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

GIOVANNA SPERANZA, PAOLA GRAMATICA,† GIUSEPPE DADA and PAOLO MANITTO†

Centro Studi Maria Branca, Via E. Porro 1, 20158 Milano, Italy; †Istituto di Chimica Organica dell'Università e Centro di Studio delle Sostanze Organiche Naturali del CNR, Via Venezian 21, 20133 Milano, Italy

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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 Curacao 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

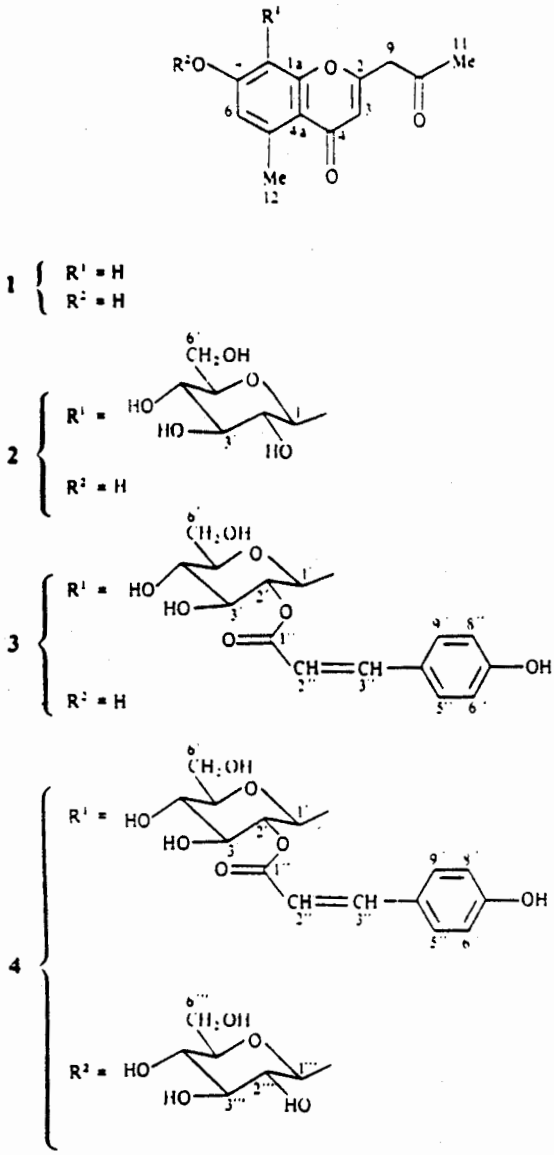
Aloeresin C was obtained in 0.85% yield from Cape aloe via methanol extraction followed by flash chromatography and finally, HPLC (reverse-phase) purification. Inspection of its UV spectrum revealed strong resemblances with the absorption pattern of 7-hydroxy-5-methylchromones [7].

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].



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 (δ 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 μm; 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 μm; 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 λ_{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. $C_{34}H_{38}O_{16}\cdot 4H_2O$ 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 μm; 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 μm; 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. ^1H NM

Assignment
H-3
H-6
$-CH_2-CO-$
CH_3-CO-
Me–Ar
H-2″
H-3″
H-5″, H-9″ (2H
H-6″, H-8″ (2H
H-1′
H-2′
H-1‴

*After deute
groups were ob
δ 10.0–11.5 whe
†The singlet
‡Obscured b
§Related to

Table 2. ^{13}C NMR chem
aloesin (2), aloeresin A (
values. D

Carbon No.	1	
2	160.2*	16
3	112.7	1
4	177.9	17
4a	114.2	1
5	141.3	1-
6	116.5	1
7	160.8*	1
8	100.4	1
1a	159.0	1
9	47.4	
10	202.1	2
11	29.7	
12	22.3	
1′	—	
2′	—	
3′	—	
4′	—	
5′	—	
6′	—	
1″	—	
2″	—	
3″	—	
4″	—	
5″, 9″	—	
6″, 8″	—	
7″	—	
1‴	—	
2‴	—	
3‴	—	
4‴	—	
5‴	—	
6‴	—	

