REVIEW ARTICLE

THE STORY OF MUSCARINE

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Muscarine, first isolated from the fly agaric (*Amanita muscaria*), is one of the foundation stones of modern pharmacology. It was, indeed, among the first substances known to possess an action which more or less faithfully reproduces some of the effects of stimulation of the autonomic nervous system. The free base is almost certain to be a valuable heuristic tool—it has, for instance already given clear confirmation[1] of the presence of atropine-like actions, originally recorded by Tedeschi[2], in the anti-cholinesterase drug 284C51. But unless an abundant natural source is found* muscarine is not likely to be available in reasonable quantities until it can be synthesised, and its synthesis is unlikely to precede the discovery of its structure. It is surprising, therefore, to find that this small molecule, which has played such an important role, has defied all but the most recent attempts to unravel its structure.

Early History

The earliest attempts to isolate the active toxic principles of the fly agaric, and of other fungi, were made by Braconnot[4–6] and by Schrader[7]. Braconnot was unable to relate the poisonous properties of any fungus to the acrid principle he obtained. Schrader looked for the active principle in the red-coloured material which was the only part he found toxic to birds. Vauquelin[8] suspected that the toxic substance was in the fatty contents of the mushroom. Letellier[9] believed that there were two active principles—the "acrid" and the "narcotic"—but both he and Braconnot were more concerned with fungi in general than with the fly agaric. Although Letellier did not succeed in isolating either the acrid or the narcotic principle in a pure state he found that the acrid principle was easily destroyed by boiling, by drying, by alcohol, by alkali, and by dilute acid; the narcotic principle—which he called amanitine—was resistant to these treatments. His acrid principle was, therefore, unlike muscarine which is stable in acid or alkaline solution and which is usually extracted in alcohol. As he attributed the narcotic properties of fungi to amanitine, and believed that amanitine was the toxic principle of *A. phalloides*, it is unlikely that he used the name amanitine for the substance eventually called muscarine. Indeed, the name amanitine is now applied to one of the main toxic principles of *A. phalloides*[10]. Eventually it became apparent that it was chiefly choline that Letellier had obtained[11]; probably the choline was contaminated with other active principles of the fungi investigated.

The first successful researches into the active principles of the fly agaric were those of Schmiedeberg and Koppe[12] who obtained a deliquescent

*Eugster[3] has apparently found such a source in *Inocybe patouillardi*.

syrupy base which stopped the isolated frog heart in diastole. This base was produced by precipitation with potassium bismuth iodide or with potassium mercuric iodide. They called it muscarine, but according to Harnack it was still mixed with choline.

Soon after this Harnack[11] isolated the aurichloride of a material which he called amanitine after Letellier but which he eventually showed to be choline, one of the most abundant constituents of the fly agaric. Harnack also obtained some muscarine aurichloride to which he gave the empirical formula $C_5H_{12-14}O_2N.AuCl_4$. As Harnack's aurichlorides of muscarine and of choline were similar in appearance, and as he had 8 g. of the so-called muscarine for analysis, his material was more probably choline aurichloride contaminated with muscarine aurichloride[13]. Furthermore, Harnack's muscarine was equal in potency on the frog heart to that prepared by Schmiedeberg and Koppe, which he had already claimed to be mainly choline.

Schmiedeberg and Harnack[14] were the first to obtain comparatively pure muscarine. They thought that it was a hydrated betaine aldehyde and proposed for it the structure: $Cl^-Me_3N^+CH_2CH(OH)_2$.

"*Synthetic Muscarine*"

Schmiedeberg and Harnack claimed to have confirmed this structure by synthesis. By oxidising choline with nitric acid, they obtained a material which they thought had the required empirical formula—although it really had one H atom less—and which they believed to have the structure proposed for muscarine. As this "synthetic muscarine" behaved pharmacologically like natural muscarine, the structure proposed by Schmiedeberg and Harnack for muscarine was accepted until Boehm[15] showed that the synthetic and natural substances, although biologically much alike, were not identical. The synthetic compound, or pseudo-muscarine, was much the weaker in most pharmacological tests. It also possessed some actions not present in the natural alkaloid and not antagonised by atropine: it had, for instance, a strong curare-like effect. Other differences also soon became apparent: Meyer[16] found that synthetic muscarine was much more potent than natural muscarine as a miotic drug in birds and that the relative potency was reversed in mammals. He also confirmed Boehm's observation of a curare-like effect in synthetic muscarine and its absence in the natural base.

