STRUCTURE OF ALOENIN, A BITTER GLUCOSIDE FROM ALOE SPECIES

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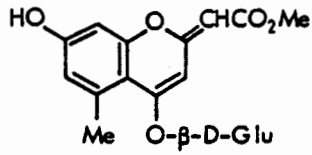
The structure of aloenin, a bitter glucoside from Aloe species, has been reinvestigated and elucidated to be 6-(2'-β-D-glucopyranosyloxy-4'-hydroxy-6'-methyl)phenyl-4-methoxy-2-pyrone (2) by a combination of the chemical and spectroscopic methods.

Recently, we isolated aloenin, a new bitter glucoside from Aloe arborescens Mill. var. natalensis Berger (Japanese name: Kidachirokai or Kidachiaroe) which is used in domestic medicine, and reported that we had elucidated its structure to be as in formula 1.[1] Aloenin has been identified with aloearbonaside, isolated independently by the Kyushu University group;[2] they have also assigned the structural formula 1 to this compound. However, intramolecular nuclear Overhauser effect (NOE)[3] studies of some derivatives of aloenin have aroused doubt about the reported structure. Hence, we have re-examined the structure of aloenin by a combination of chemical and spectroscopic methods to elucidate it unambiguously, and we report here evidence for a revised structure of aloenin.

Aloenin (2) exhibited IR absorption bands due to a conjugated ester or a lactone group.[1,4] The UV spectrum showed a bathochromic shift in an alkaline solution [λ_{max}^{EtOH} 307 nm (log ε 3.71) → $\lambda_{max}^{0.05N\ EtOH\text{-}NaOH}$ 353 (4.16)]. The IR and UV spectra closely resemble those of α-pyrone derivatives.[5] The ^{1}H NMR spectrum in acetone-d_6 showed the presence of an aromatic methyl (δ 2.19s), a methoxyl (3.86s), and four olefinic or aromatic protons (5.47d and 6.15d, J = 2.5 Hz; 6.45d and 6.62d, J = 2.2 Hz). The results of NOE measurements on its pentaacetate 3[1,4] are shown in Scheme 1.[6] The observation of a 5% NOE, [C-6'-Me (δ 2.26)] → H-5 (δ 5.95), can hardly be explained by formula 1 for aloenin.



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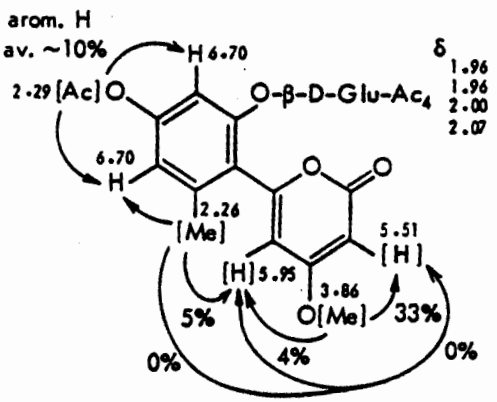
Hydrolysis of aloenin with aqueous MeOH-HCl (3%) afforded an aglycone 4 and D-glucose.[1,4] Methylation of 4 with CH_2N_2 gave its dimethyl ether 5, whose NMR spectrum in $CDCl_3$ showed signals due to an aromatic methyl, three methoxyls, and four olefinic or aromatic protons (see Scheme 1).[1,4] Permethylation of aloenin by Hakomori's method[7] yielded a hexamethyl ether 6, mp 143-143.5°, $C_{24}H_{32}O_{10}$, which could be hydrolyzed by aqueous MeOH-HCl (3%) into a dimethyl ether 7, mp 196-197.5°, $C_{14}H_{14}O_5$ [NMR (C_5D_5N) δ 2.31 (s, 3H), 3.60 (s, 3H), and 3.65 (s, 3H)], and 2,3,4,6-tetra-O-methyl-D-glucose, mp 90-94°. The latter compound was identified by comparing its IR and NMR spectra with those of an authentic sample. This suggests that the sugar moiety is linked with the aglycone through the C-1-OH group of D-glucose. Methylation of 7 with CH_2N_2 afforded 5.



