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Studies on the Constituents of *Aloe arborescens* Mill. var. *natalensis* Berger. I. The Structure of Aloearbonaside, a Glucoside of a New Type naturally Occurring Chromene[1]

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A new phenolic glucoside named aloearbonaside was isolated from the leaves of *Aloe arborescens* Mill. var. *natalensis* Berger in addition to barbaloin. The structure of aloearbonaside was established as I, methyl 2-(5-methyl-7-hydroxy-4-O-β-D-glucopyranosyl)-chromenylideneacetate, a glucoside of a new type naturally occurring chromene, on the basis of spectral and chemical evidences.

The isolation of anthraquinones,[3] barbaloin,[4] homonataloin,[5] aloinosides A[6] and B[6] from various *Aloes* together with their structural studies have been established. Recently, Haynes, *et al.*[7] reported the isolation of aloesin from bitter *Aloes* and identified it as the C-glucosylchromone, 2-acetonyl-8-glucopyranosyl-7-hydroxy-5-methylchromone. We attempted to examine the active constituents of *Aloe arborescens* Mill. var. *natalensis* Berger (Japanese name: Kidachiaroe) which was extensively used as domestic medicine in Japan. This report deals with the isolation of phenolic compounds, barbaloin and a new glucoside named aloearbonaside, and the evidence leading to the structure (I) for this. This is a glucoside of a new type naturally occurring chromene to be described.

Since the fresh leaves were used as antiphlogistics for burns and wounds, or as carminatives, the investigation of the original substance was of significance. The following three samples were prepared, and examined by thin-layer chromatography (TLC). 1) The methanol extract of fresh leaves, 2) the lyophilized methanol extract of fresh leaves, and 3) the methanol extract of the powdered leaves prepared from the sun-dried chops.

The TLC (Fig. 1) showed that barbaloin was very sensitive to aerial oxidation to give aloe-emodin, and no remarkable change in the amount of aloearbonaside was observed in three samples. Accordingly, 1 and 3 were employed as starting materials for the isolation of barbaloin and aloearbonaside, respectively. The methanol extract of the sun-dried (or the fresh) leaves was worked up by repeated polyamide, silica gel chromatography and recrystallization to give two phenolic compounds, barbaloin and aloearbonaside (Chart 1).

Barbaloin, mp 137—140°, $[\alpha]_D^{25}$ −13.0°, $C_{21}H_{22}O_9 \cdot 2H_2O$, was identified with an authentic sample[8] by mixed melting point, infrared (IR), ultraviolet (UV) spectral[4c] and TLC comparison.

1) This work was presented at the 92nd Annual Meeting of the Pharmaceutical Society of Japan, April, 1972. "Abstracts of Papers" II, p. 221.
2) Location: *Katakasu, Higashi-ku, Fukuoka.*
3) W. Karrer, "Konstitution und Vorkommen der organishen Pflanzenstoffe," Birkhäuser verlag, Basel und Stuttgart, 1958, pp. 503—528.
4) *a*) H. Mühlmann, *Pharm. Acta Helv.*, 27, 17 (1952); *b*) A.J. Birch and F.W. Donovan, *Aust. J. Chem.*, 8, 523 (1955); *c*) J.E. Hay and L.J. Haynes, *J. Chem. Soc.*, **1956**, 3141.
5) L.J. Haynes, J.E. Henderson, and J.M. Tyler, *J. Chem. Soc.*, **1960**, 4879.
6) L. Hörhammer, H. Wagner, and G. Bittner, *Z. Naturforsh.*, **19b**, 222 (1964).
7) L.J. Haynes, D.K. Holdsworth, and R. Russel, *J. Chem. Soc.*, **1970**, 2581.
8) The authentic sample was prepared from the commercial "Aloin®" (Merck).

Aloearbonaside (I), mp 135°, $[\alpha]_D^{11}$ −23.5°, $C_{19}H_{22}O_{10}\cdot H_2O$, indicated the positive reaction for hydroxamic acid, Echtblau salz B-KOH[9] and Fehling reagent. I showed the ester band at 1710 cm^{-1} and the hydroxy band at 3300 cm^{-1} (br) in IR spectrum (in dioxane), and the benzenoid absorption at 302 mμ in UV spectrum. On acetylation with Ac_2O and pyridine (I) afforded pentaacetate (Ia), mp 91°, $C_{29}H_{32}O_{15}$, and on methylation by Hakomori's method[10] I gave pentamethyl ether (Ib), mp 140°, $C_{24}H_{32}O_{10}$, possesing the anomeric proton signal with coupling constant (J=6 Hz) at δ 4.80 ppm in nuclear magnetic resonance (NMR) spectrum. On hydrolysis using 1% HCl in H_2O, followed by silica gel chromatography, I provided aglycone A (II), mp 257—260° (sublimate at 200°), $C_{11}H_{10}O_3$, revealing the strong fluorescence by KOH solution, together with aglycone B(III), mp 201°, $C_{13}H_{12}O_5\cdot\frac{1}{2}H_2O$, and D-glucose. On hydrolysis using emulsin, I only gave an aglycone B as aglycone. And aglycone B was quantitatively led to aglycone A under treatment with 3% HCl in EtOH. Accordingly, I was determined to be monoglucoside of aglycone B.

