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Chemotaxonomic Significance of Anthraquinones in the Roots of Asphodeloideae (Asphodelaceae)

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Key Word Index—*Asphodelus*; *Asphodeline*; *Bulbine*; *Bulbinella*; *Kniphofia*; Asphodeloideae; Asphodelaceae; roots; anthraquinones; chemotaxonomy.

Abstract—The distribution of seven anthraquinones in the roots of some 46 species belonging to the genera *Asphodelus, Asphodeline, Bulbine, Bulbinella* and *Kniphofia* was studied by TLC and HPLC. 1,8-Dihydroxyanthraquinones based on a chrysophanol unit are the main constituents of the subterranean metabolism in the subfamily Asphodeloideae. The genera *Bulbine, Bulbinella* and *Kniphofia* elaborate knipholone-type compounds. These compounds appear to be characteristic constituents for the three genera *Bulbine, Bulbinella* and *Kniphofia* and support the idea that *Kniphofia* is not related to the Alooideae.

Introduction

The subfamily Asphodeloideae (Asphodelaceae) comprises nine genera with approximately 261 species (Smith and Van Wyk, 1991). There have been different views on the relationships amongst the various genera of the Asphodelaceae *sensu* Dahlgren *et al.* (1985). Both Hutchinson (1959) and Cronquist (1981) classified *Kniphofia* with the Alooideae genera and not with the Asphodeloideae, which includes among others *Asphodelus, Asphodeline, Eremurus, Bulbinella* and *Bulbine.* Chemotaxonomic investigations (Rheede Van Oudtshoorn, 1963, 1964) on Asphodeleae and Aloineae (Liliaceae) *sensu* Hutchinson (1959) have shown that the genera *Bulbine, Asphodelus, Asphodeline* and *Eremurus* are linked by the presence of 1,8-dihydroxyanthraquinones. We present here a further example of the value of anthraquinones in establishing the relationships among the various genera of the Asphodeloideae. This study concentrated on lipophilic anthranoid aglycones, produced by the subterranean metabolism of the plants.

Materials and Methods

Plant materials. Root samples from 46 species belonging to the genera *Asphodelus, Asphodeline, Bulbine, Bulbinella* and *Kniphofia* were collected from various sources as listed in Table 1, the most important being the live collection and the herbarium collections of the National Botanical Institute, Pretoria, South Africa. Voucher numbers or collecting localities are indicated in Table 1. Abbreviations are as follows: Gardens of the National Botanical Institute at Pretoria (NBI), the National Herbarium, Pretoria (PRE) and the Herbarium of the Rand Afrikaans University (JRAU).

Procedures. Fresh roots were air-dried, powdered (*ca* 1 g) and extracted by percolation in acetone for 12 h. After removal of the solvent, the crude extracts were taken up in MeCN and passed through C_{18} cartridges to remove substances of high R_f. Samples were dissolved in a MeCN/H_2O (1:1) mixture and injected into the HPLC system. A Phenomenex Spherisorb 3 ODS 2 column was used (C_{18} reverse phase, 3 μm particle size, 100×4.6 mm i.d.; flow rate 1 ml min^{-1}; 20 μl sample loop). The solvent system comprised a 10–100% non-linear gradient of A in B. A: MeCN; B: MeCN/H_2O; 45:55 (R_f-values: **1** = 12.22; **2** = 5.12; **3** = 4.62; **4** = 13.25; **5** = 9.2; **6** = 8.74; **7** = 8.9). The crude extracts were also analysed by TLC using the following solvent systems: $CHCl_3$/petrol (1:1), $CHCl_3$/EtOAc (1:1) and benzene/petrol/EtOAc (1:2:1) on silica gel (Merck) plates. Chromatographic zones were detected under UV light (254 and 366 nm). Identification of compounds was achieved by direct TLC and HPLC comparisons with authentic reference samples (R_f-values, visibility/colour

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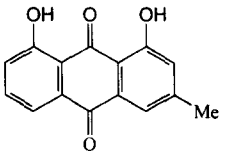
TABLE 1. DISTRIBUTION OF ANTHRAQUINONES IN SOME GENERA OF THE SUBFAMILY ASPHODELOIDEAE (ASPHODELACEAE)

