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CHEMOTAXONOMIC INVESTIGATIONS IN ASPHODELEAE AND ALOINEAE (LILIACEAE)

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Abstract—Seven species from two genera (*Aloe*, *Kniphofia*) of the tribe Aloineae and eight species from four genera (*Eremurus*, *Asphodelus*, *Asphodeline*, *Bulbine*) of the tribe Asphodeleae were investigated with regard to the presence of 1,8-dihydroxyanthraquinone derivatives and non-volatile organic acids in roots and leaves. Anthraquinones were found in all the genera investigated, except *Kniphofia*. Isocitric acid was detected only in species of *Aloe*. The results are discussed in relation to taxonomic arrangements of the taxa investigated. The plant in which homonataloin was demonstrated to be present and which was called *Aloe africana* in a preliminary communication[4] has now been found to be *Aloe speciosa* Baker.

INTRODUCTION

THE SYSTEMATIC arrangement of many taxa of the large family of Liliaceae is far from definitive. Anatomists and embryologists suggested, for instance, that a rearrangement within the tribe Asphodeleae of Krause[1] should be considered (for discussion and literature see Hegnauer[2]). According to Schnarf[3] the subtribes Anthericinae and Asphodelinae should be rearranged. *Paradisia* should be placed in Anthericinae (not Asphodelinae) and the genera *Bulbine*, *Bulbinella*, *Bulbinopsis*, *Alectorurus* and perhaps others not yet carefully investigated, should be removed from Anthericinae and placed along with *Asphodelus*, *Asphodeline* and *Eremurus* in Asphodelinae. It was furthermore suggested that the subtribe Asphodelinae should rather be placed near Aloinae in the tribe Aloineae than near Anthericinae in the tribe Asphodeleae.

The arguments of Schnarf[3] for these proposals were based on embryological and anatomical observations. The Asphodelinae, as defined by him, contain idioblasts in the vascular bundles of the leaves which resemble the well known aloin cells (containing aloin and chemically related compounds) of *Aloe* and related genera. Schnarf observed that the content of the idioblasts of the Asphodelinae gave some microchemical reactions believed to be characteristic of aloin-like compounds. Definite proof for the presence of anthraquinone derivatives in this taxon was however lacking.

In previous work[4] we have shown that derivatives of 1,8-dihydroxyanthraquinones are indeed present in *Bulbine*. We were able to isolate aloe-emodin and chrysophanol from *Bulbine annua* Willd. and to demonstrate the presence of the same two quinones in *Bulbine asphodeloides* Spreng. by means of chromatographic methods. This is in complete agreement

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[1] K. KRAUSE, in Engler-Prantl, *Die natürlichen Pflanzenfamilien*, 2. Aufl., Band 15a, Leipzig (1930).
[2] R. HEGNAUER, *Chemotaxonomie der Pflanzen*, Band II, pp. 275, 307 and 311, *Birkhäuser Verlag*, Basel (1963).
[3] K. SCHNARF, *Österr. bot. Z.* **93**, 113 (1944).
[4] M. C. B. VAN RHEEDE VAN OUDTSHOORN, *Planta Med.* **11**, 332 (1963).

with the proposal made by Schnarf and the statements of Hegnauer[2] (pp. 307 and 311). It seemed worth while to extend our investigation along the same lines including other genera of Asphodeleae and Aloineae.

Using a specific enzymatic method, Soderstrom[5] investigated 58 succulent plant species with regard to the accumulation of isocitric acid. He also included 3 genera of Liliaceae (*Aloe*, *Haworthia* and *Sansevieria*). Representatives of *Aloe* and *Haworthia* proved to be accumulators (up to 19·4% isocitric acid in the dry leaves of *Aloe saponaria* [Ait.] Haw.). We decided to investigate the nature of the non-volatile organic acids in our plants in order to find out whether accumulation of isocitric acid is restricted to the pronounced succulent genera of the groups considered here or whether it is a character of wider taxonomic importance.

RESULTS

We had various amounts of plant material at our disposal. In some instances we were restricted to herbarium specimens. Therefore all identifications were carried out by means of paper and thin-layer chromatography of suitably prepared extracts. In some instances, however, the results of our chromatographic investigation were checked by the isolation of pure compounds. Chrysophanol was isolated from the roots of *Eremurus robustus* Regel. Aloin was isolated from the dried leaf juice of *Aloe africana* Mill. and *Aloe marlothii* Berger and homonataloin from the dried juice of *Aloe speciosa* Bak. These 10-glycoanthrones were subjected to ferric chloride oxidation in order to isolate the corresponding anthraquinones to confirm their chemical nature. From *Aloe africana* Mill. a glycoside of aloin was isolated. This glycoside seems to be identical with the recently described Aloinoside B (from the crude drug Cape Aloe).[6] The compound proved to be a rhamnoside of aloin.

