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STUDIES ON THE STRUCTURE-ACTION RELATIONSHIPS OF THE CHOLINE GROUP

BY

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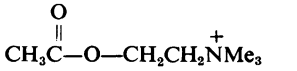
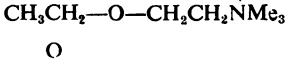
It is well known that the ester group contributes in some important but unknown way to the intense physiological activity of acetylcholine (I), and consequently the effects of changing this unit of structure are of considerable interest in any study of the structure-action relationships of the choline group. Among the simpler changes possible are : (i) replacement of the carbonyl (C=O) group by methylene as in choline ethyl ether (II) ; (ii) replacement of the linking O-atom of the ester group by methylene as in 4-keto-amyltrimethylammonium (III) ; and (iii) replacement of both the carbonyl group and the linking O-atom by methylene groups as in *n*-amyltrimethylammonium (IV). Compounds II and IV have been known for many years



(I)



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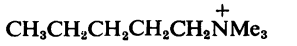
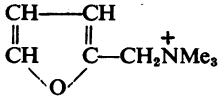


(IV)

and studied in some detail, but curiously enough 4-keto-amyltrimethylammonium (III) has only been studied recently, mainly on the isolated heart of *Venus mercenaria*, by Welsh and Taub (1951). We have prepared this ketone and tested it on a variety of preparations. There are, however, two other isomeric ketones, viz. those in which the keto-group occupies the 2- or 3-positions with respect to the N-atom, and since the position of the keto-group might well affect the type and intensity of pharmacological response we have compared all three isomeric ketones. We have also included in our studies a direct comparison of the three isomeric ethers : O-methylhomocholine, O-ethylcholine (II), and O-*n*-propylformocholine, since, although each has been studied separately, no direct comparison of them had previously been made.

Another feature of the acetylcholine structure which interested us was the length of the acetoxyethyl chain attached to the N-atom. Ing (1949) had suggested that in any homologous series of parasympathomimetic drugs of the general type $R\overset{+}{N}Me_3$, the most active member would be the one which contained a 5-atom chain (excluding

H-atoms) in the group R. Alles and Knoefel (1939) had noted the same regularity earlier.* A means of testing this " 5-atom rule " was provided by the discovery of the muscarine-like activity of furfuryltrimethylammonium iodide (" Furmethide ") by Fellows and Livingston (1940). Since the furan ring is planar and almost a regular pentagon (Beach, 1941), the " length " of the chain in furfuryltrimethylammonium (V) must approximate to that of a 4-atom acyclic chain (excluding H-atoms), but a near approach to an acyclic 5-atom chain would be found in



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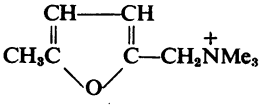


(VI)

5-methylfurfuryltrimethylammonium (VI). We have therefore prepared the iodide of this furan derivative and compared it with furfuryltrimethylammonium iodide.

Several other compounds, of less immediate interest, were included in our studies and will be mentioned below.

Methods

All the compounds prepared were tested by standard methods on the blood pressure of the cat anaesthetized with chloralose, on isolated rabbit auricles, on guinea-pig ileum, on the isolated perfused frog heart, and on the isolated frog's rectus abdominis. On the first four preparations estimates of equipotent molar ratios in terms of acetylcholine were obtained by matching the effects of varying doses with that of a fixed dose of acetylcholine. For the rectus abdominis, dose-response curves were plotted and the mean contractures produced by a fixed concentration, viz. 10^{-6}, were used to calculate equipotent molar ratios; this arbitrary method was used because most of the compounds, being stable to hydrolysis, gave steeper dose-response curves than acetylcholine did, and consequently no really satisfactory molar potencies could be calculated.

It will be obvious that the equipotent molar ratios derived by these methods (Table I) are only approximate, but it was thought that they were sufficiently illuminating to bring out the important and surprising effects of minor changes in structure, and it is hoped that the more interesting members of the various series tested will be subjected to more intensive study.

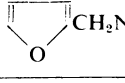
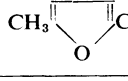
Results and Discussion

The results with most of the compounds tested are given in Table I as approximate equipotent molar ratios, i.e., as the ratio of the number of mols of the test substance producing a given effect to the number of mols of acetylcholine required to produce the same effect. Since the molecular weights of most of the compounds tested differ little from that of acetylcholine, the equipotent molar ratios differ little from the equipotent ratios by weight.

Most of the substances tested were not esters and therefore they must be immune to the hydrolytic action of cholinesterase, a circumstance which gives them an advantage in comparison with acetylcholine. In order to form some estimate of this advantage, representative members of the different series were compared with acetylcholine on rabbit auricles in the presence and in the absence of eserine. Serial Nos.

* The senior author (H.R.I.) regrets that the paper by Alles and Knoefel (1939) was unknown to him at the time that he put forward the " 5-atom rule."

