THE ALKALOID OF PITURI OBTAINED FROM *DUBOISIA HOPWOODII*

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I was recently brought a quantity of material consisting of fragments of twig and lanceolate leaves, and of a distinctly pleasant aromatic smell, with the request that I would undertake its investigation.

Those interested in it maintained that it had a wonderful influence, when smoked, of curing the pain of neuralgia, and at the same time producing a feeling of exhilaration.

I pointed out that it was desirable, before undertaking any work upon it, to have it identified botanically, partly suggesting what the active principle might be, but more particularly to safeguard against entering upon a big piece of work in an already investigated field. No information was available, however, nor could it be obtained within the month, and the work was undertaken meanwhile with the object of furnishing a preliminary report. A very short space of time was required to show the presence of an alkaloid, and an alkaloid of a very poisonous character. Further, it was apparent that the alkaloid was volatile, appearing in steam distillates, and in the smoke from the burning material.

Action on man. In the first place the fine dust from the broken material, when caught in the nose acts like snuff, causing a powerful desire to sneeze.

When smoked (which can only be done by frequently applying a lighted match to the pipe bowl) there is no great effect. The pulse is somewhat slowed, and after a half pipeful or so in my own case I had usually had enough and subsequently felt heavy-headed. I am as a rule a non-smoker, but after smoking the herb my sleep at night was less deep than usual, a result which with me also follows the smoking of tobacco. An after effect of smoking the material was a feeling that something was stuck in the oesophagus. This has followed the smoking on two separate occasions, and the unpleasant feeling has persisted for a day. Inhalation

of steam saturated with the hydrochloride of the alkaloid caused marked nausea.

Action on the Frog. The alkaloid caused death in a few minutes after injection into the dorsal lymph sac.

The frog passes into a condition of catalepsy without power of movement, and it is hard to say when death occurs. The heart on exposure half an hour later is still beating.

In smaller doses than the lethal, the paralysis sets in, and the frog when turned on its back cannot correct its position. Recovery occurs in from 20 to 40 minutes.

The same action on the frog was obtained with all the following preparations:—(a) The steam distillates, (b) the smoke collected in physiological saline solution (0.9 per cent. sodium chloride), and (c) an alkaloidal extract made with 0.2 per cent. hydrochloric acid, and subsequent purification by extraction with ether after rendering alkaline, and re-extraction from the ether by 0.2 per cent. hydrochloric acid.

In greater detail an injection causes a twitching of isolated muscles throughout the frog's body, then a sluggish response to stimuli so that an outstretched hind leg is only very slowly drawn up on pinching the toe, and finally complete absence of response, and paralysis within five minutes.

There is a loss of the bright green pigmentation of the skin, which takes on a darker dusky appearance.

Action on Frog's Heart. This is very characteristic. (i) A drop of solution containing the alkaloid caused, when applied to the heart externally, a cessation of beat in profound diastole. After 17 beats had been missed in this condition there came a systole, then another long diastolic interval and another isolated systole. This continues for some time, the diastolic standstills becoming of gradually less and less duration, until a slow powerful beat is established which gradually returns to the normal rate and with the former magnitude.

(ii) Further applications are now incapable of producing a new diastolic standstill. All the effect they have is to produce a slight slowing of the beat, with the accompanying increase in magnitude (fig. I).

This action of the drug upon the frog's heart is very characteristic.

(iii) If the heart be first painted with *Atropine* and the drug then applied the only effect obtainable is that of slight slowing, as in No. ii above. Consequently it may be stated that the alkaloid first stimulates

the vagus 'endings' causing prolonged diastole, and then paralyses them (escape of the heart from the diastole).

There is left, however, an action upon the heart muscle direct, which can be obtained by fresh application of the drug and after atropine.

Action on Nerve Trunks. No effect could be noted on excitability or conductivity when the nerve trunk of an ordinary sciatic-gastrocnemius preparation of the frog was employed. No elaborate apparatus was used, however.

