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Isolation of the toxic principles of *Cucumis Africanus* L.f., *Cucumis myriocarpus* Naud. emend. Schweikerdt and of *Cucumis leptodermis* Schweikerdt sp. nov. their characterisation as trilactones belonging to the "Bitter Principle" Class.

By CLAUDE RIMINGTON, M.A., Ph.D., B.Sc., A.I.C. Research Fellow under the Empire Marketing Board.

AMONG the cucurbitaceous plants native to South Africa, *Cucumis myriocarpus* has frequently been proved to be the cause of losses of stock and *C. africanus*, though less poisonous, has been shown to exert similar effects. The plants enjoy a wide distribution, often growing on the open veld, but more usually frequenting cultivated soil, such as mealie plantations, where *C. myriocarpus* is a common weed. After the harvesting of the mealie crop, stock animals are often turned into the pastures to graze the stubble and it is under these circumstances that most cases of *Cucumis* poisoning occur. The toxic principle appears to be confined to the juicy pulp of the ripe fruits. *C. myriocarpus* occurs also in Australia where it is recognised as a stock-poisoning plant (Dept. Agric. W. Australia, 1926), and is known popularly by the name of " paddy-melon ". Seddon (1930) reports that animals may develop an abnormal craving for the plant (depraved appetite) and may suffer from blindness as a result of chronic poisoning. In Africa, several members of the genus figure in native medicine lore, Dragendorff (1898) citing the use of *C. myriocarpus* by the kaffirs as a purgative, whilst Watt and Breyer-Brandwyk (1932) mention its use by the Sutos (Suto name monyaku) and Kwena and Chuana peoples (under the name thlaresa-mpja) as a drastic purgative which has been known to cause deaths through overdosage. *C. africanus* is used by the Xosa witch-doctors as an emetic charm and as a hydrogogue cathartic in dropsical affections (Xosa name u Thangazana). According to Atkinson (1887), who made one of the first chemical studies of *C. myriocarpus* (see also Bayley 1886), the natives warm the fruits before administering them, a procedure which lessens their toxicity. In a more recent study, Quin (1928) found an amorphous, extremely bitter toxic substance in the fruits of *C. myriocarpus* and *C. africanus*. Its pharmacological effects were fairly fully reported. Apart from the fact that his preparations were free from nitrogen and non-glucosidal in character, Quin did not advance further evidence for the purity of his product nor of its chemical composition.

BOTANICAL DESCRIPTION.

Cucumis africanus. Common names: Wild cucumber; wilde komkommer; agurkie. Nat. Herb. No. 11287.

The following is adapted from the 'Flora Capensis' (Harvey and Sonder 1894: Vol. 2: p. 495).

Cucumis africanus aerial parts annual, green, prostrate, scabrous everywhere; branches angulate; leaves deeply 3 or 5-lobed lobes entire or sublobed, denticulate, as well as the sinus rotundate, middle lobe obovate, longer than the lateral ones; ovary oblong, muricate-echinate, on a slender peduncle; pepo ovoid, densely beset with short, but sharp spines.

Two varieties are then distinguished according to whether the leaves are mostly trilobed or 5-lobed.

Stem much branched. Leaves on longish petioles, in var. α $1\frac{1}{4}$–$1\frac{3}{4}$ inch long, 1–$1\frac{1}{2}$ inch wide, the upper smaller; in var. β $1\frac{1}{2}$–$2\frac{1}{2}$ inches long, and very similar to those of *C. anguria*, L. Male flowers fascicled, very small, much shorter than the hispid petiole; female flowers on longer peduncles. Fruit $1\frac{1}{2}$ inch long, $\frac{3}{4}$ or nearly 1 inch broad. Spines 2 lines long; the ripe fruit sometimes denudate or only tubercled by the remaining base of the spines. Seeds nearly 2 lines long.

The material used in the present investigation was collected in part from the Transvaal University College Experimental Farm and in part in the grounds of the laboratory at Onderstepoort. The plants corresponded very closely to the botanical description quoted above.

Cucumis myriocarpus Naud. emend. Schweikerdt (Plate 1) Nat. Herb. No. 11405. Common names: Wild cucumber; bitter apple, bitterappel; gifappel.

The material used was obtained from the farm "Overdene" P.O. Slabberts, Cape Province.

During the course of the investigation a further supply of *Cucumis* fruits was received from the farm "Zuurvlakte", Aliwal North, Cape Province, but since there appeared to be some differences in appearance between these and the material from Slabberts, seeds of each were sown in the Poison Garden at the Laboratory and the mature plants referred to the Division of Plant Industry, Pretoria. As a result, the description of the species *Cucumis myriocarpus* has been amended and a new species *Cucumis leptodermis*, Schweickerdt, distinguished by Schweickerdt (1933) who gives a full description of the plants.

The plant coming from Aliwal North upon which the chemical investigations recorded below have been carried out is thus *Cucumis leptodermis* Schweickerdt (Plate II) Nat. Herb. No. 11418.

Cucumis myriocarpus Naud. emend. Schweickerdt.

Cucumis leptodermis Schweickerdt.

CHEMICAL EXAMINATION AND ISOLATION OF "CUCUMIN".

Feeding tests with fruits of both *Cucumis myriocarpus*, *Cucumis leptodermis* and *Cucumis africanus* confirmed the findings of Quin (1928) that toxicity is confined to the juicy pulp of the fruit characterised by its exceedingly bitter taste, the seeds and rind causing no ill effects.

Isolation of the toxic principle was accomplished in the following manner: The ripe fruits (quantities of 5 to 6 kilos were handled at a time) were subjected to pressure in a heavy, hand-operated press, the viscid, greenish juice being collected (3½ to 4 litres from 6 kilos). Basic lead acetate solution was added, whilst vigorously stirring, until no further precipitate formed, when the liquid was filtered off under suction by means of large Büchner funnels. To the clarified juice, sodium carbonate solution was then added in amount slightly more than sufficient to precipitate all the lead as carbonate. After filtration, the pale straw-coloured filtrate was shaken repeatedly with chloroform, extraction being continued until the main liquid was practically devoid of bitter taste. It was found to be imperative that the solution, once having been alkalinised by sodium carbonate, should be shaken with chloroform within a few hours otherwise the yield of "cucumin" is considerably lessened. On one occasion the solution was left standing in the laboratory for two days before extraction and in this case the yield dropped by nearly 50 per cent.

The chloroform extract, which was quite colourless, was dehydrated (12-16 hours duration) by the addition of some lumps of calcium chloride and then filtered. It was then poured with vigorous stirring into 3½ volumes of petroleum ether (B.P. 40–60°) which had been chilled to about 5° C. The voluminous white precipitate rapidly settled allowing the greater part of the supernatant liquid to be decanted, after which separation was completed on the centrifuge. After one washing with petroleum ether, the toxic material was dried over calcium chloride and paraffin wax in rapidly exhausted desiccators. As so obtained, it formed a very fine snow-white amorphous powder, odourless, but with an intensely bitter taste and irritating action upon the tissues of the nose and throat. Care should be exercised when handling it, since unpleasant thoracic symptoms were more than once experienced by the present writer, in addition to soreness of the eyes and throat, when particles of its dust were accidentally inhaled.

The supernatant liquid and centrifugates were combined, petroleum ether added up to about 4 volumes, in all, to 1 volume of original chloroform extract, the mixture filtered and set aside in stoppered bottles. In the case of the *Cucumis leptodermis* fruits, a further toxic substance crystallised out from the petroleum ether: chloroform mixture during the course of 24 to 48 hours, but in no instance could any trace of this substance be isolated from the fruits of either of the other two species.

Before the presence of this second toxic substance was appreciated, petroleum ether was generally added in amount sufficient to produce complete precipitation of the easily precipitable

amorphous substance. The filtrates from these precipitations were kept and when it was noted that those from one batch of fruits were yielding a second substance, a careful study was made of the specific gravities of the mixtures and thence, by deduction, the relative proportions of solvents which each contained.

