Results

The neuromuscular blocking activity of the cyclic analogs and congeners of succinyl dicholine is summarized in Table II. Compound **2** was the most active compound in this current series and was approximately twice as potent as succinyl choline, having a duration of action 5 times that of succinyl choline. Compound **6** was approximately equipotent to succinyl choline, although the duration of action was approximately 6 times that of succinyl choline. In each instance, the *trans* derivative was much more potent than the *cis* derivative. For example, **2** was approximately 75 times more potent than **1**, and **6** was approximately 60 times more potent than **5**. The *trans*-1,2-cyclopropane derivatives were much more potent than the *trans*-1,2-cyclobutane derivatives. For example, **2** was approximately 45 times as potent as **4**, and **6** was approximately 60 times as potent as 8. All of the compounds produced mild to marked pressor responses.

Quaternary Ammonium Compounds. V. Antiacetylcholinesterase Activity of a Series of N-*t*-Alkylpyridinium Compounds

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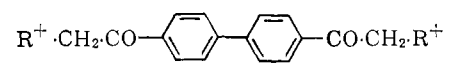
A series of N-*t*-alkylpyridinium compounds has been synthesized and the antiacetylcholinesterase activity of the compounds determined. The activities of the compounds are compared with those of an isosteric series of trimethylphenylalkylammonium compounds previously reported.[2] From these studies quaternary ammonium compounds are classified into two types, "aliphatic" and "aromatic," from the point of view of charge delocalization and stereochemistry.

Thomas and Marlow[2] have reported the antiacetylcholinesterase activities of a series of trimethylphenylalkylammonium compounds. It was found that the pattern of results obtained, as the homologous series was ascended, was fundamentally different from a "normal" series such as *n*-alkyltrimethylammonium (see Fig. 1). The fact that activity was reduced as saturated carbon atoms were introduced between the trimethylammonium group and the aromatic ring was explained in terms of the charge delocalization and stereochemistry of the compounds. From an antiacetylcholinesterase activity point of view, quaternary ammonium compounds were classified into two types, "aromatic" and "aliphatic."

A priori it might be anticipated that in a series of quaternary ammonium compounds in which the only difference between them was the structure of the onium group, the one containing the trimethylammonium group would be the most active antiacetylcholinesterase. There are two reasons for this; one is that acetylcholine, the natural substrate, contains the trimethylammonium group and in terms of "stereochemical fit" it could be expected that the enzyme surface could accommodate this group better than any other. The second reason is that in terms of the importance of the availability of the α-carbon atoms, as suggested by Thomas,[3] the trimethylammonium group is ideal. Since the evidence for the relative importance of shape and charge availability of a quaternary ammonium group has been mainly obtained from the trimethylphenylalkylammonium series in which each compound contained the trimethylammonium group, then it would be of value to examine the antiacetylcholinesterase activities of "aromatic" type compounds which did not contain this group.

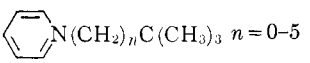
There is some evidence reported in the literature which supports the view that charge availability is more important than shape. A particularly interesting series of antiacetylcholinesterase activities, from this point of view, is one reported by Long and Schueler,[4] who determined the activities of a series of bisquaternary ammonium compounds of the type I. The compound



I

containing the trimethylammonium groups (I_{50}, 6×10^{-5}) had next to the lowest activity and the 2-methylpyridinium compound (I_{50}, 8×10^{-10}) the highest. Although the compounds reported are not strictly comparable, from the "distribution effect"[2] and potential van der Waals forces points of view, the results suggest that there is some basic difference in binding forces between "aromatic" and "aliphatic" quaternary ammonium compounds and the anionic site of acetylcholinesterase.

