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THE REACTION OF ACETYLCHOLINESTERASE WITH METHANESULFONYL ESTERS OF QUATERNARY QUINOLINIUM COMPOUNDS*

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Abstract—The methanesulfonyl esters of quaternary and diquaternary quinolinium compounds were synthesized and studied as inhibitors of purified eel acetylcholinesterase. Some derivatives produced only reversible inhibition of the enzyme; the values of their binding constants were measured. For those compounds producing irreversible (acid-transferring) inhibition, the values of the binding constants, first-order and second-order rate constants were measured. It was shown that the values of the second-order rate constants cannot be correlated with the pK_a value of the leaving group (unlike the previously studied diethylphosphoryl quinolinium compounds). The values of the binding constants for the reaction of the quaternary hydroxyl analogs with the enzyme were compared with the values measured for the respective methanesulfonyl compounds. The data indicate that, depending on position, the methanesulfonyl group has little effect on or is detrimental to non-covalent binding between enzyme and inhibitor. The potency of these compounds is generally less than their respective dimethylcarbamyl and diethylphosphoryl analogues. However, several decamethylene bridged diquaternary compounds are the most potent of the methanesulfonate anticholinesterase agents.

ACETYLCHOLINESTERASE (EC 3.1.1.7) is irreversibly inhibited by certain esters of methanesulfonic acid, e.g. methanesulfonyl fluoride, 1-methyl-3-hydroxypyridinium iodide methanesulfonate.[1,2] The mechanism has been shown to be similar to the reaction of this enzyme with certain organophosphate and carbamate compounds.[3] These three groups of compounds are often called acid-transferring inhibitors, indicating that the mechanism of inhibition is similar and involves the formation of a covalent enzyme derivative by the transfer of the inhibitor's acid group (sulfonyl, carbamyl, phosphoryl) to the enzyme's active site. Recently the tertiary, mono and diquaternary diethylphosphoryl and dimethylcarbamyl derivatives of the quinolinols and isoquinolinols were synthesized and studied.[4–6] Many of these compounds are potent inhibitors of acetylcholinesterase. The acetoxyquinolinium agents are substrates of this enzyme.[7] In a detailed study of the kinetics of these new diethylphosphoryl compounds, their potency as inhibitors was found to correlate well with the pK_a value of the leaving group.[5] The latter term applies to the portion of the inhibitor molecule that is not covalently bound to the active site of the enzyme and leaves as the first product of the hydrolytic reaction.

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The anticholinesterase activity of the dimethylcarbamyl analogues of the diethylphosphoryl compounds was not found to correlate with the pK_a values of the leaving groups. The structure of the leaving group (i.e. its molecular complementarity) was the more important determinant of carbamate activity.[6] These data were supported by the finding that the values of the binding constants for the reaction of the undissociated forms of the quaternary quinolinols qualitatively correlate with the second-order rate constant values for the inhibition of the enzyme by the respective dimethylcarbamyl analogues.[8]

To complete this study, the mono- and diquaternary methanesulfonyl derivatives of the quinolinols and isoquinolinols were prepared. In this paper we report the synthesis of these inhibitors and the values of the more important kinetic constants for their reaction with highly purified acetylcholinesterase. Some of these new compounds are acid-transferring agents and others are simple reversible inhibitors. For the acid-transferring compounds, the binding constants and rate constants were evaluated; for the reversible inhibitors, the binding constants were measured. The data indicate that these new derivatives are generally less potent than their related organophosphate and carbamate compounds. Also, these methanesulfonyl esters show marked changes in activity secondary to the position of the methanesulfonyl function on the quinoline ring. No quantitative correlation was found between the second-order rate constant values and the pK_a value of the leaving group (unlike the diethylphosphoryl analogues) or between these rate constant values and the binding constants for the undissociated species of the quinolinols (unlike the dimethylcarbamyl derivatives). It was found that the methanesulfonyl function does not increase noncovalent binding to the enzyme. The diquaternary derivatives with the $-CH_2-O-CH_2-$ bridge between nitrogens are not more potent than their monoquaternary analogues. However, the length of the bridge is apparently important, since the two compounds with the $-(CH_2)_{10}-$ bridge were found to be considerably more active than their dimethylene ether bridged analogues. These decamethylene compounds represent the most active methanesulfonyl anticholinesterase agents so far described.