Since in all, about 250 kilos of *C. leptodermis* fruits were obtained from Aliwal North and these were worked up in lots of approximately 6 kilos each, there was ample data provided from which to ascertain the best proportion of petroleum ether to chloroform to allow of separation of the two toxins. Fortunately, the solubility of the crystalline constituent is appreciable so that there is little fear of its contaminating the amorphous precipitate brought down at 3½ volums of petroleum ether. The yields obtained from the various batches of one lot were remarkably constant and on the average afforded the following figures, calculated as percentages upon the weight of fresh fruit.

	Amorphous.	Crystalline.
	%.	%.
Cucumis africanus..............................	0·013	Nil
Cucumis myriocarpus............................	0·095	Nil
Cucumis leptodermis............................	0·054	0·0015

It will thus be seen that the crystalline substance forms only about 3 per cent. of the whole of the toxic material in the one species in which it was found to occur.

Since further work revealed the fact that a simple chemical relationship existed between the amorphous and the crystalline principles, the one being $C_{27}H_{40}O_9$ and the other $C_{27}H_{38}O_8$—that is they differ by the elements of water H_2O—experiments were carried out particularly designed to ascertain whether the crystalline material was being produced from the amorphous by the chemical manipulations involved. It was demonstrated that the duration of contact between the chloroform extract and the dehydrating agent employed (calcium chloride) had no effect whatever upon the respective yields.

In some cases the yields were recorded upon the gradual addition of petroleum ether as in the following example:—

Lot B 10 from Aliwal North *C. leptodermis*.

Volume of chloroform extract 200 c.c. Poured into 200 c.c. of petroleum ether (1 vol.); slight turbidity only.

Petroleum ether increased to 2 vols. Wt. of amorphous ppt. 1·45 gm. filt. remained clear.

After 48 hours ,, ,, 3 vols. Wt. of amorphous ppt. 0·44 gm. Wt. cryst. ppt. 3·1 mgm.

,, ,, ,, ,, ,, 3½ vols. Wt. of amorphous ppt. nil. Wt. cryst. ppt. 75 mgm.

,, ,, ,, ,, ,, 4 vols. Wt. of amorphous ppt. nil. cryst. ppt. trace only.

The same amorphous principle was obtained from *Cucumis leptodermis*, *Cucumis myriocarpus* and from *Cucumis africanus* for which reason the name *Cucumin* is proposed for this substance. Although all attempts to induce it to crystallise have so far met with failure, there is very strong evidence for considering it to be a single, pure substance. This evidence is presented in the following paragraphs, wherein the determination of the physical constants of Cucumin is described.

A. *Constancy of Composition of "Cucumin".*

Elementary micro-analysis* of different preparations after drying in vacuo over P_2O_5 and petroleum wax, revealed a constancy of composition as is shown by the following figures:—

Source of material.	C %.	H %.
Cucumis africanus, T.U.C. farm....................	63·61	8·00
Cucumis myriocarpus, Lab. poison garden..........	63·64	8·52
Cucumis myriocarpus, Slabberts....................	64·02	8·00
Cucumis leptodermis, Aliwal North, Lot No. 5......	63·63	8·44
Cucumis leptodermis, Aliwal North, Lot No. 10.....	63·67	7·94
Mean..	63·71	8·16
The molecular formula $C_{27}H_{40}O_9$ requires..........	63·72	7·93

B. *Molecular Weight.*

Since the substance suffers decomposition when heated above 110° the method of Rast, used in determining the molecular complexity of the crystalline toxic principle (see later) is not in this case applicable. Barger's vapour tension method was, however, successfully employed. Solutions of azobenzol (Merck) as comparison substance and of the amorphous principle were made up in chloroform in the following proportions, employing small blown glass recepticles, like miniature flasks with narrow openings, which could be placed directly upon the balance pan.

(1) Amorphous principle 25·40 mgm. in 0·5 gm. chloroform.
(2) Azobenzol 7·50 mgm. in 0·5 gm. chlorofrom; normality 0·08.
(3) Azobenzol 9·11 mgm. in 0·5 gm. chloroform; normality 0·101.
(4) Azobenzol 12·90 mgm. in 0·5 gm. chloroform; normality 0·14.

Several capillary tubes were then filled in the usual manner, covered by water in a Petri dish, and the size of the droplets read by means of a travelling microscope. After 18 hours the lengths of the droplets were again recorded. From the figures given below, it is clear that the unknown must have had a concentration approximately equivalent to 0·1 Normal. The molecular complexity of the amorphous toxin is thus established as $C_{27}H_{40}0_9$. Such a substance would have a molecular weight of 508·3, a deci-normal solution containing 25·42 mgm. in 0·5 gm.

* All microanalyses by Dr. Backeberg, University of the Witwatersrand, to whom I wish to express my thanks.

Measurements of Droplets in Determining Molecular Weight of Cucumin by Barger's Method.

	Azobenzoi.	Cucumin.
Azobenzol solution 0·08N........................	0·66	1·19
	0·36	1·35
Difference	− 0·30	+ 0·14
,, ,, 0·101N........................	1·04	0·87
	1·09	0·85
Difference	+ 0·05	− 0·02
,, ,, 0·101N........................	0·79	0·60
	0·88	0·42
Difference	+ 0·09	− 0·18
,, ,, 0·101N........................	1·84	0·09
	1·86	0·09
Difference	+ 0·02	0
,, ,, 0·14N........................	0·34	0·97
	0·46	0·34
Difference	+ 0·12	− 0·63
,, ,, 0·14N........................	1·38	0·94
	1·59	0·56
Difference	+ 0·21	− 0·38

C. *Melting Point.*

Cucumin exhibits no sharp melting point but suffers decomposition between 111° and 115°. There is no charring, but the material foams up owing to the evolution of volatile substances. The loss in weight was found to be constant. All preparations behaved similarly.

D. *Optical Activity.*

This was constant within experimental error for all preparations. Prior to weighing, the material was dried in vacuo in an Abderhalden apparatus over calcium chloride and paraffin wax. Absolute alcohol was used as solvent. The instrument was a triple field Goertz polarimeter carrying 2 dm. tubes. The following are two typical examples:—

Wt. substance 0·2000 gm. from *Cucumis myriocarpus.*
Volume of solution 13 c.c.
Rotation observed + 1·98°.

$$\therefore [\alpha]_D^{26} = \frac{1 \cdot 98 \times 13}{0 \cdot 2 \times 2} = +64 \cdot 35^\circ$$

Wt. substance 0·1000 gm. from *Cucumis leptodermis*
Volume of solution 13 c.c.
Rotation observed + 0·98°

$$\therefore [\alpha]_D^{26} = \frac{0 \cdot 98 \times 13}{0 \cdot 1 \times 2} = +63 \cdot 69^\circ$$

The mean value was $[\alpha]_D^{26} = +64 \cdot 35^\circ$

Cucumin is easily soluble in chloroform, alcohol, acetone and pyridine, less so in ethyl acetate and benzene, soluble with difficulty in ether and only very sparingly soluble in water. Its solutions are

neutral; the action of alkalis is recorded later. It is insoluble in petroleum ether. Both Fehlings solution and ammoniacal silver nitrate are reduced on warming. Potassium permanganate is energetically decolorised. The only distinctive colour reaction which has been observed is that with concentrated sulphuric acid. An immediate and intense deep cherry-red colour is produced which persists indefinitely—the tint becoming gradually more brownish. With none of the alkaloidal reagents tried was any precipitate produced. Cucumin has an intensely bitter taste, still perceptible at a concentration of 1 in 10,000 (aqueous solution) and is highly toxic. In the presence of alkali, both bitter taste and toxicity rapidly disappear.

Physical and Chemical Properties of the Crystalline Toxic Principle, " *Leptodermin* ".

