

Structure-activity relationships in some series of 2-alkyl-1,2,3-benzotriazinium compounds

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Summary

1. The potencies of four series of *N*-alkyl-1,2,3-benzotriazinium iodides have been estimated on the frog rectus abdominis and chick biventer cervicis preparations.
2. The partition coefficients between chloroform and water were measured for all of the compounds, as well as the ionization constants for some members of two of the series.
3. The potencies of the compounds increased with the number of carbon atoms in the *N*-alkyl side chain up to a maximum at the *n*-butyl homologue while partition coefficients increased up to the *n*-pentyl homologue, and there may be some association between the lipid solubilities of the compounds and their biological activity.
4. The responses of the tissues to the benzotriaziniums were not abolished by tubocurarine, indicating that the site of action was not at acetylcholine receptors.
5. The similarity between the actions of the benzotriaziniums and those of quinine and quinidine is discussed.

Introduction

Stevens & Stevens (1970a,b) prepared a series of *N*-alkylated 1,2,3-benzotriazines which contained a quaternary ammonium ion at the 2-position in the triazine ring (Fig. 1). The structure of these compounds suggested that at least the quaternary metho compound might activate acetylcholine receptors at the neuromuscular junction or in ganglia and they have therefore been tested for ability to cause contracture of the frog rectus abdominis and chick biventer cervicis muscles. It was found that the higher homologues also caused contracture and that activity increased with chain length up to a maximum where the 2-substituent is *n*-butyl. This was unexpected, since replacement of methyl groups in quaternary ammonium compounds by larger alkyl groups almost invariably leads to a reduction in stimulating activity, and frequently to the appearance of antagonistic or blocking activity (Barlow & Hamilton, 1962; Barlow & Zoller, 1964; Barlow, Scott & Stephenson, 1967). The relationship between structure and activity seemed rather to resemble that observed in homologous series of compounds which suppress the motility of tadpoles (Brink & Posternak, 1948) or which are bacteriostatic (Albert, Rubbo, Goldacre, Davey & Stone, 1945). We have therefore measured the partition coefficients of the compounds between chloroform and water in order to see if this can be correlated with their ability to cause contracture.

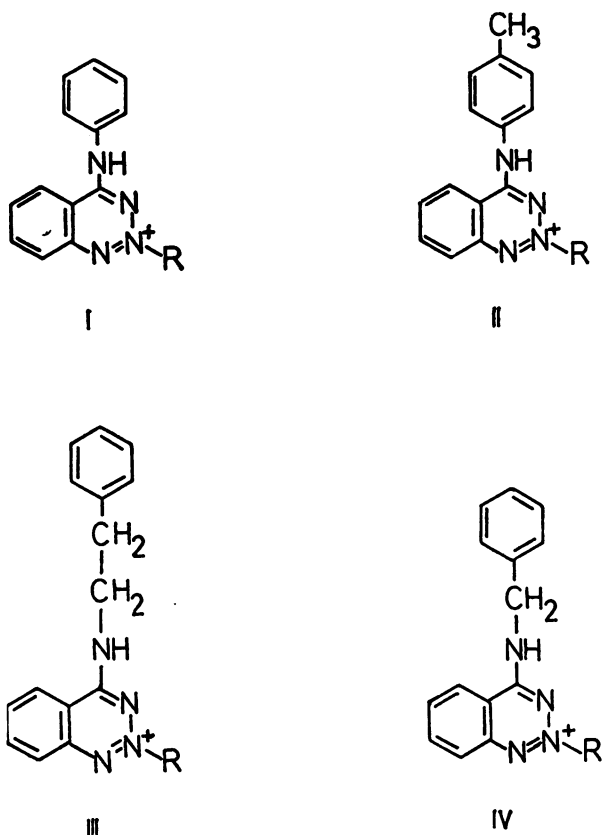


FIG. 1. Chemical structures of the benzotriazinium compounds. I=4-anilino-1,2,3-benzotriazinium; II=4-p-tolylamino-1,2,3-benzotriazinium; III=4-phenethylamino-1,2,3-benzotriazinium; IV=4-benzylamino-1,2,3-benzotriazinium. R=methyl, ethyl, *i*-propyl, *n*-propyl, *n*-butyl or *n*-pentyl. All compounds were made as the iodides.

