

The constitution of pseudo-muscarine ("synthetic muscarine") / by A.J. Ewins.

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Ewins, Arthur James.
Wellcome Physiological Research Laboratories.

Publication/Creation

London : Wellcome Physiological Research Laboratories, [1914?]

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No. 82

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PSEUDO-MUSCARINE
("SYNTHETIC MUSCARINE")

BY

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(Reprinted from "The Biochemical Journal," Vol. VIII, No. 2, 1914)

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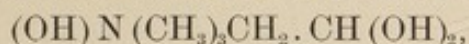


XXVII. THE CONSTITUTION OF PSEUDO-MUSCARINE ("SYNTHETIC MUSCARINE").

BY ARTHUR JAMES EWINS.

*From the Wellcome Physiological Research Laboratories, Herne Hill, S.E.**(Received March 12th, 1914.)*

Pseudo-muscarine or "synthetic muscarine" as the base has generally been termed was first obtained by Schmiedeberg and Harnack [1877]. Schmiedeberg and Koppe [1869] had obtained from the "Fly Agaric" (*Amanita muscaria*) a very powerfully active base "muscarine," which in its chemical behaviour closely resembled choline with which it was found associated in the fungus. Harnack [1876] showed that the muscarine obtained by Schmiedeberg and Koppe was still mixed with choline, and effected a purification of the base by repeated recrystallisation of the aurichloride. From the analyses of this salt he assigned to muscarine the formula $C_8H_{15}O_3N$, and on account of the close chemical relationship of the base to choline and its presence in the fungus with the latter base, suggested that its constitution could be expressed by the formula



muscarine thus being represented as a hydrated aldehyde derived from choline.

This hypothesis was apparently confirmed a little later when Schmiedeberg and Harnack [1877], by evaporating to dryness a solution of choline (or still better the platinichloride of the base) in concentrated nitric acid, obtained a base which they stated was chemically and physiologically indistinguishable from the natural muscarine obtained from *Amanita muscaria*. This base was, therefore, regarded as "synthetic" muscarine, and the supposition that muscarine was indeed an oxidation product of choline was apparently confirmed.

This straightforward solution of the problem of the constitution of muscarine was however shown to be incorrect by Boehm [1885] who found that "synthetic" muscarine, while closely resembling the natural base in physiological action, in some respects differed markedly, the most important

differences being that the synthetic base showed a powerful curare-like action which the natural base does not possess, and that the synthetic base did not constrict the mammalian pupil whereas natural muscarine does.

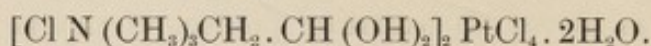
The problem was still further complicated by the fact that the aldehyde corresponding to choline, the so-called "betaine aldehyde" which was first synthesised by Berlinerblau [1884] and later by E. Fischer [1893] (who proved its constitution by oxidising it to betaine), was found to differ considerably in its action from both the natural and "synthetic" muscarines.

The problem was later re-investigated by Nothnagel [1894]. He obtained both natural and "synthetic" muscarine; the former from *Amanita muscaria*, the latter by Schmiedeberg and Harnack's method from choline. He too stated that so far as could be judged from the small amount of material available, the natural muscarine was chemically identical with the synthetic base, and confirmed Schmiedeberg and Harnack's formula for the latter. He also repeated and confirmed Berlinerblau's work. The physiological action of these bases was examined by Hans Meyer who confirmed Boehm's observations with regard to synthetic and natural muscarine and in addition pointed out further differences in their action.

Briefly then it appeared that there were two chemically indistinguishable bases which possessed different physiological actions and for this no satisfactory explanation has as yet been put forward.

Recently during an investigation into the nature of a muscarine-like base which is present in extracts of ergot, and was found to be acetyl-choline, as already described [Ewins, 1914], I had occasion to prepare some pseudo-muscarine for purposes of comparison and it occurred to me that, in view of the facts detailed above, confirmation of the constitution of this base was desirable, and that further examination might throw some light on the general problem. The preparation of the base gave no difficulty and a pure crystalline platinichloride was obtained, the base from which showed all the characteristic physiological effects¹ of synthetic muscarine as described by Boehm [1885], Hans Meyer [v. Nothnagel, 1894] and Honda [1911].

The formula assigned by Schmiedeberg and Harnack to their platinichloride and confirmed by Nothnagel was as follows:

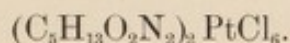


The salt thus contained two molecules of water of crystallisation. It is to be noted, however, that these were not completely removed, except by heating to a temperature of 130–135°.

¹ The physiological experiments involved in this investigation were in all cases carried out by Dr H. H. Dale.

