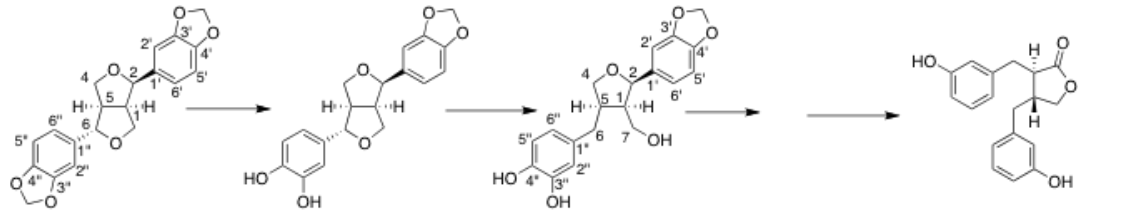
Asarinin



Metabolic processes of asarinin by human intestinal microflora

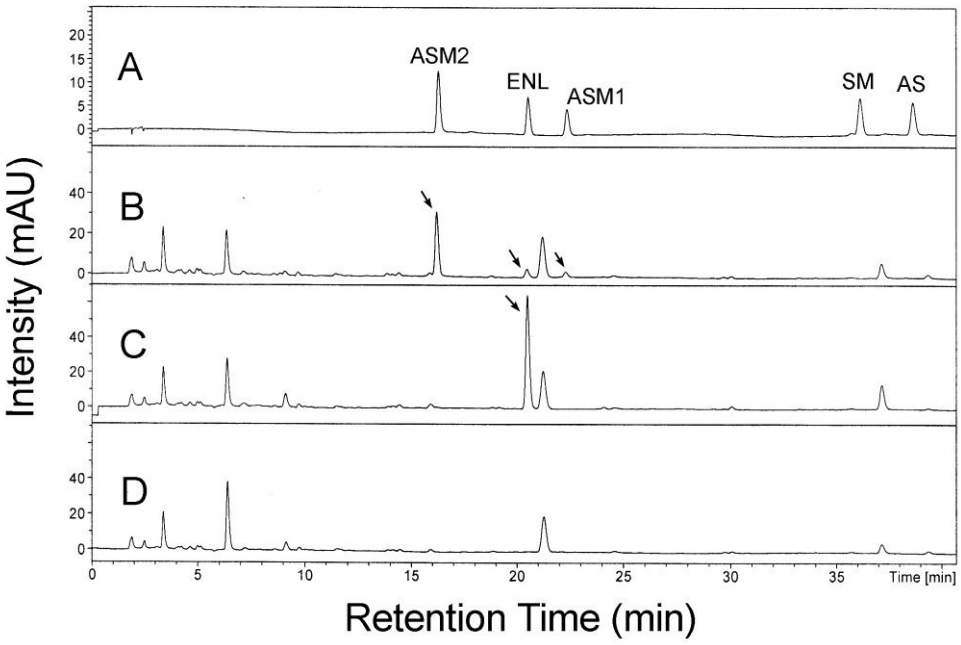


Fig. 1. HPLC elution profiles of incubation mixtures of (+)-asarinin (AS) and (+)-sesamin (SM) by human intestinal bacteria.

A, standard of (+)-AS, (-)-SM, and their metabolites; B, incubation mixture of (+)-AS; C, incubation mixture of (+)-SM; D, blank. Portions of the culture were collected at 72 h. HPLC was performed on an Agilent 1100 system (Agilent Technologies, Waldbronn, Germany) with a photodiode array detector and Agilent 1100 series binary pump, and an

Esquire 3000plus mass spectrometer (Bruker Daltonic GmbH, Bremen, Germany) coupled with an ESI interface and an ion trap mass analyzer. Analysis of transformation products of AS and SM was performed under the following conditions: column, TSK-gel ODS-80Ts (Tosoh Co., Tokyo, Japan, 4.6 mm × 150 mm); mobile phase, 0.1 % trifluoroacetic acid (solvent system A) and CH_3CN (solvent system B) in a gradient mode (B from 20 to 60% in 40 min); flow rate, 1.0 ml/min; detection, UV 280 nm; temperature, 30˚C. High-purity nitrogen was used as dry gas at a flow rate at 10 L/min, dry temperature at 360˚C. Helium was used as nebulizer at 50 psi.

Metabolic experiment

Fresh feces (5 g), obtained from a healthy subject was homogenized in 20 ml of GAM broth and the sediments were removed by decantation to give a 20% HIB mixture.