The compounds tested were all soluble in acid or neutral solution, but were insoluble in alkaline solution in which they lose a proton from the exocyclic nitrogen, and form an insoluble zwitterion. Determination of the pK_a values of some of the compounds (kindly performed by Mr. A. F. Fell) indicated that the formation of the zwitterion would be negligible at pH values more acid than 7.4, consequently all measurements of potency were performed at pH 7.0 or pH 7.2.

Methods

The frog rectus abdominis preparation

The rectus abdominis muscle from *Rana pipiens* was used in all experiments, and was mounted in modified Starling frog-Ringer solution at room temperature (20–22° C) in a 10 ml bath aerated with 5% carbon dioxide in oxygen. The modification to the Ringer solution involved a reduction in the sodium bicarbonate concentration in order to reduce the final pH of the solution to a value of 7.0. The loss of sodium ions was made good by increasing the amount of sodium chloride present. The composition of the Ringer solution was, in g/l.: NaCl, 6.12; KCl, 0.14; $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.24; NaHCO_3 , 3.7; NaH_2PO_4 , 0.01. After bubbling for 5 min with the oxygen/carbon dioxide mixture, the pH of the solution was 7.0 at 20° C.

Contractions of the muscle were recorded isotonicly on smoked kymograph paper with a frontal writing lever exerting a tension of 0.5 g. The drugs were left in contact with the tissue for 10 min, then the tissue was washed three times. Immediately after the first wash, the tissue was stretched gently under an increased tension of 2.5 g, and this tension was maintained during the second and third washes. After the third wash, the extra load was removed and the tissue allowed 5 min to regain its resting length before the next drug was added. The total time of the cycle was 45 minutes. This time was found to be necessary because the recovery of the tissue after contact with the benzotriazinium drugs was extremely slow.

The chick biventer cervicis preparation

Brown Leghorn chicks (10–15 days old) were killed by inhalation of ether and the biventer cervicis muscle from the back of the neck was dissected out by the method described by Ginsborg & Warriner (1960). The muscle was suspended in Krebs–Henseleit Ringer solution, modified by adjustment of the sodium bicarbonate concentration to produce a solution of pH 7.2 at 39° C. The composition of the Ringer solution was, in g/l.: NaCl, 7.6; KCl, 0.35; $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.28; NaHCO_3 , 1.05; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.29; KH_2PO_4 , 0.16; glucose, 2.0. The temperature was maintained at 39–40° C, which facilitated the relaxation of the tissue after drug-induced contractures (Child & Zaimis, 1960). Contractures of the muscle were recorded isotonicly on smoked kymograph paper, with a frontal writing lever exerting a tension of 0.3–0.5 g.

Potency measurements

The potency of each compound was determined by standard 2+2 assays, the reference compound being 4-anilino-2-n-propyl-1,2,3-benzotriazinium iodide in all cases.

Measurement of partition coefficients

Partition coefficients between water and chloroform were measured for all the benzotriaziniums under investigation. The concentration in the aqueous phase was measured spectrophotometrically, the concentration in the chloroform phase being calculated by difference. It was noticed that when an aqueous solution of benzotriazinium was shaken with chloroform, there was an immediate colour change in the chloroform layer, this being reddish by comparison with the frank yellow colour of the aqueous solution.

n-Hexane was also investigated as a possible lipid substitute but the concentration achieved in the hexane phase was very low; since this could lead to large errors in calculating the partition coefficient, the use of this substance was abandoned.