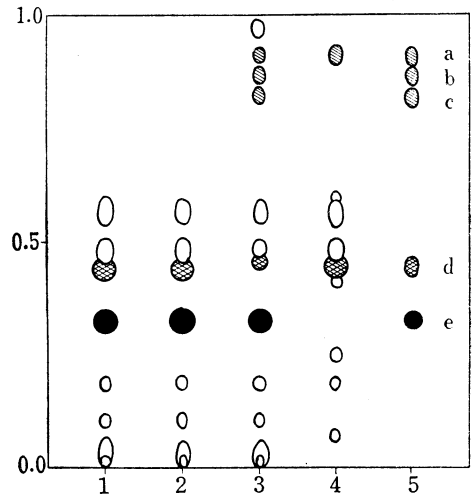


Fig. 1. Thin-Layer Chromatogram on Silica Gel[a]

1: the methanol extract of fresh leaves
2: the lyophilized methanol extract of fresh leaves
3: the methanol extract of the powdered leaves prepared from the sun-dried chops
4: the commercial aloin
5: specimen samples
a) aloe-emodin b) aglycone A c) aglycone B
d) barbaloin e) aloearbonaside
a) solvent, AcOEt:MeOH:H_2O (20:3:2); color reagent, Echtblausalz B-KOH

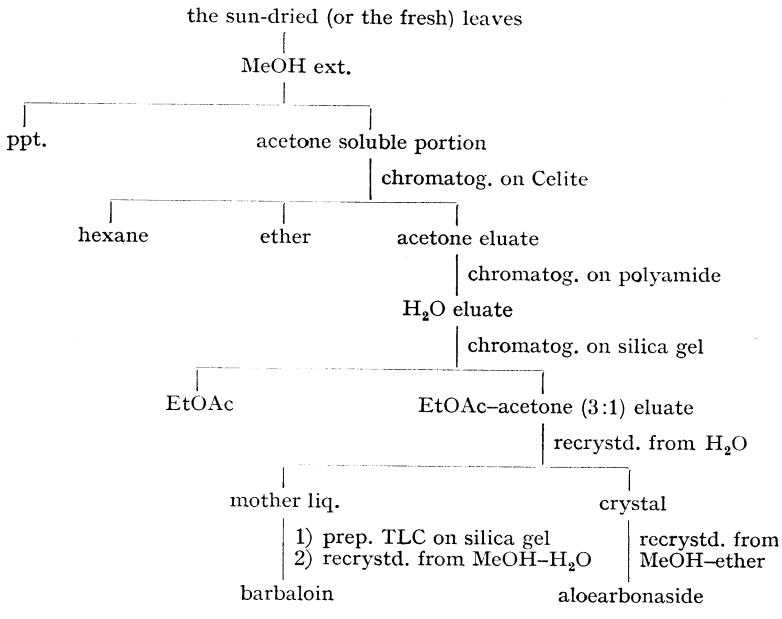


Chart 1

9) L. Hörhammer, H. Wagner, and G. Bittner, *Arzneim-Forsh.*, **13**, 537 (1963).
10) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

Acetylation of aglycone A (II) with Ac_2O and NaOAc gave monoacetate (IIa), mp 117°, $C_{13}H_{12}O_4$ and methylation of II using diazomethane afforded monomethyl ether (IIb), mp 114—115°, $C_{12}H_{12}O_3$. The IR spectrum of II indicated the characteristic absorption bands for chromone[11a] at 1660, 1615 cm^{-1} and the hydroxy group at 3240 cm^{-1} (in dioxane).

