CHEMISTRY AND PHARMACOLOGY OF MUSCARINE, MUSCARONE, AND SOME RELATED COMPOUNDS

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I. INTRODUCTION

Because of the potent pharmacological actions of muscarine, research on its chemistry and pharmacology has been stimulated for over one hundred years.

It was the first poison known to have a selective action on organs innervated by the autonomic nervous system. The findings of these investigations, however, were uncertain and inaccurate until the isolation and crystallization of muscarine chloride from *Amanita muscaria* by Eugster and Waser (30) in 1954. Investigation of the chemical structure of the pure alkaloid indicated that the structural formula proposed by Kögl and associates (64) in 1931, which was accepted and appeared in textbooks concerned with the mushroom poison, was incorrect. This proposed chemical structure was changed in several essential points, and final proof of the structure was given by Jellinek (61) in 1957 with the help of X-ray diffraction analysis. These new findings set into motion research not only on the pharmacology of muscarine, but also on that of muscarine-like substances that are structurally related to acetylcholine. Accordingly, this review will cover not only the latest developments of muscarine research, but also the structure-activity relations of muscarine-like compounds.

II. BOTANICAL ORIGIN OF MUSCARINE

For many years the presence of muscarine was suspected in numerous varieties of mushrooms. However, since biological tests without authentic standards were used for the identification of alkaloids, the results were for the most part not valid. Recently, Eugster (20, 29) gave chemical proof for the occurrence of muscarine in a few species.

Amanita muscaria (L. *ex* Fr.) Quél., *Agaricus muscarius* L., the so-called fly agaric, is a mushroom well known in Europe, northern Asia, North and South America, and South Africa. On a strong stem, bulbous at the base, sits an orange-red cap, 8 to 20 cm in diameter, spotted with yellow-white warts. When young, the mushroom is wrapped in an outer sheath, the remnants of which are represented by several white rings around the base and the spots clinging to the moist and sticky surface of the cap. These may be washed off by rain or become quite pale. The inner sheath surrounds the stem like a cuff. The gills are soft and white. The red skin peels off the cap easily, and the flesh beneath has a yellow-orange or lemon color; the other parts are pure white.

Amanita pantherina (D.C. *ex* Fr.) is a smaller variety with the same white spots on a yellow-brown cap; alternatively, the color may be lemon or olive-brown.

Amanita muscaria contains muscarine in varying amounts, depending on location, climate, age, and time of vegetation. Referring to fresh weight, different authors have found:

Harmsen (50)	0.013%
King (63)	0.0016%
Kögl, Duisberg and Erxleben (64)	0.0003%
Kögl and Veldstra (67)	0.0003%
Eugster and Waser (30)	0.0002–0.0003%

The total amount is rather small and the high values found by Harmsen and King (50, 63) are probably in error. Eugster (29) found a similar concentration

of the alkaloid in dried Mexican fly agaric. The red skin contains 0.00033% or up to 50% more muscarine than the average amount in the flesh. The old habit of peeling as a precaution against poisoning therefore seems partly justified.

Many times more poisonous are *Inocybe lateraria* (*Ricken*) syn. *Inocybe patouillardi* (*Bres.*). These mushrooms have a yellow-white or uneven brick-red cap. The surface is dry and shining, the brim split and rolled in. The stem is thick and fibrous and its colors change from white to pink or brick-red. The gills are earth-colored or olive-brown. The flesh is sweet and smells like a pear, but it has an unpleasant after-taste.

Many different varieties of *Inocybe* look very similar and contain varying amounts of muscarine. Although Fahrig (34), Mecke (77), and Loup (75) found in earlier investigations on the toxicity of this kind of mushroom concentrations as high as 0.2 to 1.6%, Eugster (20, 22) isolated much smaller amounts:

Inocybe patouillardi (Bres.)	0.037%
Inocybe fastigiata (Fr. *ex* Sch.)	0.01%
Inocybe umbrina (Bres.)	0.003%
Inocybe rimosa (Queletii)	0.0003%
Inocybe bongardi (Weinm.)	0

Muscarine was suspected in *Boletus satanas* (*Lenz*), *Russula emetica* (*Schaeff*), *Clitocybe rivulosa* (101), and even in the most deadly *Amanita phalloides*, but thus far this has not been proved by the isolation of the alkaloid.

III. ETHNOMYCOLOGY AND INTOXICATION

The fly agaric has been used by different people and tribes against frostbite, trembling, tick bites, stuttering, brain and spinal cord diseases, psychoses, and heart disease with edema. In Siberia it is used by the medicine men of the Kamchaka tribes (Koryaks and Chukchi) for their enjoyment and religious rites, as it produces delirious excitation and sleep with hallucinations (52). The active principle is eliminated unchanged in the urine and drunk again for reasons of economy. The incomparable fury of the fighting Berserkers in Iceland and Scandinavia has been ascribed to intoxication with the fly agaric (33). In Mexico drunkenness and exaltation caused by the black Mexican variety of Amanita is well known (83).

Poisoning with these mushrooms is rare, since they are easily recognized. However, they have been mistaken as edible by foreigners and immigrants. For example, Italian workers in Switzerland, and Balkan prisoners interned during the wars in western Europe were poisoned relatively frequently by them. The edible *Amanita caesaria* has a very similar appearance to *Amanita muscaria*, except that it has no warts and the yellow color of the flesh is absent. *Inocybe lateraria* has often been taken to be *Tricholoma gambosum* (Maipilz) or as a wood mushroom (*Psalliota lanipes*), both of which are harmless.

The symptoms of intoxication with mushrooms rich in muscarine, especially *Inocybe*, are very typical:

1. The symptoms start early, after one-quarter to two hours, with headache,

nausea, vomiting, and constriction of the pharynx. Then salivation, lacrimation, and diffuse perspiration set in, combined with miosis, disturbed accommodation, and reduced vision. Gastric and small bowel colic leads to diarrhea, and there is a painful urge for urination. Bronchoconstriction leads to asthmatic attacks and severe dyspnea, and bradycardia combined with marked hypotension and vasodilation results in circulatory shock. Death after 8 to 9 hours has been reported in about 5% of the cases, but can be avoided completely by prompt diagnosis and treatment with atropine. The prognosis is good.

2. Usually after eating *Amanita muscaria* or its toxic varieties, central symptoms predominate, depending on the geographical localization of the mushroom. Thus *mycetismus cerebralis* may be combined with the above-mentioned symptoms of muscarine poisoning. It starts with headache and somnolence, which suddenly change to unrest, nausea, blurred vision and ataxia. Then psychic symptoms begin with anxiety, hallucinations, a furious delirium with dancing and singing, later convulsions and tonic spasms, and even opisthotonus and trismus may develop. These manic symptoms may pass away after some hours, and give place to complete coma with shock, defective respiration, and death after 5 to 24 hours, or they may change to deep sleep, followed by awakening without any symptoms or signs except for a retrograde amnesia.

Thus, as a result of the combination of peripheral and central actions, signs and symptoms may change unexpectedly: miosis or mydriasis, slowing or acceleration of the pulse rate, mania or sedation. Poisoning with fly agaric is very different from intoxication with muscarine because of these psychic effects. Atropine antagonizes only the peripheral actions of muscarine. The central symptoms, depending on the state of excitation, are controllable with hypnotics or sedatives.

There are several possible explanations for these differences in symptoms. They are usually attributed to different types of compounds in *Amanita muscaria*. 1. *Muscarine*, with peripheral parasympathomimetic actions, augmented by *choline* and *acetylcholine*, all of which have been isolated. 2. *Muscaridine*, an atropine-like acting substance, especially in the *Amanita* species of northern Europe and Siberia, with predominantly central action, which has recently been isolated (66a). 3. *Capillary toxin* and a volatile active principle (*Pilztoxin* of Harmsen), absent in dried mushrooms. 4. *Muscarufine*, which causes the red color of the skin of the mushroom, and which has been isolated as a quinone-like oxytricarbonic acid (65), but its nature is still uncertain (23).

The existence of different antagonistic principles in the same mushroom seems unusual. One of the unknown substances could be an indole alkaloid, but serotonin and bufotenin have not been identified with certainty (100). Possibly the active principle is a derivative of 4-hydroxy indole, similar to psilocybin (23). Different amino acids or unknown peptides may be responsible for the central actions. Although muscarine is a quaternary amine, it might pass the blood-brain barrier, perhaps in combination with an amino acid or lecithin, and exert strong central actions.

IV. CHEMISTRY OF MUSCARINE AND MUSCARONE

A. Isolation

In 1869 Schmiedeberg and Koppe (88) published the first fundamental paper on muscarine. The alkaloid was prepared by precipitation of an alcohol-water extract of *Amanita muscaria* with potassium bismuth- and potassium mercury iodide. The concentrate was tested on cats and frogs and shown to have parasympathomimetic activity. In 1875, Harnack (51) separated choline from muscarine as the chloroaurate. He probably had only 20% muscarine in his preparation. Later (87), he oxidized choline with nitric acid and obtained a base, which he assumed to be identical with muscarine. In 1885 Boehm (10) demonstrated that this "synthetic choline-muscarine" had a strong curariform action that was not antagonized by atropine, indicating that it was not pharmacologically identical with muscarine. Betainaldehyde was also found to be quite different in its actions by Meyer in 1893 (78). In 1907, Straub and Führer (40, 93) investigated the antagonism between muscarine and atropine on the isolated frog heart. Their quantitative method is still used today. Honda in 1911 (56) attempted again to prepare pure muscarine as the tartrate, but obtained only an enriched preparation of muscarine still mixed with choline. In 1914 Ewins (32) definitely proved that "synthetic choline-muscarine" was choline nitrite: $(CH_3)_3N^+CH_2CH_2ONO$.

King (63) was able to isolate muscarine in 1922 by its solubility in absolute alcohol, insolubility with basic lead acetate and precipitation with mercuric chloride. He fractionated muscarine as the bitartrate from choline, and finally obtained nearly pure muscarine-chloroaurate. It was tested on the isolated rabbit ileum and hearts of toads and frogs.

Kögl *et al.* (64) began their work in the autumn of 1928 and published their first paper in 1931. They cleaned and extracted in one night 1250 kg of fly agaric and peeled the skin of 500 kg for work on muscarufine! During the course of the work, some of the investigators developed symptoms of intoxication: tears, sweating, and enteric colic, presumably from absorption of the alkaloids through the skin. Because of an insufficient supply of alcohol during the first three days, the washed fly agarics were moistened with alcohol and stored in a cold room. This procedure probably resulted in the destruction of a considerable portion of the muscarine. The alcoholic extract was concentrated by vacuum distillation. Kögl (64) separated the mixture in a system of water, alcohol, and ether. Muscarine remained in the alcoholic solution. The residue was again dissolved in water, the fat extracted with ether, and muscarine adsorbed on charcoal. This procedure resulted in a 9-fold concentration. Choline and similar bases were separated by adsorption on a permutite column and precipitated with mercuric chloride. This gave a 170-fold concentration of muscarine. The Reinecke salt was biologically active; the tetrachloroaurate had a melting point of 115 to 117°. Elemental analysis indicated the empiric formula $C_8H_{18}O_2N$.

The isolation of pure, crystalline muscarine chloride was accomplished by Eugster and Waser (30) in a similar way in 1954. Muscarine and other bases were

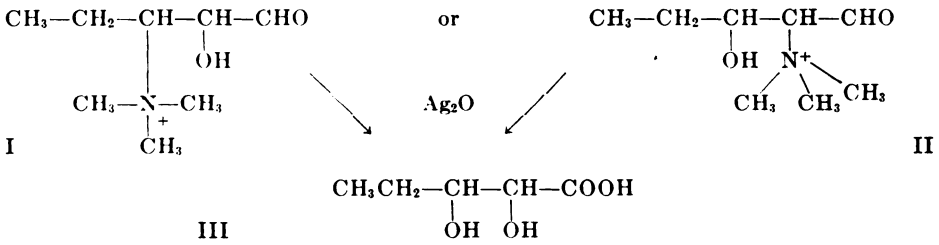
precipitated in the alcoholic extract of *Amanita muscaria* by Reinecke acid, the salts decomposed by the Kapfhammer method to the chlorides, and the raw chlorides chromatographed on cellulose columns with different elution systems. The biological activity of the elution fluid was tested with the isolated frog heart, and later by colorimetric methods (Dragendorff reagent, iodine vapor, bromcresol green). Pure muscarine was crystallized first as the tetrachloroaurate and later as the chloride. From 124 kg of mushrooms, 260 mg of pure muscarine chloride was isolated, which represents a concentration of 480,000-fold, and a yield of 70%.

Muscarine is very soluble in alcohol and insoluble in ether (22, 23). It is stable in weakly alkaline aqueous solution, but easily oxidized in acid. It is only weakly adsorbed by charcoal. It is precipitated by phosphotungstic acid, Reinecke acid, $KBiI_4$, $KHgI_4$, and sodium tetraphenyl boron.

B. Determination of structure

Combustion analysis gave the empiric formula $C_9H_{20}O_2N^+Cl^-$, often with one-half or one molecule of water. The melting point was 181 to 182°C, and the optical rotation in water: $\alpha_D^{20.5}$ + 6.7°. Muscarine iodide (m.p. 149°) is much less hygroscopic than muscarine chloride; both are highly soluble in water.

The elucidation of the structure of muscarine was chiefly due to Eugster (18, 19), Eugster and Waser (30, 31), and Kögl *et al.* (66). In their early investigation Kögl *et al.* (64) found that after Hofmann degradation with silver oxide in the cold, trimethylamine was in the distillate, and α,β-dihydroxyvalerianic acid (III) in the residue. Since muscarine is stable with alkali, this degradation was never confirmed. In contrast to the results of Kögl *et al.* later work showed muscarine to have a negative aldehyde reaction (Tollens, Schiff, Fehling, Angeli-Rimini). Therefore the old formulas (I, II) are incorrect.