In order to obtain further evidence that there is a difference in the coulombic component of the total force of adsorption between "aliphatic" and "aromatic" quaternary ammonium compounds, a homologous series of pyridinium compounds (II) has now been examined. The pyridinium series of compounds (II) are isosteric with the trimethylphenylalkylammonium series reported previously[2] and so any differences in the pattern of change of activity between the two series of compounds, as the homologous series are ascended, should be a reflection of differences in the coulombic component of the total adsorption force of the two series.



II

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(2) J. Thomas and W. Marlow, *J. Med. Chem.*, **6**, 107 (1963).

(3) J. Thomas, *ibid.*, **3**, 309 (1961).

(4) J. P. Long and F. W. Schueler, *J. Am. Pharm. Assoc. Sci. Ed.*, **43**, 79 (1954).

TABLE I

N-ALKYLPYRIDINIUM COMPOUNDS[a]

Pyridinium (halide)	Alkyl halide	Pyridine	Solv., conditions used	Solv. for recrysta.	M.p., °C. Compd.	M.p., °C. Deriv.	Derivative formula recrysta solv.	Analysis of derivative: % Calcd. C	% Calcd. H	% Calcd. Pt	% Found C	% Found H	% Found Pt
Isopropyl (iodide)[b]	Isopropyl iodide (4.3 g., 0.025 mole)	16 g., 0.2 mole	EtOH (30 ml.), reflux, 4 hr.	EtOH	104–105[c]	198–199	Picrate sodium picrate addnct $C_{20}H_{16}NaN_7O_{11}$ EtOH	39.9	2.7		39.6	2.7	
Isobutyl (iodide)	Isobutyl iodide (5 g., 0.027 mole)	2.2 g., 0.025 mole	No solv.,[d] 115°, 2 hr.	Acetone ppt. with ether at −25°	67–69	173–174	Chloroplatinate $C_{18}H_{28}Cl_6N_2Pt$ acetonitrile	30.9	4.3	28.0	30.9	4.4	27.6
1-(3,3-Dimethylbutyl) (bromide)[e]	1-Bromo-3,3-dimethylbutane (5.2 g., 0.03 mole)	2.5 g., 0.03 mole	No solv., 115°, 2 hr.	Et·Me·CO ppt. with ether at −25°	69–70.5	208–209	Chloroplatinate $C_{22}H_{36}Cl_6N_2Pt$ acetonitrile	35.8	4.9	26.5	35.6	4.9	26.7
1-(4,4-Dimethylpentyl) (bromide)	1-Bromo-4,4-dimethylpentane (7.6 g., 0.03 mole)	4.6 g., 0.09 mole	No solv., 115°, 3 hr.		51–54	173–174	Chloroplatinate $C_{24}H_{40}Cl_6N_2Pt$ acetonitrile	37.7	5.4	25.5	36.6	5.4	25.5
1-(5,5-Dimethylhexyl) (bromide)[g]	1-Bromo-5,5-dimethylhexane (9.7 g., 0.05 mole)	3.95 g., 0.05 mole	Et·Me·CO, reflux, 6 hr.	Et·Me·CO	80–83	169–170.5	Chloroplatinate $C_{26}H_{44}Cl_6N_2Pt$ acetonitrile	39.4	5.6	24.6	39.2	5.8	24.6
1-(6,6-Dimethylheptyl) (bromide)	1-Bromo-6,6-dimethylheptane (4.2 g., 0.02 mole)	3.2 g., 0.04 mole	No solv., 115°, 3 hr.		71–73	173–174	Chloroplatinate $C_{28}H_{48}Cl_6N_2Pt$ acetonitrile	41.0	5.8	23.8	40.2	5.5	23.6

