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Chromones and anthrones from *Aloe marlothii* and *Aloe rupestris*

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Abstract

A phytochemical investigation of the leaf exudate of *Aloe marlothii* has resulted in the isolation of a new chromone (7-*O*-methylaloeresin A) and a new anthrone (5-hydroxyaloin A 6′-*O*-acetate). Furthermore 7-*O*-methylaloesin was isolated as a natural product for the first time from the leaf exudate of *Aloe rupestris*. The structure elucidation of these compounds was based on spectral data including 2D NMR. The chemotaxonomic value of 7-*O*-methylaloesin in *Aloe* series *Asperifoliae* and section *Pachydendron* is discussed. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Aloe marlothii* Berger; *Aloe rupestris* Bak.; Asphodelaceae; Chromone; Anthrone; 5-Hydroxyaloin A 6′-*O*-acetate; 7-*O*-Methylaloesin; 7-*O*-Methylaloeresin A; Chemotaxonomy

1. Introduction

Previously, we have reported the isolation and structure determination of anthrones and oxanthrones from South African *Aloe* (Dagne et al., 1996, 1997, 1998). Investigations on *A. marlothii* and *A. rupestris* have now resulted in the isolation and characterization of five compounds, of which three are new. *Aloe marlothii* is a large single-stemmed plant occurring in South Africa, Botswana, Mozambique and Zimbabwe. It was used in the past as a source of drug aloes but it is no longer commercially used. *A. rupestris* is also widely distributed in Southern Africa and has become a popular garden plant (van Wyk and Smith, 1996). Previous HPLC analysis has shown that aloesin and aloeresin A occur in the leaf exudate of *A. marlothii* (van Der Bank et al., 1995). However to date there is no phytochemical report on *A. rupestris*.

2. Results and discussion

2.1. Structure elucidation of compounds 1–3

Compounds **1**–**5** were isolated as described in Section 3. Compound **1** was obtained as a yellow amorphous substance. The HR-positive FAB mass spectrum of compound **1** gave a pseudomolecular ion at *m*/*z* 555.1854 $[M+H]^+$, corresponding to the molecular formula $C_{29}H_{30}O_{11}$ (calcd 555.1866). Compound **1** exhibited absorption maxima in its UV spectrum that are typical of C-glucosylated 5-methylchromones (Sen and Bagchi, 1959). Analysis of the ^{1}H NMR spectrum of **1** indicated the presence of two singlets at δ 6.15 and δ 6.82 assignable to olefinic (H-3) and aromatic (H-6) protons respectively. The presence of a coumaroyl unit as part of the molecule was evident from the ^{1}H and ^{13}C NMR (see Table 1) spectral data. Further evidence was obtained from its EI-mass spectrum, which shows the presence of coumaroyl fragment ions at *m*/*z* 147 and 119. The NOESY correlation between H-6 and OMe in **1** confirmed the placement of the latter at C-7. The position of the coumaroyl group at C-2′ of the glucose moiety follows from the downfield shift of the signal of H-2′ in comparison with that for the corresponding proton in **3** (i.e. from δ 5.65 in **1** to δ 3.25 in **3**). Furthermore hydrolysis of compound **1** under acidic conditions gave compound **3**. From the data presented above, the structure of **1** was therefore deduced as 7-*O*-methylaloeresin A.

Compound **2** was obtained as a yellow amorphous substance. The negative-ion FAB mass spectrum of **2** showed a pseudomolecular ion at *m*/*z* 475 $[M-H]^-$, suggesting the molecular formula $C_{23}H_{24}O_{11}$, which was consistent with ^{1}H and ^{13}C NMR spectral data (Table

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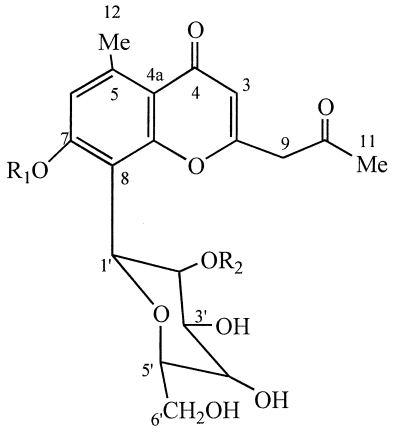
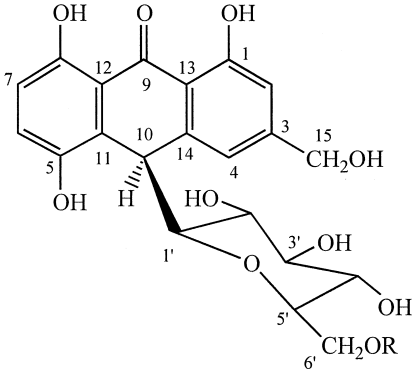


