Metabolism of Aloesin and Related Compounds by Human Intestinal Bacteria: A Bacterial Cleavage of the *C*-Glucosyl Bond and the Subsequent Reduction of the Acetonyl Side Chain

Qing-Ming CHE,[a] Teruaki AKAO,[b] Masao HATTORI,*[,a] Kyoichi KOBASHI,[b] and Tsuneo NAMBA[a]

Research Institute for Wakan-Yaku (Traditional Sino-Japanese Medicines)[a] and Department of Pharmaceutical Sciences,[b] Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan. *Received August 10, 1990*

By anaerobic incubation with a bacterial mixture from human feces, aloesin (aloeresin B; 1) was converted to 2-acetonyl-7-hydroxy-5-methylchromone (aloesone; 3) and *dl*-7-hydroxy-2-(2′-hydroxypropyl)-5-methylchromone (aloesol; 4a + 4b) through a cleavage of the *C*-glucosyl bond, followed by reduction of the acetonyl side chain. An analogous compound, aloeresin A (2), was converted to *p*-coumaric acid and aloesin (1), the latter being subsequently transformed to aloesone (3) and *dl*-aloesol (4a + 4b). On the other hand, 7-*O*-methylated derivatives (7, 5a and 5b) of aloesin and of 8-*C*-glucosylaloesol were not cleaved to the corresponding aglycones, suggesting the importance of a free hydroxy group adjacent to the *C*-glucosyl group in the molecule for the bacterial cleavage of aloesin derivatives. This is the first report on the cleavage of the *C*-glycosyl bond of chromone *C*-glucosides by intestinal bacteria.

Keywords aloe; aloesin; aloeresin A; aloesol; aloesone; *C*-glycosylchromone; *C*-glycosyls cleavage; human intestinal bacteria; metabolism

Five types of *C*-glycosyls, namely *C*-glycosides of anthrones, flavonoids, xanthones, chromones and gallic acids, are known to occur in a variety of plants, and they are resistant to acidic hydrolysis and enzymatic hydrolysis in contrast with the corresponding *O*-glycosyls.[1]

In the course of our studies on the metabolism of crude drug components by intestinal bacteria, we have found that barbaloin (anthrone *C*-glycoside),[2] homoorientin (flavonoid *C*-glycoside),[3] mangiferin (xanthone *C*-glycoside)[4] and bergenin (*C*-glycosylated gallic acid)[4] are transformed to the corresponding aglycones by human intestinal bacteria. In addition, we have isolated an *Eubacterium* species capable of transforming barbaloin to aloe-emodin-9-anthrone.[5]

In the present paper, we report the cleavage of the *C*-glycosyl bond of a chromone *C*-glycoside, aloesin (formerly aloeresin B, **1**) and aloeresin A (**2**) from aloe,[6-9] and the subsequent reduction of the acetonyl side chain by human intestinal bacteria.

Results

Analysis of Bacterial Metabolites of Aloesin (1) and Aloeresin A (2) by High-Performance Liquid Chromatography (HPLC) Aloesin (**1**) and its 2″-*O*-(*E*)-*p*-coumaroyl derivative, aloeresin A (**2**), were anaerobically incubated with a bacterial mixture from human feces. Five days after incubation, BuOH extracts of the respective incubation mixtures were analyzed by means of HPLC using an octadecyl silica (ODS) column. Figure 1 shows the typical elution profiles of the extracts. Aloesin (**1**) was converted to two metabolites, while aloeresin A (**2**) was converted to four metabolites including aloesin (**1**), two of which had identical retention times ($t_R = 4.3$ min and $t_R = 5.3$ min) with those obtained in the metabolism of aloesin (**1**).

Isolation and Identification of the Metabolites For the purpose of determining the structures of metabolites, aloesin (**1**) was anaerobically incubated in a large scale with a bacterial mixture from human feces in an anaerobic box, and the products were extracted with an organic solvent

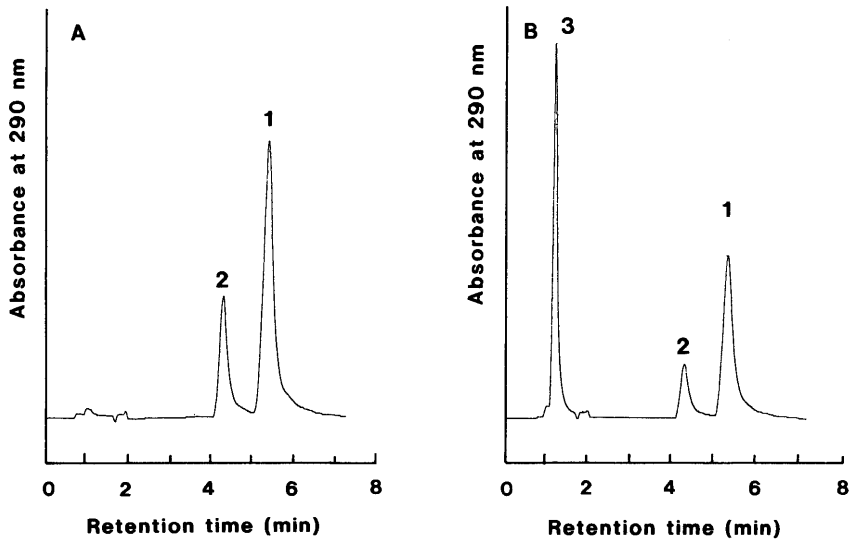


Fig. 1. Elution Profiles of the Metabolites of Aloesin (**1**) (A) and Aloeresin A (**2**) (B) Obtained by Anaerobic Incubation with a Bacterial Mixture from Human Feces