Results

Potency measurements

The results of the potency measurements for the four series of benzotriaziniums tested on the frog rectus and chick biventer cervicis are shown in Table 1 and

TABLE 1. Activity of benzotriaziniums on frog rectus abdominis and chick biventer cervicis

Nature of 2-substituent	Anilino series	Benzylamino series	Phenethylamino series	<i>p</i> -Tolylamino series
Frog rectus				
Methyl	9.94±0.28 (10)	10.57±0.27 (9)	10.66±0.37 (9)	6.80±0.21 (9)
Ethyl	2.43±0.08 (10)	4.95±0.15 (9)	4.44±0.20 (9)	1.68±0.03 (9)
<i>i</i> -Propyl	1.23±0.02 (9)	2.72±0.07 (6)	2.26±0.12 (6)	0.58±0.02 (9)
<i>n</i> -Propyl	1	2.22±0.18 (6)	1.21±0.03 (9)	0.67±0.03 (10)
<i>n</i> -Butyl	0.89±0.02 (10)	1.87±0.06 (6)	1.00±0.04 (6)	0.64±0.05 (10)
<i>n</i> -Pentyl	1.16±0.05 (10)	2.65±0.17 (6)	1.28±0.09 (6)	0.87±0.05 (9)
Chick Biventer				
Methyl	3.86±0.10 (10)	4.60±0.17 (10)	4.86±0.21 (9)	1.56±0.17 (11)
Ethyl	1.81±0.07 (10)	3.22±0.05 (9)	3.28±0.19 (9)	0.93±0.04 (9)
<i>i</i> -Propyl	1.76±0.04 (9)	3.06±0.17 (6)	2.72±0.10 (6)	0.55±0.01 (9)
<i>n</i> -Propyl	1	2.03±0.09 (10)	1.58±0.08 (9)	0.59±0.01 (9)
<i>n</i> -Butyl	0.69±0.03 (9)	1.98±0.13 (6)	1.05±0.06 (6)	0.55±0.02 (9)
<i>n</i> -Pentyl	1.98±0.10 (6)	2.29±0.29 (6)	1.39±0.13 (6)	0.91±0.06 (9)

Values, which are equipotent molar ratios relative to 4-anilino-2-*n*-propyl-1,2,3-benzotriazinium iodide, are given with standard errors, and the number of tissues used (in parentheses).

