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Phytochemical Study on *Aloe vera*

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From the freeze dried leaves of *Aloe vera*, aloe-emodin, feralolide, a mixture of aloins A and B, elgonica dimers A and B were isolated and characterized based on spectral data.

Key words : *Aloe vera*, Dihydroisocoumarin, Anthraquinone dimer

INTRODUCTION

Aloe plants have been used as herbal medicine for centuries and the name aloe means the dried exudate from the cut leaves of *Aloe ferox* Mill. (Cape aloe, Liliaceae), *Aloe ferryi* Baker. (Socotrine aloe), *A. bainesii* Th. Dyer. (Natal aloe) and *Aloe vera* L. (Curacao aloe) (Namba, 1986). Among them, *Aloe vera* has not only been one of the most used natural drug well known for its cathartic properties, but also has been widely used as raw materials of cosmetics and health foods (Leung, 1978, Hoffenberg, 1979).

Although previous investigations showed that the leaves of *A. vera* contain a number of anthracene and chromone derivatives such as aloin A, aloin B, 1, 3,6,8-tetra-nitro-4,5-dihydroxy-2-hydroxymethyl anthraquinone and 7-hydroxy-aloin, its chemical composition is far from being completely investigated (Hoffenberg, 1979, Rauwald and Voetig, 1982).

As a part of our chemical investigations on the constituents of aloe, we report chemical investigation of the freeze dried ground leaves of *A. vera* which is led to the isolation of five compounds from the ethyl acetate soluble fraction of the methanolic extract.

MATERIALS AND METHODS

Melting points were determined on a Electrothermal digital micro melting point apparatus without correction. IR spectra were recorded on a Shimadzu IR-400 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were determined at 300 MHz and 75.5 MHz, respectively, on a Brucker AM 300 spectrometer with tetramethylsilane as the internal standard. In the case of compound 2, the chemical shifts were referenced to residual solvent peaks (3.3 ppm in ^{1}H-NMR (500 MHz) and 49 ppm in ^{13}C-NMR (125 MHz)) and were recorded in δ values. Multiplicities of ^{1}H- and ^{13}C-NMR signals are indicated as s (singlet), d (doublet), and t (triplet). Column chromatography was done with silica gel (Merck; 70-230 mesh). TLC was carried out on pre-coated Merck Kieselgel 60 F_{254} plates (0.25 mm), and spots were detected under UV light using 50% H_2SO_4 reagent.

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Plant materials

Freeze dried ground leaves of *Aloe vera* (W1) was kindly provided by Namyang Aloe Co. Ltd..

Isolation

Freeze dried *Aloe vera* (W1, 5.5 kg) was refluxed with methanol three times for three hours each time. The methanol layer was filtered and concentrated *in vacuo* (W1M1, 850 g). The methanol extract (W1M1) was extracted with dichloromethane (W1M1D1, 120 g), ethyl acetate (W1M1E1, 132 g), *n*-butanol (W1M 1B1, 144 g) and water (W1M1W1, 380 g), successively. The ethyl acetate extract (W1M1E1, 40 g) was subjected to column chromatography on silica gel and eluted with CH_2Cl_2-MeOH gradually increasing polority. The elutes were collected in 100 ml portions, monitored by TLC, and finally combined into 14 fractions. Recrystallization with MeOH yielded compound 1 (250 mg) from fraction 2, compound 2 (80 mg) from fraction 3, and compound 3 (350 mg) from fractions 6 and 7. Fraction 10 was rechromatographed on a silica gel column, with CH_2Cl_2-MeOH (10 : 1), to give **4** (40 mg) and **5** (15 mg).

Compound 1 (aloe-emodin) : Yellowish needles

from MeOH, mp 220~2°C, ^{1}H-NMR (DMSO-d_6, 300 MHz) δ; Table I, ^{13}C-NMR (DMSO-d_6, 75.5 MHz) δ; Table II

