

LXXIX.—*The Constitution of Pilocarpine. Part I.*

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IN a previous communication (this vol., 494), it was shown that the constitutional formula previously proposed for pilocarpine by Hardy and Calmels must be abandoned, and an account was given of certain preliminary experiments on the constitution of isopilocarpine. Since then, Pinner and Kohlhammer (*Ber.*, 1900, 33, 1424) have confirmed these conclusions, and have described several compounds of pilocarpine with bromine, as well as a new crystalline product, bromocarpinic acid, $C_{10}H_{15}O_4N_2Br$. The present paper contains a full account of the isolation and identification of the products formed by the oxidation of isopilocarpine with permanganate, and of the reactions of the base with soda lime, fused caustic potash, and methyl iodide.

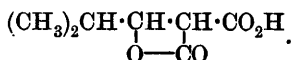
In attacking the problem of the constitution of pilocarpine, two difficulties are encountered, first, the remarkable stability of isopilocarpine towards many reagents, for example, caustic potash or nitric acid, and secondly, the high price of the alkaloid, which renders it necessary to work with small quantities of material. Although it is not yet possible to propose a formula for the alkaloid, yet, from the results recorded in this paper, the existence of certain groups in the molecule can be shown to be highly probable.

From the fact that isopilocarpine only with difficulty forms salts with metallic hydroxides, such as sodium, barium, and copper hydroxides, as well as from its behaviour towards hot alkali, it is legitimate to conclude that it contains a lactone group. In many reactions, the nitrogen is eliminated as ammonia and methylamine, and when isopilocarpine methiodide is treated with caustic potash, methylamine alone is formed. Di-alkyl derivatives of isopilocarpine could not be prepared, thus confirming Chastaing's observation (*Compt. rend.*, 1885, 101, 507), and since methyl iodide may be supposed to act as an acid towards a base, it must be assumed that of the two nitrogen atoms, the one which confers the basic character on isopilocarpine is evolved as ammonia, whilst the other is split off as methylamine, and does not act as basic nitrogen. Herzig and Meyer (*Monats.*, 1898, 19, 56) have stated that one methyl group is attached to a nitrogen atom, and since methylisopilocarpine cannot be further methylated, it must be inferred that the nitrogen atoms occur as $:NH$ and $:N \cdot CH_3$, the former being that to which the basic character of isopilocarpine is due.

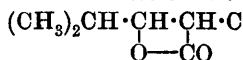
Further, the facts, that isopilocarpine is stable towards acids or alkalis and at high temperatures, and that when methylisopilocarpine

methiodide is decomposed by potash, methylamine and not dimethylamine is formed, show that these groups are attached to the rest of the molecule by a much stronger affinity than is the case in tropine, in which, by similar reactions, the nitrogen atom is eliminated, after successive methylations, as trimethylamine by simple heating with water. By distillation of isopilocarpine with soda-lime, a picoline, probably 3-methylpyridine, is formed in addition to ammonia and methylamine, but only in very small quantity. As the preparation of a sufficient quantity of the methylpyridine for its complete identification would have necessitated the use of large quantities of valuable material, and as its production in such small quantity, by so drastic a reaction, would not afford much clue to the constitution of the alkaloid, the investigation of this reaction was not further pursued.

By oxidation with permanganate, in addition to acetic acid, an acid, $C_7H_{10}O_4$, has been isolated, which proved on titration to be lactonic. It was not identical with any acid of this formula previously described, but, from the fact that the corresponding unsaturated dibasic acid yielded isobutyric acid on ultimate oxidation, the most probable formula would appear to be



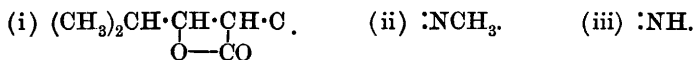
This would necessitate the existence of the complex



in isopilocarpine.

The formation of isobutyric acid by fusion of isopilocarpine with caustic potash, which is proved in this paper, and the lactonic nature of the alkaloid would thus be explained.

