Anthraquinones of Certain Egyptian *Asphodelus* Species

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The study of the anthraquinones of *Asphodelus fistulosus* and *A. microcarpus* resulted in the isolation of chrysophanol, aloe-emodin, anhydrorugulosin, three others bianthraquinones (the cleavage products of which are either 1,8-dehydroxyanthraquinone or chrysophanol) and chrysophanol-8-mono-β-D-glucoside. The obtained results revealed a qualitative difference in the anthraquinone content of the different parts (leaves, seeds and tubers).

Introduction

Several species of the family Liliaceae were found to contain quinones [1]. Of these, anthraquinones represent the major constituents, followed by naphthoquinones. On the other hand, only one benzoquinone *viz.* polygonaquinone was reported in the family. Van Rheede van Oudtschoorn [2] detected anthraquinones in several genera of Liliaceae including *Asphodelus albus*. The study of the anthraquinones of the tubers of *A. microcarpus*, growing in Egypt, was carried out by the authors [3]. Two new bianthraquinones, asphodelin and microcarpin, were recently isolated from *A. microcarpus* [4].

The present work comprises the study of the anthraquinones of *Asphodelus fistulosus* as well as the qualitative comparison of these constituents in the different parts of *A. fistulosus* and *A. microcarpus*.

Results and Discussion

The anthraquinones, either in the free or in the glycoside form, of both *A. fistulosus* and *A. microcarpus* were studied. The method used for the preparation of the free anthraquinones envolves extraction of the plant material with ethanol, followed by treating with alkaline solution and extraction of the liberated anthraquinones, from the acidified medium, with ether.

The column chromatographic technique, using silica gel succeeded only in separating two anthraquinones in a pure form (Table I), while on applying preparative TLC (a common method for the separation of the anthraquinones) and using silica gel (benzene-methanol 90:10) for two developments), six anthraquinone components were obtained in a pure form. The remaining constituents, having very close R_F values, could not be separated on trying several solvent systems and were found to be present in relatively small amounts (anthraquinone components **1–3** in both species and M in *A. microcarpus*). The anthraquinone mixture (M) consists of another 4 components. The identity of these latter components as anthraquinones was

Table I.

Eluting solvent	Fraction No.	Component	R_F *	Detection by NH_3 DL	Detection by NH_3 UV
Hexane-Benzene (50:50)	1–19	**8**	0.83	R.	Or.
Hexane-Benzene (20:80)	20–48	**9**	0.85	R.	R.Br.
Benzene	49–85	**7**	0.74	R.	Y.Or.
		6	0.60	R.	R.Br.
		9	0.85	R.	R.Br.
Benzene-Chloroform (70:30)	86–102	**6**	0.60	R.	R.Br.
		7	0.74	R.	Y.Or.
		9	0.85	R.	R.Br.
Benzene-Chloroform (50:50)	103–157	**4**	0.45	R.	O.Br.
		1–3	—	Br.	R.
			0.50	Or.	Br.
Benzene-Chloroform (30:70)	158–195	**5**	0.50	Or.	Br.
		4	0.45	R.	O.Br.
		1–3		Br.	R.
Chloroform	196–210	M	—	R.	Or.
		1–3	—	Br.	R.
Chloroform-Methanol (50:50)	211–224	M	—	R.	Or.

Y., Yellow; R., Red; Br., Brown and Or., Orange.
* Adsorbent: Silica gel G.; Solvent system: Benzene-methanol (80:20).

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also confirmed (Börntrager test and magnesium acetate solution).

On applying preparative TLC technique, several precautions (including devoid of light) were taken in consideration because of the sensitivity of some anthraquinones in laboratory conditions [1]. Moreover, the qualitative picture of the anthraquinones, prepared either from the air-dried plant by Soxhlet extraction of from fresh plant at room temperature (without any heat) was found to be the same, proving that none of the anthraquinones detected may be an artefact.

Of the isolated six anthraquinone components, one (**4**) was unstable, even when all precautions were taken, and gave as decomposition product 1,8-dihydroxyanthraquinone. The fact that the latter is the sole decomposition component of **4** led to the belief that the original component is probably 1,8-dihydroxy-dianthraquinone which decomposed rapidly.

