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A *C*-GLUCOSYLATED 5-METHYLCHROMONE FROM KENYA ALOE*

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Key Word Index—*Aloe ferox*; Liliaceae; Kenya aloe; 5-methylchromones; *C*-glucoside; aloeresin D.

Abstract—A new bitter *C*-glucoside, aloeresin D, was isolated from a commercial sample of Kenya aloe. Its structure was assigned as 8-*C*-β-D-[2-*O*-(*E*)-*p*-coumaroyl]glucopyranosyl-2-[(*R*)-2-hydroxy]propyl-7-methoxy-5-methylchromone by spectral data and chemical transformations.

INTRODUCTION

In continuing our chemical investigations on aloe [1, 2], the dried latex of the leaves of *Aloe* sp., we now wish to report the occurrence of a new bitter *C*-glucoside in a commercial sample coming from Kenya. As Cape aloe, Kenya aloe is obtained from *Aloe ferox* Miller and its hybrids [3]. The structure of the compound, which we propose to name aloeresin D [2], was established as **1** on the basis of spectral as well as chemical evidence.

RESULTS AND DISCUSSION

A combination of droplet counter current (DCCC) and flash chromatography of the aqueous methanol extract of aloe afforded aloeresin D in 12.5% yield. The compound, $C_{29}H_{32}O_{11}$ (by elemental analysis and FD mass spectrometry) exhibited UV, IR and mass spectra characteristic of *C*-glucosylated 5-methylchromones [2, 4, 5]. In addition, peaks were observed in the EI mass spectrum indicating the presence of a *p*-coumaroyl group (fragments at *m/z* 410 $[M-146]^+$, 392 $[M-164]^+$) and of a hydroxypropyl side chain in the 2-position of the chromone nucleus (fragments at *m/z* 512 $[M-44]^+$, 366 $[M-146-44]^+$ and 348 $[M-164-44]^+$ due to the rearrangement shown in **7**). These data, together with those obtained by ^1H and ^{13}C NMR spectral analysis (Tables 1 and 2) allowed structure **1** to be assigned to aloeresin D (apart from the absolute configuration of C-10).

Assignments in Tables 1 and 2 were mainly based on analogies of chemical shifts and coupling constants with those found for the chromone derivatives of the aloeresin series, i.e. aloesin (formerly aloeresin B, **2**), aloeresin A (**3**) and aloeresin C (**4**), previously isolated from Cape aloe [2]. Proton–proton coupling constants were confirmed by homonuclear decouplings and ^{13}C assignments were supported by off-resonance, proton-coupled and DEPT spectra. The intensity of the proton-decoupled ^{13}C signal at δ115.8 corresponds to 3 carbons (i.e. C-4a, C-6″, C-8″) as proved by a gated decoupled (NOE suppressed) experiment.

Inspection of the ^1H and ^{13}C NMR chemical shifts of compounds **1**–**6** (Tables 1 and 2 and ref. [2]) clearly reveals the effects of two structural modifications, namely the methylation of the phenolic hydroxyl at C-7 and the reduction of the side chain carbonyl group to secondary alcohol. Thus, going from **2** and **3** [2] to **1**, **5** and **6**, the 'methylation' effect causes significant Δδs: upfield for C-6 (4–5) and downfield for H-6 (*ca* 0.3), C-7 (*ca* 1), C-4a (*ca* 1) and C-8 (*ca* 2) (the effect on C-8 appears to be reduced when the hydroxyl at C-2′ is acylated as in the pair **1** and **3** [6, 7]). The presence of an alcoholic function at C-10 in place of a keto group markedly influences the chemical shifts of C-2, C-3 and of H-3 (Δδ *ca* +4.5, −1.3, −0.15, respectively).

