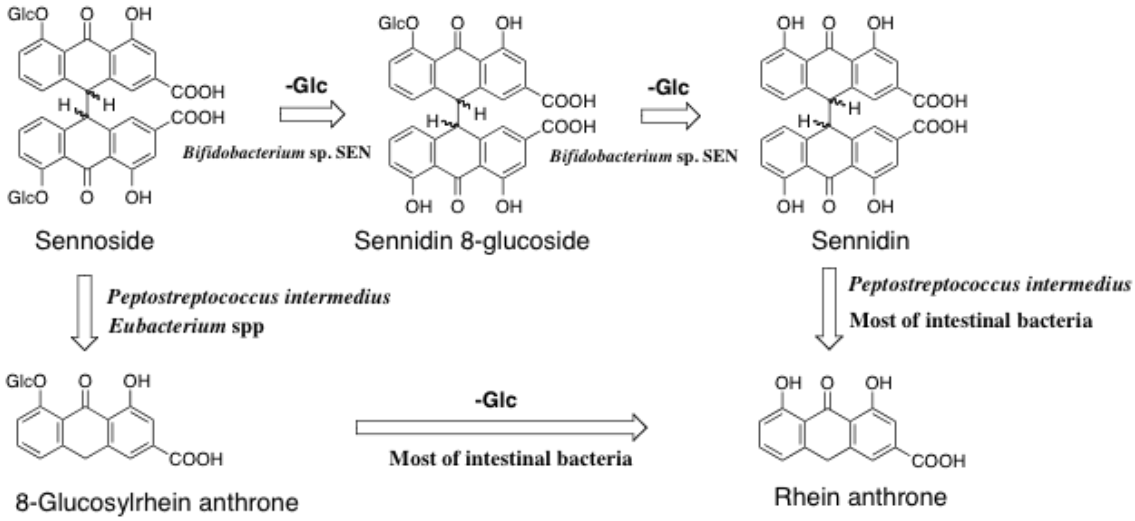
Sennoside A



Metabolic processes of sennoside by human intestinal bacteria

代謝実験

腸内細菌代謝　ヒト腸内細菌株 *Clostridium sphenoides, Eubacterium rectale*

動物代謝　ラット

単一化合物　sennoside A

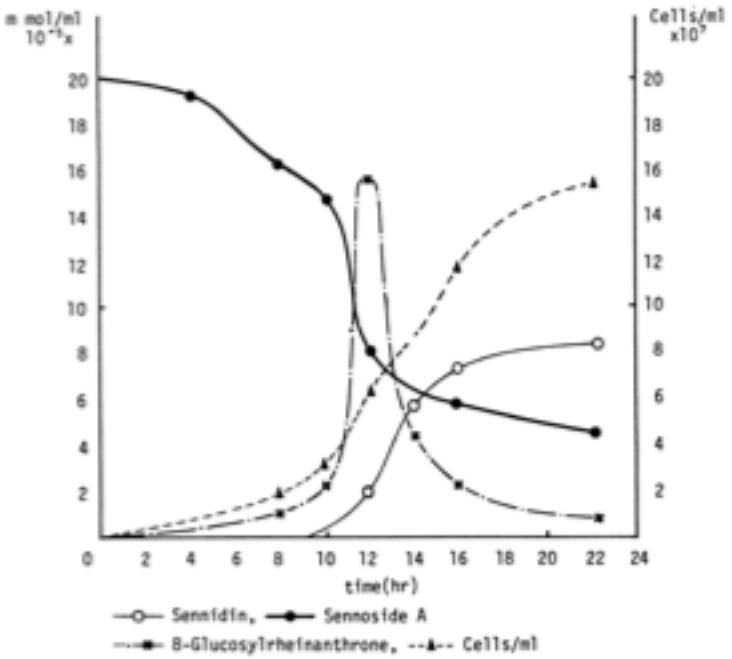


Fig. 1. Metabolism of sennoside A by *Clostridium sphenoides*.

Fifty ml of a sterilized GAM solution was mixed with 0.8 ml of a sterilized sennoside A solution (10 mg/ml). The culture medium (45 ml) was inoculated with 1.0 ml of *Clostridium sphenoides* precultured in GAM (2 x 10 viable cells/ml) and divided into 9 parts in test tubes (5.0 ml/tube). These tubes and one control tube without inoculation were incubated at 37° in an anaerobic jar in an atmosphere of CO_2 in the presence of activated steel wool. Test tubes were centrifuged at various times of incubation at 0° to obtain the supernatant fluid, which was kept in a frozen state. After 22 hours incubation, all the samples were quantitatively analyzed for metabolites by thin-layer chromatography/densitometry. [Kobashi *et al*., *Planta Med.*, **40**, 225-236 (1980)]

