Structure of Aloenin, a New Biologically-active Bitter Glucoside from *Aloe arborescens* var. *natalensis*

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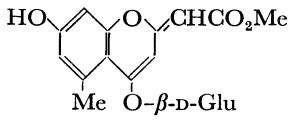
Aloenin, a new bitter glucoside with an inhibitory activity for the gastric juice secretion of rats, was isolated from the leaves of *Aloe arborescens* Mill. var. *natalensis* Berger, and the structure was confirmed to be 4-methoxy-6-(2-β-D-glucopyranosyloxy-4-hydroxy-6-methylphenyl)-2-pyrone by means of chemical, spectroscopic, and X-ray crystallographic methods. Ambiguity in the assignment of 2-pyrone-ring carbon signals was clarified by examination of the ^{13}C-NMR spectra of aloenin and the related compounds.

The plants of Aloe species have been widely used for folk remedies, and their chemical constituents have been studied by several workers.[1–11] Recently, Soeda[12] and Yamamoto[13] have tested the effectiveness of "Cape Aloe" as material for medical treatment. In Japan, *Aloe arborescens* Mill. var. *natalensis* Berger (Kidachirokai or Kidachiaroe in Japanese) has been traditionally used as material for folk remedies for gastro-intestinal disturbances, burns, insect bites, athlete's foot, *etc.* In the course of chemical and biochemical examinations of this plant, a new bitter glucoside, named aloenin, was isolated, its structure being postulated to be a chromene derivative (**1**).[8] Aloenin was identified with aloearbonaside which was independently isolated and named by Makino *et al.*[9] They also assigned the same structure **1** to this compound. It was decided upon mutual agreement to use thereafter the name "aloenin".[8] However, doubts concerning the proposed structure were aroused by anomalous intensity enhancement found in intramolecular nuclear Overhauser effect (NOE)[14] of some derivatives of aloenin, as well as ambiguity in assignments of the ^{13}C-NMR spectrum. The structure of aloenin was reexamined by means of chemical, spectroscopic, and X-ray crystallographic methods, and a report given on a revised structure of aloenin.[15–17] We wish to describe *en bloc* the results of studies on the revised structure **2** for aloenin, the assignment of 2-pyrone-ring carbon signals in the ^{13}C-NMR spectra of aloenin and its derivatives, and tests of the inhibitory activity to the gastric juice secretion of rats.



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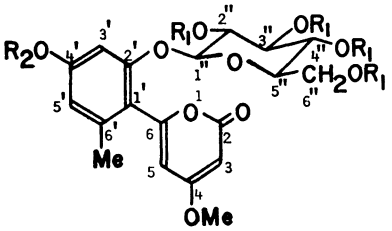
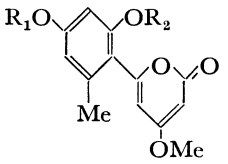
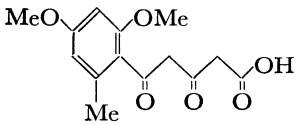


2: $R_1=R_2=H$
5: $R_1=R_2=Me$
10: $R_1=H$, $R_2=Me$
18: $R_1=R_2=Ac$
19: $R_1=Ac$, $R_2=Me$



3: $R_1=R_2=H$
4: $R_1=R_2=Me$
6: $R_1=Me$, $R_2=H$
20: $R_1=Me$, $R_2=Ac$
21: $R_1=R_2=Ac$



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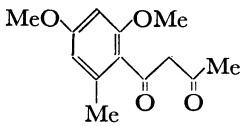

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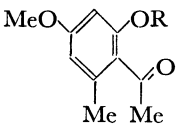
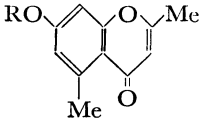
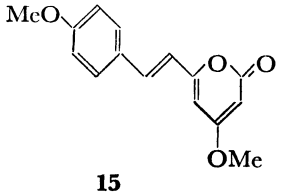


9: R=Me
11: R=β-D-glucosyl
12: R=H
16: R=Ac
17: R=tetra-*O*-acetyl-β-D-glucosyl



13: R=H
14: R=Me



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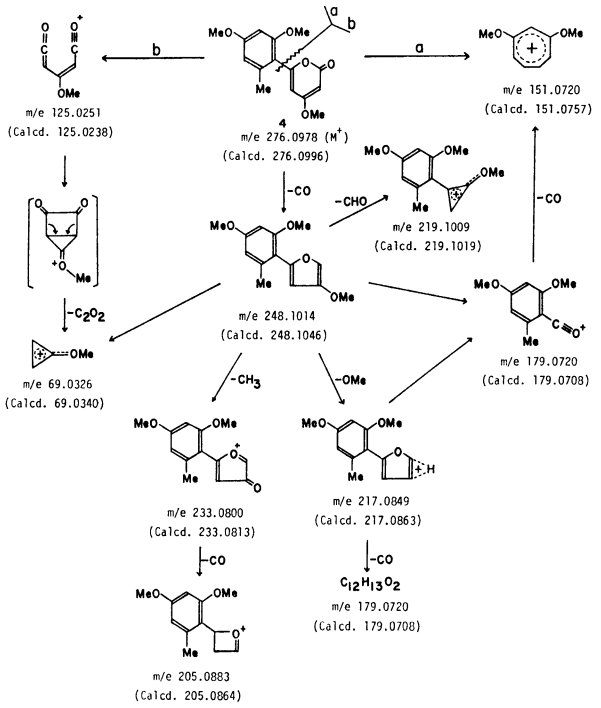
Results and Discussion

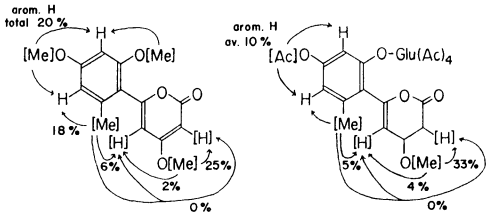
Structure Elucidation. Aloenin (**2**)** was isolated as a major component by chromatography on silica gel from the leaf juice of the Aloe plants cultivated in pots. The IR spectrum of aloenin exhibited bands (1714 and 1642 cm^{-1}) due to a conjugated ester or a lactone group. The UV spectrum in an alkaline solution showed a bathochromic shift by 46 nm. The IR and UV spectra closely resemble those of 2-pyrone derivatives.[18] The PMR spectrum in acetone-d_6 showed the presence of an aromatic methyl, a methoxyl, and four olefinic or aromatic protons.