The anthraquinone components **5**, **6** and **8** were identified as dianhydrorugulosin, aloe-emodin and chrysophanol respectively. On the other hand, components **7** and **9** were found to be bianthraquinones. **7** was obtained in small amounts and its cleavage product is identified as 1,8-dihydroxyanthraquinone; while that of **9** was proved to be only chrysophanol. The data obtained (m.p., IR, UV, MS, tetraacetate and tetramethyl ether derivatives) of the antraquinone **9** are different from those of the other known bianthraquinones, as well as from those recently separated from *A. microcarpus* [4], and seems that it is a new natural bianthraquinone. Further investigation of the latter two bianthraquinones are in progress. The R_F values of the isolated anthraquinones and the available authentic references are shown in Table II.

The above anthraquinones (**1**–**9**), were isolated from both species. The qualitative investigation of the anthraquinones in the different parts of the two species, as carried out by two-dimensional TLC (Fig. 1) revealed certain differences (Table III). The leaves and tubers of *A. microcarpus* have the same qualitative picture *i. e.* the 9 anthraquinones in addition to the mixture M, while the seeds of the same species lack components **4**, **5** and **9**. On the other hand, the antraquinone mixture (M) is not detected in *A. fistulosus*; moreover its seeds lacked, in addition, **7** and **9**. Chrysophanol-8-mono-β-D-glucoside was detected in all parts of the two species. No C-glycosides was detected in both species.

The percentage of the total free anthraquinones (Table IV) varies from 0.009 in the seeds of *A.*

Table II. The R_F values of the isolated anthraquinones and the available authentic references.

Anthraquinone Component	Silica gel					Polyamide		Detection			
								NH_3		$Mg(OAc)_2$	
	A.	B.	C.	D.	E.	F.	G.	DL	UV	DL	UV
Decomposition of **4**	0.58	0.69	0.19	0.79	0.84	0.23	0.04	Or.	R.	R.	R.V.
5	0.18	0.77	—	0.50	0.78	0.08	—	Or.	Br.	R.	R.
6	0.25	0.46	—	0.60	0.78	0.54	0.12	R.	R.Br.	R.	R.
7	0.62	0.64	0.04	0.74	0.84	0.59	0.04	R.	Y.Or.	R.	R.
8	0.67	0.79	0.26	0.83	0.85	0.46	0.08	R.	Or.	R.	R.
9	0.69	0.83	0.11	0.85	0.86	0.42	0.05	R.	R.Br.	R.	R.Br.
Rhein	—	0.60	—	0.71	0.23	0.03	—	R.	R.	R.	R.
Quinalizarin	—	0.41	—	0.03	0.23	—	—	V.	V.	V.	V.
1-Hydroxy-anthraquinone	0.62	0.69	0.25	0.78	0.86	0.53	0.15	R.	R.	R.	R.Or.
1,4-Dihydroxy-anthraquinone	0.58	0.70	0.25	0.76	0.83	0.41	0.07	R.	R.	V.	P.
1,8-Dihydroxy-anthraquinone	0.58	0.69	0.19	0.79	0.84	0.23	0.04	Or.	R.	R.	R.V.
Emodin	0.33	0.49	—	0.64	0.80	0.14	0.02	R.	R.	R.	R.
Chrysophanol	0.67	0.79	0.26	0.83	0.85	0.46	0.08	R.	Or.	R.	R.
Catenarin	0.26	0.47	—	0.60	0.81	0.06	—	V.	V.	V.	V.
Aloe-emodin	0.25	0.46	—	0.60	0.78	0.54	0.12	R.	R.Br.	R.	R.
Aloin	—	0.42	—	0.16	0.31	0.81	0.60	Br.	Or.	R.	R.Br.

Br., brown; Or, orange; P., pink; R., red; V., violet; Y., yellow. Solvents: A: Benzene-ether (4:1 v/v); B: Benzene-acetic acid (80:20 v/v); C: Benzene-carbontetrachloride (1:1 v/v); D: Benzene-methanol (80:20 v/v); E: Ethyl acetate-methanol-water (100:16:14 v/v); F: Methanol-benzene (90:10 v/v); G: Ethanol-water (6:4 v/v).