Action on Nerve endings in Skeletal Muscle. The classical experiment for testing the action of curare on the frog was employed. The alkaloid was injected into the lymph sac of a frog, one of whose legs (the right) was protected from the drug by a tight ligature passing under the sciatic nerve and around the thigh.

Both legs were then skinned, and the sciatic of the left leg also exposed, and this and the right sciatic nerve stimulated alternately with tetanic shocks. After a time there was a loss of response in the left leg, but not in the right leg (which was protected by the ligature).

The alkaloid thus paralyses motor nerve endings, for a response can still be obtained by stimulating the muscles directly.

Action on Mammals. Rabbit. The doses administered proved fatal very rapidly. The animal was convulsed, the convulsions being of the clonic type, and death was apparently due to failure of respiration, for the heart could be seen beating when only a snatched and shallow respiration was occurring at long intervals.

Isolated Rabbit's Heart. A perfusion of the heart with oxygenated Ringer's Solution was performed according to Locke and Rosenheim.¹ The heart was beating very poorly indeed, but owing to several previous failures it was decided to make an attempt to perfuse the alkaloid. A couple of cubic centimetres of a purified extract were added to the circulating Ringer solution with an astounding result. At first the heart was slowed but showed beats of increasing amplitude. Suddenly it escaped from the vagus control and commenced beating at an excessive pace and with tremendous vigour. So great was the pace that a period followed in which beats kept being dropped with a consequent beat of very great excursion. This continued for some time, and then the heart slowly quietened down again (see fig. II).

Dog. A small dose injected subcutaneously caused a very frisky

1. Locke and Rosenheim, *Journal of Physiology*, Vol. XXXVI, p. 205.

terrier to become unsteady when balancing on his hind legs, then to quieten down, and finally to retch. Vomiting did not occur, however.

Action on Blood Pressure. A series of blood pressure tracings were taken. Morphine, chloroform and ether, and chloroform, were administered. The vagi were divided in the earlier experiments, not in those with curare. The drug was injected into the jugular vein and immediately gave rise to convulsions (fig. III). In later experiments curare was therefore used.

An injection is accompanied by a marked rise in blood pressure, with vaso-constriction as shown by an intestinal plethysmograph. The heart is slowed, and the magnitude of the beat increased, as the result of the first injection, but in a very short interval of time the beat becomes extremely rapid. Subsequent injections are (if the first injection was strong) without a renewal of the slowing effect. The renewed rise of blood pressure with each fresh injection of the alkaloid is due to the marked vaso-constriction in the first place (see figs. V and VI).

The plethysmograph lever, however, shows a rise with the continued high blood pressure. Careful analysis points to the vaso-constriction period corresponding with the slowing of the heart period. The heart then becomes very rapid, and this is the time when the plethysmograph tracing rises. Owing to a fault in the plethysmograph the excursions of the lever were only about half what they should have been.

The above pharmacology is very suggestive of nicotine, and in no single point is any contradiction apparent. Further, the volatility of the alkaloid is also in agreement.

The chemistry of the alkaloid was therefore investigated, and the conclusion arrived at that the alkaloid is indeed nicotine. At this point a study of Kobert's *Lehrbuch der Intoxikationen*, page 1071, led to a true recognition of the nature of the material I was handling.

Under Nicotine is to be found a reference to Pituri (*Duboisia hopwoodii*) v. Muller, 'Pituri . . . welches wie ein Gemisch von Kautabak und Kokablättern wirken soll. Man vermutet, dass, das darin enthaltene Alkaloid "*Piturin*," mit Nikotin verwandt, oder identisch ist.'

Immediately, some of the material was handed to Professor Ewart for comparison with specimens of *Duboisia hopwoodii* in the Melbourne Herbarium. The reply was an absolute confirmation of their identity. A short historical account of the previous work on the alkaloid of Pituri (*Duboisia hopwoodii*) will now be given.

The use of Pituri by the natives of Queensland and the Northern Territory has been described and is still described by those who come into contact with the far back tribes.

The first to describe its pharmacological action was Bancroft,¹ who, however, does not give any suggestion of the true nature of the alkaloid contained.