Hydrolysis of aloenin with 3% methanolic hydrochloric acid afforded an aglycone **3** and D-glucose. Methylation of **3** with diazomethane gave a trimethyl ether **4**, whose PMR spectrum in $CDCl_3$ showed signals due to an aromatic methyl, three methoxyl, and four olefinic or aromatic protons. Exhaustive methylation of aloenin by Hakomori's method[19] yielded a hexamethyl ether **5**, which could be hydrolyzed with 3% methanolic hydrochloric acid to a dimethyl ether **6** and 2,3,4,6-tetra-*O*-methyl-D-glucose. This suggests that the sugar moiety is linked with the aglycone through the C-1 hydroxyl group of D-glucose. Methylation of **6** with diazomethane afforded **4**. Treatment of **4** with 5% methanolic potassium hydroxide at room temperature yielded potassium salt of the corresponding β-diketo acid, which on acidification with dilute hydrochloric acid gave an unstable β-diketo acid **7**. The structure of **7** was assigned by the fact that it



Scheme 1. Fragmentation patterns in the mass spectrum of **4**.

** In this paper, for convenience, the carbon atoms of aloenin (**2**) and its derivatives are numbered as shown in the structural formulae.



4 **20**

Fig. 1. NOE values of **4** and **20** in $CDCl_3$.

underwent facile decarboxylation on heating to yield 1-(2,4-dimethoxy-6-methylphenyl)butane-1,3-dione (**8**), which was identified by comparison with an authentic sample synthesized from 3,5-dihydroxytoluene by acetoacetic ester condensation. The results demonstrate the presence of a C-4 hydroxylated 2-pyrone skeleton.[20] The product **8** possesses a new acetyl group which is not present in the parent compound **4**. The absence of the acetyl group in **4** warrants a carboxyl group in **7** to be attached to the end of the side chain of **8**. This indicates that the structure of **4** should be represented as 4-methoxy-6-(2,4-dimethoxy-6-methylphenyl)-2-pyrone. A typical mass-spectral fragmentation pattern of C-6 substituted 4-methoxy-2-pyrone derivatives[21] was observed for **4**. The fragments were completely characterized by high-resolution mass-spectral measurements as shown in Scheme 1. The results of the NOE experiments are in line with structure **4** for the dimethyl ether of aglycone **3** as shown in Fig. 1.

Structure **4** was further confirmed by synthesis according to the following procedure. β-Diketone (**8**) prepared from 2,4-dimethoxy-6-methylacetophenone (**9**)[22] was first converted into **7** by treatment with sodium amide in liquid ammonia followed by carbon dioxide.[23] Cyclization of **7** in the presence of acetic anhydride gave 2-pyrone derivative,[23] which was then converted into trimethyl ether **4** with diazomethane. Trimethyl ether **4** derived from aloenin was found to be identical with the synthesized trimethyl ether (**4**). In the course of synthetic studies of **4**, we found that some 2,4,6-trisubstituted acetophenones had been erroneously correlated[24] with their PMR and IR spectra, *i.e.*, the 2,4,6-trisubstituted acetophenones prepared from 3,5-dihydroxytoluene and acetyl chloride by the Friedel-Crafts reaction were identified as 2,6-dimethoxy-4-methyl-, 2,4-dimethoxy-6-methyl-, 2-hydroxy-6-methoxy-4-methyl-, and 2-hydroxy-4-methoxy-6-methyl-acetophenones. However, the acetophenone derivatives were established to be 2,4-dimethoxy-6-methyl-, 2,6-dimethoxy-4-methyl-, 2-hydroxy-4-methoxy-6-methyl-, and 2-hydroxy-6-methoxy-4-methyl-acetophenones, respectively, by reexamination of the signal assignments in the PMR spectra and the conversion of the acetophenone derivatives into known *o*- or *p*-orsellinates.[22]

The alkaline degradation of a monomethylated product **10** of aloenin by refluxing with 5% methanolic potassium hydroxide yielded a glucoside **11**, which could be hydrolyzed to 2-hydroxy-4-methoxy-6-methylacetophenone (**12**)[22] and D-glucose. This shows that the glucose moiety in aloenin is located on the C-2' hydroxyl group of the aglycone **3**. The aglycone (**3**) was converted into 2,5-dimethyl-7-hydroxychromone

TABLE 1. ATOMIC PARAMETERS[a)]

Atoms	x/a	y/b	z/c
C (1)	0.6099 (5)	0.1251 (3)	0.1062 (2)
C (2)	0.5120 (5)	0.0190 (3)	0.1261 (2)
C (3)	0.3271 (5)	0.0134 (3)	0.0950 (2)
C (4)	0.2339 (5)	0.1148 (4)	0.0436 (3)
C (5)	0.3301 (4)	0.2153 (3)	0.0252 (2)
C (6)	0.2608 (4)	0.3284 (3)	−0.0262 (2)
C (7)	0.1785 (4)	0.3207 (3)	−0.1214 (2)
C (8)	0.1097 (4)	0.4264 (3)	−0.1718 (2)
C (9)	0.1237 (4)	0.5396 (3)	−0.1250 (2)
C (10)	0.2029 (4)	0.5496 (3)	−0.0306 (2)
C (11)	0.2727 (4)	0.4448 (3)	0.0194 (2)
C (12)	0.3541 (5)	0.4571 (4)	0.1216 (2)
C (13)	0.3049 (6)	−0.1883 (4)	0.1592 (3)
O (1)	0.5157 (3)	0.2206 (2)	0.0555 (2)
O (2)	0.7755 (3)	0.1420 (3)	0.1296 (2)
O (3)	0.2201 (4)	−0.0833 (3)	0.1084 (2)
O (4)	0.1782 (4)	0.2059 (3)	−0.1626 (2)
O (5)	0.0559 (5)	0.6423 (3)	−0.1743 (2)

a) E.s.d. in parentheses ($\times 10^4$).

