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ANTHRAQUINONES, PRE-ANTHRAQUINONES AND ISOELEUTHEROL IN THE ROOTS OF *ALOE* SPECIES

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Key Word Index—*Aloe*; Aloeaceae; roots; anthraquinones; pre-anthraquinones; isoeleutherol; chemotaxonomy.

Abstract—Comparative TLC analysis of the root extracts of 32 *Aloe* species showed that chrysophanol, asphodelin, chrysophanol-8-methyl ether, aloechrysone, helminthosporin, aloesaponol III, aloesaponarin I, aloesaponol I, aloesaponarin II, aloesaponol II and laccaic acid D-methyl ester are widely distributed in these plants. Isoeleutherol, which is reported here for the first time as a natural product, occurs only in the *Saponariae* series of *Aloe*. The chemotaxonomic implication of the distribution of these compounds for the genus *Aloe* is discussed.

INTRODUCTION

There are more than 360 *Aloe* species described so far and these are known to occur mainly in Africa [1]. Some authors [2, 3] have treated the family Liliaceae in the wider sense and, therefore, place the genus *Aloe* in this family, while others have used narrow family concepts and consider the genus as a member of the Asphodelaceae [4, 5] or the Aloeaceae [1, 6] as distinct from the Liliaceae. At present it seems that the breaking up of the large Liliaceae family into smaller and more homogenous families has gained wider acceptance. However, the issue of whether to place the genus *Aloe* in the Asphodelaceae or the Aloeaceae is still unresolved. Moreover, it has been pointed out that the subgeneric grouping of the genus *Aloe* in Africa is not satisfactory [7].

Various workers including Reynolds [8, 9] have attempted to use chemical information to aid taxonomic classification. Because of the wide medicinal and cosmetic uses, the leaves of *Aloe* have been the focus of chemical studies, including chemotaxonomic oriented investigations. So far, roots and subterranean stems of only *A. saponaria* [10–13], *A. berhana* [14] and *A. graminicola* [15] have been investigated. The compounds reported from these species are anthraquinones and pre-anthraquinones most of which have not been reported from other sources.

The work presented herein describes the distribution of 12 polyketide-derived compounds in the roots of 32 *Aloe* species. One of these compounds, isoeleutherol, is reported here for the first time from a natural source. The chemotaxonomic significance of these compounds is also discussed. The study is mainly based on TLC comparison of the root extracts using in most cases authentic samples as standards.

RESULTS AND DISCUSSION

The roots of *Aloe* were extracted with acetone by percolation at room temperature. TLC analysis of the extracts revealed that these are rich in pigments. Chrysophanol (**1**), asphodelin (**3**), aloesaponarin I (**14**), aloesaponol I (**15**), aloesaponarin II (**16**), aloesaponol II (**17**) and laccaic acid D-methyl ester (**18**) were detected in all the species analysed. The distribution of the other compounds is shown in Table 1.

The anthraquinones discussed here appear to have been derived through two parallel biogenetic routes of the polyketide pathway, differing by the way the octaketide chain folds [16]. Condensation of the polyketide chain in the manner shown in Figs 1 and 2 leads to 1,8-dihydroxy- and 1-methyl-8-hydroxyanthraquinones, respectively.

1,8-*Dihydroxyanthraquinones*

Chrysophanol (**1**) has been reported to occur in the leaves of some *Aloe* species [17] and the subterranean stem of *Aloe saponaria* [11]. Other genera in the family Asphodelaceae such as *Kniphofia* [18], *Bulbine*, *Eremurus*, *Asphodelus* and *Asphodeline* [17] were also reported to contain chrysophanol. Prechrysophanol (**2**) has recently been isolated from the subterranean stems of *A. graminicola* [15]. Asphodelin (4,7'-bichrysophanol, **3**) was first isolated from *Asphodelus microcarpus* [19] and later from *Aloe saponaria* along with asphodelin-9'-anthrone and two other related bianthracene derivatives [13].