Nothnagel[17] repeated the so-called synthesis of muscarine by the method of Schmiedeberg and Harnack and confirmed their structure for synthetic muscarine. He recognised that choline nitrous ester was formed in this synthesis but considered that it was an intermediate product in the formation of synthetic muscarine.

Here was a very unsatisfactory situation: two bases, which had obviously different biological actions, could not be distinguished by chemical methods. This situation might never have arisen had Schmiedeberg and Harnack, or Nothnagel, determined the nitrogen content of their synthetic compound!

The true identity of synthetic muscarine was not known until Ewins[18]

showed that the product obtained on oxidising pure choline with nitric acid was choline nitrous ester—or choline nitrite, $Cl^{-}Me_3N^{+}CH_2CH_2$-ONO. Dale[19] showed that this substance was very much less active than acetylcholine as a depressor agent in the cat.

Isolation of Muscarine

Varying success has attended other attempts to isolate natural muscarine. Inoko[20] extracted it from *Amanita pantherina*. Nothnagel[17], who accepted the structure proposed by Schmiedeberg and Harnack, claimed to have isolated 500 mg. of the platinum salt of muscarine from many hundredweights of the fresh fungus; but King doubted the purity of this material. Harmsen[21], and Honda[22] also isolated some muscarine, but they were more interested in its pharmacology than in its chemical structure. Heinisch and Zellner[23], Zellner[24], and Küng[25] failed to obtain muscarine.

King[13] eventually obtained the first really pure muscarine, which he crystallised as the aurichloride from a mixture of muscarine and choline aurichlorides. The method depended upon the solubility of muscarine in absolute ethanol, its non-precipitation by basic lead acetate, and its precipitation by aqueous and by alcoholic mercuric chloride and by phosphotungstic acid. King found that there was twenty times as much choline as muscarine in the fly agaric. From assays on the toad heart and rabbit gut, he estimated that each kilogram of fresh *A. muscaria* contained about 16 mg. muscarine chloride; he crystallised 80 per cent of this. He did not give a melting point. King's muscarine, which he said was not adsorbed by charcoal, had a molecular weight of about 210, was stable to boiling in decinormal acid or alkali, and was about equal in potency to Honda's preparation in stopping the frog's heart in diastole. It was, however, more potent than other earlier samples, being twenty times more potent than the materials isolated by Schmiedeberg and Koppe and by Harnack; it was five times more active than acetylcholine, and seven times more active than arecoline, on isolated rabbit gut. These results are supported by others on a recently isolated, highly pure sample of muscarine chloride (Fig. 1) prepared by Dr. S. Wilkinson of the Wellcome Research Laboratories; it was about four or five times more active than acetylcholine on isolated rabbit auricles in the absence of an anticholinesterase drug. When the relative potencies were determined in the presence of neostigmine, muscarine and acetylcholine were about equal (Mogey, unpublished work). Fraser[26] obtained similar results

Fig. 1. Crystals of muscarine chloride (× 165) prepared by Dr. S. Wilkinson.

although muscarine and acetylcholine did not give parallel dose-response curves. Thus, presumably, muscarine is not hydrolysed by cholinesterase; as muscarine is also stable in alkali it cannot be a choline ester.

King's muscarine aurichloride crystallised as large delicate leaflets quite unlike choline aurichloride crystals. It therefore resembled the sample prepared by Nothnagel—the biological potency of which has not been recorded—but was totally unlike the material, crystallising as long or short prismatic shapes, which Harnack regarded as muscarine, and which he could not distinguish in crystal form from choline.

Another step in the elucidation of the structure of muscarine was taken by Kögl and his colleagues[27]. They treated the fresh fungi with ethanol, and removed fats with ether and other impurities with charcoal. Choline was adsorbed on Permutit, and other substances were precipitated by suitable adjustment with mercuric chloride. The muscarine was precipitated from acetone as the reineckate and converted to the chloride. Their yield of base was 2·8 mg./kg. in one experiment and 1·3 mg./kg. in another.

