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The ganglion blocking activity of diastereoisomeric dimethylaminobornyl acetates and their methiodides

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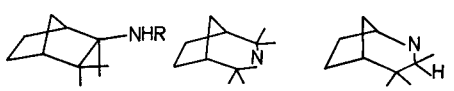
Some diastereoisomeric dimethylaminobornyl acetates and their methiodides have been prepared and tested for ganglion blocking activity. Included in these compounds was an enantiomeric pair and associated quaternary salts. These optical antipodes displayed virtually no difference between their actions at the ganglion. Differences between the activities of the least and most potent diastereoisomers was limited to a factor of about five. Assays were made upon the cat superior cervical ganglion and also the guinea-pig vas deferens preparation the successful quantitative use of which is described.

In 1956, Stone, Torchiana & others reported that mecamylamine (I, R = Me) possessed ganglion blocking activity comparable with hexamethonium. This finding suggested that secondary amines might prove to be classes of compounds with biological activities comparable with those of bis quaternary ammonium salts of the hexamethonium type. Further work (Lee, Wragg & others, 1958) on isomers II and III of mecamylamine and normecamylamine (I, R = H) led to investigations on simpler six membered structures where it was found that 1,2,2,6,6-pentamethyl piperidine (pempidine) exhibited ganglion blocking activity to a high degree (Corne & Edge, 1958; Spinks, Young & others, 1958; Spinks & Young, 1958).



I II III

With the activity of these amines established it was decided to compare the biological activities of three diastereoisomeric dimethylaminobornyl acetates (1–3) and their methiodides (1M–3M). To enlarge the scope of the present work the enantiomers (4 and 4M) of the *trans* isomers (3 and 3M) were also prepared and studied.

These compounds combine bridge-ring features associated with high affinity together with an acetoxy group, present in the transmitter substance acetylcholine. Possibly this would lead to compounds with high activity. From the affinities of the compounds it might, in addition, be possible to make deductions about how they are bound to receptors. The molecules are rigid and so changes in affinity are unlikely to arise from any change in the preferred conformation of the drug, only from differences in ability to fit groups in the receptor.

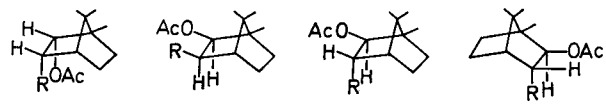
CHEMISTRY

The preparation of the three diastereoisomeric dimethylaminobornyl acetates (1–3) derived from 1*R*, 4*R*-(+)-camphor has been described elsewhere (Chittenden & Cooper, 1970).

Compound (4). Enantiomer of (3). This was prepared from 1*S*, 4*S*-(−)-camphor by an exactly similar procedure to that used in the preparation of (3). Hydrochloride m.p. 279–281° (put on at 270°). (Found: C, 61·0; H, 9·5; N, 5·0; $C_{14}H_{26}NO_2Cl$ requires C, 61·0; H, 9·5; N, 5·1%.) $[\alpha]_D$ − 9·0°.

Quaternary methiodides (1M–4M). The free, tertiary bases (1–4) were dissolved in acetone to which a little sodium bicarbonate had been added. A 3 molar excess of methyl iodide was added and the mixture refluxed for 5 h. The inorganic material was filtered off and ether added to precipitate the quaternary salts. These were crystallized from an ether–methanol mixture.

The formulae of the compounds studied are shown in Fig. 1 and the physical constants of the methiodides are listed in Table 1.



1 2 3 4

FIG. 1. Formulae of compounds.

Compound No. Compounds derived from 1*R*, 4*R*-(+)-camphor

1

1 R = NMe_2: *cis*, *endo*-2-acetoxy-3-dimethylaminobornane hydrochloride.
1M R = N^+Me_3: *cis*, *endo*-2-acetoxy-3-dimethylaminobornane methiodide.

2

2 R = NMe_2: *cis*, *exo*-2-acetoxy-3-dimethylaminobornane hydrochoride.
2M R = N^+Me_3: *cis*, *exo*-2-acetoxy-3-dimethylaminobornane methiodide.

3

3 R = NMe_2: 2-*exo*-acetoxy-3-*endo*-dimethylaminobornane hydrochloride.
3M R = N^+Me_3: 2-*exo*-acetoxy-3-*endo*-dimethylaminobornane methiodide.

Compounds derived from 1*S*, 4*S*-(—)-camphor.

4

4 R = NMe_2: 2-*exo*-acetoxy-3-*endo*-dimethylaminobornane hydrochloride.
4M R = N^+Me_3: 2-*exo*-acetoxy-3-*endo*-dimethylaminobornane methiodide.

