Aloeresin I, an Anti-Inflammatory 5-Methylchromone from Cape Aloe

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Abstract

A new diglucoside having a 5-methylchromone moiety was isolated from a commercial sample of Cape aloe, the dried exudate from *Aloe ferox* Miller, and named aloeresin I. Its structure was established as **1** on the basis of spectral and chemical evidence. Aloeresin I (**1**) (1 μmol/cm^2) reduces *in vivo* the oedematous response (39%) induced by Croton oil in the mouse ear with the same potency as aloesin, one of the most abundant Cape aloe constituents, and to a higher extent than aloeresin H (**2**). Indomethacin (0.3 μmol/cm^2), the reference anti-inflammatory compound, provokes 61% oedema inhibition.

5-Methylchromones are common metabolites of *Aloe* spp. [1]. Typically, they bear a glucosyl residue at the 8-position which, in turn, is frequently acylated with cinnamoyl residues [1]. Some of them exhibit significant anti-inflammatory [2], [3] and antioxidant [3], [4] activities, thus stimulating the search for new biologically active chromones from natural sources [5]. Continuing our studies on Cape aloe, the dried latex from the leaves of *Aloe ferox* Mill. [6], we describe here the isolation, structural elucidation and evaluation of the anti-inflammatory activity of a new diglucoside having a 5-methylchromone moiety, named aloeresin I (**1**).

Aloeresin I (**1**) was isolated from a commercial sample of Cape aloe in ca. 0.15% yield. Its mono- and bidimensional ^{1}H- and 13C-NMR spectra (Table **1**) were strongly reminiscent of those of aloeresin H (**2**) recently isolated from Cape aloe [5]. Additional signals, assignable to an (*E*)-*p*-coumaroyl group, were also present. That the *p*-coumaroyl group was involved in an ester linkage at the *O*-2 position of the C-19 glucose could be inferred from the marked "acylation effect" observed on the chemical shifts of H-2″, C-1″, C-2″, and C-3″ [7] with respect of those of aloeresin H (**2**) [5] (Table **1**). The D-configuration of the glucose units in **1** was deduced from the base-catalysed hydrolysis to give aloeresin H (**2**) which, in turn, was chemically related to aloesin [5] (conformational studies of aloesin derivatives based on NOE experiments and CD spectra indicated the D-configuration of glucose in aloesin [8]).

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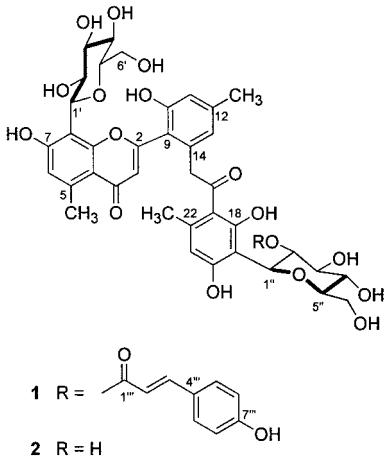

1 R =

2 R = H

The topical anti-inflammatory activity of aloeresin I (**1**) was evaluated using the Croton oil ear test in mice [9] in comparison with that of aloeresin H (**2**) and that of aloesin [10]. The three chromones were found to possess significant anti-inflammatory activities (Table **2**), although lower than that of the NSA reference drug, indomethacin. Aloesin and aloeresin I (**1**) showed similar anti-inflammatory activity, while aloeresin H (**2**), devoid of the *p*-coumaroyl group, exhibited a slightly inferior activity. Concerning aloesin, it can be noticed that its *in vivo* anti-inflammatory activity had not been reported till now, although this chromone represents one of the most abundant constituents of Cape aloe (ca. 20% of the drug) [11]. The mechanism of anti-inflammatory action of aloesin remains to be defined since previous *in vitro* studies [3] did not give significant and clear information: the reported radical scavenging activity [3] seems to be too moderate to completely justify the *in vivo* observed anti-inflammatory properties.

