THE EXPERIMENTAL PRODUCTION OF KRIMPSIEKTE IN SHEEP WITH *TYLECODON GRANDIFLORUS* (BURM. F.) TOELKEN AND SOME OF ITS BUFADIENOLIDES

L. A. P. ANDERSON, J. P. J. JOUBERT, L. PROZESKY, T. S. KELLERMAN, R. ANITRA SCHULTZ, J. PROCOS and PHILIPPA M. OLIVIER*, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

ANDERSON, L. A. P., JOUBERT, J. P. J., PROZESKY, L., KELLERMAN, T. S., SCHULTZ, R. ANITRA, PROCOS, J. & OLIVIER, PHILIPPA M., 1983. The experimental production of krimpsiekte in sheep with *Tylecodon grandiflorus* (Burm.f.) Toelken and some of its bufadienolides. *Onderstepoort Journal of Veterinary Research*, 50, 301–307 (1983)

Six bufadienolides were isolated from *Tylecodon grandiflorus* (Burm.f.) Toelken. The paretic syndrome, krimpsiekte, could be induced in sheep either by repeated oral administration of small quantities of plant material or the intravenous injection of small quantities of certain bufadienolides. A mild to moderate, acute to subacute, multifocal cardiomyopathy was evident in sheep poisoned by both the plant and the bufadienolides.

INTRODUCTION

Krimpsiekte can be caused by plants in the genera *Tylecodon, Cotyledon* and *Kalanchoe* (Henning, 1926; Steyn, 1949) as well as some bufadienolide cardiac glycosides isolated from *Tylecodon wallichii* (Naudé & Schultz, 1982) and *Kalanchoe lanceolata* (Anderson, Schultz, Joubert, Prozesky, Kellerman, Erasmus & Procos, 1983). This paretic syndrome differs completely from typical cardiac glycoside poisoning induced by higher doses of the plant or the bufadienolides (Naudé, 1977; Naudé & Schultz, 1982; Anderson *et al*. 1983).

The toxicity of *Tylecodon grandiflorus* and the isolated components was investigated as an extension of the research on cardiac glycosides.

DISTRIBUTION AND DESCRIPTION OF THE PLANT

Family: Crassulaceae

Name: *Tylecodon grandiflorus* (Burm.f.) Toelken

Synonyms: *Cotyledon tuberculosa* Lam.

               Cotyledon purpurea Haw.

               Cotyledon curviflora Sims.

Common name: 'Rooisuikerblom'

Distribution. Where known the specific locality is cited (Fig. 1), otherwise only districts are listed.

Cape Peninsula: Between lighthouses, Cape Point; Simon's Town; Table Mountain, near Hout Bay Nek; Near Kommetjie; Lions Head; Camps Bay; Slopes around Cape Town.

Bellville District: Durbanville.

Stellenbosch.

Malmesbury District: Bokbaai.

Hopefield District: Matjiesfontein, north of Hopefield.

Piketberg District: Between Redelinghuys and Leipoldtville.

Clanwilliam District.

T. grandiflorus has a fairly restricted distribution. It is largely confined to the winter rainfall region of the Cape, where it is found on the western slopes of the Cape Peninsula, around Cape Town, and northwards along the coast to the Clanwilliam area. It occurs most frequently at altitudes of between 20 and 70 m, but has also been found as high as 1 000 m on the slopes of Table Mountain. It favours sandy soils, dry rocky hillsides, crevices in granite boulders and has also been collected from salt pans and sand flats in Coastal Sandveld/Coastal Fynbos transition vegetation.