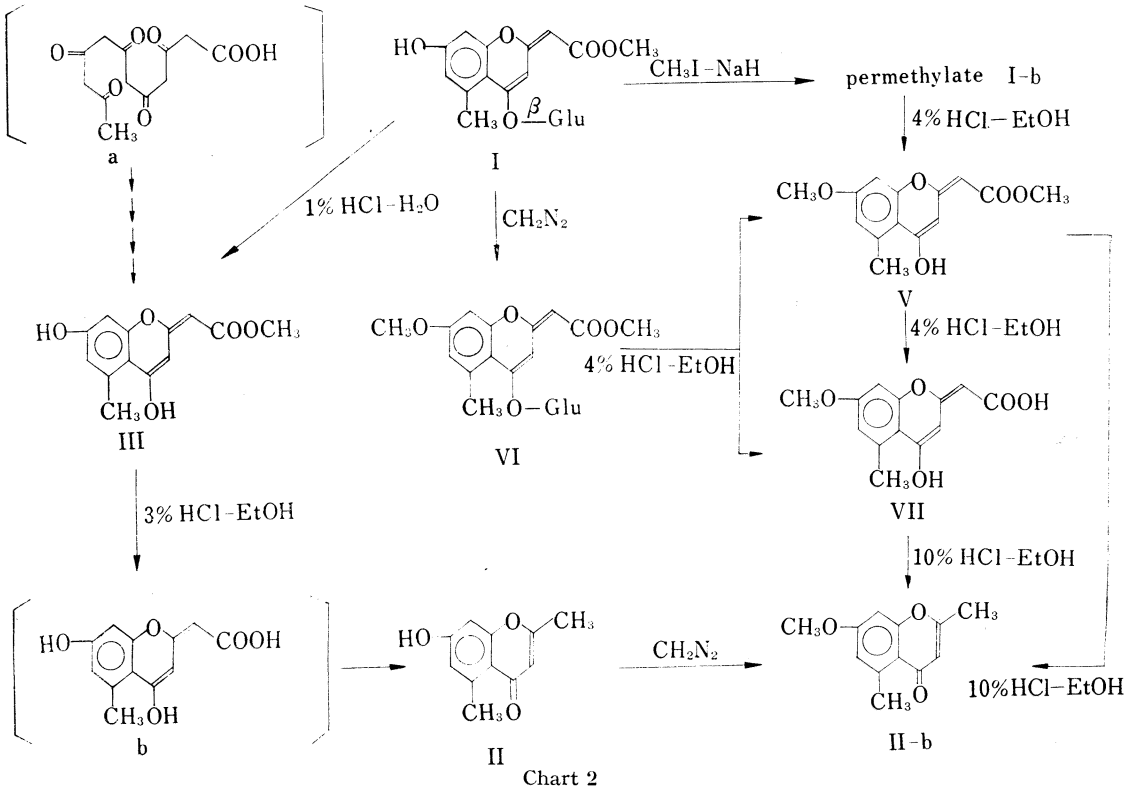


Chart 2

TABLE I. The NMR Data[a]

Compounds	Solvent	C_2-CH_3	C_5-CH_3	C_3-H	C_6-H	C_8-H	-CH=C_2-C_3H=C_4-	
I	$(CD_3)_2SO$	—	2.14	—	6.35	6.47	5.59	6.23
Ia	$CDCl_3$	—	2.26	—	6.70	6.70	5.50	6.00
Ib	$CDCl_3$	—	2.28	—	6.50	6.62	5.51	6.12
II	$(CD_3)_2SO$	2.27	2.65	5.98	6.62	6.62	—	
IIa	$CDCl_3$	2.33	2.84	6.07	6.85	7.05	—	
IIb	$CDCl_3$	2.29	2.80	6.00	6.64	6.64	—	
III	acetone-d_6	—	2.19	—	6.34	6.34	5.53	6.21
IIIa	$CDCl_3$	—	2.20	—	6.88	6.94	5.55	6.00
IIIb	$CDCl_3$	—	2.28	—	6.38	6.38	5.52	6.02
IV	acetone-d_6	—	2.23	—	6.47	6.47	5.38	6.06
V	acetone-d_6	—	2.26	—	6.40	6.40	5.52	6.09
VI	$(CD_3)_2SO$	—	2.18	—	6.54	6.62	5.60	6.24
VII	acetone-d_6	—	2.23	—	6.39	6.39	5.36	6.12
Deacetylaloesin[b]	$(CD_3)_2SO$	2.29	2.64	5.98	6.70	—	—	
Deacetylaloesin acetate[b]	$CDCl_3$	2.39	2.80	6.09	6.80	—	—	

a) δ in ppm *b*) Ref. 7

11) *a*) F.M. Dean, "Naturally Occurring Oxygen Ring Compounds," Butterworths Scientific Publication, London 1963, p. 251; *b*) S. Arora, H. Deymann, R.D. Tiwari, and E. Winterfeldt, *Tetrahedron*, **27**, 981 (1971); *c*) K. Sen and P. Bagchi, *J. Org. Chem.*, **22**, 1618 (1957); *d*) M.M. Badawi and M.B.E. Fayez, *Ind. J. Chem.*, **5**, 93 (1967).

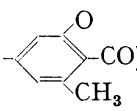
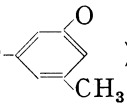
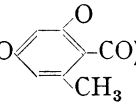
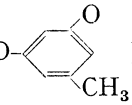
By the comparison of the UV spectra of II with those of deacetylaloesin,[7] 2-methyl-5-acetonyl-7-hydroxychromone[11b] and other 2-methylchromone derivatives,[11c] II was assumed to be dimethyl-monohydroxychromone. The NMR spectrum of II, IIa and IIb presented two three-proton signals which can be assigned to C-methyl groups and in each case one signal is at low field δ 2.65, 2.84, and 2.80 ppm, respectively. These signals are comparable with those assigned to the shield 1-methyl group (δ 2.78 ppm) in 1,3-dimethylanthraquinone, and this position corresponds to the 5-position in a chromone. Furthermore, chromones with a proton at C_5 normally show a signal at δ 8.0 ppm, but there is no proton signals at lower field than δ 7.05 ppm besides a hydroxy proton, in NMR specrta of II, IIa and IIb. Therefore, the shield C-methyl signals were assigned to C_5 in II, IIa, and IIb. The other C-methyl protons at δ 2.27—2.33 ppm in II, IIa, and IIb were assigned to C_2-position in chromone by the comparison of deacetylaloesin,[7] 2-methyl-5-acetonyl-7-hydroxychromone[11b] and other 2-methylchromone derivatives.[11d] Accordingly, two C-methyl groups of II should locate at C_2 and C_5 in chromone.

