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# *Psilocaulon absimile* N.E.Br. as a Stock Poison.

## II. Isolation of the Toxic Alkaloidal Constituent and its Identification as Piperidine Hydrochloride.

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*Common Names:* Asbos, loogbos.

*Origin:* Prieska.

In a recent issue of this Journal, Rimington and Steyn (1932) described preliminary investigations into the toxic principles present in the plant *Psilocaulon absimile* N.E.Br. Two different principles were detected, an organic acid and an alkaloid, both capable of causing death when administered in sufficient amount. The acid was identified as oxalic acid which is present in the plant to the extent of 8·6 per cent. of the dry weight and in the paper referred to a complete analysis was recorded of the organic acid fraction and of the ash.

In the present communication, the isolation is described of the poisonous alkaloid and its identification as *piperidine*, the base being present in the plant largely as the hydrochloride.

*Psilocaulon absimile* is a member of the family Aizoaceae. It is a succulent plant somewhat resembling the *Mesembryanthema* (Fig. 1) and enjoys a wide distribution in the drier parts of the Union being found in the Willowmore District and northwards throughout the karroid areas to Prieska. The writer has seen it growing abundantly in the Victoria West-Loxton District where during the war 1914-18 it was gathered and burnt for the sake of its alkaline ash which was disposed of commercially. As previously recorded, the plant is not a frequent cause of death to stock but losses due to its ingestion have been known to occur (Steyn, 1931).

## Isolation of the Toxic Alkaloid.

Preliminary experiments indicated that the alkaloid was readily extracted by water or by chloroform but was not soluble in ether. A method for its isolation was worked out as follows:—

The fresh plant material was partially dried in the sun and then in a warm oven until friable. It was then ground to a fine powder. The M.L.D. of this powder was about 33 gm. when drenched to rabbits weighing about 2 Kg. The symptoms of intoxication were laboured respiration, accelerated heart beat, salivation and symptoms of paralysis. Death appeared to be due to asphyxia. When sub-lethal doses were administered, the animals became drowsy and paralysed, with greatly diminished respiration but recovered completely without ill-effects.

100 gm. of the plant powder was extracted in a Soxhlet extractor with chloroform for 4 to 5 hours, the solvent was then evaporated off and the dark waxy residue extracted repeatedly with warm water until the iodine reaction was negative. The waxy residue was soluble in benzene and, by fractionation, a substance melting at 110° and crystallising in jagged plates was obtained (Fig. 2). Being non-toxic, it was not further investigated. The watery extract was evaporated to dryness on the water bath and taken up in chloroform, rejecting any insoluble material. The chloroform solution which was coloured light brown was concentrated until masses of crystals began to separate. The dark mother liquid, still containing much alkaloid, was drained away and added to a subsequent batch or worked up separately. The light yellow crystals (Fig. 3) were dissolved in water, decolorised by boiling with charcoal, the solution evaporated to dryness and again taken up in chloroform. This solution was poured into about 5 volumes of dry ether or, preferably, petroleum ether (B.P. 40°) when the toxic substance was precipitated in micro-crystalline condition (Fig. 4). With petroleum ether, the crystals are smaller but are more easily dried in vacuo (over paraffin wax and sulphuric acid or calcium chloride) since even in the pure condition they are somewhat hygroscopic. The material so obtained proved to be the hydrochloride of the alkaloid, whence it may be deduced that a part at least is present in the plant in this form. Considerable difficulty was experienced in removing all coloured impurities, but it was found that these substances could be removed by washing repeatedly with pyridine, in which the hydrochloride of the base is only sparingly soluble, followed by precipitation from chloroform-petroleum ether and thorough washing with the latter solvent. As so obtained, it formed a snow-white micro-crystalline powder the crystals being short rectangular prisms.

## Properties of Alkaloidal Hydrochloride.

Slightly hygroscopic. Very soluble in water, alcohol and chloroform. Insoluble in ether and petroleum ether, very sparingly soluble in hot benzene. When heated it sublimes very readily making determination of melting point difficult; melts * at 237°. Optically inactive.

* All melting points recorded on Kofler electrically-heated micro-melting point apparatus: they are therefore " corrected ".