Description. A semi-succulent perennial. *Stems* decumbent, pale coloured, sparingly branched, 4–20 cm long, 2–3 cm thick, during spring, with a tuft of leaves at the apex, in summer, covered with withered leaf bases which subsequently fall off leaving tubercules with hard spinescent tips. *Leaves* spirally arranged, deciduous, with a rounded, tuberculate, protruding base, soft and fleshy green, linear to oblanceolate, flat or concave above, 40–80(–120) mm × 4–14 mm, entire and glabrous. *Inflorescence* a pedunculate thyrse produced after leaves have withered and fallen, flowers spirally arranged along central axis, maturing acropetally; peduncle erect, unbranched, angular, 150–400 mm long, laxly furnished with bracts which wither with age, the base persistent; peduncle base glabrous, apex and pedicels glandular-pubescent with pale rust-coloured hairs; pedicels erect, 10–20 mm long. *Flowers* 4–10, zygomorphic, suberect, glandular-pubescent. *Calyx* 8–18 mm long, linear, acute, bases almost free. *Corolla* orange to red, sometimes yellow with red streaks, 35–65 mm long, two thirds a curved tube, slightly constricted just above calyx and just below corolla lobes, dilated at centre; tube opening asymmetrically, lobes oblong-lanceolate, recurved and spreading to become somewhat bilabiate. *Stamens* 10, inserted 1 cm from base of corolla tube, one set of 5 shorter and reaching mouth of tube, the other 5 2–3 mm longer and exserted, anthers purple, the shorter 5 ripening first. *Carpels* slender, slightly shorter than corolla tube, style cylindric, squamae oblong with a slightly constricted middle, 5 to many ovules (Fig. 2, 3 & 4).

Flowering time: From January to March but most commonly in February. *T. grandiflorus* is easily distinguished from the other species of *Tylecodon* and *Cotyledon* with similar distribution and habitat by its large, irregular flowers and the appearance of the leaves and flowers at different seasons (Adamson & Salter, 1950; Dyer, 1948; Harvey & Sonder, 1862; Schonland, 1915; Schonland & Baker, 1902; Von Poellnitz, 1937).

In 1978, Toelken recognized and described the genus *Tylecodon* as being separate from *Cotyledon* on the basis of its spirally arranged leaves and several other characteristics. The name *T. grandiflorus* (Burm.f.) Toelken is thus a recent one, this plant having previously been known as *C. grandiflora* Burm.f. The names under which this plant has been illustrated and described are listed under 'Synonyms'. This plant is figured in: The flowering plants of Africa (Dyer, 1948).

MATERIALS AND METHODS

Isolation of the toxic principles

Fresh *T. grandiflorus* plants (405 kg) from Stellenbosch and Durbanville areas were minced and extracted 3 times in a Waring blender with ethyl acetate and the
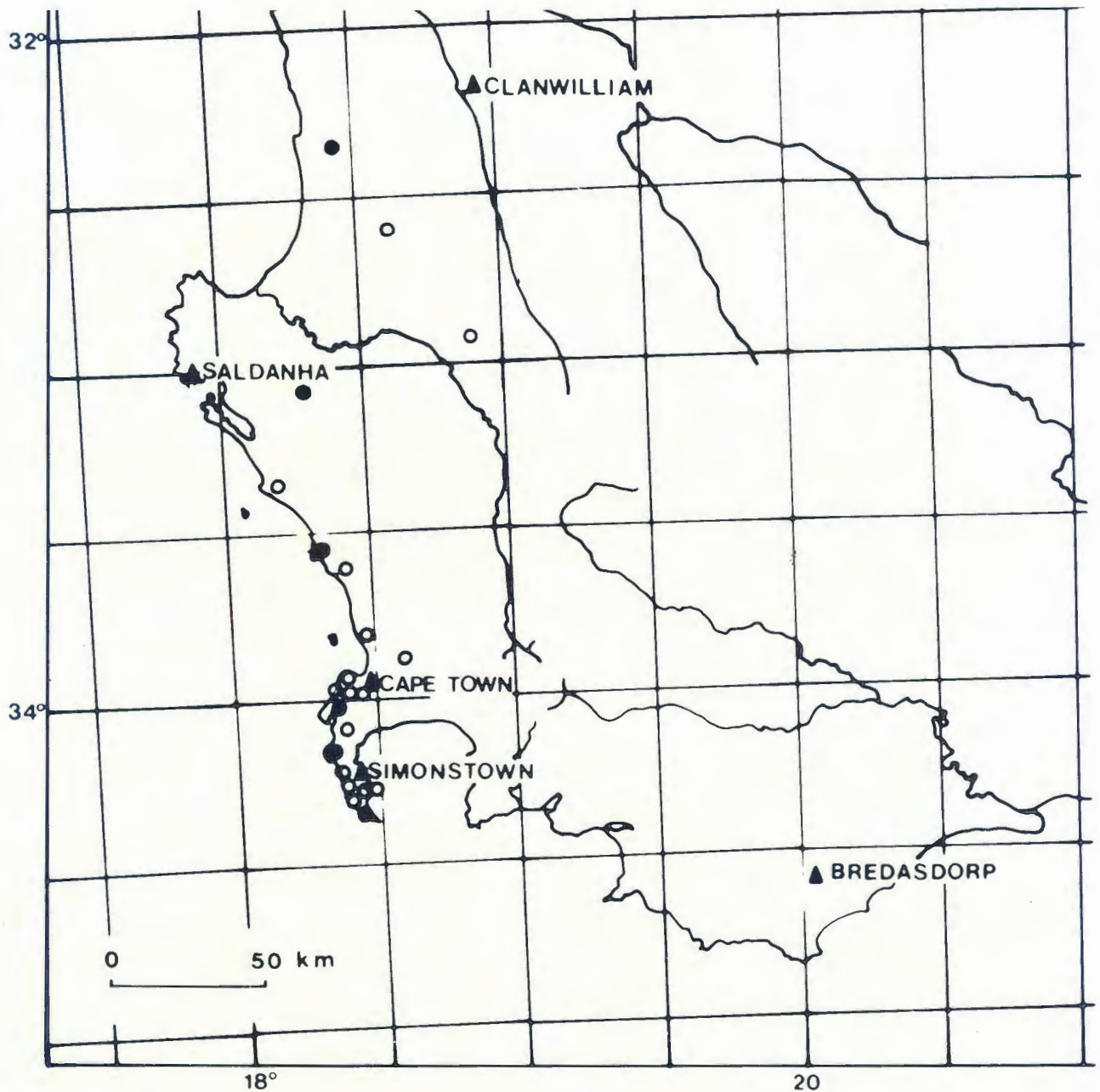
FIG. 1 Distribution of *T. grandiflorus* (Burm.f.) Toelken in the Republic of South Africa.

