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Further structure-activity relations of heterocyclic analogues of hemicholinium-3

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The importance of the 3-methyl group on the pyridinium ring of bis-quaternary nitrogen salts for hemicholinium-3-like activity having been conditionally established (Benz & Long, 1969a,b), derivatives containing classical isosteres of the 3-methyl group and the three oxidation states of the 3-methyl group were examined. Oxidation of the 3-methyl group to $-CH_2OH$ decreased activity tenfold. Subsequent oxidation to –CHO and –COOH further decreased activity. When the 3-methyl group was replaced by a halogen, activity was maintained by the iodo-derivative but decreased as the size of the halogen decreased and as the electronegativity increased. Substitution of an ethyl group for the 3-methyl decreased activity twofold, whereas replacement with –OH eliminated activity.

It has previously been shown (Benz & Long, 1969a) that the hemiketal moiety of hemicholinium-3 (HC-3) can be replaced by the 3-methylpyridinium cationic substituent without loss of activity. That the *m*-methyl group enhanced activity was demonstrated by the fact that both the *ortho* and *para* isomers were inactive as was the unsubstituted pyridinium ring. It was hoped that the same structural specificity would extend to the isomeric piperidinium series. However, this was not found to be so. The 4-methylpiperidinium derivative was equipotent to HC-3, whereas the 3-methylpiperidinium analogue was much less active. The low activity of the 3-methylpiperidinium analogue was tentatively explained on the basis of enantiomeric dilution coupled with a possible low yield of the active conformer on quaternization (Benz & Long, 1969a). This hypothesis is presently being investigated using nmr spectroscopy.

To further investigate the structural requirements for activity, a series of dimethylalkylammonium and dimethylpyridinium analogues was synthesized (Benz & Long, 1969b). It was thought that perhaps the carbon unit linking the quaternary nitrogen to the *m*-methyl group on the 3-methylpyridinium system could be mimicked by a simple alkyl chain. Thus, a three carbon chain with one double bond should be optimal for activity. This expectation was realized since highest activity was observed with the allyl derivative. Activity decreased as the carbon chain was lengthened, shortened or if the double bond was deleted. In the dimethylpyridinium series it was shown that addition of a second methyl group to the ring, in addition to the 3-methyl group, decreased activity in all cases. This was especially surprising for the 3,5-dimethylpyridinium derivative. This compound had a methyl group in each of its *meta*-positions, thereby increasing the statistical likelihood of the *m*-methyl group coming in contact with its receptor. However, this compound was only 1/20 as active as its monomethyl isomer.

The purpose of this communication is to report the results of our experiments with another series of heterocyclic HC-3 analogues, and to discuss their structure-activity relation.

EXPERIMENTAL

Chemistry

The compounds were synthesized according to the procedure described for hemicholinium (Long & Schueler, 1954). Carbon, hydrogen and nitrogen analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y., or by Galbraith Laboratories, Inc., Knoxville, Tenn. All combustion analyses fell within $\pm 0{\cdot}3\%$ of the calculated percentages. Infrared spectra were obtained with a Beckman model IR-10 recording infrared spectrometer as 1% mixtures of the quaternary salt in potassium bromide. Nuclear magnetic resonance spectra were obtained on a Varian A-60 spectrometer. Compounds were run as 1 to 10% solutions in d_6-dimethyl sulphoxide with tetramethylsilane as an internal standard.

Pharmacology

The 24-h LD50 for each compound was determined using three groups of 10 mice, each weighing 20–25 g. The animals received the compound intraperitoneally and were observed for the type and time for onset of symptoms. The LD50 and its 95% fiducial limits were estimated by the method of Litchfield & Wilcoxon (1948).