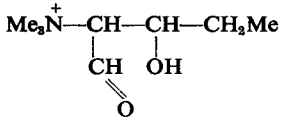
Properties of Muscarine

By this time a good deal had been published about the chemical and physical properties of muscarine. It was known to be soluble in ethanol and in water, slightly soluble in chloroform, and insoluble in ether; according to King, it was not adsorbed by charcoal or kaolin, and was stable in alkaline solution; Kögl and others[27] stated that it soon decomposed in acid solution but Ewins[28] has recorded that the muscarinic activity of extracts of *A. muscaria* is not appreciably reduced by boiling in acid or in alkali, and King[13] confirmed this. Kögl, Salemink, Schouten, and Jellinek[29] now state that muscarine is stable to boiling in acid or in alkali.

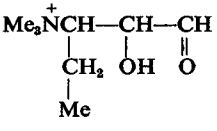
Although the presence of an aldehyde group has been denied[30], Kögl, and others[27] concluded—from positive reactions with Schiff's reagent and the nitroxyl reagent of Angeli-Rimini—that there was such a group in muscarine. As a benzoyl derivative was obtained, they believed that there was also a hydroxyl group. Trimethylamine was produced by the action of silver oxide: the base was therefore thought to contain a trimethylammonium group. Muscarine is optically active*; therefore there is probably at least one asymmetric carbon atom present. The chloride had a molecular weight of 195·5 according to Kögl and Veldstra[31] or about 210 according to King. Kögl and Veldstra gave the melting point of the aurichloride as 115–117°†. Kögl and his colleagues[27] concluded that the earlier formula of Schmiedeberg and Harnack was too small; they proposed $C_8H_{18}O_2N$, and suggested that muscarine was structure (I) or possibly (II).

* $[\alpha]_D^{20}$ + 1·57° (water; chloride; Kögl and others[27]). $[\alpha]_D^{25}$ + 8·1° (ethanol; chloride; Kuehl, Lebel and Richter[34]), $[\alpha]_D^{20.5}$ + 6·7° (water; reineckate; Eugster and Waser[35]).

† Later, however, Eugster and Waser[35] gave 121–121·5° as m.p., Kuehl and others[34] gave it as 116–119° and Kögl and others[29] as 120–121° for the same salt.



(I)



(II)

Pfeiffer[32] preferred the second formula on the basis of the distances between the nitrogen and the oxygen atoms.

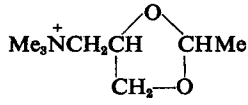
Kögl and Veldstra synthesised the first of their proposed structures, but the synthetic material had only about one-forty-thousandth of the activity of natural muscarine. Kögl and Veldstra, however, suggested that the discrepancy in activity between their synthetic compound and natural muscarine might be explained by a very weak activity in all but one of the isomers present in the synthesised racemate. According to Kögl, Salemink, and others[29] van der Laan[33] failed to resolve the various isomers. But what other compound shows such stereospecificity? If a completely inert material is mixed with an equal weight of an active material, A, the mixture will have half the activity of A. Common ratios for the potencies of optical isomers are 20 (tubocurarine), 17 (adrenaline), and 4 (methadone): 40,000 is surely too much; and this, moreover, is the ratio of the synthetic racemate, prepared by Kögl and Veldstra, and consisting of the presumed active and inactive isomers, to the natural alkaloid. Furthermore the stereoisomers of simple ammonium compounds show no marked differences in muscarinic activity[36].

The formula put forward by Kögl and Veldstra is, therefore, unlikely to be correct. But it is still quoted in some textbooks as the accepted structure of muscarine.

More Synthetic Approaches

Other attempts to synthesise molecules which might be identical with that of natural muscarine have also failed. Because muscarine was supposed to be a hydrated betaine aldehyde, it was reasonable to expect betaine aldehyde, $Cl^{-}Me_3N^{+}CH_2CHO$, to show some muscarine-like actions. It was synthesised by Berlinerblau[37], and later by Fischer[38], but Meyer[16] found that betaine aldehyde and muscarine were quite different pharmacologically and that the former was inactive on the pigeon's pupil. Bode[39], Nothnagel[17] and Ewins[28] all failed in their attempts to make muscarine by synthetic means. None of the synthetic compounds was as active as the natural alkaloid and none was without the nicotine-curare type of action, which is absent in muscarine[19].

