

## CHOLINE PHENYL ETHERS AS INHIBITORS OF AMINE OXIDASE

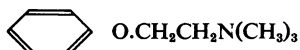
BY

BARBARA G. BROWN AND P. HEY\*

From the Department of Pharmacology, University of Leeds

(RECEIVED OCTOBER 26, 1955)

The parent compound of this series, choline phenyl ether,



was first studied by Hunt and Renshaw (1929), who showed it to be a powerful ganglion stimulant. Hey (1952), while investigating the nicotine-like stimulant activities of nuclear-substituted choline phenyl ethers, noticed that choline *p*-tolyl ether (TM6), which had a very weak ganglion-stimulant action, potentiated the effects of adrenergic nerve stimulation, as indicated by an increase in the tone and amplitude of contraction of the nictitating membrane in response to preganglionic stimulation of the cervical sympathetic nerve (Fig. 1). The compound was also found to potentiate the effects of injected adrenaline and noradrenaline on the blood pressure (Fig. 2) and on the nictitating membrane of cats.

It seemed unlikely that these potentiations were related to actions at ganglia; they could, however, be accounted for—at least in part—if the compound delayed the destruction of adrenaline and noradrenaline. Work on adrenaline inactivation was then in progress in the laboratory, and we were soon able to show that choline *p*-tolyl ether was in fact a powerful inhibitor of adrenaline inactivation *in vitro*, and that this was because it inhibited amine oxidase. Other related compounds which produced similar potentiating effects were also shown to inhibit amine oxidase.

Whether amine-oxidase inhibition fully accounts for the phenomena which prompted us to look for it will not be considered further here. But, having been led to the discovery of these new inhibitors, it seemed worth while to make a quantitative study of their relative potencies, and to see if we could determine any relations between chemical structure and amine-oxidase-inhibitory activity. This paper describes that work.

By a method about to be detailed, we have sought to correlate amine-oxidase-inhibitory activity and chemical structure in the parent compound and in eighteen nuclear-substituted choline phenyl ethers, and in some related compounds in which the cationic head was modified, and the chain length and structure were altered. We have also compared the activities of these new drugs with well-known inhibitors of amine oxidase such as ephedrine, amphetamine, and cocaine. The nature of the inhibition—whether competitive or non-competitive—has also been determined.

Amine oxidase has usually been studied *in vitro* by manometric methods—for example, by Hare (1928); Blaschko, Richter, and Schlossmann (1937b); Philpot (1940); and Tickner (1951). Blaschko, Richter, and Schlossmann (1937a), however, followed biologically the inactivation

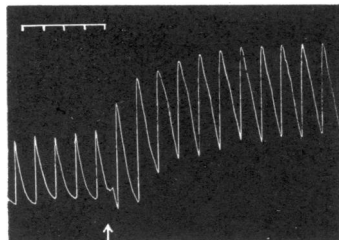
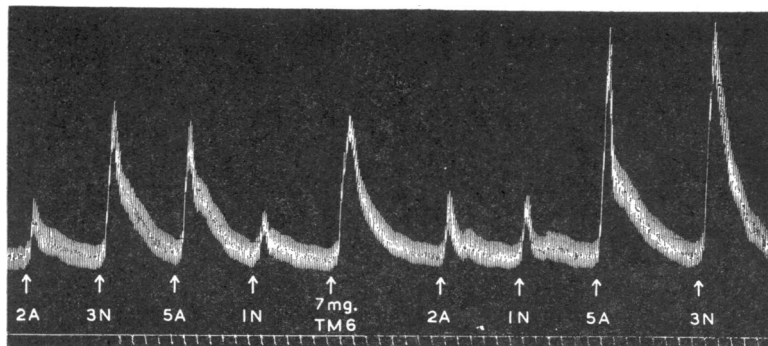


FIG. 1.—Showing the increase in tone, and amplitude of contraction, of the electrically stimulated nictitating membrane produced by the intravenous injection of 10 mg. choline *p*-tolyl ether bromide (TM6), in a cat under chloralose anaesthesia. Preganglionic stimulation 5 sec. in each minute. Time, min.

of adrenaline by liver slices and liver extracts, assaying the remaining adrenaline on the cat blood pressure and the rabbit intestine. The method used here to determine the degree of inhibition of amine oxidase depends upon the biological assay of adrenaline, and is a modification of that used by Bain, Gaunt, and Suffolk (1937) to follow the inactivation of adrenaline by mammalian tissue slices *in vitro*.

\*Present address: Royal College of Medicine, Baghdad, Iraq.

FIG. 2.—Showing the increase in response of the carotid blood pressure to adrenaline and noradrenaline after the intravenous injection of 7 mg. choline *p*-tolyl ether bromide (TM6). A,  $\mu$ g. adrenaline; N,  $\mu$ g. noradrenaline. Spinal cat. Time 30 sec. The immediate pressor effect of TM6 results from ganglionic stimulation (Hey, 1952).



