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# Isolation of Ethanol Metabolizing Enzyme Inhibitors from *Aloe* spp.[1]

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Abstract—In the course of evaluation of hepatoprotective components against alcohol-induced toxicity from *Aloe* spp., the methanol extract was found to cause a significant inhibition of rat liver cytosolic alcohol dehydrogenase activity. Systematic fractionation of active fractions monitored by bioassay led to isolation of four compounds; aloe-emodin, aloenin, ethylidene-aloenin and β-sitosterol, which were estimated as active principles for inhibition of c-ADH and c-ALDH activities *in vitro*.

Keywords—*Aloe* · ethanol metabolism inhibitors · ADH · ALDH · aloe-emodin · aloenin · ethylidene-aloenin · β-sitosterol

In a previous communication for the purpose of evaluating hepatoprotective components against alcohol-induced toxicity from *Aloe* spp., we demonstrated that water soluble fraction, with a single oral administration to rats, caused a significant decrease in the serum ethanol concentration as well as enhancement of liver cytosolic alcohol dehydrogenase (ADH) activity(Shin, *et al.*, 1995). On the other hand, the fraction soluble in methanol was found to cause an increase in the serum ethanol concentration and inhibit ADH activity, which strongly suggested that there should exist some components inhibiting alcohol metabolizing enzymes in these plants.

The present report deals with isolation and characterization of alcohol metabolism inhibitors from *Aloe* spp. Systematic fractionation of the methanol extract monitoring with bioassay led to the isolation of four components which were identified as potential inhibitors of alcohol metabolism *in vitro*.

## Experimental

**Plant materials** - A whole part of fully grown hot-air dried leaves of *Aloe arborescens* (AA) and freeze dried powders of aereal parts of *Aloe vera* (AV) were supplied from Kim Jeong Moon *Aloe* Co. Ltd.

A voucher specimen is deposited at the herbarium of the company.

**Reagents** - 2-mercaptoethanol, sodium deoxycholate, propionaldehyde, pyrazole, pargyline, NAD and semicarbazide were purchased from Sigma Chem. Co. Ltd. and other organic chemicals of reagent grade quality were commercially available.

Livers for preparation of enzyme source were obtained from male Sprague-Dawley rats weighing 200-250g bred in the animal facility of this institute.

**Fractionation and isolation of compounds** - The dried powders of *Aloe* spp.(450 g each) were extracted with hot methanol for 3 hr. 8

[1]Part 3 in the series: "Studies on the effect of *Aloe* spp. on ethanol metabolism" (See references Shin, *et al.*, and Woo, *et al.*).

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times and concentrated under reduced pressure to give a methanol extract(102.2 g and 114 g for AA and AV), which were again fractionated into n-hexane, chloroform, ethylacetate and n-butanol fractions. Repeated column chromatographic separation of chloroform fraction over silica gel by eluting chloroform-methanol (gradient) led to isolation of β-sitosterol and aloe-emodin, and ethylacetate fraction by eluting chloroform-methanol (gradient) gave ethylidene-aloenin. Gel filtration of subfractions from ethylacetate fraction on Sephadex LH-20 eluting with methanol led to isolation of aloenin. The identity of these four compounds were confirmed by the method described previously(Woo, *et al.*, 1994).

**Preparation of enzyme sources** - The entire liver of normal rats was removed and rinsed gently with 0.25 M sucrose solution containing 2 mM 2-mercaptoethanol and 10 mM sodium phosphate pH 7.4 at 4°C, and homogenized with approximately 7 volumes of 0.25 M sucrose solution in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 600 xg for 10 min, followed by further centrifugation of the supernatant at 10,000 xg for 10 min, and the resulting supernatant was used for the measurement of cytosolic ALDH activity. A portion of 10,000 xg supernatant was further centrifuged at 105,000 xg for 60 min to obtain the cytosolic fraction, which was used for the measurement of c-ADH activity *in vitro*.