TABLE I

	Serial No.	Name and formula	Approx. equipotent molar ratios (ACh = 1)				
			Cat's blood pressure	Rabbit auricles	Guinea-pig ileum	Frog heart	Frog rectus
Ketone series	1	4-Keto-amyltrimethylammonium iodide $CH_3CO.CH_2CH_2CH_2NMe_3\}I$	purely pressor	50	80	416	1.1
	2	3-Keto-amyltrimethylammonium iodide $CH_3CH_2CO.CH_2CH_2NMe_3\}I$	purely pressor	250	670	1,670	1.3
	3	2-Keto-amyltrimethylammonium iodide $CH_3CH_2CH_2CO.CH_2NMe_3\}I$	84 pressor after atropine	1,660	330	5,010	153
	4	3-Keto-2-methylamyltrimethyl-ammonium iodide $CH_3CH_2CO.CH(CH_3)CH_2NMe_3\}I$	4,500 depressor after atropine	4,700	584	20,000	700
	5	3-Ketobutyltrimethylammonium iodide $CH_3CO.CH_2CH_2NMe_3\}I$	150 pressor after atropine	70	167	230	0.7
Ether series	6	O-Methylhomocholine iodide $CH_3OCH_2CH_2CH_2NMe_3\}I$	39	44	98	294	23
	7	O-Ethylcholine iodide $CH_3CH_2OCH_2CH_2NMe_3\}I$	10	22	10	22	42
	8	O-*n*-Propylformocholine perchlorate $CH_3CH_2CH_2OCH_2NMe_3\}ClO_4$	49	39	61	392	27
	9	*n*-Amyltrimethylammonium iodide $CH_3CH_2CH_2CH_2CH_2NMe_3\}I$	66	44	8	325	13
Furfuryl series	10	Furfuryltrimethylammonium iodide 	10–30	16	12	126	505
	11	5-Methylfurfuryltrimethylammonium iodide 	1–3	1.2	0.34	15	7,000
	12	Acetoxyethyldimethylsulphonium picrate $CH_3CO.OCH_2CH_2SMe_2\}C_6H_2O_7N_3$	50	18	30	96	15

1, 3, 4, 5, 9, 11, and 12 (Table I) were tested in this way. Remarkably uniform results were obtained : compounds 1, 3, 5, 9, and 11 were all about one-tenth as active (in terms of acetylcholine = 1), when compared with acetylcholine in the presence of eserine, as when compared in the absence of eserine ; for compound 4 the factor was one-eighth and for compound 12 one-seventh. These figures should be remembered by the reader in scanning the results recorded in Table I.

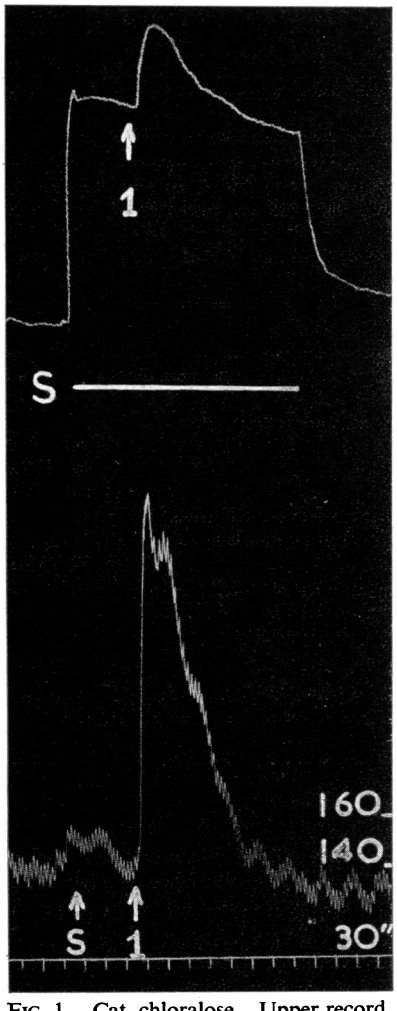


FIG. 1.—Cat, chloralose. Upper record, nictitating membrane. Lower record, arterial blood pressure. At S, cervical sympathetic stimulated (2 volt, 20/sec.) during period marked by horizontal line. At arrow labelled 1, 50 μg. 4-keto-amyl-compound injected. Note pure rise of blood pressure and increased contraction of the nictitating membrane. Time marker: 30 sec. (Experiment of Dr. Brenda Schofield.)

All the compounds listed in Table I were also tested as inhibitors of the cholinesterases of dog's caudate nucleus and horse serum. None of them produced appreciable inhibition of the hydrolysis of acetylcholine by either enzyme when present in a concentration of 10^{-3}M.

Ketone series

The outstanding feature of the three isomeric keto-amyl-compounds (Serial Nos. 1–3) was their nicotine-like activity ; this was particularly intense for the 4- and 3-keto-compounds, both of which produced only a *rise* of blood pressure in the cat ; this effect (see Fig. 1 for a typical result with the 4-keto-compound) was pronounced when they were administered in doses of 0.1 to 0.2 mg., the 4-keto-compound being somewhat more potent than its 3-keto-isomer. Both compounds also were approximately equipotent with acetylcholine in producing contracture of the frog's rectus abdominis. The 2-keto-amyl-compound had some depressor action on the cat's blood pressure in a dose of 0.2 mg. (57 μg./kg.) ; increasing the dose to 0.5 mg. (143 μg./kg.) led to a slightly greater fall, followed by a small rise of blood pressure. After atropine, doses of 0.5 mg. had no effect, but large doses (e.g. 50 mg.) produced a sharp and considerable rise of blood pressure (Fig. 2*b*). The 2-keto member had therefore a less pronounced nicotine-like action on blood pressure than its 4- and 3-keto-isomers ; it was also considerably weaker than its isomers on the rectus abdominis (Table I). These results are in general agreement with those of Welsh and Taub (1951), who found that the equipotent

molar ratios of the three isomers on *Venus mercenaria* heart were : 4-keto : 3-keto : 2-keto = 12 : 160 : 620 (acetylcholine = 1). The toxicities (LD50 values for mice) followed a similar order (Welsh and Taub, 1951).