TABLE 2. ANISOTROPIC THERMAL PARAMETERS[a)]

Atoms	B_{11}	B_{22}	B_{33}	B_{12}	B_{23}	B_{31}
C-1	2.36	2.20	2.81	0.03	−0.53	0.15
C-2	2.71	2.38	2.58	−0.12	−0.15	0.25
C-3	2.62	2.16	2.84	−0.40	0.31	0.12
C-4	2.46	2.69	3.20	−0.34	−0.38	0.43
C-5	2.05	2.53	2.42	0.14	−0.43	0.04
C-6	2.09	2.53	2.29	0.20	−0.37	0.17
C-7	2.23	2.58	2.74	0.07	−0.37	−0.13
C-8	2.48	2.96	2.28	0.04	−0.48	0.19
C-9	1.81	2.57	2.58	−0.12	−0.31	0.50
C-10	2.34	2.48	2.27	−0.04	−0.06	0.19
C-11	1.88	2.57	2.02	−0.18	−0.03	0.20
C-12	3.13	3.50	1.98	0.26	−0.37	0.12
C-13	4.17	2.51	3.82	−0.08	0.64	0.75
O-1	2.00	2.17	3.14	0.09	−0.51	0.49
O-2	2.29	2.70	5.49	0.00	−1.16	0.67
O-3	2.92	2.69	4.63	−0.70	0.10	0.98
O-4	4.44	2.84	3.42	0.77	−1.68	−1.05
O-5	3.67	2.30	3.42	−0.34	−1.12	0.95

a) In the expression $F_o \cdot \exp[-1/4(B_{11}h^2a^{*2} + \cdots\cdots + B_{13}2hla^*c^*)]$.

(**13**)[25] by treatment with 5% hydrochloric acid. On the other hand, a similar treatment of **6** gave 2,5-dimethyl-7-methoxychromone (**14**). This suggests the presence of a hydroxyl group attached to the C-4′ position of the aromatic ring in aloenin. The authentic chromones, **13** and **14**, were obtained by the Pechman condensation, followed by decarboxylation, of *p*-orsellinic acid with ethyl acetoacetate.[25] Sethna and Shah[26] reported that such reactions as above yield 4,5-dimethyl-7-hydroxycoumarin. We reexamined the reported structure by spectroscopic and chemical methods in connection with the syntheses of the chromones, **13** and **14**, and found that the product is 2,5-dimethyl-7-hydroxychromone, not coumarin.

TABLE 3. BOND DISTANCES[a)]

Bonds	Distances	Bonds	Distances
C (1)-C (2)	1.404 (5)	C (6)-C (11)	1.407 (4)
C (1)-O (1)	1.378 (4)	C (7)-C (8)	1.395 (5)
C (1)-O (2)	1.233 (4)	C (7)-O (4)	1.366 (5)
C (2)-C (3)	1.372 (5)	C (8)-C (9)	1.384 (5)
C (3)-C (4)	1.427 (5)	C (9)-C (10)	1.392 (5)
C (3)-O (3)	1.340 (4)	C (9)-O (5)	1.360 (5)
C (4)-C (5)	1.343 (5)	C (10)-C (11)	1.388 (4)
C (5)-C (6)	1.468 (5)	C (11)-C (12)	1.501 (5)
C (5)-O (1)	1.376 (4)	C (13)-O (3)	1.430 (5)
C (6)-C (7)	1.407 (5)		

a) E.s.d. in parentheses ($\times 10^3$).

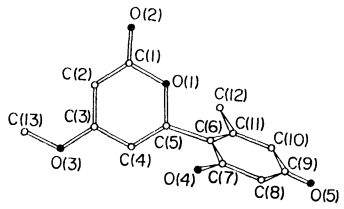


Fig. 2. Atomic numbering in aglycone **3** for X-ray analysis. The atoms indicated with ○ and ● denote carbon and oxygen atoms, respectively.

In order to establish the complete structure of aloenin, we examined the structure by X-ray crystallographic analysis. However, due to the lack of a good single crystal of aloenin, the analysis was carried out with aglycone **3**. Cell dimentions were obtained by least-squares calculations from 2θ values of 13 well-centered, resolved Cu $K\alpha$ diffraction peaks. Crystal data: monoclinic (space group $P2_1/c$), four molecules per unit cell with dimensions $a=7.422(2)$, $b=10.719(4)$, $c=14.393(4)$ Å, $\beta=99.54(2)°$; $U=1129.1$ Å^3; $D_c=1.46$ g cm^{-3}. A total of 2292 reflections were collected on a four circle automatic diffractometer; 198 reflections were smaller than 1.96 times of the standard deviations in intensity and were recorded as "unobserved." The phases of 153 strong reflections with $|E| > 1.85$ were determined by direct methods using the program MULTAN.[27] The E map for the best solution showed the 18 non-hydrogen atoms as the largest peaks. The structure was refined by full-matrix least-squares method. Four cycles of anisotropic refinement for carbon and oxygen atoms and of isotropic refinement for hydrogen atoms reduced the R index to 0.073. The final atomic coordinates and anisotropic thermal parameters of the non-hydrogen atoms are given in Tables 1 and 2, and bond distances and angles in Tables 3 and 4, with atoms designated as shown in Fig. 2. The results indicate that the structure of the aglycone is of structural formula **3**.