Compound 2 (feralolide) : mp 175~6°C, IR ν_{max} (KBr); 3,200 (br.), 1,650, 1,610, 1,575, 1,454, 1,380, 1, 260, 1,150, UV λ_{max}^{MeOH} (log ε) nm; 268.0 (4.18), 305.0 (4.02), EI-MS (*m/z*); 344 (M^+), 326 (M^+-H_2O), 308, 284, 270, 179, 177 (base peak), 151, 150, ^{1}H-NMR (CD_3OD, 500 MHz) δ; 2.53 (3H, s, -COCH$_3$), 2.82 (1H, dd, 2nd order, CHa), 2.86 (1H, dd, 2nd order, CHb), 2.94 (1H, dd, J=14.1, 5.5, H-4), 3.05 (1H, dd, J= 14.1, 7.8, H-4), 4.70 (1H, dddd, J=7.8, 5.5, 12.0, 5.5, H-3), 6.17 (1H, d, J=2, H-4'), 6.18 (1H, d, J=2, H-6'), 6.24 (1H, d, J=2.0, H-5), 6.27 (1H, d, J=2.0, H-7), ^{13}C-NMR (CD_3OD, 125 MHz) δ; 169.1 (C-1), 79.4 (C-3), 37.6 (C-4), 109.8 (C-5), 157.5* (C-6), 100.9 (C-7), 163.4* (C-8), 120.9 (C-9), 137.1 (C-10), 141.9 (C-1'), 101.3 (C-2'), 159.2* (C-3'), 100.2 (C-4'), 164.5 (C-5'), 106.9 (C-6'), 32.4 (CH_2), 203.4 (-COCH$_3$), 31.9 (-COCH$_3$), *Assignments may be interchanged

Compound 3 (a mixture of aloins A and B) : Yellowish needles from MeOH, mp 134~6°C, ^{1}H-NMR (DMSO-d_6, 300 MHz) δ; Table I, ^{13}C-NMR (DMSO-d_6, 75.5 MHz) δ; Table II

Compound 4 (elgonica dimer B) : Yellowish needles from MeOH, mp 234~6°C, KOH reagent; red, IR ν_{max}(KBr); 3,350 (br., phenolic OH), 1,660, 1,635 (conjugated carbonyl), 1,605, 1,590 (aromatic), 1,080-990 (glycosidic C-O), UV λ_{max}^{MeOH}; 260, 295, 335, 390, 435 nm, ^{1}H-NMR (DMSO-d_6+D_2O, 300 MHz) δ; 7. 205 (1H, d, J=1.45 Hz, H-2), 7.661 (1H, d, J=1.45 Hz, H-4), 8.436 (1H, d, J=8.10 Hz, H-5), 7.801 (1H, d, J=8.10 Hz, H-6), 6.847 (1H, brs, H-2'), 6.891(1H, brs, H-4'), 6.597 (1H, d, J=7.80Hz, H-5'), 7.336 (1H, dd, J=8.10 & 7.80 Hz, H-6'), 6.743 (d, J=8.10 Hz, H-7'), 4.60 (2H, d, CH_2OH), 4.36 (2H, t, CH_2OH), 4.275 (1H, d, J=9.30 Hz, anomeric H), ^{13}C-NMR (DMSO-d_6, 75.0 MHz) δ; 193.272, 191.608, 181.149, 161.62, 161.428, 161.219, 159.68, 153.60, 150.98, 146.85, 145.32, 141.57, 137.91, 135.4, 133.01, 131.28, 120. 505, 119.727, 118.281, 116.924, 116.782, 115.376, 114.749, 114.335, 112.262, 82.647, 80.258, 78.474, 71.428, 69.541, 62.277, 61.908, 61.138

Compound 5(elgonica dimer A) : Amorphous powder, UV λ_{max}^{MeOH}; 260, 295, 335, 390, 435 nm, ^{1}H-NMR (DMSO-d_6, 300 MHz) δ; 12.62 (1H, br.s, -OH), 12.43 (1H, br.s, -OH), 7.21 (1H, br.s, H-2), 7.648 (1H, br.s, H-4), 8.439 (1H, d, J=7.90 Hz, H-5), 7.80 (1H, d, J=8.20 Hz, H-6), 6.602 (1H, s, H-2'), 6.744 (1H, s, H-4'), 6.88 (1H, d, J=7.70 Hz, H-5'), 7.397 (1H, dd, J=8.10 & 7.70 Hz, H-6'), 6.90 (d, J=8.10 Hz, H-7'), 4.58 (2H, d, CH_2OH), 4.25 (1H, d, J=8.10 Hz, anomeric H), ^{13}C-NMR (DMSO-d_6, 75.0 MHz) δ; 193.