In the NMR spectrum of monoacetate (IIa) two aromatic proton signals with *meta* coupling (J=2.5 Hz) were observed at δ 6.85 and 7.05 ppm, and it was assumed that the acetoxy group in IIa attached at C_7. This assumption was verified on the basis of comparison with the chemical shifts of the C_6,C_8-proton of IIa and those of the flavonoids[12] whose hydroxyl group located at C_7. In addition, a proton signal attached to C_3 (δ 6.07, 1H, s) was superimposed to that of 7-acetoxy-2,5-dimethylchromone.[7]

Thus, the structure of aglycone A was proved to be 2,5-dimethyl-7-hydroxychromone.

Aglycone B(III) showed the presence of 12 protons including one methoxycarbonyl and two hydroxy groups by NMR and IR spectral analyses, and was in good agreement with the molecular formula $C_{13}H_{12}O_5$, confirmed by elemental analyses and mass spectrum.

On methylation using CH_2N_2, III gave dimethyl ether (IIIb), mp 126—128°, $C_{15}H_{16}O_5$. IIIb exhibited an aromatic methyl group (δ 2.28, 3H, s) and two aromatic ptorons(δ 6.38, 2H, s), besides a methoxycarbonyl group (δ 3.86, 3H, s) and two methoxy groups (δ 3.78, 3H, s; δ 3.82, 3H, s) in NMR spectrum. On acetylation with Ac_2O and pyridine, III afforded diacetate (IIIa), mp 141—142°, $C_{17}H_{16}O_7$. IIIa indicated an aromatic methyl group (δ 2.20, 3H, s) and two aromatic protons (δ 6.88, 1H, d; δ 6.94, 1H, d, J=2.5), in addition to a methoxycarbonyl group (δ 3.87, 3H, s) and two acetoxy groups (δ 2.30, 3H, s; δ 2.34, 3H, s) in NMR spectrum. Since two aromatic protons with *meta* coupling in IIIa shifted by δ 0.54 and 0.60 ppm from those of the parent phenol (III) at δ 6.88 and 6.94 ppm in NMR spectrum, these two protons must be located at C_6 and C_8, or at C_5 and C_7.

In the mass spectra were observed the peaks at *m/e* 150 (HO-  -CO), 122 (HO- ) in II and III, and those at *m/e* 164 (CH_3O-  -CO), 136 (CH_3O- ) in IIb and IIIb, which resulted that the benzene portion in II and III was virtually unaffected under acid conversion of III to II. Aglycone B, lacking the typical chromone absorption bands in IR and UV spectra, revealed the chromenyl absorption bands[13] at 1645 cm^{-1} (IR) and 310 mμ (UV). The existence of normal aromatic methyl signals at δ 2.19—2.28 ppm in III, IIIa, and IIIb suggested the absence of a ketone group at C_4, and was in good accord with the fact that a sharp or a long range coupled, less than J=1.0 Hz, proton signal due to C_3-H in 2-methylchromone[11d] was not demonstrated in these compounds.

12) J. Massicot and J.P. Marthe, *Bull. Soc. Chem. France*, **1962**, 1962; J. Massicot, J.P. Marthe, and S. Heitz, *ibid.*, **1963**, 2712.

13) L. Crombie and R. Ponsford, *J. Chem. Soc.*, **1971**, 796.

Consequently, the structure of aglycone B was established to be the chromenyl compound whose hydroxyl groups located at C_4 and C_7. These findings were reasonably supported by the formation of γ-pyrone system in II resulted from the acid treatment of III. The presence of a methoxycarbonyl group in III was verified by hydrolysis of dimethyl ether (IIIb) followed by silica gel chromatography, to give the carboxylic acid (IV), mp 185°, $C_{14}H_{14}O_5$. The another characteristic NMR spectrum presented the long-range coupled two proton signals ($-CH=\overset{\curvearrowleft}{C}_2-C_3H=\overset{\curvearrowleft}{C}_4-$) or the terminal methylene ones ($CH_2=\overset{\curvearrowleft}{C}_2-\overset{\curvearrowleft}{C}_3=\overset{\curvearrowleft}{C}_4-$) at δ 5.36—6.24 ppm (J=2.5 Hz) in the following substances, I, Ia, Ib, III, IIIa, IIIb, IV, V, VI, and VII. The absence of a hydrogen bonding between methoxycarbonyl group and hydroxy group in III was proved by the following evidences: the prompt methylation with diazomethane at room temperature to give dimethyl ether IIIb, the presence of a methoxycarbonyl group at 1730 cm^{-1} (in dioxane) and the lack of a terminal methylene absorption band at 890 cm^{-1} in IR spectrum. These observations lead to conclude that the methoxycarbonyl group of aglycone A attaches at the C_2-terminal methylene rather than C_3 of the pyran portion.

