Pilocarpine and the alkaloids of jaborandi leaves / by Hooper Albert Dickinson Jowett.

XLVII.—*Pilocarpine and the Alkaloids of Jaborandi Leaves.*

By Hooper Albert Dickinson Jowett, D.Sc.

Pilocarpine, the principal alkaloid contained in jaborandi leaves, was discovered by Hardy (*Bull. Soc. Chem.*, 1875, [ii], 24, 497) and by Gerrard (*Pharm. J.*, 1875, [iii], 5, 865, 965; 7, 225) independently in 1875, and crystalline salts were prepared by the latter chemist, but no detailed description of the salts was given nor were they analysed. In 1876, Kingzett (this Journ., 1876, ii, 30,367) published an analysis of a platinichloride which had not been purified, and proposed the formula $C_{23}H_{35}O_4N_4$; his results, however, agree within the limits of experimental error with the simpler formula, $C_{11}H_{16}O_2N_2$. Harnack and Meyer (*Annalen*, 1880, 204, 67) later announced that a second

alkaloid was present in jaborandi leaves, and named it jaborine, its formula being given as $C_{11}H_{16}O_2N_2$. This base was amorphous and formed amorphous salts. These chemists also stated that pilocarpine on dry distillation with soda-lime yields trimethylamine. Methods of preparation of pilocarpine and a determination of its specific rotatory power were described by Petit (*Ber.*, 1877, **10**, 896) and by Poehl (*Jahresb.*, 1880, 993, 1074). Chastaing (*Compt. rend.*, 1883, **97**, 1435; 1885, **100**, 1593) published a series of papers dealing with the chlorination and bromination of pilocarpine and its reaction with alkyl iodides. In 1885, Harnack and Meyer (*Chem. Centr.*, 1885, 628) described a third alkaloid in jaborandi leaves, which they named pilocarpidine and ascribed to it the formula $C_{10}H_{14}O_2N_2$. This base, although amorphous, gave a crystalline nitrate, melting at 129·2°, and no precipitate with auric chloride.

Between 1885 and 1887, Hardy and Calmels (*Compt. rend.*, **102**, 1116, 1251, 1562; **103**, 277; **105**, 68) published a series of papers dealing with the description of various salts of pilocarpine and pilocarpidine, details of the conversion of the former into the latter, and experiments on the constitution of the alkaloid. They represented the base as a derivative of β-pyridinelactic acid and trimethylamine, $C_5NH_4 \cdot C(CH_3)\langle \begin{matrix} CO \\ N(CH_3)_3 \end{matrix} \rangle O$, and claimed to have completed the proof of the constitution by a partial synthesis of the alkaloid. The account of their work, however, is very unsatisfactory and incomplete, and no reliance can be placed on their results: no description or analysis of the various crystalline salts is given and no proof is furnished of the identity of the product said to result from the decomposition of the alkaloid.

Nothing further was published till 1896, when Merck (*Merck's Ber.*, 1896, 11), and shortly afterwards Kundsen (*Ber. Pharm.*, 1896, **6**, 164) reported their failure to confirm Hardy and Calmels' results. In this year also, Paul and Cownley (*Pharm. J.*, 1896, [iv], **3**, 1, 437) drew attention to the unsatisfactory state of our knowledge of the subject, and noted the results of a few experiments on this group of alkaloids.

The first complete account of pilocarpine and its salts, as also of pilocarpidine, was given by Petit and Polonowsky (*J. Pharm.*, 1897, [vi], **5**, 370, 430, 475; **6**, 8). In addition to the physical constants and analyses of the salts, important observations were recorded as to the optical behaviour of the bases under varying conditions. These chemists were able to convert pilocarpine into pilocarpidine by heat or by the influence of alkali, proved that the latter was isomeric with the former, and asserted, although on very slender experimental evidence, that the pilocarpidine so prepared was identical with that found in the leaves. They also pointed out several inaccuracies in Hardy and

Calmels' work, particularly as regards the experiments on the synthesis of the base.

In 1898, Herzig and Meyer (*Monatsh.*, 19, 56) criticised Petit and Polonowsky's results, and stated that the relationship between pilocarpine and pilocarpidine was not so simple as was supposed. Merck (*Arch. Pharm.*, 1898, 236, 141) contended that the pilocarpidine of the latter chemists was simply altered pilocarpine, and showed that pilocarpidine (natural) underwent a similar change on heating. To these criticisms, Petit and Polonowsky replied that the pilocarpidine used by Merck was not a single substance, but was really impure pilocarpine.

It must be pointed out that a confusion of names has arisen, since Harnack and Merck use the name pilocarpidine for an alkaloid of formula $C_{10}H_{14}O_2N_2$, whilst Petit and Polonowsky apply it to a base isomeric with pilocarpine, $C_{11}H_{16}O_2N_2$, but as just stated do not establish conclusively its identity with the alkaloid accompanying pilocarpine in jaborandi leaves. Merck, in his last paper, relies entirely on his analytical results for the proof that no pilocarpidine is formed from pilocarpine, but if his formula is wrong and these bases are really isomeric, his experiments, instead of disproving Petit and Polonowsky's results, actually support them.

It will thus be seen from this brief summary of the work on pilocarpine that our knowledge of this group of alkaloids is unsatisfactory, and with the view of remedying these defects, the present investigation was undertaken.

The pilocarpine used in this investigation was obtained from the following sources: (i) as nitrate in unbroken packages from two manufacturers; (ii) prepared by myself from *Pilocarpus microphyllus* or Maranham jaborandi; (iii) from a very small sample of true jaborandi leaves, *P. jaborandi*, of which I have been unable to obtain further supplies. The purified alkaloids obtained from these sources proved to be identical.

It may be desirable to state here that throughout this investigation the greatest care has been taken to work with pure materials, and where the results differ from those of Petit and Polonowsky, the determinations have been repeated several times with different preparations. These authors give the melting points as uncorrected, and do not indicate that any special means were taken to ensure the purity of their products; such precautions are particularly necessary, owing to the ease with which pilocarpine is converted into isopilocarpine during the processes of regeneration, &c., and of conversion into different salts.

The results communicated in the present paper may be briefly summarised as follows:

1. The physical constants and descriptions of the salts of pilocarpine

as given by Petit and Polonowsky are generally confirmed with a few exceptions, the most important discrepancy being found in the melting point of the picrate.

2. The optical behaviour of pilocarpine has been thoroughly studied and some of Petit and Polonowsky's figures have been corrected, although their general conclusions are confirmed.

3. The acid character of pilocarpine has been investigated, and the previous work on this subject by Hardy and Calmels corrected.

4. The existence of a base isomeric with pilocarpine, produced from it by the action of heat or alkali, as previously stated, is confirmed. Since this base is isomeric with, and bears a close relation to pilocarpine, it is proposed to call it *isopilocarpine*. It is shown that the base can be distilled unchanged in a vacuum.

5. Some of the physical constants of isopilocarpine and its salts as given by Petit and Polonowsky for pilocarpidine are confirmed, and others corrected.

6. Proof is given of the existence of isopilocarpine in jaborandi leaves and in the pilocarpine nitrate of commerce.

7. The existence of the pilocarpidine of Harnack and Merck and their statements regarding its composition are confirmed; hence the use of the name pilocarpidine for isopilocarpine as suggested by Petit and Polonowsky must be abandoned. Some of the salts of this base are described.

8. The absence of pilocarpidine in the pilocarpine nitrate of commerce and in the varieties of jaborandi leaves at present on the market is proved.

