Pilosine : a new alkaloid from Pilocarpus microphyllus / by Frank Lee Pyman.

CCXXXVII.—*Pilosine: A New Alkaloid from* Pilocarpus microphyllus.

By Frank Lee Pyman.

Pilocarpine, $C_{11}H_{16}O_2N_2$, its stereoisomeride *iso*pilocarpine, and pilocarpidine, $C_{10}H_{14}O_2N_2$, are the only alkaloids from the different varieties of jaborandi leaves which have been thoroughly characterised.

Pilocarpine and *iso*pilocarpine are found in most varieties. Pilocarpidine is obtained from *Pilocarpus jaborandi*, but according to Jowett (Trans., 1900, **77**, 473) does not occur in *P. microphyllus*.

Now, Herzig and Meyer (*Monatsh.*, 1898, **19**, 56) have shown that pilocarpidine does not contain an *N*-methyl group, and it is probable that this alkaloid is the parent base of pilocarpine containing an imino-group in the place of the *N*-methyl group. In accordance with this view it has been found that pilocarpidine, in common with other glyoxalines containing a free imino-group, gives a deep red coloration with sodium diazobenzene-*p*-sulphonate (Pauly's histidine reagent). It was then found that the mother liquors from *Pilocarpus microphyllus* containing the alkaloids remaining after the separation of pilocarpine and *iso*pilocarpine also gave a well-marked red coloration with sodium diazobenzene-*p*-sulphonate. A large quantity of such residues (representing many tons of leaves) being available it was thought of interest to subject them to a fresh investigation following the movements of the base containing the free imino-group with the help of this colour reaction.

After a lengthy and tedious process of separation, depending largely on the different solubilities of the bases in water, the residues gave (1) resinous matter, (2) a sparingly soluble oil giving an intense Pauly reaction, (3) a sparingly soluble crystalline base, which has been designated *pilosine*—this base, which was obtained in a yield amounting to 0·007 per cent. of the leaves, did not give the Pauly reaction; (4) a mixture of pilocarpine and *iso*pilocarpine; and (5) the mother liquor from (4), which contained the remaining readily soluble bases, and gave only a moderate Pauly reaction.

Pilocarpidine if present in these leaves would have been in fraction 5, but it was clear that the base responsible for the Pauly reaction was mainly concentrated into fraction 2, and it seemed not unlikely that the colour given by fraction 5 was due to the same compound. In any case, all attempts to isolate pilocarpidine from the last fraction were unsuccessful, and Jowett's statement that *P. microphyllus* does not contain pilocarpidine is thus confirmed.

The base contained in fraction 2, which gives the Pauly reaction, has not yet been isolated in a crystalline form, but the new crystalline alkaloid from the third fraction, *pilosine*, has been characterised and investigated.

Constitution of Pilosine and its Derivatives.

Pilosine has the empirical formula $C_{16}H_{18}O_3N_2$, and is a monacid optically active base. Its salts do not crystallise readily, but the following have been obtained in crystalline form: *sulphate*, $(C_{16}H_{18}O_3N_2)_2,H_2SO_4$; *hydrogen tartrate*, $C_{16}H_{18}O_3N_2,C_4H_6O_6$, and *aurichloride*, $C_{16}H_{18}O_3N_2,HAuCl_4$.

Pilosine gives no coloration with sodium diazobenzene-*p*-sulphonate; it contains an *N*-methyl, but no methoxyl group. It also contains a lactonic grouping, which accounts for two of the three oxygen atoms. The third oxygen atom occurs in the form of a hydroxyl group, for pilosine yields on treatment with acetic anhydride a new unsaturated base, *anhydropilosine*, $C_{16}H_{16}O_2N_2$, by the removal of the elements of water.

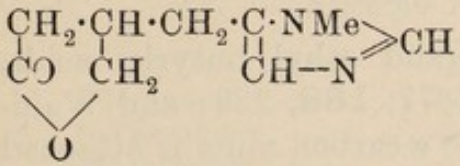
Anhydropilosine is a monacid, optically active base, forming beautifully crystalline salts, of which several have been prepared, and contains a lactonic grouping.

