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# ALOERESIN C, A BITTER *C,O*-DIGLUCOSIDE FROM CAPE ALOE*

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**Key Word Index**—*Aloe ferox*; Liliaceae; Cape aloe, 5-methylchromones; *C,O*-diglucoside; aloeresin C

**Abstract**—A new bitter *C,O*-diglucoside, aloeresin C, was isolated from commercial Cape aloe. Its structure, 2-acetonyl-7-*O*-β-D-glucopyranosyl-8-*C*-β-D-[2′-*O*-(E)-*p*-coumaroyl]glucopyranosyl-5-methylchromone, was established by spectral and chemical methods.

## INTRODUCTION

Aloe is the dried latex of the leaves of *Aloe ferox* Miller known commercially as Cape aloe, or of *Aloe vera* Miller, known as Curaçao aloe [1]. So far, two epimeric 10-*C*-β-D-glucopyranosyl aloe-emodin anthrones, viz. aloins A and B [2, 3], and three 2-acetonyl-7-hydroxy-5-methylchromones, viz. aloesone (**1**) [4], aloesin (**2**) (formerly aloeresin B) [5] and aloeresin A (**3**) [6], have been isolated from the latex. We report here a chemical investigation of a commercial sample of Cape aloe which resulted in the isolation of a new bitter constituent we named aloeresin C. Its structure was proved to be the 7-*O*-glucopyranoside of aloeresin A (**4**) on the basis of spectral as well as chemical evidence.

## RESULTS AND DISCUSSION

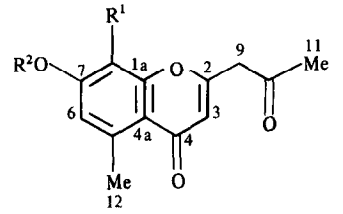
Aloeresin C was obtained in 0.85% yield from Cape aloe via methanol extraction followed by flash chromatography and finally, HPLC (reverse-phase) purification. Inspection of its UV spectrum revealed strong resemblances with the absorption pattern of 7-hydroxy-5-methylchromones [7].

Peaks at 703 $[M+1]^+$, 541 ($703-C_6H_{10}O_5$, equivalent to protonated **3**), 395 (equivalent to protonated **2**) and 233 (equivalent to protonated **1**) were observed in the fast atom bombardment (FAB) mass spectrum of aloeresin C, thus suggesting a diglucoside structure of the aloesin series. This was supported by further spectral evidence, as shown in Tables 1 and 2, in which $^1H$ and $^{13}C$ NMR chemical shifts of aloeresin C are listed together with those of the other structurally related 5-methylchromones occurring in aloes. Aloesin (**2**) and aloeresin A (**3**) were isolated from Cape aloe using HPLC (see Experimental), whereas aloesone (**1**) was synthesized (unpublished results). Complete lists of NMR data have previously been reported only for aloesin **2** [5, 10]. Assignments in Tables 1 and 2 are mainly based on analogies of chemical shifts and coupling constants with those found for the corresponding signals in coumarins [8] and flavones *C*- and *O*-glucosides [9, 10]. Proton couplings were confirmed by double-resonance experiments and $^{13}C$ assignments supported by both off-resonance and selective proton irradiations (e.g. the signals of C-1′, C-3 and C-6 in **2** as well as those of C-1′ and C-2′ in **3** were detected by simultaneous irradiation of the corresponding protons). An NOE experiment performed on aloesin (**2**) proved unequivocally that the glucosyl residue is attached to ring A at C-8 position (*ca* 15% increased intensity of the H-6 proton at δ6.69 by irradiating the aromatic-methyl singlet). This information was needed since previous evidence in favour of structure **2** [5] could not be regarded as conclusive for choosing between C-8 and C-6 substitution. It must also be pointed out that structure **3** for aloeresin A and, as described below, structure **4** for aloeresin C, substantially depend on chemical correlation with aloesin (**2**). In addition, the proton coupled $^{13}C$ spectrum of **2** and **3** showed a quartet of doublets ($^1J_{CH} = 130$ Hz, $^3J_{CH} = 6$ Hz) centred at δ22.5 and 22.7, respectively, thus confirming both the absence of a substituent at C-6 and the assignment of the above signal to Me-5. This signal was erroneously attributed to C-11 by Markham *et al.* [10] in a proton-decoupled spectrum of aloesin (**2**).