Table 1. *Physical constants of the methiodides of the dimethylaminobornyl acetates studied.*

Methiodide	M.p.	$[\alpha]_D$ (*c* in EtOH)	Microanalysis* C	H	N	Yield (%)
1M	259–261°	—17·9 (2·07)	47·55	7·1	3·8	70
2M	248–249°	+22·9 (1·88)	47·0	7·1	3·6	35
3M	185–187°	+ 7·6 (2·1)	47·3	7·15	3·8	82
4M	186–188°	— 5·1 (2·45)	47·6	7·5	3·6	45

* $C_{15}H_{28}NO_2I$ requires C, 47·25; H, 7·4; N, 3·7%.

PHARMACOLOGY

The compounds were tested on preparations containing parasympathetic ganglia (guinea pig isolatedileum) and sympathetic ganglia (stimulated guinea-pig vas deferens and cat superior cervical ganglion preparations) and for their ability to stimulate

receptors in the neuromuscular junction (the isolated semispinalis preparation of the chick).

Guinea-pig isolated ileum

Approximately 2 cm of ileum was taken from a freshly killed guinea-pig at a point 5 cm from the ileo-caecal junction. This was suspended in a 5 ml organ bath containing Ringer Tyrode solution at 37° gassed with 5% carbon dioxide in oxygen. Contractions of the ileum were recorded on a kymograph, using a frontal lever, the magnification being approximately 8:1. The compounds were tested for their ability to depress submaximal contractions produced by the following agonists added to the bath: acetylcholine, nicotine, 5-hydroxytryptamine and barium chloride. The test compound was added to the bath 1 min before the agonist.

Guinea-pig isolated vas deferens preparation, stimulated through the hypogastric nerve and transmurally

Male guinea-pigs, 400–800 g, were stunned by a blow on the head and bled. The vas deferens was dissected (Huković, 1961) and set up on a Perspex holder in a 50 ml bath, containing McEwan solution (1956) maintained at 29° and gassed with 5% carbon dioxide in oxygen. Conventional silver electrodes (unshielded) were used to stimulate the hypogastric nerve and parallel wire electrodes were used for transmural stimulation (Birmingham & Wilson, 1963). For both types of stimulation the stimulus parameters were identical at a frequency of 25 Hz for 10 s and a pulse width of 0·1 ms. Supramaximal voltage was usually 80–100 V for transmural stimulation and 25–40 V for the hypogastric nerve. Contractions were recorded by means of a Devices 2LDO1 linear displacement optical wedge transducer connected to a Smiths Servoscribe pen recorder. For quantitative studies the nerve was stimulated every 3 min, and the preparation stimulated transmurally once before, and 12 min after the addition of the drug to the bath (see Fig. 2). The reduction in response to nerve

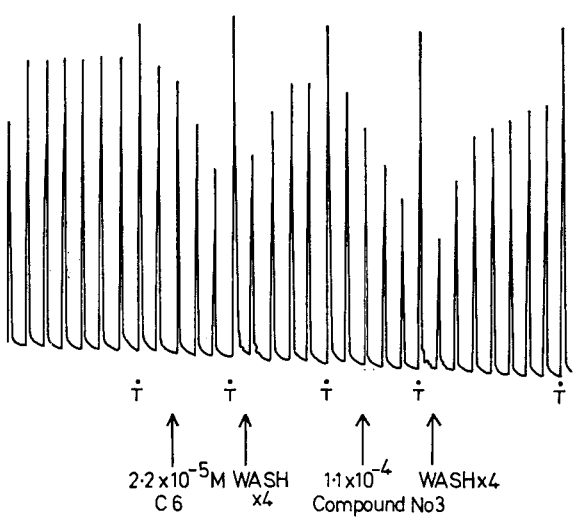


FIG. 2. Guinea-pig isolated vas deferens preparation. Part of record obtained during bioassay of 2-*exo*-acetoxy-3-*endo*-dimethylaminobornane HCl (Compound No. 3) against hexamethonium (C_6). All contractions were elicited by stimulation of the hypogastric nerve for a period of 10 s. T indicates contractions elicited by transmural stimulation. A time cycle of 3 min was used between each stimulation.

and transmural stimulation was measured 9 and 12 min respectively after contact with the drug, and plots of percentage reduction in response against log doses were made. In some cases reduction in response to nerve stimulation was also measured 30 min after contact with the drug. Two doses were chosen lying on the straight line portion of the log dose response curve, and a latin square 2 × 2 point assay was made using hexamethonium as standard drug. As full recovery did not always occur after washing out, it was not possible to complete the latin square (16 doses) on a single preparation; results from two or three preparations were combined to complete the procedure.

