Characterisation of a nataloin derivative from *Aloe ellenbeckii*, a maculate species from east Africa

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Abstract

6′-Malonylnataloin, a malonylated derivative of the rare anthrone nataloin, is characterised for the first time from *Aloe ellenbeckii* A. Berger. Anthrone *C*-glycosides are among a suite of chemical constituents of systematic importance in *Aloe*. The compound is of interest as a putative phytochemical marker for the east African taxa in the maculate species complex.

Article Outline

1. Introduction

The genus *Aloe* L. (Aloaceae) is an exclusively Old World group comprising ca. 400 species, with centres of diversity in southern and east Africa, the Arabian Peninsula and Madagascar (Newton, 2004). The phytochemical constituents and bioactivity of *Aloe* spp. have attracted research interest since the trade in ‘drug aloes’, prepared from the leaf exudate, expanded rapidly in the nineteenth century (Yeats, 1870). Today, the principle sources of these natural products are wild populations of *A. ferox* Mill. in South Africa, and *A. scabrifolia* L.E. Newton & Lavranos, *A. secundiflora* Engl. and *A. turkanensis* Christian in east Africa (Oldfield, 2004). In contrast, *A. vera* (L.) Burm.f., the source of the leaf parenchyma known as ‘aloe gel’, is widely cultivated. Harvesting for the natural products industry is a significant threat which has resulted in all species of *Aloe*, with the exception of *A. vera*, being protected by national as well as international conventions such as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

Besides being of pharmacological importance, the leaf chemistry of *Aloe* spp. bears systematic significance, particularly at the infrageneric rank. Secondary metabolite profiles have been used in the evaluation of infrageneric groups such as series *Longistylae* Berger (Van Heerden et al., 1996), section *Pachydendron* Haw. (Reynolds, 1997), section *Anguialoe* Reynolds and series *Purpurascentes* Salm-Dyck (Viljoen and Van Wyk, 2001). Phytochemical data may offer insights into the maculate species complex, an assemblage of about 40 species so-named for their conspicuous leaf markings. Although it is widely regarded as a well-supported group, infrageneric boundaries and species delimitation in the maculate complex are problematic.
The present investigation yielded a malonylated nataloin derivative, 6′-malonylnataloin (**1**), from *Aloe ellenbeckii* A. Berger (Fig. 1). This compound had previously been detected in *A. ellenbeckii* and several related east African species by high performance liquid chromatography-photodiode array (HPLC-PDA) analysis (Wabuyele, 2006), but remained uncharacterised. Anthrones, particularly *C*-glycosylanthrones, have been recognised for their systematic significance in *Aloe* ([Chauser-Volfson and Gutterman, 1998] and [Viljoen et al., 1998]). In addition to the relevance of **1** as a putative marker for east African taxa in the maculate species complex, it may prove informative regarding affinities with other infrageneric groups in *Aloe*.

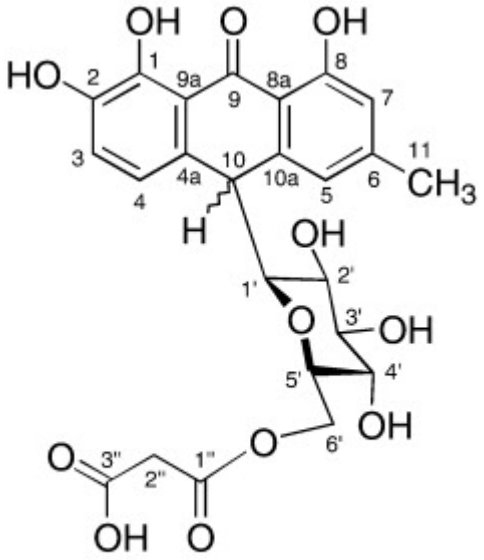

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Fig. 1. 6′-Malonylnataloin from *Aloe ellenbeckii* (anthrone core numbered according to IUPAC).

2. Materials and methods

Whole fresh leaves (992 g) of *A. ellenbeckii*from the Living Collections of the Royal Botanic Gardens, Kew (accession 1973–2107), were thinly sliced and extracted with 2.2 L ethyl acetate for 48 h on an orbital shaker. The extract was filtered through filter paper (Whatman No 1) before and after treatment with sodium sulphate anhydrate, and the solvent evaporated under reduced pressure at 40 °C. The residue (2 g) was dissolved in 20 mL methanol (MeOH), of which an aliquot was subjected to HPLC-PDA and subsequently on-line mass spectrometric analysis (LC-UV-MS).

