Biologically Active Constituents of Leaves and Roots of *Aloe arborescens* var. *natalensis*

Toshifumi Hirata and Takayuki Suga

Department of Chemistry, Faculty of Science, Hiroshima University

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Aloe Plant (Liliaceae), Biologically Active Substances, Inhibitory Activity on Gastric Juice Secretion

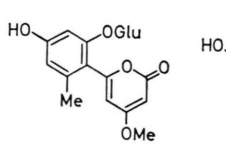
Several biologically active substances, such as aloenin (**1**), magnesium lactate, aloe-emodin (**4**), barbaloin (**5**), and succinic acid, were found to be contained in the leaf juice of *Aloe arborescens* Mill. var. *natalensis* Berger, which has widely been used in domestic medicines. Aloenin (**1**) and magnesium lactate were elucidated to exhibit an inhibitory action on the gastric juice secretion of rats. Various constituents other than the above bioactive substances were found in the leaves and the roots of the plant.

The chemical constituents of the plants of Aloe species used widely in domestic medicines have been investigated by several groups of workers [1–16], and the anthraquinone derivatives of the constituents have been reported to be effective as a peptic or a laxative [1, 2]. In this country, *Aloe arborescens* Mill. var. *natalensis* Berger (Japanese name: Kidachirokai or Kidachiaroe) has traditionally been used as the materials for folk remedies for gastro-intestinal disturbances, burns, insect bites, athlet's foot, and etc. We recently isolated a new bitter glucoside, named aloenin (**1**), as a major constituent from the leaf juice of this plant, and elucidated its structure [8, 10, 14, 17] and biosynthetic pathway [18]. Nishioka *et al.*, on the other hand, found 2″-O-feruloylaloesin (**2**) [12], 2″-O-*p*-coumaloylaloesin (**3**) [12], and aloearbonaside [9] in the leaves of the plant; the last compound was identified with aloenin (**1**). Since chemical constituents other than these compounds, **1**, **2**, and **3**, have not been examined yet with the *Aloe arborescens*, we have now investigated the constituents of the leaves and the roots to clarify whether the Aloe plants contain the biologically active substances which are effective for the folk remedies materials.

Results and Discussion

The leaves of the Aloe plant were minced and squeezed to give a green juice and a residue. We isolated eight constituents from the leaf juice on chromatographic separation, and identified them as aloenin (**1**) [17], 2″-O-feruloylaloesin (**2**) [12], aloe-emodin (**4**) [1], barbaloin (**5**) [1], aloesin (**6**) [6], succinic acid, D-glucose, and magnesium lactate. Although the anthraquinone derivatives, (**4**) and (**5**), and aloesin (**6**) have been reported to be present in other Aloe species [1, 6], this has not been the case for *Aloe arborescens*. The dry-incinerated sample of the leaf juice was further found to contain the metal ions composed mainly of potassium and sodium atoms on spectroscopic analyses. In addition, a methanol extract of the leaf residue consisted of *n*-alkanes, *n*-triacontanol, *n*-dotriacontanol, sitosterol, free fatty acids, and fatty acid methyl esters. On the other hand, a methanol extract of the roots was found to be composed of *n*-alkanes, aloe-emodin (**4**), sitosterol, 1-linoleyl monoglyceride, fatty acids, fatty acid methyl esters, 3-O-[β-D-glucopyranosyl]-sitosterol, D-glucose, and magnesium lactate.

Since the leaf materials of the Aloe plant are frequently used as the folk remedies materials for gastro-intestinal disturbance, an effect of the constituents on the gastric juice secretion was tested following Shay's method [19]. As the result, aloenin (**1**)



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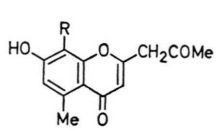
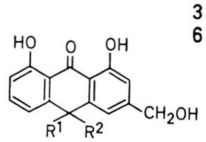


2: R=2″-O-feruloylglucosyl
3: R=2″-O-*p*-coumaroylglucosyl
6: R=glucosyl



4: $R^1, R^2 = O$
5: R^1 = glucosyl, R^2 = H

Requests for reprints should be sent to Prof. T. Suga, Department of Chemistry, Faculty of Science, Hiroshima University, Higashisenda-machi, *Hiroshima 730*, Japan.

Table I. Inhibition of gastric juice secretion by some constituents of Aloe species.