Genera and species	Voucher number/locality	Major compounds (see list below) 1	2	3	4	5	6	7
Asphodelus								
A. acaulis Desf.	*d'Alleizette s.n. sub PRE 44604* (PRE)	+	+	+	–	–	–	–
A. albus Mill.	*Porta 7475* (PRE)	+	+	+	–	–	–	–
A. fistulosus L.	*Cardoso 26* (PRE)	+	+	+	–	–	–	–
	Donner 10602 (PRE)	+	+	+	–	–	–	–
Asphodeline								
A. liburnica Reichb.	*Porta & Rigo 235* (PRE)	+	+	+	–	–	–	–
Bulbine								
A. alooides (L.) Willd.	NBI SV19643	+	–	+	+	–	–	–
B. angustifolia V. Poelln.	*De Castro 168* (JRAU)	+	–	–	–	+	+	+
B. capitata V. Poelln.	*De Castro 169* (JRAU)	+	–	–	–	–	+	+
B. filifolia Bak.	*De Castro 262* (JRAU)	+	–	–	–	–	–	–
B. frutescens (L.) Willd.	Olifantskop	+	–	–	–	–	+	+
B. lagopus (Thunb.) N.E. Br.	20 km S of Cradock	+	–	–	–	+	+	+
	Standford	+	–	–	–	–	+	+
	7 km N of Jansenville	+	–	–	–	+	+	+
	Coegakop	+	–	–	–	+	+	+
B. latifolia (L.f.) Roem. & Schult.	Olifantskop	+	–	+	–	–	+	+
	ex hort. NBG (Addo)	+	–	–	–	–	+	+
	De Castro 255 (JRAU)	+		+	–	–	+	+
B. latifolia aff.	ex hort NBG (Umtamvuna)	+	–	+	–	–	+	+
B. mesembryanthemoides Haw.	NBI SV23899	+		+	+	–	–	–
B. narcissifolia Salm-Dyck	*Brits s.n.* (JRAU)	+	–	+	–	–	+	+
B. natalensis Bak.	Durban Municipal Nursery	+	–	+	–	–	+	+
	Durban Municipal Nursery	+	–	+	–	–	+	+
	ex hort. NBG (Kouga Dam)	+	–	+	–	–	+	+
B. praemorsa (Jacq.) Roem. & Schult.	NBI SV25563B	+	–	+	+	–	–	–
B. sedifolia Schltr. ex V. Poelln.	NBI SV19661	+	–	+	+	–	–	–
B. succulenta Compton	NBI SV19536	+	–	–	–	–	+	+
Bulbinella								
B. barkerae P.L. Perry	*Muir 2685* (PRE)	–	+	–	–	–	–	–
B. cauda-felis (L.f.) Dur. & Schinz	*Perry 3371* (PRE)	–	–	–	–	–	–	–
B. ciliolata Kunth	*Perry 3548* (PRE)	+	–	+	–	–	–	–
B. divaginata P.L. Perry	*Perry 3265* (PRE)	+	–	–	–	–	+	+
	Perry 3315 (PRE)	+	–	–	–	–	+	+
B. eburnifolia P.L. Perry	*Perry 3463* (PRE)	–	+	–	–	–	–	–
B. elata P.L. Perry	*Steiner 1494A* (PRE)	+	–	–	–	–	+	–
	Oorlogskloof	+	–	–	–	–	+	+
B. elegans Schltr. ex P.L. Perry	*Perry 3323* (PRE)	+	+	+	–	–	–	–
	Perry 3470 (PRE)	+	+	+	–	–	–	–
B. graminifolia P.L. Perry	*Perry 3025* (PRE)	+	+	–	–	–	–	–
B. latifolia Kunth var. *latifolia*	Arendskraal	+	–	–	–	+	+	+
B. nutans (Thunb.) Dur. & Schinz var. *nutans*	*Acocks 16888* (PRE)	–	–	–	–	–	+	+
	Acocks 17182 (PRE)	+	–	–	–	–	+	+
B. nutans (Thunb.) Dur. & Schinz var. *turfosicola* P.L. Perry	*Andreae 547* (PRE)	+	–	–	–	–	+	+
B. punctulata A. Zahlbr.	*Perry 3140* (PRE)	–	–	–	–	–	+	–
B. trinervis (Bak.) P.L. Perry	*Liebenberg 7202* (PRE)	–	+	–	–	–	+	–
B. triquetra (L.f.) Kunth	*Acocks 24501* (PRE)	–	–	+	–	–	+	–
	Baijnath s.n.b. (PRE)	+	–	+	–	–	+	–
Kniphofia								
K. albescens Codd	*Parkhouse s.n. sub PRE 36917*	+	+	+	–	–	+	–
K. brachystachya (Zahlbr.) Codd	*Roux 1463* (PRE)	+	+	+	+	–	+	–