Of the compounds recently synthesised, none has excited more interest than the acetal derivative of Fourneau and his colleagues[40]. They prepared 2268F (III), and other related compounds, because of Fourneau's hypothesis that the difference between Kögl's synthetic compound and natural muscarine might be due to the formation "d'une liaison interne du type acetal".



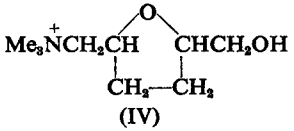
(III) 2268F.

This compound is more potent than acetylcholine in many respects and is practically devoid of nicotinic actions; it is one of the most potent muscarinic substances known, being even more active than carbachol, which, unlike acetylcholine, is not sensitive to destruction by cholinesterase.

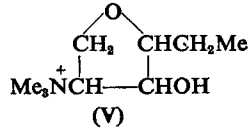
But 2268F is not muscarine: in large doses (6 mg./kg.) it produces, for instance, a pressor response in atropinised, anaesthetised cats[41], an action not present in muscarine according to Ambache[42]. It also stimulates the frog's rectus abdominis; we now know that muscarine also has this action[26] but large doses are needed; the effect is antagonised by tubocurarine. Compound 2268F also has a weak ganglion-stimulating effect, an action also possessed by muscarine[43–44]; this ganglion stimulation is easily blocked by atropine.

The molecule of 2268F is, furthermore, too small for it was based on Kögl's formula for muscarine, $C_8H_{18}O_2N$, which would give a molecular weight of 195·5 for the chloride. The molecular weight assigned by King to muscarine (about 210), suggests that Kögl's formula is one $-CH_2$ group too small. Addition of this $-CH_2$ group gives the empirical formula $C_9H_{20}O_2NCl$ (mol. wt. 209·5) which is, in fact, the formula ascribed to muscarine chloride by Eugster and Waser[35]. Kuehl and others[34] and Kögl, Salemink, and others[29] agree with this formula.

Dr. Wilkinson has written to us to say he obtained trimethylamine on Hofmann degradation of pure crystalline muscarine chloride. The trimethylamine was identified conclusively by comparison of X-ray powder photographs of the aurichloride and authentic trimethylamine aurichloride; Wilkinson thus concluded that muscarine contains a trimethylammonium group. Indeed he stuck firmly to this conclusion even after Eugster and Waser[35] (see below) had doubted the presence of this group. In 1952, Wilkinson established $C_9H_{20}O_2N$ as the empirical formula for muscarine and, concluding from the infra-red absorption spectrum that there is a tetrahydrofuran ring present in the molecule, he prepared, in 1954, two compounds (IV) and (V) for model experiments relating to the structure of muscarine.



(IV)



(V)

Wilkinson's synthetic compounds

Each compound was found by Dr. P. Fraser also of the Wellcome Laboratories to have less than one-thousandth ($\frac{1}{1000}$th) of the activity of natural muscarine on the isolated rabbit ileum.

The possibility that muscarine is an alkoxytrimethylammonium compound was suggested by Rogers, Bovet, Longo, and Marini-Bettolo[45].

They based their suggestion on the fact that certain compounds of the $R{\cdot}CH_2ON^+Me_3X$ type had marked muscarine-like actions and because they are decomposed by alkali to give trimethylammonium and the corresponding aldehyde, R·CHO. This reaction was held to resemble the Hofmann degradation of muscarine to trimethylamine and $\alpha\beta$-dihydroxyvaleric acid; silver oxide oxidised the aldehyde. Furthermore, Schiff and Angeli-Rimini reactions, believed by these authors—after Kögl and others[27]—to be given by muscarine, are also given by alkoxytrimethylammonium compounds. Rogers and others concluded that more such compounds, and particularly *n*-amyloxytrimethylammonium ($C_8H_{20}ON$), should be examined. This alkyloxytrimethylammonium type of structure is not, however, supported by the stability of muscarine free base to hydrolysis; even after drastic acid or alkaline hydrolysis no evidence for the formation of aldehydes could be obtained.