● Precise localities of *T. grandiflorus*
○ Localities for which the Grid Reference only is known

solvent evaporated on a boiling water-bath under reduced pressure. The resultant syrup was partitioned between 95 % methyl alcohol ($\pm$ 5 ℓ) and petroleum ether. Both extracts were evaporated to dryness and the residues tested on guinea-pigs for toxicity. Only the residue (711 g) from the methanolic extract was toxic. This residue was agitated with methyl alcohol (3 ℓ) and centrifuged at 4 000 rev/min for 10 minutes. The supernatant fluid was decanted and evaporated as above to yield a toxic syrup (408 g). The precipitate was non-toxic to guinea-pigs at oral doses of 500 mg/kg.

The syrup was chromatographed on a silica gel column (4,5 kg). Elution with benzene (12 ℓ) yielded non-toxic material (118 g). Elution with benzene-ethyl acetate (1:1 v/v), followed by ethyl acetate and ethyl acetate-methanol (80:20 v/v), yielded toxic fractions

(216 g), while elution with methanol yielded non-toxic material.

In establishing the toxicity of the fractions, only fractions which were lethal to guinea-pigs within 3 hours at doses of *c*. 100 mg/kg, were regarded as toxic.

The toxic fractions were recombined and evaporated to dryness to yield an amorphous residue (216 g).

