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THREE CHROMONES OF *ALOE VERA* LEAVES

NOBUYUKI OKAMURA,* NORIKO HINE, YUKIKO TATEYAMA, MOTOKO NAKAZAWA, TOSHIHIRO FUJIOKA,† KUNIHIDE MIHASHI† and AKIRA YAGI

Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Hiroshima 729-02, Japan; † Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka 814-01, Japan

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Key Word Index—*Aloe vera* (*Aloe barbadensis* Miller); Liliaceae; 8-*C*-glucosyl-(*S*)-aloesol; 8-*C*-glucosyl-7-*O*-methylaloediol; isorabaichromone.

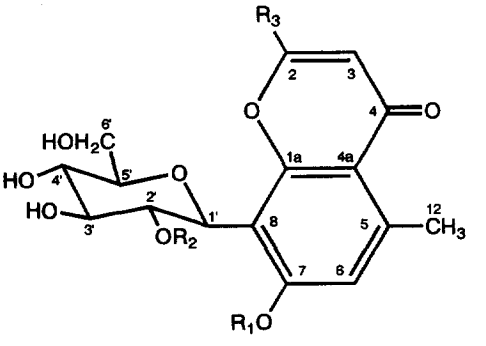
Abstract—Three new chromone components were isolated from the gel of Aloe leaf. The structures of these compounds, which we have named 8-*C*-glucosyl-(*S*)-aloesol, 8-*C*-glucosyl-7-*O*-methylaloediol and isorabaichromone, were shown to be 8-*C*-β-D-glucopyranosyl-2-[(*S*)-2-hydroxy]propyl-7-hydroxy-5-methylchromone (8-*C*-glucosyl-(*S*)-aloesol), 8-*C*-β-D-glucopyranosyl-2-(1′,2′-dihydroxylpropyl)-7-methoxy-5-methylchromone (8-*C*-glucosyl-7-*O*-methylaloediol) and 8-*C*-β-D-[2′-*O*-(*E*)-caffeoyl]glucopyranosyl-2-[(*S*)-2-hydroxy]propyl-7-methoxy-5-methylchromone, respectively (isorabaichromone), by spectroscopic methods.

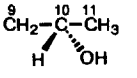
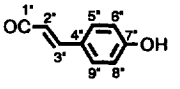
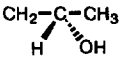
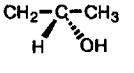
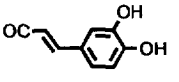
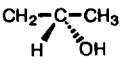
INTRODUCTION

In a previous paper, the isolation of 8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol (**1**), isoaloeresin D (**2**) and aloeresin E from *Aloe vera* was reported [1]. In a continuation of these studies, we have looked for chromone constituents showing anti-tyrosinase activity [1] from *Aloe vera* by HPLC using a photodiode-array detector. Knowledge about the spectra of unknown chromatographic peaks gave a preliminary idea about the chemical structure of the corresponding compounds. We now report on the isolation and characterization of three new chromone components of *A. vera*, which we have named 8-*C*-glucosyl-(*S*)-aloesol (**3**), 8-*C*-glucosyl-7-*O*-methylaloediol (**4**) and isorabaichromone (**5**).

RESULTS AND DISCUSSION

A. vera leaves were cut into segments prior to grinding in a blender. The resulting slurry was filtered and the filtrate treated with activated charcoal. After isolation by vacuum filtration, the charcoal-containing residue was washed with deionized water and then extracted with ethanol. The ethanol extracts were dialysed and chromatographed over MCI-gel CHP 20P using a stepwise gradient elution with water–methanol as solvent. Compounds **3** and **4** were isolated from the 10% methanol eluate by column chromatography, initially over Sephadex LH-20 with water and for subsequent purification over Cosmosil 40C_{18}-PREP. Repeated MCI-gel CHP 20P and Sephadex LH-20 chromatography of the 40% methanol eluate led to



	R_1	R_2	R_3
1	CH_3	H	
2	CH_3		
3	H	H	
4	CH_3	H	$CH(OH)CH(OH)CH_3$
5	CH_3		

* Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR spectral data for 8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol (**1**), isoaloeresin D (**2**), 8-*C*-glucosyl-(*S*)-aloesol (**3**), 8-*C*-glucosyl-7-*O*-methylaloediol (**4**), and isorabaichromone (**5**) (δ in CD_3OD)