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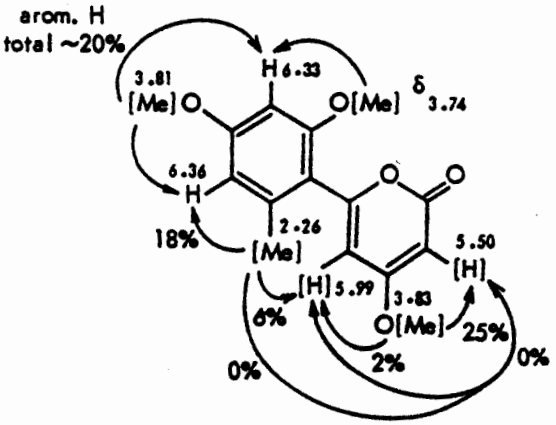

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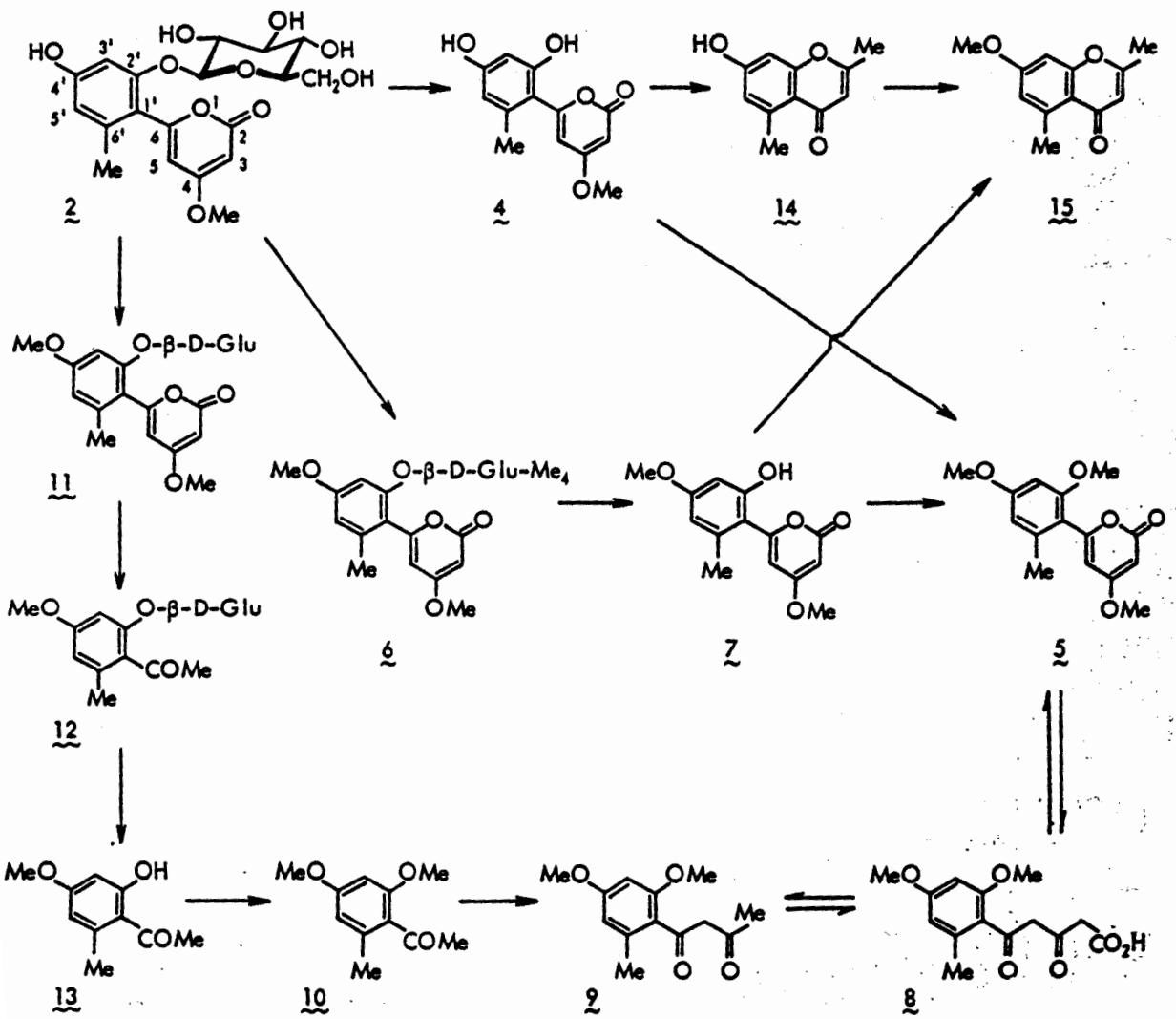
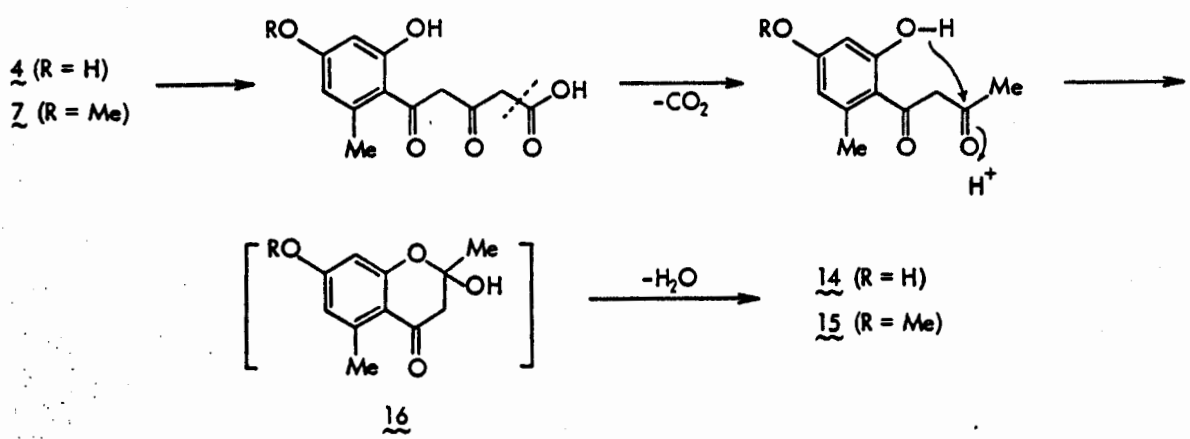
Scheme 1. The results of NOE measurements in $CDCl_3$.[6]