9. The *jaborine* of Harnack is shown to be a mixture of pilocarpidine, isopilocarpine, and possibly a trace of pilocarpine, with colouring matter. No evidence has been obtained of the existence of an alkaloid with the properties of jaborine, or of other alkaloids than those described in this paper.

10. Several experiments on the constitution of pilocarpine have been repeated and the results of Hardy and Calmels corrected. Further experiments on this subject are in progress.

11. The physiological action of the pure salts and of some derivatives and other products obtained from jaborandi leaves is being investigated by Prof. C. R. Marshall, of University College, Dundee; a brief abstract of the results obtained is given in this paper.

I. Pilocarpine.

Salts of Pilocarpine.

Pilocarpine Nitrate, $C_{11}H_{16}O_2N_2,HNO_3$, occurs in well-defined crystals and was recrystallised repeatedly from alcohol and from water

until the melting point and specific rotation were constant. It melts at 178° (corr.) and a determination of its specific rotation gave the following result :

$$\alpha_D = +15{\cdot}87°;\ l = 2 \text{ dcm.};\ c = 9{\cdot}572;\ [\alpha]_D = +82{\cdot}90°.$$

Determinations of the solubility of the salt in water and in absolute alcohol gave the following results :

(1.) In water : 6·89 of solution at 20° gave 0·925 salt ; hence 1 part is soluble in 6·4 parts of water at 20°.

(2.) In absolute alcohol : 11·191 of solution at 20° gave 0·0414 salt ; hence 1 part is soluble in 269·3 parts of absolute alcohol at 20°.

The dry salt was analysed with the following results :

0·1712 gave 0·305 CO_2 and 0·0994 H_2O. C = 48·6 ; H = 6·45.

NH_3 from 2 grams of salt neutralised 15·2 c.c. N acid. N = 10·6.

$C_{11}H_{16}O_2N_2,HNO_3$ requires C = 48·65 ; H = 6·32 per cent.

" " for N in base, N = 10·3 "

Pilocarpine Hydrochloride, prepared by the usual methods, is easily obtained pure, and in very large crystals by crystallisation from strong alcohol. The pure salt melts at 204—205°, and a determination of its specific rotation gave the following result :

$$\alpha_D = +18{\cdot}21°;\ l = 2 \text{ dcm.};\ c = 9{\cdot}924;\ [\alpha]_D = +91{\cdot}74°.$$

On analysis, the following result was obtained :

1·0614 gave 0·6216 AgCl. Cl = 14·48.

$C_{11}H_{16}O_2N_2,HCl$ requires Cl = 14·48 per cent.

Pilocarpine Hydrobromide is obtained in beautiful crystals by the method employed for the hydrochloride. The pure salt melts at 185° (corr.), and a determination of its specific rotation gave the following result :

$$\alpha_D = +15{\cdot}5°;\ l = 2 \text{ dcm.};\ c = 10{\cdot}058;\ [\alpha]_D = +77{\cdot}05°.$$

These figures are higher than those given by Petit and Polonowsky, but the salt was recrystallised three times without alteration of the melting point ; the lower figures previously given must have been those for an impure salt, due perhaps to a small quantity of isopilocarpine hydrobromide formed during the preparation.

On analysis, the following result was obtained :

0·5154 gave 0·3334 AgBr. Br = 27·58.

$C_{11}H_{16}O_2N_2,HBr$ requires Br = 27·64 per cent.

Pilocarpine Sulphate is somewhat difficult to obtain in crystals owing to its extreme solubility in water or absolute alcohol, but it can be obtained by adding anhydrous ether to a solution of the dried

salt in absolute alcohol and allowing to stand in a closed vessel. The pure salt melts at 132° (corr.) and a determination of its specific rotation gave the following result:

$$\alpha_D = +6{\cdot}2°;\ l = 1 \text{ dcm.};\ c = 7{\cdot}318;\ [\alpha]_D = +84{\cdot}72°.$$

Pilocarpine Picrate is obtained by adding a solution of picric acid to an aqueous solution of the hydrochloride, and can easily be crystallised from hot water or alcohol. It melts at 147° (corr.) and is particularly useful for identifying the base, even when mixed with isopilocarpine, as the picrates are somewhat easily separated by fractional crystallisation from water.

Pilocarpine Aurichloride, $C_{11}H_{16}O_2N_2,HAuCl_4,H_2O$.—Hardy and Calmels have described four compounds of gold chloride with pilocarpine, namely, $B,HAuCl_4$, $B,AuCl_3$, $B(AuCl_3)_2$, $B,HAuCl_4,AuCl_3$ and have specified the conditions under which they were formed, but give no analyses of the products. I have repeated their experiments, using excess of base, auric chloride, and hydrochloric acid respectively, and in every case obtained the normal aurichloride as the sole product.

On adding auric chloride to an aqueous solution of a salt of pilocarpine, a precipitate is obtained which quickly becomes crystalline. The air-dried salt melts indefinitely at about 100°. On analysis:

0·1764 gave 0·0612 Au. Au = 34·69.

$C_{11}H_{16}O_2N_2,HAuCl_4,H_2O$ requires Au = 34·82 per cent.

When the salt is dried for some time in a water-oven below 100°, it loses its water of crystallisation; the anhydrous compound melts indefinitely at 117—130°. On analysis:

0·1496 gave 0·0538 Au. Au = 35·96.

$C_{11}H_{16}O_2N_2,HAuCl_4$ requires Au = 35·96 per cent.

The melting points of these salts are not at all sharp, and cannot be used for purposes of identification.

Pilocarpine Gold Chloride, $C_{11}H_{16}O_2N_2,AuCl_3$.—If the normal aurichloride is boiled with water or alcohol, it first dissolves, but crystals very quickly separate from the hot liquid, which is now strongly acid. These, when dry, melt sharply at 163° (corr.), thus differing considerably from the normal aurichloride. The dried salt was analysed with the following result:

0·09 gave 0·0344 Au. Au = 38·22.

$C_{11}H_{16}O_2N_2,AuCl_3$ requires Au = 38·51 per cent.

On adding a solution of auric chloride to the hydrobromide, a red precipitate was obtained, probably $C_{11}H_{16}O_2N_2,HBr,AuCl_3$, but the substance was not further examined.

Pilocarpine Platinichloride is obtained by adding platinic chloride to a solution of pilocarpine hydrochloride; it forms silky plates, which can be recrystallised from hot acidified water. The recrystallised salt melts with decomposition at about 218° (corr.). On analysis:

0·0594 gave 0·0144 Pt. Pt = 24·24.

$(C_{11}H_{16}O_2N_2)_2,H_2PtCl_6$ requires Pt = 23·58 per cent.

No compound of the composition $(C_{11}H_{16}O_2N_2)_2PtCl_4$, as described by Hardy and Calmels, could be isolated.

Pilocarpine Methiodide is formed on dissolving dry pilocarpine in excess of methyl iodide, when the clear solution gradually deposits an oil, which, however, could not be crystallised.

Pilocarpine Ethiodide is obtained by dissolving pure dry pilocarpine in excess of ethyl iodide and allowing to stand, when an oil separates which slowly becomes crystalline. The compound may be recrystallised from alcohol and ether. It forms cubical crystals, very soluble in water or alcohol, and melts at 114° (corr.). On analysis:

0·3742 required 10·4 c.c. *N*/10 $AgNO_3$ for precipitation. I = 35·25.

$C_{11}H_{16}O_2N_2,C_2H_5I$ requires I = 34·84 per cent.

A determination of the specific rotation gave the following result:

$\alpha_D = +2·71°$; $l = 1$ dcm.; $c = 4·158$; $[\alpha]_D = +65·2°$.

