PHYTOCHEMICAL INVESTIGATIONS OF *BULBINEABYSSINICA, BULBINENATALENSIS* AND *TRACHYANDRA DWARICATA*

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THE REQUIREMENTS FOR THE DEGREE OF
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BY
GIZACHEW NIGUSSIE

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dddedicated to: My mothery 1Ityij. sister and 7Idty brother.

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ABSTRACT

PHYTOCHEMICAL INVESTIGATIONS OF *BULBINE ABYSSINICA*, *BULBINE NATALENSIS* AND *TRACHYANDRA DIVARICATA*

Advisor: Dr. Wendimagegn Mammo

Co-advisor: Prof. Sebsebe Demissew

Phytochemical studies were carried out on *Bulbine abyssinica*, *Bulbine natalensis* and *Trachyandra divaricata*. All three plants belong to the family Asphodelaceae.

B. abyssinica is the only *Bulbine* species knowen to occur in Ethiopia. *B. natalensis* and *T. divaricata* are of South African origin.

The extract of the roots of *B. abyssinica* afforded knipholone-6'-methyl ether (12) chrysophanol (14), knipholone anthrone (20), isoknipholone (22), knipholone (24), aloe-emodin (2), 8-hydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione (16) and 5,8-dihydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione (18).

The extract of the roots of *B. natalensis* gave chrysophanol (14), knipholone anthrone (20), isoknipholone (22), knipholone (24), aloe-emodin (2), 8-hydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione (16) and 5,8-dihydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione (18).

The occurence of chrysophanol (14), aloe-emodin (2), chryslandicin (48) and islandicin (58) in the roots of *T. divaricata* was ascertained by direct TLC comparison with authentic samples. No knipholone-type compounds were detected in the extract of the roots of *T. divaricata*.

The structures of the compounds were elucidated based on their ^{1}H NMR, IR, UV and mass spectra and in some cases, by direct comparison with authentic matrials.

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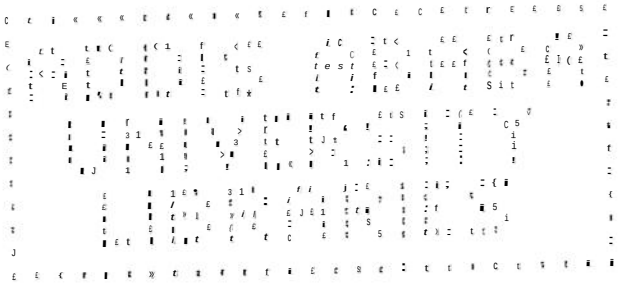

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1.0 INTRODUCTION

1.1 General

Secondary metabolites have been considered as having no direct function in the biochemical activities that support the growth, development, and reproduction of the organism in which they occur [1]. It is now believed that these compounds have, if not crucial, important role for the continuation of life on this planet. Without these compounds, which are used as defense, communication, genetic coding and many more applications, life would have been very difficult [2]. Mankind used these naturally occurring compounds for thousands of years as medicines, cosmetics, dyes, preservatives, poisons, etc. [3].

In recent years, in addition to the numerous uses cited above, they are also investigated for academic or scientific purpose. Secondary metabolites are now vital source of information in some newly emerging multi-disciplinary sciences such as chemical ecology, chemotaxonomy, etc. [2].

Due to this, a new bridge has been formed between Biology and Chemistry, specially natural product chemistry. The occurrence or absence of structurally similar compounds could be used to confirm or rule out the placement of plants under the same systematic group [4]. The field of chemotaxonomy generates ample information in this regard to strengthen taxonomic studies of plants. In recent years such studies

have gone deep into understanding the interrelationship of the living system which led to the emergence of chemical ecology [2].

1.2 Chemotaxonomy and its significance

Chemical plant taxonomy or chemotaxonomy of plants may be defined as a scientific investigation of the potentialities of chemical characters for the study of problems of plant taxonomy and plant phyiogeny. Plant taxonomy is the science of classifying, describing and naming appropriately taxa and arranging them in a natural system of plants.

The principles of chemotaxonomy were elaborated in the past century by A. P. De Condolle and by Greshoff [5]. De Condolle put forward two postulates.

I. Chemical characteristics of plants will be most valuable to plant taxonomy in the future.

II. Plant taxonomy will be the most useful guide to man in his search for new industrial and medicinal plants.

Now his postulates have come true, both in delimitation of plants and in the search for new medicinal plants. Much work is being done and hence a large bulk of knowledge has been acquired in this field.

The position of many taxa in the natural system of plants is still highly uncertain. The position of *Kniphofia*, *Bulbine* and *Trachyandra* in the family of Asphodelaceae could

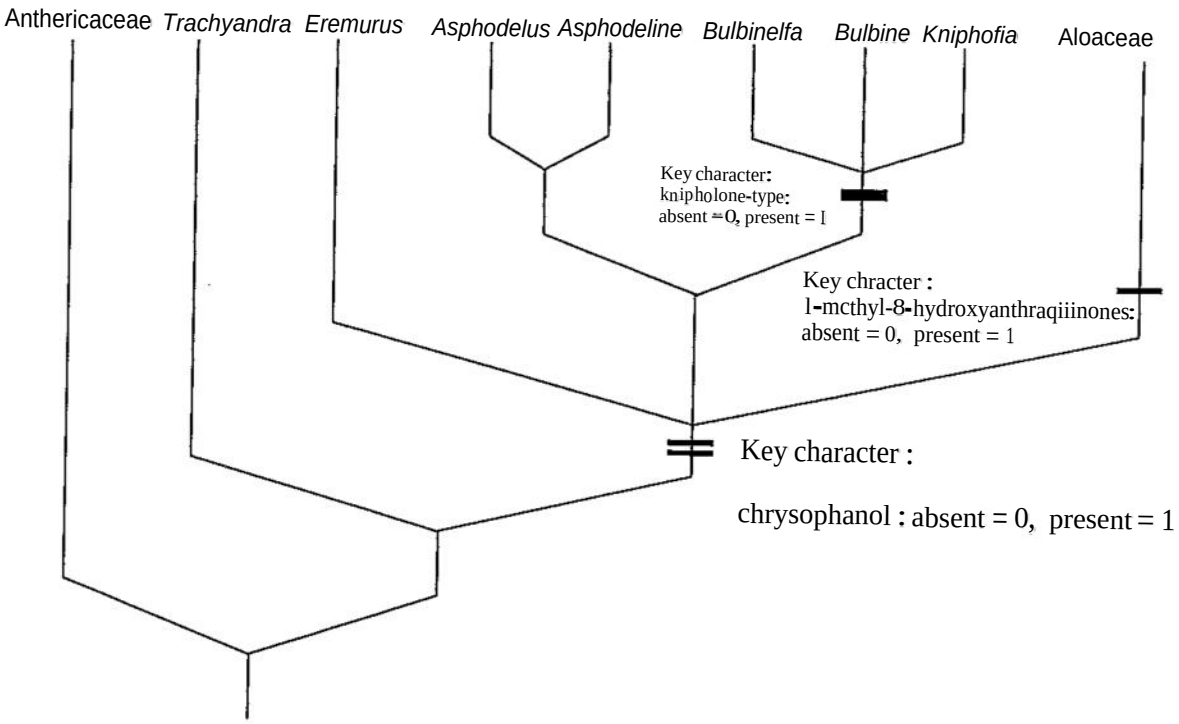
be taken as an example [6,7,8]. Such problem appears in all levels of taxonomic categories, e.g.; species in a genus; genera in a family; families in an order and even orders in a class.

Varying interpretation and evaluation of morphological characters very often result in disagreement regarding classification, e.g., morphological similarities of members of the genus *Bulbine* and some members of the subfamily Alooideae was one such encounter [9]. In such instances taxonomists as a rule look for characters other than morphological ones. Generally anatomical, embryological, paiynoiogical and cytological characters are considered first. Sometimes these characters do not generate complete information regarding plant taxa. In such situations chemical characters may become very useful guide to taxonomists. At present one important task of chemotaxonomy consists of procuring additional evidence in all cases of obscure relationships of plants. So far little has been done in delimitation of the family Asphodelaceae reinforced with chemotaxonomic evidences [10].