1: $R_1 = Me$; $R_2 = p$-coumaroyl
3: $R_1 = Me$; $R_2 = H$
5: $R_1 = H$; $R_2 = H$



2: R = Ac
4: R = H

2). The IR spectrum exhibited absorptions due to OH groups (3373 cm^{-1}), unconjugated ester carbonyl (1724 cm^{-1}) and chelated carbonyl (1636 cm^{-1}). The ^1H NMR spectral data of anthrone **2** revealed two H-bonded phenolic OH singlets at 11.36 and 11.74 ppm. The ^1H and ^{13}C NMR (see Table 2) spectral data of **2** were similar to those of **4** except for the signals due to the presence of an acetyl moiety in **2**. Acid hydrolysis of **2** yielded the known 5-hydroxyaloin A (**4**). The downfield shift of the signals corresponding to the C-6′-methylene protons in **2** (from δ 3.45, 3.58 in **4** to δ 3.81, 4.14 in **2**) is in agreement with the esterification of this hydroxyl group, thus placing the acetate unit at C-6′ of the glucose moiety. These data along with the ^1H–^1H COSY and NOESY are in accord with 5-hydroxyaloin A 6′-*O*-acetate (**2**) as the structure for the new compound.

Compound **3** was obtained as colourless needle-shaped crystals from MeOH. The positive ion FAB mass spectrum of compound **3**, showed a pseudomolecular ion peak at m/z 409 $[M+H]^+$. This together with ^1H and ^{13}C NMR data including DEPT (Table 1) was in agreement with the molecular formula $C_{20}H_{24}O_9$. The IR, UV ^1H and ^{13}C NMR spectra of compound **3** revealed close similarity with aloesin (**5**), with the difference being the additional signals in the ^1H and ^{13}C NMR spectra of **3** for the OMe group. Assignments for the other protons are given in Table 1. Thus, the structure of **3** was established as 7-*O*-methylaloesin, which was further confirmed by methylation of aloesin with diazomethane to give a substance identical to **3** (co-TLC, FAB-MS).

This is the first report of **3** as a natural product. It was previously made by methylation of aloesin with CH_2N_2 and described as "bright yellow solid obtained as foam which would not crystallize" mp 121–122°C (Haynes and Holdsworth, 1970) and 122–124°C (Speranza et al., 1986). In our case the crystalline natural product **3**, gave a higher mp, i.e. 134–136°C. The difference may be due to the fact that **3** was obtained as needle crystals.

Table 1
NMR assignments for compound **1** (in Me_2CO-d_6) and **3** (in MeOH-d_4)[a]

	1		**3**	
Atoms	δ_H	δ_C	δ_H	δ_C
2	–	162.5	–	160.8
3	6.15 *s*	113.2	6.20 *s*	113.0
4	–	180.7	–	178.9
4a	–	116.0	–	115.9
5	–	144.3	–	141.3
6	6.82 *s*	113.1	6.98 *s*	111.9
7	–	161.9	–	160.3
8	–	113.3	–	113.1
1a	–	159.7	–	157.3
9	3.71 *s*	49.9	3.35 *s*	47.8
10	–	203.8	–	202.5
1	2.31 *s*	31.1	2.33 *s*	30.0
12	2.72 *s*	24.2	2.84 *s*	23.0
7-OMe	3.91 *s*	57.6	3.99 *s*	56.5
1′	5.13 *d* (10.2)[b]	72.8	4.98 *d* (9.8)	72.9
2′	5.65 *t* (9.6)	73.9	3.25	71.1
3′	3.71 *t*	78.6	3.45	79.1
4′	3.43 *m*	72.8	3.32	70.7
5′	3.51 *m*	82.9	3.36	81.8
$6'_a$	3.88 *dd*	63.9	3.85 *dd*	61.8
$6'_b$	3.46 *dd*	63.9	3.40 *dd*	61.8
1″	–	167.3		
2″	6.04 *d* (15.6)	114.3		
3″	7.32 *d* (15.6)	146.1		
4″	–	127.6		
5″, 9″	7.43 *d* (8.4)	131.7		
6″, 8″	6.84 *d* (8.4)	117.5		
7″	–	161.4		