Properties of Pilocarpine.

Pilocarpine is best obtained by adding excess of ammonia to an aqueous solution of one of its salts, and extracting two or three times with chloroform; after washing to remove ammonia, the chloroform solution, on evaporation or distillation, yields a colourless oil, which can be freed from the last traces of the solvent by leaving it in an open dish for several days. As thus obtained, pilocarpine is a thick oil, freely soluble in water, alcohol, or chloroform, but almost insoluble in ether or light petroleum. All efforts to crystallise it by ordinary methods failed. It can be distilled in a vacuum, but during this process undergoes change into isopilocarpine: thus, pure pilocarpine, after distillation at about 260° under 5 mm. pressure, was found to form a nitrate, which melted at 146°, this being the melting point of a mixture of pilocarpine and isopilocarpine, containing chiefly the latter.

Petit and Polonowsky state that pilocarpine and isopilocarpine are not extracted by chloroform from a solution of the salt rendered alkaline with caustic soda. The following experiment shows that it is possible

under those circumstances to extract an appreciable quantity of the bases.

Pilocarpine nitrate was dissolved in water, excess of soda added, and the liquid extracted four times with chloroform, 52·8 per cent. of the base being thus removed. The alkaline liquid was now rendered acid, excess of ammonia added, and the rest of the alkaloid extracted by shaking once with chloroform. Petit and Polonowsky's statement therefore requires modification. When the base is set free with caustic soda, it is extracted slowly and incompletely by chloroform; ammonia should, therefore, always be used.

Specific Rotation of Pilocarpine.

Petit and Polonowsky found the specific rotation of the base to be +106°, Poehl having previously obtained the value $[\alpha]_D$ +101·6°. But although aware of the fact that the base retains the last traces of solvent with great avidity, they do not indicate how this difficulty was overcome. In the following experiments, after the rotation had been taken, a known quantity of the liquid (for example, 4 or 5 c.c.) was placed in a tared dish, exactly neutralised with very dilute aqueous nitric acid, and evaporated to dryness in a vacuum desiccator. In this way, from the amount of nitrate found, the concentration of the solution used can be calculated. The following results were obtained:

α_D = +10° ; l = 0·5 dcm. ; c = 19·83 ; $[\alpha]_D$ = +100·8°
α_D = +14·3° ; l = 1 dcm. ; c = 14·188 ; $[\alpha]_D$ = +100·8°
α_D = + 2·1° ; l = 2·1 dcm. ; c = 1·0641 ; $[\alpha]_D$ = +100°
Mean of three determinations, $[\alpha]_D$ = +100·5.°

Petit and Polonowsky have shown that caustic alkali affects the rotation of pilocarpine in a peculiar manner, and explain this by supposing that the base is the anhydride of an acid with a lower rotation: on treatment with caustic soda, the salt is formed which gives the rotation of the acid. They boiled pilocarpine with excess of caustic soda, then titrated back with decinormal acid, so that there was exactly a molecule of caustic soda present to each molecule of pilocarpine, and, on examining the resulting solution, found that the specific rotation had fallen to +23·8°. In the same paper, however, they point out that pilocarpine is converted into isopilocarpine by boiling with caustic soda, and as the latter has a lower rotation than the former, their experiment is invalid.

To ascertain the effect of alkali, mixtures of pilocarpine and caustic soda in varying proportions were made, one being kept for a month, whilst another was treated with hot caustic soda. The following results were obtained:

(1.) 4 mols. of base to 1 mol. caustic soda.
$\alpha_D = +5{\cdot}08°$; $l = 1$ dcm.; $c = 5{\cdot}949$; $[\alpha]_D = +85{\cdot}40°$.
(2.) 2 mols. of base to 1 mol. caustic soda.
$\alpha_D = +3{\cdot}716°$; $l = 1$ dcm.; $c = 5{\cdot}949$; $[\alpha]_D = +62{\cdot}47°$.
(3.) 1 mol. of base to 1 mol. caustic soda.
$\alpha_D = +1{\cdot}916°$; $l = 1$ dcm.; $c = 5{\cdot}949$; $[\alpha]_D = +32{\cdot}21°$.
(4.) 1 mol. of base to 4 mols. caustic soda.
$\alpha_D = +1°$; $l = 1$ dcm.; $c = 2{\cdot}9745$; $[\alpha]_D = +33{\cdot}62°$.
This, after standing one month, gave $[\alpha]_D = +31{\cdot}5°$.
$\alpha_D = +0{\cdot}6°$; $l = 1$ dcm.; $c = 2{\cdot}8376$; $[\alpha]_D = +21{\cdot}15°$.
(5.) Boiled with excess of caustic soda for 10 minutes.

These experiments show that the specific rotation of pilocarpine with excess of the alkali is $+31{\cdot}5°$, and that the figure obtained by Petit and Polonowsky, namely, $+23{\cdot}8°$, is due to a slight decomposition into isopilocarpine. It was also found that this conversion of pilocarpine into the acid of lower specific rotation may take place, to a limited extent, in aqueous solution in the absence of alkali. Thus the specific rotation of pilocarpine in aqueous solution, after standing for 3 weeks, was:

$$\alpha_D = +5{\cdot}5°;\ l = 1 \text{ dcm.};\ c = 7{\cdot}094;\ [\alpha]_D = +77{\cdot}53°.$$

This solution gave a nitrate of melting point 178° (corr.), so that the base was pure pilocarpine.

An attempt was made to determine the rotation of free pilocarpic acid by preparing it from the barium salt, but low figures were obtained, which were found to be due to the partial conversion of pilocarpine into isopilocarpine by the alkali.

Compounds of Pilocarpine with Metals.

Hardy and Calmels stated that pilocarpine might be regarded as the anhydride of a hypothetical pilocarpic acid, and described the sodium, potassium, barium, copper, and silver salts. Petit and Polonowsky confirm this statement, describing, however, only the sodium and barium salts and the acid, which rapidly passed into the anhydride.

Sodium Pilocarpate is formed as a varnish by evaporating to dryness a solution of pilocarpine in water, to which a molecular proportion of soda has been added. As stated by Petit and Polonowsky, chloroform does not dissolve this salt or extract any alkaloid from it when dry; if, however, it is dissolved in water, an appreciable amount of the alkaloid can be removed by shaking with chloroform. The alkaloid can be titrated with alkali by boiling, and using phenolphthalein as an indicator; the end reaction, however, is not sharp.

The liquid remains colourless until about half the theoretical quantity of alkali has been added, and then gradually develops colour, but a decided red is not observed until practically the theoretical quantity of alkali has been added. This behaviour indicates the extraction of about 50 per cent. of the alkaloid from soda solution, as previously described.

Barium Pilocarpate can be obtained, as stated by earlier workers, by evaporating to dryness equivalent proportions of pilocarpine or the nitrate with baryta water, and extracting the residue with 90 per cent. alcohol. I attempted to prepare free pilocarpic acid from this salt by decomposing it with exactly the right amount of dilute sulphuric acid, and obtained a product having the following specific rotation:

$$\alpha_D = +1{\cdot}5^\circ;\ l=1;\ c=8{\cdot}73;\ [\alpha]_D = +17{\cdot}18^\circ.$$

The melting point of the nitrate of this base was 164°, showing that the treatment with baryta converted some of the pilocarpine into isopilocarpine.

Copper Pilocarpate.—Hardy and Calmels stated that they prepared this salt by boiling a solution of barium pilocarpate to which a solution of a copper salt has been added, when it separated as a green powder, and they gave it the formula B_2Cu. I have obtained a similar result, but, on analysing the product, found it to be copper hydroxide:

0·1982 gave 0·1610 CuO. Cu = 64·90.

