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Nicotinic stimulant action of some tolyl and xylyl analogues of 1,1-dimethyl-4-phenylpiperazinium (DMPP)

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Summary

1. The nicotinic stimulant properties of DMPP have been compared with those of its *ortho*-, *meta*- and *para*-tolyl analogues, and its 2,6- and 3,5-xylyl analogues, on the blood pressure of the cat and on the semispinalis cervicis muscle of the chick.

2. A single *ortho*-methyl group does not significantly change the nicotinic activity, but two such groups greatly reduce or abolish activity. *Meta*- or *para*-methyl substitution reduces activity, but does not abolish it.

3. The effect on nicotinic activity of methyl substitution in the phenyl ring of DMPP closely parallels the effect of ring-methyl substitution in phenyl choline ether.

4. *Ortho*-methyl substitution has been shown spectroscopically to alter markedly the spatial orientation of the phenyl ring of DMPP relative to the piperazine ring. The effect of these conformational changes on nicotinic activity is discussed in relation to a recent hypothesis concerning the conformation of acetylcholine required for interaction with nicotinic receptors.

5. The compounds tested had no muscarinic activity on the isolated guinea-pig ileum.

Introduction

Since the discovery by Chen, Portman & Wickel (1951) of the potent nicotinic stimulant properties of 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), this compound has been widely used as a ganglionic stimulant. Crystalline DMPP has the conformation shown in Fig. 1 (Chothia & Pauling, 1970). The plane of the benzene ring is believed to be maintained at right angles to the mirror plane of the

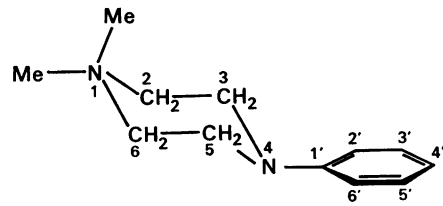


FIG. 1. Conformation of DMPP in crystals of its iodide salt.

piperazine ring by conjugation between the aromatic π-electrons and the unshared electron pair on the adjacent nitrogen atom. From observations on space-filling molecular models, it appeared that insertion of methyl groups into the two *ortho* positions (that is 2′ and 6′ in Fig. 1) would cause the phenyl ring to twist through an angle of 90° around the Ph-N bond, as a result of the very strong steric repulsion between these methyl groups and the methylene hydrogen atoms on C_3 and C_5 of the piperazine ring. A similar, although smaller, twist would also be induced by a single *ortho*-substituent, but *meta*- or *para*-substituents should not affect the relative orientation of the two rings. Chothia & Pauling (1970) pointed out a close stereochemical relationship between the conformations of DMPP and other nicotinic agonists, including acetylcholine. If this stereochemical correlation is mechanistically significant, changes in the conformation of DMPP brought about by ring substitution should be reflected as changes in the nicotinic stimulant potency. To test this hypothesis, we have synthesized all three tolyl analogues of DMPP and its 2,6- and 3,5-xylyl analogues, and studied their conformations and nicotinic stimulant properties. Some of the results obtained with the *ortho*-tolyl and 2,6-xylyl compounds have been reported previously (Green & Marshall, 1970).

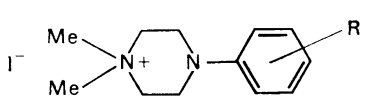
Methods

Chemical

Test compounds

The 1-methyl-4-arylpiperazine bases were prepared by heating the appropriate toluidines or xylidines with N-methylmorpholine and concentrated hydrochloric acid as described by Fujii, Tomino & Watanabe (1954) for 1-methyl-4-phenylpiperazine. Yields were generally low, particularly with 2,6-xylidine, which gave only about 2% of the desired product. The bases were then quaternized by allowing them to stand at room temperature with a slight excess of methyl iodide in ethanol or ether solution. Structures, empirical formulae, molecular weights, solvents of crystallization, uncorrected melting points and analytical data are listed in Table 1.

