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The chemotaxonomic value of the diglucoside anthrone homonataloside B in the genus *Aloe*

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Abstract

The chemotaxonomic value of the only known diglucoside anthrone from *Aloe*, homonataloside B, is discussed. This compound has been detected in only 14 species in a chemotaxonomic survey of 380 *Aloe* species. The homonataloside B-accumulating species are only found in Africa with none of the Malagasy endemics producing this compound. A summary of the morphological variation is presented together with the taxonomic distribution of this unique anthrone. The representatives of this distinct chemotype occupy disparate positions in the largely artificial hierarchy of the present classification system. Species included in this chemotype provide chemical evidence of hybridisation in *Aloe*.

Keywords: *Aloe*; Aloaceae; Homonataloside B; Chemotaxonomy

1. Introduction

As part of a series of papers emanating from a broad chemotaxonomic screening of almost all species in the genus *Aloe* (Viljoen, 1999) we report here the occurrence of an unique compound, homonataloside B in 14 species in a survey of 380 species. Homonataloside B was isolated from *A. lutescens* (Van Heerden et al., 2001) and

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with the exception of a single species it is always associated with the homonataloin isomers. The presence of this compound illustrates how the chemical patterns encourage a reinvestigation of morphological similarities, often with convincing support for possible taxonomic relationships between species. This compound, like many others identified in a broader study (Viljoen, 1999), is instrumental in defining possible taxonomic relationships between species that have previously not been associated with one another. The value of chemotaxonomic characters in *Aloe* has been emphasised in a series of papers (Viljoen et al., 1996, 1998, 1999, 2001; Viljoen and Van Wyk, 1998, 1999, 2000) and accentuates the need to explore additional characters of taxonomic value in a large genus of ca. 420 species characterised by perplexing morphological variation, hybridisation and a reticulate mode of evolution.

2. Materials and methods

Leaf exudate was collected in situ and at the National Botanical Institute, Pretoria (NBI), Johannesburg Botanical Garden (JBG), National Botanical Garden, Kirstenbosch (NBG), Royal Botanical Gardens, Kew (RBG). Samples from east Africa are from the aloe collections of L.E. Newton, P. Favell and A. Ellert. Species included in the discussion and voucher details are listed in Table 1.

The exudate was investigated with HPLC. Samples were dissolved in methanol and passed through C_{18} cartridges to remove substances of high retention time.

Table 1
Distribution of major leaf exudate compounds co-occurring with homonataloside B[a]

		1	2	3	4	5	6	7	8	9
A. abyssicola Lavranos & Bilaidi	NBI 15813	–	–	+	–	–	–	–	+	–
A. amicorum L.E. Newton	L.E. Newton 3217	–	–	+	+	–	+	–	+	–
A. bargalensis Lavranos	NBI 16949	–	–	+	–	–	+	–	+	–
A. breviscapa Reynolds & P.R.O. Bally	NBI 17034	+	+	+	+	–	+	–	+	–
A. citrina S. Carter & P. Brandham	ex hort P. Favell	+	+	+	+	–	+	–	+	–
A. cryptopoda Baker	A. Ellert 8 & ex hort NBI	+	–	+	–	+	–	–	+	+
A. dhufarensis Lavranos	RBG, Kew 409-77	+	–	+	+	–	–	–	+	–
A. erensii Christian	RBG, Kew 29558	–	+	+	–	–	+	–	+	–
A. krapohliana Pole-Evans	J. Lavranos 29442	+	–	+	–	–	–	–	+	–
A. lutescens Groenewald	JBG 855332 & Kingskloof	+	–	+	–	+	–	–	+	+
A. mendesii Reynolds	NBI 11992	+	–	+	–	–	–	+	+	–
A. molederana Lavranos & Glen	NBI 11194	+	+	+	–	–	+	+	–	–
A. tomentosa Deflers	RBG, Kew 305-70-02870 & NBI 21758	+	+	+	–	–	+	–	+	–
A. wickensii Pole-Evans	ex hort NBG & JBG 855336	+	–	+	–	+	–	–	+	+

[a] 1 = aloesin, 2 = 7-*O*-methylaloesin, 3 = homonataloside B, 4 = aloeresin A, 5 = 3′-*O*-coumaroylaloesin, 6 = aloeresin D 7 = aloin A, B, 8 = homonataloin A, B and 9 = 3′,6′-di-*O*-coumaroylaloesin.

