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DIASTEREOMERIC *C*-GLUCOSYLANTHRONES OF *ALOE VERA* LEAVES

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Key Word Index—*Aloe vera*; Liliaceae; 8-*O*-methyl-7-hydroxyaloin A; 8-*O*-methyl-7-hydroxyaloin B; 10-hydroxyaloin A; 10-hydroxyaloin B.

Abstract—The leaves of *Aloe vera* afforded, together with 10-hydroxyaloins A and B, a new pair of diastereoisomeric *C*-glucosylanthrones whose structures were established as 8-*O*-methyl-7-hydroxyaloin A and 8-*O*-methyl-7-hydroxyaloin B from spectroscopic studies.

INTRODUCTION

Aloe has been widely used as a folk medicine for centuries. Two kinds of products are provided from aloe leaves. One is a colourless and tasteless gel obtained from parenchyma cells used in the treatment of skin diseases [1]. The second, a yellow exudate from the leaves, is a well known purgative [2], the active phenolic components of which are abundant in the inner epidermal cell layers. The purgative principles from aloe have been identified as *C*-glucosylanthrone, aloin A, aloin B, homonataloin A and homonataloin B.

On investigation of the leaves of *Aloe vera* by HPLC using a photodiode-array detector [3], we found four unidentified peaks, corresponding to two pairs of diastereoisomeric *C*-glucosylanthrones, together with aloins A (barbaloin) and B (isobarbaloin) as major *C*-glucosylanthrones. Photodiode-array detection gave considerable structural information by comparison of R_fs and UV spectra with those of aloin-related standards. We report here on the isolation and structure elucidation of these compounds, namely the new compounds 8-*O*-methyl-7-hydroxyaloin A (**1**) and 8-*O*-methyl-7-hydroxyaloin B (**2**), and the related known compounds, 10-hydroxyaloins A (**3**) and B (**4**).

RESULTS AND DISCUSSION

HPLC analysis of the leaf extract showed four peaks with similar characteristic UV-VIS spectra to those of aloins A and B. The R_fs of **1** and **2**, showing the same UV-VIS spectra, were 10.62 and 10.37 min, respectively, both of which were almost overlapped. These findings suggested that **1** and **2** were a diastereoisomeric pair differing in configuration at C-10 in the anthrone moiety. The aloins A and B had R_fs of 21.08 and 20.43 min, respectively.

Powdered ethanol extracts of *A. vera* gel, which had been absorbed on activated charcoal, was dialysed and chromatographed over an MCI-gel CHP 20P column using stepwise gradient elution with water–methanol mixtures. The isolation of compounds **1**–**4** was achieved by repeated MCI-gel CHP 20P CC and Sephadex LH-20 CC.

Compound **1** (8-*O*-methyl-7-hydroxyaloin A), obtained as yellowish needles, showed UV absorption maxima at 206, 221, 294 and 345 nm. The HR-positive FAB-mass spectrum showed $[M+H]^+$ at m/z 449.1448, suggesting the molecular formula $C_{22}H_{25}O_{10}$.

Compound **2** (8-*O*-methyl-7-hydroxyaloin B), obtained as a yellowish amorphous solid, showed UV absorption maxima at 204, 221, 294 and 345 nm. The positive FAB-mass spectrum exhibited a parent ion peak at m/z 449 $[M+H]^+$.

The ^1H and ^{13}C NMR spectral data of **1** and **2** were closely related to those of homonataloins A (10*S*, 1′*S*; $[\alpha]+27.6°$) and B (10*R*, 1′*S*; $[\alpha]-82.6°$) [4], a pair of diastereoisomeric *C*-glucosylanthrones, except that the methyl at C-3 was replaced by a hydroxymethylene group (δ_H 4.49, 4.54, δ_H 4.50, 4.54; δ_C 63.1, 63.1), as shown in Tables 1 and 2. In the ^1H NMR spectra the D-glucopyranosyl linkage in **1** and **2** was shown to be in the β-configuration, as determined by the large