With Wagner's reagent an immediate amorphous precipitate, even in high dilution. With Mayer's reagent an amorphous white precipitate from concentrated solutions. Auric chloride gives a microcrystalline precipitate when added to a concentrated solution. Neither platinic chloride nor picric acid give precipitates under the usual conditions. Nessler's reagent gives a cream-coloured precipitate.

When alkali, or even silver hydroxide suspension, was added to an aqueous solution of the hydrochloride a strongly ammoniacal but somewhat fishy smell developed. It was found that this odour was characteristic of the free base which could be distilled in steam and the hydrochloride recovered quantitatively and unchanged by trapping the distillate in dilute hydrochloric acid.

This finding greatly facilitated the purification of the alkaloid and led to the adoption of the following method of preparation which also afforded a reliable determination of the quantity present in the plant.

A weighed quantity of the plant powder was extracted with warm dilute (N/10) hydrochloric acid in successive portions. The combined extracts were rendered alkaline and steam distilled, the volatile bases being absorbed in an excess of standardised dilute hydrochloric acid. Titration of an aliquot afforded a measure of the total volatile bases.

The solution was then evaporated to dryness and extracted with dry chloroform, thereby rejecting ammonium chloride, and the alkaloidal salt precipitated by pouring into petroleum ether. A typical experiment is the following:—

Weight of plant taken = 10 gm.

Total volatile base = 43·4 cc. N/10 = 63·4 mgm. N.

Ammonium chloride = 8·53 cc. N/10 = 11·93 mgm. N.

Weight of alkaloidal salt recovered without recrystallisation = 0·4722 gm. = 54·3 mgm. N.

Weight of alkaloidal salt recovered after recrystallisation = 0·4158 gm. = 47·9 mgm. N.

The dried plant therefore contains approximately 4·5 per cent. of the alkaloid, calculated as hydrochloride, equivalent to 3·3 per cent. of free base.

Micro-analysis * afforded the following figures:—

| | C | H | N | Cl |
|---|---|---|---|---|
| required for $C_5H_{12}NCl$ | 49·40 | 9·88 | 11·53 | 29·22 |
| Found | 49·89 | 9·38 | 11·21 | 29·32 |

* All micro-analyses by Dr. Backeberg of the University of the Witwatersrand, Johannesburg, to whom I wish to extend my sincere thanks.

*Molecular weight determination:*

This was carried out on a carefully dried specimen by the cryoscopic method using water as solvent.

Wt. of solvent = 14·93 gm.

Wt. of material = 0·1302 gm.

Depression of F.P. = 0·273°.

∴M. Wt. (assuming complete ionisation) = 118·2.

$C_5H_{11}N$. HCl requires 121·5.

The composition of the substance is therefore correctly given by its empirical formula.

For a base with the formula $C_5H_{11}N$ several possibilities are open. It might be aliphatic, a methyl-pyrrolidine or piperidine.

A negative carbylamine test excluded primary amines. A strongly positive Legal test—deep blue colour when the free base is mixed with sodium nitroprusside solution and acetaldehyde, fading gradually through purple to bluish red—showed that it was a secondary base.

When heated alone, no reddening of a pine splinter occurs but when the hydrochloride of the base is heated with zinc dust, the vapours afford a positive, intense pyrrol reaction.

It was suspected that the substance was piperidine hydrochloride which supposition proved to be correct when derivatives were made and compared with those similarly prepared from a pure specimen of piperidine (Merck).

PREPARATION AND DESCRIPTION OF DERIVATIVES.

All the salts of piperidine are readily soluble in water, a fact which renders their preparation and purification difficult.

*Picrate.*—This salt was prepared by adding 25 mgm. of the alkaloidal hydrochloride to 45·8 mgm. of picric acid dissolved in 2 c.c. of hot water. On cooling in the ice-chest the picrate separated in small golden coloured prisms. These were collected, washed sparingly with cold water, and dried on absorbent material. They had M.P. 148° and after recrystallisation from a very small quantity of hot water, from which it separated in fragile aggregations of small prisms, melted at 149-150°.

The M.P. of piperidine picrate is given as 145° but a specimen prepared as above for comparison also melted at 149-149·5°. Mixed M.P. 148°.