Two frequencies of nerve stimulation were used in the sciatic nerve-gastrocnemius muscle preparation of Duch rabbits. The rabbits, weighing 1 to 2 kg, were anaesthetized with phenobarbitone sodium, 200 mg/kg, administered intravenously. Interrupted tetanic stimulation (high frequency) of 250 Hz, with a pulse duration of 1 ms and maximal voltage (10–15 V), was applied to the sciatic nerve for 0·2 s every 10 s. A Grass stimulator (model S-4C) with appropriate circuit interrupter was used in these studies. The other experiments (low frequency) utilized similar preparations, except single shock stimulations every 10 s with a pulse duration of 5 ms and a voltage of 10–15 V were used. Application of a stimulus with these parameters elicited maximal muscle contraction. Only the most active compounds at the higher frequency of stimulation were tested with single shock. The relative potencies and 95% fiducial limits for the higher frequency stimulations were calculated by the method of Finney (1952) using a 2×2 parallel line assay. In these assays five animals were used for each dose and the doses were varied by a 0·48 log interval. The ED50 was estimated by using a log dose-probability plot. The ED50 was not determined at the lower frequency of stimulation since our only purpose for testing the most active agents was to qualitatively demonstrate their relative inactivity at single shock with respect to their activity at interrupted tetanic stimulation. All drugs were administered via the marginal ear vein.

The ability of the compounds to inhibit human red blood cell acetylcholinesterase was measured with a thermostatic recording pH stat (S-30240, E. H. Sargent). The specific methods have been published previously (Benz & Long, 1969a,b).

RESULTS

Neuromuscular inhibition in rabbit

In this preparation HC-3 causes a neuromuscular blockade characterized by its slow onset (approximately 5 min after i.v. administration), long duration (approximately 2–3 h), dependence on the frequency of nerve stimulation (the higher the frequency, the greater the blockade) and specific antagonism by choline (1–5 mg/kg).

Any compound causing a neuromuscular blockade with these characteristics will be termed HC-3-like.

Compound 1 showed only weak HC-3-like activity. Maximum blockade occurred 15–30 min after intravenous administration with a duration of action of about 1h. Its effects were only partially antagonized by choline, 10 mg/kg. An initial drop in twitch tension occurred immediately, followed by the slow developing HC-3 effect. Compounds 2 and 3 demonstrated qualitatively the same effects.

In the halogen series, HC-3-like properties became more pronounced as the atomic size of the halogen was increased to iodine. The time for maximum blockade was 10–20 min for the chloro-(compound 4), 30–40 min for the bromo-(compound 5) and 35–45 min for the iodo-derivative (compound 6). The effect of the iodo-derivative was readily reversed by choline, 1–2 mg/kg, whereas the effect of the chloro-derivative was less so. The slowly developing blockade caused by compounds 4 and 5 was always preceded by a small initial drop in twitch tension. This was not true with compound 6. However, compound 6 did elicit the initial drop in twitch tension at higher doses. This transient initial phase of action has been observed previously (Benz & Long, 1969a,b) and is thought to be due to the compounds' ability to inhibit acetylcholinesterase. Compounds 7 and 10 exhibited HC-3-like properties, but compounds 8, 9 and 11 did not.

At high frequency nerve stimulation, compounds 6 and 7 were the most active agents studied. Their activity was evaluated in seven preparations at low frequency nerve stimulation and was found to be significantly less, as would be expected for an HC-3-like agent. Doses of 1 mg/kg were necessary to produce a blockade which was rapid in onset and was accompanied by salivation, lacrimation and defaecation. At lower doses these compounds enhanced neuromuscular transmission. The latter two effects are explainable on the basis of the compounds' ability to inhibit acetylcholinesterase (Table 1).

Mouse toxicity (Table 1)

Toxicity in mice paralleled the various compounds' ability to block neuromuscular transmission in the rabbit. The most active HC-3-like agents in the rabbit elicited symptoms of HC-3 poisoning upon intraperitoneal administration to mice as outlined by Long & Schueler in 1954. The most active acetylcholinesterase inhibitors caused marked salivation, urination, defaecation and fasciculations before death occurred. Fasciculations continued even after respiratory movements ceased.