The results of the present investigation therefore indicate the existence of the following groups in pilocarpine and isopilocarpine :



EXPERIMENTAL.

Distillation of Isopilocarpine with Soda Lime.

Four grams of isopilocarpine nitrate were treated with 50 grams of soda-lime as previously described (*loc. cit.*, 494), and the ammonium salt separated from the hydrochlorides soluble in absolute alcohol. The latter were dissolved in water, excess of auric chloride added, and the crystalline precipitate recrystallised from hot dilute hydrochloric acid. The filtrate from the precipitated aurichloride contained methylamine, which was isolated as the platinichloride and analysed.

0.0358 fused on heating and gave 0.0148 Pt. Pt = 41.34.

$(\text{CH}_3 \cdot \text{NH}_2)_2, \text{H}_2\text{PtCl}_6$ requires Pt = 41.3 per cent.

The aurichloride, amounting to less than 0.2 gram, melted at 185–187° (corr.), contained Au = 45.71 per cent. (methylpyridine aurichloride requires Au = 45.64 per cent.), and yielded a crystalline picrate which melted at about 130°, and a crystalline platinichloride melting indistinctly at 200°. When oxidised with permanganate, an acid was formed which gave a precipitate with copper acetate, but no coloration with ferrous sulphate; the quantity obtained was too small to admit of identification. These properties agree best with those of 3-methylpyridine.

The bases formed by distillation with soda-lime, therefore, are ammonia and small amounts of methylamine and (probably 3-) methylpyridine.

Interaction of Isopilocarpine with Methyl Iodide.

As isopilocarpine under certain conditions loses ammonia, and as it forms a methiodide, experiments were undertaken similar to those carried out on tropine (Merling, *Ber.*, 1891, 24, 3108) to see whether, by successive methylation, the hydrogen atoms attached to the nitrogen could be replaced by methyl, and possibly some of the bonds broken. Pure isopilocarpine methiodide was treated with the theoretical amount of moist silver oxide and filtered, a solution of isopilocarpine methyl-hydroxide being obtained, which gave no precipitate on the addition of picric acid or platinic chloride. After boiling with these reagents and cooling, crystalline salts separated, however, and the same result was obtained if the solution of the methyl hydroxide was previously boiled with dilute hydrochloric acid. On boiling, therefore, isopilocarpine methyl-hydroxide loses water, becoming converted into methyl-isopilocarpine, which is capable of forming salts.

Methylisopilocarpine Picrate crystallises in beautiful, orange needles from hot water or alcohol, being sparingly soluble in these solvents at the ordinary temperature. The crystals melt sharply at 136° (corr.) to a clear liquid, and the melting point is not altered by further recrystallisation.

Methylisopilocarpine Platinichloride is obtained in well shaped, orange cubes which melt sharply at 218° (corr.) to a clear liquid without decomposition. It can be recrystallised from hot acidified water. On analysis:

0.1846 gave 0.0422 Pt. Pt. = 22.87.

0.2494 „ 0.0572 Pt. Pt. = 22.93.

$[\text{C}_{11}\text{H}_{15}(\text{CH}_3\text{O}_2\text{N}_2)_2], \text{H}_2\text{PtCl}_6$ requires Pt = 22.87 per cent.

The hydrochloride was obtained by boiling the methyl-hydroxide with dilute hydrochloric acid, but neither this salt nor the nitrate has yet been crystallised. The base is soluble in water but insoluble in chloroform, thus differing from isopilocarpine, which is miscible with this solvent.

Methylisopilocarpine was heated with excess of methyl iodide, and the latter removed by distillation; the syrupy residue was then treated with moist silver oxide and filtered. After boiling with dilute hydrochloric acid, it yielded a crystalline picrate and platinichloride melting at 129° and 213° respectively, or a few degrees lower in each case than the corresponding salts of methylisopilocarpine. On analysis, it was found that further methylation had not taken place and that the base was unaltered methylisopilocarpine.

0.225 platinichloride gave 0.0514 Pt. Pt = 22.84.

