THE HISTORY AND CHEMISTRY OF MUSCARINE

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THE fungus *Amanita muscaria* (L. ex Fr.) Quél., with the unmistakable brilliant orange-red cap, flecked with white fragments of its volva, is found in birch and pine woods from summer to late autumn. The epithet, *muscaria*, refers to its former use as a fly poison and hence the common name "fly agaric". The genus *Amanita* is said to be probably responsible for the majority of cases of mushroom poisoning and, although the deadly *A. phalloides* causes most fatalities, occasionally *A. muscaria* has proved fatal. The fungus contains a number of basic substances including choline, acetylcholine, and the intriguing alkaloid muscarine. Muscarine is a foundation stone of modern pharmacology, being one of the first substances known which reproduced faithfully some of the responses to stimulation of the parasympathetic nervous system. Dixon[1] was so impressed by this activity that he advanced the hypothesis that the vagus nerve liberated a muscarine-like substance which acted as a chemical transmitter of its impulses and this was shown to be substantially correct by Hunt and Taveau[2] and by Dale[3] in their classical studies of acetylcholine.

The similarity between the actions of acetylcholine and muscarine on smooth muscles and glands gave rise to the definition "muscarinic" actions of acetylcholine, to distinguish them from the effects on ganglia and voluntary muscles, the so-called "nicotinic" actions. It was obviously of fundamental importance that muscarine should be isolated and characterised so that comparative pharmacological studies and absolute assessment of its potency could be made. Muscarine, however, proved extraordinarily elusive and only recently, almost 150 years after the first approaches were made in 1811 by Braconnot[4] and Schrader,[5] have its isolation and the determination of its structure been achieved.

Although one tends to associate muscarine with *A. muscaria*, it has been isolated from, or muscarinic activity demonstrated in, a variety of other fungi. It has invariably been fly agaric, which is prolific, that has been extracted, but far higher concentrations of muscarine occur in fungi of

[1] Dixon, *Med. Mag.*, 1907, **16**, 454.
[2] Hunt and Taveau, *Brit. Med. J.*, 1906, II, 1788.
[3] Dale, *Science*, 1939, **90**, 393; Dale, Feldberg, and Vogt, *J. Physiol.*, 1936, **86**, 353.
[4] Braconnot, *Ann. Chim. (France)*, 1811, **79**, 265; 1813, **87**, 237.
[5] Schrader, cited by Harmsen, *Arch. exp. Path. Pharmak.*, 1903, **50**, 311.

other genera. As far as the Reviewer is aware, *A. pantherina* D.C. ex Fr. is the only other species of *Amanita* from which the alkaloid has been isolated.[6] Activity has been found in extracts of *Boletus luridus* Schaeff. ex Fr.,[7] *Russula emetica* Schaeff ex Fr.,[8] *Clitocybe rivulosa* Pers. ex Fr., and other *Russula*[9] and *Clitocybe*.[10] The widest distribution, however, seems to occur in the genus *Inocybe*. Wicki[11] and Loup[12] have recorded activity in 26 out of 33 species. *I. asterospora, rimosa, cookei,* and *umbrina* were stated to have potent muscarinic activity.[13] On the basis of pharmacological assays of *I. lateraria* Ricken (syn. *I. patouillardi* Bres.) and *I. napipes* Lange, incredibly high concentrations of muscarine[11,12,14,15] were recorded, probably as a result of the simultaneous assay of acetylcholine. The most reliable figures have recently been obtained by Eugster and his colleagues[16] by actually isolating the muscarine. Their following percentage yields show the wide variation in the different species: *A. muscaria*, 0·0002%;* *I. patouillardi*, 0·037%; *I. fastigiata*, 0·01%; *I. umbrina*, 0·003%; *I. bougardi*, 0·0%.

The early work has been reviewed previously,[17] but in order to present a clear picture it is essential that this phase of the problem be described briefly. In reality the story of muscarine revolves round the presentation of three formulae, two erroneous ones $C_5H_{14}O_2N^+$ and $C_8H_{18}O_2N^+$, and the correct one $C_9H_{20}O_2N^+$, and this Review is sub-divided to follow these trends. It is noticeable that with the advent of more refined chemical techniques, particularly chromatography and ion-exchange, many of the principle difficulties associated with the isolation of a minute amount of muscarine from a large mass of material and its separation from closely related compounds were resolved. Many of the doubts and controversies which arose repeatedly were the result of statements which related to chemically impure preparations and based on inadequate analytical data. Once pure muscarine had been obtained the elucidation of its structure and its synthesis followed very quickly.

* Average yield from batches of *A. muscaria* collected over a period of 3 years.

[6] Inoko, *Annalen med. fac. Jap. Univ. Tokyo*, 1887—1889, **1**, 227; *J.*, 1892, **62**, 232.

[7] Boehm, *Arch. exp. Path. Pharmak.*, 1885, **19**, 87.

[8] Kobert, *St. Petersburg med. Wochenschr.*, 1891, **51**, 463, cited by Harmsen (see ref. 5); Kobert, "Lehrbuch der Intoxikationem." Vol. II., Euke, Stuttgart, 1906, p. 1288.

[9] Carter, *Amer. J. Physiol.*, 1901, **5**, 158.

[10] Wicki and Loup, *Trav. Lab. Thèrap. exp. Genève*, 1930—1932, **13**, 9; Wicki, *Schweiz. Z. Pilzk.*, 1930, **8**, 42; 1931, **9**, 78; Wicki and Roch, *Rev. méd. Suisse*, 1935, **55**, 896.

[11] Wicki, *Bull. Soc. Mycol. Genève*, 1928, **11**, 14.

[12] Loup, Thesis 114, Geneva, 1938.

[13] Yasumari, Ishida, and Kozu, *J. Appl. Mycol.*, 1949, **3**, 118; Ford and Sherrick, *J. Pharmacol.*, 1911, **2**, 549; 1913, **4**, 321.

[14] Mecke, *Arch. exp. Path. Pharmak.*, 1934, **175**, 23.

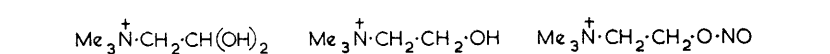
[15] Fahrig, *Arch. exp. Path. Pharmak.*, 1920, **88**, 227.

[16] (*a*) Eugster, *Helv. Chim. Acta*, 1957, **40**, 886; (*b*) Eugster and Muller, *ibid.*, 1959, **42**, 1189.

[17] Bowden and Mogey, *J. Pharm. Pharmacol.*, 1958, **10**, 145; Salemink, *Pharm. Weekblad*, 1960, **95**, 165, 197.