Materials and Methods

1H- and 13C-NMR spectra: Bruker Avance 400 spectrometer; HR ESI MS: Bruker Daltonics FT-ICR APEX-II instrument. Aloesin was isolated from Cape aloe as described in [7].

The commercial Cape aloe used in this investigation was purchased from D. Ulrich Spa (Nichelino, Italy). It was produced in the Port Elisabeth region (Cape Town, South Africa). A voucher specimen (NP-CA 036) is kept at the Dipartimento di Chimica Organica e Industriale, Università di Milano.

Powdered Cape aloe (350 g) was extracted with EtOAc-$CHCl_3$-MeOH as previously described [5]. The extract (40 g) was fractionated by flash chromatography (silica gel 230–400 mesh, 1 kg, 8×40 cm column, EtOAc-MeOH, 1 : 1, 750 mL fractions). Fractions 3–6 were combined and concentrated under vacuum. The residue (ca. 15 g) was further fractionated by flash chromatography (silica gel, 750 g, 8×30 cm column, $CHCl_3$-MeOH gradient from 4 : 1 to 1 : 4, 200 mL fractions). Fractions 8–12 (2.2 g) were flash chromatographed (silica gel, 300 g, 5×30 cm column, 25 mL fractions) eluting with EtOAc-EtOH-H_2O, 100 : 20 : 13, to give aloeresin I (**1**) (fractions 11–23, 800 mg) showing ca. 90% purity by TLC (silica gel 60 F254 aluminium sheet, Merck, EtOAc-EtOH-H_2O, 100 : 20 : 13, Rf: 0.48). Final purification was achieved on a Sephadex LH-20 column (4×30 cm) using MeOH-H_2O, 1 : 1 as eluent (1 drop/3 s, 10 mL fractions). Aloeresin I (**1**) (fractions 35–54, 550 mg) was obtained as an amorphous powder, pure by TLC and analytical HPLC (Merck LiChrospher 100 RP-18 column,

Table **1** NMR data of aloeresin I (**1**) in DMSO-d_6 at 400 MHz (^{1}H) and 100 MHz (^{13}C)[a,b]

Position	*δ_H (J, Hz)*	*δ_C*	*HMBC (H → C)*
2		159.8	
3	6.02 s	114.1[c]	C-2, C-4a
4		178.2	
4a		114.9	
5		139.9[d]	
Me-5	2.69 s	21.8	C-4a, C-5, C-6
6	6.67 s	116.4	C-4a, Me-5, C-8
7		159.4	
8		110.5	
8a		155.1[e]	
9		118.4	
10		155.9[e]	
11	6.69 s	114.8	C-9, Me-12, C-13
12		140.0[d]	
Me-12	2.24 s	20.6	C-11, C-12, C-13
13	6.52 s	121.9	
14		134.8	
15	4.08, 4.13, AB system (16.8)	48.3	
16		202.4	
17		120.3	
18		156.8[e]	
19		107.6	
20		157.9[e]	
21	6.07 s	109.3	C-17, C-19, Me-22
22		136.9	
Me-22	1.83 br s	19.3	
1′	4.71 d (9.8)	73.7	C-8, C-2′, C-3′
2′	3.84 dd (9.8)	71.0	
3′	3.14–3.21 m	78.3	
4′	3.42–3.50 m	69.9[f]	
5′	3.35–3.40 m	81.2	
6′	3.61–3.68 m; 3.73 dd (11.8, 2.0)	60.1[g]	
1″	4.95 d (10.0)	72.5	C-19, C-2″, C-3″
2″	5.17 dd (10.0)	72.4	
3″	3.55 dd (10.0)	75.4	
4″	3.42–3.50 m	69.6[f]	
5″	3.14–3.21 m	80.8	
6″	3.61–3.68 m; 3.42–3.50 m	60.9[g]	
1‴		164.8	
2‴	6.16 d (15.9)	114.3[c]	C-1‴, C-4‴
3‴	7.40 d (15.9)	143.6	C-1‴, C-2‴, C-5‴
4‴		125.0	
5‴, 9‴	7.46 d (8.6)	129.5	C-3‴, C-7‴, C-5‴′
6‴, 8‴	6.78 d (8.6)	115.5	C-4‴, C-7‴
7‴		159.6	

[a] δ in ppm vs. solvent signal as internal reference (DMSO-d_6: δ_H = 2.50, δ_C = 39.50); spectra recorded at 50 °C.

b Signals of glucose hydroxy groups were observed in the range δ = 3.0–5.0 and broad signals of phenolic groups were at δ = 9.93, 9.75, 9.40, 9.32.