Ringer and Murrell² next had the opportunity of examining a small amount of the alkaloid as regards its action on the cat, and they discuss its possible relationship and intermediate position between atropine on the one hand, pilocarpine and muscarine on the other.

Fraser³ considers that the alkaloid is not nicotine owing to a difference in action on the pupil. He describes the effect on the blood pressure, the increased systole of the heart, and the vagus effects, as similar to the effects produced by nicotine.

Langley and Dickenson⁴ give a most comprehensive account of the pharmacology, and sum up their experiments with the clear and definite statement that 'Its physiological action is identical with nicotine.'

From the chemical side Petit⁵ identified the alkaloid as nicotine on the following evidence:—(a) Rotation in alcohol, — 123·9; for nicotine (Buignet), — 121·9. (b) Power of neutralising sulphuric acid. (c) One analysis of the platinochloride, Pt 34 per cent., Cl 36 per cent. (d) Evidence of a qualitative nature.

Liversidge considered the work of Petit inconclusive. As the result of numerous analyses of the platinochloride, he shows that there is no fixed composition. The platinum varied between 34·15 per cent. and 38·40 per cent., while the chlorine varied between 31·32 per cent. and 36·86 per cent. He concludes, as the result of combustion analysis, that the alkaloid has the formula $C_{12}H_{16}N_2$, and not $C_{10}H_{14}N_2$ (nicotine). It will thus be seen that in spite of much previous work the exact nature of the alkaloid is still in doubt.

My own investigations are now published as an independent confirmation of much that has already been done, and as proof, especially on the chemical side, *that the alkaloid of pituri is nicotine*. It agrees with nicotine in the following points:—(1) Its pharmacology;

1. Bancroft, *Journal Queensland Phil. Soc.*, March, 1872.
2. Ringer and Murrell, *Journal of Physiology*, Vol. I, p. 377, 1879.
3. Fraser, *Proc. Roy. Soc. Edinburgh*, p. 200, 1879.
4. Langley and Dickenson, *Journal of Physiology*, Vol. XI, p. 265, 1890.
5. Petit *Pharmaceutical Journal*, Vol. IX, p. 819, 1878-1879.

(2) its volatility; (3) the positive iodo-nicotine test; (4) the solubility in water, and more particularly in the way an opalescent warm solution will clear on cooling; (5) its unwillingness to yield crystalline salts, e.g., the hydrochloride and sulphate.

In an early attempt to prepare the hydrochloride it was noticed how on evaporation the alkaloidal salt was carried over in the steam, and it is an observation which has been made with nicotine that the hydrochloride is more volatile than the free base. The hydrochloride was left on evaporation as a thick brown syrup, which after a week in a vacuum over strong sulphuric acid showed star clusters of long radiating needles. When removed from the desiccator, water was absorbed and the crystals re-dissolved.

It may here be mentioned that several times on adding strong hydrochloric acid to the watery solutions of the free alkaloid a fine red colour developed fairly rapidly. No characteristic absorption bands were shown, only general absorption of the violet, blue and green.

Schwebel¹ mentions the same reaction in connection with nicotine. (6) *The oxalate* was prepared by adding an ethereal solution of the free base to an ethereal solution of oxalic acid. The precipitate was re-dissolved in a little warm 96 per cent. alcohol, and ether added until a fine opalescence was formed. Pearly crystal plates separated on standing. M.Pt. 77° C. becoming 109°-111° after re-solidifying; decomposition, 115° C.²

(7) *The platino chlorides*. Two have been prepared, and correspond to those of nicotine.

- (a) *Orange salt* crystallising beautifully from solutions containing excess of hydrochloric acid. Absolute identity of crystalline form with the same salt prepared from nicotine tartrate (Merck).
- (b) *Yellow salt*. Generally obtained as a light yellow powdery deposit after the separation of (a) and when the excess of hydrochloric acid has somewhat diminished.