Compounds	Origins	Dose [mg/kg]	pH	Gastric juice [ml/100 g body wt.]	Inhibition [%]
Control	—	—	1.8	4.7	0
Aloenin (**1**)	*A. arborescens*	100	1.8	3.6	23
Aloe-ulcin [16]	Cape Aloe	100	2.0	3.1	34
Magnesium lactate	*A. arborescens*	50	2.0	3.2	32
Magnesium lactate	Commercially	100	1.8	3.0	36

and magnesium lactate were proved to exhibit the inhibitory activity on the gastric juice secretion, as shown in Table I. Here, we wish to point out that a substance, named aloe-ulcin, with an inhibitory action on histidine decarboxylase has been reported to be isolated from "Cape Aloe" [16], but it has remained unidentified. However, it has now been found that the substance should be magnesium lactate on the ground of complete agreement of its infrared spectrum with that of magnesium lactate. In conclusion, it is fascinating to note that the leaves of *Aloe arborescens* were found to contain aloenin (**1**) and magnesium lactate which both exhibit an inhibitory activity on the gastric juice secretion and the latter an inhibitory action on histidine decarboxylase, aloe-emodin (**4**) and barbaloin (**5**) which are used in the laxatives, and succinic acid which is effective for arthritis and rheumatic fever in a combination with salicylate.

Experimental

Mps were determined by means of a hot plate and are uncorrected. GLC analyses were performed on an instrument attached with FID and a column (2 m × 3 mm) packed with 10% PEGS or 2% SE-30 on Celite (60 ~ 80 mesh) and 2% OV-17 on Chromosorb AW-DMCS (80 ~ 100 mesh).

Extraction and isolation. After cuttings of the lateral buds (ca. 5 cm long) of the Aloe plant had been planted and then cultivated on pots for 3 ~ 4 years, the leaves (3.7 kg) were collected in late April, minced mechanically and squeezed on a three fold gauze to give a green leaf juice. The leaf juice on evaporating to dryness on a steam bath afforded a brown viscous mass, which was subjected to column chromatography on silica gel with $CHCl_3$ – MeOH mixture with MeOH increasing 0 to 100% and then with H_2O, and then to preparative TLC on silica gel with $CHCl_3$ – MeOH (4 : 1) to give aloe-emodin (**4**) (205 mg), barbaloin (**5**) (120 mg), 2″-O-feruloylaloesin (**2**) (38 mg), aloenin (**1**) (1.78 g), aloesin (**6**) (13 mg), succinic acid (1.68 g), D-glucose (521 mg), and magnesium lactate (419 mg) in order of their polarity. The leaf residue separated from the leaf juice, on the other hand, was immersed in MeOH at room temp. for one month. Removal of the solvent from the methanol solution gave a brown viscous sirup, which on diluting with ether was treated with 2% $NaHCO_3$ to separate an acidic fraction (0.63 g) and a neutral fraction (5.72 g). The acidic fraction, upon methylation with CH_2N_2 and then chromatography on a gas chromatograph, was found to be composed majorly of fatty acids. A part (2.0 g) of the neutral fraction was chromatographed on a silica gel column with *n*-hexane-EtOAc mixture with EtOAc increasing 0 to 30% to give *n*-alkanes (10 mg), fatty acid methyl esters (75 mg), *n*-triacontanol (21 mg), *n*-dotriacontanol (9 mg), and sitosterol (15 mg).

The roots (3.5 kg) of the above Aloe plant were immersed in MeOH at room temp. for one month. A methanol extract obtained by removing the solvent from the methanol solution was extracted with ether to give an ether soluble fraction and an ether insoluble one. The ether soluble fraction (581 mg) was subjected to preparative TLC (SiO_2) with *n*-hexane-EtOAc (7 : 3) to give *n*-alkanes (6 mg), fatty acid methyl esters (226 mg), sitosterol (53 mg), aloe-emodin (**4**) (12 mg), 1-linoleyl monoglyceride (85 mg), and fatty acids (131 mg). The ether insoluble fraction on removal of the solvent gave a viscous oil (2.82 g), which was chromatographed on a silica gel column with $CHCl_3$ – MeOH mixture with MeOH increasing 0 to 100% to give 3-O-[β-D-glucopyranosyl)-sitosterol (72 mg), D-glucose (580 mg), and magnesium lactate (1.12 g).

Identification of the compounds. Aloenin (**1**). m.p. 145 ~ 147 °C (lit.[10] 145 ~ 147 °C); $[\alpha]_D^{25}$ −26.8° (*c* 2.2, MeOH); IR (KBr) ν_{max} 3400, 1713, 1640, 1605, 1560 cm^{-1}; PMR (Acetone-d_6) δ 2.19 (s, arom. Me), 3.86 (s, OMe), 5.47 (d, J = 2.5 Hz. >C=CH–), 6.15 (d, J = 2.5 Hz, >C=CH–), 6.45 (d, J = 2.2 Hz, arom. H), 6.62 (d, J = 2.2 Hz, arom. H); UV (EtOH) λ_{max} 232 nm (log ε 3.87), 245 (3.81), 307 (3.91); direct comaparison (co-TLC, IR, PMR, UV, and mmp).