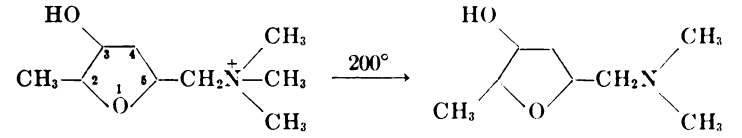


The presence of a *trimethylammonium group* was confirmed not only by liberation of trimethylamine in molten alkali but also by pyrolysis of muscarine chloride at 200° *in vacuo*. Normuscarine (V) is formed by splitting off methyl-chloride. It is soluble in ether and alcohol, and is inactive on the frog heart. Determination of N-alkyl groups showed muscarine to possess 3, and normuscarine 2 N-methyl groups. Normuscarine was quantitatively converted back to biologically active muscarine by quaternization.

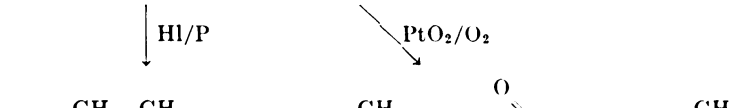
Since muscarine (IV) cannot be reduced nor can it be degraded by periodic acid, one oxygen belongs to a secondary hydroxy group which may be acetylated

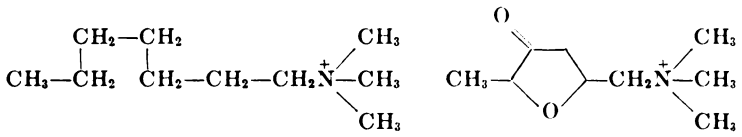
to acetylmuscarine, or oxidized by PtO_2 in O_2 to the corresponding ketone, muscarone (VI). The second oxygen atom was shown by infrared spectroscopy to be most probably an ether-oxygen in a five-membered ring (tetrahydrofurane).

Elucidation of the carbon skeleton was more difficult. Degradation by chromic acid gave only acetic acid and no higher fatty acids. Muscarine treated with PBr_3 and then reduced with zinc or lithium aluminum hydride gave propionic acid and traces of valerianic acid. Finally, degradation with hydriodic acid and phosphorus resulted in n-hexyl-trimethylammonium iodide (VII), a product containing the whole carbon chain.



Muscarine IV — nor-Muscarine V

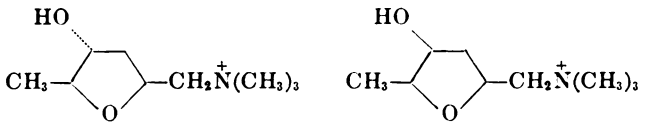




VII — Muscarone VI

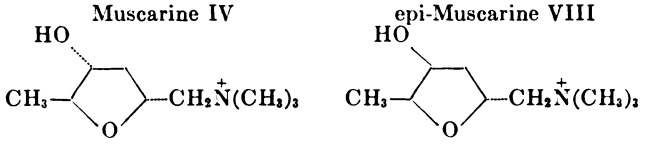
X-ray diffraction analysis of muscarine by Jellinek (61) showed the constitution with the steric position of all substituents on the tetrahydrofurane ring (Fig. 1).

Because of the three asymmetric centers in muscarine, four pairs of enantiomorphs exist. All have been synthesized and their steric configurations proven by oxidation to the corresponding ketones, and by infrared spectroscopy of the tertiary bases. In the following formulas, side-chains below the ring plane are marked by a dotted line.



Muscarine IV

epi-Muscarine VIII



allo-Muscarine IX

epiallo-Muscarine X

C. Synthesis of muscarine and its isomers

A stereospecific synthesis was achieved by Hardegger and Lohse (48). Starting with L-glucosaminic acid (XI), they converted the compound by diazotization to

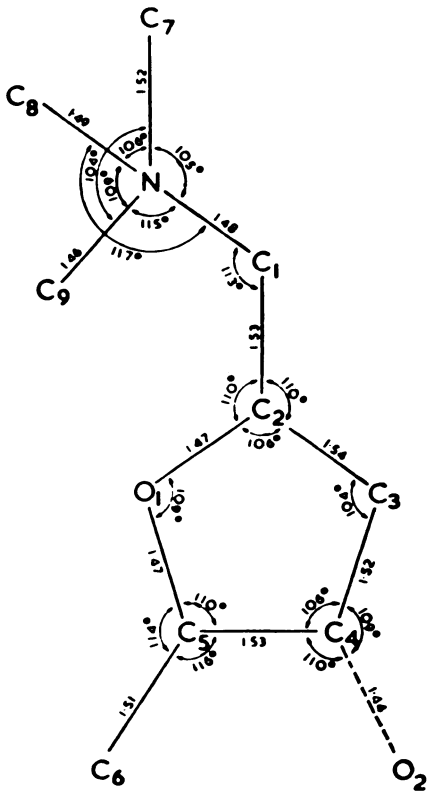
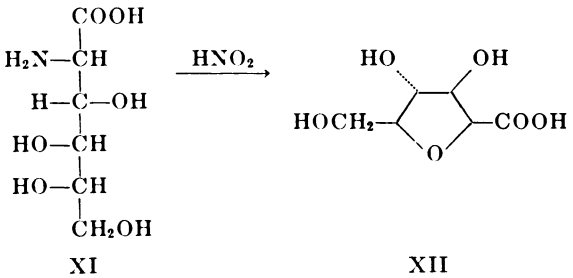
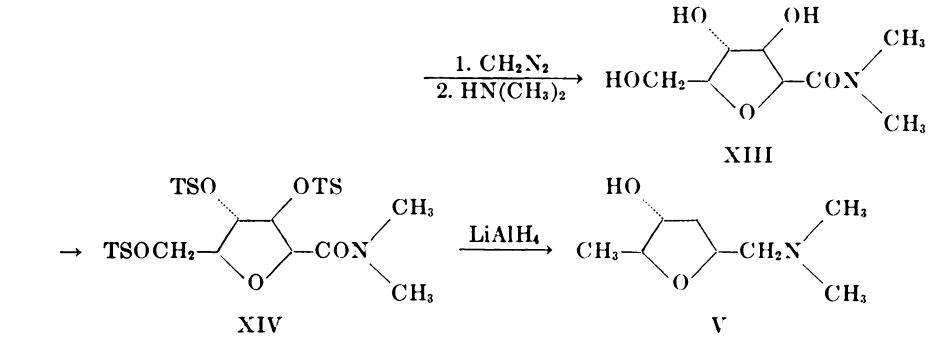


FIG. 1. Structure of muscarine, determined by X-ray analysis (Jellinek, 61). Bond distances in Å and angles within the muscarine ion. (With permission of the author.)

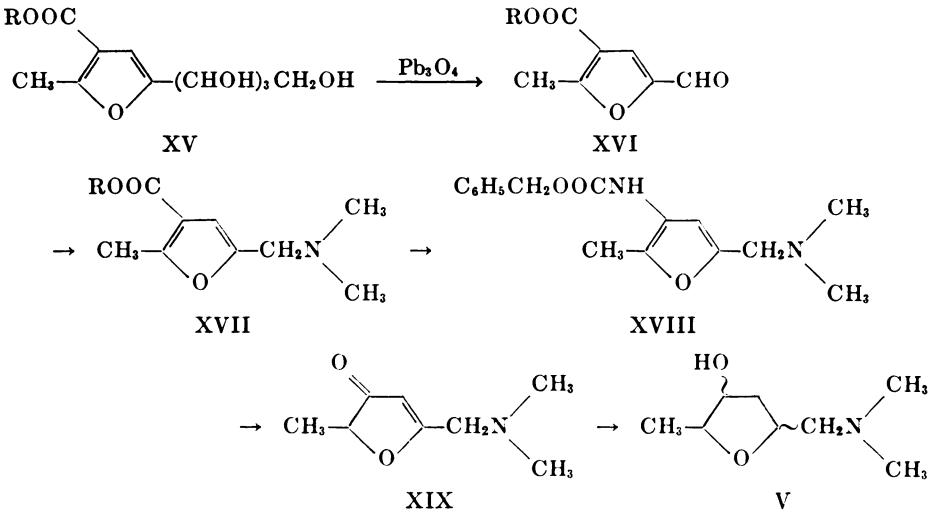
L-chiatric acid (XII), the methyl-ester of which reacted with dimethylamine to give the dimethylamide (XIII). Its tritosylate (XIV) was then reduced by lithium aluminum hydride to normuscarine (V), which was finally methylated to L(+)-muscarine (IV). Starting with D-chiatric acid, they obtained in the same way the enantiomorph D(−)-muscarine (14).



XI XII



Eugster *et al.* (21, 25–28) developed a method of synthesis for all enantiomorphs. They started by condensing glucose with acetoacetic ester to 2-methyl-5-tetrahydroxybutyl-furan-(3)-carboxylic ester (XV). Oxidation by Pb_3O_4 gave a 5-formyl compound. Condensation with dimethylformamid/formic acid introduced the dimethyl side chain (XVII). Its hydrazide was transformed to the azide, and in benzyl alcohol to the benzyl urethane (XVIII). Acid saponification led directly to the key compound, dihydrofuranone (XIX), which was reduced in different ways to all four diastereoisomers of nor-muscarine.



V. PHARMACOLOGY OF L(+)-MUSCARINE

The only previous review on muscarine is that by Fühner (1923) (41) in Heffter's "Handbuch der experimentellen Pharmakologie." It presents an interesting and comprehensive account of all experimental results known at that time. Unfortunately, the term muscarine was applied not only to the alkaloid extracted and first described in its pharmacological actions by Schmiedeberg and Koppe in 1869 (88), but also to "synthetic muscarine," which was choline nitrite. The fore-

going pages have shown muscarine to be chemically entirely different from choline, and to possess no ester-linkage. Muscarine itself had not been obtained in a pure form at that time. Therefore, it has to be kept in mind that the older results were due not only to the alkaloid, but also to other amines and acetylcholine, which are normally present in *Amanita muscaria*. Since the isolation and synthesis of pure muscarine, different groups of pharmacologists have reinvestigated its actions, and this review will mainly deal with their findings.

A. Actions on the intact animal

1. Toxicity and general actions (Table 1). The i.v. LD50 of muscarine chloride in the mouse was found to be 0.23 mg/kg, which is 143 times more potent than acetylcholine chloride (33 mg/kg) (39). Signs of poisoning were similar for both drugs, with the difference that at the LD50 acetylcholine was lethal almost immediately but muscarine only after three to ten minutes. Gyermek and Unna (46) found 0.42 mg muscarine chloride/kg to be lethal within 30 to 60 seconds as the result of respiratory failure; the heart was still beating after respiration had ceased. The bronchi were filled with secretions, and there was marked salivation, lacrimation, and defecation.

An interesting experiment on the *monkey* showed muscarine to have little activity by mouth. An intraperitoneal injection of 0.5 to 2 mg was rapidly followed by profuse mucous salivation and vomiting (39). The animal became prostrate with apparently severe abdominal discomfort and miosis. The respiration was deep and slow with uncoordinated abdominal and thoracic movements. Atropine antagonized these signs and symptoms, with the exception of the miosis, within five minutes. Mydriasis was observed forty minutes later. The same monkey was given 2 mg by mouth a fortnight later without effect during the next five hours. Although muscarine was shown to be stable to boiling and to pepsin, no action following oral administration of this dose was found in the monkey, despite the fact that the amount given was many times that which causes poisoning by the ingestion of *Amanita muscaria* in the human being.

2. Cardiovascular effects. Most intravenous experiments on whole animals have been performed on rabbits, cats, and dogs. Fraser (39) found muscarine chloride to be four times more potent than acetylcholine in lowering the blood pressure of these animals; the minimal active doses were 5.0, 1.0, and 0.5 μg/kg, i.v., respectively. Gyermek and Unna (46) found that the threshold doses for lowering the blood pressure of dogs and cats varied from 0.05 to 0.2 μg/kg. Waser (96) demonstrated a much higher activity. The minimal depressive dose was 0.002 to 0.004 μg/kg, or half the effective dose of acetylcholine (0.005 to 0.01 μg/kg). Higher doses (0.1 to 10.0 μg/kg) lowered the blood pressure markedly, and 1 to 10 μg/kg depressed it to the lowest value compatible with life. The decrease of pressure curve was as rapid as with acetylcholine, but the recovery after high doses was slower. Fraser (39) claimed that the onset of action was slow. As will be explained later, a prolonged action is characteristic of unesterified structure, such as muscarine.

Heart rate is slowed considerably, and cardiac arrest may occur when the

action of muscarine is not antagonized by atropine. Higher doses of muscarine are needed for lowering blood pressure after atropine. In an atropinized animal, no increase of blood pressure occurred after 330 to 500 μg/kg of muscarine chloride or muscarine iodide (46, 96). This finding led to the conclusion that muscarine has little or no nicotinic action. Vagotomy had no effect on the depressor action. The effect of stimulating the peripheral vagus was not abolished by muscarine; rather, the vagal response summated with the depressor action of the alkaloid. In summary, muscarine exerts the same effects as acetylcholine and many other cholinomimetic drugs. It is, however, more potent and its action is often of a longer duration.

3. Respiratory effects. Intravenous doses from 0.02 to 1.0 μg of muscarine per kg, i.v., produced a marked increase in the volume and rate of respiration in cats (96). This action may have been due to stimulation of the chemoreceptors of the carotid body, but this has not yet been proven conclusively. Amounts over 1 to 10 μg/kg stopped the respiration by bronchoconstriction. At the same time, the tracheal cannula often was obstructed by a profuse secretion of mucus. In this action, muscarine was four times more active than acetylcholine in cats and rabbits (39). In the guinea pig and rabbit, bronchoconstriction was obtained in the same manner and the intrathoracic pressure was decreased. The bronchoconstrictor effects were readily prevented by atropine. Recovery from muscarine was slower than from acetylcholine. Death usually occurred by failure of respiration and blood pressure.