Cat nictitating membrane (superior cervical ganglion) preparation

13 cats, 2–2·5 kg, were anaesthetized with chloralose (0·1 g/kg intravenously) after induction by ether. The assay procedure for determining the ganglion blocking activity was essentially that described by Fakstorp & Pederson (1954). The trachea was intubated and a deep cervical well was prepared by forward reflection of the oesophagus and larynx. The cervical sympathetic trunk was dissected free from the vagus nerve and cut at a point approximately 2 cm caudal to the superior cervical ganglion. The cervical well was filled with liquid paraffin and for preganglionic stimulation the cut cervical sympathetic nerve was stimulated by means of bipolar silver electrodes. The nerve was stimulated once every 2 min with stimulus parameters of 25 Hz for 6 s and a pulse width of 0·25 ms. Voltage was adjusted to give a supramaximal contraction which was obtained between 3–5 V. The resting tension in the nictitating membrane was 4 g and contractions were recorded in a manner similar to those of the isolated vas deferens preparation. The compounds were injected intravenously into the femoral vein and the maximum reduction in height of the nictitating membrane was measured. Dose levels giving approximately 25 and 50% reduction in height were used in a latin square 2 × 2 point assay using hexamethonium as standard. The drugs were injected only when the nictitating membrane contractions had returned to their original height. One assay could be completed on one preparation as full recovery from doses of the aminobornylacetates and hexamethonium occurred within 20 and 30 min respectively. The results obtained from two preparations in the assay of mecamylamine against hexamethonium were combined owing to the lengthy recovery time for mecamylamine (80–100 min).

The effects of the compounds on contractions evoked by post-ganglionic stimulation were also studied at dose levels that reduced by 50% contractions evoked by preganglionic stimulation. The post-ganglionic trunk was exposed approximately 5 mm from the ganglion and a monopolar silver electrode was hooked under the nerve; the indifferent electrode was placed on one of the neck muscles. The stimulus parameters used were identical to those used for preganglionic stimulation.

Isolated semispinalis muscle of the chick

The semispinalis muscle was removed from 3–10 day old chicks anaesthetized with ether (Child & Zaimis, 1960). Recordings of the contractions of the muscle were made in the manner described for the guinea-pig ileum. Submaximal contractions were produced by 2 μM solution of nicotine and the test compounds were studied for their ability to produce contractions relative to nicotine.

Drugs

Mecamylamine hydrochloride was purchased from Merck Sharpe and Dohme Ltd., and hexamethonium bromide was purchased from May & Baker Ltd.

RESULTS

Effects on guinea-pig isolated ileum

At a relatively high bath concentration of 100 μM the eight aminobornyl acetate compounds did not produce contraction of the ileum, neither did they affect contractions produced by acetylcholine, 5-hydroxytryptamine and barium chloride. At a bath concentration of 10 μM they reduced the contraction produced by nicotine, demonstrating that all the aminobornyl acetate compounds possessed ganglion blocking activity. The guinea-pig ileum proved inadequate for precise quantitative estimates of ganglion blocking activity as the degree of reproducibility of assays was poor owing mainly to the preparation failing to contract consistently under stimulation by nicotine. It has been reported (Fakstorp & Pederson, 1954) that 1,1-dimethyl-4-phenylpiperazinium (DMPP) is a more suitable agonist than nicotine when assaying for ganglion blocking activity on the ileum. In our experience, however, DMPP has not shown any advantage over nicotine.

Effects on guinea-pig isolated vas deferens

Birmingham & Wilson (1963) published convincing pharmacological evidence for the presence of ganglia in the hypogastic nerve. In the present work compounds under investigation were assayed for their ganglion blocking activity by reducing the contractions of the vas deferens elicited by preganglionic stimulation of the hypogastric nerve. The results are shown in Table 2 where activities of the compounds