The crystal structure consists of two stackings of the planes of phenyl and pyrone groups of the aglycone molecules, which are packed along the a-axis and the c-axis, respectively (Fig. 3), the dihedral angle between the planes being 117°. One carbonyl and two hydroxyl groups participate in the formation of the

Table 4. Bond angles[a]

Angles	θ	Angles	θ
C (2) - C (1) - O (1)	118.4 (3)	C (6) - C (7) - C (8)	121.4 (3)
C (2) - C (1) - O (2)	126.1 (3)	C (6) - C (7) - O (4)	116.4 (3)
O (1) - C (1) - O (2)	115.5 (3)	C (8) - C (7) - O (4)	122.1 (3)
C (1) - C (2) - C (3)	119.5 (3)	C (7) - C (8) - C (9)	117.9 (3)
C (2) - C (3) - C (4)	120.6 (3)	C (8) - C (9) - C (10)	121.9 (3)
C (2) - C (3) - O (3)	124.9 (3)	C (8) - C (9) - C (5)	117.6 (3)
C (4) - C (3) - O (3)	114.5 (3)	C (10) - C (9) - O (5)	120.5 (3)
C (3) - C (4) - C (5)	119.0 (3)	C (9) - C (10) - C (11)	120.3 (3)
C (4) - C (5) - C (6)	127.5 (3)	C (6) - C (11) - C (10)	119.2 (3)
C (4) - C (5) - O (1)	120.5 (3)	C (6) - C (11) - C (12)	121.3 (3)
C (6) - C (5) - O (1)	112.0 (3)	C (10) - C (11) - C (12)	119.5 (3)
C (5) - C (6) - C (7)	120.1 (3)	C (1) - O (1) - C (5)	122.1 (3)
C (5) - C (6) - C (11)	120.5 (3)	C (3) - O (3) - C (13)	117.5 (3)
C (7) - C (6) - C (11)	119.3 (3)		

a) E.s.d. in parentheses ($\times 10$).

Table 5. ^{13}C-Chemical shifts of aloenin (**2**) and its derivatives, **4**, **6**, **18**, **19**, **20**, and **21**

Carbon No.	**2**[a]	**4**[b,e]	**6**[c]	**18**[c,d,e]	**19**[c,e]	**20**[c]	**21**[c]
2	165.5	165.2 (+2.6)	166.2	164.3 (+2.0)	164.7 (+2.0)	164.5	164.2
3	88.4	88.2 (+0.4)	88.2	88.5 (+0.4)	88.2 (+0.3)	88.6	88.9
4	171.7	171.2 (+2.0)	171.7	170.7 (+1.3)	171.1 (+0.9)	170.7	170.5
5	105.0	104.6 (+1.4)	104.8	105.0 (+1.0)	105.0 (+0.8)	104.1	104.4
6	159.3	158.9 (+1.2)	156.4	156.4[f] (+0.8)	157.4 (+0.7)	157.7	156.8
1′	114.7	114.8 (+0.5)	113.0	120.3 (+0.3)	115.6 (0.0)	118.4	123.3
2′	157.8	158.9 (+1.3)	159.2	155.1[f] (+0.7)	155.5 (+0.6)	150.0	149.4
3′	102.0	96.1 (+0.8)	99.5	106.2 (+0.8)	99.7 (+0.4)	106.1	114.2
4′	161.0	161.8 (+1.4)	161.6	152.1 (+0.8)	161.5 (+0.6)	161.2	151.8
5′	112.4	107.0 (+1.1)	108.1	117.7 (+0.7)	108.9 (+0.6)	114.0	121.1
6′	140.0	139.9 (+0.8)	139.8	140.2 (+0.6)	140.2 (+0.3)	140.0	140.1
1″	102.7	—	—	98.7 (+0.1)	98.8 (+0.1)	—	—
2″	74.5	—	—	70.5 (+0.8)	70.6 (+0.7)	—	—
3″	78.1	—	—	72.5 (+0.6)	72.6 (+0.4)	—	—
4″	71.0	—	—	68.2 (+0.7)	68.3 (+0.6)	—	—
5″	78.1	—	—	72.0 (+0.4)	72.0 (+0.3)	—	—
6″	62.3	—	—	62.0 (+0.6)	62.0 (+0.5)	—	—
4-OMe	56.0	55.8 (+1.0)	56.0	55.9 (+0.7)	55.9 (+0.5)	55.9	56.0
4′-OMe	—	55.3 (+0.4)	55.2	—	55.3 (+0.3)	55.5	—
6′-Me	20.2	20.2 (0.0)	20.4	19.7[g] (0.0)	20.3 (0.0)	20.3	20.1[h]
2′-OMe	—	58.8 (+0.4)	—	—	—	—	—
4′-OCOMe	—	—	—	21.2[g]	—	20.8	{20.7[h] 21.1[h]}
4′-OCOMe	—	—	—	168.5	—	169.2	168.7×2

a) Determined in C_5D_5N at 60°. b) Determined in $CDCl_3$-CD_3OD (9 : 1). c) Determined in $CDCl_3$ (TMS = 0). d) The δ_c values for the tetraacetyl groups at glucose moiety are 20.5×4, 170.1, 169.7, 169.1, and 168.8 ppm. e) Figures in parentheses are shift values observed in $CDCl_3$: CD_3OH(1 : 1) in ppm. f), g), and h) Assignments are interchangeable.

intermolecular hydrogen bond. One chain is formed through O(2)-carbonyl and O(5)-hydroxyl groups. The other chains O(4)- and O(5)-hydroxyl groups belonging to the neighboring molecules are correlated by a 2_1 screw axis. The crystal structure is stabilized by the networks of these hydrogen bonds.

The β-glucopyranosyloxyl structure was established by the enzymic hydrolysis of **2** with β-glucosidase (emulsin) and the J-value of 5.5 Hz obtained from the anomeric proton signal at δ 4.78 in the PMR spectrum of **5**. The structure of aloenin has now been established as 4-methoxy-6-(2-β-D-glucopyranosyloxy-4-hydroxy-6-methylphenyl)-2-pyrone (**2**).

^{13}C-NMR Study. In the ^{13}C-NMR spectroscopic study of aloenin (**2**) and its derivatives during the course of structure confirmation, it was found that preliminary assignments[15] in accord with reported results[30] differ from those for yangonin (**15**).[28] In order to clarify the ambiguity and disagreement[28-31] in the signal assignment of C-2, C-4, and C-6 in 6-

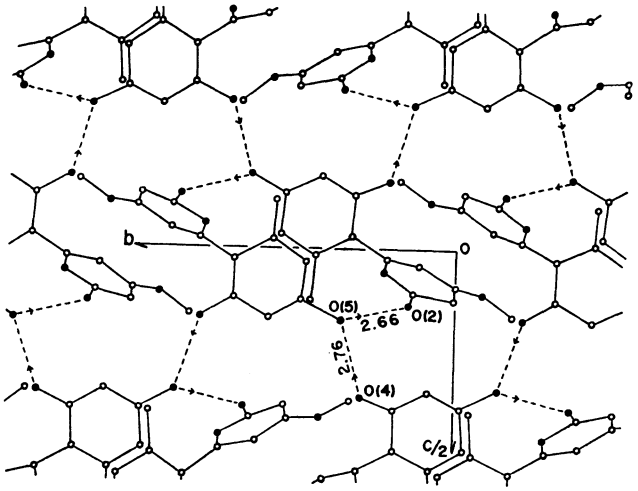


Fig. 3. The projection of aglycone **3** viewed along the a-axis. Hydrogen bonds are shown by a broken line. The atoms indicated with ○ and ● denote carbon and oxygen atoms, respectively.