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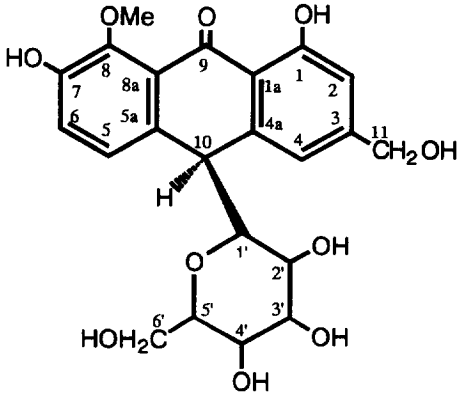

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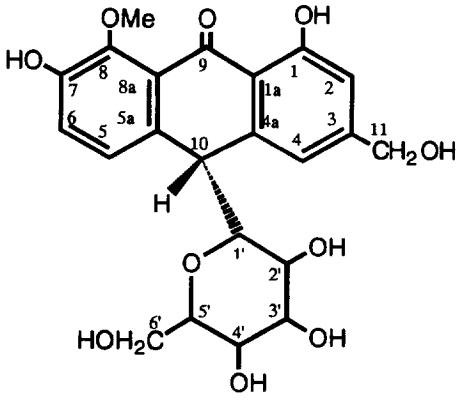

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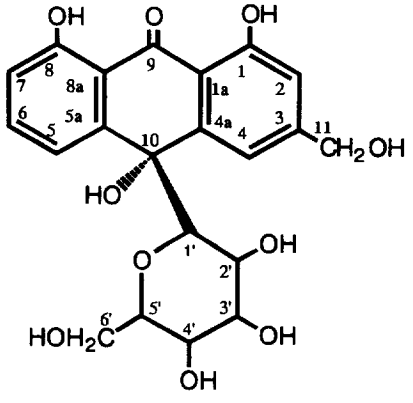

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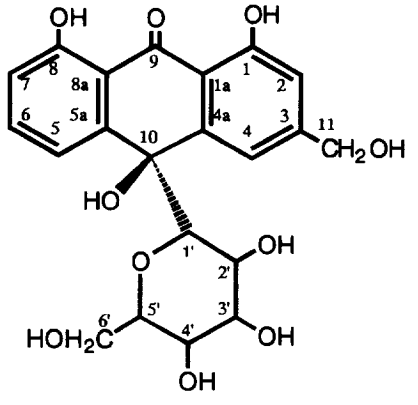

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coupling constants ($J = 9.5$ Hz) of the anomeric proton. The differences between the chemical shifts of the 2′-hydroxyl protons in **1** and **2** may be due to the 10*S* and 10*R* configuration, respectively. Furthermore, the difference between the chemical shifts due to C-4a and C-5a in **1** and **2** suggests that it is due to the adjacent C-2′ hydroxyl group. In the NOESY spectra of **1** and **2** in DMSO-d_6, the methoxy proton showed no correlation to the neighbouring aromatic proton, indicating that the methoxy group was connected to C-8 (Fig. 1). A NOESY cross-peak was observed for H-4/H-2′ in **1** and the C-10 configuration of **1** was inferred to be (*S*). On the other hand, the NOESY spectrum of **2** revealed a cross-peak for H-5/H-6′$_1$, and the presence of this cross peak established C-10 of **2** to be (*R*) [6]. The optical rotation of **1** and **2** showing $+27.2°$ and $-0.7°$, respectively, corresponded to that of aloins A (10*S*, 1′*S*; $+21°$) and B (10*R*, 1′*S*; $-19°$) [7]. Therefore, the structure of **1** and **2** was established as 8-*O*-methyl-7-hydroxyaloins A and B, respectively.

On HPLC analysis, yellowish crystals **3** and yellowish needles **4**, gave the R_ts of 15.06 and 12.24 min, respectively, and had the same UV spectrum pattern. The optical rotations of **3** and **4** were $+5.1°$ and $-49.2°$, respectively, establishing that they were C-10 epimers. Therefore, **3** and **4** were determined to be 10-hydroxyaloins A and B, respectively, by comparing the ^{1}H and ^{13}C NMR spectra with those of the literature data [6].