Microanalysis:—

| | N |
|---|---|
| Found | 17·95 |
| $C_{11}H_{14}N_4O_7$ requires | 17·89 |

*Aurichloride.*—30 mgm. was dissolved in a few drops of water and 1 c.c. of a 30 per cent. solution of gold chloride added. On

standing in the ice chest the aurichloride separated in fine orange-yellow crystals, which were again seen to be aggregates, of very minute prisms or needles (Fig. 5). These were filtered off, washed sparingly and dried. The salt from piperidine hydrochloride was in every way similar. The M.P. was unchanged by recrystallisation.

M.P. 173-175° decomp.
Piperidine aurichloride M.P. 173-176° decomp.
Mixed M.P. 174-177° decomp.

The melting point of piperidine aurichloride, B' $HAuCl_4$, is given in Watt's Dictionary of Chemistry (1914) as 206°, but this is clearly inaccurate unless under the conditions used here a different derivative is produced. The mixed melting point left no doubt whatsoever about the identity of the two materials.

Microanalysis:—

| | Au |
|---|---|
| Found | 46·43 |
| $C_5H_{12}NAuCl_4$ requires | 46·39 |

*Picrolonate.*—Piperidine picrolonate has not, as far as the writer is aware, been described hitherto. It was prepared as follows:—

12·2 mgm. of base hydrochloride was added to 26 mgm. of picrolonic acid dissolved in 1 c.c. of hot 96 per cent. alcohol. Almost immediately the separation of beautifully formed crystals commenced and continued in the ice chest. They were filtered off, washed with a little alcohol and dried. Identical crystals were prepared from piperidine and from the *Psilocaulon* alkaloid. Piperidine picrolonate crystallises from alcohol in well formed, golden-coloured hexagonal tables (see Fig. 6). The hexagon may be equilateral or take the shape of a triangle with truncated apices.

M.P. 230-232°
Piperidine picrolonate M.P. 231-234°
Mixed M.P. 231-234°

Microanalysis:—

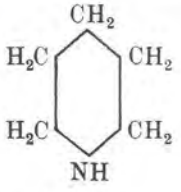
| | C | H | N |
|---|---|---|---|
| Found | 52·31 | 5·48 | 19·88 |
| $C_{15}H_{19}N_5O_5$ requires | 51·58 | 5·45 | 20·05 |

*Platinichloride.*—Difficulty was experienced in preparing a specimen of the platinichloride owing to its high solubility in both water and alcohol. 1 c.c. of 10 per cent. aqueous platinic chloride solution was added to about 30 mgm. of alkaloidal hydrochloride dissolved in a few drops of water and the mixture left in a small vessel in a dessicator for several days. As the liquid evaporated, a small deposit of reddish crystalls formed, M.P. 188°. These were removed and dried on an absorbent surface. Piperidine platinichloride similarly prepared had M.P. 189°. Mixed M.P. 188°.

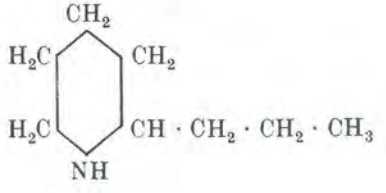
Microanalysis:—

| | Pt |
|---|---|
| Found | 33·62 |
| $C_{10}H_{24}N_2PtCl_6$ requires | 33·65 |

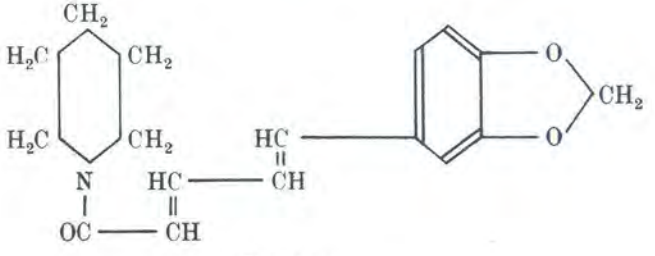
The benzoyl derivative was also prepared and crystallised in colourless prisms having the correct M.P. 48°. It is thus established that the toxic alkaloid present in *Psilocalon absimile* is piperidine $C_5H_{11}N$. It appears to occur in the form of the hydrochloride. Piperidine has not hitherto been found in vegetable material (except possibly in the husks of pepper seeds) although it is a component part of several important, naturally-occurring alkaloids. Thus, coniine is α-n-propyl-piperidine; whilst piperine is the acid amide of piperidine and piperic acid. The following constitutional formulae will render these relationships clear:—



Piperidine.