$[C_{11}H_{13}(CH_3)O_2N_2]_2 \cdot H_2PtCl_6$ requires Pt = 22.87 per cent.

Further heating with methyl iodide and subsequent decomposition produced a similar result, the platinichloride melting at 212° and containing Pt = 22.54 per cent. It follows therefore that by the action of methyl iodide only monomethylisopilocarpine can be produced.

The methiodide prepared as above, was treated with 20 per cent. caustic potash solution in a sealed tube for 4 hours at 150° , and the volatile bases collected and converted into the hydrochloride. The salt was entirely soluble in absolute alcohol and was precipitated in four fractions with platinic chloride. These platinichlorides were analysed with the following results:

Fraction 1. 0.2226 gave 0.0916 Pt. Pt = 41.15.

„ 2. 0.3258 „ 0.134 Pt. Pt = 41.13.

„ 3. 0.1788 „ 0.074 Pt. Pt = 41.38.

„ 4. 0.1654 „ 0.0688 Pt. Pt = 41.59.

$(CH_3 \cdot NH_2)_2 \cdot H_2PtCl_6$ requires Pt = 41.3 per cent.

The base was therefore homogeneous and was methylamine.

The explanation of these results would seem to be that there exists in isopilocarpine the group $\cdot NH$, which reacts with methyl iodide, forming $R \cdot NH \cdot CH_3I$; this with moist silver oxide yields $R \cdot NH \cdot CH_3 \cdot OH$, which on boiling with dilute acids loses water and forms $R \cdot N \cdot CH_3$.

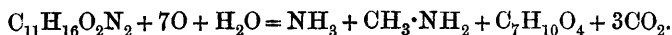
The methylated base can react with methyl iodide, forming $R \cdot NCH_3 \cdot CH_3I$, but on further treatment, the methyl iodide is eliminated, leaving the original base $R \cdot NCH_3$. With caustic potash, the nitrogen of this complex is eliminated as methylamine, together with the same base formed from the other nitrogen group.

The non-basic product of the action of potash on methylisopilocarpine is under investigation.

Nitrous acid appears to have no action on isopilocarpine.

Oxidation of Isopilocarpine with Permanganate.

In the preliminary account of this reaction (*loc. cit.*), it was shown that the bases formed were ammonia and methylamine, and that two oils had been isolated, one having the properties of a lactone. Further investigation has shown that, in addition to a small quantity of acetic acid, an almost theoretical yield of a new acid, $C_7H_{10}O_4$, is obtained. Experiments were made with varying amounts of permanganate, but the best results were obtained when 6 molecular proportions were used, the yield then amounting to 70 per cent. of the theoretical. The reaction may be expressed by the following equation :



The details of one experiment may be given. Fifty-four grams of pure isopilocarpine nitrate, dissolved in a litre of water, were oxidised at 80° by the gradual addition of 188 grams of permanganate dissolved in 5 litres of water, the whole being kept thoroughly stirred by a turbine. The manganese dioxide was removed by filtration and thoroughly washed with hot water, the colourless filtrate and washings evaporated to a low bulk, made alkaline with caustic soda, and then distilled until the distillate was no longer alkaline. The residue was neutralised with hydrochloric acid, evaporated to dryness, mixed with sand, thoroughly dried, and then extracted with hot absolute alcohol in a Soxhlet apparatus. The alcoholic liquid, after saturation with hydrogen chloride, was allowed to stand, then boiled in a reflux apparatus for 2 hours, and distilled on the water-bath.

The distillate had the distinct odour of ethyl acetate, so the first fractions were collected separately. The residue, after removal of the alcohol by distillation, was thrown into water, the acid liquid extracted several times with ether, the ethereal liquid washed with water until free from acid, dried over calcium chloride, and distilled to remove the ether.

The first fraction distilled from the alcoholic solution was hydrolysed with caustic potash, and the alcohol removed by evaporation ; the residue was then dissolved in water, acidified with dilute sulphuric acid, and distilled. The crystalline barium salt obtained from the acid distillate was converted into the crystalline silver salt by treatment with silver nitrate solution, and on analysis the following result was obtained :

0.0584 gave 0.0376 Ag. Ag = 64.4.