EXPERIMENTAL

General. Optical rotations UV-VIS: MeOH; ^{1}H and ^{13}C NMR: TMS as int. standard; Positive FAB-MS (JEOL HX-110): glycerol as matrix; HR FAB-MS (JEOL HX-110): polyethylene glycol as matrix; CC: MCI-gel CHP 20P (75–150 μm, Mitsubishi Chemical Industries) and Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemicals); HPLC: detection by UV-8000 UV-VIS detector (Tosoh) set at 290 nm and a Model 991J photodiode-array detector (Waters).

Plant material. Aloe vera (*A. barbadensis*) was collected in the field of Aloecorp (Texas, U.S.A.), and a voucher specimen is deposited at the Plant Resources Center Herbarium of the University of Texas at Austin (U.S.A.). Powdered EtOH extracts of *A. vera* gel, which was treated with an activated charcoal, were also provided by Aloecorp.

HPLC analysis. The column used was a Wakosil-II

Table 1. ^{1}H NMR spectral data for 8-*O*-methyl-7-hydroxyaloin A (**1**) and 8-*O*-methyl-7-hydroxyaloin B (**2**) (δ in DMSO-d_6)

Proton	1	2
H-2	6.79 *d* (0.8)	6.76 *d* (0.8)
H-4	6.94 *d* (0.8)	6.90 *d* (0.8)
H-5	7.10 *d* (7.5)	7.10 *d* (8.0)
H-6	7.13 *d* (7.5)	7.15 *d* (8.0)
H-10	4.43 *d* (2.0)	4.47 *d* (2.0)
8-OMe	3.79 *s*	3.78 *s*
3-C$\underline{H_2}$OH	4.49 *dd* (14.0, 5.5)	4.50 *dd* (15.0, 5.5)
	4.54 *dd* (14.0, 5.5)	4.54 *dd* (15.0, 5.5)
3-CH$_2$O$\underline{H}$	5.31 *t* (5.5)	5.31 *t* (5.5)
1-OH	12.17 *s*	12.17 *s*
7-OH	9.38 *s*	9.41 *s*
2′-OH	5.15 *d* (5.5)	5.26 *d* (5.5)
3′-OH	4.87 *d* (5.0)	4.86 *d* (5.0)
4′-OH	4.71 *d* (5.0)	4.71 *d* (5.0)
6′-OH	3.51 *t* (5.5)	3.48 *t* (5.5)
H-1′	3.17 *dd* (9.5, 2.0)	3.12 *dd* (9.5, 2.0)
H-2′	2.78 *dt* (9.5, 9.5, 5.5)	2.81 *dt* (9.5, 9.5, 5.5)
H-3′	3.10 *dt* (9.5, 9.5, 5.0)	3.10 *dt* (9.5, 9.5, 5.0)
H-4′	2.66 *dt* (9.5, 9.5, 5.0)	2.68 *dt* (9.5, 9.5, 5.0)
H-5′	2.74 *dt* (9.5, 5.5, 2.5)	2.72 *dt* (9.5, 5.0, 2.0)
H-6′$_1$	3.38 *dt* (11.5, 5.5, 2.5)	3.35 *dt* (11.5, 5.5, 2.0)
H-6′$_2$	3.11 *dt* (11.5, 5.5, 5.5)	3.13 *dt* (11.5, 5.5, 5.0)

Table 2. ^{13}C NMR chemical shifts of 8-*O*-methyl-7-hydroxyaloin A (**1**) and 8-*O*-methyl-7-hydroxyaloin B (**2**) (δ in CD_3OD)

Carbon no.	1	2
1	161.8	161.9
2	113.9	113.7
3	150.7	151.2
4	118.4	116.6
5	122.4	121.2
6	124.7	126.1
7	149.6	150.6
8	148.1	148.5
9	191.7	191.7
10	45.3	45.5
1a	119.8	119.6
4a	141.5	145.8
5a	137.2	133.2
8a	128.0	128.5
11	63.1	63.1
8-OMe	62.0	62.0
1′	84.9	85.2
2′	71.6	71.7
3′	79.6	79.7
4′	72.5	72.7
5′	81.1	80.9
6′	64.3	64.5

