CHAPTER III

RESULTS

Part I. Determination of Monoamine Oxidase Activity in the Rat Liver Mitochondrial Preparations by Using Oxygen Consumption as an Indicator.

The specific activities of MAO for various substrates (norepinephrine, benzylamine, B-phenylethylamine tryptamine and dopamine) in different concentrations are shown in Fig. 3, 7, 11, 15, 19. In general, MAO activities increased correspondingly to substrate concentrations up to the maximum after which the enzymatic activities remain somewhat constant. The exception is, however, the enzymatic activity with B-phenylethylamine which declines after the maximal point has been attained. This characteristic phenomenon had been reported to be due to the substrate inhibition of the MAO. In these experiments, the rat liver mitochondrial preparations were suspended in a medium containing phosphate buffer pH 7.0, the temperature was maintained at 37° C. The oxygen consumption rate was determined from the slope of oxygraph's tracings and specific activities of MAO were calculated from differences in oxygen consumption rates at the times before and after substrate addition and represented in term of microatoms of oxygen consumed per milligram of mitochondrial protein per 4 minutes of incubation. The assay method is apparently specific and applicable to MAO activity due

to the fact that addition of pargyline, an irreversible monoamine oxidase inhibitor (MAOI), into the assay mixture suppressed oxygen consumption rate (in the presence of amine substrate) to the baseline levels. Moreover, mitochondrial preparations were always frozen initially and then thawed just before the experiment and the incubation medium was made unflavorable to mitochondrial respiration. These conditions, taken together, will limit the contribution of mitochondrial oxidative phosphorylation in oxygen consumption to the least extent. Therefore, the assay method adopted in these experiments can be regarded as a convenient and reliable approach for the study of MAO activity in mitochondrial samples. The concentrations of monoamine substrates providing maximal enzymatic activities were shown to be 166.40 ± 0.0013 mcM for norepinephrine, 20.80 ± 0.0011 mcM for benzylamine, 2.60 ± 0.0019 mcM for B-phenylethylamine, 5.20 ± 0.0022 mcM for tryptamine, and 41. 60 ± 0.0036 mcM for dopamine (all values are mean ± S.E.M).

Part II. Effect of the *in vitro* Addition of Piperine to Monoamine Oxidase Activity in the Rat Liver Mitochondrial Preparations.

The inhibitory effects of piperine to MAO activities toward different monoamine substrates (norepinephrine, benzylkamine, B-phenylethylamine, tryptamine and dopamine) are illustrated in Figures 4, 8, 12, 16, 20, 23. Evidently, the inhibitory effect of piperine were markedly exhibited to every

substrates tested and, in general cases, the effect was concentration-dependent. However, at higher concentrations of piperine, the inhibitory effect tended to be reversed as considering from the disappearance rate of oxygen in the assay medium. This finding was applicable to most substrates tested, except B-phenylethylamine. Regarding to the specificity of piperine effect, the results obtained did not suggest the preferential inbition to any specific type of MAO.

Part III Kinetics of Monoamine Oxidase Inhibition by Piperine *in vitro*.

The kinetic behaviour of MAO inbition by piperine was assesses by determining MAO activities toward different substrate concentrations in the presence of fixed concentration of piperine. The results are shown in Figures 6, 10, 14, 18, 22 and Tables 2-6. The kinetic parameters obtained by Double Reciprocal Plot are tabulated in Table 7. Apparently, the profile of enzyme inhibition was quite complex and inconsistent. The kinetic behaviour of MAO inhibition toward B-phenylethylamine, tryptamine and dopamine as revealed by kinetic parameters was more or less competitive in nature inhibition kinetics whereas that of benzylamine and norepinephrine was somewhat complex. The kinetics of MAO inhibition by piperine in this study may be dependent on specific substrate used and possibly other unidentifable factors.

Part IV Effect of the *in vitro* Addition of Piperine to Monoamine Oxidase Activity in the Rat Brain Mitochondrial Preparations.

The inhibitory effects of piperine to MAO activities toward different monoamine substrates (norepinephrine, B-phenylethylamine, and dopamine) are illustrated in Table 8. The results obtained thus far were not satifactory and suggested non-significant effect to MAO activity. Due to the methodological difficulty in preparing rat brain mitochondrial fraction in sufficient quantity for the assay, the study was not continued and further elaboration concerning the exact effects of piperine to brain MAO activity should be investigated.

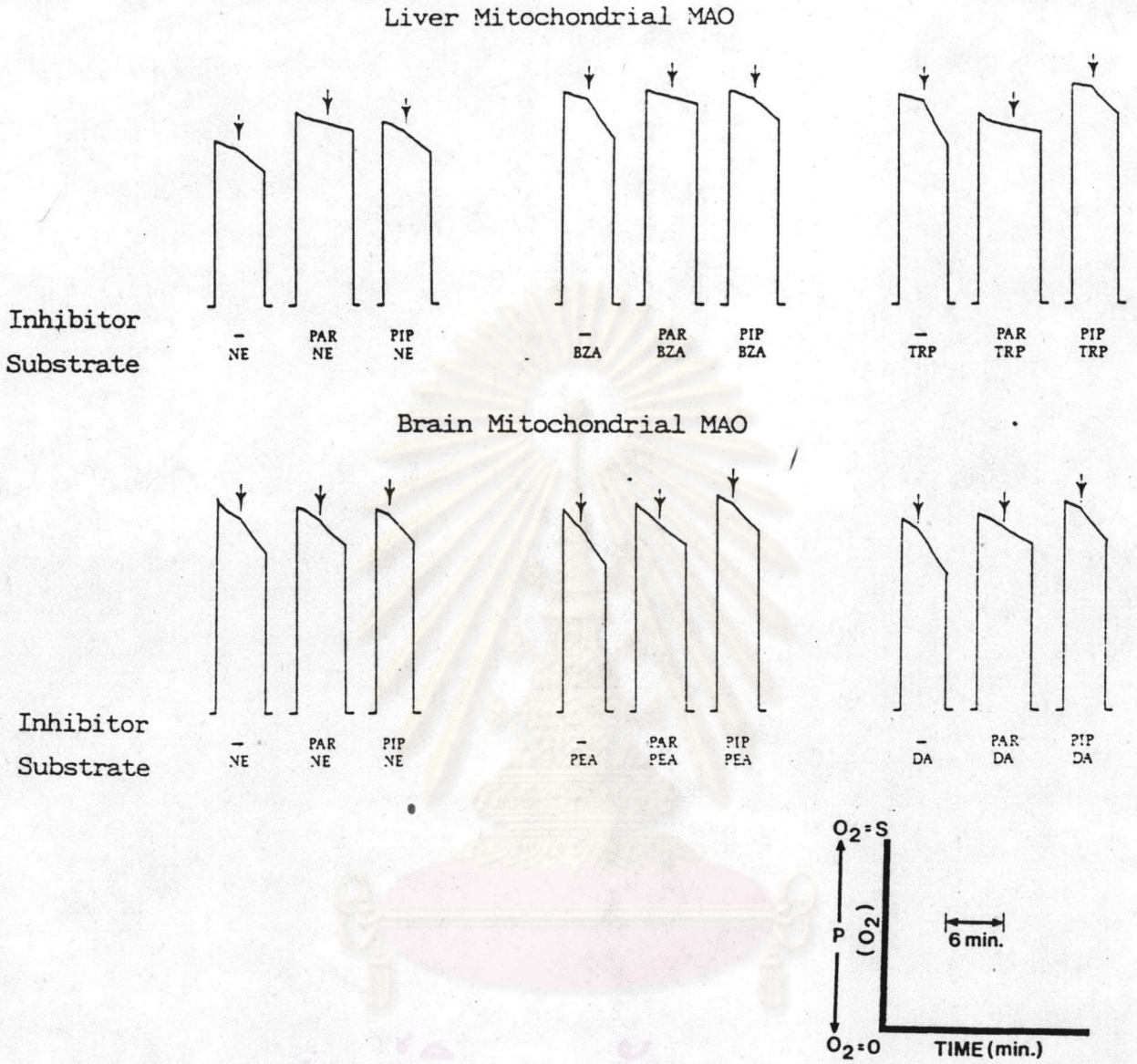

FIGURE // Typical tracings obtained from the measurement of MAO activity towards various substrates in rat liver and brain mitochondrial preparations. Arrows indicate the addition of inhibitors into the incubation mixture (phosphate buffer, pH 7.0 at 37 ^{o} C). Detailed procedures are described in the Appendix

TABLE I

INHIBITION OF MAO ACTIVITIES BY PIPERINE *(IN VITRO)*

(PER CENT INHIBITION)

SUBSTRATE: MONOAMINES

SUBSTRATE	PIPERINE CONCENTRATION (mcM)										
	.25	.33	.49	.65	.98	1.30	1.95	2.60	3.90	5.20	10.40
BZA			17.27	30.41	50.55	62.00	72.18	76.70	71.30	59.05	
PEA				30.26	45.70	59.06	66.97	68.63			
NE				24.36	39.50	52.32	64.87	49.36			
DA	17.41	31.83	47.78	59.09	71.01	65.95					
TRP		26.15		30.36		35.63		35.92		25.66	

All substrates were used at the concentrations rendering maximal MAO activities in normal assay conditions.

