THE GANGLION-BLOCKING ACTIVITY OF AMINOBIICYCLO-[2,2,1]HEPTANES (CONGENERS OF MECAMYLAMINE) AND BICYCLO[3,2,1]AZAOCCTANES (BRIDGED CONGENERS OF PEMPIDINE)

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The structural requirements for strong ganglion-blocking activity and long duration of action amongst some lower homologues of mecamlamine, together with the discovery of similar activities amongst isomers with enlarged ring structures, are described. In both series of compounds it was found that the successive introduction of *C*-methyl groups surrounding the nitrogen atom resulted in a progressive increase in ganglion-blocking activity and duration of action.

The pharmacology of the potent ganglion-blocking compound pempidine, 1,2,2,6,6-pentamethylpiperidine, has recently been described (Corne and Edge, 1958; Spinks, Young, Farrington, and Dunlop, 1958).

The independent discovery of the properties of this compound by Spinks and Young (1958) originated from their observation that secondary and tertiary aliphatic amines in which the basic group is sterically hindered by attachment to a tertiary carbon atom have a modest level of ganglion-blocking activity.

By contrast, we first studied structure-activity relationships amongst certain aminobicyclo[2,2,1]-heptanes which were lower homologues of

which the nitrogen atom was endocyclic, instead of exocyclic. Structure-activity relationships amongst these new compounds led us to discover the ganglion-blocking activity of the structurally simpler, unbridged compound pempidine. This work, which has already been very briefly outlined (Lee, Wragg, Corne, Edge, and Reading, 1958), is now reported in some detail.

METHODS

Chemical

The first four isomers of mecamlamine examined in the present work (Table I) were prepared as follows: *N*-methyl-*d*(-)-isobornylamine was prepared by the method of Wegler and Frank (1936). *N*-methyl-*d*(+)-bornylamine was prepared by the method of Forster (1899). *N*-methylfenchylamine was prepared in 20% yield by the Leuckhart reaction on fenchone. Hydrochloride sublimed >300°. (Found: C, 64.4; H, 10.8; N, 6.9. Required for C₁₁H₂₁N, HCl: C, 64.8; H, 10.8; N, 6.87.) *N*-methylcamphidine was prepared by the method of Auwers (1868).

Most of the remaining compounds were prepared from nitrobicyclo[2,2,1]heptenes obtained via classical Diels-Alder reactions. Mild catalytic reduction of these intermediates gave the corresponding nitrobicyclo[2,2,1]heptanes. Catalytic reduction under more drastic conditions gave the expected aminobicyclo[2,2,1]heptanes, which could be *N*-alkylated by well-established methods to give products which undoubtedly had the structures shown as (V), (VII), and (IX) in Table II (Lee and Wragg, 1960a).

aminobicyclo-
[2,2,1]heptanes.
N atom is exocyclic

bicyclo[3,2,1]azaoctanes.
N atom is endocyclic

mecamlamine. During this work we unexpectedly encountered a chemical route to bicyclo[3,2,1]azaoctanes, isomeric compounds in

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The compounds (X; $R_1 = -C_6H_5$, $R_2 = -NH.CH_3$, $-N(CH_3)_2$, or $-NH.CH_2.C_6H_5$) and (X; $R_1 = \text{cyclohexyl}$, $R_2 = -NH.CH_3$) were obtained similarly. The compounds (XI; $R_1 = R_2 = -CH_3$, $R_3 = -NH_2$) and (XI; $R_1 = -C_6H_5$, $R_2 = -H$, $R_3 = -NH.CH_3$) were synthesized via reactions between the appropriate nitrobicyclo[2,2,1]heptanes and formaldehyde. The compound (IV) in Table II was made by reducing the corresponding nitro-derivative chemically.

We were able to compare some of the foregoing aminobicyclo[2,2,1]heptanes (congeners of mecamlamine), in which the nitrogen atom is exocyclic, with the corresponding isomeric bicyclo[3,2,1]azaoctanes (structures (VI) and (VIII), in Table II; bridged congeners of pempidine), in which the nitrogen atom is endocyclic, as a result of a discovery made during a concurrent systematic study (Lee and Wragg, 1960b) of the aluminium lithium hydride reduction of crude 3-nitroisocamphane (XII). The basic fraction of the product mainly consisted of a single primary amine (M & B 4333) and, quite unexpectedly, two secondary amines (M & B 5561 and M & B 5562), all three of which were isomeric with the expected product (XIII).