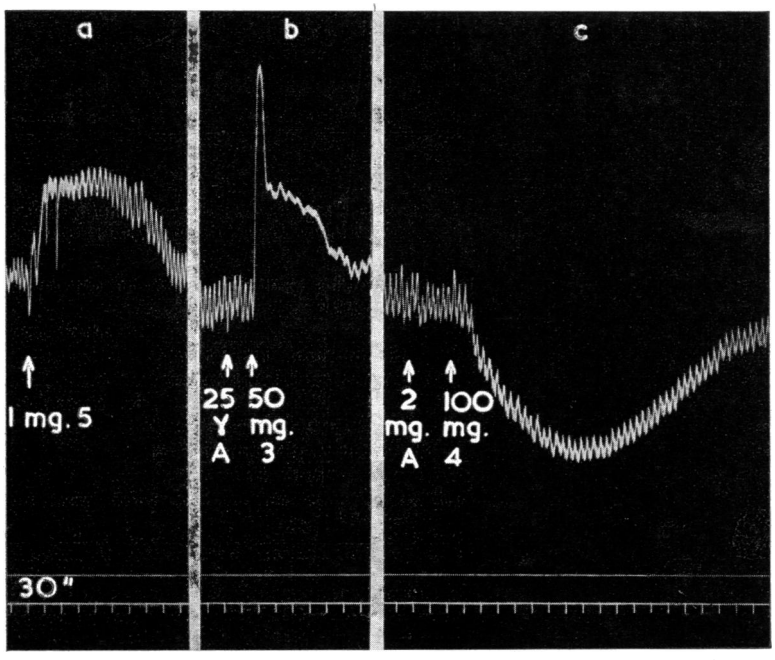


FIG. 2.—Cat, 3.5 kg., chloralose. Blood pressure record. (*a*) At arrow 1 mg. 3-ketobutyl-compound (No. 5); note slight fall of B.P., followed by prolonged rise. (*b*) At first arrow, 25 μg. atropine, and at second arrow, 50 mg. 2-keto-amyl-compound (No. 3). (*c*) At first arrow, 2 mg. atropine, and at second arrow, 100 mg. 3-keto-2-methylamyl-compound (No. 4). Time marker: 30 sec. (Experiment of Professor J. H. Burn.)

The nicotine-like activity of the 4-keto-amyl-compound was also demonstrated by its ability, when injected intravenously or painted directly on the superior cervical ganglion, to contract the cat's nictitating membrane (see Fig. 1).

All three keto-amyl-compounds produced muscarine-like effects on rabbit auricles, guinea-pig ileum, and the frog heart, and the 4-keto-compound was consistently the most potent of the three. At first sight it may seem curious that the 4-keto-compound, with its intense nicotine-like properties, should be considerably more potent on these isolated tissues than the 2-keto-compound, which alone of the three keto-amyl-compounds had some muscarine-like action on blood pressure. However, the seemingly muscarine-like effects of the 4-keto-compound appear to be largely due to a stimulant nicotine-like action on ganglion cells ; thus, whereas hexamethonium does not reduce the response of guinea-pig ileum to acetylcholine, it did reduce that to the 4-keto-compound to about 30 per cent of its original value (mean of six estimates, range 20–40 per cent). A typical result is shown in Fig. 3. Similarly the response of isolated rabbit auricles to the 4-keto-compound was reduced to about the same extent by the presence of hexamethonium in the bath, whereas that to

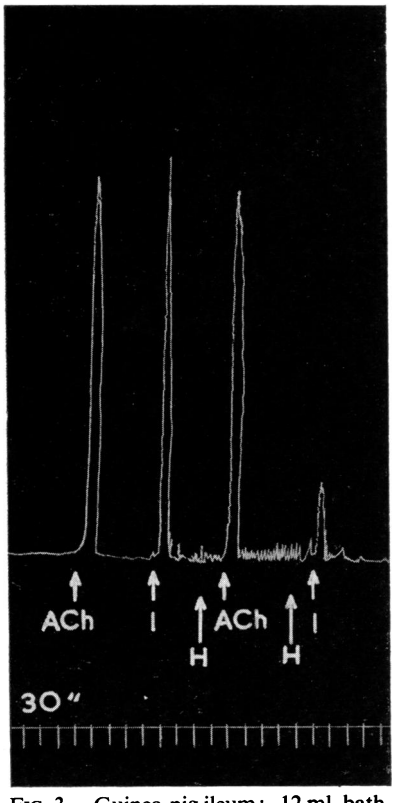


FIG. 3.—Guinea-pig ileum; 12 ml. bath. Test doses: ACh = 1 μg. acetylcholine; 1 = 100 μg. 4-keto-amyl-compound; H = 1 mg. hexamethonium iodide immediately preceding test dose and left in bath. Note the reduction of the response to the 4-keto-amyl-compound after hexamethonium. (Experiment of Dr. S. R. Kottegoda.)

acetylcholine was unchanged (Fig. 4); the residual muscarine-like action of the 4-keto-compound could then be abolished by atropine. It seems therefore that the effects of the 4-keto-compound on these isolated tissues are not purely muscarine-like, but are largely due to its outstanding nicotine-like properties. A more detailed analysis of the apparently muscarinic effects of this compound and of the other members of the ketone series is being carried out in this laboratory.

