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SOME OBSERVATIONS UPON A NEW INHIBITOR OF MONOAMINE OXIDASE IN BRAIN TISSUE

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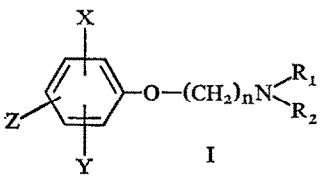
Abstract—The inhibition of monoamine oxidase (MAO) *in vitro* and *in vivo* by *N*-methyl-*N*-propargyl-3(2,4-dichlorophenoxy) propylamine hydrochloride (M&B 9302) is described. The kinetics of the MAO-M&B 9302 reaction show a unique abnormality as compared with known inhibitors. The plot of percentage inhibition against concentration of M&B 9302 does not show a simple sigmoid curve, but reveals a pair of sigmoid curves joined by a horizontal section where the inhibition is invariant. The hypothesis that in the enzyme prepared, the MAO is a binary system of enzymes each of which has a detectably different sensitivity to this particular inhibitor, is put forward and discussed. Evidence after dialysis supports this hypothesis.

DURING the examination of new potential inhibitors of monoamine oxidase in brain during 1961–1962, some compounds were found which gave unusual kinetics. It is the object of this communication to describe the behaviour of these compounds, and to present the evidence in support of the hypothesis that this enzyme in brain from rat, rabbit, dog and man is more complex than had hitherto been believed. During the course of this work, initiated about 5 yr ago, suggestions have appeared in the literature indicating that liver monoamine oxidase enzymes may be more complex than had previously been believed.[1–3]

The compounds concerned belonged to the general class represented by structure I. The member of the series which was finally selected for detailed studies is represented by structure II, and is called M&B 9302.



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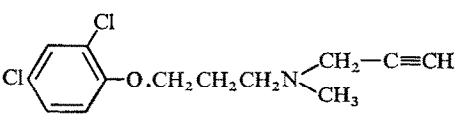

II M&B 9302 ,HCl

where X, Y, Z are mainly halogen or lower alkyl; $n = 2$–3; R_1 and R_2 are lower alkyl.

MATERIALS AND METHODS

Chemicals

M&B 9302 is *N*-methyl-*N*-propargyl-3-(2,4 dichlorophenoxy) propylamine hydrochloride. It has a molecular weight 308·5 and contains 88 per cent active cation. It is a

colourless microcrystal line solid, m.p. 115–117°, readily soluble in water. A 1·0 per cent solution has a pH of 4·4.

Reagents used were AnalaR quality unless otherwise stated.

Tests used

Two main screening tests have been used. The first is a modification of that of Maxwell, Gray and Taylor,[4] being a test *in vitro* in which a compound is examined for its inhibition of the oxidative deamination of tyramine by MAO from rat brain mitochondria. Activity is expressed either as the I_{50} (the concentration causing 50 per cent inhibition of the enzyme) or as a potency ratio (P.R.) relative to iproniazid where P.R. = I_{50} for iproniazid/I_{50} for the compound.

The second is a similar *in vitro* examination following *in vivo* administration of the drug to rats, rabbits and dogs.

Details of in vitro *test*

Female adult albino rats, killed with chloroform, were used throughout. Both M&B strain rats and Wistar and Sprague–Dawley animals obtained from outside suppliers have had to be used. Weights ranged from 80 to 250 g.

Enzyme preparation

Brains from freshly killed rats were plunged into ice-cold sucrose solution (0·25M). When cold they were mopped dry, weighed and homogenised with 4 vol. ice-cold sucrose, in a top driven MSE homogeniser at full speed for 3 min. Volume of homogenising flask was 30 ml. The pooled homogenates were then centrifuged at 800 *g* for 5 min and the precipitate was rejected (unbroken cells, cell debris, nuclei, etc.). The supernatant was then recentrifuged at 25000 *g* for 15 min and the precipitate (largely mitochondria) resuspended in a volume of phosphate buffer (Na_2HPO_4-NaH_2PO_4, 0·067M to PO_4; pH 7·2) twice that of the original weight of brain (ml/g). The suspension was then 'aged' at 37° for ½ hr and stored at 4° as the stock enzyme mitochondrial preparation.