In this study chrysophanol (**1**) and asphodelin (**3**) were detected in the roots of all species analysed. The occurrence of asphodelin in *Aloe* as well as in *Asphodelus* species could further strengthen the previously suggested

taxonomic and chemical affinity between Aloinae and Asphodelinae [17] or between Aloeaceae and Asphodelaceae in the present sense [6].

Chrysophanol-8-methyl ether (**4**) was reported from some *Senna* (*Cassia*) species (Leguminosae), *Ventilago calyculata* (Rhamnaceae) [19] and *Aloe berhana* [14]. The corresponding pre-anthraquinone, aloechrysone (**5**) was also reported from *A. berhana*. In this study, compounds **4** and **5** were found to occur in the roots of most of the *Aloe* species analysed. However, it is interesting to

Table 1. The distribution of some anthraquinones, pre-anthraquinones and isoeleutherol in the roots of *Aloe*

Species	Voucher no.	Compounds‖				
		4	**5**	**6**	**7**	**21**
Group 4						
A. pirottae Berger	SD2166*	+	+	+	—	—
A. pirottae Berger	SD2328*	+	+	+	—	—
A. rugosifolia Gilbert & Sebsebe	SD2205*	—	+	—	+	—
Group 6						
A. duckerii Christian	N2550†	—	—	+	+	+
A. dumetorum Mathew & Brandham	N3087†	—	—	+	+	+
A. dumetorum Mathew & Brandham	N3487†	—	—	+	+	+
A. graminicola Reynolds	N3539†	—	—	+	+	+
A. graminicola Reynolds	N3085†	—	—	+	+	+
A. graminicola Reynolds	AY34‡	—	—	+	+	+
A. greatheadii Schonl.	M1870§	—	—	+	+	+
A. kefaensis Gilbert & Sebsebe	SD2208*	—	—	—	+	+
A. kilifiensis Christian	N3631†	—	—	+	+	+
A. lateritia Engler	N3614†	—	—	+	+	+
A. lateritia Engler	N3620†	—	—	+	+	+
A. lateritia Engler	N3422†	—	—	+	+	+
A. lateritia Engler	N2496†	—	—	+	+	+
A. macrocarpa Tod.	SD2380*	—	—	+	+	+
Group 8						
A. chabaudii Schonl.	GN4681§	+	+	+	+	—
A. rivae Bak.	SD2201*	+	+	+	+	—
Group 9						
A. otallensis Bak.	SD2206*	—	+	—	+	—
A. pubescens Reynolds	M1874§	+	+	+	+	—
A. trichosantha Berger	SD2269*	+	+	+	+	—
Group 11						
A. cryptopoda Bak.	GN3769§	+	+	—	+	—
Group 12						
A. Christianii Reynolds	M1871§	+	+	+	+	—
Group 13						
A. calidophila Reynolds	SD2165*	+	+	+	+	—
A. calidophila Reynolds	SD2198*	+	+	+	+	—
A. camperi Schweinf.	SD2213*	+	+	+	+	—
A. camperi Schweinf.	SD2214*	+	—	+	+	—
A. gilbertii Sebsebe & Brandham	SD2207*	+	+	+	+	—
A. sinana Reynolds	SD2210*	+	+	+	+	—
A. sinana Reynolds	M1876§	+	+	+	+	—
Group 14						
A. globuligemma Pole Evans	GN12069§	+	+	+	+	—
A. orthologha Christian	GN4687§	+	+	+	+	—
A. secundiflora Engler	SD2196*	+	—	+	+	—
Group 15						
A. aculeata Pole Evans	GN4822§	+	+	+	+	—
Group 16						
A. berhana Reynolds	SD2209*	+	+	+	+	—
A. harlana Reynolds	SD2338*	—	+	—	+	—
Group 17						
A. megalacantha Bak.	SD2282*	+	+	+	+	—
A. schelpei Reynolds	SD2391*	—	+	—	+	—
A. schelpei Reynolds	M1878§	+	+	+	+	—

Table 1. *Continued*

Species	Voucher no.	Compounds‖ 4	5	6	7	21
Group 19						
A. arborescens Mill.	GN10417§	+	+	+	+	—
A. catengiana Reynolds	M1880§	+	+	+	+	—
Group undetermined						
A. pulcherima Gilbert & Sebsebe	SD2384*	+	+	+	+	—

*Specimen deposited at the National Herbarium, Addis Ababa University, Addis Ababa, Ethiopia.