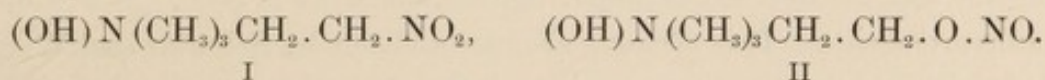
As a preliminary step it was decided to attempt to confirm the above formula. The platinichloride was obtained "air dry" by standing in an ordinary desiccator over calcium chloride. The salt then remained unaltered in weight when placed *in vacuo* in a desiccator over sulphuric acid, and when heated under ordinary pressure in a steam bath (98°). Loss of weight occurred when the salt was heated to a temperature of about 115° , but it was found that by heating to $130-135^{\circ}$ the loss of weight was considerably greater than that required for the loss of two molecules of water, and it was evident that the salt was undergoing decomposition. Finally it was found that the air dry platinichloride could be heated *in vacuo* at 100° for some hours without undergoing any loss of weight; a fact which afforded conclusive evidence of the absence of water of crystallisation.

An examination of the analytical data recorded by Schmiedeberg and Harnack, as well as by Nothnagel, further showed that there was no record of a determination of the nitrogen content of the molecule, although an otherwise complete analysis had been carried out. The platinichloride was accordingly analysed for nitrogen according to Dumas' method, when figures were obtained showing the presence of four nitrogen atoms in the molecule, i.e. two atoms of nitrogen in each molecule of free base. Accepting the remaining analytical data as more or less accurate for the supposedly hydrated platinichloride the actual formula for this salt was presumably the following:



Subsequently complete analysis proved this to be correct.

For the constitution of this base the two most probable formulae were the following:



Formula (I) is that of an aliphatic nitro-derivative, the nitrogen being directly linked to a carbon atom while (II) represents the base as the choline ester of nitrous acid. That formula (II) correctly represents the constitution of the base is shown by the following facts:

In the first place the base gives a typical Liebermann's nitroso-reaction with phenol and sulphuric acid, which would not be the case if it were a nitro derivative. In the second place, on hydrolysis of the base by means of dilute acid or alkali, nitrous acid is very readily liberated, and choline may be recovered from the solution.

This base was actually obtained and recognised as such by Nothnagel

[1894] (who termed it "nitroso-choline") in the preparation of pseudo-muscarine by Schmiedeberg and Harnack's method. He considered this substance, however, to be a bye-product of the reaction, chiefly on account of a difference of crystalline form of the platinichlorides of the base and of pseudo-muscarine respectively. It was, however, remarked by Schmiedeberg and Harnack [1877] that the platinichloride of pseudo-muscarine (synthetic muscarine) which crystallises ordinarily in octahedra might also form "Federfahne ähnliche Aggregaten," a description which corresponds with that of "federbartartige Krystallen" recorded by Nothnagel for the platinichloride of "nitroso-choline."

That the supposed difference of constitution of these two bases in reality had very little foundation is supported by the analytical figures recorded by Nothnagel [1894] and shown in the following table:

Pseudo-muscarine platinichloride $[N(CH_3)_3CH_2 \cdot CH(OH)_2]_2$ $PtCl_6 \cdot 2H_2O$ Found	Nitroso-choline platinichloride $[N(CH_3)_3CH_2CH_2O \cdot NO]_2$ $PtCl_6 \cdot 2H_2O$ Found	Choline nitrous ester platinichloride (anhydrous) Calculated
C = 17.66	17.69	17.8
H = 4.92	3.70	3.85
N —	8.31	8.31
Cl = 31.07	—	31.6
Pt = 28.54, 28.60, 28.44	28.73	28.93
Aurichloride	Aurichloride	Aurichloride
Au = 42.66	Au = 41.79	Au = 41.7

It will be observed that Nothnagel also formulates the "nitroso-choline" platinichloride as containing two molecules of water of crystallisation which, he states, were not removed by long heating at 100° . Nevertheless after drying the salt at 100° he obtained figures which, as is seen, agree well for the anhydrous salt. This fact was also pointed out by Schmidt and Wagner [1904], who obtained "nitroso-choline" by the prolonged action of concentrated nitric acid on choline at the ordinary temperature. The identity of this base with pseudo-(synthetic) muscarine is further supported by the similarity of the melting points of the aurichlorides ("pseudo-muscarine" aurichloride m.p. 234° after sintering from 174° ; "nitroso-choline" aurichloride m.p. 240°) and by the fact stated by Nothnagel that the solubilities of the platinichloride are very similar.

In view, therefore, of the foregoing facts, it must be accepted that by the action of concentrated nitric acid upon choline as originally described by Schmiedeberg and Harnack, only one base yielding a platinichloride comparatively little soluble in cold water is produced. This base, which, as will

be shown in detail elsewhere by Dale, produces all the physiological effects described by various authors as brought about by pseudo-muscarine (synthetic muscarine), is, in fact, the choline ester of nitrous acid, and the constitution originally suggested for this base by Schmiedeberg and Harnack must be considered to be disproved.