TABLE 1. *Structures, formulae, analytical data, solvents of crystallization and melting points of the 1,1-dimethyl-4-arylpiperazinium iodides*



R	Empirical formula	Molecular weight	Analysis C Calc.	C Found	H Calc.	H Found	Solvent of crystallization	Melting point (°C with decomposition)
H(DMPP)	$C_{12}H_{19}N_2I$	318	45·3	45·4	6·0	6·1	aq. EtOH	233–235
o-CH_3	$C_{13}H_{21}N_2I$	332	47·0	47·1	6·4	6·4	aq. EtOH	269–271
m-CH_3	$C_{13}H_{21}N_2I$	332	47·0	47·1	6·4	6·3	iPrOH/MeOH	197–198
p-CH_3	$C_{13}H_{21}N_2I$	332	47·0	47·2	6·4	6·5	iPrOH	199–200
2,6-$(CH_3)_2$	$C_{14}H_{23}N_2I$	346	48·5	48·3	6·7	6·7	EtOH	252–254
3,5-$(CH_3)_2$	$C_{14}H_{23}N_2I$	346	48·5	48·1	6·7	6·8	EtOH	215

Ultraviolet spectra

Since iodide ions absorb light at wavelengths below 260 nm, aqueous solutions of the 1,1-dimethyl-4-arylpiperazinium iodides were first converted into the corresponding chlorides by shaking them gently with freshly precipitated silver chloride. After removal of the precipitate of silver chloride and iodide by filtration, the filtrates were diluted with water to make the concentration of the piperazinium chlorides up to 0.1 mM or 1 mM. The spectra were then measured with a Unicam SP800 spectrophotometer. At wavelengths above 260 nm, the spectra of the chlorides and iodides were identical.

Pharmacological

Experiments in cats

Cats were anaesthetized with a mixture of chloralose (80 mg/kg) plus pentobarbitone (2·5 mg/kg) injected intraperitoneally. Drugs were given through a cannula into a femoral vein, and the blood pressure was recorded with a Statham pressure transducer from a cannula in a common carotid artery. In some cats twitch tension was recorded isometrically from the tibialis anterior muscle. The sciatic nerve was stimulated with rectangular pulses of 100 μs duration at a frequency of 0·1 Hz and at a strength sufficient to induce a maximal muscle twitch. Before administration of test compounds all the cats were injected with atropine sulphate (1 mg/kg).

Chick semispinalis cervicis muscle preparation

Semispinalis cervicis muscles were removed from chicks between 2 and 10 days old, and mounted in oxygenated double-glucose Tyrode solution at 40·5° C (Child & Zaimis, 1960). Test compounds were added to the bath at 10 min intervals in increasing concentrations until the maximum response to the particular compound was produced. Each concentration of the drug was left in contact with the tissue until the response reached a plateau (60 s) before being washed out. Absence of desensitization was checked by periodic addition of a control concentration of suxamethonium.

Guinea-pig ileum preparation

Sections of isolated guinea-pig ileum were mounted in oxygenated Tyrode solution at 32° C. In all experiments the bath fluid contained hexamethonium bromide (10 μg/ml) in addition to the test compound.

Drugs

The compounds used, other than the test compounds above, were acetylcholine chloride, atropine sulphate and chloralose (BDH), pentobarbitone sodium (Abbott), mecamylamine hydrochloride (Merck, Sharp & Dohme), edrophonium bromide (Roche), suxamethonium bromide (Allen & Hanbury) and hexamethonium bromide (May & Baker). The concentration quoted for each of these drugs refers to the corresponding salt.

Results

Ultraviolet spectra

The spectrum of DMPP is compared in Fig. 2 with those of its *ortho*-tolyl and 2,6-xylyl analogues. DMPP chloride has three absorption bands with maxima at 278, 239 and 203 nm. A single *ortho*-methyl substituent decreases the intensity of the two longer wavelength bands without greatly affecting the shortest wavelength band. The introduction of two *ortho*-methyl groups abolishes the longest wavelength band, and reduces the intensity of both the other bands. This reduction in intensity is accompanied by a shift of the peaks to longer wavelengths.

Meta- or *para*-methyl substitution caused only minor changes in the spectrum of DMPP. Absorption maxima and the logarithms of the molar extinction coefficients (ε) are given in Table 2 for all six compounds studied.

Pharmacological studies

Nicotinic stimulant activity

Each drug was given to three atropine treated anaesthetized cats in a range of doses, the highest of which produced a rise in systolic blood pressure of about 120 mmHg (1 mmHg≡1·333 mbar). The dose producing a 100 mm rise in each cat was obtained by interpolation. The mean dose and the molar potency ratio

TABLE 2. *Peak wavelengths* (λ_{max}) *and corresponding value of log* ε *for the three main ultraviolet absorption bands of the* 1,1-*dimethyl*-4-*arylpiperazinium chlorides* (*for structures, see Table* 1)