Repeated chromatography of the toxic residue on silica gel using benzene-ethyl acetate (25:75 v/v $\rightarrow$ ethyl acetate), chloroform-acetone-methanol (75:25:1 v/v/v) and chloroform-ethyl acetate-methanol (70:30:1 v/v/v), yielded 5 crystalline components, Tyl A (392 mg), Tyl B (60 mg), Tyl C (1,35 g), Tyl E (137 mg) and Tyl F (773 mg), as well as one amorphous component Tyl D (720 mg).
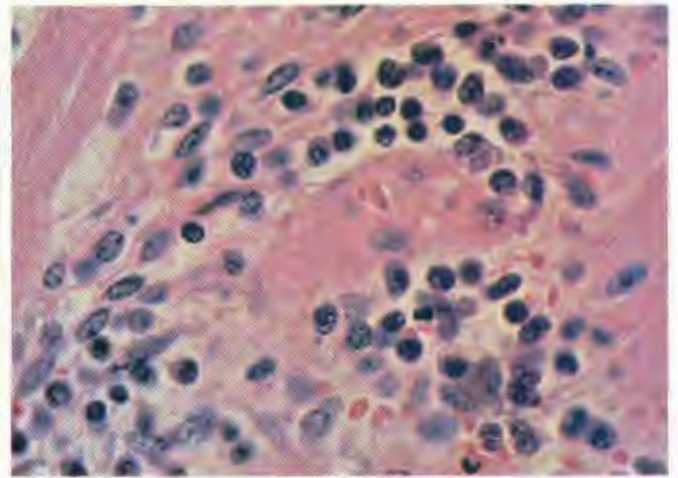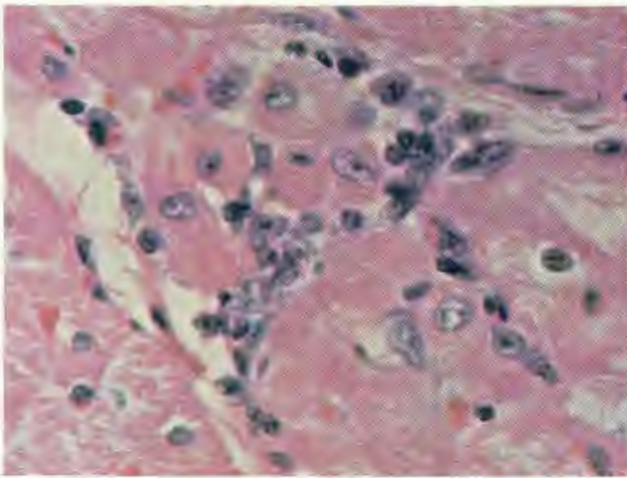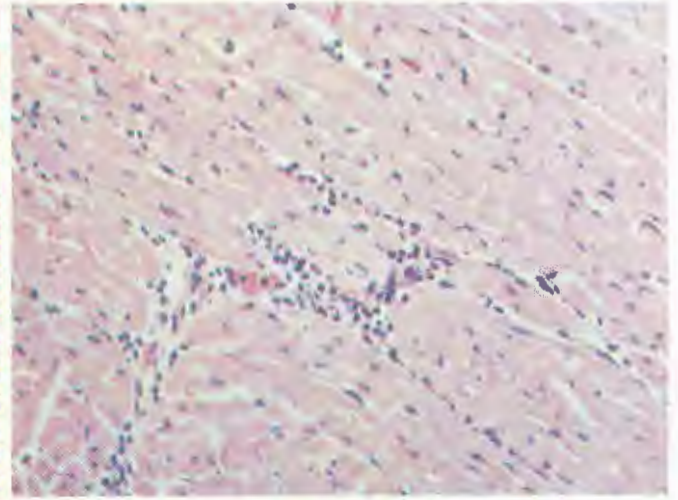
FIG. 2 The leaves of *T. grandiflorus* form in spring before they flower

FIG. 3 Flowers of *T. grandiflorus*

FIG. 4 Flowering plants after the leaves have fallen

FIG. 5 An infiltration of round cells into the myocardium of a sheep dosed with *T. grandiflorus*: HE × 200

FIG. 6 A higher magnification of an interstitial infiltration of round cells in the myocardium of a sheep dosed with plant material: HE × 1200

FIG. 7 Necrosis of myocardial fibres with a mononuclear cellular infiltration: HE × 1200

Reagents and apparatus

Melting points are uncorrected and were determined with a Büchi melting point apparatus. Ultraviolet (UV) absorption refers to methanol, nuclear magnetic resonance (NMR) to CDC1$_3$ solutions and infra-red (IR) to KBr discs. UV absorptions were measured with a Beckman DK-2A spectrometer, IR spectra with a Perkin Elmer 257 spectrometer, mass spectra with a AEI MS-9 mass spectrometer and NMR spectra on a Varian XL 100 spectrometer. Merck silica gel 60, particle size 0,06–0,20 mm, was used for column chromatography. Merck pre-coated silica gel 60 F 254 aluminium sheets (thickness 0,2 mm) were used for thin layer chromatography and the spots were visualized by spraying with 85 % sulphuric acid and heating at 120 °C for 1–2 minutes.