The crystalline toxic material separating out of the chloroform-petroleum ether mother liquors after the precipitation of the amorphous principle was removed by filtration, washed with petroleum ether and dried. In this way the bulky, fluffy agglomerations of crystals were reduced to fine felted masses, somewhat difficult to handle owing to tendency (electrification?) to adhere to glass rods, the walls of vessels, etc. Microscopical examination showed the crystals to be exceedingly fine, long needles (see Fig. 1) which, under lesser magnification were seen to be grouped in silky fan-shaped tufts (see Fig. 2). The material was snow-white in colour and odourless, further resembling cucumin in its intensely bitter taste. Recrystallisation presented some difficulty, but was accomplished by adding petroleum ether to a dilute chloroform solution until precipitation commenced, filtering and allowing the filtrate to stand for a few days in a stoppered vessel. The crystals formed in rosettes of exceedingly fine snow-white needles having the same melting point as the original preparation.

The first few samples which were prepared for micro-analysis were dried in vacuo at room temperature, over paraffin wax and calcium chloride, as in the case of cucumin, but the analytical figures were so irregular as to arouse suspicion that, owing to the peculiar physical structure of the material, traces of the solvents, particularly the higher boiling fractions of the petroleum ether, were being retained. Drying was then conducted by heating in an oven at 105–110° when, immediately, satisfactory results were obtained.

Micro-combustion results afforded the following figures:—

Material	C%	H%
Sample B 10x	66·19	7·75
Sample B 6x	66·11	7·49
The molecular formula $C_{27}H_{38}O_8$ requires	66·12	7·76

The molecular weight was determined by Rast's camphor method

Sample C x	488
Sample B 6 x	486
Sample B 10 x	477
$C_{27}H_{38}O_8$ requires	490

On account of its occurrence, it is proposed to call this crystalline toxic principle " Leptodermin ".

Fig. 1.—Leptodermin crystallised from chloroform-petroleum ether. ×135.

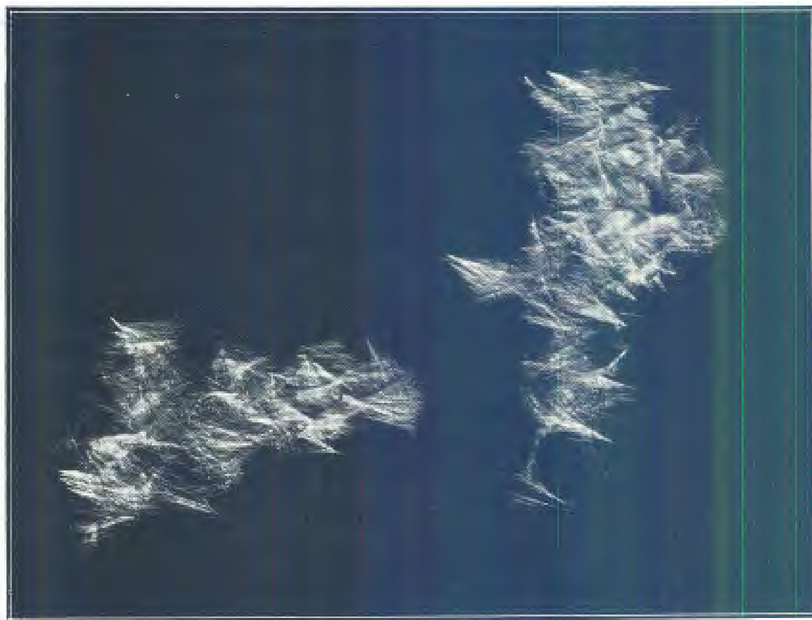

Fig. 2.—Leptodermin crystallised from chloroform-petroleum ether. ×35.

Melting point.

Observed under the Kofler micro-melting point apparatus the crystals were seen to melt sharply at 184°.

Optical Activity.

This was determined, as in the case of Cucumin, in absolute alcohol in a 2 dm. tube.

Wt. of substance 0·0740 gm.
Vol. of solution 13 c.c.
Rotation observed 0·734°

$$\therefore [\alpha]_D^{26} = \frac{+0\cdot734 \times 13}{0\cdot074 \times 2} = +64\cdot46^\circ$$

It is somewhat surprising that the optical activity is the same as that of cucumin. The two substances, notwithstanding the sharp melting point of the one at 184° and the decomposition of the other at 111–115°, are obviously closely related as the following general properties and the comparison table which concludes them testify.

Leptodermin exhibits the same general solubilities as cucumin being, however, very sparingly soluble in petroleum ether. It also reduces warm Fehling's solution and ammoniacal silver nitrate and decolorises potassium permanganate. The same cherry-red colour is produced with sulphuric acid as is given by Cucumin. Alkaloidal reagents produce no percepitate. It is gradually dissolved by alkalis with loss of bitter taste and of toxicity.

COMPARISON OF PROPERTIES OF "CUCUMIN" AND "LEPTODERMIN".

	Cucumin.	Leptodermin.
Structure....................	Amorphous	Crystalline: fine needles.
M.P.........................	Decomposes at 111–115° with constant loss of weight	184°.
$[a]^{26}_{D}$..................	+ 64·35°	+ 64·46°.
Formula....................	$C_{27}H_{40}O_9$	$C_{27}H_{38}O_8$.
Mol. wt......................	508 (Barger's method)	490 (Rast's method).
Fehlings solution............	Reduced on warming	Reduced on warming.
Ammoniacal silver nitrate....	Reduced on warming	Reduced on warming.
Colour reaction with H_2SO_4..	Cherry-red	Cherry-red.

The similarity may be further emphasised by anticipating the findings to be described later, that both substances are trilactones and that they are equally toxic towards fish and towards rabbits (intravenous injection). The action of dilute acid leads to the production of the same substance from each.

The difference of the elements of water, H_2O, between the molecular formulae of cucumin and leptodermin taken together with the close chemical and pharmacological similarity between these substances, would suggest that they probably possess the same essential structure but that in leptodermin closure has occurred, of the lactone or of some other type, between a hydroxyl group and an adjacent hydrogen atom, resulting in the elimination of water.

In these circumstances, if the closure is of the lactone type, a free carboxyl group would be expected in the cucumin molecule and one more lactone ring in leptodermin than in cucumin. That this is not the case was demonstrated as described below. If an internal condensation has occurred, it must involve some other mechanism, as for example the removal of hydrogen and hydroxyl leaving a cyclic structure or a double bond.

An approximately N/30 alcoholic potassium hydroxide solution was made up in 92 per cent. alcohol and then standardised against sulphuric acid. 15 c.c. alc. KOH required 5·32 c.c. H_2SO_4 (0·968N) ∴ Normality 0·03434. 51·6 mgm. of cucumin was dissolved in 15 c.c. alc. KOH, phenol phthalein added and the mixture immediately back titrated by N/10 H_2SO_4. Required = 5·2 c.c.
∴ Neutralised = 0·12 c.c.

There is thus no free carboxyl group in cucumin.

A similar mixture was titrated after standing 24 hours at room temperature:—

H_2SO_4 required = 2·2 c.c.
∴ Neutralised = 3·02 c.c. of N/10.
Theory for 2 lactone groups = 3·05 c.c.

In order to determine whether further lactone groups are opened up upon boiling with alkali, 52·6 mgm. of cucumin was refluxed for 2 hours with 15 c.c. of alc. KOH. and then back titrated.

H_2SO_4 required = 2·25 c.c.
∴ Neutralised = 3·07 c.c. of N/10.
Theory for 3 lactone groups = 3·09 c.c.

After 6 hours boiling and 45 hours at room temperature a figure of 3·02 c.c. was obtained as against the theoreticals 3·05 c.c. for 2 lactone groups. Aqueous sodium hydroxide gradually dissolved cucumin at room temperature, 3 equivalents of alkali being neutralised as determined by back titration. There is thus no evidence for the existence of more than 3 lactone groups in the cucumin molecule.