Further Analysis

Although muscarine contains a quaternary nitrogen, Eugster and Waser[35] could not show that it was a trimethyl quaternary grouping, for—unlike Kögl and others[27] and Wilkinson—they obtained volatile bases but no trimethylamine on Hofmann degradation. From the negative colour tests, and the infra-red absorption spectrum, they concluded that there is neither aldehyde nor ketone group in the molecule. On oxidation with chromic acid they got acetic acid and no $\alpha\beta$-dihydroxyvaleric acid, which Kögl and his colleagues did obtain.

The results obtained by Eugster and Waser thus differ radically from those of Kögl and others[27]. If the C_9 formula is correct, and if there are no double or triple bonds in muscarine, it must of necessity contain one ring[46].

As muscarine was completely inert to periodate oxidation it was assumed by Kuehl and colleagues[34] that there are no vicinal hydroxyl groups or adjacent hydroxyl and ketone groups. Acetylation gave a monoacetyl derivative which possessed, according to infra-red spectroscopy, one ester-group; as muscarine was obtained again on deacetylation there was no internal rearrangement of the molecule on acetylation. There is thus one, but not two, hydroxyl groups. Chemical reagents failed to demonstrate an aldehyde group and no carbonyl group was detected by infra-red spectroscopy. The Zeisel methoxyl test was negative—therefore no methyl ether is present. The inertness of the second oxygen suggested that it might be an ether. Kuehl and colleagues obtained no trimethylamine on Hofmann degradation of natural muscarine under a variety of conditions including those described by Kögl and others[27]. Neither did they detect any acid substances after these procedures. All they got was unchanged muscarine.

Eugster[47] repeated that on oxidation of muscarine with chromic acid he obtained acetic acid only, and claimed that Hofmann degradation with silver oxide gave no trimethylamine though fusion with potassium hydroxide gave an unspecified amount. He showed that any structure which would give rise to a carbonyl group in acid solution is impossible. As muscarine is, furthermore, not sensitive to reduction or hydrogenation

by sodium amalgam, lithium aluminium hydride, sodium borohydride, or hydrogen and platinum it cannot be an acetal derivative as was assumed by Fourneau. It is possible that a ring structure might help to stabilise the acetal grouping, but it is unlikely that it would give the negative carbonyl reaction with dinitrophenylhydrazine reported by Eugster and Waser.

Kögl, Salemink, and others[29] have modified some of the original claims of Kögl and others[27] and Kögl and Veldstra[31]. They now state that muscarine is stable to boiling for 3 hours in 2N HCl or 3N NaOH or for 8 hours in HCl at pH 4 or below pH 1; that as tests with phenylhydrazine, dinitrophenylhydrazine and semicarbazide all gave negative results there can be no carbonyl group; that negative results were also obtained with Schiff's reagent and with the nitroxyl reagent of Angeli-Rimini whereas earlier Kögl and others[27] had reported positive results with these reagents; that—like their previous results, but unlike the more recent work of Eugster and of Kuehl and colleagues—treatment, under very vigorous conditions, of a large quantity of muscarine (100 mg.) with silver oxide yielded trimethylamine. The muscarine isolated by Kögl, Salemink, and colleagues[29] was one-quarter as active as acetylcholine on the Straub frog heart; Fraser[26] reported that Wilkinson's muscarine was two-thirds as active as acetylcholine on the Hartung-Clark frog-heart preparation.

As Kögl, Salemink, and colleagues[29] discovered acetylcholine in their extracts of *A. muscaria* it is possible that the 1931 sample of muscarine was contaminated with this, and that it was the acetylcholine which disappeared on boiling and which gave the reactions leading to the erroneous conclusion that muscarine contained a carbonyl group. But this does not explain how Wilkinson (private communication) and Kögl, Salemink, and others[29] obtained a good yield of trimethylamine whereas Kuehl and colleagues[34] and Eugster[47] did not. The discrepancy is more likely to be due to the degradative procedure, Kögl, Salemink and colleagues[29] using vigorous conditions and much muscarine, whereas the American authors used gentler conditions. Eugster only obtained small quantities of trimethylamine after fusion of muscarine with potassium hydroxide; under similar conditions he obtained material chromatographically similar to trimethylamine from morpholine compounds.

