Journal of Medicinal and Pharmaceutical Chemistry

VOL. 3, No. 1 (1961)

Quaternary Ammonium Compounds—I. The Antiacetylcholinesterase Action of a Series of Spiran Quaternary Ammonium Compounds

J. THOMAS, *Department of Pharmacy, University of Manchester, Manchester*

Introduction

Many quaternary ammonium compounds can inhibit acetylcholinesterase and attempts have been made to correlate the antiacetylcholinesterase activity of the ions with their structure.[1-3] The majority of the quaternary ammonium compounds which have been examined have had the general formula $(R'R''R'''R''''N)^+ X^-$, where the four R groups have been similar or dissimilar organic radicals. Cyclic nitrogenous compounds such as 1-alkylpyridinium salts and 1,1-dialkylpiperidinium salts have also received attention. From a stereochemical point of view these three types of compounds have a common feature: they possess at least one carbon to nitrogen single bond about which there is free rotation. As a result of this freedom of movement, the configuration of these compounds is not 'fixed'. It would be useful, therefore, if a series of quaternary ammonium compounds were examined in which the configuration of the molecule was 'rigid' and known. This can only be achieved in molecules where the rotation about the carbon to nitrogen bond is restricted. Compounds with this characteristic are spiran quaternary ammonium salts in which two rings are linked together by a common pentavalent nitrogen atom (Fig. 1). Such a spiran

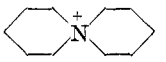

Fig. 1

quaternary ammonium system is potentially interesting because of the fine steric control which could be exercised over substituents in the rings. The present preliminary investigation has been

Table I. Spiran quaternary ammonium compounds prepared

No.	Compound molecular formula	Cyclic amine	α,ω-Dihalo polymethylene	Solvent (500-ml portion)	Reflux time, h	Solvent for recrystallization	m.p., °C	Analysis		Reference
								Calcd.	Found	
1	$C_{10}H_{20}NBr$	Piperidine (8·6 g, 0·1 mole)	1,5-Dibromopentane (11·5 g, 0·05 mole)	Chloroform	6	Chloroform[c]	309(d.)	C, 51·3 H, 8·5 Br, 34·1	C, 51·2 H, 8·3 Br, 34·2	5
2	$C_9H_{18}NBr$	Pyrrolidine (7·1 g, 0·1 mole)	1,5-Dibromopentane (11·5 g, 0·05 mole)	Chloroform	15	Ethyl methyl ketone–chloroform	260–1	Br, 36·4	Br, 36·6	6
3	$C_{11}H_{22}NBr$[f]	2,6-Dimethyl piperidine (11·3 g, 0·1 mole)	1,4-Dibromobutane (10·8 g, 0·05 mole)	Chloroform	6	Chloroform–methanol[e] Chloroform–ethyl methyl ketone	285–6	Br, 32·3	Br, 32·2	
4	$C_8H_{16}NBr$	Pyrrolidine (7·1 g, 0·1 mole)	1,4-Dibromobutane (10·8 g, 0·05 mole)	Chloroform	24	Acetone[a]	261–2	Br, 38·8	Br, 38·6	10
5	$C_{12}H_{24}NBr$[f]	2,6-Dimethyl piperidine (11·3 g, 0·1 mole)	1,5-Dibromopentane (11·5 g, 0·05 mole)	Chloroform	6	Chloroform–ethyl methyl ketone	254–5	Br, 30·5	Br, 30·7	
6	$C_9H_{18}NOBr$	Piperidine (17·2 g, 0·2 mole)	2,2′-Dichloro-diethyl ether (14·3 g, 0·1 mole)[b]	Chloroform	12	Chloroform[a]	276–8	Br, 33·9	Br, 33·5	11
7	$C_8H_{16}NO_2Cl$	Morpholine (17·4 g, 0·2 mole)	2,2′-Dichloro-diethyl ether (14·3 g, 0·1 mole)	Chloroform	12	Chloroform[e]	298–9(d.)	Cl, 18·4	Cl, 18·6	7
8	$C_8H_{16}NOBr$	Morpholine (8·7 g, 0·1 mole)	1,4-Dibromobutane (10·8 g, 0·05 mole)	Chloroform	12	Chloroform–ethyl methyl ketone[d]	188–9	Br, 36·0	Br, 35·97	11

