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FIVE CHROMONES FROM *ALOE VERA* LEAVES

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Key Word Index—*Aloe vera*; Liliaceae; leaves; 8-*C*-glucosyl-noreugenin; 4′-*O*-glucosylisoaloeresin DI; 4′-*O*-glucosyl-isoaloeresin DII; 8-*C*-glucosyl-(2′-*O*-cinnamoyl)-7-*O*-methylaloediol A; 8-*C*-glucosyl-(2′-*O*-cinnamoyl)-7-*O*-methylaloediol B.

Abstract—Five new chromone components were isolated from the gel of *Aloe vera* leaves. Their structures have been established as 8-*C*-glucosyl-noreugenin, 4′-*O*-glucosyl-isoaloeresin DI, 4′–*O*-glucosyl-isoaloeresin DII, 8-*C*-glucosyl-(2′-*O*-cinnamoyl)-7-*O*-methylaloediol A and 8-*C*-glucosyl-(2′-*O*-cinnamoyl)-7-*O*-methylaloediol B, from spectroscopic studies.

INTRODUCTION

The phenolic constituents of *Aloe* leaf can be generally classified into two main groups, namely chromones, such as aloesin, and anthraquinones, such as aloins A and B. We have screened chromone constituents showing anti-tyrosinase activity [1] from *Aloe vera*. In previous papers, the isolation of 8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol, isoaloeresin D (**1**), aloeresin E, 8-*C*-glucosyl-(*S*)-aloesol (**2**), 8-*C*-glucosyl-7-*O*-methylaloediol (**3**) and isorabaichromone from *A. vera* was reported [1, 2]. 8-*C*-Glucosyl-7-*O*-methyl-(*S*)-aloesol, **1**, aloeresin E, **2**, and isorabaichromone, which had the (*S*)-configuration at C-10, were specifically recognized only in *A. vera*, while the corresponding diastereomers which had the (*R*)-configuration were not found [3]. These compounds may be usable as chemotaxonomic markers. On investigation of the leaves of *A. vera* by HPLC using a photodiode-array detector, we found five unidentified peaks, corresponding to chromones. Such detection gave us considerable structural information by comparison of R_ts and UV spectra with those of aloesin-related standards. We now report on the isolation and structural elucidation of five new chromones, which we have named 8-*C*-glucosyl-noreugenin (**4**), 4′-*O*-glucosyl-isoaloeresin DI (**5**), 4′-*O*-glucosyl-isoaloeresin DII (**6**), 8-*C*-glucosyl-(2′-*O*-cinnamoyl)-7-*O*-methylaloediol A (**7**) and 8-*C*-glucosyl-(2′-*O*-cinnamoyl)-7-*O*-methylaloediol B (**8**).

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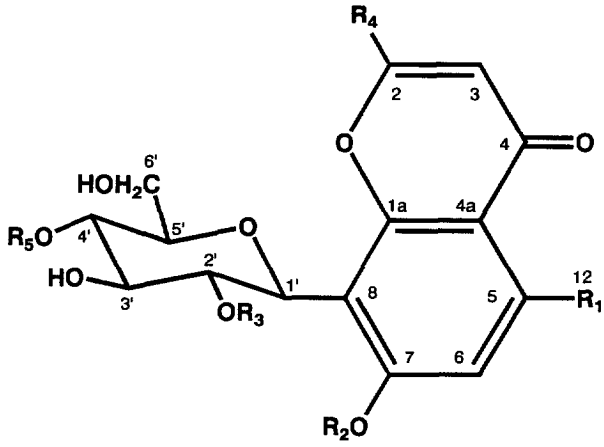
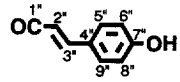
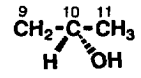
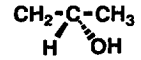
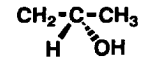
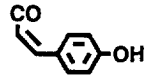
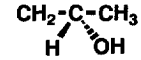
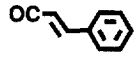
RESULTS AND DISCUSSION