1.3 Chemotaxonomy of the family Asphodelaceae

In the last few years taxonomic problems of the family Asphodelaceae have been investigated. Delimitation of members of this family have been problematic due to similarity in morphological characters between *Aloe* and *Kniphofia*; *Asphodelus* and *Asphodeline*; *Aloe* and *Bulbine*, etc. The pioneering work on this subject was that of Hegnauer [11] and Rheede van Oudtshoorn [12] that used chemical characters to

establish the relation of the Alooideae and Asphodeloideae, Asphodeloideae and Anthericaceae at gross family level. But this required further search within and between the families. In this regard the contribution of the groups led by Prof. Ermias Dagne and Prof. Sebsebe Demissew at Addis Ababa University and the work of the group led by Ben-Erik van Wyk in South Africa enabled to propose the following partially resolved ciadogram (Scheme-1) of the generic relationships in the Asphodelaceae [13,14,15].



Scheme-I Partially resolved ciadogram of generic relationships in the Asphodelaceae, Anthericaceae and Aloaceae.

Wide occurrence of knipholone (24) and absence of 1-methyl-8-hydroxy anthraquinone derivatives in the genus *Kniphofia* [16], but widespread occurrence of the later in the *Aloe* species [17] helped to establish the position and relationship of the genus

Kniphofia with that of the subfamily Alooideae and Asphodelaceae. Here knipholone (24) was suggested as taxonomic marker for the genus *Kniphofia* [10].

Some species of *Bulbine,* such as *B. latifolia* have karyotypes and morphologies similar to certain taxa of the subfamily Alooideae. But still some argue that *Bulbine* should be excluded from Alooideae, mainly on the basis of flower structure and lack of nectar production [9].

The evidence to clarify this controversy has to come from chemotaxonomic investigation of some members of the Asphodelaceae. Based on the presence of knipholone and its derivatives in some species of the genera *Bulbinella, Bulbine* and *Kniphofia,* conclusion has been drawn that these three genera form a monophyletic unit within the family Asphodelaceae [10,18].

In addition to the three genera mentioned above, absence of knipholone-type compounds in the roots of *Asphodelus* and *Asphodeline* species but existence of chrysophanol (14) and aloe-emodin (2) was also reported [10]. It is worth noting that no report appeared in the literature regarding the genus *Trachyandra.*

1.4 The family Asphodelaceae

The family Asphodelaceae comprises eleven genera namely; *Asphodeline*, *Asphodelus*, *Bulbine*, *Bulbinella*, *Eremurus*, *Hemiphylacus*, *Jodrellia*, *Kniphofia*, *Paradisea*, *Simethis* and *Trachyandra* [19] with more than 261 species in the old world. The main distribution of the family is from the Mediterranean west to central Asia and Africa, particularly South Africa. It is represented by 5 genera and 12 species in Ethiopia and Eritrea [19]. This family is closely related to Anthericaceae and may sometimes be difficult to distinguish on gross morphology, especially the genus *Trachyandra* which until recently was even referred to the genus *Anthericum.*

1.4.1 The genus *Bulbine*

The genus *Bulbine* Wolf includes about 50 species worldwide, mostly in South Africa and two in Australia [19]. Some species in the genus have found traditional medicinal applications for the treatment of various ailments that probably arise from bacterial and fungal infections [20, 21]. Watt and Breyer-Brandwijk recorded that *B. latifolia* and *B. capitata* are used in southern Africa for the treatment of diarrhoea, dysentery and rheumatism [22].

B. abyssinica A. Rich. is the only *Bulbine* species that is known to exist in Ethiopia. It occurs in the south, east and central parts of Ethiopia as well as in Somalia, Kenya, Uganda, Tanzania, Burundi, Rwanda and Congo Democratic republic [19].

1.4.2 The genus *Trachyandra*

Trachyandra Kunth, is a predominantly South African genus with about 50 species, most of which are endemic in the winter rainfall areas in southwestern Cape. Only one species reaches northeast Africa and has its northern most limit in Yemen. In the past the genus had been placed in the family Anthericaceae. But recently it has been placed in the family Asphodelaceae [19].

Only *T. saltii* is known to occur in Ethiopia. This species is very variable and has been subdivided into three varieties, var. *saltii* (Baker)Oberm, var. *secunda* (Krause and Dinter)Oberm and var. *oatesii* (Baker)Oberm. It is widely distributed in its different forms mostly in eastern and southern part of the country [19].

1.5 Anthraquinones and related compounds of the Asphodelaceae

Anthraquinones and naphthoquinones constitute important classes of compounds with important biological properties [23, 24, 25]. These compounds, elaborated both by higher and lower plants, are also among the most well known naturally occurring pigments.

Anthraquinones and related compounds are located in all parts of a plant, including roots, aerial parts, flowers and seeds.

Anthraquinones occur widely distributed in the family Asphodelaceae. This has been used to distinguish this family from Anthericaceae which has very close morphological similarity with some members of the family [27]. Anthraquinonones could be considered as useful chemical characters to delimitate plant families [25]. It has been shown that such compounds were important taxonomic markers for some members of the family Asphodelaceae [25]. Tables 1-3 show anthraquinones isolated from the family Asphodelaceae. No anthraquinone was reported so far from the Anthericaceae [26].

The co-existence of anthraquinones and naphthoquinones in plants is no longer rare and they appear to have a common precursor. New types include isofuranonaphthoquinones and naphthoquinones linked to coumarin units with dimers of various kinds, and benzoisochromanquinones form substantial sub-groups [25]. Even though naphthoquinones are widely distributed both in lower and higher plants along with anthraquinones, the information available at present reveals that naphthoquinones seem to be of little taxonomic value [47]. Table-4 shows naphthoquinones isolated from the family Asphodelaceae.

Table-1. Anthraquinones isolated from the family Asphodelaceae

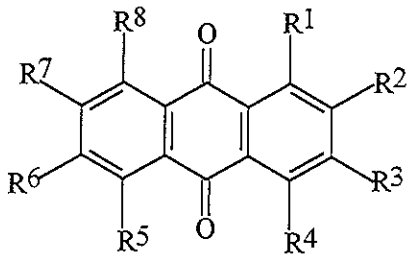
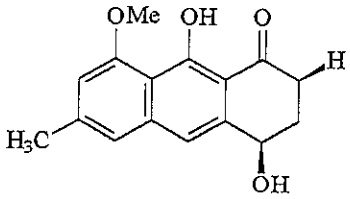
Compounds	Source	Structure								Ref.
										
		R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	
Aloe-emodin (**2**)	*Asphodelus microcarpus*, *A. fistulosus*	OH	H	CH_2OH	H	H	H	H	OH	[28]
Aloe-emodin ω-acetate (**4**)	*Kniphofia foliosa*	OH	H	CH_2OAc	H	H	H	H	OH	[29]
Chrysophanol (**14**)	*Asphodelus*	OH	H	CH_3	H	H	H	H	OH	[32, 33]
Rhein (**6**)	*Kniphofia uvaria*	OH	H	COOH	H	H	H	H	OH	[39]
8-O-methylaloesaponol III (**8**)	*K. foliosa*									[10]

Table-2. Knipholone-type anthraquinones isolated from the family Asphodelaceae

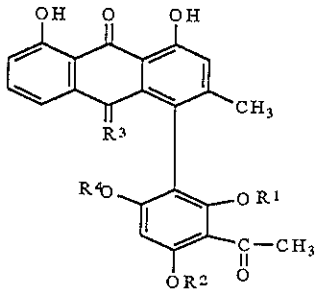
Compounds	Source	Structure				Reference
						
		R^1	R^2	R^3	R^4	
Knipholone (**24**)	*K. foliosa, Bulbine frutescens, B. latifolia*	H	CH_3	O	H	[17,20,35,36]
Isoknipholone (**22**)	*K. foliosa*	CH_3	H	O	H	[10]
Knipholone anthrone (**20**)	*K. foliosa*	H	CH_3	H_2	H	[10]
Isoknipholone anthrone (**26**)	*K. foliosa*	CH_3	H	H_2	H	[10]
4'-demethylknipholone (**28**)	*Bulbine capitata* (aerial part)	H	H	O	H	[49]
6'-O-Methylknipholone (**12**)	*B. capitata*	H	CH_3	O	CH_3	[20]

Table-2. Continued.

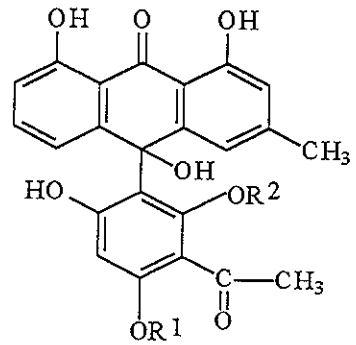
Compounds	Source	Structure		Reference
				
		R^1	R^2	
Foliosone (**32**)	*Kniphofia foliosa*, *Bulbine capitata*	OCH_3	H	[10, 49]
Isofoliosone (**34**)	*K. foliosa*	H	OCH_3	[10]

Table-3. Dimmeric anthraquinones isolated from the family Asphodelaceae.