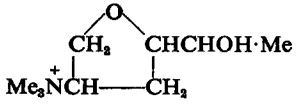
So muscarine is $C_9H_{20}O_2N$. It contains no aldehyde or ketone—as Scelba[30] claimed before Kögl and others[27] stated that some such group was present. It has one ring in its structure and no double bond. It is dextrorotatory. There is only one hydroxyl group—a secondary alcohol*—and the second oxygen function is probably an ether although not a methyl ether; it may be in a tetrahydrofuran ring (Dr. S. Wilkinson, private communication and ref. 29). Eugster and Waser[35] could not

* That this hydroxyl group is a secondary alcohol was first suggested by Eugster[47] because muscarine gave a positive response to the iodoform test. He therefore suggested that the side chain –CHOH·Me was present in muscarine. In reality the positive iodoform test was given by the other oxygen of the tetrahydrofuran ring and so did not prove the presence of the –CHOH·Me group. The evidence for a secondary alcohol group therefore rests on the X-ray crystallographic data[49] (see below).

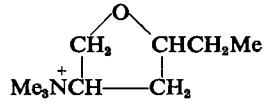
confirm that there are three methyl groups on the nitrogen, but Wilkinson (unpublished work), Eugster[47], and Kögl, Salemink and colleagues[29] decided that muscarine contains a trimethylammonium grouping.

The nature of the oxygen functions in the molecule is one of the most interesting points. It now seems fairly clear that one is in a hydroxyl group and that the other is most probably an ether but not a methyl or ethyl ether; infra-red absorptiometry seems to indicate that it is in a tetrahydrofuran ring. The nature of this atom may not be important, however, for cells may not be able to distinguish clearly between an ether, an acetal, or a carbonyl oxygen: choline ethyl ether[19], 2268F, and acetylcholine all have high muscarinic activities. The relative position of the oxygen is probably more important. Many active compounds have one oxygen function about 3–4 Ångstrom units away from the nitrogen and the second about 1–2 Å further off, e.g. 2268F and acetylcholine.

Eugster[47] has recently suggested that muscarine is the trimethylammonium salt of 2-(1-hydroxyethyl)-4-aminotetrahydrofuran (VI).



(VI) Structure of muscarine after Eugster[47]

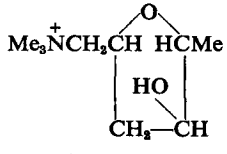


(VII) Eugster's desoxymuscarine

He synthesised the related desoxy compound (VII) from which, on degradation with silver oxide, he obtained almost a 47 per cent yield of trimethylamine, whereas from natural muscarine he obtained practically none. It seemed to him, therefore, that the introduction of the hydroxyl group—as in his proposed structure for muscarine—produced some special orientation preventing the production of trimethylamine. Thus Eugster also took shelter in the last refuge of the organic chemist—stereospecificity—and so joined the company of those who had seriously tackled this problem before him. Eventually he showed that his proposed structure was incorrect[48].

The Structure of Muscarine

From the infra-red absorption spectrum Kögl, Salemink and colleagues[29] concluded that muscarine contains a tetrahydrofuran ring, thus settling the nature of the second oxygen function. By the action of hydrogen iodide followed by hydrogenation they obtained trimethylhexylammonium iodide thus showing the carbon skeleton of muscarine. They suggested that muscarine is the quaternary trimethylammonium salt of 2-methyl-3-oxy-5-(aminomethyl)-tetrahydrofuran (VIII)



(VIII) Muscarine according to Kögl, Salemink and colleagues[29]

X-ray crystallographic data were held to confirm this structure. Details of the bond distances and angles in the muscarine ion are shown in Figure 2. The standard deviation of the measurement of the bond lengths was 0·08 Å and that for the angles was 6°. The values are therefore all within normal limits.

In the tetrahydrofuran ring of Figure 2 all the atoms except C(3) are in the one plane. C(3) lies on the same side of this plane as do C(1) and C(6) whereas O(2) is on the opposite side. The sequence C(3), C(2), C(1), N, and C(7) is virtually in a single plane; O(2) is only 0·05 Å away from this plane and the mean distance of C(3), C(2), C(1), N, and C(7) from the plane is 0·06 Å[49]. Thus O(2) can be regarded as being in the plane of the chain enumerated.