R	λ_{max}	log ε	λ_{max}	log ε	λ_{max}	log ε
H(DMPP)	278	2·98	239	3·94	203	4·15
o-CH_3	271	2·93	236	3·74	206	4·14
m-CH_3	281	2·99	242	3·88	208	4·20
p-CH_3	283	3·00	238	3·97	203	4·16
2,6-$(CH_3)_2$	—	—	248	3·34	210	3·96
3,5-$(CH_3)_2$	282	2·93	243	3·85	213	4·21

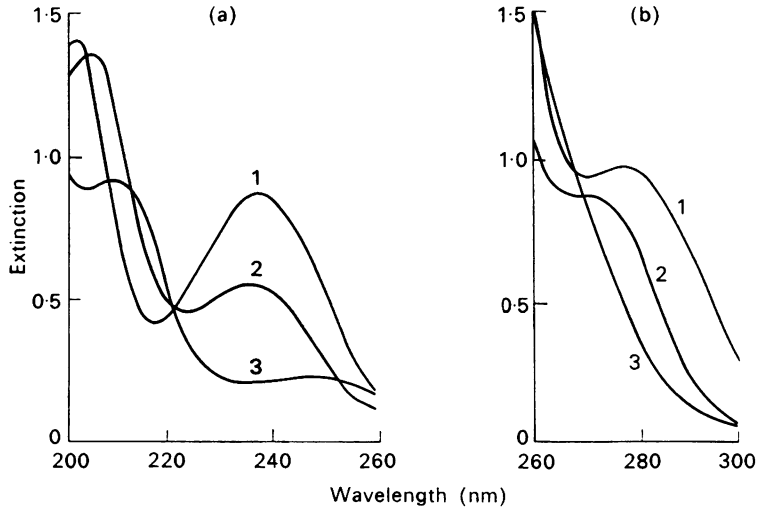


FIG. 2. Ultraviolet spectra of aqueous solutions of the chloride salts of DMPP (1) and its *o*-tolyl (2) and 2,6-xylyl (3) analogues. The concentrations of all three compounds were 0·1 mM in (a) and 1 mM in (b). Path length 1 cm.

relative to DMPP are shown in Table 3. Low doses (up to 6 μmol/kg) of 1,1-dimethyl-4-(2,6-xylyl)piperazinium had no effect on the blood pressure; a higher dose (15 μmol/kg) caused the blood pressure to fall, possibly as a result of the drug's adrenergic neurone blocking action (Green & Marshall, 1970). Low doses (1 μmol/kg) of 1,1-dimethyl-4-*p*-tolylpiperazinium produced rises in blood pressure of up to 60 mmHg, but on increasing the dose to 3 μmol/kg the pressor effect declined. This compound also has adrenergic neurone blocking activity *in vitro*, and this action may partially obscure any ganglionic stimulation at higher doses. Mecamylamine (2 mg/kg) abolished the pressor responses produced by all the test compounds.

In one cat the blood supply to the adrenal glands was interrupted, and the potencies of all the compounds were redetermined. From Table 3 it can be seen that the dose of compound required to produce a 100 mmHg rise in blood pressure was increased about 3-fold in each case. This confirms previous findings (Jones, Gomez Alonso de la Sierra & Trendelenburg, 1963) that DMPP exerts its pressor action in the cat primarily by stimulation of the adrenal medulla rather than the sympathetic ganglia, and also indicates that the substituted DMPP derivatives act in a similar fashion. This is in contrast with the pressor action of substituted phenyl choline ethers, which is unaffected by adrenalectomy (Hey, 1952).

During the testing of the two most potent compounds (DMPP and its *ortho*-tolyl analogue) on the cat blood pressure, a short-lived (10–15 s) cessation of respiration was noticed, accompanied by intense skeletal muscle fasciculations. When tested on the cat tibialis anterior muscle-sciatic nerve preparation, both compounds at doses higher than 0·1 μmol/kg produced neuromuscular blockade of the depolarizing type, as was also noted for DMPP by Ling (1959). The depression of maximal twitch height was preceded by twitch augmentation, the tension in response to tetanic nerve stimulation (50 Hz for 5 s) was maintained throughout the period of stimulation, and the neuromuscular block was unaffected by edrophonium (100 μg/kg). These observations indicated that the DMPP analogues also exerted a nicotinic stimulant action at the skeletal muscle neuromuscular junction. This action was studied quantitatively on the semispinalis cervicis muscle of the chick by com-

TABLE 3. *Nicotinic stimulant activities of the* 1,1-*dimethyl*-4-*arylpiperazinium iodides* (*see Table* 1 *for structures*)

