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ANTHRAQUINONES AS TAXONOMIC MARKERS IN ETHIOPIAN *KNIPHOFIA* SPECIES

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Key Word Index—*Kniphofia*; Liliaceae; chemotaxonomy; anthraquinones; aloe-emodin acetate; chrysophanol; islandicin; knipholone.

Abstract—From the rhizomes, leaves, flowers and fruits of five *Kniphofia* species aloe-emodin, aloe-emodin acetate, chrysophanol, islandicin and knipholone were isolated and characterized. The taxonomic significance of the variations in the anthraquinone compositions is discussed.

INTRODUCTION

The genus *Kniphofia* (Liliaceae) is, except for one species recorded from the Yemen and two from the Malagasy Republic, exclusively confined to the continent of Africa. Out of a total of 67 species, 45 species are recorded from South Africa, a place considered to be the primary centre of diversity for the genus. The remaining 22 species are confined to tropical Africa. Six *Kniphofia* species have so far been recorded from Ethiopia namely, *K. foliosa* Hochst., *K. insignis* Rendle, *K. pumila* (Ait.) Kunth., *K. hildebrandtii* Cufod and *K. schimperi* Baker, all of which are endemic to Ethiopia except *K. pumila*.

One of the main taxonomic problems of the genus *Kniphofia* is the delimitation of boundaries between the different species [1]. Cufodontis described *Kniphofia* as 'genus difficile' [2]. Similar difficulties were encountered by Codd during the revision of the South African species of *Kniphofia* [3]. Moreover, the presence of hybrid populations such as between *K. pumila* and *K. schimperi* in Ethiopia adds further problems to the identification of certain *Kniphofia* species [4].

During investigations of the chemical constituents of *K. foliosa*, a plant used in Ethiopian traditional medicine as a home remedy for abdominal cramps, several known as well as new anthraquinones were characterized by our group [5, 6]. An extension of this work to other *Kniphofia* species in the country revealed that anthraquinones constitute the major secondary metabolites of the genus. In the present study we have attempted to distinguish the five species so far investigated on the basis of their anthraquinone constituents.

RESULTS AND DISCUSSION

The results of the present study of *K. foliosa*, *K. insignis*, *K. isoetifolia*, *K. pumila* and *K. schimperi* are presented in Table 1. The only anthraquinone previously reported from the genus *Kniphofia* is rhein (**1**) [5]. However, the present investigation of the rhizomes, leaves, flowers and fruits of *K. foliosa* revealed the presence of several anthraquinones of which knipholone (**2**) is the major anthraquinone in all parts of the plant. The unique structural feature of knipholone is that it arises as a result of coupling of chrysophanol (**3**) with an acetylphloroglucinol unit [5]. Chrysophanol has also been detected in all parts of the plant.

Interestingly islandicin (**4**), an anthraquinone rarely isolated from higher plants was found in the rhizomes and leaves of *K. foliosa*. Islandicin is commonly found in fungi notably in *Penicillium islandicum*. The only report to our knowledge of its occurrence in higher plants is in the heartwood of *Maesopsis eminii* (Rhamnaceae) [7]. The rhizomes of *K. foliosa* yielded a bright red pigment, designated as Kf_8, with molecular formula $C_{30}H_{20}O_8$, which upon sodium dithionite cleavage [8] gave islandicin and chrysophanol indicating that the pigment is a bisanthraquinone composed of chrysophanol and islandicin. In addition, a yellow pigment, Kf_7, $C_{30}H_{20}O_7$, was detected in the rhizomes. The structural details of these bisanthraquinone pigments will be reported later.

Furthermore the well-known anthraquinone aloe-emodin (**5**) known to exhibit antileukemic properties [9] was isolated from the fruits of *K. foliosa* and it was also detected in the leaves and flowers of the same plant. We have earlier reported the isolation of aloe-emodin acetate (**6**) from the leaves of *K. foliosa* [6]. We have since detected this compound in the fruits and flowers of the same species. The presence of a wide array of anthraquinones in *K. foliosa* shows that this species is significantly rich in this class of compounds. Interestingly, emodin (**7**) and physcion (**8**) which sometimes co-occur with chrysophanol are completely absent from *K. foliosa* as well as the other *Kniphofia* species studied.

As shown in Table 1, knipholone, chrysophanol, islandicin and the bisanthraquinone pigments designated as Kf_7 and Kf_8 were present in the rhizomes of all *Kniphofia* species investigated. Both chrysophanol and islandicin were minor components and by far the most abundant anthraquinone was knipholone. The brightly coloured red pigment Kf_8 gave the characteristic orange pigment to the rhizomes. The bisanthraquinones Kf_7 and Kf_8 are restricted to the rhizomes whereas knipholone,

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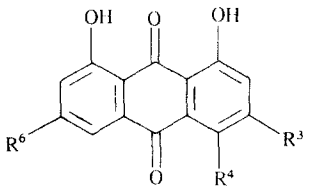
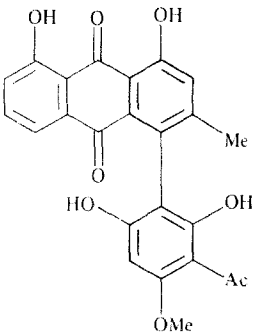
Table 1. Distribution of anthraquinones in Ethiopian *Kniphofia* species