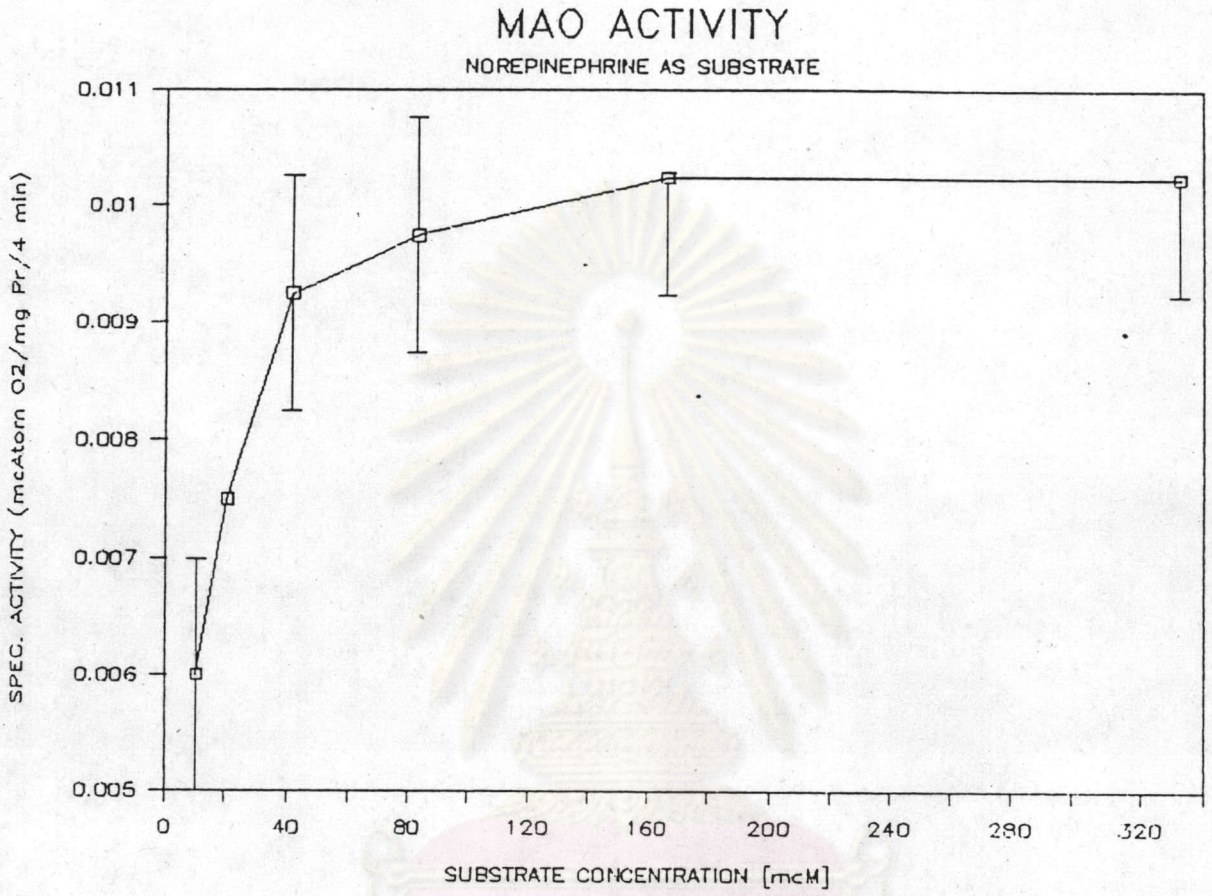

FIGURE III Substrate-activity relationship of MAO to norepinephrine, a preferential substrate for MAO-A activity.

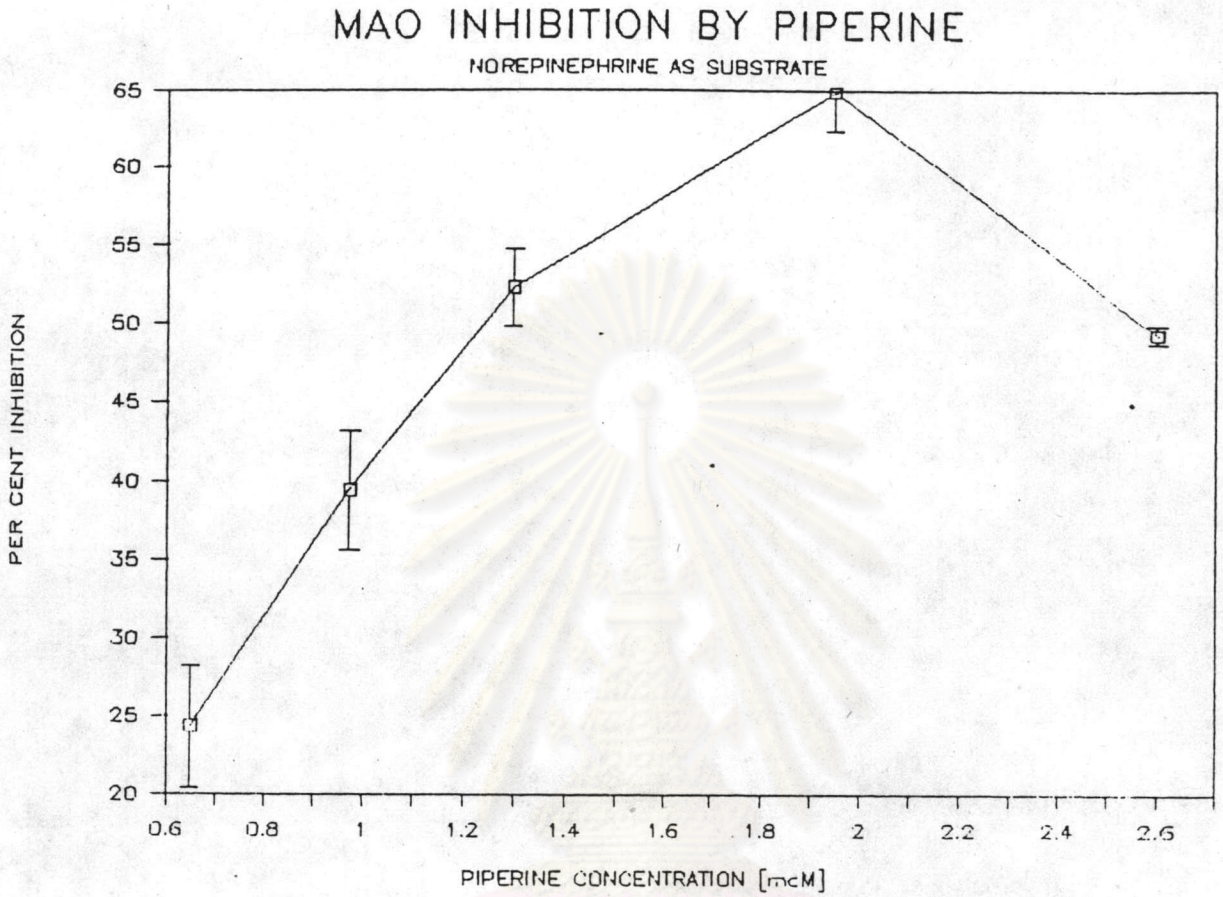

FIGURE *IV* Concentration-response relationship of MAO inhibition by piperine. Substrate (norepinephrine) concentration used was that provided maximal activity of MAO in the assay system.

TABLE II

SPECIFIC ACTIVITY OF MONOAMINE OXIDASE

(MicroAtom O2/mg Protein/4 Minutes)

SUBSTRATE: NOREPINEPHRINE

	SUBSTRATE CONCENTRATION (mcM)					
	10.40	20.80	41.60	83.20	166.40	332.80
Control:						
MEAN:	.0093	.0175	.0245	.0303	.0390	.0450
SEM:	.0006	.0013	.0016	.0021	.0029	.0027
+ Piperine (1.30 mcM):						
MEAN:	-	-	.0050	.0095	.0128	.0180
SEM:	-	-	.0006	.0005	.0003	.0012

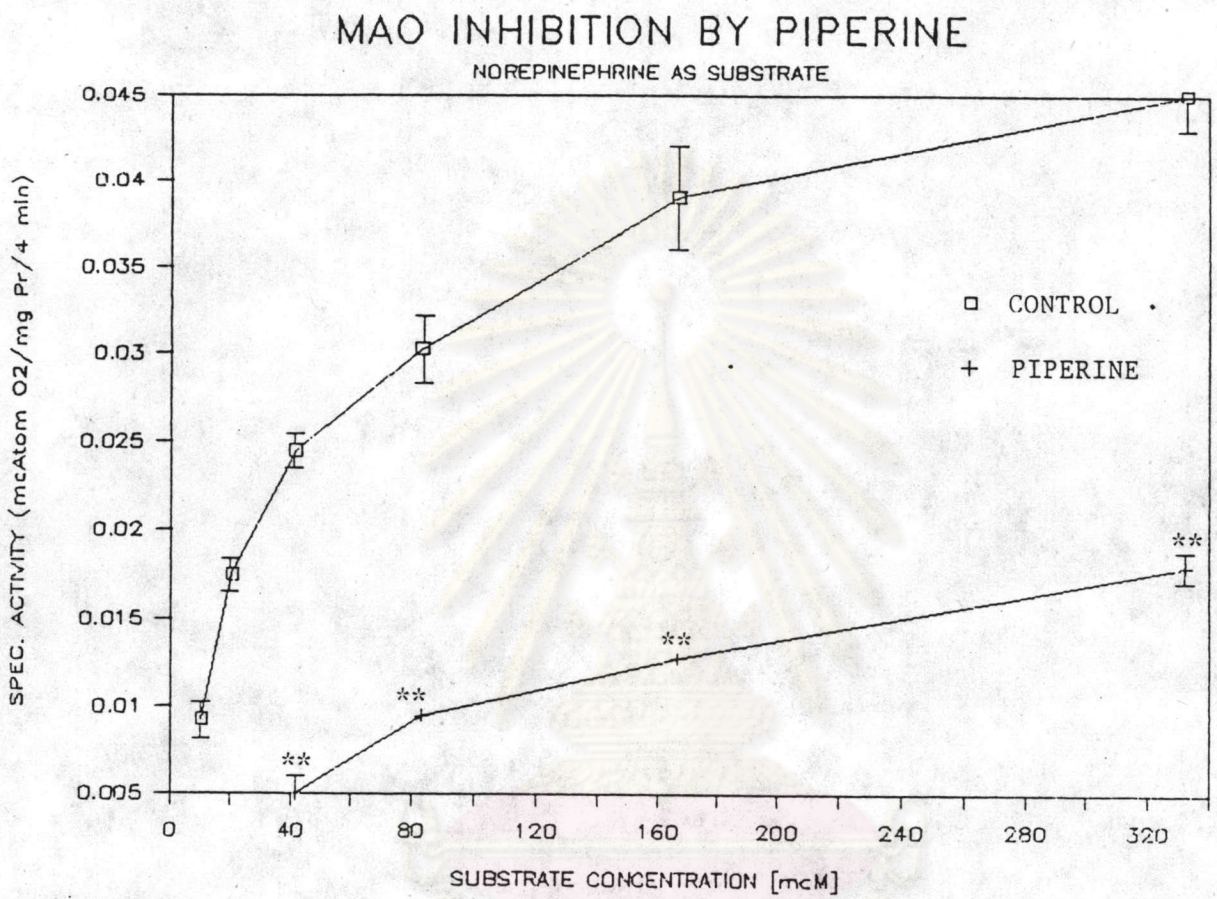

FIGURE *V* Substrate-activity relationship of MAO to norepinephrine in the presence of piperine. Concentration of piperine used in this experiment was that provided approximately 50% inhibition of maximal MAO activity in the assay system.

