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Aloeresin H, a new polyketide constituent of Cape aloe☆

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Abstract—A new constituent, aloeresin H (**1**), was isolated from Cape aloe, a bittering and laxative agent. Its structure was elucidated by degradation experiments combined with 1D and 2D NMR data. Aloeresin H represents the first *C*,*C*-diglucoside discovered in commercial samples of the drug and its polyketide origin can be interpreted in terms of two-chain condensation. Possible conformations of the virtual aglycone were obtained as energy minima by quantum mechanical calculations and were found to be consistent with particular NOE associations observed in the original glucoside.

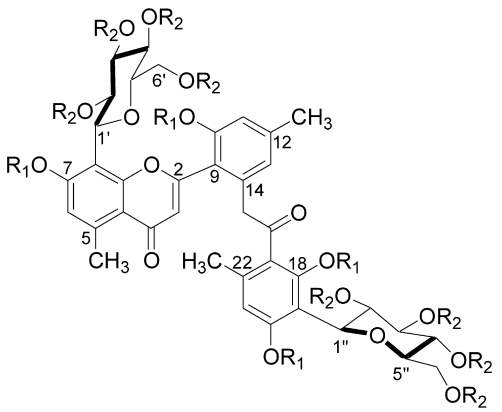
1. Introduction

The drug known as Cape aloe, which is the dried exudate from the cut leaves of *Aloe ferox* Miller (fam. Liliaceae),[1] is widely used for its cathartic properties[2] and as a bittering agent in alcoholic beverages.[3] It has been shown to be a complex mixture of secondary metabolites,[4] synthesized through the acetate-malonate pathway,[5] as well as of a small number of process products.[4b,6] The main natural products so far isolated amount to 50–60% in weight of the drug and belong to the biogenetic families of hexaketides (e.g. 1-methyltetralins),[7] heptaketides (e.g. 5-methylchromones),[8a–f] octaketides (e.g. 1,8-dihydroxy-9-anthrones),[9a] and nonaketides (3-benzylisocoumarins).[10] An additional feature of these compounds is the frequent C-glucosylation,[4a] aloesin (**4**, formerly aloeresin B)[8a,c] being a representative in this regard.

In continuation of our chemical investigation into Cape aloe we report here the structural elucidation of a new constituent we named aloeresin H. This name was suggested by the presence in the molecule of a 8-*C*-β-D-glucopyranosyl-7-hydroxy-5-methylchromone portion (cf. **4**) which is common to all aloeresins (A–G) described so far.[4–8a–f,i] Aloeresin H (**1**) represents the first *C*,*C*-diglucosylated molecule discovered in *Aloe* spp. exudates or extracts.

2. Results and discussion

Aloeresin H (**1**) was obtained as an amorphous pale yellow powder from EtOAc–MeOH–$CHCl_3$ extracts of different commercial samples of Cape aloe (ca. 0.1% yield based on the solid drug) by means of combined chromatographic techniques.



1 $R_1 = R_2 = H$

2 $R_1 = Me$ $R_2 = H$

3 $R_1 = Me$ $R_2 = Ac$

Its positive- and negative-ion FABMS spectra indicated a molecular weight of 770 Da, and the corresponding molecular formula $C_{38}H_{42}O_{17}$ was deduced as follows. By treatment with dimethyl sulfate aloeresin H (**1**) furnished a tetra-*O*-methyl derivative (**2**) which in turn gave an octa-acetate (**3**) by acetylation with Ac_2O/Py (pseudomolecular

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Keywords: aloe; polyketide; 5-methylchromones; conformation; quantum mechanical calculations.

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Table 1. NMR data of aloeresin H (**1**) and its tetramethyl ether (**2**) in DMSO-d_6 at 600 MHz (^{1}H) and 150 MHz (^{13}C)

No. C	**1**		**2**	
	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C
2		159.58		160.08
3	5.98 s	114.30	6.07 s	113.94
4		178.50		178.44
4a		114.97		116.25
5		140.20		141.89
5-Me	2.71 s	22.16	2.76 s	22.53
6	6.70 s	116.50	6.96 s	112.72
7		159.30		161.84
7-OMe			3.89 s	56.28
8		110.50		113.54
8a		155.52		157.19
9		118.32		120.26
10		155.30		159.75
10-OMe			3.81 s	56.28
11	6.72 s	114.80	6.92 s	111.07
12		140.20		141.01
12-Me	2.28 s	20.60	2.32 s	21.18
13	6.58 s	122.80	6.72 s	123.18
14		135.20		134.56
15a	4.27 d (17.2)	48.21	4.15 brs	48.60
15b	4.09 d (17.2)			
16		202.80		202.89
17		120.54		128.58
18		155.52		158.01
18-OMe			3.61 s	63.34
19		109.40		119.40
20		157.32		160.20
20-OMe			3.72 s	55.93
21	6.14 s	109.70	6.59 s	110.63
22		136.30		135.68
22-Me	1.88 s	18.97	1.95 s	18.41
1′	4.71 d (9.7)	73.42	4.72 d (9.8)	72.66[a]
2′	3.81 dd (9.7)	70.51	[f]	70.56[b]
3′	3.13–3.20 m	78.33	[f]	78.99[c]
4′	3.25–3.32 m	70.20	[f]	70.10[b]
5′	3.13–3.20 m	81.12	[f]	81.48[d]
6′	3.56 br d (11.4)	60.02	[f]	61.02[e]
	3.67 br d (11.4)			
1″	4.68 d (9.6)	74.79	4.67 d (10.0)	73.37[a]
2″	3.47 dd (9.6)	71.93	[f]	70.56[b]
3″	3.25–3.32 m	77.77	[f]	78.52[c]
4″	3.25–3.32 m	69.45	[f]	70.77[b]
5″	3.25–3.32 m	81.06	[f]	81.00[d]
6″	3.56 br d (11.4)	60.02	[f]	61.64[e]
	3.67 br d (11.4)			