It was thought to be of interest to determine whether all these equivalents of alkali neutralised were bound by the cucumin or whether, possibly, one equivalent of carbon dioxide was evolved in the course of the reaction. For this purpose the assembly shown in Fig. 3 was used. To the mixture of alcoholic potash and saponified cucumin, 1 c.c. of N/10 sulphuric acid was added in excess of that required to neutralise the potassium hydroxide originally present, the mixture was transferred to the wide-necked flask and the contents distilled under reduced pressure into a Büchner flask containing 5 c.c. of N/10 sodium hydroxide. A slow stream of air, purified by H_2SO_4 and soda-lime, was admitted from the capillary. At the conclusion of the distillation a little CO_2-free water was let in from tbe funnel to carry over the last traces of any volatile acids present. The contents of the Büchner flask was then back titrated in the usual way using phenol phthalein and deci-normal sulphuric acid. After recording the titration figure, a known excess of acid was added and a stream of CO_2-free air bubbled through the solution for some hours to remove any CO_2 present. Sodium hydroxide solution was then run in until neutrality was reached. The reliability of the method and apparatus was checked by distilling dilute acetic acid.

Fig. 3.—Apparatus for determination of volatile acid in alkaline hydrolysis of cucumin.

15 c.c. alc. KOH + 2 c.c. of approx, N/10 acetic acid + 6·5 c.c. of N/10 H_2SO_4 distilled into 5 c.c. of N/10 NaOH.

Neutralised by the acetic acid 2·06 c.c.

Theory 2·15 c.c.

50·8 mgm. cucumin + 15 c.c. alc. KOH left overnight and then distilled

NaOH (0·09523N) 5 c.c.
Acid (1·0190N) back 3·7 c.c.
Neutralised 1·25 c.c. of N/10
Due to CO_2 0·37 c.c. of N/10
∴ Derived from cucumin 0·87 c.c.
Theory for 1 equivalent of volatile acid 1·00 c.c.

In a second experiment 50·8 mgm. cucumin were refluxed for 2 hours with 15 c.c. of alc. KOH and the determination then carried out as above.

Alkali neutralised by volatile acid	1·40 c.c.
Due to CO_2	0·50 c.c.
∴ Derived from cucumin	0·90 c.c.
Theory for 1 equivalent	1·00 c.c.

An attempt was made to titrate the residue left in the reaction flask, but low values were obtained indicating either that relactonisation of this portion of the molecule had, in part, taken place, or that further decomposition had occurred.

The residue was tested for toxicity in the following manner: 10 mgm. cucumin plus 3 c.c. alc. KOH were left overnight at room temperature, the equivalent quantity of acid necessary to neutralise the KOH added was then introduced and the resulting solution of the de-lactonised toxin injected intravenously into a 1½ kilogram rabbit. No toxic symptoms were produced.

Although mild acid hydrolysis of cucumin causes some alteration in the structure of the molecule it would appear that this is not of very drastic nature, since the resultant product maintains to the full the toxicity of the original cucumin. Precisely the same is true of leptodermin. 50·8 mgm. cucumin + 10 c.c. of 96 per cent. alcohol + 5 c.c. of N/10 H_2SO_4 were refluxed for 6 hours. Back titration with N/10 NaOH required 5·22 c.c. of alkali indicating no more than a very slight possible development of acid groups in the toxin molecule. After evaporation of most of the alcohol, the solution was shaken with chloroform and the extracted product precipitated by addition of petroleum ether. Recovered 43 mgm. of a white amorphous substance in all respects resembling cucumin.

10 mgm. injected intravenously into a 1¾ kilogram rabbit killed the animal in 1½ hours with the typical symptoms of cucumis poisoning.

Micro-analysis gave: C 65·46; H 8·15. Molecular weight 477 (Rast's method).

It had M.P. 136–137°.

It is of interest to note, parenthetically, that the residue left after heating cucumin to a temperature of 120° also retains, undiminished the toxic properties of the original substance and is very similar in composition to that produced by the mild action of acids.

Effect of Alkali upon Leptodermin.

Experiments were conducted with the same apparatus as was used for cucumin and in the same manner. It was found that, as in the case of cucumin, three equivalents of alkali are neutralised by leptodermin, and that one equivalent of the acid so formed is volatile but is not carbon dioxide. The precise nature of this constituent has not as yet been determined. 49 mgm. of leptodermin dissolved in 15 c.c. of alc. KOH were left in a thermostat at 37°.

The amount of alkali neutralised was 2·92 c.c. N/10
Theory for three equivalents 3·00 c.c.
On distillation 1·05 c.c. of N/10 alkali were neutralised of which 0·20 c.c. of N/10 alkali were due to CO_2
∴ Derived from leptodermin 0·75 c.c. of N/10 alkali
Theory for one equivalent 1·00.

The difficulty must be emphasised of obtaining absolutely quantitative results when working with such small quantities in a relatively large apparatus.

Effect of Alkali upon the Residue obtained by Heating at 120°.

Once again it was found that three equivalents of alkali were neutralised and one equivalent of volatile acid produced. 46·5 mgm. of the residue, obtained by heating cucumin at 115–120° until no further loss in weight occurred, was dissolved in 15 c.c. alc. KOH and the solution left for 2 days in a thermostat at 37°. It was then titrated to phenol phthalein by deci-normal sulphuric acid.

H_2SO_4 required = 2·38 c.c.
∴ Neutralised = 2·94 c.c.
Theory for 3 lactone groups = 3·0 c.c.

After distillation of the mixture in the usual way,
1·0 c.c. of N/10 alkali were neutralised of which
0·1 c.c. of N/10 alkali was due to CO_2
∴ Derived from the substance 0·90 c.c. of N/10 alkali
Theory for 1 equivalent 1·00 c.c.

Leptodermin when refluxed with dilute sulphuric acid in a manner similar to that described in the case of cucumin, yielded a toxic product apparently identical with that afforded by cucumin.

	C.	H.	M.P.
Substance derived from Cucumin ...	65·46	8·15	136–137°
Substance derived from Leptodermin	65·44	8·20	137–138°

Micro-methoxyl determinations showed that the CH_3O group is not present in either cucumin or leptodermin.

Attempts to demonstrate the presence of hydroxyl groups in these substances by methylation, using diazomethane and ether-chloroform solutions of cucumin and leptodermin were unsatisfactory.

No methoxyl groups were introduced. When, however phenylisocyanate was employed, phenylurethanes were formed.

It is noteworthy, however, that urethane formation does not take place readily, and it may be recalled that the methylation of the hydroxyl group of tubaic acid, a decomposition product of rotenone, is only accomplished with great difficulty (Takei 1928, 1929) whilst according to Clark (1931) tephrosin, though containing an hydroxyl group, gives extremely poor yields or fails entirely to form acyl or alkyl derivatives. The action of acetic anhydride upon cucumin did not lead to the production of a simple acetyl derivative but to another substance which is being further investigated.

Certain considerations demand that the possibility should be entertained that phenylisocyanate reacts with Cucumin and Leptodermin by opening up those same linkages which are attacked by alkali and further suggest that the salt-forming groups are phenolic in character and not carboxylic. Thus, should a simple lactone structure be assumed in addition to three independently situated hydroxyls, the sum of the oxygen atoms in the molecule, including one which is ketonic, would be equal to 10. Cucumin contains 9 oxygen atoms and Leptodermin only 8, so that such an interpretation is clearly impossible. It is preferable to regard the hydroxyl groups forming phenylurethanes as identical with those, three in each molecule, combining with alkali and to conclude that the oxygen atoms unaccounted for are, in all probability, part of cyclic systems.

The Demonstration of Hydroxyl Groups in "Cucumin" and "Leptodermin" by means of Phenylisocyanate.