Additional support for the spectroscopic determination of structure **1** came from the chemical relationship that we established between aloeresin D and aloesin (**2**), according to the following procedure. Aloesin (**2**) [2] was treated with diazomethane to give the methyl ether **5** [8], which was then reduced with sodium borohydride. The resulting product, when analysed by HPLC and ^{13}C NMR, was found to be a *ca* 1:1 mixture of **6** and its epimer at C-10, which were separated by prep. HPLC (reverse phase). The more mobile one was shown to be identical in all respects with the product arising from alkaline hydrolysis of aloeresin D (**1**), i.e. **6** for the reason given below.

Finally, the (*R*)-configuration of C-10 in aloeresin D (**1**) was proved by drastic degradation of its deacylated derivative **6** with hot sodium hydroxide [4] followed by isolation of crude 3-hydroxybutanoic acid (**8**). This, after conversion into its methyl ester (**9**) by reaction with diazomethane, was treated, without further purification, with (*R*)-(+)-1-phenylethyl isocyanate. The resulting carbamate (**10**) was then compared by capillary GC with analogous derivatives prepared from the methyl esters of (*R*)- and (*RS*)-3-hydroxybutanoic acid [9]. The carbamates of both the authentic methyl (*R*)-3-hydroxybutanoate and the crude ester **9** (obtained by degradation of **6**) co-eluted with one of the two well separated diastereomers arising from the reaction of isocyanate with racemic methyl-3-hydroxybutanoate.

*Part 3 in the series "Studies on *Aloe*". For part 2 see ref. [2].

Table 1. ^{1}H NMR spectral data for aloeresin D (**1**), **5** and **6** (δ-values, DMSO-d_6)*

Assignment	1†	5	6
H-3	6.04	6.18	6.05
H-6	6.86	6.99	6.97
H-9	2.67 (2)‡	§	2.58 *br d* (2, J = 6.0)
H-10	4.26 *t q* (J, J' = 6.0)	—	4.15 *m*
H-11	1.21 *d* (3, J = 6.0)	2.21 (3)	1.15 *d* (3, J = 6.0)
H-12	2.67 (3)	2.74 (3)	2.72 (3)
H-2″	6.12 *d* (J = 15.0)		
H-3″	7.33 *d* (J = 15.0)		
H-5″, H-9″	7.49*d* (2, J = 8.5)		
H-6″, H-8″	6.80*d* (2, J = 8.5)		
H-1′	4.98 *d* (J = 10.0)	4.72 *d* (J = 10.0)	4.76 *d* (J = 10.0)
H-2′	5.61 *dd* (J, J' = 10)		
OMe	3.85	3.89	3.87

*After deuterated water exchange; unmarked signals are singlets, integral values different from 1 and coupling constants (Hz) are given in parentheses.

†The phenolic proton could be observed at δ 9.95 before D_2O exchange.

‡Coupling cannot be determined due to signal overlapping.

§Obscured by sugar protons.

Table 2. ^{13}C NMR chemical shifts of aloeresin D (**1**), **5** and **6** (δ-values, DMSO-d_6)

Carbon N°	1	5	6
2	165.0*	160.6*	164.9
3	111.3	112.4	111.2*
4	178.8	178.7	178.7
4a	115.8	115.8	115.9
5	141.8	141.1	141.0
6	111.3	111.7	111.5*
7	159.8†	160.2*	160.0
8	110.7	113.0	112.8
1a	157.5	157.1	157.2
9	43.3	47.7	43.2
10	63.9	202.3	63.8
11	23.7	29.8	23.8
12	22.9	22.8	22.8
7-OMe	56.5	56.4	56.4
1′	70.6‡	72.8	73.0
2′	72.3	70.9	70.9
3′	75.9	78.7	78.8
4′	70.9‡	70.5	70.8
5′	82.0	81.6	81.8
6′	61.6	61.7	61.7
1″	165.4*		
2″	114.0		
3″	144.4		
4″	125.0		
5″, 9″	130.3		
6″, 8″	115.8		
7″	159.6†		

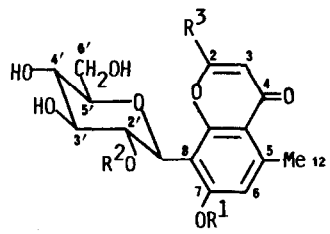
*†‡Signals are interchangeable.