Dosing trials

(1) *Guinea-pigs*. The toxicity of the plant extracts was monitored by dosing orally the various fractions to weaned guinea-pigs.

The toxicity and cumulative effect of 5 bufadienolides were assayed in young (150–250 g) male albino guinea-pigs (Table 1). The approximate subcutaneous LD$_{50}$ of each bufadienolide was determined (2 animals/dose) and the cumulative effect by the subcutaneous injection of 25% and 50 % LD$_{50}$ day, until the guinea-pigs died (4 animals/dose).

(2) *Sheep*. Eleven Merino, Dorper and Blackhead Persian sheep (milktooth–8 tooth) of both sexes and with live masses varying between 27 and 41 kg were dosed per stomach tube or injected intravenously as set out in Tables 2 and 3. The sheep were examined daily and electrocardiograms were recorded in some of them.

Pathology

Three guinea-pigs and 11 sheep were autopsied (Tables 1, 2 & 3). Specimens from various organs including the brain, spinal cord, heart, lungs, liver, spleen, kidneys and skeletal muscles were collected in 10 % formalin. Tissue blocks were routinely processed and stained with haematoxylin and eosin (HE). Special staining techniques employed were Masson's trichrome technique and Dahl's Alizarin method (Lee, 1968).

TABLE 1 Observations on guinea-pigs intoxicated by subcutaneous injection of bufadienolides isolated from *T. grandiflorus*

Bufadienolide	LD$_{50}$ mg/kg	Cumulative effect	Histopathology of cumulative cases
Tyl A	c. 0,12	*	Brain +
Tyl C	c. 0,2	o	Not examined
Tyl D	c. 0,12	*	Brain +
Tyl E	c. 0,2	o	Not examined
Tyl F	c. 0,18	*	Brain +

o = Not cumulative

* = Cumulative effect demonstrated

+ = Mild lesions

RESULTS

Toxic principles

The compounds, apart from the amorphous Tyl D, were all crystallized from chloroform-ether, and had the following melting points: Tyl A 297–299 °C, Tyl B 301–302 °C, Tyl C 217–220 °C, Tyl E 237–239 °C and Tyl F 209–213 °C.

None of these compounds gave a green anthrone colour reaction (Cheronis & Entikrin, 1958), but spectral data confirmed their glycosidic nature.

All 6 compounds were identified as bufadienolides by means of characteristic spectral data (Anderson & Koekemoer, 1968), namely, maximum UV absorptions at 299 nm, IR absorptions at c. 1 710, 1 630, 1 540, 1 240 and 840 cm^{-1} and double doublets in the 'H NMR spectra at c. δ 6,2, 7,2 and 7,8 (characteristic for the 3 protons of the α-pyrone ring).

None of these compounds were identical with cotyledoside (Van Wyk, 1975).

Clinical signs

Plant material. The findings are summarized in Table 2. Sheep were chronically intoxicated by the administration of varying doses over several days. In all the animals gastrointestinal changes were amongst the first clinical signs noticed, followed by locomotory/postural changes, the most comspicuous being nodding of the head, reluctance to stand and/or sternal recumbency with the head and neck resting on the floor. Higher doses were associated with forced respiratory movements and mild terminal cardiac changes, namely, bradicardia and tachycardia (Sheep 2), ST depression and increased amplitudes of the T wave (Sheep 3 & 6).

Bufadienolides. The results are summarized in Tables 1 & 3.

(1) *Guinea-pigs*. The occurrence of clinical signs or death after administration of 3 × 50 % or 5–6 × 25 % LD$_{50}$ of a bufadienolide (Tyl A, Tyl D and Tyl F) were indicative of their cumulative effect. The animals dosed with Tyl C and Tyl E recovered before the next dose was given and these compounds were, therefore, not cumulative.

The clinical signs noticed were muscular tremors, neck paresis and paralysis.

(2) *Sheep*. In all the animals gastro-intestinal as well as postural/locomotory changes were seen. Mild cardiac changes were noticed in 1 sheep only.