4. Organs with smooth muscle. Muscarine causes contraction of the nonpregnant *uterus* of the rabbit, of the urinary *bladder* of the cat and the dog, and of the circular and longitudinal muscle of the *gut* of the cat or dog (39). The organs of the dog are very sensitive to muscarine as compared to acetylcholine, and the activity ratio of the two compounds for this species was approximately 40 to 500 in different organs. Whereas the threshold dose of muscarine was 0.1 to 0.5 μg/kg for the dog and 0.5 to 5.0 μg/kg for the cat, no response from the uterus and gut of the cat was obtained with acetylcholine below 20 μg/kg. The action of acetylcholine on the cat's bladder was greatly reduced after muscarine. Atropine prevented all the responses obtained with both drugs.

Generally speaking some smooth muscle seems to be highly sensitive to muscarine, and pretreatment with this drug may change the type of reaction to other cholinomimetic drugs, as will be discussed more extensively in connection with preparations of isolated organs.

5. Miscellaneous autonomic effectors. Miosis and *salivation* were observed in the cat with a minimal intravenous dose of 1.0 μg/kg; they were proportional to the injected dose and were antagonized by atropine (97). Experiments on mice, with intraperitoneal injection, showed the sphincter muscle of the iris and the salivary glands to be sensitive to approximately the same doses (minimal dose 60 to 130 μg/kg) (46). *Chromodacryorrhoea* was produced in rats by subcutaneous or intraperitoneal injection of 20 to 35 μg/kg of muscarine chloride (72).

6. Autonomic ganglia. No change of synaptic transmission through the superior cervical ganglion (assessed by stimulation of the preganglionic sympathetic nerve

TABLE 1

Activity in the whole animal (μg/kg, i.v., MCl = muscarine chloride, MI = muscarine iodide)

	Rabbit	Cat	Dog	Mouse	Rat	Guinea Pig	References
Toxicity, LD50				230 MCl			39
				420 MCl 830 MI			46
		5.0 MCl					96
Depression of blood pressure	5.0 MCl	1.0 MCl	0.5 MCl				39
		0.02 MCl 0.2 MCl					46
		0.002— 0.004 MCl 0.004 MI					96 97
Respiration stimulation		0.02 MCl 0.02 MI					96 97
Bronchoconstriction	2.0 MCl	2.0 MCl				2.0 MCl	39,96
Depression	5.0 MCl	5.0 MCl 5.0 MI					96 97

Smooth muscle organs Uterus	10.0 MCl	5.0 MCl	0.5 MCl				39
Bladder		0.5 MCl	0.1 MCl				
Gut (circular) Gut (longitudinal)		5.0 MCl 2.0 MCl	0.5 MCl 0.2 MCl				
Miosis and salivation		1.0 MCl		60–130 i.p.			97 46
Skeletal muscle		>500 MCl	>500 MCl				46
fasciculation or paralysis		>330 MCl >330 MI					97
Autonomic ganglia Contraction of nictitating membrane		1–4.0 1.0 MCl					46 96
Blockade		>330 MCl					97

and recording the contraction of the nictitating membrane) was observed with high doses (500 μg/kg) of muscarine. Stimulation of the peripheral vagus nerve after different doses of the alkaloid always gave a proportional response of blood pressure (96). Muscarine, 1 μg/kg, produced a slow contracture of the nictitating membrane, which was very marked with 5 to 10 μg/kg. This was abolished by 30 to 50 μg atropine/kg. As atropine has a blocking action on the perfused isolated ganglion only in high doses (69), this antagonism was considered to be mainly peripheral. More recently, a direct action of muscarine on ganglionic synapses has been shown in the perfused preparation (*vide infra*).

7. *Skeletal muscle.* In all experiments on atropinized cats, muscarine in intravenous doses up to 500 μg/kg never showed a neuromuscular blocking or enhancing action on the gastrocnemius muscle stimulated through the sciatic nerve (30, 96). This seems to be one major difference between muscarine and many muscarine-like substances (see later), and also between muscarine and the "synthetic muscarine-like" choline nitrite (10).

8. *Central nervous effects.* Because of the predominantly peripheral action of muscarine, it is difficult to measure its central effects. Gyermek and Unna (46) attempted to eliminate the peripheral actions by blocking the cholinergic receptors with 5 mg of atropine-methyl-bromide per kg intraperitoneally fifteen to twenty minutes before the administration of muscarine. By this procedure the minimal lethal dose of d,l-muscarine iodide was elevated from 1 mg/kg to over 160 mg/kg.

B. Actions on isolated organs

1. *Heart.* Straub's method of the isolated frog heart proved helpful in screening the activity of pure muscarine chloride in different extracts from *Amanita muscaria* (30, 46, 92). The contraction of the heart of *Rana temporaria* was reduced in its amplitude to 75% by 0.0042 ± 0.0003 μg muscarine chloride/ml. The rate of beat was slowed. Kögl's impure muscarine was 27% less potent in experiments on hearts of *Rana esculenta.* These cardiac actions were promptly antagonized by 10^{-8} atropine. It has been demonstrated by different authors that acetylcholine is more active than muscarine chloride on the frog heart. The potency relative to muscarine has been reported as follows: 2 times in *Rana temporaria* (2, 30), 1.25 times in *R. pipiens* (46), 1.5 times in *R.* (species unspecified) (39), 4.0 times in *R. esculenta* (66). Muscarine iodide was one-ninth as active as acetylcholine chloride (97), in terms of the salts. The activity of acetylcholine depends greatly on the activity of acetylcholinesterase, which may be inhibited by neostigmine. When this is given, a more accurate relationship can be obtained, since muscarine is not attacked by this enzyme.

After washing, recovery usually was complete; however, Fraser (39) found recovery to be slow and never quite complete. He investigated the action of the alkaloid on the isolated beating auricle of the guinea pig and the rabbit. Muscarine and acetylcholine were equiactive in their actions on the frequency and amplitude of contraction of the isolated guinea pig auricle. Muscarine was 5.5 times more active than acetylcholine on the rabbit auricle, and fibrillation was converted by

TABLE 2

Spasmogenic activity of muscarine and acetylcholine on isolated intestinal muscles

Species	Organ	Activity Ratio of Muscarine to Acetylcholine (=1)	References
Frog	Duodenum	33	39
Mouse	Ileum	0.83	39
Guinea pig	Ileum	4.1	39
	Ileum	2.5	74
Rat	Ileum	1.7	46
	Duodenum	2.0	46
	Colon	0.75	39
Rabbit	Ileum	1.6	39
		2.0	30
		2.5	46
Dog	Ileum (longitudinal)	4.1	39
Cat	Ileum	1.0	30
Horse	Ileum (longitudinal)	22.5	39
	Ileum (circular)	111.0	39
Monkey	Ileum (longitudinal)	10.0	39

both drugs to a slow, regular beat. Muscarine was 10 times more potent than acetylcholine and after washing the effect persisted 14 times as long as that of acetylcholine.

2. Smooth muscle. Most work which has been done with muscarine on smooth muscle organs has been for the purpose of comparing its action with that of acetylcholine. Muscarine has generally proved to be more potent and to produce a slightly different type of response. Muscarine spasm usually develops more slowly than that following acetylcholine and recovery takes longer after washing the preparation. The sensitivity of different species varies considerably.

a. Intestine. Except on the mouse ileum and the rat colon, muscarine was 1.6 to 111 times more active than acetylcholine in producing spasm (39). A concentration of *ca.* 3×10^{-8} M muscarine produced a submaximal contraction of the guinea pig ileum which was equivalent to that following 5×10^{-8} M acetylcholine (74). Physostigmine or neostigmine usually increased the sensitivity of the muscle to acetylcholine until the activities of both compounds were equal. Only in two exceptional preparations did physostigmine not always cause an increase in the sensitivity to acetylcholine (39).

Dibenamine (4×10^{-4} M) blocked the action of both compounds to the same degree. This may be explained by the occupation of the same receptors by both cholinomimetic drugs (74). On rabbit ileum, muscarine chloride was effective in concentrations of 0.001 to 0.02 μg/ml (45) (see Table 2).

Contraction elicited by acetylcholine is rapid, and the action is maximal within sixty seconds. This agent is destroyed by the tissue cholinesterases, and the effect disappears within fifteen to thirty minutes without washing. Muscarine has a biphasic action: an initial fast contraction, followed within thirty seconds by a slow phase. Small movements of the intestine also may occur at this time.

TABLE 3

Spasmogenic activities of muscarine and acetylcholine on isolated uterine muscles

Species	Muscle	Activity Ratio of Muscarine to Acetylcholine (=1)	References
Mouse	(Longitudinal)	1.0	39
Guinea pig	(Longitudinal)	5.3	39
Rabbit	(Longitudinal)	0.43	39
	(Longitudinal)	0.9	46
Rat	(Longitudinal)	2.27	39
	(Longitudinal)	3.0	46
Horse	(Longitudinal)	8.35	39
	(Circular)	9.7	39
Dog	(Longitudinal)	10.0	39

Maximal contraction is reached after five to ten minutes. The relaxation following washing is at least two times slower than that after acetylcholine. Muscarine is not destroyed by cholinesterases; accordingly its action persists until the drug is removed by washing. After a period of relaxation, a second contraction may follow, which is reversible by washing. Repeated application of muscarine damages the intestine, which then contracts slowly and incompletely, and shows contractures and "twitches" (74).

b. Uterus. The action of muscarine has been investigated on strips of longitudinal and circular uterine muscle of six different species (Table 3). As on the gut, the spasm caused by muscarine was slower in onset, and recovery after washing was retarded as compared to that following acetylcholine (39). Except on the uterus of mouse and rabbit, muscarine was more active than acetylcholine. After recovery from a muscarine-induced spasm, the response of the rabbit uterus preparation to acetylcholine was temporarily abolished. Atropine always prevented the action of muscarine.

c. Bladder. Extensive investigations with the pelvic nerve-bladder preparation of the dog have been conducted by Gyermek and Unna (45, 46). The bladder of female dogs was cannulated and connected with a water manometer; the pelvic nerve was then stimulated electrically every ten seconds to avoid spontaneous movements of the bladder, and compounds were administered through the iliac artery into the abdominal aorta. The effective dose range of muscarine for potentiation of the response to nerve stimulation was between 0.025 and 0.2 μg/kg, and that of (−)-muscarone was between 0.01 and 0.05 μg/kg. While the atropinized (1 to 3 μg/kg) bladder preparation remained more or less sensitive to pelvic nerve stimulation and to ganglionic stimulant drugs, it was insensitive to 10,000 times higher dose of muscarine (>1.0 mg) by intraarterial injection. Muscarine produced persistent contractions of the bladder, whereas acetylcholine (2 μg/kg) caused rapid, brief contractions which resemble those following nicotine. The cat bladder was twice as sensitive to muscarine as was the dog bladder.

Isolated longitudinal strips of bladder from different species were also more

TABLE 4

Spasmogenic activities of muscarine and acetylcholine on isolated muscles from the urinary bladder (39)

Species	Muscle	Activity Ratio of Muscarine to Acetylcholine (=1)
Guinea pig	(Longitudinal)	20.8
Rabbit	(Longitudinal)	24.9
	(Circular)	51.5
Dog	(Longitudinal)	88.5
Rat	(Longitudinal)	6.3
Frog	(Longitudinal	4.5
Horse	(Longitudinal)	10.0
	(Circular)	8.8
Monkey	(Longitudinal)	200.0

sensitive to muscarine than to acetylcholine (39) (Table 4). Muscarine responses were slower, and were prevented by atropine.

d. Other isolated organs. Muscarine was approximately 150 times as active as acetylcholine in causing spasm of the *tracheal chain* preparation of the guinea pig and of the rabbit. Its action was slow and had a latent period of about two to three minutes, a condition not seen with acetylcholine (39). The *ureter* of the horse was twenty-nine times as sensitive to the alkaloid as to acetylcholine, and recovery of the preparation was rapid after washing out either compound (39).

The *dorsal muscle of the leech* gave no response to muscarine, even in relatively high concentrations (1 μg/ml); acetylcholine was active only after physostigmine (39). Different preparations of *arteries* have been used for the assay of muscarine. Small spiral strips were used by Kuenzle and Waser (74) for differentiating receptors. Muscarine (2×10^{-4} M bath concentration) chloride caused strong contractions of the circular muscles, and its potency was ten times greater than that of acetylcholine even when neostigmine and trimethaphan camphorsulfonate (Arfonad) were present. That muscarine occupied the same receptor sites as acetylcholine was indicated by the complete cross protection which was obtained. The *carotid artery* chain of the horse had a latent period of five minutes after muscarine was added to the bath. The spasmogenic potency was three times that of acetylcholine, which acted without a time lag (39). In the isolated perfused rabbit ear, muscarine and acetylcholine produced the same degree of constriction or dilatation of the blood vessels.

The actions of muscarine chloride, acetylcholine, and epinephrine (adrenaline) upon isolated *iris* muscles were studied thoroughly by Kuenzle and Waser (73). In this procedure rings of sphincter muscles of pigs and sectors of rabbit dilator muscles were suspended in oxygenated Tyrode solution, and their contractions recorded isometrically. Muscarine had a more potent but slower contractor effect upon sphincter and dilator muscles than acetylcholine. The effect of acetylcholine on the sphincter and the response to mechanical stimulation were sensitized by muscarine. Even the dilator was sensitized by muscarine to adrenaline

and noradrenaline; sensitization by muscarine was markedly stronger than by acetylcholine. The action of muscarine was blocked less effectively by atropine than was that of acetylcholine. Neostigmine and eserine intensified contractions produced by acetylcholine, but hardly by muscarine. Phentolamine (Regitine) depressed only slightly the actions of muscarine and acetylcholine on the dilator muscle.