In vitro *estimations*

The I_{50} (i.e. the concentration of compound required to produce 50 per cent inhibition of the mitochondrial preparation) was measured by a standard Warburg technique. (Final phosphate buffer concentration 0·067M, pH 7·2; final substrate (tyramine) concentration 0·009M; gas phase—oxygen and temperature 37°.) Flasks were shaken at 37° for half an hour before 'tipping' and commencing readings. In the *in vitro* test this allows iproniazid inhibition to develop, in the *in vivo* test it reduces the no-substrate blank, which would otherwise be rather high. The activity of the preparation was ascertained for each run using an enzyme blank; at least two flasks containing known concentrations of iproniazid were included in each set of flasks. This latter checked the I_{50} for iproniazid—the reference compound—which was found to vary slightly between experiments. The remaining flasks in the run contained selected dilutions of the substance under test. The I_{50}'s of these were obtained from a plot of percentage inhibition against concentration. As mentioned, estimates of the potency ratio (P.R.) of the compound were obtained for each Warburg run using the formula P.R. — (I_{50} for iproniazid)/(I_{50} for compound under test).

In vivo *injection followed by* in vitro *estimation*

Rats were injected with the compound under test dissolved in saline, water or dimethyl formamide, by an intramuscular route. Suitable control animals were also prepared. After 1 or 17 hr the rats were killed by chloroform, the brains removed on to filter paper and brought to 0°. They were weighed and homogenised with 5 ml of phosphate buffer. The dose given was in a volume of 1 ml/kg, i.e. 0·2 ml for 200 *g* rat. After homogenisation the homogenate was then made up to 12·5 ml with ice-cold phosphate buffer, and the MAO activity measured in the Warburg using 2·5 ml of homogenate and 0·5 ml of substrate in buffer. The conditions of the estimation are otherwise identical with those in the *in vitro* test. Inhibition was measured by calculating activity found in μl O_2/hr/g wet wt. of brain and referring this to the mean value obtained with suitable controls. A plot of percentage inhibition against dose was used to obtain the ID_{50} (i.e. the dose in mg/kg required to cause 50 per cent inhibition of the brain MAO). The potency ratio was calculated by dividing the comparable value of the ID_{50} for iproniazid by the ID_{50} obtained as described. The ID_{50}'s for iproniazid at 1 and 17 hr were found to be 16 and 4 mg/kg respectively.

Brain catechol amine concentrations

Albino rats were used. Drugs were given by the i.m. route and controls were treated in an identical fashion except for the injection of drug solvent instead of the drug in solution. In our early work on reference compounds, noradrenaline was measured by the method of Shore and Olin.[5] In later experiments including some on M&B 9302, both noradrenaline and 5-hydroxytryptamine (5-HT) were estimated.[6] Two tests were used. In the first the drug was administered by the i.m. route, the animal killed 17 hr later and the amine estimation was then carried out. In the second, reserpine was administered (1 mg/kg i.p.) and the test compound given 2 hr later. Subsequent procedure was then exactly as in the first test.

Other animals used as sources for enzymes as well as human sources

Rabbit work was carried out using New Zealand Whites and dog results were obtained from pure bred whippets. Human material was obtained at post-mortem a few hours after death and the tissue was deep frozen until worked up.

In rat and human *in vitro* brain experiments mitochondrial preparations were used. In the rat *in vivo* experiments and in all work with the dog and the rabbit, however, homogenates were assayed.

Note on MAO assays

In the enzyme activity as measured by a standard Warburg method, the cyanide recommended[7, 8] was omitted from the reaction mixture as was semicarbazide.[8] Their addition was found to interfere with the action of certain inhibitors studied. With M&B 9302 as inhibitor and tyramine as substrate no significant increase above theoretical in the total oxygen uptake per mole of substrate was detected in the absence of these. Some slight excess oxygen uptake was found with tryptamine and 5-HT, but this did not involve any change in I_{50}, or any abolition of the binary nature of the inhibition curve with tryptamine (vide infra).

RESULTS

Toxicity: lethal doses

The mean lethal doses LD_{50} mg/kg are for the *mouse* i.v. 94; s.c. 400, p.o. 430. *Rat* i.v. 62, p.o. 210 with subacute toxicity p.o. > 7·00, LD_{50} > 100 mg/kg/day.

Monoamine oxidase activity

Comparisons of this with other monoamine oxidase inhibitors are shown below in Tables 1 and 2. It may be said at once that M&B 9302 is about 1000 times more active than iproniazid.

