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THREE CHROMONE COMPONENTS FROM *ALOE VERA* LEAVES

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Key Word Index—*Aloe vera*; Liliaceae; 8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol; isoaloeresin D; aloeresin E: inhibition of mushroom tyrosinase.

Abstract—Three new chromone components, 8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol, isoaloeresin D and aloeresin E were isolated from the leaves of *Aloe vera*. Their structures have been established from spectroscopic studies; the structures of 8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol, isoaloeresin D and aloeresin E were shown to be 8-*C*-β-D-glucopyranosyl-2-[(*S*)-2-hydroxy]propyl-7-methoxy-5-methylchromone, 8-*C*-β-D-[2′-*O*-(*E*)-*p*-coumaroyl]glucopyranosyl-2-[(*S*)-2-hydroxy]propyl-7-methoxy-5-methylchromone and 8-*C*-β-D-[2′-*O*-(*E*)-cinnamoyl]glucopyranosyl-2-[(*S*)-2-hydroxy]propyl-7-methoxy-5-methylchromone, respectively. The inhibitory action of these compounds against tyrosine oxidation by mushroom tyrosinase was examined.

INTRODUCTION

Aloe is the dried latex obtained from the exudate of cut leaves of *Aloe vera*, known in commerce as Curaçao Aloes, or of *A. ferox* and hybrids of this species with *A. africana* and *A. spicata*, known in commerce as Cape Aloes [1]. In Japan, *A. vera* has also recently attracted attention as a health food.

Using HPLC analysis [2], several unidentified peaks, together with aloesin (**1**) (formerly aloeresin B), barbaloin (aloin A) and isobarbaloin (aloin B) as major phenolic constituents, were found in the leaves of *A. vera*. We now wish to report the isolation and characterization of three new chromone components of *A. vera*, which we have named 8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol (**2**), isoaloeresin D (**3**) and aloeresin E (**4**), and the inhibition of tyrosinase by these compounds.

RESULTS AND DISCUSSION

Leaves of *A. vera* were extracted with MeOH at room temperature. HPLC analysis of the extracts revealed that there were three unidentified components (**2–4**). *Aloe vera* gel extracts which were absorbed in activated charcoal contained **2–4** when compared with the non-treated material. The adsorbates were eluted from the activated charcoal with EtOH. Repeated Sephadex LH-20 and MCI-gel CHP 20P chromatography of the EtOH eluate led to the isolation of **2–4**. On HPLC analysis using photodiode-array detection, peaks **2–4** showed similar UV–VIS spectra to those of **1** and aloesol. The R_ts of **2–4** were 6.93, 20.43 and 30.90 min, respectively.

Compound **2** (8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol), obtained as a yellowish amorphous substance, showed absorption maxima at 214, 226, 243, 252 and 293 nm in the UV–VIS spectrum. The HR-positive FAB-mass spectrum showed a $[M + H]^+$ at *m/z* 411.1651, suggesting the molecular formula $C_{20}H_{26}O_9$. The ^{1}H NMR spectral data were closely related to those of **1**, except for other proton signals due to a methoxyl (δ 3.93) attached to the aromatic ring at C-7 and a methine (δ 4.26) bonded to hydroxyl group (Table 1). In addition, comparison of ^{13}C NMR chemical shifts of **2** with those of **1** clearly indicated methylation of the phenolic hydroxyl at C-7 and reduction of the side-chain carbonyl group at C-10 to secondary alcohol (Table 2).

Compound **3** (isoaloeresin D) obtained as white amorphous substance, showed absorption maxima at 213, 228, 242, 252 and 300 nm in the UV–VIS spectrum. The positive and negative FAB-mass spectra exhibited fragment ion peaks at *m/z* 557 $[M + 1]^+$ and *m/z* 555 $[M - 1]^-$, respectively. The ^{1}H NMR spectral data of **3** were similar to those of **2**, except that a simple AA′BB′ system for the aromatic protons of a *p*-coumaric acid ester was present (Table 1). Definitive proof of the principal structure was obtained from the result of enzymatic hydrolysis of **3** with pancreatin in phosphate buffer, which afforded **2** and *p*-coumaric acid, identified by HPLC. The position of the *p*-coumaroyl group was determined by comparison with the ^{13}C NMR spectra of **2**. Acylation effects [3] (a downfield shift of 1.0 ppm for the C-2′, and an upfield shift by 2.8 and 2.6 ppm for C-1′ and C-3′, respective-