Analytical HPLC was carried out with a Waters system (600 pump, 717plus autosampler and 2996 PDA detector) and a reversed phase column (Jones Chromatography, Genesis C_{18}, dp 4 μm, 4.6 mm i.d. × 250 mm) at 30 °C. The solvent system comprised a linear gradient of 24–99% MeOH in water, containing 1% formic acid (HCOOH) throughout, over 30 min, followed by isocratic elution for 10 min until re-equilibration of the column, at a flow rate of 1 mL/min. The eluate was monitored between 200 and 500 nm at 1.2 nm resolution. A prominent component eluting at 19.0 min with UV absorption maxima (λ_{max}) 273, 307 and 355 nm was observed. These UV spectral data compared well to those reported by Wabuyele (2006), and those of nataloin ($C_{21}H_{22}O_9$, M_r 418), previously isolated from the leaf exudate of the non-maculate Kenyan species *A. kedongensis* Reynolds [= *A. nyeriensis* var. *kedongensis* (Reynolds) S. Carter] (Conner et al., 1987). The relative molecular mass of the compound corresponding to the component eluting at 19.0 min was deduced from mass spectrometric data, acquired with a Waters Alliance HPLC system coupled with a PDA detector (Waters 2996) and a Micromass ZQ mass detector. A Phenomenex Luna C_{18} column (dp 5 μm, 3 mm i.d. × 150 mm) was used at 30 °C. The mobile phase comprised a gradient of aqueous acetonitrile, 10–100% containing 0.1% HCOOH throughout, over 20 min, followed by isocratic elution for 5 min, at a flow rate of 0.5 mL/min. The eluate was monitored at 200–500 nm, followed by electrospray (ES) and atmospheric pressure chemical (APC) ionisation using an ESCi multiprobe in positive and negative modes. The *m*/*z* values at 505 $[M + H]^+$ and 527 $[M + Na]^+$ in the positive mode, and 503 $[M-H]^-$ in the negative mode, indicated a relative molecular mass of 504. The presence of a free carboxylic acid was indicated by a fragment with *m*/*z* 459 detected in the negative mode $[M-H-CO_2]^-$, as well as marked sharpening of the peak and prolonged retention in the presence of acid (1% HCOOH) during HPLC analysis.

The crude ethyl acetate extract was applied to a polyamide column (30 × 340 mm), packed and eluted with MeOH. Fractions containing a high proportion of **1** were identified by HPLC-PDA analysis, combined and the solvent evaporated under reduced pressure. The residue was re-dissolved in 2 mL MeOH and applied to a column of Sephadex LH-20 equilibrated in MeOH. Nuclear magnetic resonance (NMR) spectral data (1D ^1H, 1D ^{13}C, 1D selective NOE, COSY, HSQC and HMBC experiments) of the combined fractions containing **1** were acquired in deuterated methanol (CD_3OD) at 30 °C on a Bruker Avance 400 MHz spectrometer.