Pilosine dissolves in warm 5 per cent. aqueous potassium hydroxide, and can be recovered from this solution mainly unchanged, even after boiling for half an hour, although an odour of benzaldehyde indicating some decomposition is produced. When pilosine is distilled, however, with aqueous potassium hydroxide, kept at a strength of 20 per cent., for several hours, benzaldehyde passes over, and a new base, *pilosinine*, $C_9H_{12}O_2N_2$, remains in the alkaline liquid, the reaction being expressed by the following equation:

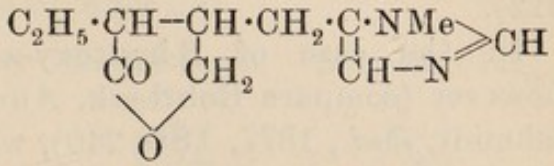
$$C_{16}H_{18}O_3N_2 = C_6H_5{\cdot}CHO + C_9H_{12}O_2N_2.$$

Pilosinine is a monacid, optically active, crystalline base, forming a well-crystallised *nitrate*, $C_9H_{12}O_2N_2,HNO_3$, and *hydrochloride*, $C_9H_{12}O_2N_2,HCl$. It contains an *N*-methyl group, and a lactonic grouping. It can be distilled unchanged, does not immediately

decolorise aqueous acid permanganate, but decolorises bromine in chloroform solution. Its chemical properties are thus very similar to those of pilocarpine and *iso*pilocarpine; its solubilities, and physiological properties are also similar, and its parent compound, pilosine, occurs in the same plant which produces these glyoxaline derivatives. It is therefore highly probable that pilosinine itself is not only a glyoxaline derivative, but a lower homologue of pilocarpine and *iso*pilocarpine, having the constitutional formula given below:



Pilosinine.

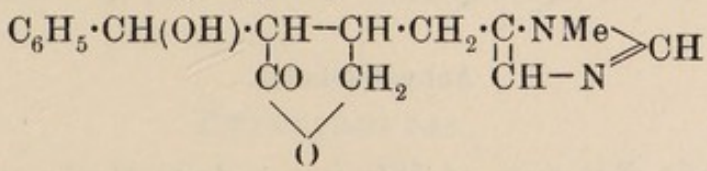


Pilocarpine.

Pilosinine is here represented as a 1:5-glyoxaline derivative owing to its connexion with pilocarpine and *iso*pilocarpine; there is, however, no direct evidence that it is a 1:5- rather than a 1:4-glyoxaline derivative.

The above formula satisfactorily accounts for all the known properties of pilosinine, and explains the similarity of its behaviour with that of pilocarpine and *iso*pilocarpine. It also permits of the construction of a probable formula for pilosine.

Since pilosine is decomposed into benzaldehyde and pilosinine on boiling with strong aqueous sodium hydroxide, and yields anhydropilosine when dehydrated by means of acetic anhydride, it must contain the grouping $C_6H_5 \cdot CH(OH)$ attached to a carbon atom, which also bears a hydrogen atom. This grouping must, therefore, displace a hydrogen atom in one of the three methylene groups of the pilosinine molecule in such a way as to give rise to a formula for pilosine capable of explaining the ready decomposition of this alkaloid into pilosinine and benzaldehyde. This condition is fulfilled only in the case where pilosine is represented as derived from pilosinine by the displacement by $C_6H_5 \cdot CH(OH)$ of one of the hydrogen atoms of the methylene group in the α-position with reference to the carboxylic group:



Pilosine.

for, if the $C_6H_5 \cdot CH(OH)$ group substituted either of the other two methylene groups, it would give rise to compounds which would not be expected to decompose into benzaldehyde and pilosinine.