$C_2H_3O_2Ag$ requires Ag = 64.67 per cent.

The acid was therefore *acetic acid*, and examination of the other portions of the alcoholic distillate failed to reveal the presence of any other acid.

The ethereal residue was fractionated first in a vacuum and finally under atmospheric pressure, the following fractions being obtained :

1. Boiling at 280—299°. Yield = 5 per cent.
2. " " 299° " = 90 "
3. " " 200—210° under 10 mm. pressure. Yield = 5 per cent.

The liquid boiling at 299° was therefore pure, and on further fractionation was found to distil almost completely at this temperature. It was quite free from nitrogen. Analyses were made of a number of specimens, with the following results :

1. 0·1608 gave 0·34 CO₂ and 0·1106 H₂O. C = 57·83 ; H = 7·65.
 2. 0·1822 " 0·393 CO₂ " 0·1266 H₂O. C = 58·83 ; H = 7·72.
 3. 0·22 " 0·4712 CO₂ " 0·153 H₂O. C = 58·41 ; H = 7·73.
 4. 0·239 " 0·511 CO₂ " 0·164 H₂O. C = 58·32 ; H = 7·62.
- C₉H₁₄O₄ requires C = 58·07 ; H = 7·53 per cent.

The specific rotation of the pure liquid was determined with the following result :

$$\alpha_{15.0} = +22^{\circ} ; l = 50 \text{ mm.} ; d \ 15^{\circ}/15^{\circ} = 1.1053 ; [\alpha]_{15.0}^{20} = +39.8^{\circ}.$$

The liquid was insoluble in water, but soluble in ether or alcohol, and had a peculiar, but not unpleasant, smell. On hydrolysis, it was found to be the ethyl ester of a dibasic or lactonic acid, as with phenolphthalein as indicator it required 2 molecular proportions of alkali to effect neutralisation.

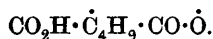
Acid C₇H₁₀O₄, formed by Oxidation.

The acid was formed from the ester by hydrolysing either with caustic potash or aqueous hydrochloric acid. It is a thick, slightly yellow oil which does not solidify at -21°, boils at 210—220° under 10 mm. pressure, and is freely soluble in water, ethyl alcohol, or benzene. All attempts to crystallise it failed. On analysis :

- 0·1826 gave 0·3514 CO₂ and 0·1094 H₂O. C = 52·48 ; H = 6·65.
 C₇H₁₀O₄ requires C = 53·16 ; H = 6·33 per cent.

When titrated in the cold with *N*/10 alkali, using phenolphthalein as indicator, 0·1678 required 10·7 c.c. *N*/10 alkali. Calculated 10·6 c.c. When titrated by boiling, first with excess of alkali, and then titrating back with acid, while still hot, 0·2234 required 26·00 c.c. *N*/10 alkali. Calculated 28·2 c.c.

The acid is therefore lactonic, and the formula may be written



A neutral solution of the potassium salt gave white, gelatinous precipitates with lead acetate and silver nitrate, the precipitate with the latter undergoing slow reduction. It gave no reaction with barium or calcium chloride, or copper acetate.

Formation of the Unsaturated Diethyl Ester.

A preliminary experiment having shown that phosphorus pentabromide reacted with the ethyl ester, the whole of the ester at disposal was treated in this way. Twelve grams of the ester and 60 grams of phosphorus pentabromide were placed in a reflux apparatus, and, after the pasty mass had gradually liquefied, the whole was gently heated. Ethyl bromide was first given off, and then hydrogen bromide. As the object of the reaction was to obtain, not the bromo-derivative, but the unsaturated ester, the heating was continued until no more hydrogen bromide was evolved. The product was then gradually added to an excess of absolute alcohol, warmed to remove ethyl bromide, poured into ice-cold water, and the aqueous liquid extracted several times with ether. The ethereal solution, after washing with water, was dried over calcium chloride and distilled. In this way, 16.6 grams of crude product was obtained, which was fractionated in a vacuum. Considerable difficulty was experienced during distillation owing to frothing of the liquid, but ultimately three fractions were obtained, boiling respectively at 165—170°, 170—195°, and 195—200° under .20 mm. pressure. There were thus at least two substances present, so the first and third fractions were examined.

