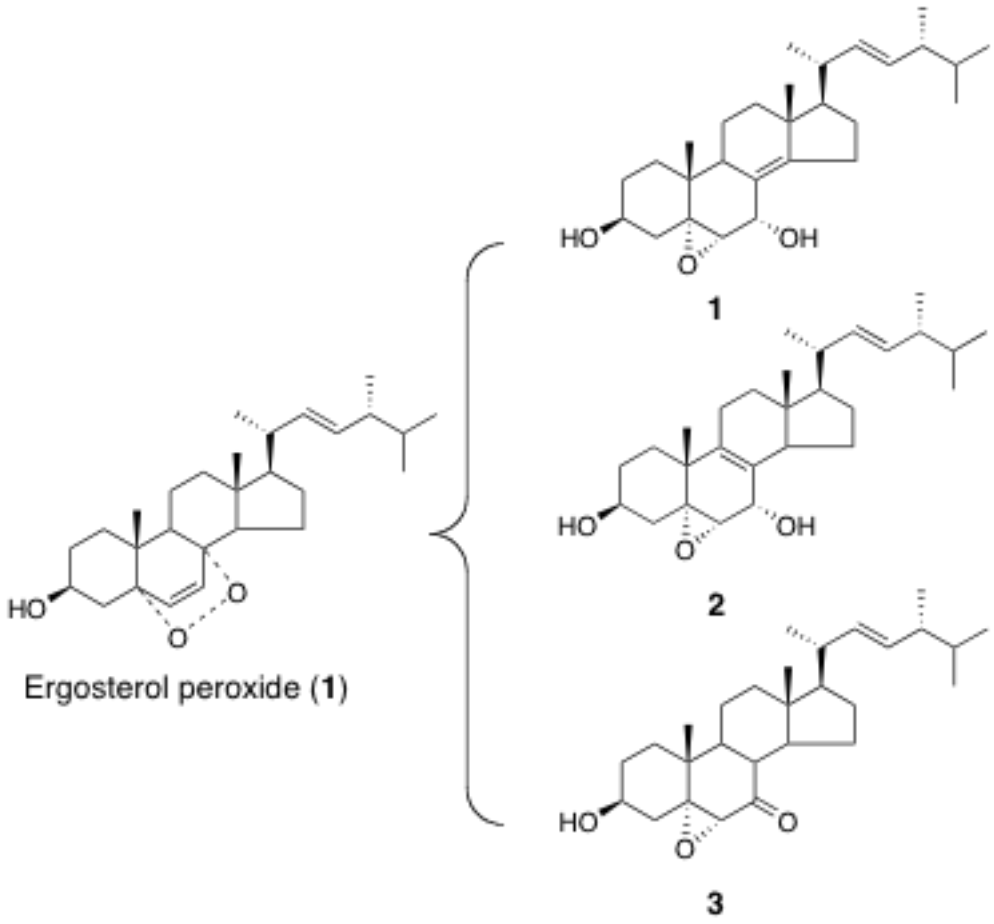
Ergosterol Peroxide



Transformation of ergosterol peroxide by rat fecal flora

代謝実験

腸内細菌代謝　ヒト及びラット腸内細菌フローラ

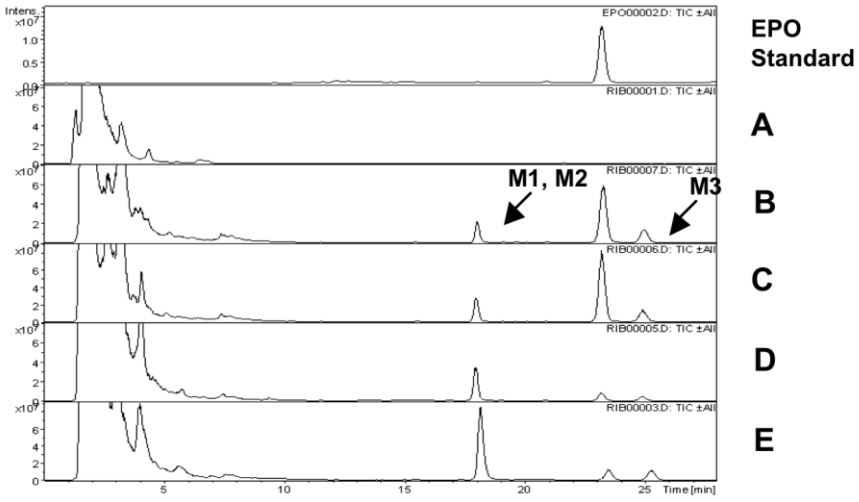


Fig. 1. Total ion current (TIC) chromatograms of cultures of rat intestinal flora with ergosterol peroxide (EPO).