The metabolites were extracted with BuOH and analyzed by HPLC. HPLC was performed with an ODS-5 column (Nomura Chem. Co.) under conditions: mobile phase, CH_3CN–H_2O (1 : 3); flow rate, 1.0 ml/min; detection at 290 nm. 1, metabolite A (aloesone, **3**); 2, metabolite B (*dl*-aloesol, **4a** + **4b**); 3, metabolite C (*E*- and *Z*-*p*-coumaric acids).

TABLE I. ^{13}C-NMR Spectral Data for Metabolites and Aloesin Derivatives

Carbon	3[a]	4[b]	5a[a]	5b[a]	7[a]	8[c]
2	161.0 (s)	167.9 (s)	159.5 (s)	159.4 (s)	160.6 (s)	159.6 (s)[d]
3	112.9 (d)	113.3 (d)	111.1 (d)	111.0 (d)	112.4 (d)	113.7 (d)
4	178.1 (s)	182.7 (s)	178.6 (s)	178.6 (s)	178.7 (s)	179.1 (s)
5	141.6 (s)	144.5 (s)	140.0 (s)	139.9 (s)	141.1 (s)	144.1 (s)
6	116.6 (d)	118.8 (d)	116.2 (d)	116.2 (d)	115.8 (d)	118.5 (d)
7	160.5 (s)	163.8 (s)	164.4 (s)	164.4 (s)	160.2 (s)	159.0 (s)[d]
8	100.5 (d)	102.6 (d)	110.8 (s)	110.8 (s)	111.8 (s)	106.2 (s)
9	159.2 (s)	162.3 (s)	157.7 (s)	157.6 (s)	157.2 (s)	159.6 (s)[d]
10	114.3 (s)	116.8 (s)	114.7 (s)	114.6 (s)	113.0 (s)	113.9 (s)
5-CH_3	22.3 (q)	23.9 (q)	22.5 (q)	22.4 (q)	22.8 (q)	23.1 (q)
7-O-CH_3					56.4 (q)	
1′	47.4 (t)	45.0 (t)	43.0 (t)	43.0 (t)	47.7 (t)	48.2 (t)
2′	202.7 (s)	67.2 (d)	64.0 (d)	64.0 (d)	202.3 (s)	200.7 (s)
3′	29.8 (q)	24.3 (q)	23.6 (q)	23.3 (q)	29.8 (q)	30.2 (q)
1″			73.7 (d)	73.6 (d)	72.8 (d)	68.1 (d)
2″			71.1 (d)	71.1 (d)	71.0 (d)	73.7 (d)
3″			78.7 (d)	78.6 (d)	78.7 (d)	73.7 (d)
4″			70.5 (d)	70.4 (d)	70.5 (d)	70.2 (d)
5″			81.5 (d)	81.5 (d)	81.5 (d)	76.6 (d)
6″			61.4 (t)	61.4 (t)	61.7 (t)	61.6 (t)
O-CO-CH_3						168.9 (s)
						169.4 (s)
						170.3 (s)
						170.6 (s)
O-CO-CH_3						20.7 (q) (×4)

Measured at 22.5 MHz in *a*) DMSO-d_6, *b*) CD_3OD or *c*) $CDCl_3$. *d*) Assignments may be interchanged in each column.

and separated into two metabolites (metabolites A and B) by means of column chromatography.

Metabolite A, $t_R = 5.3$ min on HPLC, was obtained as colorless needles, mp 218—221 °C. Its mass spectrum (MS) showed a molecular ion at *m/z* 232, corresponding to the molecular formula $C_{13}H_{12}O_4$. The proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra showed that the chromone nucleus and the acetonyl side chain were intact but the *C*-glucopyranosyl moiety was missing. The spectroscopic data agreed with those of aloesone (2-acetonyl-7-hydroxy-5-methylchromone, **3**).[10]