Thus, the structure of aglycone B was confirmed to be methyl 2-(5-methyl-4,7-dihydroxychromenylidene)acetate.

Upon the biogenetic view-point, the structure of III could be well speculated by a cyclization process *via* a polyketide chain (a).[14] As illustrated in Chart 2, it was concluded that the chromenyl compound (III) was converted into chromone (II) *via* (b).

The location of D-glucose moiety in I has been elucidated as base on the following evidences. I afforded monomethyl ether (VI), mp 148—150°, $C_{20}H_{24}O_{10}$, by the methylation with diazomethane at room temperature. VI was hydrolyzed with 4% HCl in EtOH to give V, mp 192° (sublimate at 160°), $C_{14}H_{14}O_5$, and an acid (VII), mp 211 —213°, $C_{13}H_{12}O_5$. On the basis of spectral data V and VII were suggested to the monomethyl ether of III and the corresponding acid of V, respectively, and V was hydrolyzed with 4% HCl in EtOH to afford VII. On the other hand, pentamethyl ether (Ib) was hydrolyzed with 4% HCl in EtOH to yield V, mp 192° (sublimate at 160°). Furthermore, V and VII respectively were treated with 10% HCl in EtOH to give 2,5-dimethyl-7-methoxychromone, mp 113—114° (sublimate at 105°), $C_{12}H_{12}O_3$, which was identified by the direct comparison (UV, IR, NMR, and mixed melting point) with an authentic sample (IIb).

Thus, the location of D-glucose was determined at C_4-OH in I. The NMR data were summerized in Table I.

In conclusion, the structure of aloearbonaside (I) was established to be methyl 2-(5-methyl-7-hydroxy-4-O-β-D-glucopyranosyl)-chromenylideneacetate.

Along with the investigation of other phenolic compounds, the pharmacological and biogenetic studies of III are in progress.

Experimental[15]

Isolation of Barbaloin——The homogenate of the fresh leaves (3 kg) collected in Fukuoka (July, 1971) was extracted with MeOH (4 liters) and the filtrate was evaporated. The resinous residue (120 g) was treated

14) A.J. Birch, *Science*, **156**, 202 (1967).

15) Melting points were determined on a Kofler block and uncorrected. IR spectra were obtained with a KOKEN DS-301 and UV spectra were recorded with a Shimadzu SV-50A. NMR spectra were taken with a Nihondenshi C-60H. Chemical shifts were expressed in ppm from Me_4Si as internal reference and coupling constants (J) in Hz. Mass spectra were determined on a JEOL-01 double forcus high resolution spectrometer. TLC were performed on silica gel G (Kiesel gel G, Merck) employing the following solvent systems: 1) EtOAc:MeOH:H_2O (20: 3: 2) for glycosides, carboxylic acids and their derivatives, 2) $CHCl_3$:EtOAc (1: 2) for aglycones A, B and their derivatives. As spraying reagent 1% Echtblau salz B-KOH solution was used and the blue fluorescence under UV light, when alcoholic alkali was sprayed, was used for detection. Paper chromatography (PPC) for sugar was run on Toyo Roshi No. 50 using the upper layer of BuOH:AcOH:H_2O (4:1: 5) and BuOH:pyridine:H_2O (6: 4: 3) by ascending method with double development technique and a coloring reagent was aniline hydrogenphthalate.

with acetone (2 liters) to remove the uncharacterized precipitates and the acetone soluble portion (7 g) was chromatographed on a Celite column using hexane, EtOAc and acetone as solvent. The acetone eluate (3.8 g) was chromatographed on a polyamide column using H_2O and MeOH as solvent. The H_2O eluate (1.84 g) was rechromatographed on a silica gel column using EtOAc–acetone (3:1) as solvent. After evaporating the solvent, the residue (0.87 g) was recrystallized from H_2O to yield crude aloearbonaside. Preparative TLC of the mother liquid followed by recrystallization (MeOH–H_2O) gave pale yellow prisms, barbaloin (21.4 mg, Yield: 0.007% from the fresh leaves).

Barbaloin——mp 137—140° (decomp.), $[\alpha]_D^{25}$ −13.0° (MeOH, $c=0.7$). *Anal.* Calcd. for $C_{21}H_{22}O_9 \cdot 2H_2O$: C, 55.50; H, 5.77. Found: C, 55.05; H, 5.47. IR ν_{max}^{KBr} cm^{-1}: 3400, 1635, 1620. UV λ_{max}^{MeOH} mμ (log ε): 261 (3.85), 269 (3.92), 297 (3.98), 362 (4.08). UV $\lambda_{max}^{MeOH\text{-}KOH}$ mμ: 269, 302, 379.

Isolation of Aloearbonaside (I)——Chops of the leaves were dried for one week to give the brown chips in the yield of 5%. The powder (1 kg) prepared from the sun-dried chips was percolated with MeOH (20 liters) and the solvent was evaporated to give the resinous residue (160 g). After working up in the same way as for barbaloin, the crude aloearbonaside was recrystallized from H_2O, then MeOH–H_2O to afford colorless needles, aloearbonaside (2.8 g. Yield: 0.28% from the sun-dried leaves).