Compounds	Source	Structure	Reference
Asphodelin (**36**)	*Asphodelus microcarpus*	10'= O	[31]
1,1',8,8'-Tetrahydroxy-3,3'-dimethyl-4,7'-9,9,'10-trione-10'-C-α-$_L$-arabinopyranosyl (**38**)	*A. ramosus*	10'= α-$_L$-Arabinopyranosyl	[40]
1,1',8,8'-Tetrahydroxy-3,3'-dimethyl-4,7'-9,9,'10-trione-10'-C-α-$_L$-arabinopyranosyl (**40**)	*A. ramosus*	10'= 6-Deoxy-β-$_D$-glucopyranoside	[40]

Table-3. Continued.

Compounds	Source	Structure	Reference
1,1',8,8'-Tetrahydroxy-3,3'-dimethyl-4,7'-9,9,'10-trione-10'-C-(6-deoxy-β-$_D$-gulopyranoside) (**42**)	*A. ramosus*	10'= 6-Deoxy-β-$_D$-gulopyranoside	[40]
1,1',8,8'-Tetrahydroxy-3,3'-dimethyl-4,7'-9,9,'10-trione-10'-C-α-$_L$-arabinopyranosyl (**44**)	*A. ramosus*	10'= α-$_L$-Rhamnopyranosyl	[40]
1,1',8,8'-Tetrahydroxy-3,3'-dimethyl-4,7'-9,9,'10-trione-10'R-C-β-$_D$-xylopyranoside (**46**)	*A. ramosus*	10'= β-$_D$-Xylopyranoside	[40]

Table-3 continued.

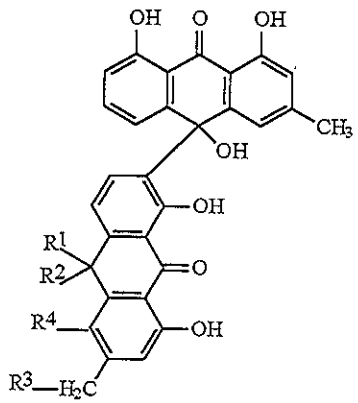
Compounds	Source	Structure				Reference
						
		R^1	R^2	R^3	R^4	
Chryslandicin (**48**)	*Kniphofia sp*	R^1+R^2= O	-	H	OH	[34]
Chrysalodin (**50**)	*K. foliosa*	R^1+R^2= O	-	OH	H	[34]
Ramosin (**52**)	*Asphodelous ramosus*	OH	Glc	H	H	[38]

Table-3 continued.

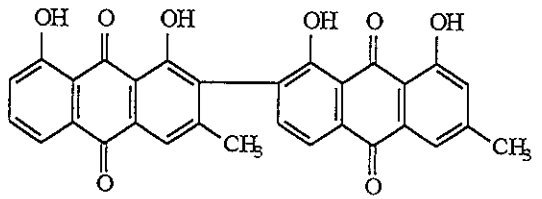
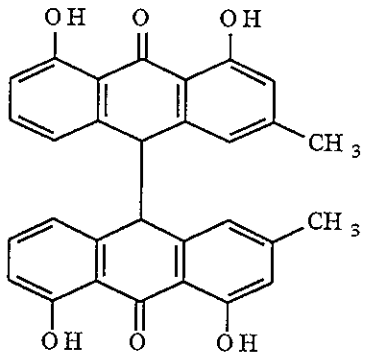
Name of Compound	Source	Structure	Reference
Ararobinol (**54**)	*Asphodelus micocarpus*		[30]
Microcarpin (**56**)	*A. microcarpus*		[37]

Table-4. Isofuranonaphthoquinones isolated from *Bulbine capitata*.

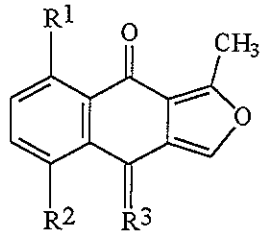
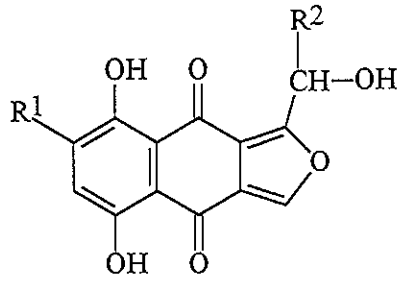
Name of compound	Source	Structure 			Reference
		R^1	R^2	R^3	
5,8-Dihydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione (**18**)	*Bulbine capitata*	OH	OH	O	[20]
8-Hydroxy-1-methylnaphtho[2,3-c]furan 4,9-dione (**16**)	*B. capitata*	OH	H	O	[20]
8-Hydroxy-1-methylnaphtho[2,3-c]furan 9(4H)-one (**62**)	*B. capitata*	OH	H	H_2	[20]
5-Hydroxy-8-methoxy-1-methylnaphtho[2,3-c]furan-4,9-dione (**64**)	*B. capitata*	OCH_3	OH	O	[20]
8-Hydroxy-5-methoxy-1-methylnaphtho[2,3-c]furan-4,9-dione (**66**)	*B. capitata*	OH	OCH_3	O	[20]

Table-4 continued.

Name of compound	Source	Structure		Reference
				
		R^1	R^2	
5,8-Dihydroxy-1-hydroxy methylnaphtho[2,3-c]furan-4,9-dione (**68**)	*B. capitata*	H	H	[20]
Isofuranonaphthoquinone (**70**)	*B. capitata*	OCH_3	CH_3	[20]

1.6 Biogenesis and distribution of anthraquinones in the family Asphodelaceae

Anthraquinones have been repeatedly used in the classification of plant taxa. Many examples could be cited to show the usefulness of anthraquinones as chemical characters with in a family [41, 42].

The fact that anthraquinones are derived by different pathways may be particularly misleading if anthraquinones are used to deduce relationships among plant taxa. Only anthraquinones derived by the same pathway can be considered equivalent. It is therefore mandatory to compare pathways leading to anthraquinones rather than the final quinonoid formulas [43, 44].

Generally the biosynthesis of anthraquinones is believed to follow two routes, the acetate-malonate and the shkimate-mevalonate pathway [41]. Based on their structures and biosynthesis, anthraquinones are either the emodin type or the alizarin-type. Those anthraquinones with substituent on both benzenoid rings, follow the acetate-malonate biogenetic route. They are chracterized by having substitution on both rings A and B, and are the emodin type. Antraquinones of the alizarin-type are totally devoid of substituents on one benzenoid ring and they arise by the shikimate-mevalonate pathway.

The majority of the anthraquinones which are assumed to be elaborated by the acetate-

malonate pathway conform to the emodin (**1**) pattern and this is considered to arise by suitable folding and condensation of a polyketide chain derived from eight acetate units. Numerous variations of this basic structure exist resulting from O-methylation, dimmerization and the introduction or omission of nuclear hydroxyl groups [41,45].

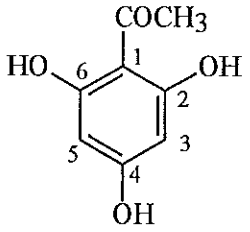
Anthraquinones of the Asphodelaceae have substituents on both rings [41]. Therefore the most plausible biogenetic route for anthraquinones in the Asphodelaceae appears to be of the acetate-malonate origin. This was supported by labeling experiments [46].

The co-existence of chrysophanol (**14**), aloe-emodin (**2**) and rhein (**6**) in most species of the family may be indicative for step-wise oxidation of the methyl group of chrysophanol (**14**) leading all the way to rehin (**6**) through aloe emodin [28, 32, 33, 39]. Oxidation of chrysophanol at position-4 may be considered as the path to islandicin (**58**).

Oxidative coupling, a very common modification in the biosynthesis of anthraquinones, could also be observed in anthraquinones isolated from Asphodelaceae. Oxidative coupling between two chrysophanol units could lead to asphodeline (**36**) [31]. Oxidative coupling followed by glycosylation or *vice versa* could also be predicted for the formation of dimeric compounds isolated from *A. ramosus* [38, 40].

Another unique oxidative coupling elaborated in some members of the family Asphodelaceae could lead to the biosynthesis of knipholone-type compounds [10, 17, 18, 20, 35, 36].