Metabolite B, $t_R = 4.3$ min on HPLC, was also obtained as colorless needles, mp 175—178 °C. Its MS showed a molecular ion peak at *m/z* 234, two mass units higher than that of metabolite A. The infrared (IR) spectrum showed no C=O stretching band due to the acetonyl group present in aloesin (**1**). The signals at δ 1.27 (3H, d, $J = 6.3$ Hz), 2.65 and 2.68 (each 1H, each dd, $J = 14.2$, 7.6 Hz and $J = 14.2$, 5.6 Hz, respectively), 4.19 (1H, m) in the ^{1}H-NMR spectrum indicated the presence of a CH_3–CH(OH)–CH_2– group in the molecule. On the basis of the above findings and the ^{13}C-NMR spectral data (Table I), the structure of metabolite B was determined as 7-hydroxy-2-(2′-hydroxypropyl)-5-methylchromone (aloesol). This compound was obtained as a racemic mixture ($[\alpha]_D$ 0°) though the optically active 2′*S*-form ($[\alpha]_D$ +38.4°) has been reported to occur in rhubarb.[11]

Similarly, anaerobic incubation of aloeresin A (**2**) with an intestinal bacterial mixture, followed by solvent extraction and column chromatography led to the isolation of four metabolites. These metabolites were identified as aloesin (**1**), aloesone (**3**), *dl*-aloesol (**4a**+**4b**) and *p*-coumaric acid on the basis of their spectral data. The last compound was determined to be a mixture of *E*- and *Z*-forms in a ratio of 9:1 by means of ^{1}H-NMR.

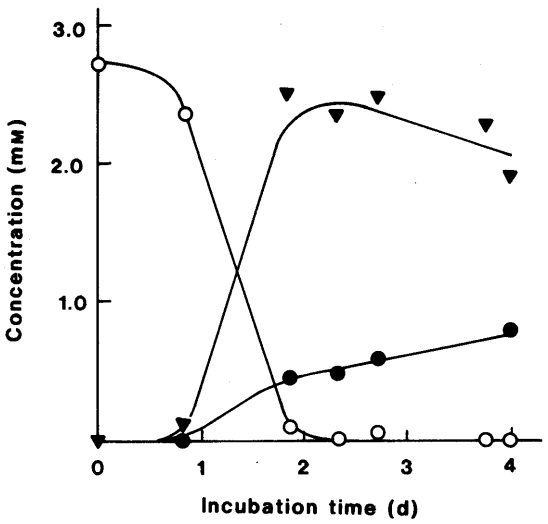


Fig. 2. Time Course of the Metabolism of Aloesin (**1**)

Aloesin (**1**) was anaerobically incubated with an intestinal bacterial mixture at 37 °C. The metabolites were analyzed by means of TLC-densitometry. (○) aloesin (**1**), (▼) aloesone (**3**), (●) *dl*-aloesol (**4a**+**4b**).

Time Courses of the Metabolisms of Aloesin (1) and Aloeresin A (2) by a Bacterial Mixture from Human Feces Figure 2 shows the time course of the bacterial transformation of aloesin (**1**). Aloesin (**1**) started to be converted to aloesone (**3**) and *dl*-aloesol (**4a**+**4b**) 20 h after incubation with the bacterial mixture, and disappeared completely within approx. 50 h as aloesone (**3**) reached a maximum concentration (*ca.* 80% in yield). Aloesone (**3**) then decreased in amount, while *dl*-aloesol (**4a**+**4b**) gradually increased. On further incubation, the amount of *dl*-aloesol (**4a**+**4b**) exceeded that of aloesone (**3**) as will be mentioned later. Transformation of aloesone (**3**) to *dl*-aloesol (**4a**+**4b**) was also confirmed by a separate

experiment; aloesone (3) itself was transformed slowly to *dl*-aloesol (**4a**+**4b**) through the entire time of incubation with an intestinal bacterial mixture in peptone yeast extract Fildes (PYF) broth, *ca.* 10% of the starting material being transformed to *dl*-aloesol (**4a**+**4b**) within 5 d. However, on anaerobic incubation with the bacterial suspension in phosphate buffer, *ca.* 60% of aloesone (3) was converted to *dl*-aloesol (**4a**+**4b**) within 3 d.

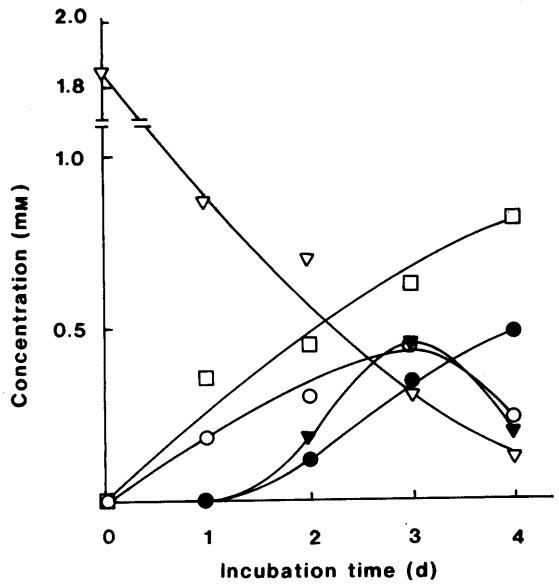


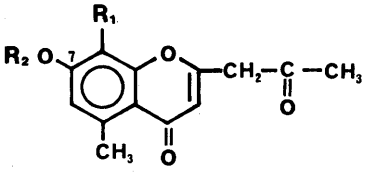
Fig. 3. Time Course of the Metabolism of Aloeresin A (2)

Aloeresin A (2) was anaerobically incubated with an intestinal bacterial mixture at 37 °C. The metabolites were analyzed by TLC-densitometry. (▽) aloeresin A (2), (□) *p*-coumaric acid, (○) aloesin (1), (▼) aloesone (3), (●) *dl*-aloesol (**4a**+**4b**).