The primary amine did not have the structure (XIII) because its *N*-methyl derivative (M & B 4334) was not identical with mecamlamine (I, Table II) (compare Stein, Slettinger, Arnold, Reinhold, Gaines, and Pfister, 1956); moreover, both M & B 4333 and M & B 4334 were inactive when tested for ganglion-blocking activity and their actual chemical structures have not been investigated.

On the other hand, chemical evidence (Lee, Lunt, Wragg, and Barber, 1958; Lee, Wragg, Corne, Edge, and Reading, 1958) revealed that the two secondary amines had the structures (II; $R=H$) and (III; $R=H$) in Table II or geometrical isomers thereof. On the basis of chemical evidence, infra-red spectral data and the estimates of ganglion-blocking activity (see Results), we have provisionally assigned the structure (II; $R=H$) to M & B 5561 and the structure (III; $R=H$) to M & B 5562. Both compounds were *N*-methylated, giving M & B 5199 (II; $R=CH_3$) and M & B 5200 (III; $R=CH_3$) respectively, both of which are isomeric with

mecamlamine. In practice, the two secondary amines formed in the aluminium lithium hydride reduction of (XII) were isolated as a mixture of 85% M & B 5561 and 15% M & B 5562, which could be separated into its components only by preparative gas chromatography. The unseparated mixture (M & B 4364) upon *N*-methylation gave M & B 4348A which consisted of 75% of the tertiary amine, M & B 5199, and 25% of its isomer, M & B 5200. M & B 4348 obtained in another experiment consisted of 55% M & B 5199 and 45% M & B 5200. These mixtures were studied as such because their separation into pure components by preparative gas chromatography was feasible only on a small scale.

We then proceeded to reduce with aluminium lithium hydride three nitrobicyclo[2,2,1]heptanes which had previously been reduced catalytically. Gas chromatography indicated that these reductions, in contrast to similar reduction of crude 3-nitroisocamphane (XII), each yielded only one product. The resulting single secondary amines were then *N*-methylated. Chemical and infra-red spectral evidence, together with the levels and duration of ganglion-blocking activity observed, led us to assign provisionally the symmetrical ring-enlarged structures (VI and VIII, analogous to II) (Table II) to the products thus obtained.

Pharmacological

The methods used were as described by Corne and Edge (1958), with the following exceptions and additions:

Most of the determinations of ganglion-blocking activity on the superior cervical ganglion-nictitating membrane preparation of the anaesthetized cat were performed using continuous (10/sec.) supramaximal pre-ganglionic stimulation. A few determinations were performed using intermittent (50/sec. for 5 sec./min.) stimulation. Several of the compounds had a short duration of action (comparable to that of hexamethonium). Greater reliance is placed on the potency estimates assigned to these compounds than to those with a long duration of action (comparable to that of mecamlamine), whose potency is difficult to estimate (Corne and Edge, 1958).

Acute oral and intravenous toxicities were studied in groups of 5 or 10 mice. Mydriatic responses in groups of 5 mice were observed after intraperitoneal, as well as oral, administration. Urinary excretion determinations were performed as described by Muggleton and Reading (1959).

The compounds were studied as the hydrohalides or acetates and all activity and toxicity determinations are expressed in terms of the cations. Other compounds used were hexamethonium bromide, acetylcholine chloride, adrenaline hydrochloride, histamine acid phosphate, nicotine hydrogen tartrate, and pilocarpine nitrate. With the exception of hexamethonium and adrenaline, all doses and concentrations of these compounds are expressed in terms of the salts.

RESULTS

Four Structural Isomers of Mecamylamine

The activity of these compounds when tested on the pre-ganglionically stimulated nictitating membrane of the cat was only 4 to 7% of that of hexamethonium (Table I). None of these results was thought worth following up. A compound with an identical planar formula to *N*-methyl-*d*(-)-isobornylamine and *N*-methyl-*d*(+)-bornylamine but having an unspecified steric structure

TABLE I

BLOCKING ACTIVITY OF 4 ISOMERS OF MECAMYLAMINE ON THE PRE-GANGLIONICALLY STIMULATED NICTITATING MEMBRANE OF THE CAT

Compound	Structure	Activity (Hexamethonium = 100)
<i>N</i> -Methyl- <i>d</i> (-)-isobornylamine		5
<i>N</i> -Methyl- <i>d</i> (+)-bornylamine		4
<i>N</i> -Methylfenchylamine		7
<i>N</i> -Methylcamphidene		6

was reported by Rubinstein, Pedersen, Fakstorp, and Rønnov-Jessen (1958) to have a weak sympathetic ganglion-blocking activity and to be mildly hypertensive in the anaesthetized cat. A compound with an identical planar formula to *N*-methylfenchylamine but prepared by an unspecified route was also reported by Rubinstein *et al.* (1958) to have appreciable sympathetic ganglion-blocking activity. As our compound was short-acting and that studied by Rubinstein *et al.* was long-acting, it is unlikely that the two were identical chemically.