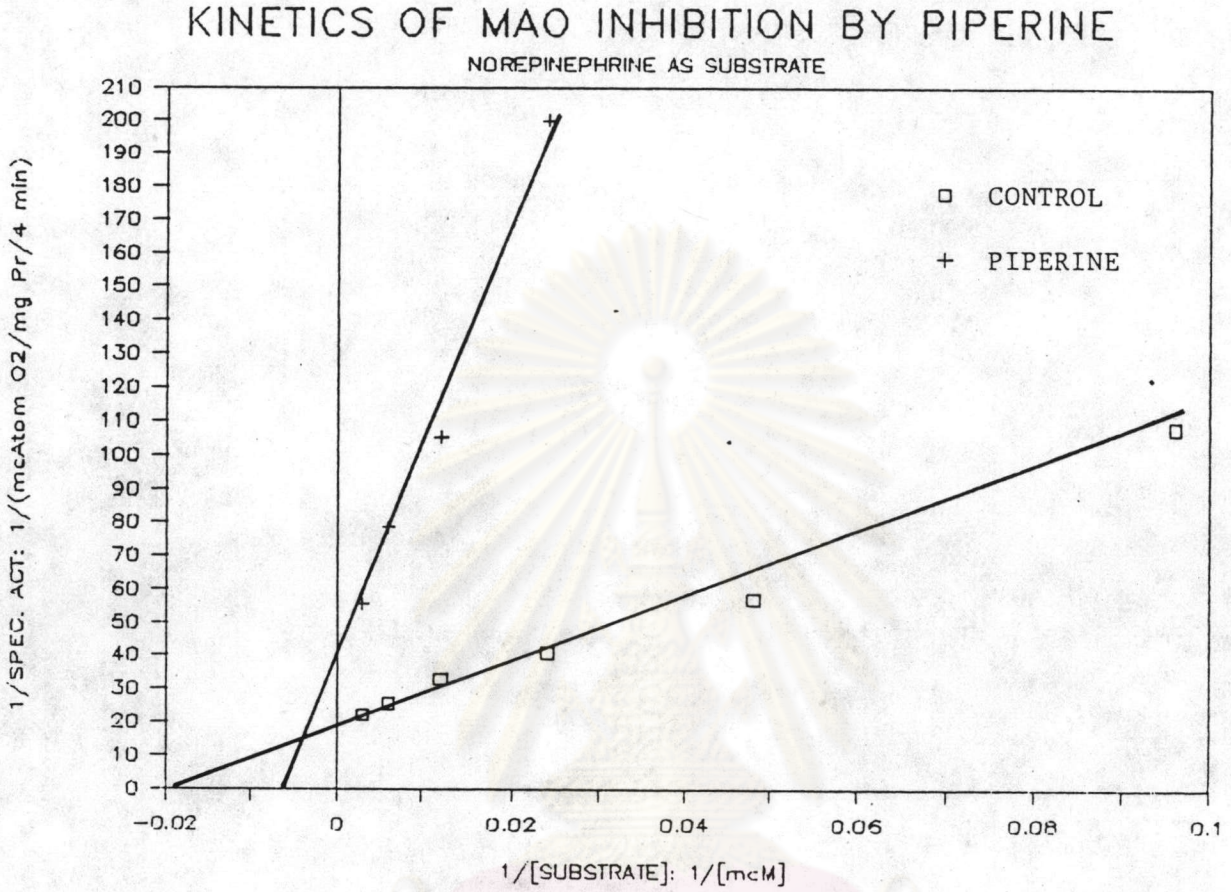

FIGURE VI Kinetic behaviour of MAO inhibition by piperine as considered by double reciprocal plot. Norepinephrine was used as a preferential substrate for MAO-A activity.

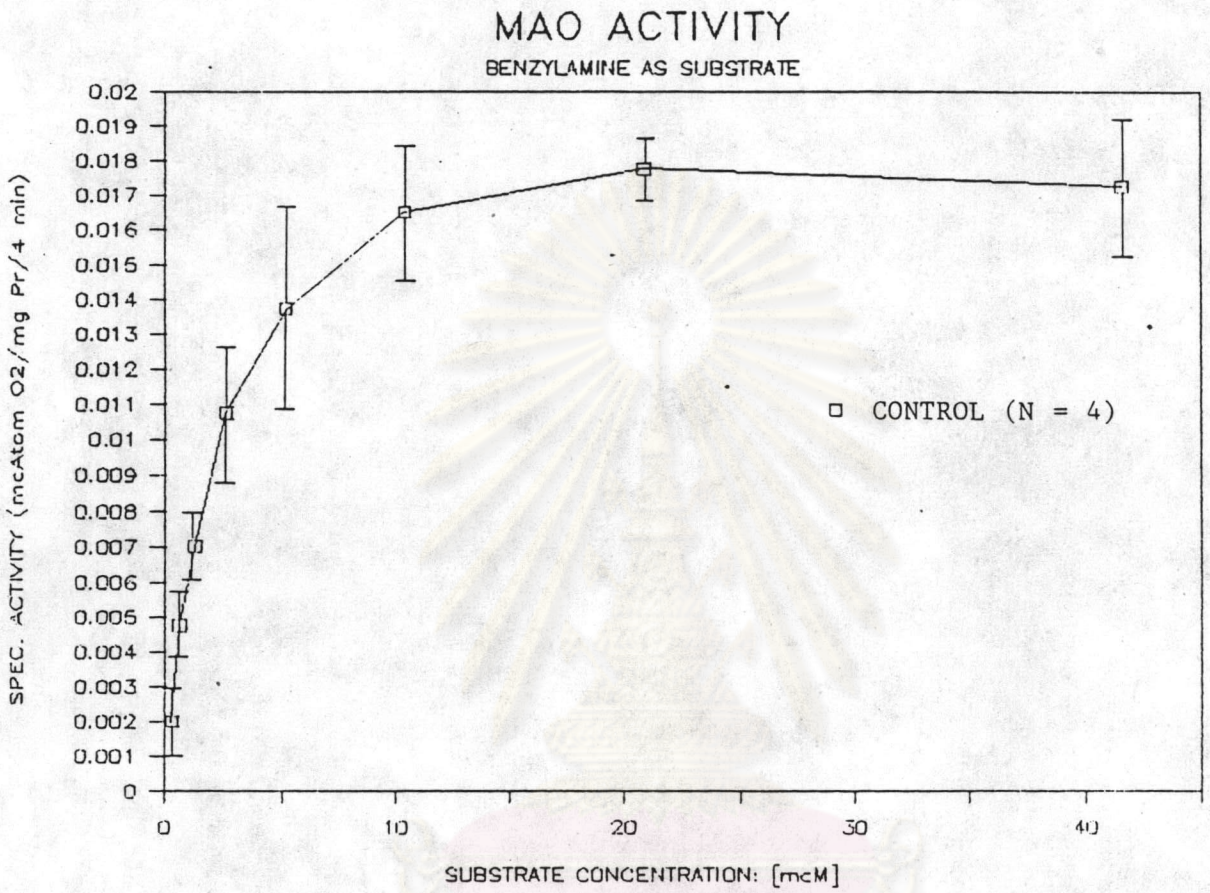

FIGURE *VII* Substrate-activity relationship of MAO to benzylamine, a preferential substrate for MAO-B activity.