According to the dictionaries of Organic Chemistry—Beilstein's 'Handbuch der Organischen Chemie' and Fehling's 'Neues Handwörterbuch'—(a) would have the formula $C_{10}H_{14}N_2, 4HCl, PtCl_2$; (b) the formula $C_{10}H_{14}N_2, 2HCl, PtCl_2$, in the case of nicotine.

The percentages of platinum and chlorine would be:—for (a)

1. Schwebel, *Ber. Deut. Chem. Ges.*, Vol. XV, p. 2850.

2. Parenty and Grasset, *Bull. Soc. Chem. Paris*, Vol. XIII, p. 299.

Nicotine, 4HCl PtCl_2 , platinum 33·91 per cent., chlorine 37·18 per cent.; for (b) Nicotine, 2HCl PtCl_2 , platinum 38·8 per cent., chlorine 28·4 per cent.

A third platino-chloride has:—Nicotine, 2HCl PtCl_4 , platinum 34·03 per cent., chlorine 37·30 per cent.

The following analyses were made on the platino-chlorides. A pure yellow salt was not obtained in sufficient quantity for analysis, but always had some orange salt admixed:—

<i>Preparation</i>		<i>Calculated Mol. Wt. of Base Minus x hydrogen atoms</i>
(1) Yellow (b), first preparation well washed, but not recrystallized	Platinum 36·02 % Chlorine 32·2 %	172
(2) Yellow (b), a distinct preparation from (1)	Platinum 36·08 % Chlorine 32·29 %	172
(3) Yellow (b), completely independent preparation to (1) or (2)	Platinum 35·79 % Chlorine 32·07 %	174
(4) <i>Pink Salt</i> , identical with orange salt (a), but coloured by the red HCl reaction previously described	Platinum 34·25 % Chlorine 36·1 %	168
(5) <i>Orange Salt</i> (a), a lovely sample beautifully crystallized	Platinum 34·46 % Chlorine 34·60 %	174
(6) Orange Salt (a), lovely crystals	Platinum 33·76 % Chlorine 36·52 %	171·6
(7) Pure orange nicotine platinum chloride from nicotine tartrate (Merck)	Platinum 34·06 % Chlorine 35·72 %	172
(8) *Orange nicotine platinochloride from nicotine tartrate (Merck)	Platinum 33·70 % Chlorine 35·96 %	177

*Note.—The actual amount of nicotine platinochloride analysed in this case was only 0·18 gram. Hence the slightest experimental losses are magnified in the calculated Mol. Wt. of base.

The figures for the Mol. Wt. of base are obtained by calculating the amount of platinochloride and chlorine corresponding to one atom of platinum and then subtracting the platinum and chlorine from the weight of platinochloride so obtained.

Examples from (6):—

<i>Platinochloride</i>	<i>Platinum</i>	<i>Chlorine</i>
0·3729 gram	0·1256 gram	0·1362 gram
576·6 salt	194·3 platinum	210·7 chlorine
576·6 - (194·3 + 210·7) = 171·6 the weight of base + x hydrogens.		

The number of hydrogen atoms (x) will be between 2 and 4.

The above estimations were carried through by Wallach's¹ method

1. Wallach, *Ber. deut. Chem. Gesellsch.*, p. 753 (1881).

for the simultaneous estimation of platinum and chlorine in double platinochlorides.

The results show the platinum tending to be slightly on the high side, the chlorine not inconsiderably on the low side, as required by theory. Petit and Liversidge's analyses show the same error, which, as Liversidge suggests, may be due to the platinochlorides not showing a constant composition.

Refractive Index of Alkaloid. This was obtained using a sample of alkaloid distilled in a current of hydrogen. The alkaloid passed over in a colourless condition at a temperature in the close neighbourhood of 240° C. The distillate in bulk had the faintest yellow tinge.

A Zeiss apparatus (for examination of a single drop) was employed.

Temperature			Refractive Index
28° C.	...		1.5220
25° C.	...		1.5240
20° C.	...		1.5265
16° C.	...		1.5275
13° C.	...		1.5290
10° C.	...		1.5305

This gives for 15° C. a refractive index 1.5283

For nicotine $N_D = 1.52828$ (Llandolt, *Ann. Chem.*, Vol. CLXXXIX, p. 317).