Coniine.



Piperine.

Incidentally, it may be remarked that commercial piperidine is prepared by the somewhat expensive process of reducing pyridine or crude boil oil bases, by means of sodium-amalgam or electrolytically (E. Merck D.R.P. 90308) but is usually contaminated by its homologues. It is listed (British Drug Houses) at about £6. 8s. per pound. One pound could be obtained from about 10 kilos of dried *Psilocaulon absimile* by the simple process outlined in the present paper and requiring only such cheap chemicals as caustic soda and hydrochloric acid, chloroform and light petroleum. Piperidine is used in pharmaceutical chemistry, the dyestuff and chemical industries and in the vulcanising of rubber. The wide distribution of the plant and its uselessness as fodder would seem to invite contemplation of its commercial exploitation.

## Pharmacology.

Following the administration of extracts of *Psilocaulon absimile* from which the oxalic acid has been removed, to rabbits, per os, the symptoms developing are as follows: Within 10 minutes to half an hour after dosing, salivation, laboured respiration, weak and accelerated pulse, general muscular weakness and occasional convulsive tremors passing over the whole body. Death is due to

asphyxia, the respiratory movements ceasing entirely before the onset of the pre-mortal convulsions. At post-mortem the heart is found to be dilated and engorged with blood. The following experiments record the action of the pure alkaloidal hydrochloride isolated from the plant.

Rabbit 1 weighing 2 kilos dosed 0·9 gm. of alkaloidal hydrochloride dissolved in water per os. Within half an hour the animal became restless, shaking its head and moving about in the cage. These symptoms rapidly subsided and were followed by torpor. The head sank to one side and the animal gradually became weaker until it was half-prostrate and comatose. Pronounced salivation was noticed. After remaining in this condition for about an hour it made a gradual recovery.

Rabbit 2 weighing 1·34 kilos was dosed in the same manner with 1·2 gm. of the alkaloidal salt at 9.25 a.m. This quantity corresponds to about 33 gm. of plant, the amount found to be fatal when administered suspended in water.

Symptoms developed as follows:—

10.0. Slightly restless, salivating.

10.25. Uncomfortable in cage, hunched up, breathing laboured, twitching muscular tremors of head and neck. Head inclined to sink.

10.30. Intense salivation. Head falling. Pronounced convulsive muscular tremors over the whole body.

10.32. Violent convulsions, the animal standing almost on its head in the cage whilst convulsive tremors excited every muscle in the body. Finally it collapsed and lay prostrate very cyanotic and with saliva dripping from the open mouth. Respiration ceased. Intense peristalsis.

10.34. Died 1 hour 8 minutes after dosing.

Post-mortem findings: Heart greatly dilated, auricles engorged. Lungs slightly hyperaemic. Liver very slightly hyperaemic. Mesenteric vessels injected. Stomach normal. Small intestine normal. Large intestine distended with semi-fluid contents but not inflamed. All other organs normal.

The action on frogs is also characteristic. A pond frog obtainable locally, identified for me as *Bufo regularis* Reuss., was given 25 mgm. of alkaloidal hydrochloride dissolved in about 2 c.c. of Ringer solution by injection into the dorsal lymph-sac. Symptoms supervened as follows: Within 2-4 minutes very irritable, clambering about and jumping violently. After 7-10 minutes respiration shallow, weak and intermittent. After 20 minutes and onwards comatose but apparently conscious, prostrate, breathing only at 10 seconds intervals or longer. Limbs remain in any position in which they are placed. When turned upon its back the animal makes only a few feeble movements in a vain attempt to right itself. After 40 minutes, slight limb tremors were seen. The heart beat was approximately 13 beats in 10 seconds. Died overnight.

A frog which received 5 mgm. suffered no observable ill-effects.