[a–e] Signals of glucose hydroxyl groups were observed in the range 3.0–5.0 δ and for **1** broad signals of phenolic groups in the range 9.5–10.5 δ. Signals with the same superscripts are interchangeable.
[f] Overlapping signals in the range 3.0–4.4 δ.

ions at m/z 827 and 1163, respectively, in positive FABMS). Mono- and bi-dimensional NMR analyses of aloeresin H (**1**) revealed the presence of two *C*-β-D-glucopyranosyl residues. Proton and carbon-13 signals due to either one of the residues were distinguished by ^{1}H–^{1}H COSY, HMQC and TOCSY[11] experiments (see Table 1).

The values of ^{1}H and ^{13}C chemical shifts and ^{1}H–^{1}H coupling constants belonging to each set of resonances were consistent with two glucose units,[12] both attached to an sp^2 carbon atom through the β-bond (J_{HH} ca. 9.7 Hz)[13] of the anomeric carbon (δ_H 4.68 and 4.71; δ_C 74.79, 73.42).[14] Therefore, the molecular formula $C_{26}H_{22}O_7$ could be assigned to the aglycone (two hydrogen atoms replacing the glucosyl residues). COSY, HMQC, HMBC and differential NOE experiments performed on aloeresin H (**1**) and on its tetra-*O*-methyl ether (**2**) supported the existence of the molecular fragments represented in Figure 1. An additional singlet at δ 5.98 and 6.07 in the ^{1}H NMR spectra of **1** and **2**, respectively, could be assigned to a fifth olefinic proton (see Table 1 for the corresponding ^{13}C chemical shifts). Evidence for a methylene group having readily exchangeable protons was provided by the disappearance of the signals of an AB-system in the spectrum of **1** recorded in DMSO-d_6 (see Table 1) and in acetone-d_6 (δ_A=4.52, δ_B=4.04, J=16 Hz) after D_2O addition. The APT spectrum of aloeresin H (**1**) in DMSO-d_6 (see Table 1) displayed two signals characteristic of carbonyl groups (δ 178.50 and 202.8) and fifteen signals of quaternary sp^2-carbon atoms (including those of fragments **A**, **B** and **C** shown in Fig. 1). Considering that four enolic functions were demonstrated by the conversion of **1** into a tetra-*O*-methyl ether (**2**)-but six oxygen-bearing quaternary carbons were apparent in the δ 150–160 of the APT spectrum-an ethereal oxygen had to be added to the C, H, O atoms previously identified in order to account for the molecular formula of the aglycone.

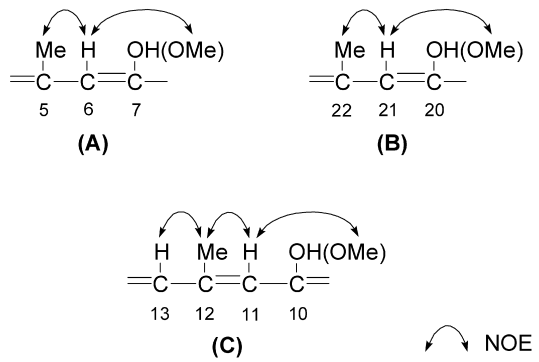


Figure 1. Structure fragments of aloeresin H (**1**) and of its tetra-*O*-methyl ether (**2**) as identified by spectroscopic data (see Table 1 for δ assignments).

A number of spectroscopic data of aloeresin H (**1**) were reminiscent of the 7-hydroxychromone system (Table 2). In particular, the electronic spectrum resembled that of aloesin (**4**) both in its maxima and in the corresponding extinction coefficients, except for the broad absorption band at higher wavelength which appeared to be more prominent than in aloesin. In addition, the chemical shift values of the olefinic proton at the highest field, of the methyl protons at δ 2.71, and of the other atoms of the fragment **A** in Figure 1 (Table 1) were very similar to those of the 7-hydroxy-5-methylchromones occurring in Cape aloe.[8a–f] The significant low field resonance of the methyl group could be attributed to the deshielding effect of the carbonyl group (δ_C=178.50, ν=1651 cm^{-1}) of the chromone nucleus.[8a–g] The indication of a 7-hydroxy-5-methylchromone as a molecular portion connected to the remnant of the molecule through the carbon-2 was corroborated by a chemical correlation between aloesin (**4**) and aloeresin H (**1**) (Fig. 2). It was found that 7-*O*-methylaloesin pentaacetate, predominantly in the 9*Z*-configuration (**5**) as shown by its NMR data, and tetra-*O*-methyl aloeresin H octaacetate (**3**), when subjected to ozonization followed by CrO_3-oxidation, gave the same degradation product **6**, whose structure was confirmed by spectroscopic data.