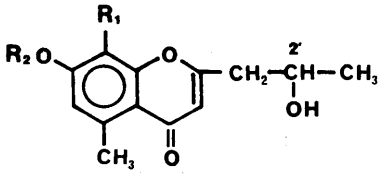
TABLE II. Transformation of Aloesin (1) and Related Compounds by Human Intestinal Bacteria

Substrate	Yield of metabolite (%)	
	Aloesone	Aloesol
Aloesin (**1**)	17	43[a]
Aloeresin A (**2**)	27	59[a]
Aloesone (**3**)		9[a]
(2′*R*)-8-*C*-Glucocylaloesol (**5a**)		49[b]
(2′*S*)-8-*C*-Glucosylaloesol (**5b**)		44[c]
(2′*R*)-8-*C*-Glucosyl-7-*O*-methylaloesol (**6a**)	0	0
(2′*S*)-8-*C*-Glucosyl-7-*O*-methylaloesol (**6b**)	0	0
7-*O*-Methylaloesin (**7**)	0	0
Aloesin 2″,3″,4″,6″-tetra-*O*-acetate (**8**)	0	0

Aloesin (1) and related compounds (2—8) (2—6 mM) were anaerobically incubated with a bacterial mixture from human feces in PYF medium for 3 d at 37 °C. The products were extracted with BuOH and quantitatively analyzed by TLC densitometry. *a*) *dl*-form; *b*) 2′*R*-form; *c*) 2′*S*-form.



1: R_1 = *C*-Glc, R_2 = H
2: R_1 = *C*-Glc—(2)—*p*-Coum., R_2 = H
3: R_1 = R_2 = H
7: R_1 = *C*-Glc, R_2 = Me
8: R_1 = *C*-Glc (Ac)$_4$, R_2 = H



4a: R_1 = R_2 = H (2′*R*)
4b: R_1 = R_2 = H (2′*S*)
5a: R_1 = *C*-Glc, R_2 = H (2′*R*)
5b: R_1 = *C*-Glc, R_2 = H (2′*S*)
6a: R_1 = *C*-Glc, R_2 = Me (2′*R*)
6b: R_1 = *C*-Glc, R_2 = Me (2′*S*)

Chart 1. Structures of Aloesin (1) and Related Compounds (2—8)

Figure 3 shows the time course of the transformation of aloeresin A (2). One day after incubation, aloesin (1) and *p*-coumaric acid were produced but no aloesone (3) and *dl*-aloesol (**4a**+**4b**) were detected in the incubation mixture, indicating that enzymic hydrolysis of the *p*-coumaroyl moiety proceeded prior to the cleavage of the *C*-glucosyl in aloeresin A (2). After that, aloesone (3) and *dl*-aloesol (**4a**+**4b**) appeared gradually. Aloesone (2) became its maximal concentration (*ca.* 25% in yield) 3 d after incubation and then decreased on further incubation, while *dl*-aloesol (**4a**+**4b**) was continuously produced during the incubation. *p*-Coumaric acid was also continuously liberated, accompanied by a decrease in the amount of aloeresin A (2).

Screening of Intestinal Bacterial Strains for Ability of Metabolizing Aloesin (1) Twenty different kinds of defined bacterial strains from human feces were assayed for their ability to metabolize aloesin (1). However, none of the bacteria examined could cleave the *C*-glycosyl bond of aloesin (1) and the substrate was completely recovered 2 d after cultivation with each bacterium in general anaerobic medium (GAM) or PYF broth (data not shown).

Metabolism of Aloesin Derivatives by Human Intestinal Bacteria For comparing substrate specificity in the cleavage reaction of the *C*-glucosyls and in the reduction of the acetonyl side chain, aloesin (1), aloeresin A (2) and various aloesin derivatives synthesized chemically were incubated with a bacterial mixture from human feces under similar conditions. Both (2′*R*)- and (2′*S*)-8-*C*-glucosylaloesols (**5a** and **5b**) were transformed to (2′*R*)- and (2′*S*)-aloesols (**4a** and **4b**) in yields of 49 and 44%, respectively, 3 d after incubation, while (2′*R*)- and (2′*S*)-8-*C*-glucosyl-7-*O*-methylaloesols (**6a** and **6b**), 7-*O*-methylaloesin (**7**) and aloesin 2″,3″,4″,6″-tetra-*O*-acetate (**8**) were not metabolized (Table II).

Discussion

Anaerobic incubation of aloesin (1) with fecal flora of humans resulted in the cleavage of the *C*-glycosyl bond of aloesin (1) to give aloesone (3), which was subsequently reduced to *dl*-aloesol (**4a**+**4b**) (Chart 2).