Ring substituent	Dose (nmol/kg ± S.E.M.) required to produce 100 mmHg rise in b.p. (a) Normal cats	(b) Ligated adrenals	Relative molar potency‡ (a)	(b)	ED50 (μmol/litre) on chick semispinalis cervicis muscle	Relative molar potency
H(DMPP)	35 ± 3§	95	1·00 (1)	1·00 (1)	1·85	1·00
o-CH_3	25 ± 2§	90	1·32	1·05	1·62	1·14
m-CH_3	195 ± 9	525	0·18 (0·13)	0·18 (0·14)	9·6	0·19
p-CH_3	*	*	<0·03 (0·004)	<0·02 (0·005)	22	0·08
2,6-$(CH_3)_2$	†	n.t.	†	n.t.	p.a.	<0·01
3,5-$(CH_3)_2$	535 ± 23	1,600	0·06 (0·05)	0·06 (0·05)	26	0·07

n.t. not tested; p.a. partial agonist (see text); * produced 60 mmHg rise in blood pressure at a dose of 1 μmol/kg in normal cats, and at a dose of 5 μmol/kg in a cat with ligated adrenals (see text); † fall in blood pressure (see text); ‡ figures in parentheses are the relative molar potencies (dose required to produce a 60 mmHg rise in blood pressure) for the corresponding substituted phenyl choline ethers (Hey, 1952); § by Student's *t* test these values differ significantly at $P=0·1$, but not at $P=0·05$.

parison with suxamethonium, which acts as a pure agonist on this preparation (Marshall, 1968).

Log dose-response curves for all the compounds are shown in Fig. 3, and ED50 values and molar potency ratios (DMPP = 1) are included in Table 3. From Fig. 3 it can be seen that 1,1-dimethyl-4-*p*-tolylpiperazinium, which produced submaximal responses on the cat blood pressure, acted as an agonist capable of producing a maximal response on the semispinalis cervicis muscle. The 2,6-xylyl analogue, which had no pressor effect in cats, acted as a partial agonist on the semispinalis cervicis muscle. The results in Table 3 show that the order of potency of DMPP and its analogues is the same in the two test systems, and, apart from the two most weakly active compounds just mentioned, the molar potency ratios are similar in both systems, indicating that the compounds probably act in a similar fashion at both types of receptor.

Muscarinic stimulant activity

At concentrations up to those required to produce a maximal response on the chick semispinalis cervicis muscle, none of the compounds contracted the isolated guinea-pig ileum in the presence of hexamethonium (10 μg/ml).

Discussion

Conformation of the DMPP analogues

The ultraviolet absorption of DMPP at wavelengths above 200 nm is due to the dialkylated aniline moiety. The changes in the spectrum of DMPP brought about by *ortho*-methyl substitution (Fig. 2) are similar to those which occur on the introduction of *ortho*-substituents into N,N-dimethylaniline (I) (Remington, 1945; Klevens & Platt, 1949) and may be interpreted in the same way. Steric repulsion between the

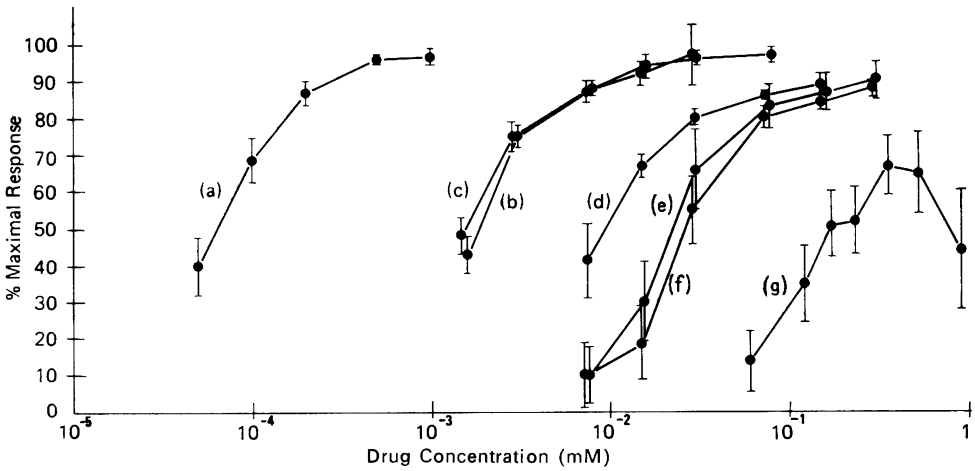
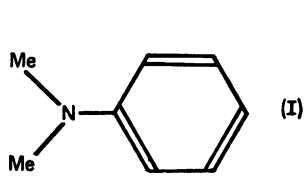
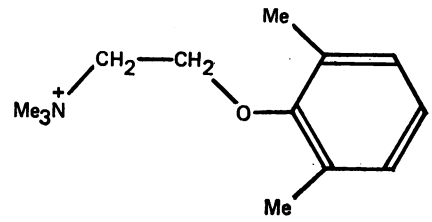


