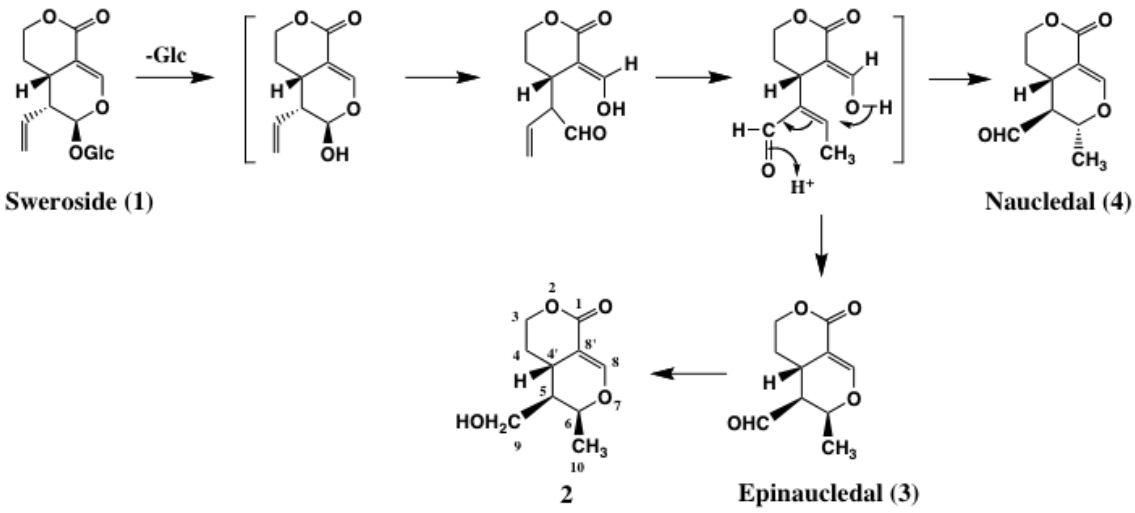
Sweroside



Metabolic processes of sweroside by human intestinal bacteria

代謝実験

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Screening of defined strains for ability to metabolize sweroside (1)

Each pre-cultured bacterial suspension (0.2 ml) was added to GAM broth (10 ml) and cultured for 24 h at 37°C in an anaerobic jar, according to the steel wool method. Sweroside (8.4 mg, **1**) was added to each culture, and the mixture was incubated for 24 h under anaerobic conditions and then extracted with EtOAc (10 ml). After evaporation of the EtOAc, $CHCl_3$-MeOH (1:1, 0.5 ml) was added and an aliquot (30 μl) of the solution was applied to a silica gel TLC plate, which was developed with $CHCl_3$–MeOH (17:1). The spots separated on the plate were quantitatively analyzed with a TLC-scanner at 245 nm. After the EtOAc extraction, the remaining aqueous solution was evaporated *in vacuo* to give a residue, to which MeOH (0.5 ml) was added. An aliquot (20 μl) of the MeOH solution was spotted on a TLC plate and the plate was developed with $CHCl_3$–MeOH (5:1). The amount of sweroside (**1**) recovered was quantitatively analyzed by TLC-densitometry. [Adel *et al*., Shoyakugaku Zasshi **44**, 122-126 (1990)]

Metabolism of sweroside (1) by *Proteus mirabilis*

A precultured bacterial suspension (100 ml) of *P. mirabilis* was added to GAM broth (900 ml) and cultured for 24 h at 37°C under anaerobic conditions. The bacterial culture was centrifuged at 7000 rpm for 10 min. The pellets were suspended in 0.1 m phosphate buffer (100 ml, pH 7.3). Sweroside (200 mg, **1**) was added to the suspension (80 ml). The mixture was anaerobically incubated for 22 h at 37°C and extracted 3 times with EtOAc (100 ml each). The combined EtOAc phases were evaporated to dryness *in vacuo*. The residue was chromatographed on preparative TLC plates with $CHCl_3$ (17:1), and the metabolite (metabolite A; **2**) obtained was purified further by high-performance liquid chromatography (HPLC; Chemo Pak, nucleosil 50-5, 250 mm x 4.6 mm ID) with $CHCl_3$–MeOH (17:1) as a mobile phase. Yield after purification, 3.5 mg. [Adel *et al*., Shoyakugaku Zasshi **44**, 122-126 (1990)]

Naucledal (4)

R_f =0.61 on a TLC plate with $CHCl_3$-MeOH as a developing solvent.
(17:1); $[\alpha]_D$= +8.8° (*c*, 0.02, MeOH); yield, 1.5 mg). [Adel *et al*., Shoyakugaku Zasshi **44**, 122-126 (1990)]

Epinaucledal (3)

R_f =0.45; $[\alpha]_D$=-37.7° (*c*, 0.01, MeOH); yield, 2.5 mg). The ^{1}H-NMR data of both compounds agreed well with those reported. [Adel *et al*., Shoyakugaku Zasshi **44**, 122-126 (1990)]

Metabolite 2

Colorless syrup; R_f =0.30 on TLC with $CHCl_3$–MeOH (17:1); MS *m/z* (rel. int.): 198 (100, M^+), 180 (29, M^+–H_2O), 167 (59, M^+–CH_2OH), 165 (73, M^+–H_2O–CH_3), 140 (79); IR ν cm^{-1}: 3400 (OH), 1690 (C=O), 1602(C=O); UV λ_{max} nm: 248; $[\alpha]_D$= -6.7° (*c*, 0.02, MeOH); ^{1}H NMR(270 MHz, $CDC1_3$): δ 1.21 (3H, d, J=6.6 Hz, H_3-10), 1.63 (1H, m, Hax-4), 1.85 (1H, m, H-5), 2.05 (1H, m, Heq-4), 2.38 (1H, dddd, 2.0, 4.2, *ca*. 12.6, *ca*. 12.6, H-4a), 3.59 (1H, dd, *J*=10.5, 8.4 Hz, Ha-9), 3.88 (1H, dd, *J*=10.5, 5.1 Hz,

Hb-9), 4.27 (1H, ddd, *J*=11.5, 2.4, 12.7 Hz, Hax-3), 4.45 (1H, ddd, *J*=11.5, 2.0, 4.6 Hz, Heq-3), 4.65 (1H, dq, *J*=4.2, 6.6 Hz, H-6), 7.67 (lH, d, *J*=2.0 Hz, H-8). [Adel *et al*., Shoyakugaku Zasshi **44**, 122-126 (1990)]

参考文献

1) Adel I. El-Sedawy A. I., Hattori M., Kobashi K., and Namba T.: Metabolism of sweroside from *Swertia japonica* by human intestinal bacteria. Shoyakugaku Zasshi **44**, 122-126 (1990).