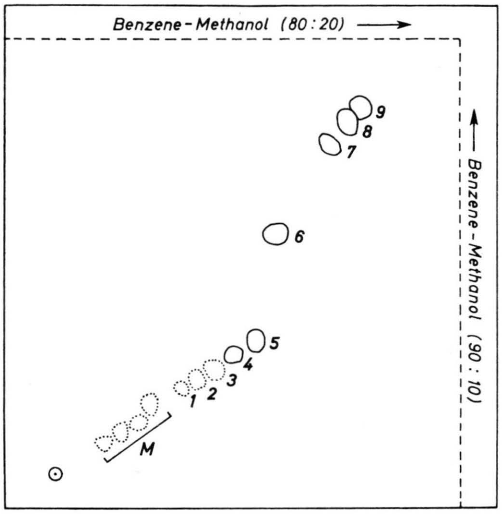

Fig. 1. Two-dimensional thin-layer chromatogram of the anthraquinones of the tubers (collected in September). Adsorbent: Silica gel G.

Tab. III. Qualitative comparison of the anthraquinones in the different parts of the *Asphodelus* species.

Species	M	1–3	4	5	6	7	8	9	10
A. fistulosus									
leaves		+	+	+	+	+	+	+	+
seeds		+	+	+	+		+		+
A. microcarpus									
leaves	+	+	+	+	+	+	+	+	+
seeds	+	+			+	+	+		+
tubers	+	+	+	+	+	+	+	+	+

Table IV. The percentages * of the anthraquinones in the different parts of *Asphodelus* species.

Species	Plant part	Free	Glycoside
A. fistulosus	leaves	0.06	0.0218
	seeds	0.009	0.0013
	leaves	0.12	0.0125
A. microcarpus	seeds	0.02	0.0021
	tubers	2.01	0.059

* Calculated on the air dried material.

fistulosus to 2.31 in the tubers of *A. microcarpus*. The anthraquinone glycosides are represented in small amounts.

Experimental

Material

A. fistulosus L. var. *tenuifolius* Cav. and *A. microcarpus* Salzm. et Vivi were collected from Dakhla (New Valley) and Burg El-Arab respectively; leaves and tubers in April and seeds in June. The plants were kindly authenticated by Dr. K. H. Batanouny, Faculty of Science, Cairo University.

Thin-layer chromatography

Adsorbents: silica gel G and polyamide. Solvent systems: several solvents [3, 5–9] were used (Table II). Visualization was carried out by UV or by spraying with alcoholic magnesium acetate [10] or KOH solution [11] or by exposing to ammonia vapours and reexamining under UV.

Anthraquinones of A. fistulosus

Preparation of the anthraquinones

The procedure used for the preparation of both the free anthraquinones and the glycosides have been previously reported in detail [3]. The anthraquinone mixture was fractionated by preparative TLC (1 mm thick) using benzene-methanol 90:10 two developments. Components **8** and **9** were found to posses very close R_F values in the above solvent, thus they were scraped from the chromatoplates as one zone and then re-fractionated by another preparative TLC using benzene. Development of the plates was carried out in chromatographic jars covered with black paper to devoid light. Components **1**, **2** and **3** were proved to be anthraquinones; however the quantities obtained are comparatively small, and trials to get any of them in a crystalline form were unsuccessful.

Anthraquinone No. **4**: It was found to be unstable. The decomposed product was identified as 1,8-dihydroxyanthraquinone (m. p., m. m. p).
$C_{14}H_8O_4$

Calcd:	C 70.00	H 3.33,
Found:	C 70.01	H 3.21.

Dianhydrorugulosin (**5**) [3]: It decomposed at 318 – 319 °C.
$C_{30}H_{18}O_8$

Calcd:	C 71.14	H 3.56,
Found:	C 71.11	H 3.54.

Tetraacetate m. p. 244 – 248 °C. Cleavage of **5** (50 mg treated with 10% KOH, 50 mg sodium dithionite were added and the mixture was heated for 30 min at 90 °C, then the cold mixture was acidified with 10% HCl and the formed precipitate was extracted with chloroform) gave chrysophanol (TLC, m. m. p., IR). MS of **5** showed m/e 506 ($C_{30}H_{18}O_8$) and m/e 253.