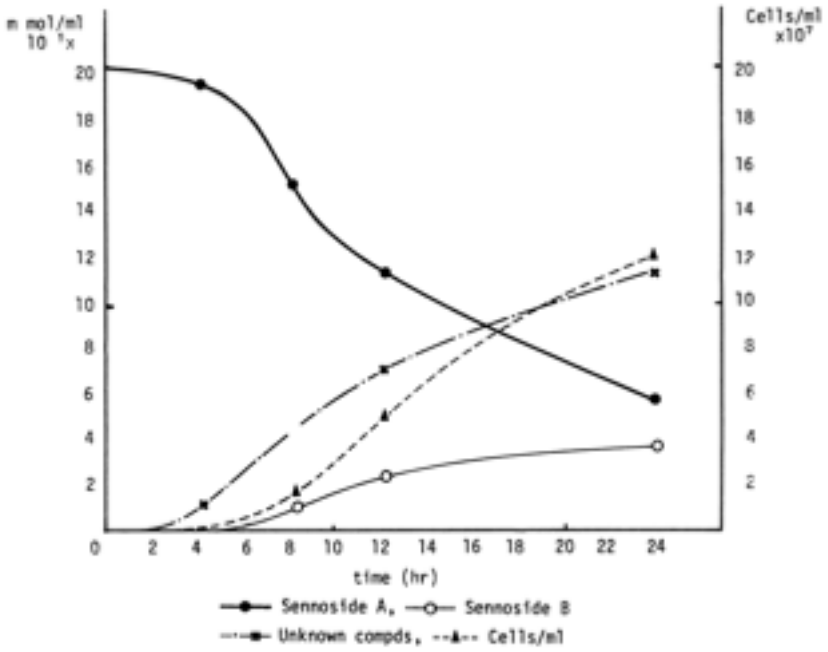


Fig. 2. Metabolism of sennoside A by *Eubacterium rectale*.
[Kobashi *et al*., *Planta Med.*, **40**, 225-236 (1980)]

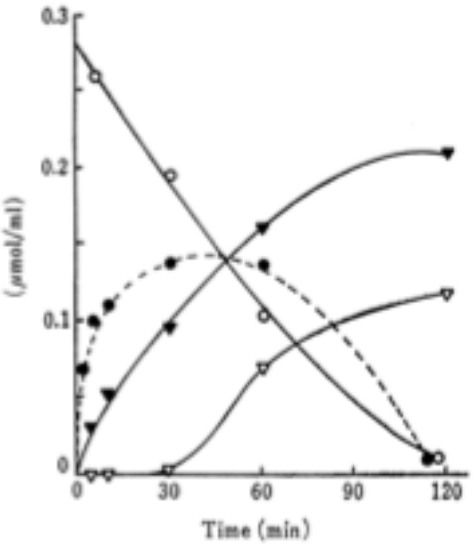


Fig. 3. Metabolism of sennoside A by a suspension of rat feces.

○, sennoside A; ●, sennidin A 8-glucoside; ▼, sennidin A; ▽, sennidin B
[Hattori *et al*., *Chem. Pharm. Bull*., **30**, 1338-1346 (1982)]

Preparation of a suspension of rat feces and its supernatant fluid

Fresh feces (20 g) of Wistar rats (female, 180—220 g body weight) were suspended in 100 mM phosphate buffer (200 ml, pH 7.3) containing 0.05% cysteine, which had previously been bubbled through with carbon dioxide to eliminate air. The supernatant fluid was prepared by centrifuging the suspension at 10000 rpm for 10 min.
[Hattori *et al*., *Chem. Pharm. Bull*., **30**, 1338-1346 (1982)]

Incubation of sennoside A with rat feces and quantitative analysis of its metabolites

To 5 ml of a suspension of rat feces was added 500 μl of a sennoside A solution (1 mg/ml, dissolved in 100 mM phosphate buffer, pH 7.3). After replacing air in the test tube with carbon dioxide, the mixture was incubated at 37°C for the indicated periods of time. The tube was then immediately cooled and centrifuged at 10000 rpm for 10 min. Next, 2% ethylenediaminetetraacetic acid (EDTA) (0.5 ml), 0.5 n HC1 (0.5 ml) and *n*-BuOH (2 ml) were added to 2 ml of the upper layer. After vigorous shaking, the mixture was centrifuged at 3000 rpm for 10 min to separate it into two layers. Five μl of the upper layer was applied on a silica gel thin-layer plate (Merck Silica gel 60 F254,

layer thickness 0.25 mm). The plate was then developed with a solvent system A, *n*-PrOH–AcOEt–H_2O (4: 4: 3, v/v) containing a few drops of AcOH. The spots on chromatogram were detected under ultraviolet (UV) light and analyzed quantitatively by using a Shimadzu CS-910 chromatoscanner (Shimadzu Seisakusho Ltd., Kyoto). [Hattori *et al*., *Chem. Pharm. Bull*., **30**, 1338-1346 (1982)]

Isolation of sennidin A 8-glucoside

Sennoside A (1 g) was incubated with a suspension (2.5 1) of rat feces at 37°C for 30—60 min, followed by centrifugation at 10000 rpm for 20 min. The supernatant fluid was acidified with hydrochloric acid to pH 3, and extracted with BuOH (2.5 1). The extract was washed with water, neutralized with triethylamine, and concentrated *in vacuo* below 37°C to a small volume (1—2 ml), which was then applied to a column (3 x 41 cm) of Sephadex LH 20 (Pharmacia Fine Chemicals) and eluted with 70% methanol. Fractions of 2 ml/tube were collected, and the absorbance at 370 nm was monitored. Fractions were pooled and evaporated to dryness *in vacuo*. Peak I contained substances mostly derived from feces. Peak II and Peak IV were identified as sennoside A and sennidin A, respectively, by comparing R_f values on TLC and absorption spectra with those of authentic samples. Peak III was further purified by repeated Sephadex LH 20 column chromatography to give 2 mg of a chromatographically homogeneous compound (sennidin A 8-glucoside): R_f =0.43 on TLC developed with solvent system A, UV-VS: λ_{max}, pH 1: 218, 270, 300 (shoulder), 387 nm; pH 7: 220, 270, 365 nm; pH 13: 225, 297 (shoulder), 422 nm. [Hattori *et al*., *Chem. Pharm. Bull*., **30**, 1338-1346 (1982)]

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