On the other hand, aloeresin A (2) was first subjected to hydrolysis of the (*E*)-*p*-coumaroyl moiety by bacterial esterase to give aloesin (1) and *p*-coumaric acid. The latter was obtained as a mixture of (*E*)- and (*Z*)-forms. The aloesin (1) produced was further metabolized to aloesone (3), *dl*-aloesol (**4a**+**4b**). The products were essentially not altered by repeated experiments but their relative ratios or

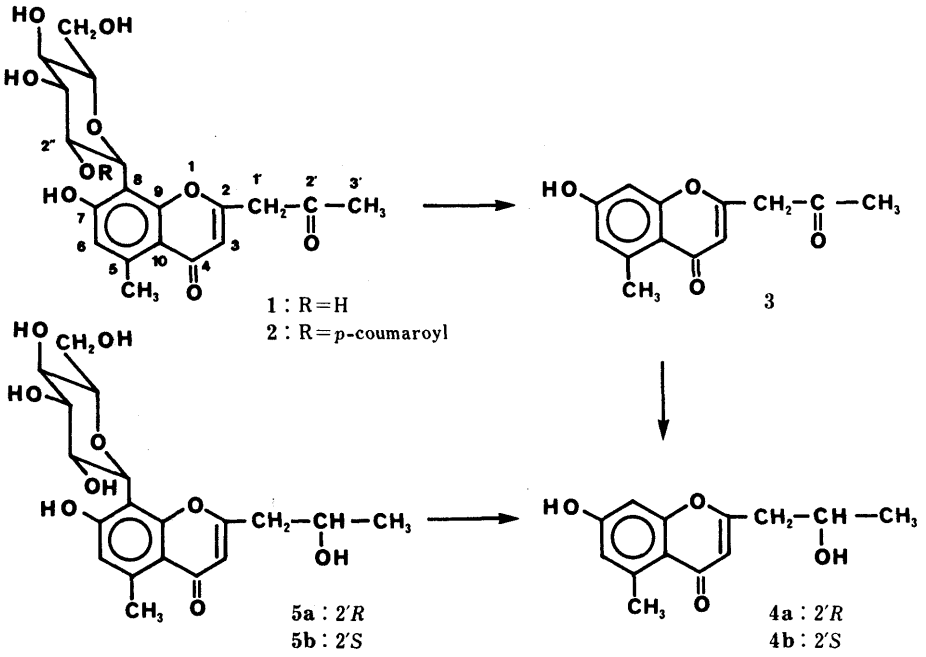


Chart 2. Metabolic Processes of Aloesin (**1**) and Aloeresin A (**2**) by Human Intestinal Bacteria

yields varied significantly with feces, incubation time and culture media.

(2'*R*)- and (2'*S*)-8-*C*-glucosylaloesols (**5a** and **5b**) were metabolized to (2'*R*)- and (2'*S*)-aloesols (**4a** and **4b**), respectively, by fecal flora. However, tetraacetylaloesin (**8**) was not metabolized, possibly due to its sparing solubility in the medium and its poor permeability to bacterial cells. Furthermore, 7-*O*-methylaloesin (**7**) and (2'*R*)- and (2'*S*)-8-*C*-glucosyl-7-*O*-methylaloesols (**6a** and **6b**) were not transformed to the corresponding aglycones, suggesting the necessity of a free hydroxyl group adjacent to the *C*-glucosyl group for cleaving the *C*-glycosyl bond.

During the incubation of aloesin (**1**), no 8-*C*-glucosylaloesols (**5a** and **5b**) were detected in the metabolic mixture when analyzed by means of HPLC and thin-layer chromatography (TLC). Further, reduction of aloesone (**3**) to *dl*-aloesol (**4a**+**4b**) proceeded quite slowly in the separate experiment. These findings indicate that the cleavage of the *C*-glycosyl bond of aloesin (**1**) occurrs prior to the reduction of the acetonyl group by intestinal bacteria.

On the isomerization of (*E*)-form to (*Z*)-form in *p*-coumaric acid, it is not clear whether the reaction was proceeded by the action of intestinal bacteria or not, because this type of isomerization may be effected by exposure to light during incubation and extraction procedures.

An attempt to find out bacteria capable of metabolizing aloesin (**1**) from our stock strains of intestinal bacteria was unsuccessful but a preliminary experiment to isolate such bacteria from human feces revealed that some bacteria had potent ability to cleave the *C*-glycosyl bond of aloesin (**1**). As reported previously,[5] *Eubacterium* sp. BAR has potent ability to transform barbaloin to aloe-emodin-9-anthrone by cleaving the *C*-glycosyl bond, but this bacterium had no ability to metabolize aloesin (**1**). This suggests that intestinal bacteria produce some *C*-glycosyl-cleaving enzymes specific for individual substrates as mentioned in the previous paper.[5]

Experimental

Instruments Melting points were determined on a Yanagimoto micro-melting point apparatus and uncorrected. ^{1}H- and ^{13}C-NMR spectra were measured with JEOL JNM-FX 270 (^{1}H, 270 MHz) and JEOL JNM-FX 90Q (^{13}C, 22.5 MHz) spectrometers and chemical shifts are represented as δ values relative to tetramethylsilane (TMS). MS were measured with a JEOL JMS DX 300 mass spectrometer at an ionization voltage of 70 eV. Densitometric profiles of TLC were recorded on a Shimadzu CS-910 dual wavelength TLC scanner. Optical rotations were measured with a Jasco DIP-4 automatic polarimeter at 21 °C.