These purified samples were dissolved in methanol–water (1 : 1) and injected into the HPLC system. Operating conditions were as follows: A Phenomenex IB-Sil column was used (C_{18} reverse phase, 5 μm particle size, 250 × 4.6 mm internal diameter, flow rate 1 ml min^{-1}, 20 μl sample loop). The solvent system comprised 30–60% linear gradient of methanol in water over 25 and 3 min isocratic, 100% in 2 and 4 min isocratic. Detection was by diode array detector, using two channels (A set at 275 ± 70 nm; B set at 365 ± 40 nm). Compounds were identified by comparison of the retention times and UV/VIS spectra with reference samples. Authentic reference samples were available through previous studies; aloesin, aloeresin A and D were supplied by Prof G. Speranza (isolated from Cape aloes), 7-hydroxyaloesin (from *A. rupestris*) and aloin (from *A. megalacantha*) were supplied by Prof. E. Dagne while homonataloin, isolated from *A. speciosa* was used as reference standard. Homonataloside B, 3′-*O*-coumaroylaloesin and 3′,6′-di-*O*-coumaroylaloesin were isolated from *A. lutescens* as novel compounds (Van Heerden et al., 2001).

3. Results and discussion

3.1. Leaf exudate chemistry

The HPLC profiles of the leaf exudates of a selection of species accumulating homonataloside B are shown in Fig. 1 and a summary of the results is given in Table 1. Homonataloside B, 3′-*O*-coumaroylaloesin and 3′,6′-di-*O*-coumaroylaloesin were isolated from *A. lutescens* (Van Heerden et al., 2001). Initially the monocoumaroyl ester present in *A. lutescens* was incorrectly identified as aloeresin A. However, the structure of the dicoumaroyl ester of aloesin was unequivocally established as 3′,6′-di-*O*-coumaroylaloesin, and, as it was considered unlikely that the 2′-ester would co-occur with a 3′,6′-diester, the structure of the monoester was reinvestigated. Careful inspection of the NMR data of the monoester revealed that the compound was indeed 3′-*O*-coumaroylaloesin. As can be expected, the UV spectra and HPLC retention times of this compound and aloeresin A are virtually identical.

Four species included in this chemotype also show the presence of aloeresin A (based on R_t and UV absorbance). It is possible that this could well be 3′-*O*-coumaroylaloesin, but as 3′,6′-di-*O*-coumaroylaloesin is not present in these two species it would be at least provisionally correct to identify this compound as aloeresin A, which is very common in the leaf exudate of *Aloe* species. All species in this group, except for *A. molederana*, contain the anthrone isomers homonataloin A and B.

The rare exception of aloin co-occurring with homonataloin is found in *A. mendesii*. These two pairs of anthrones are mutually exclusive and were only found to co-occur in three species in our greater survey of 380 species.

Homonataloside B, the defining compound for this group is the only diglucoside anthrone reported from *Aloe*, a chemical anomaly implying a possible taxonomic value.