If the formula ascribed by Harnack [1876] to the natural base be accepted as correct the difference in physiological action of the two bases is easily accounted for by their different chemical constitutions. It is, however, by no means certain that this formula can be accepted as correctly representing the constitution of the base. The facts that many choline-esters have a high degree of activity [Hunt and Taveau, 1911], which in the case of acetylcholine has been shown to approximate to the muscarine type of action [Dale, 1914], and that the so-called pseudo-muscarine is the choline-ester of nitrous acid, make it possible that muscarine itself may be a base of this class. In such a case recrystallisation of the aurichloride might well be accompanied by partial hydrolysis of the base.

With these possibilities in mind I prepared the choline-ester of nitric acid $(\text{OH})\text{N}(\text{CH}_3)_3\text{CH}_2\cdot\text{CH}_2\cdot\text{O}\cdot\text{NO}_2$ by the method described by Schmidt and Wagner [1904]. This base, again, is possessed of very considerable activity and in fact its action in some respects resembles that of natural muscarine much more closely than does that of pseudo-muscarine; nevertheless it still produces a marked curare effect upon frogs. How far these facts have any bearing upon the question of the constitution of natural muscarine must, however, for the present remain an open question.

EXPERIMENTAL.

Preparation of pseudo-muscarine. 3.8 grams of pure choline platinichloride were dissolved in about 5 cc. of concentrated nitric (D 1.4) on the water bath and then rapidly evaporated just to dryness on a sand bath. The reaction product was washed successively with absolute alcohol and once or twice with small quantities of cold water to remove the bulk of the unchanged choline platinichloride. The residue was crystallised from a little hot water. The platinichloride of pseudo-muscarine separated on standing as extremely well formed octahedra. The product was homogeneous and melted sharply with decomposition at $250\text{--}251^\circ$ (bath at 200° and temperature slowly raised). Recrystallisation from water caused no alteration either of crystalline form or of melting point. The salt was anhydrous. Analysis of this salt gave the following results:

0.1043 g.; 0.0300 g. Pt. Pt=28.76.
 0.1071 g.; 0.0312 g. Pt. Pt=29.13.
 0.1384 g.; 0.0401 g. Pt. Pt=28.97.

*0.1641 g.; 0.1114 g. CO₂; 0.0654 H₂O; C=18.5; H=4.4.
 0.1320 g.; 0.0846 g. CO₂; 0.0475 H₂O; C=17.5; H=4.0.
 *0.1346 g.; 9.0 cc. N₂ (moist) at 766 mm. and 11°. N=8.0.
 0.1184 g.; 8.5 ,, ,, 753 ,, 13°. N=8.38.

Calculated for [C₅H₁₃O₂N₂]₂PtCl₆. Pt=28.93; C=17.80; H=3.86; N=8.31.

The analysis of this salt for C, H, and N gave some difficulty since NO was readily evolved. Unless special precautions were taken and doubly long reduced copper or silver spirals employed some of the oxides of nitrogen escaped reduction and results were obtained such as those instanced above and marked with an asterisk. In such cases nitrous acid was readily shown to be present in the potash bulbs or nitrometer.

The platinichloride was converted into the aurichloride by decomposing the former by means of excess of potassium chloride, evaporating the solution to dryness, extracting with alcohol, evaporating off the alcohol and precipitating the concentrated aqueous solution of the residue with an aqueous solution of gold chloride. The aurichloride thus formed was recrystallised from water, in which it is moderately soluble, and was obtained in the form of plates which melted with decomposition at 256° after sintering from about 200°.

Analysis :

0.1753 g.; 0.0727 g. Au; Au=41.47.

Calculated for [C₅H₁₃O₂N₂]₂AuCl₄. Au=41.73.

Hydrolysis of pseudo-muscarine. 0.4 gram of the platinichloride of pseudo-muscarine was boiled under reflux for 2 hours with 5 cc. of 10% hydrochloric acid. The solution was then concentrated and on standing there was obtained 0.07 gram of a platinichloride crystallising in needles and hexagonal plates, melting at 237–238°.

Analysis gave the following :

0.0646 g.; 0.0199 g. Pt. Pt=30.8.

Calculated for [C₅H₁₄ON]₂PtCl₆. Pt=31.6 per cent.

The mother liquors from the platinichloride were decomposed by potassium chloride and the aurichloride of the base obtained as described above. This was moderately soluble in hot water and melted at 261–262°.

Analysis :

0.1387 g.; 0.0610 g. Au. Au=44.0.


Calculated for [C₅H₁₄ON]AuCl₄. Au=44.4 per cent.

The mother liquors from the aurichloride were decomposed by H_2S and on treatment with mercuric chloride gave a crystalline salt melting sharply at 249° which when mixed with choline mercuric chloride showed no alteration of melting point.

On hydrolysis therefore pseudo-muscarine yields choline. The formation of nitrous acid on hydrolysis was readily shown by boiling a solution of the chloride of the base for a few moments with dilute alkali, acidifying the solution, and adding to a solution of potassium iodide and starch. The dark blue colour of the starch-iodine complex at once appeared.

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