**Enzyme assay** - Cytosolic ADH and ALDH activities were assayed by measuring the rate of production of NADH at 340 nm spectrophotometrically(Boehringer, 1970 ; Lebsack *et al.*, 1977).

ALDH reaction mixtures contained 0.2 M Tris-HCl buffer(pH 8.3), 1 M KCl, 0.1 M-pyrazole, 1 M 2-mercaptoethanol, 0.1 M propionaldehyde, 0.1 M NAD in 0.01 M HCl and 0.1 ml of enzyme sources in a total volume of 2.5 ml.

After 5 min for temperature equilibration in the cuvette in a thermostatic bath, the reactions were started by the addition of substrate. Changes in absorbance at 340 nm were measured with a Hitachi U-3210 spectrophotometer incubating at 25°C for 8 min. Blanks without aldehyde were run with all reactions.

ADH assays were run in 0.1 M Tris-HCl buffer(pH 8.5), 0.2 M ethanol, 0.05 M semicarbazide HCl, 0.1 M NAD in 0.01 M HCl, in a total volume of 3.0 ml. Reactions were started by the addition of 0.1 ml of enzyme source incubating at 30°C. One unit of enzyme is defined as the amount of enzyme that catalizes the formation of 1 μM of NADH per min. Proteins were determined by Lowry method(Lowry, *et al.*, 1951) with bovine serum albumin as protein standard.

## Results and Discussion

Various fractions obtained by partitioning the methanol soluble extracts from AV and AA successively with *n*-hexane, chloroform, ethylacetate and *n*-butanol were subjected to assay for ADH inhibitory activity *in vitro*. As shown in Table 1, the chloroform soluble fractions from both species were found to exhibit the most potent inhibitory activity, the $IC_{50}$

Table 1. $IC_{50}$ values of various fractions from *Aloe* spp. against rat ADH

| Fraction | IC50(μg/ml)[a] | |
|---|---|---|
| | AV | AA |
| *n*-Hexane Fr. | >>133 | 1000 |
| $CHCl_3$ Fr. | 65 | 17 |
| EtOAc Fr. | 90 | 39 |
| n-BuOH Fr. | 500 | 90 |
| $H_2O$ Fr. | 1500 | 900 |
| Hexane-Insol. Fr. | 270 | 26 |
| Pyrazole | 0.9 μM | |

Assay conditions are as described in the text.
$IC_{50}$ values were calculated by regression analysis of data plotted on semi-log paper.
a) Final concentrations in the reaction mixtures.

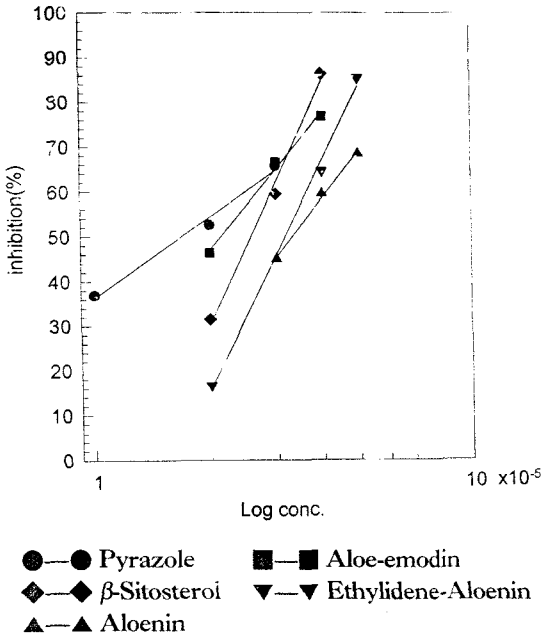



Fig. 1. Effects of *Aloe* components on c-ADH *in vitro*.