[a] All the pyridinium compounds were hygroscopic and difficult to crystallize. All the reagents and solvents were dried before use and reaction mixtures were protected from the atmosphere. The compounds were handled in a drybox under nitrogen. [b] Calcd.: I, 50.9. Found: I, 50.8. [c] E. M. Kosower and J. A. Skorcz, *J. Am. Chem. Soc.*, **82**, 2195 (1960), report m.p. 119.6–121°; A. B. Prescott, *ibid.*, **18**, 91 (1896), report m.p. 114–115°. [d] Oil obtained which crystallized after a standing for 2 weeks at 0°. [e] Calcd.: Br, 32.7. Found: Br, 31.9. [f] The product was a pasty mass. It was washed with ether in a drybox and kept over P_2O_5 for 2 months. All attempts to recrystallize it produced oils. [g] Calcd.: C, 57.4; H, 8.1. Found: C, 57.5; H, 8.2. [h] The product was an oil which set to a brown solid after 2 days over P_2O_5. [i] The product was an oil which solidified on standing for 3 weeks over P_2O_5. All attempts to recrystallize it were unsuccessful.

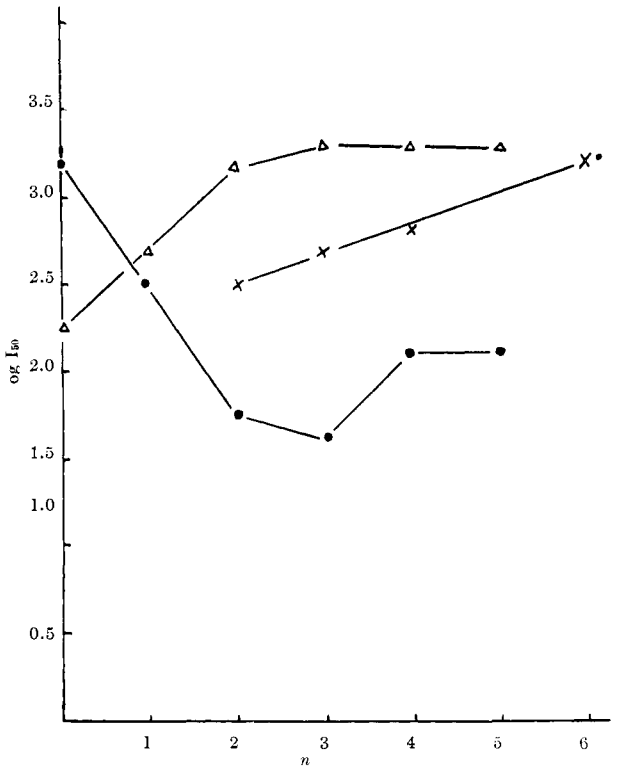

Fig. 1.—Antiacetylcholinesterase activities of three homologous series of quaternary ammonium compounds: ×—×, trimethylalkylammonium[12]; ●—●, trimethylphenylalkylammonium[1]; ▲—▲, N-alkylpyridinium.

Experimental

Chemical.[5]—All the pyridinium compounds were prepared by condensing the appropriate alkyl halide with pyridine under the conditions shown in Table I. The alkyl halides were prepared as indicated below.

1-Bromo-3,3-dimethylbutane[6,7] was characterized as the thiuronium picrate, m.p. 186–187.5°.

Anal. Calcd. for $C_{13}H_{12}N_5O_7S$: C, 48.0: H, 5.7. Found: C, 48.0; H, 5.9.

1-Bromo-4,4-dimethylpentane[8,9]: thiuronium picrate, m.p. 169–170°.

Anal. Calcd. for $C_{14}H_{21}N_5O_7S$: C, 41.7; H, 4.9. Found: C, 42.0; H, 5.2.

1-Bromo-5,5-dimethylhexane[10]: thiuronium picrate, m.p. 151–152°.

Anal. Calcd. for $C_{15}H_{23}N_5O_7S$: C, 43.2; H, 5.6. Found: C, 43.1; H, 5.6.

1-Bromo-6,6-dimethylheptane[10]: thiuronium picrate, m.p. 151–152°.

Anal. Calcd. for $C_{16}H_{25}N_5O_7S$: C, 44.6; H, 5.8. Found: C, 45.0; H, 5.8.