TABLE 1. *IN VITRO* INHIBITION OF BRAIN MITOCHONDRIA BY M&B 9302 AND ELEVEN REFERENCE COMPOUNDS

Compound	Man					Rat				
	pI^{A}_{50}	pI^{B}_{50}	pI^{AB}_{50}	25S75	No. Est.	pI^{A}_{50}	pI^{B}_{50}	pI^{AB}_{50}	25S75	No. Est.
M&B 9302	9·2	5·4	—	3·9	4	8·78	5·15	—	3·6	16
Dexamphetamine			3·1	0·8	1			3·7	1·1	5
Etryptamine								3·9	1·4	1
Pargyline			7·4	0·9	4			7·6	1·0	1
Tranylcypromine			6·3	0·8	3			6·5	1·0	4
Procaine								2·5	1·0	1
Phenoxypropazine			5·8	1·1	3			5·6	0·7	2
Isocarboxazid			5·2	0·9	2			5·4	1·0	12
Nialamide			6·0	0·9	3			5·4	0·9	2
Phenelzine			6·1	0·9	1			5·8	0·6	13
Pheniprazine			6·1	1·1	1			6·4	0·8	5
Iproniazid			5·3	1·1	3			4·92	0·79	122

Please see text for explanation of inhibition constants. The formulae of the reference compounds are given in the appendix.

TABLE 2. *IN VIVO* RAT BRAIN INHIBITION BY M&B 9302 AND NINE REFERENCE COMPOUNDS

Compound	Time									
	1 hr					17 hr				
	ID_{25}	ID_{50}	ID_{75}	25S75	No. Est.	ID_{25}	ID_{50}	ID_{75}	25S75	No. Est.
M&B 9302	0·1	4	8	80	70	0·1	16	32	320	47
Dexamphetamine	200	~200			10					
Pargyline	0·3	0·8	2·0	7·4	12	0·2	0·5	1·2	6·7	11
Tranylcypromine	0·2	0·4	0·8	4·0	11	0·08	0·1	0·2	2·5	23
Phenoxypropazine	0·2	0·4	1·0	5·0	15	0·3	0·5	1·0	3·3	12
Iproniazid	6·0	16	32	5·3	19	2·0	4·0	6·0	3·0	14
Nialamide	18	25	36	2·0	23	19	22	30	1·6	24
Isocarboxazid	0·8	1·0	1·4	1·8	11	0·2	0·3	0·4	2·0	6
Phenelzine	2·5	3·8	5·6	2·2	12	2·5	6·0	15	6	12
Pheniprazine	0·1	0·4	0·9	9·0	11	0·09	0·3	0·8	9	12

Please see text for explanation of inhibition constants. The formulae of the reference compounds are given in the appendix.

In addition to the activity mentioned, the drug shows an abnormal dose response curve. When the per cent inhibition was plotted against the dose or concentration, instead of the normal sigmoid type of curve two sigmoid curves were obtained, separated by a plateau. Some analytic discussion of this phenomenon is essential for a proper understanding of the results presented later.

Abnormal dose response curve. Theoretical considerations
Normal MAO kinetics

In this case a plot of the percentage inhibition against concentration of an inhibitor gives the simple sigmoid curve shown in Fig. 1a. All known inhibitors of MAO used as reference compounds gave this. To describe such a plot only two major values are required. It is essential to be able to assess the power of the inhibitor, and this is done by finding the concentration required to produce a fixed degree of inhibition of the enzyme. By convention the inhibitor strength is assessed as the concentration (moles) causing 50 per cent inhibition of the enzyme. This inhibitory power is then the I_{50}. In practice, however, this often notationally inconvenient value is expressed in a manner analogous to the pH scale by using the 'pI_{50}'—this being defined as $-\log_{10}$ of the I_{50}. This is more concise, and though sometimes a trifle confusing at first, considerably aids the plotting and calculation of results.

One other parameter is sometimes needed. We have now dealt with the pK position of the curve and it is only necessary to deal with its slope. Suppose that 75 per cent rather than 50 per cent of the enzyme must be inhibited before a pharmacological result in which we are primarily concerned appears, it is then in the pI_{75} and not in the pI_{50} that we are really interested. Fig. 1b shows how two theoretical enzymes 'X' and 'Y' may have the same pI_{50} (6·0), but may be of very different inhibitory powers at higher concentrations. Enzyme 'X' has a pI_{75} of 5·5 and this means that a 75 per cent enzyme loss will be produced by minus antilog$_{10}$ 5·5 mole or a concentration of about 3×10^{-6} Molar. Enzyme 'Y', on the other hand, has a pI_{75} of 4·5 and this means that the larger concentration of 3×10^{-5} Molar will be required to produce 75 per cent inhibition of the enzyme and the postulated pharmacological effect in which we are interested. This inhibitory property is best expressed as a slope (as mentioned above) and is conveniently measured as I_{75}/I_{25} (or in the log nomenclature pI_{25}–pI_{75}) = 25S75. The vast majority of enzymes give a slope of approximately 1·0. Such a value has the dual purpose of showing that the enzyme kinetics follow, or at least resemble, the normal Michaelis-Menten pattern and show that no complicated inhibition problems are to be expected because of an inhibition curve with an abnormal. slope. For the vast majority of enzymes and their inhibitors these two inhibition measurements, pI_{50} and 25S75 are completely adequate.