Phase 1. The formula $C_5H_{14}O_2N^+$

Vauquelin[18] suspected that the toxicity was associated with the fatty contents of the fungus. Letellier[19] considered that there was a toxic principle common to species of *Amanita*, which he referred to as amanitine, a name now applied exclusively to toxin isolated from *A. phalloides*.[20] Although several subsequent investigators[21] realised that toxicity was related to the basic constituents, the first classical studies, both chemical and pharmacological, were made in 1869 by Schmiedeberg and Koppe[22] who isolated a syrupy alkaloid having potent physiological activity and arresting the isolated heart in diastole. This preparation was obviously impure and, by fractionating the mixture of chloroaurates obtained from it, Harnack[23] isolated choline and a substance to which he assigned the formula $C_5H_{14}AuCl_4NO_2$. To this material he gave the name muscarine and subsequently[24] suggested that it had the structure (1), whose relation to choline (2) is obvious.



(1) (2) (3)

This structure was apparently substantiated by synthesis. From choline, by the action of nitric acid, was obtained the so-called "synthetic muscarine" to which structure (1) was assigned and which was reputed to be identical with natural muscarine.[25] Nothnagel[26] repeated this work with equally convincing proof of identity.

However, as techniques became more refined, pharmacologists began to doubt these observations. Boehm[27] showed that the "synthetic muscarine" had a curarising activity not found in the natural preparation, and equally subtle differences were described by Meyer[28] and Schmidt.[29]

This doubt existed until 1914, but in the meantime varying success had attended other attempts to isolate muscarine. Inoko[6] prepared an active

[18] Vauquelin, *Ann. Chim.* (*France*), 1813, **85**, 25.
[19] Letellier, Inaug. Thesis, Paris, 1826, cited by Kobert, "Lehrbuch des Intoxikationem," Enke, Stuttgart, 1906, Vol. II, p. 1288.
[20] Wieland and Hallermayer, *Annalen*, 1941, **548**, 1.
[21] Sicard and Schoras, *Compt. rend.*, 1865, **60**, 847; Letellier and Speneux, *Ann. hyg. publ. et méd. légale*, 1876, **27**, 71; Apoiger, *Buchners Reperf. f. d. Pharm.*, 1851, **7**, 289; Kaiser, *Inaug. diss. Göttingen*, 1862; Boudier, "Des Champignons au Point de Vues de leurs Caractères Usuelles Chimiques et Toxicologiques," Paris, 1869.
[22] Schmiedeberg and Koppe, "Das Muscarin, das giftige Alkaloid des Fliegenpilzes," Vogel, Leipzig, 1869.
[23] Harnack, *Arch. exp. Path. Pharmak.*, 1875, **4**, 168.
[24] Harnack and Schmiedeberg, *Zentr. med. Wissensch.*, 1875, **36**, 598; *Arch. exp. Path. Pharmak.*, 1877, **6**, 101.
[25] Schmiedeberg, *Arch. exp. Path. Pharmak.*, 1881, **14**, 376.
[26] Nothnagel, *Ber.*, 1893, **26**, 801; *Arch. Pharm.*, 1894, **232** ,261; *J.* 1893. **64**, 297; 1894, **66**, 437.
[27] Boehm, *Arch. exp. Path. Pharmak.*, 1885, **19**, 60.
[28] Meyer, *Ber.*, 1893, **26**, 803.
[29] Schmidt, *Annalen*, 1904, **337**, 37.

fraction from *A. pantherina*, and potent material was obtained by Harmsen[30] and Honda,[31] whilst further extensive pharmacological comparisons were made by Harmsen,[30] Straub,[32] and Fühner.[33] Finally, however, Ewins[34] re-examined the method of preparation of "synthetic muscarine" and proved that in fact the product was the choline nitrous ester (3) and not the derivative (1). The pharmacological properties of the nitrous ester were clearly shown by Dale[35] and independently confirmed by Weinhagen[36] as distinct from those of muscarine and identical with those described earlier for "synthetic muscarine".

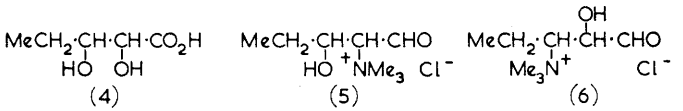
Phase 2. The formula $C_8H_{18}O_2N^+$

In 1922 King,[37] after critically examining previous procedures and being concerned with the preponderance of choline in the basic fractions, drastically modified existing methods of isolation and obtained 90 mg. of a non-crystalline muscarine chloride from 25·5 kg. of fly agaric. With the possible exception of Honda's preparation,[31] with which it was equiactive, this was undoubtedly the purest sample which had as yet been obtained. King, however, other than establishing the equivalent weight of 210 for the base by estimating the gold in the chloroaurate and hence eliminating the $C_5H_{14}O_2N^+$ formula, added little to the chemical knowledge. He could find no evidence that muscarine was quaternary and was therefore in sympathy with those who doubted its formulation as a trimethylammonium base.[38]

Until 1931 there was a further period of inertia, then Kögl and his colleagues,[39] using Permutit as a means of concentration and fractionation of the reineckates as a method of purification, isolated from 1250 kg. of fungus 137 mg. of a crystalline reineckate which they considered to be the pure salt of muscarine. A crystalline chloroaurate was prepared and the analytical figures for these two salts corresponded to the formula $C_8H_{18}O_2N^+$. The chloride regenerated from the reineckate by the method of Kapfhammer and Bischoff[40] was optically active $\{[\alpha]_D^{20} + 1{\cdot}57°$ (in water)$\}$ and was remarkably stable to alkali, but its activity was reduced by one half on prolonged contact with acid. In contrast to Scelba's earlier observations,[41] the chloride was found to give reactions

[30] Harmsen, *Arch. exp. Path. Pharmak.*, 1903, **50**, 361.
[31] Honda, *Arch. exp. Path. Pharmak.*, 1911, **65**, 454; *Chem. Abs.*, 1912, **6**, 529.
[32] Straub, *Pflüg. Arch. ges. Physiol.*, 1907, **119**, 127.
[33] Fühner, *Arch. exp. Path. Pharmak.*, 1908, **59**, 179.
[34] Ewins, *Biochem. J.*, 1914, **8**, 209.
[35] Dale, *J. Pharmacol.*, 1914, **6**, 147; Dale and Ewins, *J. Physiol.*, 1914, **48**, 24.
[36] Weinhagen, *Z. physiol. Chem.*, 1919, **105**, 249; 1921, **112**, 13; *J. Amer. Chem. Soc.*, 1920, **42**, 1670.
[37] King, *J.*, 1922, **121**, 1743.
[38] Guth, *Monatsh.*, 1925, **45**, 631; Küng, *Z. physiol. Chem.*, 1914, **91**, 241; Heinisch and Zellner, *Monatsh.*, 1904, **25**, 537; Zellner, *ibid.*, 1911, **32**, 133.
[39] Kögl, Duisberg, and Erxleben, *Annalen*, 1931, **489**, 156.
[40] Kapfhammer and Bischoff, *Z. physiol. Chem.*, 1930, **191**, 182.
[41] Scelba, *Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat.*, 1922, **31**, 518; *Chem. Abs.*, 1923, **17**, 3162.