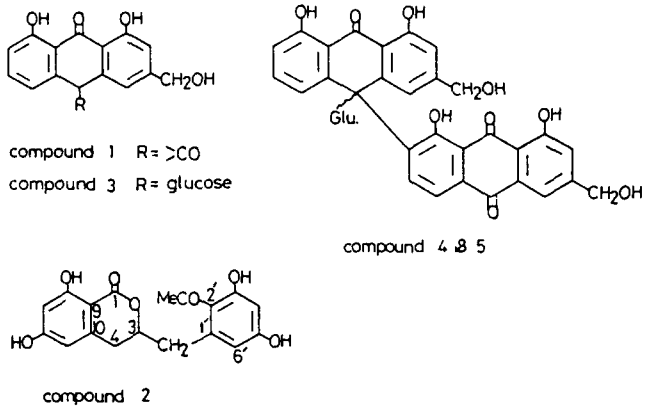

Fig. 1. The structures of compounds 1-5

Table I. ^{1}H-NMR chemical shift values for aloe-emodin, aloin A, compound 1 (aloe-emodin) and compound 3 (aloins a+b) in DMSO-d_6

Carbon	aloe-emodin[1)]	aloin A[2)]	Compound 1 (aloe-emodin)	Compound 3 (aloins A+B)
2	7.29	6.86s	7.17s	6.81-6.83 m
4	7.69	7.04s	7.56s	6.99s
5	7.72	7.08d (8.0)	7.60d	7.02-7.06 m
6	7.80	7.57dd (8.0)	7.71dd	7.47-7.54 m
7	7.38	6.89d (8.0)	7.26d	6.84-6.87 m
10		4.57d (2.0)		4.53s
CH_2OH	4.63	4.56d (6.0)	4.56	4.53s
H-1'		3.28dd (9.5,2.0)		3.31-3.36 m
2'		2.79dd (9.5)		3.09-3.13 m
3'		3.08dd (9.5)		3.14-3.25 m
4'		2.63-2.80 m		2.68-2.82 m
5'		2.63-2.80 m		2.68-2.82 m
6'a		3.16dd (11.0,5.0)		3.31-3.36m
6'b		3.38dd (11.0,1.8)		3.99dd (14.3,7.3)

[1)](Danielsen, *et al.*, 1992), [2)](Manitto, *et al.*, 1990)

Table II. ^{13}C-NMR chemical shift values for aloe-emodin, 1,8-diOH-9,10-anthraquinone, aloin A, compound 1 (aloe-emodin) and compound 3 (aloins a+b) in DMSO-d_6

Carbon	aloe-emodin[1)]	1,8-diOH-9,10-anthraquinone[2)]	aloin A[3)]	Compound 1 (aloe-emodin)	Compound 3 (aloins A+B)
1	161.72	161.07	160.8	161.98	160.80
2	120.78	123.99	112.7	121.48	112.56 (112.26)
3	153.80	137.10	151.4	153.77	152.01 (151.18)
4	117.18	119.18	117.8	117.86	117.69 (117.29)
5	119.43	119.18	118.9	120.15	120.06 (118.73)
6	137.42	137.10	136.1	138.06	135.88 (135.01)
7	124.49	123.99	115.4	125.09	115.55 (115.24)
8	161.43	161.07	161.1	161.68	161.10 (160.67)
9	191.74	192.50	193.4	198.78	193.27
10	181.59	180.88	44.2	182.18	44.13(43.93)
1a	114.57	115.49	115.8	114.85	115.73 (115.63)
4a	133.22	133.10	142.0	135.55	141.90 (141.72)
5a	133.54	133.10	145.6	133.65	145.64 (145.46)
8a	116.01	115.49	117.1	116.14	116.96 (116.14)
CH_2OH	62.15		62.5	62.61	62.31
1'			85.2		85.03(85.90)
2'			70.3		70.14(70.24)
3'			78.2		78.11(78.03)
4'			70.3		70.14(70.02)
5'			80.9		80.67(80.54)
6'			61.4		61.37

[1)](Danielsen, *et al.*, 1992), [2)](Berger and Castonguay, 1978), [3)](Manitto, *et al.*, 1990)

655, 181.628, 161.836, 161.736, 161.662, 160.004, 153.856, 151.625, 147.160, 146.201, 141.871, 138.204, 135.571, 133.492, 131.676, 121.022, 120.892, 118.571, 118.406, 117.470, 116.253, 115.929, 115.564, 114.715, 112.256, 83.571, 81.191, 78.97, 72.110, 70.323, 69.818, 62.565, 62.335, 61.619.

RESULTS AND DISCUSSION

Column chromatography on silica gel of the ethyl acetate soluble fraction of the methanolic extract furnished compounds 1, 2, 3, 4 and 5 in the order of increasing polarity.