Macroscopic pathology

No lesions were observed either in the guinea-pigs or in 2 of the sheep (Sheep 10 & 11). In the remaining sheep generalized congestion, catarrhal enteritis, ascites, lung and intermandibular oedema and a mild oedema of the abomasum were seen.

Microscopic pathology

(1) *Guinea-pigs*. A mild brain oedema affecting various areas of the brain was present in all the animals. The lesions were predominantly present in the white matter, especially of the peri-ventricular area (Table 1).

(2) *Sheep*. The cardiomyopathy (Fig. 5, 6 & 7) observed in the sheep receiving plant material (Table 2) corresponded with that reported by Anderson *et al*. (1983) in sheep dosed with *K. lanceolata*. The lesions present in sheep injected with bufadienolides isolated from *T. grandiflorus* consisted of multifocal areas of hyaline degeneration and necrosis scattered throughout the myocardium without evidence of any cellular reaction (Table 3).

An interstitial pneumonia and lung oedema were present in 2 animals, while brain oedema with a prediction for the peri-ventricular white matter was observed in 4 animals (Table 2).

TABLE 2 Observations of sheep intoxicated with *T. grandiflorus*

No.	Dosing regimen		Clinical signs	Fate	Histopathology
	Dose g/kg × n	Period dosed Day 0–Day n			
1	2,5 × 2	0–6	Decrease ruminal movements (Day 1), forced respiration and reluctance to stand (Day 7)	Died on Day 8	Heart ++
2	0,75 × 2	0–4	Decreased heart and respiratory frequency (Day 6) followed by acceleration (Day 7), inappetence, ruminal stasis, diarrhoea and reluctance to stand	Slaughtered on Day 7	Heart +++ Brain +
3	0,5 × 3	0–4	From Day 6 onwards, inappetence, ruminal stasis, transient diarrhoea, transient arrhythmia and intensified heart sounds, aimless chewing, sagging of the lower jaw, nodding of the head, walking sideways, with head held abnormally high or dangling loosely, skeletal muscles visibly tensed when forced to remain standing, sternal recumbency with head and neck on floor	Slaughtered on Day 14	Heart +++ Interstitial pneumonia
4	1,5 × 1 0,5 × 2	0 20–41	Transient forced respiration, followed by recurring transient inappetence, grinding of teeth, ruminal stasis. From Day 31 onwards also knuckling over, nodding of the head, weakness and reluctance to stand	Slaughtered on Day 48	Heart +
5	0,5 × 5 0,5 × 3	0–21 28–35	From Day 22 onwards inappetence, decreased ruminal movements, grinding of teeth. From Day 37 onwards also reluctance to stand, weakness, sternal recumbency with head on floor	Slaughtered on Day 41	Heart + Brain + Interstitial pneumonia
6	0,5 × 14 0,75 × 2	0–59 60–63	Recurring transient inappetence and decreased ruminal movements (Day 24–60), followed by forced respiration, ruminal stasis, reluctance to stand	Died on Day 69	Heart +++ Brain + Lung oedema
7	1 × 3 0,5 × 18 0,75 × 2 1 × 2	0–2 43–66 69–71 76–85	Inappetence, ruminal stasis, grinding of teeth and forced respiratory movements (Day 2–17) accompanied terminally by reluctance to stand and apathy	Died on Day 87	Heart + Brain + Lung oedema
8	0,5 × 9 0,25 × 18 0,5 × 5 0,75 × 5	0–10 51–74 77–81 84–93	Inappetence, ruminal stasis, nodding of the head, apathy (Day 11–24), accompanied by forced respiration, sternal recumbency with slight nodding of the head and muscular tremors (Day 95–97)	Died on Day 97	Decomposed

+ = Mild lesions
++ = Moderate lesions
+++ = Pronounced lesions
n = Number of daily doses

TABLE 3 Observations on sheep intoxicated with bufadienolides isolated from *T. grandiflorus*

