Onderstepoort Journal of Veterinary Research,
Volume 25, Number 4, December, 1952.
The Government Printer, Pretoria.

TRANSVAALIN, A CARDIAC GLYCOSIDE ISOLATED FROM *URGINEA BURKEI, BKR.* (TRANSVAAL SLANGKOP).

P. G. J. LOUW, Onderstepoort Laboratory.

Urginea burkei, Bkr., a liliaceous plant known in this country as "Transvaal Slangkop" (Fig. 1), has for long been known to be poisonous. It has often caused serious loss of stock, particularly in spring, in times of drought, when it may be the only green herbage attracting starving animals on the veld.

In 1916 workers at the Imperial Institute found the bulbs of this plant to contain a poisonous glycosidal substance having a digitalis-like action. Subsequent investigations by George (1925), Watt (1927), and Stephen (1934) confirmed this finding.

The isolation, from the bulbs of *Urginea burkei,* of a crystalline cardiac glycoside was recently announced (Louw, 1949). The name "transvaalin" was proposed for this substance. The purpose of the present paper is to report this work in greater detail.

For the isolation of the glycoside fresh bulbs were minced and treated with 96 per cent. alcohol. The cold alcoholic extract containing a high percentage of water, originating from the bulbs, was exhaustively extracted with a chloroform-ethyl alcohol mixture (5:1). The glycoside could be washed with water from the chloroform-alcohol fractions and thereupon purified by repeated recrystallization from 96 per cent. alcohol. The yields varied from 0·01 to 0·05 per cent. of the fresh material, depending on the time and place of collection of the bulbs.

When crystallized from 96 per cent. alcohol transvaalin was obtained in the form of shining platelets which melted without decomposition at 193–4° C.*

From the molecular weight of 672, obtained by lactone titration, and from combustion analyses undertaken on material dried to constant weight at 100° C. and 3 mm. of Hg. over P_2O_5, a molecular formula of $C_{36}H_{54}O_{14}$ was calculated. However, when dried in this manner transvaalin was found to retain one molecule of water of crystallization which could be driven off by heating at 100° C. for 6 hours over P_2O_5 in high vacuum. This suggested a molecular formula of $C_{36}H_{52}O_{13}$. Analyses of the hexa-acetyl derivative of the glycoside and results obtained in subsequent hydrolysis experiments confirmed this formula.

Transvaalin was found laevo-rotatory with $[\alpha]_D^{20} = -73{\cdot}26$ (MeOH). Its ultra-violet absorption curve, given in figure 2, revealed a maximum at 297 mμ with log E $\frac{1 \text{ mol.}}{1 \text{ cm.}}$ /liter = 3·64 in almost complete agreement with that of scilliroside.

* All melting points were determined on the Kofler micromelting point apparatus (corrected).

Received for publication on 25th July, 1951.—Editor.

Tested on the perfused rabbit heart transvaalin produced the reaction typical for cardiac glycosides. Assayed by the frog (*Xenopus laevis*) method Sapeika (1949) found its potency to be 25·6 compared with 100 for anhydrous ouabain.

In rats transvaalin produced the convulsive reaction of a raticide described by Gold and collaborators (1947) for scilliroside, which is active in a smaller dose per unit of body weight. During the first 2 or 3 hours after administering transvaalin by the stomach tube method of Lehr (1945), at the rate of 40 mg. per Kg., the rats remained normal. Paralysis, especially of the hindquarters, then occurred, and the animals showed increasing excitability. Copious salivation and a period of convulsions followed during which the animals firmly gripped the cage with their claws and teeth. Death usually ensued after 6 hours. Similar results were obtained when the dose was increased to 200 mg. per Kg.

Paralysis and convulsions also occurred in rats injected intraperitoneally with transvaalin. The m.l.d. by this route was found to be approximately the same as that *per os*, namely 40 mg. per Kg. There was no apparent difference in the reaction of male or female rats to the glycoside. In this connection it is of interest to refer to the work of Stoll and Renz (1942) who found for scilliroside, a m.l.d. of 1·2 mg./Kg. for male and 0·6 mg./Kg for female rats. They observed also that scillaren A was relatively non toxic, several hundred mg. per Kg. body-weight producing no effect in rats by the oral route.