characteristic of an aldehyde. The presence of a hydroxyl group was shown by formation of a benzoyl derivative. By Hofmann degradation were isolated the two products which have caused so much difference of opinions, namely, trimethylamine and the optically active $\alpha\beta$-dihydroxy-valeric acid (4). By re-assembling these fragments Kögl *et al.* deduced that muscarine chloride must be a quaternary salt represented by one or other of the two structures (5) and (6), of which (5) was the more probable because of its derivation from serine. The acid (4) was assumed to arise by oxidation with the silver oxide during the degradation. A racemic mixture

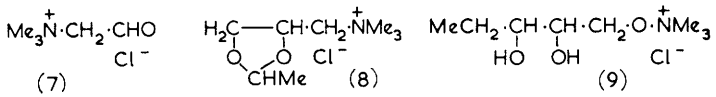


(4) (5) (6)

of aldehydes (5) was synthesised but was 40,000 times less active than muscarine.[42] Because the separation and the resolution of the two possible racemates of (5) proved impracticable, it could not be shown that this low activity was a result of stereospecificity.[43]

Phase 3. The formula $C_9H_{20}O_2N^+$

The aldehyde structure deduced by Kögl was not well received. Betaine aldehyde (7) and a number of related compounds had long been synthesised[44] and found to have the nicotine–curare type of action which is so completely divorced from that of muscarine.



(7) (8) (9)

Fourneau *et al.*[45] synthesised the dioxolan derivative (8) with high muscarinic activity but equally as non-specific as many other synthetic compounds. Rogers *et al.*[46] suggested that muscarine might have an alkoxytrimethylammonium structure such as (9), which would account for the formation of the dihydroxy-acid (4) on degradation. However, this was a period of speculation and elimination by analogies.

Close examination of Kögl's results, particularly the inadequate analytical data and absence of any proof as to the homogeneity of the fractions, clearly shows on what precarious grounds his formula was based.

[42] Kögl and Veldstra, *Annalen*, 1942, **552**, 1; Veldstra, Diss., Utrecht, 1935.
[43] Van der Laan, Diss., Utrecht, 1942.
[44] Berlinerblau, *Ber.*, 1884, **17**, 1139; Fischer, *Ber.*, 1893, **26**, 464.
[45] Fourneau, Bovet, Bovet, and Montezin, *Bull. Soc. Chim. biol.*, 1944, **26**, 516; Fourneau and Chantalou, *Bull. Soc. chim. France*, 1945, **5**, 10.
[46] Rogers, Bovet, Longo, and Marini-Bettolo, *Experientia*, 1953, **9**, 260.

Several groups of workers realised almost simultaneously that the only solution to the problem lay in isolating a sufficient amount of muscarine for comprehensive chemical and physical analyses, and moreover that the recent applications of chromatography might prove invaluable.

To Eugster and Waser, however, must be given the credit of describing the first pure crystalline muscarine chloride. In a preliminary note,[47] followed later by full chemical[48] and pharmacological details,[49] they described the successful application of partition chromatography on cellulose columns to the separation of the crude bases. The homogeneity of their fractions was evaluated by controlled toxicity tests and by paper chromatography, a modified Dragendorff's reagent[50] being used to locate the bands.

The analyses of their crystalline chloride, chloroaurate, and reineckate salts of muscarine each corresponded to a new empirical formula $C_9H_{20}O_2N^+ X^-$. The optical rotation, $[\alpha]_D^{20} + 6{\cdot}7°$ (in water) was considerably higher than that recorded by Kögl *et al.*[39] whose material must have been grossly contaminated and whose structures (5) and (6) could no longer be accommodated.

Shortly after this announcement Balénovic *et al.*[51] in Yugoslavia, using partition chromatography on cellulose columns, and Kuehl, Lebel, and Richter[52] in America, employing a preliminary fractionation on a resin I.R.C. 50 and purification by chromatography on Super-cel, isolated crystalline muscarine chloride and verified the newly established formula. Searching for new techniques for the large-scale preparation of muscarine, Balénovic *et al.*[53] found that choline and muscarine could be adsorbed on, and successively eluted from, the resin Dowex 50-X8. A similar procedure, with Amberlite I.R.A.-400, was applied later by Eugster and Muller[16b] to the isolation of muscarine from other fungal species.

Kögl and his co-workers[54] had also resumed work in this field and, realising the limitations of their previous method of fractionating the reineckates, had, by chromatography on Norite, succeeded in isolating both pure muscarine chloride and acetylcholine chloride. They withdrew their previous pronouncement in favour of the formula $C_9H_{20}ClNO_2$.

It could now be anticipated that, with identical results obtained in four independent laboratories, any deductions relating to the structure of muscarine would at least be based on the degradations of a single chemical entity.

[47] Eugster and Waser, *Experientia*, 1954, **10**, 298.
[48] Eugster, *Helv. Chim. Acta*, 1956, **39**, 1002.
[49] Waser, *Experientia*, 1955, **11**, 452.
[50] Thiele and Reuther, *Naturwiss.*, 1954, **41**, 230.
[51] Balénovic, Cerar, Găspert, and Galijan, *Arhiv Kem.*, 1955, **27**, 105.
[52] Kuehl, Lebel, and Richter, *J. Amer. Chem. Soc.*, 1955, **77**, 6663.
[53] Balénovic and Štéfanac, *Chem. and Ind.*, 1956, 23; Balénovic, Bregant, and Štéfanac, *Croat, Chem. Acta*, 1957, **29**, 45.
[54] Kögl, Salemink, Schouten, and Jellinck, *Rec. Trav. chim.*, 1957, **76**, 109; Cox, Diss., Utrecht, 1958.

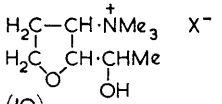
The Structure of Muscarine

In order to maintain reasonable chronological order and in spite of considerable overlapping of publications, it is felt that, because of Eugster's prior claim to publication of the correct formula, his preliminary efforts to determine the structure of muscarine should be discussed first.

