Elgonica-Dimers A and B, Two Potent Alcohol Metabolism Inhibitory Constituents of *Aloe arborescens*

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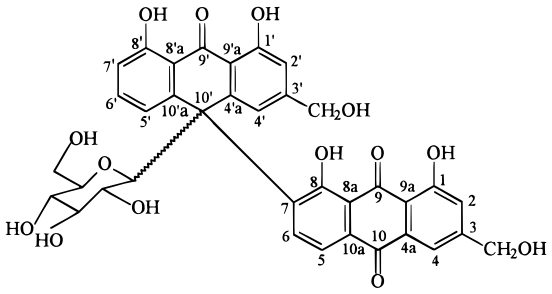
Activity-guided fractionation of the leaves of *Aloe arborescens* resulted in the isolation and characterization of the known compounds elgonica-dimers A (**1**) and B (**2**) as potent inhibitors of cytosolic alcohol dehydrogenase and aldehyde dehydrogenase activities in vitro.

Aloe arborescens Miller (Liliaceae) has been used not only as an emmenagogue, a febrifuge in pleurisy and phthisis, and as a remedy for gastrointestinal disorders, constipation, burns, and insect bites, but also is widely utilized in cosmetics and health foods.[1,2] The leaves of *A. arborescens* are known to contain a number of anthracene and chromone derivatives such as aloe-emodin, aloesin, barbaloin, and chrysophanol along with its cinnamoyl esters, as well as two phenyl α-pyrones, aloenin along with its ethylidene derivative.[3−6]

As a part of our interest in alcohol-metabolism inhibitors, it was observed that the single oral administration of a MeOH-soluble fraction of *A. arborescens* caused a significant decrease in alcohol dehydrogenase activity in rat-liver cytosol.[7] Activity-guided fractionation of the EtOAc-soluble extract led to the isolation of three active principles, aloe-emodin, aloenin, and ethylidene-aloenin, as alcohol-metabolism inhibitors in vitro.[8] In the present investigation, two known dimeric compounds, elgonica-dimers A (**1**) and B (**2**),[9] were isolated in a similar manner as very potent inhibitory principles from a *n*-BuOH-soluble extract of *A. arborescens*.

Compounds **1** and **2** were identified as elgonica-dimers A and B, respectively, by comparison of their physical and spectroscopic data (UV, negative ion FABMS, ^{1}H NMR) with literature values.[9] These two compounds are both composed of anthrone emodin-10′-*C*-*β*-D-glucopyranoside and anthraquinone aloe-emodin moieties, and analysis of their ^{1}H-NMR spectra showed them to be closely related to one another (Table 1). The ^{1}H- and ^{13}C-NMR chemical shifts of these compounds (Table 1) were assigned using a combination of 2D homonuclear correlation experiments (COSY, ROESY) and one-bond and long-range proton-detected heteronuclear correlation experiments (HMQC, HMBC) that allowed unambiguous identification of the aglycon and sugar moieties. Compound **1** differs from **2** only in the stereochemical disposition at the C-10′ quaternary carbon center and was obtained as the less polar of the two isolates. The hitherto unreported ^{13}C-NMR data for **1** and **2** are presented in Table 1. Attempts to resolve the remaining stereochemical ambiguity in the structures of **1** and **2** were unsuccessful because, despite repeated efforts, it was not possible to generate suitable crystals of these compounds for X-ray crystallography.



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Elgonica-dimers A (**1**) and B (**2**) were evaluated for their in vitro cytosolic alcohol dehydrogenase (*c*-ADH), cytosolic aldehyde dehydrogenase (*c*-ALDH), and mitochondrial aldehyde dehydrogenase (*m*-ALDH) inhibitory activities under conditions described previously.[10,11] Compounds **1** and **2** showed exceptionally high inhibitory potencies against *c*-ADH, with IC_{50} values of 0.055 and 0.011 μM, respectively, which were approximately 180 and 900 times greater than pyrazole, a positive-control substance.[11] Compounds **1** and **2** possess much higher inhibitory potencies in these bioassays than aloe-emodin, aloenin, and ethylidene-aloenin.[8] The present results, therefore, clearly indicate that elgonica-dimers A (**1**) and B (**2**) are the major active principles of *A. arborescens* in terms of the inhibition of alcohol-metabolizing enzyme systems. Potent inhibitory activities for both *c*-ALDH and *m*-ALDH by compounds **1** and **2** were obtained (Table 2). Aloe-emodin anthrone, which may be considered a decomposition product of barbaloin, one of the main constituents of *Aloe* species, has been previously reported to inhibit rat glucose-6-phosphate dehydrogenase in vitro[12,13] and Na^+,K^+-ATPase activity in the large intestine of rats.[14] From these data, together with the present results, it can be postulated that anthrone−anthraquinone dimers such as **1** and **2** might possess rather broad enzyme inhibitory activity. In vivo evaluation of compounds **1** and **2** on alcohol-metabolizing enzymes remains to be carried out.

