

Isolation of an Unusual Aloenin-acetal from Aloe

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Abstract—A hitherto unknown substance was isolated from the commercial sample of *Aloe arborescens*, which was shown to be the 4',6'-O-ethylidene-aloenin, most likely an artifact caused by hot-air drying procedure for the product preparation.

Keywords—*Aloe arborescens* • Liliaceae • acetal • 4',6'-ethylidene-aloenin

Aloe arborescens Miller has not only been used as an emmenagogue, a febrifuge in pleurisy and phthisis, and a remedy for gastrointestinal disorders, constipation, burns, insects bites^{1,2)}, but also been widely used as materials of cosmetics and health foods.

Because of the wide medicinal and cosmetic uses, the leaves of *Aloe* species have been the focus of chemical studies.

The leaves of *A. arborescens* is known to contain a number of anthracene and chromone derivatives such as chrysophanol, aloemodin, barbaloin, aloesin and its cinnamoyl esters, and a phenyl- α -pyrone, aloenin³⁻⁵⁾.

Aloenin, a major constituent, has been reported to exhibit inhibitory effects on gastric juice secretion of rats⁶⁾, on rat mast cell degranulation⁷⁾, on histamine release from rat mast cells induced by compound 48/80 or A23187⁸⁾ and on carrageenin-induced rat-paw inflammatory edema⁹⁾, and promotion effect on hair growth in depilated mice⁹⁾.

It was, recently, observed that a single oral administration of the methanol soluble fraction

from the leaves of *Aloe* species caused a significant decrease in alcohol dehydrogenase activity in rat liver cytosol¹⁰⁾.

As a part of our interest in alcohol metabolism modifiers, a systematic chemical investigation of the plants has been carried out. In the course of phytochemical examinations of *Aloe* species, a new compound, tentatively named aloenin X, was isolated from the ethylacetate soluble fraction of the methanol extract prepared from the commercial sample of *A. arborescens* leaves in addition to known compounds, aloemodin and aloenin.

The IR spectrum of aloenin X exhibited peaks at 3414(OH), 1682(conjugated ester), 1626, 1597, 1564(aromatic ring) and 1100~1000 cm⁻¹ (glycoside bond). The UV spectrum showed absorption maxima at 219.4 nm(log ϵ 4.36) and 301.6 nm(log ϵ 4.27), and in an alkaline solution showed a bathochromic shift by 40.2 nm. These spectra of aloenin X revealed strong similarities with those of 4-methoxy-2-pyrone¹¹⁾ and of aloenin⁶⁾.

The ¹H-NMR spectrum in DMSO-d₆ showed the presence of a methyl(δ 1.24, d, $J=5.01$ Hz), an aromatic methyl(δ 2.13, s), a methoxyl(δ 3.84, s), two pairs of *meta*-coupled aromatic

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protons (δ 5.59 and 6.17, d each, $J=2.03$ Hz and δ 6.40 and 6.51, d each, $J=1.69$ Hz), an anomeric proton (δ 4.96, d, $J=7.69$ Hz), seven oxymethins (δ 3.19~4.71), two alcoholic OHs (δ 3.25 and 5.22, brs each) and a phenolic OH (δ 9.78, brs).

The ^{13}C -NMR spectrum displayed 21 carbon resonances. Two methyl carbon resonances were located at δ 19.84 and 20.39, one methoxyl carbon resonance at δ 56.13, hexose carbon resonances at δ 101.14, 79.79, 74.20, 72.94, 67.39, 65.88, respectively. Judging from the DEPT experiment, it is clear that the remaining carbon resonances are due to 5 methines and 7 quaternary carbons.

The mass spectrum of aloenin X exhibited a molecular ion peak at m/z 436 in accord with the formula $\text{C}_{21}\text{H}_{24}\text{O}_{10}$ and a fragment ion peak at m/z 248 corresponding to the aglucone.

The anomeric proton was observed as a doublet at δ 4.96 and an anomeric carbon, δ 101.14, indicating an O-glycoside.

Hydrolysis of aloenin X with 3% hydrochloric acid afforded glucose as a sugar moiety and the genin, which was found to be identical with that of aloenin by direct comparison (mmp, co-TLC, EIMS) with an authentic sample.

Appearance of a three proton doublet at δ 1.24 and a quartet at δ 4.71 assignable to ethylidene group in ^1H NMR together with its molecular weight suggested that aloenin X be an ethylidene derivative of aloenin.

In comparison of the ^1H - and ^{13}C -NMR of aloenin X with those of aloenin, the signals due to the aglucone moiety of both compounds appeared at almost same positions.

C-4' and C-6' signals of aloenin X were dramatically downfield of its position of aloenin and C-3' and C-5' signals were upfield, indicating acetalation of the C-4' and C-6' oxygens of the sugar. As expected, the signals due to the sugar moiety of aloenin X appeared at

almost the same positions as those of methyl 4,6-O-ethylidene- β -D-glucopyranoside¹²⁾. The β -configuration of D-glucosyl moiety of aloenin X, was inferred by the coupling constant value of 7.69 Hz for the anomeric proton.

Therefore, the structure of aloenin X has now been established as 4-methoxy-6-[2-(4',6'-O-ethylidene-)- β -D-glucopyranosyloxy-4-hydroxy-6-methylphenyl]-2-pyrone.

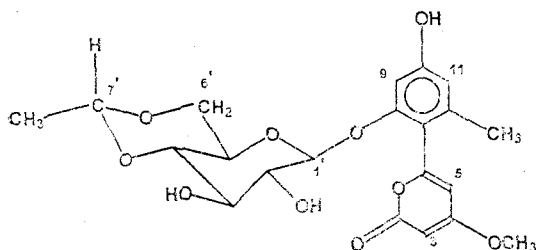
Although there were a few instances of isolation of ketals such as 4',6'-O-isopropylidene aloin B from an *Aloe* species¹³⁾, this is the first report of isolation of an acetal from plants. Nevertheless, it would be an artifact formed during hot-air drying procedure for the product preparation but the cause for its formation remains unclear.