Results and Discussion

The antiacetylcholinesterase activities (for measurements, see ref. 2) obtained with the pyridinium compounds[11] are given in Table II and shown graphically in Fig. 1. The results of Thomas and Marlow[2] on the trimethylphenylalkylammonium series and of Bergmann and Shimoni[12] on the *n*-alkyltrimethylammonium series are also included on Fig. 1, from which it can be seen that the pattern of change of activity as the three homologous series are ascended differs. Since the same technique of determining the I_{50} values and the same sample of enzyme preparation was used for both the trimethylphenylalkylammonium and the pyridinium series, then these results are directly comparable. However, the *n*-alkyltrimethylammonium series were examined under different conditions; thus these results are not directly comparable with the other two, but the pattern of change within the different series is comparable.

TABLE II

ANTIACETYLCHOLINESTERASE ACTIVITIES OF N-*t*-ALKYLPYRIDINIUM COMPOUNDS[a]

Compound	I_{50}	*n*
Isopropylpyridinium iodide	5.40×10^{-3}	0
Isobutylpyridinium iodide	1.96×10^{-3}	1
1-(3,3-Dimethylbutyl)pyridinium bromide	6.92×10^{-4}	2
1-(4,4-Dimethylpentyl)pyridinium bromide	5.00×10^{-4}	3
1-(5,5-Dimethylhexyl)pyridinium bromide	5.15×10^{-4}	4
1-(6,6-Dimethylheptyl)pyridinium bromide	5.20×10^{-4}	5

[a] I_{50} values in g.-moles/l.; ox erythrocytes used as source of enzyme; temperature 37°; substrate concentration 0.012 *M* (acetylcholine perchlorate); sodium chloride 0.1 *M*; magnesium chloride 0.04 *M*.

It may be seen from the results that the antiacetylcholinesterase activities of the pyridinium compounds rise as the homologous series is ascended, unlike the trimethylphenylalkylammonium ones which fall to a minimum and then rise. Except for the first two homologs the two series are isosteric, the only difference being the nature of the nitrogen atom. It was not possible to prepare *t*-butylpyridinium or neopentylpyridinium and so isopropylpyridinium and isobutylpyridinium iodides were prepared.

In the pyridinium series the nitrogen atom is "aromatic" in type in all cases, but in the trimethylphenylalkylammonium series it changes from "aromatic" to "aliphatic" as the homologous series is ascended.[2] Since the pyridinium compounds are in general more active than the trimethylphenylalkylammonium compounds, this then suggests that charge availability is more important than the shape of the quaternary ammonium group in determining the antiacetylcholinesterase activity of onium compounds. The leveling-off of activity with the higher homologs is unusual but it may reflect the difficulty of a longer alkyl chain with a terminal bulky *t*-butyl group coming into close association with the enzyme surface.

(5) All melting points except those of pyridinium compounds were determined on a Kofler block and are corrected.

(6) F. C. Whitmore, C. D. Wilson, J. V. Capinjola, C. A. Tongberg, C. H. Fleming, R. V. McGrew, and J. N. Crosby, *J. Am. Chem. Soc.*, **63**, 2035 (1941).

(7) F. C. Whitmore, A. M. Popkin, J. S. Whitaker, K. Mattil, and J. D. Zech, *ibid.*, **60**, 2458 (1938).

(8) F. C. Whitmore and A. H. Homeyer, *ibid.*, **55**, 4555 (1933).

(9) M. S. Karasch, C. Hannum and M. Gladstone, *ibid.*, **56**, 244 (1934).

(10) F. C. Whitmore and A. H. Homeyer, U. S. Patent 2,151,252 (1939).

(11) The pyridinium compounds were too hygroscopic to make solutions of known concentrations of them directly, so solutions were prepared of approximately the concentration for inhibition studies and then the accurate concentration was determined by analysis of the halide ion in solution.

(12) F. Bergmann and A. Shimoni, *Biochim. Biophys. Acta*, **10**, 49 (1953).