Eugster[48] obtained a yield of 0·0003% of muscarine chloride from fly agaric but much of his work was carried out on material isolated from *Inocybe patouillardi*, to which previous reference has been made. That the alkaloid was a quaternary salt was shown by its pyrolysis, with loss of methyl chloride, to the pharmacologically inactive tertiary base normuscarine, which could be reconverted into the quaternary salt, muscarine chloride. The absence of a carbonyl group was proved by non-reactivity in diagnostic tests and confirmed from the ultraviolet and infrared spectra. The presence of a hydroxyl group was indicated by formation of *O*-acetylmuscarine; and the remarkable stability of muscarine hydroxide eliminated an alkoxytrimethylammonium structure (see 9) such as that suggested by Rogers *et al.*[46]

These observations were amplified by Kuehl *et al.*[52] The presence of a hydroxyl absorption band (5·75 mμ) in the spectrum of muscarine chloride and its absence in that of acetylmuscarine suggested that only one such group was present. Although Zeisel methoxyl determinations were negative they considered that the inertness of the second oxygen atom might be the result of an ether structure. Neither Eugster nor Kuehl, admittedly working with micro-amounts, could detect either trimethylamine or any acidic product on Hofmann degradation.

By a series of micro-scale degradations of muscarine and a number of model substances, supported by evidence from infrared spectra, Eugster[55] proved that the second oxygen atom was located in a tetrahydrofuran ring and that the structures (10) and (11) accommodated his available evidence. The nature of the side chain was based on the formation of acetic acid by oxidation and of a little iodoform with hypoiodous acid. However, these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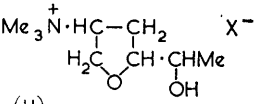
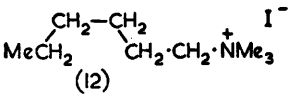

(11)

deductions proved to be wrong. In a later publication, Eugster and Waser[56] described an interesting application of catalytic oxidation which was later to prove invaluable in determining the structures of the synthetic stereoisomers of muscarine. Oxidation of muscarine chloride in the presence of reduced platinum according to the procedure of Sneedon and Turner[57]

[55] Eugster, *Helv. Chim. Acta*, 1956, **39**, 1023.
[56] Eugster and Waser, *Helv. Chim. Acta*, 1957, **40**, 888.
[57] Sneedon and Turner, *J. Amer. Chem. Soc.*, 1955, **77**, 190.

gave the oxo-derivative muscarone, whose infrared spectrum was of the cyclopentanone type, veritable proof that the hydroxyl group must be located in the tetrahydrofuran ring and not in the side chain.

In the meantime Kögl *et al.*[54] had pursued further chemical studies and by carrying out the Hofmann degradation on 100 mg. of muscarine chloride finally settled the vexing question of the nature of the products. Although the major product was normuscarine, they isolated and identified trimethylamine by paper chromatography and by *X*-ray powder analysis of the chloroaurate. In the infrared spectrum of muscarine iodide the characteristic bands of OH (3300 and 1380 cm.$^{-1}$), quaternary ammonium (1495 cm.$^{-1}$), and tetrahydrofuran (1079 cm.$^{-1}$) were located but carbonyl bands (1600 and 1830 cm.$^{-1}$) were absent. The culminating evidence relating to the disposition of the substituent groups was obtained by opening the tetrahydrofuran ring with hydriodic acid and red phosphorus, to give n-hexyltrimethylammonium iodide (12). Such a product could not arise from either of Eugster's structures (10) or (11) which would yield 2- and 3-aminohexane derivatives respectively, but must be derived from a hydroxy-derivative of the tetrahydrofuran (13). Further degradative work



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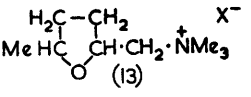
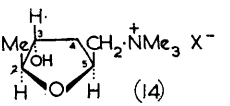


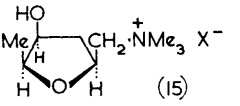
(13)

was found to be unnecessary, for from the possible variants derivable from a hydroxy-derivative of (13), the final selection was made by *X*-ray crystallographic analysis of muscarine iodide.[54,58] Differential Fourier synthesis clearly depicted the structure to be that of a quaternary ammonium salt of 5-aminomethyltetrahydro-3-hydroxy-2-methylfuran, and moreover one in which the hydroxyl group was in the *trans*-position with respect to both the methyl and the $\cdot CH_2 \cdot NMe_3^+$ side chain, as indicated in formula (14).



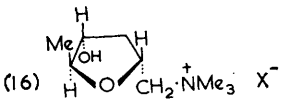
(14)

(14) Muscarine (2*S*, 3*R*, 5*S*)



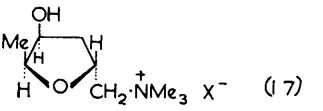
(15)

(15) Epimuscarine (2*S*, 3*S*, 5*S*)



(16)

(16) Allomuscarine (2*S*, 3*R*, 5*R*)



(17)

(17) Epiallomuscarine (2*S*, 3*S*, 5*R*)

[58] Jellinck, *Acta Cryst.*, 1957, **10**, 277; Diss., Utrecht, 1957.

Four racemic stereoisomeric forms of the basic muscarine molecule are possible, each of which can exist in two optically active forms giving a total of eight possible variants.

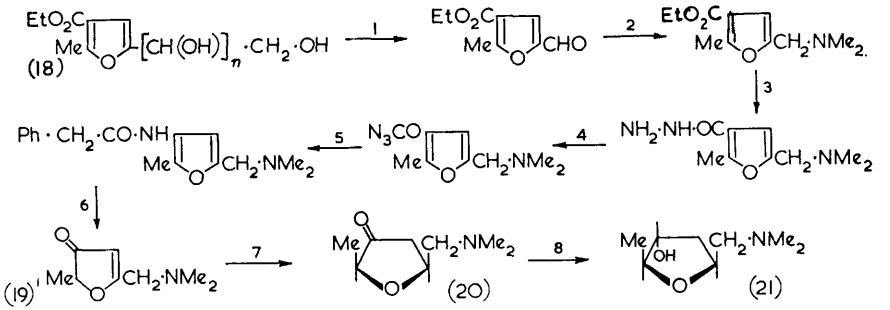
The four racemates have been named and assigned the structures (14)—(17) by Corrodi, Hardegger, and Kögl;[59] the stereochemical nomenclature, in parentheses, is based on that suggested by Cahn, Ingold, and Prelog.[60]

The two enantiomorphs of muscarine and the racemates of epi-, allo-, and epiallo-muscarine have now been synthesised.

Syntheses of Muscarine

(*A*) *Stereochemically Non-specific Syntheses.*—(±)-*Muscarine.* After publication of its structure, a number of syntheses of (±)-muscarine followed in rapid succession. From the furan derivative (18), obtained by condensing β-keto-esters with glucose or mannose, Eugster *et al.*[61] synthesised, by the series of reactions depicted (Synthesis 1), the important intermediate 5-dimethylaminomethyl-2,3-dihydro-2-methyl-3-oxofuran (19), which proved so useful in the preparation of all the stereochemical variants.

Synthesis 1.