Our results are summarized in Tables 1 and 2.

DISCUSSION

Because representatives of only six genera of the tribes Asphodeleae and Aloineae are hitherto included in this chemotaxonomical investigation, it may seem premature to draw definite conclusions concerning systematic meaning of the chemical characters studied. A few facts, however, seem worth while to be stressed.

As mentioned in the introduction our previous findings[4] were in full agreement with the observations of Schnarf[3] and the statements made by Hegnauer.[2] The results reported in this paper and summarized in Table 1, lend further support to these ideas concerning relationships in Asphodeleae and Aloineae. The genera *Bulbine*, *Asphodelus*, *Asphodeline* and *Eremurus* are indeed linked by the presence of 1,8-dihydroxyanthraquinones. These compounds may have been responsible for the microchemical reactions noticed by Schnarf[3] and may be associated with the presence of aloin cells in these plants. On the whole, the subtribe Aloinae is phytochemically related to the subtribe Asphodelinae (sensu Schnarf), anthraquinones being present in both taxa.

Interesting facts were observed with regard to the genus *Aloe*. Aloin was isolated from *Aloe marlothii* Berger and *Aloe africana* Mill. Both, together with *Aloe ferox* Mill. (which contains aloin), belong to the section Pachydendron. It would be interesting to know whether the other species of this section also contain aloin. *Aloe africana* Mill. differs from *Aloe*

[5] T. R. SODERSTROM, *Am. J. Botany*, **49**, 850 (1962).
[6] L. HÖRHAMMER, H. WAGNER and G. BITTNER, *Arzneim. Forsch.* **13**, 537 (1963).

TABLE 1. ANTHRAQUINONES AND ANTHRAQUINONE DERIVATIVES FOUND IN SPECIES OF *Aloineae* AND *Asphodelineae*

Species	Material investigated	Combined anthraquinones identified	Free anthraquinones identified*
Aloineae			
Aloe africana Mill.	Leaf juice	Aloin	Aloe-emodin
		Aloinoside B	Aloe-emodin
A. speciosa Bak.	,, ,,	Homonataloin	Nataloe-emodin†
A. marlothii Berger	,, ,,	Aloin	Aloe-emodin
A. pretoriensis Pole Evans	Leaf	Aloin	Aloe-emodin
A. saponaria (Ait.) Haw.[4]	,,	Not identified	Chrysophanol
Kniphofia burchellii Kunth	,,		
K. macowanii Bak.	,,		
Asphodelineae			
Asphodelus albus Willd.	,,	Not identified	Chrysophanol
			Aloe-emodin
Asphodeline lutea Reichb.	,,	,, ,,	Chrysophanol
			Aloe-emodin?
Eremurus robustus Regel	Leaf and root	,, ,,	Chrysophanol
E. bungei Bak.	,, ,,	,, ,,	Chrysophanol
E. × *elwesii* Micheli (= *E. elwesianus* Hort. = *E. himalaicus* × *E. robustus*)	,, ,,	,, ,,	Chrysophanol
E. himalaicus Bak.	,, ,,	,, ,,	Chrysophanol
Bulbine annua Willd.[4]	,, ,,	,, ,,	Chrysophanol
			Aloe-emodin
B. asphodeloides Spreng.[4]	,, ,,	,, ,,	Chrysophanol
			Aloe-emodin

* Before and after hydrolysis.
† Better called mono-*O*-methyl nataloe-emodin (Haynes, Henderson and Tyler[20]).