FIG. 3. Stimulant action on chick semispinalis cervicis muscle of suxamethonium (a), and the DMPP analogues—(b) DMPP, (c) *o*-tolyl, (d) *m*-tolyl, (e) *p*-tolyl, (f) 3,5-xylyl, and (g) 2,6-xylyl. The positions on the log dose-response curves are expressed as a percentage of the maximal response produced by suxamethonium in the tissue. Each point represents the mean (±S.E.M.) of between four and twelve determinations.



(I)



(II)

ortho-methyl and N-methyl (or methylene in DMPP) groups causes the benzene ring to twist out of the plane of the dialkylamino group, thus reducing the degree of conjugation between the aromatic π-electrons and the unshared electron pair on the nitrogen. In both series of compounds, disappearance of the longest wavelength absorption band on di-*ortho*-methyl substitution indicates that such conjugation is completely absent, and that the benzene ring must be coplanar with the mirror plane of the dialkylamino group. Insertion of a single *ortho*-methyl group into N,N-dimethylaniline reduced the intensity of each absorption band to an extent quantitatively consistent with a ring twist of about 50° (Klevens & Platt, 1949). Our results with 1,1-dimethyl-4-*o*-tolylpiperazinium indicate a similar partial twist about the Ar-N bond. Thus these spectral changes confirm the conclusions about the conformations of the *ortho*-methyl analogues of DMPP which had been reached from molecular models.

The conformations of DMPP and its *o*-tolyl and 2,6-xylyl analogues are illustrated in Fig. 4 in the form of Newman projections drawn looking along the C_5-C_6 and C_3-C_2 bonds of the piperazinium ring.

From similar spectroscopic studies in the phenyl choline ether series of compounds, Clark & Williams (1967) concluded that the xylyl group of xylocholine (II) also underwent a 90° twist around the Ar-O bond compared with the orientation of the phenyl group in phenyl choline ether. The full structure of xylocholine bromide has been elucidated by X-ray crystallography (Coggon, McPhail & Roe, 1969); the choline moiety is in the *gauche* conformation forming a pseudo six-membered ring, to which the xylyl group is approximately orthogonal. This con-

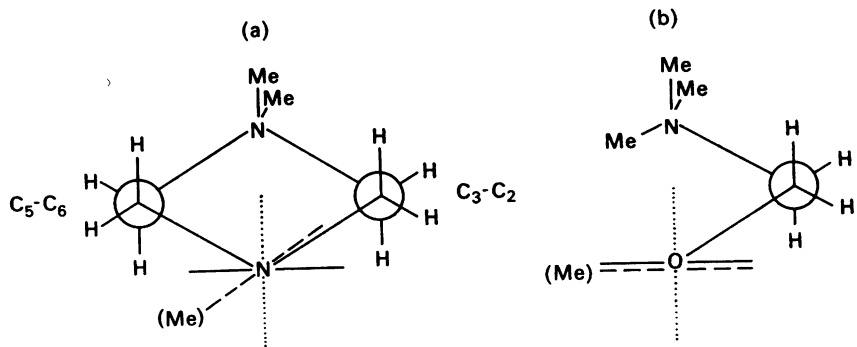


FIG. 4. Approximate conformations of (a), 1,1-dimethyl-4-arylpiperaziniums, and (b), aryl choline ethers. The circles are drawn as if looking end-on along the C_5–C_6 and C_3–C_2 bonds of the piperazine ring in (a), and along the C–C bond of the choline moiety in (b). The orientation of the planes of the benzene rings around the Ar–N bond in (a), and the Ar–O bond in (b) are shown as follows: —— phenyl, (Me) – – – *ortho*—tolyl, ···· 2,6-xylyl.

formation of xylocholine and the probable conformations of phenyl choline ether and *o*-tolyl choline ether are compared in Fig. 4 with those of the corresponding DMPP analogues.

