Biotransformation of aloenin, a bitter glucoside constituent of *Aloe arborescens*, by rats

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Summary. Aloenin has been established to be 4-methoxy-6-(2-β-D-glucopyranosyloxy-4-hydroxy-6-methylphenyl)-2-pyrone; it shows an inhibitory activity for gastric juice secretion. Rats metabolized it to 4-methoxy-6-(2,4-dihydroxy-6-methylphenyl)-2-pyrone, 2,5-dimethyl-7-hydroxychromone and glucose, which were excreted in the feces and the urine. The distribution of the radioactivity originating from ^{14}C-labeled aloenin was studied. The tracer found in the kidney and the liver reached 60% of the amount administered 24 h after feeding and decreased rapidly in the next 24 h.

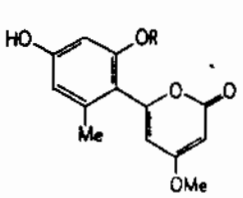
Plants of the *Aloe* genus have been used for various folk remedies, for example for gastro-intestinal disturbances, burns, insect bites and athlete's foot[2-6], and chemical constituents of the plants have been studied by many workers[2,5,6-14]. A new bitter glucoside with an inhibitory activity for the gastric juice secretion of rats[6] was isolated from *Aloe arborescens* Mill. var. *natalensis* Berger and named aloenin. We elucidated its structure as 4-methoxy-6-(2-β-D-glucopyranosyloxy-4-hydroxy-6-methylphenyl)-2-pyrone (1)[15] and also clarified its biosynthetic pathway[16]. We have now investigated the in vivo transformation of aloenin (1) in rats by tracer experiments.

^{14}C-Labeled aloenin (1) (m.p. 144.5–146.5 °C; 4.52×10^3 dpm/mg) fed to rats was prepared by uptake of acetate-U-^{14}C (8.88×10^8 dpm) into *Aloe arborescens* Mill. var. *natalensis* Berger following the reported procedure[16]. The rats used were 2-month-old males weighing 180–230 g. A suspension of the ^{14}C-labeled aloenin (10 mg) in water (1 cm^3) was fed to the rats through a stomach tube, after they had fasted overnight with free access to drinking water.

The excreta were collected 24 h or 24 and 48 h after feeding of the labeled aloenin. The feces were extracted with MeOH using a Soschlet apparatus. The urine was evaporated to dryness and extracted with MeOH. The radioactivity of these MeOH extracts indicated that during the first 24 h almost all the labeled aloenin administered was metabolized and excreted in the feces and the urine, and during the next 24 h only a very small amount, as shown in table 1. The extracts of the excreta obtained in 24 h were, separately, subjected to a combination of thin-layer radiochromatography and liquid scintillation measurement. The extract of the feces was found to comprise 2 major radioactive components, 4-methoxy-6-(2,4-dihydroxy-6-methylphenyl)-2-pyrone (2) (1.28×10^3 dpm, 26% of the total radioactivity of the crude extract)[15] and glucose (1.63×10^3 dpm, 33%). On the other hand, the extract of the urine was found to be composed of pyrone (2) (2.58×10^3 dpm, 33%) and 2,5-dimethyl-7-hydroxychromone (3) (1.64×10^3 dpm, 21%)[15,17]. The identification of these metabolites was carried out by co-TLC with solvents (a) ~ (c)[18] and co-HPLC with systems (a) and (b)[18] for 2, co-TLC with solvents (a) ~ (c) and co-HPLC with systems (a) and (c) for 3, and co-TLC with solvents (a) and (d) and co-PPC using Toyo No. 51 filter paper and n-BuOH:pyridine:H_2O = 6:4:3 as a solvent for glucose, respectively. To confirm the structures of the metabolites, non-radioactive aloenin (1) was fed to rats on a scale of 200 mg a rat, and then the corresponding metabolites were isolated by comparing their TLC and HPLC with those of the radioactive metabolites obtained above. The metabolites isolated were identified by comparing the melting points (m.p. and mixed m.p.), the chromatographic behavior (co-TLC and co-HPLC), and the spectral data (IR, UV and MS) with those of authentic samples.

Next, the distribution and/or the accumulation of aloenin (1) and its metabolites in the viscera and the blood were investigated. The rat was sacrificed 24 h or 48 h after feeding of ^{14}C-labeled aloenin; the kidney, the stomach, and the liver were removed and the blood collected. Portions (150 mg) of these viscera and the blood were separately subjected to combustion by means of a sample oxidizer to measure the radioactivity on a liquid scintillation counter. The distributions of the radioactivity in these viscera are shown in table 2, which indicates the large accumulation of the tracer in the kidney and the liver in 24 h and the rapid decrease in the radioactivity in the viscera in the next 24 h. A very low radioactivity (1.42×10^2 dpm/150 mg of the blood) was observed for the blood. The major component (1.19×10^4 dpm, 85%) of the radioactive compounds accumulated in the kidney was identified as aloenin (1) by co-TLC with solvent (a) and co-HPLC with system (c)[18]. The extract of liver contained many unknown components, which could not be identified.

Thus, it was found that aloenin (1) fed to rats was metabolized to 4-methoxy-6-(2,4-dihydroxy-6-methylphenyl)-2-pyrone (2), 2,5-dimethyl-7-hydroxychromone (3), and glucose for excretion into the feces and the urine. It was also



1 R-β-D-glucopyranosyl
2 R-H

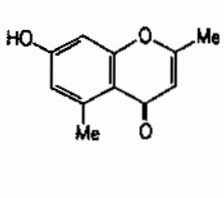


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Table 1. Distribution of radioactivity in the feces and the urine after administration of the ^{14}C-labeled aloenin (1) to rats

Exp. No.	Compound administered	Time after administration (h)	Distribution[a] Feces	Urine
1	Aloenin-^{14}C (1)	0 ~ 24	10.9	17.3
2	Aloenin-^{14}C (1)	0 ~ 24	13.0	21.5
		24 ~ 48	1.7	1.1

[a] Expressed as the percent distribution of the radioactivity based on the radioactivity of the labeled compound fed to the rats.

Table 2. Distribution of radioactivity in the organs of rats after administration of the ^{14}C-labeled aloenin (1)

Exp. No.[a]	Compound administered	Time after administration (h)	Distribution[b] Kidney	Stomach	Liver
1	Aloenin-^{14}C (1)	24	31.1	3.1	28.4
2	Aloenin-^{14}C (1)	48	1.8	1.6	7.9

[a] Corresponds to the numbers in table 1. [b] Expressed as the percent distribution of the radioactivity based on the radioactivity of the labeled compound fed to the rats.

clarified that aloenin (1) and its metabolites were accumulated in the kidney and the liver to a level of about 60% of the administered sample in 24 h after feeding, and decreased rapidly in the next 24 h.

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18 TLC analyses were performed on a glass plate (0.25 mm thick) coated with silica gel (Merck Si gel 60 G) using 4 different solvent systems [(a) $CHCl_3$:MeOH = 5:1, (b) EtOAc:hexane = 1:9, (c) $CHCl_3$:MeOH = 19:1 and (d) $CHCl_3$:MeOH = 2:3]. HPLC analyses were carried out with 3 different systems [(a) JASCO WC-03-500 column (EtOAc:hexane = 7:3), (b) JASCO SV-02-500 column (H_2O:MeOH = 4:1) and (c) JASCO SV-02-500 column (H_2O:MeOH = 9:1)].