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Short communication

# Metabolism of aloin—the influence of nutrition[1]

Angelika Koch*

*Frohme-Apotheke, Frohmestraße 14, 22457 Hamburg, Germany*

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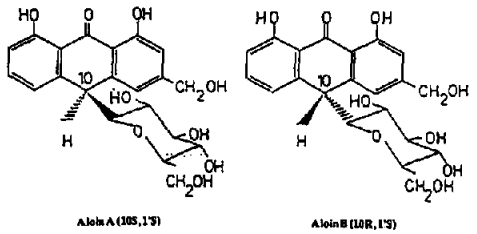
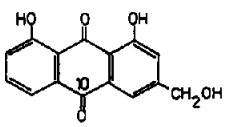
## 1. Introduction

Aloin—the C-glycosyl of aloe–emodin anthrone—is one of the main constituents of the dried leaf exudate of the laxative drug *Cape aloes* (*Aloe ferox*, *Asphodeliaceae*). Aloin occurs as two diastereomers A and B [1,2]:




Aloin A (10S,1'S)

Aloin B (10R,1'S)




Aloe-emodin

The laxative action of anthraquinone glycosides basically depends on cleavage of the corresponding free anthrones [3]. Human intestinal bacteria (*Bifidobacterium* sp.SEN) are capable of transforming the O-glycosyl of sennosides into the corresponding rhein and aloe–emodin anthrones [4]. However, participation of human bacteria in the cleavage of C-glycosyls such as aloins has not yet been clarified.

In previous papers [5,6] the present author reported on the weak purgative activity of aloin stated by the majority of volunteers in the course of experiments. Only one volunteer (C) reported severe diarrhoea and, at the same time, the analysis confirmed cleavage of aloin to aloe–emodin (anthrone). As this person differed in terms of nutrition (preference for meat products and self-medication by ferro salts vs. a vegetable diet) it was speculated that this difference in response to aloin was caused by differences in nutrition.

The objective of the present work is to investigate the laxative action of aloin when adopting ferro-salt medication (see Section 2) for the other volunteers (A and B). The expected aloe–emodin metabolite (anthrone) has to be detectable in feces [7] and urine in its oxidized form.

* Corresponding author. Fax: (+49) 40-559-3234.

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Furthermore, it was necessary to improve the experimental conditions for qualitative and quantitative analysis of aloin and its metabolites in biological materials.

When investigating fecal and urine excretions of metabolites the following major problems arise:

- the incomplete and unsatisfactory recovery of anthranoids;
- no improvement with longer and more extensive extraction of the material because it concentrates the polar by-products;
- the chemical alteration and degradation of the unstable anthrones and glycosyls;
- the bad odor of the material.

## 2. Experimental

### 2.1. Ferro-salt treatment

One week before starting the experiment with aloin every volunteer took one tablet every day of "Hermes Cevitt + $Fe^{2+}$". One tablet consists of 225 mg ascorbic acid and 85.8 mg Fe(II)gluconate ( = 10 mg $Fe^{2+}$).

### 2.2. Timetable

After ferro-salt treatment 50 mg of aloin R (Merck) was administered in a capsule to volunteers A, B and C at 10 pm. After a delay of 10–12 h the first defecations were registered in all cases and an additional 1–3 further defecations the same day.

### 2.3. Materials

The collected feces were rapidly frozen and placed for drying under low pressure into a CHRIST Alpha 1–4 Freezing and Drying unit. The dried feces ($\approx$20 g) were extracted with methanol several times in an ultrasonic bath and concentrated to 30 ml. A clean-up procedure was included by using small pre-columns of 500 mg $NH_2$ Chromabond (Machery-Nagel) Cat.No. 730 033 and 0.5 ml of sample solution. After conditioning with methanol, the columns were extracted twice with 1 ml methanol each time. The second eluates of (1 ml) methanol were concentrated to 0.5 ml. These solutions were used for chromatography.

Urine was collected for 1 day (volunteers A and B). The total quantity (A, 500 ml) was hydrolyzed with 200 ml HCl (37%) for about 20 min or (B, 2000 ml) enzymatically hydrolyzed with 1 $\mu$l $\beta$-glucuronidase–arylsulfatase (Merck Nr. 1.04114.0002) per 100 ml urine for about 3 h at 37°C, pH 5–6. With volunteer C only 20 ml of urine was collected on the first morning. The final sample preparation after neutralisation of the hydrolyzed urine was carried out on an Extrelut® column.

Other experimental conditions were summarized in Table 1.

## 3. Results and discussion

Improvements in sample processing were made by using a CHRIST Alpha 1–4 Freezing and Drying Unit. The nearly odorless powdered feces material was extracted with methanol in an ultrasonic bath. Extraction was followed by a clean-up procedure using $NH_2$ pre-columns.

Separations and quantifications were carried out with HPTLC–densitometry. In comparison with previous studies [5] the sorbent system was optimized by selecting $NH_2$ plates. Although substances such as aloin, aloe–emodin and their metabolites possess remarkable differences in polarity, $NH_2$ plates allow separation in the presented chromatographic system. Furthermore, these substances are detectable under daylight and 366 nm UV light by means of their intensive colour caused by reaction (Bornträger) between analyte and sorbent material. The recovery of the extraction process was approximately 70% for aloe–emodin and 40% for aloin.

