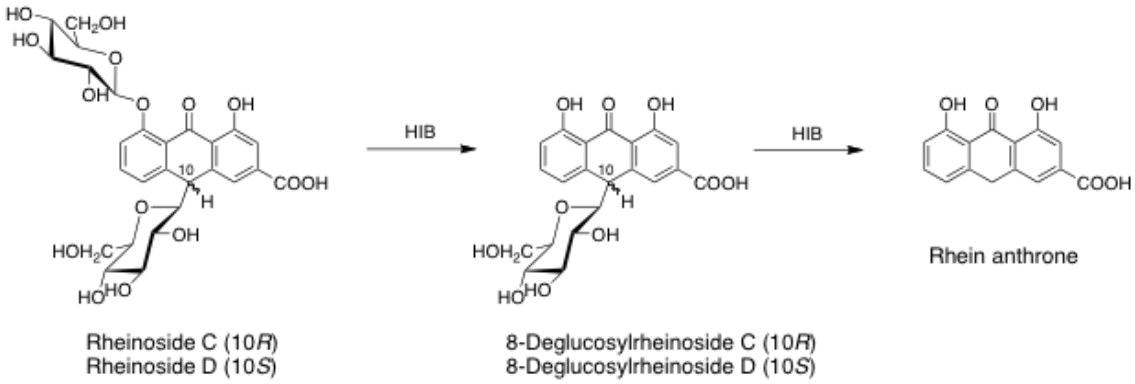
Rheinoside C, D



Metabolic processes of rheinosides C and D by human intestinal bacteria

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単一化合物　rheinoside C, D

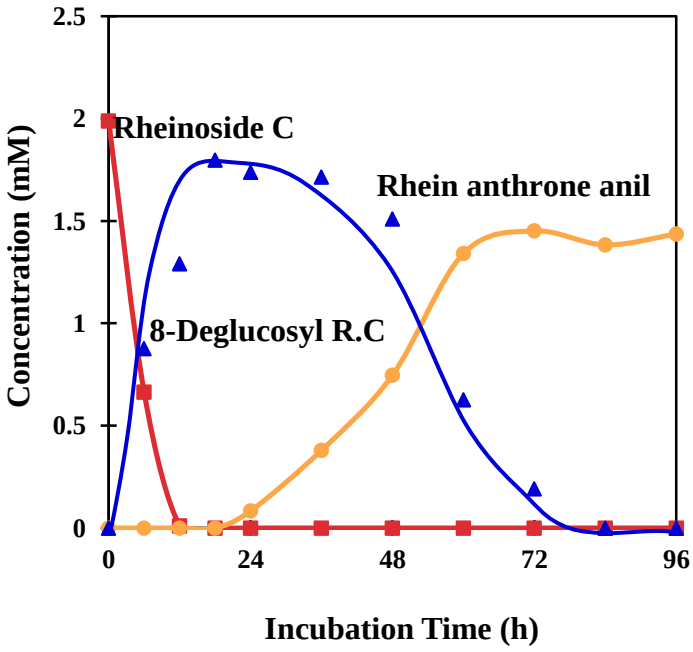

Fig. 1.　Metabolic time course of rheinoside C by human intestinal bacterial flora