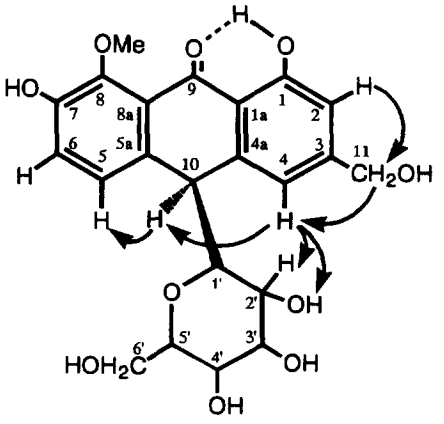
5C18 HG reverse-phase column (5 μm, 150 × 4.6 mm I.D., Wako Pure Chemical Industrials). The sepn was carried out at 45° using a linear gradient program at a flow-rate of 1 ml min^{-1}; eluent MeCN–H_2O, 0–19 min, 12–23%; 19–24 min, 23–28%; 24–39 min, 28–46% [3].

Isolation. Dried and powdered EtOH extract (530 g) was dissolved in H_2O and dialysed overnight against H_2O. The dialysate was subjected to MCI-gel CHP 20P CC using stepwise gradient elution with H_2O–MeOH as solvent. The 30% MeOH eluate was rechromatographed over an MCI-gel CHP 20P column with 40% MeOH and a Sephadex LH-20 column with MeOH–H_2O to furnish frs I, II and III. Fr. I was applied to a Sephadex LH-20 column eluting with Me_2CO to give **1** (1136 mg) and **2** (852 mg). Frs II and III were chromatographed over a Sephadex LH-20 column eluting with Me_2CO to give **3** (1874 mg) and **4** (1151 mg), respectively.

8-O-*Methyl-7-hydroxyaloin A* (**1**). Yellowish needles (Me_2CO–benzene), mp 218–224°, $[\alpha]_D^{30}$ +27.2° (MeOH; *c* 0.250). UV λ_{max}^{MeOH} nm (log ε): 206 (4.48), 221 (4.45), 294 (4.20) and 345 (3.93); HR-positive FAB-MS *m/z*: Found 449.1448 $[M+H]^+$ ($C_{22}H_{25}O_{10}$ requires 449.1439); ^{1}H and ^{13}C NMR: Tables 1 and 2.

8-O-*Methyl-7-hydroxyaloin B* (**2**). Yellowish amorphous solid, $[\alpha]_D^{25}$ −0.7° (MeOH; *c* 0.125). UV λ_{max}^{MeOH} nm (log ε): 204 (4.40), 221 (4.35), 294 (4.09) and 345 (3.82); Positive FAB-MS *m/z*: 449 $[M+H]^+$; ^{1}H and ^{13}C NMR: Tables 1 and 2.

10-*Hydroxyaloins A* (**3**). Yellowish crystals (Me_2CO–EtOAc), $[\alpha_D^{25}$ +5.1° (MeOH; *c* 0.247). UV λ_{max}^{MeOH} nm (log ε): 211 (4.44), 269 (3.82), 301 (3.83) and 368 (3.99); ^{1}H NMR (CD_3OD): δ 2.82 (1H, *dd*, J = 9.6, 8.9 Hz, H′-4), 2.94 (1H, *ddd*, J = 9.6, 6.4, 2.8 Hz, H′-5), 2.95 (1H, *dd*, J = 9.8, 8.9 Hz, H′-2), 3.24 (1H, *t*, J = 8.9 Hz, H′-3), 3.27 (1H, *d*, J = 9.8 Hz, H′-1), 3.37 (1H, *dd*, J = 11.9, 6.4 Hz, H′-6$_2$), 3.58 (1H, *dd*, J = 11.9, 2.8 Hz, H′-6$_1$), 4.67 (1H, *d*, J = 14.7 Hz,