3. Autonomic ganglia. The action of muscarine chloride injected into *perfused superior cervical ganglia* of cats has been studied by various groups (2, 3, 70). Infusions of muscarine into the normal ganglion usually caused contraction of the nictitating membrane when the dose exceeded 20 μg (70), and the alkaloid was clearly less active than acetylcholine (threshold dose 0.5 to 1 μg). Small doses of muscarine first sensitized to acetylcholine, and larger doses depressed the response to stimulant drugs and electrical stimulation. The stimulant action of muscarine was slower than that of acetylcholine; both were blocked by ganglionic infusion of atropine (1 to 5 μg), nicotine (100 μg), or hexamethonium (0.1 to 1 μg).

The chronically denervated ganglion was very sensitive to small doses (0.05 to 0.5 μg) of muscarine, which was equal in activity to acetylcholine; eserine intensified only the action of the latter. The stimulant action of muscarine was always reversibly abolished by ganglionic infusion of nicotine (50 to 100 μg) or atropine (1 to 8 μg), but not by hexamethonium (up to 10 mg) which failed also to block the action of acetylcholine (3).

Ambache (2) studied the actions of different ganglionic stimulants on autonomic neurons located within the viscera. By using botulinus toxin (20 to 30 million mouse LD50 units, Type D) on *isolated rabbit gut and guinea pig ileum*, he was able to separate ganglionic stimulating action from direct motor action on the gut. In contrast to nicotine and other predominantly neuronal stimulant drugs, the action of which on the intestine was abolished by botulinus toxin, acetylcholine and muscarine still contracted the gut by their direct action on the muscle. Any action these compounds may have on the visceral ganglia is greatly overshadowed by their powerful direct effect on the smooth muscle itself. The effects of cholinomimetic stimulants on the inferior mesenteric ganglion of the cat were studied by recording the spike potentials of postganglionic fibers (53). The order of ganglion stimulating potency in atropinized preparations was as follows: 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP) > nicotine > acetylcholine > muscarone. Muscarine neither stimulated nor blocked this ganglion in intraarterial doses up to 1.5 mg.

4. Contractural muscle. The isolated rectus abdominis muscle of frogs, the leech dorsal muscle, and the pigeon iris were used by various authors as test preparations for the nicotinic action of muscarine. Whereas some investigators (3, 46, 97) were unable to detect any stimulation even at concentrations as high as 400 to 500 μg/ml, Fraser (39) reported the production of a slow contraction of the frog rectus by 10 μg/ml, equivalent in height to the much more rapid response caused by 50 mμg acetylcholine/ml. The muscle was slow to recover after washing out the muscarine. The response to both drugs was prevented by tubocurarine.

The rat phrenic nerve diaphragm preparation was not influenced by muscarine

in concentrations of 10 μg/ml, and neuromuscular block by tubocurarine or hexamethonium was not changed (39).

C. Action on cholinesterases

Investigations by the Warburg technique on acetylcholinesterase of erythrocytes and non-specific cholinesterase of serum showed that authentic muscarine in concentrations of 10^{-4} to 10^{-8} M has no effect on the activity of either enzyme (74). This result is in agreement with another experiment, in which at a concentration of 5×10^{-4} M muscarine had no inhibitory action on either cholinesterase (39). With earlier and probably impure muscarine preparations, Ammon (4) found a marked inhibition of the serum cholinesterase of the horse by 10^{-4} M muscarine. Keeser (62) with a concentration ten times higher described strong inhibition of cholinesterase in the serum of man and rabbit. From these results, it may be concluded that muscarine does not produce its characteristic effects by inhibition of cholinesterases.

Witkop (103) found muscarine to have an inhibitory action nearly equal to that of choline on acetylcholinesterase prepared from electric eel tissue, using a constant pH-titration technique (competitive enzyme inhibitor dissociation constant: 2.2×10^{-4}).

D. Summary

Muscarine has been shown to be a very active parasympathomimetic drug which in nearly all preparations has a potency considerably higher than that of acetylcholine. This is partly due to the stability of the alkaloid, which having no ester linkage is not hydrolyzed by cholinesterases. On the other hand, muscarine does not inhibit acetylcholinesterase or non-specific cholinesterase in concentrations up to 10^{-4} M. It acts on the same receptors as acetylcholine, but its action is restricted predominantly to peripheral effector organs innervated by the autonomic nervous system. Autonomic ganglionic synapses are affected only by much higher doses. These cholinomimetic effects are antagonized by atropine.

The primary cardiovascular effects are lowering of the blood pressure and slowing of the heart rate. Among smooth muscle organs, the bladder is about ten times more sensitive to muscarine than the uterus, and three times more than the intestine. Miosis, salivation, and bronchoconstriction are very pronounced. High doses are required for ganglionic stimulation. Muscarine has no significant action on skeletal muscle.

VI. STRUCTURE-ACTIVITY RELATIONSHIPS OF MUSCARINE-LIKE COMPOUNDS

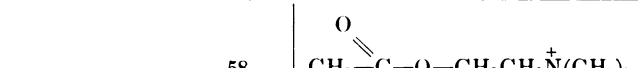
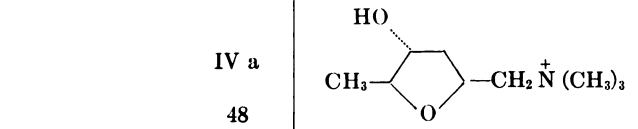
A. Isomers, homologs and derivatives of muscarine and muscarone

The actions of muscarine, muscarone, and their isomers have been investigated on various species and isolated organs (45, 46, 47, 72, 86, 95, 97, 98, 99, 104). The results are summarized in Tables 5 to 7. It is difficult to compare activities obtained with a wide range of techniques on different animals; therefore only

(Continued on page 494)

TABLE 5

Isomers of muscarine and related compounds (figures in parentheses refer to relative activity compared to acetylcholine); data taken from Waser (96–99, 104)

	Compound (Arabic numbers indicate references to syntheses)	Cat				Frog		Relative potency muscarinic/nicotinic, cat (c) or frog (f)
		Minimal i.v. effective doses, μg/kg				Bathing or perfusion concentration, μg/ml		
		Muscarinic		Nicotinic (atropinized)		Muscarinic	Nicotinic	
		Lowering of blood pressure	Stimulation of respiration (r); slow contraction of nictitating membrane (n); salivation (s)	Blockade of superior cervical ganglion	Curariform action on gastrocnemius muscle	Isolated heart	Isolated rectus muscle	
58	 Acetylcholine Cl	0.008 (1)	0.1 r 25.0 s	150 (stimulation!) (1)	250 (1)	0.002 (1)	5.0 (1)	(1)c, f
IV a 48	 L(+)-Muscarine Cl	0.003 (2.7)	0.01 r 0.7 n 1.0 s	>1,000 (<0.15)	>1,000 (<0.25)	0.01 (0.2)	>500 (<0.01)	(>14)c (>20)f
IV b 48	L(+)-Muscarine I	0.004 (2.0)	0.02 r 1.0 n 1.0 s	>1,000 (<0.15)	>1,000 (<0.25)	0.018 (0.11)	>500 (<0.01)	(>13)c (>11)f
IV c 14	D(−)-Muscarine Cl	3.0 (0.003)	20.0 r 40.0 n				>300 (<0.017)	

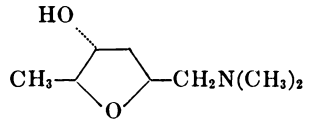
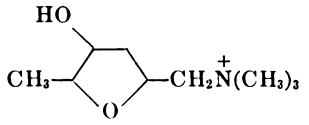
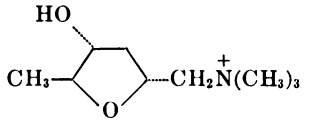
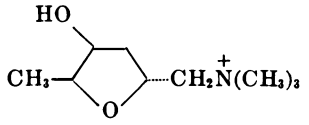
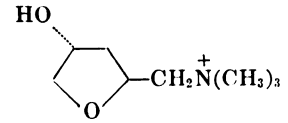
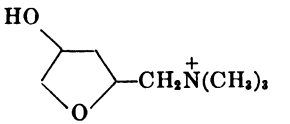
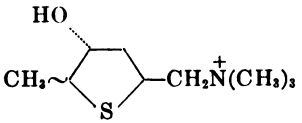
IV d 21	d,l-Muscarine I	0.01 (0.8)	0.05 r 1.2 n 2.0 s		>10,000 (<0.025)	0.032 (0.06)	>500 (<0.01)	(>30)c (>6)f
V 30	 L(+)-Nor-muscarine HCl	5.0 (0.0016)	100 r 1,000 n	>10,000 (<0.015)	>10,000 (<0.025)	5.0 (0.0004)		(>0.1)c
VIII 25	 d,l-Epi-muscarine I	3.0 (0.003)	20 r 250 n 200 s	8,000 (0.02)	>10,000 (<0.025)	214 (0.000009)	>1,000 (<0.005)	(>0.15)c (>0.002)f
IX 26	 d,l-Allo-muscarine I	1.7 (0.005)	20 r 125 n 250 s	>10,000 (<0.015)	>10,000 (<0.025)	15.0 (0.00013)	>1,000 (<0.005)	(>0.25)c (>0.03)f
X 27	 d,l-Epiallo-muscarine I	1.0 (0.008)	10 r 50 n	>500 (<0.3)	>500 (<0.5)	12.0 (0.00017)	>500 (<0.01)	(>0.02)c (>0.017)f

TABLE 5—*Continued*

Compound (Arabic numbers indicate references to syntheses)		Cat				Frog		Relative potency muscarinic/ nicotinic, cat (c) or frog (f)
		Minimal i.v. effective doses, μg/kg				Bathing or perfusion concentration, μg/ml		
		Muscarinic		Nicotinic (atropinized)		Muscarinic	Nicotinic	
		Lowering of blood pressure	Stimulation of respiration (r); slow contraction of nictitating membrane (n); salivation (s)	Blockade of superior cervical ganglion	Curariform action on gastrocnemius muscle	Isolated heart	Isolated rectus muscle	
XX 104	 d,l-Desmethyl-muscarine I	1.0 (0.008)	20 r 100 n 400 s	>10,000 (<0.015)	>10,000 (<0.025)	30 (0.00007)		(>0.4)c
XXI 104	 d,l-Desmethyl-epi-muscarine I	7.0 (0.001)	200 r 800 n	>2,000 (<0.075)	2,000 (0.125)	400 (0.000005)		(0.01)c
XXII 24	 d,l-Thiomuscarine I	15.0 (0.0005)	250 r >4,000 n 2,000 s	>4,500 (<0.03)	>4,500 (<0.06)	500 (0.000004)	>2,000 (<0.0025)	(>0.01)c (>0.0016)f

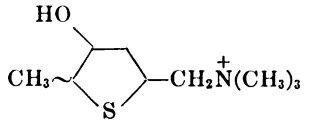
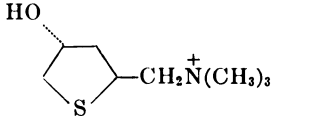
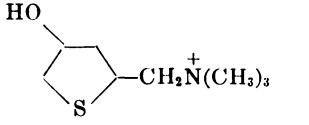
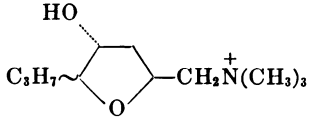
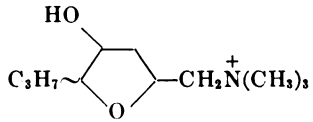
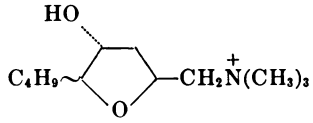
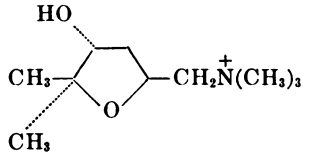
XXIII 24	 d,l-Epi-thiomuscarine I	25.0 (0.0003)	500 r >5,000 n	>5,000 (<0.03)	>5,000 (<0.05)	1,000 (0.000002)	>1,000 (<0.005)	(>0.01)c (>0.0004)f
XXIV 24	 d,l-Desmethyl-thiomuscarine I	5.0 (0.0016)	300 r 200 n	>3,000 (<0.05)	>3,000 (<0.08)	100 (0.00002)	>1,000 (<0.005)	(>0.03)c (>0.004)f
XXV 24	 d,l-Desmethyl-epi-thiomuscarine I	10.0 (0.0008)	200 r 200 n	>5,000 (<0.03)	>5,000 (<0.05)	200 (0.00001)	>1,000 (<0.005)	(>0.02)c (>0.002)f
XXVI 24	 d,l-2-Propyl-desmethyl-muscarine I	0.5 (0.016)	0.2 r >200 n	>200 (<0.75)	>200 (<1.25)	50 (0.00004)		(>0.02)c

TABLE 5—*Continued*

Compound (Arabic numbers indicate references to syntheses)		Cat				Frog		Relative potency muscarinic/ nicotinic, cat (c) or frog (f)
		Minimal i.v. effective doses, μg/kg				Bathing or perfusion concentration, μg/ml		
		Muscarinic		Nicotinic (atropinized)		Muscarinic	Nicotinic	
		Lowering of blood pressure	Stimulation of respiration (r); slow contraction of nictitating membrane (n); salivation (s)	Blockade of superior cervical ganglion	Curariform action on gastrocnemius muscle	Isolated heart	Isolated rectus muscle	
XXVII 24	 d,l-2-Propyl-desmethyl-epi-muscarine I	80 (0.0001)	>2,000 r 2,000 n	>2,000 (<0.075)	2,000 (<0.125)	1,000 (0.000002)		(>0.001)c
XXVIII 24	 d,l-2-Isobutyl-desmethyl-muscarine I	400 (0.00002)	3,000 r >4,000 n	>4,000 (<0.04)	>4,000 (<0.06)	>1,000 (<0.000002)		(>0.0004)c
XXIX 49	 d,l-2-Methyl-muscarine I	9.0 (0.0009)	1,000 r 200 n	>25,000 (<0.006)	>25,000 (<0.01)	4.0 (0.0005)	>5,000 (<0.001)	(>0.5)c (>0.5)f