An argued comparison of NMR data (Tables 1 and 2) allowed structure **4** to be assigned to aloeresin C. The presence of a β-D-glucopyranosyl residue in the 7-*O*-position of **4** rests on the $^{13}C$ chemical shifts of the sugar moiety [10], in particular on that of the anomeric carbon which is indicative of *O*-glucosylation (*ca* δ100) rather than C-glucosylation (*ca* δ70) [10], and on the coupling constant between H-1‴ and H-2‴ suggesting a β-configuration of C-1‴ [11]. In agreement with this conclusion, the C-7 signal of **4** appears to be shifted Δδ1.7 upfield with respect to aloeresin A (**3**), whilst those of C-6, C-8 and C-4a are shifted Δδ0.8, 1.2 and 0.8 downfield, respectively, (as found in 7-*O*-glucosylflavonoids) [10].

Concerning the attachment of the *p*-coumaroyl group, the involvement of the O-2′ position (i.e. of the *C*-glucosyl moiety) results from the fact that the same 'acylation' effect on the $^{13}C$ chemical shifts of C-1′, C-2′ and C-3′ was observed going from aloesin (**2**) to aloeresins A (**3**) and C (**4**) (downfield shift for C-2′ and upfield shift for C-1′ and C-3′) [10].

*Part 2 in the series "Studies on Aloe". For Part 1 see ref [6].



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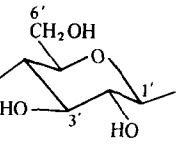

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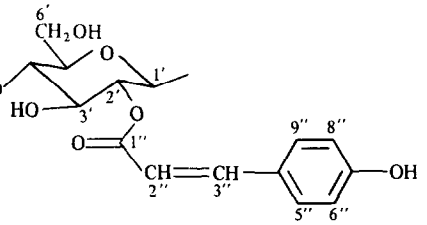

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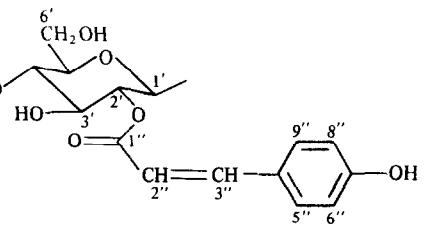

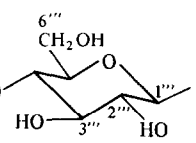

4 { $R^1$ = , $R^2$ =

Complementary and conclusive proof that aloeresin C is the 7-*O*-*β*-D-glucoside of aloeresin A arose from acid-catalysed hydrolysis experiments. In fact, aloeresin C afforded **3** and **2** in that order when heated in aqueous hydrochloric acid, whereas α- and *β*-methyl glucosides (ratio *ca* 3:1) were identified as products of acid methanolysis. By contrast, no hydrolysis of **4** was observed by treatment with emulsin. Analogous unreactivity toward *β*-D-glucosidase has been reported in the case of a 8-*C*-*β*-D-glucosylflavone 7-*O*-*β*-D-glucoside and interpreted as being due to steric or hydrogen bonding effects [12].

To our knowledge, aloeresin C represents the first example of a *C*,*O*-diglucoside of a 5-alkylchromone aglycone [13].

## EXPERIMENTAL

Commercial Cape aloe used in this investigation was purchased from the Pan-African Commercial Corporation. TLC was carried out on pre-coated silica gel $F_{254}$ plates using EtOAc–EtOH–$H_2O$ (100:20:13); chromone compounds gave fluorescent spots when observed under UV light (254 nm). Analytical and semi-prep. HPLC was performed on an instrument connected to a variable wavelength UV detector; an instrument equipped with a RI detector was used for prep. HPLC. UV–visible spectra were recorded in MeOH. $^1H$ and $^{13}C$ NMR spectra were recorded at 300 and 75.740 MHz, respectively, in DMSO-$d_6$ using the same solvent as int. standard ($\delta$2.50 and 39.50 from TMS for $^1H$ and $^{13}C$, respectively); NOE expts were carried out at 80 MHz. EIMS and FABMS were recorded on a spectrometer equipped with a combined DEI (70 eV, 270°) and FAB *ion source* (Ar as *bombarding gas*)