Optical Rotation. The free base showed a negative rotation. On carefully adding hydrochloric acid to a slight excess the rotation became positive. Thus the following figures were obtained:—

(1) <i>Base Aq.</i> + dilute HCl to double volume	1 dcm. tube	—	1.46	$\frac{1.46}{0.306} = 4.77$
	2 dcm. tube	+	0.306	
(2) <i>Nicotine Aq.</i> + HCl to double volume	1 dcm. tube	—	1.63	$\frac{1.63}{0.354} = 4.60$
	2 dcm. tube	+	0.354	
Temperature for (1) and (2), 23° C.				
(3) <i>Base Aq.</i> <i>Hydrochloride</i>	2 dcm. tube	—	1.38	$\frac{1.38}{0.27} = 5.11$
		+	0.27	
(4) <i>Base Aq.</i> <i>Hydrochloride</i>	2 dcm. tube	—	2.497	$\frac{2.497}{0.475} = 5.26$
		+	0.475	
(5) <i>Nicotine Aq.</i> <i>Hydrochloride</i>	2 dcm. tube	—	2.11	$\frac{2.11}{0.415} = 5.09$
		+	0.415	

In (3), (4) and (5) the base was first examined in a two decimetre tube. Then a given volume was made acid with a few drops strong HCl + Aq to one-eleventh of its bulk, and re-examined as hydrochloride in

a 2.2 dcm. tube, i.e., equal to a 2 dcm. tube of the original base-solution. Temperature for (3) 23° C., for (4) and (5) 32° C.

It is further evidence of the identity of the base with nicotine when the expected readings for the hydrochloride, as obtained by calculation, are compared with those actually found. For the calculation the following figures were used:—

$\alpha_D = -74.13$ for very weak water solutions of nicotine ¹					
$\alpha_D = +14.5$ for nicotine hydrochloride + 98 % water ²					
(1) Base Aq.	1 dcm. tube Rotn.	—	1.46		
Hydrochloride	Calculated	+	0.2875	Found	+ 0.306
(3) Base Aq.	2 dcm. tube Rotn.	—	1.38		
Hydrochloride	Calculated	+	0.2697	Found	+ 0.27
(4) Base Aq.	2 dcm. tube Rotn.	—	2.497		
Hydrochloride	Calculated	+	0.488	Found	+ 0.475

In these experiments the direct comparisons with nicotine were made owing to the scant figures published for the rotation of nicotine and its hydrochloride in presence of water. A Schmidt and Haensch three shadow polarimeter was used, and the majority of the readings were sharp to within $\pm 0.01^\circ$, but with the longer tubes $\pm 0.02^\circ$ was all the accuracy attainable. Averages of ten to twelve separate readings were then used.

Rotation of Pure Alkaloid (free base) in water. 0.6522 grms. of pure distilled base were dissolved in 25 c.c. distilled water and examined in a 2 dcm. tube at 21° C.

Rotn. 3.686°.

The reading was obtainable with accuracy, the solution being clear and colourless.

Translating for comparison with figures given by M. Popovici,³ 1.3044 grms. free base in 50 c.c. water gave 221' rotation in a 2 dcm. tube, which result exactly agrees (within the limits of experimental error) with Popovici's figures, which for 1.3044 grms. nicotine give 225' (by interpolation), or 219' (using the figure 1' = 0.595 mgrm. nicotine).

Combustion Analysis. These were carried out on the pure base obtained as follows:—200 grms. of pituri were made alkaline with the addition of 50 c.c. of 40 per cent. sodium hydrate and 2 litres of water, and were distilled in steam till 4 litres of distillate had been collected.

1. Llandolt, *Ann. Chem.*, p. 317.
2. Schwebel, *Ber. deut. Chem. Ges.*, Vol. XV, p. 2850.
3. Popovici, *Zeit. Phys. Chem.*, Vol. XIII, p. 447.

