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A Comparative Study of Anthraquinones in Rhizomes of *Kniphofia* Species

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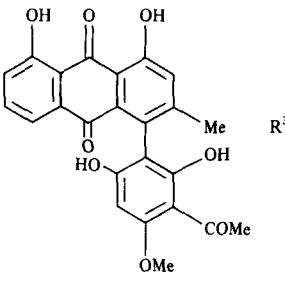
Key Word Index—*Kniphofia* spp; Liliaceae; anthraquinones; chemotaxonomy.

Abstract—The anthraquinone content of eight *kniphofia* species was compared using reversed phase HPLC method coupled with a photodiode array detector.

Introduction

Anthraquinones are medicinally important constituents of plants and are found in several plant families. We have earlier shown that the genus *Kniphofia* Moench. [Liliaceae] is rich in anthraquinones [1] and the chemotaxonomic significance of some of these compounds has been evaluated [2].

In this paper we report the analysis of seven newly investigated species of *Kniphofia*. Although many analytical methods are known for the detection of anthraquinones, little information is available on the HPLC analysis of this class of compounds [3, 4]. The recently introduced HPLC system with a computer controlled photodiode array detector provides a high potential for the manipulation of recorded chromatographic and absorption spectral data. This method has enabled us to study anthraquinone content by comparing the HPLC profile of each species with that of the previously studied plant, *Kniphofia foliosa* Hochst., and with a mixture of known anthraquinones.



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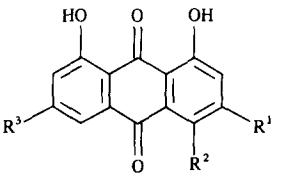
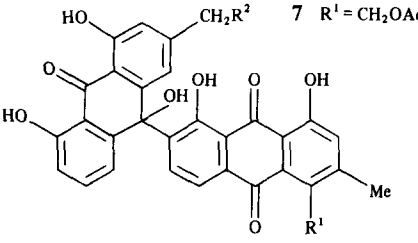


2 R^1 = Me, R^2 = OH, R^3 = H
3 R^1 = Me, R^2 = R^3 = H
5 R^1 = Me, R^2 = H, R^3 = OMe
6 R^1 = Me, R^2 = H, R^3 = OH
7 R^1 = CH_2OAc, R^2 = R^3 = H



4 R^1 = OH, R^2 = H
8 R^1 = H, R^2 = OH

Results and Discussion

The principal anthraquinone component of the rhizomes of *K. foliosa* was previously characterized as knipholone (**1**) which occurs along with other anthraquinones namely islandicin (**2**), chrysophanol (**3**) and the bianthraquinone chryslandicin (**4**) [2, 5]. The HPLC method described here allows the identification of these anthraquinones, not only in *K. foliosa*, but in other *Kniphofia* species.

Reference chromatograms of anthraquinones including physcion (**5**), emodin (**6**), aloe-emodin acetate (**7**) and chrysalodin (**8**) [2, 5] and the chromatogram of the crude acetone extract of the rhizomes of *K. foliosa* are shown in Figs 1 and 2.

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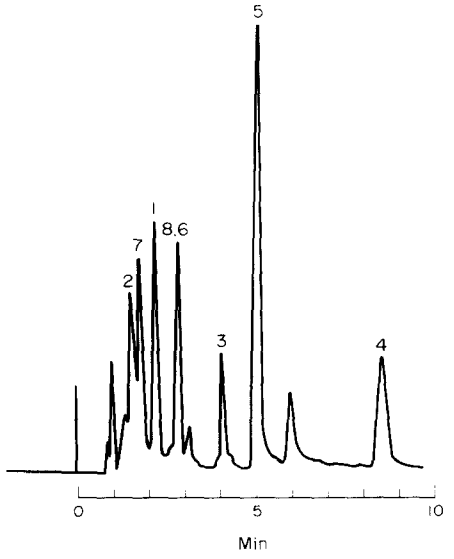

FIG. 1. HPLC PROFILE OF A CONSTITUTED MIXTURE OF SOME ANTHRAQUINONES. Column: Spherisorb ODS-2, 3μ 4.6 × 100 mm. Eluent: 75% acetonitrile and 25% water containing 0.01% phosphoric acid. Flow rate: 1 ml/min. Detection: UV 295 nm. Peaks: 1 knipholone; 2 islandicin; 3 chrysophanol; 4 chryslandicin; 5 physcion; 6 emodin; 7 aloe-emodinacetate; 8 chrysalodin.