*Isolation of aloeresin C* (**4**) Powdered Cape aloe (3 g) was treated with hot MeOH (1200 ml) and the filtrate evaporated under red. pres. to a brown residue (2 8 g) which was redissolved in hot $Me_2CO$ (1500 ml) After filtering a small amount of insoluble material and evaporating the solvent under red pres., the concentrate was adsorbed on silica gel (35–70 mesh), and chromatographed on a silica gel column (230–400 mesh, 650 g) using EtOAc–EtOH–$H_2O$ (100.20:13) as eluent. Separation was monitored by TLC Fractions containing aloeresin C as a major product ($R_f$ 0.33) were combined and further purified by semi-prep. HPLC (column: 250 × 10 mm, LiChrosorb RP-8, 7 *μ*m; flow rate: 5 ml/min; detector: *λ*340 nm; eluent. MeCN–$H_2O$, linear gradient from 10% to 25% MeCN in 25 min) The eluate of the HPLC column was lyophilized and dried under red. pres. (60°) for 2 days. An amorphous solid was obtained in 0.85% yield from starting material and shown to be pure by analytical HPLC (column: 250 × 4 mm, LiChrosorb RP-18, 10 *μ*m; flow rate: 1 ml/min; detector: *λ*300 nm; eluent: MeOH–$H_2O$, linear gradient from 30% to 60% MeOH in 25 min) and TLC Mp 199–202°; UV $\lambda_{max}^{MeOH}$ nm (log *ε*): 228 (4.56), 244 sh (4.37), 252 (4.31), 300 (4.40); $[\alpha]_D^{30}$ −48.3° (MeOH; *c* 0.06); $^1H$ and $^{13}C$ NMR see Tables 1 and 2; EIMS *m/z* (rel. int.): 394 (18.6), 376 (21 3), 298 (3 4), 261 (32 4), 245 (37 9), 203 (20.7), 164 (56.5), 163 (26.2), 147 (48.2), 120 (100.0), 119 (40.7). (Found: C, 52.55; H, 5.70 $C_{34}H_{38}O_{16}$ $4H_2O$ requires: C, 52.71; H, 5.98%.)

*Isolation of aloesin* (**2**) *and aloeresin A* (**3**). Powdered Cape aloe (20 g) was submitted to prep. liquid chromatography (column: PrePak 500/C18, 5.7 × 30 cm, particle size: 37 *μ*m; flow rate 100 ml/min.; eluent: MeOH–$H_2O$). Fractions (200 ml) were collected as follows: fractions 9–30 (MeOH–$H_2O$, 1·9) containing aloesin (single spot on TLC) and fractions 40–56 (MeOH–$H_2O$, 2·9) containing aloeresin A as a major product

After decolouration with activated charcoal, fractions 9–30 were concd under red. pres. and introduced onto an Amberlite XAD-4 column Pure aloesin (**2**) [5] was recovered by elution with MeOH (11% yield). Aloeresin A (**4**) [6] was isolated from fractions 40–56 by semi-prep HPLC (column: 250 × 10 mm, LiChrosorb RP-18, 7 *μ*m; flow rate: 5 ml/min; detector. *λ*340 nm; eluent: MeOH–$H_2O$, linear gradient from 30% to 60% MeOH in 25 min; 16% yield)

*Acid hydrolysis of aloeresin C* (**4**) Aloeresin C (5 mg) was dissolved in 1 M HCl (5 ml) and the soln kept at 100° The progress of the reaction was monitored by TLC and HPLC (analytical conditions as above). A mixture of aloesin (**2**) (*ca* 60%), aloeresin A (**3**) (*ca* 30%) and unreacted **4** (*ca* 10%) was obtained after 2 hr

Methanolysis of aloeresin C was performed by dissolving **4** (15 mg) in 3% HCl–MeOH (20 ml) and heating under reflux for 2 hr After removing the solvent under red. pres and drying *in vacuo* over KOH at room temp. overnight, the residue was silylated with BSA–TMCS–$C_5H_5N$ (1:1 5·10, v/v) and analysed by GC (FID-GC: 2 m × 3 mm i.d glass column packed with 10% Carbowax 20 M; carrier gas He at 30 ml/min; temp programmed from 120° to 170° at 2°/min, injector and FID temps, 225°)