[c g] Signals with the same superscript are interchangeable.

Table **2** Anti-inflammatory activity of aloeresin I (**1**), aloeresin H (**2**) and aloesin

Substance	*Dose (μmol/cm²)*	*N° an.*	*Oedema (mg) Mean ± S.E.*	*Oedema inhibition (%)*
Controls	–	10	6.9 ± 0.3	–
Aloeresin I (**1**)	0.3	10	5.2 ± 0.3*	25
	1.0	10	4.2 ± 0.3*	39
Aloeresin H (**2**)	0.1	10	6.4 ± 0.3	7
	0.3	10	5.6 ± 0.4*	19
	1.0	10	4.8 ± 0.3*	30
Aloesin	0.1	10	6.4 ± 0.3*	7
	0.3	10	5.3 ± 0.3	23
	1.0	10	4.2 ± 0.3*	39
Indomethacin	0.3	10	2.7 ± 0.2*	61

* $p < 0.05$ at the Student's *t*-test.

125 × 4 mm, 5 μm; MeOH-H_2O linear gradient from 30 to 90% MeOH in 30 min, 1 mL/min; detector, λ = 225 nm; Rt: 16.4 min); m.p. (uncorrected) 227 – 229 °C (dec); $[\alpha]_D^{20}$: –91.7° (*c* 0.5, MeOH); UV (MeOH): λmax (log ε) = 212 (4.72), 226 (4.66), 254 (4.38) 302 (4.57) nm; IR (KBr): *ν*max = 3392, 2923, 1699, 1648, 1603, 1454, 1380 cm^{-1}; ^1H- and ^{13}C-NMR data, see Table **1**; HR-ESI-MS: *m/z* = 917.2866280 $[M + H]^+$; calcd. for $C_{47}H_{49}O_{19}$: 917.2866558.

The topical anti-inflammatory activity was evaluated as inhibition of the Croton oil-induced ear oedema in mice [9]. Male CD-1 mice (28 – 32 g; Harlan-Italy, Udine, Italy) were kept for one week, before the experiment, at constant conditions of temperature (21 ± 1 °C) and humidity (60 – 70%), with a fixed artificial light cycle (7.00 – 19.00 h). Inflammation was induced on the inner surface of the right ear (surface: about 1 cm^2) of mice anaesthetised with ketamine hydrochloride (145 mg/kg, intraperitoneally; Virbac S.r.l., Milan, Italy) by application of 80 μg of Croton oil (Sigma, St. Louis, Missouri, USA), suspended in 42% aqueous ethanol (v/v) together with the test substances; control animals received only the irritant suspension. Aloeresin I (**1**) was tested at 0.3 and 1.0 μmol/cm^2, while aloeresin H (**2**) and aloesin were tested at 0.1 – 0.3 – 1.0 μmol/cm^2. As a reference, the non-steroidal anti-inflammatory drug (NSA) indomethacin (0.3 μmol/cm^2) (Sigma, St. Louis, Missouri, USA) was used. At the maximum of the oedematous response, six hours later, mice were sacrificed and a plug (6 mm Ø) was excised from both the treated (right) and the untreated (left) ears. Oedema was measured as the weight difference between the two plugs. The anti-inflammatory activity was expressed as percent reduction of the oedematous response in treated mice compared to the control mice. All animal experiments complied with the Italian D.L. n. 116 of 27 January 1992 and associated guidelines in the European Communities Council Directive of 24 November 1986 (86/609 ECC).

Pharmacological data were analysed by Student's *t*-test, accepting as significant a probability level lower than 0.05.

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