



The chemotaxonomic significance of the phenyl pyrone aloenin in the genus *Aloe*

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Abstract

The phenyl pyrone, aloenin was positively identified in 16 species in a greater chemotaxonomic study on 380 species of *Aloe*. A large number of species have previously been suggested to be related on the basis of their macromorphological characters. The leaf exudate composition of the 16 species are presented together with a summary of the salient morphological characters. The possible taxonomic relationships between aloenin producing species, not previously thought to be associated with one another are discussed and illustrates the need to explore additional characters of taxonomic value in this large genus of ca. 420 species where no natural classification system exists. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The phenyl pyrones have a restricted distribution in *Aloe* and up to date only five compounds belonging to this chemical class have been isolated from *Aloe*, with aloenin being the most prominent (Suga et al., 1974; Speranza and Dada, 1986; Conner et al., 1987; Woo et al., 1987). This compound occurs in 16 species, i.e. in 4.6% in a survey of 380 taxa. Previous TLC studies by Reynolds (1986, 1990) (P19 in his TLC zones) and Rauwald et al. (1991) documented the occurrence of aloenin in a number of species but the possible value of this compound at the infrageneric level of

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taxonomy is not discussed. An ‘all-encompassing’ chemotaxonomic survey of almost all species of *Aloe* (Viljoen, 1999) has illustrated the valuable contribution of leaf exudate compounds as useful for chemotaxonomic characterization at the infrageneric level (Viljoen et al., 1996, 1998, 1999, 2000; Viljoen and Van Wyk, 1999). The presence of aloenin (always in co-occurrence with aloin) furthermore reveals a possible taxonomic alignment between species which have previously been overlooked. It is generally accepted that the system created by Reynolds (especially his 1966 publication) is based on utility and does not necessarily reflect natural relationships between taxa. Judging by the perplexing range of morphological characters prevalent in *Aloe*, it would seem virtually impossible to suggest natural relationships between taxa without seeking additional taxonomic evidence, the leaf exudate compounds (e.g. aloenin) being one such instrumental character.

2. Materials and methods

Leaf exudate was collected in situ and at the National Botanical Institute, Pretoria (NBI), Royal Botanical Gardens, Kew (RBG) and the Botanische Staatssammlung München (BSM). Samples from east Africa were from the aloe collection of L.E. Newton and other specialists of succulent plant. Species included in the discussion, accession numbers and voucher details are listed in Table 1.

The exudate was investigated with HPLC. Samples were dissolved in methanol and passed through C₁₈ cartridges to remove substances of high retention time. These purified samples were dissolved in methanol–water (1 : 1) and injected into the HPLC

Table 1
Vouchers, accession numbers, locality details and distribution of species containing aloenin

Species	Voucher	Distribution
<i>A. arborescens</i>	3 individuals from 18 populations (see Viljoen, 1999)	South Africa, Mozambique, Malawi, Zimbabwe
<i>A. brachystachys</i>	NBI 17388 (<i>A. schliebenii</i>)	S Tanzania
<i>A. brandhamii</i>	Carter et al. 2600 (type material)	S Tanzania
<i>A. bussei</i>	RBG, Kew 1990–1816 and ex hort P. Favell	NE Tanzania
<i>A. cheranganiensis</i>	ex hort P. Favell and ex hort B. Kemble	Uganda and Kenya
<i>A. classenii</i>	Newton 3910	S Kenya
<i>A. dawei</i>	RBG, Kew 1951–35710 and ex hort BSM	Sudan, Kenya and Uganda
<i>A. dorothaeae</i>	RBG, Kew 295-58-29212 and NBI 17305	NE Tanzania
<i>A. gossweileri</i>	ex RBG, Kew	Angola
<i>A. kedongensis</i>	NBI 11210	Kenya
<i>A. leachii</i>	RBG, Kew 1990–1820	NE Tanzania
<i>A. leptosiphon</i>	RBG, Kew 1990–1812	NE Tanzania
<i>A. monticola</i>	ex hort P. Favell	N Ethiopia
<i>A. nyeriensis</i>	Gil–Gil and Rumuruti	Kenya
<i>A. secundiflora</i>	Newton 4016 and Sebsebe 219	Sudan, Ethiopia, Kenya Tanzania
<i>A. tororoana</i>	ex hort P. Favell	Uganda