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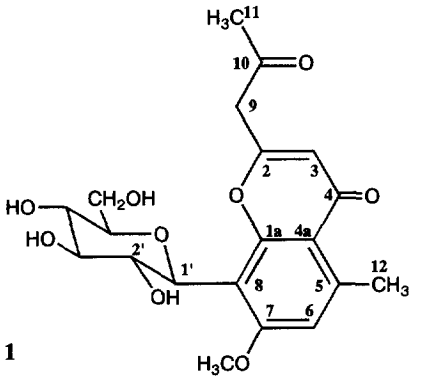

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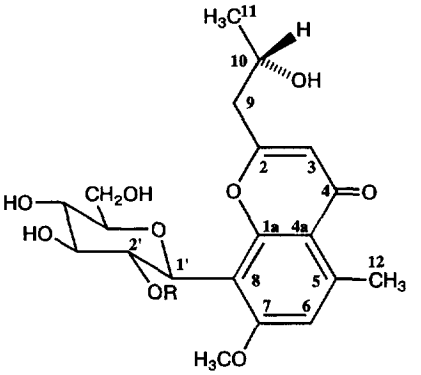

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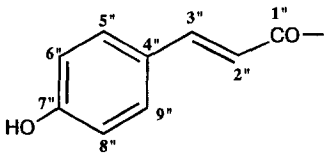
3 **R =**



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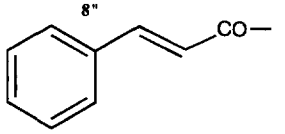

Table 2. ^{13}C NMR chemical shifts of aloesin (**1**), 8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol (**2**), isoaloeresin D (**3**) and aloeresin E (**4**) (δ in CD_3OD)

C	1	2	3	4
2	162.1	167.0	167.2	167.1
3	113.1	111.9	111.9	111.9
4	181.6	181.9	182.0	181.9
4a	115.9	117.0	116.9	116.9
5	142.9	143.7	144.3	144.3
6	117.9	112.6	112.4	112.3
7	161.3	162.1	161.6	161.6
8	110.9	113.1	111.3	111.6
1a	159.3	158.9	159.1	159.3
9	*	44.2	44.3	44.5
10	204.6	66.3	66.5	66.6
11	29.9	23.6	23.6	23.5
12	23.1	23.6	23.3	23.5
7-OMe		56.7	57.0	56.9
1′	75.3	74.6	71.8	71.9
2′	72.7	72.7	73.7	74.0
3′	79.7	80.0	77.4	77.6
4′	71.5	71.9	72.4	72.4
5′	82.2	82.4	82.4	82.7
6′	62.6	63.0	62.7	62.9
1″			167.8	167.2
2″			114.2	118.1
3″			146.4	146.1
4″			126.7	135.3
5″, 9″			130.9	128.9
6″, 8″			116.6	129.7
7″			160.7	131.3

*Overlapped with solvent.

ly) in the glucose moiety showed the presence of a *p*-coumaroyl group at O-2′ in **3** (Table 2).

Compound **4** (aloeresin E), obtained as yellowish amorphous substance, showed absorption maxima at 205, 217, 223, 244, 252 and 281 nm in the UV–VIS spectrum. The HR-positive FAB-mass spectrum showed a $[M+H]^+$ at *m/z* 541.2073, suggesting the molecular formula $C_{29}H_{32}O_{10}$. The ^1H and ^{13}C NMR signals were similar to those of **3**, except that the

Table 1. ^1H NMR spectral data for aloesin (**1**), 8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol (**2**), isoaloeresin D (**3**) and aloeresin E (**4**) (δ in CD_3OD)