Through the kindness of Dr. Blaschko it has been possible to compare some of the results obtained by this biological assay technique with those obtained by the more conventional manometric method.

A short account of some of the observations has already been published (Brown and Hey, 1952). Since then Schayer and his colleagues (Schayer, Wu, Smiley, and Kobayashi, 1954) have shown, in studies with  $^{14}\text{C}$ -labelled adrenaline and other substrates, that choline *p*-tolyl ether is a potent inhibitor of amine oxidase in intact mammals.

#### METHODS

Adrenaline was incubated with liver slices in defibrinated cat blood at  $37.5^\circ\text{C}$ . The adrenaline concentration was determined at various times by assaying the mixture on the blood pressure of spinal cats. Bain *et al.* (1937) used, as a measure of the rate of adrenaline inactivation, the time taken to inactivate half of the original amount present; Bain and Batty (1952, 1956) took the regression coefficient of log adrenaline concentration against time. We have estimated the rate of inactivation by calculating the amount of adrenaline destroyed in 30 min. To do this, four or more blood and adrenaline samples are removed for assay at times ranging from 5 to about 90 min. The adrenaline assay and the estimation of the adrenaline content of the mixtures are described in detail by Bain *et al.* (1937) and by Bain and Batty (1956). When the log adrenaline concentration is plotted against time, as in Fig. 3, a straight line results. The regression coefficient of log adrenaline concentration against time is then calculated; from this the amount of adrenaline remaining after 30 min., and thus the amount destroyed in that time, is determined.

The activity of a compound as an inhibitor of amine oxidase is found by comparing the amount of adrenaline destroyed in 30 min., in the presence of the inhibitor, with the amount destroyed during the same time in its absence. This difference is calculated as a percentage reduction, and it is this which is taken as a measure of amine-oxidase-inhibitory

activity. The collection of blood and the preparation of liver slices are described in the paper by Bain *et al.* (1937).

**Adrenaline Solutions.**—A concentrated stock solution containing 10 mg./ml. of adrenaline base was prepared by dissolving 100 mg. of the base in 5.46 ml. of 0.1 N-HCl and adjusting the volume to 10 ml. with distilled water. From this a dilute stock solution was made containing 1 mg./ml. of adrenaline base.

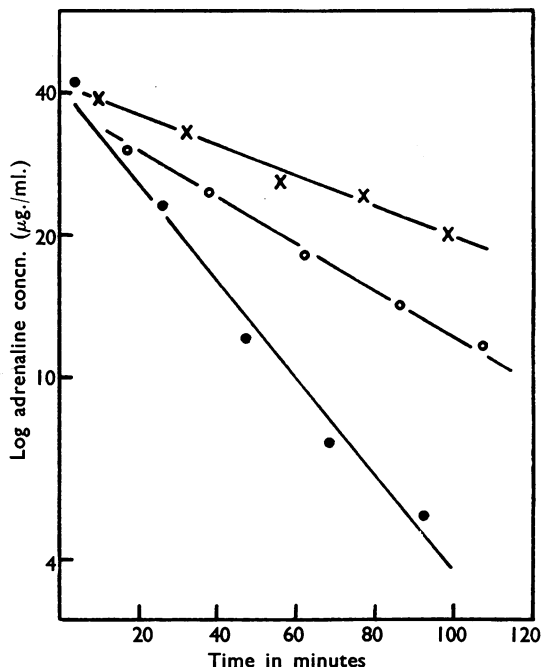


FIG. 3.—The destruction of adrenaline by guinea-pig liver slices and its inhibition by two nuclear-substituted choline phenyl ethers. Initial adrenaline concentration  $40\ \mu\text{g./ml.}$  Ordinate, remaining adrenaline concentration, assayed on the blood pressure of the spinal cat; abscissa, time in min. after the addition of adrenaline. Control liver,  $\bullet$ — $\bullet$ ; liver plus choline 2:4-xylyl ether (TM9)  $10\ \mu\text{g./ml.}$ ,  $\circ$ — $\circ$ ; liver plus choline 4-chlorophenyl ether (TM2)  $10\ \mu\text{g./ml.}$ ,  $\times$ — $\times$ .

**Inhibitor Solutions.**—To determine the inhibitory activity of the choline phenyl ether compounds at concentrations of 10 and 100  $\mu\text{g./ml.}$ , solutions were prepared containing 200  $\mu\text{g./ml.}$  or 2 mg./ml. respectively. For the weaker inhibitors, such as ephedrine and cocaine, solutions containing up to 20 mg./ml. were necessary.