†At the East African Herbarium, Nairobi, Kenya.

‡At the Herbarium, Botany Department, University of Nairobi, Kenya.

§At the Botanic Garden Museum, Harare, Zimbabwe.

‖Chrysophanol-8-methyl ether (**4**), aloechrysone (**5**), helminthosporin (**6**), aloesaponol III (**7**), isoeleutherol (**21**).

Chrysophanol (**1**), asphodelin (**3**), aloesaponarin I (**14**), aloesaponol I (**15**), aloesaponarin II (**16**), aloesaponol II (**17**) and laccaic acid D-methyl ester (**18**), were detected in all samples analysed. The grouping used above is according to Reynolds [7].

note that these compounds were not detected in any of the species belonging to Group 6, i.e Series *Saponariae* (Table 1).

Yagi *et al.* [11] have earlier reported the occurrence of helminthosporin (**6**), aloesaponol III (**7**), isoxanthorin (**8**) and aloesaponol IV (**9**) in the subterranean stem of *A. saponaria*. In this study, helminthosporin (**6**) was detected in the roots of most of the samples analysed, while aloesaponol III (**7**), which appears to be the precursor of **6**, occurs in the roots of all species analysed, except *A. pirottae*.

Interestingly, the purgative *C*-10-glucosides, barbaloin (**10**) and homonataloin (**11**), which are commonly found in the leaves of several *Aloe* species [8, 9, 17] have not been detected in the roots of any of the species analysed here. It is worth noting that all the 1,8-dihydroxyanthraquinones found in *Aloe* are not oxygenated at C-6 as is the case with emodin (**12**) and physcion (**13**), commonly found in many families of higher plants.

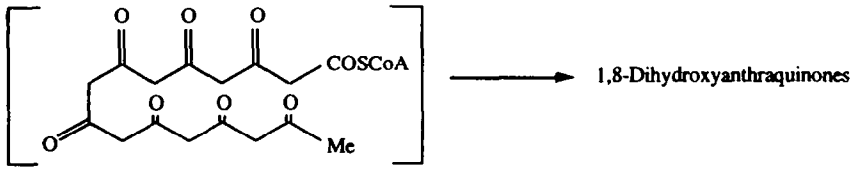
1-*Methyl*-8-*hydroxyanthraquinones*

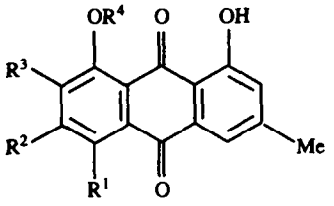
Aloesaponarin I (**14**), aloesaponol I (**15**), aloesaponarin II (**16**), aloesaponol II (**17**), laccaic acid D-methyl ester (**18**) and deoxyerythrolaccin (**19**) were isolated from the subterranean stems of *Aloe saponaria* [10]. The biogenetic relationship among aloesaponarin I, aloesaponol I and laccaic acid D-methyl ester was also established [20]. 1-Methyl-8-hydroxyanthraquinones are not commonly found in plants. Apart from *Aloe* [10, 14, 15], the only plants from which such compounds were reported are *Eleutherine americana* (Iridaceae) [21], *Gladiolus segetum* (Iridaceae) [22], *Rheum* sp. (Polygonaceae) [23], *Araliorhamnus vaginata* (Rhamnaceae) [24] and *Rhamnus fallax* (Rhamnaceae) [25].