In a preliminary experiment, 50 mgm. of cucumin in 2 c.c. of chloroform was treated with approximately 0·1 c.c. phenylisocyanate and 1 c.c. of petroleum ether added. The mixture was warmed and set aside in a closed vessel for 48 hours. Excess of pertoleum was then added and the product centrifuged off, washed well with petroleum ether, and dried. Analysis showed that it contained 1·5 per cent. N. It possessed a slightly bitter taste and had not a sharp melting point. In order to bring the reaction to completion, the following procedure was finally adopted. To 50 mgm. of cucumin in 5 c.c. of dry chloroform was added 1 c.c. of phenylisocyanate and the mixture refluxed for 4-5 hours, then left to stand for 48 hours. Excess of petroleum ether was added after any diphenylurea had been filtered off and the product thoroughly washed with petroleum ether. The resulting substance was devoid of bitter taste, and melted at 158–160°.

Micro-analysis

	C	H	N
Found	67·71	6·49	4·74
$C_{48}H_{55}O_{12}N_3$ (for 3 OH groups) requires	66·59	6·41	4·85

Leptodermin similarly treated yielded a product which had M.P. 161–163°

Micro-analysis

	C	H	N
Found	68·01	6·33	5·67
$C_{48}H_{55}O_{11}N_3$ (for 3 OH groups) requires	67·53	6·22	5·63

It would thus appear that both cucumin and leptodermin contain 3 hydroxyl groups.

The Demonstration of Ketonic Groups in Cucumin and Leptodermin.

Both Cucumin and Leptodermin react readily with Brady's reagent at room temperature yielding 2:4 dinitrophenylhydrazones. Since aldehydic reactions are not given by either substance the oxygen function must be ketonic.

The derivatives were prepared as follows: 50 mgm. of either Cucumin or Leptodermin were dissolved in 1 c.c. of absolute alcohol, 4 c.c. of water rapidly added and then 1 c.c. of concentrated hydrochloric acid affording a perfectly clear solution about 2N in acid. To this solution was added 3 c.c. of a warm solution containing 0·5 gm. of 2:4 dinitrophenylhydrazine dissolved by the aid of heat in 30 c.c. of 2N hydrochloric acid. The mixture was rotated for about a minute. Precipitation commenced almost immediately and the derivative soon assumed an easily centrifugeable flocculent form. It was washed on the centrifuge, firstly with 2N hydrochloric acid and then repeatedly with water, and finally crystallised from the minimal quantity of hot 60 per cent. alcohol. The cucumin derivative did not crystallise well; it formed an orange powder with M.P. 211–2°Leptodermin 2:4 dinitrophenylhydrazone was obtained in orange-coloured spear-shaped, flat prisms with M.P. 225°.

Micro-analysis

	C	H	N
Cucumin 2:4 dinitrophenylhydrazone ...	57·24	6·61	7·74
$C_{33}H_{44}O_{12}N_4$ requires	57·51	6·45	8·13
Leptodermin 2:4 dinitrophenylhydrazone	59·26	7·02	7·80
$C_{33}H_{42}O_{11}N_4$ requires	59·07	6·31	8·35

The results demonstrate that cucumin and leptodermin each contain one ketonic oxygen atom.

Examination of Cucumin for the presence of an isopropyl side-chain. Isolation of a volatile oxidation product as the 2:4 dinitrophenylhydrazone.

Several bitter principles contain an isopropyl side-chain which, under suitable conditions, may be removed by oxidation and transformed into acetone. An attempt was made to carry out such a degradation of Cucumin but although a volitile substance reacting with Brady's reagent was obtained, this proved not to be acetone. By varying the conditions somewhat, a second reaction product was obtained in better yield.

The exact light which these observations throw upon constitution of Cucumin it is at present somewhat difficult to assess. In the preliminary experiment, 1 gm. of cucumin dissolved in 5 c.c. of glacial acetic acid was placed in a kjeldahl microdistillation flask and a brisk current of steam passed whilst chromic acid-acetic acid mixture was admitted drop by drop in such a manner that excess of oxidising agent at any time was carefully avoided. Reduction of the chromium was at first rapid, but subsequently slowed down considerably whilst a tarry material separated in the flask. The whole oxidation extended over nearly two hours. The distillate was

collected in an ice-cooled receiver. At the conclusion of the experiment, it was neutralised by sodium hydroxide and again steam distilled the first and second 20 c.c. portions being separately collected. The greater part of the volatile material was contained in the first portion. This had a peculiar smell reminiscent of cucumber but rather more pungent and with a suggestion of rancidity.

To the liquid was added 4 c.c. of hydrochloric acid and then an excess of warm Brady's reagent, 0·5 gm. 2:4 dinitrophenylhydrazine dissolved in 30 c.c. of 2N hydrochloric acid. Immediate precipitation took place. The derivative was centrifuged and washed firstly with 2N hydrochloric acid and then with water. After repeated recrystallisation from boiling 60 per cent. alcohol, it was obtained in the form of tangerine-red, elongated flat plates with M.P. 166–9°. The yield was only 3·2 mgm.

Microanalysis:

	C	H	N
Found	51·38	5·65	21·41
$C_{12}H_{16}O_4N_4$ requires	51·42	5·71	20·00

In an endeavour to increase the yield, the conditions were altered, 1 gm. of Cucumin in 5 c.c. of acetic acid being refluxed, whilst chromic acid oxidation mixture was dropped in as before from the top of the vertical condenser. The oxidation lasted 5 hours. After steam distilling, neutralising the distillate and again distilling the solution was treated as before with Brady's reagent. A copious precipitate formed which was finally obtained from 60 per cent. alcohol with constant M.P. 123–4°. The form of the crystals varies somewhat according to the speed, etc., of crystallisation, being either long, narrow, needle-like prisms or more plate-like rectangular flat prisms. The colour was orange-yellow. The yield was 0·1742 gm. Mixed M.P. with Acetone 2:4 dinitrophenyl hydrazine 123–4°.

Microanalysis:

	C	H	N
Found	46·46	4·55	23·05
$C_9H_{10}O_4N_4$ requires	45·37	4·20	23·53

An isopropyl group is therefore present. The substance first obtained was probably either

$$\begin{matrix}CH_3\\CH_3\end{matrix}{>}CH{\cdot}CH_2{\cdot}CH_2{\cdot}CHO \text{ or } \begin{matrix}CH_3\\CH_3\end{matrix}{>}C{=}C{\cdot}CH_2{\cdot}GHO.$$

suggestive of a side-chain similar to that present in alpha-camphorene and santalene.

Degradation of Cucumin by Alkaline Fusion.

As noted previously the action of dilute sodium hydroxide upon cucumin is to open up three linkages capable of being titrated by alkali. When boiling 40 per cent. sodium hydroxide was employed much more deep seated changes occurred, a variety of phenolic and phenolic-acidic substances being produced, but no definite degradation product could be isolated in any quantity.

An attempt was therefore made to bring about decomposition by a very drastic means in the hope that the molecule would be resolved into relatively simple substances.

1·25 gm. cucumin was mixed with 10 gm. potassium hydroxide and a little water in a nickel crucible. The temperature was raised gradually to 250° at which point it was maintained for 2 hours. After cooling, the melt was treated with water affording a brown solution with a cresol-like odour and giving a strongly positive reaction with diazobenzenesulphonic acid. The reaction mixture was made acid by hydrochloric acid, excess of potassium carbonate added, and the mixture then extracted by ether in a continuous extractor. The ether extract was shaken with 1 per cent. sodium hydroxide solution which removed practically all the colour (solution B). The residual ether after washing was dehydrated and evaporated leaving a small quantity of micro-crystalline material. This was dissolved in 96 per cent. alcohol, discolorised with a little absorbent charcoal and crystallised. The prisms so obtained were dried on a porous tile, washed well with water and again crystallised from ether, yielding a crop of long, fine, colourless prisms (see Fig. 4). This material gave a brown-red coloration with sulphuric acid, but no diazo reaction or colouration with ferric chloride. It was sparingly soluble in ethyl acetate from which is was recrystallised M.P. 218°.