EPO standard, blank (GAM + rat intestinal flora) (A), 1 hour after cultivation (B), 4 hours after cultivation (C), 8 hours after cultivation (D), 32 hours after cultivation (E).

Preparation of a bacterial mixture of rat feces

Fresh feces (5 g), obtained from male Wistar strain rats (8 weeks old, Sankyo Laboratory Service, Tokyo, Japan), was homogenized in 100 ml of GAM broth and the sediments were removed by cotton gauze to give a 5% rat intestinal bacterial (RIB) mixture. [Lee *et al.*, *Biol. Pharm. Bull.*, **31**, 949-954 (2008)]

LC-MS analysis for the transformation of EPO by rat intestinal bacteria (RIB)

Fifty μl of 100 mM EPO in MeOH and 500 μl of an RIB or human intestinal bacteria (HIB) mixture were added to 4.45 ml of GAM broth, and the mixture was incubated at 37°C under anaerobic conditions. A 200 μl aliquot was removed at intervals and extracted with ethyl acetate. After evaporation of ethyl acetate with nitrogen gas, the

residue was dissolved in 0.5 ml of MeOH. The MeOH solution was filtered through a 0.45 μm membrane filter, and a 10 μl portion was injected into a column for LC-MS analysis. [Lee *et al.*, *Biol. Pharm. Bull*., **31**, 949-954 (2008)]

Analytical conditions of LC/MS

HPLC separation was performed on a COSMOSIL 5C_{18}-MS-Ⅱ Waters column (particle size, 5 μm; 4.6×150 mm i.d.) (Nacalai Tesque, Kyoto, Japan). The mobile phase contained a gradient of water and MeOH (0 min, 1:1, 10 min, 1:9, 28 min, 1:9 (v/v)) at a flow rate of 1 ml/min at 35°C. The flow rate was reduced by a splitter to 0.2 ml/min for the MS detection. The standard positive ion mode was selected for measurement under the following conditions: full scan range, *m/z* 50 to 950; nebulizer, 50 psi; dry gas, 10.0 l/min; and dry temperature, 360°C, APCI-temperature, 450°C. Analysis mode was full scan acquisition resulting in a typical total ion current (TIC) plot. [Lee *et al.*, *Biol. Pharm. Bull*., **31**, 949-954 (2008)]

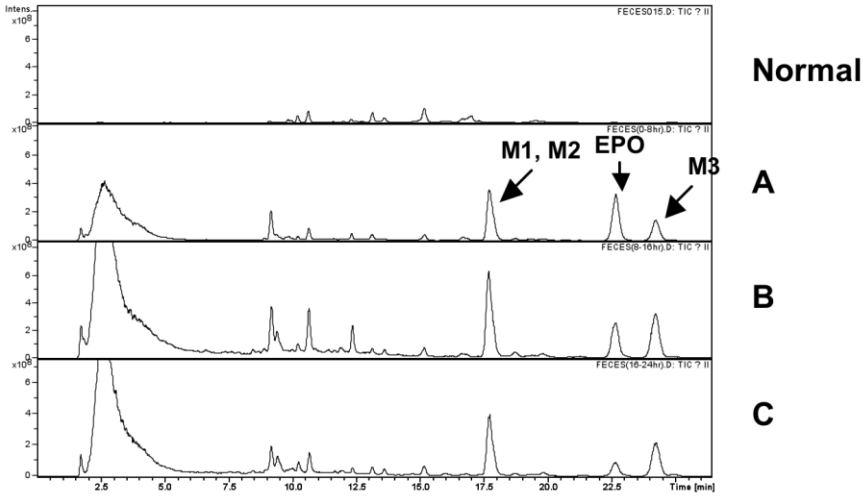


Fig. 2. TIC chromatograms of rat feces after p.o. administration of ergosterol peroxide (EPO).

EPO was administered at a dose of 100 mg/kg. Normal rat feces (Normal), 0—8 h after oral administration (A), 8—16 h after oral administration (B), 16—24 h after oral administration (C). [Lee *et al.*, *Biol. Pharm. Bull*., **31**, 949-954 (2008)]

参考文献

1) Lee J., Ma C., Park D., Yoshimi Y., Hatanaka M. and Hattori M.: Transformation of ergosterol peroxide to cytotoxic substances by rat intestinal bacteria. *Biol. Pharm. Bull*., **31**, 949-954 (2008).