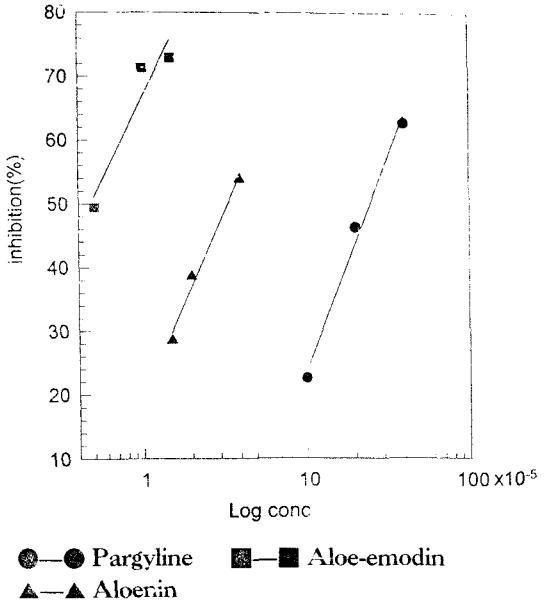



Fig. 2. Effects of *Aloe* components on c-ALDH *in vitro*.

values of AV and AA, the concentration of the inhibitor which caused 50% inhibition of the enzyme activity, being 65 and 17 μg/ml, respectively. The inhibitory potency was decreased in the order of ethylacetate, n-butanol and n-hexane fractions similarly in both species.

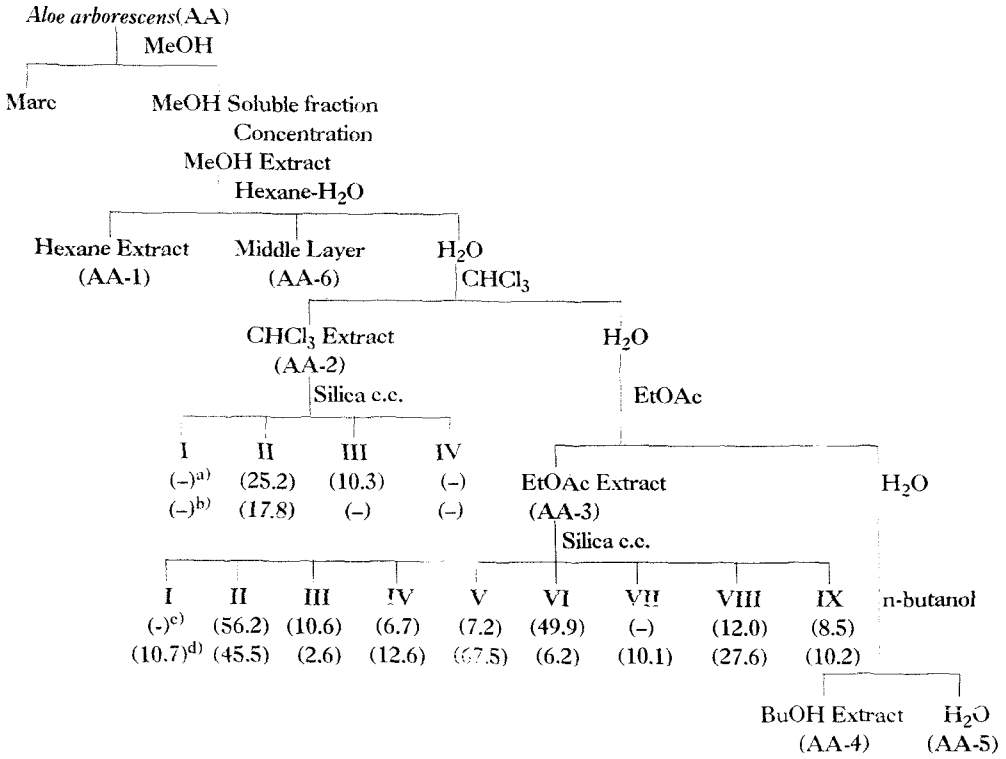
Further fractionation of the chloroform and ethylacetate soluble fractions by silica gel column chromatography monitored by both liver cytosolic ADH and ALDH activities gave 13 subfractions in case of AA (Scheme 1) and 12 subfr. in case of AV, respectively, in which subfr. such as AA-2-II, AA-3-II, AA-3-V, AA-3-VI, AA-3-VIII, AV-2-III, AV-3-II, AV-3-IV, AV-3-V and AV-3-VI were found to exhibit significant enzyme inhibitory activities.

