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Anticholinesterase activity and charge delocalisation in "aliphatic" and "aromatic" quaternary ammonium compounds

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The anticholinesterase activities of a homologous series of trimethyl(phenylalkyl)-ammonium bromides have been determined and compared with anti-acetylcholinesterase activities previously reported. The pattern of results obtained with the two enzymes differs widely. The surface activities of the compounds at a constant molar concentration was determined in order to investigate the influence of the "distribution effect" on the anticholinesterase activities of the compounds. The anticholinesterase activities of a series of polycyclic aromatic quaternary compounds have also been determined and compared with their previously reported anti-acetylcholinesterase activities. With these compounds, the pattern of results obtained with the two enzymes was similar.

UNDER standardised conditions the degree of inhibition of acetylcholinesterase by quaternary ammonium compounds is dependant upon the forces of attraction between the onium ion and the active site of the enzyme. The total force of adsorption between onium ions and acetylcholinesterase is comprised of the following constituent forces (Bergmann, 1955, Bernhard, 1955): (*a*) coulombic interaction between the positive charge of the quaternary ammonium group and the anionic site of the enzyme; (*b*) Van der Waal's forces between the hydrocarbon moiety of the quaternary ammonium ion and the enzyme surface.

From a study of the inhibition of acetylcholinesterase by quaternary ammonium compounds, Thomas & Marlow (1963) have suggested that onium ions may be divided into "aromatic" and "aliphatic" types based on their anti-acetylcholinesterase activity. The characteristics of the two types of compound are: (*a*) "aromatic"; the positive charge is delocalised around a flat aromatic ring so it is considered that practically all of the charge is available for coulombic interaction with the anionic site of the enzyme. (*b*) "aliphatic"; the positive charge is delocalised among the four α-carbon atoms arranged tetrahedrally around the nitrogen atom. It is believed that such a situation leads to only a portion of the unit positive charge being available for coulombic interaction between an onium ion and the enzyme anionic site.

The basic difference between the two types of compound is their interaction with the anionic site of acetylcholinesterase. Since there is conflicting evidence about the presence and nature of an anionic site in cholinesterase (Adams & Whittaker, 1950; Bergmann, 1955; Bergmann & Segal, 1963) it was thought of interest to compare the anticholinesterase activities of a series of quaternary ammonium compounds, in which both "aromatic" and "aliphatic" types were represented, with their anti-acetylcholinesterase activities. A homologous series of trimethyl-(phenylalkyl)ammonium salts has therefore been examined. Also, the anticholinesterase activities of quinolizinium bromide and of some of its

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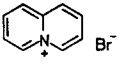
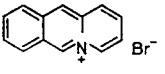
benzo-homologues have been determined and compared with the results obtained with the same compounds using erythrocyte acetylcholinesterase (Thomas, 1963). These results give an indication of the effect of the size of the condensed ring system on the anticholinesterase activity of "aromatic" quaternary compounds.

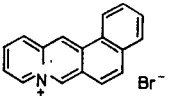
N-Methylpyridinium iodide



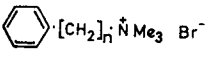
Quinolizinium bromide



Benzo[*b*]quinolizinium bromide



Naphtho[2,1-*b*]quinolizinium bromide



Trimethyl(phenylalkyl)ammonium bromide

Experimental

CHEMICAL

With the exceptions of trimethylphenylammonium bromide and trimethylbenzylammonium bromide, all compounds used have been previously prepared by Thomas & Marlow (1963) and Thomas (1963). The two compounds have now been synthesised by condensing the appropriate dimethylamine with methyl bromide.

Trimethylphenylammonium bromide, m.p. 214·5° (McDowell & Kraus, 1951, report 215°). Found: C, 50·2; H, 6·9. Calc. for $C_9H_{14}BrN$: C, 50·0; H, 6·5.