Aminobicyclo[2,2,1]heptane Derivatives (N Atom Exocyclic, as in Mecamylamine)

All of the phenyl and cyclohexyl derivatives of structures (X) and (XI) above had a very low activity, less than 3% of that of hexamethonium.

The ganglion-blocking activity, duration of action, and oral and intravenous toxicity of the nor derivatives of mecamylamine (structures IV, V, VII, and IX) are shown in Table II. Of these compounds, only two (M & B 4086 and M & B 4620) had a prolonged duration of action on the pre-ganglionically stimulated nictitating membrane, and M & B 4086 was selected for a brief examination of its mode of action.

Properties of M & B 4086.—Mecamylamine and M & B 4086 act specifically at the ganglion since a large intravenous dose (8 mg./kg.) of either compound completely inhibited the effect of pre-ganglionic stimulation on the nictitating membrane, but had no inhibitory action on post-ganglionic stimulation or on intravenously injected adrenaline (Fig. 1).

On the isolated guinea-pig ileum, M & B 4086 in a concentration of 0.08 mg./ml. completely inhibited the effect of nicotine-induced contractions and caused a slight reduction in the response to acetylcholine but had no effect on histamine- or pilocarpine-induced contractions. The nicotine response was slow to recover after removal of the antagonist from the bath. A concentration of 0.8 mg./ml. had an initial stimulant action and blocked the effect of all four agonists.

M & B 4086 had about 50% of the activity of, but a similar duration of action to, mecamylamine when compared by the mydriatic response in mice after intraperitoneal injection.

An oral dose of 5 mg./kg. in a group of six rats showed an identical urinary excretion pattern with that shown by mecamylamine over a period of 24 hr. Thus recoveries of M & B 4086 at 2, 4, 6, and 24 hr. were 3.3, 9.6, 16.5, and 48.7% respectively. The corresponding figures for mecamylamine were 1.3, 8.8, 15.2, and 45.6%.

TABLE II

BLOCKING ACTIVITY ON THE PRE-GANGLIONICALLY STIMULATED NICTITATING MEMBRANE OF THE CAT AND TOXICITY IN MICE OF SOME AMINOBICYCLO[2,2,1]HEPTANE AND BICYCLO[3,2,1]AZAOCANE DERIVATIVES

VS=Very short duration of action (shorter than hexamethonium). S=Short duration of action (similar to hexamethonium). M=Medium duration of action (longer than hexamethonium, shorter than pempidine). L=Long duration of action (similar to pempidine). Where the results were very variable, the range of activity is given in parentheses.

M & B No.	Structure	Activity (Hexamethonium = 100)	Number of Experiments	Duration of Action	LD50 mg./kg.	
					Oral	Intra-venous
	Mecamylamine ← (I)	120 (60-190)	5	L	98	12.9
5561	(II)	134 (85-170)	4	L		29.9
5199		100 (50-200)	5	L		28.3
5562	isomers (III)	75	3	VS		30.3
5200		40 (25-50)	5	M		32.5
4364	Mixture of 85% M & B 5561 and 15% M & B 5562	140 (120-190)	5	L	470	35.0
4348A	Mixture of 75% M & B 5199 and 25% M & B 5200	125	2	L	214	37.0
4348	Mixture of 55% M & B 5199 and 45% M & B 5200	170 (100-210)	6	L	L	
4315	(IV)	15	1	S		

TABLE II—continued

M & B No.	Structure	Activity (Hexamethonium = 100)	Number of Experiments	Duration of Action	LD50 mg./kg.	
					Oral	Intra-venous
4058	$R_1 = R_2 = H$	15	2	S	≈ 135	46.0
4086	$R_1 = CH_3, R_2 = H$ ←	35	2	L	138	33.8
4620	$R_1 = R_2 = CH_3$	50	2	L	≈ 165	18.1
4126	$R_1 = CH_2.C_6H_5, R_2 = H$	< 1	1			
4387	← isomers	50	3	L	≈ 415	48.5
4188	$R = CH_3$	25	2	S		68.5
4269	$R = C_2H_5$ ← isomers	25	2	S		34.2
4443	$R = CH_3$	25	2	S	≈ 425	109
4442	$R = C_2H_5$ ← isomers	20–25	2	S	≈ 267	54.5
4280	←	15	2	S	405	44.5