Table 2. UV data (λ_{max} in nm, log ε in parentheses) of aloeresin H (**1**) and selected reference compounds

	EtOH					0.01 M aqueous NaOH			
Aloeresin H (**1**)	230 infl. (4.37)	244 (4.29)	254 (4.25)		299 (4.22)	260 (4.47)	320 infl. (4.25)	344 (4.43)	
Aloesin (**4**)[a]		246 (4.17)	254 (4.16)		298 (391)	265 (4.25)	316 (3.93)		390[b] (4.32)
7-Hydroxy-2,5-dimethylchromone[c]		242 (4.10)	250 (4.12)		295 (3.97)	258 (4.31)	320 (3.97)		
7-Hydroxy-2-methylchromone[d]		242 (4.24)	249 (4.32)		296 (4.04)				
Resacetophenone (**10**)[e]	231 (3.93)			278 (4.14)	315 (3.91)	256 (3.90)		336 (4.42)	
Orcacetophenone (**11**)[f]	233 (3.88)			282 (3.99)	320 (3.78)			330 (4.07)	

An intense band was present at λ_{max} 210–230 nm (log ε 4.18–4.54) in all spectra.
[a] Lit.[8a] λ_{max} (EtOH) (log ε) 244 (4.14), 252 (4.18), 296 nm (3.90).
[b] Presumably due to enolization of the acetonyl side chain.
[c] Synthesized[8a] and isolated from Cape aloe.[8f]
[d] Ref. 8h
[e] Lit.[17b] λ_{max} (pH 3) (log ε) 228 (3.97), 275 (4.14), 312 nm (3.83); (pH 11) 248 (3.81), 328 nm (4.41); cf. also Ref. 16b,17a,c,d.
[f] Refs. 16b,17a,e.

The presence of a second *C*-glucosylated aromatic ring incorporating the fragment (**B**) of Figure 1 was established by isolation of compound **7** from the reaction mixture of ruthenium-mediated oxidation[15] of aloeresin H tetra-*O*-methyl ether octaacetate (**3**) (Fig. 2). Structure **7** resulted from spectroscopic analyses (see NOE associations in Fig. 2); it was further substantiated by comparison of UV[16a,b] and NMR[16c] data with those reported for 2,4-dimethoxyphenylglyoxylic acids **8** and **9** (see Section 4).

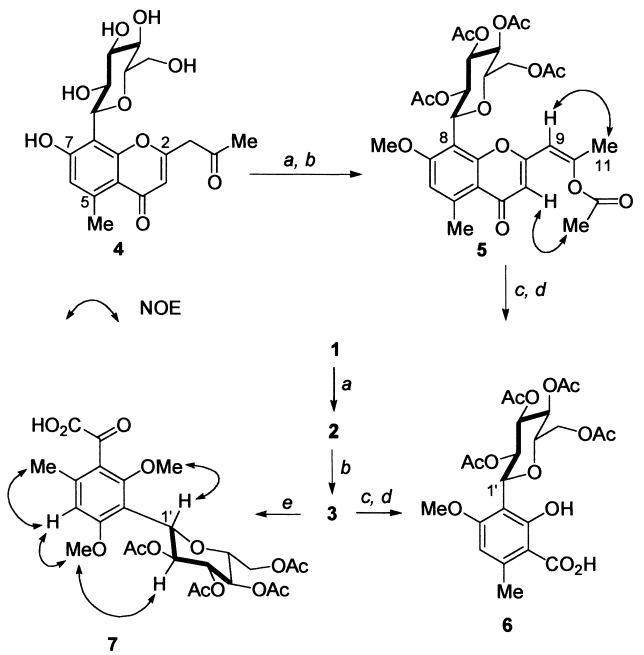
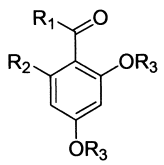


Figure 2. Oxidative degradations of 7-*O*-methylaloesin pentacetate (**5**) and aloeresin H tetra-*O*-methyl ether octaacetate (**3**). Significant NOE associations are indicated. (a) Me_2SO_4, K_2CO_3, DMF–acetone; (b) Ac_2O, Py; (c) O_3, CH_2Cl_2; (d) Jones reagent; (e) $RuO_2{\cdot}nH_2O$, $NaIO_4$, CCl_4–CH_3CN–H_2O (3:3:4).