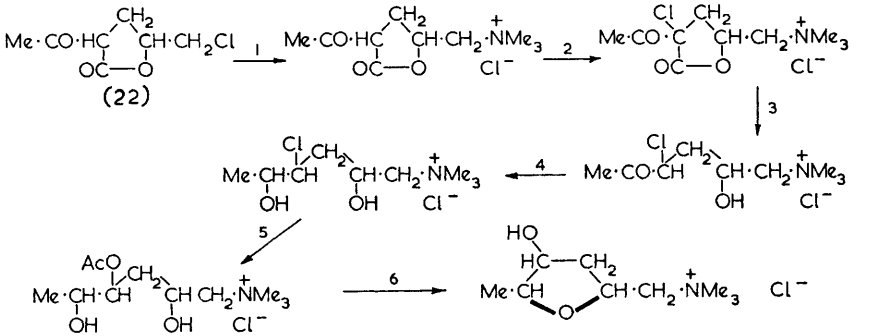
Reagents: 1, Pb_3O_4. 2, Leuckart reaction. 3, N_2H_4. 4, HNO_2. 5, $Ph{\cdot}CH_2{\cdot}OH$. 6, 2_N-HCl at 100°. 7, Pd—H_2. 8, $LiAlH_4$. 9, KBH_4.

Catalytic hydrogenation of the ketone (19) gave (±)-normuscarone (20) which was further reduced by lithium aluminium hydride to (±)-normuscarine (21). Alternatively, one-stage reduction of the ketone (19) with potassium borohydride gave a mixture of racemates of the normuscarines (see later section on isomers of muscarine) from which (±)-normuscarine (21) was isolated by chromatography on deactivated alumina. The infrared spectrum of the quaternary (±)-muscarine iodide, obtained from the

[59] Corrodi, Hardegger, and Kögl, *Helv. Chim. Acta*, 1957, **40**, 2454.
[60] Cahn, Ingold, and Prelog, *Experientia*, 1956, **12**, 81.
[61] Eugster, *Helv. Chim. Acta*, 1957, **40**, 2462; Eugster, Häfliger, Denss, and Girod, *ibid.*, 1957, **40**, 205.

base (21), was almost identical with that of natural (+)-muscarine iodide and its biological activity one-half of that of the natural isomer.

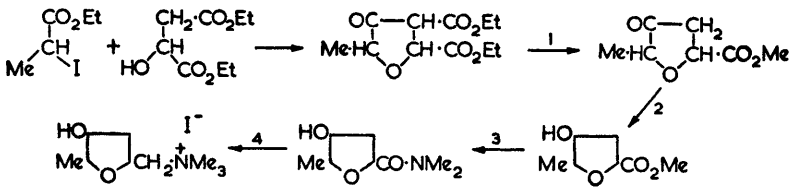
Synthesis 2.



Reagents: 1, NMe_3. 2, SO_2Cl_2. 3, 20% HCl. 4, $NaBH_4$. 5, AgOAc. 6, 20% H_2SO_4, then conc. H_2SO_4.

Kögl, Cox, and Salemink[62] described a straight-forward synthesis from δ-chloro-α-acetyl-γ-valerolactone (22) which followed the course indicated in Synthesis 2. The final ring closure, carried out according to the procedure described by Reppe,[63] gave a mixture of isomers from which about 30% of (±)-muscarine chloride was isolated by chromatography on Norite. The product, which did not crystallise, had an activity of about one-third of that of the natural alkaloid.

Synthesis 3.



Reagents: 1, H_2SO_4, then CH_2N_2. 2, $NaBH_4$. 3, $NHMe_2$. 4, $LiAlH_4$, then MeI.

A second reputed synthesis by Kögl *et al.*[64] (see Synthesis 3) was shown later[59] to give little or no muscarine but essentially the racemate of allo-muscarine.

A mixture of muscarine and allomuscarine was obtained by a method devised by Natsumoto and Maekawa.[65]

[62] Kögl, Cox and Salemink, *Experientia*, 1957, **13**, 137; *Annalen*, 1957, **608**, 81.
[63] Reppe, *Annalen*, 1955, **596**, 90, 118.
[64] Corrodi, Hardegger, Kögl, and Zeller, *Experientia*, 1957, **13**, 138.
[65] Natsumoto and Maekawa, *Angew. Chem.*, 1958, **70**, 507.

(*B*) *Resolution of* (±)-*Muscarine.*—Two rather conflicting procedures have been described for the resolution of (±)-muscarine.

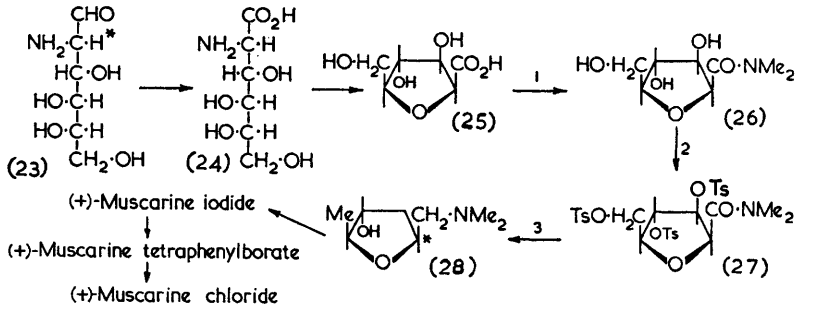
By partition chromatography on powdered cellulose, Kögl *et al.*[66] improved the separation of muscarine from the mixture of racemates obtained in their synthesis 2 and effected its resolution by means of (−)-di-*p*-toluoyltartaric acid. The salt of (+)-muscarine separated first and the regenerated (+)-muscarine chloride was found to be identical with natural muscarine chloride.

In contrast, however, Eugster *et al.*[67] found that the less soluble salt of (−)-di-*p*-toluoyltartaric acid was that of the unnatural isomer (−)-muscarine.

(+)-Muscarine chloride, m.p. 178—179°, $[\alpha]_D$ + 8·1° (in EtOH), was obtained from the less soluble salt of (+)-di-*p*-toluoyltartaric acid. The (−)-muscarine chloride, m.p. 179—180°, $[\alpha]_D$ − 8·4° (in EtOH), had only 5% of the biological activity (blood pressure in cats) of its enantiomorph.

(*C*) *Specific Stereochemical Syntheses of* (+)- *and* (−)-*Muscarine.*—An elegant stereospecific synthesis of (+)-muscarine chloride was devised by Hardegger and Lohse[68] (see Synthesis 4). This started from L-glucosamine (23) and proceeded *via* L-glucosaminic acid (24), L-chitaric acid (25)

Synthesis 4.