It is well known that derivatives of β-methylcholine usually have scarcely any nicotine-like properties and consequently it is of interest that the introduction of a methyl group into the β- or 2-position of the 3-keto-amyl-compound leads to a large decrease in nicotine-like activities. Whereas the 3-keto-amyl-compound had a purely pressor effect in the cat, the 3-keto-2-methylamyl-compound (Serial No. 4) had a small depressor effect in doses of 1 to 2 mg. (0.6 to 1.2 mg./kg.); after atropine, which abolished the depressor effect of small doses, no rise of blood pressure was observed; indeed, after a full dose of atropine (2 mg. in a 3.5 kg. cat) a large dose of the 3-keto-2-methylamyl-compound (100 mg.) produced a slow but considerable fall of blood pressure (Fig. 2*c*). The absence of a demonstrable nicotinic action on blood pressure is perhaps even more striking in this 3-keto-2-methylamyl-compound than it is in acetyl-β-methylcholine (Simonart, 1932), since the former is a β-methyl derivative of an intensely nicotinic substance. The introduction of the β-methyl group appears to have largely blocked the characteristic properties of the 3-keto-amyl-compound without conferring any fresh properties upon the new compound; thus all its actions, except that on guinea-pig ileum, were considerably weaker than those of the 3-keto-amyl-compound (see Table I); on the rectus abdominis it was nearly seven hundred times weaker than the 3-keto-amyl-compound.

The 3-ketobutyl-compound (Serial No. 5) had a slight depressor effect upon the cat's blood pressure, less than that of the 2-keto-amyl-compound; in doses of about 0.6 mg. per kg. it produced a slight fall, followed by a prolonged rise of blood pressure (Fig. 2*a*); after atropine, similar doses produced a pure rise of blood pressure. In all other tests it was more active than its higher homologue, the 3-keto-amyl-compound; it might be thought, therefore, that this 3-ketobutyl-compound, with its 4-atom chain, provides an exception to the " 5-atom rule " mentioned above;

however, this rule, as originally postulated (Ing, 1949), applied only to parasympathomimetic (i.e. muscarinic) properties, and only further investigation can reveal whether the effects of this compound on isolated tissues are purely muscarinic or, as seems more probable, like those of the 4-keto-amyl-compound, largely nicotinic.

The occurrence of almost purely nicotine-like properties in the members of the ketone series (except in Serial No. 4) is remarkable, and could scarcely have been predicted from a consideration of the structure of acetylcholine. The contrast between the 4-keto-amyl-compound and the predominantly muscarinic ethyl ether

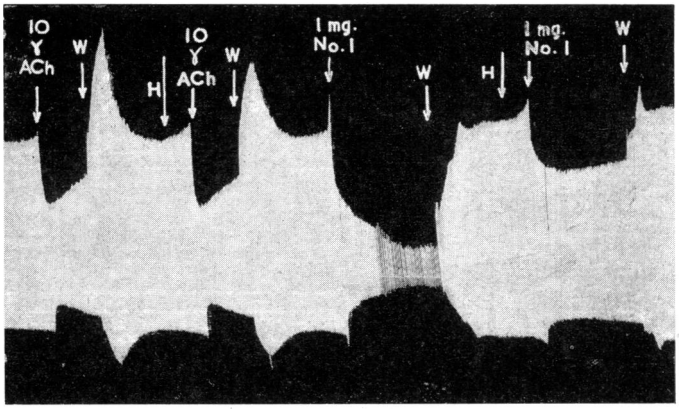
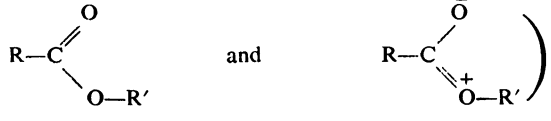


FIG. 4.—Rabbit auricles; 75 ml. bath. W = bath washed out. H = 10 mg. hexamethonium iodide added to bath. Note that hexamethonium did not reduce the effect of 10 μg. acetylcholine (ACh), but it did reduce that of 1 mg. 4-keto-amyl-compound (No. 1).

of choline (Dale, 1914) is particularly striking in view of the close structural relationship which they both bear to acetylcholine (see formulae I, II and III on p. 103). It might be concluded that the dual nature of the physiological effects, which acetylcholine is well known to produce, was in some way linked to the presence in its structure of both carbonyl and ether groups. It would, however, be rash to draw this conclusion because the ester group is a resonating system (usually assumed to be a hybrid of the structures :



and its properties cannot be regarded as a summation of those of a ketone and an ether.