**Blood-Liver-Adrenaline Mixtures.**—The composition of these mixtures in all experiments, unless stated otherwise, was: 4.55 ml. defibrinated cat blood; 1.0 g. sliced guinea-pig liver; 0.2 ml. adrenaline solution containing 1 mg./ml. base. In addition, each control liver system contained 0.25 ml. 0.9% NaCl solution, and each experimental system 0.25 ml. of the appropriate inhibitor solution. These mixtures were placed in 25 ml. Jena glass vaccine bottles and incubated in a water-bath at 37.5° C. with constant mechanical agitation.

**Other Methods.**—Some other methods, used in experiments to determine particular points, are described at the most appropriate places in the text.

**Compounds Studied.**—These are listed in Tables I, III, and IV.

## RESULTS AND DISCUSSION

### The Effects of Structural Changes in the Choline Phenyl Ether Molecule

The following changes were made in the parent compound:

- (1) substitution in the benzene nucleus
- (2) modification of the cationic head
- (3) alteration of the side chain between the ether oxygen and the nitrogen atom.

**Substitution in the Benzene Nucleus.**—The structures of the eighteen nuclear substituted compounds studied, together with their activities, are shown in Table I. Seventeen have various combinations of methyl groups and the halogens chlorine and bromine; the remaining one has an isopropyl group in the *ortho* position. For convenience in experimental work, code names and numbers have been given to the compounds; these are indicated in Table I, and the compounds are hereafter referred to by these codes.

With the exception of the isopropyl derivative, there is a highly significant difference between compounds, taking 0.05% limits of probability ( $P < 0.001$ ). For most of the compounds, however, there were insufficient observations to make possible a direct comparison of one with another; they were therefore treated as groups, so that those having only *ortho* substituents were compared with those having only *meta* or *para* substituents. The difference in activity between those compounds having one *ortho* or one *para* group is insignificant ( $P = 0.8-0.9$ ), as is also the difference between

TABLE I  
THE ACTIVITIES OF NUCLEAR-SUBSTITUTED CHOLINE PHENYL ETHERS AS INHIBITORS OF ADRENALINE INACTIVATION BY GUINEA-PIG LIVER

Initial concentration of adrenaline 40  $\mu\text{g./ml.}$  Concentration of inhibitors 10  $\mu\text{g./ml.}$

Code Number (TM)	$\begin{array}{c} \text{O.CH}_2\text{CH}_2\text{N}^+(\text{CH}_3)_3\text{Br}^- \\ \diagup \quad \diagdown \\ \text{C}_6\text{H}_4 \\ \diagdown \quad \diagup \\ \text{C}_2\text{H}_5 \end{array}$					% Reduction in Amount of Adrenaline Destroyed in 30 Min.
	2	3	4	5	6	
5		Br		Br		6.0
20	CH(CH <sub>3</sub> ) <sub>2</sub>			CH <sub>3</sub>		7.5
3		Cl				12.5
8		CH <sub>3</sub>		CH <sub>3</sub>		14.0
4		Br				17.5
-10	CH <sub>3</sub>				CH <sub>3</sub>	19.5
7		CH <sub>3</sub>	CH <sub>3</sub>			19.5
13	CH <sub>3</sub>	CH <sub>3</sub>				24.5
14	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>			25.0
9	CH <sub>3</sub>		CH <sub>3</sub>			26.0
1				CH <sub>3</sub>		28.0
19	CH <sub>3</sub>				CH <sub>3</sub>	33.0
16			Cl			36.5
6			CH <sub>3</sub>			37.5
15			Br			38.0
12	Cl					38.5
18	CH <sub>3</sub>					40.0
17	CH <sub>3</sub>		CH <sub>3</sub>		CH <sub>3</sub>	40.5
2			Cl			64.5

those with one or with two *meta* groups ( $P = 0.2$ ). When, however, compounds having an *ortho* or *para* group are compared with those having one or two *meta* groups, there is a highly significant difference between the two ( $P < 0.001$ ). The activity of those compounds possessing an *ortho* or *para* group is significantly different from those with a *meta* group and an *ortho* or *para* group ( $P < 0.001$ ).

These results are summarized in Table II, from which it will clearly be seen that *ortho* or *para* substitution increases the activity of the compounds over that of the parent compound, choline phenyl ether (TM1); that *meta* substitution depresses it; and that when *ortho*, or *para*, and *meta*

TABLE II  
THE EFFECTS OF NUCLEAR SUBSTITUTION ON THE AMINE-OXIDASE-INHIBITORY ACTIVITY OF CHOLINE PHENYL ETHER

Activity is expressed as the percentage decrease in the amount of adrenaline destroyed in 30 min. by guinea-pig liver slices in presence of 10  $\mu\text{g./ml.}$  of inhibitor

Substitution	Amine-oxidase-inhibitory Activity
<i>Ortho</i> or <i>para</i> .. .. .	39.5
None .. .. .	28.0
<i>Ortho</i> or <i>para</i> , and <i>meta</i> .. .. .	27.5
<i>Meta</i> .. .. .	18.5

groups are combined in the same molecule the activity becomes that of the unsubstituted compound.