Further repeated chromatographic (CC, PTLC, VLC) separation of these active fractions resulted in the isolation of four pure compounds and their structure elucidation demonstrated that those four compounds were aloe-emodin, aloenin, ethylidene-aloenin and β-sitosterol already separated from this plant (Woo, *et al.*, 1994).

The four compounds isolated were subjected to a test for ADH and ALDH inhibitory activity *in vitro* and the results were shown in Fig. 1 and 2. All of four compounds and pyrazole known as a typical ADH inhibitor exhibited an inhibition of ADH activity in a concentration-dependent manner.

Aloe-emodin and aloenin inhibited ALDH activity in a concentration-dependent manner, however, in case of ethylidene-aloenin and β-sitosterol, the enzyme activity could not be estimated at a concentration higher than $1 \times 10^{-5}$ M due to occurrence of precipitation.

Based on these results, for comparison of their inhibitory potencies, $IC_{50}$ values were calculated from regression equations and indicated in Table 2. A similar potency of ADH inhibition was shown in aloe-emodin, aloenin and ethylidene-aloenin, but interestingly β-sitosterol was almost equipotent to pyrazole, a reference ADH inhibitor.

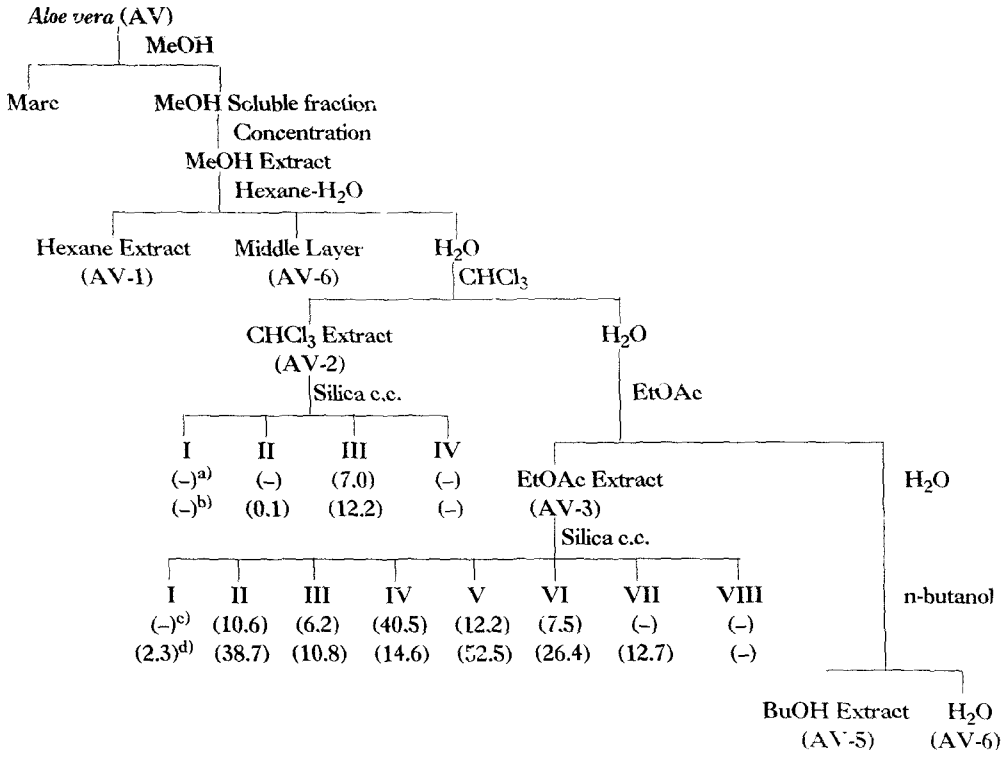



**Scheme I.** Effect of subfractions of *Aloe arborescens* on ADH and ALDH activities.
Enzyme assay conditions are as described in the text ; a) % inhibition of c-ALDH at 10 μg/ml, b) % inhibition of c-ADH at 10 μg/ml, c) % inhibition of c-ALDH at 20 μg/ml, d) % inhibition