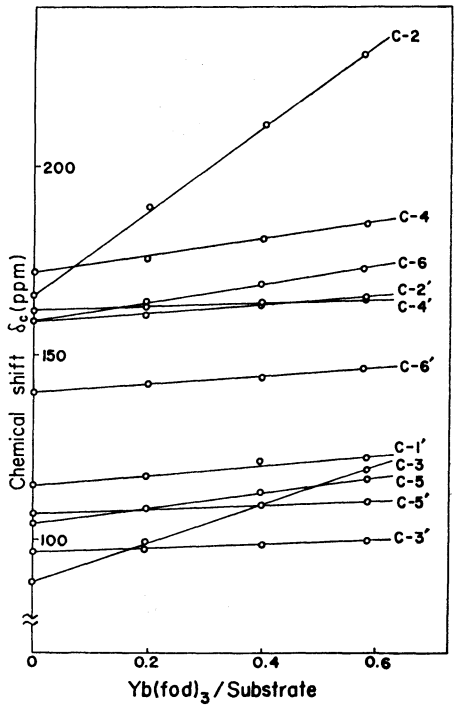


Fig. 4. Variations in the ^{13}C chemical shift of **4** in $CDCl_3$ with increasing concentration of $Yb(fod)_3$.

substituted 4-methoxy-2-pyrone rings, we investigated natural-abundance ^1H noise-decoupled ^{13}C FT NMR spectra of aloenin (**2**) and its derivatives (Tables 5 and 6).

Signals due to C-2, C-4, and C-6 in the 2-pyrone ring were distinguished as follows. Selective ^1H decoupling irradiated at H-3, H-5, and 4-OCH_3 frequencies revealed the C-2, C-4, and C-6 signal assignments, since $^2J_{C(2)-C(3)-H(3)} < {}^2J_{C(6)-C(5)-H(5)}$ is known for 2-pyrone.[32] Hydrogen-bonding shifts[33] of the C-2 carbonyl peaks were distinguished when a $CDCl_3$–CD_3OH (1 : 1) mixed solvent was used for **4**, **18**, and **19**

Table 6. ^{13}C-Chemical shifts[a] of acetophenone derivatives, **9**, **12**, **16**, and **17**

Carbon No.	**9**	**12**	**16**	**17**
1	124.1	115.2	127.2	125.8
2	158.5	167.2	148.7	154.9
3	96.1	99.1	105.8	100.6
4	161.2	164.4	160.5	160.8
5	107.4	111.8	114.2	109.8
6	137.9	141.9	137.8	137.5
COMe	204.4	203.9	202.1	204.1
COMe	32.4	33.0	31.9	32.4
4-OMe	55.3	55.3	55.4	55.4
6-Me	20.0	25.1	20.1	19.6

a) Determined in $CDCl_3$ (TMS=0).

Table 7. Inhibition of gastric juice secretion of rats by aloenin (**2**)

Compound	Dose (mg/kg)	pH	Secretion (ml/100 g body weight)	Inhibition (%)
Control	—	1.8	4.7	0
Aloenin (**2**)	100	1.8	3.6	23

(Table 5). The lanthanide-induced shift (LIS) method[34,35] was applied to **4** employing $Yb(fod)_3$ in $CDCl_3$. The largest lanthanide-induced shift value was obtained for the C-2 peak as indicated in Fig. 4.

For assignments of the benzene ring carbons, we examined the substituent effects[36] of some acetophenone derivatives, **9**, **12**, **16**, and **17** (Table 6). The methylation and acetylation shifts in *o*-hydroxyacetophenone[37,38] were utilized for assigning the benzene ring carbons of all the compounds examined. The deuteration effect[39] (−0.6 ppm) was detected for C-2 of **12**, which has an abnormal chemical shift due to an intramolecular hydrogen-bond. The substituent effects were useful for assigning the benzene-ring carbons of aloenin derivatives (Table 5).

The signals due to the sugar moiety of **2** appear at almost the same positions as those of methyl β-D-glucopyranoside[32] in pyridine-d_5 except for the anomeric C-1 signal which was shifted upfield by about −3 ppm from the position of the C-1 signal of methyl glucoside. A similar behavior was observed for the sugar moieties of **17**, **18**, and **19** and methyl tetra-*O*-acetyl-β-D-glucopyranoside in $CDCl_3$. On the other hand, anomeric carbon signals in sugar moieties of some steroid and triterpenoid glycosides were found to resonate at almost the same position as that in the corresponding methyl glycosides.[40] Chemical shift changes in aromatic aglycone carbons due to glycosidation (**6**→**19** as shown in Table 5) will also be useful for structure determination of natural glycosides having aromatic aglycones.

Thus, the assignments for all carbon atoms of aloenin (**2**) and its derivatives were established as shown in Tables 5 and 6; the present ^{13}C-NMR data on derivatives, **4**, **6**, **9**, **12**, and **16**—**21**, are in line with the structure of aloenin (**2**).

Test of Inhibitory Action on Gastric Juice Secretion. Since Aloe juice is widely used as domestic medicine for gastro-intestinal disturbance, the action of aloenin (**2**) on the gastric juice secretion of rats was tested by the method of Shay *et al.*[41] (Table 7). Aloenin (**2**) was found to exhibit an inhibitory action on gastric juice secretion.