The *first fraction* (b. p. 165—170°) contained a large amount of bromine, but also instantly decolorised cold alkaline permanganate solution. It consisted chiefly of the bromoethyl ester, with a small quantity of the unsaturated compound. On analysis, the following results were obtained :

0.1702 gave 0.293 CO₂ and 0.097 H₂O. C = 46.94 ; H = 6.34.

C₅H₉Br(CO₂C₂H₅)₂ requires C = 44.74 ; H = 6.44 per cent.

The *third fraction* (b. p. 195—200°) contained bromine and readily decolorised cold alkaline permanganate. It consisted chiefly of the unsaturated ethyl ester, and on analysis gave the following result :

0.2934 gave 0.0654 AgBr. Br = 9.5.

C₁₁H₁₉BrO₄ requires Br = 27.1 per cent.

In order to complete the debromination, the whole of the liquid was heated with two molecular proportions of diethylaniline in a reflux apparatus on a sand-bath for 13 hours, when, on cooling, crystals of diethylaniline hydrobromide separated. The coloured liquid was then poured into excess of dilute hydrochloric acid and extracted with

ether. The ethereal solution was washed successively with dilute acid, dilute sodium carbonate solution, and water, dried over calcium chloride, and the ether removed by distillation. The residue was distilled in a vacuum, and on fractionation the greater part came over at 155° under 10 mm. pressure, a small quantity of a liquid of higher boiling point being apparently present.

Hydrolysis of the Unsaturated Ester and Oxidation of the Acid.

As the quantity of the ester was insufficient to admit of satisfactory fractionation, the unsaturated acid was isolated, purified as far as possible, and then oxidised by Crossley and Le Sueur's method (*Trans.*, 1899, 75, 161), in the hope that identification of the products of oxidation would give a clue to the constitution of the acid.

The ester boiling at 155° under 10 mm. pressure was dissolved in methyl alcohol and treated with an equal weight of caustic potash in methyl alcoholic solution. On the addition of alkali, a red colour was produced, changing to brown. The alcoholic liquid was heated on a water-bath for $2\frac{1}{2}$ hours in a reflux apparatus, the alcohol removed by evaporation, and the residue acidified and extracted with ether. The ethereal solution, after washing with water, was dried over calcium chloride, and allowed to evaporate spontaneously. No crystals separated, but a light coloured oil was obtained, having the odour of a fatty acid. The oil distilled between 180° and 200° under 10 mm. pressure, and dissolved in sodium carbonate solution with effervescence; this solution at once decolorised permanganate at the ordinary temperature, thus affording proof of its unsaturated character (von Baeyer, *Annalen*, 1888, 245, 146). On oxidation with cold permanganate, the acid required 98 c.c. *N/10* solution (calculated 110 c.c.). After removal of the manganese dioxide, the filtrate was oxidised by chromic acid, distilled with steam and the volatile acid, which smelt strongly of a butyric acid, converted into the silver salt. On analysis, the following result was obtained:

0.126 gave 0.111 CO_2 ; 0.037 H_2O ; and 0.069 Ag. C = 24.05;
H = 3.26; Ag = 54.76.

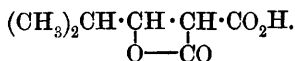
$\text{C}_4\text{H}_7\text{O}_2\text{Ag}$ requires C = 24.61; H = 3.59; Ag = 55.38 per cent.

The neutral solution gave a precipitate with calcium chloride which dissolved on warming, and separated on cooling.

The acid, therefore, is isobutyric acid; the amount is small, but additional indirect proof of its identity is furnished by the fact that isobutyric acid is formed by fusion of pilocarpine with potash.