Powdered ethanol extracts of *A. vera* gel [1, 2], which had been adsorbed on activated charcoal, were dialysed and chromatographed over an MCI-gel CHP 20P column using stepwise gradient elution with water–methanol mixtures. The isolation of compounds **4**–**8** was achieved by repeated MCI-gel CHP 20P CC and Sephadex LH-20 CC and by subsequent purification over Wakosil-II 5C18 HG Prep. On HPLC analysis using photodiode-array detection, peaks **4**–**8** showed similar UV-vis spectra to those of **1**–**3**.

Compound **4** obtained as pinkish yellow amorphous solid, showed UV absorption maxima at 209, 251, 257 and 296 nm. The HR-positive FAB-mass spectrum showed a $[M+H]^+$ ion at m/z 355.1032, suggesting the molecular formula $C_{16}H_{19}O_9$. The ^1H and ^{13}C NMR spectral data were closely related to those of **2**, except that a hydroxypropyl side-chain in the 2-position of the chromone nucleus was replaced by methyl group (δ_H 2.40; δ_C 20.3) (Tables 1 and 2). The *peri*-hydroxyl proton signal (δ 13.0) and the marked high-field shift (C-4a; δ 13.4, C-6: δ 11.8) of the carbon signals at C-4a and C-6, compared with those of **2**, indicated that the hydroxyl group is attached at C-5. The position of the glucose moiety in **4** was determined by comparison with ^{13}C NMR spectra of noreugenin (2-methyl-5,7-hydroxychromone) [4]. A down-field shift of the C-8 was observed by 11.2 ppm. On the basis of the above results, **4** was determined to 8-*C*-glucosyl-noreugenin.

Compound **5** obtained as yellowish amorphous solid, showed UV absorption maxima at 212, 226, 243, 252 and 295 nm. The HR-positive FAB-mass



	R_1	R_2	R_3	R_4	R_5
1	CH_3	CH_3			H
2	CH_3	H	H		H
3	CH_3	CH_3	H	$CH(OH)CH(OH)CH_3$	H
4	OH	H	H	CH_3	H
5	CH_3	CH_3			Glc
6	CH_3	CH_3			Glc
7	CH_3	CH_3		$CH(OH)CH(OH)CH_3$	H
8	CH_3	CH_3		$CH(OH)CH(OH)CH_3$	H

spectrum showed a $[M+Na]^+$ ion at m/z 741.2361, suggesting the molecular formula $C_{35}H_{42}O_{16}Na$. The ^1H and ^{13}C NMR spectral data were closely related to those of **1**, except for additional signals due to the presence of a β-hexose moiety (Tables 1 and 2). Definitive proof of the principal structure was obtained from the results of enzymatic hydrolysis of **5** with β-glucosidase, giving **1** and glucose. The position of the glucose moiety in **5** was determined to be C-4′ from a glycosylation shift by 5.9 ppm by comparison with ^{13}C NMR spectra of **1**. Therefore, the structure of **5** was established as 4′-*O*-glucosyl-isoaloeresin DI.

Compound **6** obtained as yellowish amorphous solid, showed UV absorption maxima at 212, 226, 243, 252 and 293 nm. The HR-positive FAB-mass spectrum showed a $[M+Na]^+$ ion at m/z 741.2369, suggesting the molecular formula $C_{35}H_{42}O_{16}Na$. The ^1H and ^{13}C NMR spectral data were similar to those of **5**, but there were the major differences between the ^1H NMR spectra of the two isomers regarding the chemical shifts and coupling constants of the protons at C-2″ and C-3″ of the *p*-coumaroyl group (Tables 1 and 2). The R_fs of **6** and **5** were 13.94 and 11.93 min, respectively, but the product arising from pancreatic lipase [5] hydrolysis of **6** had the same R_f as that of **5**. These data established the structure of **6**, namely 4′-*O*-glucosyl-isoaloeresin DII, in which the *trans-p*-coumaroyl group in **5** is replaced by a *cis-p*-coumaroyl group.

