Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 18, Numbers 1 and 2, July and October, 1943.

Printed in the Union of South Africa by the Government Printer, Pretoria.

The Isolation of the Toxic Principle "Potassium Cymonate" from "Gifblaar" *Dichapetalum cymosum* (Hook) Engl.

By J. S. C. MARAIS, Section of Pharmacology and Toxicology, Onderstepoort.

It has long been known that "Gifblaar" *Dichapetalum cymosum* (*Chailletia cymosa*) is one of the most poisonous plants of Southern Africa. The plant is mainly distributed throughout the Northern and Western Transvaal, Bechuanaland and South-West Africa. A good summary of our present knowledge in respect of poisoning by Gifblaar is given by Steyn (1928) with reference to various attempts to isolate the toxic principle. In 1935 Rimington reported on the chemical investigation of the plant giving an account of the different methods employed by him in endeavouring to isolate the toxic principle.

In the present investigation the methods described by Rimington were used to prepare a concentrate of the toxin. By further manipulations and purification it was found possible to prepare an amorphous hygroscopic powder which was highly toxic to rabbits, viz., 5 mgm. per kilogram body-weight. On investigating this concentrate it proved to contain some sodium acetate. In attempts to remove the acetate by liberating the acetic acid it was observed that the toxic principle behaved in very much the same way as the acetic acid. Thereupon the methods of extraction were further investigated and modified until the following was evolved as the most suitable method of extraction.

Method for the Isolation of the Toxic Principle.

Ten kilograms of the dried and finely ground plant material are continuously extracted with 96 per cent. alcohol for 36 to 48 hours in a large soxhlet extraction apparatus. After extraction, the alcohol is distilled off under diminished pressure. The extract, which now consists of a syrupy mass, containing a large amount of chlorophyll, is taken up in about 4 litres of water and after acidifying with 500 c.c. of a 10 per cent. sulphuric acid solution, it is left to stand for a day or two, to allow the chlorophyll and other precipitated material to settle. The solution is then filtered, using a large buchner filter and applying suction. This gives a clear although slightly brown coloured filtrate. After dividing in suitable portions the filtrate is repeatedly shaken out with ether. The ether shakings are neutralized with N potassium hydroxide, using phenolphthalein as indicator. By this means the acid extracted by the ether is converted into the potassium salt, which is insoluble in the ether and goes, therefore, into the water solution. After separation of the potassium salt solution from the ether extract, the ether solution is distilled to remove dissolved chlorophyll. Thus the recovered

ether may be used over and over again for shaking out the acid filtrate. This process of shaking out with ether and neutralizing with potassium hydroxide is continued until only negligible amounts of acid are extracted by the ether.

The combined potassium salt solution is concentrated to about 300 c.c. by distilling off under diminished pressure on a waterbath. The free acid is again liberated by adding the equivalent amount of dilute sulphuric acid. This solution is now repeatedly shaken out with ether until only negligible amounts of acid are extracted by the last ether shakings, as tested by neutralizing an aliquot against $\frac{N}{10}$ potassium hydroxide solution, using phenolphthalein as an indicator. The total ether shakings are decolorized by activated charcoal and dried overnight by adding anhydrous sodium sulphate. The sodium sulphate and charcoal are filtered off and the ether solution concentrated by gently distilling off the ether. As soon as nearly all the ether has been distilled off, the concentrate is rinsed with small amounts of ether into a small distillation flask and again distilled, collecting in fractions of 10 degrees up to 160° C. At this stage only a small viscid dark brown residue, which hardens on cooling, remains in the distillation flask. All the fractions as well as the initial ether distillate are now carefully neutralized with N potassium hydroxide using phenolphthalein as indicator. The potassium salt solutions are now separately evaporated to dryness on a waterbath. The residues are washed with acetone, dried at 100° C. and crystallized from 96 per cent. alcohol. The lower boiling fractions yield only small amounts of crystals and the ether distillate none at all. The best yields of the crystalline potassium salt are obtained from the fractions 110°–160° C. All of the crystalline material is collected, pooled and further purified by recrystallization from 96 per cent. alcohol. The name suggested for this crystalline potassium salt is potassium cymonate.

Experimental Results.

Using the above described method for the extraction of 10 kilograms of plant material, the initial ether shakings required altogether 600 c.c. N. potassium hydroxide ($f = 0{\cdot}83$). After again acidifying with the equivalent amount of sulphuric acid it was found necessary to shake out 16 times with ether before all of the acid had been extracted. The yields of potassium cymonate from the different fractions were as follows:—

Fraction.	c.c. N. KOH ($f = 0{\cdot}83$), required for neutralization.	Yield of Crystalline K salt	M.P. of Crystalline K salt
1. Ether distillate 40°	31·0 c.c.	None	—
2. 40°– 50°	1·8 c.c.		
3. 50°– 60°	1·5 c.c.	0·0280 gm.	185°–203°
4. 60°– 90°	4·0 c.c.		
5. 90°–100°	5·5 c.c.		
6. 100°–110°	17·5 c.c.	0·2586 gm.	200°–208°
7. 110°–120°	14·2 c.c.		
8. 120°–130°	21·5 c.c.	0·7534 gm.	200°–213°
9. 130°–140°	24·4 c.c.	0·6000 gm.	206°–213°
10. 140°–150°	24·7 c.c.	0·8270 gm.	208°–213°
11. 150°–160°	46·7 c.c.	1·1870 gm.	208°–213°
	Total.....	3·6540 gm.	