8 $R_1 = CO_2H$ $R_2 = H$ $R_3 = Me$
9 $R_1 = CO_2H$ $R_2 = R_3 = Me$
10 $R_1 = Me$ $R_2 = R_3 = H$
11 $R_1 = R_2 = Me$ $R_3 = H$

That the two carbon atoms of the side-chain of **7** corresponded to a $-CO-CH_2$-group in the original molecule before oxidative degradation (the carboxylic function of **7** arising from the methylene group) was inferred by a number of spectral analogies between aloeresin H (**1**) and 2,4-dihydroxyacetophenones. In fact, the UV spectra of aloeresin H (**1**), resacetophenone (**10**) and orcacetophenone (**11**) in basic solution all display a broad band at 344, 336 and 330 nm, respectively, due to the marked bathochromic shift of the maxima in the range 280–300 nm, exhibited by these compounds in ethanol solution (Table 2). Furthermore, the ^{13}C chemical shift of the non-chromone carbonyl group of aloeresin H (δ_C=202.80) and the frequency of its IR absorption (broad band centered at 1605 cm^{-1} including phenyl ring absorption) appear similar to those shown by the acetophenones **10**[17d,g] and **11**,[17f] which reflect the characteristic chelation occurring in *o*-hydroxy aromatic ketones and aldehydes.[18] To account for all ^1H and ^{13}C NMR data (Table 1) and the molecular formula, a third benzene ring having the four contiguous carbon atoms of the fragment (**C**) (Fig. 1) and the two remaining quaternary carbons at δ 118.32 and 135.20 had to be inserted into the molecule of aloeresin H (**1**).

In agreement with a biogenetic explanation (see infra), the most reasonable assemblage of the three identified blocks of atoms, each containing an aromatic ring, leads to the formula **1** for aloeresin H. This structure was definitely confirmed by HMBC and NOESY experiments summarized in Figure 3. Among them, the three-bond correlations of H-3 with C-9 and of H-13 with C-15 are of particular relevance to define the connections between the aromatic rings.

The particular NOE associations indicated in Figure 3 can

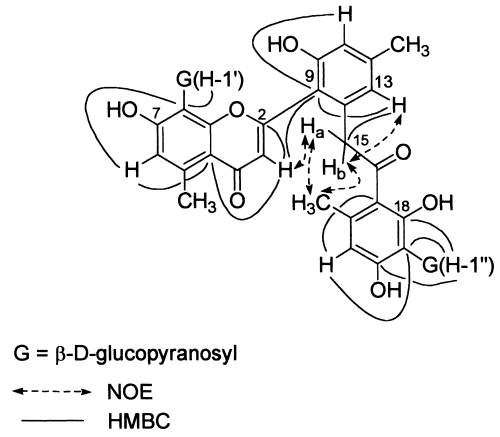


Figure 3. Relevant HMBC and NOE correlations of compound **1** in acetone-$d_6$at 600 MHz (additional NOE correlations are indicated in Fig. 1).

be explained considering the minimum energy structures generated for the virtual aglycone of aloeresin H by quantum mechanical calculations using the program Gaussian 94[19] at the semi-empirical level AM1.[20] Substitution of hydrogen atoms for the two glucose residues (at C-8 and C-19) was assumed to be of negligible influence on the conformational preferences of the molecule core. This is a major assumption, but it seemed to be justified under the circumstances. On the other hand, such a substitution allowed computational efforts to be greatly reduced. Eight minima were found, having energy differences within 1.5 kcal mol^{-1} (see Table 3 in Section 4). Among them, the two conformations pictured in Figure 4 appear to be the most favourable for ^{1}H NOE associations across the methylene group and the aromatic nuclei.

These conformations differ almost exclusively in the sign of the dihedral angle C3–C2–C9–C14 (see Table 3 in Section 4). In both of them the relative chemical shifts of the two methylene protons involved in NOE associations (Fig. 3 and Table 1) are also consistent with their position relative to the the adjacent aromatic ring. Calculated dihedral angles C9–C14–C15–Ha and C9–C14–C15–Hb are ca. 1° and −118°, respectively, in both conformers. Consequently, Ha is almost coplanar with the *m*-cresol ring and therefore more deshielded than Hb. It can be pointed out that the curvatures of the minima are extremely low: normal mode analysis reveals that low vibrational frequencies (about 10 cm^{-1} or less) are associated to torsional movements around C14–C15 and C15–C16. These findings suggest a high flexibility of the molecule with regard to the connection between the 2-arylchromone and orcacetophenone moieties.

3. Origin of aloeresin H

The formation of the aloeresin H molecule can be rationalized in terms of intra- and intermolecular aldol condensations (followed by dehydration) involving two identical decarboxylated heptaketide chains[4b,5] one of which can be replaced by aloesin itself (**4**) in Figure 5. In any case, this hypothesis accounts for the hydroxylation pattern of the aromatic nuclei as well as for the position of the three methyl groups, of the two *C*-glucosyl residues, and of the ketomethylene C_2-unit connecting the *meta*-cresol and the resorcinol rings. Aloeresin H (**1**) could be a process product resulting from thermal condensation reactions between two aloesin molecules (**4**) with concomitant opening of the γ-pyrone ring of one of them. It should be pointed out that the commercial drug is prepared by heating