The formula for pilosine given above, however, represents pilosine

as the lactone of a β-hydroxy-carboxylic acid, and it is well known that in certain circumstances β-hydroxy-acids are capable of decomposition in this manner.

Thus, β-hydroxy-acids, in which the α-carbon atom is doubly alkylated, yield on distillation an aldehyde and a carboxylic acid, for instance,

$$CH_3\cdot CH(OH)\cdot CEt_2\cdot CO_2H = CH_3\cdot CHO + CHEt_2\cdot CO_2H$$

(compare Schnapp, *Annalen*, 1880, **201**, 70; Jones, *ibid.*, 1884, **226**, 287; and Blaise and Marcilly, *Bull. Soc. chim.*, 1904, [iii], **31**, 312).

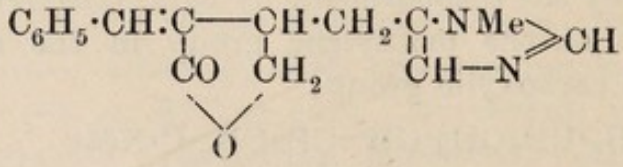
In the case of β-hydroxy-α-methyl(and ethyl)-butyric acids, however (compare Rohrbeck, *Annalen*, 1877, **188**, 229; and Waldschmidt, *ibid.*, 1877, **188**, 240), where the α-carbon atom is attached to a hydrogen atom—as in the case of pilosine—distillation leads to the formation of the corresponding unsaturated acid:

$$CH_3\cdot CH(OH)\cdot CHMe\cdot CO_2H = CH_3\cdot CH{:}CMe\cdot CO_2H + H_2O.$$

It appears, nevertheless, that the mode of decomposition of β-hydroxy-acids, of which the α-carbon atoms are attached to a hydrogen atom, is influenced by the presence of a negative substituent such as the phenyl group; thus, Perkin (Trans., 1886, **49**, 160) has shown that an odour of benzaldehyde is developed on heating β-hydroxy-β-phenyl*iso*butyric acid. This indicates that the decomposition of this compound proceeds in some degree at least in accordance with the following equation:

$$C_6H_5\cdot CH(OH)\cdot CHMe\cdot CO_2H = C_6H_5\cdot CHO + CH_2Me\cdot CO_2H.$$

The formula for pilosine given above is thus established with some degree of probability, and it is interesting to note that the $C_6H_5\cdot CH(OH)$ group in this alkaloid occupies the same position with regard to the rest of the molecule that the C_2H_5 group occupies in the case of pilocarpine and *iso*pilocarpine. This formula satisfactorily accounts for all the known properties of pilosine, and leads to the following formula for anhydropilosine:



Anhydropilosine.

Specific Rotation of Pilosine and its Derivatives.

Pilocarpine, *iso*pilocarpine, and pilocarpidine are dextrorotatory in the form of base or salts with acids, and less dextrorotatory in the form of the alkali salts of the corresponding hydroxy-acid.

Pilosine and pilosinine are also dextrorotatory in the form of

both base and salts with acids, whilst anhydropilosine is dextrorotatory as base, but lævorotatory as salt; all three alkaloids are lævorotatory in the form of the alkali salts of the corresponding hydroxy-acid.

The specific rotation of the three alkaloids as base, salt with acid, and salt with alkali calculated in each case for base is given below, together with the corresponding values for pilocarpine, *iso*pilocarpine, and pilocarpidine, calculated from Jowett's determinations.

Specific Rotation of Base as:

	Base.	Salt with acid.	Salt with alkali.
Pilocarpine.....	+100·5°	+108·0°	+31·5°
*iso*Pilocarpine.....	+42·8	+46·5	±0·0
Pilocarpidine	+81·3	+97·0	+35·2
Pilosine	+39·9	+24·6	−67·6
Anhydropilosine ...	+66·2	−20·8	−132·7
Pilosinine	+14·2	+5·8	−5·8

Jowett has shown that the difference between pilocarpine and *iso*pilocarpine is due to the change of sign of one of the two optically active carbon atoms, pilocarpine readily yielding *iso*pilocarpine on treatment with alkali. Now pilosine can be recovered mainly unchanged after boiling for some time with dilute aqueous sodium hydroxide, and it is therefore clear that pilosine and its derivatives correspond in stereochemical relations rather with *iso*pilocarpine than with pilocarpine.