Table 2. *Ganglion blocking activity of compounds studied.*

	Equipotent molar ratios relative to hexamethonium with 95% confidence limits		
	Guinea-pig isolated vas deferens prepn		
Compound	9 min Contact time	30 min Contact time	Cat superior cervical ganglion
1	3·57 (2·3 –4·17)	3·03 (2·17–3·84)	7·7 (4·54 –11·1)
1M	3·03 (2·27–4·17)	3·03 (2·7 –3·45)	7·7 (6·67 – 9·1)
2	4·00 (1·27–9·10)	—	12·5 (8·3 –20·0)
2M	1·37 (0·61–2·27)	—	4·0 (3·85 – 4·17)
3	5·00 (3·70–7·70)	—	20·0 (16·7 –25·0)
3M	2·33 (1·69–3·13)	—	6·3 (4·17 – 8·3)
4	5·56 (4·17–6·67)	—	16·7 (11·1 –25·0)
4M	2·70 (1·96–3·70)	—	4·76 (4·17 – 5·56)
Mecamylamine	0·31	—	1·17 (0·93 – 1·44)

are expressed as equipotent molar ratios relative to hexamethonium. Ganglion blocking activity was assayed against hexamethonium and not mecamylamine as standard because the latter drug proved very difficult to wash out of the preparation and has been classified as a non-competitive antagonist (Rossum & Ariens, 1959; Trendelenburg, 1961).

A portion of a record obtained in the assay procedure (Fig. 2) shows that after contact with the drug a reduction in contraction proceeds with time but the contractions showed no signs of reaching a plateau within 9 min. In any assay procedure

it is advisable to measure the reduction in response at the plateau where the drug has reached equilibrium with the receptor, since measurements at the plateau eliminate differences in activity caused by different diffusion rates. It was found that with the aminobornyl acetates a plateau of the contractions was reached after a 25–30 min contact time, and slopes of log dose response curves were reasonably parallel to those obtained with hexamethonium (a known competitive antagonist). These findings indicate that the aminobornyl acetate compounds appear to be acting as competitive antagonists. A comparison of the results obtained with compounds 1 and 1M assayed at contact times of 9 and 30 min showed no significant difference, and the use of a 9 min contact time for the assay of all the other compounds was therefore considered justified. The use of a 30 min contact time throughout would have been time consuming and results would have had to be combined from a number of preparations.

The vas deferens preparations was also used to study the degree of specificity of ganglion blockade by stimulating the preparation preganglionically through the hypogastric nerve and postganglionically by stimulation transmurally. Dose response curves for pre- and post-ganglionic stimulation obtained in the assay of compound 1 and 1M are shown typically in Fig. 3. The tertiary aminobornyl acetates were

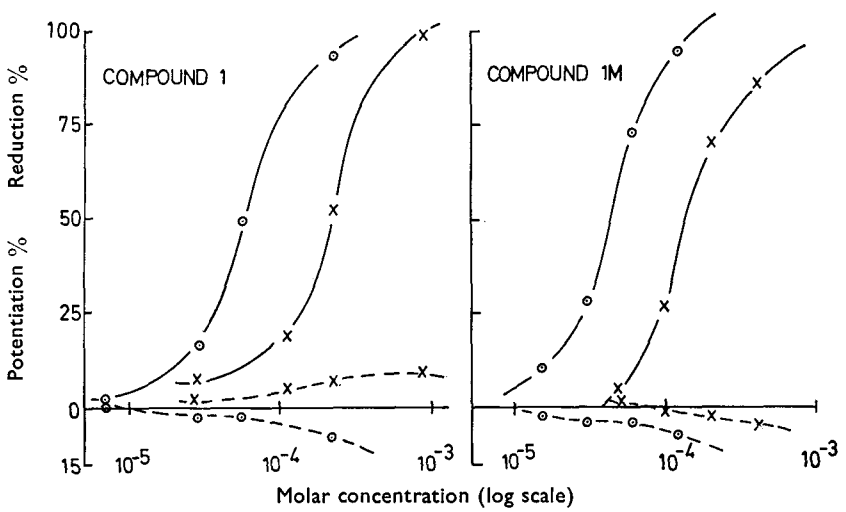


FIG. 3. Dose response curves obtained in bioassay of *cis-endo*-2-acetoxy-3-dimethylaminobornane HCl (Compound 1) and its methiodide (Compound 1M) against hexamethonium using guinea-pig isolated vas deferens preparation. (×——×) preganglionic stimulation with test compounds. (⊙——⊙) preganglionic stimulation with hexamethonium. (×— — —×) postganglionic (transmural) stimulation with test compounds. (⊙— — —⊙) postganglionic stimulation with hexamethonium.

slightly less specific in their ganglion blocking activity than were their respective methiodides. This was demonstrated by the tertiary compounds producing a slight depression of contractions elicited by post-ganglionic stimulation whereas no depression, but sometimes a slight potentiation, was observed with the quaternary compounds.

Effects on cat superior cervical ganglion

Table 2 shows the ganglion blocking activities measured using this type of assay. Experiments using post-ganglionic stimulation to test the specificity of the compounds corroborate the results obtained on the vas deferens preparation. Thus, no reduction in response to post-ganglionic stimulation was obtained with the methiodides whereas a 5–7% reduction in response was manifested by the tertiary bases.