Dixon, in Heffter's Handbuch der experimentellen Pharmakologie (1924, Vol. 2 ii), describes the action of piperidine as being similar to, although weaker than that of nicotine and coniine. In general it may be said to have a peripheral action, paralysing the neuro-muscular junctions. It also acts as a depressant on the Vagus centres thereby causing the respiration to diminish in frequency and the pulse to accelate, the latter symptom being augmented by a slight degree of pressor action (about 1/20 that of an equal quantity of nicotine). Piperidine also produces a direct excitation of the motor cells of the spinal cord.

As pointed out in the first paper upon *Psilocaulon absimile* (Rimington and Steyn, 1933), it would appear that acute deaths from the ingestion of this plant in the field are to be ascribed for the most part to the toxic action of the alkaloidal constituent. Chronic poisoning can no doubt also occur, but this is almost certainly attributable to the very large amount of oxalic acid which the plant contains.

It is of interest to remark that, as a general rule, those plants secreting large quantities of organic acids, the so-called " acid plants " of Ruhland and Wetzel (see Rimington and Steyn, 1933), also contain reserves of mobile nitrogen in the form of ammonium salts, whilst in non-acidic plants, amides such as asparagine take the place of ammonia. In the case of *Psilocaulon absimile*, a typical " acid plant ", one finds ammonia very largely replaced by the equally strongly basic piperidine. It would be of no small interest to discover what exigencies of metabolism have led to this substitution.

## Other Alkaloids of the Aizoaceae.

Piperidine has not been described previously as a naturally-occurring alkaloid.

It would appear that with the exception of Hartwich and Zwicky's (1914) " Mesembrine " this is also the first alkaloid to be isolated from any member of the family Aizoaceae.

" Mesembrine " was not isolated in a state of purity nor were any derivatives or salts prepared. Hartwich and Zwicky assigned to it the probable formula $C_{16}H_{19}O_4N$ and considered it to be identical with the substance reported on by Meiring (1898). They stated that it had a cocaine-like action. " Mesembrin " was found in *Mesembryanthemum expansum*, L., and *M. tortuosum* whilst 23 out of 37 *Mesembryanthemum* species examined were stated to give positive tests for the presence of alkaloids.

It is of interest that *Scelatium anatomicum* (Harv.) Bolus (*Mesembryanthemum anatomicum* Harv., *Mesembryanthemum emarcidum* Thunb.) is related by Watt and Breyer-Brandwijk (1932) to be used by native peoples as an excitant and deliriant (small doses) or as a sedative (larger doses) actions which are reminiscent of the effects of piperidine salts as quoted in the literature and as recorded in the present work.

It would appear that the family Aizoaceae might well repay closer chemical study.

## Summary.

The alkaloid present in *Psilocaulon absimile*, N.E.Br. has been isolated and identified as *Piperidine*. It occurs in the plant in part as the hydrochloride and is present to the extent of approximately 4·5 per cent. of the dry weight.

Piperidine picrolonate has been prepared and described. It crystallises in golden-yellow hexagonal tables with M.P. 232-234°. The melting point of piperidine picrate is recorded as 149-149·5° and that of the gold salt as 173-176°.

Piperidine has not hitherto been found as a naturally occurring alkaloid in plants, although it is a component of certain important alkaloids such as, for example, piperine and coniine.

Only one other alkaloid has been reported in the family Aizoaceae, the alkaloid "Mesembrin," which was not definitely characterised as a pure substance.

Death due to acute poisoning following the ingestion of *Psilocaulon absimile* by grazing stock is to be attributed in large measure to the action of the alkaloid. Chronic poisoning is almost certainly due to the effects of the large quantity of oxalic acid which the plant also contains. The toxicological effects upon rabbits and frogs of preparations of piperidine hydrochloride obtained from the plant are briefly described in the present paper. Good quality piperidine is only obtainable commercially at a high price, about £6. 8s. per lb., and it is suggested that the plant *Psilocaulon absimile*, which is widely distributed in the Union and useless as a fodder, might serve as a cheap alternative source for the manufacture of this material.

## Acknowledgments.

I wish to thank Dr. D. G. Steyn for his kindness in procuring me material and for his interest in this investigation, and the Division of Plant Industry, Pretoria, for identification of the specimen.

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## EXPLANATION OF FIGURES.

Fig. 1*a*. *Psilocaulon absimile*.