[a] Signal assignments are based on ^1H–^{13}C COSY spectrum.
[b] Coupling constants (J in Hz) in parentheses.

Table 2
NMR assignments for compound **2** (in Me_2CO-d_6)[a]

Atoms	δ_H	δ_C
1	11.74 *s*	161.6
2	6.91 *d* (0.8)[b]	113.1
3	–	151.3
4	7.10 *d* (0.8)	116.9
5	–	146.8
6	6.79 *d* (8.8)	116.7
7	7.19 *d* (8.8)	124.8
8	11.36 *s*	156.2
9	–	194.9
10	4.83 *d* (2.0)	39.8
11	–	117.6
12	–	127.1
13	–	116.8
14	–	145.2
15	4.71 *s*	64.0
1′	3.25 *dd*	84.5
2′	3.12 *t*	71.2
3′	3.34 *t*	78.0
4′	2.83 *t*	72.3
5′	3.07 *m*	78.1
$6'_a$	4.14 *dd* (11.6, 2.0)	63.6
$6'_b$	3.81 *dd* (11.6, 6.8)	63.6
$OCCH_3$	–	171.1
$OC\underline{C}H_3$	2.29 *s*	20.0

[a] Signal assignments are based on ^1H-^{13}C COSY spectrum.
[b] Coupling constants (*J* in Hz) in parentheses.

2.2. Chemotaxonomic values of compounds *1–3*

A chemotaxonomic study of the genus *Aloe* (Viljoen, 1999) included a population study of *A. marlothii*. Leaf exudates of 50 individual plants from each of 54 populations (2700 samples) were studied by TLC revealing significant variation in both the chromone and anthrone compositions. Some individuals in a single population are observed to produce the anthrone isomers homontaloin A and B, while others accumulate aloin A and B. This mosaic pattern of variation (aloin and homonataloin individuals within a single population) seems to be erratic without any geographical correlation, and is repeated in all populations of *A. spectabilis*, indicating a chemical identity with *A. marlothii* (Viljoen and van Wyk, 1996). The 7-*O*-methylaloeresin A and 5-hydroxyaloin A 6′-*O*-acetate chemotype of *A. marlothii* described here seems to be restricted to a single population in the Northern Province of South Africa. The presence of **2** suggests that at least part of the chemical complexity is due to various acetate derivatives, as was shown for *A. succotrina* (Rauwald and Diemer, 1986) and for *A. littoralis* (Dagne et al., 1996).

In a chemotaxonomic study of 380 species of *Aloe* (Viljoen, 1999) **3** was detected in 40 species. An interesting chemotaxonomic pattern of **3** is its presence in most species of *Aloe* series *Asperifoliae* Berger (Viljoen et al., 1996) and in four species of *Aloe* section *Pachydendron* Haw. The *Asperifoliae* is considered to be a southern clade of tropical origin (Viljoen et al., 1996), with *A. littoralis* Bak. as the basal species. It is interesting to note that the presence of **3**, in *A. littoralis* supports this hypothesis. In the section *Pachydendron*, **3** is found only in *A. africana* Mill., *A. excelsa* Berger, *A. rupestris* and *A. thraskii* Bak. The latter three species are known to be very closely related (Reynolds, 1950, 1966) so that the chemical similarity comes as no surprise. These results also indicate that *A. africana* is closely associated with these species.

3. Experimental

3.1. General

Mps: uncorr.; Optical rotation: MeOH; UV: MeOH; IR: KBr discs; ^1H NMR and ^{13}C NMR (Bruker AMXR 300, 300 and 75 MHz, respectively): Me_2CO-d_6 and MeOH-d_4 with TMS as int. standard; FAB-MS (Finnigan MAT 95Q double focussing MS with cesium gun): glycerol matrices; TLC solvent system I (EtOAc–MeOH–H_2O; 77:13:10).