Compound **7** obtained as white amorphous solid, showed UV absorption maxima at 217, 222, 245, 252 and 282 nm. The HR-positive FAB-mass spectrum showed a $[M+Na]^+$ ion at m/z 579.1841, suggesting the molecular formula $C_{29}H_{32}O_{11}Na$. The ^1H and ^{13}C NMR spectral data were closely related to those of **3**,

Table 1. ^{1}H NMR spectral data for 8-*C*-glucosyl-(*S*)-aloesol (**2**), 8-*C*-glucosylnoreugenin (**4**), isoaloeresin D (**1**), 4′-*O*-glucosyl-isoaloeresin DI (**5**), 4′-*O*-glucosyl-isoaloeresin DII (**6**), 8-*C*-glucosyl-7-*O*-methylaloediol (**3**), 8-*C*-glucosyl-(2′-*O*-cinnamoyl)-7-*O*-methylaloediol A (**7**) and 8-*C*-glucosyl-(2′-*O*-cinnamoyl)-7-*O*-methylaloediol B (**8**) (δ in CD_3OD)

H	2	4	1	5	6	3	7	8
3	6.06	6.07	6.14	6.13	6.17	6.32	6.35	6.39
6	6.66	6.23	6.79	6.81	6.82	6.94	6.81	6.82
9	2.73†	2.40	2.81†	2.78 *dd* (14.2, 5.3) 2.86 *dd* (14.2, 7.7)	2.76 *dd* (14.6, 5.4) 2.85 *dd* (14.6, 7.9)	4.29 *d* (4.6)	4.36 *br*, *d* (4.2)	4.46 *br*, *d* (3.4)
10	4.26 *m*		4.38 *m*	4.37 *m*	4.35 *m*	4.24 *m*	4.31 *m*	4.43 *m*
11	1.27 *d* (6.1)		1.31 *d* (5.9)	1.31 *d* (6.4)	1.29 *d* (6.1)	1.26 *d* (6.4)	1.33 *d* (6.1)	1.35 *d* (6.4)
12	2.69		2.73	2.73	2.75	2.81	2.75	2.75
2″			6.05 *d* (15.6)	6.15 *d* (16.1)	5.65 *d* (12.5)		6.28 *d* (15.9)	6.26 *d* (15.9)
3″			7.37 *d* (15.6)	7.41 *d* (16.1)	6.73 *d* (12.5)		7.46 *d* (15.9)	7.45 *d* (15.9)
5″, 9″			7.34 *d* (8.5)	7.46 *d* (8.7)	6.97 *d* (8.7)		7.30–7.52	7.30–7.50
6″, 8″			6.75 *d* (8.5)	7.08 *d* (8.7)	6.84 *d* (8.7)		7.30–7.52	7.30–7.50
7″							7.30–7.52	7.30–7.50
1′	5.03 *d* (9.8)	3.87 *d* (11.6)	5.18 *d* (10.3)	5.19 *d* (10.1)	5.13 *d* (10.4)	4.99 *d* (9.7)	5.20 *d* (10.1)	5.20 *d* (10.1)
2′	§	§	5.73 *dd* (10.3, 9.8)	5.73 *dd* (10.1, 9.6)	5.71 *dd* (10.4, 9.8)	§	5.70 *dd* (10.1, 9.5)	5.70 *dd* (10.1, 9.6)
H-1‴				4.94 *d* (7.0)	4.92 *d* (8.5)			
OMe			3.88	3.89	3.89	3.99	3.88	3.90

† Coupling not determined due to signal overlapping.
§ Glucosyl protons appear between δ 3.45–4.00.

except for the presence of a cinnamic acid ester group in **7** (Tables 1 and 2). Definitive proof of the structure was obtained from the result of pancreatic lipase hydrolysis of **7**, which afforded **3** and cinnamic acid, identified by HPLC. The position of the cinnamoyl group in **7** was determined to be at C-2′ by comparison with the corresponding carbon of **3** in the ^{13}C NMR spectrum. A high-field shift of 2.2 and 2.6 ppm for C-1′ and C-3′, respectively, and a down-field shift of 1.6 ppm for C-2′ were observed. Accordingly, these data established the structure of **7**, namely 8-*C*-glucosyl-(2′-*O*-cinnamoyl)-7-*O*-methylaloediol A, in which the C-2′ proton in **3** is replaced by a cinnamoyl group.