Physiological Action of Pilosine and its Derivatives.

Dr. P. P. Laidlaw, of the Wellcome Physiological Research Laboratories, kindly tested the physiological action of these alkaloids with the following results:

Pilosine has a very weak pilocarpine action, since it produces a typical but very weak inhibition of the cat's heart when given in 20 milligram doses. Anhydropilosine shows a similar behaviour.

Pilosinine has a mild pilocarpine action. It inhibits the heart of frogs and cats, and causes a fair reaction of saliva. It is somewhat stronger in this respect than pilosine, but it is still very much weaker than pilocarpine.

Experimental.

Isolation of Pilosine from Pilocarpus microphyllus.

The residual syrup from several tons of *Pilocarpus microphyllus* leaves from which the pilocarpine and *iso*pilocarpine had been removed as completely as possible during manufacture, was diluted with water, and fractionally precipitated with ammonia. This

produced a precipitation, first of dark-coloured, then of light-coloured oil; the mother liquors, which gave no further turbidity on the addition of ammonia, were then extracted with chloroform, which removed a considerable amount of water-soluble alkaloid, from which pilocarpine and *iso*pilocarpine were isolated in the usual way, the mother liquor then returning to the fractionation process.

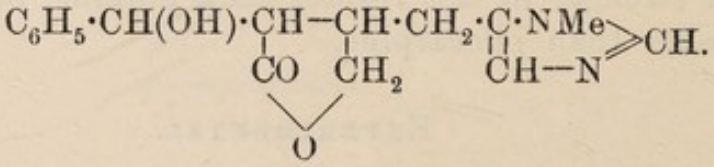
The dark- and light-coloured oils mentioned above were dissolved in dilute acid and again fractionally precipitated with ammonia, when the coloured impurities became largely concentrated in the earliest fractions. The later fractions of the sparingly soluble oil, when dissolved in a little alcohol, readily deposited crystals of a new alkaloid, *pilosine*, and the aqueous mother liquor gave up further quantities of water-soluble alkaloid to chloroform. The pilosine was purified by recrystallising from alcohol, and the mother liquors when they no longer deposited crystals were returned to the fractionation process, and the whole of these operations were again repeated many times.

Eventually, the following products were obtained: (1) non-basic or faintly basic, black, resinous matter; (2) a viscid syrup, sparingly soluble in water, giving an intense red coloration with Pauly's reagent; (3) a quantity of pilosine; these were obtained from the fractions precipitated as sparingly soluble oils on the addition of ammonia to the aqueous solutions of their salts.

From the chloroform extract of the mother liquors there were obtained: (4) a quantity of the mixed nitrates of pilocarpine and *iso*pilocarpine, and (5) a syrupy residue which gave a moderate red coloration with Pauly's reagent.

The results of the investigation, therefore, show that *Pilocarpus microphyllus*, which yields up to 0·99 per cent. of pilocarpine nitrate (Carr and Reynolds, *Pharm. J.*, 1908, **80**, 542) and small quantities of *iso*pilocarpine, contains also about 0·007 per cent. of pilosine. No other alkaloid is present in greater amount than 0·003 per cent. A sparingly soluble base giving the Pauly reaction is present, but this does not appear to be pilocarpidine.

Pilosine,



Pilosine crystallises from alcohol in large, colourless plates, which melt at 187° (corr.). It suffers no loss at 100°:

0·1516 gave 0·3722 CO_2 and 0·0864 H_2O. C = 66·9; H = 6·4.

0·1514 „ 12·6 c.c. N_2 at 24° and 773 mm. N = 9·8.