Aloearbonaside (I)——mp 135°, $[\alpha]_D^{11}$ −23.5° (MeOH, $c=3.3$). *Anal.* Calcd. for $C_{19}H_{22}O_{10} \cdot H_2O$: C, 53.27; H, 5.65. Found: C, 53.72; H, 5.85. IR ν_{max}^{KBr} cm^{-1}: 3350, 1665, 1610, 1555. IR $\nu_{max}^{dioxane}$ cm^{-1}: 3300, 1710, 1640, 1610, 1555. UV λ_{max}^{MeOH} mμ (log ε): 302 (3.81). NMR (DMSO-d_6)ppm: 3.20—5.00 (m, sugar), 3.80 (3H, s, $COOCH_3$), 10.80 (1H, s, OH), and the other signals are given in Table I.

Pentaacetyl Aloearbonaside (Ia)——I (50 mg) was acetylated with Ac_2O (3 ml) and pyridine (10 ml) for 12 hr at room temperature. The crude product was worked up as usual and recrystallized from ether to give colorless (Ia), mp 91°, (60 mg). *Anal.* Calcd. for $C_{29}H_{32}O_{15}$: C, 56.13; H, 5.20. Found: C, 55.98; H, 5.21. IR ν_{max}^{KBr} cm^{-1}: 1755, 1730, 1650, 1570. UV λ_{max}^{MeOH} mμ (log ε): 225 (sh.), 284 (4.02). NMR ($CDCl_3$) ppm: 1.98 (6H, s, 2×OAc), 2.03 (3H, s, OAc), 2.09 (3H, s, OAc), 2.29 (3H, s, OAc), 3.86 (3H, s, $COOCH_3$), 4.18 (2H, m, sugar), 5.10 (5H, m, sugar), and the other signals are given in Table I.

Pentamethyl Aloearbonaside (Ib)——According to the Hakomori's method, I (360 mg) in dimethyl sulfoxide (DMSO) (10 ml) was mixed with NaH (508 mg) in DMSO (30 ml) at room temperature for 1 hr. To the reaction mixture CH_3I (30 ml) was added and was allowed to stand for 1 hr at room temperature. After dilution with H_2O the reaction mixture was extracted with $CHCl_3$ and $CHCl_3$ layer was washed with H_2O, dried and evaporated to drynesss. The oily product (680 mg) was chromatographed on a silica gel column using hexane–EtOAc as solvent. The hexane–EtOAc (3:2) eluate was recrystallized from hexane–ether to give colorless needles (Ib), mp 140°, (51 mg). *Anal.* Calcd. for $C_{24}H_{32}O_{10}$: C, 59.81; H, 6.70. Found: C, 59.99; H, 6.71. IR ν_{max}^{KBr} cm^{-1}: 3100—2840, 1725, 1650, 1615, 1570. UV λ_{max}^{MeOH} mμ (log ε): 295 (3.99), 284 (sh.). NMR ($CDCl_3$) ppm: 3.24 (3H, s, CH_3O), 3.40—3.65 (9H, s, 3×CH_3O), 3.82 (3H, s, CH_3O), 3.40—3.65 (6H, m, sugar), 3.85 (3H, s, $COOCH_3$), 4.80 (1H, d, $J=6$, anomeric), and the other signals are given in Table I.

Acid Hydrolysis of I——I (525 mg) was hydrolyzed with 1% HCl in H_2O (100 ml) for 1 hr under reflux, followed by the usual work-up. A sugar, D-glucose was detected by PPC. The hydrolysate (193 mg) was chromatographed on a silica gel column using hexane–EtOAc as solvent. The hexane–EtOAc (10:3) eluate was recrystallized from acetone to yield pale yellow needles aglycone A (57 mg). The hexane–EtOAc (2:1) eluate was recrystallized from EtOAc–ether to yield colorless needles aglycone B (39 mg).

Aglycone A (II)——mp 257—260° (sublimate at 200—205°). *Anal.* Calcd. for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30. Found: C, 69.35; H, 5.28. IR ν_{max}^{KBr} cm^{-1}: 3100—2600, 1655, 1630, 1570—1540. IR $\nu_{max}^{dioxane}$ cm^{-1}: 3240, 1660, 1615, 1585. UV λ_{max}^{MeOH} mμ (log ε): 220 (4.57), 242 (4.54), 250 (4.57), 290 (4.35). NMR (DMSO-d_6) ppm: 10.65 (1H, s, OH), and the other signals are given in Table I. Mass Spectrum *m/e*: 190 (M^+), 162 (M^+–CO), 150, 122.

Aglycone B (III)——mp 201°. *Anal.* Calcd. for $C_{13}H_{12}O_5 \cdot 1/2H_2O$: C, 60.69; H, 5.09. Found: C, 61.19; H, 5.06. IR ν_{max}^{KBr} cm^{-1}: 3400, 1670, 1630, 1605, 1560. IR $\nu_{max}^{dioxane}$ cm^{-1}: 3300, 1730, 1645, 1610, 1575. UV λ_{max}^{MeOH} mμ (log ε): 250 (3.72), 286 (3.81), 310 (3.87). NMR (acetone-d_6) ppm: 3.90 (3H, s, $COOCH_3$), 8.53 (2H, s, 2×OH), and the other signals are given in Table I. Mass Spectrum *m/e*: 248 (M^+), 220 (M^+-CO), 190, 150, 122.