EXPERIMENTAL

General experimental procedures—Melting points were measured on a Mitamura-Riken apparatus and are uncorrected. IR measurements were recorded in KBr pellets on a JASCO FT/IR-5300 spectrometer and EI-MS spectra were obtained on a Hewlett Packard Model 5985 B GC/MS System. A Bruker CXP-300 spectrometer was used to record ^1H (300 MHz) and ^{13}C (75.08 MHz) NMR spectra in $\text{DMSO}-d_6$ with TMS as internal standard and chemical shifts are reported in δ (ppm) and coupling constants are in Hz.

Plant material—*Aloe arborescens* sample used in this investigation was a commercial product prepared in KJM Aloe Co., Ltd.

Isolation of aloenin X—The sample (100 g) was refluxed with MeOH. The MeOH extract (21 g) was partitioned with hexane (1.7 g), CHCl_3 (1.3 g), EtOAc (2.4 g), successively. The EtOAc soluble portion was subjected to column chromatography over Si gel eluting with CHCl_3 and then CHCl_3 containing increasing amounts of MeOH. Separation was monitored by TLC



and fractions containing aloenin X as a major component were combined. A further purification by flash chromatography (eluent: CHCl_3 -MeOH = 9 : 1) afforded aloe-emodin (1.8 mg), aloenin X (47.4 mg) and aloenin (2.2 mg), which were found to be pure on TLC.

Structural elucidation of known compounds—The structures of the known compounds, aloenin, its aglucone and aloe-emodin were each confirmed by comparing their MS and NMR data with those described in the literature^{14,15}.

Aloenin X—Needles from MeOH, mp 246~247°; UV, IR and $^1\text{H-NMR}$: see text, $^{13}\text{C-NMR}$: see Table I.

Aloe-emodin—Needles from MeOH, mp 216~217°; $^1\text{H-NMR}$ (DMSO- d_6) δ : 4.62 (2 H, brs, 3- CH_2), 7.30 (1 H, s, H-2), 7.37 (1 H, d, $J=7.8$ Hz, H-7), 7.66 (1 H, s, H-4), 7.72 (1 H, d, $J=7.8$ Hz, H-5), 7.79 (1 H, t, $J=7.8$ Hz, H-6), 11.95 (2 H, brs, 2 x OH); EIMS m/z (rel. int., %) 270 (95), 241 (100).

Aloenin—Needles from MeOH, mp 147~148°; UV, λ_{max} 230, 303; (+NaOH) 247, 346 nm; $^1\text{H-NMR}$ (DMSO- d_6) δ : 2.13 (3 H, s, 12-Me), 3.04~3.69 (6 H, m), 3.84 (3 H, s, 4-OMe), 4.80 (1 H, d, $J=7.4$ Hz, H-1'), 4.96 (2 H, brs, OH), 5.59 (1 H, d, $J=2.2$ Hz, H-3), 6.22 (1 H, d, $J=2.2$ Hz, H-5), 6.38 (1 H, brs, H-11), 6.48 (1 H, d, $J=2.0$ Hz, H-9), 9.81 (1 H, brs, OH); EIMS m/z (rel. int., %) 262 ($\text{C}_{14}\text{H}_{14}\text{O}_5^+$, 19.3) 248 (100), 151 (25.5), 150 (24).

Acid hydrolysis of aloenin X—Aloenin X (10 mg) dissolved in 3% HCl (5 ml) had been heated under reflux for 2 hr, the mixture was

Table I. $^{13}\text{C-NMR}$ spectral data of aloenin X and related compounds

Carbon No.	1	2	3
2	164.28	163.78	
3	88.00	87.91	
4	171.11	170.79	
5	104.44	104.20	
6	157.90	157.44	
7	113.85	114.85	
8	156.33	156.40	
9	100.70	100.80	
10	159.57	160.96	
11	111.30	109.21	
12	139.30	138.95	
12- CH_3	19.84	19.66	
4- OCH_3	56.13	56.13	
10- OCH_3 (1'- OCH_3)		55.15	56.7
1'	101.14	99.31	104.1
2'	74.20	73.17	74.2
3'	72.94	76.72	72.9
4'	79.79	69.78	79.8
5'	65.88	77.11	65.9
6'	67.37	60.73	67.7
7'	98.76		99.4
7'- CH_3	20.39		19.6

1: Aloenin X at 75.08 MHz in DMSO- d_6 .

2: Methylaloeenin at 75.47 MHz in DMSO- d_6 .¹⁶⁾

3: Methyl-4,6-O-ethylidene- β -D-glucopyranoside at 15.08 MHz in CDCl_3 - CD_3OD =4 : 1.¹²⁾

diluted with water and extracted with ether. The ether extract gave the aglucone (4 mg). The aqueous layer was neutralized and the neutral solution on concentration under reduced pressure gave D-glucose, which was confirmed by TLC analysis.

Aglucone—Amorphous solid, mp 213~214°, $^1\text{H-NMR}$ (DMSO- d_6) δ : 2.08 (3 H, s, 12-Me), 3.82 (3 H, s, 4-OMe), 5.59, 6.11 (1 H, d, $J=2.2$ Hz, H-5), 6.16 (1 H, brs, H-11), 6.23 (1 H, d, $J=2.0$ Hz, H-9), 9.59 (1 H, brs, OH), 9.65 (1 H, brs, OH); EIMS m/z (rel. int., %) 248 (100), 151 (40), 150 (40).

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