Abnormal MAO kinetics with M & B 9302

In the case of M&B 9302, the kinetics of the MAO-M&B 9302 reaction show a unique abnormality. The plot of percentage inhibition of MAO against concentration or dose of M&B 9302 does not show a simple smooth plot, but reveals a pair of sigmoid curves joined by a horizontal section where the inhibition is invariant (Figs. 1c and 1d). The simplest hypothesis to explain this complexity is that in the enzyme prepared, the MAO is a binary system of enzymes each of which has a detectably different sensitivity to this particular inhibitor. The experimental plots are then

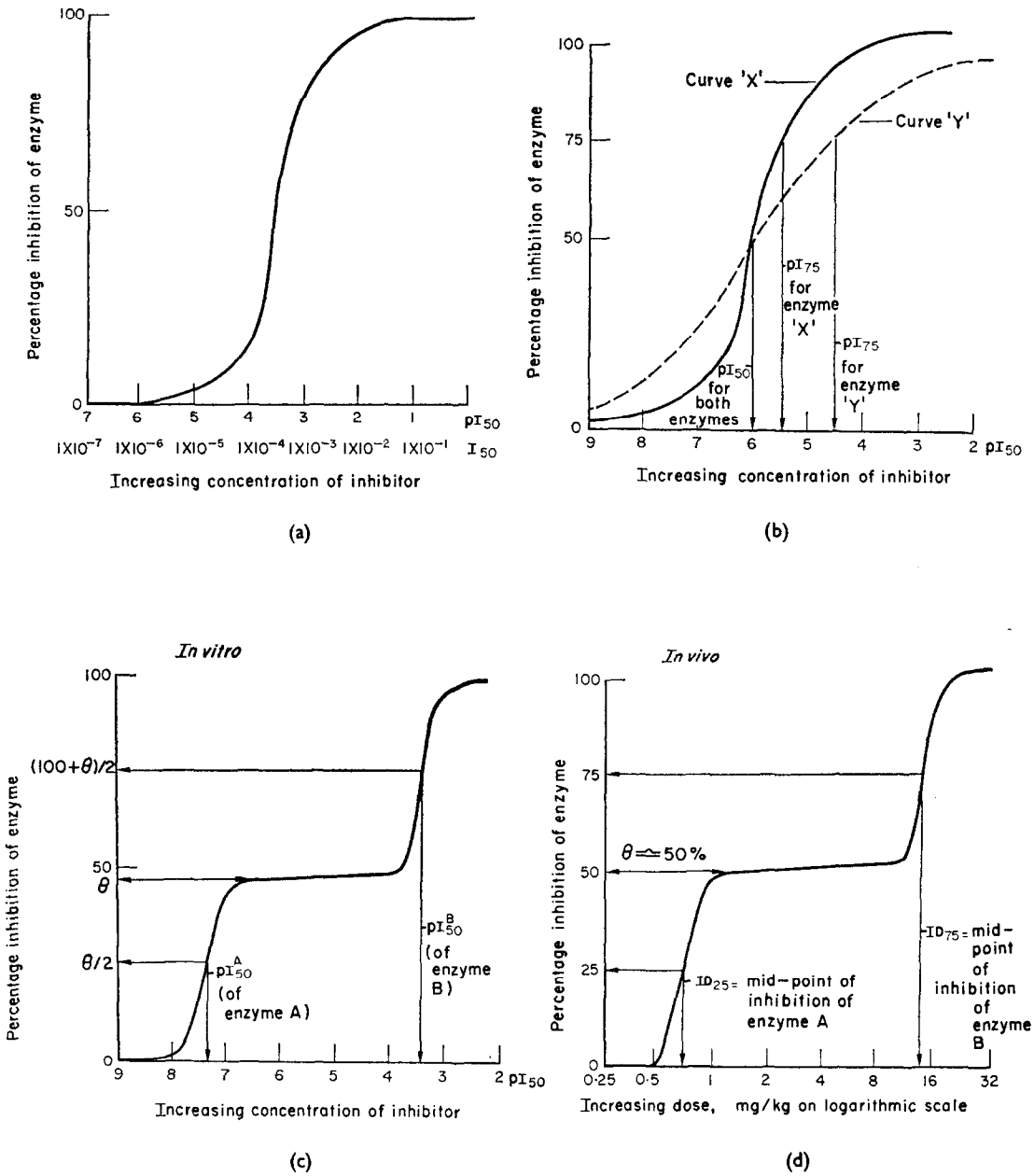

FIG. 1. Theoretical figures showing normal and 'M&B 9302 type' inhibition curves. (a) Normal enzyme inhibition curve; (b) Normal inhibition curves for two enzymes X and Y showing the effect of the slope of the curve on the pI_{75}; (c) *In vitro* inhibition curve for 'M&B 9302 type' of inhibition; (d) *In vivo* inhibition curve for 'M&B 9302 type' of inhibition.

explained as being due to the fact that the first and left hand curve represents the inhibition of a relatively sensitive enzyme (A) and the horizontal section then represents a stage where enzyme A is fully inhibited but the more resistant enzyme B is as yet unaffected. Finally, the second curve indicates the stages of inhibition of enzyme B. Clearly, two measures of inhibitory strength of the pI_{50} type will be required to express the inhibition constants of two enzymes. The more sensitive enzyme A will be 50 per cent inhibited at the midpoint of the left hand sigmoid curve or where the percentage inhibition reaches a value of $\theta/2$. (θ is the value of the inhibition achieved at the horizontal stretch of the curve, where enzyme A is completely inhibited. At the point $\theta/2$, enzyme A has clearly been half inhibited.) The log concentration where enzyme A is 50 per cent inhibited can conveniently be called the pI^{A}_{50}. Similarly, the -log concentration at the mid-point of the right hand sigmoid curve (the -log concentration when the enzyme inhibition reaches a value of $(100—\theta)/2$) gives the inhibition constant of the relatively more resistant enzyme B—the pI^{B}_{50}.