The free base was fixed by oxalic acid, and the distillate then concentrated in vacuo.

It was now made alkaline, and as the alkaloid did not separate as an oil, it had to be extracted by repeated quantities of ether.

The ether was removed, and the residue distilled in hydrogen. 1st fraction distilled at 98° C. (chiefly water); 2nd fraction distilled about 240° C. The middle portions of this fraction were used for the combustions. Suitable amounts were immediately filled into small tubes, which were then sealed, and weighed, to be used in due course.

RESULTS

<i>Found</i>	<i>Pituri Body</i>			<i>Nicotine</i>	<i>Calculated for Liversidge</i>
				$C_{10}H_{14}N_2$	$C_{12}H_{16}N_2$
Carbon	71.90 %	74.23 %	73.14 %	74.06 %	76.59 %
Hydrogen	8.56 %	8.60 %	9.02 %	8.64 %	8.51 %
Nitrogen	16.2 %	17.7 %	17.5 %	17.3 %	14.9 %

These results uphold the nicotine formula, and completely discount the formula suggested by Liversidge.

The Amount of Nicotine in Pituri. 20 grms. of the powdered material dried at 60° C. for two hours, and submitted to the usual process for nicotine estimations yielded 250 c.c. distillate requiring 31 c.c. $\frac{N}{10}$ H_2SO_4 for neutralisation (Rosolic Acid as indicator); next 120 c.c. distillate requiring 2 c.c. $\frac{N}{10}$ H_2SO_4 ; total, 33 c.c. $\frac{N}{10}$ H_2SO_4 = 2.67 grms. nicotine per 100 grms. of pituri.

Throughout the experimental work evidence for the existence of another alkaloid has been distinctly absent.

After removal of the volatile nicotine there is no fixed alkaloid remaining (confirmation of Liversidge), and any other alkaloid present must therefore be volatile.

Until large quantities of the crude base can be fractionally distilled, there is no likelihood of such a possible alkaloid being separated.

The pharmacology and the analytical data indicate that such an alkaloid or alkaloids could only be present in small quantities, and even then would in all probability be closely allied to the nicotine. These statements are made on account of a belief, quite unsupported by any observations I have been able to make, that pituri has a peculiar 'cocaine-like' action. At all events, there is no doubt that *the alkaloid* of pituri (*Duboisia hopwoodii*) is *Nicotine*.

SUMMARY

- (1) The alkaloid of pituri is nicotine.
- (2) No evidence is forthcoming that there is any exhilarating or cocaine-like action, or that there is any pharmacological action, other than what nicotine can produce.
- (3) There is no fixed alkaloid.
- (4) There is no evidence to suggest any other volatile alkaloid than nicotine, though small quantities of allied alkaloids, as in tobacco, may exist.

Fig. I. FROG'S HEART

Painted with dilute alkaloid hydrochloride solution at A. 1, 2 and 3, consecutive. Interval between 3 and 4, and between 4 and 5 = 50 beats of heart.
At B, again painted heart with the alkaloid.

Fig. II. RABBIT'S HEART

Isolated and perfused with oxygenated Ringer solution. At A the heart receives Ringer solution, to which a couple of c.c. of dilute alkaloid hydrochloride has been added.

FIG. III. DOG. VAGI CUT

At A, 2 c.c. dilute alkaloid hydrochloride injected into jugular vein. Convulsions followed.

FIG. IV. SMALL DOG UNDER CURARE

Artificial respiration. At A, 2 c.c. dilute alkaloid hydrochloride injected into jugular vein.

↑ A

FIG. V. DOG. CURARE AND ARTIFICIAL RESPIRATION.

At A, 1 c.c. of steam distillate, *i.e.* containing free alkaloid injected into jugular vein. Note rise of B.P. and slowing of heart, accompanied by fall of plethysmograph lever. Rapid heart follows, and passive rise of plethysmograph.

↑ A

FIG. VI. DOG. CURARE.

At A, 1 c.c. steam distillate into jugular vein. The lever recording Intestinal Volume was slightly ahead of that recording B.P.