Comparison of the activity of the compound having an *ortho*-isopropyl group (TM20) with that of the corresponding methyl substituted compound (TM19) shows that the introduction of a larger group into the *ortho* position has markedly reduced the activity.

**Modification of the Cationic Head.**—The cationic head was changed in two ways; the modified compounds, together with their code names and numbers and amine-oxidase-inhibitory activities, are shown in Table III.

(i) Changing the trimethyl cationic head to triethyl, as in TE2 and TE6, leads to an approximate tenfold decrease in activity compared with the corresponding trimethyl compounds TM2 and TM6 of Table I.

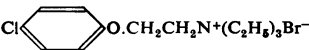

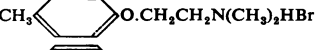
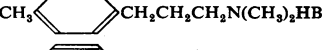
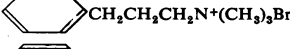
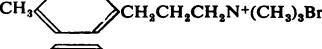
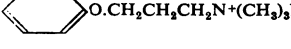
(ii) The effect of replacing the quaternary ammonium cationic head with a tertiary amino group, as in TTM6, is also to reduce the activity to one-tenth that of the corresponding compound in the original series.

**Alteration of the Side Chain.**—These compounds are also shown in Table III.

TABLE III

THE EFFECT OF ALTERATIONS IN THE CATIONIC HEAD AND CHAIN STRUCTURE OF CHOLINE PHENYL ETHER ON AMINE-OXIDASE-INHIBITORY ACTIVITY

Activity is expressed as the percentage reduction in the amount of adrenaline destroyed in 30 min. by guinea-pig liver slices

Code Name and No.	Formula	Amine-oxidase-inhibitory Activity at Concentrations ( $\mu\text{g.}/\text{ml.}$ ) of	
		10	100
TE2		16.5	34.5
TE6		4.5	33.0
TTM6		19.0	35.5
TDM6		5.0	63.0
DM1		20.0	42.0
DM6		12.0	42.0
CTM1		1.0	37.5

(i) Replacement of the ether oxygen by a methylene group—DM1 and DM6—reduces the inhibitory activity on amine oxidase to approximately one-tenth that of TM1 and TM6.

(ii) An increase in chain length of one methylene group, as in CTM1, also reduces the activity considerably, though not so drastically as do changes in the cationic head of the molecule.

The other tertiary amino compound, TDM6, which has a modified side chain, can be compared with the corresponding quaternary compound DM6. There is little difference in the activities of these two compounds—suggesting that the reduction in their activity, as compared with TM6, results from the modification in the side chain rather than from the change in the cationic head.

These results may be summarized by saying that all the structural modifications mentioned here have resulted in compounds with lower activities against amine oxidase than had the original choline phenyl ether series. On the other hand, none of the modified compounds is completely devoid of activity.

It is possible to draw from the observations certain general conclusions concerning the relations between structure and amine-oxidase-inhibiting power in derivatives of choline phenyl ether. Thus, a cationic head consisting of three methyl groups is apparently necessary for maximum activity. Replacing the three methyl groups with three ethyl groups, or changing the quaternary ammonium group to a tertiary amino group, reduces the activity to approximately one-tenth of that of the compound having a trimethyl cationic head. A side chain of the structure  $-\text{O.CH}_2\text{CH}_2-$  also confers maximum activity, though replacement of the ether oxygen by a methylene group does not drastically reduce the activity, nor does increasing the straight chain length by one methylene group. Finally, in the choline phenyl ether series itself, maximum activity is conferred by halogen or methyl group substitution in the *ortho* or *para* position. *Meta* substitution, and the introduction of larger groups in the *ortho* position, appears to reduce rather than to increase the activity relative to the unsubstituted compound.

#### Comparison with Established Inhibitors of Amine Oxidase

The following compounds have been shown by previous workers to inhibit amine oxidase *in vitro*: ephedrine (Blaschko *et al.*, 1937b); amphetamine (Blaschko, 1940); cocaine and procaine (Philpot, 1940). We have studied these compounds by our method in concentrations ranging from 10  $\mu\text{g.}/\text{ml.}$

to 1 mg./ml. These results are shown in Table IV, in which is also included an active inhibitor of the choline phenyl ether series, TM6.

TABLE IV

THE MEAN ACTIVITIES OF SOME ESTABLISHED INHIBITORS OF AMINE OXIDASE, TOGETHER WITH THAT OF CHOLINE *p*-TOLYL ETHER BROMIDE

Activity is expressed as the percentage reduction in the amount of adrenaline destroyed by guinea-pig liver slices in 30 min.