$C_{16}H_{18}O_3N_2$ requires C = 67·1; H = 6·4; N = 9·8 per cent.

0·1272, titrated with dilute sulphuric acid using methyl-orange, required 4·4 c.c. $N/10$-H_2SO_4; whence equivalent = 289.

$C_{16}H_{18}O_3N_2$ requires M.W. = 286.

Pilosine is sparingly soluble in cold, fairly readily so in hot, water; sparingly soluble in cold, and easily so in hot, alcohol; and very sparingly soluble in boiling chloroform, ether, ethyl acetate, acetone, or benzene.

It dissolves readily in dilute acids, but not in cold dilute alkalis; it is soluble in hot aqueous sodium hydroxide. It gives no coloration with sodium diazobenzene-*p*-sulphonate.

Pilosine contains one NMe but no OMe group, since, on heating with hydriodic acid, methyl iodide is not eliminated below about 280°:

0·4436 gave 0·3598 AgI. NMe = 10·0.

$C_{15}H_{15}O_3N(NMe)$ requires NMe = 10·1 per cent.

The presence of a lactonic grouping was shown as follows:

A suspension of the base in cold water was rendered alkaline to phenolphthalein on the addition of a single drop of $N/10$-sodium hydroxide. After boiling, however, with excess of $N/10$-sodium hydroxide and titrating with $N/10$-sulphuric acid, it was found that 0·2328 required 8·1 c.c. $N/10$-NaOH, 8·1 c.c. being the required figure for one lactonic grouping, and the base was eventually recovered unchanged.

Pilosine, like pilocarpine and *iso*pilocarpine, is dextrorotatory, determinations of its specific rotation giving the following results:

in chloroform: α_D +0·94°; l = 2 dcm.; c = 1·168; $[\alpha]_D$ +40·2°;
in absolute alcohol: α_D +0·66°; l = 2 dcm.; c = 0·827; $[\alpha]_D$ +39·9°.

Pilocarpine is converted into its stereoisomeride *iso*pilocarpine by prolonged boiling with aqueous sodium hydroxide, and in order to determine whether pilosine underwent a similar change a quantity was boiled with excess of 5 per cent. aqueous sodium hydroxide for half an hour, then acidified with hydrochloric acid, and precipitated with ammonia. The base, which was obtained in nearly quantitative yield, was examined without further purification, when it melted at 187° (corr.), and gave the following result in chloroform solution:

α_D +0·61°; l = 2 dcm.; c = 0·762; $[\alpha]_D$ +40·0°.

Pilosine, therefore, is not readily altered by treatment with alkali, behaving in this respect like *iso*pilocarpine.

The specific rotatory power of pilocarpine falls from +100·5° to +31·5°, and that of *iso*pilocarpine from +42·8° to *nil* after remaining with excess of alkali, owing to the formation of salts of the corresponding hydroxy-acids; a similar change occurs even more

markedly in the case of pilosine. After heating pilosine for ten minutes on the water-bath with two molecules of potassium hydroxide in aqueous solution, the following result was obtained:

α_D −5·10°; l=2 dcm.; c=3·774; $[\alpha]_D$ −67·6°.

The salts of pilosine do not crystallise readily on the whole, but the sulphate, acid tartrate, and aurichloride have been obtained in crystalline form. The hydrochloride, hydriodide, nitrate, picrate, and acid oxalate failed to crystallise on keeping.

Pilosine Sulphate.—On evaporating a neutral aqueous solution of pilosine sulphate to a viscid syrup, and stirring with absolute alcohol, this salt is obtained in crystalline form, and may be recrystallised from absolute alcohol. It separates in clusters of plates, which melt at 194—195° (corr.). This salt is very easily soluble in water, very sparingly so in cold absolute alcohol, and is anhydrous:

0·1486 gave 0·3128 CO_2 and 0·0780 H_2O. C=57·4; H=5·87.

0·1596 „ 0·0568 $BaSO_4$. SO_4=14·6.