No.	Isolated bufadienolide	Dosing regimen		Clinical signs	ECG changes	Fate	Histopathology
		Dose mg/kg × n	Period dosed Day 0–Day n				
9	Tyl C	0,02 × 6 0,02 × 1 0,01 × 4	0–8 13 14–17	Recurring transient inappetence, decreased ruminal movements, dyspnoea and grinding of teeth. From Day 9 onwards, head and ears hung, it frequently changed position of feet, the back was arched and feet planted close together, it weakened, lay in sternal recumbency with neck on floor	Tachycardia (Day 8 and Day 9), increased QRS wave amplitude and ST depression (Day 10), arrhythmia (Day 13), ST elevation (Day 14 and 16)	Slaughtered on Day 21	Supected white muscle disease
10	Tyl D	0,012 × 4	0–3	Locomotory/postural changes from Day 3 onwards. Walked with difficulty keeping head down and back arched, paretic, lay down often. Terminal inappetence, ruminal stasis	Nothing unusual	Slaughtered on Day 7	Heart +
11	Tyl A	0,012 × 4 0,012 × 1	0–3 8	Days 3 and 4 decreased ruminal movements. After exercise frequently changed position of feet, tremors, reluctant to stand. From Day 7 onwards signs appear without exercise, sternal recumbency with neck stretched on floor	Nothing unusual	Slaughtered on Day 9	Heart +

+ = Mild lesions
+ + = Moderate lesions
+ + + = Pronounced lesions

DISCUSSION

The presence of cardiac glycosides in *T. grandiflorus* was confirmed by the isolation of 6 toxic bufadienolides. Since only small amounts of bufadienolides were obtained, not all 6 were administered to sheep.

In this investigation particular attention was paid to the chronic syndrome of cardiac glycoside poisoning induced by the repeated administration of small doses of the plant or its bufadienolides. The clinical signs observed in the subacute plant poisoning elicited corresponded with those reported by Naudé & Schultz (1982), whereas the chronic poisoning more closely resembled krimpsiekte (Henning, 1926). Symptoms comparable with the krimpsiekte syndrome were reluctance to stand, nodding of the head and recumbency with the head and neck on the floor. In this study torticollis, tetaniform convulsions (Henning, 1926) and typical krimpsiekte posture (Naudé & Schultz, 1982) were not observed in any of the animals. These differences may be ascribed to variations in the manifestation of the same syndrome. Furthermore, it must be remembered that these sheep were kept in a stable where the deleterious effects of the sun (Henning, 1926) and exercise (Henning, 1926; Naudé & Schultz, 1982) were absent.

Even more typical signs of krimpsiekte were induced by the intravenous injection of bufadienolides A and D, both of which had a cumulative effect in guinea-pigs. A similar correlation between the cumulative effect in guinea-pigs and inducement of krimpsiekte was observed by Anderson *et al.*, 1983.

The histopathological examination revealed a mild to moderate, acute to subacute, multifocal cardiomyopathy in animals receiving either plant material or bufadienolides. There was no direct correlation between the amount of plant material consumed or the clinical signs and the extent of the myocardial lesions. The severest cardiac lesions were seen in 3 sheep (Sheep 2, 3 & 6) that had been given plant material and the mildest in 2 sheep (Sheep 10 & 11) that had received bufadienolides. The fact that ECG changes were present only in the former might indicate that the severity of the histopathological lesions and degree of ECG changes was correlated.

Multifocal myocardial hyaline degeneration and necrosis have been observed in many conditions affecting man and animals. These changes have been reported in humans dying of brain lesions or following surgery; in rats suffering from potassium deficiency alone or from both a potassium and a sodium deficiency; and in humans or laboratory animals after catacholamine release (Reichenbach & Benditt, 1970; Selye & Bajusz, 1959). Moreover, Emberson & Muir (1969) reported the disintegration of myocardial fibrils with the loss of thin filaments in rats perfused with hypokalemic solutions. They deduced that a decrease in myocardial potassium content increased the susceptibility of the myocardium to myofibrillar degeneration. Schatzman (1953) and Joyce & Weatherall (1953) (both sources cited by Glynn, 1964) showed that small concentrations of cardiac glycosides can inhibit the active movement of potassium and sodium across cell membranes. At both therapeutic and/or toxic levels the enzyme responsible for the active transport of ions across membranes is inhibited (Joubert, 1981). If one considers the important role of potassium ions in maintaining the structure and functions of cell membranes (*vide supra*), it is suggested that one of the causes of the cardiomyopathy in sheep receiving plant material or bufadienolides is the altered transfer of ions across the sarcolemma which renders it more susceptible to injury.