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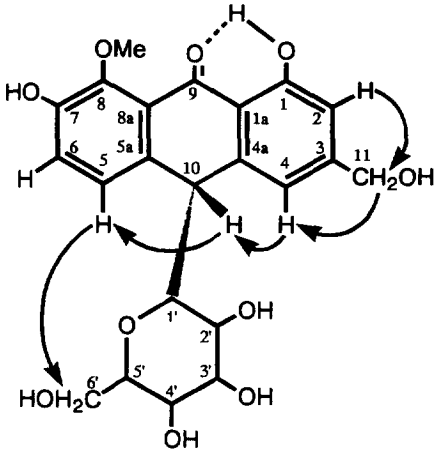

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Fig. 1. NOESY correlations for compounds **1** and **2**.

3-C$\underline{H}_2$OH), 4.71 (1H, *d*, J = 14.7 Hz, 3-C$\underline{H}_2$OH), 6.92 (1H, *dd*, J = 8.2, 1.2 Hz, H-7), 6.95 (1H, *br*, *d*, J = 1.5 Hz, H-2), 7.38 (1H, *br*, *d*, J = 1.5 Hz, H-4), 7.47 (1H, *dd*, J = 7.6, 1.2 Hz, H-5), 7.57 (1H, *dd*, J = 8.2, 7.6 Hz, H-6); ^{13}C NMR (CD_3OD): δ 63.3 (C-6′), 64.7 (C-11), 71.7 (C-4′), 73.0 (C-2′), 76.9 (C-10), 79.6 (C-3′), 81.7 (C-5′), 85.2 (C-1′), 115.3 (C-2), 115.9 (C-1a), 116.3 (C-4), 117.8 (C-8a), 118.3 (C-7), 118.9 (C-5), 136.4 (C-6), 146.7 (C-4a), 149.2 (C-5a), 152.4 (C-3), 163.0 (C-8), 163.1 (C-1), 194.5 (C-9).

10-*hydroxyaloins B* (**4**). Yellowish needles (MeOH), $[\alpha]_D^{25}$ −49.2° (MeOH; *c* 0.260). UV λ_{max}^{MeOH} nm (log ε): 211 (4.37), 269 (3.75), 301 (3.76) and 368 (3.93); ^{1}H NMR (CD_3OD): δ 2.83 (1H, *dd*, J = 9.8, 8.9 Hz, H′-4), 2.92 (1H, *ddd*, J = 9.8, 6.1, 2.4 Hz, H′-5), 2.99 (1H, *dd*, J = 9.2, 8.9 Hz, H′-2), 3.25 (1H, *t*, J = 8.9 Hz, H′-3), 3.26 (1H, *d*, J = 9.2 Hz, H′-1), 3.36 (1H, *dd*, J = 11.6, 6.1 Hz, H′-6_2), 3.56 (1H, *dd*, J = 11.6, 2.4 Hz, H′-6_1), 4.65 (1H, *d*, J = 14.5 Hz, 3-C$\underline{H}_2$OH), 4.69 (1H, *d*, J = 14.5 Hz, 3-C$\underline{H}_2$OH), 6.93 (1H, *dd*, J = 8.2, 1.2 Hz, H-7), 6.93 (1H, *br*, *d*, J = 1.5 Hz, H-2), 7.39 (1H, *dd*, J = 7.6, 1.2 Hz, H-5), 7.48 (1H, *br*, *d*, J = 1.5 Hz, H-4), 7.58 (1H, *dd*, J = 8.2, 7.6 Hz, H-6); ^{13}C NMR (CD_3OD): δ 63.4 (C-6′), 64.7 (C-11), 71.8 (C-4′), 73.0 (C-2′), 76.8 (C-10), 79.6 (C-3′), 81.8 (C-5′), 85.3 (C-1′), 115.4 (C-2), 116.6 (C-1a), 117.0 (C-4), 117.3 (C-8a), 118.0 (C-7), 118.1 (C-5), 137.2 (C-6), 147.0 (C-4a), 148.9 (C-5a), 151.8 (C-3), 162.7 (C-8), 163.3 (C-1), 194.6 (C-9).

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