Aloe-emodin (**4**). m.p. 220～221 °C (decomp.) (lit.[1] 216～219 °C); IR(KBr) ν_{max} 3400, 1670, 1620 cm^{-1}; PMR (C_5D_5N) δ 5.00 (s, $-CH_2-OH$) and 7.1～7.6 (m, arom. H); UV(EtOH) λ_{max} 253 nm (log ε 4.65), 288 (4.28), 432 (4.24); direct comparison (co-TLC, IR, PMR, UV, and mmp). *Barbaloin* (**5**). m.p. 148～149 °C (decomp.) (lit.[1] 148～148.5 °C); $[\alpha]_D^{25}$ −15.1° (*c* 0.5, MeOH); IR(KBr) ν_{max} 3420, 1640, 1620 cm^{-1}; PMR (Acetone-d_6) δ 5.03 (s, $-CH_2OH$) and 7.1～7.6 (m, arom. H); UV(MeOH) λ_{max} 260 nm (log ε 3.80), 269 (3.95), 298 (4.00), 360 (4.03); direct comparison (co-TLC, IR, PMR, UV, and mmp). *Aloesin* (**6**). m.p. 142～144 °C (lit.[6] 143～144 °C); $[\alpha]_D^{25}$ +59.2° (*c* 0.23, EtOH); IR(KBr) ν_{max} 3300, 1715, 1662, 1596 cm^{-1}; UV(MeOH) λ_{max} 216 nm (log ε 4.30), 250 (4.15), 254 (4.20), 298 (4.00); PMR(Acetone-d_6) δ 2.20 (s, arom. Me), 2.62 (s, $-CO-Me$); direct comparison (co-TLC, IR, PMR, UV, and mmp). *2″-O-Feruloylaloesin* (**2**). m.p. 152～156 °C (lit.[12] 153～156 °C); $[\alpha]_D^{25}$ −73.2 °C (*c* 0.78, MeOH); IR(Nujol) ν_{max} 3400, 1715, 1650, 1600 cm^{-1}; UV(MeOH) λ_{max} 240 nm (log ε 4.00), 300 (4.20); PMR(Acedone-d_6) δ 2.33 (s, arom. Me), 2.75 (s, $-CO-Me$), 3.92 (s, OMe); direct comparison (co-TLC, IR, PMR, UV, and mmp). *Sitosterol.* m.p. 138～139 °C; $[\alpha]_D^{25}$ −30.5° (*c* 0.6, MeOH); PMR ($CDCl_3$) δ 5.35 (m, $>C=CH-$), 3.50 (m, $>CH-O-$); direct comparison (co-TLC, co-GLC, IR, PMR, and mmp). *n-Triacontanol.* m.p. 82～83 °C; IR(KBr) ν_{max} 3300, 1050 cm^{-1}; PMR($CDCl_3$) δ 0.93 (b.t, Me), 1.33 (b.s, $-CH_2-$), 3.65 (t, $-CH_2OH$); MS *m/e* 420 (M-H_2O). *n-Dotriacontanol.* m.p. 82～83 °C; IR(KBr) ν_{max} 3300, 1050 cm^{-1}; PMR ($CDCl_3$) δ 0.91 (b.t, Me), 1.30 (b.s, $-CH_2-$), 3.62 (t, $-CH_2OH$); MS *m/e* 448 (M-H_2O). *1-Linoleyl monoglyceride.* $C_{21}H_{38}O_4$ (Found: C, 71.00; H, 10.70%); $[\alpha]_D^{25}$ −0.5° (*c* 0.7, MeOH); IR(Liquid) ν_{max} 3400 (OH), 1735 cm^{-1} ($-CO-O-$); PMR($CDCl_3$) δ 2.77 (b.t, J = 5.0 Hz, 2H, $-CH_2-CO-O-$), 3.13 (b,s, 2H, OH), 3.6～4.3 (m, 5H, $>CH-O-$ and $-CH_2-O-$), 5.40 (m, 4H, 2× $-CH=CH-$); MS *m/e* (rel. intensity) 354 (M^+, 5), 262 (100). The acetate derivative: IR(Liquid) ν_{max} 1734 cm^{-1} ($-CO-O-$); PMR($CDCl_3$) δ 2.07 (s, 6H, 2×OAc), 2.73 (b.t, J=5.0 Hz, 2H, $-CH_2-CO-O-$), 4.0～4.2 (m, 4H, 2× $-CH_2-O-$), 5.32 (m, 1H, $>CH-O-$), 5.35 (m, 4H, 2× $-CH=CH-$). Saponification of the original monoglyceride with 5% KOH−MeOH for 12 hr at room temp. gave linoleic acid, which on methylation with CH_2N_2 was identified by direct comparison (co-TLC, co-GLC, IR, and PMR). *3-O-[β-D-Glucopyranosyl]-sitosterol.* $C_{35}H_{60}O_6$ (Found: C 72.67; H, 10.29%); m.p. 280～282 °C (lit.[20] 280～282 °C); $[\alpha]_D^{25}$ −38.1° (*c* 0.32, MeOH), IR(Nujol) ν_{max} 3400 (OH), 1650 (C=C), 1020 cm^{-1} (C−O). The PMR spectrum was not obtained because this compound was practically insoluble in the solvents commonly used for PMR measurements. The acetate derivative: m.p. 166～167 °C (lit.[20] 166～167 °C); IR(Liquid) ν_{max} 1740 cm^{-1} ($-CO-O-$); PMR ($CDCl_3$) δ 0.8～1.4 (m, Me), 2.00 (s, OAc), 2.01 (s, OAc), 2.05 (s, OAc), 2.07 (s, OAc), and 5.36 (m, $>C=CH-$). Hydrolysis of the original glucosyl sitosterol with 5% HCl gave D-glucose and sitosterol. D-*Glucose.* m.p. 145～146 °C; $[\alpha]_D^{25}$ +88.0° → +43.0° (*c* 2.0, H_2O); direct comparison (co-TLC, co-PC, IR, PMR, and mmp). *Magnesium lactate.* $Mg(C_3H_5O_3)_2 \cdot 3H_2O$ (Found: C, 28.09; H, 6.29; Mg, 9.47%). Determinations of water of crystallization and magnesium were performed on a differential thermal and thermal gravity analyzer and an atomic absorption spectrometer, respectively; IR(Nujol) ν_{max} 1610 cm^{-1} (carboxylate ion); PMR(D_2O) δ 1.32 (d, J=6.5 Hz, CH−Me), 4.15 (q, J=6.5 Hz, $>CH-Me$); direct comparison (IR and PMR); the emission spectrum exhibited bright lines at 2795.5, 2802.7, and 2852.2 Å, which are characteristic of a magnesium atom. *Metal ions.* The emission spectroanalysis and the atomic absorption analysis of the dry-incinerated sample of the leaf juice indicated the presence of potassium (57.1% in total metal components), sodium (31.7), manganese (8.8), magnesium (2.3), calcium (1.3), and copper atoms (0.01). *Fatty acids and fatty acid methyl esters.* Determinations of the fatty acid compositions of the free fatty acid and the fatty acid methyl ester fractions were performed by means of GLC. However, the free fatty acids, after methylation with CH_2N_2, were subjected to the chromatography. The results are given in Table II. *n-Alka-*