		Kniphofia species					
		K. foliosa	*K. insignis*	*K. isoetifolia*	*K. pumila*	*K. schimperi*	*K. foliosa from Bale*
Chrysophanol	Rh	+	+ (tr)	+	+	+	+
	Lf	+	–	+	–	*	+
	Fl	+	–	+	–	+ (tr)	+
	Fr	–	–	*	*	*	+
Islandicin	Rh	+	+ (tr)	+	+	+ (tr)	+
	Lf	+	–	–	–	*	–
	Fl	+	–	–	–	–	–
	Fr	–	–	*	*	*	–
Aloe-emodin	Rh	–	–	–	–	–	–
	Lf	+	–	–	–	*	+
	Fl	+	+	+	–	+	+
	Fr	+	–	*	*	*	+
Aloe-emodin acetate	Rh	–	–	–	–	–	–
	Lf	+	–	–	–	*	+
	Fl	+	–	+	–	–	+
	Fr	+	–	*	*	*	+
Knipholone	Rh	+	+ (tr)	+	+	+ (tr)	+
	Lf	+	–	–	–	*	+
	Fl	+	–	–	+ (tr)	–	+
	Fr	+	–	*	*	*	+
Kf_2	Rh	–	–	–	–	–	–
	Lf	–	–	–	–	*	+
	Fl	–	–	–	–	–	–
	Fr	–	–	*	*	*	–
Kf_7	Rh	+	+	+	+	+	+
	Lf	–	–	–	–	*	–
	Fl	–	–	–	–	–	–
	Fr	–	–	*	*	*	–
Kf_8	Rh	+	+	+	+	+	+
	Lf	–	–	–	–	*	–
	Fl	–	–	–	–	–	–
	Fr	–	–	*	*	*	–

*Sample not checked.
Rh, Rhizomes; Lf, leaves; Fl, flowers; Fr, fruits, tr, trace.



1 R^3 = COOH; R^4 = R^6 = H
3 R^3 = Me; R^4 = R^6 = H
4 R^3 = Me; R^4 = OH; R^6 = H
5 R^3 = CH_2OH; R^4 = R^6 = H
6 R^3 = CH_2OAc; R^4 = R^6 = H
7 R^3 = Me; R^4 = H; R^6 = OH
8 R^3 = Me; R^4 = H; R^6 = OMe



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chrysophanol and islandicin were also found in other parts of the plant. On the other hand aloe-emodin and aloe-emodin acetate were absent from the rhizomes of all species but were detected in the leaves, flowers and fruits.

Of all the *Kniphofia* species we studied, two species *K. pumila* and *K. insignis* were not rich in anthraquinones. The latter species characterized by its white flowers, had the least number of anthraquinones. Aloe-emodin was the only anthraquinone found in the flowers and no anthraquinones were detected in the leaves and fruits of this species; and even in the rhizomes chrysophanol, islandicin and knipholone were present only in trace quantities. Similarly the flowers of *K. pumila* contained only knipholone while the leaves apparently produced no anthraquinones.

Using the data shown in Table 1 it is possible to distinguish the five *Kniphofia* species studied. As summarized in Table 2, the anthraquinone composition of the flowers is especially useful in separating the species from one another. Thus, the five species may be separated into two groups based on the presence or absence of chrysophanol in the flowers and further distinguished by the presence of aloe-emodin in *K. insignis*, knipholone in *K. foliosa* and aloe-emodin acetate in *K. isoetifolia*.

A *Kniphofia* species collected from Bale region, Ethiopia and identified by botanists as *K. foliosa* showed sufficient variation from *K. foliosa* collected from other regions to warrant discussion. The fruits of *K. foliosa* from Bale did not contain chrysophanol, and islandicin was absent in both the flowers and leaves (see Table 1). On the other hand *K. foliosa* from the other regions contained chrysophanol in its fruits and islandicin was also detected in both leaves and flowers. In addition the presence of the bisanthraquinone designated as Kf_2 in the leave of *K. foliosa* from Bale further distinguished this collection. The difference in anthraquinone composition between *K. foliosa* samples from various regions and the one collected from Bale is currently under investigation. Interestingly, Kf_2 which by reductive cleavage using alkaline sodium dithionite gave rise to aloe-emodin and chrysophanol, was found in no other *Kniphofia* species so far studied. The structural details of Kf_2 will be reported later.

EXPERIMENTAL

General. Mps are uncorr. MS were recorded at 70 eV on AEI MS 30 and MS 50 instruments. ^{1}H NMR spectra were measured at 60 MHz using Bruker WH 90 instrument with TMS as the int. standard. TLC: silica gel; solvent systems: hexane–Me_2CO (9:1, solvent 1), EtOAc–C_6H_6 (1:4, solvent 2), petrol–Me_2CO (9:1, solvent 3), MeOH–$CHCl_3$ (1:1, solvent 4) and EtOAc–C_6H_6 (3:7, solvent 5). Prep. TLC: silica gel 2 mm thick; detection: visually and UV 254 nm.