When hydrolysed with 0·5 per cent. sulphuric acid transvaalin yielded scillaridin A and scillabiose as the disaccharide, the same products as those derived from the hydrolysis of scillaren A. Scillaridin A was identified by physical properties such as solubility, crystal form, melting point and specific rotation. When mixed with authentic scillaridin A, kindly supplied by Prof. A. Stoll, Basle, no depression of melting point was observed. The biose was identified as scillabiose by its rotation and the melting point of the acetyl derivative. Hydrolysis of the biose yielded d-glucose and rhamnose. These findings may be represented as follows:—

$$\underset{\text{Transvaalin}}{C_{36}H_{52}O_{13}} + H_2O = \underset{\text{Scillaridin A}}{(C_{24}H_{32}O_4)} + \underset{\text{Scillabiose}}{C_{12}H_{22}O_{10}}$$

$$(C_{24}H_{32}O_4) \xrightarrow{-H_2O} C_{24}H_{30}O_3$$

$$C_{12}H_{22}O_{10} \xrightarrow{+H_2O} C_6H_{12}O_5 + C_6H_{12}O_6$$

Rhamnose Glucose.

The question immediately arose as to whether transvaalin and scillaren A are isomeric or identical. The difference in toxicity of the two substances supports the possibility of their being isomers. However, the raticidal property of transvaalin suggested the possibility that it might be not a homogeneous glycoside but, in fact, a mixture of scillaren A and scilliroside, the cardiac glycosides in white and red squill, respectively. This alternative was discredited by the observation that transvaalin could be hydrolysed quantitatively into scillaridin A and scillabiose.

Transvaalin, purified with freshly precipitated lead hydroxide, was thereupon fractionated by the methods of purification and extraction developed by Stoll and Renz (1942) for the separation of scilliroside and scillaren A from mixtures of the two glycosides. Neither scilliroside nor a glycosidal fraction with increased potency

for rats, could be isolated in this manner, the toxicity of the transvaalin for rats remaining unchanged. These results do not support the view that transvaalin is not a homogeneous glycoside.

Mention has previously been made of the fact that transvaalin melted at 193–4° C. without decomposition. After processing with chloroform-butanol, according to the Stoll and Renz methods mentioned above, and recrystallization from 96 per cent. alcohol it was obtained in rosettes of platelets which softened at 194° C. and started to decompose at 250° C, melting completely with decomposition at 270° C. From 50 per cent methyl alcohol transvaalin crystallized in rosettes of long hexagonal plates, similar in appearance to scillaren A crystallized from the same solvent. When heated on the Kofler these crystals melted completely without decomposition at 194° C., recrystallized at about 200° C., started to decompose at 240° C. and melted completely with decomposition at 270° C. Under identical conditions authentic scillaren A and a mixture of scillaren A and transvaalin behaved in a manner similar to transvaalin.

Transvaalin, scillaren A (both crystallized from methyl alcohol) and a mixture of the two glycosides, softened at 180° C. and melted with decomposition at 225–235° C. (uncorr.), when heated in sealed capillaries in a sulphuric acid bath.

It is concluded that transvaalin is either isomeric with scillaren A or a very stable complex of this glycoside and scilliroside which could not be separated by the ordinary methods of fractionation.

Experimental.

(1) *Extraction and Purification of Transvaalin.*

10 Kg. of freshly minced bulbs in the early flowering stage were mixed with 96 per cent. alcohol, stirred up occasionally and left overnight. The fluid was expressed and the extraction with alcohol repeated. A dark red alcoholic extract of a thick consistency was obtained. In this way any enzyme action was eliminated.

The alcoholic extract, containing a high percentage of water derived from the bulbs, was directly shaken with half its volume of chloroform. The chloroform layer was separated and the extraction repeated six times with a chloroform-ethyl alcohol mixture (5 : 1). The chloroform-alcohol fractions were then washed several times with water. The chloroform, on further addition of alcohol, was used over again.

The watery solutions were concentrated by fanning, care being taken to prevent formation of moulds.