H	1	2	3	4
H-3	6.14	6.09	6.14	6.12
H-6	6.70	6.91	6.79	6.81
H-9	3.83	2.73*	2.81*	2.81*
H-10		4.26 *m*	4.38 *m*	4.37 *m*
H-11	2.29	1.26 *d* (J = 5.9)	1.31 *d* (J = 5.9)	1.30 *d* (J = 5.9)
H-12	2.70	2.78	2.73	2.73
H-2″			6.05 *d* (J = 15.6)	6.25 *d* (J = 15.7)
H-3″			7.37 *d* (J = 15.6)	7.45 *d* (J = 15.7)
H-5″, H-9″			7.34 *d* (2, J = 8.5)	7.30–7.56
H-6″, H-8″			6.75 *d* (2, J = 8.5)	7.30–7.56
H-1′	4.97 *d* (J = 9.0)	4.99 *d* (J = 9.8)	5.18 *d* (J = 10.3)	5.19 *d* (J = 10.3)
H-2′	†	†	5.73 *dd* (J, J' = 9.8)	5.73 *dd* (J, J' = 9.8)
OMe		3.93	3.88	3.89

*Coupling cannot be determined due to signal overlapping.

†Glucosyl protons appear at a range of δ 3.45–4.00.

aromatic portions of the *p*-coumaric acid ester were replaced by those of a cinnamic acid ester (Tables 1 and 2). Definitive proof of the principal structure was obtained from the result of pancreatin hydrolysis of **4** which afforded **2** and cinnamic acid, identified by HPLC. These data indicated that the *p*-coumaric acid ester of **3** was replaced by a cinnamic acid ester (**4**). The position of the cinnamoyl group in **4** was determined to be at O-2′ by comparison with the corresponding carbon of **2** in the ^{13}C NMR spectrum (Table 2); an upfield shift by 2.7 and 2.4 ppm for the C-1′ and the C-3′, respectively, and a downfield shift of 1.3 ppm for the C-2′, were observed [3].

From its ^{1}H and ^{13}C NMR spectra, **3** may be regarded as aloeresin D. However, the specific rotation **2** was different from the product [4] arising from alkaline hydrolysis of aloeresin D. Aloeresin D which was isolated from *A. ferox* has the (*R*)-configuration at C-10. Therefore, we synthesized 8-*C*-glucosyl-7-*O*-methyl-aloesol from aloesin [4]. The synthesized 8-*C*-glucosyl-7-*O*-methylaloesol was found to be an approximately 1:1 mixture (a diastereomeric pair at C-10) on HPLC. The R_t of the more mobile component was identical to that of the hydrolysate of aloeresin D, while the R_t of **2** was in agreement with that of the less mobile component (estimated by co-HPLC). Compound **3** and aloeresin D exhibited different R_ts [2], and the product arising from pancreatin hydrolysis of aloeresin D had a different R_t from that of **2** described above on HPLC. For this reason we assigned the (*S*)-configuration at C-10 to **2–4**.

The inhibitory action of the compounds for mushroom tyrosinase is shown in Fig. 1. Compounds **3** and **4** inhibited the tyrosine oxidation by mushroom tyrosinase, whereas **2** did not show any activity. Because cinnamic acid and *p*-coumaric acid showed inhibitory action, it is thought that the existence of these esters in **3** and **4** contributes to the inhibitory activity.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR were recorded at 100 and 25 MHz, respectively; chemical shifts are given in δ values with TMS as int. standard. Positive and negative ion FAB-MS were recorded using glycerol as matrix. HR FAB-MS were recorded using polyethylene glycol as matrix.

Plant material. Aloe vera (*A. barbadensis* Miller) 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, was also provided by Aloecorp.

Tyrosinase assay. The assay was performed using a modified method of ref. [5]. Samples were dissolved in 1 ml of dist. H_2O or 10% DMSO and dild to 2 ml with 1/15 M Pi buffer (pH 6.8). To the test soln (2 ml), 2 ml of 1/15 M Pi buffer, 0.5 ml of tyrosine (0.1 mg ml^{-1}) and 0.5 ml of mushroom tyrosinase (48 units ml^{-1}, Sigma) were added, and the reaction mixt. incubated for 1 hr at 37°. The amount of dopachrome in the reaction mixt. was measured from A at 475 nm. The % inhibition of tyrosinase reaction was calcd as follows: % inhibition = $[(AB) - (C - D)]/(A - B) \times 100$, A: A at 475 nm without test sample after incubation, B: A at 475 nm without test sample before incubation, C: A at