Effects on chick isolated semispinalis muscle

None of the compounds produced a contracture when given at a 100 μM bath concentration, thus demonstrating the lack of any depolarizing action at the neuromuscular junction (Child & Zaimis, 1960).

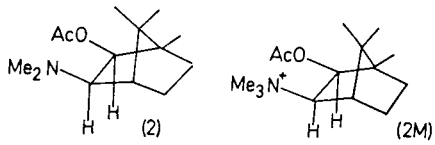
DISCUSSION

The results show that all of the eight compounds tested are weak, but specific, ganglion blocking agents and indicate a competitive type of action. When compared with those obtained from the vas deferens preparation, the individual equipotent molar ratios (EMR's) derived from the nictitating membrane vary by a factor of between two to four. However the EMR's obtained by the two techniques are significantly related by a correlation coefficient of 0·99 although the cat nictitating membrane preparation is less sensitive to these compounds than the guinea-pig vas deferens.

With the exception of the *cis, endo* compound (1), there is a significant increase in potency upon quaternizing each of the tertiary aminoacetates, the weakest quaternary salt being more potent than the strongest tertiary compound. By both assay techniques the most potent compound is the quaternary salt (2M); the least potent is compound (4) (from the vas deferens results) or compound (3) (from the nictitating membrane). The ratio between the least potent and most potent is 4·1 (vas) or 5·0 (nictitating membrane). The low values for this ratio (bearing in mind the biological variation shown by the 95% confidence limits) probably reflects the comparative unimportance of stereochemical variations in this group of compounds.

Similarly, Stone, Torchiana & others (1962) noted only small differences between the gangliolytic activity of the *exo* and *endo* epimers of (±)-mecamylamine (*ca* 1·5, based on antagonism to nicotine convulsions in mice). In addition they found no difference between the (+)- and (±)-forms of mecamylamine. Our observations upon the activities of the pairs of enantiomers (3) and (4), (3M) and (4M), confirm, as with mecamylamine, the lack of enantiomeric dependence of ganglion blocking activity in bicyclo (2.2.1) heptane compounds.

Larger differences in activity between isomers have been recorded for compounds whose actions at ganglia proceed via a depolarizing mechanism. For example, the enantiomers of nicotine (Barlow & Hamilton, 1965) display a difference of about fourteen between their equipotent molar ratios derived from the cat superior cervical ganglion. Acetylcholine itself has a depolarizing action at the ganglion and it could be argued that in converting the tertiary dimethylaminobornyl acetates to their methiodides, e.g. (2) to (2M), structures more closely aligned with acetylcholine



would be produced. In consequence a depolarizing action at the ganglion could well have been expected together with a definite stereochemical dependence of blocking activity. The results of the chick semispinalis muscle and guinea-pig ileum experiments suggest that the present compounds are not operating via a depolarizing mechanism as no contractions occurred, and the small stereochemical dependence of ganglion blockade is perhaps not surprising.

In the compounds tested there is only a small dependence of gangliolytic activity upon stereochemical variation. This small dependence of the cholinergic receptor in sympathetic ganglia upon the chiral properties of the antagonist is reflected in the small difference in the activities of the enantiomers of trimetaphan. The (+)-isomer is only about twice as active as its enantiomer (Randall, Peterson & Lehman, 1949). In view of such observations it is likely that the drug-receptor complex in such ganglia is not asymmetric to any observable extent. With molecules like pempidine and mecamylamine the presence of large groups close to the onium nitrogen atom appear to be important for high affinity at the ganglion receptor (Barlow 1964). The redistribution of the methyl groups from the 2- and 3-positions in mecamylamine to the 1- and 7-position in the present compounds, together with the introduction of the acetate function has resulted in an overall decrease in activity.

Early work on the Huković isolated nerve vas deferens preparation was based on the assumption that the hypogastric nerve contains mainly post-ganglionic nerve fibres. Sjöstrand (1962) and Birmingham & Wilson (1963) provided evidence which suggested that this assumption was invalid and concluded that contractions in the Huković preparation were initiated mainly by stimulation of preganglionic fibres. This strongly suggested the presence of ganglionic synapses in the hypogastric nerve. The significant correlation demonstrated for ganglion blocking activity by the two procedures used in the present study confirms pharmacologically the presence of such synapses in the hypogastric nerve of the guinea-pig. To date the guinea-pig vas deferens preparation has been used only qualitatively but the results presented in this paper show that this preparation may be used quantitatively.

Acknowledgement

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