Ether series

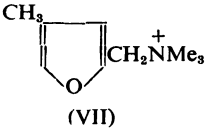
The three ethers (Serial Nos. 6, 7, and 8) have all been studied previously, O-methylhomocholine by Simonart (1928), O-ethylcholine by Dale (1914) and Simonart (1932), and O-propylformocholine by Ewins (1914) and Hunt and Renshaw (1932b). The results in Table I show clearly, what might have been anticipated, that O-ethylcholine is the most potent in muscarine-like activities. The potencies of O-methylhomocholine and O-propylformocholine are remarkably alike

on all five preparations, and it is noteworthy that both ethers are nearly twice as active as O-ethylcholine on the rectus abdominis. Dale (1914) was the first to draw attention to the fact that the muscarine-like properties of O-ethylcholine were more prominent than its nicotine-like properties ; this appears not to be true of the other two isomeric ethers, which on all preparations except guinea-pig ileum resemble amyltrimethylammonium (Serial No. 9) more closely than they do O-ethylcholine.

The furfuryl series

The chief point of interest in this series was that the 5-methylfurfuryl-compound (Serial No. 11) was considerably more active in its muscarine-like effects than furmethide (Serial No. 10) ; thus it was about ten times more active on the cat's blood pressure, and even more so on some isolated tissues (see Table I). Both compounds appear to be primarily muscarine-like drugs, but they do display some nicotine-like effects, particularly on the superior cervical ganglion and on the denervated gastrocnemius of the cat. The 5-methylfurfuryl-compound produced a considerable contraction of the cat's nictitating membrane when injected intra-arterially in a dose of 0.1 mg.; it was also effective when a 1 mg./ml. solution was painted on the superior cervical ganglion. In the perfused superior cervical ganglion of the cat it was about one-quarter as effective as acetylcholine or 2268F (acetal of 3 : 2-dihydroxypropyltrimethylammonium iodide). On the denervated gastrocnemius of the cat the 5-methylfurfuryl-compound was again about one-quarter as active as acetylcholine and 2268F in eliciting a muscle twitch ; the furfuryl-compound (Serial No. 10) was less active, being about one-twentieth as active as acetylcholine. On the other hand, neither the 5-methylfurfuryl-compound nor furmethide produced a rise of blood pressure in the cat after atropine, even in high doses.* Both compounds were also considerably weaker than acetylcholine in producing contracture of the frog's rectus abdominis (Table I). The 5-methylfurfuryl-compound has also been shown by Grewal (1951) to be some 13.5 times as powerful a miotic in the mouse as furmethide, and to be as potent in this respect as 2268F. It is also a more potent drug in constricting the bladder than carbachol, but at the same time has a lower acute toxicity (Lourie, 1952).

The synthesis of the 5-methylfurfuryl-compound was undertaken as a means of testing the " 5-atom rule." The fact that the compound is considerably more active in its muscarinic effects than furmethide is in agreement with the rule. Two other isomeric methylfurfuryl compounds are, however, possible, namely the 4-methylfurfuryl-compound (VII) and the 2-methylfurfuryl-compound (VIII), of which the former (VII) may be regarded as having the equivalent of a 5-atom acyclic chain.



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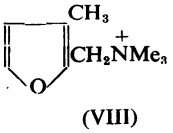


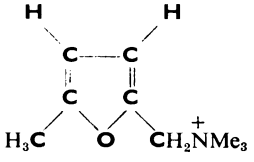
(VIII)

Until these new isomers have been prepared and tested it would be unwise to draw firm conclusions from the present results. The higher activity of the 5-methylfurfuryl-

* We are indebted to Dr. E. M. Lourie for these results on the nicotinic properties of the 5-methylfurfuryl-compound. His more detailed investigations on this compound will be published separately.

compound might conceivably be due to the effect of the methyl group on the furan ring, which would be to reduce somewhat the aromatic character and stability of the ring.

The 5-atom rule had a purely empirical basis, but it involves the notion that spatial factors play an important part in determining the *intensity* of muscarinic effects. Consequently it is worth drawing attention to the spatial characteristics of these furan derivatives. In both furmethide and the 5-methylfurfuryl-compound the O-atom and all the C-atoms, except those of the N-methyl groups, will lie in the same plane ; thus in the 5-methylfurfuryltrimethylammonium cation all the atoms printed in bold type in formula (IX) will be coplanar. In acetylcholine too, owing



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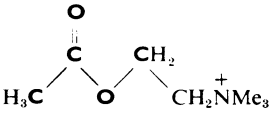
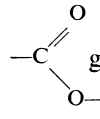
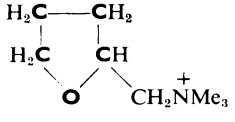


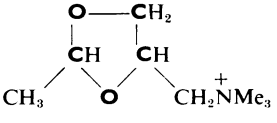
(X)

to resonance in the  group, flattening of the ester group will occur and the

mean positions of all the atoms printed in bold type in formula (X) will be coplanar. It is possible that the substantial flatness of the cations of the furfuryl- and 5-methylfurfuryl-compounds may contribute to their effectiveness at parasympathetic nerve endings. In this connexion it is interesting that tetrahydrofurfuryltrimethylammonium iodide, in which only the atoms composing the ring will be coplanar (cf. formula XI), has about one-tenth of the parasympathomimetic activity of furmethide (Fellows and Livingston, 1940). At the same time it must be remembered



(XI)



(XII)

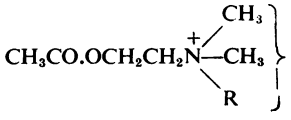
that 2268F (XII ; coplanar atoms in bold type) is one of the most potent synthetic parasympathomimetic compounds known.