The other inhibition parameters that can be quoted are θ and the general slope of the plot of inhibition against concentration or dose. Quoting values for θ is obviously of lesser importance, but they do indicate behaviour as a binary mixture of enzymes and do measure the respective quantities of enzyme A and of enzyme B in the enzyme complex. In fact, values of θ are remarkably constant in falling between 40 and 50 per cent inhibition mark. In addition to pI^{A}_{50}, pI^{B}_{50} and θ some indication of the general slope of the double curve has been mentioned as worth reporting. The value of I_{75}/I_{25} or $pI_{25}–pI_{75}$ is a good as any, as before expressed as 25S75. Provided that the slopes of the two individual sigmoid curves are at all reasonable, a doubled curve must mean an increase in the value $pI_{25}–pI_{75}$ due to the interpolation of the horizontal stretch in the curve. With certain congeners of M&B 9302 in fact, it has been found that this value is increased from the 'normal' of about 1 to over 9 log units.

For *in vivo* graphs θ is fortunately approximately 50 and here the equivalent of the pI^{A}_{50} is the mid-point of the left sigmoid curve where the percentage inhibition is about $\theta/2$ and to all intents and purposes is given by the ID_{25} or dose causing 25 per cent inhibition. The equivalent of the pI^{B}_{50} of *in vitro* work is then the ID_{75} or dose causing 75 per cent inhibition (see Fig. 2). For inhibitors which give single smooth curves the inhibition constant is the mid-point—the ID_{50}—or the dose causing 50 per cent inhibition of the enzyme complex.

The equivalent estimate of slope for *in vivo* plots is the ID_{75}/ID_{25}, but even with reference compounds this can and does occasionally show some degree of variability because the slope of inhibition plots *in vivo* are subject to many complicating factors such as rates of absorption, excretion, metabolism of inhibitors, etc. so that much less weight can be given to these figures. Slopes with M&B 9302 and its congeners tend to be much larger and even more variable and therefore are included for reasons of completeness.

Details of inhibition of the rate and human brain enzyme

The inhibitory activity of M&B 9302 and a number of reference compounds on rat brain MAO is shown in Tables 1 and 2. The *in vitro* activity against enzyme A is very high indeed, considerably exceeding that of any of the marketed reference inhibitors. Its action on enzyme B lies, however, near the bottom of the range of

pI^{AB}_{50}s of the reference compounds. Fig. 2 shows an experimental curve with tyramine as substrate giving standard deviations of the points.