Inhibitor	Amine-oxidase-inhibitory Activity at Concentrations ( $\mu\text{g./ml.}$ ) of					
	3	10	30	100	300	1,000
Choline <i>p</i> -tolyl ether Br (TM6) ..	31.5	37.5	54.5	61.0		
Amphetamine $\text{H}_2\text{SO}_4$ ..		15.5	33.5	61.0		
Ephedrine HCl ..			4.0	12.5	36.5	
Procaine HCl ..				9.5	19.5	46.0
Cocaine HCl ..				1.0	11.5	31.0

It will be seen that 10  $\mu\text{g./ml.}$  TM6 causes an inhibition of 37.5%, and 30  $\mu\text{g./ml.}$  amphetamine sulphate an inhibition of 33.5%; hence, weight for weight, TM6 is approximately three times more active. Similarly, 300  $\mu\text{g./ml.}$  of ephedrine hydrochloride is required to produce an inhibition equivalent to 10  $\mu\text{g./ml.}$  of TM6, which is therefore thirty times more active. Procaine and cocaine are even less active than ephedrine, 1 mg./ml. of cocaine hydrochloride being needed to give an inhibition equal to that produced by 3  $\mu\text{g./ml.}$  of TM6. Thus, choline *p*-tolyl ether bromide has approximately three times the amine-oxidase-inhibitory power of amphetamine sulphate, thirty times that of ephedrine hydrochloride, and three hundred times that of procaine hydrochloride and of cocaine hydrochloride.

#### The Nature of the Inhibition

The method of Lineweaver and Burk (1934) was used to determine whether the inhibition of amine oxidase by the choline phenyl ethers is competitive or non-competitive. The velocity of the reaction in which adrenaline is destroyed was investigated at various concentrations of substrate in the presence of different concentrations of inhibitor.

The substrate concentrations in a typical experiment were 40, 100, 200, and 300  $\mu\text{g./ml.}$  of adrenaline. To obtain these concentrations slight modifications were necessary in the composition of the blood-liver-adrenaline mixtures previously described. As before, 4.55 ml. of cat blood was used; the amounts of adrenaline solution added were, respectively, 0.2 ml. of solution containing 1 mg./ml. base; 0.05 ml. of 10 mg./ml. solution; 0.1 ml. of 10 mg./ml. solution; and 0.15 ml. of 10 mg./ml. solution. The control liver system was made up to a volume of 5 ml. with 0.9% NaCl solution. To the experimental systems

were added 0.25 ml. of the appropriate inhibitor solution and sufficient 0.9% NaCl to bring the final volume to 5.0 ml.

The inhibitor used was choline 2:4:6-mesityl ether bromide (TM17); the velocity of the reaction was taken as the amount of adrenaline destroyed in 30 min.

Fig. 4 illustrates such an experiment, in which the concentration of inhibitor was 10  $\mu\text{g./ml.}$  From this and other similar experiments, there is no doubt that the substrate-concentration reaction-velocity curves for the destruction of adrenaline by guinea-pig liver, in the absence and in the presence of inhibitor, are not parallel, but are convergent and meet at the ordinate. It is thus clear that the inhibition is competitive.

#### Manometric Experiments

The activities of TM6 and amphetamine sulphate as inhibitors of amine oxidase were compared by a manometric technique, and the nature of the inhibition by TM6 was determined.

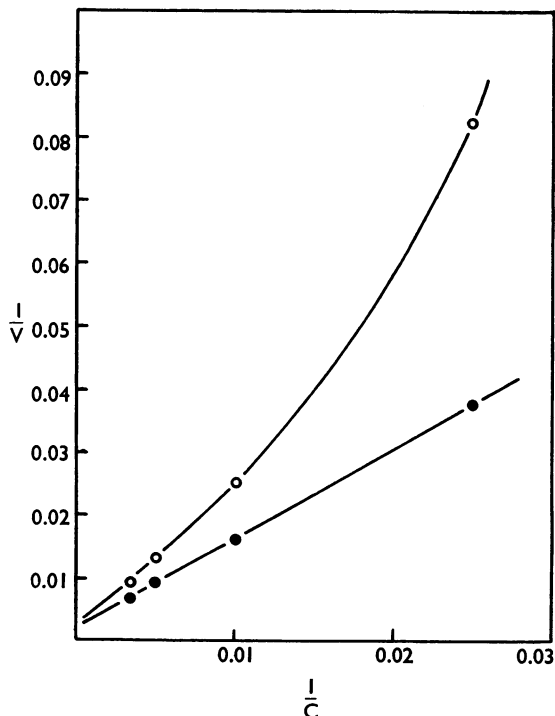


FIG. 4.—Showing the competitive nature of the inhibition of amine oxidase by choline 2:4:6-mesityl ether bromide (TM17). The method used is that described by Lineweaver and Burk (1934), the reaction velocity being taken as the amount of adrenaline destroyed by guinea-pig liver slices in 30 min. Ordinate, reciprocal of the reaction velocity; abscissa, reciprocal of the substrate concentration. Control liver, ●—●; liver plus TM17 10  $\mu\text{g./ml.}$ , O—O.