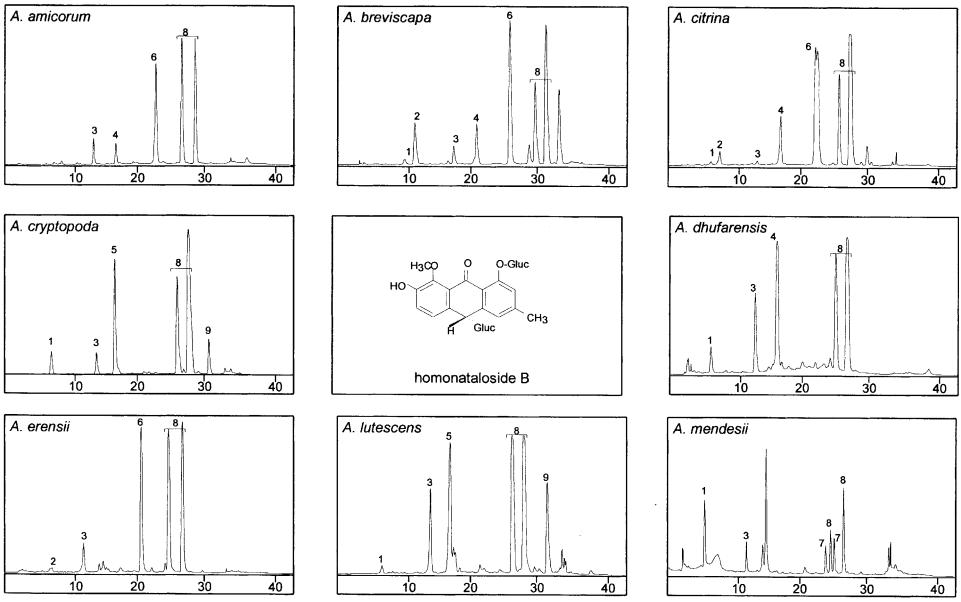

Fig. 1. HPLC chromatograms of eight species containing homonataloside B. Number of peaks corresponds to compounds in Table 1. The chemical structure of homonataloside B is shown in the centre block.

3.2. Morphological characters

The salient morphological characters are briefly discussed below.

3.2.1. Habit characters (caulescence, branching and orientation)

Wide ranges of habit characters are represented in this morphologically heterogeneous group. The three pendent species (*A. abyssicola, A. amicorum* and *A. mendesii*) usually hang from rock faces. In the case of *A. amicorum* the stem is very long while the other two species produce much shorter stems. None of the species in this group produces tall erect stems with the plants occurring as single individuals. Some of the species occur in groups, while *A. bargalensis* and rarely *A. citrina* form dense groups as a result of suckering.

3.2.2. Leaf characters (orientation, texture, thorns and maculation)

Almost all species bear their leaves in an erect or spreading manner. In some species the leaves are somewhat incurved (e.g. *A. breviscapa*). No species produces leaves that are strongly deflexed and canaliculate. For most species the leaves are smooth with the exception of *A. amicorum* where the leaves have a rough texture and *A. bargalensis* where leaves are characteristically furrowed. With the exception of the *Latebracteatae*-group, these aloes generally lack the prominent large pungent thorns

characteristic for many species of *Aloe*. In some species the leaf margin is completely entire (e.g. *A. molederana*, *A. tomentosa*, *A. dhufarensis* and *A. breviscapa*). The leaves are mostly immaculate except for *A. erensii* and *A. citrina* where the leaves are copiously spotted.

3.2.3. Inflorescence and flower characters

In all species the peduncle is erect except for the pendent species where it is arcuate-ascending. The inflorescence varies from simple (e.g. *A. bargalensis*) to branched (e.g. *A. erensii*). In most species the raceme is cylindrical. *Aloe amicorum* and to a lesser extent *A. erensii*, are exceptions as the flowers are secundly disposed. The bracts and pedicels of these species are generally small except for the species pertaining to series *Latebracteatae* (including *A. krapohliana*) where the bracts and pedicels are larger than the average for this group of species. The flowers vary from being glabrous (e.g. series *Latebracteatae*) to those where the perianth is covered in a conspicuous bloom (e.g. *A. erensii* and *A. breviscapa*) to species where the flowers have a prominent pubescent perianth surface (e.g. *A. tomentosa*, *A. citrina* and *A. molederana*). It is here suggested that these three species with a distinct tomentose perianth and the presence of homonataloside B (*A. citrina*, *A. molederana* and *A. tomentosa*) are related. The hairy perianth is only restricted to a small number of species in the genus. It is unlikely that a hairy perianth together with the diglucoside anthrone, homonataloside B, would have evolved independently in these three species. Although this morphologically unique character (hairy perianth) probably only evolved once it would be presumptuous to suggest that all the species (not discussed here) are related. Being guided by the total leaf exudate composition for these species there is reason to believe that some species with a hairy perianth have been involved in hybridisation events obscuring relationships. This is also clearly demonstrated in Viljoen (1999) where the presence of the 'hybrid compound' 8-*O*-methyl-7-hydroxyaloin has been detected in *Aloe pubescens*, another species with a hairy perianth.