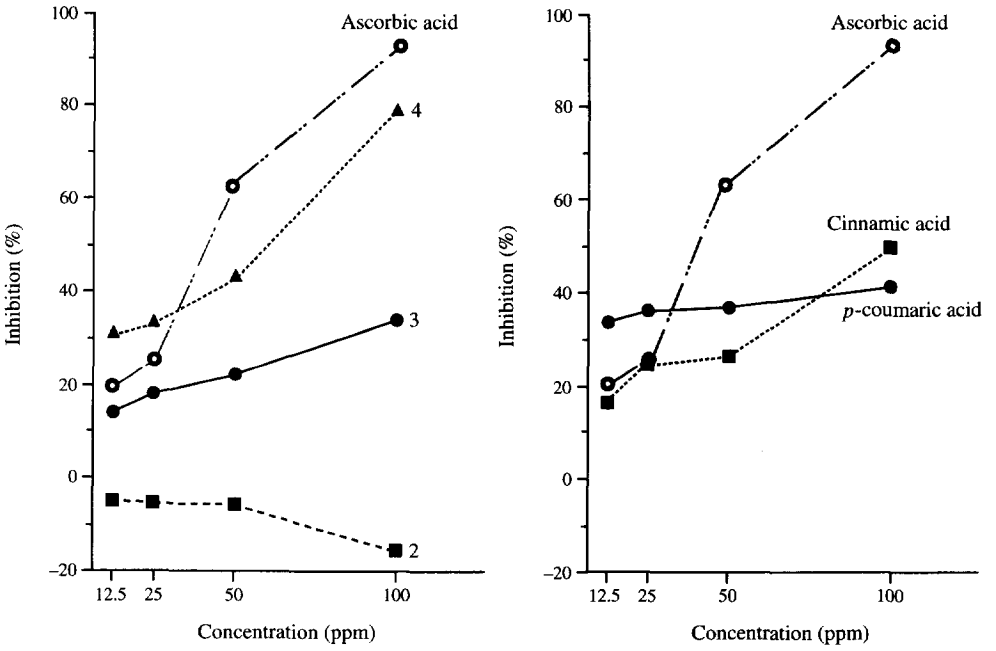

Fig. 1. Inhibitory action of compounds **2–4**, cinnamic, *p*-coumaric and ascorbic acids against tyrosine oxidation by mushroom tyrosinase.

475 nm with test sample after incubation, D: A at 475 nm with test sample before incubation.

HPLC analysis. HPLC was carried out on a Wako Wakosil-II 5C18 HG reverse-phase column (Osaka, Japan). The sepn was carried out using a linear gradient prog. (column: 5 μm, 150 × 4.6 mm I.D.; flow rate: 1 ml min^{-1}; detector: 290 nm; column temp.: 45°; eluent MeCN–H_2O, 0–19 min, 12–23%; 19–24 min, 23–28%; 24–39 min, 28–46%) [2].

Isolation. Dried and powdered EtOH extract (100 g) was dissolved in 50% MeOH and chromatographed over a Sephadex LH-20 column (25–100 μm) eluting with 50% MeOH to afford 2 frs, 1 (19.2 g) and 2 (12.5 g). Fr. 1 was subjected to MCI-gel CHP 20P CC (75–150 μm, Mitsubishi) using a stepwise gradient elution with H_2O–MeOH as solvent. The 15% MeOH eluate was rechromatographed over a MCI-gel CHP 20P column eluting with 10% MeOH to give **2** (74 mg). The 80% MeOH eluate was rechromatographed on a MCI-gel 20P column eluting with 60% MeOH to give **4** (51 mg). Fr. 2 was rechromatographed over a Sephadex LH-20 column with 50% MeOH and a MCI-gel CHP 20P column with 60% MeOH, and recrystallized from EtOAc–Me_2CO to yield **3** (151 mg).

8-C-*Glucosyl*-7-O-*methyl*-(S)-*aloesol* (**2**). Yellowish amorphous. $[\alpha]_D^{30}$ +9.1° (MeOH; *c* 1.00). UV λ_{max}^{MeOH} nm (log ε): 214 (4.16), 226 (4.17), 243 (4.07), 252 (4.05) and 293 (3.91). HR-positive FAB-MS *m/z*: found 411.1651 $[M+H]^+$ ($C_{20}H_{27}O_9$ requires 411.1654). ^1H and ^{13}C NMR: Tables 1 and 2.