In this study, we detected compounds **14–18** in the roots of all species analysed, indicating that 1-methyl-8-hydroxyanthraquinones are characteristic constituents of the *Aloe* genus. It would be of interest to investigate whether such compounds are also produced by other closely related genera in the Aloeaceae (such as *Gasteria, Haworthia, Lomatophyllum* and *Poellnitzia*) or the Asphodelaceae (such as *Asphodeline, Asphodelus, Bulbine, Bulbinella, Eremurus, Hemiphlacus, Jodrellia, Kinphofia, Paradisea, Simethis* and *Trachandra*) [6]. Such a study may help to resolve some of the unsettled taxonomic issues in this area.

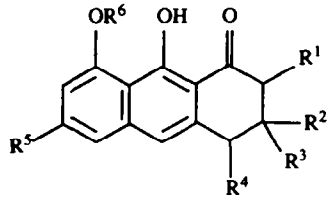
Isoeleutherol-4-*O*-glucoside (**20**) has been isolated previously by Yagi *et al.* [12] from the subterranean stem of *A. saponaria* and yielded isoeleutherol (**21**) upon hydrolysis. In this study, we have isolated and characterized **21** from the roots of *A. graminicola* and this appears to be the first report of its occurrence in nature. The spectroscopic data of **21** were in close agreement with those reported for isoeleutherol in the literature [12]. Confirmation for the placement of the OMe group at position 4 was achieved by a NOE experiment, in which irradiation of the signal at δ1.78 (methyl at C-3) resulted in enhancement of the signals at δ4.13 (OMe) and 5.88 (H-3), while irradiation of the methoxyl resonance at δ4.13 resulted in enhancement of the signals for the C-3 methine (δ5.88), C-3 methyl (δ1.78) and C-5 hydroxyl (δ9.37) protons.

Reynolds [7] made 20 subgeneric groupings of the tropical African *Aloe*. Some of these groups are heterogenous including assemblies of unrelated taxa, while others are relatively homogenous. One such homogenous group is Group 6 (Series *Saponariae*) characterized by pronounced basal swelling of the perianth with abrupt constrictions above the ovary. Our study shows that only members of this group of *Aloe* produce isoeleutherol (**21**), indicating that **21** may be chemotaxonomic marker of the group (Table 1).

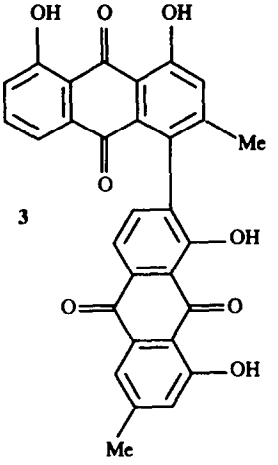


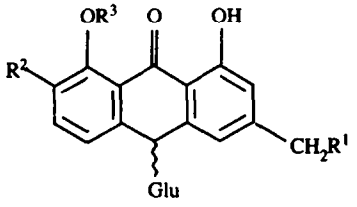


	R^1	R^2	R^3	R^4
1	H	H	H	H
4	H	H	H	Me
6	OH	H	H	H
8	OH	H	OMe	H
12	H	OH	H	H
13	H	OMe	H	H



	R^1	R^2	R^3	R^4	R^5	R^6
2	H	OH	Me	H	H	H
5	H	OH	Me	H	H	Me
7	H	H	H	OH	Me	H
9	OMe	H	H	OH	Me	H





10 $R^1 = OH, R^2 = R^3 = H$
11 $R^1 = H, R^2 = OH, R^3 = Me$

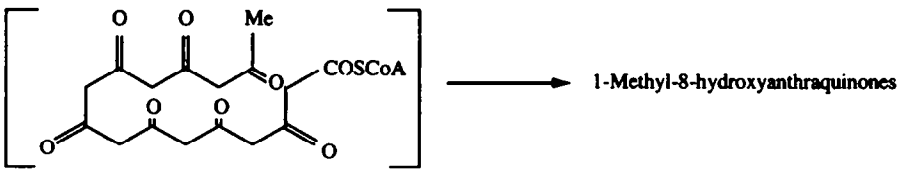
Fig. 1. 1,8-Dihydroxyanthraquinones and their precursors.