9	$C_{11}H_{22}NOCl$[f]	2,6-Dimethyl piperidine (11·3 g, 0·1 mole)	2,2′-Dichloro-diethyl ether (7·15 g, 0·05 mole)	Chloroform	20	Chloroform–ethyl methyl ketone	301–2	Cl, 16·2	Cl, 16·4	
10	$C_{14}H_{20}NBr$	1,2,3,4-Tetra-hydroquinoline (6·7 g, 0·05 mole)	1,5-Dibromopentane (11·5 g, 0·05 mole)	Ethanol 90%	20	Isopropanol	228–9(d.)	Br, 28·36	Br, 28·1	
11	$C_{14}H_{20}NBr$	1,2,3,4-Tetra hydroisoquinoline (6·7 g, 0·05 mole)	1,5-Dibromopentane (11·5 g, 0·05 mole)	Ethanol 90%	20	Isopropanol	201–2	Br, 28·36	Br, 28·35	8
12	$C_{13}H_{18}NBr$	Indoline (3 g, 0·025 mole)	1,5-Dibromopentane (4·75 g, 0·025 mole)	Ethanol 90% (250 ml)	20	Isopropanol	156–7	Br, 29·8	Br, 29·6	
13	$C_{13}H_{18}NBr$	Piperidine (8·5 g, 0·1 mole)	*o*-Xylylene dibromide (13·2 g, 0·05 mole)	Chloroform	30	Chloroform–ethyl methyl ketone	244–5	Br, 29·8	Br, 29·66	9
14	$C_8H_{18}NBr$	Diethylamine (14·6 g, 0·2 mole)	1,4-Dibromobutane (21·6 g, 0·1 mole)	Chloroform (600 ml)	12	Ethanol (absolute)–ethyl methyl ketone	303	Br, 38·5	Br, 38·2	
15	$C_9H_{20}NBr$	Diethylamine (14·6 g, 0·2 mole)	1,5-Dibromopentane (23 g, 0·1 mole)	Chloroform (600 ml)	20	Ethanol (absolute)–ethyl methyl ketone	299	Br, 36·0	Br, 35·8	
16	$C_{10}H_{18}NBr$	1,2,3,4-Tetra-hydropyridine (16·6 g, 0·2 mole)	1,5-Dibromopentane (23 g, 0·1 mole)	Chloroform (800 ml)	16	Chloroform[e]	286(d.)	Br, 34·5	Br, 34·2	
17	$C_{10}H_{24}NBr$	Diethylamine (14·6 g, 0·2 mole)	*n*-Propyl bromide (49·2 g, 0·4 mole)	Ethanol 70% (700 ml)	24	Ethyl methyl ketone	227–9	Br, 33·6	Br, 33·5	

[a] The crude product was purified by extraction with acetone in a Soxhlet extractor.
[b] Spiran chloride was produced by this method. The crude material was converted to the bromide by dissolving it in water, adding a large excess of hydrobromic acid and distilling the solution to dryness under reduced pressure.
[c] Crystallized from the chloroform when being extracted.
[d] Crystallized slowly by evaporating the solvent under reduced pressure without heat.
[e] Two recrystallizations required from the different mixed solvents.
Cis-isomer.[4]
All the compounds, except 10, 11, 12 and 17, were prepared by Method A.

carried out to determine whether or not spiran formation *per se* has any effect on the antiacetylcholinesterase action of quaternary ammonium compounds.

A series of spiran quaternary ammonium compounds has been prepared (Table I) and their antiacetylcholinesterase activity determined (Table II).

