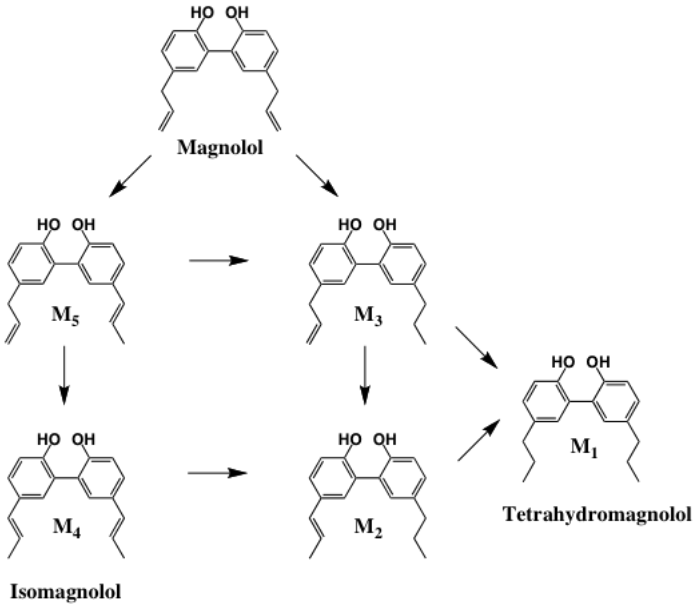
Magnolol



Metabolic processes of magnolol in rats

代謝実験
腸内細菌代謝　ヒト腸内細菌フローラ
動物実験　ラット
単一化合物　magnolol、[ring–^{14}C]magnolol

Administration of magnolol to rats

A suspension of magnolol in arabic gum (2 ml, 25 mg/ml) was orally administered daily (at 4:30 p.m.) to the rats (10 male, Wistar rats, 4 weeks of age, weighing 218—252 g) for 6d. During the experiments, food and drinking water were allowed *ad libitum*. The feces and urine excreted were collected daily (at 4:30 p.m.). [Hattori *et al*., *Chem. Pharm. Bull*., **32**, 5010-5017 (1984)]

Isolation of the fecal metabolites

The feces (24—50 g), which had been collected at 24-h intervals after the administration of magnolol, were extracted three times with MeOH (200 ml) and the solution was concentrated *in vacuo*. The residue was suspended in water (200 ml) and

extracted three times with benzene (200 ml), which was then evaporated off *in vacuo*. The residue was dissolved in $CHC1_3$ (10 ml) and applied to a column of alumina (Merck, Art. 1077, 100g). The column was washed with MeOH (100ml) and eluted with BuOH–AcOH–H_2O (3:1:1). The eluate was evaporated to dryness *in vacuo,* dissolved in ether (200 ml) and washed three times with 5% Na_2CO_3 (100 ml), then dried over Na_2SO_4. The solution was concentrated, and the residue was dissolved in $CHCl_3$–MeOH (1:1) and adsorbed on C-18 SEP-PAK (Waters Associates, Milford, Mass.), which was eluted with CH_3CN–H_2O (1:1). The eluate was evaporated to dryness *in vacuo*, and the residue was dissolved in MeOH (1.0 ml). The solution was repeatedly chromatographed on TSK-ODS-120A (25 cm x 7.2 mm i.d.) at a pressure of 110 kg/cm^2. The metabolites, Ml, M2, M3, M4, M5 and M6, were isolated and the structures were determined on the basis of the physical properties and direct comparison with authentic samples. [Hattori *et al*., *Chem. Pharm. Bull*., **32**, 5010-5017 (1984)]

M1: colorless prisms from benzene–hexane, mp 142—143 °C, EI-MS *m/z*: 270 (M^+, base peak), UV λ_{max}: 291 nm. Ml was identical with an authentic sample of tetrahydromagnolol on the basis of comparisons of mp, IR, ^{1}H-NMR (see Table I) and retention time of HPLC.

M2: EI-MS *m/z*: 268 (M^+, base peak), UV λ_{max}: 277 nm.

M4: EI-MS *m/z*: 266 (M^+, base peak), UV λ_{max}: 243 nm.

M5: EI-MS *m/z*: 266 (M^+, base peak), UV λ_{max}: 245, 291 nm.

M6: EI-MS *m/z*: 266 (M^+ , base peak), UV λ_{max}: 291 nm.

Incubation of magnolol with rat feces

Fresh feces (1 g) of rats were suspended in 104 volumes of dilution medium. An aliquot (0.3 ml) of the suspension was added to GAM broth (10 ml) containing 0.13 mg of magnolol and anaerobically incubated at 37 °C for 48 h. The culture was then extracted three times with benzene (10 ml). The benzene layer was evaporated to dryness *in vacuo* and the residue was dissolved in MeOH (0.3 ml). A 2 m1 aliquot of the methanolic solution was then analyzed by HPLC and LC-MS.