The light-brown concentrate was again fractionated with chloroform-alcohol (5 : 1), as above, and the final aqueous extract fanned until dry to yield 10 gm. of a straw-coloured product. This was rubbed with water to give a water-insoluble portion (6·5 gm.) which, on solution in 96 per cent. alcohol and subsequent concentration crystallized in aggregates with a light-yellow tinge. After repeated recrystallization from 96 per cent. alcohol, rosettes of colourless platelets which melted at 193–4° C. without decomposition were obtained.

By the above extraction method the yield of pure glycoside varied from 0·01 to 0·05 per cent. on the basis of the original wet material.

(2) *Transvaalin.*

Solubility properties.

Transvaalin was found sparingly soluble in cold water but more so in hot water. It was insoluble in ether, chloroform, benzene and petrol ether, slightly soluble in ethyl acetate and readily soluble in methyl-alcohol, ethyl-alcohol, acetone, dioxan and pyridine.

Analyses.

A. Dried over P_2O_5 at 100° C. and 3 mm. Hg for 2 hours.

4·090 mg.:* 9·155 mg. CO_2; 2·820 mg. H_2O.
4·085 mg.: 9·160 mg. CO_2; 2·900 mg. H_2O.
4·025 mg.: 9·025 mg. CO_2; 2·860 mg. H_2O.
3·980 mg.: 8·900 mg. CO_2; 2·760 mg. H_2O.

Found: C = 61·06, 61·17, 61·17, 61·01 per cent.
H = 7·71, 7·94, 7·95, 7·75 per cent.

Calculated for $C_{36}H_{54}O_{14}$: C = 60·82 per cent, H = 7·65 per cent.

B. Dried over P_2O_5 at 100° C. and high vacuum for 6 hours.

3·252 mg.: 7.30 mg. CO_2; 2·19 mg. H_2O.
3·118 mg.: 7·01 mg. CO_2; 2·17 mg. H_2O.

Found: C = 62·68, 62·22 per cent.
H = 7·71, 7·79 per cent.

Calculated for $C_{36}H_{52}O_{13}$: C = 62·41, H = 7·57 per cent.

Rotation.

237·5, 205·3 mg. transvaalin dried over P_2O_5 at 100° C. and 3 mm. Hg for 2 hours dissolved in 10 ml. methanol gave the following rotations when a 1 dm. tube was used.

$$\Theta = -1.74°, -1.506°$$

$$\therefore [\alpha]_D^{20} = -73.26°, -73.35°$$

Lactone titration.

113·4, 100·0 mg. transvaalin (dried over P_2O_5 at 100° C. and 3 mm. Hg) were dissolved in 20 ml. methyl alcohol. To the solution 10 ml. 0·1 N NaOH were added and the excess alkali back-titrated after 48 hours with 0·1 N H_2SO_4 using phenolphthalein as indicator.

ml. 0·1N NaOH used — 1·76, 1·487.

Found: molecular weight: 644·3, 672·3.

Calculated for $C_{36}H_{52}O_{13}.H_2O$: 710·8.

Colour tests.

Transvaalin gave a negative Legal test corresponding to the scilla group of cardiac glycosides having six-membered lactone rings.

* Micro-analyses by Drs. G. Weiler and F. B. Strauss, Oxford, England.

When transvaalin was dissolved in ethyl acetate and a mixture of sulphuric acid and acetic anhydride (50:1) added, the solution turned carmine-red and slowly changed to a permanent green colour. (Liebermann test).

(3) *Hydrolysis of transvaalin.*

1·5 Grammes transvaalin were suspended in 250 ml. 0·5 per cent. sulphuric acid and heated in a boiling waterbath for 30 minutes with stirring. The glycoside slowly dissolved and after a while a crystalline product separated. The reaction mixture was cooled and the precipitate centrifuged down, washed and dried, yielding 0·764 gm. of the aglucone. Calculated for $C_{24}H_{30}O_3$ (scillaridin A): 0·773 gm.

Scillaridin A.