B_2Cu requires Cu = 13·2; $Cu(OH)_2$ requires Cu = 65·1 per cent.

Evidence, however, was obtained of the existence of copper pilocarpate, although the salt could not be isolated in a pure form.

When freshly precipitated copper hydroxide in excess is boiled with a solution of pilocarpine, part of it dissolves, and a dark blue solution is formed, which, when filtered and evaporated to dryness, gives a dark blue varnish. On analysis, the following result was obtained:

0·1912 gave 0·0136 CuO. Cu = 5·67 per cent.

A portion, well washed with chloroform to remove any free alkaloid, was also analysed:

0·1598 gave 0·019 CuO. Cu = 9·49.

$(C_{11}H_{15}O_3N_2)_2Cu$ requires Cu = 12·47 per cent.

The product was therefore copper pilocarpate, with a little pilocarpine adhering to it.

Silver Pilocarpate, as described by Hardy and Calmels, was silver oxide, but an impure salt may be obtained by treating pilocarpine with silver hydroxide, filtering, and evaporating the solution to dry-

ness. The product, however, as in the case of the copper salt, contained a large quantity of pilocarpine.

Pilocarpine Argento-nitrate, $(C_{11}H_{16}O_2N_2)_2,AgNO_3$.—Hardy and Calmels state that on mixing aqueous solutions of silver nitrate and pilocarpine, two crystalline substances are obtained, having either the formula $B,AgNO_3$ or $B,(AgNO_3)_2$, according to whether excess of the base or silver salt is used. I have found that under both these conditions only one salt is formed, and analysis shows it to have a different composition from that previously assigned to it. The crystals melt at 141° (corr.) sharply, and the results of the analyses were as follows:

(1.) Salt prepared by adding excess of silver nitrate,

0·1378 gave 0·0254 Ag. Ag = 18·43.

(2.) Salt prepared by adding excess of pilocarpine,

0·1652 gave 0·0302 Ag. Ag = 18·28.

$(C_{11}H_{16}O_2N_2)_2,AgNO_3$ requires Ag = 18·41 per cent.

These experiments are more in accord with the view that pilocarpine is a lactone and not a betaine as suggested by Hardy and Calmels.

II. Isopilocarpine.

Formation of Isopilocarpine from Pilocarpine.

The formation of this base from pilocarpine by the action of heat or alkali was first noted by Petit and Polonowsky. Although its existence has been questioned by Merck, I am able to fully confirm Petit and Polonowsky's observation, but, as in the case of pilocarpine, some of the physical constants on record require modification. The name pilocarpidine, given to this base by Petit and Polonowsky is, however, inadmissible, since that name should be retained for a base, $C_{10}H_{14}O_2N_2$, found in jaborandi leaves, which has been partly described by Harnack and by Merck. As the new alkaloid is isomeric with pilocarpine and easily formed from it, I propose to term it *isopilocarpine*. This base, being so readily formed by the action of heat or alkali on pilocarpine, is very often present in it as an impurity, and many of the discrepancies between previous figures and my results are due to this fact. If pilocarpine hydrochloride is heated for half an hour at a few degrees above its melting point, or if some pure pilocarpine is distilled in a vacuum, a product is obtained which yields a crystalline nitrate melting at 146°, and this melting point is not affected by two or more recrystallisations of the salt. It is possible, however, to separate pilocarpine and isopilocarpine from the mixture. The base

is best obtained by heating an alcoholic solution of pilocarpine with alcoholic soda for some hours on a water-bath in a reflux apparatus, and on regenerating the base one or two crystallisations of the nitrate will yield a pure product melting at 159° (corr.). Most of the salts have been prepared from the base obtained both by the action of heat and of alkali on pilocarpine. The physical constants of these salts proved that the base formed by either method is isopilocarpine. These results necessitate a revision of the statement frequently met with, based on Hardy and Calmels' work, that "pilocarpidine, on heating, yields pilocarpidine, jaborine, and jaboric acid."

Salts of Isopilocarpine.

Isopilocarpine Nitrate can be obtained in very long prisms from water, or in short prisms from alcohol, and is insoluble in ether, chloroform, or acetone. It melts sharply at 159° (corr.), and one of many determinations of its specific rotation gave the following result:

$$\alpha_D = +4{\cdot}7^\circ\ ;\ l = 2\ \text{dcm.}\ ;\ c = 6{\cdot}586\ ;\ [\alpha]_D = +35{\cdot}68^\circ.$$

The purity of the salt was determined by the fact that, after three recrystallisations from alcohol and from water, the melting point and specific rotation were unchanged. Determinations of the solubility in water and in absolute alcohol gave the following results:

(1.) In water: 4·402 solution at 19° gave 0·4696 salt; hence 1 part is soluble in 8·4 parts of water at 19°.

(2.) In absolute alcohol: 10·3924 solution at 20° gave 0·029 salt; hence 1 part is soluble in 357·3 parts of absolute alcohol at 20°.

Petit and Polonowsky give the solubility in 95 per cent. alcohol as 1 in 135, and of pilocarpine nitrate as 1 in 146. If this were so, the order of solubility of these salts in alcohol would be the reverse of that in water, which is improbable, and, as above shown, is not the case. On combustion, the dry salt afforded the following figures:

0·2578 gave 0·462 CO_2 and 0·1494 H_2O. C = 48·87; H = 6·43.
$C_{11}H_{16}O_2N_2,HNO_3$ requires C = 48·65; H = 6·32 per cent.

Isopilocarpine Hydrochloride can be obtained from the nitrate by the usual methods, and is best crystallised by adding ether to an alcoholic solution, as the salt is extremely soluble both in water and in absolute alcohol. The pure air-dried salt melts at 127° (corr.), and, unlike pilocarpine hydrochloride, is not hygroscopic, but stable in the air. It contains half a molecular proportion of water of crystallisation as shown by the following analysis:

0·3374, dried at 110° until constant, lost 0·012 H_2O. H_2O = 3·5.
$(C_{11}H_{16}O_2N_2,HCl)_2,H_2O$ requires H_2O = 3·55 per cent.

A determination of its specific rotation gave the following result:

$$\alpha_D = +1{\cdot}93^\circ;\ l = 1 \text{ dcm.};\ c = 4{\cdot}974;\ [\alpha]_D = +38{\cdot}8^\circ.$$

The anhydrous salt melts at 159° (corr.).

Isopilocarpine Hydrobromide is obtained in a similar manner to the hydrochloride by adding ether to an alcoholic solution. The crystals are anhydrous and melt at 147° (corr.). A determination of its specific rotation gave the following result:

$$\alpha_D = +1{\cdot}5^\circ;\ l = 2 \text{ dcm.};\ c = 2{\cdot}288;\ [\alpha]_D = +32{\cdot}8^\circ.$$

Isopilocarpine Picrate, prepared by adding picric acid to the aqueous solution of the hydrochloride and recrystallising from water or alcohol, forms long, brilliant yellow needles which melt at 161° (corr.).

Isopilocarpine Aurichloride is formed by adding a solution of auric chloride to a solution of pure isopilocarpine hydrochloride, and allowing the amorphous precipitate to become crystalline. The crystals are anhydrous and melt at 158—159° (corr.). On analysis:

0·1888 gave 0·0678 Au. Au = 35·91.

$C_{11}H_{16}O_2N_2,HAuCl_4$ requires Au = 35·96 per cent.

Isopilocarpine Gold Chloride is prepared by boiling the aurichloride in water, when crystals separate exactly as in the case of the corresponding pilocarpine salt; these melt at 185—186° (corr.). On analysis:

0·1564 gave 0·0604 Au. Au = 38·61.