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Table 1. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) Data of Compounds **1** and **2** and Observed HMBC Correlations (DMSO-d_6)

	1			**2**		
position	δ_H (mult., J in Hz)	δ_C	HMBC (H to C)	δ_H (mult., J in Hz)	δ_C	HMBC (H to C)
1		162.6	OH-1		161.6	OH-1
2	7.21(s)	120.9	H-4	7.22 (s)	120.6	H-4, CH_2OH-3
3		151.9	CH_2OH-3		151.4	CH_2OH-3
4	7.66 (s)	117.3	H-2	7.67 (s)	117.3	H-2
5	7.82 (d, 9.7)	118.6		7.81 (d, 8.9)	118.4	H-6
6	8.43 (d, 8.2)	138.2		8.44 (t 8.9)	138.5	
7		142.5	H-5		141.7	H-5, H-6
8		160.6	H-6, OH-8		159.8	OH-8
9		192.5			193.2	
10		182.2	H-4, H-5		182.3	H-4, H-5
4a		131.4	H-4		131.5	H-4
8a		116.4	H-5		116.0	H-5
9a		115.2	H-2, H-4		115.6	H-2, H-4
10a		133.1	H-6		133.3	
1′		162.4	OH-1′		161.5	OH-1′
2′	6.90 (s)	112.6	H-4′	6.74 (s)	114.5	
3′		154.5	CH_2OH-3′		153.8	CH_2OH-3′
4′	6.85 (s)	118.8	H-2′	6.59 (s)	117.5	H-2′
5′	6.60 (d, 8.1)	119.7	H-7′	6.87 (d, 8.8)	120.8	H-6′, H-7′
6′	7.34 (t, 8.1)	135.8		7.38 (t, 8.8)	135.4	H-5′
7′	6.77 (d, 8.1)	115.1		6.89 (t, 8.8)	115.4	
8′		162.2	H-6′		161.5	H-6′, OH-8′
9′		194.2			194.3	
10′		53.0	H-6, H-4′, H-5′		52.3	H-6, H-4′, H-5′
4′a		146.3			145.9	H-4′
8′a		117.8	H-7′		117.4	H-7′
9′a		115.4	H-2′, H-4′, OH-1′		115.7	H-2′, H-4′
10′a		147.8	H-6′		146.9	H-6′
CH_2OH-3	4.59	62.2		4.60	62.2	
CH_2OH-3′	4.39	62.6		4.36	62.4	
OH-1	12.57			12.59		
OH-8	12.45			12.40		
OH-1′	12.16			12.16		
OH-8′	11.56			11.59		
1″	4.23 (d, 8.8)	82.9		4.25 (d, 8.5)	83.3	
2″	2.39	71.8		2.40	71.9	
3″	3.08	78.7		3.09	78.8	
4″	2.75	69.9		2.74	70.1	
5″	3.42	80.4		3.38	80.9	
6″	3.80	61.5		3.76	61.4	

Table 2. Inhibitory Potencies of Compounds **1** and **2** Against Rat *c*-ADH, *c*-ALDH, and *m*-ALDH

	IC$_{50}$ (μM)		
compd	c-ADH	c-ALDH	m-ALDH
1	0.055	0.16	0.36
2	0.011	0.53	0.72
aloe-emodin[a]	24.4	6.0	
aloenin[a]	33.1	27.0	
ethylidene-aloenin[a]	34.3	(34.8)[b]	
pargyline[c]		300.0	5.6
pyrazole[d]	9.8		

[a] See Shin *et al.*[8] [b] Percent inhibition at 1×10^{-5} M of the inhibitor. Dose−response data could not be obtained at higher concentrations. [c] ALDH inhibitor used as a positive control. [d] ADH inhibitor used as a positive control.

Experimental Section

General Experimental Procedures. Melting points were measured on a Mitamura-Riken melting point apparatus and are uncorrected. Optical rotations were determined on a Perkin-Elmer model 241 polarimeter. The UV and IR spectra were recorded on a Hitachi 3100 UV-vis and JASCO FT-IR-5300 spectrophotometer, respectively. A Bruker CXP-300 spectrometer was used to record NMR spectra (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) with TMS as an internal standard in DMSO-d_6. FABMS were obtained in a glycerol matrix in the negative-ion mode on a VG70-VSEQ mass spectrometer. Column chromatography was performed on Merck Si gel 60 (70−230 mesh) and Sephadex LH-20 (25−100 μm; Pharmacia Fine Chemicals, Piscataway, NJ). Precoated Kieselgel 60 F_{254} plates (thickness 0.2 mm; E. Merck, Darmstadt, Germany) were used for TLC, with visualization conducted by spraying with 10% v/v aqueous H_2SO_4 followed by heating at 110 °C for 10 min. 2-Mercaptoethanol, sodium deoxycholate, propionaldehyde, pyrazole, pargyline, NAD, and semicarbazide were purchased from Sigma Chemical Co. (St. Louis, MO).