From absolute alcohol the aglucone crystallized in the typical form of scillaridin A, and when heated in a sealed capillary tube in a sulphuric acid bath, the crystals turned brown at 205° C. and melted with decomposition at 245–250° C.

Rotation.

Using a 1 dm. tube, 200 mg. aglucone dissolved in 25 ml. chloroform-methanol (4:1) gave a rotation of $-\cdot 50°$.

$$\therefore [\alpha]_D^{29} = -62.5° \ (CHCl_3 - MeOH)$$

$$\{[\alpha]_D^{20} \text{ for scillaridin A} = -62.6° \ (CHCl_3 - MeOH)\}$$

Analysis.

Dried over P_2O_5 at 100° C. and 3 mm. Hg:

3·385 mg.: 9·745 mg. CO_2; 2·540 mg. H_2O.
4·260 mg.: 12·210 mg. CO_2; 3·130 mg. H_2O.
3·790 mg.: 10·860 mg. CO_2; 2·830 mg. H_2O.

Found: C = 78·56, 78·20, 78·21 per cent.
H = 8·40, 8·22, 8·36 per cent.

Calculated for $C_{24}H_{30}O_3$: C = 78·63, H = 8·25 per cent.

Scillabiose.

After separation of the aglucone from the hydrolysis mixture, the filtrate was neutralized with $BaCO_3$, filtered and evaporated in front of a fan. The sugar was obtained as a colourless non-crystalline product (0·59 gm.).

Rotation.

0·59 Gramme sugar was dissolved in 10 ml. water and the rotation determined using a 1 dm. tube.

Rotation = $-1·39°$.

$$\therefore [\alpha]_D^{30} = -23.56° \text{ (water)}.$$

Compare Scillabiose: $[\alpha]_D^{20} = -24.8$ (water).

Hydrolysis of the biose.

236 Mg. of the biose were dissolved in 8 ml. 1 per cent. sulphuric acid and heated on a steambath. The rotation of the solution changed from $[\alpha]_D^{30} = -23{\cdot}56$ to a constant value of $[\alpha]_D^{28} = +29{\cdot}16$ after 20 hours, which corresponded with the value for an equimolecular mixture of rhamnose and d-glucose.

Identification of d-glucose and rhamnose.

After neutralization of the hydrolysis mixture with barium carbonate and filtration, an aliquot of the sugar filtrate was incubated with bakers yeast at 37° C. for 12 hours. The mixture was centrifuged and to the clear solution a few drops of phenylhydrazine and acetic acid added and then heated in a boiling waterbath. The crystalline osazone obtained was recrystallized from benzene. It melted at 184° C. and gave no depression of melting point with authentic rhamnosazone.

Another portion of the sugar filtrate was treated with phenylhydrazine and acetic acid in a boiling waterbath. The crystalline osazone obtained was dried and treated with a small amount of acetone leaving a portion of the osazone undissolved. This was recrystallized from acetone. It melted at 208° C., and was found to be identical with the osazone of d-glucose.

When a drop of the hydrolysis mixture was chromatographed on a strip of filter paper by the method of Partridge (1948), using phenol as solvent, only two constituents having Rf values 0·39 and 0·59, coinciding with those of d-glucose and rhamnose, were obtained.

Hexa-acetyl scillabiose.

The neutralized sugar filtrate from the hydrolysis of 1·5 gm. of transvaalin was evaporated to dryness, dissolved in 15 ml. pyridine and after addition of 4 ml. acetic anhydride, left at room temperature for 4 days. The mixture was then poured into ice water, the white precipitate dissolved in the minimum amount of absolute alcohol and a little petrol ether added. The solution became turbid. On leaving for several days, rosettes of fine needles formed. After recrystallization from absolute alcohol these melted at 96° C.

(4) *Hexa-acetyl derivative of transvaalin.*

100 Mg. transvaalin were dissolved in 2 ml. pyridine and 1·5 ml. acetic anhydride added. The mixture was left for 24 hours at room temperature and then poured into a large volume of ice water yielding a clear white precipitate. This was filtered off, washed and dried. Yield 120 mg.