Compound 1, mp 220~2°C and compound 3, mp 134~6°C, were readily elucidated as aloe-emodin and a mixture of aloin A(barbaloin) and aloin B (isobarbaloin), respectively, by comparison with reported spectroscopic data, and finally confirmed by comparison with authentic samples (Tables I and II).

Compound 2, mp 175~6°C, obtained as amorphous powder showed hydroxyl (3,200 cm^{-1}), α,β-unsaturated ketone (1,650 cm^{-1}) and aromatic ring (1,610 and 1,575 cm^{-1}) absorption bands in its IR spectrum and showed absorption peaks characteristic of a dihydroisocoumarin at 268 and 305 nm in its UV spectrum (Grove, 1972, Grove and Pople, 1979). The MS spectrum showed a molecular ion at *m/z* 344 (16%) corresponds to $C_{18}H_{16}O_7$ and other fragment peaks at *m/z* 326 (96%) formed by loss of H_2O from the molecular ion, *m/z* 179 (65%), 177 (100%), 151 (95%) and 150 (15%). It can be noticed that the peaks at *m/z* 179, 151 and 150 in the MS spectrum of **2** are consistent with the fragmentation pattern reported for dihydroisocoumarins (Grove, 1972, Grove and Pople, 1979).

The ^{1}H-NMR spectrum of **2** in methanol-d_4 exhibited the presence of an acetyl (δ 2.53), two methylenes (δ 2.86 and 2.82, and 3.05 and 2.94), a methine (δ 4.70) and four aromatic protons ascribable to two pairs of meta-coupled ones (δ 6.24 and 6.27, and 6.17 and 6.18, J=2.0 Hz). The signals at 2.94 and 3.05 were assignable to the equatorial proton and the axial proton of a methylene group, respectively, which were coupled to the vicinal methine proton appearing at δ 4.70 as a double doublet with J values of 14.1 and 5.5, and 14.1 and 7.84, respectively. Unresolved peaks due to the second methylene protons appeared at δ 2.86 and 2.82; Each peak showed coupling with vicinal methine proton. Assuming that **2** had a dihydroisocoumarin skeleton, its structure was most likely feralolide which is recently isolated only from a commercial sample of Cape aloe, *Aloe ferox*. The identity was confirmed by comparison of its physical properties and spectral data with those reported in the literature (Speranza *et al.*, 1993). Its structure was further confirmed by detailed analysis of the ^{1}H and ^{13}C-NMR spectra, aided by HMQC (Summers *et al.*, 1986) and HMBC (Bax and

Summers, 1986) experiments (Fig. 2). This is the second report of its occurrence in nature.

Compound 4, mp 234~6°C, showed characteristic positive color test(KOH and Molisch) for hydroxyanthraquinone glycoside and showed the presence of a hydroxyl (3,350 cm^{-1}), two kinds of ketones (1,660 and 1,635 cm^{-1}) and a glycoside bond (990-1,080 cm^{-1}), indicating that compound 4 is 1,8-dihydroxyanthraquinone glycoside (Thomson, 1987).

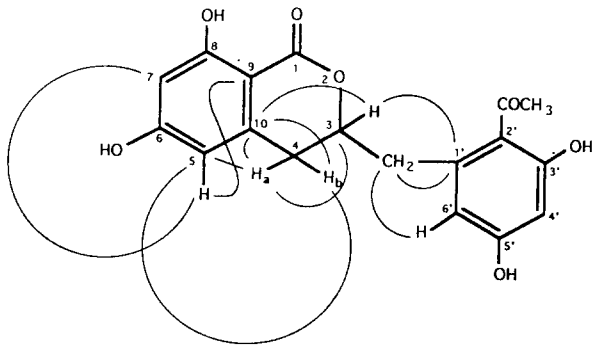

Fig. 2. HMBC corelations of compound 2

The ^{13}C-NMR spectrum showed absorption for thirty six carbones, two of which appeared in the region characteristic of hydrogen bonded carbonyls (δ 191.608 and 193.272), a carbonyl (δ 181.149), five oxygen-bearing tetrahedral carbons (δ 60-85) and a hydroxymethyl (δ 62.277) indicating C-bonded glucosidic nature, and two of which were signals for another hydroxymethyl group (δ 61.908 and 61.138). Therefore, **4** was suggested to be a anthraquinone dimer having D-glucose.