The biogenetic relationship of anthraquinones isolated from the family Asphodelaceae could be illustrated as given on Scheme-II.



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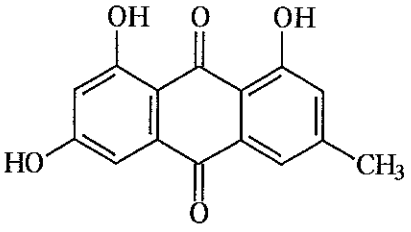

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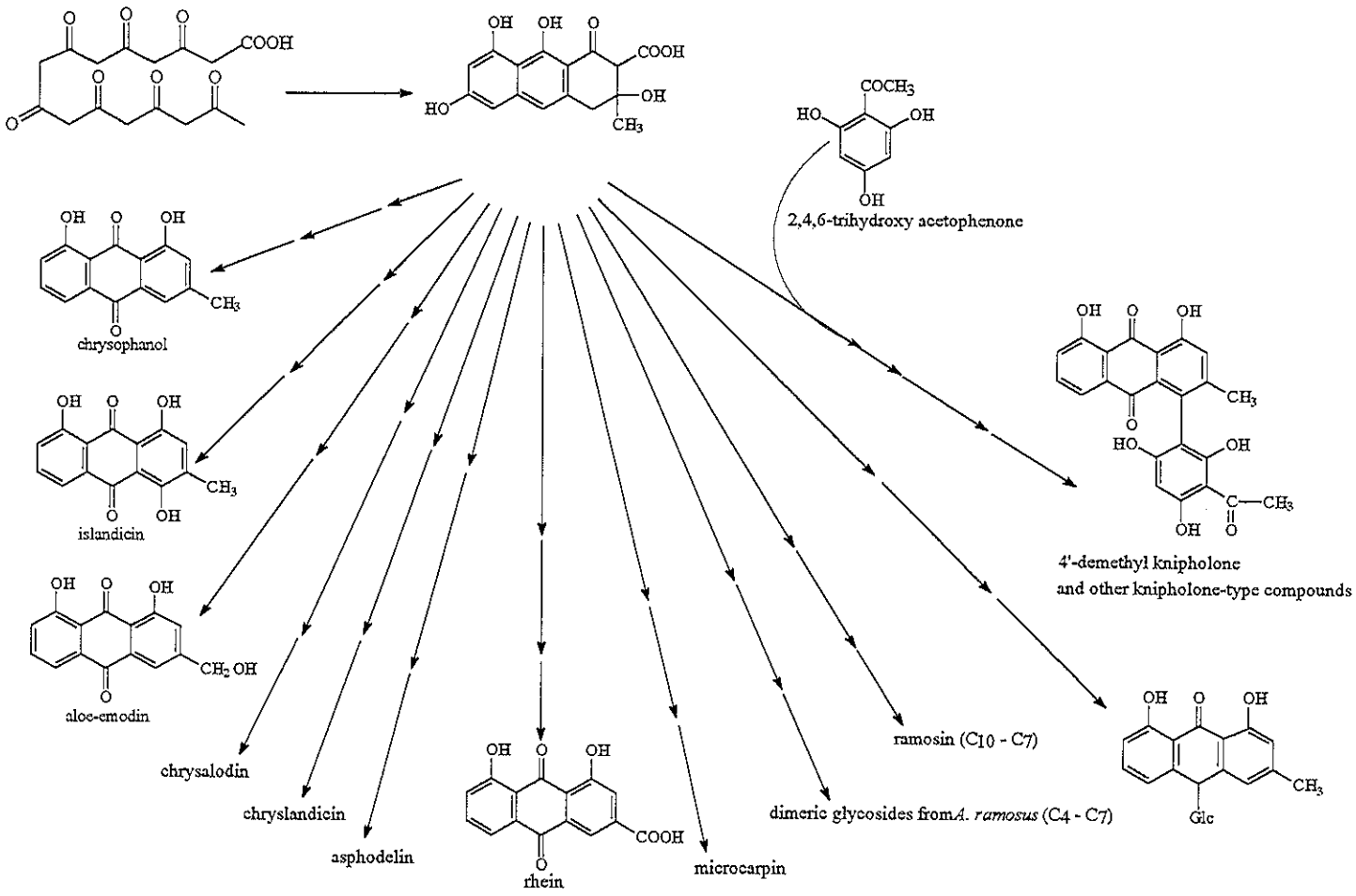
Scheme-II. Biogenetic relationship of anthraquinones of the Asphodelaceae.



1.7 Objective of the Project

The main objective of this study is to carryout phytochemical investigations of *Bulbine abyssinica*, *Bulbine natalensis* and *Trachyandra divaricata*. The chemical information generated in this work may be used in the proper placement of these plants in the family Asphodelaceae.

2.0 RESULTS AND DISCUSSION

2.1 General

The chemical ivestigation of three plants belonging to the family Asphodelaceae was conducted in this study. These are *Bulbine abyssinica*, *Bulbine natalensis* and *Trachyandra divaricata*. The source, voucher numbers and origin of plant materials included in the discussion are listed in Table-5.

Table-5. Source, voucher numbers and origin of the plant materials.

Species	Voucher number	Origin
B. abyssinica A. Rich	Sebsebe and Friis 5585	Showa, Ethiopia
B. natalensis Baker	Dagne S562	Aroboretum, Chemistry Dept., AAU
T. divaricata Kunth	Sebsebe 5619	South Africa

2.2 Extraction and isolation

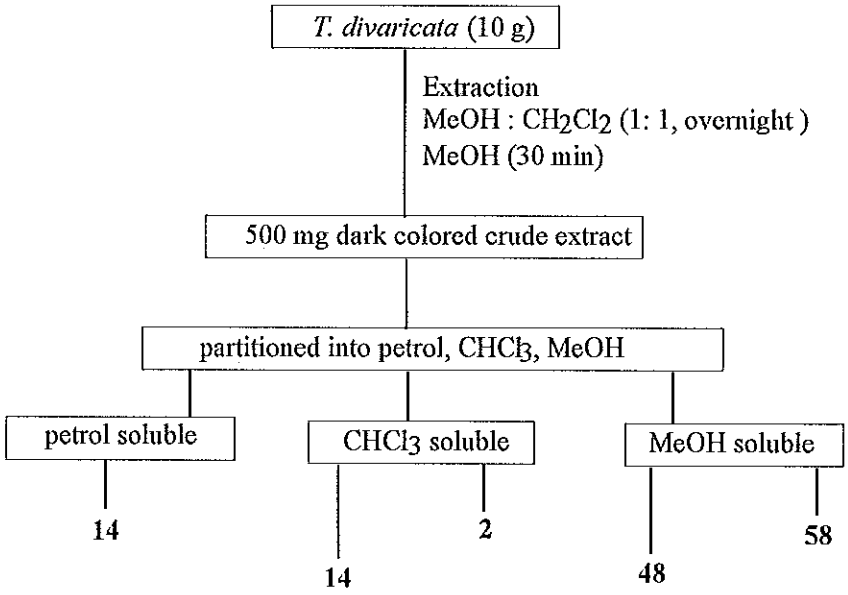
Extraction

In all cases (*Bulbine abyssinica, Bulbine natalensis and Trachyandra divaricata*) extraction was done by soaking the air dried and powdered roots of the plants in MeOH :CH_2Cl_2 (1:1) overnight followed by soaking in MeOH for 30 min. The combined extracts gave dark colored residues after removing the solvent by reduced pressure distillation.