TABLE 2. NON-VOLATILE ORGANIC ACIDS FOUND IN LEAVES OF *Aloineae* AND *Asphodelineae*

Species	Acids identified*			
	Citric	Malic	Isocitric	Succinic
Aloineae				
Aloe saponaria (Ait.) Haw.	+	+	+	+
A. mitriformis Mill.	+	+	+	+
A. pretoriensis Pole Evans	+	+	+	+
A. speciosa Bak.	+	+	+	+
Kniphofia burchellii Kunth	+	+	−	−
K. macowanii Bak.	+	+	−	−
Asphodelineae				
Asphodelus albus Willd.	+	+	−	−
Asphodeline lutea Reichb.	+	+	−	−
Eremurus robustus Regel	+	+	−	?
E. bungei Bak.	+	+	−	−
E. × *elwesii* Micheli†	+	+	−	−
E. himalaicus Bak.	+	+	−	−
Bulbine annua Willd.	+	+	−	−
B. asphodeloides Spreng.	+	+	−	−

* Calcium oxalate was detected in all the plants investigated.
† See Table 1.

marlothii Berger in that the latter contains no aloinoside B. We have previously reported[4] that *Aloe africana* contains homonataloin. The leaves we worked with were supplied by the Botanical Garden of Leiden from a non-flowering plant. Chemically this material proved to be virtually identical with leaf juices from *Aloe speciosa* Baker received from Southern Africa. Careful rechecking of the botanical characters (vegetative characters only) of the plant in the Botanical Garden of Leiden made it highly probable that this plant belongs in fact to *Aloe speciosa* Baker. Therefore it seems necessary to withdraw *Aloe africana* Mill. from the list of homonataloin-bearing species.

We were able to isolate homonataloin from the dried leaf juice of *Aloe speciosa* Bak. and chrysophanol from dried leaves of *Aloe saponaria* (Ait.) Haw. It is worth while to note that *Aloe speciosa* and *Aloe saponaria* are placed in the section *Eualoe*. Both species do not contain aloin. On the other hand, aloin was isolated from the leaves of *Aloe pretoriensis* Pole Evans, a species placed likewise in the section *Eualoe*. These observations make it rather probable that the large section *Eualoe* is very heterogenous with regard to the accumulation of anthraquinone derivatives.

A thorough chemical investigation of many species of this large genus may lead to a more natural classification. It was stated by Reynolds[7] that more details are needed for a revised grouping of species in *Aloe*.

Leaves of the two species of *Kniphofia* investigated by us were devoided of anthraquinone derivatives. This is in agreement with the observations of Münkner.[8] It should be remembered, however, that recently Boross[9] isolated rhein from the seeds of *Kniphofia uvaria*. It seems possible, therefore, that in this genus the accumulation of anthraquinones is restricted to organs other than leaves.

We were able to detect isocitric acid in all species of *Aloe* investigated, but were unable to detect this acid in the other genera. It seems probable that the accumulation of isocitric acid is mainly associated with succulency.

EXPERIMENTAL

Plant Material

Unless otherwise stated all plant organs were dried at 50° and stored in glass containers above silica gel. The sources and herbarium numbers of plants are given in Table 3.

A. Extraction Procedures

(*a*) *Free anthraquinones.* Extraction of the free anthraquinones was performed as previously described.[4]

(*b*) *Combined anthraquinones.* Dried *Aloe* juice was dissolved in methanol to form a 10% solution. Leaf and root material was extracted with methanol in a Soxhlet apparatus. The methanol solutions were used for chromatographic identifications. For the identification of the anthraquinones released by acid hydrolysis from the combined anthraquinones the following method was used: 10 ml of a 10% methanolic solution was evaporated, and the residue taken up in 10 ml 3 N hydrochloric acid and refluxed for 30 min on a boiling water bath. Only in the case of *Aloe* was it found necessary to add 4% ferric chloride and extend the heating time to about 3 hr. This indicates the presence of ordinary glycosides in all genera

[7] G. W. REYNOLDS, *The Aloes of South Africa*, published by the Trustees The Aloes of South Africa Book Fund, Johannesburg, 1950.
[8] H. MÜNKNER, *Beitr. Biol. Pflanz.* **16**, 217 (1928).
[9] L. BOROSS, *Acta Chim. Hung.* **35**, 195 (1963).

except *Aloe*. The acid hydrolysates were shaken with 2 × 5 ml ether and the ether layer was chromatographed with the solvents for free anthraquinones.[4]

(*c*) *Organic acids*. (i) 0·5 g leaf material was acidified with 1 ml 4 N sulphuric acid and set aside for 12 hr. Afterwards this pulp was mixed with 1 g Celite 545 and extracted with ether