Chromatography Wakogel 200 (Wako Pure Chem. Ind. Co., Osaka) was used for column chromatography and Merck Kieselgel 60 F_{254} (E. Merck, Darmstadt; layer thickness, 0.25 mm) for TLC.

Chemicals Aloesin (**1**) and aloeresin A (**2**) were isolated from crude aloin powder (Wako Pure Chem. Ind. Co.) and purified by column chromatography on silica gel followed by crystallization from EtOH. 7-*O*-Methylaloesin (**7**) was prepared by treating aloesin (**1**) with diazomethane.[6]

Culture Media PYF solution broth was prepared according to the procedure of Mitsuoka.[12] GAM was purchased from Nissui Co. (Tokyo, Japan).

Reduction of Aloesin (1) with $NaBH_4$ $NaBH_4$ (2.0 g) was added to a solution of aloesin (**1**) (1.0 g) in MeOH (20 ml). The mixture was stirred for 3 h at room temperature and acidified to pH 3 with 2 N HCl. The solution was extracted with BuOH. The BuOH solution was evaporated to dryness *in vacuo* and the residue was chromatographed on silica gel (column size, 30 cm × 2 cm i.d.; solvent, $CHCl_3$–MeOH (6:4)) to give a mixture of (2'*R*)- and (2'*S*)-8-*C*-glucosylaloesols (**5a** and **5b**) in a yield of 900 mg. The diastereomers were separated by preparative HPLC under the following conditions: column, ODS-10 (Nomura Chem. Co.); mobile phase, CH_3CN–H_2O (3:40); flow rate, 2.0 ml/min; detection, 290 nm.

(2'*S*)-8-*C*-Glucosylaloesol (**5b**): t_R = 7.0 min on HPLC (MeOH). EI-MS *m/z*: 397 (M^++1). ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 1.13 (3H, d, J=6.1 Hz, 3'-H_3), 2.60 (1H, dd, J=5.9, 13.7 Hz, 1'-H_a), 2.63 (3H, s, 5-Me), 2.65 (1H, dd, J=7.3, 13.7 Hz, 1'-H_b), 3.1—5.0 (sugar-H), 4.10 (1H, m, 2'-H), 4.73 (1H, d, J=9.5 Hz, 1''-H), 5.98 (1H, s, 3-H), 6.64 (1H, s, 6-H). In an anaerobic incubation with a bacterial mixture from human feces, the compound was converted to an aglycone, (2'*S*)-aloesol: $[\alpha]_D$ +39.1° (c=0.11, MeOH). The optical rotation value was in agreement with that of the naturally occurring 2'*S*-form.[11]

(2'*R*)-8-*C*-Glucosylaloesol (**5a**): t_R = 8.1 min on HPLC. $[\alpha]_D$ −14.8° (c=0.13, MeOH). EI-MS *m/z*: 397 (M^++1). ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 1.16 (3H, d, J=6.1 Hz, 3'-H_3), 2.58 (2H, d, J=6.1 Hz, 1'-H_2), 2.63 (3H, s, 5-Me), 3.1—5.0 (sugar-H), 4.13 (1H, m, 2'-H), 4.73 (1H, d, J=9.5 Hz, 1''-H), 5.97 (1H, s, 3-H), 6.64 (1H, s, 6-H). In an anaerobic

incubation with a bacterial mixture from human feces, the compound was converted to an aglycone, (2′*R*)-aloesol: $[\alpha]_D$ −30° (*c*=0.31, MeOH).

Acetylation of Aloesin (1) Aloesin (**1**) was acetylated with acetic anhydride in pyridine to give 2″,3″,4″,6″-tetra-*O*-acetylaloesin (**8**). The physical and spectral data of **8** were as follows: EI-MS *m/z* (rel. int.): 562 (M^+, 20%), 340 (13%), 337 (30%), 261 (21%), 43 (100%). ^{1}H-NMR (270 MHz, $CDCl_3$) *δ*: 1.74 (3H, s, 3′-H_3), 2.03 (3H, s, OAc), 2.10 (3H, s, OAc), 2.17 (3H, s, OAc), 2.38 (3H, s, OAc), 2.78 (3H, br d, *J*=0.7 Hz, 5-H_3), 3.62, 3.70 (each 1H, AB q, *J*=16.2 Hz, 1′-H_2), 3.94 (1H, ddd, *J*=11.1, 2.2, 3.4 Hz, 5″-H), 4.16 (1H, dd, *J*=12.6, 2.2 Hz, 6″-H_a), 4.33 (1H, dd, *J*=12.6, 3.4 Hz, 6″-H_b), 5.2—5.4 (sugar-H), 6.07 (1H, s, 3-H), 6.69 (1H, d, *J*=0.7 Hz, 6-H).