system. Operating conditions were as follows: A Phenomenex IB-Sil column was used (C_{18} reverse phase, 5 μm particle size, 250 mm \times 4.6 mm internal diameter; flow rate 1 ml min^{-1} ; 20 μl sample loop). The solvent system comprised a 30% to 60% linear gradient of methanol in water over 25 min, 3 min isocratic, 100% in 2 min, 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*) aloenin (from *A. debrana*), aloin (from *A. megalacantha*) and nataloin (from *A. pulcherrima*) was supplied by Prof E. Dagne.

3. Results and discussion

3.1. Leaf exudate chemistry

The single chemical compound which represents a ‘unifying chemical character’ between the species listed in Table 1 is the phenyl pyrone, aloenin. This compound is confined to a small number of species (16), in a broader chemotaxonomic survey of 380 species of *Aloe*. The total leaf exudate composition for all the species are shown in Table 2 and a selection of HPLC profiles are illustrated in Fig. 1. The chromones,

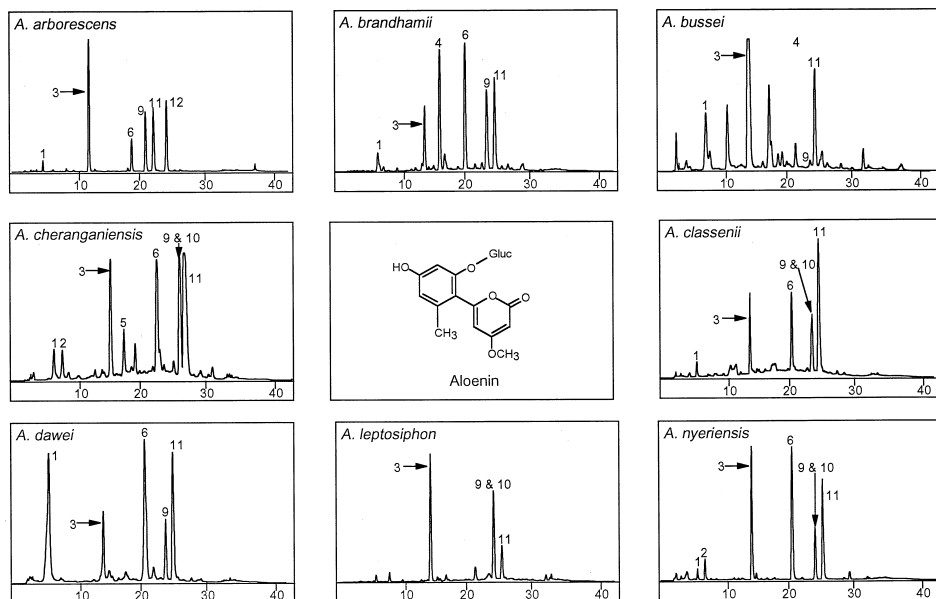


Fig. 1. HPLC chromatograms of eight species containing aloenin. Numbers of peaks correspond to compounds in Table 2. The structure of aloenin (3) is shown in the centre block.

aloesin and aloeresin D occur in a large number of species in this group. These compounds are very widely distributed in the genus and possibly of little taxonomic value. In all, except one species the major anthrone which is accumulated is aloin A and B. The presence of nataloin A and B in *A. kedongensis* is noteworthy as this compound is restricted to only a few species in *Aloe* and seems to be erratic in distribution and thus of limited taxonomic value. It has also been shown by Viljoen (1999) that nataloin is a 'hybrid compound' which is readily synthesized when an aloin and homonataloin species hybridize, thus indicative of the possible ancient hybrid origin of *A. kedongensis* between an aloin- and homonataloin-producing species.