Table II. Antiacetylcholinesterase activity of spiran quaternary ammonium compounds and some analagous open chain compounds. I_{50} values in gram moles per litre. Erythrocyte stromata used as source of acetylcholinesterase. Inhibitions carried out at pH 6·3 and pH 7·4 with substrate concentrations of 0·0033 M and 0·0166 M respectively. Temperature 37°.

Compound	pH 6·3	pH 7·4	Compd. no.
1,1′-Spirobipiperidinium bromide	$1{\cdot}4 \times 10^{-2}$	$4{\cdot}9 \times 10^{-2}$	1
Spiro(piperidine-1,1′-pyrrolidinium) bromide	$1{\cdot}3 \times 10^{-2}$	$4{\cdot}84 \times 10^{-2}$	2
2,6-Dimethylspiro(piperidine-1,1′-pyrrolidinium) bromide	$2{\cdot}6 \times 10^{-2}$	$6{\cdot}96 \times 10^{-2}$	3
1,1′-Spirobipyrrolidinium bromide	$2{\cdot}7 \times 10^{-2}$	$5{\cdot}4 \times 10^{-2}$	4
2,6-Dimethyl-1,1′-spirobipiperidinium bromide	$3{\cdot}33 \times 10^{-3}$	$7{\cdot}2 \times 10^{-3}$	5
Spiro(piperidine-1,4′-morpholinium) bromide	$5{\cdot}0 \times 10^{-2}$	[a]	6
4,4′-Spirobimorpholinium chloride	[b]	[c]	7
Spiro(morpholine-4,1′-pyrrolidinium) bromide	$8{\cdot}8 \times 10^{-2}$	$1{\cdot}9 \times 10^{-1}$	8
2,6-Dimethylspiro(piperidine-1,4′-morpholinium) chloride	$2{\cdot}28 \times 10^{-2}$	$6{\cdot}8 \times 10^{-2}$	9
Spiro(1,2,3,4-tetrahydroquinoline-1,1′-piperidinium) bromide	$7{\cdot}72 \times 10^{-4}$	$4{\cdot}38 \times 10^{-4}$	10
Spiro(1,2,3,4-tetrahydroisoquinoline-1,1′-piperidinium) bromide	$1{\cdot}69 \times 10^{-3}$	$1{\cdot}51 \times 10^{-3}$	11
Spiro(indoline-1,1′-piperidinium) bromide	$1{\cdot}15 \times 10^{-3}$	$1{\cdot}89 \times 10^{-3}$	12
Spiro(isoindoline-1,1′-piperidinium) bromide	$5{\cdot}92 \times 10^{-3}$	$1{\cdot}11 \times 10^{-2}$	13
N,*N*-Diethylpyrrolidinium bromide	$1{\cdot}37 \times 10^{-2}$	$2{\cdot}25 \times 10^{-2}$	14
N,*N*-Diethylpiperidinium bromide	$6{\cdot}6 \times 10^{-3}$	$2{\cdot}27 \times 10^{-2}$	15
Spiro(1,2,5,6-tetrahydropyridine-1,1′-piperidinium) bromide	$6{\cdot}3 \times 10^{-3}$	$2{\cdot}21 \times 10^{-2}$	16
Diethyl-di-*n*-propylammonium bromide	$1{\cdot}2 \times 10^{-2}$	$6{\cdot}45 \times 10^{-3}$	17
Tetraethylammonium bromide[e]	[d]	[f]	18

[a] 32% inhibition at $1{\cdot}29 \times 10^{-1}$. [b] 34% inhibition at $1{\cdot}57 \times 10^{-1}$. [c] 31% potentiation at $6{\cdot}15 \times 10^{-2}$. [d] 50% potentiation at $2{\cdot}52 \times 10^{-2}$. [e] Obtained commercially. [f] 25% inhibition at $9{\cdot}07 \times 10^{-2}$.

Experimental

Chemical

Two general methods of preparing spiran quaternary ammonium compounds have been used.