$C_{11}H_{16}O_2N_2,AuCl_3$ requires Au = 38·5 per cent.

Isopilocarpine Platinichloride, obtained by the usual method, crystallises in orange scales, which melt at 226—227° (corr.) with blackening and effervescence. On analysis:

0·2508 gave 0·0592 Pt. Pt = 23·60.

$(C_{11}H_{16}O_2N_2)_2,H_2PtCl_6$ requires Pt = 23·58 per cent.

Isopilocarpine Methiodide is obtained by dissolving pure dry isopilocarpine in excess of methyl iodide, allowing it to stand, then removing the excess of methyl iodide, and recrystallising the residue from absolute alcohol until pure. It melts at 114° (corr.). A determination of the specific rotation gave the following result:

$$\alpha_D = +0{\cdot}83^\circ;\ l = 1 \text{ dcm.};\ c = 2{\cdot}734;\ [\alpha]_D = +30{\cdot}4^\circ.$$

On analysis, the following result was obtained:

0·2597 required 7·6 c.c. $N/10$ silver nitrate for precipitation. I = 37·1.

$C_{11}H_{16}O_2N_2,CH_3I$ requires I = 36·23 per cent.

Properties of Isopilocarpine.

Isopilocarpine can be obtained by a similar method to that used for pilocarpine, and the two bases possess very similar properties. Petit and Polonowsky have described it as forming large crystals, which are very hygroscopic, and soluble in all proportions in water and in alcohol I have not obtained it in a crystalline form, but have found that it can be distilled in a vacuum without decomposition, and forms a colourless, viscid oil, becoming quite fluid on warming. It boils at 261° under 10 mm. pressure, and the distillate yields a nitrate melting at 159°, thus proving that the base distils unchanged. The specific rotation was observed with the same precautions as detailed under pilocarpine, with the following results :

$\alpha_D =$ +5°; $l = 1$ dcm.; $c = 11{\cdot}652$; $[\alpha]_D =$ +42·91°.
$\alpha_D =$ +2·8°; $l = 1$ dcm.; $c =$ 6·555; $[\alpha]_D =$ +42·7°.
Mean of two determinations $[\alpha]_D =$ +42·8°.

Three determinations of the specific rotation of the base, to which a molecular proportion of sodium hydroxide had been added, showed the solution to be inactive, and on adding excess of alkali no activity was observed.

Copper Isopilocarpate could not be obtained pure, though evidence of its existence was obtained. An aqueous solution of pure isopilocarpine was boiled with an excess of freshly precipitated copper hydroxide, filtered, and the blue filtrate evaporated to dryness ; this was extracted with chloroform, the chloroform solution precipitated with ether, and the mother liquor evaporated to dryness. All three products were then analysed, with the following results :

(1). Portion insoluble in chloroform :
0·0744 gave 0·0068 CuO. Cu = 7·3 per cent.
(2). Portion soluble in chloroform, but precipitated by ether :
0·0888 gave 0·01 CuO. Cu = 8·99 per cent.
(3). Portion soluble in the mixture of chloroform and ether :
0·7238 gave 0·0192 CuO. Cu = 2·12 per cent.
$(C_{11}H_{15}O_3N_2)_2Cu$ requires Cu = 12·47 per cent.

The last product was chiefly isopilocarpine, but the others contained copper isopilocarpate.

Isopilocarpine Argento-nitrate.—On adding excess of silver nitrate in aqueous solution to an aqueous solution of isopilocarpine, an oily precipitate was obtained which contained silver, but could not be obtained crystalline.

Existence of Isopilocarpine in the Alkaloids obtained from Jaborandi Leaves and in the Pilocarpine Nitrate of Commerce.

The settlement of this question was one of the most important points in this inquiry, and unexpectedly proved to be a most difficult and tedious task. Harnack, and more recently Merck, have stated that the alkaloid yielding the crystalline nitrate accompanying pilocarpine is pilocarpidine, $C_{10}H_{14}N_2O_2$, and it has been generally assumed that any impurity in pilocarpine nitrate would be pilocarpidine nitrate, or less likely jaborine nitrate. Petit and Polonowsky, on the other hand, state that the impurity existing in the pilocarpine nitrate of commerce, sometimes to the extent of 50—60 per cent., is isopilocarpine nitrate, and that this alkaloid is found in the leaves. There are thus apparently two opposite statements, both supported by experimental proof. The only proof adduced by Petit and Polonowsky as to the identity of isopilocarpine prepared from pilocarpine with that obtained from the leaves, or from the pilocarpine nitrate of commerce, is that auric chloride behaves in precisely the same manner with isopilocarpine obtained from the first two sources.

My experiments prove conclusively that the impurity existing in the pilocarpine nitrate *at present* on the market, and in the alkaloid prepared from jaborandi leaves *at present* obtainable is isopilocarpine, identical with that formed from pilocarpine, and further that isopilocarpine exists ready formed in the leaves.

Two samples of pilocarpine nitrate from different manufacturers were used, and a quantity of impure pilocarpine nitrate was prepared from the jaborandi leaves at present in the market, called Maranham jaborandi. The process of purification employed was one of repeated fractional crystallisation until a product of constant melting point was obtained. All three products behaving similarly, they were at a certain stage mixed, and the results obtained therefore relate to all three specimens.

By repeated fractional recrystallisation, a nitrate was obtained of melting point 146°, which was not changed by two recrystallisations, and when obtained in two fractions both melted at 146°; this was regarded as a pure product, and therefore was converted into the hydrochloride, from which, by recrystallisation from absolute alcohol, and finally by fractional recrystallisation from alcohol and ether, a quantity of pure pilocarpine salt was separated. A hydrochloride, however, was obtained melting at 150°, which was unchanged by two recrystallisations from alcohol and ether, and by collection in two fractions. From this product, the aurichloride, platinichloride, picrate, and base were prepared, and the hydrochloride, auri-

chloride, and platinichloride analysed. In all cases, melting points and specific rotations were obtained, differing but slightly from those of the corresponding isopilocarpine salts, and the analytical results agreed exactly with those required for the latter base. On recrystallisation of the hydrobromide and nitrate, however, the product was found to still contain pilocarpine; and from the nitrate—which was expected to melt at 146°, but which actually melted at 150°—by recrystallising it seven times, pure isopilocarpine was finally obtained. It had thus been necessary to recrystallise the nitrate and hydrochloride about 20 times and in about 40 fractions before obtaining a pure product and a satisfactory result.

The proof of the identity of the base with isopilocarpine was furnished by the physical constants and analyses of the base, nitrate, hydrochloride, hydrobromide, picrate, aurichloride, gold chloride, and platinichloride.

In view of the importance of this point, the analytical data are here given; the melting points in all cases agreed exactly with those recorded for the salts of isopilocarpine. The agreement of these figures likewise affords proof of the correctness of the constants previously given.

Base obtained from impure pilocarpine nitrate.

Specific Rotation of Base:

$$\alpha_D = +1{\cdot}5°;\ l = 1 \text{ dcm.};\ c = 3{\cdot}549;\ [\alpha]_D = +42{\cdot}2°.$$

With excess of alkali, $\alpha_D = 0°$.

Nitrate.—Specific rotation:

$$\alpha_D = +6{\cdot}1°;\ l = 2 \text{ dcm.};\ c = 8{\cdot}784;\ [\alpha]_D = +34{\cdot}72°.$$

The solubility in water and in alcohol was determined, with the following results:

Solubility in water: 6·401 of solution at 19° gave 0·7262 salt; hence 1 part is soluble in 7·8 parts of water at 19°.