Experimental

MS analyses were performed on Hitachi mass spectrometers, Model RMU-7L (for the high-resolution mass spectrum measurements) and Model RMS-4 (for the usual measurements), ionizing at the order of 70 eV. The 100 MHz and 60 MHz PMR spectra were recorded on Varian HA-100 and Hitachi Perkin-Elmer R-20 spectrometers, respectively, using TMS as an internal standard. The natural-abundance ^{13}C FT NMR spectra were taken on a Varian NV-14 FT NMR spectrometer operating at 15.087 MHz at 30 °C, using TMS as an internal reference ($\delta_c=0$) in a 8-mm spining tube, precision of δ_c being *ca.* ±0.1. The ^{13}C signals were assigned using known chemical shift rules,[33] ^1H single-frequency off-resonance (SFORD) and/or selective decoupling techniques[33] in combination with C_6D_6-induced ^1H shifts.[42] The NOE experiments were carried out on a Varian HA-100 spectrometer operating at 100 MHz in the frequency-swept and internal-TMS-locked mode. Samples were prepared in concentration of 5% (w/v) in $CDCl_3$ and carefully degassed just prior to measurements. The NOE values are represented by an increase in integrated intensities, precision of the values being *ca.* $\pm2\%$. Crystallographic analysis was performed on a Syntex $P2_1$ diffractometer using ω-scan technique with Cu $K\alpha$ radiation and a Ni-filter. The differential thermal and thermal gravimetric analyses were carried out on a Rigakudenki 8002 DTA-TGA analyzer. Melting points determined with a micro hot stage apparatus are uncorrected.

Extraction and Isolation. The leaves (3.7 kg) of *Aloe arborescens* Mill. var. *natalensis* Berger, which had been cultivated in pots for 3—4 years after planting the cuttings of lateral buds *ca.* 5 cm long, were collected in late April, minced mechanically, and squeezed on a three fold gauze to give a green leaf juice. The leaf juice on evaporation of water on a steam bath afforded a brown viscous mass, which was subjected to column chromatography on silica gel with a $CHCl_3$–MeOH mixture with MeOH increasing from 0 to 100% and then preparative TLC on silica gel with a $CHCl_3$–MeOH (3:1) mixture to give aloenin (**2**) (1.78 g).

*Aloenin (**2**).* Mp 145—147 °C (monohydrate) and 204—205 °C (anhydrous): the melting points and water of crystallization were determined by differential thermal and thermal gravimetric analyses; $[\alpha]_D^{25}$ −26.79° (*c* 2.2, MeOH); IR (dioxane) 3300, 1714, 1642, 1608, 1560 cm^{-1}; UV (EtOH) λ_{max} 307 nm (log ε 3.91), 245 (3.81), 232 (3.87), (0.05 M KOH-EtOH) 353 nm (log ε 4.16), 253 (4.14), 225 (3.98); PMR (Acetone-d_6) δ 2.19 (s, 3H, C(6′)-Me), 3.86 (s, 3H, C(4)-Me), 5.47 (d, J=2.5 Hz, 1H, C(3)-H), 6.15 (d, J=2.5 Hz, 1H, C(5)-H), 6.45 (d, J=2.2 Hz, 1H, arom. H), 6.62 (d, J=2.2 Hz, 1H, arom. H); ^{13}C-NMR (C_5D_5N) (Table 5). Found: C, 53.02; H, 5.76%. Calcd for $C_{19}H_{22}O_{10}\cdot H_2O$: C, 53.27; H, 5.65%.

*Dimethyl Ether **10**.* Methylation of **2** (100 mg) with diazomethane gave dimethyl ether **10** (102 mg): Mp 117—118 °C; IR (KBr) 3400, 1680, 1635, 1610, 1561 cm^{-1}; PMR (C_5D_5N) δ 2.29 (s, 3H, C(6′)-Me), 3.54 (s, 3H, C(4′)-OMe), 3.71 (s, 3H, C(4)-OMe), 5.60 (d, J=2.5 Hz, 1H, C(3)-H), 6.05 (d, J=2.5 Hz, 1H, C(5)-H). Found: C, 56.35; H, 5.65%. Calcd for $C_{20}H_{24}O_{10}$: C, 56.60; H, 5.70%.

*Pentaacetate **18**.* A pentaacetate derivative (**18**) of **2** was prepared by treatment with acetic anhydride in pyridine for 1 day at room temp: Mp 192—193 °C; IR ($CHCl_3$) 1758, 1714, 1698, 1645, 1608, 1567 cm^{-1}; PMR ($CDCl_3$) δ 1.98 (s, 6H, 2×OAc), 2.02 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.27 (s, 3H, C(6′)-Me), 2.29 (s, 3H, C(4′)-OAc), 3.86 (s, 3H, C(4)-OMe), 5.52 (d, J=2.5 Hz, 1H, C(3)-H), 5.99 (d, J=2.5 Hz, 1H, C(5)-H), 6.72 (bs, 2H, arom. 2H), 3.5—5.3 (7H); NOE (Fig. 1); ^{13}C-NMR ($CDCl_3$) (Table 5). Found: C, 49.60; H, 4.52%. Calcd for $C_{29}H_{32}O_{20}$: C, 49.72; H, 4.60%.

*Exhaustive Methylation of Aloenin (**2**).* Aloenin (**2**) (120 mg) was treated according to Hakomori's method[19] with sodium hydride and methyl iodide in dimethyl sulfoxide to give hexamethyl ether **5** (67 mg): Mp 143—143.5 °C; IR (KBr) 3050, 1720, 1648, 1615, 1572 cm^{-1}; PMR ($CDCl_3$) δ 2.22 (s, 3H, C(6′)-Me), 3.35 (s, 3H, OMe), 3.38 (s, 3H, OMe), 3.49 (s, 3H, OMe), 3.58 (s, 3H, OMe), 3.76 (s, 3H, OMe), 3.78 (s, 3H, OMe), 4.78 (d, J=5.5 Hz, 1H, C(1″)-H), 5.46 (d, J=2.0 Hz, 1H, C(3)-H), 6.08 (d, J=2.0 Hz, 1H, C(5)-H), 6.46 (d, J=2.0 Hz, 1H, arom. H), 6.59 (d, J=2.0 Hz, 1H, arom. H). Found: C, 59.71; H, 6.75%. Calcd for $C_{24}H_{32}O_{10}$: C, 59.99; H, 6.71%.