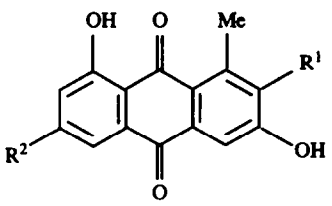
EXPERIMENTAL

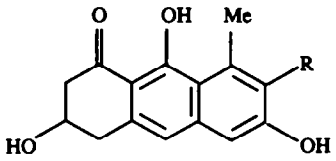
Plant material. The plant materials used in this study were collected from various localities in Ethiopia, Kenya, Tanzania, Zimbabwe, Angola and Namibia. Voucher specimens were deposited at the National Herbarium (ETH), Addis Ababa University, Addis Ababa, Ethiopia, the East African Herbarium (EAH), Nairobi, Kenya and at the National Herbarium (SRGH), Harare, Zimbabwe. Voucher numbers are given in Table 1.

Extraction and TLC analysis. Dried and ground roots (1 g each) of the *Aloe* species indicated in Table 1 were extracted with Me_2CO by percolation at room temp. for 2 days. The extracts were concd under red. pres. and applied to pre-coated silica gel (Merck) plates which were then developed using the solvent systems: petrol–$CHCl_3$ (1:1) (S-1), $CHCl_3$ (S-2), $CHCl_3$–EtOAc (7:3) (S-3), $CHCl_3$–EtOAc (1:1) (S-4) and EtOAc–MeOH–H_2O (200:33:27) (S-5). The chromatograms were viewed under UV light (254, 366 nm) and also sprayed with ethanolic KOH and fast blue salt B spray reagents. Identification of the major chromatographic zones was achieved by direct comparison (co-TLC) with authentic samples.

Isolation and characterization of isoeleutherol (**21**). Powdered roots (30 g) of *A. graminicola* (Voucher no. AY34) were extracted at room temp. with petrol and $CHCl_3$, successively. The $CHCl_3$ extract (2.5 g) was then applied to a column packed with oxalic acid-impregnated silica gel which was eluted with petrol–$CHCl_3$ mixtures



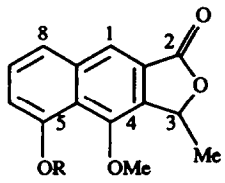




14 $R^1 = CO_2Me$, $R^2 = H$
16 $R^1 = R^2 = H$
18 $R^1 = CO_2Me$, $R^2 = OH$
19 $R^1 = H$, $R^2 = OH$

15 $R = CO_2Me$
17 R = H

Fig. 2. 1-Methyl-8-hydroxyanthraquinones and their precursors.



20 R = Glu
21 R = H

of increasing polarities. The fraction obtained by elution with 5% $CHCl_3$ in petrol showed one major spot on TLC (S-1). Purification of this fraction by circular prep. TLC (using S-1 as eluent) afforded **21** (5 mg).

Isoeleutherol (**21**). Blue fluorescence on TLC under UV_{366}; amorphous (lit. mp [12] 162–164°); found $[M]^+$ 244.0759; $C_{14}H_{12}O_4$ requires 244.0736; UV λ_{max}^{MeOH} nm: 252, 300, 320, 360; ^1H NMR ($CDCl_3$, 400 MHz): δ9.37 (1H, *s*, OH), 8.22 (1H, *s*, H-1), 7.55 (1H, *dd*, $J = 8.0$, 2.0 Hz, H-8), 7.50 (1H, *t*, $J = 8.0$ Hz, H-7), 7.18 (1H, *dd*, $J = 8.0$, 2.0 Hz, H-6), 5.88 (1H, *q*, $J = 8.0$ Hz, H-3), 4.13 (3H, *s*, OMe), 1.78 (3H, *d*, $J = 8.0$ Hz, Me); EIMS *m/z* (rel. int.): 244 $[M]^+$ (72), 229 $[M - Me]^+$ (100), 201 (43), 173 (26), 158 (48).

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