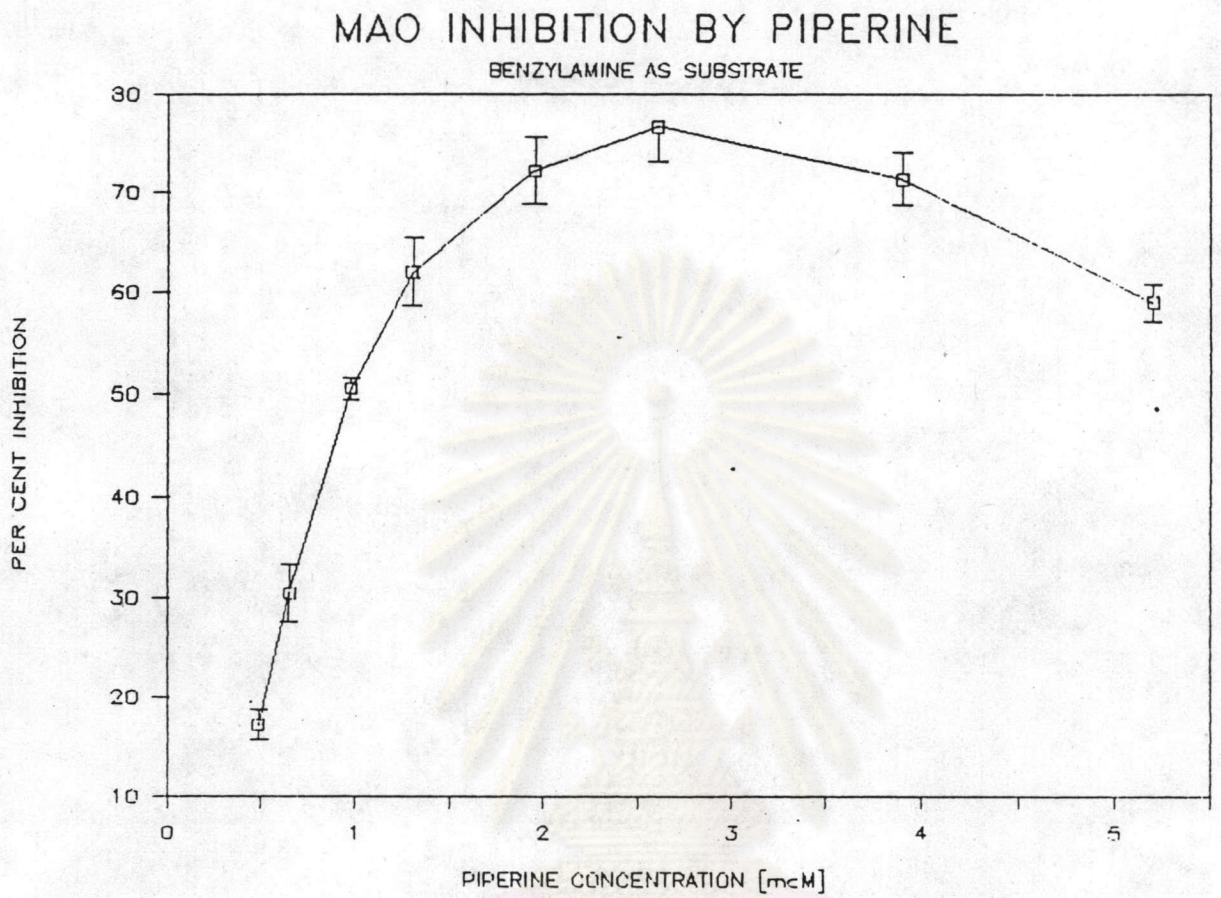

FIGURE *VIII* Concentration-response relationship of MAO inhibition by piperine. Substrate (benzylamine) concentration used was that provided maximal activity of MAO in the assay system.

TABLE III

SPECIFIC ACTIVITY OF MONOAMINE OXIDASE

(MicroAtom O2/mg Protein/4 Minutes)

SUBSTRATE: BENZYLAMINE

	SUBSTRATE CONCENTRATION (mcM)					
	1.300	2.600	5.200	10.400	20.800	41.600
Control:						
MEAN:	.0103	.0165	.0240	.0305	.0378	.0433
SEM:	.0011	.0008	.0012	.0025	.0013	.0028
+ Piperine (0.98 mcM):						
MEAN:	-	.0030	.0038	.0060	.0083	.0145
SEM:	-	.0008	.0004	.0007	.0012	.0014

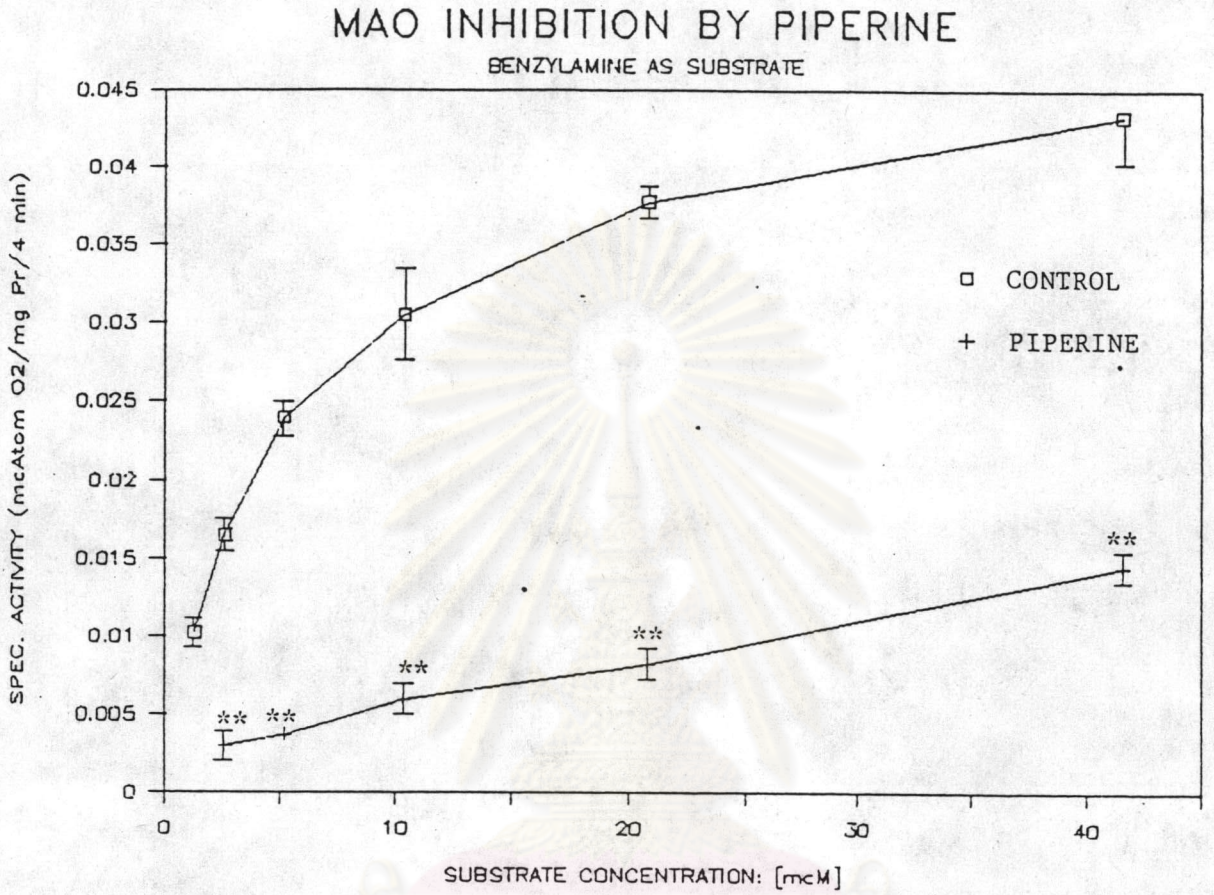

FIGURE IX Substrate-activity relationship of MAO to benzylamine in the presence of piperine. Concentration of piperine used in this experiment was that provided approximately 50% inhibition of maximal MAO activity in the assay system.

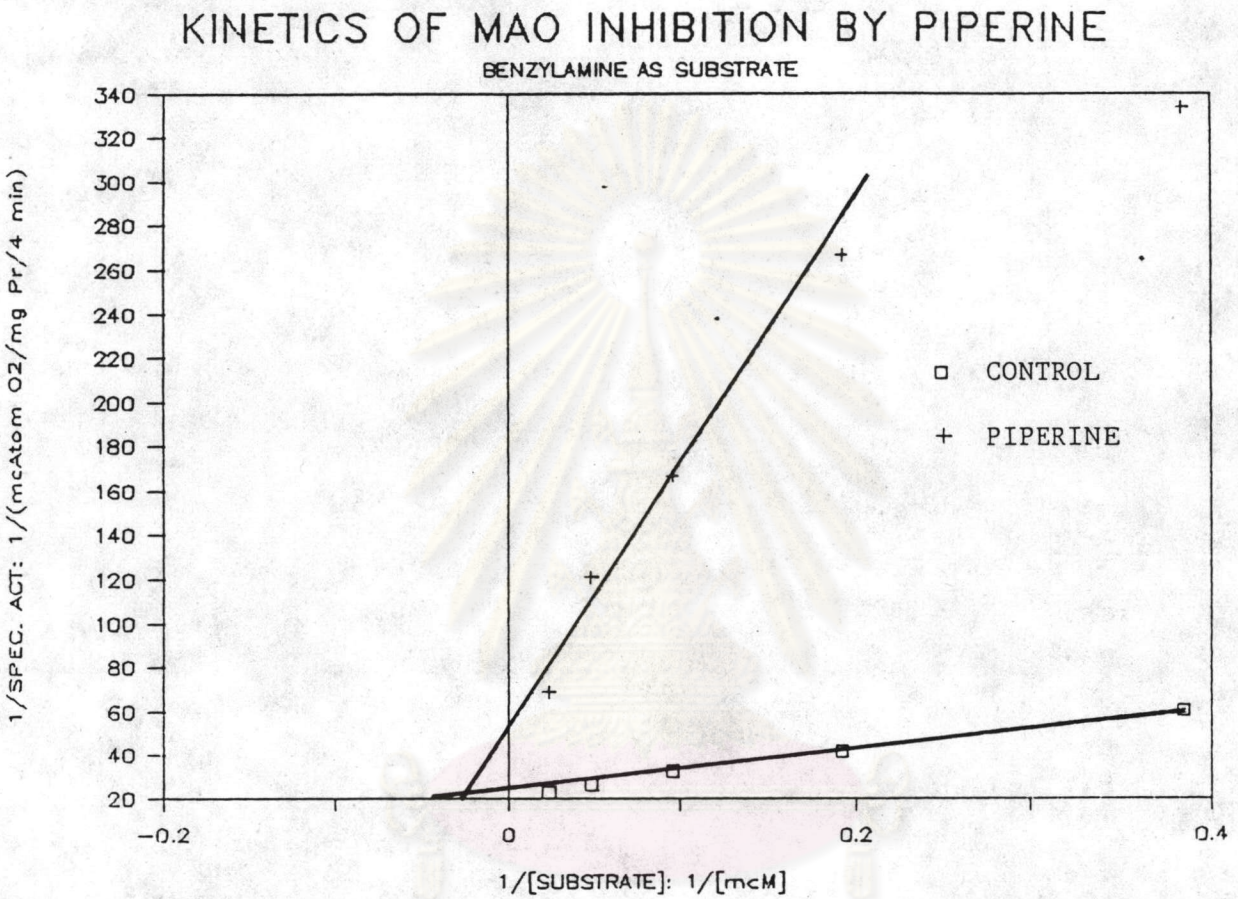

FIGURE X Kinetic behaviour of MAO inhibition by piperine as considered by double reciprocal plot. Benzylamine was used as a preferential substrate for MAO-B activity.