METHODS

Synthesis

Methanesulfonyl quinolinols. Quinolinols were dissolved in pyridine (100 ml for each 0·1 mole) and methanesulfonylchloride (0·14 mole) was added. After stirring at room temperature for 15 min, the mixture was heated on a steam bath for 10–20 min to complete the reaction. The cooled solution was distilled in a vacuum to eliminate pyridine. Water was added to the residue and the pH of the mixture was adjusted to 8 with dilute NaOH and then extracted with benzene. After washing the benzene extract with H_2O, it was dried and decolorized. The crude residue (yields 80–85 per cent) was sufficiently pure to be used for quaternization. The melting points were taken on compounds purified by recrystallization from ether or benzene/petroleum ether.

Quaternization. The *N*-methyl quaternary salts were prepared in the usual manner by heating the methanesulfonyl esters (dissolved in acetone) with an excess of methyl iodide on a steam bath for 30–60 min. The product was collected and recrystallized from methanol or ethanol. Yields ranged from 25 to 80 per cent.

Diquaternary compounds containing an N,N′-*dimethylene ether bridge. Bis* (chloroethyl) ether was transformed to the iodo-derivative with excess sodium iodide in acetone. Sodium chloride was filtered off. The methanesulfonyl quinolinol in acetone was added to the di-iodo ether and the mixture was left at room temperature for 2–3 hr. The precipitated product was collected and recrystallized from methanol. Yield ranged from 20 to 80 per cent.

Diquaternary compounds containing an N,N′-*decamethylene bridge.* Methanesulfonyl quinolinols with 1,10 dibromodecane in dimethylformamide were heated on a steam bath for 4 hr. The quaternary derivatives were precipitated with ether. As purification of the bromides was difficult, they were dissolved in hot water or aqueous methanol and transformed into their picrates by addition of an aqueous solution of sodium picrate. The picrate compounds were recrystallized from a mixture of methanol and acetone. Yields were low, about 5–15 per cent. Elemental analysis data for all quaternary compounds appear in Table 1.

Enzyme. Acetylcholinesterase (EC 3.1.1.7) was the same preparation from *Electrophorus electricus* used in the previous related studies.[5,6,8,9] The specific activity of this preparation was 90 m-moles acetylcholine hydrolyzed/ml/hr when measured by automatic titration with a pH-stat. The medium was 0·1 M NaCl, 0·02 M $MgCl_2$, 0·005% gelatin, 1×10^{-5} M EDTA, 0·001 M acetylcholine bromide at pH 7 and 25°. A value of $1{\cdot}1 \times 10^{-4}$ M was measured for K_m.

Assay techniques. Enzyme activity was measured as the decrease in the initial concentration of $1{\cdot}8 \times 10^{-3}$ M acetylcholine bromide. The hydroxamic acid method was used to measure the acetylcholine concentration in a medium of 0·02 M sodium phosphate buffer, 0·1 M NaCl, 0·02 M $MgCl_2$, 0·005% gelatin, 1×10^{-5} M EDTA at pH 7·25 and 25°.[10]

Inhibition

For those compounds producing irreversible (acid-transferring) inhibition, the reaction was initiated by the addition at zero time of appropriate amounts of inhibitor to a solution of enzyme in the medium and under the conditions described under Assay. The rate of progressive inhibition was followed by sampling aliquots of the reaction mixture at 7–20 time intervals from time zero. The relative amount of enzyme activity in each aliquot was assayed in the presence of acetylcholine for 2 min as described. From 5 to 14 inhibitor concentrations were tested for each compound.