TABLE 3. SOURCES OF PLANT MATERIAL

Species	Type of sample*	Source	Herbarium number, L.E.P. no.†
Aloineae			
Aloe africana Mill.	Dried leaf juice; sample a	Grahamstown, S.A.	Herbarium specimen not yet received
A. africana Mill.	,, ,, ,, sample b	,,	
A. africana Mill.	,, ,, ,, sample c	,,	
A. speciosa Bak.	Dried leaf juice; sample a	,,	Herbarium specimen not yet received
A. speciosa Bak.	,, ,, ,, sample b	,,	
A. speciosa Bak.	Leaves	Botanical Garden, Leiden	00433
A. marlothii Berger	Dried leaf juice; sample a	Pietersburg, S.A.	Herbarium specimen not yet received
A. marlothii Berger	,, ,, ,, sample b	,,	
A. saponaria (Ait.) Haw.	Leaves	Botanical Garden, Leiden	00434
A. mitriformis Mill.	,,	,,	—
A. pretoriensis Pole Evans	,,	,,	00435
Kniphofia burchellii Kunth	,,	,,	00066
K. macowanii Bak.	,,	,,	00055
Asphodelineae			
Asphodelus albus Willd.	Herbarium specimen	Southern France	00436
Asphodeline lutea Reichb.	Leaves	Botanical Garden, Leiden	—
Eremurus robustus Regel	Whole plant	Commercially grown, Leiden	00073
E. bungei Bak.	,, ,,	,,	00076
E. × *elwesii* Micheli (= *E. elwesianus* Hort. = *E. himalaicus* × *E. robustus*)	,, ,,	,,	00077
E. himalaicus Bak.	,, ,,	,,	00063
Bulbine annua Willd.	Whole plant	Cultivated in Leiden	00089
B. asphodeloides Spreng.	Herbarium specimen	Potchefstroom, S.A.	00073

* No differences in chemical composition were observed between different samples (a, b, c) of one species.
† L.E.P. no.: Herbarium of the Laboratorium voor Experimentele Plantensystematiek der Rijksuniversiteit Leiden.

in a Soxhlet apparatus for at least 18 hr. The ether extract was concentrated to a volume of 10 ml and 0·01–0·03 ml of this used for chromatography in solvent systems A, B, C and D.

(ii) 5 g leaf material was extracted on a boiling water bath with 50 ml of 80% ethanol under reflux for at least 45 min. The alcoholic digest was cooled and filtered through a Buchner funnel and washed with 2 × 10 ml 80% ethanol. The filtrate was diluted to 250 ml with water and passed through a column of 5 g Amberlite IRA 400 (CO_3^{2-} form, Ranson[10]). The column

[10] S. L. RANSON, in Paech and Tracey, *Modern Methods of Plant Analysis*, Vol. II, p. 539, Springer Verlag, Heidelberg (1955).

(0·5 × 10 cm) was washed with 300 ml distilled water and the acids eluted with 90 ml 6 N formic acid at a rate of 1·5 ml per min. The eluate, or aliquot part, was concentrated under reduced pressure to a volume of 5 ml; 0·01–0·03 ml of the concentrate was chromatographed in solvent systems A, B, C and D.

Using isocitric acid (prepared from isocitric acid lactone[11] supplied by British Drug Houses, England) as a test substance we observed that it was partially converted into the lactone during the extraction using methods (i) and (ii). By using *Aloe saponaria* (Ait.) Haw. we found isocitric acid lactone present in a rather high amount in the dried leaves. It can be expected that isocitric acid was partially converted into the lactone under the conditions used for drying and storage as well as during the extraction.

The conversion of isocitric acid to its lactone presents a convenient method for the detection of isocitric acid, because no solvent system was found which gives a clear separation between isocitric acid and citric acid. Three of the solvents (A, B and D) give, however, a clear separation between citric acid, malic acid and isocitric acid lactone. The presence of isocitric acid was therefore detected by the presence of its lactone. It can furthermore be confirmed by saponification of the lactone to give isocitric acid.

If there was any doubt as to the presence of isocitric acid lactone, an aliquot part of the acid eluate obtained under (ii) was evaporated under reduced pressure and heated *in vacuo* for 1 hr at 100°.[10] The dried residue was taken up in 25% ammonia. According to Ranson[10] this method results in the formation of an unknown isocitric–ammonia compound which can readily be separated from the unaltered citric acid with solvent D.

We used method (i) for herbarium samples and (ii) when a larger quantity of material was available. The extract obtained by method (i) has the disadvantage of a high concentration of oxalic acid which tends to streak on the chromatograms.

