CHAPTER 5 CHEMOSYSTEMATICS

[1] Supplementary spectral data are provided in Appendix C on the enclosed CD.

Introduction

The succulent-leaved genus *Aloe* L. (Asphodelaceae) is an Old World monocot group of over 500 species occurring throughout Africa, the Arabian Peninsula and western Indian Ocean Islands (Newton, 2001). At least a quarter of *Aloe* species are valued for traditional medicine (Grace et al., 2009) while a small number are wild harvested or cultivated for natural products prepared from the bitter leaf exudate or gel-like leaf mesophyll; *A. vera* is particularly common in cultivation and supports a global natural products industry. The genus has been the subject of considerable phytochemical research during the past century, intended to characterise and authenticate natural products from species in trade and identify their bioactive properties. The purgative effects of *Aloe* leaf exudate ('drug aloes') have been attributed to anthraquinone *C*-glycosides, notably barbaloin and aloins A and B (Reynolds, 1985; Chauser-Volfson and Gutterman, 2004; Steenkamp and Stewart, 2007). The mucilaginous leaf mesophyll ('aloe gel') is rich in acylated polysaccharides possessing anti-inflammatory properties (Steenkamp and Stewart, 2007).

Besides bioactivity, compounds in the leaves and roots of *Aloe* spp. are of potential taxonomic value (Viljoen et al., 1999). Phytochemical characters have been useful in the discussion of a natural (evolutionary) infrageneric classification of *Aloe*, since several groups in the existing classification are seemingly highly artificial, their taxonomy lacking predictive strength. For instance, the presence of bitter phenolic compounds in the brown floral nectar of *A. spicata* L.f., *A. castanea* Schönland and *A. vryheidensis* Groenewald (Johnson et al., 2006) supports their circumscription in the same infrageneric group. At the generic rank, nectar sugar composition has assisted to clarify relationships among *Aloe* and related genera (Van Wyk et al., 1993). Over 200 species of *Aloe* have been chemically characterised (Reynolds, 1985; Dagne et al., 2000; Reynolds, 2004), yet a number of taxonomically problematic groups of species are among those for which chemical data are lacking. One such example is section *Pictae* Salm-Dyck (= section *Maculatae* Baker; series *Saponariae* Berger), a distinctive yet poorly resolved taxonomic entity loosely referred to as the "maculate species complex". In the broadest sense, the section comprises about 40 infraspecific taxa characterised by the shape of the perianth (tubular with a pronounced constriction above a bulbous basal swelling) and patterned leaf surfaces. The adaxial, and sometimes abaxial, leaf surface is densely adorned with conspicuous or obscure white or pale yellow-green spots, which may converge in transverse bands or longitudinal striae. Whilst these morphological characters make representatives of section *Pictae* distinctive, species relationships within the group are

puzzling. To date, chemical characters shared by representatives of section *Pictae* have been evaluated at the supraspecific level only. The presence of isoeleutherol in the roots of maculate taxa has been interpreted as a taxonomic marker for them (Yenesew and Dagne, 1993; Van Wyk et al., 1995), while an anthrone *C*-glycoside was similarly interpreted for East African maculate taxa (Wabuyele, 2006). Here, we report on the first comprehensive comparative study of UV-absorbing leaf constituents in section *Pictae*, and the systematic significance of selected chemical characters in resolving inter- and infraspecific relationships in the group and its sectional circumscription.

Materials and methods

Plant material for chemical analyses was collected from wild populations in South Africa and plants of wild provenance kept in glasshouses at the Royal Botanic Gardens, Kew. Voucher specimens were deposited in the herbarium at Kew (K) and the National Herbarium (PRE) in South Africa (Table 5.1). Mature leaves were removed close to the stem axis and sliced thinly; where possible, the mucilaginous mesophyll was removed. Material was extracted for 24–48h in MeOH, filtered through filter paper (Whatman No. 1) and the filtrate air-dried. Residues were dissolved in MeOH; aliquots were centrifuged for 5–10 min at 100 rpm and the supernatant analysed.