$(C_{16}H_{18}O_3N_2)_2H_2SO_4$ requires C=57·3; H=5·7; SO_4=14·3 per cent.

A determination of the specific rotation in aqueous solution gave the following result:

α_D +1·87°; l=2 dcm.; c=4·454; $[\alpha]_D$ +21·0°.

Pilosine hydrogen tartrate remains as a syrup on evaporation of its aqueous solution; this becomes crystalline on stirring with absolute alcohol, and melts at 135—136° (corr.). It tends to separate from hot absolute alcohol at first as an oil, which gradually changes into prismatic crystals:

0·1792 gave 0·3606 CO_2 and 0·0912 H_2O. C=54·9; H=5·7.

$C_{16}H_{18}O_3N_2,C_4H_6O_6$ requires C=55·1; H=5·6 per cent.

A determination of its specific rotatory power gave the following result:

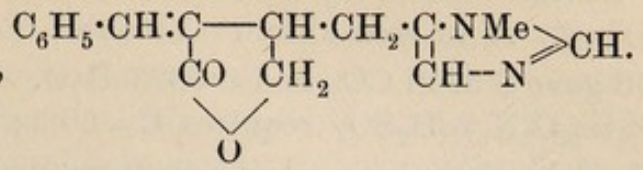
α_D +1·85°; l=2 dcm.; c=3·838; $[\alpha]_D$ +24·2°.

Pilosine aurichloride was precipitated from an aqueous solution of the hydrochloride on the addition of gold chloride as an oil, which became partly crystalline on keeping. It crystallises from glacial acetic acid in clear, golden, wedge-shaped plates, which melt at 143—144° (corr.). This salt is very sparingly soluble in water or cold glacial acetic acid, and is anhydrous:

0·2047 gave 0·0641 Au. Au=31·3.

$C_{16}H_{18}O_3N_2,HAuCl_4$ requires Au=31·5 per cent.

Anhydropilosine,



Pilosine loses the elements of water when boiled for a short time with acetic anhydride; if undiluted acetic anhydride is used, a certain amount of charring takes place, and the quality and quantity of the resulting product, anhydropilosine, is unsatisfactory. On the other hand, boiling with glacial acetic acid does not dehydrate pilosine. By boiling pilosine, however, for a short time with a mixture of equal volumes of acetic anhydride and glacial acetic acid, anhydropilosine is readily obtained in nearly theoretical yield.

Ten grams of pilosine, 20 c.c. of glacial acetic acid, and 20 c.c. of acetic anhydride were boiled for half-an-hour under a reflux condenser. The product was diluted with water, and mixed with a slight excess of ammonia, when most of the anhydropilosine was precipitated, and became crystalline on seeding and stirring; the remainder was obtained by extracting the liquor with chloroform. Anhydropilosine crystallises from ethyl acetate in clusters of colourless rods, which melt at 133—134° (corr.). It suffers no loss at 100°:

0·1682 gave 0·4400 CO_2 and 0·0896 H_2O. C = 71·4; H = 6·0.

$C_{16}H_{16}O_2N_2$ requires C = 71·6; H = 6·0 per cent.

Anhydropilosine is sparingly soluble in cold, readily so in hot, water; it is very readily soluble in alcohol, acetone, benzene, chloroform, or warm ethyl acetate; fairly readily so in cold ethyl acetate, and sparingly soluble in ether. Unlike pilosine, it immediately decolorises aqueous permanganate.

The presence of a lactonic grouping in this compound was shown, as in the case of pilosine:

0·1805 required 6·4 c.c. *N*/10-NaOH, 6·7 c.c. being required for one lactonic grouping.

Anhydropilosine is dextrorotatory, a determination of its specific rotation in 95 per cent. alcohol giving the following result:

α_D +4·73°; l = 2 dcm.; c = 3·571; $[\alpha]_D$ +66·2°.

After heating anhydropilosine for ten minutes in the water-bath with two molecules of potassium hydroxide in aqueous solution, the following result was obtained:

α_D −8·34°; l = 2 dcm.; c = 3·142; $[\alpha]_D$ −132·7°.