In the experiments in which the two inhibitors were compared, a homogenate of guinea-pig liver was used, since this was the tissue employed in the work described earlier in this paper. The nature of the inhibition by TM6 was determined with a homogenate of rabbit liver. The preparation of the extract was the same in both instances. The tissue was frozen solid and then ground to a thin paste, to which was added twice its weight of 0.066M-sodium phosphate buffer pH 7.4. This mixture was dialysed overnight against running water; the next morning one-tenth its volume of 0.2M-sodium phosphate buffer, pH 7.4, was added. Open manometers and Warburg manometer flasks were used, the centre tubes of which contained 0.3 ml. N-KOH. The gas phase was oxygen and the temperature 37.5° C. The substrate was 0.01M(-)-*p*-sympatol hydrochloride. The rate of oxidation was followed manometrically, readings being taken at 5 min. intervals for 30 min. The inhibition was measured as the percentage reduction in oxygen uptake at the end of 30 min. In each experiment six manometer flasks were used (1-6); the main chamber of each contained 0.7 ml. of liver homogenate and 0.9 ml. of 0.066M-sodium phosphate buffer pH 7.4; the volume was made up to 1.8 ml. with either 0.2 ml. of distilled water, in flasks 1 and 2, or with 0.2 ml. of inhibitor solution in flasks 3, 4, 5, and 6. The side bulbs of flasks 1, 3, and 5 contained 0.2 ml. of distilled water; those of 2, 4, and 6 contained 0.2 ml. of 0.1M(-)-*p*-sympatol HCl.

*A Comparison of Amphetamine and TM6.*—Table V records the inhibition produced by various concentrations of TM6 and amphetamine. The 55% inhibition produced by  $3.2 \times 10^{-4}$ M-amphetamine lies midway between those of 77% and 28% produced by  $10^{-4}$ M- and  $10^{-5}$ M-TM6

TABLE V

TO SHOW THE PERCENTAGE REDUCTION OF OXYGEN UPTAKE AFTER 30 MIN. IN GUINEA-PIG LIVER EXTRACT-INHIBITOR MIXTURES

Substrate 0.01M(-)-*p*-sympatol

Inhibitor	Concentration (M)			
	$3.2 \times 10^{-4}$	$10^{-4}$	$3.2 \times 10^{-5}$	$10^{-5}$
Amphetamine H <sub>2</sub> SO <sub>4</sub> Choline <i>p</i> -tolyl ether Br (TM6)	55	77	9	28

respectively. Therefore, the concentration of TM6 having the same effect as  $3.2 \times 10^{-4}$ M, or 59 μg./ml., amphetamine is approximately  $5 \times 10^{-5}$ M, or 13.7 μg./ml. Thus amphetamine has, weight for weight, only one-quarter the activity of the choline ether. By the biological method described earlier, the choline ether inhibitor was found to be three

times more active than amphetamine. There is thus close agreement between the results obtained by the two methods.

*The Nature of the Inhibition.*—An extract of rabbit liver was incubated with two concentrations of substrate, 0.02M- and 0.002M(-)-*p*-sympatol HCl, alone and in the presence of the inhibitor,  $10^{-3}$ M-TM6. The oxygen consumption was measured over a period of 22 min., at which time the percentage reduction in oxygen uptake in the presence of inhibitor was calculated.

TABLE VI

THE NATURE OF THE INHIBITION OF RABBIT LIVER AMINE OXIDASE BY CHOLINE *P*-TOLYL ETHER BR. (TM6)

Substrate	Extra O <sub>2</sub> Consumption (μl.) after 22 min.		% Inhibition
	Substrate Alone	Substrate + $10^{-3}$ M-TM6	
0.02M(-)- <i>p</i> -sympatol HCl	29	22	24
0.002M(-)- <i>p</i> -sympatol HCl	13	4	69

Table VI shows that the degree of inhibition is dependent on the concentrations of both inhibitor and substrate. This confirms the earlier observations made by the biological method, that inhibition by choline phenyl ethers is competitive.

## CHEMICAL SECTION

Melting and boiling points are uncorrected.

1. 2-aryloxyethyl bromides were prepared by reacting the appropriate phenol in alkaline solution with ethylene dibromide and these were then condensed with trimethylamine in acetone solution, or with triethylamine, to yield the quaternary compound as described in a previous paper (Hey, 1952). New 2-aryloxyethyl bromides are recorded in Table VII, and new quaternary compounds in Table VIII.