Method A. 1,1′-Spirobipiperidinium bromide was prepared by refluxing a solution of piperidine (8·6 g, 0·1 mole) and 1,5-dibromopentane (11·5 g, 0·05 mole) in chloroform (500 ml) for 6 h. The solution was distilled to dryness under reduced pressure on a steam bath, the solid residue dissolved in water, sodium hydroxide (2 g, 0·05 mole) added and the solution evaporated to dryness under reduced pressure. The resulting solid was extracted with chloroform in a Soxhlet extractor. 1,1′-Spirobipiperidinium bromide crystallized from the chloroform. Yield, 10·1 g (86 per cent), m.p. 309°(d.).

Anal. Calcd. for $C_{10}H_{20}BrN$: C, 51·3; H, 8·5; Br, 34·1. Found: C, 51·2; H, 8·3; Br, 34·2.

Method B. Spiro(1,2,3,4-tetrahydroquinoline-1,1′-piperidinium) bromide was prepared by refluxing a solution of 1,2,3,4-tetrahydroquinoline (6·7 g, 0·05 mole), 1,5-dibromopentane (11·5 g, 0·05 mole) and sodium hydroxide (2 g, 0·05 mole) in ethanol (500 ml, 90 per cent) for 24 h. The solution was distilled to dryness under reduced pressure on a steam bath. The residue was refluxed with chloroform (150 ml) for 10 min and the solution filtered. The filtrate was evaporated to dryness to produce a black hygroscopic mass. Crystallization from isopropanol gave a white crystalline product. Yield, 1·8 g (12 per cent), m.p. 228–229°(d.).

Anal. Calcd. for $C_{14}H_{20}BrN$: Br^-, 28·36. Found: Br^-, 28·1.

Antiacetylcholinesterase

The antiacetylcholinesterase activity of the compounds was determined by the standard Warburg manometric technique. Details of the conditions used are given in Table II.

Results and Discussion

The antiacetylcholinesterase activities of the spiran quaternary ammonium compounds are given in Table II.

It appears that the spiran system or ring closure confers no

special antiacetylcholinesterase properties on the quaternary ammonium system in the case of six-membered rings. 1,1′-Spirobipiperidinium bromide, *N*,*N*-diethylpiperidinium bromide and the corresponding open chain compound diethyl-di-*n*-propylammonium bromide have similar activities. However, in the case of the series tetraethylammonium bromide, *N*,*N*-diethylpyrrolidinium bromide and 1,1′-spirobipyrrolidinium bromide there is a marked difference in activity between the open chain compound and the cyclized ones, cyclization increasing the activity considerably. The introduction of an ether oxygen into the ring system reduces the activity in all cases and two oxygen atoms practically removed activity completely. This is possibly due to the solvating effect of the oxygen atoms.

The introduction of *cis*-2,6-dimethyl groups increased the activity of 1,1′-spirobipiperidinium bromide and of the corresponding morpholine compound.

The most active compounds of the series were those with an aromatic system present in the spiran. In both the tetrahydroquinoline and indoline pairs of compounds the isomer with the nitrogen atom adjacent to the aromatic ring was the more active.

If it is considered that differences in the I_{50} values of the compounds reflect differences in the binding of the spiran quaternary ammonium compounds to the anionic site of acetylcholinesterase, then it can be seen that small changes in the structure of the ammonium ions can affect the forces of binding between the onium ion and the anionic site on the enzyme. Detailed discussion of these differences in binding in terms of charge distribution and stereochemistry of the compounds will be presented in subsequent communications.

Summary. A series of spiran quaternary ammonium compounds has been prepared and their antiacetylcholinesterase activity determined. It has been shown that spiran formation does not confer any particular properties on the antiacetylcholinesterase action of quaternary ammonium compounds, but slight changes in the structure of these ions modify the binding of spiran quaternary ammonium ions to the anionic site of acetylcholinesterase.

Acknowledgements. The author wishes to thank Professor K. Bullock for the antiacetylcholinesterase determinations.

(*Received 9 May, 1960*)

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