UV-absorbing components in methanolic leaf extracts (50mg ml^{-1} in 50% MeOH) were separated and detected by analytical HPLC (Grace et al., 2008). Chromatograms were extracted at 254 nm and 335 nm; data were recorded with Waters Empower software. UV spectra, retention times (R_t) and the surface areas of peaks showing at least half the UV absorbance of the most-absorbing peak (100%) were recorded from each chromatogram. Peak area, calculated from integrals measured in uV sec^{-1}, was used to quantify the presence of these major components. The relative molecular mass (*M*r) of compounds of interest in leaf extracts was determined by LC-MS (Grace et al., 2008). A Thermo Finnigan Surveyor LC system coupled to a quadrupole ion trap mass spectrometer (Thermo Finnigan LCQ Classic) was used to acquire mass spectral data for compounds of interest. Samples were separated on a column (Phenomenex Ltd., Luna C_{18}, dp 5µm, 4.6 × 150 mm) at 30 °C with a mobile phase comprising a linear gradient of MeOH: water:5 % methanolic acetic acid (t=0, 0:80:20; t=20, 80:0:20; t=27, 0:80:20; t=37, 0:80:20). The eluate was monitored at 200–500nm prior to the positive APCI mode and scanned in the range 125–1200 *m/z*. The most prevalent ions in each scan were isolated and collision induced dissociation (CID) spectra obtained of their ions.

Data were recorded with Thermo Scientific Xcalibur software, and compounds were identified by comparison of UV- and mass spectral data to reference samples and the literature (Viljoen et al., 1998; Viljoen et al., 1999).

Results and discussion

Spectral data indicated that UV-absorbing constituents in 34 representatives of section *Pictae* are of systematic interest (Table 5.2). The present discussion is focused on compounds which may be particularly relevant, the flavones isoorientin and isovitexin and the anthrone *C*-glycoside 6′-malonylnataloin.

Isoorientin (luteolin-6-*C*-glucoside) was the major constituent in leaf extracts of 13 of the 20 species in which it was detected, including the type species for section *Pictae*, *A. maculata* (Table 5.2). It was present in highest concentrations (log_{10} peak area 6.12–6.96 $uVsec^{-1}$) in individuals of *A. umfoloziensis* collected from four populations over an area of approximately 100 km^2 (Table 5.1). Isoorientin was detected in species occurring throughout the pan-African range of the representatives of the maculate species complex. These included the widespread *A. greatheadii* var. *greatheadii*, *A. macrocarpa* and *A. zebrina*, as well as species found only within local regions of high species richness in southern and East Africa.

Isovitexin (apigenin 6-*C*-glucoside) was less prevalent among representatives of the maculate species complex. It was the major constituent in leaf extracts of four southern African taxa (*A. greenii*, *A. parvibracteata*, *A. pruinosa* and *A. striata*), but was absent in the East African species surveyed. The highest concentrations of isovitexin were identified in *A. parvibracteata* (log_{10} peak area 6.1–6.8 $uVsec^{-1}$). Significantly, isoorientin was observed in similarly high concentrations in two populations of *A. parvibracteata* sampled over approximately 120 km^2. Similar concentrations of isoorientin were observed in the closely related and co-occurring *A. umfoloziensis*, but the absence of isovitexin in the latter supports the retention of two separate species. The absence of isovitexin in *A. maculata* also contests the hypothesis that *A. umfoloziensis* is conspecific with the type species of section *Pictae*. The single other taxon in which both isovitexin and isoorientin were recorded was *A. macrocarpa*, a geographically disjunct representative of section *Pictae*.

Table 5.1 Plant material used for phytochemical analysis of *Aloe* section *Pictae*

Taxon (names reduced to synonymy in parentheses)	Voucher number	Origin
Aloe affinis A.Berger	Grace 87	Mac Mac Falls, South Africa
A. amudatensis Reynolds	RBG 1977-6734	Weiwei, Kenya
A. barbertoniae Pole-Evans	Grace 85	Barberton, South Africa
A. branddraaiensis Groenew.	RBG 1957-14502	South Africa
A. burgersfortensis Reynolds	Grace 89	Burgersfort, South Africa
A. burgersfortensis Reynolds	RBG 1965-72105	Lydenburg, South Africa
A. chabaudii Schönland	RBG 1996-1526	Buffel's Drift, Zimbabwe
A. dewetii Reynolds	Grace 83	Alpha, South Africa
A. ellenbeckii A.Berger	RBG 1973-2107	Nairobi, Kenya
A. ellenbeckii A.Berger	RBG 1977-2441	Marsabit, Kenya
A. ellenbeckii A.Berger (*A. dumetorum*)	RBG 1977-3962	Marsabit, Kenya
A. fosteri Pillans	Grace 88	Ohrigstad, South Africa
A. fosteri Pillans	RBG 2003-1796	South Africa
A. grandidentata Salm-Dyck	RBG 1973-2520	Orange Free State, South Africa
A. greatheadii Schönland	RBG 1996-1525	Harare, Zimbabwe
A. greatheadii var. *davyana* (Schönland) Glen & D.S. Hardy (*A. graciliflora* Groenew.)	Grace 67	Tonteldoos, South Africa
A. greatheadii var. *davyana* (Schönland) Glen & D.S. Hardy (*A. longibracteata* Pole-Evans)	Grace 66	Lydenburg, South Africa
A. greatheadii var. *davyana* (Schönland) Glen & D.S.Hardy	RBG 1965-12201	Pretoria, South Africa
A. greatheadii var. *davyana* (Schönland) Glen & D.S.Hardy (*A. davyana*)	RBG 1973-2542	Pretoria, South Africa
A. greatheadii var. *greatheadii*	Grace 58	Louis Trichardt, South Africa
A. greatheadii var. *greatheadii*	Grace 61	Boyne, South Africa
A. greenii Baker	Grace 74	eShowe, South Africa
A. immaculata Pillans	Grace 62	Chuniespoort, South Africa