Bicyclo[3,2,1]azaoctane Derivatives (N Atom Endocyclic, as in Pempidine)

The activity, duration of action, and toxicity of these compounds (structures II, III, VI, and VIII) are shown in Table II. One of the two isomers in each of the mixtures designated M & B 4364 and

M & B 4348A had a higher activity and a longer duration of action than the other, and this finding considered together with the results obtained amongst mecamlamine congeners strengthened the chemical and infra-red spectral evidence in assigning the structures as shown. The effects of

FIG. 1.—Cat, 2.4 kg., chloralose anaesthesia. Contractions of nictitating membrane. At arrows, 10 μ g. adrenaline was injected intravenously; the continuous lines indicate pre-ganglionic stimulation at 10/sec.; the discontinuous lines indicate post-ganglionic stimulation at 10/sec.; at the black dot, 8 mg./kg. M & B 4086 was injected intravenously. Time, 60 sec.

FIG. 2.—Cats, (a) 3.25 kg., (b) 2.5 kg., (c) 3.0 kg., (d) 2.3 kg., chloralose anaesthesia. Contractions of nictitating membrane. Continuous line indicates pre-ganglionic stimulation at 10/sec. Kymograph was stopped between periods of stimulation. Intravenous doses in μ g./kg. at times shown. HEX=hexamethonium. Time, 60 sec.

these two pairs of isomers in comparison with hexamethonium on the pre-ganglionically stimulated nictitating membrane are shown in Fig. 2. When compared with hexamethonium on the pupillary diameter of the anaesthetized cat after removal of the superior cervical ganglion, M & B 5199 and M & B 5200 had activities of 250% and 70% respectively. When compared with mecamlamine in mice injected intraperitoneally, respective mydriatic activities of 100% and 50% were obtained. Some of the pharmacological properties of the mixture (M & B 4348A) of these two isomers were examined in more detail.

Properties of M & B 4348A.—An intravenous dose of 6.7 mg./kg. of M & B 4348A completely blocked the effect on the nictitating membrane of pre-ganglionic stimulation but had no effect on the contraction produced by post-ganglionic stimulation or by an intravenous injection of adrenaline. In the same preparation a further dose of 6.7 mg./kg. caused a small rise in blood pressure. In other experiments the pressor response to nicotine (0.1 mg./kg.) was reduced about 50% by a dose of 0.05 mg./kg., and 0.1 mg./kg. caused an almost total inhibition of the effect on the blood pressure of peripheral vagal stimulation. Doses of up to 6.7 mg./kg. had no effect on blood pressure responses to small doses of acetylcholine or histamine, and the response to adrenaline was potentiated. Respiratory arrest occurred after a total dose of 31 mg./kg. On the isolated Langendorff rabbit heart preparation doses of 2.0 to 8.0 mg. injected into the aortic cannula caused a slowing of the beat.

Oral doses of 3.3 mg./kg. and 4.0 mg./kg. of M & B 4348A and M & B 4364 respectively caused a marked mydriasis in mice within 10 min. of administration and the response was of prolonged duration.

On the isolated guinea-pig ileum a concentration of 0.067 mg./ml. of M & B 4348A completely inhibited the response to nicotine and reduced the response to pilocarpine, but had no effect on acetylcholine or histamine responses (Fig. 3). The compound was removed after being in contact with the gut for 14.5 min. and 50% recovery of the nicotine response occurred about 3 hr. later (Fig. 3).

On the isolated frog rectus abdominis muscle a concen-

tration of 0.67 mg./ml. inhibited responses to acetylcholine without causing a contracture.

When examined for its potency on nicotine-induced convulsions in mice M & B 4348A injected intraperitoneally was found to have an ED₅₀ of 0.065 mg./kg. The lower, less active, homologue (VI; M & B 4387) had an ED₅₀ of 0.25 mg./kg. Consequently, these two compounds had respectively 1.66 and 0.43 times the activity of mecamlamine (ED₅₀=0.108 mg./kg.) and 23 and 6 times the activity of hexamethonium (ED₅₀=1.5 mg./kg.) in this test. The greater potency of amine, as compared with quaternary, ganglion-blocking compounds in this test has been described for pempidine by Corne and Edge (1958), and Stone, Meckelnburg, and Torchiana (1958) have found that peripheral ganglion-blocking activity and anticonvulsant potency are not necessarily related in structurally dissimilar compounds.