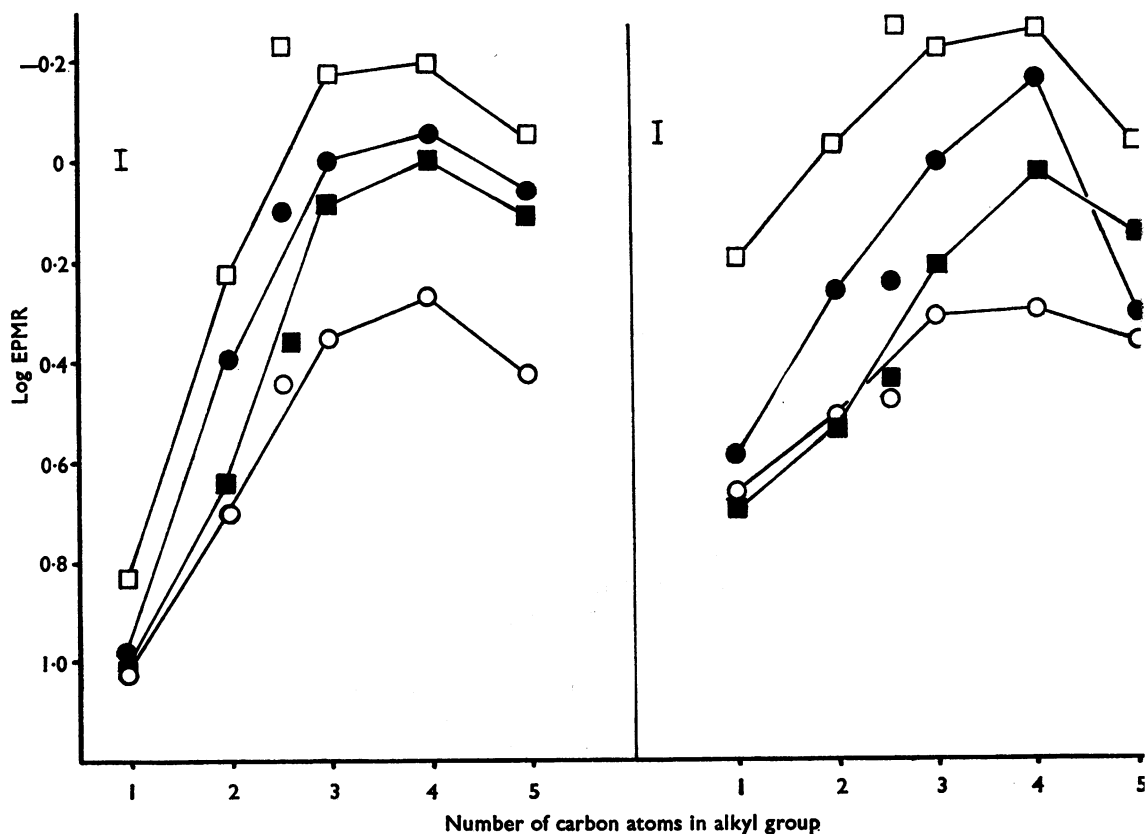


FIG. 2. Plot of activity against number of carbon atoms attached to quaternary nitrogen atom. Activity is expressed as the logarithm of the equipotent molar ratio (EPMR). Since high values of log EPMR indicate low activity, the values on the ordinate scale have been inverted. The values for the *i*-propyl compounds have been inserted to the left of those for the *n*-propyl compounds for reasons of clarity. The vertical bar indicates the size of the mean standard error for all points. Frog rectus on the left, chick biventer on the right. ○=Benzylamino series, ●=anilino series, □=*p*-tolylamino series, ■=phenethylamino series.

are illustrated in Fig. 2 which shows the relationship between activity and the number of carbon atoms in the side chain attached to the quaternary nitrogen. Examination of the plot of activity against chain length (Fig. 2), shows a similar pattern of activity for the four series of compounds on both frog and chick

TABLE 2. Partition coefficients (K) and ionization constants (pK_a) of benzotriaziniums

Nature of 2-substituent	Anilino series		<i>p</i> -Tolylamino series		Benzylamino series	Phenethylamino series
	K	pK_a	K	pK_a	K	K
Methyl	0.42	8.3	1.13	8.5	0.50	0.56
Ethyl	1.03	8.5	3.16	8.5	0.68	2.51
<i>i</i> -Propyl	2.33	8.5	33.52	—	2.71	4.88
<i>n</i> -Propyl	2.38	8.3	8.40	8.9	2.53	3.60
<i>n</i> -Butyl	15.36	8.4	25.67	—	5.33	10.50
<i>n</i> -Pentyl	33.86	—	96.22	—	13.93	27.57

K values are the means of two determinations. pK_a values are the means of three experiments: estimated error was of the order of ± 0.1 pH unit. The *n*-pentyl member of the anilino series and the *i*-propyl, *n*-butyl and *n*-pentyl members of the *p*-tolylamino series were unstable at the pH used in the determination.