Treatment of 5 with 1N MeOH-KOH at room temperature yielded the corresponding potassium salt, which on treatment with HCl gave an unstable β-diketo acid 8. The structure of 8 was assigned by the fact that it underwent facile decarboxylation on heating to yield 1-(2',4'-dimethoxy-6'-methyl)phenylbutane-1,3-dione (9), mp 72-73°, $C_{13}H_{16}O_4$, which was identified by direct comparison with a synthetic sample.[8] These reactions demonstrate the presence of a C-4 hydroxylated α-pyrone skeleton.[10] The product 9 possesses a new acetyl group which is not present in the parent compound 5. The absence of the acetyl group in 5 warrants a carboxyl group in 8 to be attached to the end of the side chain of 9. These facts clearly indicate that the structure of 5 should be represented as 6-(2',4'-dimethoxy-6'-methyl)phenyl-4-methoxy-2-pyrone. A typical mass-spectral fragmentation pattern of C-6 substituted 4-methoxy-2-pyrone derivatives[11] was observed for 5 and these fragments were completely characterized by high-resolution mass-spectral measurements. The NOE experiments were also carried out on 5. The results obtained (see Scheme 1) as well as those with 3 are consistent with the structure 2 for aloenin.[12]

Structure 5 was further confirmed by synthesis by the following procedure. β-Diketone 9 prepared from 2,4-dimethoxy-6-methylacetophenone (10)[8,9] was first converted into 8 by treatment with sodium amide in liquid ammonia, followed by carbon dioxide.[13] Cyclization of 8 in the presence of acetic anhydride[13] gave an α-pyrone derivative, which was subsequently converted into a methyl ether with CH_2N_2. This methyl ether was shown to be identical with 5 derived from aloenin.