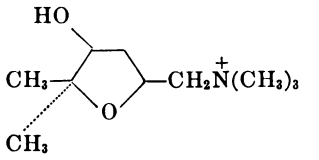
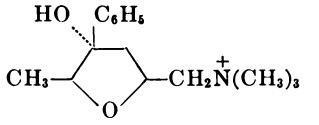
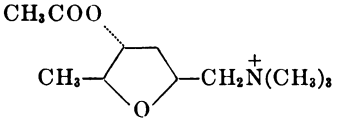
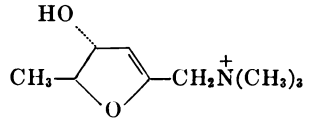
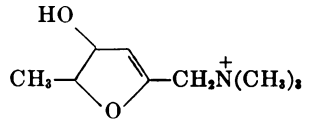
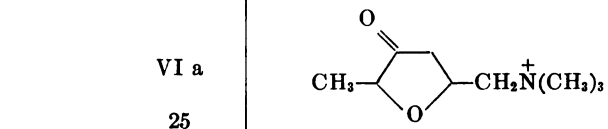
XXX 49	 d,l-2-Methyl-epi-muscarine I	15.0 (0.0005)	200 r 200 n	>25,000 (<0.006)	>25,000 (<0.01)	6.0 (0.0003)	>5,000 (<0.001)	(>0.06)c (>0.3)f
XXXI 49	 d,l-3-Phenyl-muscarine Cl	0.006 (1.3)	0.07 r 0.5 n	>2 (<75)	>2 (<125)	0.04 (0.05)	25 (0.2)	(>0.01)c (0.25)f
XXXII 18	 Acetyl-(+)-muscarine Cl	0.006 (1.3)	0.1 n					
XXXIII 16	 d,l-4,5-Dehydro-muscarine I	0.01 (0.8)	0.6 r 3.0 n 2.0 s	>500 (<0.3)	>500 (<0.5)	0.2 (0.01)	50 (0.1)	(>2.0)c (>0.1)f
XXXIV 16	 d,l-4,5-Dehydro-epi-muscarine I	0.02 (0.4)	1.0 r 5.0 n 10.0 s	>500 (<0.3)	>500 (<0.5)	0.4 (0.005)	>100 (<0.05)	(>1.0)c (>0.1)f

TABLE 6

Muscarone and related compounds (figures in parentheses refer to relative activity compared to acetylcholine; data taken from Waser (96–99, 104)

Compound (Arabic numbers indicate references to syntheses)		Cat				Frog		Relative potency muscarinic/nicotinic, cat (c) or frog (f)
		Minimal i.v. effective doses, μg/kg				Bathing or perfusion concentration, μg/ml		
		Muscarinic		Nicotinic (atropinized)		Muscarinic	Nicotinic	
		Lowering of blood pressure	Stimulation of respiration (r); slow contraction of nictitating membrane (n); salivation (s)	Blockade of superior cervical ganglion	Curariform action on gastrocnemius muscle	Isolated heart	Isolated rectus muscle	
58	 Acetylcholine Cl	0.008 (1)	0.1 r 25.0 s	150 (stimulation!) (1)	250 (1)	0.002 (1)	5.0 (1)	(1)c, f
VI a 25	 D(−)-Muscarone I	0.001 (8)	0.02 r 0.2 n	10 (15)	30 (8.3)	0.0075 (0.27)	2.5 (2)	(0.7)c (0.13)f
VI b 25	L(+)-Muscarone I	0.003 (2.7)	0.1 r 0.2 n 1.0 s		50 (5)		10 (0.5)	(0.5)c

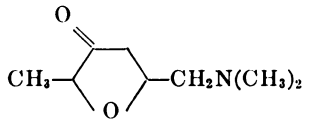
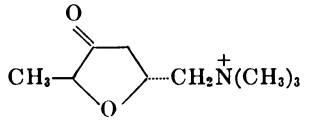
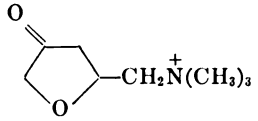
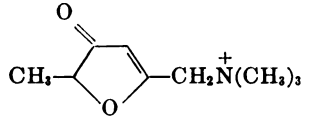
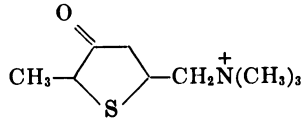
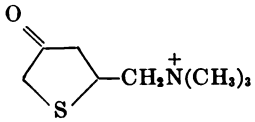
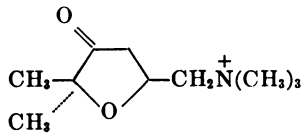
VI c 25	d,l-Muscarone I	0.0015 (5.3)	0.03 r 0.2 n	20 (7.5)	40 (6.3)	0.01 (0.2)	2.5 (2)	(0.7)c (0.1)f
XXXV 25	 d,l-Nor-muscarone HCl	1.0 (0.008)						
XXXVI 26	 d,l-Allo-muscarone I	0.003 (2.7)	0.03 r 0.4 n 0.8 s	30 (5)	70 (3.6)	0.02 (0.1)	1.5 (3.3)	(0.6)c (0.03)f
XXXVII 104	 d,l-Desmethyl-muscarone I	0.06 (0.13)	20 r 30 n 40 s	>100 (<1.5)	30 (8.3)	8.0 (0.00025)		(0.015)c
XXXVIII 21	 d,l-4,5-Dehydro-muscarone I	0.0015 (5.3)	0.03 r 0.5 n 0.5 s	20 (7.5)	30 (8.3)	0.01 (0.2)	0.9 (5.5)	(0.7)c (0.04)f

TABLE 6—*Continued*

Compound (Arabic numbers indicate references to syntheses)		Cat				Frog		Relative potency muscarinic/nicotinic, cat (c) or frog (f)
		Minimal i.v. effective doses, μg/kg				Bathing or perfusion concentration, μg/ml		
		Muscarinic		Nicotinic (atropinized)		Muscarinic	Nicotinic	
		Lowering of blood pressure	Stimulation of respiration (r); slow contraction of nictitating membrane (n); salivation (s)	Blockade of superior cervical ganglion	Curariform action on gastrocnemius muscle	Isolated heart	Isolated rectus muscle	
XXXIX 24	 d,l-Thiomuscarone I	0.04 (0.2)	5 r 5 n	>500 (<0.3)	150 (1.7)	1.0 (0.002)	50 (0.1)	(0.1)c (0.02)f
XL 24	 d,l-Desmethyl-thiomuscarone I	0.2 (0.04)	10 r 10 n 100 s	>1,000 (<0.15)	200 (1.25)	4.0 (0.0005)	50 (0.1)	(0.03)c (0.005)f
XLI 49	 d,l-2-Methyl-muscarone I	0.05 (0.16)	0.2 r 3.5 n	>200 (<0.75)	>200 (<1.25)	1.5 (0.0013)	30 (0.17)	(>0.15)c (0.008)f

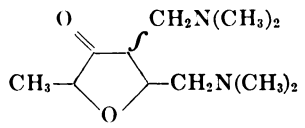
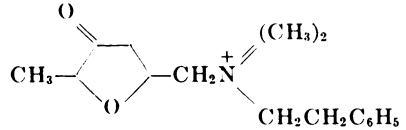
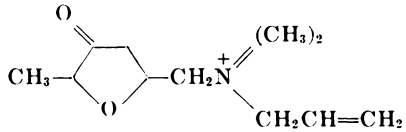
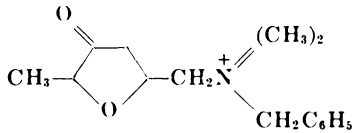
XLII 49	 d,l-4-Dimethylaminomethyl-nor-muscarone 2HCl	15.0 (0.00053)	350 r 800 n	>1,000 (<0.15)	>1,000 (<0.25)	29 (0.00007)	>2,900 (<0.0017)	(>0.003)e (>0.04)f
XLIII 49	 d,l-N-Phenethyl-nor-muscarone Cl	5.0 (0.0016)	100 r 200 n	4,000 (0.04)	4,000 (0.06)	300 (0.000007)	>1,300 (<0.004)	(0.03)e (>0.002)f
XLIV 49	 d,l-N-Allyl-nor-muscarone Cl	14.0 (0.00057)	80 r 60 n	1,400 (0.11)	>2,800 (<0.09)	700 (0.000003)	>1,400 (<0.0035)	(0.005)e (>0.001)f
XLV 49	 d,l-N-Benzyl-nor-muscarone Br	270 (0.00003)	700 r >2,000 n	2,000 (0.075)	3,200 (0.08)	>3,200 (<0.0000006)	>1,600 (<0.003)	(0.0004)e (>0.0002)f

TABLE 7

Potency (relative to acetylcholine) of muscarine, muscarone, and derivatives on isolated organs, mouse pupil and production of chromodacryorrhoea in rats (investigated by Gyermek and Unna [45–47], van Rossum [86] and Küng† [72])*

Compound	Ileum		Uterus		Bladder	Pupil	Chromodacryorrhoea Rat
	Rabbit	Rat	Rabbit	Rat	Dog	Mouse	
Acetylcholine Cl	1	1	1	1	1		
(+)-Muscarine Cl	2.5	1.7	0.9	3.0	50	1	1†
(−)-Muscarine Cl	0.00625						0.02†
d,l-Muscarine I	0.80	1.0*				0.2	0.7†
d,l-Epi-muscarine I	<0.0035	0.0018*				0.3	<0.01†
d,l-Allo-muscarine I	0.0055	0.0053*					<0.01†
d,l-Epiallo-muscarine I	0.00375	0.016*					<0.01†
(−)-Muscarone I	13.25	7.8	2.25	6.0	120	5.2	
(+)-Muscarone I	5.5	2.55	0.9	2.31	40	3.1	
d,l-Muscarone I	6.25	3.4	1.53	3.9	60	5.0	5.0†
d,l-Allo-muscarone I	3.0	1.7	0.63	3.0	26	1.0	
d,l-4,5-Dehydro-muscarone I	1.65	0.85	0.3	0.54	12	0.3	
d,l-4,5-Dehydropropyl-muscarone I	<0.00625	0.085	0.009	<0.009	0.04	<0.01	

measurements done with the same technique are tabulated. It has to be kept in mind, for instance, that frogs change their sensitivity towards drugs according to the season. The results have been calculated using acetylcholine as a common standard, since this substance is the physiological neurotransmitter. The disadvantage of its rapid destruction by cholinesterases in blood and tissues may be overcome by simultaneous application of anticholinesterase drugs. As this precaution makes it uncertain how much of the action is due to injected or endogenous acetylcholine, we preferred to calculate the intravenous doses for ganglionic (15, 68) and neuromuscular action (13) in the cat from published data obtained from observations on isolated organs. They are comparable to doses of closely related drugs as methacholine or carbachol (57, 71, 91).

"*Muscarinic*" actions on the cat characterized in Tables 5 to 7 include the fall of carotid blood pressure, slow peripherally induced contraction of the nictitating membrane, salivation, and brief stimulation of respiration, probably through the carotid body. Muscarinic effects on isolated organs include paralysis of the frog heart, contraction of smooth muscle of the ileum, uterus, and bladder, contraction of the mouse pupil, and chromodacryorrhoea in rats.

Typical "*nicotinic*" actions on the atropinized cat are rapid contraction of the nictitating membrane induced by ganglionic stimulation, and fasciculation of skeletal muscle, followed by ganglionic block and curare-like paralysis. Increase of blood pressure after atropine is not regularly observed with muscarone and related compounds, and is not included in the tables because doses reported were often not high enough to produce this effect. This action has been noted after

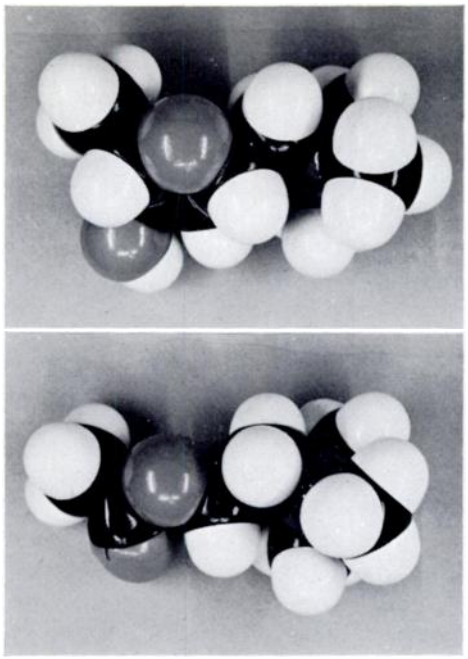


FIG. 2. Stuart Briegleb models of L(+)-muscarine and acetylcholine.

20 to 400 μg muscarone/kg, 50 μg thio-muscarone kg, 200 to 500 μg desmethyl-thiomuscarone/kg, and 100 μg 2-propyl-desmethylmuscarine/kg. Nicotinic action has been tested on different isolated organs: contraction of the frog rectus muscle (listed in the tables) and stimulation of the atropinized dog bladder (45, 46, 47). Muscarone caused liberation of epinephrine and smaller amounts of norepinephrine from the suprarenal glands of rats (85).

1. Stereoisomers. As mentioned previously there exist four pairs of enantiomorphs of muscarine, all of which have been synthesized and investigated pharmacologically (45, 46, 97). The correlation of their activity to stereostructure is of great importance, because these compounds have a more stable steric localization of their functional groups than other cholinomimetic agents, such as acetylcholine, the molecules of which have flexible carbon chains and freely rotating functional groups (Fig. 2).