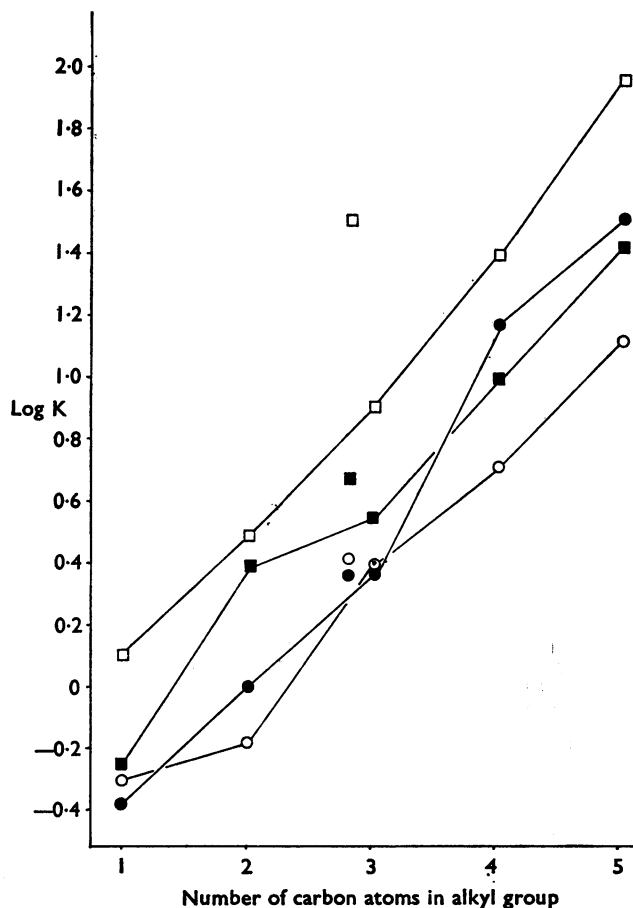


FIG. 3. Plot of log partition coefficient against number of carbon atoms attached to quaternary nitrogen atoms. The values for the *i*-propyl compounds have been inserted to the left of those for the *n*-propyl compounds for reasons of clarity. Symbols as in Figure 2.

preparations. On both preparations, in the anilino, phenethylamino and benzylamino series, activity increases with the number of carbon atoms attached to the quaternary nitrogen, up to a maximum at the *n*-butyl member. However, in the *p*-tolylamino series, the *i*-propyl member is the most active homologue on both preparations. The general order of potency between the four series, on both tissues, is: *p*-tolylamino > anilino > phenethylamino > benzylamino.

Partition coefficients and ionization constants

The shape of the u.v. spectra was the same for all the members of each series of compounds examined. The results of the chloroform/water partition coefficients (*K*) and ionization constants for those compounds investigated are shown in Table 2. The relationship between log partition coefficients and number of carbon atoms on the quaternary nitrogen is illustrated in Figure 3. The general order of log *K* between the four series is *p*-tolylamino > anilino = phenethylamino > benzylamino. The ionization constants indicate that, in the anilino series, the percentage of compound in the zwitterionic form would be 3–4% at pH 7.0, and 5–7% at pH 7.2; in the *p*-tolylamino series, the percentages would be 1–3% and 2–5% respectively. Since it was determined in pilot experiments that the zwitterionic form of the compounds is biologically inactive (presumably due to its low solubility), the low concentrations of zwitterion in the solutions tested should not seriously affect the estimates of potency. Although *pK_a* values for the benzylamino and phenethylamino series have not yet been determined due to technical problems associated with their stability at alkaline pH, preliminary experiments indicate that their values are likely to be greater than those of the anilino and *p*-tolylamino series. Therefore the percentage zwitterion formation would be much lower, and estimates of potency almost totally unaffected.

One feature of the contractions produced by all of the benzotriaziniums on both

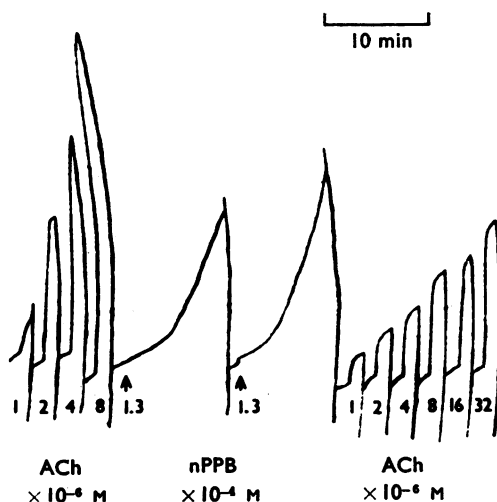


FIG. 4. Responses of the frog rectus abdominis to acetylcholine (ACh) and 4-phenethylamino-2-*n*-propyl 1,2,3-benzotriazinium iodide (nPPB). These responses were recorded isototically with a gimbal mounted side-writing lever and show the reduction in size of the acetylcholine responses after two doses of the benzotriazine. Drug contact time for acetylcholine was 90 s, for benzotriazine, 10 minutes. Concentrations refer to the final concentration in the organ bath. (Retouched trace.)