Enzymic Hydrolysis of I——I (10.6 mg) suspended in H_2O (5 ml) was stirred with emulsin (30 mg) at 39° for 32 hr. The reaction mixture was extracted with EtOAc and only (III), as the aglycone, was detected on TLC.

Acid Treatment of Aglycone B (III) yielding Aglycone A (II)——III (21 mg) was refluxed with 3% HCl in EtOH (10 ml) for 1.5 hr. The reaction mixture was diluted with H_2O, extracted with EtOAc and the solvent was evaporated to dryness to yield a crude product (13.2 mg). The product was chromatographed on a silica gel column using hexane–EtOAc as solvent and the hexane–EtOAc (2:1) eluate was recrystallized from acetone to yield colorless needles (II), mp 254—256°.

Monoacetylaglycone A (IIa)——II (14.7 mg) was acetylated with Ac_2O (2 ml) and AcONa (2 mg) for 2 hr at 50°, followed by usual work-up. The product was recrystallized from ether to give colorless needles

(IIa), (10 mg), mp 117°. Mass Spectrum: Calcd. for $C_{13}H_{12}O_4$: 232.074; Found: 232.069. IR ν_{max}^{KBr} cm^{-1}: 1755, 1660, 1630. UV λ_{max}^{MeOH} mμ (log ε): 226 (4.03), 241 (3.81), 248 (3.78), 266 (sh.), 299 (3.50). NMR ($CDCl_3$) ppm: 2.31 (3H, s, OAc), and the other signals are given in Table I. Mass Spectrum *m/e*: 232 (M^+), 190, 161, 150, 122.

Monomethylaglycone A (IIb)——The solution of II (10 mg) dissolved in MeOH–$CHCl_3$ (1:1) (20 ml) was methylated with CH_2N_2 solution for 4 hr at 30°. The product was recrystallized from ether to yield colorless needles (IIb), mp 114—115° (sublimate at 105°), (8 mg). Mass Spectrum: Calcd. for $C_{12}H_{12}O_3$: 204.075; Found: 204.079. IR ν_{max}^{KBr} cm^{-1}: 3100—2800, 1660, 1610, 1575. UV λ_{max}^{MeOH} mμ (log ε): 225 (4.24), 242 (4.20), 249.5 (4.21), 285 (3.98). NMR ($CDCl_3$) ppm: 3.86 (3H, s, OCH_3), and the other signals are given in Table I. Mass Spectrum *m/e*: 204 (M^+), 176 (M^+—CO), 164, 161, 149, 136, 122.

Diacetylaglycone B (IIIa)——III (20 mg) was acetylated with Ac_2O (1 ml) and pyridine (10 ml) for 12 hr at room temperature, followed by the usual work-up. The product was recrystallized from ether to give colorless fine needles (IIIa), mp 141—142°, (29 mg). *Anal.* Calcd. for $C_{17}H_{16}O_7$: C, 61.44; H, 4.85. Found: C, 61.37; H, 4.86. IR ν_{max}^{KBr} cm^{-1}: 1765, 1730, 1650, 1620, 1575. UV λ_{max}^{MeOH} mμ (log ε): 290 (3.92). NMR ($CDCl_3$) ppm: 2.30 (3H, s, $COCH_3$), 2.34 (3H, s, $COCH_3$), 3.87 (3H, s, $COOCH_3$), and the other signals are given in Table I. Mass Spectrum *m/e*: 332 (M^+), 290, 248, 220, 190, 150, 122.

Dimethylaglycone B (IIIb)——The solution of III (620 mg) dissolved in MeOH–$CHCl_3$ (1:1) (100 ml) was methylated with CH_2N_2 solution for 4 hr at room temperature. The product was recrystallized from hexane–ether to give colorless needles (IIIb), mp 126—128°, (552 mg). *Anal.* Calcd. for $C_{15}H_{16}O_5$: C, 65.12; H, 5.84. Found: C, 65.37; H, 6.01. IR ν_{max}^{KBr} cm^{-1}: 2980, 1710, 1645, 1330, 1150. UV λ_{max}^{MeOH} mμ (log ε): 278 (3.87), 299 (3.89). NMR ($CDCl_3$) ppm: 3.78 (3H, s, CH_3O), 3.82 (3H, s, CH_3O), 3.86 (3H, s, $COOCH_3$), and the other signals are given in Table I. Mass Spectrum *m/e*: 276 (M^+), 248, 234, 220, 164, 136.