The ^{1}H-NMR spectrum of **4** revealed signals for nine aromatic protons, two hydroxymethyl groups, and a hexose. Coupling patterns among the aromatic protons indicated two pairs of *meta*-coupled protons (δ 6.847 and 6.891, and 7.661 and 7.205), one pair

Table III. ^{13}C-NMR chemical shift values for aloe-emodin, chrysophanol, aloin A, compound 4 and compound 5 in DMSO-d_6

Carbon	aloe-emodin[1)]	chryso-phanol[1)]	aloin A[2)]	A[a)]	B[b)]	Compound 4	Compound 5
1	161.72	161.67	160.8	161.1	161.6	161.2	161.5
2	120.78	124.16	112.7	123.8	123.8	120.5	120.8
3	153.80	149.26	151.4	149.1	149.4	150.1	151.3
4	117.18	120.64	117.8	120.4	120.3	120.5	120.4
5	119.43	119.41	118.9	118.8	118.8	118.3	118.3
6	137.42	137.41	136.1	132.8	132.8	135.4	135.4
7	124.49	124.49	115.4	148.0	148.1	146.9	146.8
8	161.43	161.41	161.1	157.7	157.9	159.7	159.7
9	191.74	191.72	193.4	191.7	191.9	191.6	191.7
10	181.59	181.57	44.2	181.0	181.3	181.1	181.2
1a	114.57	113.85	115.8	113.5	113.8	114.9	114.3
4a	133.22	133.10	142.0	132.6	132.6	131.1	131.3
5a	133.54	133.40	145.6	132.8	133.0	133.1	133.0
8a	116.01	115.94	117.1	115.4	115.7	115.4	115.4
CH_2OH	62.15	21.7	62.5	62.2		62.3	62.3
CH_3				21.5	21.7 21.9	62.0	61.9
1'			85.2	161.4	161.6	161.6	161.5
2'			70.3	116.8	120.4	116.8	117.5
3'			78.2	153.0	148.6	153.6	153.7
4'			70.3	113.3	116.6	112.3	112.3
5'			80.9	119.3	119.3	119.7	117.0
6'			61.4	136.8	136.8	138.0	138.3
7'				116.3	116.3	116.9	115.9
8'				161.4	161.3	161.4	161.4
9'				192.7	192.8	193.3	193.3
10'				69.6	69.7	69.5	70.2
1'a				112.9	112.4	112.3	111.6
4'a				141.7	141.9	141.6	141.6
5'a				147.8	147.8	145.3	145.8
8'a				114.3	114.4	114.7	114.4

[1)](Danielsen, *et al.*, 1992), [2)](Manitto, *et al.*, 1990)

[a)]1,1',8,8',10'-pentahydroxy-methyl-3'-hydroxymethyl-7,10'-bianthracene-9,9',10-trione(chrysalodin)

[b)]1,1',8,8',10'-pentahydroxy-3,3'-dimethyl-7,10'-bianthracene-9,9',10-trione

of ortho-coupled protons (δ 8.436 and 7.801, J=8.10 Hz) and an ABC system (6.597 (d, J=7.8 Hz), 6.743 (d, J=8.1 Hz) and 7.336 (dd, J=7.8 and 8.1 Hz)). The deshielded nature of the ortho coupled protons, which is indicative of H-5 and H-6 of an anthraquinone, and the absence of any H-10 proton in the anthrone required a C-10 to C-7 linkage. The anomeric proton of the hexose was observed as a doublet (J=9.3 Hz) at δ 4.275 indicating a C-glycoside. These spectral data were in agreement with those for the structure of elgonica-dimer B, previously known from *Aloe elgonica* (Conner, *et al.*, 1990).

Compound 5 obtained as amorphous powder. The IR, ^{1}H-NMR and ^{13}C-NMR spectral data was similar to that for compound 4 except in the anthrone moiety where appreciable variations were observed. These differences, which can only be attributed to a different arrangement of substituents around C-10. Accordingly, the structure of compound 5 was identified as elgonica dimer A, isomer in configuration at C-10 of **4**, previously known from *Aloe elgonica* (Conner, *et al.*, 1990).

Previous workers (Conner, *et al.*, 1990) reported that these two dimers, named elgonica-dimers A and B, isolated by circular preparative TLC on Si gel. However, we obtained these two compounds by column chromatography over Si gel eluting with CH_2Cl_2-MeOH (10 : 1). Elgonica dimer B was obtained as a major component from the early eluting fractions. The ^{13}C-NMR of **4** and **5** have been elucidated ambiguously for the first time (Table III). Compounds 4 and 5 have not been isolated from this plant by any of the earlier authors.

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