frog and chick tissues is that they are completely unaffected by concentrations of tubocurarine ($1.27 \times 10^{-5}\text{M}$ – $1.27 \times 10^{-3}\text{M}$) which cause complete block of the responses to acetylcholine ($5 \times 10^{-6}\text{M}$ – $5 \times 10^{-4}\text{M}$). It was found that contractures of the frog rectus induced by quinine ($5 \times 10^{-4}\text{M}$) and contractures of the chick biventer induced by quinidine ($1.6 \times 10^{-3}\text{M}$) were also resistant to the above concentrations of tubocurarine. Another feature of the action of the benzotriaziniums was that members of the benzylamino and phenethylamino series ($1.3 \times 10^{-4}\text{M}$) after repeated application to the frog rectus, reduced the size of the contractures produced by subsequent doses of acetylcholine (Fig. 4). Although no quantitative measurements were made, the extent of the antagonism appeared to be related to the number of doses of benzotriazinium applied. Representatives of the anilino and *p*-tolylamino series ($2.5 \times 10^{-5}\text{M}$ – $1 \times 10^{-4}\text{M}$) did not produce any block of subsequent acetylcholine contractures.

Discussion

The results of the potency determinations and partition coefficient measurements indicate that there is some association between lipid solubility and activity on both tissues used. It is clear that lipid solubility increases as the alkyl group attached to the quaternary nitrogen is lengthened in each benzotriazinium series, and that lipid solubility is associated with the biological activity of the compounds.

Alkyl groups are electron donating, and when they are added to the aromatic group attached to the exocyclic nitrogen atom, electron density on this nitrogen is increased. The order in which electron density increases is: phenethylamino > benzylamino > *p*-tolylamino > anilino. The order of activity, however, is *p*-tolylamino > anilino > phenethylamino > benzylamino, so there is no obvious correlation between biological activity and the inductive effect.

When it was observed that the benzylamino series was less active than the anilino series, it was expected that the phenethylamino series, in which the benzene ring is further separated from the benzotriazine ring, would be even less active. However, the activity of the phenethylamino series was almost the same as the anilino series.

Baker & Ho (1966) noted a similar order of activity in a series of substituted dihydro-s-triazines. They found that a benzyl derivative showed much weaker binding activity to dihydrofolic reductase than the phenyl derivative, but that the corresponding phenethyl derivative was equipotent with the phenyl analogue.

Table 2 shows that the *i*-propyl homologue in the *p*-tolylamino series has a high partition coefficient by comparison with those of the other three series. This was unexpected, since it had been thought that the partition coefficients of the *i*-propyl and *n*-propyl compounds would be very similar. It may be that the high partition coefficient of the *i*-propyl member of this series is in some way related to the rather high activity exhibited on the frog and chick muscles.

It seems unlikely that the benzotriaziniums produce contracture in these tissues by activating acetylcholine receptors because their responses are unaffected by high concentrations of tubocurarine. Also, it is unusual for the activity of a homologous series of quaternary ammonium compounds acting on acetylcholine receptors to increase with the size of the alkyl group attached to the quaternary nitrogen atom.

It may be that the benzotriazinium compounds produce their effects by mechan-

isms similar to those of quinine and quinidine. As well as producing tubocurarine-resistant contractions of the frog and chick muscle, the benzotriazines cause facilitated responses in curarized rat diaphragm muscle stimulated directly (Cull & Scott, unpublished observations) a property which is also exhibited by quinine and quinidine, and which is attributed to a prolongation of the active state of muscle contraction (Lammers & Ritchie, 1955). It would be interesting to prepare series of *N*-alkyl derivatives of quinine and quinidine, to see if these compounds showed similar changes in biological activity with changes in chemical structure.

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