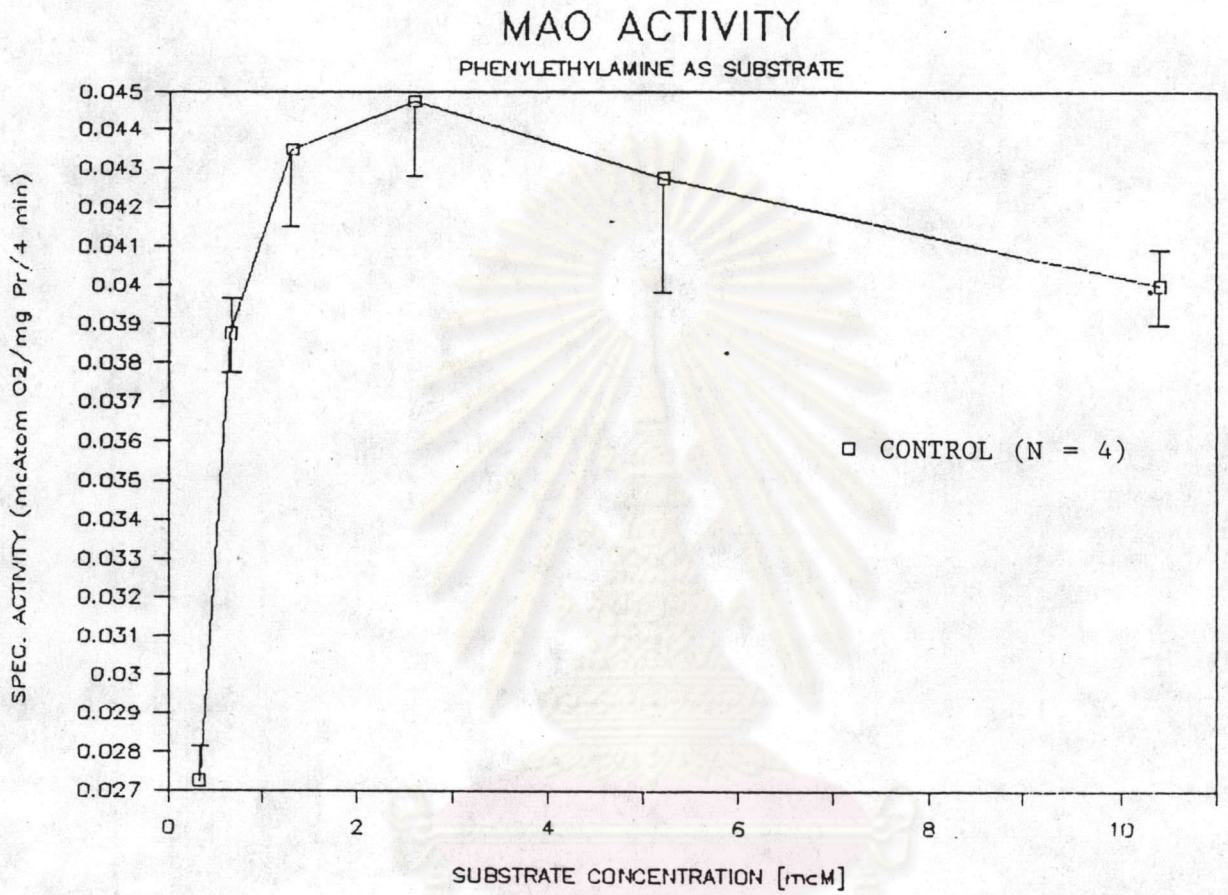

FIGURE XI Substrate-activity relationship of MAO to B-phenylethylamine, a preferential substrate for MAO-B activity.

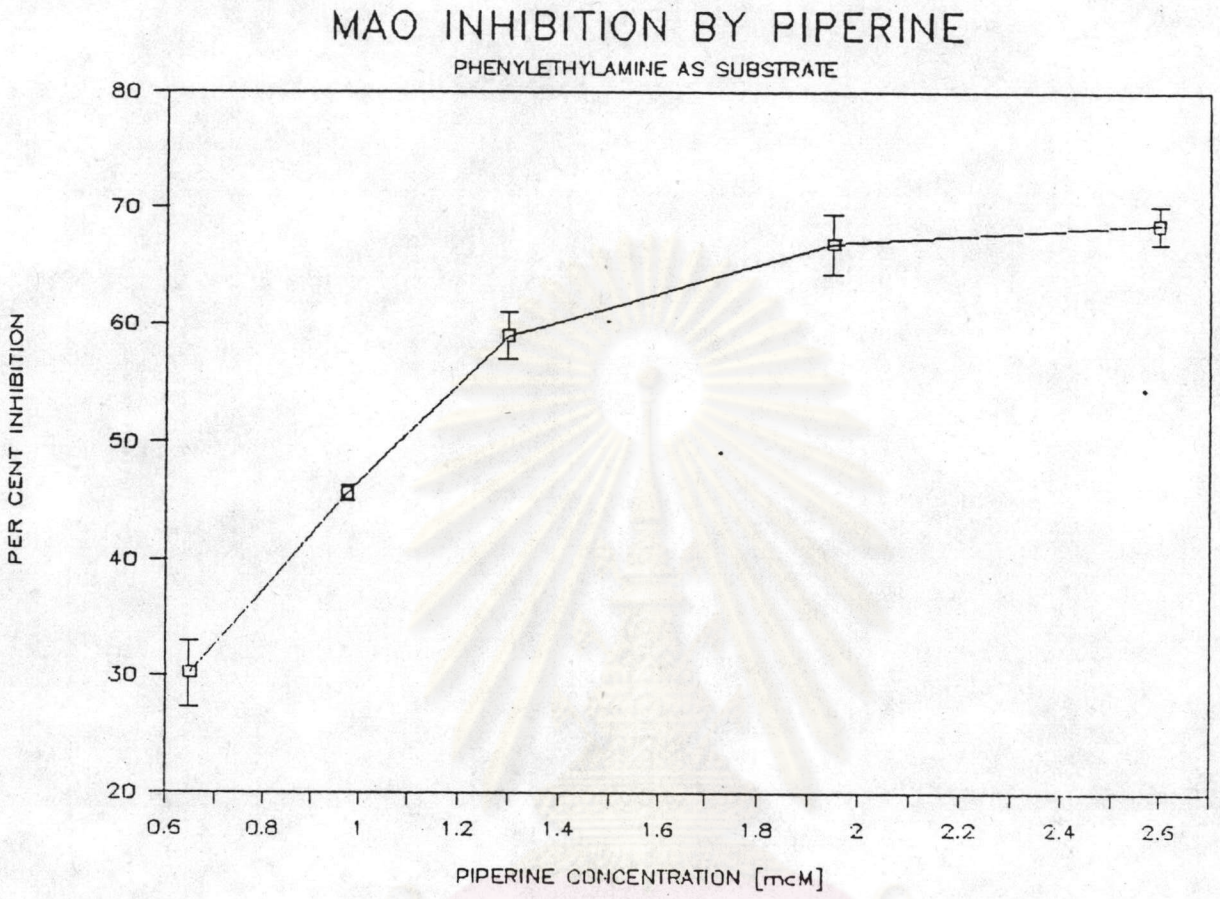

FIGURE XII Concentration-response relationship of MAO inhibition by piperine. Substrate (B-phenylethylamine) concentration used was that provided maximal activity of MAO in the assay system.

TABLE IV

SPECIFIC ACTIVITY OF MONOAMINE OXIDASE

(MicroAtom O2/mg Protein/4 Minutes)

SUBSTRATE: PHENYLETHYLAMINE

	SUBSTRATE CONCENTRATION (mcM)					
	.325	.650	1.300	2.600	5.200	10.400
Control:						
MEAN:	.0203	.0330	.0475	.0513	.0490	.0465
SEM:	.0022	.0015	.0028	.0021	.0013	.0027
+ Piperine (0.98 mcM):						
MEAN:	-	.0110	.0143	.0273	.0400	.0480
SEM:	-	.0024	.0027	.0063	.0083	.0091