The acetyl derivative was obtained in rosettes of fine needles after recrystallization from absolute alcohol. Heated on the Kofler apparatus it melted sharply at 200° C. without decomposition, recrystallizing again and finally melting with decomposition at 247–251° C.

Analysis.

(Dried over P_2O_5 at 100° C. and 3 mm. Hg for 2 hours).

3·802 mg.: 8·460 mg. CO_2; 2·400 mg. H_2O.
3·345 mg.: 7·420 mg. CO_2; 2·140 mg. H_2O.
4·025 mg.: 8·940 mg. CO_2; 2·610 mg. H_2O.

Found: C = 60·71, 60·53, 60·60 per cent.
H = 7·06, 7·16, 7·25 per cent.

Calculated for
hexa-acetyl transvaalin: $C_{48}H_{64}O_{19}$: C = 61·01, H = 6·83 per cent.

Titration.

84·6, 87·4 mg. acetyl-transvaalin, recrystallized from methyl alcohol and dried over P_2O_5, were dissolved in 20 ml. methyl alcohol and 10 ml. 0·1N NaOH added. After 48 hours the excess alkali was back titrated with 0·1N H_2SO_4 using phenolphthalein as indicator.

ml. 0·1 N NaOH used = 6·39, 6·60.

Calculated (for 6 acetyl groups and one lactone group);

6·27, 6·47 ml. 0·1N NaOH.

Rotation.

97·2, 101·0 mg. acetyl derivative dried over P_2O_5 were dissolved in 10 ml. methyl alcohol and the rotation using a 1 dm. tube determined.

$$\Theta = -\cdot 72°, -\cdot 76°$$

$$\therefore [\alpha]_D^{21} = -74\cdot 06°, -75\cdot 08° \text{ (MeOH)}.$$

(5) *Fractionation of transvaalin with chloroform and butanol.*

(*a*) *Fractionation of the alcoholic extract of* U. burkei.

20 Kg. minced bulbs were extracted with 96 per cent. alcohol and the extract processed with chloroform and alcohol as described for the preparation of transvaalin, giving 1,400 ml. of a light-brown aqueous concentrate containing 2·4 gm. dissolved solids per 100 ml. The dissolved solids produced typical ratpoisoning and had a m.l.d. of ca. 150 mg./Kg. when dosed *per os* to rats.

1,200 ml. of the concentrate were shaken out four times with 1,000 ml. amounts of chloroform-butanol (95 : 5). The chloroform shakes were concentrated *in vacuo* and dried in a vacuum exsiccator yielding 2·04 gm. of a straw-coloured product which on dosing to rats produced the symptoms of rat-poisoning and had a m.l.d. of ca. 135 mg./Kg., from which it is evident that the rat-poisoning principle had not been concentrated.

The aqueous concentrate was now shaken out six times with a chloroform-butanol mixture (4 : 1) yielding 12·8 gm. of a yellow resinous product.

6 Gm. of the resinous extract were dissolved in 400 ml. 25 per cent. alcohol and then treated with a suspension of lead hydroxide which had been freshly prepared and washed free of alkali, giving a yellow precipitate and a light-yellow filtrate.

The filtrate was concentrated to 215 ml. (containing 3·70 gm. solid) and was then shaken out eight times with 400 ml. amounts of chloroform-butanol (95 : 5). After removal of the chloroform under diminished pressure and drying *in vacuo*, 1·61 gm. of a slightly yellow product was obtained. Dosed *per os* to rats, this product produced typical rat-poisoning symptoms, the m.l.d. being ca. 80 mg./Kg.

(*b*) *Attempted fractionation of transvaalin.*

9 Gm. transvaalin crystallized from 96 per cent. alcohol, melting at 194° C. and having a m.l.d. of 40 mg./Kg. for rats, were suspended in 600 ml. water and then shaken out eight times with 500 ml. amount of chloroform-butanol (95 : 5).

The chloroform-butanol was removed under reduced pressure and the residue dried *in vacuo*. Treated with 96 per cent. alcohol the colourless product (1·2 gm.) spontaneously crystallized in rosettes of platelets. After recrystallization from 96 per cent. alcohol it softened at 195° C. and started to decompose at 250° C. and melted completely at 270° C. When recrystallized from 50 per cent. methanol, it was obtained in rosettes of hexagonal plates which melted at 194° C., recrystallized again, started to decompose at 250° C. and melted completely at 270° C.