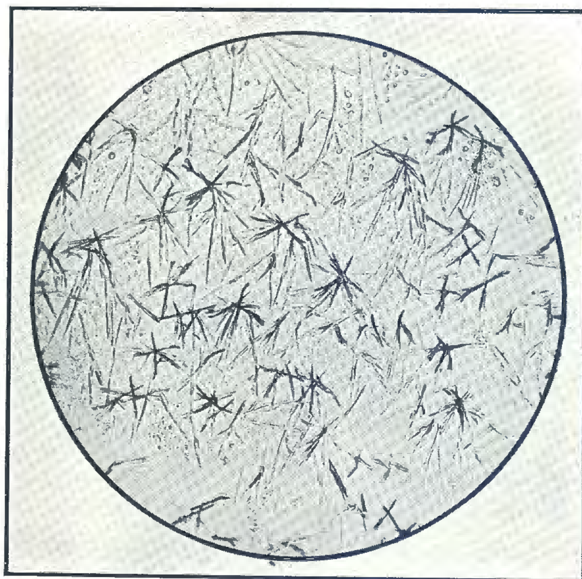

Fig. 4.—Substance M.P. 218° from KOH melt of cucumin crystallised from ethyl acetate. ×140.

The material extracted by the 1 per cent. sodium hydroxide solution (solution B) was transferred to ether by acidifying and shaking with the solvent. This ethereal solution when dried and concentrated left a dark-brown residue which was taken up in 96 per cent. alcohol and boiled with charcoal. The pale yellow solution was slightly acid to litmus. It was evaporated to dryness and the residue exhausted with boiling petroleum ether, ethyl acetate and cold glacial acetic acid in turn.

Varnish-like materials were obtained in each case from which nothing definite could be isolated.

The main solution of the melt was now acidified and exhaustively extracted by ether. On evaporating the solvent, a dark tarry residue was obtained from which boiling petroleum ether (B.P. 40°) extracted a small quantity of crystalline material. This was recrystallised from hot ethyl acetate and obtained in the form of tufts of fine, prismatic rods. M.P. 63°. No other crystalline degradation product could be isolated.

The oxidation of cucumin in acetone solution by potassium permanganate was also carried out, but no substance capable of throwing light upon the constitution of the material could be obtained. Similarly alkaline reduction by means of hot sodium hydroxide and zinc dust led only to complex mixtures from which no crystalline material could readily be obtained. In view of the difficulty encountered in these preliminary experiments, it is proposed to defer work upon chemical constitutional lines until at some later date a more favourable opportunity presents itself.

Toxicity Tests upon Fish.

The majority of the substances used as fish poisons by native peoples are complex lactones belonging to the general class of "bitter principles". It seemed likely that cucumin and leptodermin might behave in the same way and, as the method of determining toxicity by the use of fish is capable of giving very exact results with the use of only small quantities of material, comparative toxicity tests were carried out as described below. A quantitative comparison of the toxicity of the two cucumis principles was of especial interest in view of the many chemical resemblances between them. Their general similarity was found to extend also as far as their pharmacological action, since no appreciable difference could be found between the limiting toxic concentration necessary to kill fish either of cucumin, leptodermin or of the substance derived from cucumin by the action of heat.

A variety of carp, *Tilatia starrmani*, Smith* obtainable locally was employed, the tests being carried out in a manner similar to that described by Gersdorff (1930), the fish chosen being as nearly uniform in size as possible.

Since cucumin, etc., are only soluble with difficulty in water, the requisite quantity of material was first of all dissolved in 96 per cent. alcohol and the solution added drop by drop to the proper volume of water whilst shaking vigorously. A short period on the shaking machine served to complete solution. The lower concentrations were prepared by dilution from the higher. Control experiments showed that the highest quantity of alcohol used (0·15 to 0·2 per cent.) was without effect in 48 hours.

Surprisingly constant values were obtained for the survival time considering the chances of individual variation inherent in biological material. From the results the three curves (Figs. 5, 6 and 7) were constructed from which it is apparent that all three

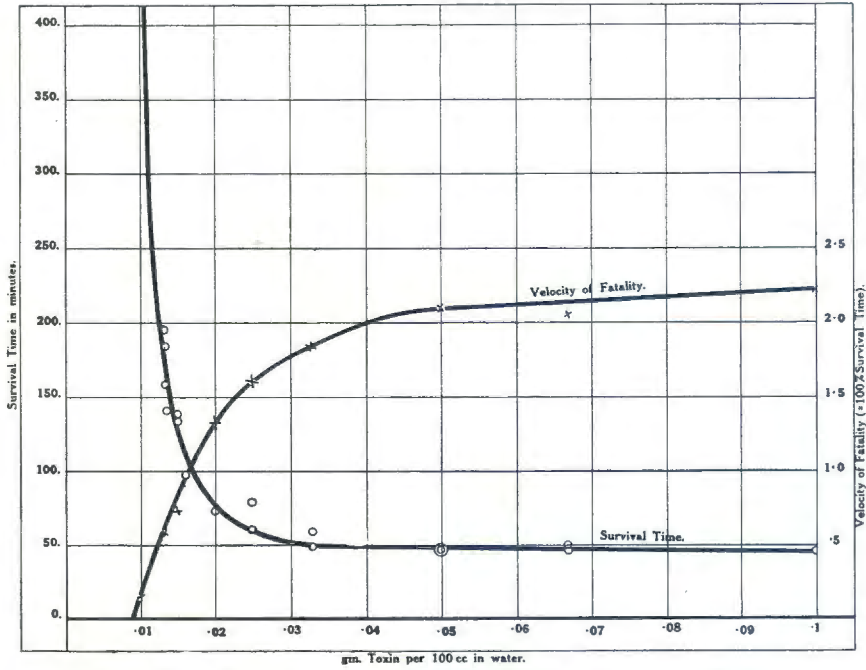

Fig. 5.—Toxicity of cucumin towards fish (*Tilatia starrmani*, Smith). Temperature 24° C.

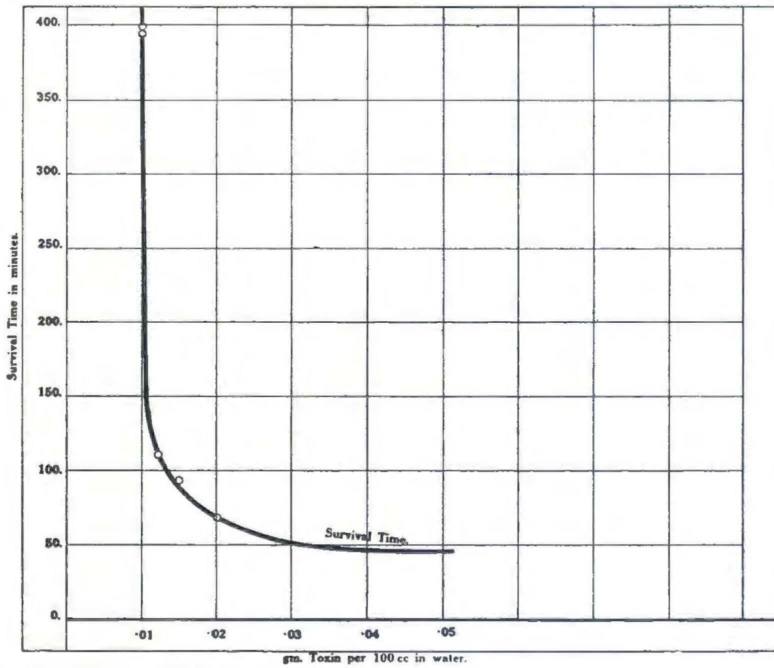

Fig. 6.—Toxicity of Leptodermin towards fish. Temperature 25° C.

materials are practically equally toxic towards fish under the conditions of the experiment. The residue obtained by heating cucumin is fatal in rather lower concentration than are the other two, but 100 grams of this material represents approximately 110 grams of the original cucumin.