H	1	3	4	2	5
3	6.09	6.06	6.32	6.14	6.13
6	6.91	6.66	6.94	6.79	6.81
9	2.73*	2.73*	4.29 *d* (4.6)	2.81*	2.82*
10	4.26 *m*	4.26 *m*	4.24 *m*	4.38 *m*	4.37 *m*
11	1.26 *d* (5.9)	1.27 *d* (6.1)	1.26 *d* (6.4)	1.31 *d* (5.9)	1.31 *d* (6.4)
12	2.78	2.69	2.81	2.73	2.74
2″				6.05 *d* (15.6)	5.98 *d* (15.9)
3″				7.37 *d* (15.6)	7.31 *d* (15.9)
5″				7.34 *d* (8.5)	6.93 *d* (2.1)
6″				6.75 *d* (8.5)	
8″				6.75 *d* (8.5)	6.73 *d* (8.2)
9″				7.34 *d* (8.5)	6.84 *dd* (8.2, 2.1)
1′	4.99 *d* (9.8)	5.03 *d* (9.8)	4.99 *d* (9.7)	5.18 *d* (10.3)	5.18 *d* (10.1)
2′	†	†	†	5.73 *dd* (10.3, 9.8)	5.71 *dd* (10.1, 9.2)
OMe	3.93		3.99	3.88	3.89

* Coupling cannot be determined due to signal overlapping.
† Glucosyl protons appear at a range of 3.45–4.00 ppm.

the isolation of **4**. On HPLC analysis using photodiode-array detection, peaks **3**–**5** showed similar UV-VIS spectra to those of **1** and **2**.

Compound **3** (8-*C*-glucosyl-(*S*)-aloesol) showed UV absorption maxima at 215, 224, 244, 252 and 293 nm. The HR-positive FAB-mass spectrum showed the $[M+H]^+$ ion at *m/z* 397.1499, suggesting the molecular formula $C_{19}H_{25}O_9$. The ^{1}H and ^{13}C NMR spectral data were closely related to those of **1**, except for the absence of a methoxy group attached to the aromatic ring at C-7 (Tables 1 and 2). Additional support for the spectroscopic determination of structure **3** was obtained from methylation of **3** with diazomethane, which afforded **1**, identified by co-HPLC. As **1** and 8-*C*-glucosyl-7-*O*-methyl-(*R*)-aloesol exhibited different R_fs [1], we assigned the (*S*)-configuration to C-10 of **3**.

Compound **4** (8-*C*-glucosyl-7-*O*-methylaloediol) showed UV absorption maxima at 214, 227, 244, 252 and 294 nm. The HR-positive FAB-mass spectrum showed the $[M+H]^+$ ion at *m/z* 427.1608, suggesting the molecular formula $C_{20}H_{27}O_{10}$. The ^{1}H NMR spectral data of **4** were similar to those of **1** in terms of chemical shift and proton signal patterns, but there were some differences in the chemical shift ($\Delta\delta + 1.56$) and coupling (doublet) of C-9 (Table 1). In the assignment of the ^{13}C NMR signals by DEPT, a methine group at δ 76.2 was assigned to C-9 implying that a hydroxy group was attached to C-9 (Table 2), and the H-9/H-10 coupling constant (J = 4.6 Hz) confirmed the configuration. These data established the structure of **4**, in which a C-9 proton in **1** had been replaced by a hydroxy group.

Compound **5** (isorabaichromone) showed UV absorption maxima at 220, 244, 251, 296 and 330

Table 2. ^{13}C NMR chemical shifts of 8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol (**1**), isoaloeresin D (**2**), 8-*C*-glucosyl-(*S*)-aloesol (**3**), 8-*C*-glucosyl-7-*O*-methylaloediol (**4**), and isorabaichromone (**5**) (δ in CD_3OD)

	1	3	4	2	5
2	167.0	167.1	169.2	167.2	167.6
3	111.9	112.2	110.8	111.9	112.2
4	181.9	182.3	182.4	182.0	182.3
4a	117.0	118.5	117.6	116.9	117.3
5	143.7	143.1	144.1	144.3	144.6
6	112.6	112.2	113.1	112.4	112.6
7	162.1	162.3	162.7	161.6	162.0
8	113.1	116.1	113.7	111.3	111.9
1a	158.9	160.0	159.1	159.1	159.6
9	44.2	44.3	76.2	44.3	44.6
10	66.3	66.7	69.5	66.5	66.8
11	23.6	23.6	19.7	23.6	23.6
12	23.6	23.3	23.7	23.3	23.6
7-OMe	56.7		56.9	57.0	57.1
1′	74.6	76.0	74.9	71.8	72.1
2′	72.7	73.2	72.9	73.7	74.0
3′	80.0	80.1	80.3	77.4	77.9
4′	71.9	71.8	72.2	72.4	72.7
5′	82.4	82.7	82.6	82.4	82.9
6′	63.0	62.8	63.3	62.7	63.1
1″				167.8	168.2
2″				114.2	114.5
3″				146.4	147.1
4″				126.7	127.5
5″				130.9	115.1
6″				116.6	149.9*
7″				160.7	146.9*
8″				116.6	116.6
9″				130.9	123.0

* Assignments may be reversed.