Anhydropilosine forms salts which crystallise readily.

Anhydropilosine sulphate separates from absolute alcohol in clusters of prisms, which melt at 174° (corr.) after sintering a few

degrees earlier. It is anhydrous, and is very easily soluble in water, but sparingly so in cold absolute alcohol:

0·1658 gave 0·3652 CO_2 and 0·0823 H_2O. C=60·1; H=5·5.

$(C_{16}H_{16}O_2N_2)_2,H_2SO_4$ requires C=60·5; H=5·4 per cent.

A determination of its specific rotatory power in aqueous solution gave the following result:

α_D −1·43°; l=2 dcm.; c=4·064; $[\alpha]_D$ −17·6°.

Anhydropilosine nitrate crystallises from water in large, tabular prisms, which melt and decompose at 153—154° (corr.). It is anhydrous, and is very readily soluble in water, but sparingly so in cold alcohol:

0·1605 gave 0·3405 CO_2 and 0·0734 H_2O. C=57·8; H=5·1.

$C_{16}H_{16}O_2N_2,HNO_3$ requires C=58·0; H=5·2 per cent.

A determination of its specific rotatory power in aqueous solution gave the following result:

α_D −1·38°; l=2 dcm.; c=3·806; $[\alpha]_D$ −18·1°.

Anhydropilosine hydrogen oxalate crystallises from alcohol in clusters of flat needles, which melt at 153—154° (corr.). It is anhydrous, and is very easily soluble in water, but sparingly so in cold alcohol:

0·1726 gave 0·3802 CO_2 and 0·0800 H_2O. C=60·1; H=5·2.

$C_{16}H_{16}O_2N_2,C_2H_2O_4$ requires C=60·3; H=5·1 per cent.

A determination of its specific rotatory power in aqueous solution gave the following result:

α_D −1·46°; l=2 dcm.; c=4·093; $[\alpha]_D$ −17·8°.

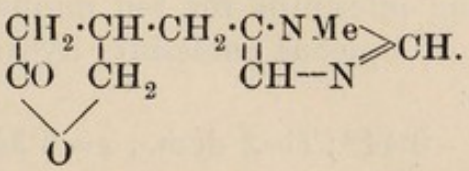
Action of 20 per Cent. Potassium Hydroxide on Pilosine. Formation of Pilosinine and Benzaldehyde.

Twenty grams of pilosine were distilled with a solution of 50 grams of potassium hydroxide in 250 c.c. of water, the level of the liquid in the distillation flask being kept constant by the addition of water from a tap-funnel. The condensed distillate contained drops of benzaldehyde during the first few minutes only, but after eleven hours' continuous distillation, when some 4 litres had been collected, the distillate still had the characteristic odour of benzaldehyde, and gave a faint turbidity with aqueous phenylhydrazine acetate.

The distillation was stopped at this point, and the whole of this distillate mixed with an excess of aqueous phenylhydrazine acetate, when 9·6 grams of benzaldehydephenylhydrazone (m. p. 158°. Found, C=79·5; H=6·2. Calc., C=79·5; H=6·2 per cent.) were obtained, that is, 70 per cent. of the theoretical yield.

The colourless alkaline distillation residue was acidified by the addition of 100 grams of 50 per cent. sulphuric acid, boiled for five minutes (to cause lactone formation), then made alkaline with ammonia, and filtered from a little insoluble matter. The ammoniacal solution was then completely extracted with chloroform, and the latter distilled, when a quantity of nearly pure pilosinine base was obtained. This was crystallised as nitrate, and gave 10·0 grams of the pure salt. The ammoniacal liquor still contained a further quantity of pilosinine as the ammonium salt of the corresponding hydroxy-acid, and was therefore again boiled with dilute mineral acid, made alkaline with ammonia, and extracted with chloroform, this process being again repeated. The additional quantities of pilosinine thus obtained gave another 2·8 grams of the pure nitrate, so that 12·8 grams of this salt were obtained in all, that is, 75 per cent. of the theoretical yield. The ultimate mother liquors when again distilled with concentrated aqueous potassium hydroxide and worked up as before, gave a further quantity of pilosinine nitrate.