Isoaloeresin D (**3**). Amorphous (EtOAc–Me_2CO), mp 156–160°. $[\alpha]_D^{27}$ −156.8° (MeOH; *c* 0.25). UV λ_{max}^{MeOH} nm (log ε): 213 (4.52), 228 (4.58), 242 sh, 252 (4.34), 300 (4.54). Positive FAB-MS spectra *m/z*: 557 $[M+1]^+$; negative FAB-MS *m/z*: 555 $[M-1]^-$. ^1H and ^{13}C NMR: Tables 1 and 2.

Aloeresin E (**4**). Yellowish amorphous. $[\alpha]_D^{26}$ −112.6° (MeOH; *c* 0.55). UV λ_{max}^{MeOH} nm (log ε): 205 sh, 217 (4.44), 223 (4.43), 244 (4.26), 252 (4.30), 281 (4.39). HR-positive FAB-MS *m/z*: found 541.2073 $[M+H]^+$ ($C_{29}H_{33}O_{10}$ requires 541.2073). ^1H and ^{13}C NMR: Tables 1 and 2.

Enzymatic hydrolysis of **3** *and* **4**. Each sample (1 mg) dissolved in 0.01 M Pi buffer (2 ml, pH 8.0) was incubated with pancreatin (1 mg, Biocatalyst Ltd, U.K.) at 37° for 2–7 days. The hydrolysate was filtered through a membrane filter and injected for HPLC (columns: 5 μm, 150 × 4.6 mm I.D., Wako Wakosil-II 5C18 HG; flow rate: 1 ml min^{-1}; detector: 290 nm; column temp.: 45°; eluent MeCN–0.1% H_3PO_4, 0–9 min, 15%; 9–14 min, 15–35%; 14–20 min, 35%). Identification of chromatographic peaks was advanced using a photodiode-array detector.

Synthesis of 8-C-*glucosyl*-7-O-*methyl-aloesol* [2]. A soln of CH_2N_2 in Et_2O made from 1-methyl-3-nitro-1-nitrosoguanidine (Aldrich) was added to **1** (500 mg) in MeOH (50 ml) and the reaction mixt. kept for 2 hr. After removal of solvent, the residue was submitted to MCI-gel CHP 20P CC (37–75 μm) with 20% MeOH to give pure 7-*O*-methylaloesin (127 mg). $NaBH_4$ (40 mg) was added to a soln of 7-*O*-methylaloesin (25 mg) in MeOH (5 ml) with stirring and the reaction mixt. allowed to stand for 10 min. After addition of dil. HCl, the reaction mixt. was evapd to dryness under red. pres. and the residue was applied to MCI-gel CHP 20P CC (37–75 μm) with 20% MeOH to give a mixt. of 8-*C*-glucosyl-7-*O*-methyl-aloesol (20 mg). The mixt. of 8-*C*-glucosyl-7-*O*-methyl-aloesol was analysed by HPLC: LiChrosorb RP-8 (Merck) (column: 7 μm, 125 × 4 mm I.D.; flow rate: 1 ml min^{-1}; detector: 280 nm; eluent MeCN–H_2O, linear gradient from 5% to 10% MeCN in 15 min; two peaks of similar intensity with R_t 12.8 and 14.2 min). Compound **2** showed the longer R_t, while the hydrolysate of aloeresin D showed the shorter R_t.

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REFERENCES

1. U.S. Pharmacopeia: XX–The National Formulary XVII (1990), p. 37, United States Pharmacopeial Convention, Rockville.
2. Okamura, N., Asai, M., Hine, N. and Yagi, A. (1996) *J. Chromatogr.*, in press.
3. Markham, K. R., Chari, V. M. and Mabry, T. J. (1982) in *The Flavonoids – Advances in Research* (Harborne, J. B. and Mabry, T. J., eds), p. 19. Chapman & Hall, London.
4. Speranza, G., Dadà, G., Lunazzi, L., Gramatica, P. and Manitto, P. (1986) *Phytochemistry* **25**, 2219.
5. Pomerantz, S. H. (1963) *J. Biol. Chem.* **238**, 2351.