nm. The HR-positive FAB-mass spectrum showed the $[M+H]^+$ ion at m/z 573.1967, suggesting the molecular formula $C_{29}H_{33}O_{12}$. The ^1H NMR spectral data of **5** were similar to those of **2**, except that an AA′BB′ pattern for the aromatic protons due to a *p*-coumaric acid ester was replaced by an ABD pattern for three protons (Table 1). The positive FAB-mass spectrum exhibited a fragment ion peak at m/z 163 $[C_9H_7O_3]^+$. Definitive proof of the structure was obtained from the result of pancreatin hydrolysis of **5**, which afforded **1** and caffeic acid, identified by HPLC. These results indicated that the *p*-coumaric acid ester of **2** had been replaced by a caffeic acid ester (**5**). The position of the caffeoyl group in **5** was determined to be at O-2′ by comparison with the corresponding carbons of **1** in the ^{13}C NMR spectrum (Table 2). An upfield shift of 2.5 and 2.1 ppm for the C-1′ and the C-3′, respectively and a downfield shift of 1.3 ppm for the C-2′ were observed [2]. From the ^1H and ^{13}C NMR spectra, **5** may be regarded as rabaichromone which has the (*R*)-configuration of C-10 [3]. However, the hydrolysate of **5** showed the same R_t as that of **1** having the (*S*)-configuration at C-10 on HPLC. Therefore, the configuration of **5** at C-10 was assigned to the (*S*)-configuration.

EXPERIMENTAL

General. Optical rotations and UV-VIS: MeOH; ^1H 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), Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemicals) and Cosmosil $40C_{18}$-PREP (Nacalai Tesque Inc.); 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 had been 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 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 [4]: eluent MeCN–H_2O, 0–19 min, 12–23%; 19–24 min, 23–28%; 24–39 min, 28–46%.

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 a stepwise gradient elution with H_2O–MeOH as solvent. The 10% MeOH eluate was chromatographed over a Sephadex LH-20 column eluting with H_2O followed by Cosmosil $40C_{18}$-PREP CC with 10% MeOH to give **3** (27 mg) and **4** (38 mg). The 40% MeOH eluate was rechromatographed over a MCI-gel CHP 20P column eluting with 50% MeOH followed by Sephadex LH-20 CC eluting with 50% MeOH to give **5** (772 mg).

8-C-*Glucosyl*-(S)-*aloesol* (**3**). White amorphous solid, $[\alpha]_D^{28}$ +40.0° (MeOH; *c* 0.495). UV λ_{max}^{MeOH} nm (log ε): 215 (4.28), 224 (4.25), 244 (4.23), 252 (4.26) and 293 (3.99); HR-positive FAB-MS m/z: Found 397.1499 $[M+H]^+$ ($C_{19}H_{25}O_9$ requires 397.1498); ^1H and ^{13}C NMR: Tables 1 and 2. R_t (HPLC-1) was 23.98 min.

8-C-*Glucosyl*-7-O-*methylaloediol* (**4**). White amorphous solid, $[\alpha]_D^{27}$ +40.8° (MeOH; *c* 0.25). UV λ_{max}^{MeOH} nm (log ε): 214 (4.26), 227 (4.26), 244 (4.22) 252 (4.21) and 294 (4.01); HR-positive FAB-MS m/z: Found 427.1608 $[M+H]^+$ ($C_{20}H_{27}O_{10}$ requires 427.1603); ^1H and ^{13}C NMR: Tables 1 and 2. R_t (HPLC-1) was 26.53 min.

Isorabaichromone (**5**). Yellowish amorphous solid, $[\alpha]_D^{28}$ −180.7° (MeOH; *c* 0.25). UV λ_{max}^{MeOH} nm (log ε): 220 (4.59), 244 (4.51), 251 (4.48), 296 (4.45) and 330 (4.33); HR-positive FAB-MS m/z: Found 573.1967 $[M+H]^+$ ($C_{29}H_{33}O_{12}$ requires 573.1971); Positive FAB-MS m/z: 573 $[M+H]^+$, 163 $[C_9H_7O_3]^+$; ^1H and ^{13}C NMR: Tables 1 and 2. R_t (HPLC-1) was 17.06 min.

Enzymatic hydrolysis of (**5**). A sample (1 mg) of **5** dissolved in 0.01 M phosphate buffer (2 ml, pH 8.0) was incubated with pancreatin (1 mg, Biocatalyst Ltd, U.K.) at 37° for 7 days. The hydrolysate was filtered through a membrane filter and analysed by HPLC (HPLC-2). Identification of chromatographic peaks was helped using a photodiode-array detector. R_t of the hydrolysate (**1**): 6.93 min ($k' = 3.12$); R_t of 8-*C*-glucosyl-7-*O*-methyl-(*R*)-aloesol: 5.12 min ($k' = 2.05$).

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