For those compounds which did not produce acid-transferring inhibition, the degree of reversible anticholinesterase activity was assessed as previously described.[8] The technique involves measuring the amount of enzyme activity at increasing concentrations of substrate. A pH-stat apparatus was used in this study, the usual constant temperature and CO_2-free techniques prevailing. The medium was 0·1 M NaCl, 0·02 M $MgCl_2$, 0·005% gelatin, 1×10^{-5} M EDTA and acetylcholine bromide varied from 5×10^{-5} M to 1×10^{-3} M.

Kinetics

The scheme for the reaction of acetylcholinesterase with the acid-transferring methanesulfonyl anticholinesterase agents has been previously described.[1] Briefly:

$$\underset{E}{HE} + \underset{I}{CH_3SO_2X} \overset{K_I}{\rightleftharpoons} \underset{E\cdot I}{E\cdot CH_3SO_2X} \overset{k_3}{\rightarrow} \underset{E'}{ECH_3SO_2} + \underset{P_1}{HX}$$

A.

$$\underset{E'}{ECH_3SO_2} + H_2O \xrightarrow{k_4} \underset{E}{HE} + \underset{P_2}{CH_3SO_2OH}$$

where E and I have their usual meanings, X is the leaving group of the inhibitor, $E{\cdot}I$ is the enzyme–inhibitor complex, E' is methanesulfonylacetylcholinesterase, P_1 and P_2 are the products of the reaction, K_1 is the dissociation constant and k_3 and k_4 are rate constants. Preparations of the inhibited enzyme with proper controls were extensively diluted in the phosphate buffer medium for periods of several days and no enzyme activity was measurable. Thus k_4 must be very small and therefore it is not necessary to consider the reaction indicated by step B.

The equations have previously been derived from this scheme[1] and

$$\ln \frac{(\varepsilon)}{E^\circ} = -\frac{k_3 t}{1 + K_I/(I)} \tag{1}$$

where

$$E^\circ = (E) + (E{\cdot}I) + (E')$$
$$(\varepsilon) = (E) + (E{\cdot}I)$$

For the condition

$$(I) \gg E^\circ \text{ and } (I) > K_I$$

$$k = \frac{k_3}{1 + K_I/(I)} \tag{2}$$

and

$$\frac{1}{k} = \frac{1}{k_3} + \frac{K_I}{k_3} = \frac{1}{(I)} \tag{3}$$

where k is the apparent rate constant.
For the condition

$$(I) \ll K_I$$

$$k = \frac{k_3(I)}{K_I} \tag{4}$$

and

$$k_3 = \frac{k'_3}{K_I} \tag{5}$$

where k'_3 is the bimolecular (second-order) rate constant.

Some compounds did not demonstrate irreversible inhibition progressive with time, but rather were reversible anticholinesterase agents. The simple reaction scheme for enzyme and reversible inhibitor is given by

$$\underset{E}{HE} + \underset{I}{CH_3SO_2X} \overset{K_I}{\rightleftharpoons} \underset{E{\cdot}I}{HE{\cdot}CH_3SO_2X}$$

The binding constant, K_I, for this reversible inhibition reaction in the presence of acetylcholine was evaluated from data plotted according to the expression which has been previously described and defined:[8, 11]

$$\frac{1}{V} = \frac{1}{kE^\circ}\left[1 + \frac{(I)}{K_I' 1 + k_4/k_3}\right] + \frac{K_M}{kE^\circ}\left(1 + \frac{(I)}{K_I}\right) \cdot \frac{1}{S} \qquad (6)$$

RESULTS AND DISCUSSION

Compounds 1, 3, 4, 6, 8, 9, 11 and 12 listed in Table 1 demonstrate irreversible inhibition progressive with time. Data were plotted in accordance with equation (1). A typical graph of data for compound 12 appears as Figure 1. A bimolecular reaction is indicated for those experiments where the rate of inhibition is directly proportional to the inhibitor concentration. The second-order rate constant values (k'_3) were calculated from these data in accordance with equations (4) and (5) and are recorded in Table 2. When all times for 50 per cent inhibition of the enzyme are plotted as a function of inhibitor concentration, the expected exponential curve is developed. Figure 2 is a typical representation of these types of data. Appropriate apparent rate constant values and inhibitor concentrations were also plotted in a double reciprocal manner according to equation (3). A typical plot appears in Fig. 3. All slopes intercepted the y axis, indicating that reversible complexes are found between the inhibitor and enzyme.[1] The binding constant, K_I, was evaluated from this type of plot. The value of this constant was not measured in the previous phosphate and carbamate studies because of the high order of activity of those agents.