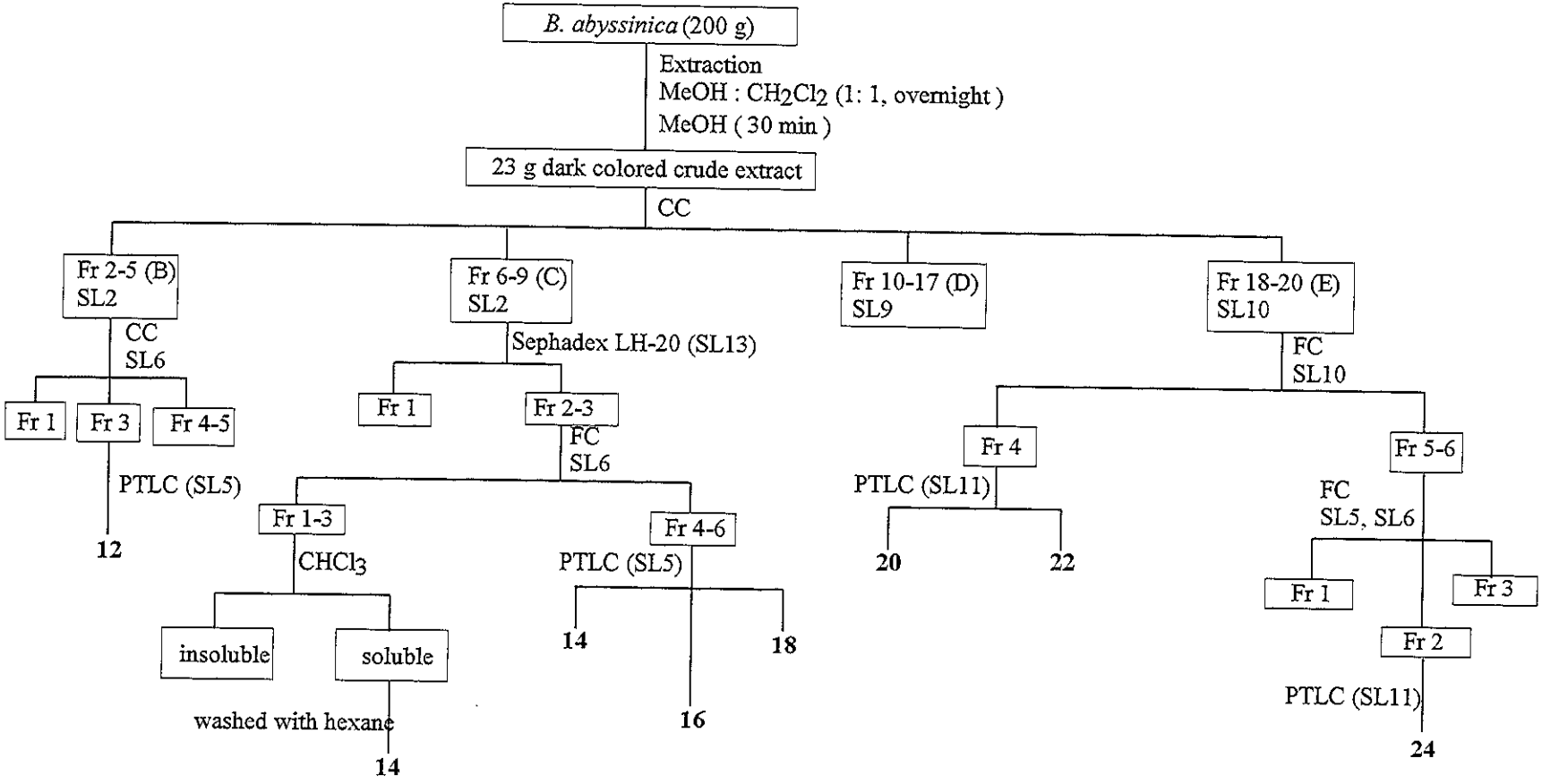
Isolation

Isolation of the pure compounds was performed by column chromatography of the extracts on silica gel. The progress of the separation was monitored by TLC. The components were detected by exposing TLC plates to UV light and by spraying with 5% KOH in methanol. Those fractions which showed positive test for quinones were rechromatographed on column chromatography (silica gel) and Sephadex LH-20. Further separation and purification was done using PTLC. A number of pure compounds were isolated, for selected compounds, ^{1}H NMR, MS, UV-Vis, IR and MP data were obtained and others were identified based on TLC comparison with authentic samples (see Schemes-III-V for isolation procedure and refer exprimental part for solvent sytem code).

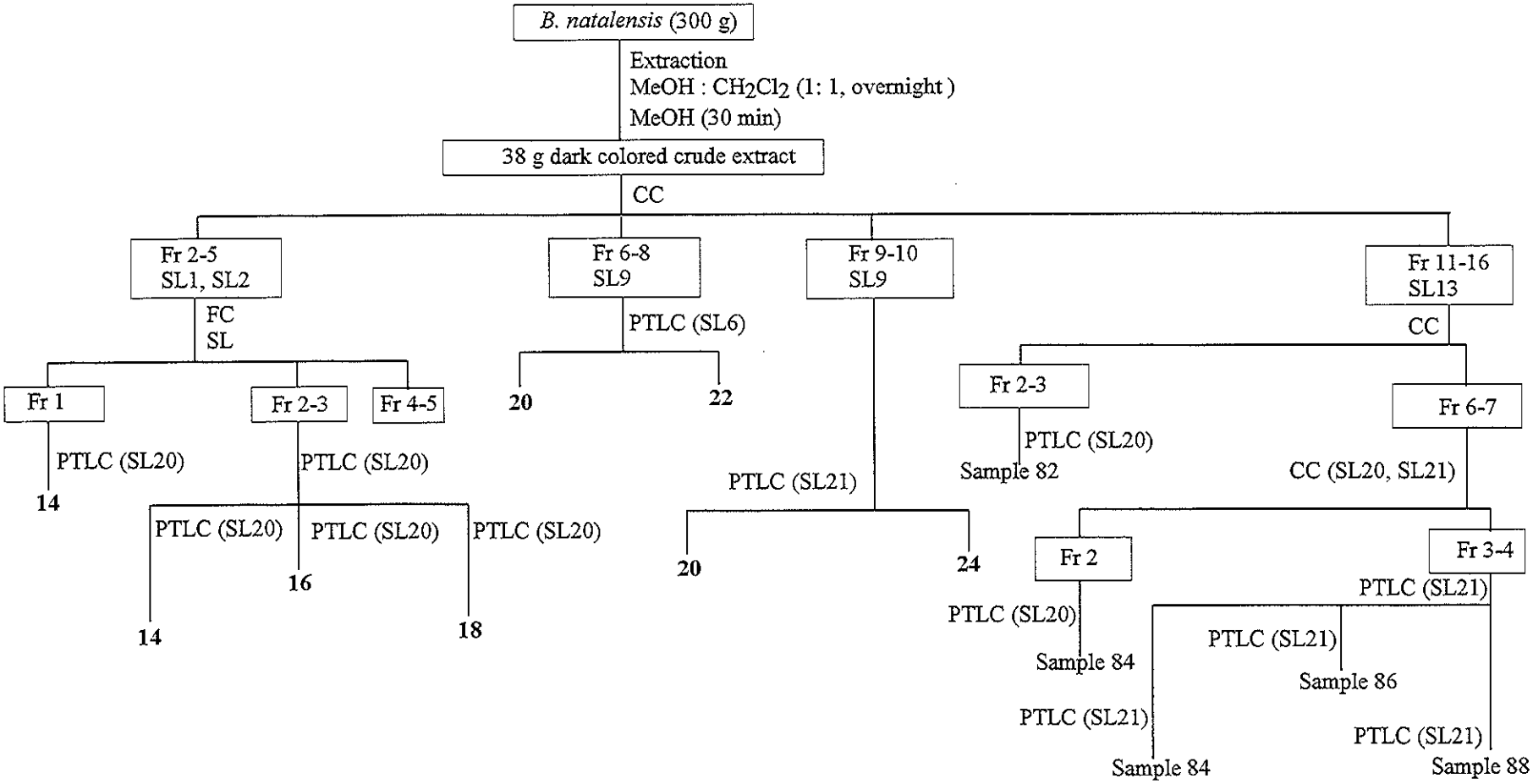
Scheme-III. Extraction and fractionation procedure for *T. divaricata*



Scheme-IV. Extraction and isolation procedure for *B. abyssinica*



Scheme-V. Extraction and isolation procedure for *B. natalensis*



2.2.1.1 Knipholone-6'-methyl ether (**12**)

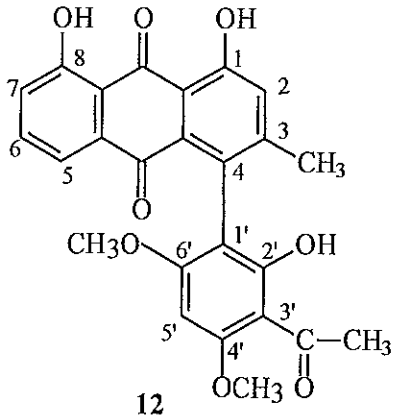
Compound **12** was obtained in pure form after column chromatography and PTLC on silica gel of the extract of *B. abyssinica* as described in the Experimental Section. The compound had a yellow color on TLC which changed to redish brown upon spraying the TLC plate with 5% KOH in methanol.