Acid Hydrolysis of IIIb yielding IV——IIIb (92 mg) was hydrolyzed with 3% HCl in EtOH (10 ml) for 1 hr under reflux, and after evaporating the solvent, the residue was extracted with ether. The ether solution was extracted with 5% $NaHCO_3$ and the alkaline layer was acidified with 5% HCl, and then extracted with EtOAc. The EtOAc extract was chromatographed on a silica gel column using EtOAc as solvent. The EtOAc eluate was recrystallized from hexane–acetone to yield colorless prisms (IV), mp 185° (decomp.), (13 mg). Mass Spectrum: Calcd. for $C_{14}H_{14}O_5$: 262.084. Found: 262.083. IR ν_{max}^{KBr} cm^{-1}: 3000—2000, 1655, 1630, 1610, 1570. IR $\nu_{max}^{dioxane}$ cm^{-1}: 2750, 1720., 1645, 1610, 1580. UV λ_{max}^{MeOH} mμ (log ε): 286 (sh.), 300 (3.82). NMR (acetone-d_6) ppm: 3.78 (3H, s, CH_3O), 3.83 (3H, s, CH_3O), and the other signals are given in Table I. Mass Spectrum *m/e*: 262 (M^+), 247, 220.

Methylation of I with CH_2N_2 yielding VI——The solution of I (160 mg) dissolved in MeOH–$CHCl_3$ (1:1) (60 ml) was methylated with CH_2N_2 for 8 hr at 30°. The solvent was evaporated and the residue (171 mg) was chromatographed on a silica gel column using hexane–acetone as solvent. The hexane–acetone (2:1) eluate was recrystallized from EtOAc–ether to yield colorless prisms (VI), mp 148—150° (84 mg). *Anal.* Calcd. for $C_{20}H_{24}O_{10}$: C, 56.60; H, 5.70. Found: C, 56.32; H, 5.79. IR ν_{max}^{KBr} cm^{-1}: 3400, 1695, 1640, 1610, 1565. UV λ_{max}^{MeOH} mμ (log ε): 300 (3.75). NMR (DMSO-d_6) ppm: 3.20—4.90 (11H, m, sugar), 3.79 (3H, s, CH_3O), 3.84 (3H, s, $COOCH_3$), and the other signals are given in Table I.

Acid Hydrolysis of VI yielding VII and V——VI (113 mg) was hydrolyzed with 4% HCl in EtOH (20 ml) under reflux for 1 hr and after evaporating the solvent, the residue was extracted with EtOAc. The EtOAc solution was extracted with 5% $NaHCO_3$, and the aqueous layer was neutralized and extracted with EtOAc to give acidic substance (20.5 mg). The EtOAc layer was evaporated to give neutral substance (73 mg). The crude acidic substance was chromatographed on a silica gel column using hexane–EtOAc (1:2) to afford (VII), pale pink prisms (from acetone), mp 211—213° (decomp.), (7.3 mg). Mass Spectrum: Calcd. for $C_{13}H_{12}O_5$: 248.068. Found: 248.068, *m/e*: 248 (M^+), 206. IR ν_{max}^{KBr} cm^{-1}: 3100—3000, 1730—1700, 1650, 1610, 1590. UV λ_{max}^{MeOH} mμ (log ε): 285 (sh.), 304 (3.77). NMR (acetone-d_6) ppm: 3.76 (3H, s, CH_3O), 8.58 (1H, s, OH), and the other signals are given in Table I. The crude neutral substance was chromatographed on a silica gel column using hexane–EtOAc as solvent and the hexane–EtOAc (4:1) eluate was recrystallized from ether to afford colorless needles (V), mp 192° (sublimate at 160°), (32 mg). *Anal.* Calcd. for $C_{14}H_{14}O_5$: C, 64.11; H, 5.38. Found: C, 63.87; H, 5.42. IR ν_{max}^{KBr} cm^{-1}: 3240, 1670, 1610, 1550. UV λ_{max}^{MeOH} mμ (log ε): 306 (3.92). NMR (acetone-d_6) ppm: 3.79 (3H, s, CH_3O), 3.93 (3H, s, $COOCH_3$), 8.60 (1H, s, OH), and the other signals are given in Table I. Mass Spectrum *m/e*: 262 (M^+), 234 (M^+—CO), 220.

Acid Hydrolysis of Ib yielding V——Ib (10 mg) was hydrolyzed with 4% HCl in EtOH (10 ml) under reflux for 1 hr. After evaporating the solvent, the residue was extracted with EtOAc and evaporated to dryness. The residue (6 mg) was chromatographed on a silica gel column using hexane–EtOAc as solvent. The hexane–EtOAc (7:3) eluated was recrystallized from hexane–EtOAc to yield colorless needles (V), mp 192° (sublimate at 160°), (3 mg).

Conversion of V and VII into IIb——A solution of V (60 mg) in 10% HCl in EtOH (20 ml) was refluxed for 3 hr. After evaporating the solvent, the residue was extracted with EtOAc and the solvent was evaporated to dryness. The residue (30 mg) was chromatographed on a silica gel column using hexane–EtOAc as solvent. The hexane–EtOAc (5:1) eluate was recrystallized from ether–hexane to give colorless needles

(IIb), mp 113—114° (sublimate at 105°) (10 mg). A solution of VII (2 mg) in 10% HCl in EtOH (2 ml) was treated in the same way, and the product was identified with IIb on TLC.

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