Table 5.1 *(continued)*

Taxon (names reduced to synonymy in parentheses)	Voucher number	Origin
A. immaculata Pillans	Grace 64	Chuniespoort, South Africa
A. lateritia var. *graminicola* (Reynolds) S.Carter	RBG 1973-2058	Thompson's Falls, Kenya
A. lateritia var. *graminicola* (Reynolds) S.Carter (*A. lateritia* var. *solaiana*)	RBG 1973-2070	Nanyuki, Kenya
A. leptosiphon A.Berger (*A. greenwayi)*	RBG 1967-16201	Abercorn, Zambia
A. lettyae Reynolds	Grace 60	Haenertsburg, South Africa
A. macrocarpa Tod.	RBG 1972-4103	Adamitulla, Ethiopia
A. maculata All.	Grace 82	Ngome, South Africa
A. maculata All.	Grace 84	Carolina, South Africa
A. maculata All. (*A. saponaria* var. *ficksburgensis*)	RBG 1982-268	Ficksburg, South Africa
A. maculata All. (*A. saponaria*)	RBG 1990-1902	Cape Province, South Africa
A. monotropa I.Verd.	Grace 65	Mmafefe, South Africa
A. mudenensis Reynolds	RBG 1947-52506	Natal, South Africa
A. parvibracteata Schönland	Grace 77	Jozini, South Africa
A. parvibracteata Schönland	Grace 78	iNgwavuma, South Africa
A. parvibracteata Schönland	Grace 79	Pongola, South Africa
A. parvibracteata Schönland	Grace 80	Pongola, South Africa
A. petrophila Pillans	RBG 1973-2501	Transvaal, South Africa
A. prinslooi I.Verd. & D.S.Hardy	Grace 68	Colenso , South Africa
A. pruinosa Reynolds	Grace 69	Ashburton, South Africa
A. simii Pole-Evans	Grace 86	White River, South Africa
A. striata Haw.	RBG 1985-4082	Karoo, South Africa
A. suffulta Reynolds	RBG 1961-56203	Mozambique
A. swynnertonii Rendle	Grace 59	Thohoyandou, South Africa
A. swynnertonii Rendle	RBG 1970-2395	Livingstone Falls, Malawi

Table 5.1 *(continued)*

Taxon (names reduced to synonymy in parentheses)	Voucher number	Origin
A. umfoloziensis Reynolds	Grace 71	eShowe, South Africa
A. umfoloziensis Reynolds	Grace 72	eShowe, South Africa
A. umfoloziensis Reynolds	Grace 73	eShowe, South Africa
A. umfoloziensis Reynolds	Grace 75	eShowe, South Africa
A. umfoloziensis Reynolds	Grace 76	eShowe, South Africa
A. vanbalenii Pillans	Grace 81	Nongoma, South Africa
A. vanrooyenii G.F.Sm. & N.R.Crouch	Grace 70	Muden, South Africa
A. vogtsii Reynolds	Grace 57	Louis Trichardt, South Africa
A. wollastonii Rendle (*A. lateritia* var. *kitaliensis*)	RBG 1973-1982	Kitale, Kenya
A. zebrina Baker (*A. ammophila* Reynolds)	Grace 63	Chuniespoort, South Africa
A. zebrina Baker (*A. ammophila* Reynolds)	RBG 1973-2574	Potgietersrus, South Africa

Chemosystematic similarities among *A. greenii*, *A. parvibracteata* and *A. pruinosa* may have biogeographical significance, since these species occur in the eastern sub-tropical savanna regions of South Africa. Indeed, the similarity between *A. greenii* and *A. pruinosa* is unsurprising, as they are remarkably alike in features of gross morphology (leaf shape, flower colour, pruinose flowers). However, these species can be separated by plant size, stem length, surculose growth habit, and the restricted distribution of *A. pruinosa* to the vicinity of Pietermaritzburg in KwaZulu-Natal.