The rat *in vivo* results, however, are less impressive. The activity against enzyme A ($\approxeq$ ID_{25}) is about as good as the best of the reference compounds. Its inhibition of enzyme B ($\approxeq$ ID_{75}) is reversible and its action, not impressive at 1 hr, has become weak at 17 hours.

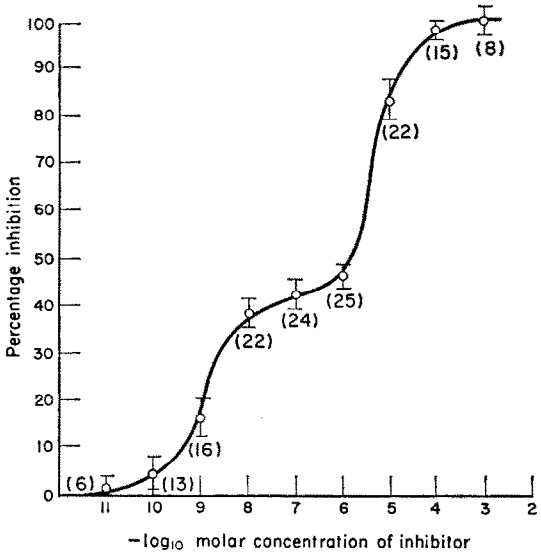

FIG. 2. Inhibition curve of M&B 9302 against rat brain monoamine oxidase *in vitro* using tyramine as a substrate. Points refer to the mean inhibition of tyramine oxidation and the vertical lines to the standard deviations. The figures in brackets indicate the number of experiments from which the mean is calculated.

Effect of choice of substrate

Table 3 gives the pI_{50} value for M&B 9302 and two reference compounds in an experiment where inhibition was compared using three different substrates. Within experimental error there is no significant difference in any inhibition constant depending on the choice of substrate. (There is excellent evidence that only enzyme A acts on 5-HT, hence the lack of a pI^{B}_{50} for M&B 9302 with this substrate.)

TABLE 3. EFFECT OF CHOICE OF SUBSTRATE ON INHIBITION OF RAT BRAIN MAO BY M&B 9302, TCPR AND IPN

Substrate	Inhibitor			
	M&B 9302		TCPR	IPN
	pI^{A}_{50}	pI^{B}_{50}	pI^{AB}_{50}	pI^{AB}_{50}
Tyramine	8·8	5·2	6·5	4·9
Tryptamine	8·9	5·4	6·2	4·4
5-HT	8·6	—	6·0	4·6

TCPR = Tranylcypromine; IPN = Iproniazid.
Please see text for definition of inhibition constants.

Detailed comparison of M&B 9302 with TCPR in four species

Tranylcypromine (TCPR) is probably the marketed inhibitor showing up best in the animal screening tests—especially if irreversible *in vivo* inhibition is given weight (17 hr figures). Table 4 makes a comparison of M&B 9302 and TCPR in four species and two tissues.

It might be pointed out that M&B 9302 appears as a 'differentiator' in all four species, though not necessarily in all tests or tissues. A 'differentiator' is here used in the sense that the compound treats MAO as a mixture of two enzymes A and B. In no case does TCPR behave as a differentiator. It is likely that *in vivo* as well as *in vitro*, M&B 9302 is a better inhibitor of the brain A enzyme in all species than is TCPR. The reverse probably holds for the B enzyme. *In vivo* M&B 9302 is a relatively poor inhibitor of liver enzyme.

Brain noradrenaline and 5-HT concentrations

M&B 9302 and a variety of control compounds were examined to find what changes in concentrations of noradrenaline and/or 5-HT they might cause in the brain. Most were also examined for their ability to augment, reduce, abolish or reverse the fall caused by a previous injection of reserpine.