EXPERIMENTAL

Commercial Kenya aloe used in this investigation was purchased from the Saamer Int. Ltd. (Mombasa, Kenya). TLC was performed on silica gel F_{254} pre-coated plates (0.25 mm layer) using EtOAc–EtOH–H_2O (100:20:13) as eluent, unless otherwise indicated; spots were visualized by exposing to UV light (254 nm) or by spraying with Methyl Orange in the case of acidic compounds. Analytical and semi-prep. HPLC was performed on an instrument connected to a variable wavelength UV detector. DCCC was carried out with an apparatus equipped with 300 glass tubes (400 × 2.7 mm). FID-GC: 2 m × 3 mm i.d. glass column packed with 10% FFAP; carrier gas He 30 ml/min; temp. prog. from 120 to 190° at 4°/min; inj. and FID temps, 250°. Capillary GC: 25 m × 0.32 mm cross-linked silicone OV-1701 fused silica WCOT column; carrier gas H_2 1.5 ml/min; temp. prog. from 80 to 200° at 3°/min; split mode; inj. and FID temps, 250°. ^{1}H and ^{13}C NMR spectra were run at 300 and 75.47 MHz, respectively, in DMSO-d_6 using the same solvent as int. standard (δ2.50 and 39.50 from TMS for ^{1}H and ^{13}C, respectively). EIMS and FDMS were recorded on an apparatus equipped with a combined EI (70 eV, 270°) and FD ion source.

Isolation of aloeresin D (**1**). Powdered Kenya aloe (2 g) was dissolved in 15 ml of the DCCC solvent (1:1 ratio of the two phases formed from $CHCl_3$–MeOH–H_2O, 7:13:8). Both phases were filtered together on cotton wool and submitted to DCCC in the ascending mode (mobile phase: upper layer; flow rate 40 ml/hr); 7 ml fractions were collected and analysed by TLC. Fractions containing a major spot with R_f 0.61 were combined and further purified by CC (silica gel, 230–400 mesh, 130 g) using EtOAc–EtOH–H_2O (100:20:13). On removal of solvent under red. pres. the column fractions yielded a yellowish compound (250 mg) which was found to be pure on TLC and analytical HPLC (column: 250 × 4 mm, LiChrosorb RP-18, 5 μm; flow rate: 1 ml/min; detector: λ 280 nm; eluent: MeOH–H_2O, linear gradient from 30% to 60% MeOH in 25 min; R_t 17 min). Mp 143–145°; UV λ_{max}^{MeOH} nm (log ε): 226 (4.53), 242 sh (4.31), 252 (4.24), 300 (4.47); IR ν_{max}^{KBr} cm^{-1}: 3400, 1705, 1645; $[\alpha]_D^{30}$ −163° (MeOH; c 0.24); ^{1}H and ^{13}C NMR: see Tables 1 and 2; EIMS m/z (rel. int.): 556 $[M]^+$ (0.6), 538 $[M-H_2O]^+$ (1.3), 512 (1.7), 410 (1.8), 392 (2.4), 366 (1.5), 348 (2.0), 277 (5.6), 259 (21.1), 217 (11.3), 177 (9.8), 164 (28.2), 147 (100), 120 (33.8). (Found: C, 61.52; H, 5.77. $C_{29}H_{32}O_{11} \cdot 0.5\ H_2O$ requires: C, 61.59; H, 5.84%.)