The UV spectrum of this compound had absorption maxima at 254, 280 and 439 nm showing the presence of a quinonoid chromophore. The IR spectrum displayed absorption bands at 3472, 1657 and 1619 cm^{-1} that indicate the presence of hydroxyl groups, non chelated and chelated carbonyl groups, respectively.

The mass spectrum showed a molecular ion peak at m/z 448 (86%) in agreement with the molecular formula $C_{24}H_{20}O_8$ for compound **12** . Major fragment ions appeared at m/z 433 (100%) and 417 (38%).

The singlets at δ13.91, 12.62 and 12.10 in the ^1H NMR spectrum show three chelated hydroxyl groups. The signals at δ7.61 (*dd*, J=1.9, 7.7 Hz), 7.57 (*t*, J=7.7 Hz) and 7.22 (*dd*, J=1.9, 7.7 Hz) are attributed to an ABC aromatic proton pattern of the quinonoid system. The singlet at δ2.68 indicates the presence of COMe group attached to an aromatic ring and the two singlets at δ4.0 and 3.68 indicate methoxy groups attached to an aromatic ring. The singlet at δ3.68 indicates an OMe at C-4' while the one at δ4.0 can be attributed to an OMe at C-2' or C-6'.

The spectroscopic data discussed above suggest that this compound has structure **12**. Table-6 shows ^{1}H NMR data for compound **12** and the reported ^{1}H NMR values for knipholone-6'-methyl ether.



12

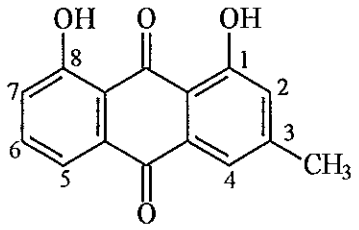
Table-6. ^{1}H NMR data of compound **12** and knipholone-6'-methyl ether

H	Compound **12** (400MHz, $CDCl_3$) δ ppm	Knipholone-6'-methyl ether (300MHz, $CDCl_3$) [20] δ ppm
OH-1	13.91(*s*)	13.92 (*s*)
OH-8	12.62 (*s*)	12.61 (*s*)
OH-2'	12.10 (*s*)	12.08 (*s*)
H-5	7.61 (*dd*, 1.8, 8 Hz)	7.61 (*dd*, 1.9, 7.7 Hz)
H-6	7.57 (*t*, 8 Hz)	7.56 (*t*, 7.7 Hz)
H-2	7.25 (*s*)	7.26 (*s*)
H-7	7.22 (*dd*, 1.8, 8 Hz)	7.21 (*dd*, 1.9, 7.7 Hz)
H-5'	6.10 (*s*)	6.13 (*s*)
OMe-6'	4.00 (*s*)	4.00 (*s*)
OMe-4'	3.68 (*s*)	3.77 (*s*)
COMe-3'	2.68 (*s*)	2.67 (*s*)
Me-3	2.11 (*s*)	2.10 (*s*)

Comparison of the spectral data generated for compound **12** with those reported for knipholone-6'-methyl ether [16] revealed that the two compounds are identical. According to Bezabih and co-workers [16] the placement of an OMe at position 6' was confirmed based on NOE studies. Knipholone-6'-methyl ether was first reported from *B. capitata* [16].

2.2.1.2 Chrysophanol (**14**)

This yellow pigment was isolated from the early fractions (see the Experimental Section) of the column chromatography of the extract of *B. abyssinica*. The IR spectrum of this compound displayed absorption bands at 2956, 1676 and 1627cm^{-1} that indicate the presence of hydroxyl groups, non chelated carbonyl and chelated carbonyl groups, respectively. The UV-Vis spectrum of the compound had absorption maxima at 254, 280 and 450 nm suggesting a quinonoid chromophore. The presence of two chelated hydroxyl groups (δ12.14, *s*; δ12.04, *s*), five aromatic protons [δ7.67 (*t*, J=12 Hz); 7.83 (*dd*, J=4,12 Hz); 7.66 (*d*, J=4 Hz); 7.11 (*dd*, J=4 Hz) and 7.30 (*dd*, J=4,12 Hz)] and one aromatic methyl group (δ2.48, *s*) was evident from the ^{1}H NMR spectrum. The melting point of compound **14** was in agreement with the reported value for chrysophanol (mp 192-4 ^{0}C, lit.[34] mp 193-5 ^{0}C). The identity of compound **14** as chrysophanol was further established by comparison with an authentic sample using TLC.



14

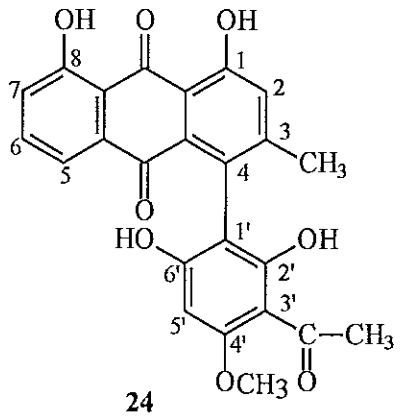
2.2.1.3 Knipholone (**24**)

It is a brick red pigment which turned into a deep red color upon spraying with 5% KOH in methanol on TLC. The UV-Vis spectrum of **24** had absorption maxima at 257, 293, 350 and 450 nm showing the presence of quinonoid chromophore. The IR spectrum displayed absorption bands at 3388, 2902, 1644 and 1603 cm^{-1} that indicate the presence of hydroxyl groups, non chelated and chelated carbonyl groups. From its ^1H NMR spectrum, three chelated hydroxyl groups were observed at δ14.20 (*s*), 12.62 (*s*) and 11.98 (*s*). The signals at δ2.17 (*s*), 2.67 (*s*) and 3.96 (*s*) were due to aromatic methyl, an acetyl methyl and methoxy groups, respectively, attached to aromatic systems. Aromatic proton signals appeared at δ6.18 (*s*), 7.25 (*dd*, J=1.4, 7.7 Hz), 7.31 (*s*), 7.61 (*dd*, J=1.4, 8 Hz) and 7.62 (*t*, J=8 Hz). The ^1H NMR, IR, UV-Vis and MS data of **24** agree very well with reported values for knipholone [16, 35, 36]. Hence the structure **24** was proposed for this compound. Table-7 compares the ^1H NMR data for compound **24** with the reported values for knipholone [16].

Knipholone (**24**) was first isolated from *Kniphofia foliosa* [35] and since then has been reported from other *Kniphofia*, *Bulbine* and *Senna* species [17, 20, 36, 56].

Table-7. ^{1}H NMR data of compound **24** and knipholone.

H	Compound **24** (400 MHz, $CDCl_3$) δ ppm	Knipholone (300 MHz, $CDCl_3$) [20] δ ppm
OH-1	14.20 (*s*)	14.10 (*s*)
OH-8	12.62 (*s*)	12.45 (*s*)
OH-2', 6'	11.98 (*s*)	11.95 (*s*)
H-5	7.61 (*dd*, 1.4, 8 Hz)	7.47 (*dd*, 1.1, 8 Hz)
H-6	7.62 (*t*, 8 Hz)	7.73 (*t*, 8 Hz)
H-2	7.31 (*s*)	7.36 (*s*)
H-7	7.22 (*dd*, 1.4, 8 Hz)	7.23 (*dd*, 1.1, 8 Hz)
H-5'	6.18 (*s*)	6.17 (*s*)
OMe-4'	3.96 (*s*)	3.90 (*s*)
COMe-3'	2.67 (*s*)	2.69 (*s*)
Me-3	2.17 (*s*)	2.16 (*s*)



24

2.2.2 *Bulbine natalensis*

Chemical investigation of the MeOH:CH_2Cl_2 (1:1) extract of the roots of *B. natalensis* afforded five anthraquinones **2, 14, 20, 22** and **24** and two isofuranonaphthoquinones **16** and **18**.

The identification of **2, 14, 20,** and **22** as aloe-emodin (**2**), chrysophanol (**14**), knipholone anthrone (**20**) and isoknipholone (**22**) was based on TLC comparison with authentic samples. Compound **24** was identified as knipholone based on ^{1}H NMR, IR, UV-Vis, MS data and TLC comparison with authentic sample. Compounds **16** and **18** were identified to be 8-hydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione and 5,8-dihydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione, respectively, based on their ^{1}H NMR, UV-Vis, IR and MS data.