5-Hydroxymethylfurfuryltrimethylammonium iodide (in which the 5-methyl group of formula VI is replaced by $HOCH_2-$) was also prepared and tested, but its activities on the five preparations used were too weak to make its detailed study of any interest.

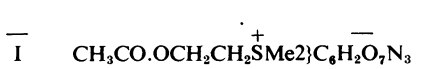
Miscellaneous compounds

Holton and Ing (1949) reported the relatively high activity of acetoxyethyldimethylethylammonium iodide (XIII ; $R = C_2H_5$) ; this compound, in which one of the N-methyl groups of acetylcholine is replaced by ethyl, was between a half and a

fifth as active as acetylcholine. In discussing the theoretical implications of this result, Holton and Ing pointed out that, although compounds of type (XIII) contain the two N-methyl groups, which they regarded as essential for high activity in compounds of this type, it was impossible to predict how the nature of the group R would influence activity, and they recalled the results of Hunt and Renshaw (1929) with the compound in which R was benzyl and those of Hunt and Taveau (1911) with the compound in which R was *iso*amyl ; both these compounds had activities comparable with that of choline. We have since prepared the compounds in which the R group



(XIII)

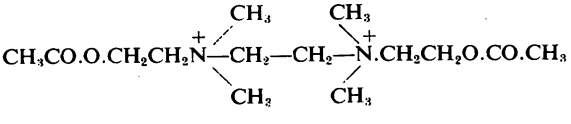


(XIV)

of formula (XIII) is *n*-propyl and *n*-butyl. Both these compounds proved to be exceptionally weak on all five preparations used and certainly not more active than choline itself. The conclusion seems to be inevitable that groups larger than ethyl in structure (XIII) interfere with the attachment of the cation to the excitable structure, which normally responds to the impact of acetylcholine, to such an extent as to overshadow the effect of the presence of two N-methyl groups in the cation.

In the same paper Holton and Ing discussed the results of Welch and Roepke (1935) with the phosphorus and arsenic analogues of acetylcholine. At that time the sulphur analogue had not been tested, although Hunt and Renshaw (1925, 1932a) had studied the sulphur analogues of choline phenyl ether and acetylformocholine. We have now prepared and tested acetoxyethyldimethylsulphonium picrate (XIV) and our results are included in Table I. If our results are compared with those of Welch and Roepke (see Holton and Ing, 1949) it will be seen that acetylsulphocholine is consistently less active than acetylphosphocholine and not strikingly more active than acetylarsenocholine. Two factors may contribute to this result : the presence of only two, instead of three, methyl groups on the S-atom and the size of the latter. The S—C bond length (1.82A) differs little from that of the P—C bond length (1.87A) (see Pauling, 1939), but some uncertainty exists about the angle between the bonds of tricovalent sulphur, so that a reliable estimate of the carbon to carbon distance between two methyl groups in a sulphonium compound cannot be given.

One other compound was prepared, namely N : N′-bis-acetoxyethyl-N : N′-dimethylethylene diamine dimethochloride, the relationship of which to acetyl-



(XV)

choline is made plain in formula (XV). This substance was, however, extremely feeble in its action on all five preparations and certainly less active than choline ; it was also extremely easily hydrolysed in aqueous solution.

CHEMICAL SECTION

All melting points uncorrected. Analyses by Drs. Weiler and Strauss.

Ketone series

The three keto-amyltrimethylammonium iodides were prepared from the corresponding chloroketones by reaction with aqueous dimethylamine and subsequent treatment of the dimethylaminoketones with methyl iodide in ether at a low temperature. β-Dimethylaminoethyl ethyl ketone, being a Mannich base, reacts violently with methyl iodide, even at 0° C., with the formation of tetramethylammonium iodide, but if methyl iodide is added to an ethereal solution of the base, cooled in a solid CO_2-acetone mixture, and the solution is allowed to warm up slowly as the CO_2 evaporates nearly theoretical yields of the quaternary salt are obtained. γ-Dimethylaminopropyl methyl ketone and dimethylaminomethyl propyl ketone react smoothly with methyl iodide in ethereal solution at −10° to 0° C.

The chloroketones.—γ-Chloropropyl methyl ketone was prepared by passing hydrogen chloride into an acetic acid solution of acetobutyrolactone at 85–90° C., the lactone being obtained from ethyl acetoacetate and ethylene oxide in aqueous alcoholic caustic soda solution.

β-Chloroethyl ethyl ketone was obtained by bubbling ethylene into a violently-stirred solution of propionyl chloride and aluminium chloride in nitromethane at 0° C. (see Cardwell and McQuillin, 1949).

Chloromethyl *n*-propyl ketone was prepared by the Arndt-Eistert synthesis from propionyl chloride and diazomethane.

4-Keto-amyltrimethylammonium iodide crystallized from acetone-ethanol, m.p. 100–102° C.; hygroscopic. Found: C, 35.75; H, 6.65. Calc. for $C_8H_{18}ONI$: C, 35.45; H, 6.65%. Welsh and Taub (1951) record m.p. 87–90° C.

3-Keto-amyltrimethylammonium iodide crystallized from ethanol, m.p. 146° C. Found: C, 35.3; H, 6.50. Calc. for $C_8H_{18}ONI$: C, 35.45; H, 6.65%. Welsh and Taub (1951) record m.p. 142–144° C.