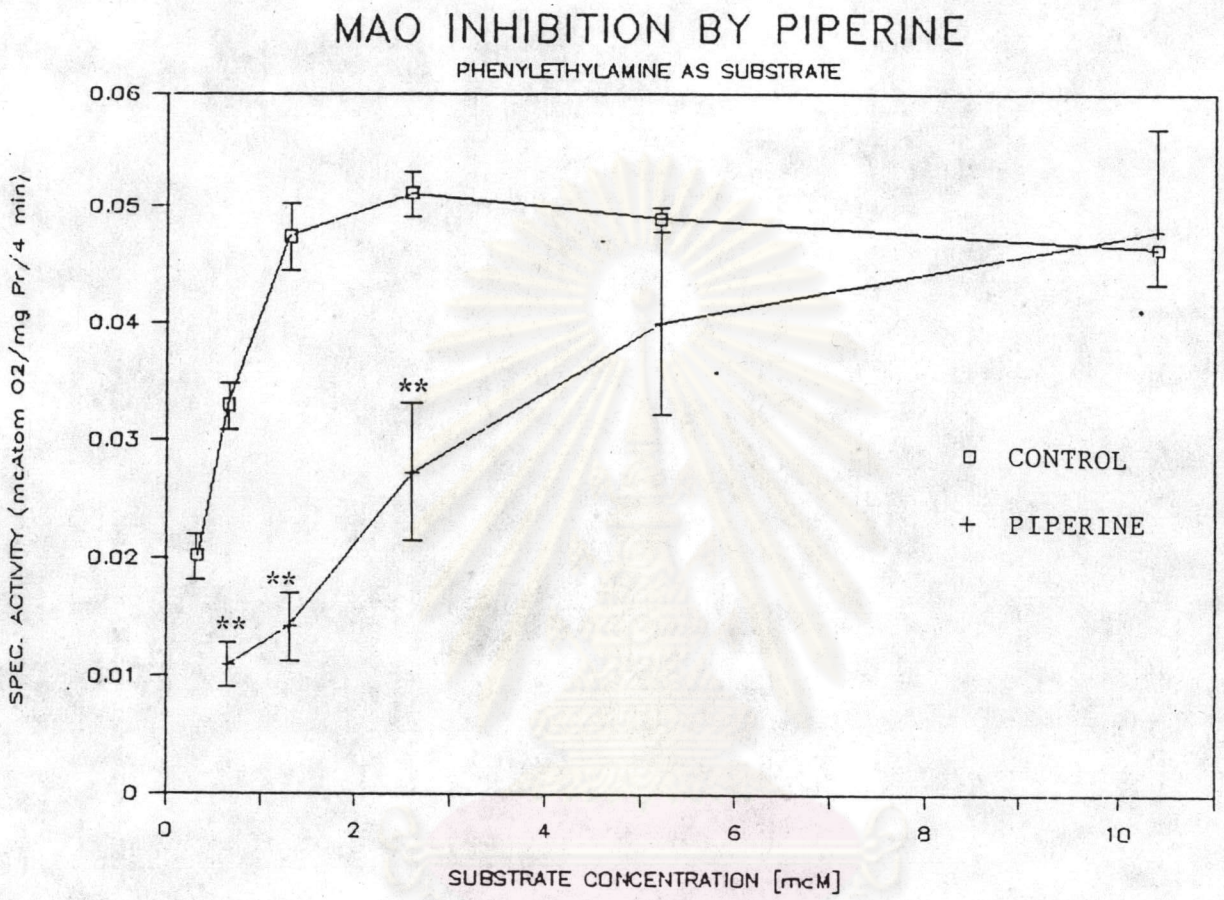

FIGURE XIII Substrate-activity relationship of MAO to B-phenylethylamine in the presence of piperine. Concentration of piperine used in this experiment was that provided approximately 50% inhibition of maximal MAO activity in the assay system.

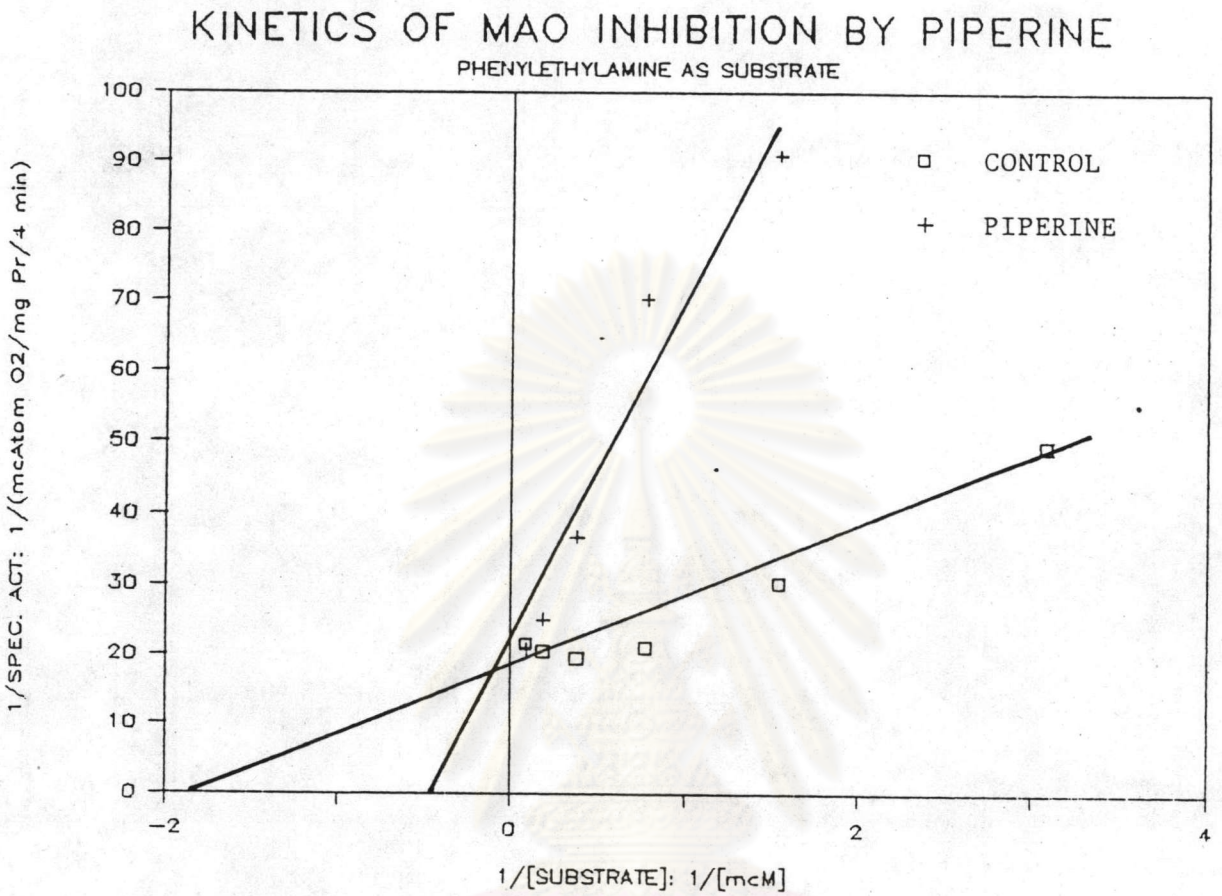

FIGURE XIV Kinetic behaviour of MAO inhibition by piperine as considered by double reciprocal plot. B-Phenylethylamine was used as a preferential substrate for MAO-B activity.

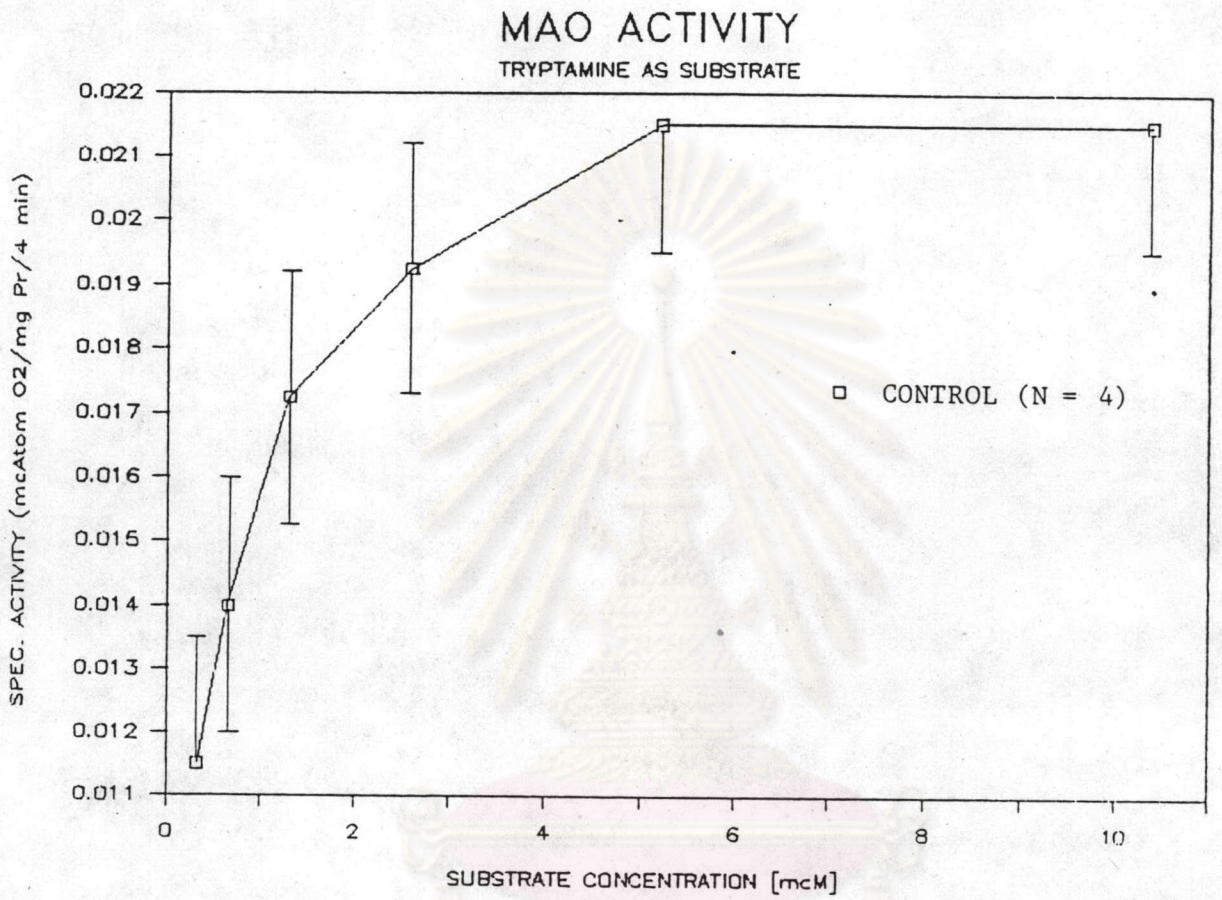

FIGURE XV Substrate-activity relationship of MAO to tryptamine, a preferential substrate for MAO-B activity.