The presence of isovitexin as a major constituent of *A. striata* is noteworthy. Berger (1908) included this species in his concept of the maculate group (series *Saponariae* Berger) on account of its floral morphology, but, due to its striking glaucous leaves with entire, red margins, *A. striata* and its close relatives (*A. buhrii*, *A. karasbergensis*, *A. komaggasensis* and *A. reynoldsii*) have since been recognised in section *Paniculatae* (Reynolds, 1950; Glen and Hardy, 2000). *A. striata* hybridises readily with *A. maculata*, with which it shares a similar range and flowering period (Smith 2003). Future taxonomic assessment may confirm the relationship between *A. striata*, or indeed *Paniculatae* in its entirety, and section *Pictae.* Isovitexin has, however, been detected in several basal infrageneric groups related distantly to section *Pictae*; it is a major constituent of grass-like species of *Aloe* in sections *Leptaloe* A.Berger and *Graminaloe* Reynolds, species with a rambling habit in series *Macrifoliae* A.Berger, as well as species with berried fruits in the segregate genus *Lomatophyllum* (Viljoen et al., 1998).

These are, to our knowledge, the first records of isoorientin and isovitexin in the maculate species complex of *Aloe*. Flavonoids were absent from the few maculate taxa included in a previous screening for this compound class in *Aloe* (Viljoen et al. 1998), while uncharacterised luteolin and apigenin derivatives have been reported in East African maculate taxa (Wabuyele, 2006).

In addition to flavonoids, plicataloside, a naphthalene derivative widespread in *Aloe*, was detected for the first time as a minor constituent of *A. greatheadii*. The co-occurrence of a naphthalene derivative and a flavone (isoorientin, the major constituent detected in *A. greatheadii*) is unusual (Viljoen et al., 1999).

A malonylated anthrone *C*-glycoside of systematic interest was detected in five species included in our survey. The compound, 6′-malonylnataloin (7-hydroxychrysaloin 6′-*O*-malonate), was characterised from *A. ellenbeckii* (Grace et al., 2008) after it was proposed as a

marker for maculate species occurring in East Africa (Wabuyele, 2006). It was the major constituent detected in *A. ellenbeckii*, and was also present in another East African species, *A. lateritia* var. *graminicola.* However, the detection of 6′-malonylnataloin in southern African representatives of section *Pictae*, *A. mudenensis* and *A. vogtsii*, as well as non-maculate species *A. ciliaris* and *A. vanbalenii*, diminished the value of this compound as an informative taxonomic character at the infrageneric level. Indeed, due to the instability of *C*-glycosylanthrones and likelihood of the malonyl moiety being lost during extraction, we surmise that malonylated anthrone *C*-glycosides may be more common in *Aloe* than presently appreciated.

Table 5.2 Isoorientin, isovitexin and 6′-malonylnataloin in *Aloe* section *Pictae* and related species

Infrageneric taxon[a]	Major constituents (++), presence (+) and absence (–)		
	Isoorientin	Isovitexin	6′-Malonylnataloin
Aloe affinis	+	–	–
A. amudatensis	++	–	–
A. barbertoniae	+	–	–
A. branddraaiensis	–	–	–
A. burgersfortensis	–	–	–
A. burgersfortensis	–	+	–
A. dewetii	–	–	–
A. ellenbeckii (*A. dumetorum*)	–	–	+
A. ellenbeckii	–	–	++
A. fosteri	+	–	–
A. fosteri	++	–	–
A. grandidentata	++	–	–
A. greatheadii	–	–	–
A. greatheadii var. *davyana* (*A. davyana*)	–	–	–
A. greatheadii var. *davyana* (*A. verdoorniae*)	–	–	–
A. greatheadii var. *davyana* (*A. graciliflora*)	+	–	–
A. greatheadii var. *davyana*	++	–	–
A. greatheadii var. *davyana* (*A. longibracteata*)	+	–	–
A. greatheadii var. *greatheadii*	++	–	–
A. greatheadii var. *greatheadii*	++	–	–