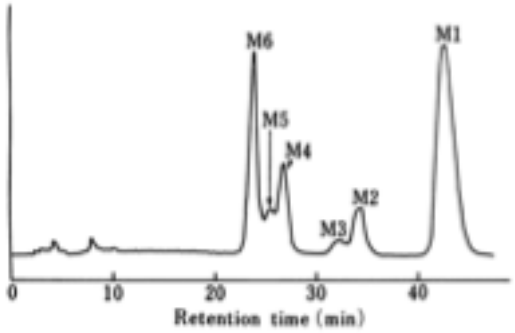


Fig. 1. A chromatogram of the fecal metabolites following oral administration of magnolol

HPLC was carried out by using a semi-micro column (25 cm x 1.5 mm i.d.) of μs-Finepak SIL C18; solvent, $CH_3CN–H_2O$ (50:50); flow rate, 100/d/min; ultraviolet trace at 250 nm; 3 ml of the sample was injected.

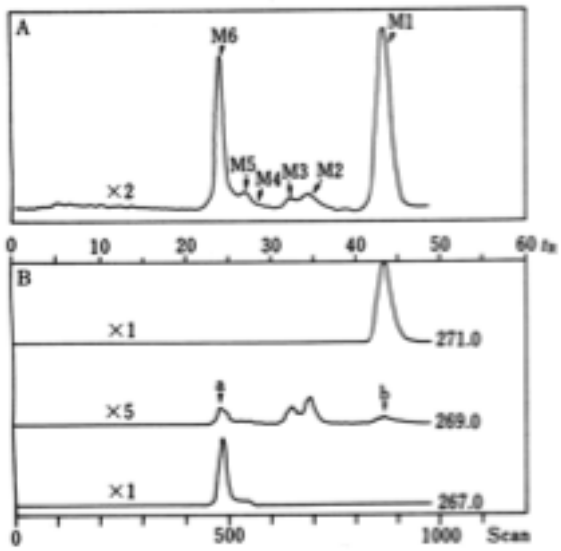


Fig. 2. A reconstructed ion current chromatogram (RIC, A) and mass chromatograms (B) of metabolites excreted in feces

A sample was chromatographed on the FAMILIC-300 system. The column was 25 cm x 1.5 mm i.d. μs-Finepak SIL C18 and the solvent was $CH_3CN–H_2O$ (50:50), flow rate 100 m1/min. The mass spectrometer was a JMS-D 300 equipped with a direct inlet LC–MS interface, operated in the positive chemical ionization mode, a, attributable to a

weak $(M + 3)^+$ ion of magnolol (4.3% of the QM^+ ion); b, attributable to a weak $(M-2)^+$ ion of tetrahydromagnolol (1.4% of the QM^+ ion). [Hattori *et al*., *Chem. Pharm. Bull*., **32**, 5010-5017 (1984)]

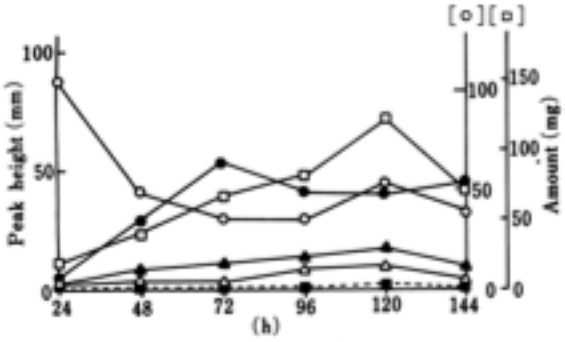


Fig. 3. Changes of the fecal metabolites following the repeated oral administrations of magnolol to rats

A dose of 50 mg was administered daily to each of ten rats and the feces were collected and combined at 24-h intervals. The benzene-soluble metabolites were analyzed by HPLC. Excreted amounts of Ml and M6 per 10 rats were calculated at 24-h intervals after administration (the right Y-axis). Variations in the amounts of other metabolites as well as Ml and M6 are also represented as relative changes in peak height (the left Y-axis). (□), Ml; (△), M2; (■), M3;(●), M4; (▲), M5; (○), M6. [Hattori *et al*., *Chem. Pharm. Bull*., **32**, 5010-5017 (1984)]

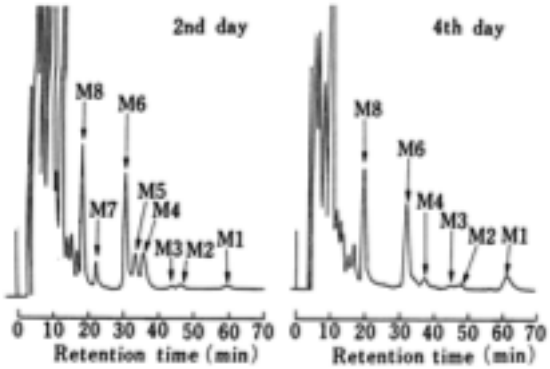


Fig. 4. HPLC chromatograms of the urinary metabolites following repeated oral administrations of magnolol