By substituting appropriate values for K_I and k'_3 into equation (5), the value of k_3 can be calculated. The latter is the first-order rate constant for the conversion of the reversible enzyme–inhibitor complex ($E{\cdot}I$) to the irreversibly inhibited enzyme (E') at high concentrations of inhibitor. At sufficiently low inhibitor concentrations, the mechanism is bimolecular and k'_3 is the second-order rate constant.

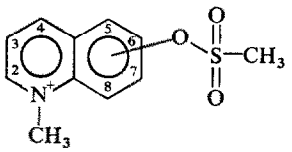
Compounds 2, 5, 7 and 10 did not produce progressive inhibition, even when the enzyme was exposed to relatively high concentrations (1×10^{-3} M) for several days; they did function as reversible inhibitors. The binding constant values for the reaction of these compounds with acetylcholinesterase were calculated from the slopes developed when enzyme activities at various substrate concentrations and in the presence of the inhibitor were plotted in a double manner according to equation (6). Figure 4 is a graph of such data measured for compound 2.

The binding constant and second-order rate constant values are listed in Table 2. Appropriate data from previous studies are included to facilitate comparisons. It is apparent from comparing the rate constant values that changes in the position of the methanesulfonyl group on the quinoline ring do not result in major fluctuations of activity. All compounds have remarkably similar values for the constants, unlike their diethylphosphoryl and dimethylcarbamyl counterparts, where differences of 100.000-fold were observed.

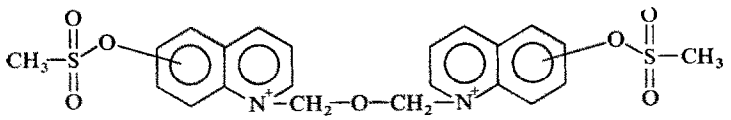
In the previous study of the diethylphosphoryl compounds, the second-order rate constant values were directly correlated with the pK_a value of the leaving group.[5] This was not true of the dimethylcarbamates nor was this relationship found for the methanesulfonates. The activity of the monoquaternary carbamates was qualitatively