3.3. Taxonomic arrangement and affinities between homonataloside B-containing species as represented in Fig. 2

Visual assessment of the distribution of homonataloside B superimposed on the present classification is disparate and not confined to any infrageneric group using the system of Reynolds (1950, 1966). This could partly be ascribed to the fact that many species containing this unique dianthrone were described after the publications of Reynolds. The taxonomic distribution and affinities between the species are discussed, starting with the *Latebracteatae* group and following in an anticlockwise direction (Fig. 2). All three species placed in series *Latebracteatae* accumulate the characteristic anthrone homonataloside B. It has been debated strongly if these taxa should enjoy species status as the morphological distinction between the taxa seems to be very vague considering the clinal geographical variation (Bullock, 1974; Kamstra, 1975). In his monograph on the tropical aloes Reynolds (1966) created group 11 (series *Latebracteatae pro parte*). Two species, *A. macrosiphon* and *A.*

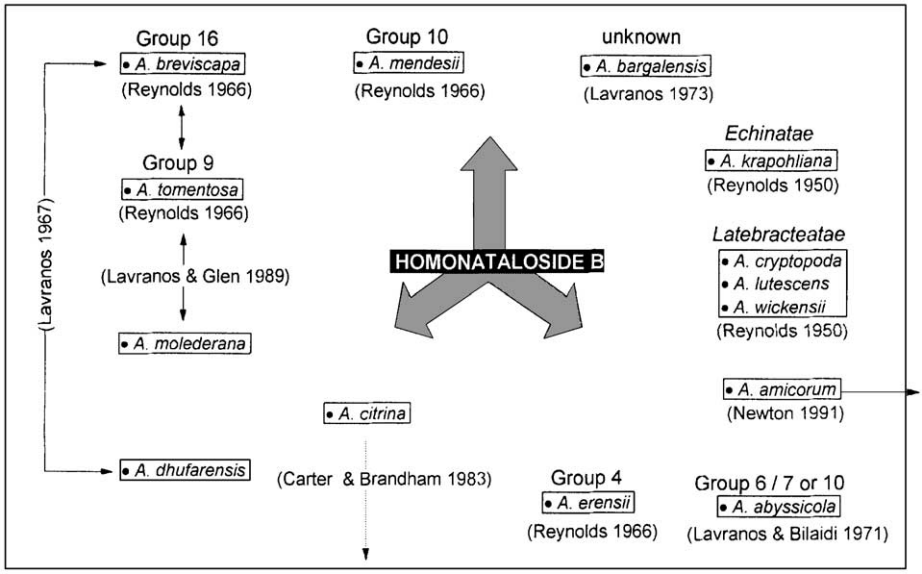

Fig. 2. Taxonomic distribution of the homonataloside-containing species. The groupings of Reynolds (1950, 1966) has been used.

compacta, which are included in this group, were analysed. (These two species have since been reduced to synonym in Carter, 1994.) They were found to be virtually identical in leaf exudate composition when compared to one another, yet they produced a completely different exudate profile compared to that of the *A. lutescens*–*A. wickensii* complex.

Aloe krapohliana is placed together with *A. humilis* and *A. melanacantha* in series *Echinatae*. All three species in this group have very different chemical profiles. *Aloe krapohliana* shows a leaf exudate composition virtually identical to that of *A. lutescens* with which it also shares the presence of peculiar transverse bands that are often found on the leaves of these two species (Viljoen and Van Wyk, 1998). *Aloe humilis* is a flavonoid producing species (Viljoen et al., 1998) while *A. melanacantha* accumulates aloeresin E and F, which are the chemical markers for series *Mitriformes* (Viljoen and Van Wyk, 1999).

In his species description of *Aloe bargalensis*, Lavranos (1973) does not suggest any taxonomic relationships. He regards the leaf character to be unique and characteristic of this Somalian species. *Aloe mendesii* is a pendent species, hence its placement by Reynolds (1966) in group 10 which houses all the pendent to semi-pendent species. Reynolds suggests the closest ally of this species to be *A. veseyi* from Zambia, the leaf exudate of the latter species is very different from that of *A. mendesii*. *Aloe breviscapa* occupies a position in group 16 (Reynolds, 1966), a group containing plants with 'compact rosettes'. Reynolds includes comments, that based on habit and growth characters this species resembles *A. tomentosa*. The latter