Solubility in absolute alcohol: 15·48 of solution at 19° gave 0·045 salt; hence 1 part is soluble in 343 parts of absolute alcohol at 19°.

Hydrochloride.—Specific rotation:

$$\alpha_D = +2{\cdot}7°;\ l = 1 \text{ dcm.};\ c = 7{\cdot}036;\ [\alpha]_D = +38{\cdot}3°.$$

0·7278 lost 0·0242 H_2O when the air-dried salt was dried until of constant weight. $H_2O = 3{\cdot}32$ per cent.

Hydrobromide.—Specific rotation:

$$\alpha_D = +0{\cdot}75°;\ l = 1 \text{ dcm.};\ c = 2{\cdot}182;\ [\alpha]_D = +34{\cdot}3°.$$

Aurichloride.—Analysis: 0·1956 gave 0·0704 Au. Au = 35·99.

$C_{11}H_{16}O_2N_2,HAuCl_4$ requires Au = 35·96 per cent.

Gold Chloride.—Analysis : 0·0516 gave 0·0198 Au. Au = 38·37.
$C_{11}H_{16}N_2O_2,AuCl_3$ requires Au = 38·51 per cent.

Platinichloride.—Analysis : 0·12 salt gave 0·0282 Pt. Pt = 23·5.
$(C_{11}H_{16}O_2N_2)_2,H_2PtCl_6$ requires Pt = 23·58 per cent.

These figures, together with the melting points, afford conclusive proof of the identity of the base obtained from impure pilocarpine nitrate with isopilocarpine prepared from pilocarpine by the action of heat or alkali.

The mother liquors from the nitrate last obtained were then examined, and a base isolated which at first was thought to be a new alkaloid with a nitrate melting at 144°, as this constant was not altered by four recrystallisations. By preparing the picrate, however, and recrystallising several times, the base was found to be a mixture of pilocarpine and isopilocarpine.

In order to obtain further evidence that these mixtures, which in some respects behaved as a pure substance, consisted of pilocarpine and isopilocarpine, four mixtures of the nitrates were made in certain proportions and recrystallised from alcohol. The results fully confirmed those previously obtained.

(1) A mixture of 90 per cent. of pure pilocarpine nitrate and 10 per cent. of pure isopilocarpine nitrate, after one crystallisation, melted at 170°, and after five recrystallisations yielded pure pilocarpine nitrate.

(2) A mixture of equal parts of the two nitrates melted indefinitely at 144—147°, and after five recrystallisations yielded an impure product melting at 160—165°, which seemed to be unaltered by further recrystallisation.

(3) A mixture of 33 parts of pilocarpine nitrate and 66 parts of isopilocarpine nitrate melted at 144°, and this melting point was not altered by three recrystallisations.

(4) A mixture of 90 per cent. isopilocarpine nitrate and 10 per cent. pilocarpine nitrate melted first at 157°, and after two recrystallisations yielded pure isopilocarpine nitrate.

These experiments fully explain the difficulties above-mentioned, and also a statement that pure pilocarpine nitrate melting at 141·7° was not changed by recrystallisation (Paul and Cownley, *loc. cit.*). The alkaloid prepared by me from the leaves was not subjected to any conditions that would convert any appreciable amount of the pilocarpine into isopilocarpine; the latter, however, was found to be present, although only in small quantity, and it certainly exists in the leaves.

III. Pilocarpidine.

Having failed to obtain pilocarpidine from either the pilocarpine nitrate of commerce or from the leaves, I procured a small quantity of the nitrate from Merck, and after verifying Merck's statements regarding its composition and properties, made another very careful search for the base both in pilocarpine nitrate of commerce and in jaborandi leaves, but with negative results.

Salts and Properties of Pilocarpidine.

Pilocarpidine Nitrate, $C_{10}H_{14}O_2N_2,HNO_3$, as obtained from Merck was in large, prismatic crystals which melted at 135° (corr.). It was purified by recrystallisation from absolute alcohol until of constant melting point, and then melted at 137° (corr.). The salt is anhydrous, and determinations of its specific rotation and solubility gave the following results :

$$\alpha_D = +5{\cdot}2°\ ;\ l = 1\text{ dcm.}\ ;\ c = 7{\cdot}104\ ;\ [\alpha]_D = +73{\cdot}2°.$$

Solubility in water : 0·935 solution gave 0·3154 salt ; hence 1 part is soluble in 2 parts of water at 15°.

Solubility in absolute alcohol : (i) 5·6152 solution gave 0·0672 salt ; hence 1 part is soluble in 82·56 parts of absolute alcohol at 15°.

(ii) 6·0958 solution gave 0·0756 ; hence 1 part is soluble in 79·63 parts of absolute alcohol at 15°. Mean = 1 in 81·1.

It was noted that this nitrate was much more easily decomposed by heat than that of the other bases.

Pilocarpidine Aurichloride.—On adding auric chloride to a somewhat dilute solution of pilocarpidine nitrate, no precipitate was obtained, but crystals separated on spontaneous evaporation. These were recrystallised from glacial acetic acid, and melted at 124—125° (corr.).

Pilocarpidine Platinichloride was prepared in the usual way, and was obtained in yellow needles, which, when air-dried, melted at 88—89° (corr.), but when rendered anhydrous by heating at 80° for some time, then just above 100°, melted at 187° (corr.). The air-dried salt gave, on analysis, the following result :

0·1796, dried at 80° until constant, lost 0·1048 H_2O. $H_2O = 8{\cdot}3$.

0·1508 gave 0·0342 Pt. Pt = 22·67 per cent.

$(C_{10}H_{14}O_2N_2)_2,H_2PtCl_6,4H_2O$ requires $H_2O = 8{\cdot}28$; Pt = 22·39 per cent.

The anhydrous salt was also analysed :

0·1648 gave 0·0402 Pt. Pt = 24·39.

$(C_{10}H_{14}O_2N_2)_2,H_2PtCl_6$ requires Pt = 24·41 per cent.

Pilocarpidine Picrate came down as an oil on attempting to crystallise from hot water the precipitate obtained by adding picric acid to the aqueous solution of the nitrate, and all attempts to crystallise it failed. This behaviour is very different from that of the picrates of pilocarpine and isopilocarpine, which crystallise with ease.

Specific Rotation of the Base.—An endeavour was made to determine this constant by a similar method to that employed for pilocarpine, but owing to the readiness with which pilocarpidine undergoes decomposition, a satisfactory result was not obtained. The nitrate was therefore dissolved in a little water, a molecular proportion of caustic soda added, the solution made up to a definite volume, and the rotation observed; then another molecular proportion of alkali was added, and the rotation again taken. The results were as follows:

$$\alpha_D = +1{\cdot}25^\circ;\ l = 1 \text{ dcm.};\ c = 1{\cdot}5374;\ [\alpha]_D = +81{\cdot}3^\circ.$$

with alkali:

$$\alpha_D = +0{\cdot}5^\circ;\ l = 1 \text{ dcm.};\ c = 1{\cdot}419;\ [\alpha]_D = +35{\cdot}2^\circ.$$

Pilocarpidine thus behaves similarly to the other bases examined.

Non-existence of Pilocarpidine in Pilocarpine Nitrate of Commerce and in Jaborandi Leaves.

The properties which differentiated pilocarpidine from the other bases were the great solubility of the nitrate in water or alcohol, and the formation of an amorphous picrate. The nitrate was repeatedly fractionated from absolute alcohol and from mother liquors, and the product having the lowest melting point was carefully examined for pilocarpidine by forming the platinichloride and the picrate.