Aloe-emodin exhibited exceptionally potent inhibition of ALDH ($IC_{50}$ = 6 μM), its inhibitory potency, being approximately 50 times as potent as that of pargyline, a typical c-ALDH inhibitor(Lebsack, *et al.*, 1977). Ethylidene-aloenin and β-sitosterol showed a far weaker inhibitory activity of c-ALDH.

In a previous report (Shin, *et al.*, 1995), the water soluble high molecular weight fractions from both AV and AA have been demonstrated to exhibit enhancing activities of alcohol metabolism, whereas the fractions soluble in organic solvent were found to cause an inhibition of alcohol metabolism. These results and together with the present results strongly suggest that *Aloe* spp. not only possess active principles enhancing but also those suppressing ethanol metabolism.

Although the compounds isolated have been demonstrated to exhibit inhibition ADH and ALDH activity *in vitro* in the present experiment, they are not considered as main active components of *Aloe* representing the alteration of alcohol metabolizing enzyme system, because the relative yield of chloroform or ethylacetate fraction were rather lower compared to those of other fractions ; especially water soluble high molecular weight fraction. The leaves of *Aloe arborescens* were reported to contain a number of anthracene and chromone derivatives (Yamamoto, *et al.*, 1991 ; Kodym, 1991 ; Yagi, *et al.*, 1987), and aloenin has been demonstrated to exhibit various pharmacological activities such as inhibition of gastric juice secretion (Hirata and Suga, 1978), histamine release (Nakagomi, *et al.*, 1987) and carrageenin-induced edema in rats (Yamamoto, *et al.*, 1991), etc.




Scheme II. Effect of subfractions of *Aloe vera* on ADH and ALDH activities.
Enzyme assay conditions are as described in the text ; a) % inhibition of c-ALDH at 10 μg/ml, b) % inhibition of c-ADH at 10 μg/ml, c) % inhibition of c-ALDH at 20 μg/ml, d) % inhibition of c-ADH at 20 μg/ml, (–) inactive.

Table 2. $IC_{50}$ values of *Aloe* components against rat ADH and ALDH

| Compounds | $IC_{50}$ ($M \times 10^{-5}$) | |
|---|---|---|
| | c-ADH | c-ALDH |
| Pyrazole | 1.9 | – |
| Pargyline | – | 30.0 |
| Aloe-emodin | 2.4 | 0.6 |
| Aloenin | 3.3 | 2.7 |
| Ethylidene-aloenin | 3.4 | (34.8)[a] |
| β-Sitosterol | 2.2 | (33.3) |

Assay conditions are as described in the text.
$IC_{50}$ values were calculated from regression analysis of data plotted on semi-log papers.
[a]% inhibition at $1.0 \times 10^{-5}$ M of inhibitors.
Dose-response data could not be obtained at higher concentrations.

Aloe-emodin anthrone which is considered as a decomposition product of barbaloin, one of main components of *Aloe*, inhibited glucose-6-phosphate dehydrogenase of rat liver *in vitro* (Rychener and Steiger, 1989 ; Cotton and Rossum, 1975) and also inhibited $Na^+$, $K^+$ ATPase activity in large intestinal membrane in rats(Ishii, *et al.*, 1990). From these results, it can be postulated that the compounds isolated possess rather broad substrate specificity and thus the compounds might also have inhibitory activities on some other enzymes relating to a pharmacological and/or biological activities of these compounds.

To confirm these and to elucidate the mode of activities of the compounds isolated more precisely, *in vivo* effects of these compounds on alcohol metabolizing enzymes are currently

under investigation and the results will be reported elsewhere.

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