B. Paper Chromatography

Whatman No. 1 chromatographic paper (unwashed) was used in all experiments. Chromatography was carried out at 18–23° by the ascending technique unless otherwise stated. An accommodation time of 1 hr was found to be sufficient. Papers were dried at room temperature.

(i) *Solvents for* 1,8-*dihydroxyanthraquinone derivatives.* The solvents were the same as previously described.[4]

(ii) *Solvents for organic acids.*

A. Ether–formic acid–water (10:4:1 v/v).[12]

R_f values: citric acid 0·38; isocitric acid 0·37; isocitric acid lactone 0·60; malic acid 0·50; succinic acid 0·84.

B. Ethylmethylketone–acetone–water–formic acid (80:4:12:2 v/v).[13]

R_f values: citric acid 0·51; isocitric acid 0·49; isocitric acid lactone 0·77; malic acid 0·60; succinic acid 0·88.

C. Butylformate–formic acid–water (10:4:1 v/v).[14]

R_f values: citric acid 0·24; isocitric acid 0·25; isocitric acid lactone 0·36; malic acid 0·36; succinic acid 0·63.

[11] R. POHLOUDEK-FABINI, C. WOLLMANN and H. WOLLMANN, *J. Chromatog.* **2**, 525 (1959).
[12] J. FITELSON, *J. Assoc. Offic. Agric. Chemists* **45**, 246 (1962).
[13] L. REIO, *J. Chromatog.* **1**, 338 (1958).
[14] H. A. W. BLUNDSTONE, *Nature*, **197**, 377 (1963).

D. Propanol–ammonia (6:4 v/v).[10]

R_f values: citric acid 0·19; isocitric–ammonia compound 0·24; malic acid 0·33; succinic acid 0·40.

(iii) *Solvents for sugars.*

E. Ethylacetate–pyridine–water (7:2:1 v/v) descending.

F. Butanol–acetic acid–water (4:1:5 v/v) descending.

Detection of Constituents

Free anthraquinones were detected by using the magnesium acetate spray[15] and combined anthraquinones as previously described.[4] Organic acids were detected with bromophenol-blue indicator[11] and aniline/glucose.[16] Sugars were detected using the following spray: 1·66 g phthalic acid and 0·93 g aniline were dissolved in 100 ml water-saturated *n*-butanol. After spraying, papers were heated for 10 min at 105°.

C. Thin-layer Chromatography

(i) 1·8-*Dihydroxyanthraquinone derivatives.* Preparation of thin-layer chromatoplates and the solvents used were the same as described formerly.[4]

(ii) *Sugars.* Thin-layer chromatoplates of Cellulose MN 300 (Macherey, Nagel and Co, Düren) were prepared as described by Schweiger.[17] The following solvents were used:

G. Ethylacetate–pyridine–water (2:1:2 v/v).[17]

H. Phenol (water saturated)–1 % ammonia.[17]

For detection of the sugars the spray reagent described under paper chromatography was used.

D. Isolations

General. Melting points were taken with a Kofler micro-melting point apparatus. Infra-red spectra were determined in potassium bromide discs on a Perkin-Elmer Infracord 137. Ultra-violet spectra were determined on a Beckman spectrophotometer Model DU; methanol was used as solvent. Microanalyses were carried out by the Laboratorium voor Organische Scheikunde der Universiteit Amsterdam, Micro-analytische afdeling.

(*a*) *Aloin from* Aloe africana *Mill. and* Aloe marlothii *Berger.* Using the calcium method described by Harders[18] we were able to isolate aloin from the dried juice of the above-mentioned species. The crude ppt. was recrystallized from *iso*-propanol and twice from methanol. *Aloe africana* yielded 6% aloin, m.p. 146–148°, and *Aloe marlothii* 9% aloin, m.p. 146–148°. The u.v. and i.r. spectra were identical with those of aloin isolated from the crude drug, Aloe capensis, in the same way. Ferric chloride oxidation[19] of aloin from both species gave aloe-emodin, m.p. 225° (crystallized from methanol). No depression of m.p. with authentic aloe-emodin.

(*b*) *Aloinoside B from* Aloe africana *Mill.* During an effort to isolate aloin from *Aloe africana*, using Léger's method,[20] we noticed a crude white residue. The extract was filtered and the residue dried above P_2O_5 (m.p. 225°). Crystallized from methanol it gave almost white needles which had a m.p. of 234° after drying in the above manner (yield 3%). The u.v.