TABLE 4. FULL COMPARISON OF INHIBITION RESULTS FOR M&B 9302 AND TRANYLCYPROMINE IN THE BRAIN AND LIVER OF FOUR SPECIES

Tissue and inhibitor	Rat			Dog			Rabbit			Man		
	pI^{A}_{50}	pI^{AB}_{50}	pI^{B}_{50}	pI^{A}_{50}	pI^{AB}_{50}	pI^{B}_{50}	pI^{A}_{50}	pI^{AB}_{50}	pI^{B}_{50}	pI^{A}_{50}	pI^{AB}_{50}	pI^{B}_{50}
Brain												
M&B 9302	8·8	—	5·15	6·7	—	4·6	7·9	—	4·0	9·2	—	5·3
TCPR	—	6·5	—	—	6·5	—	—	3·6	—	—	6·3	—
Liver												
M&B 9302	7·7	—	5·15	—	4·6	—	—	3·6	—	7·7	—	5·15
TCPR	—	5·7	—	—	5·5	—	—	5·4	—	—	5·5	—
In vivo	ID_{25}	ID_{50}	ID_{75}	ID_{25}	ID_{50}	ID_{75}	ID_{25}	ID_{50}	ID_{75}	ID_{25}	ID_{50}	ID_{75}
Brain												
M&B 9302	0·1	16	32	—	5	10	18	30	45	—	—	—
TCPR	0·08	0·12	0·16	—	—	~0·5	—	35	75	—	—	—
Liver												
M&B 9302	35	190	—	15	30	—	—	35	75	—	—	—
TCPR	0·25	0·45	0·75	—	0·5	2	—	—	45	—	—	—

TCPR = Tranylcypromine
Please see text for explanation of inhibition constants.

The main results are summarised in Table 5. Additional and similarly expressed values for brain noradrenaline 17 hr after injection of a test compound alone (50 mg/kg) are: cocaine, 166; α-ethyltryptamine, 157; chlorpromazine, 98; dexamphetamine, 106. The controls show that known inhibitors of MAO cause rises in both amines. The validity of the test is suggested by the fact that no significant rise was obtained with the very weak inhibitor dexamphetamine and with the non-inhibitors chlorpromazine and imipramine. Whether pre-treatment with reserpine is used or not,

M&B 9302 is clearly a most potent compound as judged by its ability to increase brain noradrenaline and 5-HT.

FURTHER EXPERIMENTAL POINTS AND DISCUSSION

The multiple enzyme hypothesis and dialysis

In the attempt to find other possible explanations for the abnormal curves than the presence of two enzymes, several other hypotheses have been examined. At the outset, it should be mentioned that with the substrate tyramine, an examination of the kinetics was made before and after dialysis. In this experiment, a large enzyme preparation was made and aliquots were dialysed for different times against 0·067M phosphate buffer, pH 7·2. Before dialysis the typical binary plot was obtained with the substrate tyramine and M&B 9302 as inhibitor. After 40- and 60-hr dialysis however, the enzyme activity was approximately halved and the 'inhibition' plot with the inhibitor showed a single sigmoid curve, which is best interpreted on the view that the activity of enzyme A had disappeared and with it the complications in the inhibition curves found at dilute inhibition concentration. After 16-hr dialysis an intermediate state of affairs was found (see Fig. 3). On the other hand, with tryptamine as substrate,

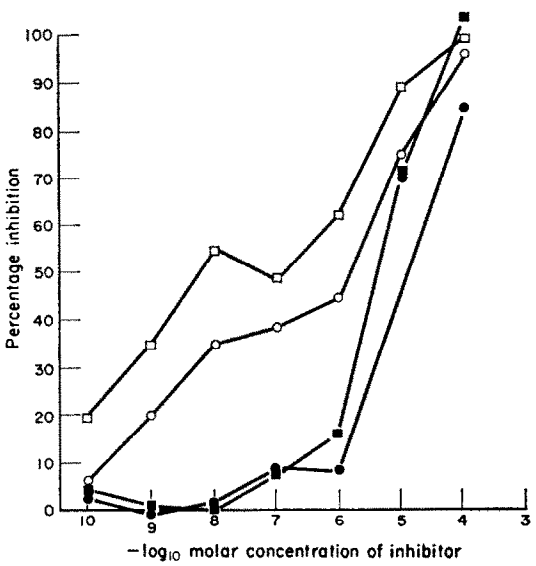

FIG. 3. Inhibition curves showing the effect of dialysis on rat brain monoamine oxidase inhibited by M&B 9302. Tyramine was used as substrate. □—□ = undialysed. ○—○ = dialysed for 16 hr. ■—■ = dialysed for 40 hr. ●—● = dialysed for 60 hr.

there was no qualitative difference between the curve obtained with undialysed enzyme and that obtained with enzyme which had been dialysed for 16-hr, though in the latter case the enzyme activity was reduced as with tyramine. When the enzyme was dialysed for 60 hr, activity had fallen so low that reliable readings could not be obtained. This is consistent with the suggestion that enzyme A is responsible for most of the deamination of the tryptamine. The dialysis experiments supported the idea of the presence of two enzymes.

Several other possible explanations have been considered and rejected. Among these were the presence of an insoluble impurity, the formation of an active metabolite, the formation of a substrate inhibitor compound, a two-phased reaction of the inhibitor with the enzyme, and destruction of an activator or optimal co-enzyme. None of these ideas fitted the facts so well as the multiple hypothesis.