HPLC was carried out using a column (50 cm x 2.1mm i.d.) of TSK-ODS-120A; mobile phase, $CH_3CN–H_2O–AcOH$ (50:50:0.5); flow rate, 0.4 ml/min; ultraviolet trace ratio of the metabolites varies depending on the period after the repeated administrations of

magnolol. [Hattori *et al*., *Chem. Pharm. Bull*., **32**, 5010-5017 (1984)]

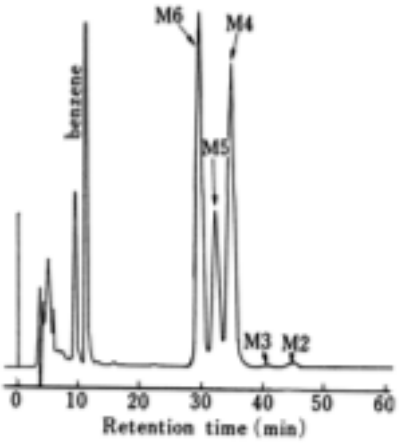


Fig. 5. A chromatogram of the metabolites obtained by *in vitro* incubation with rat feces

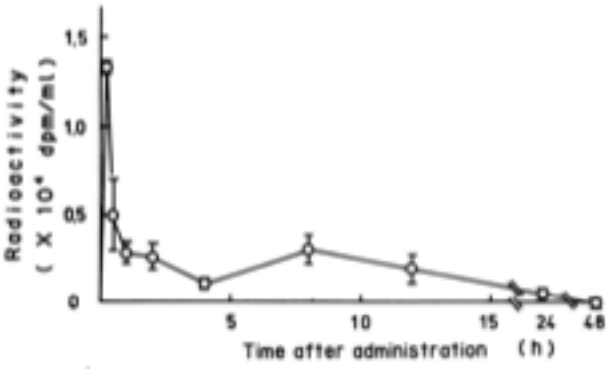


Fig. 6. Blood levels of radioactivity after oral administration of [ring–^{14}C]magnolol to rats (n = 3). [Hattori *et al*., *Chem. Pharm. Bull*., **34**, 158-167 (1986)]

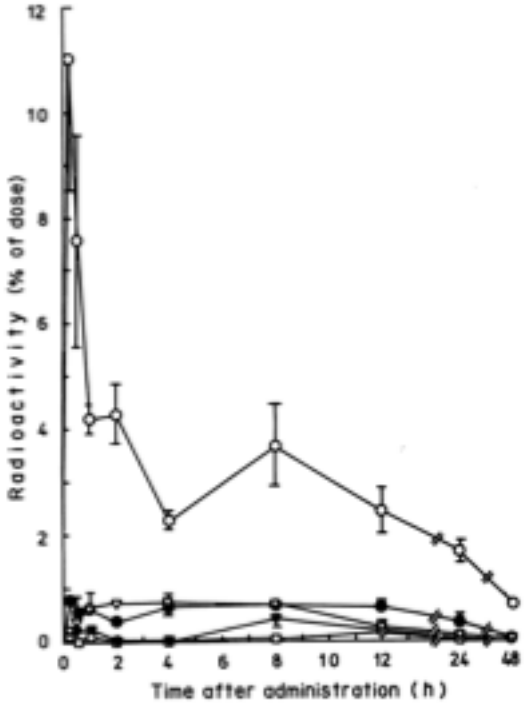


Fig. 7. Time courses of distribution of radioactivity in the organs after oral administration of [ring–^{14}C]magnolol to rats (n = 3)

(○), liver; (▽), pancreas; (●), kidney; (▼), lung; (□), heart. [Hattori *et al*., *Chem. Pharm. Bull*., **34**, 158-167 (1986)]