Table II. Fatty acid compositions of the free fatty acid and the fatty acid methyl ester fractions isolated from the leaves and the roots.

Acid	Leaves		Roots	
	Free acid [%]	Methyl ester [%]	Free acid [%]	Methyl ester [%]
Myristic	0.2	1.0	0.1	0.1
Palmitic	18.2	2.8	49.1	46.3
Stearic	7.1	2.2	20.7	23.5
Oleic	5.3	2.1	6.1	8.2
Linoleic	27.5	35.3	10.2	10.3
Linolenic	35.2	50.4	8.9	8.1
Unknown	6.5	6.2	4.9	3.5

nes. The *n*-alkane compositions of the alkane fractions on determination by means of GLC were as follows: for the leaf alkanes, C_{12} (0.7% in total alkanes), C_{12} (0.8), C_{14} (0.5), C_{15} (1.6, C_{16} (3.3), C_{17} (1.7, C_{18} (1.7), C_{19} (2.4), C_{20} (2.8), C_{21} (3.0), C_{22} (3.2), C_{23} (8.4), C_{24} (0.7), C_{25} (2.8), C_{26} (1.3), C_{27} (1.1), C_{28} (0.2), C_{29} (22.7), C_{30} (0.1), C_{31} (20.3), and C_{32} (6.5) and for the root alkanes, C_{12} (0.4), C_{13} (9.7), C_{14} (1.9), C_{15} (10.6), C_{16} (1.4), C_{17} (8.5), C_{18} (1.9), C_{19} (10.9), C_{20} (1.2), C_{21} (9.1), C_{22} (0.7), C_{23} (7.3), C_{24} (0.6), C_{25} (2.9), C_{26} (1.3), C_{27} (0.1), C_{28} (0.1), C_{29} (12.3), C_{30} (0.1), C_{31} (8.1), and C_{32} (2.8).

An inhibitory action of several components on the gastric acid secretion. An inhibitory action of several components, such as given in Table I, on the gastric juice secretion was tested following Shay's method[19]. Male rats of Donryu strain 130 ~ 170 g in body weight were used for the bioassay. The volume and pH of the gastric juice secreted during 4 hr after oral administration of the samples through a gastro-tube are shown in Table I.

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