Trimethylbenzylammonium bromide, m.p. 236·5–237·5° (Kharasch, Williams & Nudenberg, 1955, report 235°). Found: C, 52·0; H, 7·1. Calc. for $C_{10}H_{16}BrN$: C, 52·2; H, 6·96.

MEASUREMENT OF ANTICHOLINESTERASE ACTIVITY

An electrically heated, thermostatically controlled water-bath adjusted to 37° was used. A beaker (100 ml) was supported in the water-bath and into it was placed a glass electrode, a glass stirrer, one arm of an agar bridge and the tip of a microburette. The other end of the agar bridge dipped into a saturated potassium chloride solution into which also dipped a calomel electrode. Both glass and calomel electrodes were connected to a pH meter, the temperature compensator of which was set at 37°.

A solution of the inhibitor (x ml) of selected concentration, was pipetted into the beaker followed by distilled water (43·5 — x ml). The enzyme preparation (1·5 ml) was added and the mixture incubated at 37° for 30 min. Acetylcholine perchlorate (5 ml of 0·12M solution) was added and the pH of the solution quickly adjusted to 7·4 with CO_2-free 0·02N sodium hydroxide solution. The constantly stirred mixture was then maintained

at pH 7·4 for 15 min by addition of further sodium hydroxide solution, the volume used being recorded. The determination was repeated using different concentrations of inhibitor until inhibition values from 30 to 70% were obtained. The pI50 values were obtained from graphs drawn with the ordinates representing the -ve log of the concentration of inhibitor and the abscissae representing % inhibition. To check that the apparatus was working correctly (particularly the response of the glass electrode), a determination was made without an inhibitor before and after a series of experiments on a particular compound. The results were corrected for non-enzymic hydrolysis of the acetylcholine, the rate of which was determined by replacing the enzyme preparation by a buffer solution (pH 7·4, 1·5 ml) and noting the volume of 0·02N sodium hydroxide added every 5 min for 45 min to maintain the pH at 7·4. All conditions were those used in inhibition experiments. A plot of the volume of alkali added against time was a straight line and the volume of the sodium hydroxide solution consumed in 15 min was obtained from the graph.

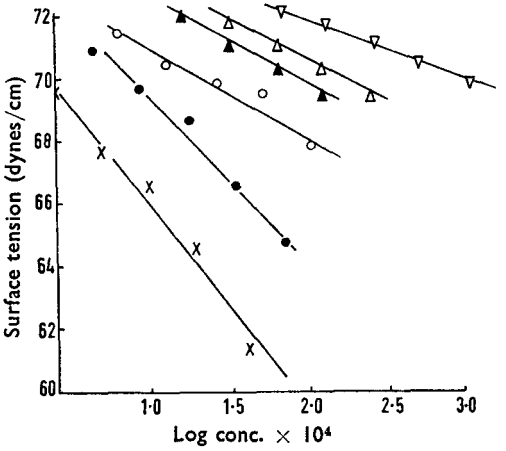


FIG. 1. Surface tension/concentration curves for trimethyl(phenylalkyl)ammonium bromides.

- ▽ Trimethylphenylammonium bromide.
- △ Trimethylbenzylammonium bromide.
- ▲ Trimethylphenethylammonium bromide.
- ○ Trimethyl(3-phenylpropyl)ammonium bromide.
- ● Trimethyl(4-phenylbutyl)ammonium bromide.
- × Trimethyl(5-phenylpentyl)ammonium bromide.

SOURCE OF CHOLINESTERASE

Horse serum obtained from commercial sources and preserved with chloroform was used as it has been shown that it has a high cholinesterase activity and that the chloroform has no effect on the activity (Stedman, Stedman & White, 1933). Also, even though horse serum contains an ali-esterase this has no action on acetylcholine (Richter & Croft, 1942).

MEASUREMENT OF SURFACE TENSION OF SOLUTIONS OF TRIMETHYL(PHENYLALKYL)AMMONIUM COMPOUNDS

The surface tension of solutions of the trimethyl(phenylalkyl)ammonium bromides was determined as described by Thomas & Clough (1963) and the results shown in Fig. 1. From this, surface tensions of the solutions of constant molar concentration were obtained.