Relationship between structure and nicotinic stimulant activity

On the basis of the three-dimensional conformations of some nicotinic agonists, as established by X-ray crystallography, Chothia (1970) suggested that the conformation of acetylcholine required for interaction with nicotinic receptors must be close to that illustrated in Fig. 5, and that the essential parts of the molecule through which this interaction occurs are the trimethylammonium group and the carbonyl oxygen. The powerful nicotinic stimulant properties of DMPP and of phenyl choline ether (Hunt & Renshaw, 1936) can be explained if the 1′:2′-C-C-bond of the phenyl group (see Fig. 1) is regarded as being equivalent spatially and electronically to the carbonyl group of acetylcholine. Comparison of the Newman projections in Figs. 4 and 5 shows that in both DMPP and phenyl choline ether this spatial equivalence is lost on di-*ortho*-methyl substitution. This is consistent with the low nicotinic stimulant potency of 1,1-dimethyl-4-(2,6-xylyl)piperazinium and of xylocholine (Clark & Jana, 1966). In contrast to this effect of di-*ortho* substitution, insertion of a single *ortho*-methyl substituent into DMPP marginally enhances the nicotinic stimulant potency. *Meta*- or *para*-methyl substituents, which do not affect the orientation of the phenyl group in DMPP, reduce the nicotinic stimulant activity, but not to the same extent observed on di-*ortho*-methyl substitution. This difference between the marginal rise in potency found on insertion of a single *ortho*-methyl substituent and the considerable fall in potency found on either *meta*- or *para*-methyl substitution suggests that the orientation of the aryl group in the *ortho*-tolyl compound is such that it fits the receptor more closely than does the phenyl group of DMPP. Although the benzene ring of DMPP and of its *meta*- or *para*-tolyl analogues can be readily rotated around the Ar-N bond to bring it into the same alignment as is present in the *ortho*-tolyl compound, the energy needed to do this must be provided at the expense of the drug-receptor binding energy. In the aryl choline ether series, where the benzene rings in the phenyl and *ortho*-tolyl compounds have the same orientation (Clark & Williams, 1967) (see Fig. 4b), the nicotinic stimulant activity of all three tolyl choline ethers is less than that of phenyl choline ether, the order of potency being *ortho*>*meta*>*para* (Hunt & Renshaw, 1936).

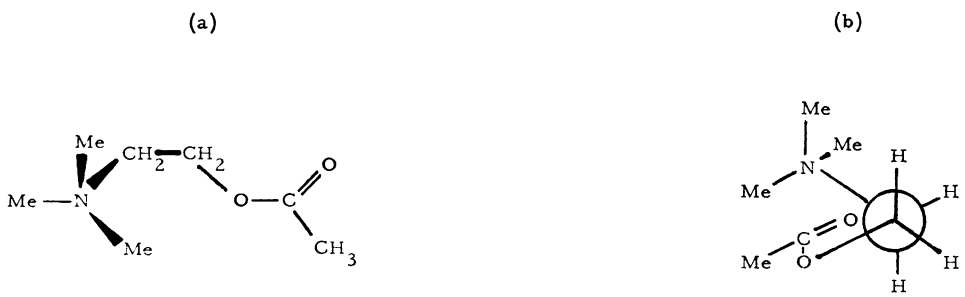


FIG. 5. Conformation of acetylcholine. In (a) the $CH_2CH_2OCO.CH_3$ moiety is approximately in the plane of the paper, with the $\overset{+}{N}Me_3$ group rising out of the plane at an angle of 75°. In (b) the circle is drawn as if looking along the choline C–C bond.

The striking quantitative parallelism between the reduction in activity found on insertion of *meta*-methyl, *para*-methyl or 3,5-dimethyl substituents into the phenyl ring of DMPP and that observed (Hey, 1952) when the same substituents are inserted into phenyl choline ether (see Table 3) provides further evidence that the two series of compounds interact with nicotinic receptors in a common conformation. The $-\overset{+}{N}CH_2CH_2N$-moiety in DMPP is restricted to the *gauche* conformation by being incorporated into the piperazine ring; this implies that the choline moiety in phenyl choline ether also interacts with the nicotinic receptor in the *gauche* rather than in the fully staggered conformation. This conclusion, although contrary to that reached recently by Clark & Smith (1971) from studies with some weakly active and inactive analogues of phenyl choline ether, is in agreement with Chothia's model (Chothia, 1970) for the interaction of nicotinic agonists with the acetylcholine receptor.

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