Aloe-emodin (**6**): Anthraquinone **6** was identified as aloe-emodin [3] (m. p., m. m. p. 221 °C, TLC, triacetate, trimethylether, UV, IR).

Anthraquinone No. **7**: It was obtained in small amounts, it decomposed at 267 °C. Cleavage of **7** with sodium dithionite gave 1,8-dihydroxyanthraquinone (TLC, m. p.). UV (in ethanol) showed λ_{max} at 228, 262, 293 and 382 nm.

*Chrysophanol (***8***)*: Anthraquinone **8** was identified as chrysophanol[3] (m. p., m. m. p., 194 – 195 °C),

Calcd:	C 70.87	H 3.95,
Found:	C 70.78	H 3.89.

(TLC, acetate, UV, IR, MS.)

Anthraquinone No. **9**: It decomposed at 253 °C. $C_{30}H_{18}O_8$

Calcd:	C 71.14	H 3.56,
Found:	C 70.98	H 3.64.

MS showed that it is a bianthraquinone revealing M^+ 506 and a fragment at 253 which corresponds to half M^+. Cleavage of **9** with sodium dithionite gave chrysophanol (TLC, m. p.). The tetraacetate m. p. 203 °C showed M^+ 674 which corresponds to

Bianthraquinones	m.p. [°C]	tetra-acetate m.p. [°C]
Asphodelin	274–277	284–289
Dianhydrorugulosin	318–319	244–248
Microcarpin	315–319	305–310
Bianthraquinone (**9**)	253	203

$C_{38}H_{26}O_{12}$ and a fragment at 337 which corresponds to half the M^+. Cleavage of the tetraacetate with sodium dithionite gave chrysophanol diacetate (m. m. p., TLC). The tetramethyl ether derivative melted at 195 °C.

$C_{34}H_{26}O_8$

Calcd:	C 72.59	H 4.62,
Found:	C 72.52	H 4.38.

*Chrysophanol-8-mono-β-D-glucoside (***10***)*: Preparation of the anthraquinone glycosides revealed the presence of only **10**. Its identification was proved by m. p., TLC, acid hydrolysis at 100 °C with 2 N HCl and detection of chrysophanol (TLC, m. m. p.) and glucose (paper chromatography).

Identification and fractionation of the anthraquinone content of the seeds was carried out as mentioned above.

Anthraquinones of A. microcarpus

About 2.4 g of the total anthraquinone mixture (obtained from tubers) were chromatographed on a column of silica gel (250 g). Elution was made with hexane-benzene, benzene, benzene-chloroform, chloroform and chloroform-methanol mixtures, collecting fractions each of 100 ml (Table III). Further fractionation of the components was carried out by preparative TLC.

Investigation of the anthraquinones of the leaves and seeds was carried out in the usual manner.

Two-dimensional TLC

The qualitative comparison of the anthraquinones in the different parts of the two studied species was further carried out to obtain a precise check using benzene-methanol 80:20 in one direction and 90:10 in the other direction.

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[1] R. H. Thomson, Naturally Occurring Quinones, 2nd ed., Academic Press, London-New York 1971.
[2] M. C. B. van Rheede van Oudtschoorn, Phytochem. **3**, 383 [1964].
[3] A. M. Rizk, F. M. Hammouda, and M. M. Abdel-Gawad, Phytochem. **11**, 2122 [1972].
[4] A. G. González, R. Freire, R. Hernández, J. A. Salazar, and E. Suàrez, Chem. and Ind. (17), 851 [1973].
[5] H. Wagner and H. P. Hörhammer, Deutsche Apoth.-Ztg. **108**, 633 [1968].
[6] G. Hauschild, M. Steiner, and K. W. Glombitza, Planta Medica **20**, 1 [1971].
[7] E. Leistner, Phytochem. **10**, 3015 [1971].
[8] T. Furuya, H. Koiima, and T. Katsuta, Phytochem. **11**, 1073 [1972].
[9] Z. F. Ahmed, H. Rimpler, A. M. Rizk, F. M. Hammouda, and S. I. Ismail, Phytochem. **9**, 1595 [1970].
[10] T. J. McCarthy, Planta Medica **16**, 348 [1968].
[11] R. P. Labadie and A. B. Svendsen, Pharm. Weedblad. **102**, 615 [1967].