*Acid Hydrolysis of Aloenin (**2**).* After aloenin (**2**) (100 mg) dissolved in 3% methanolic hydrochloric acid (5 ml) had been heated under reflux for 2 h, the mixture was diluted with water and extracted with ether. The ether extract gave the aglycone (**3**) (41 mg): Mp 213—214 °C; IR (KBr) 3350, 1670, 1628, 1600, 1562 cm^{-1}; PMR (C_5D_5N) δ 2.33 (s, 3H, C(6′)-Me), 3.61 (s, 3H, C(4)-OMe), 5.67 (d, J=2.5 Hz, 1H, C(3)-H), 6.38 (d, J=2.5 Hz, 1H, C(5)-H). Found: C, 62.75; H, 4.81%. Calcd for $C_{13}H_{12}O_5$: C, 62.90; H, 4.87%. The aqueous layer was passed through an Amberlite resin IR-45 (OH) column until all chloride ions had been removed. The neutral solution on concentration under reduced pressure gave D-glucose (30 mg): mp 144—145 °C, $[\alpha]_D^{25}$ +109°→+49° (*c* 0.2, H_2O).

*Enzymic Hydrolysis of Aloenin (**2**).* A suspension of aloenin (**2**) (20 mg) and emulsin (50 mg) in a phosphate buffer solution (pH 6.5) (5 ml) was stirred at 35 °C for 2 days. Formation of the aglycone (**3**) and glucose was confirmed by TLC and PPC analyses.

*Trimethyl Ether **4**.* Methylation of aglycone (**3**) (20 mg) with diazomethane gave the trimethyl ether **4** (20 mg): Mp 133—134 °C; IR (Nujol) 1725, 1641, 1613 cm^{-1}; PMR ($CDCl_3$) δ 2.26 (s, 3H, C(6′)-Me), 3.74 (s, 3H, C(2′)-OMe), 3.81 (s, 3H, C(4′)-OMe), 3.83 (s, 3H, C(4)-OMe), 5.50 (d, J=2.5 Hz, 1H, C(3)-H), 5.99 (d, J=2.5 Hz, 1H, C(5)-H), 6.34 (s, 2H, C(3′)- and C(5′)-H); NOE (Fig. 1); MS *m/e* (rel intensity) 276 (M^+, 100), 261 (5), 248 (52), 233 (33), 217 (12), 205 (44), 191 (13), 189 (17), 179 (29), 178 (30); High-resolution MS (Scheme 1); ^{13}C-NMR ($CDCl_3$) (Table 5). Found: C, 65.00; H, 5.72%. Calcd for $C_{15}H_{16}O_5$: C, 65.21; H, 5.84%.

*Acid Hydrolysis of the Hexamethyl Ether (**5**).* The hexamethyl ether (**5**) (100 mg) was refluxed with 5 ml of 3% methanolic hydrochloric acid for 2 h and then the mixture, after being diluted with water, was extracted with chloroform. The chloroform extract gave a product **6** (35 mg) [mp 194—195 °C; IR (KBr) 1665, 1605, 1546, 1504, 844, 810, 750 cm^{-1}; PMR ($CDCl_3$) δ 2.32 (s, 3H, C(6′)-Me), 3.78 (s, 3H, C(4′)-OMe), 3.85 (s, 3H, C(4)-OMe); ^{13}C-NMR ($CDCl_3$) (Table 5)] and 2,3,4,6-tetra-*O*-methyl-D-glucose (mp 90—94 °C). Methylation of the product (**6**) (10 mg) with diazomethane gave trimethyl ether (**4**) (10 mg). The tetra-*O*-methyl-D-glucose was identified by comparing the mp and IR spectrum

with those of a synthetic sample.

*Acid Hydrolysis of the Dimethyl Ether (**10**).* The dimethyl ether (**10**) (50 mg) was refluxed with 3 ml of 3% methanolic hydrochloric acid for 2 h. The same treatment as in the case of **5** gave the compound **6** (17 mg) [mp 194—195 °C; IR (KBr) 1665, 1605, 1546, 1504 cm^{-1}] and glucose.

*Conversion of **3** and **6** into Chromone Derivatives.* The aglycone (**3**) (50 mg) was heated with 5% hydrochloric acid (5 ml) under reflux for 1 h, giving 2,5-dimethyl-7-hydroxychromone (**13**) (30 mg; mp 244—245 °C).[25] The same treatment of **6** as above gave 2,5-dimethyl-7-methoxychromone (**14**) (32 mg; mp 116—117 °C).

*Alkaline Cleavage of the Trimethyl Ether (**4**).* A solution of trimethyl ether (**4**) (200 mg) in 5% methanolic potassium hydroxide (20 ml) was stirred at room temp in an atmosphere of nitrogen. Crystals which started to appear after 12 h were filtered after 2 days and washed with absolute ethanol and then chloroform. They showed red coloration with ethanolic ferric chloride. A solution of the crystals (132 mg) in water (10 ml) was acidified carefully with a few drops of dilute hydrochloric acid and then immediately extracted with ether to give β-keto acid **7** (110 mg): IR (liquid) 3500—2500, 1725, 1600 cm^{-1}; PMR ($CDCl_3$) δ 2.33 (s, 3H, arom. Me), 3.73 (s, 6H, 2×OMe), 6.62 (s, 2H, arom. 2H); the reddish violet coloration is characteristic of β-keto acids with ethanolic ferric chloride. The keto acid (**7**) underwent facile decarboxylation during the course of purification by recrystallization. Without any purification, **7** (80 mg) dissolved in $CHCl_3$ (10 ml) was heated under reflux for 5 h in a stream of nitrogen to undergo decarboxylation. This was confirmed by the formation of barium carbonate in a barium hydroxide trap attached to the reflux apparatus. Removal of the solvent from the chloroform solution gave 1-(2,4-dimethoxy-6-methylphenyl)butane-1,3-dione (**8**) (61 mg; mp 72—73 °C, M^+ 236), which was identified by direct comparison with a synthetic specimen described below.