TABLE 5. RAT BRAIN NORADRENALINE AND 5-HT LEVELS* 17 HR AFTER I.V. INJECTION OF 50 MG/KG OF TEST COMPOUNDS. EXPERIMENTS WITH AND WITHOUT PREVIOUS RESERPINE INJECTIONS

Compounds	M&B 9302	Controls					
		A	B	C	D	E	F
Noradrenaline							
Compound alone	198	153	145	176	153	98	55
Compound after reserpine†	101	67	94	61	103	34	
5-HT							
Compound alone	235	214	212	169	159	—	56
Compound after reserpine*	123	158	136	93	85	—	—

* As percentage of normal.

† Compound injected 2 hr after 1 mg/kg reserpine i.p.

A = Pargyline
B = Tranylcypromine
C = Iproniazid
D = Nialamide
E = Imipramine
F = Reserpine alone

General points

(1) M&B 9302 is a fantastically active inhibitor of enzyme A. Zeller[9,10] writing in 1960 claimed for tranylcypromine that it had the highest pI_{50} ever recorded in the open literature for an inhibitor of any enzyme in any species or tissue. M&B 9302 generally exceeds the values cited by Zeller of 6·7–7·2 by a factor of 10^2–10^3.

(2) M&B 9302 is a poor inhibitor of the B enzyme, both compared with its own performance against the A enzyme and with that of marketed reference compounds against the whole enzyme complex. Fortunately, the results of other tests, thought to measure anti-depressive activity (e.g. the pharmacological anti-reserpine screening test) appear in the case of differentiators to correlate with the compound's ability to inhibit enzyme A and to bear no relation to the effect on enzyme B.

(3) M&B 9302 shows evidence of being a differentiator in at least some tests in all four species examined.

(4) Its *in vivo* effect on liver enzyme is much weaker than that on the brain.

(5) Studies on the pharmacological properties of M&B 9302 in various species of animals indicate that it possesses the pharmacological properties normally associated with inhibitors of monoamine oxidase. In particular, it is very effective in preventing the sedative properties of reserpine in mice, rabbits, and rats; in potentiating the actions of tryptamine both centrally and on the isolated rat fundal strip preparation; and in prolonging the cardiovascular actions of tyramine (Maxwell, D. R., personal communication).

It will be realised that M&B 9302 was found during a continuous research effort in which various compounds were synthesized as the structure-activity pattern took form.

These aspects and the pharmacology and toxicology will be dealt with in other publications to be submitted to the Journal of Pharmacy and Pharmacology and to the British Journal of Pharmacology and Chemotherapy.

After the preparation of these results for publication, the paper by L. Maître[11] appeared, in which attention was drawn to the new compound Su 11,739. This is a non-hydrazine MAO inhibitor with a very high potency *in vitro* and interesting effects on brain tissue. The differences observed in the MAO activity in various tissues were considered to be evidence that tissues of the same species contain more than one single monoamine oxidase. It is to be noted that the author did not observe experimentally the presence of more than one MAO in rat brain tissue, as has been done by the present author.

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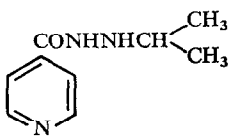
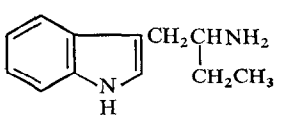
APPENDIX

Iproniazid

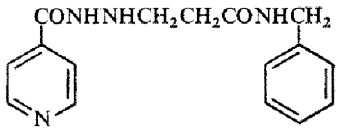


Isocarboxazid

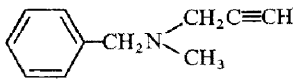
Etryptamine



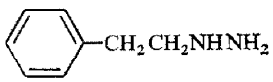
Nialamide



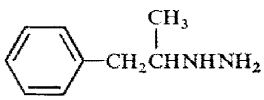
Pargyline



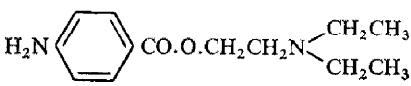
Phenelzine



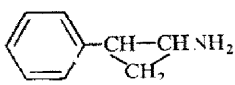
Pheniprazine



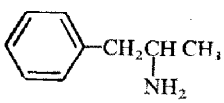
Procaine



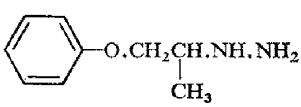
Tranylcypromine



Dexamphetamine



Phenoxypropazine



Imipramine