DISCUSSION

Considering first the structure-activity relationships observed amongst mecamlamine congeners (IV, V, VII, and IX in Table II) it is apparent that the presence of a double bond in the 5,6-position had no effect on activity (compare M & B 4315 and M & B 4058). Activity was also unaffected by the substitution of a C-ethyl for a C-methyl group (compare M & B 4188 and M & B 4269). On the other hand, replacement of the N-methyl group in M & B 4086 by an N-benzyl group (M & B 4126) eliminated activity. Removal of one C-methyl group from the 3-position of mecamlamine (I) giving M & B 4280 (IX) resulted in a pronounced drop in activity and in a shortened duration of action. Rubinstein, Pedersen, Fakstorp, and Rønnev-Jessen (1958) have reported that a compound with this planar

FIG. 3.—Contractions of isolated guinea-pig ileum in a 10 ml. bath. At A, 0.15 μ g./ml. of acetylcholine; N, 5.0 μ g./ml. of nicotine; H, 0.5 μ g./ml. of histamine; P, 0.5 μ g./ml. of pilocarpine. Between arrows 67 μ g./ml. of M & B 4348A was present. Time in min. after removal of antagonist from the bath shown below nicotine responses.

formula, but prepared by an unspecified route, paralleled mecamylamine closely in sympathetic ganglion-blocking activity and in having a long duration of action. The pharmacological properties of our product make its chemical identity with the compound studied by Rubenstein *et al.* (1958) unlikely. By contrast, removal of a C-methyl group from the 2-position of (I) giving M & B 4086 (V; $R_1 = -CH_3$, $R_2 = -H$) whilst lowering activity did not shorten the duration of action. The corresponding primary amine, M & B 4058, was less active than M & B 4086, whereas the corresponding tertiary amine, M & B 4620, was similar both in activity and in duration of action to M & B 4086. A similar level of activity in related secondary and tertiary amines was later encountered again, both in the endocyclic series (for example M & B 5561 and M & B 5199), and in pempidine and the corresponding secondary amine (Corne and Edge, 1958; Spinks *et al.*, 1958).

Of the two possible structures (II; $R = -CH_3$) and (III; $R = -CH_3$) for M & B 5199, the former appears more likely because an endocyclic nitrogen atom attached on each side to alkyl-substituted carbon atoms is a grouping which, in pempidine and its congeners, has since been found to be associated frequently with a high level of ganglion-blocking activity. Since infra-red spectral analysis eliminated the possibility that M & B 5200 is a geometrical isomer of M & B 5199, it probably has the structure (III; $R = CH_3$). The fact that M & B 5200 is not only less active but also has a shorter duration of action than M & B 5199 is not incompatible with the suggested assignment of structure, because subsequent work has indicated that an adjacent bridge-end α -carbon atom is less likely to confer ganglion-blocking activity on an endocyclic nitrogen atom than is a gem-dimethyl group.

Perhaps the most significant implication of our results as a whole was that it had been possible to pass from the aminobicycloheptane structure, in which the nitrogen atom is exocyclic, to the isomeric bicycloazaoctane structure, in which the nitrogen atom is endocyclic, without loss in ganglion-blocking activity. Moreover, the biological results (Table II) on the pairs of isomers, M & B 4269 and M & B 4442, M & B 4188 and M & B 4443, M & B 4086 and M & B 4387, and mecamylamine and M & B 5199, conformed to a remarkably systematic pattern. In the first place, the individual compounds in each pair have both a closely similar level of ganglion-blocking activity and a similar duration of action, independent of whether the C-substituents are methyl or ethyl groups. Secondly, the successive introduction of

C-methyl groups into M & B 4188 or M & B 4443 results in a stepwise increase both in activity and in duration of action in the resulting homologous series.

It thus became apparent that a high level of ganglion-blocking activity and a long duration of action would probably be exhibited by (XIV) in which the introduction of methyl groups surrounding the endocyclic nitrogen atom had been taken one step further than in M & B 5199.

(XIV)

(XV)

In practice we decided to synthesize instead the corresponding unbridged compound, pempidine (XV), which did in fact exhibit high activity and prolonged action.

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