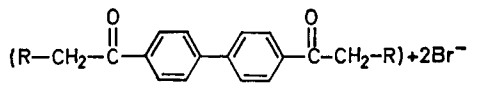
Acetylcholinesterase inhibition (Table 1)

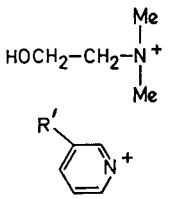
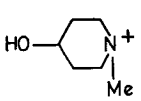
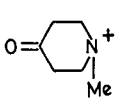
All compounds demonstrated the ability to inhibit human red blood cell acetylcholinesterase. A qualititative difference in the manner in which these compounds inhibited the enzyme, as compared to neostigmine, was observed. Neostigmine required an induction period of a few minutes before maximum inhibition occurred, whereas the present compounds inhibit maximally, immediately upon being added to the incubation medium.

DISCUSSION

It has previously been shown that placement of a methyl group specifically in the 3-position on the pyridinium ring or in the 4-position on the piperidinium ring greatly

Table 1. *Biological data for substituted nitrogen heterocyclic derivatives of* HC-3



Compound	Structure R =	Neuromuscular inhibition in rabbit Interrupted tetanic R.P.[a]	Neuromuscular inhibition in rabbit Interrupted tetanic ED50 (mg/kg)	Toxicity in mice LD50 (mg/kg)	Acetylcholinesterase inhibition ID50 (M)
		1·0	0·013	0·12 (0·09–0·17)	
1	R′ = CH_2OH	0·10 (0·09–0·13)	0·142	1·3 (1·1–1·5)	$3·8 \times 10^{-8}$
2	R′ = CHO	0·05 (0·03–0·07)	0·290	2·3 (1·4–3·7)	$1·3 \times 10^{-7}$
3	R′ = COOH	<0·03	>0·5	>20·0	
4	R′ = Cl	0·19 (0·06–0·32)	0·072	0·35 (0·31–0·41)	$1·2 \times 10^{-8}$
5	R′ = Br	0·24 (0·13–0·82)	0·053	0·27 (0·20–0·35)	$2·5 \times 10^{-8}$
6	R′ = I	0·98 (0·56–1·68)	0·013	0·13 (0·11–0·14)	$1·5 \times 10^{-8}$
7	R′ = Et	0·49 (0·22–1·43)	0·027	0·21 (0·15–0·28)	$2·2 \times 10^{-8}$
8	R′ = OH	<0·03	>0·5	>20·0	$5·6 \times 10^{-6}$
9		<0·03	>0·5	6·3 (5·6–7·1)	$3·8 \times 10^{-7}$
10		0·09 (0·04–0·25)	0·150	1·10 (0·77–1·57)	$6·9 \times 10^{-7}$
11		<0·03	>0·5	3·00 (2·5–3·5)	$2·1 \times 10^{-8}$
	Neostigmine	—	—	—	$5·2 \times 10^{-7}$

[a] R.P. = relative potency with respect to HC-3 = 1·00 and 95% fiducial limits.

enhanced the HC-3-like neuromuscular blocking activity of these cationic substituents (Benz & Long, 1969a). To rule out the possibility that one of the oxidation products of the methyl group was responsible for the activity, compounds 1–3 were evaluated. The most active agent was compound 1. Its activity was approximately 1/10 that of the 3-methylpyridinium analogue (compound 2, Benz & Long, 1969a), which is equipotent to HC-3. None of these products can account for the high activity of the 3-methylpyridinium system. Another series of this type has been studied previously (Marshall & Long, 1959).

Since the methyl group may be regarded as an inert space filler (Schatz, 1960), it was of interest to apply the principles of bioisosterism to this series. Four classical isosteres of the methyl group, three halogens and the hydroxyl group, were evaluated. Activity increased from the chloro-derivative (compound 4) which was 1/5 as active, through the bromo-derivative (compound 5) which was 1/4 as active, to the iodo derivative (compound 6) which was equipotent to HC-3. In the literature, it has been estimated that the methyl and chloro-groups occupy similar volumes (Burger & Foggio, 1956). However, the van der Waals radii for the various halogens and the methyl group should give a better indication of the sphere of influence of these groups, since this is the optimum distance from the nucleus beyond which the electron cloud of a non-bonded atom cannot easily advance. The van der Waals radius of a methyl group has been found by a number of investigations to approach 0·2 nm (Cromer, Ihde & Ritter, 1951; Steinfink, Post & Fankuchen, 1955). The van der Waals radii of chlorine, bromine and iodine are 0·180, 0·195 and 0·215 nm respectively.* Thus, bromine or perhaps iodine would best represent the volume of a methyl group. However, since bromine is more electronegative than iodine, it is not unreasonable to expect highest activity with 3-iodo-derivative (compound 6), since it would be most capable of mimicking a methyl group as an inert space filler.