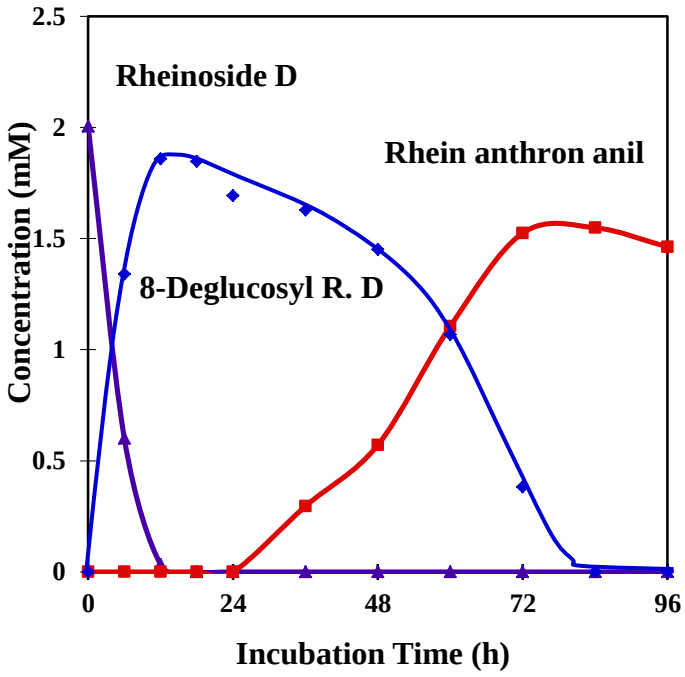

Fig. 2. Metabolic time course of rheinoside D by human intestinal bacterial flora

Metabolism of rheinosides C and D by human intestinal flora

A fecal bacterial suspension (0.5 ml) precultured for 24 h was inoculated into PYF broth (4.5 ml) containing 2 mM rheinoside C or D, and the mixture was anaerobically incubated for 24 h.

Quantitative determination of rheinosides and 8-deglucosylrheinosides

A 50 µl portion of the culture and 50 µl of MeOH containing 0.1% AcOH were vigorously stirred and centrifuged at 8800 x g for 1 min to separate a supernatant and precipitates. A portion of the supernatant was applied to a normal phase TLC plate of silica gel and developed with a mixed solvent $CHCl_3$-MeOH-H_2O (6 : 4 : 1). The spots of rheinoside and 8-deglucosylrheinoside were quantitatively determined by TLC-densitometry at a wavelength of 350 nm.

Quantitative determination of rhein anthrone

A 50 µl portion of the culture, 20 µl of 1% *N*, *N*-dimethyl-*p*-nitrosoaniline solution in pyridine and 50 µl of BuOH saturated with H_2O containing 0.1% AcOH were mixed

and centrifuged at 8800 x g for 1 min. A portion of the BuOH phase was spotted onto a polyamide TLC sheet, and developed with $CHCl_3$-MeOH-H_2O (7:3:0.5). Rhein anthrone anil was quantitatively determined by TLC-densitometry at 660 nm, using a standard line of an authentic compound.

Metabolism of 8-deglucosylrheinoside D by *Eubacterium* sp. BAR

A 0.5 ml portion of a bacterial suspension of *Eubacterium* sp. BAR cultured in GAM broth for 24 h at 37°C was inoculated into PYF broth (4.5 ml) containing 8-deglucosylrheinoside D (final concentration of 1 mM), and the mixture was anaerobically incubated at 37°C. The formation of rhein anthrone was quantitatively monitored by TLC densitometry, after treatment with *N*, *N*-dimethyl-*p*-nitrosoaniline. Bacterial growth was monitored at 540 nm after 10-fold dilution of the culture.