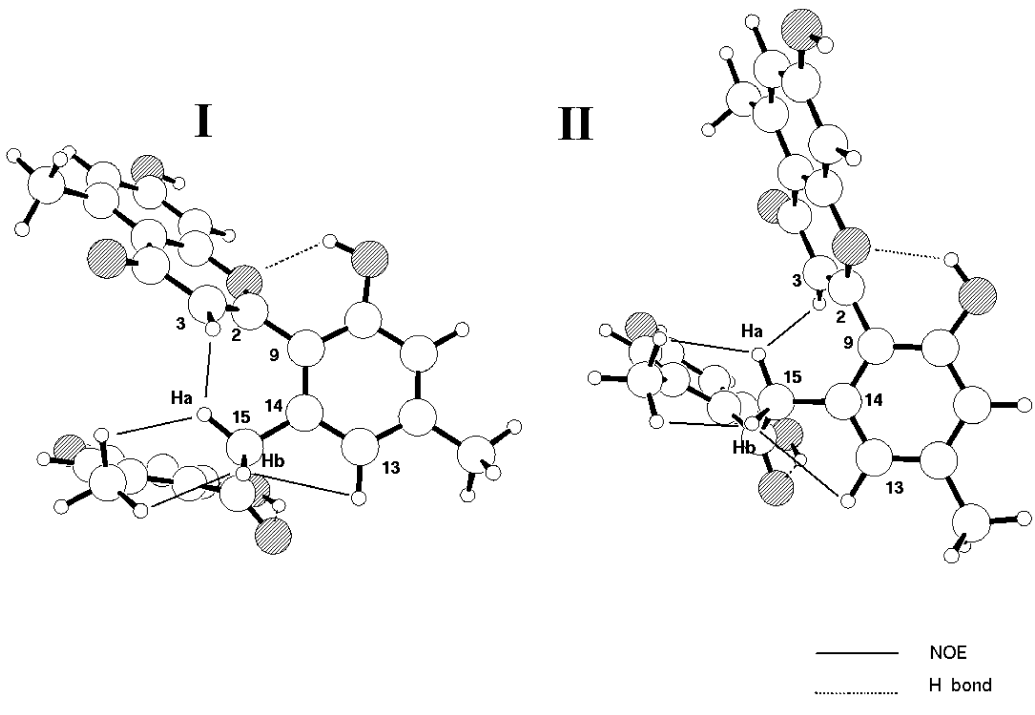


Figure 4. Representative structures of the global minima calculated for the aglycone of aloeresin H (aloeresin H has two glucosyl residue at C-8 and C-19). Relevant NOESY correlations and possible hydrogen bonds are indicated (*cf.* Tables 3 and 4 for geometry and energy data).

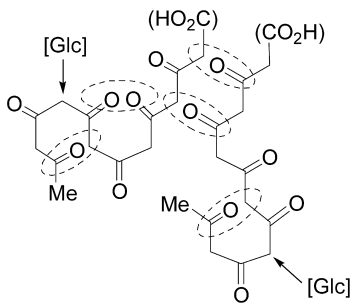


Figure 5. Possible origin of aloeresin H through condensation of two polyketide units.

for a few hours on an open fire the juice collected from the cut leaves of *Aloe* plants.[4b]

4. Experimental

4.1. General procedures

Melting points were determined on a SMP3 apparatus (Stuart Scientific) and are uncorrected. Microanalyses were obtained with a Perkin–Elmer 240 elemental analyzer. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. UV spectra were recorded on a Hewlett Packard 8452A Diode Array Spectrophotometer, CD spectra on a Jasco J-500 instrument, and IR spectra on a Perkin–Elmer FT-IR 1725 X spectrometer. ^{1}H and ^{13}C NMR spectra were acquired at 599.19 and 150.858 MHz on a Bruker DRX-600 spectrometer using the UXNMR software package and at 300.135 and 75.469 MHz on a Bruker AC 300 equipped with an ASPECT 3000 data system. Chemical shifts (δ) are given in ppm and were referenced to the solvent signal: δ_H 2.50, δ_C 39.5; δ_H 3.30, δ_C 49.0; δ_H 2.05, δ_C 30.5 and 205.1 from TMS for DMSO-d_6, CD_3OD and acetone-d_6, respectively. FAB MS spectra were recorded on a VG 7070 EQ mass spectrometer. Analytical TLC was performed on silica gel 60 F_{254} aluminum sheet (Merck) using EtOAc–EtOH–H_2O, 100:20:13 as eluent, unless otherwise stated. Components were detected by spraying with 0.5% Fast Blue B salt, followed by heating at 140°C; preparative TLC on pre-coated silica gel 60 F_{254} plates (1 mm layer thickness, Merck). HPLC was carried out on a Waters Model 600 E liquid chromatograph connected to a HP 1050 Diode-Array detector (Hewlett–Packard); chromatographic conditions: column, LiChrospher 100 RP-18 (5 μm, 125×4 mm, Merck); flow rate, 1 mL/min; detector, λ 225 nm; mobile phase, MeOH–H_2O, linear gradient from 30 to 90% MeOH in 30 min. Silica gel 60, 63–200 μm and 40–63 μm (Merck) was used for column and flash chromatography, respectively. Aloesin was isolated from Cape aloe as reported in Ref. 8d. Resacetophenone was from Aldrich.

4.2. Drug samples

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 is kept at the Dipartimento di Chimica Organica e Industriale, Università di Milano.