The tabulated actions (Tables 5 and 6) are best discussed under different aspects of structural characteristics. The stereoisomers of muscarine are several hundred times less active than the natural alkaloid. Epi-muscarine (VIII), with all three substituents on the same side of the tetrahydrofurane ring, is the weakest; allo-muscarine (IX), with the large methyltrimethylammonium side-chain in the *cis*-position to the hydroxy group is a little more active; epi-allo-muscarine (X), with only the small methyl group on the same side of the ring as the hydroxy group, is the most active of all the isomers. The same relationship was found with various isolated organs (see Table 7). (+)-Muscarine, with its

hydroxy group in the *trans*-position to the other two ring substituents, is consistently by far the most potent compound. The synthetic enantiomorph, (−)-muscarine (IV c), has an activity of only 0.1% of that of the natural alkaloid. Stereospecificity in this group is indeed remarkably high.

We may conclude that steric hindrance of the ether-oxygen linkage, the hydroxy group, or both, plays an important role in the contact of at least three functional groups of these molecules with the cholinergic receptor. The larger is the sterically interfering group, with the consequent hindrance of bond-formation by the active pharmacophore groups with the receptor, the less potent is the action of the compound.

Corresponding conclusions were reached by Witkop (103) in his investigation of the inhibitory activity of muscarine and muscarone derivatives on acetylcholinesterase. The differences in the anticholinesterase activities of the isomers were not as great as the differences in their pharmacological potencies, but they showed the alkaloids of the natural series (muscarine and epi-muscarine) to have a closer approach to coplanarity of the hetero ring with both the quaternary N and hydroxyl O-atoms than in the case of the allo-series (allo- and epiallo-muscarine). Nearly the same anticholinesterase activity was shown by 4,5-dehydro-muscarine (XXXIII) and 4,5-dehydro-epi-muscarine (XXXIV). Muscarone and allo-muscarone (XXXVI) were highly active and seemed stereochemically comparable to muscarine, whereas 4,5-dehydro-muscarone (XXXVIII) has considerably less activity. The most potent inhibitory compound was O-acetyl-muscarine (XXXII), which is not hydrolyzed by the enzyme. These results suggest that the binding strengths of these compounds with acetylcholinesterase are the result of the net steric influences on three-pointed bonding, afforded by the three substituted ring positions.

Another interesting investigation of the atropine-like action of the stereoisomers of muscarine by van Rossum (86) demonstrated the purely parasympathomimetic action of d,l-muscarine and d,l-epiallo-muscarine. On the other hand, d,l-epi-muscarine was parasympatholytic on the frog heart, and d,l-allo-muscarine had a dual action, being a partial stimulant and a competitive antagonist with parasympatholytic properties. Atropine-like action on the frog heart was found with propyl-desmethyl-muscarine (XXVI), 4,5-dehydro-epi-muscarine (XXXIV), thiomuscarine (XXII), epi-thiomuscarine (XXIII), desmethyl-thiomuscarine (XXIV), desmethyl-epi-thiomuscarine (XXV) (Waser, unpublished observations).

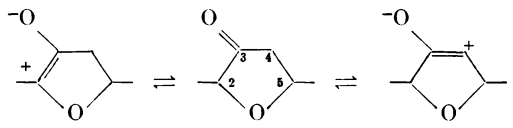
The intrinsic activity, representing the ability of these drugs to produce an effect, was the same on the more sensitive receptors of the rat intestine. The affinities of the isomers to the receptors were of the order muscarine > epiallo-muscarine > allo-muscarine > epi-muscarine.

2. Hydroxy and carbonyl groups. All muscarone derivatives closely resemble acetylcholine in their structure and actions. They (Table 6) are more potent than muscarine and its derivatives and exhibit strong stimulating and blocking effects

on ganglionic synapses and neuromuscular junctions, as indicated by contraction of the nictitating membrane and fasciculation of skeletal muscle. In atropinized animals, higher doses block the sympathetic ganglia and the endplates in a curare-like fashion.

Here stereospecificity is less important, as L(+)-muscarone (VI b) and d,l-allo-muscarone (XXXVI) are only two or three times less active than D(−)-muscarone (VI a) and d,l-muscarone, respectively. It is noteworthy that the enantiomorphic (−)-muscarone form, related to inactive (−)-muscarine, is more active! Nearly all muscarine compounds with the exception of 3-phenyl-muscarine (XXXI) and the 4,5 dehydro-derivatives (XXXIII, XXXIV), have muscarinic and no nicotinic action, whereas all muscarone derivatives show both actions.

The change of the hydroxy group in muscarine to the carbonyl group in muscarone causes several important changes in the properties of the molecule. Concerning the steric position, the oxygen of the carbonyl group lies in the ring plane and is less influenced by substituents on either side of the tetrahydrofurane ring. The carbonyl group has a marked polar character which enables it, like the electrophilic carbon of an ester, to form a covalent bond with some basic group in the receptor. This mechanism has been proposed for the binding of acetylcholine to acetylcholinesterase by Wilson (102). Compared to this, the binding force of the hydroxy group in muscarine must be relatively weak. Even the acetylated muscarine compound (XXXII) and 3-phenyl-muscarine (XXXI) have a muscarinic activity comparable to that of acetylcholine on blood pressure. Acetyl-muscarine is not hydrolyzed by acetylcholinesterase (103), but perhaps is by unspecific esterases in the tissue (74). It must be kept in mind that the keto-form of muscarone may change into the enol form with a coplanar hydroxy group and the formation of a double bond at the C_2—C_3 or C_3—C_4 position in the ring. Racemization at carbon 2 has to be expected.



3. Ring oxygen (ether group). Replacing the tetrahydrofurane ring in muscarine by tetrahydrothiophene (XXII to XXV) results in a markedly reduced muscarinic activity, to $\frac{1}{1500}$ the original potency. The difference is much less ($\frac{1}{30}$) in muscarone compounds (XXXIX, XL). At the same time, the stereospecificity of muscarine is not present in the thio-compounds (XXIII, XXV). This may be explained by the inability of the sulfur atom to form strong hydrogen bonds with some cholinoceptive groups, as the electro-negativity of sulfur (2.5) is much less than that of the ring oxygen (3.5) (80). The increase of ring size by about 35% naturally affects the position and biological function of the neighboring groups and the fit of the molecule on the receptor. The hundred times weaker

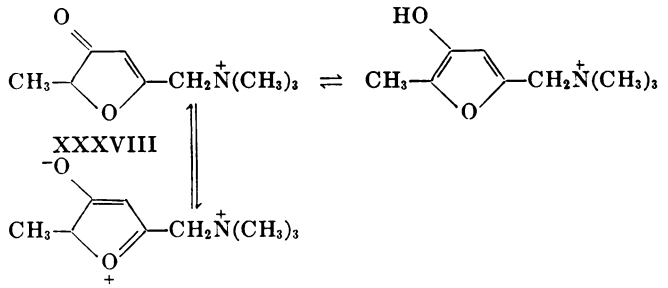
muscarinic action of acetylthiocholine compared to acetylcholine has been known for a long time (84).

4. Chain length. Diminishing the chain length ($^{+}$N—C—C—O—C—C) from six atoms to five in desmethyl-muscarine (XX, XXI), and to a lesser extent in muscarone derivatives (XXXVII), results in decrease of cholinomimetic action with the exception of desmethyl-thiomuscarine (XXIV) and desmethyl-epi-thiomuscarine (XXV), which are three times more active than the methylated compounds. This effect may again be caused by the large sulfur atom's pushing neighboring carbon atoms out of their places in the furane ring. The inserting of two methyl groups at ring carbon 2 diminishes the activity of muscarine (XXIX, XXX) by 3,000 to 10,000 times, but that of muscarone (XLI) only 10 to 50 times.

When the chain is lengthened by introducing propyl or *iso*butyl groups instead of the methyl group (XXVI to XXVIII), the decrease in activity is again very marked.

5. Aromatization. Introduction of a 4,5 double bond into the molecules of muscarine and muscarone alters slightly the shape of the furane ring. As mentioned before, the substituent at C_5 enters into a coplanar position and the charge distribution in the ring is changed. 4,5-Dehydro-muscarine (XXXIII) and 4,5-dehydro-epi-muscarine (XXXIV) are almost as active at peripheral cholinoceptive sites (muscarinic action) as muscarine. The potency of epi-muscarine is increased one hundredfold by dehydrogenation at C_4—C_5. 4,5-Dehydro-muscarone (XXXVIII) is even as potent as d,l-muscarone. All three dehydro-compounds show a stronger nicotinic action on ganglionic and neuromuscular synapses than do the corresponding saturated compounds (47, 97, 98).

In compounds with a furane ring, the oxygen acquires a partial positive charge and the whole ring system has an unsaturated electronegative character. Formation of hydrogen-bonds will be more difficult, but the charge distribution will favor binding on the ring. All substituents in the ring are coplanar, and therefore the molecule is flattened. It has been reported that a variety of furane derivatives have a higher muscarinic potency than do their saturated analogs (35, 60), whereas the nicotinic action is not changed (47, 95). 4,5-Dehydro-muscarone (XXXVIII) might consist partially of an aromatic furane ring due to enolization and a hybrid structure which makes total racemization possible. Other unsaturated muscarine compounds have not been investigated in this respect.



6. Cationic ammonium group. Nor-muscarine (V) and nor-muscarone (XXXV), which lack a quaternary nitrogen atom, are nearly inactive. The cationic part of the molecule is very important for the action on the cholinergic receptor, presumably because the positively charged part of the molecule may be bound to an anionic site on the receptor. The substitution of two tertiary amine groups in muscarone (XLII) does not augment its activity, and replacing one methyl in the trimethylammonium group by a large aliphatic or aromatic substituent (XLIII to XLV) generally has a more alternating effect on action than in aliphatic cholinomimetic compounds (55).

B. Derivatives of furane, glycol, and propanediol

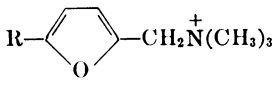
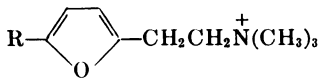
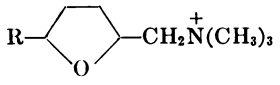
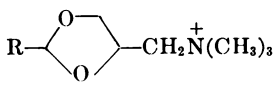
Nearly twenty years before the structure of muscarine was known, Fellows and Livingston (35) demonstrated the high muscarinic potency of furane and tetrahydrofurane compounds with a methylammonium group in the α-position to the ring oxygen (XLVI) (Table 8). Their activity was comparable to that of acetylcholine, methacholine (Mecholyl), and carbamylcholine (carbachol), where the ammonium head carried three methyl groups. Replacing one of these groups by an ethyl group reduced the activity to one-tenth, and with larger alkyl groups as substituents the action was strongly diminished. The hydrogenated derivatives (LI, LII) were roughly one-tenth as active as their corresponding furane compounds without a corresponding change in the toxicity. The marked fall of blood pressure, negative inotropic and chronotropic effects on isolated rabbit hearts, increased flow of saliva, and increased tone of the intestine and bladder were overcome or prevented by atropine. The strong parasympathomimetic action of furfuryl-trimethylammonium iodide (XLVI) on man was also investigated (79). Since the furane ring is planar and almost a regular pentagon, the length of the chain in the most active compound of this series, furtrethonium (Furmethide), must approximate that of a 4-atom acylic chain.

Ten years later Ing and co-workers (5, 60) tested their 5-atom rule by synthesizing the 5-methylfurtrethonium (XLVII), which proved as active as acetylcholine (Table 8). In the perfused superior cervical ganglion and on the denervated gastrocnemius of the cat, it was about one-quarter as effective as acetylcholine, but, like furtrethonium, considerably weaker in producing contracture of the frog's rectus abdominis. The miotic action in the mouse was thirteen times as powerful as that of furtrethonium (44). Anticholinesterase agents (neostigmine, eserine) were far more active as miotics. Lourie (76) proved 5-methyl-furtrethonium to be more active than carbachol on the bladder, both in the organ bath and *in situ* after intravenous injection, and much less toxic than carbachol to different animals, probably because in high doses it caused a less protracted bronchoconstrictor effect. Neither the 5-methylfurfuryl compound nor furtrethonium produced a rise of blood pressure in the cat after atropine, even in high doses.