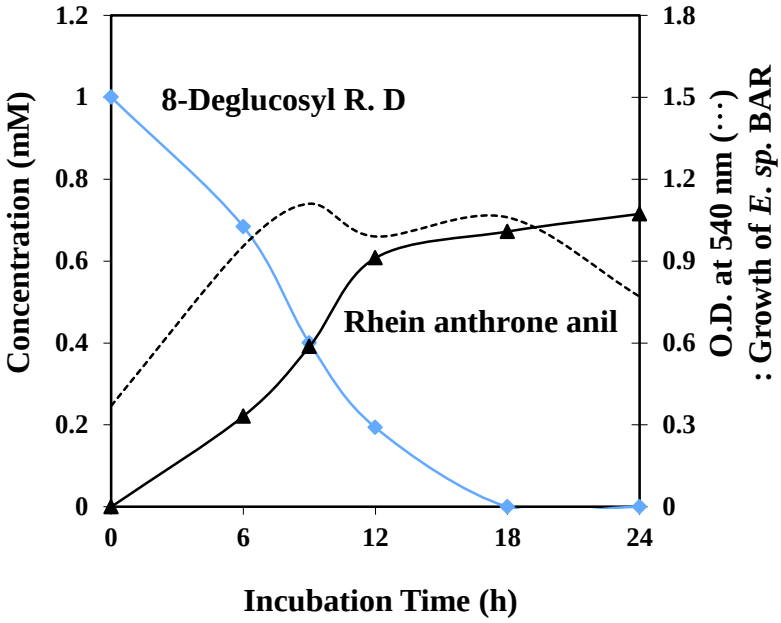

Fig. 3. Metabolic time course of 8-deglucosylrheinoside D by *Eubacterium.* sp BAR

Rheinoside C

Pale yellow amorphous powder, FAB-MS: *m/z* 595 $[M+H]^+$. ^{1}H-NMR (D_2O, 500 MHz)

δ: 7.55 (1H, dd, *J*=7.6, 8.2 Hz, H-6), 7.19 (1H, d, *J*=8.2 Hz, H-5), 7.18 (1H, brs, H-4), 7.03 (1H, d, *J*=7.6 Hz, H-7), 7.00 (1H, brs, H-2), 5.15 (1H, d, *J*=7.7 Hz, H-1″), 4.19 (1H, brs, H-10), 3.95 (1H, brd, *J*=12.4 Hz, H_a-6″), 3.81－3.76 (2H, m, H-2″, H_b-6″), 3.69－3.66 (2H, m, H-4″, H-5″), 3.58 (1H, t, *J*=9.4 Hz, H-3″), 3.45 (1H, brd, *J*=12.4 Hz, H_a-6′), 3.33 (1H, m, H_b-6′), 3.26 (1H, t, *J*=9.0 Hz, H-3′), 3.21 (1H, brd, *J*=9.8 Hz, H-1′), 2.84－2.93 (3H, m, H-2′, H-4′, H-5′). ^{13}C-NMR (D_2O, 75 MHz) δ: 189.9 (C-9), 168.8 (COOH), 158.2 (C-1), 156.7 (C-8), 143.2 (C-4a), 141.3 (C-10a), 135.3 (C-3 or C-6), 134.8 (C-3 or C-6), 123.5 (C-5), 122.4 (C-8a, C-9a), 119.0 (C-4), 116.4 (C-2), 114.7 (C-7), 100.4 (C-1″), 83.0 (C-1′), 79.4 (C-5′), 77.8 (C-3′), 76.2 (C-5″), 75.7 (C-3″), 72.9 (C-2″), 70.5 (C-2′), 69.3 (C-4′, C-4″), 60.9 (C-6′), 60.6 (C-6″), 44.5 (C-10).

Rheinoside D

Pale yellow amorphous powder, FAB-MS : *m/z* 595 $[M+H]^+$. ^{1}H-NMR (D_2O, 500 MHz) δ: 7.67 (1H, t, *J*=8.5 Hz, H-6), 7.35 (1H, d, *J*=8.5 Hz, H-5), 7.31 (1H, brs, H-4), 7.20 (1H, brs, H-2), 7.20 (1H, d, *J*=8.5 Hz, H-7), 5.11 (1H, d, *J*=7.7 Hz, H-1″), 4.15 (1H, brs, H-10), 3.99 (1H, brd, *J*=12.4 Hz, H_a-6″), 3.84 (1H, dd, *J*=5.3, 12.4 Hz, H_b-6″), 3.73 (1H, t, *J*=8.6 Hz, H-2″), 3.58－3.66 (3H, m, H-3″, H-4″, H-5″), 3.30 (1H, brd, *J*=12.4 Hz, H_a-6′), 3.26－3.35 (3H, m, H-1′, H-3′, H_b-6′), 2.83－2.90 (2H, m, H-4′, H-5′), 2.79 (1H, t, *J*=9.4 Hz, H-2′). ^{13}C-NMR (D_2O, 75 MHz) δ: 190.3 (C-9), 168.8 (COOH), 158.7 (C-1), 157.3 (C-8), 145.2 (C-4a), 139.4 (C-10a), 136.0 (C-6), 134.6 (C-3), 123.4 (C-5), 123.0 (C-8a), 122.8 (C-9a), 120.5 (C-4), 117.1 (C-2, C-7), 102.7 (C-1″), 83.0 (C-1′), 79.7 (C-5′), 78.0 (C-3′), 76.7 (C-5″), 75.5 (C-3″), 73.3 (C-2″), 70.5 (C-2′), 69.7 (C-4′, C-4″), 61.3 (C-6′), 61.0 (C-6″), 44.3 (C-10).