Hydrolysis of aloeresin D (**1**). Aloeresin D (**1**, 100 mg) dissolved in MeOH (20 ml) and 0.1 N NaOH (2 ml) was refluxed for 1 hr



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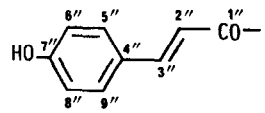
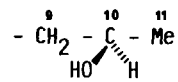
1	Me		
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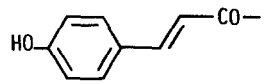
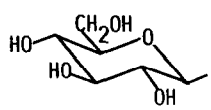
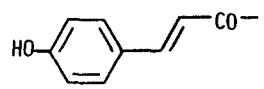
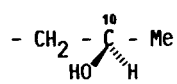
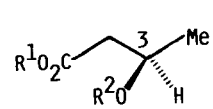
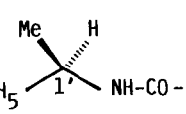
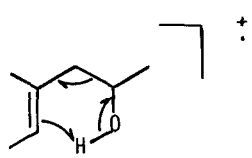
3	H		$-CH_2-CO-Me$
4			$-CH_2-CO-Me$
5	Me	H	$-CH_2-CO-Me$
6	Me	H	



8 $R^1 = H$ $R^2 = H$

9 $R^1 = Me$ $R^2 = H$

10 $R^1 = Me$ $R^2 =$ 



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under He. After cooling, the reaction mixture was neutralized with dil HCl and evapd to dryness. The residue was then treated with hot H_2O (20 ml) and desalted by passing through a column of Amberlite XAD-7 (100 ml). Elution with MeOH (150 ml) followed by evapn of solvent afforded a crude product which was purified by semi-prep. HPLC (column: 250 × 25 mm; LiChrosorb RP-18, 7 μm, flow rate: 15 ml/min; detector: λ 340 nm; eluent MeOH–H_2O, linear gradient from 30% to 60% MeOH in 18 min; R_t 5 min). After lyophilization compound **6** was obtained as an amorphous powder (50 mg). TLC: R_f 0.37; mp 141–143°; UV λ_{max}^{MeOH} nm (log ε): 228 (4.36), 244 (4.29), 252 (4.30), 294 (4.12); IR ν_{max}^{KBr} cm^{-1}: 3400, 1650, 1600; $[\alpha]_D^{30}$ −39.5° (MeOH; *c* 0.21); ^{1}H and ^{13}C NMR see Tables 1 and 2; EIMS (probe) 70 eV, *m/z* (rel. int.): 410 $[M]^+$ (26.1), 392 (14.8), 366 (14.2), 277 (44.5), 261 (34.8), 259 (100), 246 (18.7), 233 (83.2), 217 (27.7). (Found: C, 58.24; H, 6.15. $C_{20}H_{26}O_9$ requires: C, 58.53; H, 6.38%.)

7-O-*Methylaloesin* (**5**). A slow stream of CH_2N_2 prepared from Diazald (Aldrich, 2.5 g, N_2 as carrier) [10] was bubbled for 2 hr into a soln of aloesin (**2**) [2] (300 mg) in MeOH (50 ml) at 25°. Evapn of solvent left a residue which was submitted to semi-prep. HPLC (conditions as above) to give pure **5** (200 mg). TLC: R_f 0.43; mp 122–124° (lit. [8] 121–122°); UV λ_{max}^{MeOH} nm (log ε): 227 (4.33), 244 (4.30), 252 (4.29), 296 (4.09); IR ν_{max}^{KBr} cm^{-1}: 3400, 1715, 1660, 1612, 1595; $[\alpha]_D^{30}$ +18.3 (MeOH; *c* 0.23); ^{1}H and ^{13}C NMR: see Tables 1 and 2; EIMS (probe) 70 eV, *m/z* (rel. int.): 408 $[M]^+$ (19.5), 390 (1.7), 366 (5.2), 318 (2.9), 317 (7.1), 301 (3.9), 300 (0.65), 289 (8.4), 288 (4.9), 275 (92.2), 259 (53.2), 247 (16.9), 246 (5.2), 233 (100), 232 (15.6), 217 (15.6). (Found: C, 57.61; H, 5.98. Calc. for $C_{20}H_{24}O_9 \cdot 0.5\ H_2O$: C, 57.55; H, 6.04%.)