In many of the species included in this group a series of unidentified compounds has been recorded. These compounds all show the same UV absorbance spectra and have not been found in any other non-aloenin-producing species.

Aloe arborescens was widely sampled throughout the southerly part of its distributional range and three individual plants from 18 populations were analysed. The results show that compounds 1, 3, 6, 9 and 11 (Table 2 and Fig. 1) are always present but only certain populations produced the late-eluting chromone which is probably a coumaroyl chromone. Reynolds and Herring (1991) used this variation to demonstrate the South African origin of *A. arborescens* growing in Gibraltar. The same variation study was carried out on *A. nyeriensis* which was collected in Kenya at two separate populations and showed no variation between and within the two populations sampled. The compound 10 ($R_f = 23.61$) shown in Table 2 co-elutes with aloin B and can only be detected by comparing the UV spectra recorded within the time of elution of this 'single' peak.

Aloe gossweileri from Angola has a unique combination of compounds as it is the only species in this survey that accumulated both the aloin and 10-hydroxyaloin B anthrones. The latter compound has been isolated from the widespread *A. littoralis* (Dagne et al., 1996) and Viljoen et al. (1996) illustrated that this compound is a chemotaxonomic marker for *Aloe* series *Asperifoliae*. One is tempted to explain the unlikely combination of compounds as the product of a possible hybridization event between this species in the southern parts of Angola with one of the 10-hydroxyaloin-containing species of *Aloe* which are widely distributed in the south western arid parts of southern Africa.

3.2. Morphological characters

The salient morphological characters for species belonging to the aloenin chemotype are briefly discussed below.

3.2.1. Habit characters (caulescence, branching and orientation)

Using the habit characters the 16 species allow themselves to be clustered into one of four groups:

Group 1: Most species in the aloenin-containing group are distinctly caulescent, occurring in groups (arborescent) and usually not suckering from the base. This group represents all the species included in Reynolds' group 19 (*A. arborescens*, *A. cheranganiensis*, *A. dawei*, *A. tororoana*, *A. nyeriensis*, *A. gossweileri* and *A. kedongensis*).

Table 2
Presence of major leaf exudate compounds of species in the aloenin chemical group^a

R _f (min)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	5.49	8.23	13.95	16.24	16.26	20.03	21.55	23.48	23.61	23.61	24.07	24.12	25.41	26.55
<i>A. arborescens</i>	■		■			■			■		■	■		
<i>A. brachystachys</i>	■		■						■		■			
<i>A. brandhamii</i>	■		■	■		■			■		■			
<i>A. busseti</i>	■		■						■		■			
<i>A. cheranganiensis</i>	■	■	■	■	■	■			■	■	■			
<i>A. classenii</i>	■		■			■			■		■			
<i>A. dawei</i>	■		■			■			■		■			
<i>A. doratheae</i>	■		■	■					■		■			
<i>A. gossweileri</i>	■		■				■		■		■			
<i>A. kedongensis</i>	■	■	■					■			■	■		■
<i>A. leachii</i>	■		■	■		■			■		■			
<i>A. leptosiphon</i>	■		■						■	■	■			
<i>A. monticola</i>	■		■			■			■	■	■			
<i>A. nyeriensis</i>	■		■			■			■	■	■			
<i>A. secudiflora</i>	■		■	■					■	■	■			
<i>A. tororoana</i>	■	■	■						■	■	■			

^a(1) aloesin, (2) 7-O-methylaloesin, (3) aloenin, (4) unidentified compound, (5) aloeresin A, (6) aloeresin D, (7) unidentified anthrone, (8) unidentified compound, (9) aloin B, (10) unidentified compound, (11) aloin A, (12) unidentified chromone, (13) nataloin B, (14) nataloin A.

Group 2: Includes four species which are usually acaulescent and solitary, suggesting that they do not form suckers and hence do not occur in dense groups (*A. brachystachys*, *A. leachii*, *A. monticola* and *A. secundiflora*).