3.2. Plant material

Leaf exudate of *A. marlothii* was collected from plants near the town of Vivo in the Northern Province of South Africa, while exudate of *A. rupestris* Bak. was collected near the town of Muden in Kwazulu-Natal. Both populations of these very distinctive aloes were identified by one of us (BEVW) and there are no other species at the two localities with which *A. marlothii* and *A. rupestris* can possibly be confused.

3.3. Extraction and isolation

Leaf exudate of *A. marlothii* (2 g) was taken up in MeOH, which upon concentration gave 1.6 g of a yellow mixture. This was subjected to flash CC over silica gel eluting with EtOAc and MeOH gradients. The polar frs were further purified by prep. TLC which resulted in the isolation of **1** (120 mg), **2** (95 mg) and **4** (50 mg).

The dried leaf exudate of *A. rupestris* (*ca.* 14 g) when extracted with Me_2CO yielded 6.6 g of acetone soluble portion, 2.2 g of which was subjected to flash CC over silica gel, eluting with CH_2Cl_2 and MeOH gradients. Monitoring the separation with TLC and combining the frs in which a substance with R_f 0.2 (Solvent system I) was the major component, and recrystalization from MeOH gave 260 mg of colourless needle-shaped crystals of **3**. The last two frs were further purified by prep. TLC to yield compound **5** (100 mg).

3.4. 7-O-Methylaloeresin A (*1*)

Yellow amorphous; $[\alpha]_D^{23}$ −93° (*c* 0.01, MeOH); UV λ_{max}^{MeOH} nm: 220, 250, 290; IR ν_{Max}^{KBr} cm^{-1}: 3360, 2932, 1714, 1652, 1597, 1505, 1457, 1383; ^1H and ^{13}C NMR (see Table 1);. +VE HR-FABMS: *m/z* 555.1854 $[M+H]^+$ (calcd 555.1866); EIMS *m/z* (rel. int.): 554 (16), 408 (7), 276 (21), 275 (100), 259 (27), 233 (74), 193 (19), 147 (43), 119 (9).

3.5. 5-Hydroxyaloin A 6′-O-acetate (*2*)

Yellow amorphous; $[\alpha]_D^{23}$ −17° (*c* 0.02, MeOH); UV λ_{max}^{MeOH} nm: 270, 290, 360; IR ν_{max}^{KBr} cm^{-1}: 3373, 2919, 1724, 1636, 1616, 1578, 1465, 1378; ^1H and ^{13}C NMR (see Table 2); -VE FABMS: *m/z* 475 $[M-H]^-$.

3.6. 7-O-Methylaloesin (*3*)

Colourless needle-shaped crystals from MeOH, m.p. 134–136°C; $[\alpha]_D^{23}$ +16.0 (*c* 0.2, MeOH). R_f 0.2 (Solvent system I); UV λ_{max}^{MaOH} nm: 245, 253, 295; IR ν_{max}^{KBr} cm^{-1}: 3278, 1719, 1651, 1597; ^1H and ^{13}C NMR (see Table 1); +ve FAB-MS: *m/z* 409 $[M+H]^+$.

3.7. Known compounds *4* and *5*

The structure of the known compounds **4** and **5** were established, as 5-hydroxyaloin A (Rauwald and Beil, 1993; Dagne et al., 1997) and aloesin (Gramatica et al., 1982) respectively, by comparison of spectral and other physical data with those of authentic samples.

3.8. Acid hydrolysis of *1* and *2*

Compounds **1** and **2** (10 mg each) in MeOH were treated with 1% methanolic HCl (2 ml) and stirred for 8 h at room temp. After the usual work up, compound **1** gave a product (3 mg) identical with **3** (co-TLC and FAB-MS); similarly **2** was converted to **4** (4 mg) (co-TLC, ^1H NMR and FAB-MS).

3.9. Methylation of *5*

Compound **5** (5 mg) was methylated using CH_2N_2 in Et_2O at 0°C by keeping the mixture in ice for 1 h and then allowing it to stand overnight at room temp resulting in a substance (3 mg) identical to **3** (co-TLC, FAB-MS).

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