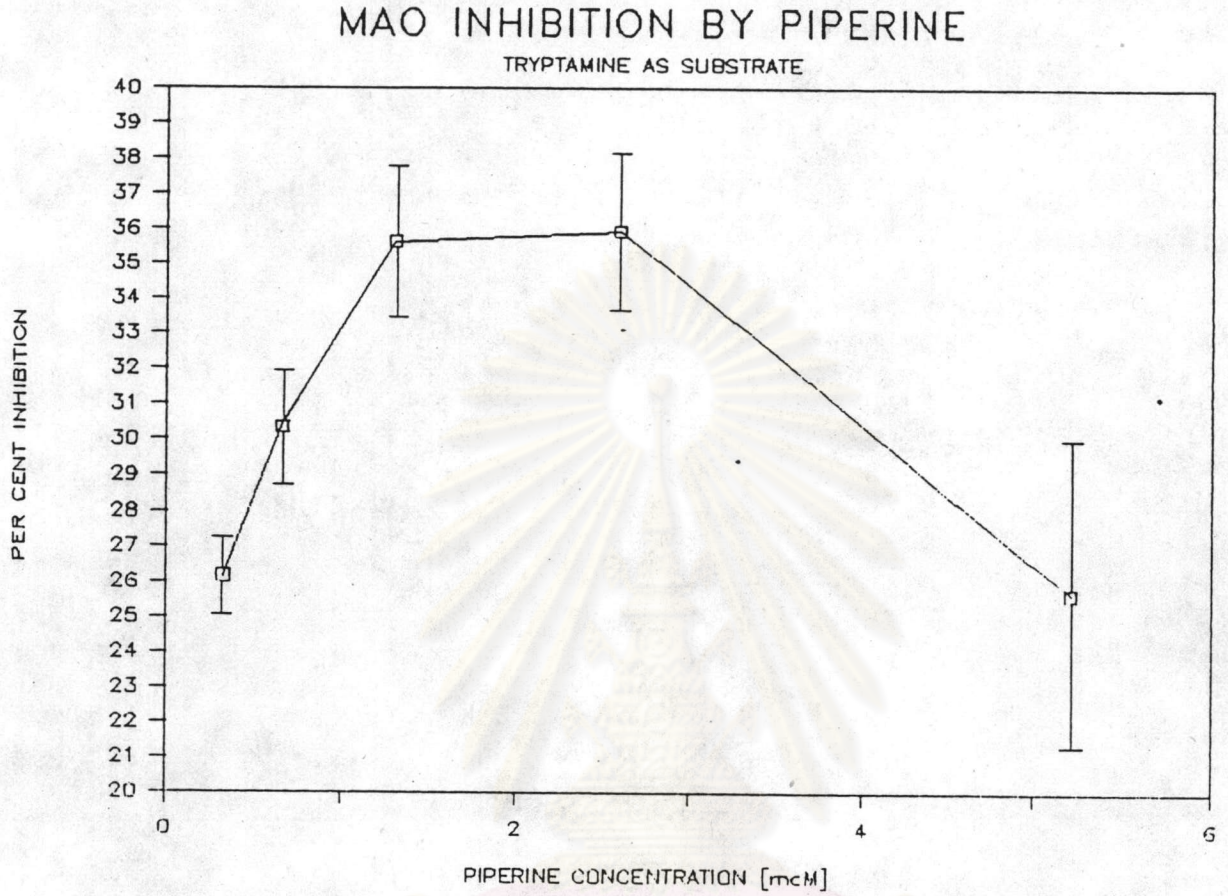

FIGURE XVI Concentration-response relationship of MAO inhibition by piperine. Substrate (tryptamine) concentration used was that provided maximal activity of MAO in the assay system.

TABLE V

SPECIFIC ACTIVITY OF MONOAMINE OXIDASE

(MicroAtom O2/mg Protein/4 Minutes)

SUBSTRATE: TRYPTAMINE

	SUBSTRATE CONCENTRATION (mcM)					
	.650	1.300	2.600	5.200	10.400	20.800
Control:						
MEAN:	.0253	.0323	.0373	.0405	.0390	.0360
SEM:	.0026	.0046	.0029	.0038	.0035	.0022
+ Piperine (1.30 mcM):						
MEAN:	.0093	.0185	.0248	.0323	.0340	.0378
SEM:	.0010	.0027	.0021	.0031	.0041	.0042

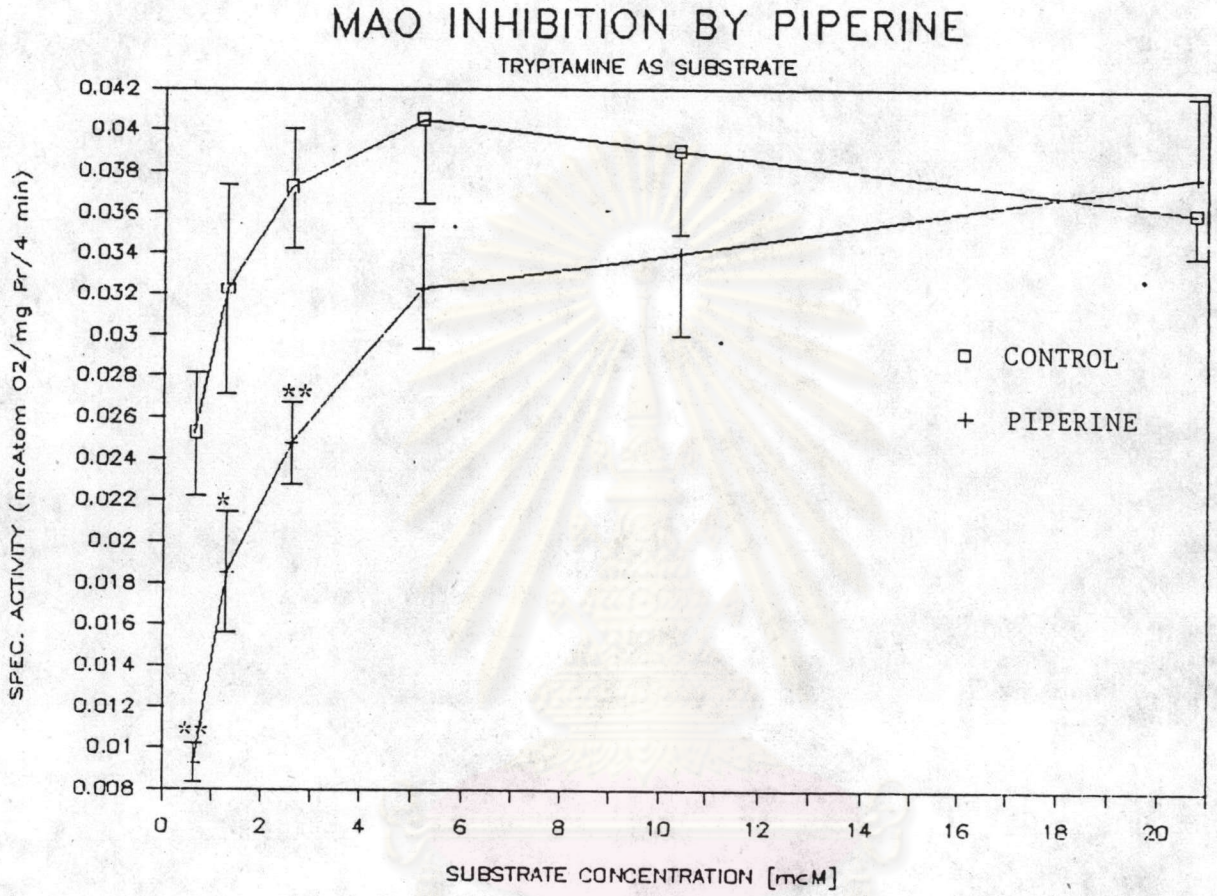

FIGURE XVII Substrate-activity relationship of MAO to tryptamine in the presence of piperine. Concentration of piperine used in this experiment was that provided approximately 50% inhibition of maximal MAO activity in the assay system.