Group 3: Two species; *A. brandhamii* and *A. classenii* are usually shortly caulescent and occur in groups as a result of suckering.

Group 4: The last group consists of three species represented in Reynolds' group 5 (*A. bussei*, *A. dorotheae* and *A. leptosiphon*) which are usually acaulescent and form dense groups resulting from suckering.

The groupings above are almost in complete agreement with the groups suggested by Reynolds (1966), with the exception that the demarcated 'groups' are not considered to be taxonomically allied to one another.

3.2.2. Leaf characters

In general, the leaf characters seem to be of little taxonomic value. In all cases the leaves are smooth and in most cases soft except in the *A. bussei* group, where the leaves are very hard and tough (Lavranos, 1970). Most species in this chemical group bear their leaves in a spreading and deflexed fashion. The majority of these species also have distinct thorns which are firm and pungent in most taxa. *Aloe bussei* and *A. brachystachys* have small teeth which are cartilaginous in the case of *A. bussei*. The leaves are mostly without marking or sculpturing. Only the leaves of *A. dorotheae*, *A. leptosiphon* and variably *A. bussei* together with *A. tororoana* are spotted.

3.2.3. Inflorescence and flower characters (inflorescence structure, perianth shape, perianth markings, flower orientation)

In all the species, the inflorescence is erect and varies from simple to 1–4 branches or a much-branched panicle. The racemes are mostly cylindrical and varies from densely to laxly flowered. In most cases the perianth is straight and cylindrical with the exception of *A. gossweileri* and *A. kedongensis* where the perianth is curved. The perianth of *A. dawei* and *A. secundiflora* is spotted while the perianth of *A. classenii* has a slight surface bloom. In most species the flowers are arranged symmetrically around the floral axis except in the case of *A. secundiflora*, *A. leachii* and *A. brandhamii*, where the flowers are secund on the floral axis.

In summary, aloenin occurs in the taxonomic extremes of the genus. It is found in the tall arborescent, shrubby aloes (group 19) and in a contrasting group of small acaulescent aloes from Tanzania (group 5) demonstrating the disadvantage of creating a 'classification system' using a single macro-morphological character. Although Reynolds' group 5 and group 19 are completely different with respect to habit characters, they share similarities in inflorescence and leaf characters. The perplexing range of morphological characters as illustrated above warrants a search for additional, more conservative taxonomic characters to explore possible phylogenetic relationships within the genus *Aloe*.

3.3. The taxonomic arrangement of aloenin-containing species as represented in Fig. 2

The present taxonomic arrangement of the 16 aloenin-accumulating species are shown in Fig. 2 according to the groupings created by Reynolds (1966). Reynolds included six

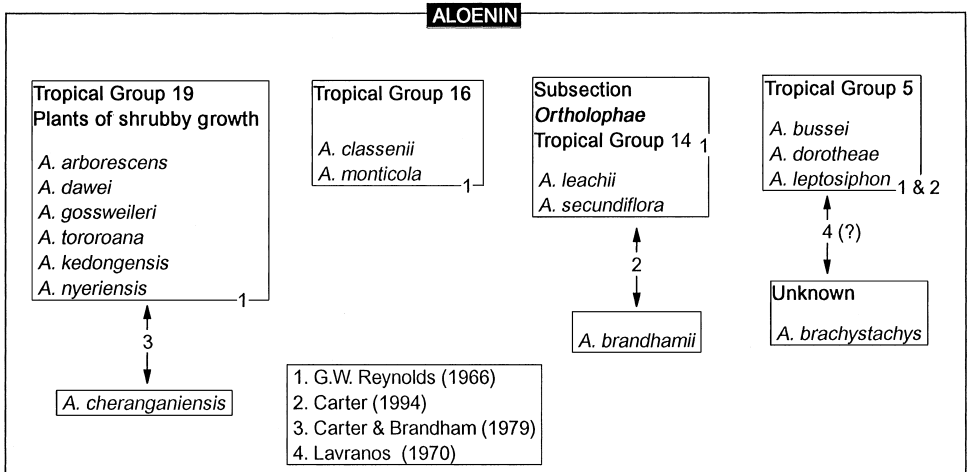


Fig. 2. A taxonomic 'affinity-diagram' showing possible relationships between the taxa as previously suggested. Numbers correspond to literature references where these relationships between the taxa have been discussed.