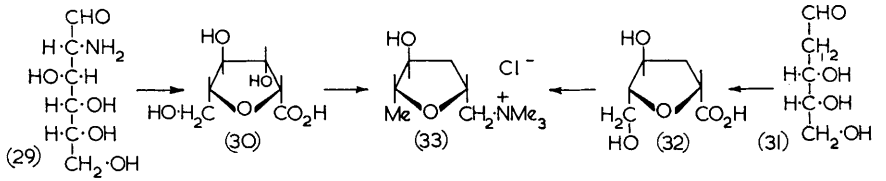
Ts = *p*-$C_6H_4Me{\cdot}SO_2$
Reagents: 1, CH_2N_2, then $NHMe_2$. 2, *p*-$C_6H_4Me{\cdot}SO_2Cl$. 3, $LiAlH_4$.

and its dimethylamide (26). Reduction of the tritosyl derivative (27) with lithium aluminium hydride produced a low yield of (+)-normuscarine (28) which was quaternised, and the (+)-muscarine isolated as the tetraphenylborate, which was converted into the chloride with cæsium chloride.

Since the absolute configuration and relation of L-glucosamine to L-glyceraldehyde has been established, natural muscarine must be

[66] Cox, Hardegger, Kögl, Leitchti, Lohse, and Salemink, *Helv. Chim. Acta*, 1958, **41**, 229.
[67] Eugster, Häfliger, Denss, and Girod, *Helv. Chim. Acta*, 1958, **41**, 886.
[68] Hardegger and Lohse, *Helv. Chim. Acta*, 1957, **40**, 2383.

2,5-anhydro-1,3,6-trideoxy-L-*ribo*-hexityltrimethylammonium chloride in which $C_{(5)}$ (marked with an asterisk) is the Rasanov carbon with the L_G-configuration, and it has been described as L-(+)-muscarine.

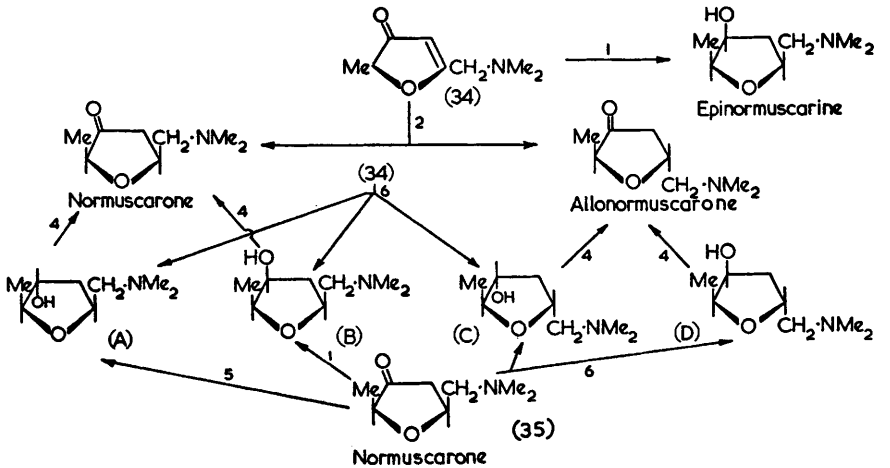


Two comparable specific syntheses of (−)-muscarine chloride were also carried out. The first[66] proceeded from D-glucosamine (29) *via* D-chitaric acid (30), and in the second[69] 2-deoxy-D-ribose (31) was converted into 3-deoxy-D-chitaric acid (32). By analogous series of reactions to those used in the preparation of the (+)-isomer, both these acids gave (−)-muscarine chloride (33) which was devoid of activity on the isolated frog heart.

Synthesis of Epi-, Allo-, and Epiallo-muscarine, etc.

As mentioned previously, the ketone (34) proved to be a valuable intermediate. The reduction of this ketone was investigated at considerable length and for various conditions, (±)-muscarine and its three racemic stereoisomers being isolated.[70].

Synthesis 5.



Reagents: 1, H_2–Raney Ni. 2, H_2–Pd. 3, KBH_4. 4, Pt–O_2. 5, $LiAlH_4$. 6 $Al(OPr^i)_3$.

A, Normuscarine. B, Epinormuscarine.
C, Allonormuscarine. D, Epiallonormuscarine.

[69] Hardegger, Furter, and Kiss, *Helv. Chim. Acta*, 1958, **41**, 2401.
[70] Eugster, Häfliger, Denss, and Girod, *Helv. Chim. Acta*, 1958, **41**, 205, 583, 705.

The various courses of reduction, as shown in Synthesis 5, invariably led to a series of mixed racemates of the normuscarines, which were separated by chromatography on alumina and subsequently quaternised.

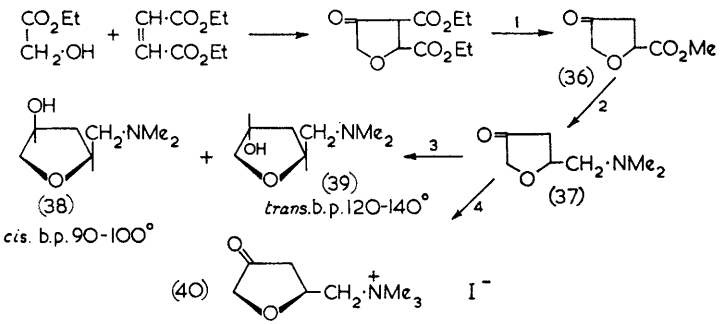
The nature of the products was conveniently established as follows.

The four isomers fall into two natural groups according as to which keto-derivative (normuscarone) they give on catalytic oxidation, thus:

Nor- and epinor-muscarine	→ Normuscarone
Allonor- and epiallonor-muscarine	→ Allonormuscarone

In each of the two pairs, therefore, the individuals differ only with respect to the configuration of the hydroxyl group. That the hydroxyl group of muscarine was *trans* to both the Me and the $CH_2{\cdot}NMe_3^+$ group was known from the *X*-ray crystallographic work and from the synthesis from L-glucosamine, and further proof was derived from the infrared spectra. Epimuscarine iodide gave a band at 3165 cm.$^{-1}$, which was absent from the spectrum of muscarine iodide and is characteristic of hydrogen bonding. This implied that the hydroxyl and the NMe_3^+ group were *cis* with respect to each other in epimuscarine and *trans* in muscarine. Similarly, the infrared spectra of allo- and epiallo-muscarine showed this discriminating difference associated with hydrogen bonding in the allomuscarine molecule.

The pharmacology of the muscarines and muscarones has been studied in great detail. Before reviewing their stereospecificity, brief mention must be made of the syntheses by Eugster and his colleagues of two series of derivatives, the demethyl- and the dehydro-muscarines, which are of considerable importance in such a discussion because in each case the number of asymmetric centres has been reduced from three to two.