2-Keto-amyltrimethylammonium iodide crystallized from ethanol, m.p. 154° C. Found: C, 35.45; H, 6.58. Calc. for $C_8H_{18}ONI$: C, 35.45; H, 6.65%. Welsh and Taub (1951) record m.p. 148–151° C.

3-Keto-2-methylamyltrimethylammonium iodide.—5-Dimethylamino-3-keto-4-methylpentane was prepared by a Mannich condensation of diethyl ketone, dimethylamine hydrochloride, and paraformaldehyde in ethanol; its methiodide was prepared by direct reaction with methyl iodide in a flask cooled by a solid CO_2-acetone mixture. The iodide crystallized from ethanol in needles, m.p. 158° C. Found: C, 37.95; H, 6.98. $C_9H_{20}ONI$ requires C, 37.90; H, 7.13%.

3-Ketobutyltrimethylammonium iodide.—Methyl vinyl ketone (1 mol.) and anhydrous dimethylamine (2 mol.) were mixed in a bottle cooled in a CO_2-acetone mixture. The bottle was stoppered (wired on) and left at room temperature for 48 hr. β-Dimethylaminoethyl methyl ketone was isolated by fractional distillation and converted into its methiodide as described for other Mannich bases; it crystallized from ethanol in prisms, m.p. 115° C. Found: C, 31.80; H, 6.36. $C_7H_{16}ONI, \frac{1}{2}H_2O$ requires C, 31.80; H, 6.39%.

Ether series

O-Methylhomocholine iodide.—The monomethyl ether of trimethylene glycol, prepared by the method of Noyes (1897), was added slowly to a chloroform solution of phosphorus tribromide, prepared by running the theoretical amount of bromine into a

suspension of red phosphorus in chloroform under reflux. γ-Methoxypropyl bromide, so obtained, was shaken with excess aqueous dimethylamine at room temperature ; dimethylaminopropyl methyl ether, isolated by fractional distillation, reacted rapidly with methyl iodide in ether solution to give the quaternary iodide ; prisms from ethanol, m.p. 221° C. Found : C, 32.60 ; H, 6.55. $C_7H_{18}ONI$ requires C, 32.44 ; H, 6.95%.

O-Ethylcholine iodide.—Dimethylaminoethyl ethyl ether, prepared from ethoxyethyl bromide and aqueous dimethylamine at about 80°, was converted into its methiodide in ether. The salt crystallized from acetone-ethyl acetate, m.p. 159°. Found : C, 32.25 ; H, 6.76. Calc. for $C_7H_{18}ONI$ requires C, 32.44 ; H, 6.95%. Knorr (1904) records m.p. 160–165° C.

The corresponding *β-ethoxyethyldimethylethylammonium iodide* was obtained by allowing a solution of dimethylaminoethyl ethyl ether and ethyl iodide to stand for several days. It was crystallized by carefully pouring dry ether on to the surface of its solution in acetone-ethyl acetate and allowing the solution to stand undisturbed for a few days ; needles, m.p. 58°. Found : C, 35.0 ; H, 7.23. $C_8H_{20}ONI$ requires C, 35.18 ; H, 7.33%.

O-Propylformocholine perchlorate.—Chloromethyl *n*-propyl ether, prepared from *n*-propanol, formaldehyde, and hydrogen chloride (Henze, Duff, Matthews, Melton, and Forman, 1942) was treated with anhydrous trimethylamine in dry ether at a low temperature (CO_2-acetone freezing mixture) ; reaction was rapid and the solid iodide separated as a very hygroscopic powder. Its concentrated solution in dry ethanol was treated with the theoretical amount of 60 per cent perchloric acid at –10° C. and the perchlorate which separated recrystallized from ethanol ; felted needles, m.p. 97°–99° C. Found : C, 36.42 ; H, 7.96. $C_7H_{18}O_5NCl$ requires C, 36.28 ; H, 7.78%.

Furfuryl series

The furfuryl compounds were prepared by two different methods.

(i) 2-Methylfuran and 2 : 5-dimethylfuran were converted into the respective furfuryl bromides by means of N-bromosuccinimide in dry carbon tetrachloride (Buu-Hoi and Lecocq, 1946). No attempt was made to isolate these highly reactive bromides ; their solutions in carbon tetrachloride were cooled in a CO_2-acetone freezing mixture and treated with the theoretical amount of anhydrous trimethylamine, when an immediate reaction ensued. The quaternary bromides were converted to picrates by treating their aqueous solutions with excess calcium picrate solution, and the picrates recrystallized from water.

(ii) Furfuryldimethylamine was prepared from furfural as described by Moore (1949) and 5-methylfurfuryldimethylamine from 2-methylfuran (sylvan) by the method of Holdren and Hixon (1946), except that paraformaldehyde was used instead of formalin and the reaction was performed in alcoholic solution (yield 60%). 5-Hydroxymethylfurfuryldimethylamine was prepared from furfurol in a similar way. The tertiary bases were converted into methiodides in the usual way in ether solution.

Furfuryltrimethylammonium picrate, yellow needles from water, m.p. 161.5°. Found : C, 45.70 ; H, 4.0. $C_{14}H_{16}O_8N_4$ requires C, 45.67 ; H, 4.35%. The iodide crystallized from ethanol-ethyl acetate, m.p. 118–120° (Holdren and Hixon record 118–119°).