8-Deglucosylrheinoside C

Pale yellow amorphous powder, FAB-MS: *m/z* 433 $[M+H]^+$. ^{1}H-NMR (CD_3OD, 500 MHz) δ: 7.65 (1H, d, *J*=1.7 Hz, H-4), 7.50 (1H, dd, *J*=7.3, 8.1 Hz, H-6), 7.42 (1H, d, *J*=1.7 Hz, H-2), 7.08 (1H, d, *J*=7.3 Hz, H-5), 6.88 (1H, d, *J*=8.1 Hz, H-7), 4.67 (1H, brs, H-10), 3.55 (1H, dd, *J*=2.6, 11.5 Hz, H_a-6′), 3.35 (1H, dd, *J*=5.6, 11.5 Hz, H_b-6′), 3.39 (1H, dd, *J*=2.1, 9.7 Hz, H-1′), 3.28 (1H, t, *J*=8.5 Hz, H-3′), 2.91 (1H, m, H-5’), 2.98 (1H, t, *J*=8.5 Hz, H-4′), 2.88 (1H, dd, *J*=8.5, 9.7 Hz, H-2′). ^{13}C-NMR (CD_3OD, 125 MHz) δ: 195.5 (C-9, C=O), 168.6 (COOH), 163.3, 162.6, 146.9, 142.9, 138.4, 136.8, 121.4 (2C, C-5)**,** 120.3 (C-4), 118.9, 117.9 (C-2), 117.3 (C-7), 86.2 (C-1′), 81.8 (C-5′), 79.9 (C-3′),

72.0 (C-2′ or C-4′), 71.9 (C-2′ or C-4′), 63.2 (C-6′), 45.9 (C-10).

8-Deglucosylrheinoside D

Pale yellow amorphous powder, FAB-MS: *m/z* 433 $[M+H]^+$. ^{1}H-NMR (CD_3OD, 500 MHz) δ: 7.66 (1H, d, *J*=1.3 Hz, H-4), 7.52 (1H, dd, *J*=7.3, 8.1 Hz, H-6), 7.44 (1H, d, *J*=1.3 Hz, H-2), 7.09 (1H, d, *J*=7.3 Hz, H-5), 6.88 (1H, d, *J*=8.1 Hz, H-7), 4.69 (1H, d, *J*=2.1, H-10), 3.57 (1H, dd, *J*=2.1, 11.4 Hz, H_a-6′), 3.38 (1H, dd, *J*=5.5, 11.4 Hz, H_b-6′), 3.41 (1H, dd, *J*=2.1, 9.5 Hz, H-1′), 3.23 (1H, t, *J*=9.5 Hz, H-3′), 2.93 (1H, m, H-5′), 2.96 (1H, t, *J*=9.5 Hz, H-4′), 2.88 (1H, t, *J*=9.5 Hz, H-2′). ^{13}C-NMR (CD_3OD, 75 MHz) δ: 195.3 (C-9, C=O), 169.0 (COOH), 162.9, 162.6, 146.4, 143.3, 137.4, 136.6, 121.5**,** 121.3, 120.0, 118.6, 118.0, 116.9, 86.3, 81.7, 79.9, 72.0, 71.9, 63.2, 46.0 (C-10).

参考文献

牧野圭吾修士論文『ヒト腸内細菌による anthrone 及び oxyanthrone C-配糖体の C-グルコシル結合の開裂について』 (2000) 富山医科薬科大学