Compound **8** obtained as white amorphous solid, showed UV absorption maxima at 217, 222, 244, 252 and 281 nm. The HR-positive FAB-mass spectrum showed a $[M+Na]^+$ ion at *m/z* 579.1843, suggesting the molecular formula $C_{29}H_{32}O_{11}Na$. The ^{1}H and ^{13}C NMR spectral data were quite similar to those of **7**, but there were some differences in the chemical shifts of C-9 ($\Delta\delta_H+0.10$, $\Delta\delta_C-0.5$) and C-10 ($\Delta\delta_H+0.12$, $\Delta\delta_C-0.6$). The R_fs of **8** and **7** were 29.51 and 28.67 min, respectively, and the product arising from pancreatic lipase hydrolysis of **8** had a R_f different from that of **3**. NOE experiments to determine the absolute configuration in the stabilized conformers of **7** and **8** were carried out, but no interaction between a proton at C-3 and those at C-9 and C-10 was observed. On the basis of the results obtained, the structure of **8**, namely 8-*C*-glucosyl-(2′-*O*-cinnamoyl)-7-*O*-methylaloediol B, was determined as a configurational isomer of **7** at the C-9 and C-10 hydroxyl groups.

EXPERIMENTAL

General

Optical rotations and UV-vis: MeOH. ^{1}H and ^{13}C NMR: TMS as int. standard. Positive FAB-MS: glycerol as matrix. HR FAB-MS: polyethylene glycol as matrix. TLC of sugars: Funacel SF plates (Funakoshi) with the upper layer of *n*-BuOH–pyridine–H_2O (6 : 2 : 3) + pyridine (1) as solvent and aniline hydrogen phthalate as spray reagent. CC: MCI-gel CHP 20P (75–150 μm, Mitsubishi Chemical Industries), Sephadex LH-20 (25–100 μm, Pharmacia), Cosmosil 40C_{18}-PREP (Nacalai Tesque Inc.) and Wakosil-II 5C18 HG Prep (150 × 20 mm I.D., Wako Pure Chemical Industrials). HPLC: detection at 290 nm and a photodiode-array detector.

Plant material

Aloe vera L. (*A. barbadensis* Miller) was collected in the field at 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 activated charcoal, was also provided by Aloecorp.

HPLC analysis

The column used was a Wakosil-II 5C18 HG reverse-phase column (5 μm, 150 × 4.6 mm I.D., Wako

Table 2. ^{13}C NMR chemical shifts of 8-*C*-glucosyl-(*S*)-aloesol (**2**), 8-*C*-glucosylnoreugenin (**4**), isoaloeresin D (**1**), 4′-*O*-glucosyl-isoaloeresin DI (**5**), 4′-*O*-glucosyl-isoaloeresin DII (**6**), 8-*C*-glucosyl-7-*O*-methylaloediol (**3**), 8-*C*-glucosyl-(2′-*O*-cinnamoyl)-7-*O*-methylaloediol A (**7**) and 8-*C*-glucosyl-(2′-*O*-cinnamoyl)-7-*O*-methylaloediol B (**8**) (δ in CD_3OD)