The inactivity of the *m*-hydroxy isomer (compound 8) can be similarly explained. It is well known that phenols are stronger acids than alcohols, due to the fact that the oxygen anion can delocalize into the ring system. This is even more true for compound 8 since the hydroxyl group is *meta* to a quaternary nitrogen atom which will withdraw electrons from the oxygen atom by induction, rendering the hydroxyl group more acidic. A model compound for comparison would be 3-hydroxy-*N*-methylpyridinium which has a $pK_a = 4{\cdot}99$ (Bridges, Davies & Williams, 1966). Since this molecule is ionized at pH 7·4, compound 8 may be also. The oxygen anion thus formed may be too polar and thus, does not mimic the methyl group.

From the above discussion, and as one would expect, the receptor site for the methyl group seems to be hydrophobic in nature, as compounds with non-polar substituents on the ring have higher activity. This theory was further confirmed by the relatively high activity of the 3-ethylpyridinium analogue (compound 7). This compound was approximately 1/2 as active as the 3-methyl derivative, which is equipotent to HC-3. It is apparent that the receptor can accept the longer 3-ethyl group, but its activity is less than that of the 3-methyl derivative. Compound 7 presented the necessary hydrophobic group to the methyl receptor, but this group was too large for ideal binding and therefore its activity was reduced.

From the low activity exhibited by the piperidinium analogues (compounds 9–10), it appears that the binding site for the 4-methyl group on the piperidinium system

* From Framework Molecular Models, Prentice Hall, Inc., Englewood Cliffs, N.J.

(compound 6, Benz & Long, 1969a) is also specific, lending further evidence that its binding site and that of the 3-methyl group on the pyridinium system may be the same or of the same type.

It has previously been suggested (Benz & Long, 1969a) that another factor may be considered in relating structure with biological activity in compounds of this type. This hypothesis focuses on the acidity of the protons on the methylene group joining the carbonyl group to the quaternary nitrogen. According to this hypothesis one might conjecture that activity may be associated with the acidity of such protons or with an interaction of the anion thus formed.

The importance of increased acidity of the methylene protons, which join the carbonyl and quaternary nitrogen head, in relation to the biological activity of the molecule is minimized when one reflects on the relative activity of the various analogues in this and the previous series. In the monomethyl pyridinium and piperidinium series (Benz & Long, 1969a) this theory would predict highest activity for the unsubstituted heterocycles, when in fact the 3-methylpyridinium and 4-methylpiperidinium analogues were found to be the most active. Likewise, in the present series, this theory would predict high activity for the 3-hydroxy (compound 8) and the 3-chloro (compound 4) derivatives since they would decrease the electron density about the quaternary nitrogen, thereby increasing the acidity of the methylene protons the most. Yet, the 3-hydroxy derivative was inactive and the 3-chloro- was much less active than the 3-iodi-derivative. The 3-iodo- and 3-methyl analogues were equipotent biologically, yet the former exerted a —Inductive effect on the quaternary nitrogen, whereas the 3-methyl exerted a +Inductive effect.

These inductive effects of the halogens and the methyl group on the pyridinium ring system have been adequately demonstrated by Brown and co-workers (Brown & McDaniel, 1955). Therefore, it seems unlikely that the heterocyclic ring substituents exert their effects via increasing or decreasing electron density on the quaternary head which in turn would influence acidity of the methylene group. Stereochemical and volume factors of the ring substituents appear to be more important.

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