TABLE 1. TERTIARY, MONO- AND DIQUARTERNARY METHANESULFONYL ESTERS OF QUINOLINOLS AND ISOQUINOLINOLS

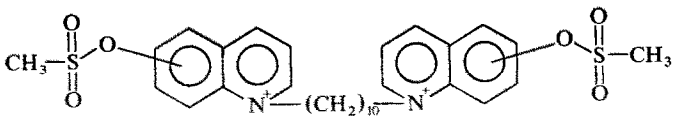
Group A



Group B



Group C



Cmpd. No.	T*	X⁻†	m.p.‡°C	Formula	Calcd. (%) C	H	N	S	Found (%) C	H	N	S
					Group A							
1	3	iodide	180 (56)§	$C_{11}H_{12}NSO_3I$	36·18	3·31	3·84	8·78	36·11	3·03	3·97	8·71
2	5	iodide	170 (91)	$C_{11}H_{12}NSO_3I$	36·18	3·31	3·84	8·78	36·01	3·15	3·96	8·51
3	6	iodide	189 (85)	$C_{11}H_{12}NSO_3I$	36·18	3·31	3·84	8·78	36·06	3·15	3·95	8·48
4	7	iodide	182 (79)	$C_{11}H_{12}NSO_3I$	36·18	3·31	3·84	8·78	36·09	3·21	3·96	8·56
5	5 iso	iodide	203 (90)	$C_{11}H_{12}NSO_3I$	36·18	3·31	3·84	8·78	36·32	3·33	3·77	9·03
					Group B							
6	3	iodide	166	$C_{22}H_{22}N_2S_2O_7I_2$	35·50	2·98	3·76	8·61	35·83	3·11	3·90	8.57
7	5	iodide	187	$C_{22}H_{22}N_2S_2O_7I_2$	35·50	2·98	3·76	8·61	36·08	2·98	3·72	8·44
8	6	iodide	202	$C_{22}H_{22}N_2S_2O_7I_2$	35·50	2·98	3·76	8·61	35·76	3·01	3·96	8·50
9	7	iodide	193	$C_{22}H_{22}N_2S_2O_7I_2$	35·50	2·98	3·76	8·61	36·24	3·03	3·78	8·76
10	5 iso	iodide	219	$C_{22}H_{22}N_2S_2O_7I_2$	35·50	2·98	3·76	8·61	36·08	2·98	3 76	8·44
					Group C							
11	6	picrate	155	$C_{42}H_{42}N_8O_{20}S_2$	48·37	4·06	10·75	6·15	47·62	3·99	10·84	6·11
12	5 iso	picrate	118	$C_{42}H_{42}N_8O_{20}S_2$	48·37	4·06	10·75	6·15	48·74	3·93	10·61	6·02

* Number refers to the position of substitution on the ring; iso indicates the compound is an isoquinolinium.

† X^- = anion.

‡ Melting points were taken on a Uni-Melt apparatus.

§ Number in parentheses is the melting point of the tertiary analogue.

related to the value of the dissociation constant (K_I) for the reaction of the undissociated form of the leaving group with the enzyme.[8] These correlations were not present in the phosphate or methanesulfonate studies.

The values of the binding constants for the methanesulfonates can be compared with the values for the K_I of their respective leaving groups (hydroxyl derivatives)

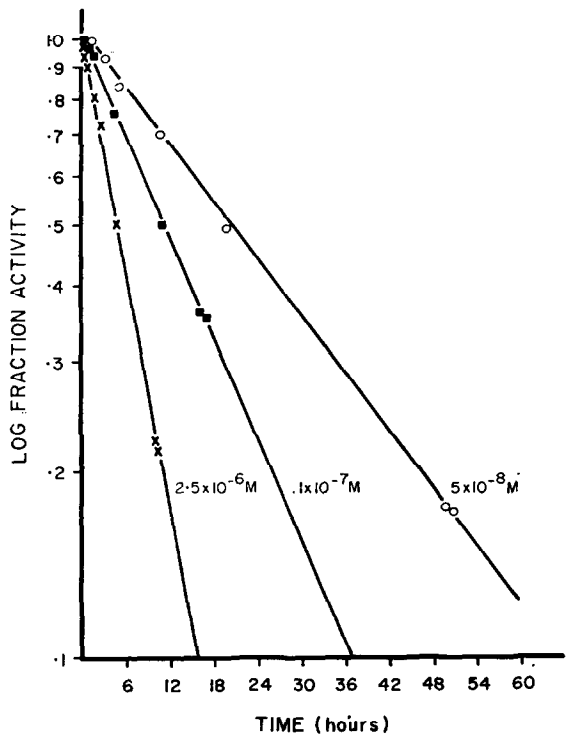


FIG. 1. The development of irreversible (acid-transferring) inhibition progressive with time for the reaction of acetylcholinesterase with several concentrations of compound 12 at pH 7 and 25°. Data are plotted in accordance with equation (1). Second-order rate constant values were calculated from those plots where the half-time of the reaction was inversely proportional to inhibitor concentration (see text).

previously measured. The values indicate that the methanesulfonyl function is generally detrimental to good binding (compounds 2, 6, 7 and 10) or has little effect (remaining compounds). In no instance did a methanesulfonate compound demonstrate better non-covalent binding than its hydroxy-substituted analogue. Generally those agents which are acid-transferring inhibitors have binding constant values of the same order as their respective hydroxyl-substituted analogues. For those compounds acting as simple reversible inhibitors, the values of the binding constant were several orders of magnitude less than their appropriate hydroxyl derivatives. Thus, good binding characteristics appear essential for the acid-transferring activity of the methanesulfonate agents.