C	2	4	1	5	6	3	7	8
2	167.1	169.3	167.2	167.6	166.8	169.2	169.6	169.7
3	112.2	108.7	111.9	112.2	112.2	110.8	110.7	110.5
4	182.3	184.3	182.0	182.3	182.3	182.4	182.3	182.4
4a	118.5	105.1	116.9	117.2	117.3	117.6	117.6	117.5
5	143.1	158.4	144.3	144.6	144.8	144.1	144.7	144.8
6	112.2	100.4	112.4	112.6	112.7	113.1	112.7	112.7
7	162.3	162.8	161.6	161.0	162.2	162.7	161.9	162.1
8	116.1	104.9	111.3	111.9	111.9	113.7	112.2	111.8
1a	160.0	165.4	159.1	159.6	159.8	159.1	159.4	159.4
9	44.3	20.3	44.3	44.6	44.6	76.2	76.2	75.7
10	66.7		66.5	66.8	66.7	69.5	69.8	69.2
11	23.6		23.6	23.7	24.0	19.7	19.8	19.7
12	23.3		23.3	23.6	23.5	23.7	23.7	23.7
7-OMe			57.0	57.1	57.1	56.9	57.1	57.1
1′	76.0	75.4	71.8	72.6	72.6	74.9	72.7	72.8
2′	73.2	72.9	73.7	74.1	73.6	72.9	74.5	74.1
3′	80.1	80.1	77.4	77.8	77.6	80.3	77.7	77.8
4′	71.8	71.9	72.4	78.3	78.1	72.2	72.1	72.3
5′	82.7	82.5	82.4	82.9	82.9	82.6	82.8	83.0
6′	62.8	63.0	62.7	63.0	63.0	63.3	63.1	63.1
1″			167.8	167.8	167.7		167.5	167.4
2″			114.2	116.5	117.6		118.4	118.4
3″			146.4	146.0	144.7		146.5	146.4
4″			126.7	129.8	129.7		135.6	135.7
5″, 9″			130.9	130.8	132.6		129.2	129.2
6″, 8″			116.6	118.0	116.7		130.1	130.1
7″			160.7	163.8	159.7		131.6	131.6
1‴				101.9	101.7			
2‴				74.8	74.9			
3‴				72.1	72.6			
4‴				71.3	71.2			
5‴				78.0	77.9			
6‴				62.5	62.4			

* Overlapped with solvent.

Pure Chemical Industrials). The sepn was carried out at 45° using an isocratic and a linear gradient programme at a flow-rate of 1 ml min^{-1}. HPLC-1 : eluent 5% MeCN–H_2O. HPLC-2 [3]: eluent MeCN–H_2O, 0–19 min, 12–23% ; 19–24 min, 23–28% ; 24–36 min, 28–46%. HPLC-3: eluent MeCN–0.1% aq. phosphoric acid, 0–9 min, 15% ; 9–14 min, 15–35% ; 14–20 min, 35%.

Isolation

Dried and powdered EtOH extract (530 g) was dissolved in H_2O and dialyzed overnight against H_2O. The dialysate was subjected to MCI-gel CHP 20P CC using a stepwise gradient elution with H_2O–MeOH. The 10% MeOH eluate was rechromatographed over a MCI-gel CHP 20P column eluting with 30% MeOH and a Sephadex LH-20 column eluting with H_2O and 80% Me_2CO followed by Wakosil-II 5C18 HG Prep CC with 5% MeCN to give **4** (44 mg). The 30% MeOH eluate was rechromatographed over a MCI-gel CHP 20P column eluting with 40% MeOH and a Sephadex LH-20 column eluting with Me_2OH and H_2O followed by Wakosil-II 5C18 HG Prep CC with 15% MeCN to give **5** (44 mg) and **6** (36 mg). The MeOH eluate was chromatographed over a Sephadex LH-20 column eluting with 50% MeOH followed by Cosmosil 40C_{18}-PREP CC with 30% MeOH to afford two frs. Fr. 1 was subjected to repeated chromatography over a Sephadex LH-20 column eluting with Me_2CO to give **7** (231 mg). Fr. 2 was chromatographed over a Sephadex LH-20 column eluting with Me_2CO followed by Wakosil-II 5C18 HG Prep CC with 25% MeCN to give **8** (17 mg).