Results and Discussion

TRIMETHYLPHENYLALKYLAMMONIUM COMPOUNDS

The results of the anticholinesterase determinations are compared in Fig. 2, with a curve similarly obtained by Thomas & Marlow (1963) on the same compounds using red blood cell acetylcholinesterase. Since different enzyme preparations were used to determine the activities, the pI50 values are not comparable, but as it is the pattern of change *within* each series which is compared, rather than absolute pI50 values, differences in the enzyme preparations used are of little consequence to the interpretation of the results.

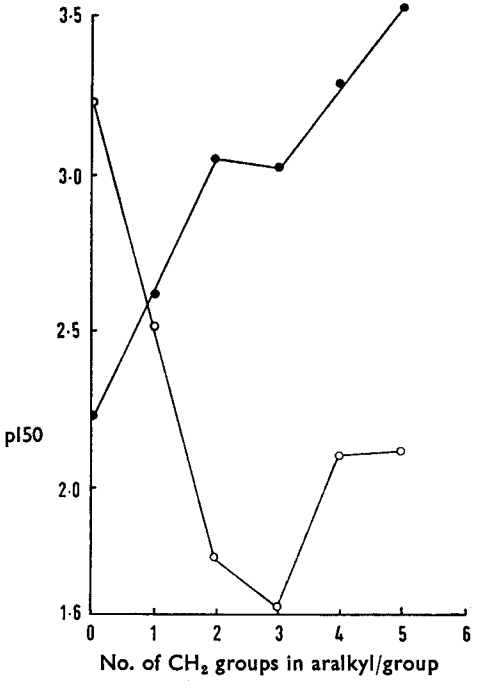


FIG 2. Anticholinesterase and antiacetylcholinesterase activities of trimethyl-(phenylalkyl)ammonium compounds. Anticholinesterase activities —●—: anti-acetylcholinesterase activities —○— (values taken from Thomas & Marlow, 1963).

It can be seen from Fig. 2 that the change in activity towards the two enzymes differs widely in the homologous series. The pattern of results

obtained with red blood cell acetylcholinesterase has been explained by Thomas & Marlow (1963). The essential point in their explanation of a fall in activity followed by a rise as the series is ascended is that the compounds change from being "aromatic" in type to "aliphatic." Consequently, there is a decrease in coulombic interaction between the positive charge of the quaternary ammonium group and the anionic site of the enzyme. However, the Van der Waal's component of the total adsorption force increases as the series is ascended and so two factors are operating to change the activities of successive homologues; the first tending to decrease activity and the second tending to increase it as the series is ascended.

The pattern of results now obtained with the horse serum cholinesterase is quite different. The activity rises progressively as the the number of methylene groups in the aralkyl group increases, except with phenethyl and 3-phenylpropyl compounds, in which the number changes from 2 to 3. This pattern poses two questions: (i) why are the results so different wth the two enzymes and (ii) why have trimethylphenethylammonium bromide and trimethyl(3-phenylpropyl)ammonium bromide the same activity? Since an increase in the number of methylene groups in the aralkyl group should cause a regular increase in Van der Waal's forces of attraction with both enzymes, and this has been shown to be so with the trimethylalkylammonium series (Bergmann, 1955), it appears that the differences between the behaviour with the two esterases must be ascribed to the coulombic component of the total adsorption force. The most reasonable assumption is that the difference is a reflection of variations in the anionic sites in the two enzymes but precisely what these are is obscure.

