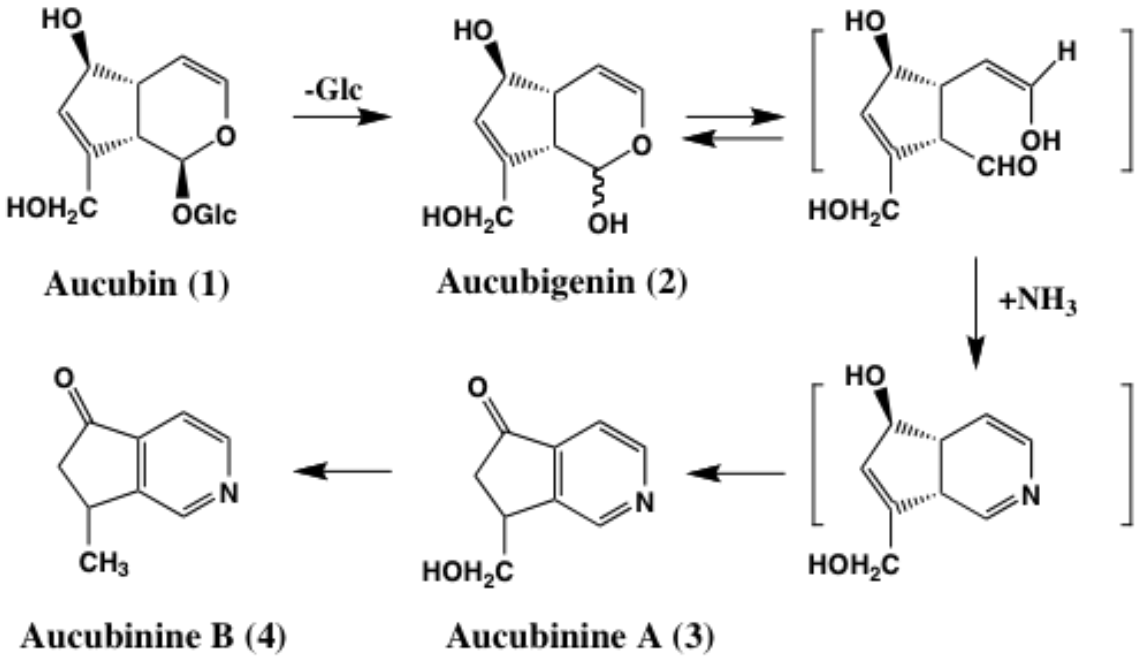
Aucubin



代謝実験

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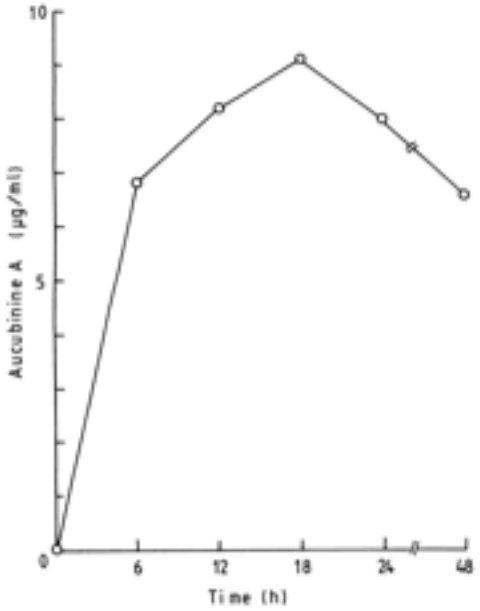

Fig. 1. Time course of the formation of aucubinine A (**3**) from aucubin (**1**) by *Klebsiella pneumoniae.*

Aucubin (1 mg/mL) was anaerobically incubated with a cell suspension of *K. pneumoinae* in 0.1 m phosphate buffer (pH 7.4). The products were analyzed by TLC-densitometry. [Hattori *et al*., *Phytother. Res*., **4**, 66-70 (1990)]

Incubation of aucubin with *Bacteroides fragilis*

A strain of *B. fragilis* was anaerobically cultured in GAM broth (50 mL x 5) for 20 h at 37 °C in an anaerobic jar, in which air had been replaced by oxygen-free CO_2 gas in the presence of steel wool (steel wool method). The culture was centrifuged at 5700 x g for 5 min. The precipitates were washed with a saline solution and suspended in 0.1M phosphate buffer (pH 7.4, 200 mL). Aucubin (200 mg, **1**) was then added. The mixture was anaerobically incubated for 12 h at 37 °C and extracted with water-saturated BuOH (50 mL x 3). The combined BuOH solutions were pooled and evaporated *in vacuo* to give an oily residue. The residue was applied to a column of silica gel (45 cm x 2.1 cm ID). The column was eluted with hexane, hexane + $CHCl_3$, and $CHCl_3$ with in creasing amounts of MeOH. Three metabolites (A1, A2 and A3) obtained from these fractions

were further purified by repeating preparative TLC. [Hattori *et al*., *Phytother. Res*., **4**, 66-70 (1990)]