Reduction of 7-O-*methylaloesin* (**5**). $NaBH_4$ (100 mg) was added under stirring to a soln of **5** (50 mg) in MeOH (15 ml) and the reaction mixture kept at 25° for 10 min. After addition of dil HCl and evapn to dryness under red. pres. the residue was redissolved in H_2O (10 ml) and adsorbed on Amberlite XAD-7. Elution with MeOH gave the reduction product in 80% yield. This product, appearing as a single spot by TLC, was shown to be a mixture of two compounds by HPLC (column: 250 × 4 mm, LiChrosorb RP-8, 10 μm; flow rate: 1 ml/min; detector: λ 280 nm; eluent MeCN–H_2O, linear gradient from 10% to 20% MeCN in 15 min; two peaks of similar intensity with R_t 10.2 and 11.2 min). The compound of shorter R_t, when isolated by HPLC, was shown to be identical with **6** (co-HPLC, UV and ^{1}H NMR).

Alkaline degradation of **6**. Compound **6** (200 mg) was refluxed with 0.3 N NaOH (50 ml) for 4.5 hr. After neutralization with 1 N HCl and lyophilization, the residue was treated with H_2O (10–15 ml) and the ppt filtered off. The filtrate was acidified with a few drops of conc H_2SO_4 and extracted with Et_2O in a Soxhlet for 16 hr. After evapn of solvent under red. pres., the product, having the same TLC R_f (Me_2CO, visualization Methyl Orange) as an authentic sample of 3-hydroxybutanoic acid (**8**), was treated with an excess of CH_2N_2–Et_2O. The crude ester **9**, checked by GC using Me 3-hydroxybutanoate as ref., was converted into the carbamate **10** by treatment with excess (*R*)-(+)-1-phenylethyl isocyanate (0.2 ml) in toluene (1 ml) containing catalytic amounts of 4-(*N,N*-dimethylamino)pyridine (2 hr at 100° and 2 hr under reflux after addition of 5 ml MeOH) [11]. On removal of solvent under red. pres. the reaction mixture gave a residue which was subjected to CC (silica gel 230–400 mesh, 50 g) using hexane–$CHCl_3$–EtOAc (5:5:2) as eluent. Fractions showing one spot with R_f 0.3 by TLC in the same eluent as used for CC were collected and evapd. The residue was found to be the pure carbamate **10** by comparison (capillary GC) with an authentic sample prepared from Me (*R*)-3-hydroxybutanoate (Fluka). Mp 54–55° (after crystallization from MeOH–H_2O); $[\alpha]_D^{25}$ +36.5 ($CHCl_3$; *c* 0.975); IR ν_{max}^{KBr} cm^{-1}: 3330 (s), 1730 (s), 1680 (s), 1535 (s), 1440 (m), 1385 (m), 1330 (m), 1255 (s), 1200 (s); ^{1}H NMR (80 MHz, $CDCl_3$): δ 1.28 (3H, *d*, *J* = 7.0 Hz, Me–C(O–)H–), 1.45 (3H, *d*, *J* = 7.0 Hz, Me–C(NH–)H), 2.53 (2H, $-CH_2-$, AB part, $J_{AX} = J_{BX}$ = 7.0 Hz, J_{AB} = 15.0 Hz), 3.60 (3H, *s*, COOMe), 4.78 (1H, *br q*, C_6H_5–CH–), 5.15 (1H, *qt*, $J_{AX} = J_{BX}$ = 7.0 Hz, *J* = 7.0 Hz), 7.30 (5H, *s*, arom. H). (Found: C, 63.22; H, 7.38; N, 5.13 $C_{14}H_{19}NO_4$ requires: C, 63.38; H, 7.22; N, 5.28%.) The 1:1 mixture of **10** and its C-3 epimer, obtained by derivatization of Me (*R,S*)-3-hydroxybutanoate as described above, exhibited two peaks of R_t 31.9 min (**10**; 3*R*,1′*R*) and 31.3 min (3*S*, 1′*R*).

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