*Alkaline Cleavage of the Dimethyl Ether (**10**).* A solution of the dimethyl ether (**10**) (100 mg) in 5% methanolic potassium hydroxide (10 ml) was refluxed for 12 h. The mixture, after neutralization with hydrochloric acid and subsequent removal of the solvent, was subjected to preparative TLC on silica gel to give an oily product **11** (32 mg): IR (Nujol) 3450, 1600, 1180 cm^{-1}, which was treated with acetic anhydride in pyridine at room temp to afford tetraacetate **17**: mp 144—146 °C; IR (KBr) 1753, 1690, 1610, 1382, 1320, 1239 cm^{-1}; PMR ($CDCl_3$) δ 2.00 (s, 3H, OAc), 2.05 (s, 6H, 2×OAc), 2.10 (s, 3H, OAc), 2.22 (s, 3H, Me), 2.40 (s, 3H, COMe), 3.78 (s, 3H, OMe), 6.52 (s, 2H, arom. 2H); ^{13}C-NMR (Table 6). Found: C, 64.37; H, 6.70%. Calcd for $C_{24}H_{30}O_8$: C, 64.56; H, 6.77%. Hydrolysis of **11** (20 mg) with 3% methanolic hydrochloric acid (5 ml) gave 11 mg of 2-hydroxy-4-methoxy-6-methylacetophenone (**12**) (mp 78.0—78.5 °C; *m/e* 180 (M^+)), a trace of 2,5-dimethyl-7-methoxychromone (**14**), and glucose. The products, **12** and **14**, were identified by comparison with an authentic specimen,[22,25] glucose being detected by TLC and PPC analyses.

*2-Acetoxy-4-methoxy-6-methylacetophenone (**16**).* Acetylation of **12** (66 mg) with acetic anhydride in pyridine at room temp gave a methylacetophenone derivative (**17**) (80 mg): IR (liquid) 1760, 1680, 1605 cm^{-1}; PMR ($CDCl_3$) δ 2.28 (s, 3H, OAc), 2.33 (s, 3H, Me), 2.44 (s, 3H, COAc), 3.78 (s, 3H, OMe), 6.52 (d, J=2.0 Hz, 1H, arom. H), 6.65 (d, J=2.0 Hz, 1H, arom. H); ^{13}C-NMR (Table 6).

*Tetraacetate **19**.* Acetylation of the dimethyl ether (**10**) (50 mg) with acetic anhydride in pyridine at room temp gave tetraacetate **19** (53 mg): Mp 182—183 °C; IR (KBr) 1755, 1720, 1650, 1615, 1574 cm^{-1}; PMR ($CDCl_3$): δ 1.98 (s, 6H, 2×OAc), 2.03 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.30 (s, 3H, arom. Me), 3.82 (s, 3H, OMe), 3.87 (s, 3H, OMe), 5.50 (d, J=2.5 Hz, 1H, C(3)-H), 5.98 (d, J=2 Hz, 1H, C(5)-H), 6.50 (s, 2H, C(3′)- and C(5)-H); ^{13}C-NMR (Table 5).

*Methoxyacetate **20**.* Acetylation of the compound **6** (61 mg) with acetic anhydride in pyridine at room temp gave methoxyacetate **20** (58 mg): Mp 159—160 °C; IR (KBr) 1765, 1735, 1635, 1620, 1560 cm^{-1}; PMR ($CDCl_3$) δ 2.20 (s, OAc) 2.32 (s, Me), 3.83 (s, C(4′)-OMe), 3.86 (s, C(4)-OMe), 5.52 (d, J=2.0 Hz, C(3)-H), 5.95 (d, J=2.0 Hz, C(5)-H), 6.53 (d, J=2.0 Hz, arom. H), 6.72 (d, J=2.0 Hz, arom. H); ^{13}C-NMR (Table 5).

*Diacetate **21**.* Acetylation of the aglycone (**3**) (100 mg) with acetic anhydride in pyridine at room temp gave diacetate **21** (32 mg): Mp 151—152.5 °C; IR (KBr) 1764, 1725, 1640, 1614, 1565 cm^{-1}; PMR ($CDCl_3$) δ 2.20 (s, 3H, OAc), 2.30 (s, 3H, OAc), 2.33 (s, 3H, Me), 3.87 (s, 3H, OMe), 5.52 (d, J=2.0 Hz, 1H, C(3)-H), 5.98 (d, J=2.0 Hz, 1H, C(5)-H), 6.87 (m, 2H, arom. 2H); ^{13}C-NMR (Table 5).

*Synthesis of 1-(2,4-Dimethoxy-6-methylphenyl)butane-1,3-dione (**8**).* Sliced metallic sodium (25 mg) was added under ice-cooling to a mixture of ethyl acetate (0.5 ml) and 2,4-dimethoxy-6-methylacetophenone (**9**) (100 mg), which was prepared from 3,5-dihydroxytoluene by acetoacetic ester condensation.[22] The reaction mixture was left to stand at room temp for 5 min and then heated at 110 °C for 2 h. The mixture, after cooling and addition of water (5 ml), was extracted with ether to remove the unchanged substance. The separated alkaline solution was acidified with dilute acetic acid and extracted with ether to yield dione **8** (55 mg): Mp 72—73 °C; IR (KBr) 3500—2500, 1600 cm^{-1} (enol form of β-diketone); UV (EtOH) λ_{max} 282 nm (log ε 4.04), 222 (3.92); PMR ($CDCl_3$) δ 2.15 (s, 3H, COMe), 2.34 (s, 3H, arom. Me), 3.78 (s, 6H, 2×OMe), 5.69 (s, 1H, C(2)-H (enol form)), 6.32 (bs, 2H, arom. 2H); MS *m/e* (rel intensity) 236 (M^+, 31), 219 (30), 205 (20), 179 (100), 152 (36). Found: C, 66.02; H, 6.57%. Calcd for $C_{13}H_{16}O_4$: C, 66.08; H, 6.83%. The product (**8**) gave a reddish violet coloration characteristic of a β-diketone structure by the ferric chloride test.

*Test of Inhibitory Action of Aloenin (**2**) on Gastric Juice Secretion.* Inhibitory activity to gastric juice secretion was tested by the method of Shay *et al.*[41] Male rats of Donryu strain, 130—170 g in weight, were used for the tests. Table 7 gives the amount and pH of the gastric juice secreted during a period of 4 h after oral administration of the sample through a gastro-tube, together with the results of the control experiment.

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