Isofuranonaphthoquinones are new type of naphthoquinones. There are only a few examples reported in the chemical literature. Nectriafurane (**3**) is the first example which was isolated from cultures of the fungus *Nectria haematococca* [50]. In higher plants isofuranonaphthoquinones have been isolated from *Ventilago* species (Rhamnaceae) [52-54], from *Aloe ferox* [51] and *Bulbine capitata* [20].

The elucidations of the structures of compounds **16** and **18** are discussed below.

2.2.2.2 5,8-Dihydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione (**18**)

This yellow solid showed an orange fluorescence upon exposing the TLC to long wavelength (366 nm) UV-light. The UV-Vis spectrum of **18** had absorption maxima at 254, 285, 350 and 410 nm indicating a quinonoid chromophore. The IR spectrum displayed absorption bands at 3133 and 1681 cm^{-1} due to hydroxyl and chelated carbonyl groups, respectively. The mass spectrum showed a molecular ion peak at m/z 244 that requires the molecular formula $C_{13}H_8O_5$. The ^1H NMR spectrum displayed signals at δ12.97 (*s*) and 12.81 (*s*) due to chelated hydroxyl groups. The signal at δ 7.27 (*s*), which integrated for two protons, is due to an AB aromatic proton pattern. The singlet peak at δ8.09 suggests the presence of a methine proton attached to an aromatic system. The signal at δ2.80 (*s*) is attributable to a methyl group. The spectral data discussed above suggests structure **18** for this compound. Table-9 shows a comparison of the ^1H NMR data for compound **18** with those reported for 5,8-dihydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione [20].

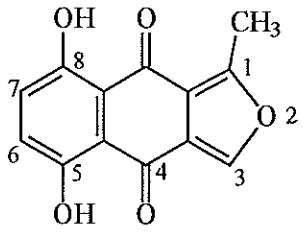


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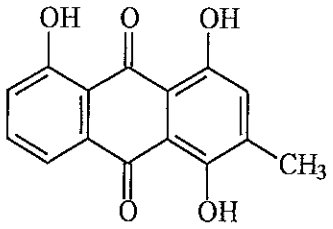
Table-9. ^{1}H NMR data of compound **18** and 5,8-dihydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione.

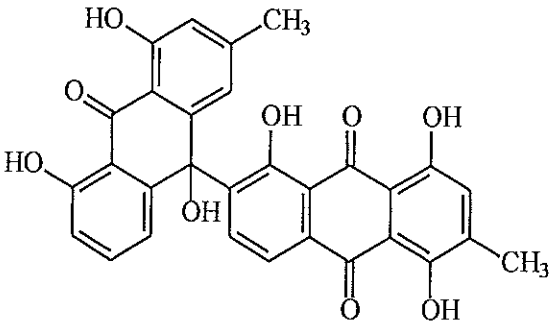
H	Compound **18** (400 MHz, $CDCl_3$) δppm	5,8-Dihydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione (300 MHz, $CDCl_3$) [20] δ ppm
OH-8	12.97 (*s*)	12.97 (*s*)
OH-5	12.81 (*s*)	12.82 (*s*)
H-3	8.09 (*s*)	8.07 (*s*)
H-6, H-7	7.27 (*s*, 2H)	7.25 (*s*)
H-1	2.80 (*s*)	2.78 (*s*)

2.2.3 *Trachyandra divaricata*

T. divaricata was found to contain four anthraquinones which were identified to be chrysophanol (**14**), aloe-emodin (**2**), chryslandicin (**48**) and islandicin (**58**) based on TLC comparison with authentic samples. Other unidentified pigments (expected to be anthraquinones) were also detected from the positive color reaction test for hydroxy anthraquinones.



58



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3.0 EXPERIMENTAL

3.1 General

Plant material

Bulbine abyssinica was collected near Debre Libanos on the road to Gojjam around the Portugese Bridge at an altitude of 2500 m. *B. natalensis* was collected from the aroboretum of the Department of Chemistry, AAU. *T. divaricata* was collected from the Cape region of the South Africa. Voucher specimen of *B. abyssinica* and *T. divaricata* have been deposited at the National Herbarium, Addis Ababa University. Sources, voucher numbers and origins of the plant materials used in these investigations are given in Table-5.

Instruments

NMR: 400 MHz spectra were recorded on a Varian VXR-5000 Spectrometer at the Chalmers University of Technology, Gothenburg, Sweden.

300 MHz spectra were recorded on a Brucker 300 MHz machine at the University of Botswana, Gaborone, Botswana.

MS: EI and CI mass spectra were recorded at the University of Botswana and on a VG ZABSPEC instrument at the Chalmers University of Technology, Gothenburg, Sweden.

IR: IR spctra were recorded on Perkin-Elmer FTIR 1600 series Spectrometer.

UV: UV-Vis spectra were recorded on Plus X Beckman, DU-65 spectrophotometer.

3.2 Extraction

3.2.1 *Bulbine abyssinica*

Air dried and powdered roots of *B. abyssinica* (200 g) were extracted with 2 L of MeOH: CH_2Cl_2 (1:1) followed by extraction with 1.5 L MeOH. The combined extract gave 23 g of dark colored resedue after removing the solvent under vacuum at 40-45 ^{0}C.

3.2.2 *Bulbine natalensis*

Air dried and powdered roots of *B. natalensis* (300 g) were extracted with 2 L of MeOH: CH_2Cl_2(1:1) followed by extraction with 1.5 L MeOH. The combined extract gave 38 g of dark colored residue after removing the solvent under vacuum at 40-45 ^{0}C.

3.2.3 *Trachyandra divaricata*

Air dried and powdered roots (10 g) of *T. divaricata* was extracted with 150 ml of MeOH:CH_2Cl_2 (1:1) followed with 100 ml of MeOH , the combined extract afforded 975 mg brown colored residue.

3.3 Isolation

3.3.1 *Bulbine abyssinica*

The crude extract (22 g) was adsorbed on silica gel and was applied on to a column (30 cm X 3 cm) packed with impregnated silica gel using solvent SL9. Successive elution with solvent SL1, SL2, SL9,SL10, SL16, SL17 and SL18 gave a total of 37 fractions, 100 ml each. The progress of the separation was followed by TLC. On the basis of TLC the following fractions were combined.

Fractions	Eluent	Code
2-5	SL2	B
6-9	SL2	C
10-17	SL9	D
18-20	SL10	E
21-27	SL16,SL17	F
31-37	SL17,SL18	G

Fraction B (6 g) was applied on a column of silica gel and eluted with solvent SL6. Five fractions (100 ml each) were collected. The first and the third fractions showed single major spots on TLC (SL6). Both yellow colored spots were changed to pink and reddish brown when sprayed with 5% KOH in methanol reagent showing positive test for hydroxy quinones. The third fraction was purified by PTLC (SL5) to afford compound **12** in a pure form which was identified as knipholone-6'-methyl ether.

Fraction C (9 g) was subjected to chromatography on sephadex LH-20 (SL13) to afford three fractions. The last two fractions showed positive color test for hydroxy quinones. The two fractions were combined and subjected to flash column chromatography on silica gel using SL6 as eluent. Six fractions were collected. Fractions 1-3 showed four major spots on TLC. The upper one, yellow in color, changed to pink when sprayed with 5% KOH in methanol. The yellow colored mixture was partitioned into $CHCl_3$ soluble and insoluble portions. The soluble portion afforded pure compound **14**. Fractions 4-6 obtained from the flash column chromatography showed three spots on TLC (SL3). The upper spot was yellow and the others are highly fluorescing bright yellow in color which turned to blue, brown and reddish brown, respectively, when sprayed with 5% KOH in methanol. Separation and purification by PTLC (SL3) afforded the upper band as pure compound **14**. The middle and the lower bands were identified as 8-hydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione (**16**) and 5,8-dihydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione (**18**) by TLC comparison with authentic samples isolated from *Bulbine natalensis*.

Fraction E (7.5 g) was subjected to flash column chromatography on silica gel using SL10 as eluent and ten fractions (100 ml each) were collected. Fraction 4 showed two widely spaced spots on TLC (SL11). Separation was made by PTLC (SL11) to obtain compounds **20** and **22** in pure form. **20** and **22** were identified as knipholone anthrone and isoknipholone, respectively, based on comparison with authentic samples. Fractions 5-6 showed complex yellow and red spots on TLC (SL6), which turned to light brown and dark brown when sprayed with 5% KOH in methanol. No simple separation

was possible hence the fractions were combined to obtain 1.3 g brown colored mass which was further subjected to flash column chromatography on silica gel using solvent systems SL5 and SL6 as eluent. Three fractions (75 ml each) were collected. Fraction 2 showed two spots on TLC (SL11). This mixture was subjected to PTLC (SL11) to afforded compound **24** (lower band).

