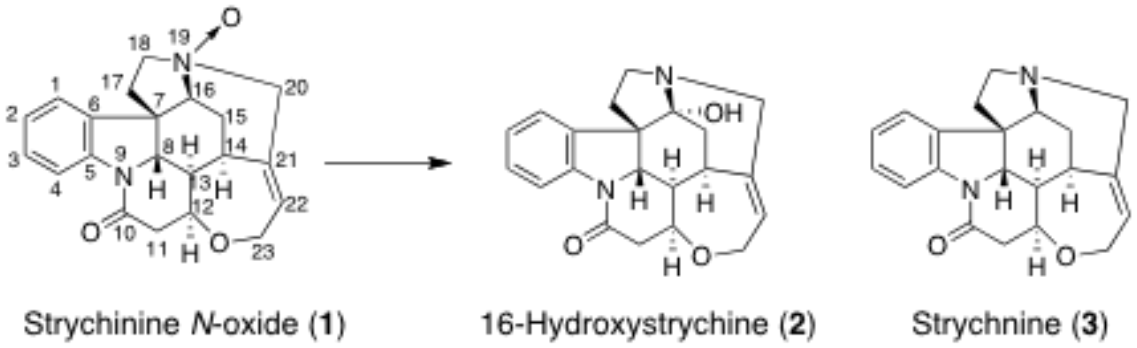
Strychinine *N*-oxide



Transformation of strychinine *N*-oxide by human intestinal bacteria

代謝実験

腸内細菌代謝　ヒト腸内細菌株　*Lactobacillus acidophilus*

Screening of bacterial strains for their ability to metabolize strychnine *N*-oxide (1)

Each precultured bacterial suspension (10 ml) was added to 100 ml of GAM broth (pH 7.3) and the mixture was incubated under anaerobic conditions at 37 °C for 24 h. The culture was centrifuged at 7800 x g for 5 min and the pellets were washed 2 times with saline and with 0.1 M phosphate buffer (pH 7.3). The bacterial cells were suspended in 0.1 M phosphate buffer (10 ml, pH 7.3) and 10 mg of strychnine *N*-oxide (**1**) in DMSO (0.1 ml) were added to each bacterial suspension. The mixture was anaerobically incubated for 48 h, and adjusted with 8 % Na_2CO_3 to pH *ca*. 9, then extracted with EtOAc (10 ml x 2). The extract was evaporated *in vacuo* to give a residue. The residue was dissolved in $CHCl_3$-MeOH (1:1 v/v, 400 μl) and the metabolites were quantitatively analyzed by HPLC using Develosil ODS Ph 5 C18 column (Nomura Co. Ltd., Seto, Japan, 250 mm x 4.5 mm i.d.); mobile phase, CH_3CN–0.03M Na_2HPO_4 (55:45); flow rate, l.0 ml/min; injection volume, 1 μl; detection, 254nm. The metabolites were analyzed quantitatively using calibration lines pre pared with authentic samples. [El-Mekkawy *et al., Planta Med*., **59**, 347-350 (1993)]

Incubation of strychnine *N*-oxide (1) with *Lactobacillus acidophilus*

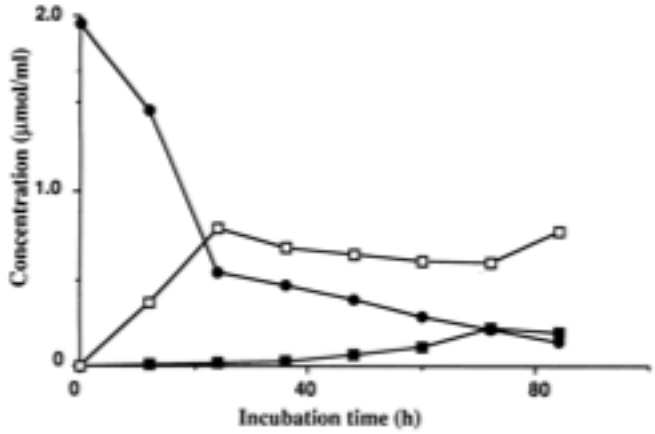
A precultured bacterial suspension (100 ml) of *L. acidophilus* was added to GAM broth (900 ml) and the mixture was cultured for 24 h at 37 °C under anaerobic conditions. The culture was centrifuged at 7800 x g for 5 min. The pellets were washed twice with saline and with 0.1 M phosphate buffer, and suspended in 0.1 M phosphate buffer (500 ml, pH 7.3). Strychnine *N*-oxide (500 mg, **1** in 5 ml of DMSO) was added to the bacterial suspension and the mixture was anaerobically incubated for 48 h at 37 °C, adjusted to pH *ca*. 9 and extracted with EtOAc (500 ml x 4). The combined solutions were evaporated to dryness *in vacuo* and the residue was applied to a column of silica gel (35 cm x 2 cm i.d.). Successive elution with hexane–$CHCl_3$–Et_2NH (9:1:0.3 and 2:8:0.3) and subsequent preparative TLC (solvent system A) afforded two metabolites (**2** and **3**). [El-Mekkawy *et al., Planta Med*., **59**, 347-350 (1993)]

Compound 2 (16-Hydroxystrychinine)

Colourless needles from MeOH (38 mg, 7.6 %), mp 270- 272 °C. $[\alpha]_D$: – 50° (c 0.05; $CHCl_3$–MeOH). MS *m/z* (rel. int.): 350 (M^+, 56%), 334 (100%). IR ν_{max} (KBr) cm^{-1}: 3400 (OH, 1650 (lactam). UV λ_{max} nm (EtOH) (log ε): 254 (4.15), 280 (3.7), 288 (3.5). The ^{1}H- and ^{13}C-NMR spectra were super imposed over that of authentic 16-hydroxy strychinine (pseudostrychnine). [El-Mekkawy *et al., Planta Med*., **59**, 347-350 (1993)]

Compound 3 (Strychinine)

Colourless needles from MeOH (18 mg, 3.6%), mp 265-266°C. $[\alpha]_D$: –153° (c 0.05; $CHC1_3$–MeOH). MS *m/z* (rel. int.): 334 (M^+, 100%). IR ν_{max} (KBr) cm^{-1}: 1655 (lactam). UV λ_{max} nm (EtOH) (log ε): 253 (4.3), 280 (3.8), 288 (3.7). The ^{1}H- and ^{13}C-NMR spectra were in agreement with the reported data for strychnine. [El-Mekkawy *et al., Planta Med*., **59**, 347-350 (1993)]



Time course for the metabolism of strychinine *N*-oxide (1)

A precultured bacterial strain of *L. acidophilus* was inoculated into GAM broth (500 ml) and cultivated for 24 h at 37 °C. The culture was centrifuged at 7800 x g for 10 min. The pellets were suspended in 0.1 M phosphate buffer (50 ml, pH 7.3). Strychinine *N*-oxide (**1**, 50 mg each) was added to each bacterial suspension and the mixture was incubated at 37 °C under anaerobic conditions. A 5 ml aliquot was taken at intervals, made basic, and extracted twice with EtOAc (5 ml). The EtOAc solution was evaporated to dryness, and the residue was dissolved in $CHCl_3$–MeOH (1:1 v/v, 200 μl) and analyzed by HPLC. [El-Mekkawy *et al., Planta Med*., **59**, 347-350 (1993)]

参考文献

1) El-Mekkawy S., Meselhy M. R., Kawata Y., Kadota S., Hattori M., and Namba T.: Metabolism of strychnine *N*-oxide and brucine *N*-Oxide by human intestinal bacteria. *Planta Med*., **59**, 347-350 (1993).