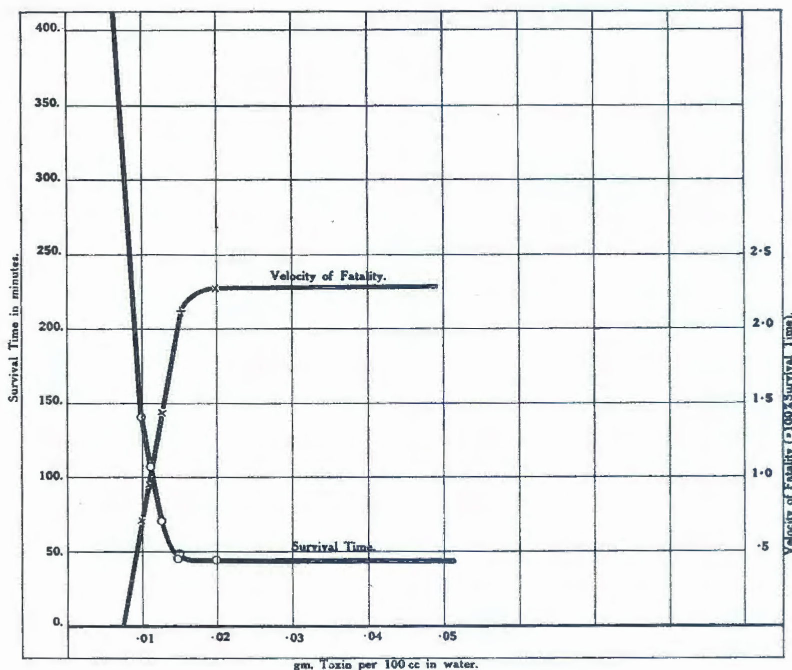

Fig. 7.—Toxicity of heat residue of cucumin towards fish.
Temperature 24° C.

The same symptoms of poisoning were observed in all cases; they were as follows. The fish commenced to swim energetically round the vessel making occasional desperate attempts to jump from the solution. Its movements then became feeble, and equilibration uncertain; later it sank to the bottom, resting there on its side whilst the gill movements were seen to be slow and laboured. Sudden darting movements were noticed at intervals, the creature swimming upside down and attempting in spasmodic rushes to reach the surface of the solution. At length, gill movements ceased altogether and the fish appeared to be dead. Upon lifting it with forceps this was sometimes found not to be the case. The procedure recommended by Gersdorff of immersing the fish in diluted hydrochloric acid in order to be certain of the death point was found to be a useful criterion.

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* I am indebted to Mr. Fitzsimmonds of the Transvaal Museum for the identification of this specimen.

Toxicity of Cucumin to Fish (Temp 24°).

Concentration.	Length of fish.	Weight of fish.	Survival time.	Velocity of fatality	$= \frac{100}{\text{survival time}}$
%.	Mm.	Gm.	Minutes.		Mean.
0·10...........	80	9·0	45	2·22	2·22
0·067..........	66	4·60	47	2·13	—
	73	5·67	51	1·96	2·05
0·05...........	65	4·12	47	2·13	—
	68	4·72	48	2·11	2·11
0·033..........	68	5·37	50	2·00	—
	70	5·78	60	1·67	1·84
0·025..........	69	5·6	61	1·64	—
	73	5·98	80	1·25	1·44
0·02...........	72	5·8	73	1·37	—
	69	4·78	77	1·30	1·34
0·016..........	72	6·16	98	—	—
0·015..........	76	6·98	135	0·741	—
	81	8·66	140	0·728	0·735
0·0133.........	67	5·41	142	0·704	—
	76	6·84	185	0·541	—
	71	6·99	160	0·625	—
	79	8·9	196	0·510	0·595
0·01...........	68	5·7	600	0·167	0·167

Toxicity of Leptodermin to Fish (Temp. 24°).

Concentration.	Length of fish.	Weight of fish.	Survival time.	Velocity of fatality	$= \frac{100}{\text{survival time}}$
%.	Mm.	Gm.	Minutes.		Mean.
0·02...........	89	10·3	71	—	—
0·015..........	88	10·25	96	—	—
0·012..........	87	10·0	113	—	—
0·010..........	90	13·41	404	—	—

Toxicity of Residue from Heating Cucumin to 115–120°
To Fish (Temp. 24–25°).

Concentration.	Length of fish.	Weight of fish.	Survival time.	Velocity of fatality	$= \frac{100}{\text{survival time}}$
%.	Mm.	Gm.	Minutes.		Mean.
0*120..........	82	8·82	44	2·27	2·27
0·015..........	86	10·57	46	2·17	—
	72	5·47	48	2·08	2·13
0·0129.........	96	13·62	70	1·43	1·43
0·0113.........	79	8·37	106	0·943	0·943
0·01...........	84	11·28	141	0·709	0·709
0·0067.........	81	10·27	508	0·197	0·197

Toxicity to Rabbits and Guinea Pigs.

The effects of the toxic preparations from *Cucumis myriocarpus* and *africanus* upon sheep, rabbits and guinea pigs have been fairly fully described by Quin (1928). The pharmacological action of the pure principles cucumin and leptodermin appears to differ but little from those recorded.

The most prompt effect is obtained by the intravenous route, the M.L.D. for the rabbit lying between 1 and 2 mgm. per Kilo, body-weight in the case of both cucumin and leptodermin. Death occurs rather suddenly 1 to 2 hours after dosing and the post-mortem findings indicate an effusion of fluid into the lungs and large intestine which is more pronounced the longer the duration of the pre-mortal agony. Pronounced injection of the mesenteric vessels and inflammation of the mucous membrane of the intestines is also observed. The effects upon guinea pigs are similar.

When administered orally by stomach tube, a much larger quantity (approximately 25 mg. per kilo for the rabbit) of the poison is required to produce death, possibly, in part, owing to its insoluble nature. The inflammation of the gastric and intestinal mucosa is more pronounced, otherwise the post-mortem picture is the same as that after intravenous injection.

The main results are presented below, the individual protocols being condensed as much as possible.

Toxicity of Cucumin and Leptodermin to Guinea Pigs (Subcutaneous).

Pig No. 1 weighing 500 gm. received 10 mgm. cucumin dissolved in dilute alcohol subcutaneously at 3.30 p.m. At 4.30 p.m. slightly apnoeic, weak, with occasional jerky tremors. Dead when next seen at 8 p.m. Post-mortem revealed hyperaemia of the subcutaneous vessels. The heart was arrested in diastole and the thoracic cacity contained some clear watery exudate. The mesenteric vessels were intensely hyperaemic, the stomach hyperaemic and of a mahogany red colour. Hyperaemia and oedema of the lungs.

Pig No. 2 weighing 460 gm. received 10 mg. of leptodermin dissolved in dilute alcohol, subcutaneously at 3.35 p.m. After about one hour the animal became incordinated and helpless with laboured respiration and occasional jerky tremors. Death ensued 1½ hours after injection. The post-mortem findings were similar to those quoted in the case of pig No. 1. A control pig No. 3 which received the same quantity of dilute alcohol as was used in the above experiments showed no symptoms.

Toxicity to Rabbits and Determination of M.L.D.

In order to test the toxicity of cucumin to the rabbit, a large animal was given 10 mgm. of cucumin dissolved in dilute alcohol by injection into the ear vein at 12.20 p.m. Symptoms developed as follows: 1.10 p.m. very restless in cage, head jerked upwards at intervals, respiration laboured. 1.20 p.m. above condition more pronounced, head falling to one side; acute dyspnoea. 1.22 p.m. two slight convulsions, very cyanotic, prostrate. 1.24 p.m. series of convulsions; irregular gasping. 1.26 p.m. tetanic spasm followed by death, 1 hours and six minutes after injection .