The influence of the side-chain position on activity was investigated in some furane derivatives synthesized by Eugster (19) (Table 9). When the ammonium group is moved from the α- to the β-position and the linking CH_2 groups thus

TABLE 8

Muscarine-like derivatives of furane, propane, and glycol-diol (configuration not known); relative activity compared to acetylcholine = 1

No.	Compound	R	Blood Pressure of Cat, Dog,* or Rabbit†	Rabbit Auricle	Ileum of Guinea Pig, Rat,* or Rabbit†	Frog Heart	Frog Rectus Abdominis (n=nicotinic)	Reference
XLVI	 Furtrethonium (Furmethide)	—H	0.03–0.1	0.044	0.083	0.008	0.002	5, 35, 60
XLVII	5-Methyl-furtrethonium	$-CH_3$	0.3 –1.0	0.83	3.3	0.067	0.00014	5, 60
XLVIII		$-CH_2CH_3$	0.0083	0.0013	0.028	0.0005–0.00026	0.0005	60
XLIX		$-CH_2OH$	very weak					60
L		—H	0.0038	0.0125	0.01	0.0025	0.043	60
LI		—H	0.003–0.01					35
LII		$-CH_2OH$			0.001†			12
LIII	 Dilvasène 2249 F	—H	0.017 0.5–2*	0.125	0.06 0.05	0.011	0.048	5 36

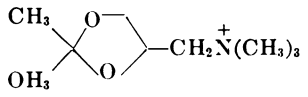
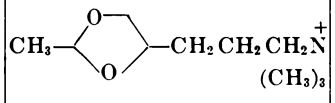
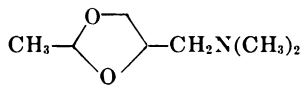
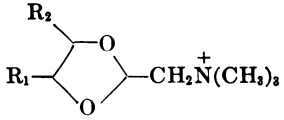
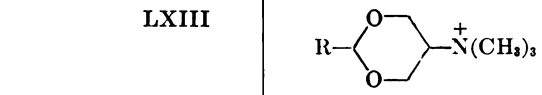
LIV	2268 F	$-CH_3$	0.63 10–50*	4.5	2.3 10	0.83 1	0.03 0.2	5 36, 37
LV	2292 F	$-CH_2CH_3$	0.1*		0.1			36
LVI	2262 F	$-C_6H_5$	0.001*		0		(n)	36, 37
LVII	 2269 F		0.01*		0.01			36
LVIII	 2301 F		0		0		n	36
LIX	 2276 F		0.01*		0.02			36
LX	 2291 F	R_1: —H R_2: H	0.01†		0.01*			38
LXI		—CH_3 H	0.013†		0.02*			38

TABLE 8—*Continued*

No.	Compound	R_1:	R_2:	Blood Pressure of Cat, Dog,* or Rabbit†	Rabbit Auricle	Ileum of Guinea Pig, Rat,* or Rabbit†	Frog Heart	Frog Rectus Abdominis (n=nicotinic)	Reference
LXII		$-CH_3$	$-CH_3$	0.005†		0.004*			38
LXIII	 2580 F	—H		1.0*		0.1†		n	94
LXIV	2581 F	$-CH_3$		100*		0.1†			94
LXV	2586 F	$-C_6H_5$		0.0004*		0†			94

incorporated in the furane ring (LXVI to LXXI, Table 9), the cholinomimetic activity is considerably diminished. The difference between the dose required for peripheral and ganglionic stimulation is much smaller (10 to 100) compared to muscarine ($>10^6$), and curarimimetic effects are observed with doses above 1 mg/kg. Neither a 5-atom chain in the furane ring nor a 2 carbon distance of the ring oxygen from the nitrogen renders these molecules distinctly muscarinic. A hydroxy-group in the side-chain (LXVIII, LXIX) or aromatization of the tetrahydrofurane ring (LXVIII) diminishes the activity.

A bisquaternary derivative of tetrahydrofurane (LXXII) had low muscarinic and only slight nicotinic activity (43). Other compounds with two methoxy groups at C_2 and C_5 (LXXI) or an ethyl group at C_2 (LXX) were inactive.

Fourneau *et al.* (36) described in 1944 the strong muscarinic activity of acetals of propanediol with a trimethylammonium group in the γ-position (Table 8). The most active compound was 2268 F (LIV), an ethylacetal, which lowered the blood pressure of the dog in a 10 to 50 times smaller dose than acetylcholine and caused cardiac arrest at a dose of 1 μg. Compound 2249 F (LIII) (Dilvasène) was 10 to 50 times less potent and considerably less toxic, and was used clinically as a vasodilator in peripheral circulation disorders. Unfortunately, this compound has some nicotinic actions, as have nearly all other propanediol derivatives mentioned here, especially when the cationic side-chain was lengthened or a phenyl group introduced (LVIII, LVI). Generally, nicotinic action was found to be much smaller for acetals than for choline esters. The cholinomimetic action was reduced in the tertiary N-dimethyl derivative (2276 F LIX) by a factor of 100 to 1000 compared to the quaternary analog 2268 F.

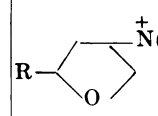
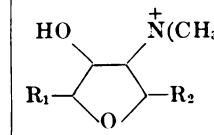
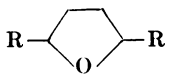
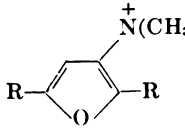
The acetal of the glycol derivative (LX) and two methylated derivatives (LXI, LXII) with symmetrical α-positions of the ring oxygen atoms to the ammonium side-chain, and some 6-membered aminoacetals (LXIII to LXV) exerted muscarinic activities, following again the 5-atom rule (94). Nicotinic activity is known only for the highly symmetrical compound, LXIII. Its methyl derivative (LXIV) was even more hypotensive in dogs than was F 2268. In this molecule 5-atom chains may be counted on either side of the heterocyclic ring. The low activities of LVII, LXII, and LXV might be due to steric interference by the substituents to binding on the receptor.

C. Isoxazolidine and morpholine compounds

A group of compounds with quaternary nitrogen in 5- and 6-membered heterocyclic ring systems different from furane (19) provided some interesting facts with respect to their cholinomimetic activities (Table 9). Nearly all *isoxazolidines* (LXXIV to LXXVIII) showed a remarkably high activity which was not specific regarding the localization of the synapse (peripheral, ganglionic, or endplate). The ring oxygen lies in the α-position to the ring nitrogen, and the active chain has between four and five C-atoms in addition to the oxygen atom. The position of the hydroxy-group in the side-chain is of great influence on the activity. *1,4-Morpholine* compounds with the side-chain in β-position relative to the nitrogen (LXXIX to LXXXII) are more active than α-substituted derivatives

TABLE 9

Muscarine-like compounds with substituted furane, isoxazolidine and 1,3 or 1,4-morpholine rings (configuration not known; diastereoisomers in regard to hydroxy group); cat: minimal effective dose, μg/kg, i.v.; isolated frog organs: μg/ml; investigated by Waser; m = muscarine, n = nicotine, c = curariform, () = weak

No.	Compound	Cat				Frog		Predominant Action	Reference (Synthesis)
		R	Lowering of blood pressure (m)	Increase of blood pressure (atropinized) (n)	Paralysis of muscle (c)	Heart (m)	Rectus muscle (n)		
LXVI		—H	30	300	1,800	980	540	(m), (n)	19
LXVII		—CH_2CH_3	1.5	300	1,000	970	330	m, (n)	19
LXVIII		—$CH(OH)CH_3$	10	5,000	>10,000	86	—	m	19
LXIX		—$CH(OH)CH_3$	15	500	3,000	24	—	m	19
LXX		R_1 R_2 —CH_2CH_3 —H —OCH_3 —OCH_3	1/1000 as active as muscarine on rabbit ileum			>20,000			12
LXXI									
LXXII		—$CH_2\overset{+}{N}(CH_3)_3$	10	>10,000	10,000				43
LXXIII		—CH_3	25	500	3,000	—	—	(m), (n)	19

LXXIV		—$CH_2CH(OH)CH_3$	0.6	50	350	0.005	83	m, n, (c)	19
LXXV		—$CH_2CH(OH)CH_3$	7	300	1,000	3.0	770?	m, (n)	19
LXXVI		—$CH_2CH(CH_3)_2$	0.7	90	80	0.84	160	m, n, c	19
LXXVII		—$CH_2CH_2CH_2CH_3$	0.5	50	100	0.19	47	m, n, c	19
LXXVIII		—$CH_2CH_2CH(OH)CH_3$	100	4,000?	>10,000	5,500	>40?	(m)	19
LXXIX		—CH_2CH_2OH	7	750	700	0.49	90	m, (n), (c)	19
LXXX		—$CH_2CH_2CH_3$	1.5	150	500	0.097	170	m, (n), (c)	19
LXXXI		—$CH_2CH(OH)CH_3$	3.0	60	1,000	0.076	50	m, n	19
LXXXII		—$CH_2CH(OH)CH_3$	20.0	220	2,000	0.99	75	m, n	19
LXXXIII		—CH_2CH_2OH	20	3,000	>3,000	1.9	>30	m	19
LXXXIV		—$CH_2CH(OH)CH_3$	—	1,500	10,000?	1,000	>33		19
LXXXV		—$CH_2CH(OH)CH_3$	20	1,500	>5,000	1,000	3,000?	(m)	19
LXXXVI		—$CH_2CH(OH)CH_3$	—	40	700	530	45	n, (c)	19
LXXXVII		—$CH_2CH(OH)CH_3$	—	700	2,500	12	1,100	(m), (n)	19

(LXXXIII to LXXXV). Stereoisomerism, although not known in detail for these compounds, has some influence on their action, perhaps because of intramolecular hydrogen bonding between hydroxy groups and oxygen. Nearly all the compounds have both muscarinic and nicotinic properties. Muscarinic activity is more variable than nicotinic. Slight curare-like effects of short duration have been noticed with the β-substituted series. *1,3-Morphine* compounds (LXXXVI, LXXXVII) with α-substitution have only weak muscarinic activity on the frog heart and some nicotinic and curare-like action in the cat.

In summary, the peripheral cholinomimetic (muscarinic) action is stronger for 5-membered than for 6-membered ring-systems, whereas the ether oxygen in the α-position to the nitrogen may be very active in isoxazolidine but not in 1,3-morpholine compounds. Compounds with a butanolic side-chain in the α-position to the ring oxygen have strong nicotinic action. A chain length of 4 as compared with 5 C-atoms is of minor importance.

D. Conclusions concerning the interaction of muscarine-type compounds with the cholinergic receptor

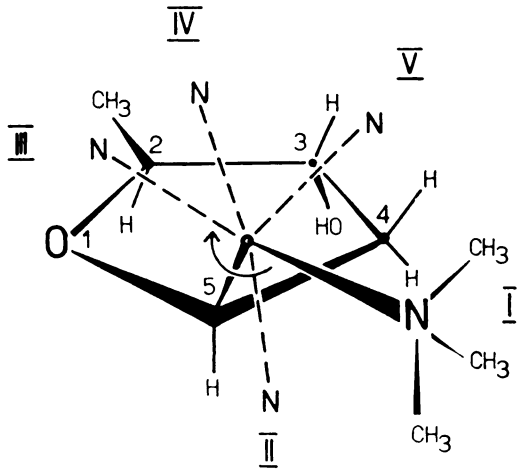
In this part we shall attempt to summarize some characteristics of the muscarine-type compounds, just discussed, which are essential for their action on the cholinergic receptor. As we know of the existence of a definite receptor unit only on the basis of the structural characteristics of the drugs which are responsible for their pharmacological action, but do not recognize its inherent nature and structure, this discussion will be theoretical and will be based on other well-known facts about characteristic groups in cholinomimetic drugs (6, 8, 11, 59, 81).

A comparison of the actions of different types of compounds in regard to their binding on the receptor area is useless, when these molecules have completely different *physicochemical properties*. They may be distributed differently in the organism according to solubility in water or lipoid material; the volume and forms of the molecules will influence their permeation through membranes, their shape and charge, the distribution in a monolayer on membranes, and so forth.

The group of compounds investigated here consists of highly water-soluble quaternary compounds, with the exception of some tertiary derivatives, the hydrochlorides of which are readily soluble in water. With few exceptions they have nearly the same molecular weight (exclusive of the anion) and a similar volume.

The active form of the molecules is not known, and as muscarine and its derivatives have a relatively flexible ammonium side-chain, different conformations are possible. The extended form has the appearance of a cylinder, whereas the folded form is more oval and tends to a globular shape. For penetration through a pore, a shape with a small diameter may be assumed, and for fixation on the receptor area another form might be active.

Flattening of the molecule by aromatization of the ring in furane derivatives generally augments muscarinic activity without influencing nicotinic action. Enlargement of the ring to tetrahydrothiophan derivatives or to 6-membered ring systems generally diminishes their activity. The shape of the molecule



L(+)-Muscarine

FIG. 3. Some possibilities of conformation of muscarine by turning the methyltrimethylammonium side-chain (positions I–V, compare Table 10).

greatly influences the close *fit* of muscarine-type compounds on the receptor. The low activity of isomers of muscarine and of derivatives with large alkyl groups at carbon-2 or in the quaternary nitrogen group may be due to this influence.

As we have seen, the C_2-methyl group plays an important activating role in keeping with the 5-atom rule of Alles and Knoefel (1) and Ing (59). These authors have suggested that in any homologous series of parasympathomimetic drugs of the general type $RN^+(CH_3)_3$, with few exceptions the most active member will be the one which contains a 5-atom chain in the group R. In the structure of muscarine we can easily identify this 5-atom chain, when we count the atoms starting from the nitrogen of the side-chain and continuing to the ether-oxygen in the furane ring. Even if we count 6 atoms in the chain by way of carbon-3, carrying the hydroxy or carbonyl group, we may assume the chain to be reduced by the ring effectively to five, as was stated by Ing *et al.* (60). The reason why molecules with a definite length exert the strongest action is not fully understood, as we cannot assign to the C_2-methyl group an explicit activating function. Van der Waals forces binding these silent groups to the receptor may perhaps give an explanation. An inductive effect on the ether oxygen may also be of some importance (7, 9).

In general, the 5-atom rule is followed by most compounds discussed in this review. The few exceptions may be explained by different factors, such as steric hindrance interfering with the formation of a drug-receptor complex.

In a discussion of structure-activity relationships, the cyclic muscarine compounds have some distinct advantages compared to acetylcholine (Fig. 2) or other aliphatic cholinomimetic compounds:

1) The side-chains have a fixed steric position on the tetrahydrofurane ring.

2) The free-moving cationic head is restricted in its position by steric hindrance of hydrogen atoms on the tetrahydrofurane ring, especially those on the neighboring carbon atoms 4 and 5. Only a few conformations have to be considered for the molecule, in solution or in the biological system, which exceed the well-defined crystal structure of Jellinek (Fig. 3).

3) The two oxygen functions are separable into two isolated groups: the ether oxygen and hydroxy group in muscarine, and the carbonyl group in muscarone.