species in his large group 19, which he defines as 'plants of shrubby growth.' *Aloe cheranganiensis* was later described by Carter and Brandham (1979) and they suggested that this species could be taxonomically related to *A. dawei*, *A. kedongensis* and *A. nyeriensis*. All three species produce aloenin. These taxa are also 'closely arranged' in the Flora treatment of Tropical East Africa (Carter, 1994). It is also interesting to note that *A. nyeriensis* together with *A. elgonica* and *A. ngobitiensis* (Carter and Brandham, 1979) are tetraploids ($2n = 28$), a rare cytological occurrence in *Aloe*. Although no exudate samples could be obtained for *A. elgonica* and *A. ngobitiensis*, Reynolds and Nicholl's (1986) and later Conner et al. (1990) reported aloenin to be present in *A. elgonica*. Two aloenin-producing species are included in Reynolds' group 16; *A. classenii* and *A. monticola*. The other species in group 16 (Reynolds, 1966) are very different and variable in terms of leaf exudate composition (Viljoen, 1999). *Aloe secundiflora* and *A. leachii* are both placed in group 14 which houses all species with secund flowers. Newton (1993) suggests that the secund flower character uniting all species in group 14 probably evolved more than once and that this character does not necessarily reflect phylogenetic relationships. Carter (1994) however suggests an affinity between *A. brandhamii* and *A. secundiflora* var. *sobolifera*, a proposal confirmed on the chemical level.

The last group consists of three species which are placed in G.W. Reynolds' group 5. This group only contains three species which are characterised by the small compact rosettes (they sucker freely to form dense groups) and the inflorescence is usually simple or sparingly branched. These three Tanzanian species, *A. bussei* (syn. *A. morogoroensis*), *A. leptosiphon* (syn. *A. greenwayi*) and *A. dorotheae* are also proposed by Carter (1994) to be closely related. The taxonomic affinities of *Aloe brachystachys*

(previously described as *A. schliebenii*) remain obscure and in the description of this species Lavranos (1970) hints on a possible taxonomic relationship with *A. seretii*, but emphasises that the relationships seems doubtful (no exudate sample could be obtained for *A. seretii*). Lavranos (1970) using inflorescence characters does however suggest that this species could occupy a position in group 5 (Reynolds, 1966). It is meaningful to note that all the species included in group 5 produce the phenyl pyrone, aloenin which is also accumulated in *A. brachystachys*.

4. Conclusions

The chemical pattern in *Aloe* is not random but shows a high degree of correlation with existing evidence of relationships in the genus. The morphological characters in this group, and for the genus as a whole, are extremely variable even within an infrageneric group. This immense variation amplifies the need to study additional characters in an attempt to discover natural relationships within *Aloe*. Although the chemical similarity reported here could be ascribed to convergence, it is just as likely that the 'core group' could well be monophyletic with species participating in recent hybridization events implying that some species in this group may be of paraphyletic origin. Chemical evidence exists to suggest the possible hybrid origins of *A. kedongensis* and *A. gossweileri*. It is reassuring to note the congruence in morphology and chemistry. The six species in group 19 are obviously all related morphologically and they all contain aloenin. The three species in group 5 form a coherent group according to morphological assessment by Carter (1994) and Reynolds (1966). The alliance between the secund-flowered *A. brandhamii*, *A. leachii* and *A. secundiflora* is reinforced by the leaf exudate chemistry. More interesting however is the discovery that these species, in their demarcated morphological groups (groups, 5, 14, 16 and 19) could be related as suggested here through chemotaxonomic evidence.

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