Metabolite A1 (aucubigenin, 2)

R_f = 035 on TLC with EtOAc–MeOH (4:1). The compound was identified as aucubigenin on the basis of the comparison of the spectral and chromatographic data with those of an authentic sample. [Hattori *et al*., *Phytother. Res*., **4**, 66-70 (1990)]

Metabolite A2 (aucubinine A, 3)

$[\alpha]_D$ (MeOH, *c* = 0.16); colorless crystals, mp 89-90°C. yield, 10 mg; R_f = 0.41 on TLC with EtOAc–MeOH (4:1). Yellow color when the TLC plate was sprayed with anisaldehyde–H_2SO_4–AcOH (0.5:1: 50) reagent, followed by heating. High-resolution MS *m/z*:, 163.0648 (M^+), Calcd for $C_9H_9NO_2$: 163.0634. IR ν_{max} (KBr) cm^{-1}: 3400, 3200 (OH), 1720 (C=O). EI-MS *m/z* (rel. int.): 163(79), 133(100), 104(60), 75(26); ^1H NMR (270 MHz, $CDC1_3$): δ 2.60 (1H, dd, *J* = 3.2, 19.5 Hz, Ha-6), 2.89 (1H, dd, *J* = 7.6, 19.5 Hz, Hb-6), 3.71 (1H, m, 7-H), 3.90 (1H, dd, *J* = 6.1, 10.5 Hz, Ha-8), 4.00 (1H, dd, *J* = 5.1, 10.5 Hz, Hb-8), 7.58 (1H, dd, *J* = 5.1, 0.9 Hz, H-4), 8.71 (1H, d, *J* = 5.1Hz, H-3), 9.06 (1H, br s, H-l). ^{13}C NMR (22.5 MHz, $CDCl_3$): δ 149.8 (C-l, d), 149.1 (C-3, d), 116.6 (C-4, d), 148.6 (C-4a, s), 205.9 (C-5, s), 39.9 (C-6, t), 39.6 (C-7, d), 143.6 (C-7a, s), 65.3 (C-8, t). [Hattori *et al*., *Phytother. Res*., **4**, 66-70 (1990)]

Metabolite A3 (aucubinine B, 4)

R_f = 0.56 on TLC. Yellow color with anisaldehyde–H_2SO_4–AcOH reagent followed by heating. IR ν_{max} (KBr) cm^{-1}: 1725 (C=O). High-resolution MS *m/z*: 147.0694 (M^+), Calcd for C_9H_9NO: 147.0685. EI-MS *m/z* (rel. int.): 147 (M^+, 100), 132 (M^+–Me, 50). ^1H NMR (270 MHz, $CDC1_3$): δ: 1.48 (3H, *J* = 7.1 Hz, H-8), 2.32 (1H, dd, *J* = 19.5, 3.7 Hz, Ha-6), 3.00 (1H, dd, *J* = 19.5, 7.5 Hz, Hb-6), 3.58 (1H, m, H-7), 7.55 (1H, dd, *J* = 5.13, 0.9 Hz, H-4), 8.71 (1H, d, *J* = 5.1 Hz, H-3), 8.98 (1H, br s, H-l). [Hattori *et al*., *Phytother. Res*., **4**, 66-70 (1990)]

Incubation of aucubin (1) with a mixture of human intestinal bacteria

a) Sixty mL of a bacterial mixture from human feces containing aucubin (100 mg, **1**) was incubated for 18 h at 37 °C. The EtOAc extract (20 mL x 4) contained aucubinines A (**3**) and B (**4**), which were identified by comparison of the chromatographic behavior and spectral properties with those of authentic samples.

b) One mL of the bacterial mixture was inoculated into 100 mL of GAM broth and anaerobically cultured for 18 h at 37 °C. The culture was then centrifuged to collect the bacterial cells. After washing with a saline solution, the cells were suspended in 40 mL of 100 mM phosphate buffer. Aucubin (100 mg, **1**) was added to the suspension. The mixture was incubated for 24 h at 37 °C and extracted with EtOAc (20 mL x 4). Aucubinines A (**3**) and B (**4**) were detected in the extract by TLC. [Hattori *et al*., *Phytother. Res*., **4**, 66-70 (1990)]

参考文献

1) Hattori M., Kawata Y., Inoue K., Shu Y. Z., Che Q. M. and Namba T. and Kobashi K.: Transformation of aucubin to new pyridine monoterpene alkaloids, aucubinines A and B, by human intestinal bacteria. *Phytother. Res*., **4**, 66-70 (1990).