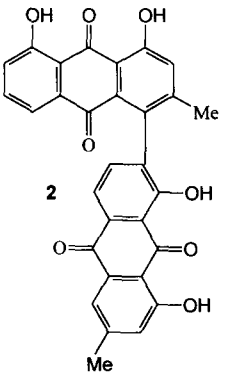
TABLE 1—*CONTINUED*

Genera and species	Voucher number/locality	Major compounds (see list below) 1	2	3	4	5	6	7
K. brevifolia Harv. ex Bak.	*Downing s.n. sub PRE 36851*	+	+	+	−	−	+	−
K. buchananii Bak.	*Acocks 13879* (PRE)	−	+	+	−	−	+	−
	Nouhuys s.n. sub PRE 36826	−	+	+	−	−	+	−
K. cf. *citrina* Bak.	*De Castro 264* (JRAU)	+	+	+	−	−	+	−
K. ensifolia Bak. subsp. *autumnalis* Codd	NBI 22808	+	+	+	−	−	+	−
K. evansii Bak.	*Killick 1653* (PRE)	+	+	+	+	−	+	−
K. gracilis Harv. ex Bak.	*Reid 5621* (PRE)	+	+	+	+	−	+	−
K. linearifolia Bak.	*De Castro 272* (JRAU)	+	+	+	−	−	+	−
K. northiae Bak.	*De Castro 267* (JRAU) 1	+	+	+	+	−	−	−
	De Castro 267 (JRAU) 2	+	+	+	+	−	−	−
K. parviflora Kunth	*Abbott 5629* (PRE)	+	+	−	−	−	−	−
	Acocks 13363 (PRE)	−	+	−	−	−	−	−
K. porphyrantha Bak.	*Winter 121* (JRAU)	+	+	+	−	−	−	−
K. typhoides Codd	*Codd 2683* (PRE)	+	−	+	+	−	−	−
	Strey 3758 (PRE)	+	−	+	+	−	−	−
K. tysonii Bak. subsp. *lebomboensis* Codd	NBI SV22341	+	+	+	+	−	+	+
K. umbrina Codd	NBI 331468	+	+	+	−	−	+	+

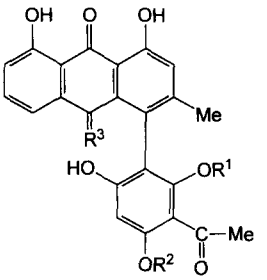
Compounds: **1**, chrysophanol; **2**, asphodeline; **3**, 10,7'-bichrysophanol; **4**, chryslandicin; **5**, knipholone anthrone; **6**, knipholone; **7**, isoknipholone.



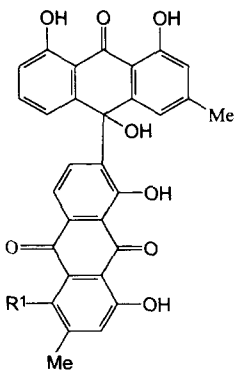
1



2



5 R^1 = H, R^2 = Me, R^3 = H_2
6 R^1 = H, R^2 = Me, R^3 = O
7 R^1 = Me, R^2 = H, R^3 = O



3 R^1 = H
4 R^1 = OH

under UV 254 and 366 nm, R_f, UV/VIS spectrum) obtained from previous studies (Dagne *et al.*, 1984, 1987, 1994).