These data tend to substantiate the opinion that the reversible binding between inhibitor and enzyme is principally the interaction of the cationic charge and structure of the leaving group with the anionic site and immediately adjacent area of the

TABLE 2. COMPARISON OF VARIOUS CONSTANTS FOR THE INHIBITION OF ACETYLCHOLINESTERASE BY SUBSTITUTED QUINOLINOLS AND ISOQUINOLINOLS*

Cmpd. No.	pK_a Hydroxyl analogue	K_I Hydroxyl analogue	K_I Methanesulfonate	k'_3 Sulfonylation	k'_3 Carbamylation	k'_3 Phosphorylation	k_3 Sulfonylation
1	5·3	$2·4 \times 10^{-7}$	9×10^{-7}	$2·4 \times 10^{3}$	$3·8 \times 10^{3}$	$1·2 \times 10^{8}$	$2·2 \times 10^{-3}$
2	6·1	$2·1 \times 10^{-8}$	$1·5 \times 10^{-5}$	0	$1·6 \times 10^{6}$	$2·4 \times 10^{6}$	
3	7·0	$1·9 \times 10^{-6}$	$1·7 \times 10^{-6}$	$1·7 \times 10^{3}$	$3·4 \times 10^{3}$	$9·3 \times 10^{6}$	$2·9 \times 10^{-3}$
4	5·7	$1·5 \times 10^{-7}$	$2·7 \times 10^{-7}$	$5·2 \times 10^{3}$	$4·2 \times 10^{5}$	$1·2 \times 10^{8}$	$1·4 \times 10^{-3}$
5	6·8	$4·3 \times 10^{-6}$	$3·0 \times 10^{-6}$	0	$3·5 \times 10^{5}$	$6·5 \times 10^{4}$	
6	4·5	$9·0 \times 10^{-7}$	$3·7 \times 10^{-5}$	$8·6 \times 10^{1}$			$3·2 \times 10^{-3}$
7	6·3	$3·4 \times 10^{-6}$	$2·5 \times 10^{-5}$	0	$2·1 \times 10^{4}$		
8	6·6	$2·2 \times 10^{-6}$	$2·5 \times 10^{-6}$	$3·6 \times 10^{3}$	$2·3 \times 10^{4}$	$2·7 \times 10^{8}$	$9·0 \times 10^{-3}$
9	5·1	$3·7 \times 10^{-7}$	$1·3 \times 10^{-6}$	$1·3 \times 10^{3}$	$5·1 \times 10^{4}$		$1·7 \times 10^{-3}$
10	6·3	$8·5 \times 10^{-7}$	$1·2 \times 10^{-5}$	0	$6·8 \times 10^{5}$	$8·1 \times 10^{5}$	
11	6·4	$1·6 \times 10^{-9}$	$1·0 \times 10^{-8}$	$1·2 \times 10^{5}$			$1·3 \times 10^{-3}$
12	6·9	$1·4 \times 10^{-7}$	$9·1 \times 10^{-8}$	$1·1 \times 10^{4}$			$1·0 \times 10^{-3}$

* Compound numbers 1 through 12 refer to the agents listed similarly in Table 1. The pK_a values of the hydroxyl derivatives (the leaving group for the acid-transferring inhibitors) were measured as previously described.[5] The values of K_I in moles/l. for the undissociated species of the hydroxyl derivatives are taken from reference 7 or measured in the same manner (compounds 11 and 12). The values in moles/l. listed in the column "K_I Methanesulfonate" are the binding constants of the methanesulfonate derivatives to acetylcholinesterase measured for both the reversible and irreversible inhibitors as described in the text. The dimensions of the second-order rate constants (k'_3) are liters·mole^{-1}min^{-1} and refer to the reaction of the methanesulfonyl, dimethylcarbamyl[6] and diethylphosphoryl[5] analogs with acetylcholinesterase. k_3 for sulfonation is the first-order rate constant for the reaction of the enzyme with high concentrations of the acid-transferring inhibitors; its dimensions are min^{-1}.