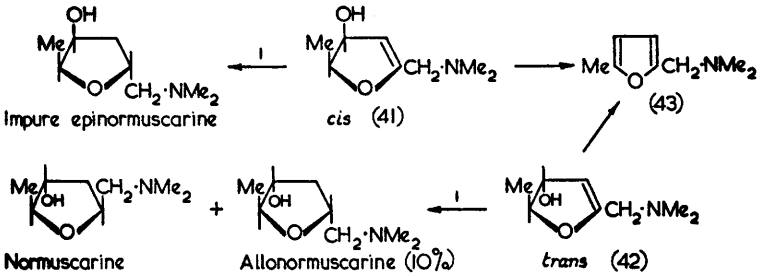
Reagents: 1, H_2SO_4, then CH_2N_2. 2, $NHMe_2$, then $LiAlH_4$. 3, KBH_4. 4, MeI.

Demethylnormuscarone (37) and its quaternary salt demethylmuscarone iodide (40) were prepared[71] from the dimethylamide derived from methyl tetrahydro-5-oxofuran-2-carboxylate (36) by first protecting the keto-group and then reducing the amide group with lithium aluminium hydride.

[71] Zwicky, Waser, and Eugster, *Helv. Chim. Acta*, 1959, **42**, 1177.

Reduction of demethylnormuscarone with potassium borohydride resulted in a mixture of *cis*- (38) and *trans*-demethylnormuscarine (39) which were separated by fractional distillation and chromatography on alumina, and were finally quaternised.

By further fractionation[72] of the mixture derived from the reduction of compound (34) with sodium borohydride (see Synthesis 5) were isolated, in addition to the normuscarine isomers, *cis*- (41) and *trans*-4,5-dehydromuscarine (42) as products of partial reduction. Both isomers were dehydrated at 200° to 2-dimethylaminomethyl-5-methylfuran (43), the quaternary salt of which had previously been synthesised and its muscarinic activity examined by Ing, Kordik, and Tudor-Williams.[73]



Reagent: 1, H_2–Raney Ni.

Pharmacological Stereospecificity in the Muscarine and Muscarone Series

One remarkable feature has transpired in the course of the very comprehensive pharmacological studies which have now been carried out with these alkaloids. Although (+)-muscarine does not possess the highest muscarinic activity, in contrast both to its stereoisomers and to such derivatives as have so far been examined, its action is highly specific and restricted to activity at postganglionic parasympathetic effector sites. Thus Waser[74] demonstrated that high doses have only peripheral parasympathetic action in the cat, and even with toxic doses up to 100 μg./kg., in the atropinised animal no blocking of sympathetic and parasympathetic ganglia or nerve-muscle transmission could be detected. By inhibiting the effect of cholinesterase he found that the activity of muscarine was enhanced ten-fold and suggested that it might be transformed in the body into an active acetyl ester. *In vivo*, in the cat and dog muscarine caused a marked drop in blood pressure.

Working with muscarine chloride isolated by the author of this Review, Fraser[75] showed that in the absence of an anticholinesterase the prepara-

[72] Denss, Girod, Häfliger, and Eugster, *Helv. Chim. Acta*, 1959, **42**, 1191.
[73] Ing, Kordik, and Tudor-Williams, *Brit. J. Pharmacol.*, 1952, **7**, 103.
[74] Waser, *Experientia*, 1955, **11**, 452; Konzett and Waser, *Helv. Physiol. Pharmacol. Acta*, 1956, **14**, 202.
[75] Fraser, *Brit. J. Pharmacol.*, 1957, **12**, 47.

tion was 4—5 times more active than acetylcholine on isolated rabbit auricles, but parallel dose–response curves were not obtained. With the same specimen, Ambache, Perry, and Robertson[76] described the absence of a pressor reponse in atropinised anæsthetised cats, a weak ganglion-stimulating effect readily blocked by atropine, and in large doses a stimulation of the frog's rectus abdominis. Gyermek and Herr,[77] however, suggest that there is a lack of ganglionic stimulation in doses 400—1000 times larger than those which stimulate post-ganglionic parasympathetic effector sites.

Fraser[75] found that muscarine was stable to pepsin and that no response could be detected on feeding it orally to monkeys in doses much greater than would be expected to cause poisoning by ingestion of *A. muscaria* in man.

The following brief summary attempts to fix the highlights of the points which have emerged from the spectrum of physiological action, but the reader is referred to the relevant publications for the extensive and specialised technical details. Tables 1 and 2 correlate the potencies of muscarine and its isomers in tests on selected pharmacological preparations.

(*a*) The enantiomorphs of (±)-muscarine differ greatly in potency, which is almost exclusively associated with the (+)-isomer.

(*b*) Waser[78] and Gyermek and Unna[79a] have clearly illustrated that there is a definite specificity of the action of the muscarines on post-ganglionic effector sites. The racemic forms of epi-, allo-, and epiallo-muscarine possess only a fraction of the potency of (+)-muscarine. Waser states they have less than one-hundredth of the effect of (+)-muscarine on the blood pressure of cats and on isolated frog hearts. Gyermek and Unna found that all muscarine isomers were devoid of significant action on skeletal muscle. These results (see Table 1*b*) lead to the conclusion that when the hydroxyl group is *cis* to either or both of the methyl and the $CH_2{\cdot}NMe_3^+$ group, as in the epi-, allo-, and epiallo-isomers, there is an enormous decrease in muscarinic activity.

(*c*) In very marked contrast there is a lack of stereospecificity in the series of related ketones, (+)- and (−)-muscarone and (±)-allomuscarone which differ only slightly in their action on smooth muscle (Table 1*c*). Although the muscarones have high muscarinic activity, they also exert effects at other synapses as well. Thus they exhibit strong nicotinic activity on the frog rectus and block ganglionic and neuromuscular transmissions in the cat. Muscarones, and in particular (−)-muscarone, were significantly more potent than (+)-muscarine and acetylcholine in stimulating post-ganglionic parasympathetic sites. In their most recent assessment Gyermek

[76] Ambache, Perry and Robertson, *Brit. J. Pharmacol.*, 1956, **11**, 442.
[77] Gyermek and Herr, *Fed. Proc.*, 1959, **18**, 399.
[78] Waser, *Experientia*, 1958, **14**, 356.
[79] Gyermek and Unna, (*a*) *Proc. Soc. Exp. Biol. Med.*, 1958, **98**, 882; (*b*) *J. Pharmacol.*, 1960, **128**, 30, 37.

TABLE 1.