Results

The distribution of the seven most common *Kniphofia* anthraquinones in 46 species of the genera *Asphodelus, Asphodeline, Bulbine, Bulbinella* and *Kniphofia* is summarized in Table 1.

Discussion

The occurrence of chrysophanol (**1**) and some of its dimers such as asphodeline (**2**) in the subfamily Asphodeloideae (Beaumont *et al.*, 1985) and also in *Aloe* species (Dagne *et al.*, 1994) has already been reported. In this study we have established the occurrence of compound **1** in all genera, while asphodelin (**2**) was detected in all but *Bulbine* (Table 1). Compounds **3** and **4** (then coded as Kf_7 and Kf_8, respectively) have been reported from the roots of six Ethiopian *Kniphofia* species (Berhanu *et al.*, 1986). The structure of Kf_8 was later elucidated as a new dimer, chryslandicin (**4**) (Dagne *et al.*, 1987). We have now identified Kf_7 as 10,7'-bichrysophanol (**3**), a compound earlier isolated from the roots of *Aloe saponaria* (= *A. maculata*) (Yagi *et al.*, 1978). In this study we have established the wide occurrence of compound **3**, while the bright red pigment **4** was identified from only four *Bulbine* and six *Kniphofia* species (Table 1).

Knipholone (**6**), in which an acetylphloroglucinol methyl ether is attached to a chrysophanol unit, was first isolated from the roots of *Kniphofia foliosa* (Dagne and Steglich, 1984) and later identified as a major compound from the roots of 14 *Kniphofia* species (Berhanu *et al.*, 1986; Yenesew *et al.*, 1988). This has led to the suggestion that knipholone could be a marker for the genus. Recently further anthraquinones, knipholone anthrone (**5**) and isoknipholone (**7**) (Yenesew *et al.*, 1994) have been isolated from the stem of *Kniphofia foliosa.*

We have now established the occurrence of knipholone (**6**) and isoknipholone (**7**) in the genera *Bulbine, Bulbinella* and *Kniphofia* (Table 1). Recently, knipholone was also reported from *Bulbine latifolia* and *Bulbine frutescens* (Van Staden and Drewes, 1994). It is interesting to note that knipholone-type compounds were not detected in the roots of *Asphodelus* and *Asphodeline* species (Table 1). This suggests that the genera *Bulbine, Bulbinella* and *Kniphofia* form a monophyletic unit within the subfamily but further studies are needed to confirm the absence of this type of compounds in other genera of the Asphodeloideae.

Conquist (1981) included the genus *Kniphofia* along with the alooid genera in the Aloaceae, presumably based on flower shape and fusion of perianth segments. On the other hand, Dahlgren *et al.* (1985) placed *Kniphofia* along with *Bulbine, Bulbinella* and others in the subfamily Asphodeloideae. This placement, distinct from Alooideae, was based on differences in the anatomical construction of the leaves and other characters. In agreement with the classification of Dahlgren *et al.* (1985), Beaumont *et al.* (1985) showed that taxa of Alooideae have a well developed cap of thin-walled parenchyma cells at the phloem pole (aloin cells), while *Kniphofia* has well-defined fibers present in a cap at both the xylem and phloem poles.

We recently reported the widespread occurrence of 1-methyl-8-hydroxy-anthraquinone derivatives in the roots of *Aloe* species (Dagne *et al.*, 1994). These compounds appear to be absent from the genus *Kniphofia.* Our results, therefore support the view of Dahlgren *et al.* (1985) that *Kniphofia* should be placed in the Asphodeloideae rather than near the Alooideae genera.

Some species of *Bulbine*, such as *B. latifolia,* have karyotypes and morphologies similar to certain taxa of the subfamily Alooideae (Smith and Van Wyk, 1991). *Bulbine* was excluded from Alooideae mainly on the basis of flowers structure and lack of

nectar production. It is now clear from our results that the similarities between *Bulbine* and some taxa of the Alooideae are only superficial.

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