Knipholone-6'-methyl ether (**12**): Red pigment; R_f 0.48 (SL8); mp 252-4^{0}C (lit. [16] mp 255-60^{0}C (dec.)); UV-Vis λ_{max} ($CHCl_3$) nm (log ε): 254 (1.6), 280 (1.7), 439 (0.7); IR ν_{max} (KBr) cm^{-1}: 3472, 2910, 1657, 1619, 1464, 1325, 1216, 1124; EIMS (probe 70 ev). m/z (rel.int.): 448 [M$^+$] (86), 433 (100), 417 (38); ^{1}H NMR (400 MHz, $CDCl_3$): δ13.91(1H, *s*, *peri* OH), 12.62 (1H, *s*, *peri* OH), 12.10 (1H, *s*, *ortho* OH), 7.6 (1H, *dd*, J=1.9, 7.7 Hz, H-5), 7.57 (1H, *t*, J=7.7 Hz, H-6), 7.25 (1H, *s*, H-2), 7.22 (1H, *dd*, J=1.9, 7.7 Hz, H-7), 6.10 (1H, *s*, H-5'), 4.00 (3H, *s*, OMe), 3.68 (3H, *s*, OMe), 2.68 (3H, *s*, Ar-COMe), 2.11 (3H, *s*, Ar-Me).

Chrysophanol (**14**): A yellow pigment; R_f 0.62 (SL3); mp192-4^{0}C, lit. [32] mp 193-5^{0}C; UV-Vis λ_{max} ($CHCl_3$) nm: 254 (2.1), 280 (2.0), 405 (2.4), 450 (2.5);

IR ν_{max} (KBr) Cm^{-1}: 2956, 2916, 1676, 1627; EIMS (probe 70 ev).m/z (rel.int.): 254[M]$^+$(100), 226 (14), 197 (12), 152 (10), 57 (17); ^{1}H NMR (400 MHz, $CDCl_3$): δ1214 (1H, *s*, peri OH), 12.04 (1H, *s*, *peri* OH), 7.83 (1H, *dd*, J=4, 12 Hz, H-5), 7.67 (1H, *t*, J=12 Hz, H-6), 7.66 (1H, *d*, J=4 Hz, H-4), 7.30 (1H, *dd*, J=4, 12 Hz, H-7), 7.11 (1H, *d*, J=4 Hz, H-2), 2.48 (3H, *s*, 3-Me).

Knipholone (**24**): Brick red solid; R_f 0.76 (SL10); mp 219-23^{0}C; UV-Vis λ_{max} ($CHCl_3$) (log ε): 257 (2.0), 293 (1.98), 350 (2.1), 450 (2.7); IR ν_{max} (KBr) cm^{-1}: 3388, 2902, 2832, 1644, 1603, 1450, 1353, 1270, 1207, 1103, 804; ^{1}H NMR (400 MHz, $CDCl_3$): δ14.20 (1H, *s*, *ortho* OH), 12.62 (1H, *s*, *peri* OH), 11.98 (1H, *s*, *peri* OH), 7.62 (1H, *t*, J=8 Hz,H-6), 7.61(1H, *dd*, J=1.4, 8 Hz, H-5), 7.31(1H, *s*, H-2), 7.25 (1H, *dd*, J=1.4, 8 Hz, H-7), 6.18 (1H, *s*, H-5'), 3.96 (3H, *s*, OMe), 2.67 (3H, *s*, Ar-COMe), 2.17 (3H, *s*, Ar-Me).

3.3.2 *Bulbine natalensis*

The dark colored crude extract (38 g) of the root part was adsorbed on oxalic acid impregnated silica gel and applied to a column (30 cm X 3 cm) packed with oxalic acid impregnated silica gel using solvent SL9. The column was eluted with solvent systems SL1, SL2, SL9, SL10 and SL13. A total of 16 fractions, 250 ml each, were collected.

Fractions 2-5, eluted with SL1 and SL2, showed five major spots on TLC (SL20). The combined fraction was subjected to flash chromatography for further separation; five fractions, 100 ml each, were collected. The first fraction showed two yellow spots on TLC (SL20) which turned to red and dark pink when sprayed with 5% KOH in methanol. Separation and purification of the lower components was achieved by PTLC (SL20) resulting in pure compound **14**. The second and third fractions showed four yellow spots on TLC (SL20). Separation and purification by PTLC (SL20) resulted in pure compounds **14**, **16** and **18**. The spots were yellow in color which turned to dark pink, brown and reddish brown, respectively, upon spraying the TLC with 5% KOH in methanol.

Fractions 6-8 of the first column chromatography which was eluted with SL9 showed four spots on TLC (SL6) out of which two were major components and they were identified to be knipholone anthrone (**20**) and isoknipholone (**22**) on the basis of TLC comparison with authentic samples.

Fractions 9-10, eluted with SL7 from the first column, showed five spots on TLC (SL20). Separation and purification of the two major components, brown and red in color, were done by successive application on PTLC(SL21). The two major components were identified as knipholone anthrone (**20**) and knipholone (**24**) by comparison with authentic samples. The brown and red spots on TLC turned to brown and dark brown, respectively, when sprayed with 5% KOH in methanol.

8-Hydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione (**16**): Bright yellow solid; R_f 0.46 (SL20); mp 183-6^{0}C (lit. [16] mp 180-3 ^{0}C); UV-Vis λ_{max} ($CHCl_3$) nm (log ε): 238 (1.82), 274 (0.67), 410 (0.41), 440 (0.43); IR ν_{max} (KBr) cm^{-1}: 3409, 2925, 2854, 1744, 1636, 1562, 1459, 1275, 1156; EIMS(probe 70 ev) m/z(rel. Int.): 228 $[M]^+$ (100), 199 (77), 129 (74), 115 (72), 71 (46); ^{1}H NMR (300 MHz, $CDCl_3$): δ12.87 (1H, *s*, OH-8), 8.06 (1H, *s*, H-3), 7.82 (1H, *dd*, J=1.1, 8 Hz, H-5), 7.65 (1H, *t*, J=8 Hz, H-6), 7.28 (1H, *dd*, J=1.1, 8 Hz, H-7), 2.79 (3H, *s*, 1-Me).

5,8-Dihydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione (**18**): Yellow solid; R_f 0.38 (SL20); mp179-83^{0}C (lit. [16] mp 176-77 ^{0}C); UV-Visλ_{max} ($CHCl_3$) nm (log ε): 254 (2.0), 285 (1.9), 350 (2.18), 410 (2.46); IR ν_{max} (KBr) cm^{-1}: 3133, 2360, 1647, 1564, 1454, 1349,1252, 1140, 884, 856; ^{1}H NMR (300 MHz, $CDCl_3$): δ12.97 (1H, *s*, OH-8), 12.81 (1H, *s*, OH-5), 8.09 (1H, *s*, H-3), 7.27 (2H, *s*, H-6 and H-7), 2.80 (3H, *s*, 1-Me).

3.3.3 *Trachyandra divaricata*

The crude extract (500 mg) was taken and partitioned into petrol, $CHCl_3$ and MeOH. The fractions obtained were checked for the presence of known compounds by comparing with authentic samples. The petrol soluble fraction was found to contain chrysophanol (**14**), the $CHCl_3$ soluble fraction was found to contain aloe-emodin (**2**) and chrysophanol (**14**). The MeOH soluble fraction was found to contain chryslandicin (**48**) and islandicin (**58**).

4.0 CONCLUDING REMARK

The isolation of knipholone-type of compounds from *B. abyssinica* and *B. natalensis*, adds evidence to the proposal forwarded by Dagne, E. and co-workers [10, 18] concerning the possibility of the genera *Kniphofia*, *Bulbinella* and *Bulbine* to show monophyletic units within the family Asphodelaceae (see Scheme-I).

van Wyk, B. E and co-workers [55] pointed out that the cladogram shown in Scheme-I was constructed based on little chemical information at hand; hence they suggested, particularly in the genera *Trachyandra*, *Eremurus*, *Asphodeline* and *Asphodelus* there is a need for more chemical data.

As indicated on the cladogram the key character that determines the place of *Trachyandra* in the family tree is the presence or absence of chrysophanol (**14**) [55].

Therefore based on our findings, the presence of chrysophanol (**14**) in *T. divaricata*, suggests that the place of *Trachyandra* in the family tree should be revised (see Scheme-I).

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