4.3. Extraction and isolation

Powdered Cape aloe (1 kg) was extracted with EtOAc–$CHCl_3$–MeOH, 10:1:2 (10 L) with vigorous mechanical stirring for two days at room temperature. After filtration, the insoluble material was taken up with MeOH (1 L), stirred for 3 h, filtered and evaporated under reduced pressure to give a brown syrup (120 g). 40 g of this residue was adsorbed on sea sand and fractioned by flash chromatography (silica gel, 1.5 kg) eluting with EtOAc containing increasing amount of MeOH. Separation was monitored by TLC. Fractions containing aloeresin H (R_f 0.36) were combined, concentrated (5 g) and further purified by flash chromatography (silica gel, 450 g) eluting with EtOAc–EtOH–H_2O, 100:20:13. Fractions were combined on the basis of TLC analysis and evaporated to dryness. The residue (ca. 700 mg) was chromatographed over a Sephadex LH-20 column (250 mL) eluted with MeOH–H_2O 1:1 to give aloeresin H (300 mg) as an amorphous powder, pure by TLC and analytical HPLC (t_R 15.4 min); mp 237–238°C; $[\alpha]_D^{20}$=+29.3 (*c* 0.5, MeOH); UV, see Table 2; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 230 (−0.76), 248 (+0.32), 340 (+0.15); IR ν_{max} (KBr) 1651, 1605, 1499, 1454, 1384 cm^{-1}, ^{1}H and ^{13}C NMR data, see Table 1. FABMS *m/z* (positive mode) 771 $[M+H]^+$, 793 $[M+Na]^+$; (negative mode) 769 $[M-H]^-$. Anal. calcd for $C_{38}H_{42}O_{17}$: C, 59.22; H, 5.49. Found: C, 58.91; H, 5.55

4.3.1. Aloeresin H tetra-*O*-methylether (2). A solution of aloeresin H (**1**) (100 mg, 0.13 mmol) in dry DMF (4 mL) was diluted with dry acetone (15 mL) and treated successively with anhydrous K_2CO_3 (500 mg) and dimethyl sulfate (0.2 mL, 2.1 mmol). The mixture was stirred at room temperature under N_2 monitoring the reaction progress by TLC (*t*-BuOH–MeCN–H_2O, 50:44:6). After 18 h, H_2O (15 mL) was added and the solution heated on a boiling water bath for 20 min, then cooled to room temperature and neutralized with 0.5 M HCl. Removal of the solvent under reduced pressure gave a residue which was dissolved in the minimum amount of water (ca. 5 mL) and desalted by passing through a column of Amberlite XAD-7 (wet volume, 75 mL) eluting with MeOH. Final purification by column chromatography (silica gel, 15 g, AcOEt–EtOH–H_2O, 100:20:5) gave **2** as an amorphous powder (92 mg, 85% yield), pure by TLC (eluent as above, R_f 0.42) and analytical HPLC (t_R 16.8 min); mp 264–265°C; UV (MeOH) λ_{max} (log ε) 216 (4.57), 226 (4.58), 244 (4.50), 250 (4.47), 294 (4.30) nm; IR ν_{max} (KBr) 1647, 1600, 1569, 1458, 1376 cm^{-1}, ^{1}H and ^{13}C NMR data, see Table 1. FABMS *m/z* (positive mode) 827 $[M+H]^+$, 849 $[M+Na]^+$; (negative mode) 825 $[M-H]^-$. Anal. calcd for $C_{42}H_{50}O_{17}$: C, 61.01; H, 6.10. Found: C, 61.33; H, 6.02.

4.3.2. 7-*O*-Methylaloesin pentaacetate (5). 7-*O*-Methylaloesin[8i] (100 mg, 0.24 mmol) prepared from aloesin (4) following the experimental procedure reported above for aloeresin H, was dissolved in dry pyridine (5 mL), cooled to 0°C and treated dropwise with acetic anhydride (10 mL) under N_2. The reaction mixture was allowed to warm to room temperature, stirred for 1 h (TLC control), and then

poured into 100 mL of ice-cold H_2O and extracted with CH_2Cl_2. The combined organic extracts were washed successively with water, 0.5 M HCl, saturated $NaHCO_3$ and with water again, and then dried over Na_2SO_4. Removal of the solvent under reduced pressure and purification by column chromatography (silica gel, 15 g, AcOEt–MeOH, 8:2) yielded **5** (130 mg, 87% yield) as a yellow crystalline solid. TLC, R_f 0.88; mp 80–82°C; ^{1}H NMR (CD_3OD, 300 MHz) δ 1.68, 1.98, 2.03, 2.05 (4×3H, 4×s, 4×CH_3COO), 2.21 (3H, br s, CH_3-11), 2.79 (3H, s, ArCH_3), 3.98 (3H, s, OCH_3), 4.08–4.35 (3H, m), 5.07–5.40 (3H, m) and 5.83 (1H, m) (Glc protons), 6.05 (1H, br s, H-9), 6.43 (1H, br s, H-3), 6.91 (1H, s, H-6); relevant NOE associations, see Figure 2; ^{13}C NMR (CD_3OD, 75 MHz) δ 20.48, 20.94, 21.30, 21.58 (CH_3COO and CH_3-11), 23.77 (ArCH_3), 57.38 (OCH_3), 63.70 (CH_2-6′), 69.52, 70.35, 71.53, 76.39, 77.55 (CH of Glc), 110.68, 111.92 and 133.21 (C-3, C-6 and C-9), 111.00 (C-8 and C-4a), 145.55 (C-5), 157.57, 159.00 and 160.42 (C-2, C-7 and C-8a), 158.14 (C-10), 169.35 (CH_3COO-10), 171.24, 171.77, 172.06 and 172.64 (CH_3COO of Glc), 182.17 (s, C-4); FABMS *m/z* (positive mode) 619 $[M+1]^+$. Anal. calcd for $C_{30}H_{34}O_{14}$: C, 58.25; H, 5.54. Found: C, 57.77; H, 5.39.