3. Results and discussion

Chemical shift values were referenced from the residual solvent resonances of CD_3OD at 3.31 ppm (^1H) and 49.1 ppm (^{13}C), with respect to TMS. The ^{13}C NMR spectral data and correlations observed in the 2D spectra (Table 1) indicated that **1** contained twelve aromatic carbons including three oxygen-bearing ones, two carbonyl functions (keto and ester groups) and six *O*-substituted sp^3 hybridised carbons, the latter suggesting the presence of a glycosidic residue. Only four protons could be observed in the aromatic region of the 1D ^1H NMR spectrum, comprising two *ortho*-coupled doublets at δ 6.89 and 7.01 ppm and two singlets at δ 6.68 and 6.82 ppm, indicating a highly substituted and/or fused ring system. The methine resonance of C–10 (δ_H 4.43; δ_C), however, showed correlations with two sets of aromatic resonances in the HMBC and selective NOE spectra. Interpretation of long-range correlations, including a weak ^4J coupling from H–4 (δ 6.89) to the C–9 carbonyl carbon (δ_C 195.9), a coupling between H–10 and H–1′ in the COSY spectrum, and NOE connectivities from H–10 to H–4, H–5 and H–1′ led to the 1,2,8-trihydroxy-6-methylanthrone core. The glycosyl residue was identified as a *C*-linked β-glucopyranose from 2D spectra. A further substitution at glucose CH_2–6′, suggested by its downfield-shifted resonances (δ_H 3.85, 4.19; δ_C 65.6), was confirmed by long-range correlations between the methylene protons to an ester carbonyl carbon C–1″ (δ 168.5). Taking into consideration the molecular mass and the presence of a free carboxylic acid, malonic acid was identified as the acylating group. The resonances for protons CH_2–2″ and carbons C–2″ and C–3″ could not be observed in the respective 1D NMR spectra, due to their exchangeable and acidic properties causing resonance broadening ([Hirakura et al., 1997] and [Schliemann et al., 2006]). DMSO-d_6 and pyridine-d_5 caused a rapid colour change of the sample from bright yellow to reddish brown. Attempts to work-up the compound of interest from polyamide column fractions using preparative HPLC were precluded by sample deterioration.

Table 1.
NMR spectral data for 6′-malonylnataloin (**1**) (CD_3OD, 30 °C, δ in ppm, J in Hz)

Position	**δ (^1H)**	**δ (^{13}C)**	**HMBC (H→C)**	**sel. NOE (H→H)**
1		145.9		
2		151.2		
3	7.01 (1H; d; 8.1)	121.2	C–1, 2, 4a	
4	6.89 (1H; d; 8.1)	120.8	C–1[a], 2, 9[a], 9a, 10	
4a		132.2		
5	6.82 (1H; s)	120.8	C–7, 8a, 10, 11	H–10, 11, 1′
6		149.2		
7	6.68 (1H; s)	117.0	C–5, 8, 8a, 11	H–11

Position	δ (^{1}H)	δ (^{13}C)	HMBC (H→C)	sel. NOE (H→H)
8		162.9		
8a		117.1		
9		195.9		
9a		119.3		
10	4.43 (1H; br d; 2.1)	45.0	C–4, 4a, 5, 8a, 9a, 10a, 1′	H–4, 5, 1′
10a		147.8		
11	2.37 (3H; s)	22.2	C–5, 6, 7	
1′	3.26 (1H; dd; 9.5, 2.0)	86.2	C–4a, 10a	
2′	3.07 (1H; m)	71.7	C–10, 1′, 3′	
3′	3.27 (1H; m)	79.7	C–1′, 2′, 4′, 5′	
4′	2.85 (1H; m)	71.9	C–2′, 3′, 5′, 6′	
5′	3.03 (1H; m)	78.8		
6′	4.19 (1H; m) 3.85 (1H; m)	65.6	C–4′, 1″ C–4′, 5′, 1″	
1″		168.5		
2″	nd[b]	nd		
3″		nd		

[a] Weak ^4J correlations.
[b] Not detected.

In spite of these shortcomings, the available evidence indicates that the compound is a new malonylated *C*-glycosylanthrone, 6′-malonylnataloin (= 7-hydroxychrysaloin 6′-*O*-malonate, $C_{24}H_{24}O_{12}$, **1**). This is, to our knowledge, the first report of a malonylated derivative of an anthrone *C*-glycoside in *Aloe*. The known instability of *C*-glycosylanthrones may account for the perceived rarity of nataloin ([Conner et al., 1987], [Chauser-Volfson and Gutterman, 1998] and [Zonta et al., 1995]) and malonylated derivatives in the genus to date.

The distribution of **1** in *Aloe* is of systematic interest. Within the maculate species complex, the compound is restricted to *A. ellenbeckii* and related east African species and may, therefore, serve as a phytochemical marker for them (Wabuyele, 2006). The compound has been detected in few maculate species occurring outside this region but has been observed in non-maculate species as diverse in form and infrageneric position as *A. ciliaris* Haw. (subsection *Macrifoliae*) and *A. vanbalenii* Pillans (subsection

Arborescentes) from South Africa. The findings will be considered with additional characters in a systematic evaluation of the maculate species complex.

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