species also contains homonataloside B, but is placed in group 9 with all the aloes having a pubescent to tomentose perianth. Lavranos (1967) hints on a resemblance of the floral characters between *A. dhufarensis* and *A. breviscapa*. He also suggests a possible relationship between *A. dhufarensis* and *A. ukambensis*; the latter species is a plicataloside-accumulating species (Viljoen et al., 1999). The Somalian species, *A. molederana* is suggested by Lavranos and Glen (1989) to be distantly related to another homonataloside containing species, *A. tomentosa*. This taxonomic relationship is based on the hairy perianth, which is also characteristic of another homonataloside B-accumulating species, *A. citrina*. The latter species was described by Carter and Brandham (1983) suggesting that it is closely allied to *A. trichosantha*, a relationship based on the pubescent perianth. *Aloe erensii*, with striped flowers is placed in group 4 together with all other species of which the perianth is distinctly or obscurely striped. Most members of group 4 produce an exudate profile containing the isomers, aloin A and B, aloinoside A and B and microdontin A and B (Viljoen and Van Wyk, 2000). *Aloe abyssicola*, another pendent species from Yemen was described by Lavranos and Bilaidi (1971) with the comment "…we find it difficult to assign precise affinities to *A. abyssicola* as indeed none are obvious". The authors do however suggest that this species could find a place in group 10 (Reynolds, 1966) using the pendent habit as diagnostic character. Newton (1991) described another pendent species, *A. amicorum* from Mount Kulal in Kenya, with comments that this species could be related to *A. inermis*. This comment is based on the inflorescence and flower characters which are described as "…quite distinct from other known pendulous species…". In summary, assessing Fig. 2 it is obvious that the distribution of this compound is taxonomically diverse. Being the only dianthrone glucoside known from *Aloe* it seems necessary to search for some measure of taxonomic coherence between the species before dismissing the occurrence of this unique anthrone as a chemotaxonomic coincidence.

The geographical distribution of the homonataloside B producing species is shown in Fig. 3 and is in agreement with other chemogeographical patterns that have emerged previously, i.e. a group of tropical origin with drought adapted species in the southern parts of Africa (Viljoen et al., 1996, 1999).

4. Conclusions

The occurrence of homonataloside B indicates a possible taxonomic relationship between species that have not previously been associated with one another, and suggests taxonomic alignments of species of which the relationships have not been clear. The leaf exudate of one of the species, *A. mendesii* immediately draws attention to caution. It is illustrated in Viljoen (1999) that the co-occurrence of homonataloin and aloin could be indicative of a previous hybridisation event. These two compounds are mutually exclusive as they are probably formed via two different biochemical pathways (Viljoen, 1999). Hybridising an aloin-producing species with a homonataloin-producing species would result in a 'species' accumulating both

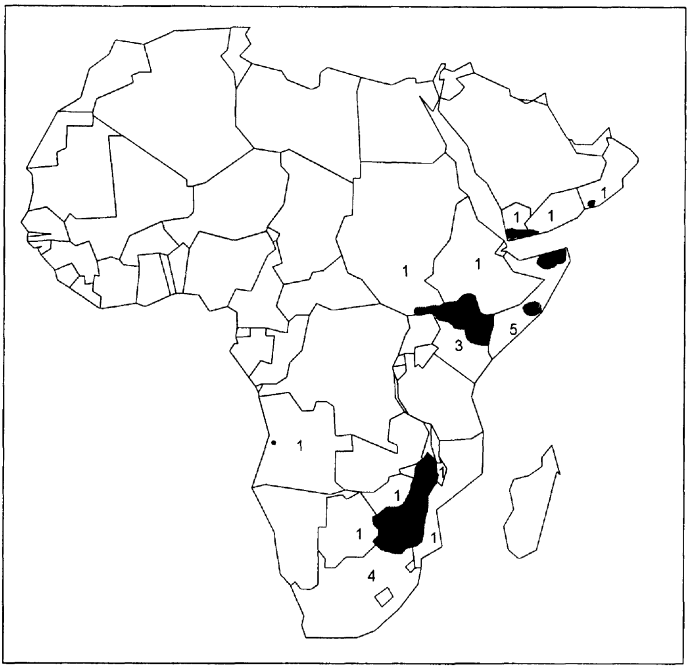

Fig. 3. Geographical distribution of species containing homonataloside B. The number in each country represents the total number of species with the characteristic chemical compound.

anthrones. As hybridisation in *Aloe* often defies morphological detection, chemical patterns have proved to be useful in this large genus where hybridisation and reticulate evolution is rampant. Chemical patterns are progressively making a contribution towards and improved understanding of possible natural relationships in *Aloe*.

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