4.3.3. Tetra-*O*-methyl aloeresin H octaacetate (3). Acetylation of tetra-*O*-methyl aloeresin H (**2**) was carried out as described above for 7-*O*-methylaloesin. After column chromatography (AcOEt–MeOH, 8:2) compound **3** was obtained (90% yield), pure by TLC (AcOEt, R_f 0.59) and analytical HPLC (t_R 19.5 min); mp 110–112°C; UV (MeOH) λ_{max} (log ε) 208 (4.76), 252 (4.41), 300 (4.28) nm; IR ν_{max} (KBr) 1755, 1652, 1600, 1571, 1458, 1372 cm^{-1}, ^{1}H NMR (CD_3OD, 300 MHz) δ 1.68 (6H, brs), 1.96 (15H, brs), 2.02 (6H, s) (8×CH_3COO and CH_3-22), 2.42 (3H, s, CH_3-12), 2.83 (3H, s, CH_3-5), 3.59 (3H, brs), 3.79 (6H, s), 3.96 (3H, s) (4×OCH_3), 3.72–4.33 (10H, m, Glc protons), 5.07 (1H, brt, J=9.6 Hz), 5.87 (1H, brt, J=9.6 Hz) (H-2′ and H-2″), 5.21 (1H, d, J=9.6 Hz), 5.29 (1H, d, J=9.6 Hz) (H-1′ and H-1″), 6.22 (1H, s), 6.58 (1H, s), 6.79 (1H, s), 6.96 (2H, s) (aromatic protons); ^{13}C NMR (CD_3OD, 75 MHz) δ 19.53, 20.31, 20.64, 20.86, 21.36, 21.94, 23.82, 24.19 (CH_3COO and ArCH_3), 56.60, 57.01 (OCH_3), 63.11, 63.65 (CH_2-6′ and CH_2-6″), 69.80, 70.16, 71.30, 71.73, 72.40, 73.39, 75.98, 76.31, 77.11 (CH of Glc), 109.62, 111.42, 112.54, 115.52, 125.10 (aromatic CH), 111.01, 117.54, 121.39, 130.88, 135.39, 139.16, 143.45, 145.33, 159.36, 159.74, 160.11, 161.81, 162.92, 163.72 (aromatic C), 170.83, 171.06, 171.35, 171.72, 172.27, 172.92 (CH_3COO), 181.64 (*C*O-4), 205.58 (*C*O-16); FABMS *m/z* (positive mode) 1163 $[M+H]^+$, (negative mode) 1161 $[M-H]^-$. Anal. calcd for $C_{58}H_{66}O_{25}$: C, 59.89; H, 5.72. Found: C, 59.51; H, 5.46.

4.3.4. Ozonization of tetra-*O*-methyl aloeresin H octaacetate (3) and of 7-*O*-methylaloesin pentaacetate (5). A solution of **3** (100 mg, 0.086 mmol) in CH_2Cl_2 (10 mL) was ozonized at −60°C until saturation (blue color, ca. 15 min) and then purged with a stream of nitrogen to remove the excess ozone. Cautious removal of the solvent under reduced pressure gave a residue which was dissolved in ice-cold acetone (5 mL) and treated dropwise with Jones reagent until persistence of a brown color. The mixture was then diluted with water (5 mL) and extracted with CH_2Cl_2. The organic extract was washed with water, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by preparative TLC (EtOAc–MeOH, 9:1) to give 5.0 mg of **6** (11% yield). TLC, R_f 0.59; UV (MeOH) λ_{max} 218, 252, 296 nm; ^{1}H NMR (CD_3OD, 300 MHz) δ 1.72 (3H, s), 1.96 (3H, s), 2.03 (6H, s) (4×CH_3COO), 2.59 (3H, s, ArCH_3), 3.84 (3H, s, OCH_3), 3.87 (1H, m, H-5′), 4.12 (1H, dd, J=11.9, 2.2 Hz, H-6′$_b$), 4.21 (1H, dd, J=11.9, 4.8 Hz, H-6′$_a$), 4.98–5.20 (2H, m, H-1′, H-4′), 5.25 (1H, dd, J'=J''=9.6 Hz, H-3′), 5.96 (1H, dd, J'=J''=9.6 Hz, H-2′), 6.30 (1H, brs, Ar-*H*); ^{13}C NMR (CD_3OD, 75 MHz) δ 20.61, 20.66 (CH_3COO), 24.23 (ArCH_3), 56.07 (OCH_3), 63.87 (CH_2-6′), 70.42, 71.19, 73.52, 76.80, 77.20 (CH of Glc), 106.23 (CH), 108.23, 145.76, 162.10, 163.99 (aromatic C), 171.38, 171.45, 171.84, 172.59 (CH_3COO), 176.46 (*C*OOH); FABMS *m/z* (positive mode) 535 $[M+Na]^+$; (negative mode) 511 $[M-H]^-$. Anal. calcd for $C_{23}H_{28}O_{13}$: C, 53.91; H, 5.51. Found: C, 54.15; H, 5.47. This compound was found to be identical to the product obtained by ozonization of **5** under the same reaction conditions.