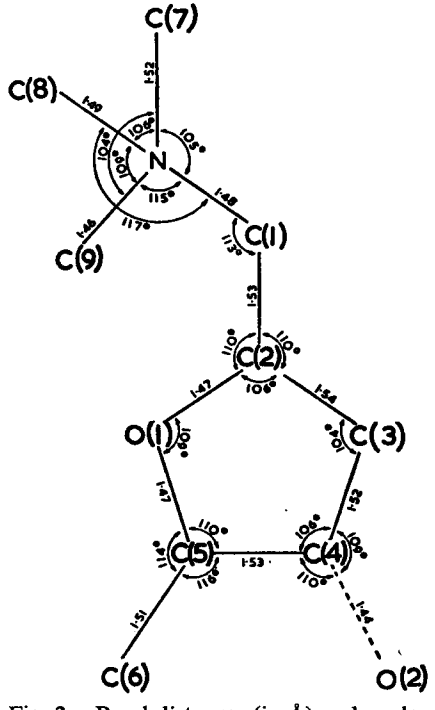


Fig. 2. Bond distances (in Å) and angles in the muscarine ion. (By kind permission of Dr. F. Jellinek and *Acta crystallographica*.)

The compound with this structure has been synthesised[50,51]. The racemate aurichloride had a melting point of 69–72°; the m.p. of natural muscarine aurichloride is 120°–121°. The synthetic racemic chloride has the same R_F value as the natural chloride. The infra-red absorption spectra of the natural and synthetic products showed a general identity although there were some differences in fine detail—particularly in C–H bands —which were taken to indicate that the synthetic material was a mixture of stereoisomers. The pharmacological activity of the synthetic material[50], tested on the frog heart, was one-third that of natural muscarine. The resolution of another synthetic racemate has apparently been tackled and the promised results[52] are awaited with interest.

Conclusion

Although the structure now proposed seems to be fairly well established there are still some slight difficulties about accepting the results unreservedly. Why do different workers obtain different results—some getting trimethylamine and $\alpha\beta$-dihydroxyvaleric acid, and some getting neither of these—on subjecting muscarine to Hofmann degradation? Can this be due solely to the vigour of the reactions? Since trimethylamine was apparently obtained by Eugster from dimethylmorpholine, can the

detection of trimethylamine from muscarine prove the presence of a trimethylammonium group? How can the tetrahydrofuran ring give a positive iodoform test, which seems to indicate the presence of a –CHOH·Me group: does the ring become hydrolysed at the oxygen? The infra-red absorption spectrum suggests that a tetrahydrofuran ring is present, but does it rule out all other ring structures?

The latest structural proposal by Kögl and his colleagues is, nevertheless, probably correct. Before final acceptance, however, the synthetic compound—satisfactorily resolved—and natural muscarine should be shown to be identical chromatographically, pharmacologically and by infra-red spectroscopy. Such a series of identities is necessary for convincing proof, because of past difficulties in elucidating the structure of this fascinating molecule.

Note added in Proof

Corrodi, Hardegger and Kögl[53] have recently stated that their claim[52] to have synthesised a mixture of muscarine and its diastereoisomers cannot be substantiated. They now believe that this product contained little or no muscarine but that it was a racemic mixture of allomuscarine. All derivatives of allomuscarine showed the same R_F values, and had nearly the same infra-red spectra, as the corresponding derivatives of muscarine. The mixture was only $\frac{1}{200}$ as active as natural muscarine on the frog heart. Thus the biological test has again proven its value—it should have been tried earlier.

Two other syntheses have also been reported. One of (±)-muscarine[54] yielded muscarine chloride m.p. 148–152° and a tetrachloroaurate m.p. 79–83° (see footnote † p. 148, and p. 154), and the other of L-muscarine[55] gave muscarine chloride m.p. 179–180°. Previously reported melting points for natural muscarine chloride were 181–182°[35] and 180–181°[29]. No biological results for the (±)-muscarine have been published; the L-muscarine was equal in activity to natural muscarine on the frog heart.

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