Table 1. $^1$H NMR spectral data for aloesone (1), aloesin (2), aloeresin A (3) and aloeresin C (4) ($\delta$-values, DMSO-$d_6$)*

| Assignment | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| H-3 | 6.05 | 6.12 | 6.18 | 6 20 |
| H-6 | 6.62† | 6.69 | 6 61 | 6.97 |
| –CH$_2$–CO– | 3.85 | 3.79 | 3.81 | ‡ |
| CH$_3$–CO– | 2.25 | 2 25 | 2.29 | 2.27 |
| Me–Ar | 2.65 | 2.67 | 2.60 | 2.62 |
| H-2″ | — | — | 6.16 *d* ($J$ = 15.0 Hz) | 6.08 *d* ($J$ = 15.0 Hz) |
| H-3″ | — | — | 7.34 *d* ($J$ = 15.0 Hz) | 7.38 *d* ($J$ = 15.0 Hz) |
| H-5″, H-9″ (2H) | — | — | 7.48 *d* ($J$ = 8.5 Hz) | 7.47 *d* ($J$ = 8.5 Hz) |
| H-6″, H-8″ (2H) | — | — | 6.77 *d* ($J$ = 8.5 Hz) | 6 75 *d* ($J$ = 8.5 Hz) |
| H-1′ | — | 4.71 *d* ($J$ = 9 0 Hz) | 4 93 *d* ($J$ = 9.0 Hz) | 5.18 *d* ($J$ = 9.0 Hz) |
| H-2′ | — | — | 5.51 *dd* ($J$ = $J'$ = 9.0 Hz) | 5 43 *dd* ($J$ = $J'$ = 9.0 Hz)§ |
| H-1‴ | — | — | — | 4.71 *d* ($J$ = 9.0 Hz) |

*After deuterated water exchange; unmarked signals are singlets; signals of glucosyl hydroxy groups were observed in the range $\delta$3–5 for 2–4 and broad signals of phenolic groups in the range $\delta$10.0–11.5 when spectra were registered without addition of deuterated water

†The singlet includes H-8.

‡Obscured by signals of sugar protons

§Related to the doublet at $\delta$5 18 by double resonance.

Table 2. $^{13}$C NMR chemical shifts of aloesone (1), aloesin (2), aloeresin A (3) and aloeresin C (4) ($\delta$-values, DMSO-$d_6$)

| Carbon No. | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 2 | 160.2* | 160.6* | 160.3* | 160.3* |
| 3 | 112 7 | 112 3 | 112 5 | 112.6 |
| 4 | 177 9 | 178.5 | 178.6 | 178.5 |
| 4a | 114.2 | 114.6 | 114.8 | 115 6 |
| 5 | 141 3 | 140.2 | 141.0 | 141.2 |
| 6 | 116.5 | 116.4 | 115.8 | 116 6 |
| 7 | 160.8* | 159.4* | 159.1* | 157 4† |
| 8 | 100 4 | 110.8 | 110.2 | 111.4 |
| 1a | 159.0 | 157 6 | 158.3 | 158.1† |
| 9 | 47 4 | 47.6 | 48.1 | 48.0 |
| 10 | 202 1 | 202 3 | 202.4 | 202.8 |
| 11 | 29.7 | 29.8 | 30 4 | 29.5 |
| 12 | 22.3 | 22.5 | 22 7 | 22.8 |
| 1′ | — | 73 5 | 70.2 | 69.6 |
| 2′ | — | 71.1 | 72.3 | 72.0 |
| 3′ | — | 78.6 | 76.0 | 75.0 |
| 4′ | — | 70 4 | 70.2 | 70.2‡ |
| 5′ | — | 81 3 | 81.8 | 81 4 |
| 6′ | — | 61.4 | 61.8 | 61 7 |
| 1″ | — | — | 165.4 | 165.4 |
| 2″ | — | — | 114.1 | 114 3 |
| 3″ | — | — | 144 4 | 143.9 |
| 4″ | — | — | 125 1 | 125.1 |
| 5″, 9″ | — | — | 130 1 | 130.1 |
| 6″, 8″ | — | — | 115.8 | 115.6 |
| 7″ | — | — | 159.6* | 159.5* |
| 1‴ | — | — | — | 101.1 |
| 2‴ | — | — | — | 73.2 |
| 3‴ | — | — | — | 76.4 |
| 4‴ | — | — | — | 70.8‡ |
| 5‴ | — | — | — | 77 1 |
| 6‴ | — | — | — | 60 5 |

*·†·‡Assignments bearing the same superscript in any one spectrum may be reversed.

Silylated $\alpha$- and $\beta$-methyl D-glucosides from aloeresin C were identified by comparison with authentic samples prepared by silylation of $\alpha$- and $\beta$-methyl D-glucosides.

*Enzymatic hydrolysis of aloeresin C* (4). A soln of aloeresin C (1 mg) in acetate buffer (0.1 M, pH 5, 3 ml) was added to a soln of $\beta$-glucosidase (400 mU) in the same buffer (8 ml) and incubated at 37° for 2 days. When analysed by TLC and HPLC only the starting aloeresin C was observed.

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