4.3.5. Preparation of compound 7. To a biphasic solution of tetra-*O*-methyl aloeresin H octaacetate (**3**) (100 mg, 0.086 mmol) in CCl_3–CH_3CN–H_2O, 2:2:5 (12 mL) were added successively with stirring $NaIO_4$ (1.5 g, 7 mmol) and $RuO_2{\cdot}nH_2O$ (5 mg), and the reaction mixture vigorously stirred for 2 days at room temperature. After removal of the precipitated sodium iodate by filtration, and addition of CH_2Cl_2 (5 mL), the two layers were separated. The upper aqueous phase was extracted with CH_2Cl_2 and the combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by preparative TLC (EtOAc–CH_2Cl_2, 1:1) to give 4.0 mg (8.4% yield) of compound **7**. TLC (AcOEt), R_f 0.63; ^{1}H NMR (CD_3OD, 300 MHz) δ 1.75, 1.94, 1.97, 2.02 (4×3H, 4×s, 4×CH_3COO), 2.35 (3H, s, ArCH_3), 3.79, 3.88 (2×3H, 2×s, 2×OCH_3), 3.89 (1H, m, H-5'), 4.14 (1H, dd, J=12.5,

Table 3. Relevant dihedral angles and relative energies of the global minima of aloeresin H aglycone

Conformation	Dihedral angles (values in degrees)				
	C3C2–C9C14	C13C14–C15C16	C14C15–C16C17	C15C16–C17C18	Energies (kcal mol^{-1})
1	55.0	73.1	−123.5	130.0	0.068
2	113.8	74.5	−130.8	130.3	1.450
3	−113.8	34.5	−108.8	125.0	1.098
4	−53.1	34.3	−110.1	125.1	0.321
5 (I)	59.7	−58.4	−117.1	127.4	0.000
6	120.2	−57.7	−114.1	123.5	0.561
7	−113.9	−61.2	−116.7	126.9	0.650
8 (II)	−62.9	−57.6	−127.7	129.3	0.580

Table 4. Relevant internuclear distances in conformers corresponding to global minima of aloeresin H aglycone

Conformation	Internuclear distances (Å)					
	Ha15–H(Me22)	Ha15–H3	Hb15–H(Me22)	Hb15–H13	(O18)H–O16	(O10)H–O1
1	2.285	2.526	2.204	2.535	2.133	2.177
2	2.200	4.764	2.251	3.749	2.130	3.267
3	2.488	4.109	2.274	3.093	2.183	3.257
4	2.469	2.610	2.277	3.083	2.181	2.159
5 (I)	2.254	2.459	2.389	2.703	2.165	2.209
6	2.338	4.010	2.458	2.713	2.191	3.314
7	2.262	3.905	2.400	2.679	2.168	3.261
8 (II)	2.215	2.600	2.272	2.734	2.148	2.247

2.4 Hz, H-6$'_b$), 4.26 (1H, dd, J=12.5, 4.5 Hz, H-6$'_a$), 5.04 (1H, d, J=10.2 Hz, H-1′), 5.15 (1H, dd, J'=J''=9.6 Hz, H-4′), 5.28 (1H, dd, J'=J''=9.6 Hz, H-3′), 5.94 (1H, brdd, J'=J''=9.6 Hz, H-2′), 6.67 (1H, s, Ar-*H*); relevant NOE associations, see Figure 2; ^{13}C NMR (CD_3OD, 75 MHz) δ 20.56 (*C*H_3COO), 22.75 (Ar*C*H_3), 56.65, 62.86 (2×O*C*H_3), 63.32 (CH_2-6′), 69.89, 71.70, 73.84, 76.37, 77.34 (CH of Glc), 111.76 (CH), 118.33, 131.67, 144.74, 160.87, 163.53 (aromatic C), 174.92 (*C*OOH); 170.95, 171.33, 171.79, 172.21 (CH_3*C*OO), 199.78 (*C*O); FABMS *m/z* (positive mode) 577 $[M+Na]^+$; (negative mode) 553 $[M-H]^-$. Anal. calcd for $C_{25}H_{30}O_{14}$: C, 54.15; H, 5.45. Found: C, 54.79; H, 5.36.

4.4. Molecular modelling calculations

The program Gaussian 94[19] at the semi-empirical level AM1[20] was used. For each calculated conformation, all geometrical parameters have been optimized and frequency calculations were performed to verify that stationary points were real minima. Owing to the many very low curvatures shown by minima, thresholds, as defined by the TIGHT option of the Gaussian program, were used in the optimisation. Preliminary calculations on the chromone–cresol and the cresol–orcacetophenone moieties were used to detect the possible torsional angles of the C2–C9 and C14–C15 bonds. Eight conformations were then optimised for the whole skeleton, as reported in Tables 3 and 4.

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