Post-mortem findings: cyanosis, slight hydrothorax, heart in diastole and enlarged. Lungs purplish in colour, hyperaemic, marked emphysema; bronchi full of frothy material. Subcutaneous and mesenteric vessels very hyperaemic, dark purplish in colour.

Stomach hyperaemic. Another animal which received 10 mgm. of leptodermin in the same manner exhibited almost identical symptoms, dying 1 hour and 4 minutes after injection. The post-mortem revealed cyanosis, dilation of the heart and coronary vessels, hyperaemia of the lungs, but no froth in the bronchi. The stomach and small intestines were hyperaemic, the latter containing a fair quantity of light yellow mucous material. Controls, which received the same quantity of alcohol only, developed no symptoms.

The above effects are typical of many experiments with cucumin and leptodermin.

In order to determine the M.L.D. for rabbits by intravenous injection, a series of rabbits were given doses of cucumin ranging from 1 to 5 mgm. per kilo body-weight in the manner already described. The M.L.D. was found to be $\pm$ 2 mgm. per kilo body-weight, although in one exceptional case even so little as 1 mgm. per kilo proved fatal within 7 hours.

The M.L.D., when the poison was administered per os, was found to be considerably higher, namely, $\pm$ 25 mgm. per kilo body-weight. The material was administered in solution in dilute alcohol by means of a stomach tube. The symptoms were somewhat similar to those following intravenous injection, restlessness and respiratory distress being almost invariably accompanied, however, by a more or less severe soft diarrhoea. Post-mortem, more pronounced catarrhal inflammation of the mucous membranes of the stomach and small intestine was observed.

The lesser effectiveness of the poison when taken in by the mouth is probably to be ascribed to the much slower rate of absorption consequent upon its insolubility.

Feeding tests with fresh *Cucumis* fruits from the Laboratory Poison Garden showed clearly that the juice is more toxic when given in this way than when the equivalent quantity of pure cucumin is drenched. Sublethal doses invariably produced diarrhoea.

Toxicity of Cucumin after Refluxing with Acid.

10 mgm. of the preparation described earlier in this paper were injected intravenously in dilute alcohol into a 1,700 gm. rabbit at 12.30 p.m. At 2 p.m. the animal was prostrate and dyspnoeic. A series of convulsions, followed by gasping and death, occurred at 2.5 p.m. The post-mortem picture was typical of cucumin poisoning.

Toxicity of Cucumin after Heating at 120° until Constant in Weight.

10 mgm. of this material was given intravenously to a 2,700 gm. rabbit at 12.30 p.m. At 1.30 p.m. prostrate and breathing heavily. Gradually increasing respiratory distress. Head bent to one side, pupils dilated. Gasping. Died at 2 p.m. The post-mortem findings were again typical of cucumin poisoning.

Testing of Cucumin for Anthelmintic Properties.

Since cucumin shows some resemblance to the lactone santonin, well known for its anthelmintic properties, it was thought to be of interest to try the effect of the administration of cucumin to sheep infested with helminthic parasites. For kindness in carrying out these tests I am much indebted to Dr. J. R. Ortlepp of this Institute. His report may be summarised as follows:—

A 0·1 per cent. aqueous solution of cucumin was found to have little or no effect *in vitro* upon the larvae of *Oesophagostomum columbianum, Haemonchus contortus*, and *Trichostrongylus* spp., the movements of the larvae merely becoming temporary suppressed. Sheep infested with the above parasites or with *Strongyloides papillosus* were dosed orally with quantities of from 0·5 to 1 gm. of cucumin. No ill-effects were observed, neither was any anthelmintic action evinced in any case.

GENERAL CONCLUSIONS.

From the description which has been given of Cucumin and Leptodermin, it is clear that these substances must be constitutionally closely related. They may be included in the class of neutral " bitter principles ".

Although certain chemical evidence is suggestive of the presence of lactone structures in their molecules, it is felt that the alternative explanation should not be excluded that the degradation products evincing acid characteristics are complex phenols rather than carboxylic acids. A similar interpretation of experimental findings was eventually adopted, it will be remembered, in the case or rotenone, the bitter principle of derris root. In the present instance, it has been shown that both cucumin and leptodermin form phenylurethanes although the union with phenylisocyanate does not take place very readily. On the basis of analytical data it may be concluded that 3 hydroxyl groups are present in each. These hydroxyl groups cannot be methylated by diazomethane and an analogy may be drawn in this connexion with certain other phenolic bitter principles. Attempted acetylation by means of acetic anhydride in pyridine solution does not lead to a triacetyl derivative, but to further more deep-seated structural changes.

Both cucumin and leptodermin contain one ketonic oxygen atom.

Gentle heating of cucumin or leptodermin with dilute acid does not destroy the toxicity of these substances but some chemical change does occur since the end products in the two cases are identical—a substance very similar to cucumin but having a molecular weight of 477. The action of heat on cucumin may lead to the same decomposition, but the identity of the product formed under these conditions has not been, as yet, established with certainty.

The action of dilute alkali very rapidly leads to the disappearance of both bitter taste and toxicity from cucumin and leptodermin. Heating at 110–120°, at which temperature cucumin suffers a constant loss of weight, in no way impairs its toxicity.

When cucumin is oxidised under certain conditions with chromic acid, a volatile substance is formed which gives rise to a well defined crystalline 2:4 dinitrophenylhydrazone. More vigorous oxidation yields acetone. An isopropyl group is therefore present in a side-chain to the molecule.

The complete elucidation of the chemical structures of cucumin and leptodermin will necessitate an extended investigation but this, it is hoped to undertake at some time in the future.

SUMMARY.

The active principles of *Cucumis africanus*, *Cucumis myriocarpus* and *Cucumis leptodermis* have been isolated and examined chemically.

In the case of *Cucumis africanus* and *Cucumis myriocarpus* only one poisonous substance was found to which the name " cucumin " has been given since the same substance has been found, so far, in all species of the genus that have been examined.

Cucumis leptodermis was found to contain in addition to cucumin a second crystalline toxic principle to which the name " *leptodermin* " has been given.

Cucumin and leptodermin are chemically very similar. Although differing widely in melting point (cucumin suffers decomposition with constant loss of weight at 110–120° whilst leptodermin melts at 184°) they possess practically the same optical rotatory power, each contain 3 hydroxyl groups and one ketonic group and yield the same product when boiled with dilute acid. They suffer similar decomposition under the action of dilute alkali, three equivalents of alkali being neutralised per molecule. Cucumin and leptodermin are equally toxic towards fish and to rabbits (intravenous route). The M.L.D. in the latter case is ± 2 mgm. per kilo body-weight.

The formula for cucumin has been established as $C_{27}H_{40}O_9$ and for leptodermin $C_{27}H_{38}O_8$. They thus differ by H_2O, the elements of water. Preliminary chemical investigations designed to throw light upon the structure of these molecules, shows that cucumin and leptodermin yield complex phenolic substances under suitable conditions. It is considered likely that certain reactions suggesting a lactone structure can be as satisfactorily explained on the assumption that the acid properties evinced are due to these phenolic groups.

Cucumin is devoid of anthelmintic properties.

ACKNOWLEDGMENTS.

I wish to thank Dr. D. G. Steyn for his continued interest in this work and for superintending the cultivation of the plants raised in the Onderstepoort Poison Garden. My thanks are also due to those who have assisted me in securing experimental material, namely, the Principal Botanist, Division of Plant Industry, Mr. R. du Toit, and Dr. J. G. Bekker.

I am also grateful to Professor M. Rindl of Grey University College for certain unpublished notes relating to *Cucumis africanus* and *Cucumis myriocarpus* and to Dr. R. J. Ortlepp for testing cucumin for anthelmintic properties.

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