Studies of Aloe. VI.\textsuperscript{1)} Cathartic Effect of Isobarbaloin

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The cathartic effect of isobarbaloin, a stereoisomer of barbaloin (compound principally responsible for the cathartic activity of Aloe), was examined in male rats by oral administration. Individual differences in sensitivity in the laxative activity of isobarbaloin and barbaloin was not found. The cathartic activity (ED\textsubscript{50}) of isobarbaloin in barbaloin positive rats was 19.2 mg/kg, nearly equal to that of barbaloin (19.5 mg/kg). Also, isobarbaloin administered orally was demonstrated to decompose to aloe-emodin-9-anthrene (active metabolite of barbaloin) as well as to barbaloin. Therefore, it is considered that the mechanism underlying the cathartic effect of isobarbaloin is the same as that of barbaloin.

Key words isobarbaloin; Aloe; barbaloin; cathartic effect mechanism; aloe-emodin-9-anthrene

We have been investigating the mechanism underlying the cathartic effect of Aloe in rats.\textsuperscript{2)} Previously,\textsuperscript{3)} we reported that barbaloin was the compound principally responsible for the cathartic activity of Aloe. After the oral administration of Aloe to rats, barbaloin is decomposed to aloe-emodin-9-anthrene (AE-anthrene) by large intestinal flora.\textsuperscript{4)} By the AE-anthrene, an increase in water content is caused in the large intestine by several mechanisms, such as the inhibition of colonic Na\textsuperscript{+}, K\textsuperscript{−}-ATPase, an increase in paracellular permeability across the colonic mucosa and the stimulation of mucus secretion, resulting in diarrhea.\textsuperscript{1,5)}

On the other hand, isobarbaloin, a stereoisomer of barbaloin, has been reported to be contained in Aloe (Chart 1).\textsuperscript{6)} Its cathartic effect has never been investigated. We thought that isobarbaloin would be decomposed to AE-anthrene by intestinal flora, in a manner similar to barbaloin, and would cause the cathartic effect to rats. Therefore, in this paper we report the cathartic activity of isobarbaloin, isolated from aloe by high-performance-liquid-chromatography (HPLC), by using rats. Then, we describe the results of our search for the decomposition products of isobarbaloin in rat large intestine after the oral administration.

![Chart 1. Structures of Barbaloin, Isobarbaloin and Those Possible Metabolic Products](image)

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**MATERIALS AND METHODS**

**Chemicals** Barbaloin and reagents used were the same as in the previous paper.\textsuperscript{1)}

**Animals** Male Wistar rats (150–200 g) were purchased from Shizuoka Laboratory Animal Center, Hamamatsu, Japan.

**Isolation of Isobarbaloin from Aloein (Merck)** Isobarbaloin was separated from aloein (Merck) by HPLC, which was operated in the reversed-phase mode, according to the modified versions of the method by Suzuki.\textsuperscript{7)} The column was a YMC-packed column SH-343-5, and the mobile phase was an acetonitrile–H\textsubscript{2}O (7:17, v/v) mixture containing 0.05% trifluoroacetic acid (TFA). The flow rate was maintained at 5.0 ml/min and isobarbaloin was monitored at 354 nm. The isobarbaloin isolated from aloe showed one peak on the chromatogram by HPLC (Fig. 1). The analytical conditions were as follows: column: Waters Nova-Pak C\textsubscript{18}, the mobile phase: an acetonitrile–H\textsubscript{2}O (5:17, v/v) mixture containing 0.05% TFA.

**Evaluation of Cathartic Activity** The cathartic response of rats to barbaloin (31.1 mg/10 ml/kg) was first checked according to the method described previously.\textsuperscript{3)} Then, rats with definite diarrhea were selected and used. The evaluation of the cathartic activity was examined according

![Fig. 1. HPLC Chromatogram of Aloein](image)

1. isobarbaloin; 2. barbaloin.

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to the method of Turumi described previously. Each group consisted of 5—10 rats. Each animal was placed in a separate cage with a filter paper mat, and isobarbaloin was administered orally. During the experiment the animals were given free access to commercial rat chow and water. At 20h after the administration, cathartic activity was judged. Not only fluid feces but also soft feces which made a clear blot on filter paper were considered as diarrhea. The cathartic activity (ED_{50} and 95% confidence limit) was determined by the Litchfield-Wilcoxon method.

Identification of AE-anthrone in Rat Cecum after Oral Administration of Isobarbaloin The detection of AE-anthrone in the rat cecum after the oral administration of isobarbaloin was practiced by the method previously demonstrated with barbaloin. The rat cecum was excised 6h after the oral administration of isobarbaloin (31.1 mg/kg) to rats positive to barbaloin. AE-anthrone-p-nitrosodimethylaniline (p-NDMA) complex was extracted with benzene from the cecum after the injection of 0.1% p-NDMA–pyridine into the cecum, and identified by TLC.

RESULTS AND DISCUSSION

Previously, we proposed that rats positive to barbaloin should be selected for the investigation of the mechanism underlying the cathartic effect of barbaloin, since the process of activation of barbaloin in the rat digestive tract by intestinal bacteria was necessary to act, but all rats did not possess the ability. We speculated that isobarbaloin was also activated through the same manner as barbaloin, because barbaloin and isobarbaloin only have different configurations at C-10 of the anthron. First, the cathartic effect of isobarbaloin was individually examined in barbaloin positive and negative rats by the oral administration (31.1 mg/kg). As shown in Table 1, all rats positive to barbaloin gave a positive response to isobarbaloin, and only a few rats negative to barbaloin exhibited a positive weak (mild) response to isobarbaloin. Individual differences in sensitivity in the laxative activity of isobarbaloin were thus observed, nearly the same as that of barbaloin. Therefore, we concluded that the selection test for rats by using barbaloin should also be carried out for studies on the cathartic effect of isobarbaloin, the same as barbaloin.

Cathartic activity (ED_{50}) of isobarbaloin in rats positive to barbaloin was examined. We had expected that an obvious difference in cathartic activity between the two compounds existed, because isobarbaloin has been reported to be chemically less stable. But, the cathartic responses of both compounds were dependent on the dose, and the ED_{50} of isobarbaloin was 19.2 mg/kg, nearly equal to that (19.5 mg/kg) of barbaloin (Table 2).

Furthermore, we examined the rat cecum contents 6h after the oral administration of isobarbaloin to examine the mechanism of its cathartic effect. As a result of this search, the decomposition products, AE-anthrone (active metabolite of barbaloin) and AE (probably artifact), were detected in the rat cecum after the oral administration of isobarbaloin just the same as barbaloin. Thus, the decomposition pathway of isobarbaloin in the rat cecum was proved to be the same as barbaloin, without any difference in optical configuration. These findings lead us to conclude that isobarbaloin causes the cathartic effect to rats by the same mechanism as that of barbaloin, and AE-anthrone is the compound responsible for the cathartic effect of isobarbaloin. We are now planning to carry out a further study to determine to what extent isobarbaloin contributes to the cathartic effect of Aloe.

REFERENCES