A 100 µl portion of a 20% HIB mixture was inoculated to 2 ml of GAM broth containing 0.2 mM (+)-AS or (+)-SM and anaerobically incubated at 37˚C for 72 h. A 100 µl aliquot was then taken out and extracted three times with 200 µl of ethyl acetate. After evaporation of the ethyl acetate *in vacuo*, the residue was dissolved in 50 µl of MeOH. The MeOH solution was filtered through a 0.2 µm membrane filter, and a 10 µl portion was injected onto a column for HPLC analysis.

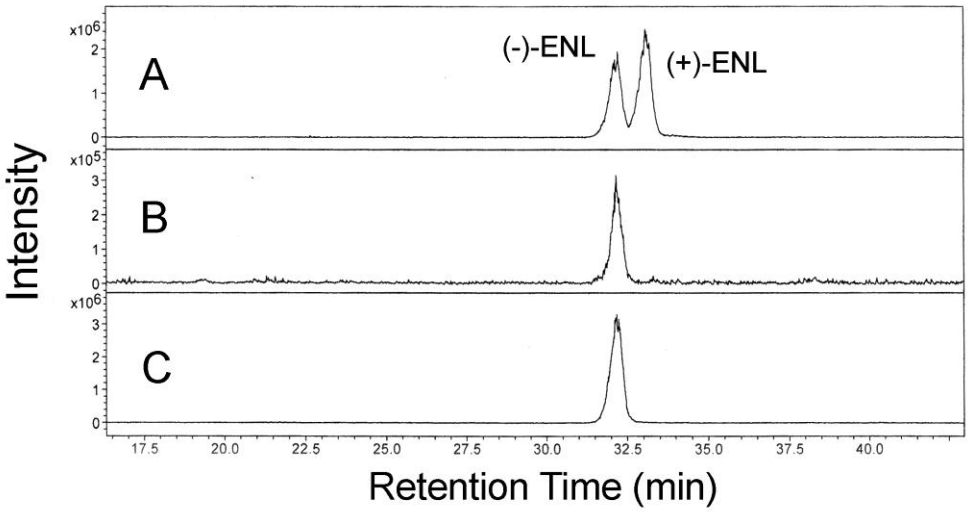


Fig. 2. Chiral LC-MS extracted ion chromatogram profiles of enterolactones (ENLs) converted from (+)-SM and (+)-AS.

A, standard of (+)- and (-)-ENLs; B, ENL from (+)-AS; C, ENL from (+)-SM. Portions of the culture were collected at 72 h. Analysis of (+)- and (-)-ENL was performed under the following conditions: column, chiral CD-Ph (Shiseido, Tokyo, Japan, 4.6 mm × 250 mm); mobile phase, 0.1 % TFA (solvent system A) and CH_3CN (solvent system B) in a gradient mode (B from 30 to 48 % for 36 min); flow rate, 0.5 ml/min; detection, UV at 280 nm; temperature, 30˚C.

Table 1. ^{1}H and ^{13}C NMR spectral data of metabolites from asarinin (AS)

Chemical shifts of metabolites					
ASM1			ASM2		
Position	δ_C	δ_H (*J*=Hz)	Position	δ_C	δ_H (*J*=Hz)
1	51.2	3.37 m	1	49.8	2.52 m
2	83.3	4.83 d (10.0)	2	84.2	5.05 d (6.5)
4a	71.9	3.81 dd (10.5, 15.5)	4a	72.8	3.79 t (7.5)
4b		4.06 d (15.5)	4b		3.84 t (8.0)
5	55.6	2.89 m	5	45.7	2.80 m
6	89.4	4.33 d (12.0)	6a	59.6	2.52 m
8a	70.4	3.22 dd (13.5, 14.5)	6b		2.89 dd (5.0, 13.5)
8b		3.76 t (14.5)	7a and 7b	34.6	3.36 dd (1.5, 5.5)
1′	133.8	−	1′	134.8	−
2′	107.3	6.87 m	2′	107.7	6.87 d (1.5)
3′	149.0	−	3′	147.9	−
4′	148.0	−	4′	148.8	−
5′	108.9	6.78 d (13.0)	5′	108.6	6.77 d (8.5)
6′	119.9	6.84 m	6′	120.3	6.81 dd (1.5, 8.5)
1″	113.8	−	1″	133.8	−
2″	114.4	6.80 d (3.0)	2″	116.6	6.67 d (2.0)
3″	146.1	−	3″	144.3	−
4″	146.4	−	4″	146.0	−
5″	116.1	6.73 d (13.0)	5″	116.2	6.68 d (8.0)
6″	118.9	6.68 dd (3.0, 13.0)	6″	120.7	6.55 dd (2.0, 8.0)
-O-CH_2-O-	102.2	5.93 s	-O-CH_2-O-	102.1	5.92 s