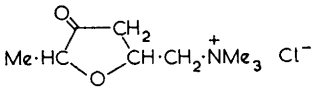
		γ/kg. *in vivo* (minimal dose) Blood pressure in cats	γ/ml. Perfusion of bath-volume Frog heart Muscarinic (M)	Frog rectus submaximal contraction. Nicotinic (N)	Quotient N/M
(*a*)	(±)-Muscarine iodide	0·01	0·032	> 500	>20,000
	(+)-Muscarine iodide	0·004	0·018	> 500	>30,000
	(+)-Normuscarine hydrochloride	5·0	5·0	—	—
(*b*)	(±)-Epimuscarine iodide	3·0	2·4	>1000	> 5
	(±)-Allomuscarine iodide	1·7	15	>1000	> 65
	(±)-Epiallomuscarine iodide	1·0	12	> 500	> 40
(*c*)	(±)-Muscarone iodide	0·0015	0·01	2·5	250
	(−)-Muscarone iodide	0·001	0·0075	2·5	330
	(±)-Allomuscarone iodide	0·003	0·02	1·5	75
(*d*)	*trans*-4,5-Dehydromuscarine iodide	0·01	0·2—0·3	50	250
	cis-4,5-Dehydromuscarine iodide	0·02	0·4	> 100	>250
	(±)-4,5-Dehydromuscarone iodide	0·0015	0·01	0·9	90
(*e*)	*trans*-Demethylmuscarine iodide	1·0	30	—	—
	cis-Demethylmuscarine iodide	7·0	400	—	—
Acetylcholine chloride		0·01	0·002	5	2500

(*a*) Concentration of sample (γ/kg.) producing fall of blood pressure equal to that given by 0·01 γ/kg. of acetylcholine, when injected intravenously into an anæsthetised cat.

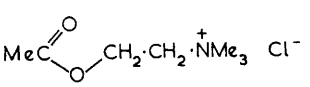
(*b*) Concentration of sample (γ/ml.) perfusing through an isolated frog's heart, which produces decreases in amplitude and rate of beat of both the auricle and ventricle, equivalent to those given with 0·002 γ/ml. of acetylcholine.

(*c*) Concentration of sample (γ/ml. of bath-volume) giving one-quarter of the maximum contraction of the isolated frog's rectus abdominis muscle.

and Unna[79b] found (−)-muscarone was 4—6 times more active than (+)-muscarine. The muscarones, therefore, resemble acetylcholine in activity and are more closely related to it structurally than is muscarine with its hydroxy-group, as illustrated.



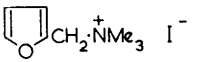
Muscarone

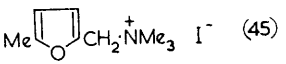


Acetylcholine

Although powerful nicotinic activity is introduced by formation of the muscarones, that this is a result of the substitution of an oxo- for a hydroxy-group cannot be concluded because of the simultaneous reduction in the number of asymmetric centres. Steric hindrance of the hydroxyl or keto-group and of the ring-oxygen atom will play important parts in determining the ease of contact with the cholinergic receptors. Moreover, since the tertiary base normuscarine is inactive, the quaternary nitrogen must be considered to be a third point of contact. According to recent studies by Gyermek and Unna[79b] all three constituents together with the ring-oxygen atom should be considered as essential pharmacophore groups.

(*d*) The introduction of a double bond, with corresponding loss of asymmetry at position 5, as in the 4,5-dehydromuscarines, had comparatively less effect in changing the pharmacological picture than in the muscarine series (Table 1*d*). Even with complete aromatisation of the ring, as in the compounds (44) and (45), high muscarinic activity had been observed.[73,80]

(44) 

 (45)

(*e*) Demethylation, with resulting loss of asymmetry at position 2 produced a marked decrease in potency (Table 1*e*).

(*f*) Witkop, Durant, and Friess[81] have examined the effect of muscarone derivatives on the inhibition of cholinesterase activity. *O*-Acetylmuscarine was found to be the most potent inhibitor and, as with the other diverse pharmacological preparations, the enzyme responded *in vitro* more markedly to stereochemical differences in the muscarine than in the muscarone series. From models of the isomers of muscarine they concluded that there appeared to be a closer approach in the natural than in the allo-series to coplanarity of the ring with both the quaternary nitrogen atom and the hydroxyl group. This could lead to additional binding strength with the receptor if the ring augmented the effect of the two polar groups.

[80] Fellows and Livingstone, *J. Pharmacol.*, 1940, **68**, 231.
[81] Witkop, Durant, and Friess, *Experientia*, 1959, **8**, 300.

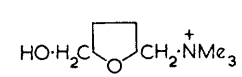
(*g*) Waser[82] recently tested a series of analogues of muscarine in which the tetrahydrofuran ring was replaced by tetrahydrothiophen. There was marked reduction in the diverse biological activities (Table 2). This he considered might be attributed to the inability of sulphur to form a hydrogen bond with the cholinergic receptor and also to the fact that the tetrahydrothiophen ring is known to be larger than the tetrahydrofuran ring[83] and could therefore affect the relative position and function of the methyl group.

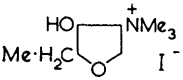
TABLE 2.

	Substance	γ/ml. Perfusion of bath-vol. Frog heart (M)	Frog rectus submaximal contraction (N)
(A)	Posn. of OH (±)	0·02	> 500
(B)	*trans*	500	>2000
	cis	1000	>1000
(C)	*trans*	30	—
	cis	400	—
(D)	*trans*	100	>1000
	cis	200	>1000
(E)	*trans*	50	—
	cis	1000	—
(F)	Mainly *trans*	>4000	>1000

In the same publication, increasing the length of the alkyl group to n- and iso-propyl was shown to decrease the activities greatly (Table 2).

The positional isomers (46) and (47) have been shown to have little if any muscarine activity.[84]

(46) 

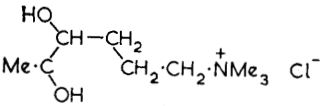
 (47)

Waser[85] has discussed the use of [^{14}C]muscarone in the elucidation of the nature of the cholinergic receptor site.

[82] Waser, *Experientia*, 1960, **16**, 347.
[83] Marsh, *Acta Cryst.*, 1955, **8**, 91.
[84] E. Gryszkiewicz-Trochimowski, O. Gryszkiewicz-Trochimowski, and 'Levy, *Bull. Soc. chim. France*, 1958, 603; Fraser, personal communication.
[85] Waser, *J. Pharm. Pharmacol.*, 1960, **12**, 577.

Other substances isolated from *A. muscaria*

Wieland, Motzel, and Merz[86] reported the presence of bufotenine in *A. muscaria*; Kögl, Salemink, and Schuller[87] have recently isolated a new alkaloid muscaridine, closely related to muscarine, which they found to be 4,5-dihydroxyhexyltrimethylammonium chloride (48). Its constitution was established by oxidation with periodate to acetaldehyde and with permanganate to the trimethylammonium derivative of β-aminobutyric acid



(48)

[86] Wieland, Motzel, and Merz, *Annalen*, 1953, **581**, 10.
[87] Kögl, Salemink, and Schuller, *Rec. Trav. chim.*, 1960, **79**, 278.