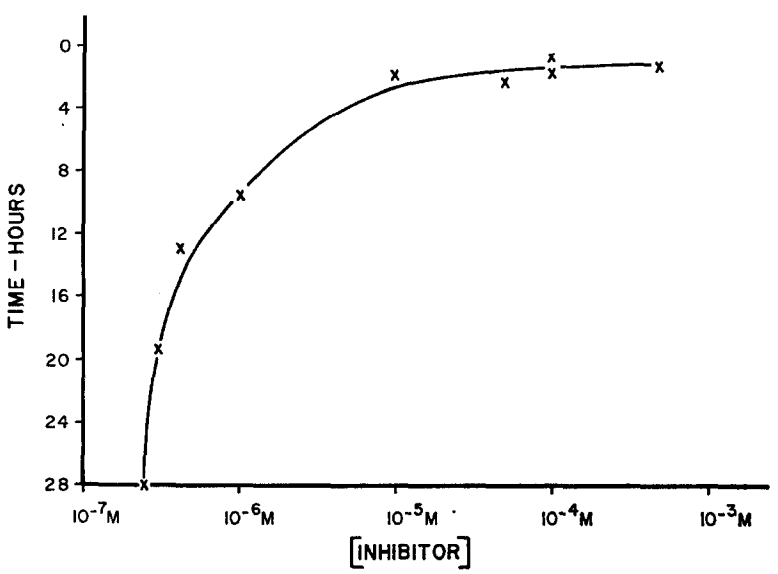


FIG. 2. The dependence of time for 50 per cent inhibition (plotted as hours on the ordinate) upon the concentration of compound 3. The curve has its origin where half-time values are inversely proportional to inhibitor concentrations and rises toward a minimum half-time value which is independent of inhibitor concentration.

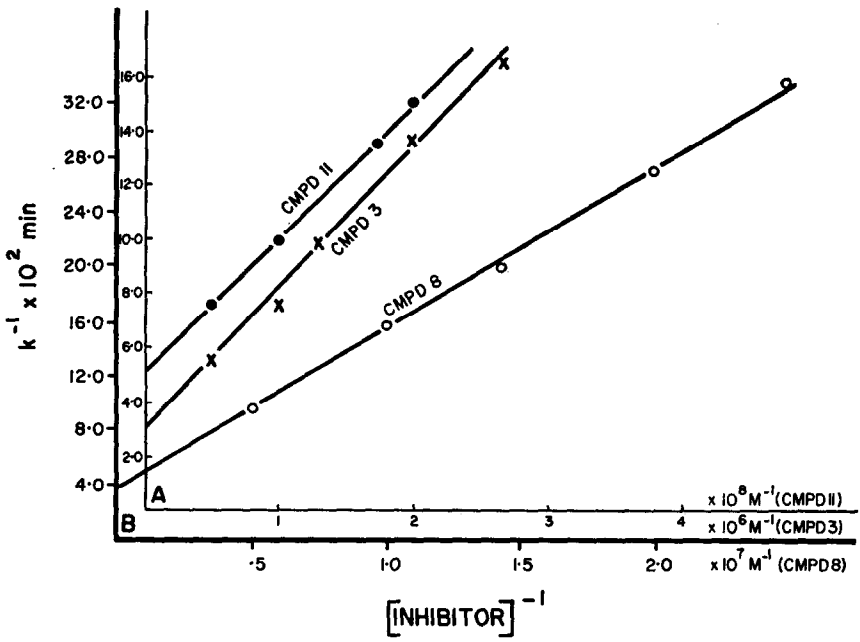


FIG. 3. This double reciprocal plot indicates the dependence of the velocity of hydrolysis upon the concentration of acetylcholine (lower plot) and in the presence of two different concentrations of compound 2 (upper plots). The compound is a reversible competitive inhibitor of acetylcholinesterase. The data, plotted in accordance with equation (6), allow calculation of the value of the binding constant K_I (upper plots) and Michaelis–Menten K_m (lower plot).