Plant Material. Fully grown inner leaves of *A. arborescens* were supplied by Kim Jeong Moon Aloe Co., Ltd., Chun Buk, Korea, in October 1995. A voucher specimen (accession no. AB-95-105) has been deposited at the herbarium of the Natural Products Research Institute, Seoul National University.

Extraction and Isolation. The air-dried and powdered leaves (1 kg) were extracted with hot MeOH for 3 h (5 ×) and concentrated under reduced pressure to give a dried MeOH residue. This extract was partitioned with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH, successively. The dried *n*-BuOH-soluble fraction (12.1 g) was chromatographed over a Si gel column using $CHCl_3$−MeOH (88:12) to give five subfractions. Subfractions 2 and 4 were further chromatographed on Sephadex LH-20 by elution with MeOH in order to purify compounds **1** (24.7 mg) and **2** (12.5 mg).

Elgonica-dimer A {10′-β-D-glucopyranosyl-1,8,1′,8′-tetrahydroxy-3,3′-bis(hydroxymethyl)[7,10′-bianthracene]-9,10,9′-trione A} (1): yellow plates ($CHCl_3$−MeOH); mp 182 °C; $[\alpha]^{25}_D$ −13.0° (*c* 0.73, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (4.53), 261 (4.48), 293 (4.23), 376 (4.21), 438 nm (4.18), (+NaOH) 237 (4.51), 298 (4.39), 374 (4.15), 514 nm (4.13); IR ν_{max} (KBr) 3428 (OH), 1618 (C=O), 1424 (C=C), 1285, 1212, 1178, 1082 cm^{-1}; ^1H- and ^{13}C-NMR data, see Table 1; negative ion FABMS *m*/*z* 777 $[M - H + glycerol]^-$ (7), 685 $[M - H]^-$ (7), 523 $[M - H - C_6H_{10}O_5]^-$ (15).

Elgonica-dimer B {10′-β-D-glucopyranosyl-1,8,1′,8′-tetrahydroxy-3,3′-bis(hydroxymethyl)[7,10′-bianthracene]-9,10,9′-trione B} (2): pale yellow plates ($CHCl_3$−MeOH); mp 176 °C; $[\alpha]^{25}_D$ −37.0° (*c* 0.56, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (4.51), 261 (4.49), 293 (4.21), 376 (4.15), 440 nm (4.13), (+NaOH) 236 (4.53), 299 (4.40), 376 (4.18), 513 nm (4.10); IR ν_{max} (KBr) 3430 (OH), 1617 (C=O), 1426 (C=C), 1260, 1218, 1156, 1073 cm^{-1}; ^1H- and ^{13}C-NMR data, see Table 1; negative ion FABMS *m*/*z* 777 $[M - H + glycerol]^-$ (3), 685 $[M - H]^-$ (7), 523 $[M - H - C_6H_{10}O_5]^-$ (18).

Preparation of Enzymes. Livers were obtained from male Sprague−Dawley rats, weighing 200−250 g, which were bred in the animal facility of the Natural Products Research Institute, Seoul National University. The *c*-ADH, the *c*-ALDH, and the *m*-ALDH were prepared from these livers.[8]

Enzyme Assays. The in vitro activities of *c*-ADH, *c*-ALDH, and *m*-ALDH were measured by procedures described previously.[10,11]

Alkaline Hydrolysis of Compounds 1 and 2. To solutions of **1** and **2** (2.0 and 1.6 mg, respectively), dissolved in 5% aqueous NaOH (2.0 mL), $Na_2S_2O_4$ (10 mg) was added and heated at 80 °C for 14 h under reflux. Each reaction mixture was acidified and extracted with $CHCl_3$. In each case, workup of the $CHCl_3$ extract gave an aglycon (0.61 and 0.48 mg, respectively), which was identified by comparison with an authentic sample of aloe-emodin by co-TLC and MS data, and by comparison with literature data.[6] The aqueous solution was concentrated under reduced pressure to give D-glucose, which was confirmed by direct comparison with an authentic sample on TLC (Si gel; pyridine−EtOAc−HOAc−H_2O; 36:36:7:21) (R_f 0.41).

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