The rotation of the product remained unchanged:

$$[\alpha]_D^{26} = -74{\cdot}5^\circ \text{ (MeOH)}.$$

On shaking out the suspension of transvaalin with further amounts of chloroform-butanol (95:5), more of the above product with the higher melting point was obtained.

The toxicity of the different fractions for rats remained unchanged, producing the typical symptoms of ratpoisoning and having a m.l.d. of 40 mg./Kg. rat.

2·0 Gm. of the transvaalin fraction with higher melting point were once more fractionated with chloroform-butanol (95:5). It was suspended in 200 ml. water and then shaken out three times with 200 ml. amounts of chloroform-butanol. The following five shakings with chloroform-butanol (9:1) yielded 1·0 gm. of a product which after three successive recrystallizations from 50 per cent. methanol had unchanged melting point. The rat-poisoning property of the product remained unchanged with m.l.d. 40 mg./Kg.

Exactly the same results were obtained by shaking out with chloroform-butanol after treatment of transvaalin with lead hydroxide.

According to the findings of Stoll and Renz (loc. cit.), any scilliroside should have been removed by the above fractionation if transvaalin was a mixture of scillaren A and scilliroside. All the different fractions obtained had unchanged activity and not a single fraction with increased rat-activity was obtained, indicating the homogeneity of transvaalin.

(6) *Watersoluble active principle.*

In the preparation of transvaalin a water-soluble product of marked activity towards rats, was also extracted in small amounts when the crude product was treated with water prior to the crystallization of transvaalin.

Injected intraperitoneally or dosed *per os* to rats, no rat-poisoning symptoms were observed. Doses of 1·0 mg. of a concentrate of this product, which could not be obtained in crystalline form, killed rats weighing 200 gm. within 45 minutes when injected intraperitoneally. The animals survived lower doses without developing any symptoms of poisoning. This product probably corresponded with the amorphous water-soluble products previously obtained from *Urginea burkei* by other workers.

SUMMARY.

(1) A cardiac glycoside, transvaalin, with molecular formula $C_{36}H_{52}O_{13}$ was isolated from *Urginea burkei* Bkr.

(2) Transvaalin has the typical raticidal properties of scilliroside when dosed *per os* or injected intraperitoneally. The m.l.d. for rats, dosed *per os*, was 40 mg./Kg.

(3) Hydrolysis of transvaalin yielded scillaridin A, $C_{24}H_{30}O_3$, and scillabiose from which it appears that transvaalin must either be isomeric with scillaren A or a very stable complex of scillaren A and a rat-poison e.g. scilliroside.

(4) Fractionation of transvaalin with chloroform-butanol yielded no scilliroside or other rat-poison, while the activity of transvaalin remained unchanged towards rats and frogs, contradicting the possibility that it is a complex of scillaren A and a rat-poison.

(5) The isolation (in small yield) of an amorphous water-soluble poisonous principle from *U. burkei*, is reported.

REFERENCES.

GEORGE, E. (1925). The active principles of the African squill *Urginea Burkei* (Slangkop) and its suitability or otherwise as a substitute for *Urginea maritina* in commerce. *Jour. S.A. Chem. Inst.*, Vol. 8, pp. 14-27.

GOLD, H. ET AL (1947). Action of Digitalis glycosides on the central nervous system with special reference to the convulsant action of red squill. *Jour. Pharmacol. Exp. Therap.* Vol. 91, pp. 15-30.

LEHR, D. (1945). Stomach-tube feeding of small laboratory animals. *Jour. Lab. Clin. Med.* Vol. 30, pp. 977-980.

LOUW, P. G. J. (1949). Two new cardiac glycosides, Rubellin and Transvaalin, from South African species of Urginea. *Nature*, Vol. 163, p. 30.

PARTRIDGE, S. M. (1948). Filter-paper partition chromatography of sugars. *Biochem. Jour.*, Vol. 42, pp. 238-250.