2. The following compounds were also prepared :

(a) 3(*p*-tolyl)propyltrimethyl Ammonium Bromide. —2(*p*-tolyl)propionic acid was prepared by methods described in the literature and converted to the methyl ester by diazomethane. The ester was quantitatively reduced to 3(*p*-tolyl)propyl alcohol by lithium aluminium hydride; b.p. 136°/16 mm.  $[n]_D^{20}$  1.5329. (Found : C, 80.0; H, 9.70. C<sub>10</sub>H<sub>14</sub>O requires C, 80.0; H, 9.40%.) (cf. Brown, 1951.) The above alcohol was treated with an ice-cold solution of phosphorus tribromide in carbon tetrachloride and gave a good yield of the bromide as a colourless liquid b.p. 127°/18 mm.  $[n]_D^{20}$  1.5412. (Found : C, 56.6; H, 6.26;

TABLE VII  
 CHARACTERISTICS OF ARYLOXYBROMIDES

Compound R-O-CH <sub>2</sub> CH <sub>2</sub> Br R=	Characters	Analysis (%)			
			C	H	Halogen
2:4 Xylyl .. ..	Solid; m.p. 45° B.P. 152°/16 mm.	Found Calc. for C <sub>10</sub> H <sub>13</sub> OBr	52.8 52.4	5.84 5.72	33.4 34.9
3:4 .. ..	Solid; m.p. 44° B.P. 149°/16 mm.	Found Calc. for C <sub>10</sub> H <sub>13</sub> OBr	52.7 52.4	5.75 5.72	33.6 34.9
2:3 .. ..	Liquid; b.p. 148°/15 mm. [n] <sub>D</sub> <sup>20</sup> 1.5478	Found Calc. for C <sub>10</sub> H <sub>13</sub> OBr	53.0 52.4	5.81 5.72	32.6 34.9
2:5 .. ..	Liquid; b.p. 135°/14 mm. [n] <sub>D</sub> <sup>20</sup> 1.5421	Found Calc. for C <sub>10</sub> H <sub>13</sub> OBr	53.2 52.4	5.76 5.72	32.5 34.9
2-Chlorophenyl ..	Liquid; b.p. 153°/15 mm. [n] <sub>D</sub> <sup>20</sup> 1.5700	Found Calc. for C <sub>8</sub> H <sub>8</sub> OBrCl	41.0 40.8	3.55 3.42	47.6 49.0
4-Bromophenyl ..	Solid; m.p. 61° B.P. 114°/0.8 mm.	Found Calc. for C <sub>8</sub> H <sub>8</sub> OBr <sub>2</sub>	34.3 34.3	2.98 2.90	54.1 57.1

 TABLE VIII  
 CHARACTERISTICS OF SUBSTITUTED CHOLINE PHENYL ETHERS AND RELATED COMPOUNDS

Compound (and Code No.) R <sup>1</sup> -O-CH <sub>2</sub> CH <sub>2</sub> N(R <sup>2</sup> ) <sub>3</sub> Br R <sup>1</sup> = R <sup>2</sup> =	M.p.	Analysis (%)					
			C	H	N	Halogen	
4-Chlorophenyl (TE2) ..	Ethyl	142°	Found Calc. for C <sub>14</sub> H <sub>23</sub> ONBrCl	50.3 49.9	6.84 6.89	3.97 4.16	34.1 34.3
4-Tolyl (TE6) .. ..	"	141°	Found Calc. for C <sub>15</sub> H <sub>23</sub> ONBr	52.4 53.3	6.80 6.63	4.05 4.03	23.4 22.9
2:4 Xylyl (TM9) ..	Methyl	191°	Found Calc. for C <sub>13</sub> H <sub>22</sub> ONBr	52.6 54.2	7.30 7.69	4.75 4.86	27.9 27.7
2-Chlorophenyl (TM12)	"	143°	Found Calc. for C <sub>11</sub> H <sub>17</sub> ONBrCl	44.9 45.0	5.63 5.84	4.56 4.77	38.9 39.3
3:4 Xylyl (TM13) ..	"	191°	Found Calc. for C <sub>13</sub> H <sub>22</sub> ONBr	54.0 54.2	7.50 7.69	4.75 4.86	27.9 27.7
2:3 Xylyl (TM14) ..	"	222°	Found Calc. for C <sub>13</sub> H <sub>22</sub> ONBr	54.0 54.2	7.57 7.69	4.75 4.86	27.9 27.7
4-Bromophenyl (TM15)	"	196°	Found Calc. for C <sub>11</sub> H <sub>17</sub> ONBr <sub>2</sub>	38.8 39.0	5.05 5.05	3.98 4.13	45.1 47.1
2:4 Dichlorophenyl (TM16)	"	176°*	Found Calc. for C <sub>11</sub> H <sub>16</sub> ONBrCl <sub>2</sub>	40.4 40.1	5.01 4.90	4.22 4.26	45.3 45.8
2:5 Xylyl (TM19) ..	"	187°	Found Calc. for C <sub>13</sub> H <sub>22</sub> ONBr	54.3 54.2	7.79 7.69	4.74 4.86	27.9 27.7

\* Jones, Metcalfe, and Sexton (1949) quote m.p. 88°-89°.