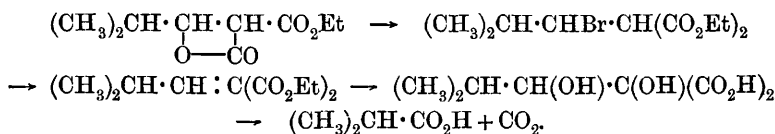
The reactions with phosphorus pentabromide and diethylaniline seem to prove conclusively the lactonic nature of the acid $\text{C}_7\text{H}_{10}\text{O}_4$ and since it does not agree with any of the known lactonic acids of

this composition, the formation of isobutyric acid would leave only one formula possible, namely,



The stability of the compound is remarkable, if it is the lactone of a β -hydroxymalonic acid; the formation of isobutyric acid, however, does not admit of the possibility of its being a γ -lactone, for if that were the case, acetone should be formed on oxidation, whereas careful search failed to detect it.

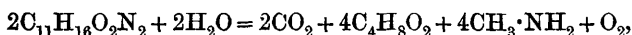
The reactions described in the preceding paragraphs may be represented as follows:



Experiments were made with pilocarpine to obtain the bromoethyl ester, by treatment with phosphorus pentabromide, but the basic character of pilocarpine interfered with the reaction, and a negative result was obtained.

Fusion of Isopilocarpine with Caustic Potash.

Chastaing (*Compt. rend.*, 1882, **94**, 223) has described the fusion of pilocarpine with caustic potash, and stated that the products of the reaction were methylamine, carbon dioxide, butyric acid, and traces of acetic acid, and that the reaction may be represented by the equation



The analytical numbers quoted for the platinichloride* prove, however, that he was working with a mixture of ammonia and methylamine, and no details are given of the identification of butyric or acetic acids.

In the preliminary account of this reaction (*loc. cit.*, 496), the bases formed were proved to be ammonia and methylamine, and the acids yielded a silver salt, which contained a higher percentage of silver than required for silver butyrate. The reaction has been further investigated. Five grams of pure isopilocarpine were fused with 50 grams of caustic potash, and the fused mass, after cooling, dissolved in water acidified with sulphuric acid, and distilled with

* Found Pt = 42.5 per cent.

$(\text{NH}_3)_2, \text{H}_2\text{PtCl}_6$ requires Pt = 43.9 and $(\text{CH}_3\cdot\text{NH}_2)_2, \text{H}_2\text{PtCl}_6$ requires Pt = 41.3 per cent.

steam. The acid liquid was extracted with ether, the ethereal solution dried over calcium chloride, and distilled.

The residual liquid, which had a strong, rancid smell, was distilled at atmospheric pressure, when a small fraction was collected below 120° , but the greater portion came over between 120° and 155° , leaving no residue. On adding a little water to the higher fraction, oily drops separated, which dissolved on the addition of more water. From the aqueous liquid, a crystalline barium salt was prepared; this was extracted with boiling alcohol, in which it was very slightly soluble.

(i) The portion dissolved by alcohol gave a white, silver salt, which was not reduced on standing. On analysis the following result was obtained :

0.0388 gave 0.0214 Ag. Ag = 55.2.

(ii) The residue after extraction with alcohol gave a crystalline silver salt, which was recrystallised from hot water. On analysis, the following result was obtained :

0.0592 gave 0.0328 Ag. Ag = 55.4.

$C_4H_7O_2Ag$ requires Ag = 55.38 per cent.

The calcium salt was soluble in warm water, but separated again on cooling. The analytical numbers, boiling point, immiscibility with water, and solubility of the calcium salt, prove conclusively that the acid formed is isobutyric acid.

The portion boiling below 120° gave a small amount of an amorphous barium salt, insoluble in alcohol, and an amorphous silver salt (Ag = 57.4 per cent.), which underwent reduction on standing. No trace of acetic acid could be found.

Further investigation of the subject is proceeding on different lines, and it is hoped, either by the use of larger quantities of material, or by synthetical methods, to bring forward further proof of the correctness, or otherwise, of the formula now proposed for the acid $C_7H_{10}O_4$.

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