[15] M. TAKIDO, *Pharm. Bull. (Japan)* **4**, 45 (1956).
[16] H. SCHWEPPE, in Linskens, *Papierchromatographie in der Botanik*, Springer Verlag, Heidelberg (1959).
[17] A. SCHWEIGER, *J. Chromatog.* **9**, 374 (1962).
[18] C. L. HARDERS, Pharm. Weekblad. **85**, 250 (1949).
[19] J. E. HAY and L. J. HAYNES, *J. Chem. Soc.* 3141 (1956).
[20] E. LÉGER, *Ann. Chim.* **6**, 318 (1916).

spectrum was virtually identical with that of aloin (λ_{max} 222, 269, 296 and 359). There is however a marked difference between the i.r. spectra of the two substances.

5 mg Aloinoside B was hydrolysed with 5 ml 5% hydrochloric acid for 30 min. Chromatography of the hydrolysate showed the presence of aloin. The acid hydrolysate was passed through columns of Amberlite IR 120 (H^+) and Amberlite IR 45 (OH^-) (0·5 × 10 cm). The eluate was concentrated under reduced pressure to a volume of 1 ml and the latter chromatographed in solvents E and F. Rhamnose was the only detectable sugar.

15 mg Aloinoside B was heated with 15 ml 4 N hydrochloric acid containing 4% ferric chloride[21] for 3 hr on a boiling water bath under reflux. After cooling, the precipitate (A) formed was filtered off and the filtrate passed through columns of Amberlite IR 120 (H^+) and Amberlite IR 45 (OH^-), both 0·5 × 10 cm. The eluate was concentrated under reduced pressure to a volume of 1 ml and again passed through above columns (to remove last traces of ferric ions), concentrated to 1 ml, and chromatographed in solvents G and H. Rhamnose and arabinose were detected. The precipitate (A) was dried at 120° and extracted with benzene in a Soxhlet apparatus. The yellow-coloured benzene extract was concentrated to a volume of 20 ml and filtered through a column of Hyflo Supercel (1 × 5 cm). The benzene was evaporated from the filtrate and the dried residue (m.p. 219°) recrystallized from methanol (5 mg; m.p. 224–226°). The u.v. and i.r. spectra were identical with those of authentic aloe-emodin. Found: C, 57·24; H, 5·85; O, 36·9%. Calculated for a monorhamnoside of aloin, $C_{27}H_{32}O_{13}$: C, 57·4; H, 5·7; O, 36·9%.

(*c*) *Homonataloin from* Aloe speciosa *Bak.* For the isolation of homonataloin from the dried juice of *Aloe speciosa* we used the method described by Haynes, Henderson and Tyler.[22] The yellow crystalline product obtained was twice recrystallized from methanol and dried at reduced pressure above P_2O_5, m.p. 200–202°. Found: C, 58·50; H, 6·02; O, 35·48; OCH_3, 7·29. Calc. for $C_{22}H_{24}O_9$, H_2O: C, 58·7; H, 5·8; O, 35·5; 1-OCH_3, 6·9%. The u.v. and i.r. spectra were identical with those of homonataloin isolated in the same manner from a sample of Natal Aloe, present in the Museum of the Farmaceutisch Laboratorium in Leiden. Ferric chloride oxidation[22] of homonataloin from *Aloe speciosa* Bak. gave mono-O-methyl-nataloe-emodin, m.p. 233°. The u.v. and i.r. spectra were identical with those of authentic material.

(*d*) *Chrysophanol from* Eremurus robustus *Regel.* Paper and thin-layer chromatography of ether extracts and hydrolysed methanol extracts from roots indicated the presence of chrysophanol and a second quinone (positive to magnesium acetate spray[15] and potassium hydroxide), the latter in a higher concentration than the first. Using the method described by Takido[23] we were able to isolate chrysophanol, m.p. 194°, from the hydrolysate of a methanol extract (yield 0·09%; no depression of m.p. on admixture with authentic chrysophanol). The different fractions collected during the isolation indicated the presence of more quinone-like compounds. Chrysophanol is obviously present as an ordinary glycoside in this species.

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[21] J. W. FAIRBAIRN and S. SIMIC, *J. Pharm. Pharmacol.* **15**, 325 (1963).
[22] L. J. HAYNES, J. I. HENDERSON and J. M. TYLER, *J. Chem. Soc.* 4879 (1960).
[23] M. TAKIDO, *Chem. Pharm. Bull. (Japan)* **6**, 397 (1958).