Microorganisms The following intestinal bacterial strains from human feces were used for the screening of bacteria capable of metabolizing aloesin (**1**): *Bacteroides fragilis* ss. *thetaotus*, *Bifidobacterium adolescentis*, *B. breve* S-2 KZ 1287, *B. bifidum* a E319, *B. pseudolongum* PNC-2-9-G, *Clostridium butyricum*, *C. innocuum* ES 24-06, *C. perfringens* To-23, *Fusobacterium nucleatum* G-0470, *Gaffkya anaerobia* G-0608, *Klebsiella pneumoniae* ATCC 13883, *Lactobacillus acidophilus* ATCC 4356, *L. brevis* II-46, *L. fermentum* ATCC 9338, *L. plantarum* ATCC 14917, *L. xylosus* ATCC 155775, *Peptostreptococcus anaerobius* 0240, *P. intermedius*, *Proteus mirabilis* S2 and *Streptococcus faecalis* II-136.

Preparation of a Bacterial Mixture from Human Feces Fresh feces from a healthy man were thoroughly suspended in 50 volume of 0.2 M phosphate buffer (pH 7.2), filtered through layers of gauze to eliminate the sediment. The filtrate was used as an intestinal bacterial mixture in the following experiments.

Time Course of the Metabolism of Aloesin (1) by Intestinal Bacteria A tube containing **1** (10.7 mg) and an intestinal bacterial mixture (10 ml) was incubated at 37 °C in an anaerobic box. A portion (0.5 ml) of the mixture was taken out at intervals, and vigorously mixed with BuOH (0.5 ml). An aliquot of the BuOH layer was applied to a TLC plate and the plate was developed with $CHCl_3$–MeOH–H_2O (50 : 10 : 1). *Rf* values of aloesin (**1**), aloesone (**3**) and *dl*-aloesol (**4a**+**4b**) were 0.06, 0.59 and 0.44, respectively. The metabolites separated on the plate were quantitatively analyzed with a TLC scanner at 290 nm in the single scan mode. The calibration line was prepared with an authentic sample.

Time Course of the Metabolism of Aloeresin A (2) by Intestinal Bacteria Similar to the case of alosin (**1**), aloeresin A (**2**) was anaerobically incubated and the metabolites were extracted with BuOH. A portion of the extract was chromatographed on a silica gel plate with $CHCl_3$–MeOH–H_2O (50 : 10 : 1) and analyzed by densitometry. *Rf* values of the products were as follows: aloesone (**3**), 0.59; *dl*-aloesol (**4a**+**4b**), 0.44; *p*-coumaric acid, 0.20; aloesin (**1**), 0.06; aloeresin A (**2**), 0.13.

Incubation of Aloesin (1) with an Intestinal Bacterial Mixture Aloesin (1.0 g, **1**) was added to an intestinal bacterial mixture (500 ml) and incubated for 3 d at 37 °C in an anaerobic box. The mixture was extracted three times with BuOH (500 ml each). The combined BuOH solutions were evaporated *in vacuo* to give a residue (*ca.* 1.8 g). The residue was applied to a column of silica gel (20 cm × 3 cm i.d.; 45 g), which was eluted successively with $CHCl_3$, $CHCl_3$–MeOH (95 : 5) to give metabolites A and B (240 and 20 mg, respectively). The metabolites were further purified by crystallization from EtOH.

Metabolite A (2-Acetonyl-7-hydroxy-5-methylchromone, **3**): Colorless needles, mp. 218—221 °C, EI-MS *m/z* (rel. int.): 232 (M^+, 62%), 190 (86%), 161 (24%), 151 (42%), 43 (CH_3CO, 100%). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1650, 1545, 1360, 1280. ^{1}H-NMR (270 MHz, DMSO-d_6) *δ*: 2.22 (3H, s, 3′-H_3), 2.66 (3H, s, 5-Me), 3.86 (2H, s, 1′-H_2), 6.04 (1H, s, 3-H), 6.59 (1H, *J*=2.4 Hz, 8-H), 6.61 (1H, br d, *J*=2.4 Hz, 6-H), 10.58 (1H, s, 7-OH).

Metabolite B (7-Hydroxy-2-(2′-hydroxypropyl)-5-methylchromone): Colorless needles, mp 175—178 °C. $[\alpha]_D$ 0° (*c*=0.044, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 1630, 1540, 1360, 1285. EI-MS *m/z* (rel. int.): 234 (M^+, 65%), 190 (85%), 161 (20%), 151 (36%), 124 (15%), 91 (13%), 45 (42%), 18 (100%). ^{1}H-NMR (270 MHz, CD_3OD) *δ*: 1.27 (3H, d, *J*=6.3 Hz, 3′-H_3), 2.65 (1H, dd, *J*=14.2, 7.6 Hz, 1′-H_a), 2.68 (1H, dd, *J*=14.2, 5.6 Hz, 1′-H_b), 2.72 (3H, s, 5-Me), 4.19 (1H, m, 2′-H), 6.06 (1H, s, 3-H), 6.63 (1H, br d, *J*=2.0 Hz, 6-H), 6.66 (1H, d, *J*=2.0 Hz, 8-H).