From 28 grams of pilocarpine nitrate of commerce, a nitrate was obtained melting at 143°, and the mother liquors from this fraction gave a platinichloride melting at 218°, this being the melting point of pilocarpine platinichloride. The fraction melting at 143° crystallised in two fractions and gave melting points of 150° and 143° respectively. There was thus no evidence obtained of the existence of pilocarpidine in this sample of pilocarpine nitrate.

From the alkaloid obtained by working up a large quantity of the leaves, 90 grams of a nitrate melting from 149—160° were obtained. This was converted into hydrochloride, recrystallised from absolute alcohol, and the alkaloid from the mother liquors converted into nitrate, which was treated as above described. The final fraction gave a nitrate melting at 143°, yielding an amorphous picrate, which afterwards, however, became mostly crystalline. The crystals proved to be chiefly pilocarpine picrate. The alkaloid was regenerated from the amorphous picrate and converted into the platinichloride, which

was amorphous, did not melt at 250° and was therefore not pilocarpidine. It was thus proved that the jaborandi leaves at present obtainable, consisting chiefly of *P. microphyllus*, contain no pilocarpidine.

IV. Jaborine.

This alkaloid has been described by Harnack and Meyer as existing in the mother liquors of pilocarpine, and as being an amorphous base yielding amorphous salts. It is stated to be less soluble in water, and more soluble in ether, than pilocarpine, and to be physiologically antagonistic to it, resembling atropine in its action. An attempt was made to isolate this alkaloid, if present, by its sparing solubility in water and its solubility in ether, since the other bases are not precipitated from their salts by ammonia, and are almost insoluble in ether. Indications of such an alkaloid had been obtained, as in some cases a precipitate was produced by ammonia. From the total alkaloid extracted by chloroform from a quantity of the leaves of *P. pennatifolius*, the only other variety obtainable besides *P. microphyllus*, resin was removed by dissolving it in alcohol and pouring into acidified water. The filtrate was then made alkaline with ammonia, the precipitate collected, redissolved in acidified water, and reprecipitated with ammonia, this operation being repeated several times, and the precipitate finally extracted with ether; a very small residue was obtained, which was not soluble in a little cold water, and did not seem to be basic. The yield of this substance was 2 per cent. of the total alkaloid, and it did not agree at all with the description given of jaborine.

A very large quantity of mother liquors from pilocarpine, prepared from *P. microphyllus*, treated in a similar manner, gave an ethereal extract which, when converted into a nitrate, yielded a crystalline salt melting at 143°, and consisted of pilocarpine and isopilocarpine, whilst the residue from the mother liquor gave only the faintest milkiness on adding ammonia.

The search for jaborine in these two varieties of leaves having thus proved to be abortive, a specimen of jaborine was obtained from Merck, and examined in the following manner. The product, which was a light brown oil smelling strongly of chloroform, was dissolved in a little absolute alcohol and poured into dilute acid, when a milkiness appeared and a slight scum separated. On adding ammonia, no precipitate was obtained; the liquid was therefore extracted three times with ether, and the ethereal extract, after washing with water, yielded on evaporation a very light coloured residue, amounting to 3 per cent. of the original substance. The aqueous liquid was further extracted with chloroform, which, on evaporation, left an

extract like the original substance. This amorphous residue was converted into nitrate, and by evaporation from alcoholic solution gave a crystalline product mixed with a little amorphous colouring matter. The crystals gave no precipitate with ammonia, melted at 142°, and on recrystallisation gave a salt melting at 148°. This formed a crystalline picrate from which, by recrystallisation, isopilocarpine picrate melting at 161° (corr.) was isolated.

The mother liquors from this salt gave an amorphous picrate, from which the base was regenerated and converted into nitrate, which contracted at 137° and melted at 141°. This alkaloid was therefore pilocarpidine, containing possibly a little pilocarpine.

It follows therefore that jaborine is not a homogeneous substance, but a mixture of isopilocarpine, pilocarpidine, and possibly pilocarpine, and, furthermore, that no alkaloid answering the description of jaborine can be found in the leaves.

It may be well at this point to offer what seems to be the correct explanation of the discrepancies existing between the statements of different chemists. When pilocarpine was first discovered, the source was *P. jaborandi*, containing pilocarpine, isopilocarpine, which was overlooked, and pilocarpidine. Within recent years, however, *P. jaborandi* has become scarce, and is not obtainable now in any quantity. It has been replaced by Maranham jaborandi from *P. microphyllus*, which contains no pilocarpidine. It has been assumed that the impurity accompanying pilocarpine nitrate was pilocarpidine nitrate, but in reality it was isopilocarpine nitrate. Petit and Polonowsky in their investigations discovered the isopilocarpine, but did not find pilocarpidine, and overlooked the possibility that pilocarpidine, as described by Harnack and by Merck, might exist, although not in the alkaloids they examined. Similarly, although Merck knew and was in possession of true pilocarpidine nitrate, he also overlooked the possibility of the existence of a third alkaloid, isopilocarpine, and described it as altered pilocarpine. All the experimental data support this explanation.

With regard to jaborine, although it is possible that such an alkaloid as described existed in *P. jaborandi*, it is certain that no such alkaloid exists in the jaborandi leaves at present obtainable, and that the jaborine of commerce is not a homogeneous substance, but a mixture of the three alkaloids with a little colouring matter. Furthermore, seeing that this alkaloid was described as amorphous, and no detailed examination made of it, it is possible that the alkaloid actually examined was an impure pilocarpine, and the balance of probability seems to be in favour of this explanation. Physiological experiments confirm the results of the chemical investigation with respect to the nature of jaborine.

V. Constitution of Pilocarpine.

Allusion was made in the introduction to this paper to the structural formula proposed by Hardy and Calmels for pilocarpine and to the unsatisfactory character of the evidence on which it was based. I have repeated some of their experiments with quite different results, and therefore give the details of these and of some further preliminary work on this question. I wish, however, to reserve the subject of the constitution of the alkaloid for a future communication. Hardy and Calmels' formula is chiefly based on the supposed production of trimethylamine from pilocarpine by certain reactions; I have been unable to obtain any evidence of the formation of this base in any of my experiments. Leaving the question of the nature of the isomerism of pilocarpine and isopilocarpine to a later date, most of the present experiments have been made with carefully purified isopilocarpine nitrate, this being the more stable of the two isomerides.

Action of Water and Alkali on Pilocarpine.

It has been stated that pilocarpine, on boiling with water, yields methyl alcohol, trimethylamine, and β-pyridinelactic acid.

0·8 gram of pure pilocarpine was heated with water in a sealed tube at 180° for 4 hours. On opening the tube, and distilling, no trace of methyl alcohol or volatile alkali was found, whilst the residual liquid gave a quantitative yield of isopilocarpine nitrate, identified by its melting point, namely, 159°.

Treatment with 20 per cent. caustic potash solution in a similar manner gave an identical result.

The only product of this action is therefore isopilocarpine.

Distillation of the Base with Soda-lime.

Harnack and Meyer state that trimethylamine is formed in this reaction. One gram of pure pilocarpine nitrate was mixed with large excess of soda-lime, then strongly heated in a Jena glass tube, and the volatile products drawn by an aspirator through two wash bottles containing a known quantity of normal hydrochloric acid.

The acid was then titrated back with normal soda, and it was found that 6 c.c. of acid were neutralised, the theoretical amount required being 7·3 c.c. acid. The liquid was then made alkaline with caustic soda and distilled, when a distinct smell of ammonia and pyridine was noted. The distillate was acidified with hydrochloric acid, evaporated to dryness, and the resulting hydrochloride extracted with

absolute alcohol. Two portions were obtained, one of which was soluble, the other insoluble, in alcohol.