The "plateau" in the regular rise in activity as the number of methylene groups in the aralkyl group changes from 2 to 3 is difficult to explain. Even though only two sets of pI50 values are reported, these two compounds have been examined four times. Different salts of the two compounds have been synthesised and examined to exclude the possibility of an error, but in all instances the two compounds have virtually the same activity. It is noticeable that the "plateau" occurs in the same part of the series as did the change in activity with acetylcholinesterase. Also in Fig. 2 the points representing the anticholinesterase activity of compounds with 0, 3, 4, 5 methylene groups in the aralkyl group lie on a straight line, suggesting that the compounds with 1 and 2 such groups have abnormally high anticholinesterase activity.

It has been suggested that the "distribution effect" will have an influence on the anticholinesterase activities of quaternary ammonium ions (Thomas & Marlow, 1963). The "distribution effect" was described by Thomas & Marlow (1963) as the increased concentration of quaternary ammonium ions at the interface of the water and enzyme surfaces, compared with the concentration in the bulk of the solution, due to the effect of water on amphipathic ions. To examine whether the "plateau" was due to the "distribution effect," the surface tension of the trimethyl(phenylalkyl)-ammonium compounds at constant molar concentration was determined. The results, given in Fig. 3, show that the surface tension values fall in a

regular manner as the number of methylene groups in the aralkyl group increases. Hence the "distribution effect" will rise regularly as the series is ascended and is not, therefore, the cause of the "plateau."

The results indicate that the adsorption of quaternary ammonium ions onto cholinesterase is not as sensitive to changes in the nature of the quaternary ammonium ion as is the case with acetylcholinesterase.

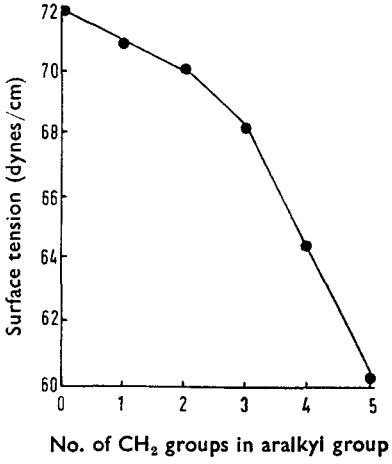


FIG. 3. Surface tension of solutions of trimethyl(phenylalkyl)ammonium bromides at constant molar concentration (log molar conc. $\times$ 10^4 = 1·9) plotted against the number of methylene groups in aralkyl groups.

QUINOLIZINIUM COMPOUNDS

The pI50 values for this series of compounds are given in Table 1, together with the values obtained by Thomas (1963) for the antiacetylcholinesterase activities of the same compounds. It can be seen that unlike the results for the trimethyl(phenylalkyl)ammonium compounds,

TABLE 1. ANTICHOLINESTERASE ACTIVITIES OF QUINOLIZINIUM COMPOUNDS. pI50 VALUES GIVEN IN g/m/l. HORSE SERUM USED AS SOURCE OF ENZYME. TEMP. 37°. SUBSTRATE CONCENTRATION 0·012M ACETYLCHOLINE PERCHLORATE. (Anti-acetylcholinesterase values taken from Thomas, 1963)

	Anticholinesterase		Antiacetylcholinesterase	
	pI50	Relative activities	pI50	Relative activities
N-Methylpyridinium iodide	1·655	0·00000084	2·485	0·00877
Quinolizinium bromide	3·023	0·000195	2·95	0·0296
Benzo[*b*]quinolizinium bromide	6·730	1·0	4·575	1·0
Naptho[2,1-*b*]quinolizinium bromide	5·712	0·0959	3·945	0·294

the pattern of results obtained with the two enzymes is similar. Activity increases as the number of rings in the compound increases up to three and then decreases. Also the increased activity of quinolizinium bromide compared with that of *N*-methylpyridinium iodide (223 times) is much less than the difference observed between benzo[*b*]quinolizinium bromide

and quinolizinium bromide (5,120 times). A similar result was obtained with acetylcholinesterase, the respective values being 3·4 times and 33 times (Thomas, 1963). The results suggest that the benzo[*b*]quinolizinium ion has optimal characteristics for adsorption onto both cholinesterase and acetylcholinesterase.

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