Screening of Bacterial Strains for Ability to Metabolize Aloesin (1) A defined stock strain of intestinal bacteria was anaerobically cultured in GAM and PYF broth (2 ml) containing aloesin (2.0 mg, **1**) for 5 d at 37 °C. The culture was extracted with BuOH (1.0 ml) and the extract was analyzed by means of HPLC under the conditions described in the legend to Fig. 1.

Incubation of Aloeresin A (2) with an Intestinal Bacterial Mixture Aloeresin A (100 mg, **2**) in dimethyl sulfoxide (DMSO) (1.0 ml) was incubated with an intestinal bacterial mixture (100 ml) for 3 d at 37 °C in an anaerobic box. The incubation mixture was extracted three times with BuOH (100 ml each) and the BuOH layer was concentrated *in vacuo*. The residue (400 mg) was applied to a column of silica gel (300 mm × 20 mm i.d.; 45 g). The column was eluted successively with $CHCl_3$ and $CHCl_3$–MeOH (95 : 5 and 90 : 10) to give aloesone (**3**) (25 mg), *dl*-aloesol (**4a**+**4b**) (5 mg), a mixture of (*E*)- and (*Z*)-*p*-coumaric acids in a ratio of 9 : 1 and aloesin (**1**) (7.3 mg). The relative ratio of *p*-coumaric acids was determined by ^{1}H-NMR on the basis of the respective peak area of the vinyl proton signals which appeared at *δ* 6.28 and 7.60 (each d, *J*=16.0 Hz) in the *E*-form and at *δ* 5.76 and 6.73 (each d, *J*=12.7 Hz) in the *Z*-form.

Incubation of (2′*R*)- and (2′*S*)-8-*C*-Glucosylaloesols (5a and 5b) with an Intestinal Bacterial Mixture A mixture of 8-*C*-glucosylaloesols (**5a** and **5b**) (50 mg) prepared by the reduction of aloesin (**1**) with $NaBH_4$ was incubated with a bacterial mixture (50 ml) for 3 d at 37 °C *in an anaerobic* box. The reaction mixture was concentrated *in vacuo*. The residue (150 mg) was chromatographed on silica gel (45 g, $CHCl_3$–MeOH (95 : 5)) to give *dl*-aloesol (**4a**+**4b**) (20 mg). Similarly, each diastereomer (**5a** and **5b**) separated by HPLC was also converted to the corresponding optically active aglycones (**4a** and **4b**) as shown in Table II.

Incubation of Aloesone (3) with an Intestinal Bacterial Mixture Aloesone (30 mg, **3**) in DMSO (0.2 ml) was incubated with an intestinal bacterial mixture (30 ml) for 3 d at 37 °C in an anaerobic box. The incubation mixture was concentrated *in vacuo*, followed by preparative TLC on silica gel with EtOAc to give *dl*-aloesol (**4a**+**4b**) in a yield of 18 mg (59%).

References

1) G. Franz and M. Gruen, *Planta Medica*, **47**, 131 (1983).
2) M. Hattori, T. Kanda, Y. Shu, T. Akao, K. Kobashi, and T. Namba, *Chem. Pharm. Bull.*, **36**, 4462 (1988).
3) M. Hattori, Y. Shu, A. I. El-Sedawy, T. Namba, K. Kobashi, and T. Tomimori, *J. Nat. Prod*, **51**, 874 (1988).
4) M. Hattori, Y. Shu, T. Tomimori, K. Kobashi, and T. Namba, *Phytochemistry*, **28**, 1289 (1989).
5) Q. Che, T. Akao, M. Hattori, K. Kobashi, and T. Namba, *Planta Medica*, in press, (1991).
6) L. J. Haynes and D. K. Holdworth, *J. Chem. Soc.* (*C*), **1970**, 2582.
7) P. Gramatica, D. Monti, G. Speranza, and P. Manitto, *Tetrahedron Lett.*, **23**, 2423 (1982).
8) K. Makino, A. Yagi, and I. Nishioka, *Chem. Pharm. Bull.*, **22**, 1565 (1974).
9) J. M. Conner, A. I. Gray, T. Reynolds, and P. G. Waterman, *Phytochemistry*, **29**, 941 (1990).
10) G. Speranza, P. Gramatica, G. Dada, and P. Manitto, *Phytochemistry*, **24**, 1571 (1985).
11) Y. Kashiwada, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **32**, 3493 (1984).
12) T. Mitsuoka, "A Color Atlas of Anaerobic Bacteria," Shobunsha Co., Tokyo, 1980, p. 325.