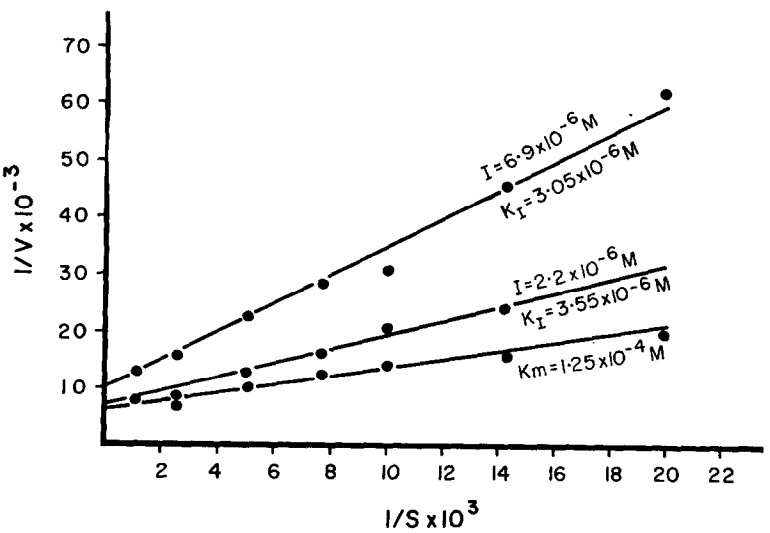


FIG. 4. The dependence of the value of the apparent rate constant (k) upon the concentrations of 1-methyl-6-hydroxyquinolinium iodide methanesulfonate (compound 3) and its *N*, *N'*, dimethylene ether bridged (compound 8) and *N,N'* decamethylene bridged (compound 11) diquaternary analogues. The data are plotted as reciprocals in accordance with equation (3), which allows calculation of the values of the binding constants (K_I). The coordinate system A is used for compounds 3 and 11 and B for compound 8. Note that the abscissa scale is different for each compound.

enzyme.[12] The methanesulfonyl group is evidently not especially complementary to the enzyme. Such data are not available for the respective diethylphosphoryl and dimethylcarbamyl analogues, but have been measured for the reaction of DFP and other organophosphates with serum cholinesterase.[13, 14] Rapid reaction equipment is required, however, to evaluate properly the binding constant values for the potent carbamate and phosphate compounds. This problem is currently under study.

The values of the second-order rate constants for sulfonylation are much lower but qualitatively parallel the values for phosphorylation of the enzyme by the monoquaternary compounds. This is not true for the carbamate agents.

For those methanesulfonate compounds producing acid-transferring inhibition, the first-order rate constant values were calculated. This is the maximum velocity of inhibition at high inhibitor concentrations. The values are remarkably uniform at $\sim 10^{-3}$ min^{-1}, paralleling the uniformity demonstrated at low inhibition concentrations (k'_3 values $\sim 10^3$ l. $mole^{-1}$ min^{-1}).

As a group, the $-CH_2-O-CH_2-$ bridged diquaternary methanesulfonate compounds tend to parallel their carbamate counterparts in that they are less potent acid-transferring agents than the monoquaternary derivatives, This is unlike the related phosphate compounds where the diquaternary derivatives studied are 20-fold more active than the appropriate mono compounds.

Only one diquaternary compound, number 8, with the *N,N'* dimethylene ether bridge is more potent than its monoquaternary analogue. The other two agents, compounds 6 and 9, are less active. It is possible that the bridge is not of appropriate length for optimum activity. To investigate this possibility, compounds 11 and 12 were synthesized. These decamethylene bridged diquaternary compounds are the most potent described. The K_I values indicate that they are on the order of 100-fold better bound than either their monoquaternary or *N,N'* dimethylene ether bridged counterparts. The better binding characteristics of these decamethylene compounds account for the increased activity at low concentrations (k_3' values), but do not significantly alter the maximum rate of sulfonylation (k_3 values).

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