Table 5.2 *(continued)*

Infrageneric taxon[a]	Major constituents (++), presence (+) and absence (–)		
	Isoorientin	Isovitexin	6′-Malonylnataloin
A. greatheadii var. *greatheadii*	++	–	–
A. greatheadii var. *greatheadii*	–	–	–
A. greenii	–	++	–
A. immaculata	++	–	–
A. lateritia var. *graminicola*	+	–	+
A. lateritia var. *graminicola* (*A. lateritia* var. *solaiana*)	+	–	–
A. leptosiphon	–	–	–
A. lettyae	+	–	–
A. macrocarpa	+	+	–
A. maculata	++	–	–
A. maculata	++	–	–
A. maculata	++	–	–
A. monotropa	–	–	–
A. mudenensis	–	–	++
A. parvibracteata	+	++	–
A. parvibracteata	–	++	–
A. parvibracteata	–	++	–
A. parvibracteata	+	++	–
A. petrophila	–	–	+
A. prinslooi	++	–	–
A. pruinosa	–	++	–
A. simii	++	–	–
A. striata	–	++	–
A. suffulta	–	–	–
A. swynnertonii	–	–	–
A. umfoloziensis	++	–	–
A. umfoloziensis	++	–	–
A. umfoloziensis	++	–	–
A. umfoloziensis	++	–	–
A. umfoloziensis	++	–	–
A. vanrooyenii	+	–	–
A. vogtsii	++	–	+
A. wollastonii (*A. lateritia* var. *kitaliensis*)	++	–	–
A. zebrina (*A. ammophila*)	++	–	–
A. zebrina (*A. ammophila*)	+	–	–

Conclusions

Aloe section *Pictae* is widely considered to be a natural or monophyletic assemblage (Groenewald, 1941) defined by a combination of synapomorphies, including distinctive perianth and leaf characters. While none of the chemical constituents identified in the present study was typical of all representatives of section *Pictae*, the detection for the first time of flavonoids in maculate members of *Aloe* is of systematic interest. The presence of flavonoids have been postulated to be a pleisiomorphic character state, restricted in *Aloe* to basal taxa in which leaf succulence and armature are not pronounced (Viljoen et al., 1998). The presence of flavonoids as the major constituents in maculate species possessing conspicuously succulent and spiny leaves, and apparently still undergoing active speciation (Glen and Hardy, 2000) introduces a new perspective to this discussion. Due to the strong selective pressures exserted on adaptive characters such as secondary metabolites, their sometimes erratic occurrence in a plant group can be accounted for not only by convergence, but also by the loss or silencing of genes coding for a biosynthetic pathway (Wink, 2003). Since a molecular phylogeny has yet to be resolved for *Aloe*, however, these possibilities are equally plausible: maculate species may be derived from basal flavonoid-containing groups in *Aloe*, or the gene encoding flavonoid biosynthesis is differentially expressed in species throughout the genus.

Within section *Pictae*, comparative data suggest that the capacity for isoorientin biosynthesis is common among tropical and sub-tropical representatives of section *Pictae*, while isovitexin is restricted to southern African maculate species. Flavonoid profiles are relevant to the problematic taxonomy, at the species and sectional levels, of this section. The presence of isoorientin in *A. striata* corresponds with the original, broad circumscription of the maculate group (Berger, 1908). It would be informative to test for the presence of isoorientin in the other members of section *Paniculatae*. Whereas 6'-malonylnataloin is typical of East African maculate species, it is not a convincing chemical synapomorphy for section *Pictae* as a whole. From a chemosystematic perspective, the distribution of flavonoids and other UV-absorbing constituents in maculate species of *Aloe* may prove useful in resolving the uncertain classification of section *Pictae*, in particular when assessed against an anticipated molecular phylogeny for the group.

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

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Short communication

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.

Keywords: *Aloe ellenbeckii*; 6′-Malonylnataloin; *C*-glycosylanthrone; Maculate aloes

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; 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*.

2. Materials and methods

Whole fresh leaves (992 g) of *A. ellenbeckii* from the Living Collections of the Royal Botanic Gardens, Kew (accession

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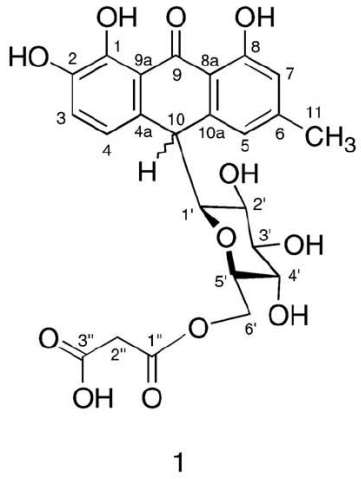

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Fig. 1. 6′-Malonylnataloin from *Aloe ellenbeckii* (anthrone core numbered according to IUPAC).

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 ^{1}H, 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 (^{1}H) 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)

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

Position	δ (^{1}H)	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
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)	65.6	C–4′, 1″	
	3.85 (1H; m)		C–4′, 5′, 1″	
1″		168.5		
2″	nd[b]	nd		
3″		nd		

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

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 ^{1}H 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; 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.

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; 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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