Fig. 1*b*. *Psilocaulon absimile*. Flowering shoot.

Fig. 2. Constituent of the wax of *Psilocaulon absimile* crystallised from benzene. Magn. 135×.

Fig. 3. Alkaloidal hydrochloride from *Psilocaulon absimile* crystallised from chloroform. Magn. 150×.

Fig. 4. Alkaloidal hydrochloride from *Psilocaulon absimile* crystallised from chloroform: ether. Magn. 135×.

Fig. 5. *Psilocaulon absimile* alkaloidal gold salt. Magn. 50×.

Fig. 6. Piperidine picrolonate crystallised from alcohol. M.P. 231–4°. Magn. 50×.

Figs. 1A and 1B.

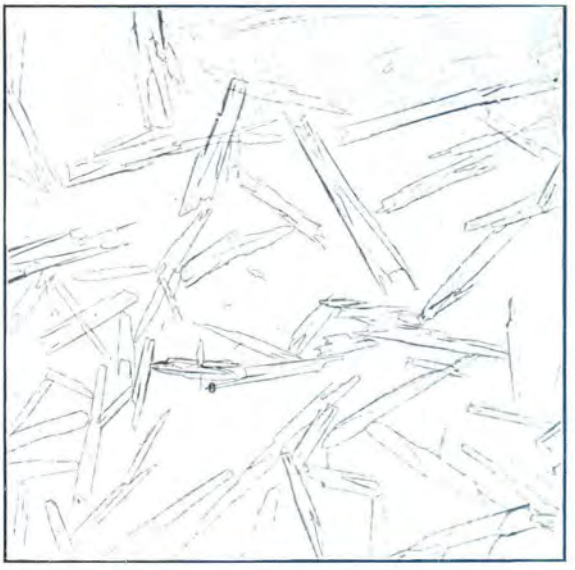

Fig. 2.

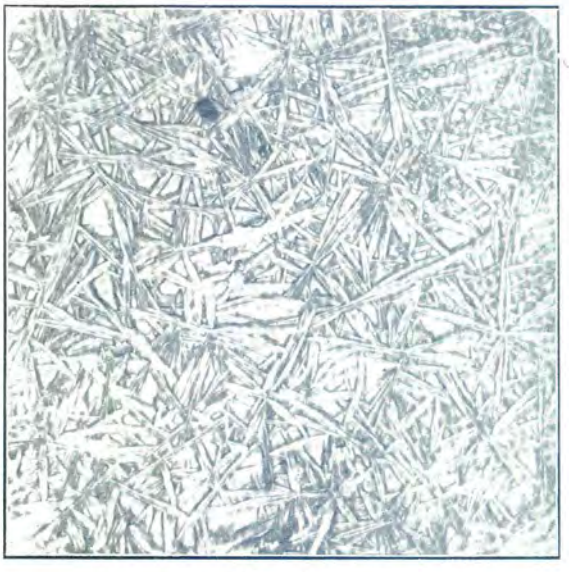

Fig. 3.

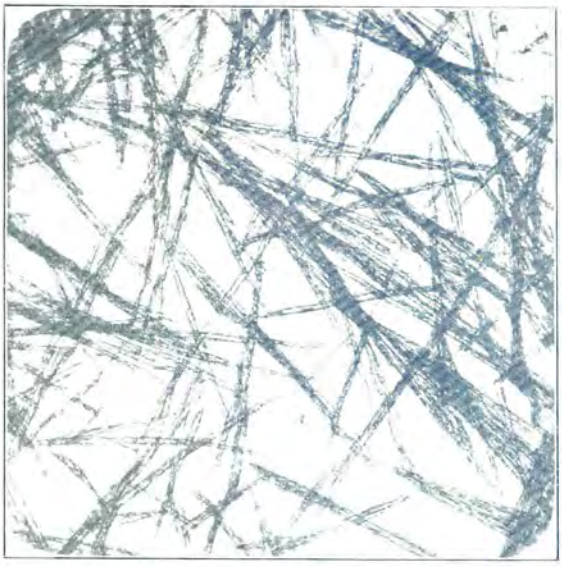

Fig. 4.

Fig. 5.

Fig. 6.