Br, 37.1. C<sub>10</sub>H<sub>13</sub>Br requires C, 56.3; H, 6.09; Br, 37.5%.) This bromide treated with an excess of trimethylamine in acetone solution at room temperature yielded the required quaternary compound m.p. 116°. (Found : C, 57.1; H, 7.90; N, 5.17; Br, 29.6. C<sub>13</sub>H<sub>22</sub>NBr requires C, 57.4; H, 8.13; N, 5.16; Br, 29.3%.)

(b) 3(*p*-tolyl)propyldimethylamine Hydrobromide.—3(*p*-tolyl)propyl bromide (above) condensed at room temperature with excess dimethylamine in ethereal solution yielding the above tertiary amine as a colourless liquid b.p. 114°/15 mm. [n]<sub>D</sub><sup>20</sup> 1.4994. (Found : C, 82.2; H, 10.9; N, 8.07. C<sub>12</sub>H<sub>19</sub>N requires

C, 81.3; H, 11.0; N, 7.93%.) The hydrobromide had m.p. 149° C.

(c) 2(*p*-toloxy)ethyl dimethylamine Hydrobromide.—2(*p*-toloxy)ethyl bromide condensed at room temperature with excess dimethylamine in ethereal solution to yield the above tertiary amine as a colourless liquid b.p. 121°/12 mm. [n]<sub>D</sub><sup>20</sup> 1.5062. (Watanabe (1949) gives b.p. 138°-155°/25.5 mm.) (Found : C, 74.2; H, 9.45; N, 8.02. C<sub>11</sub>H<sub>17</sub>ON requires C, 73.7; H, 9.56; N, 7.84%.) The hydrobromide had m.p. 134°.

Other compounds used were made by methods described in the literature.

## SUMMARY

1. A biological assay method of determining the activity of amine oxidase inhibitors *in vitro* is described.

2. Nuclear-substituted choline phenyl ethers, and related compounds, inhibit the enzymic destruction of adrenaline by guinea-pig liver.

3. The more active nuclear-substituted choline phenyl ethers—such as choline *p*-tolyl ether, choline *o*-chlorophenyl ether, and choline *o*-tolyl ether—are, weight for weight, three times more active as inhibitors of amine oxidase than amphetamine, thirty times more active than ephedrine, and three hundred times more active than cocaine.

4. Among those compounds studied, the choline phenyl ether structure imparts maximum amine-oxidase-inhibitory activity.

5. In the choline phenyl ether series, *ortho* or *para* nuclear substitution increases amine-oxidase-inhibitory activity and *meta* substitution decreases it.

6. The inhibition of amine oxidase by choline phenyl ethers is competitive.

7. The relative activity of amphetamine and a choline phenyl ether inhibitor, and the competitive nature of the inhibition by the latter, have been confirmed by a manometric method.

8. The preparation and characteristics of several new compounds used in this study are recorded.

We are indebted to Professor W. A. Bain for his help and encouragement, to Dr. J. G. Dare for statistical advice, and to Mrs. Y. Richards for semi-micro analyses.

## REFERENCES

- Bain, W. A., and Batty, J. E. (1952). *J. Physiol.*, **118**, 14P.  
— (1956). *Brit. J. Pharmacol.*, **11**, 52.  
— Gaunt, W. E., and Suffolk, S. F. (1937). *J. Physiol.*, **91**, 233.  
Blaschko, H. (1940). *Nature, Lond.*, **145**, 26.  
— Richter, D., and Schlossmann, H. (1937a). *J. Physiol.*, **90**, 1.  
— (1937b). *Biochem. J.*, **31**, 2187.  
Brown, B. G., and Hey, P. (1952). *J. Physiol.*, **118**, 15P.  
Brown, W. G. (1951). *Organic Reactions*, **6**, 504 (ref. 47). New York: John Wiley.  
Hare, M. L. C. (1928). *Biochem. J.*, **22**, 968.  
Hey, P. (1952). *Brit. J. Pharmacol.*, **7**, 117.  
Hunt, R., and Renshaw, R. R. (1929). *J. Pharmacol.*, **35**, 99.  
Jones, R. L., Metcalfe, T. P., and Sexton, W. A. (1949). *Biochem. J.*, **45**, 143.  
Lineweaver, H., and Burk, D. (1934). *J. Amer. chem. Soc.*, **56**, 658.  
Philpot, F. J. (1940). *J. Physiol.*, **97**, 301.  
Schayer, R. W., Wu, K. Y. T., Smiley, R. L., and Kobayashi, Y. (1954). *J. biol. Chem.*, **210**, 259.  
Tickner, A. H. (1951). *Brit. J. Pharmacol.*, **6**, 606.  
Watanabe, M. (1949). Jap. Pat. 172,880. (Cited in *Chem. Abstr.*, **43**, 7960.)