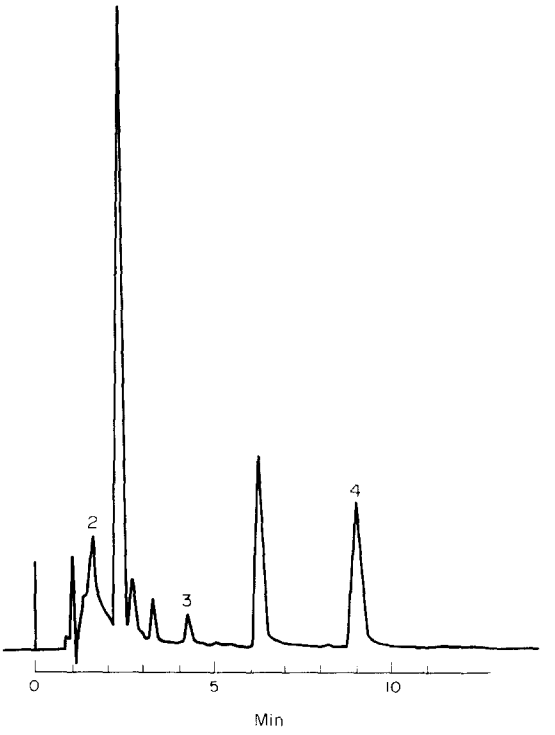

FIG. 2. HPLC CHROMATOGRAM OF ACETONE EXTRACT OF *KNIPHOFIA FOLIOSA*. See Fig. 1 for details. Peaks: 1 knipholone; 2 islandicin; 3 chrysophanol; 4 chryslandicin.

Comparison of the chromatograms of each species analysed establishes the presence or absence of the known compounds. Their identity was confirmed by UV spectra.

Knipholone (**1**) was earlier detected as the principal anthraquinone in five *Kniphofia* species from Ethiopia, namely *K. foliosa* Hochst., *K. insignis* Rendle, *K. isoetifolia* Hochst., *K. pumila* (Ait.) Kunth., and *K. schimperi* Baker [2]. We find here that it is the major anthraquinone in six out of the seven further *Kniphofia* species investigated, confirming our earlier assertion that this compound may very well be a taxonomic marker for the genus. Interestingly, as shown in Table 1 some differences were observed in the anthraquinone composition between *K. linearifolia*, obtained from Zimbabwe (near Harare) and the same specimens of material, obtained from the Royal Botanic Gardens in England. It should, therefore, be noted that there may be some differences between the chemical composition of plant material obtained from a botanic garden and that from a natural environment as there may be appreciable variation within a species.

TABLE 1. DISTRIBUTION OF ANTHRAQUINONES IN EIGHT *KNIPHOFIA* SPECIES

	Anthraquinone			
Species	Knipholone (**1**)	Islandicin (**2**)	Chrysophanol (**3**)	Chryslandicin (**4**)
K. acrae	+	—	—	—
K. caulescens	+	—	+	—
K. flammula	+	—	—	—
K. linearifolia (Kew)	+	—	+	—
K. linearifolia (Zimbabwe)	+	+	+	+
K. reynoldsii	+	+	+	—
K. rooperi	+	—	—	—
K. tysonii	+	—	—	—
K. foliosa	+	+	+	+

The above study was conducted with plant materials of less than 1 g for most of the species examined, clearly showing the value of HPLC in phytochemical, particularly chemotaxonomic, work.

Experimental

Plant materials. *K. foliosa* used in this study was collected from Addis Ababa, Ethiopia. *K. acrae* Codd, *K. caulescens* Baker, *K. flammula* Codd, *K. linearifolia* Baker, *K. reynoldsii* Codd, *K. rooperi* Lem. and *K. tysonii* Baker were kindly supplied by the Royal Botanic Gardens, Kew, England. A specimen of *K. linearifolia* Baker was obtained through Mr. S. Mavi, of the National Herbarium of Zimbabwe.

Apparatus. A microcomputer (NCR, model PC 4I) controlled liquid chromatographic system (LKB—Sweden) equipped with a photodiode array detector, variable wavelength monitor and a Rheodyne injection valve was used with a stainless steel column prepacked with Spherisorb ODS-2 3μ, 4.6 × 100 mm. The mobile phase consisted of acetonitrile–H_2O (3:1). 100 μl phosphoric acid (85%) was added per litre to avoid tailing and line broadening. Degassing was accomplished by refluxing the mobile phase for 10 min.

Extraction and analysis. The dried roots (1 g) of each *Kniphofia* species was extracted by cold percolation with acetone for 18 h. Each crude extract was passed through a silica gel (6 g) column and eluted with chloroform three times for each sample. 20 μl of the purified sample was injected as 5 mg of the purified extract in 5 ml of acetonitrile.

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