REFERENCES

ADAMSON, R. S. & SALTER, T. M., 1950. Flora of the Cape Peninsula, p. 439. Cape Town: Juta & Co. Ltd.

ANDERSON, L. A. P. & KOEKEMOER, J. M., 1968. The chemistry of *Melianthus comosus* Vahl. II. The isolation of hellebrigenin 3-acetate and other unidentified bufadienolides from the rootbark of *Melianthus comosus* Vahl. *Journal of the South African Chemical Institute*, 21, 155–158.

ANDERSON, L. A. P., SCHULTZ, R. ANITRA, JOUBERT, J. P. J., PROZESKY, L., KELLERMAN, T. S., ERASMUS, G. L. & PROCOS, J., 1983. Krimpsiekte and acute cardiac glycoside poisoning in sheep caused by bufadienolides from the plant *Kalanchoe lanceolata* Forsk. *Onderstepoort Journal of Veterinary Research*, 50, 295–300.

CHERONIS, N. D. & ENTIKRIN, J. B., 1958. Semimicro qualitative organic analysis. The systematic identification of organic compounds. New York: Interscience Publishers Inc.

DYER, R. A. (ed.), 1948. The flowering plants of Africa, 27, Plate 1046 (Artist: C. Letty). Pretoria: Government Printer.

EMBERSON, J. W. & MUIR, A. R. 1969. Changes in the ultrastructure of rat myocardium induced by hypokalemia. *Quarterly Journal of Experimental Physiology*, 54, 36–40.

GLYNN, I. M., 1964. The action of cardiac glycosides on ion movements. *Pharmacological Reviews*, 16, 381–407.

HARVEY, W. H. & SONDER, O. W. 1862. Flora Capensis, Vol. II., p. 375. Kent: Reeve & Co. Ltd.

HENNING, M. W., 1926. Krimpsiekte. *11/12th Reports of the Director of Veterinary Education and Research*, Onderstepoort, 331–364.

JOUBERT, J. P. J., 1981. Die effek van hartgifglikosiede en elektroliete op die selmembraan se natruim, kalium-adenosientrifosfatase. *Journal of the South African Veterinary Association*, 52, 225–228.

LEE, G. L., 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology. Blakistan Division, 3rd edn. New York: McGraw-Hill.

NAUDÉ, T. W. 1977. The occurrence and significance of South African cardiac glycosides. *Journal of the South African Biological Society*, 18, 7–20.

NAUDÉ, T. W. & SCHULTZ, R. ANITRA, 1982. Studies on South African cardiac glycosides. II. Observations on the clinical and haemodynamic effects of cotyledoside. *Onderstepoort Journal of Veterinary Research*, 49, 247–254.

REICHENBACH, D. D. & BENDITT, E. P., 1970. Catacholamines and cardiomyopathy: the pathogenesis and potential importance of myofibrillar degeneration. *Human Pathology*, 1, 125–150.

SCHONLAND, S., 1915. The South African species of the genus *Cotyledon*. *Records of the Albany Museum*, 3, p. 147. Grahamstown: Grocott & Sherry.

SCHONLAND, S. & BAKER, E. G., 1902. Some South African species of *Cotyledon*. *Journal of Botany*, 40, p. 23.

SELYE, H. & BAJUSZ, E., 1959. Sensitization by potassium deficiency for the production of myocardial necrosis by stress. *American Journal of Pathology*, 35, 525–534.

STEYN, D. G., 1949. Vergiftiging van mens en dier. Pretoria: Van Schaik.

TOELKEN, H. R., 1978. New taxa and new combinations in *Cotyledon* and allied genera. *Bothalia* 12, 377–393.

VAN WYK, A. J., 1975. The chemistry of *Cotyledon wallichii* Harv. III. The partial constitution of cotyledoside, a novel bufadienolide. *Journal of the South African Chemical Institute*, 28, 281–283.

VON POELLNITZ, K., 1937. Beiträge zur Kenntnis der Gattung *Cotyledon*. *Repertorium Novarum Specierum Regni Vegetabilis*, 42, 20–22.