After the removal of the potassium cymonate the residues of the different fractions were investigated. It was found that fraction 1 contained mostly potassium formate mixed with a small amount of potassium acetate, whilst the higher fractions contained potassium formate, potassium acetate and the potassium salts of higher boiling unidentified acids. After repeated recrystallization of the potassium cymonate from 96 per cent. alcohol it melted at 213° C. with decomposition and a colour change from yellow to red. The purity of the potassium cymonate was tested by recrystallizing five times from 96 per cent. alcohol. No change in melting point could be obtained.

Potassium cymonate is insoluble in anhydrous organic solvents excepting methyl alcohol. It dissolves very easily in water and is soluble with difficulty in 96 per cent. alcohol.

Attempts to isolate the free acid in a purified state failed since slight decomposition occurred when it was distilled. The potassium salt itself gave unreliable combustion analysis, so that with regard to the nature of this substance very little can be said at present.

These investigations will be continued as soon as more plant material becomes available.

Toxicity of the Potassium Cymonate.

The crystalline potassium cymonate proved to be very toxic to rabbits and gave rise to the typical gifblaar poisoning with the same general post-mortem appearances. The results of the dosing experiments are summarized in the following table:—

Rabbit.	Weight in Kilograms.	Dosed.	Mgm. K Cymonate per Kilogram.	Result.
1	1·9	Per os.....	11·5	Died within 1 hour 30 minutes.
2	2·1	„	3·2	Died within 1 hour 20 minutes.
3	1·9	„	1·9	Died within 1 hour 10 minutes.
4	2·0	„	1·0	Died within 11 hours.
5	2·0	„	0·75	Died within 3 hours 15 minutes.
6	2·3	„	0·75	Died within 8 hours.
7	2·1	„	0·75	Died within 26 hours 45 minutes.
8	2·2	„	0·50	Died within 3 hours 5 minutes.
9	2·4	„	0·50	Died within 6 hours 45 minutes.
10	2·0	„	0·50	Died within 12 hours 45 minutes.
11	2·6	„	0·50	Died overnight.
12	1·8	„	0·50	Died overnight.
13	2·4	„	0·50	Recovered.
14	2·0	„	0·50	Recovered.
15	2·1	„	0·50	Recovered.
16	2·1	„	0·25	Recovered.
17	2·2	„	0·25	Recovered.
18	2·0	„	0·25	Recovered.
19	1·8	„	0·25	Recovered.
20	3·3	Intravenously.....	0·25	Recovered.
21	1·5	„ „	0·25	Recovered.
22	1·8	„ „	0·25	Recovered.
23	2·2	„ „	0·25	Recovered.

Rabbit.	Weight in Kilograms.	Dosed.	Mgm. K Cymonate per Kilogram.	Result.
24	2·1	Intravenously.....	0·50	Died within 43 minutes.
25	3·3	„ „	0·50	Died within 2 hours 13 minutes.
26	1·9	„ „	0·50	Recovered.
27	1·6	„ „	0·50	Recovered.
28	1·5	„ „	0·75	Died within 3 hours 56 minutes.
29	1·8	„ „	0·75	Died overnight.
30	2·2	„ „	0·75	Died overnight.
31	3·3	Subcutaneously.....	0·25	Died overnight.
32	2·8	„ „	0·25	Recovered.
33	2·1	„ „	0·25	Recovered.
34	1·7	„ „	0·25	Recovered.
35	1·7	„ „	0·50	Died within 1 hour 38 minutes.
36	2·4	„ „	0·50	Died overnight.
37	2·8	„ „	0·50	Died overnight.
38	2·1	„ „	0·50	Recovered.
39	2·1	„ „	0·75	Died within 2 hours 40 minutes.
40	1·7	„ „	0·75	Died overnight.

It is apparent from these results that the M.L.D. for the rabbit is 0·5 to 0·75 mgm. of the potassium cymonate per kilogram bodyweight. It is also of interest to note that the M.L.D. *per os* is the same as that when administered intravenously or subcutaneously.

Summary.

A method for the isolation of potassium cymonate, the toxic principle of *Dichapetalum cymosum* has been described. The M.L.D. of potassium cymonate for the rabbit is 0·5 to 0·75 mgm. per kilogram bodyweight.

Acknowledgments.

I wish to express my sincere thanks to Dr. D. G. Steyn and Prof. H. L. de Waal for their keen interest throughout the course of the investigation.

LITERATURE.

RIMINGTON, C. (1935). Chemical Investigation of the "Gifblaar" *Dichapetalum cymosum* (Hook) Engl. *Onderstepoort J.*, Vol. 5, No. 1, pp. 81-95.

STEYN, D. G. (1928) Gifblaar Poisoning. A summary of our present knowledge in respect of poisoning by *Dichapetalum cymosum*. *13th and 14th Reports, Dir. of Vet. Education and Research*, Part 1, pp. 187-194.