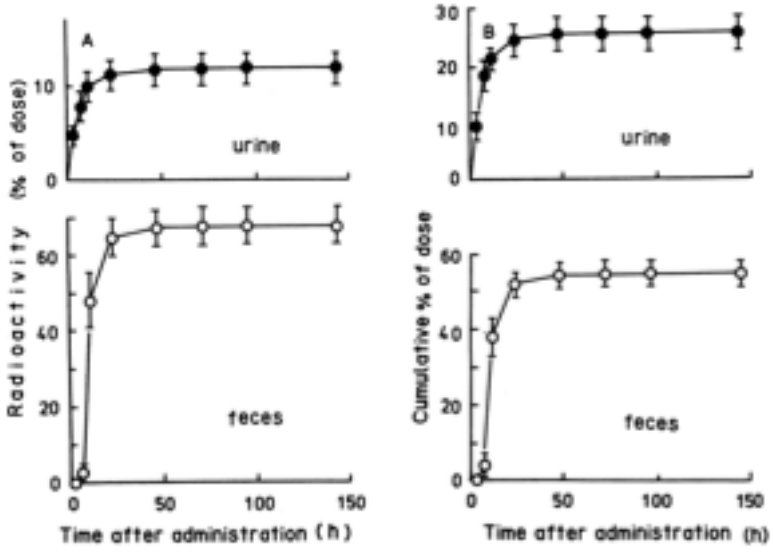


Fig. 8. Cumulative excretion of radioactivity in the feces and urine after oral and intraperitoneal administration of [ring–^{14}C]magnolol to rats (n = 6)

A, oral administration; B, intraperitoneal administration. [Hattori *et al*., *Chem. Pharm. Bull*., **34**, 158-167 (1986)]

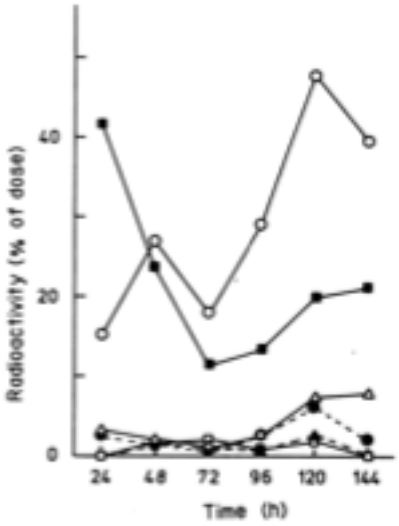


Fig. 9. Change of the fecal metabolites following repeated oral administration by [ring–^{14}C]magnolol to rats

(○), tetrahydromagnolol; (●), 5-((*E*)-l-propenyl)-5-propyl-2,2'-dihydroxybiphenyl; (▲), 5-allyl-5'-propyl-2,2'-dihydroxybiphenyl; (△), isomagnolol; (□), 5-allyl-5'-((*E*)-l-propenyl)-5,5'-dihydroxybiphenyl; (■), magnolol. [Hattori *et al*., *Chem. Pharm. Bull*., **34**, 158-167 (1986)]

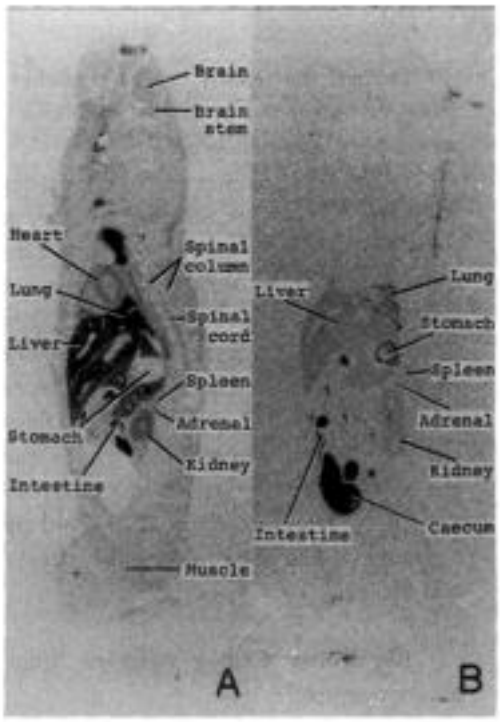


Fig. 10　Whole-body autoradiograms after intravenous administration of (ring-^{14}C) magnolol to rats.

A : one hour after administration ; B : eight hours after administration [Ma *et al*., *Shoyakugaku Zasshi*, **42**, 130-134 (1988)]

参考文献

1)　Hattori M., Sakamoto T., Endo Y., Kakiuchi N., Kobashi K., Mizuno T. and Namba T.: Metabolism of magnolol from Magnoliae Cortex. I. Application of liquid chromatography-mass spectrometry to the analysis of metabolites of magnolol in rats. *Chem. Pharm. Bull*., **32**, 5010-5017 (1984).

2)　Hattori M., Endo Y., Takebe S., Kobashi K. and Namba T.: Metabolism of Magnoliae Cortex. II. Absorption, metabolism and excretion of [ring-^{14}C] magnolol in rats. *Chem. Pharm. Bull*., **34**, 158-167 (1986).

3)　Ma Y. H., Ye J. N., Fukasaku N., Hattori M. and Namba T.: Metabolism of magnolol from Magnoliae Cortex. IV. Enterohepatic circulation and gastrointestinal excretion of [ring-^{14}C]- magnolol in rats. *Shoyakugaku Zasshi*, **42**, 130-134 (1988).