5-Methylfurfuryltrimethylammonium picrate, yellow leaflets from water, m.p. 146°. Found : C, 47.35 ; H, 4.70. $C_{15}H_{18}O_8N_4$ requires C, 47.15 ; H, 4.71%. The *iodide* formed large prisms from ethanol, m.p. 156°. Found : C, 38.90 ; H, 5.56. $C_9H_{16}ONI$ requires C, 38.45 ; H, 5.64%.

5-Hydroxymethylfurfuryltrimethylammonium iodide, crystallized from ethanol in needles, m.p. 128°. Found : C, 36.45 ; H, 5.61. $C_9H_{16}O_2NI$ requires C, 36.35 ; H, 5.39%.

Miscellaneous compounds

Acetoxyethyldimethylpropylammonium iodide was prepared by boiling an acetone solution of dimethylaminoethyl acetate and *n*-propyl iodide. The product was precipitated with dry ether and crystallized from acetone ; m.p. 107–108°. Found : C, 36.3 ; H, 6.63. $C_9H_{20}O_2NI$ requires C, 35.9 ; H, 6.64%.

Acetoxyethyldimethylbutylammonium iodide was prepared similarly from dimethylaminoethyl acetate and *n*-butyl iodide in methyl ethyl ketone solution. It was crystallized from acetone ; m.p. 88–89°. Found : C, 38.5 ; H, 6.92. $C_{10}H_{22}O_2NI$ requires C, 38.1 ; H. 6.98%.

Acetoxyethyldimethylsulphonium picrate.—S-Methylmercaptoethyl acetate reacted slowly with methyl iodide at room temperature to form a gummy salt, which was dissolved in water and treated with calcium picrate solution. The picrate crystallized from water in long needles, m.p. 135°. Found : C, 38.40 ; H, 4.06. $C_6H_{13}O_2S$, $C_6H_2O_7N_3$ requires C, 38.25 ; H, 3.98%.

N : N'-bis-acetoxyethyl-N : N'-dimethylethylene diamine dimethiodide.—N : N : N' : N'-Tetramethylethylene diamine and β-iodoethylacetate were allowed to react together in dry ethanol at room temperature until the smell of the amine was imperceptible. The solution was evaporated to dryness ; the solid residue crystallized from ethanol in silky needles. Found : C, 31.15 ; H, 5.53. $C_{14}H_{30}O_4N_2I_2$ requires C, 30.90 ; H, 5.52%.

The dimethochloride was prepared by boiling dimethylaminoethanol and ethylene dichloride in ethanol for four hours and acetylating the dry salt, obtained on evaporation of the reaction solution, with boiling acetic anhydride. After removal of excess acetic anhydride *in vacuo* the solid dimethochloride was crystallized from acetone-ethanol ; it formed hygroscopic needles, m.p. 170°. Found : C, 45.8 ; H, 8.42. $C_{14}H_{30}O_4N_2Cl_2$ requires C, 46.5 ; H, 8.32%.

SUMMARY

Several groups of substances, all related in structure to acetylcholine, have been synthesized and tested on the following five preparations : cat blood pressure, rabbit auricles, guinea-pig ileum, frog heart, and frog rectus abdominis.

Ketone series.—The three isomeric keto-amyltrimethylammonium iodides are primarily nicotine-like in their actions, the order of activity being 4-keto> 3-keto>2-keto. The apparently muscarinic effects of the 4-keto-amyl-compound on rabbit auricles and guinea-pig ileum are largely due to stimulation of ganglion cells since they are considerably reduced by hexamethonium iodide. 3-Ketobutyltrimethylammonium iodide is also primarily nicotine-like, but 3-keto-2-methylamyltrimethylammonium iodide lacks the pronounced nicotinic properties of the 3-keto-amyl-compound.

Ether series.—Direct comparison shows that O-ethylcholine is a more potent muscarinic substance than either of its isomers, O-methylhomocholine and O-*n*-propylformocholine. The activities of the two latter substances are remarkably alike and resemble those of *n*-amyltrimethylammonium more closely than those of choline ethyl ether.

Furfuryl series.—In agreement with the "5-atom rule," 5-methylfurfuryltrimethylammonium iodide is a more potent muscarinic substance than its lower homologue, furfuryltrimethylammonium iodide.

Other compounds.—Unlike acetoxyethyldimethylethylammonium iodide, its homologues, acetoxyethyldimethyl-*n*-propyl- and acetoxyethyldimethyl-*n*-butylammonium iodides, have only feeble activities, not greater than those of choline.

The activities of the sulphur analogue of acetylcholine (acetoxyethyldimethylsulphonium) are consistently less than those recorded in the literature for acetylphosphocholine.

We should like to acknowledge our good fortune in receiving indispensable help in the testing of our compounds from several members of this Department. We wish in particular to thank Professor J. H. Burn and Dr. E. M. Lourie, who undertook experiments in order to settle particular points. Professor Burn also gave us invaluable help in the design and execution of our routine tests. We wish also to thank Dr. Brenda Schofield and Dr. S. R. Kottegoda for allowing us to quote the results of individual experiments. We are indebted to Professor R. M. Hixon, of Iowa State College, for a generous gift of 2-methylfuran, prepared by Dr. F. Schultz. A grant from the Medical Research Council to the senior author enabled one of us (D.P.H.T.W.) to undertake the synthesis of several of the compounds described herein.

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