The clean-up procedures (with $NH_2$ pre-columns) are very useful to minimize "fogging" effects of emulsifiers and sugars in the samples. With these data available an HPLC separation method was also evaluated.

The DAD detector for HPLC demands greater amounts of samples to allow a good UV spec-

Table 1
Summary of experimental conditions

| | |
|---|---|
| Aloin R Merck Nr. 69685<br>Aloe–emodin Roth Art.Nr.6004 HPLC<br>Aloin standard $V_1$: | 1 mg aloin and 1 mg aloe–emodin dissolved in 10 ml of methanolic feces extract |
| Aleo–emodin standard $V_2$: | 1 mg aloe-emodin and rhein dissolved in 100 ml methanol (p.a., Merck) |
| **High performance thin layer chromatography (HPTLC)** | |
| Stationary phases | Solvent systems |
| $NH_2$-precoated HPTLC plates Merck Nr. 15647 | Ethyl acetate/2-propanol/water (100+17+13;v,v,v) |
| These plates were cut to a size of 5×5 cm | All solvents were of analytical grade |
| *Desaga TLC-Applicator AS30*<br>Conditions: | 0.5 μl per cycle, application rate: 10 s $\mu l^{-1}$ band length: 4 mm |
| *Desaga-H-Chamber 5×5 cm²*<br>Development distance (time): | 3.5 cm (4–5 min) |
| *Desaga Densitometer CD 60*<br>Feces: | Reflection mode, 370 nm (aloin), Hg lamp, and 480 nm (aloin, aloe–emodin), beam size: 0.1×2 mm ($w \times h$) |
| Urine: | Transmission mode, 510 nm, Hg lamp |
| Detection: | UV light 366 nm (feces), daylight (urine) |
| Documentation: | Reflex camera: Fuji 400 ASA UV: Filter No. 2 (orange) |
| **High performance liquid chromatography (HPLC)**<br>Apparatus: HP 1050<br>Detector: DAD HP 1040M<br>20 μl loop | |
| Columns | Solvent systems |
| $NH_2$ Hypersil APS 5 μm<br>250×8×4.6 mm³ | Gradient:<br>Ethylacetate: methanol: water<br>80+19+1 (0–3 min)<br>75+20+5 (3–5 min)<br>70+22+8 (5–12 min)<br>80+20 (5 min post-run) |
| Flow rate:<br>Column temperature: | 1 ml $min^{-1}$<br>26.5°C |
| LiChrosorb RP-18 7 μm<br>250×4 mm² | Methanol: water<br>80+20 (0–10 min)<br>100 (10–15 min)<br>all solvents are of gradient grade = LiChrosolv quality |
| Flow rate:<br>Column temperature: | 1 ml $min^{-1}$<br>25.5°C |

trum. Good results were obtained for aloe–emodine in urine with a LiChrosorb RP18 column and methanol–water, whereas aloin in feces was detectable on a $NH_2$ Hypersil APS column with an ethyl acetate–methanol–water gradient. Here, HPLC differentiates between the aloin diastereoisomers A and B and indicates degradation to 10-hydroxyaloin.

As reported in the previous papers volunteers A and B showed a striking low response to aloin when nourished with a vegetable diet (without ferro-salt medication). This agrees with the results of Hattori et al. [8]. They stated an inhibition of the metabolic activity of *Eubacterium* sp.BAR (isolated from human feces) by addition of glucose to the (in vitro) medium containing aloin. Their findings suggest that the enzyme associated with cleaving the C-glycosyl unit of aloin is inducible by the substrate. As the induction of this enzyme is suppressed by D-glucose, there is reason to believe that nutritional compounds occurring as various types of C-glycosides possibly generate the very same pool of suppressing substrate.

After ferro-salt treatment of volunteers A, B and C the samples show relatively high amounts of aloe–emodin in relation to the total weight of feces. The increasing amounts of aloe–emodin caused the laxative effect. Detection of aloe–emodin in urine is confirmation of its absorption.

According to former observations from in-vitro tests [9] outside the human intestines by mixing aloin with human feces, it is presumed that further metabolites such as bianthrones and 10-hydroxy-aloins [8] may be artifically formed during extraction and storing procedures. This was confirmed by in-vitro storage tests of aloin in different solvents [10]. The induction of enzymes for cleaving aloin cannot be responsible for the strong laxative effect caused by ferro salts. Treatments with ferro salts for volunteers without a diagnosed deficiency lead to insufficient absorption of $Fe^{2+}$. The increasing pH value in the jejunum and small intestine may cause oxidation of free $Fe^{2+}$ to $Fe^{3+}$. $Fe^{3+}$ also seems to be responsible for an increasing (non-enzymatic) oxidative cleavage of aloin to aloe-emodin.

Therefore, in general the human intestinal flora seem to be capable of metabolizing aloin, but the present results imply that nutrition plays an important role in the laxative action of aloin in human beings.

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