8-C-Glucosyl-noreugenin (4). Pinkish yellow amorphous solid. $[\alpha]_D$ +22.7° (MeOH ; *c* 0.485). UV λ_{max} nm (log ε): 209 (4.33), 251 (4.24), 257 (4.26), 296 (3.76). HR-positive FAB-MS *m/z*: found 355.1032 $[M+H]^+$ ($C_{16}H_{19}O_9$ requires 355.1035). ^1H and ^{13}C NMR : Tables 1 and 2. R_f (HPLC-1): 16.82 min.

4′-O-Glucosyl-isoaloeresin DI (5). Yellowish amorphous solid. $[\alpha]_D$ −136.4° (MeOH; *c* 0.263). UV λ_{max} nm (log ε): 212 (4.53), 226 (4.58), 243 (4.36), 252 (4.36), 295 (4.58). HR-positive FAB-MS *m/z*: found 741.2361 $[M+Na]^+$ ($C_{35}H_{42}O_{16}Na$ requires 741.2370). ^1H and ^{13}C NMR: Tables 1 and 2. R_t (HPLC-2): 11.93 min.

4′-O-Glucosyl-isoaloeresin DII (6). Yellowish amorphous solid. $[\alpha]_D$ +6.88° (MeOH; *c* 0.25). UV λ_{max} nm (log ε): 212 (4.51), 226 (4.53), 243 (4.38), 252 (4.36), 293 (4.41). HR-positive FAB-MS *m/z*: found 741.2369 $[M+Na]^+$ ($C_{35}H_{42}O_{16}Na$ requires 741.2370). ^1H and ^{13}C NMR: Tables 1 and 2. R_t (HPLC-2): 13.94 min.

8-C-Glucosyl-(2′-O-cinnamoyl)-7-O-methylaloediol A (7). White amorphous solid. $[\alpha]_D$ −91.8° (MeOH; *c* 0.29). UV λ_{max} nm (log ε): 217 (4.46), 222 (4.43), 245 (4.27), 252 (4.32), 282 (4.41). HR-positive FAB-MS *m/z*: found 579.1841 $[M+Na]^+$ ($C_{29}H_{32}O_{11}Na$ requires 579.1840). ^1H and ^{13}C NMR: Tables 1 and 2. R_t (HPLC-2): 28.67 min.

8-C-Glucosyl-(2′-O-cinnamoyl)-7-O-methylaloediol B (8). White amorphous solid $[\alpha]_D$ −164.6° (MeOH; *c* 0.225). UV λ_{max} nm (log ε): 217 (4.48), 222 (4.45), 244 (4.31), 252 (4.35), 281 (4.45). HR-positive FAB-MS *m/z*: found 579.1843 $[M+Na]^+$ ($C_{29}H_{32}O_{11}Na$ requires 579.1840). ^1H and ^{13}C NMR: Tables 1 and 2. R_t (HPLC-2): 29.51 min.

Pancreatic lipase hydrolysis

A sample (1 mg) dissolved in 0.01 M Pi buffer (2 ml, pH 8) was incubated with pancreatic lipase (1 mg, Biocatalyst Ltd., U.K.) at 37° for 7 days. The hydrolysate was filtered through a membrane filter and analysed by HPLC. Identification of chromatographic peaks was achieved using a photodiode-array detector. R_t of **3**: 6.93 min (HPLC-2); R_ts of *p*-coumaric acid and cinnamic acid: 6.15 and 17.43 min, respectively (HPLC-3).

β-glucosidase hydrolysis

A sample (1 mg) dissolved in H_2O was incubated with β-glucosidase (1 mg, Nacalai Tesque Inc.) at 37° for 2 days and the hydrolysate extracted with EtOAc. The EtOAc layer was filtered through a membrane filter and analysed by HPLC. Identification of chromatographic peaks was achieved using a photodiode-array detector. R_t of **1**: 19.27 min (HPLC-2). The aq. layer was evapd to dryness and the residue was shown by Funacel SF TLC to be glucose.

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