Pilosinine,



This base is isolated by extraction with chloroform from a solution of the nitrate made alkaline with ammonia. After removal of the solvent it remains as an oil, which becomes crystalline on stirring. It crystallises from anhydrous ethyl acetate in broad needles or plates, which melt at 78—79° (corr.):

0·1640 * gave 0·3585 CO_2 and 0·0974 H_2O. C = 59·6; H = 6·6.

$C_9H_{12}O_2N_2$ requires C = 60·0; H = 6·7 per cent.

Pilosinine is slightly deliquescent, very readily soluble in water, alcohol, chloroform, or acetone; moderately soluble in cold, and readily in hot, ethyl acetate, and sparingly soluble in dry ether. It distils in the neighbourhood of 300°/35 mm., passing over as a nearly colourless oil, which crystallises on seeding. It does not immediately decolorise aqueous acid permanganate, and gives no coloration with sodium diazobenzene-*p*-sulphonate. It decolorises bromine in chloroform solution, an insoluble, brown oil (perbromide) separating from the solution, as in the case of other glyoxaline derivatives.

The presence of a lactonic grouping in this compound was shown, as in the case of pilosine:

* Dried in a vacuum.

0·1821 required 10·1 c.c. $N/10$-NaOH, 9·7 c.c. being required for one lactonic grouping.

Pilosinine is dextrorotatory, a determination of its specific rotatory power in a freshly-made-up aqueous solution giving the following result:

$$\alpha_D +1·15°;\ l=2 \text{ dcm.};\ c=4·062;\ [\alpha]_D +14·2°.$$

The rotation of aqueous solutions of pilosinine, however, rapidly sinks on keeping, doubtless owing to the gradual conversion of the lactone into the corresponding hydroxy-acid; thus the specific rotatory power of the solution mentioned above was redetermined, after keeping, with the following results:

after twenty-four hours, $[\alpha]_D +9·8°$,
after forty-eight hours, $[\alpha]_D +7·8°$,
after five days, ... $[\alpha]_D +3·1°$.

This behaviour is analogous to that of pilocarpine, of which the rotation in 7 per cent. aqueous solution fell from $+100·5°$ to $+77·5°$ after keeping for three weeks according to Jowett (Trans., 1900, **77**, 481).

After heating pilosinine for ten minutes in the water-bath with two molecules of aqueous potassium hydroxide, the following result was obtained:

$$\alpha_D -0·44°;\ l=2 \text{ dcm.};\ c=3·941;\ [\alpha]_D -5·8°.$$

Pilosinine nitrate crystallises from water in large, clear, colourless prisms, which melt at 165—167° (corr.). It is soluble in about 2·5 parts of cold water, readily soluble in hot water, but sparingly so in alcohol. It is anhydrous:

0·1668 gave 0·2700 CO_2 and 0·0821 H_2O. C = 44·2; H = 5·5.
0·1406 „ 21·2 c.c. N_2 and 24° and 766 mm. N = 17·6.
$C_9H_{12}O_2N_2,HNO_3$ requires C = 44·4; H = 5·4; N = 17·3 per cent.

A determination of its specific rotation in aqueous solution gave the following result:

$$\alpha_D +0·73°;\ l=2 \text{ dcm.};\ c=8·412;\ [\alpha]_D +4·3°.$$

Pilosinine hydrochloride separates from water and alcohol in prismatic crystals, which melt at 218—219° (corr.). It is readily soluble in water, but somewhat sparingly so in cold alcohol:

0·2670 gave 0·1765 AgCl. Cl = 16·4.
$C_9H_{12}O_2N_2,HCl$ requires Cl = 16·4 per cent.

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