SAPEIKA, N. (1949). Personal communication.

STEPHEN, F. (1934). The isolation of the active principles of *Urginea Burkei, Bkr. Quar. Jour. Pharm. Pharmacol.*, Vol. 7, pp. 57-62.

STOLL, A. AND RENZ, J. (1942). Uber Scillirosid, ein gegen Nager spezifisch wirksames Gift der roten Meerzwiebel. *Helv. Chim. Acta.*, Vol. 25, p. 43.

THE IMPERIAL INSTITUTE (1915). Investigations of vegetable drugs and poisonous plants. *Bul. Imp. Inst.*, Vol. 13, p. 59.

WATT, J. M. (1927). Die Wirkung der aktiven Substanzen von *Urginea Burkei, Bkr. Arch. Exp. Path. Pharmak.*, Vol. 120, p. 65.

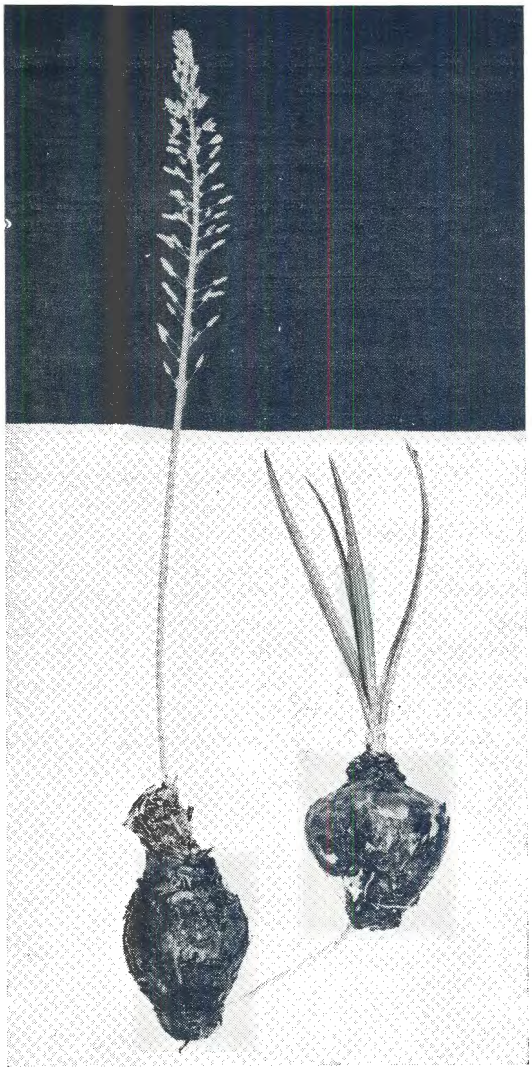

Fig. 1. *Urginea burkei*, Bkr., the "Transvaal Slangkop".

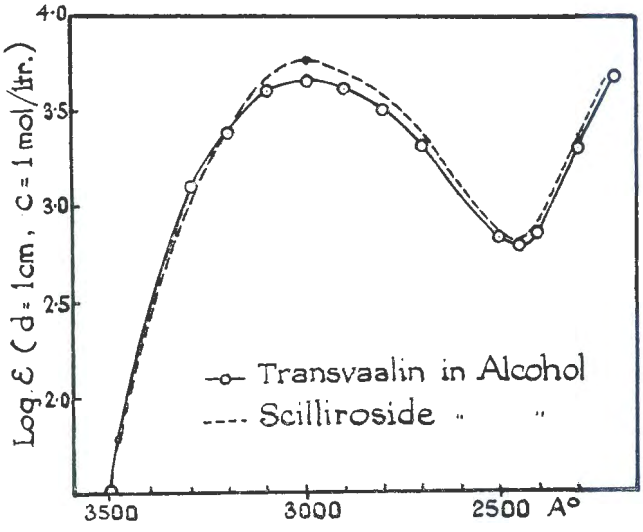
4·0
3·5
3·0
2·5
2·0
Log ε (d = 1cm , c = 1 mol/ltr.)
Transvaalin in Alcohol
Scilliroside " "
3500
3000
2500 A°

Fig. 2