(1.) The substance insoluble in alcohol was ammonium chloride. The platinum salt was made and analysed.

0·27 gave 0·118 Pt. Pt = 43·7.

$(NH_3)_2,H_2PtCl_6$ requires Pt = 43·91 per cent.

(2.) The hydrochloride soluble in alcohol. On adding auric chloride to the aqueous solution, a yellow precipitate was obtained which quickly became crystalline, and when dried melted at 185° (corr.). The salt was analysed with the following result:

0·181 gave 0·0818 Au. Au = 45·2.

$C_6NH_6,HAuCl_4$ requires Au = 45·64 per cent.

The melting point of the aurichlorides of both 2- and 3-methylpyridine is about 184°, so the bases formed are ammonia and either 2- or 3-methylpyridine.

Oxidation of Isopilocarpine with Permanganate.

Hardy and Calmels state that pilocarpine, on oxidation with permanganate, yields ammonia, a little methylamine, and β-pyridinetartronic acid, with traces of β-pyridinecarboxylic acid, which was precipitated with copper acetate. This experiment does not prove the presence of a trimethylamine group in the molecule.

4·5 grams of isopilocarpine nitrate, dissolved in a litre of water, were oxidised with 15·6 grams or 6 mols. of potassium permanganate dissolved in a litre of water, the whole warmed on a water-bath, and left overnight. The manganese dioxide was filtered off and washed thoroughly, the filtrate neutralised with hydrochloric acid, concentrated by evaporation, made alkaline with caustic soda, and distilled. The volatile bases were neutralised with hydrochloric acid, evaporated to dryness, and then separated by extraction with absolute alcohol. The larger portion was insoluble in alcohol, and proved to be ammonium chloride. The smaller portion was soluble in alcohol, and very hygroscopic; the platinichloride melted on heating, and, on analysis, proved to be the salt of methylamine.

0·077 gave 0·0324 Pt. Pt = 42.

$(CH_3{\cdot}NH_2)_2,H_2PtCl_6$ requires Pt = 41·3 per cent.

The residue, after distillation, was neutralised with sulphuric acid, evaporated to dryness, and extracted with alcohol. The alcoholic extract gave no precipitate with copper acetate, auric chloride, or potassio-mercuric iodide. Two oils, however, have been separated

from it, one of which has the properties of a lactone. These products are being further investigated.

Action of Fused Caustic Potash on Isopilocarpine.

1 gram of pilocarpine nitrate was mixed with a little aqueous potash and gradually added to 10 grams of fused caustic potash in a Jena glass tube, arranged as described in the experiment of distillation with soda-lime. The fused mass was very slightly discoloured. The volatile products, on titration, required 4 c.c. of normal hydrochloric acid. This is almost the theoretical quantity for half the nitrogen contained in pilocarpine. The hydrochloride was evaporated to dryness and heated with absolute alcohol, in which part dissolved and part was insoluble.

The portion insoluble in absolute alcohol was ammonium chloride. The platinichloride was analysed with the following result:

0·073 gave 0·0322 Pt. Pt = 44·1.

$(NH_3)_2,H_2PtCl_6$ requires Pt = 43·9 per cent.

The portion soluble in absolute alcohol was hygroscopic, and proved to be methylamine hydrochloride; it gave a fusible platinichloride, which was analysed with the following result:

0·0368 gave 0·0154 Pt. Pt = 41·8.

$(CH_3{\cdot}NH_2)_2,H_2PtCl_6$ requires Pt = 41·3 per cent.

The fused mass was now acidified with dilute sulphuric acid and distilled, when a product smelling strongly of isobutyric acid, with a trace of acetic acid, was obtained. The distillate was digested with freshly precipitated silver hydroxide, filtered, and the filtrate concentrated, when crystals were obtained which were slightly reduced. On analysis:

0·1482 gave 0·0852 Ag. Ag = 57·4 per cent.

The residue, after distillation, was extracted with ether; the ethereal extract, on evaporation, yielded an acid having the unmistakable odour of isobutyric acid. The silver salt of this product was analysed with the following result:

0·017 gave 0·0098 Ag. Ag = 57·6.

$C_4H_9O_2Ag$ requires Ag = 55·4 per cent.

Although the results of these analyses are high for isobutyric acid, yet this is probably present, with perhaps a trace of acetic acid, which would slightly raise the percentage of silver. The reaction is being further investigated.

Action of Caustic Potash on Pilocarpine and Isopilocarpine Methiodides.

About 2 grams of the methiodide were dissolved in 20 per cent. aqueous caustic potash, and heated in a sealed tube at 180° for 4 hours. The product was then distilled, the volatile bases collected, and the amount determined by titration with normal hydrochloric acid. The hydrochlorides were obtained by evaporation of the aqueous solution and examined. The liquid was now acidified with sulphuric acid and distilled, the distillate examined for volatile acids, and the acid liquid then extracted with ether for acids soluble in this medium. The liquid was afterwards made alkaline with ammonia, and extracted with chloroform for fixed bases. The residue was neutralised and evaporated to dryness, as it was intended to extract it with alcohol to see if any other product was formed, but the iodides present interfered with the operation, and it was abandoned.

The following results were obtained:

(1.) With pilocarpine.

Volatile bases. The hydrochloride was entirely soluble in absolute alcohol and was obtained in three fractions, each fraction being converted into the platinichloride and analysed, giving Pt = 41·1, 41·1, and 41·0 per cent. respectively. The base was homogeneous and was methylamine. No acid or other product was obtained and the final residue gave no precipitate with copper acetate.

(2.) With isopilocarpine.

Volatile bases. The hydrochloride was entirely soluble in absolute alcohol and the platinichloride obtained in three fractions was analysed, giving Pt = 41·2, 41·2, and 41·4 per cent. respectively, thus proving the base to be methylamine.

A trace of hydrocyanic acid was found, but no other product and no substance giving a precipitate with copper acetate could be isolated. The pyridinecarboxylic acids give a precipitate with copper acetate, and this reagent is generally used for separating and identifying them.

Acetyl chloride has no action on these alkaloids, and they are remarkably stable towards nitric acid. Cold fuming nitric acid has no action on either base, but on warming, pilocarpine is converted into isopilocarpine, which is not attacked even by warm fuming nitric acid unless the action be prolonged.

Preliminary physiological experiments on isopilocarpine, pilocarpine, and pilocarpidine have been made by Prof. Marshall, of University College, Dundee, who reports that the substances produce similar

effects on the secretory activity of the sweat and salivary glands, pilocarpine being much the most powerful. Isopilocarpine is comparatively less depressant to the hearts of rabbits, but not to those of cats or man. The jaborine of Merck possesses a mild pilocarpine effect.

Having finished the characterisation of this group of alkaloids and repeated the work previously done on the subject, I am now engaged on experiments dealing with the constitution of pilocarpine, and hope soon to be in a position to communicate the results to the Society.

THE WELLCOME CHEMICAL RESEARCH LABORATORIES.

ERRATA.

Page 481, Line 10 from top, reading :

$a_D = + 0.6°$; $l = 1$ dcm. ; $c = 2.8376$; $[a]_D = + 21.15°$,

should be brought *under* the following line instead of *above* it :

(5) Boiled with excess of caustic soda for 10 minutes.

Page 484, Line 9 from top, *for* Pilocarpidine *read* Pilocarpine.

RICHARD CLAY AND SONS, LIMITED, LONDON AND BUNGAY.