The influence of the steric position of the side-chains on the tetrahydrofurane ring on activity is very marked in muscarine, since of the eight possible isomers only muscarine is significantly active. Stereospecificity between L(+)- and D(−)-muscarine is high (factor 1,000), and the pharmacologically active L(+)-isomer shows a similar absolute configuration to that of the active L(+)-acetyl-β-methylcholine, compared to which D(−)-enantiomorph is 500 times less active (9, 17). On basis of this configurational similarity, these active molecules must possess a high degree of molecular complementarity with the receptor at least three different points (97). They most probably are represented by the three pharmacophore groups of muscarine, which presumably interact with corresponding groups of the receptor. Opposed to this, the stereospecificity of muscarone is quite low since the activity difference between its enantiomorphs is only threefold, and here the D(−)-isomer, related to inactive D(−)-muscarine, is more potent. Theoretically, low stereospecificity is possible in a symmetrical molecule when there are only two points of attachment or when two of three points of interaction are very similar in their character, distance from the third point, and other steric and electrical properties.

The molecular configuration of D(−)-muscarone is indeed similar to that of L(+)-muscarine when we look at the 6-atom chain, where the carbonyl group takes the place of the ether oxygen of muscarine. The third similarly acting group in muscarone is then the ether oxygen, which takes the place of the hydroxy group in muscarine. Although several individual factors, such as rate of absorption or destruction, differential penetration to the site of drug action, and possible racemization as mentioned before in muscarone, dominate the pharmacological action of optical isomers (82), it is remarkable that muscarone, one of the most active cholinomimetic compounds, has such a low stereospecificity.

The activity of the various muscarine-type compounds provides good evidence for the importance of three pharmacophore groups.

Changes in the quaternary ammonium group of muscarone diminish muscarinic and nicotinic activity to the same degree. Replacing one methyl group by a large alkyl or aromatic substituent has a 10 to 100 times more weakening effect than in aliphatic quaternary compounds (55). Tertiary compounds are nearly inactive. The cationic trimethylammonium group is presumably bound to an anionic anchoring site in the receptor area, without specific influence on muscarinic or nicotinic potency (43). This electrostatic bond is mainly responsible for the fixation and overall cholinomimetic activity of the molecule. Loss of the methylene link between trimethylammonium group and furane ring or incorporation of the quaternary nitrogen in the ring system clearly diminishes activity (13a).

The oxygen functions and the electron distribution in the ring system clearly have a major influence on muscarinic or nicotinic activity. The electronegative ether and the carbonyl oxygen atoms may form hydrogen bonds with the receptor, which are essential for muscarinic and nicotinic action.

Steric hindrance of bond-formation between the ether oxygen and the receptor, or replacement of the oxygen by the less electronegative sulfur in thiomuscarine diminishes muscarinic activity of muscarine-type compounds. *Trans*-position of the hydroxy group relative to other ring substituents is essential for this effect, but even steric hindrance by a large phenyl group at carbon 3 or acetylation does not influence activity. In some active furane derivatives the hydroxy group is missing. In the highly active propanediol compounds there are two equivalent ether oxygens in the ring. All these facts point to a minor, perhaps only potentiating, role of the hydroxy group.

In contrast to this, steric hindrance of hydrogen bond formation to the ether oxygen in muscarone or reduction of the electronegativity of the ring hetero-atom in thiomuscarine does not greatly influence muscarinic activity. In these derivatives the hydrogen bond may attach to the carbonyl oxygen. Thus, in muscarone-type compounds the carbonyl group must be responsible for muscarinic and nicotinic activity. Furane derivatives, such as muscarine-type compounds, without the carbonyl group have low nicotinic potency, which stresses the importance of the carbonyl group for this type of cholinomimetic action.

The charge distribution in the ring must be considered also. The saturated tetrahydrofurane ring has p-electrons over its ether oxygen and π-electrons over the carbonyl group. Therefore we find in muscarine one point with high electron density, and in muscarone two points. The polarized carbonyl group of muscarone carries a positive charge over the carbon atom. Since we know of such different compounds as nicotine and nicotinic phenol-ethers (6, 7, 42, 54) which have a similar polar charge distribution this factor might be a reason for nicotinic action. The nicotinic activity of 3-phenyl-muscarine can be explained in a similar manner. Propanediols have two ether oxygen atoms with p-electrons. Dehydrogenation of the ring system has different effects. One double bond (C_4—C_5) in muscarine or muscarone causes a slightly diminished muscarinic but distinct nicotinic action. Complete aromatization augments muscarinic potency without change of nicotinic action. Although steric change of the molecule to coplanarity has a great influence, charge distribution in the ring and dipole forces must be considered as being mainly responsible for nicotinic action (6, 89). Nucleophilic groups of the receptor protein, such as imidazole, tyrosyl, or seryl might interact with the carbonyl carbon in a similar way, as has been suggested by Wilson (102) and Krupka and Laidler (71a) for the esteratic site of acetylcholinesterase. At the same time a proton could be directed towards the carbonyl oxygen atom.

As the strongest effects of muscarine and muscarone are muscarinic, their pharmacophore groups must act on the same cholinergic receptors. These groups, therefore, must have similar interatomic distances.

We have now to investigate whether there exist conformations of muscarine and muscarone in which the active groups may be placed in corresponding distances for fixation on muscarinic receptors (90, 99). Following our theory, the

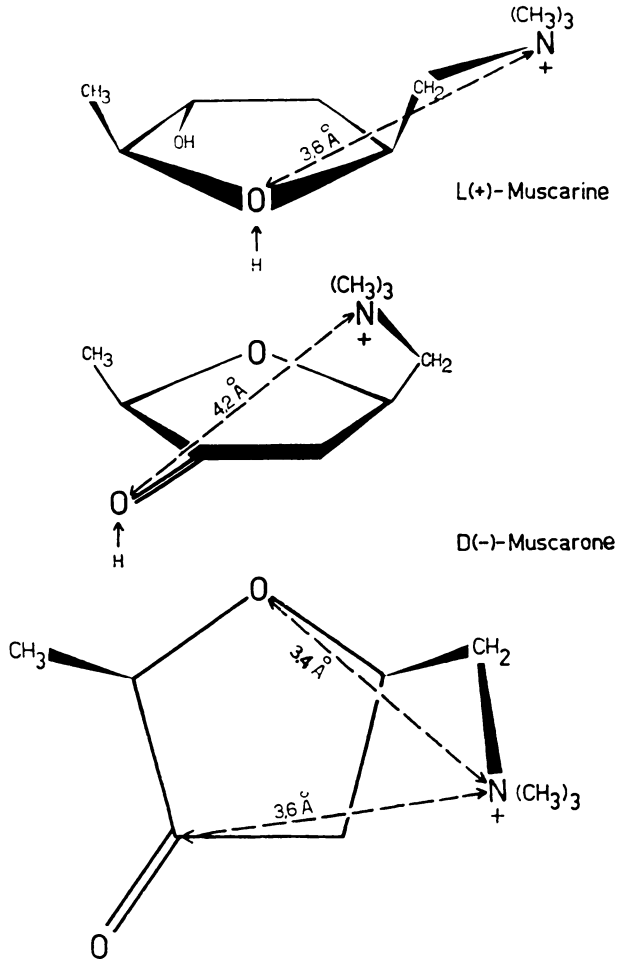


FIG. 4. Extended conformation of L(+)-muscarine (I) and folded conformation of D(−)-muscarone (V) for muscarinic action.

muscarinic action of muscarine is mainly due to ionic binding of the quaternary nitrogen group and to hydrogen bonding to the ether oxygen atom. The distance between these two groups measured in the extended chain of the Dreiding wire model, is maximally 3.8 Å, when the methyltrimethylammonium group is in the same plane as the ether oxygen and carbon 5 (Fig. 4). The trimethylammonium group may rotate, around the fixed position of its methylene linkage to the ring carbon atom, over the hydrogen atoms at carbon 5, and towards and over the ether oxygen (Fig. 3, Table 10). There is only slight steric hindrance by the hydrogen atoms of carbon 4. The minimal distance between N^+ and —O— will be 2.6 Å, and the distance in the upturned position 3.4 Å. Some conformations of the side-chain may be influenced by a hydroxy-group in the *cis*-position (epi-muscarine, allo-muscarine).

TABLE 10

Interatomic distances in various conformations of muscarine and muscarone

Conformation of Molecule (Fig. 3)	Interatomic Distances in Å (Dreiding Wire Model)			
	Muscarine and muscarone	Muscarine	Muscarone	Muscarone
	$N^+ \leftrightarrow \diagdown O \diagup$	$N^+ \leftrightarrow OH$	$N^+ \leftrightarrow O=C$	$N^+ \leftrightarrow C=O$
I } extended	3.8	5.4	5.2	4.4
II } extended	3.4	6.0	5.9	4.8
III } folded	2.6	5.6	5.2	4.2
IV } folded	2.7	5.4	5.0	3.6
V } folded	3.4	5.0	4.2	3.6

In muscarone the same conformations of the cationic side-chain are possible. Since in our theory the carbonyl oxygen is responsible for hydrogen bonding and muscarinic action, the minimal distance to the nitrogen is 4.2 Å when the chain is turned towards the ring (Fig. 4). This conformation explains also the higher activity of D-muscarone as compared to L-muscarone, because the C_2 and C_5 substituents are in a similar steric position relative to the carbonyl group as they are to the ether oxygen in the highly active L-muscarine.

In order to fit on the same muscarinic receptors, muscarine and muscarone therefore must have different conformations (Fig. 4) giving an approximate distance of 4 Å between the quaternary nitrogen and the nucleophilic oxygen functions. Pfeiffer (81) was first in proposing distances of 5.0 Å and 7.0 Å from the ether oxygen and the carbonyl oxygen, respectively, to the quaternary N-methyl group for parasympathomimetic stimulant effects.

For the nicotinic action of muscarone, the distances from the quaternary nitrogen group to the electron-dense carbon atom of the carbonyl group, and to the ether oxygen have to be considered. As an explanation of the low stereospecificity of muscarone, we have postulated these distances to be similar. When we place the quaternary ammonium side-chain in one plane with ring carbons 4 and 5, the nitrogen will be nearly equidistant from the two active ring atoms, with equivalent distances of 3.4 and 3.6 Å (Fig. 4). The quaternary nitrogen group in this position is very near to the point where it is closest to the carbonyl oxygen, and not far from the active position in muscarine. The difference between models of D- and L-muscarone is very small for this conformation of the molecule, and is mainly due to the incompletely planar ring with one carbonyl oxygen projecting on either side.

It is much more difficult to explain the low stereospecificity of muscarone by two-point binding alone (with the carbonyl and quaternary N) on the receptor, because the carbonyl group has an unsymmetrical position outside the furane ring. In the extended conformation the molecule (Fig. 5) is even less symmetrical than in the conformation proposed for muscarinic activity. Although the methyl group at C_2 might change its steric position on the ring by enolization of the carbonyl group which interacts with the receptor, the small difference in the

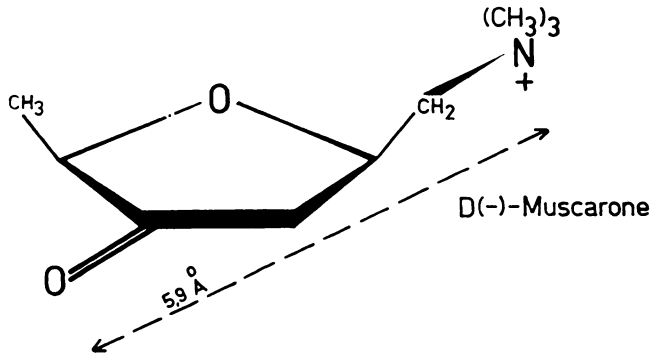


FIG. 5. Extended conformation of D(−)-muscarone (II).

nicotinic activity of the two antipodes of muscarone is not explained thereby. The distance between the quaternary nitrogen and the carbonyl oxygen is large (5.9 Å), and therefore a different type of receptor than that for muscarinic action would be expected. So far only few muscarine-like derivatives with long cationic side-chains (two or three carbon atoms) have been investigated and found to have nicotinic action. An investigation of stereoisomers of muscarone derivatives will perhaps clarify this possibility.

Beckett and co-workers (9) have proposed a rough structural picture of the cholinergic receptor based on a three-point attachment of muscarine (97) with an interatomic distance of 3.0 Å between the quaternary nitrogen and ether oxygen. Muscarone would then have its carbonyl oxygen fixed at the same place as the hydroxy group of muscarine. This part of the receptor therefore should be able to form hydrogen bonds in both ways, *i.e.*, by donating protons to the carbonyl oxygen or by accepting protons from the hydroxy group. Although this simple picture is very attractive for the explanation of muscarinic action, it accounts for neither muscarinic and nicotinic action nor for the low and inverse stereospecificity of muscarone compared to muscarine.

Unfortunately we do not know anything about the metabolism of muscarine or muscarone, but it seems unlikely that the ring is opened at the ether-oxygen carbon bonds. This has to be carefully investigated with ^{14}C-compounds, which will perhaps give new ideas about the interaction with the receptor.

In *conclusion*, maximal muscarinic action of muscarine-like compounds depends mostly on the cationic nitrogen with three methyl groups and a nucleophilic group (ether-oxygen or carbonyl-oxygen) at a distance of 4 Å. Nicotinic properties depend on the polarizability of the nucleophilic part of the molecule (carbonyl group) and the occurrence of another electron-dense part (ether-oxygen, double bond, or aromatic resonance-system) in or on the ring, in a symmetrical position with the polar group relative to the quaternary nitrogen. The same criteria explain the strong action of many other aliphatic and cyclic cholinomimetic compounds.

Finally, we must admit that while the investigation of muscarine and many similar compounds has clarified considerably the old problems of the structure-

activity relationships of cholinomimetic compounds, it has by no means solved them completely. The results may have validity for other compounds of the parasympathomimetic group, and they may give some new indications of the structures of the cholinergic receptors.

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