The alkaline degradation of a monomethylated product 11, mp 117-118°, of aloenin by refluxing with MeOH-KOH yielded a glucoside 12 (ν_{max}^{Nujol} 3480, 1600, and 1185 cm^{-1}), which could be hydrolyzed to 2-hydroxy-4-methoxy-6-methylacetophenone (13)[9] and D-glucose. This fact shows that the glucose moiety in aloenin is located on the C-2'-OH group of aglycone 4. Aglycone 4 was converted into 2,5-dimethyl-7-hydroxychromone (14)[14] by treatment with 5% HCl.[1,4] On the other hand, similar treatment of 7 gave 2,5-dimethyl-7-methoxychromone (15).[14] This fact suggests the presence of a free OH group in aloenin attached to the C-4' position of the aromatic ring. This conversion of 2-pyrone derivatives 4 and 7 into the corresponding chromones 14 and 15, respectively, may be explained by acid-catalyzed reaction via an intermediate 16 as shown in Scheme 3.



Scheme 3

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 NMR spectrum of 6. Consequently, the structure of aloenin has now been established as 6-(2'-β-D-glucopyranosyloxy-4'-hydroxy-6'-methyl)phenyl-4-methoxy-2-pyrone (2).

References and Footnotes

1) T. Suga, T. Hirata, and M. Odan, Chem. Lett., 547 (1972).
2) K. Makino, A. Yagi, and I. Nishioka, Chem. Pharm. Bull., 21, 149 (1973).
3) J. H. Noggle and R. E. Schirmer, "The Nuclear Overhauser Effect. Chemical Applications," Academic Press Inc. (1971).
4) Physical constants and spectroscopic data have already been described in the previous paper.[1] Elemental analyses of all compounds described here were satisfactory.
5) A. K. Ganguly, T. R. Govindachari, and P. A. Mohamed, Tetrahedron, 21, 93 (1965).
6) 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. Sample solutions (ca. 5% (w/v) in $CDCl_3$ and C_6D_6) were carefully degassed. The NOE values are represented by increases in integrated intensities, ±2%; [A] – B indicates that an NOE was observed on the H_B signal when the H_A frequency was saturated. The results of NOE measurements in C_6D_6 were similar to those in $CDCl_3$.
7) S. Hakomori, J. Biochem., 55, 205 (1964).
8) Compound 9 was synthesized by the acetoacetic-ester type condensation of 2,4-dimethoxy-6-methyl-acetophenone (10)[9] and ethyl acetate in the presence of sodium metal. Physical properties for 9 are as follows: ν_{max}^{KBr} 3500-2500 and 1600 cm^{-1} (β-diketone enol form); λ_{max}^{EtOH} 282 nm (log ε 4.04) and 222 (3.92); NMR ($CDCl_3$) δ 2.15 (s, COMe), 2.34 (s, arom. Me), 3.78 (s, 2 x OMe), 5.69 (s, C(2)-H enol form), and 6.32 ppm (b.s, arom. 2H); MS m/e (rel. intensity) 236 (M^+, 31), 219 (30), 205 (20), 179 (100), and 152 (36).
9) T. Suga, T. Hirata, and F. Walls, J. Sci. Hiroshima Univ., Ser. A, 38, No. 2 (1974), to be published.
10) W. B. Mors, O. R. Gottlieb, and C. Djerassi, J. Amer. Chem. Soc., 79, 4507 (1957).
11) Q. N. Porter and J. Baldas, "Mass Spectrometry of Heterocyclic Compounds," Wiley-Interscience (1971).
12) The natural-abundance ^{13}C FT NMR spectra of 5, ^1H noise-decoupled and single-frequency off-resonance decoupled, in $CDCl_3$ show signals of all carbons assignable to formula 5; that is, δ 20.1 (C-6'-Me), 48.3 (OMe), 55.8 (2 x OMe), 88.0 (C-3), 95.9 (C-3'), 104.4 (C-5), 106.8 (C-5'), 114.6 (C-1'), 139.7 (C-6'), 158.6 (C-2' and C-4'), 161.5 (C-6), 165.0 (C-4), and 170.9 (C-2) (ppm downfield from TMS). The spectra were determined by a Varian NV-14 FT NMR spectrometer at 15.09 MHz.
13) T. M. Harris and C. S. Combs, Jr., J. Org. Chem., 33, 2399 (1968).
14) T. Hirata and T. Suga, Bull. Chem. Soc. Japan, 47, 244 (1974).