Table 2. The separation of five Ethiopian *Kniphofia* species based on the anthraquinone content of the flowers

		1	2	3	4
Group 1	*K. insignis*	–	+		
	K. pumila	–	–		
Group 2	*K. foliosa*	+		+	
	K. schimperi	+		–	–
	K. isoetifolia	+		–	+

1, Chrysophanol; 2, aloe-emodin; 3, knipholone; 4, aloe-emodin acetate.

Plant material. Kniphofia foliosa was collected from Harra Wobalo, Wollo, Ethiopia, alt. 2600 m. *K. insignis* was collected from Shewa, 118 km north of Addis Ababa on the road to Gojam, alt. 2800 m. *K. isoetifolia* was collected from Shewa, 40 km from Shashemene on Goba road, alt. 2700 m while *K. pumila* was collected in the vicinity of Addis Ababa, near Bole airport, alt 2300 m and *K. schimperi* from Dejen, Gojam, alt. 2400 m. The recent collection of *K. foliosa* that showed significant variation in anthraquinone composition was made from Bale 26 km west of Dinshu, alt. 3400 m. For all the plant materials collected voucher samples were kept in the National Herbarium, Addis Ababa.

Isolation and characterization of anthraquinones. The powdered rhizomes of *K. foliosa* were extracted with Me_2CO and upon removal of the solvent the crude extract was applied to a silica gel column using solvent 1 for elution. The first four fractions, 100 ml each, mainly contained an islandicin–chrysophanol mixture. The separation of these two pigments by means of chromatographic methods was difficult and only very small amounts of each were obtained by means of prep. TLC using solvent 1. However, the chrysophanol–islandicin mixture was readily separated after acetylation. Thus 28.9 mg mixture containing the two compounds was directly acteylated with Ac_2O–pyridine. Acetylation was best effected by heating the mixture at 60–70 for 4 hr and then stirring at room temp. for 24 hr. This was followed by the usual work-up procedures. The crude acetate mixture obtained was then purified by prep. TLC using solvent 2. Recryst from MeOH yielded 15 mg chrysophanol diacetate ($R_f = 0.48$, solvent 2) and 8 mg of islandicin triacetate ($R_f = 0.37$, solvent 2).

Islandicin. Red pigment mp 212–215° (lit. 216° [10]). MS *m/z* (rel. int.) $C_{15}H_{10}O_5$ 270.0523 (M^+, 64), $C_{15}H_{10}O_4$ 254.0573 (100).

Islandicin triacetate. Pale yellow needles from MeOH, mp 203–206° (lit. 208° [10]). IR ν_{max}^{KBr} cm^{-1}: 1780, 1760, 1675, 1590. ^{1}H NMR (90 MHz, $CDCl_3$): δ2.36 (*s*, Me), 2.40 (*s*, 6H, 2 × COOMe), 2.53 (*s*, COOMe), 7.36 (*br*, H-2), 8.30 (*dd*, $J = 7.8$ and 1.4 Hz, H-5), 7.78 (*t*, $J = 8$Hz, H-6), 7.40 (*dd*, $J = 8$ and 1.3 Hz, H-7).

Aloe-emodin. Ground fruits (300 g) of *K. foliosa* collected from Bale were percolated with Me_2CO for 12 hr. Removal of the solvent yielded a greenish black residue which gave an 18 g (6%) $CHCl_3$ soluble fraction, 5 g of which was applied to a silica gel column (110 g) and was eluted with an increasing polarity of solvent 3. Fractions containing aloe-emodin along with other anthraquinones such as knipholone were combined, evaporated (0.3 g) and further chromatographed on Sephadex LH-20 (100 g) using solvent 4 to give crude aloe-emodin. Further purification by prep. TLC using solvent 2 gave 12 mg of aloe-emodin. Similarly aloe-emodin was also isolated from the fruits of *K. foliosa* collected from other regions and was characterized by comparison with an authentic sample (TLC, mp, IR, MS, NMR).

Kf_2. Powdered leaves of *K. foliosa* (104 g) collected from Bale region in Sept 1984 were soaked in Me_2CO for 12 hr and the concd extract (3 g) applied to a silica gel column (110 g) followed by gradient elution with petrol (40–60°)–Me_2CO. Fractions containing Kf_2 were combined, concd and run on prep. TLC using solvent 5. Final purification was effected by using a Sephadex LH-20 column and eluting with solvent 4.

Sodium dithionite ($Na_2S_2O_4$) reductive cleavage of Kf_2. To a soln of Kf_2 (3 mg) in 5% NaOH soln was added $Na_2S_2O_4$ (5 mg) and the mixture was heated at 80° for 1½ hr. The mixture was cooled and extracted with $CHCl_3$. Upon removal of the solvent chrysophanol and aloe-emodin were detected (TLC).

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