*,†,‡Assignments bea
any one spectrum may

ed silica gel F_{254} plates using
20:13); chromone compounds gave
observed under UV light (254 nm).
HPLC was performed on an instru-
able wavelength UV detector; an
a RI detector was used for prep.
were recorded in MeOH. ^1H and
recorded at 300 and 75.740 MHz
ing the same solvent as int. standard
for ^1H and ^{13}C, respectively; NOE
0 MHz. EIMS and FABMS were
r equipped with a combined DEI
source (Ar as bombarding gas).
4). Powdered Cape aloe (3 g) was
200 ml) and the filtrate evaporated
esidue (2.8 g) which was redissolved
After filtering a small amount of
rating the solvent under red. pres.
ed on silica gel (35–70 mesh) and
gel column (230–400 mesh, 650 g;
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r: λ340 nm; eluent: MeCN–H_2O
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s solid was obtained in 0.85% yield
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rosorb RP-18, 10 μm; flow rate
m; eluent: MeOH–H_2O, linear
MeOH in 25 min) and TLC. Mp
g α]: 228 (4.56), 244 sh (4.37), 252
48.3° (MeOH; c 0.06); ^1H and
EIMS m/z (rel. int.): 394 (18.6), 376
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C, 52.71; H, 5.98%.)
loeresin A (3). Powdered Cape aloe
liquid chromatography (column:
., particle size: 37 μm; flow rate:
-H_2O). Fractions (200 ml) were
9–30 (MeOH–H_2O, 1:9) contain-
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rate: 5 ml/min; detector: λ340 nm;
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e). A mixture of aloesin (2) (*ca*
) and unreacted 4 (*ca* 10%) was

was performed by dissolving 4
ml) and heating under reflux for
it under red. pres. and drying *in*
mp. overnight, the residue was
H_2N (1:1.5:10, v/v) and analysed
d. glass column packed with 10%
at 30 ml/min; temp. programmed
injector and FID temps, 225°.

Table 1. ^1H NMR spectral data for aloesone (1), aloesin (2), aloeresin A (3) and aloeresin C (4) (δ-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 δ3–5 for 2–4 and broad signals of phenolic groups in the range δ10.0–11.5 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 δ5.18 by double resonance.

Table 2. ^{13}C NMR chemical shifts of aloesone (1), aloesin (2), aloeresin A (3) and aloeresin C (4) (δ-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
8a	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 α- and β-methyl D-glucosides from aloeresin C were identified by comparison with authentic samples prepared by silylation of α- and β-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 β-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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REFERENCES

1. *U.S. Pharmacopeia: XX—The National Formulary XV* (1979) p. 21. Marck, Easton.
2. Hay, J. E. and Haynes, L. J. (1956) *J. Chem. Soc.* 3141.
3. Auterhoff, H., Graf, E., Eurish, G. and Alexa, M. (1980) *Arch. Pharm.* 313, 113.
4. Holdsworth, D. K. (1972) *Planta Med.* 22, 54.
5. Haynes, L. J. and Holsworth, D. K. (1970) *J. Chem. Soc. C* 2581.
6. Gramatica, P., Monti, D., Speranza, G. and Manitto, P. (1982) *Tetrahedron Letters* 2423.
7. Sen, K. and Bagchi, P. (1959) *J. Org. Chem.* 24, 316.
8. Cussans, N. J. and Huckerby, T. N. (1975) *Tetrahedron* 31, 2719.
9. Markham, K. R. and Mabry, T. J. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds) p. 62. Chapman & Hall, London.
10. 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.
11. Casu, B., Reggiani, M., Gallo, G. G. and Vigevani, A. (1964) *Tetrahedron Letters* 2839.
12. Oelrichs, P., Marshall, J. T. B. and Williams, D. H. (1968) *J. Chem. Soc. C* 941.
13. Franz, G. and Grün, M. (1983) *Planta Med.* 47, 131.