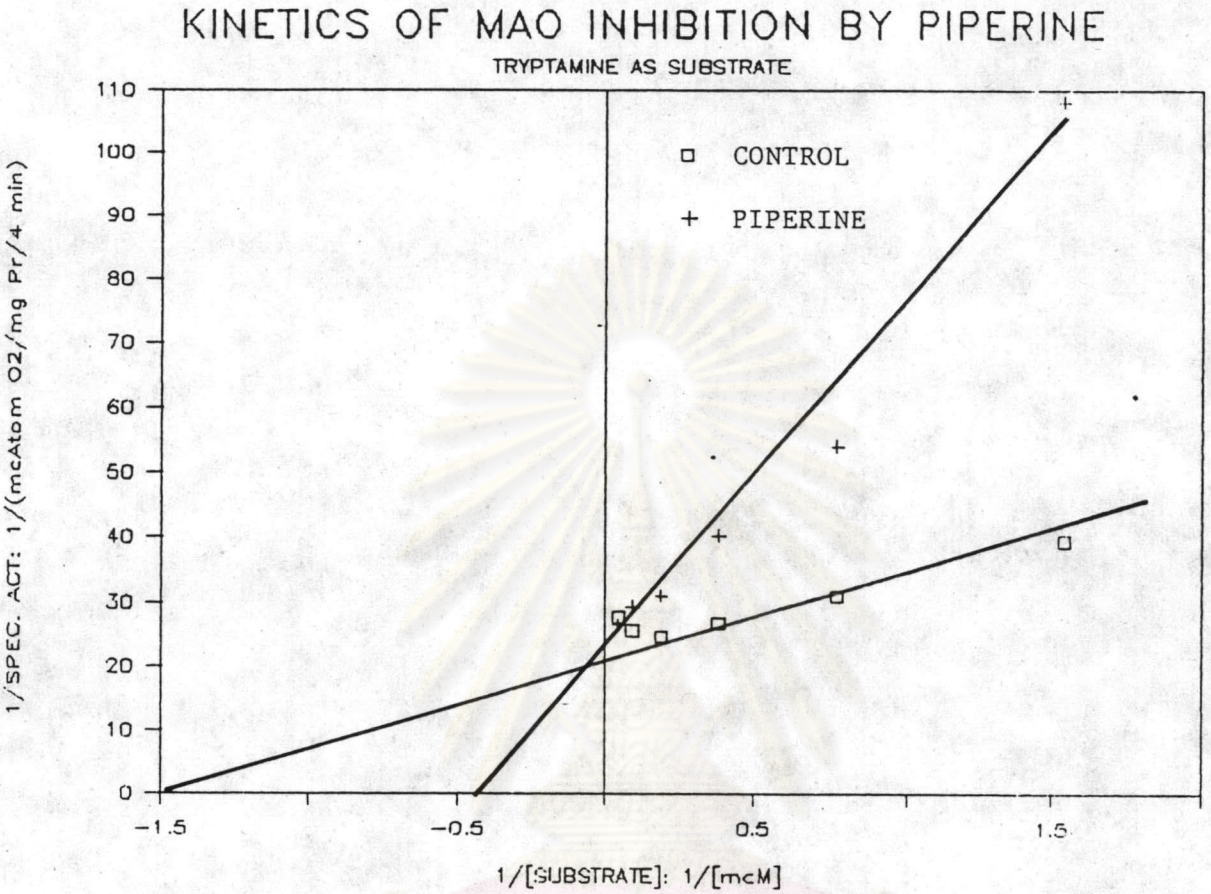

FIGURE *XVIII* Kinetic behaviour of MAO inhibition by piperine as considered by double reciprocal plot. Tryptamine was used as a preferential substrate for MAO-B activity.

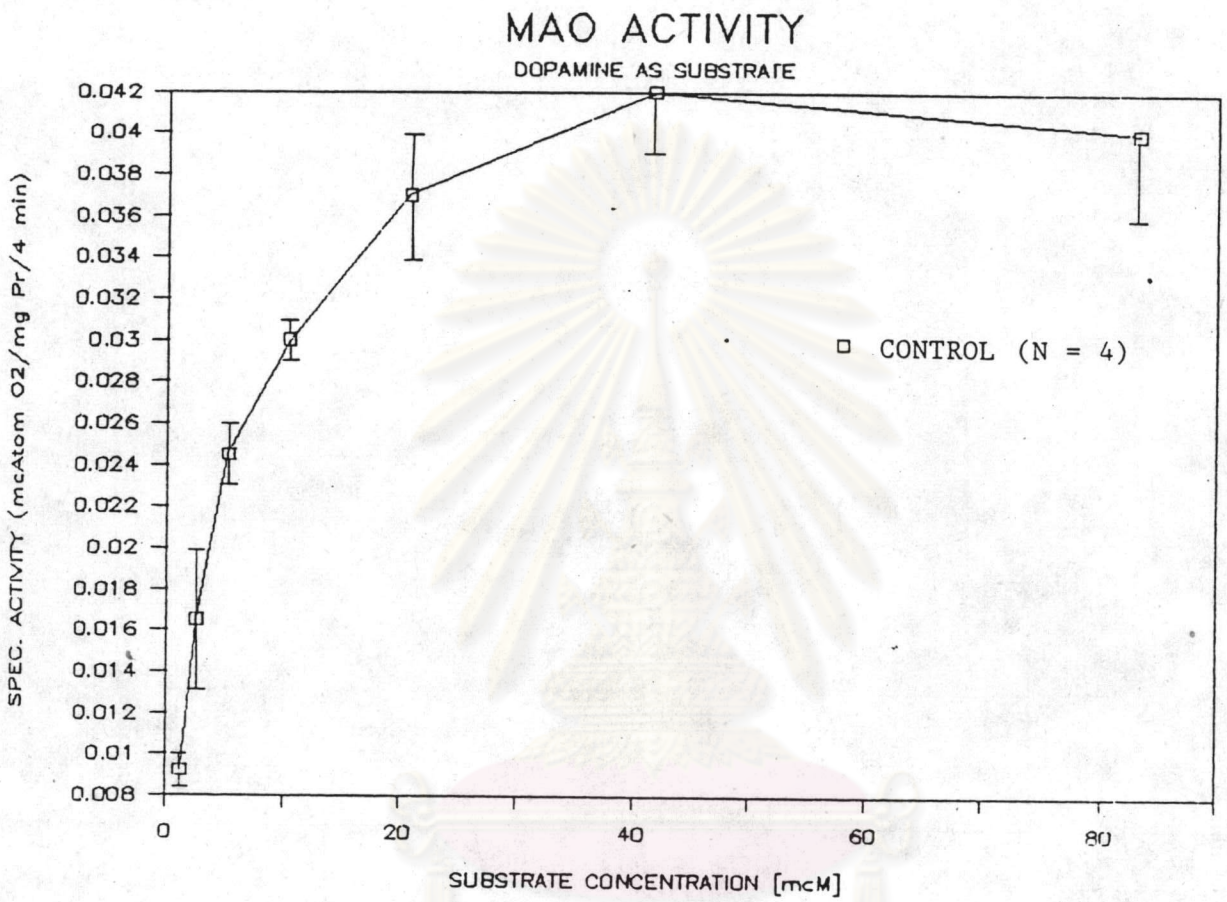

FIGURE *XIX* Substrate-activity relationship of MAO to dopamine, a common substrate for both types of MAO activity.

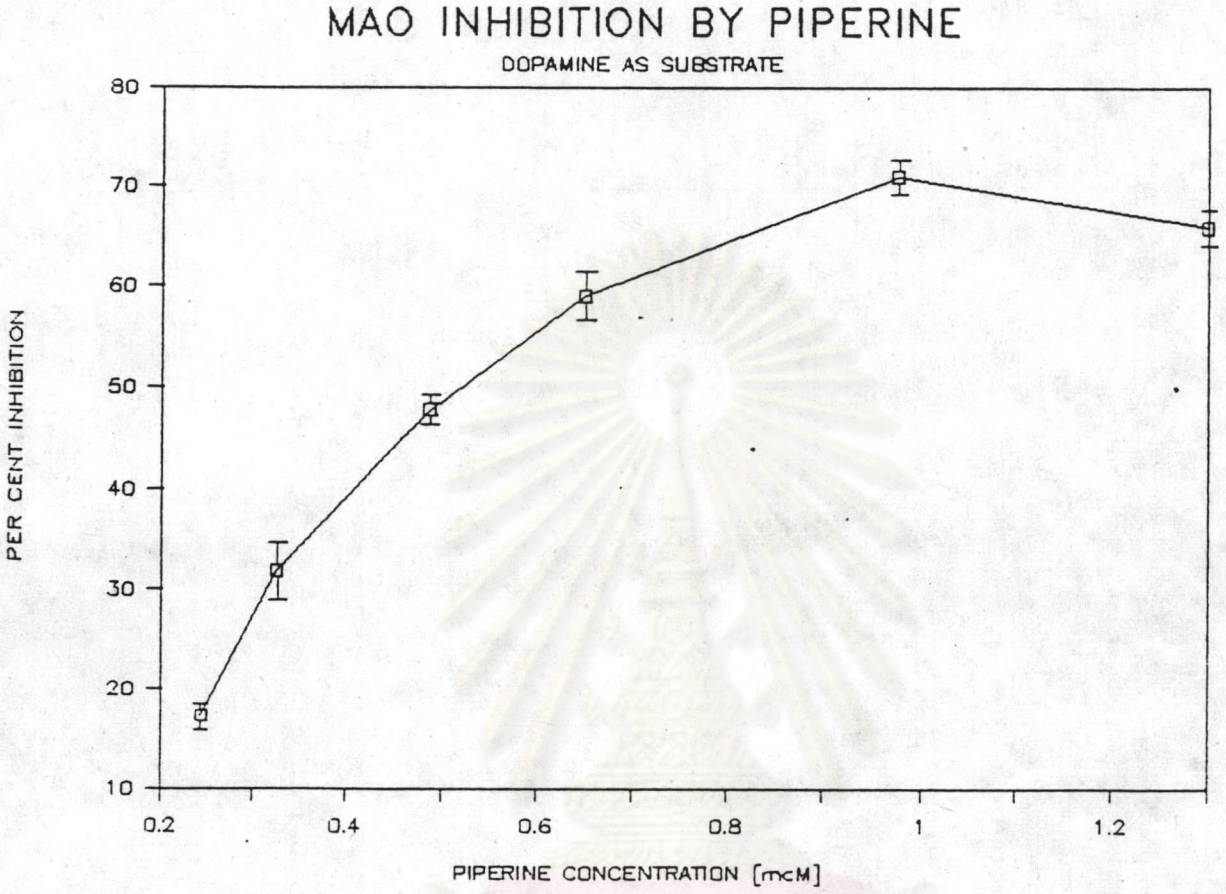

FIGURE XX Concentration-response relationship of MAO inhibition by piperine. Substrate (dopamine) concentration used was that provided maximal activity of MAO in the assay system.

TABLE VI

SPECIFIC ACTIVITY OF MONOAMINE OXIDASE

(MicroAtom O2/mg Protein/4 Minutes)

SUBSTRATE: DOPAMINE

	SUBSTRATE CONCENTRATION (mcM)				
	5.20	10.40	20.80	41.60	83.20
Control:					
MEAN:	.0230	.0313	.0388	.0448	.0508
SEM:	.0044	.0066	.0063	.0066	.0074
+ Piperine (0.49 mcM):					
MEAN:	.0083	.0125	.0210	.0390	.0523
SEM:	.0020	.0026	.0050	.0096	.0115

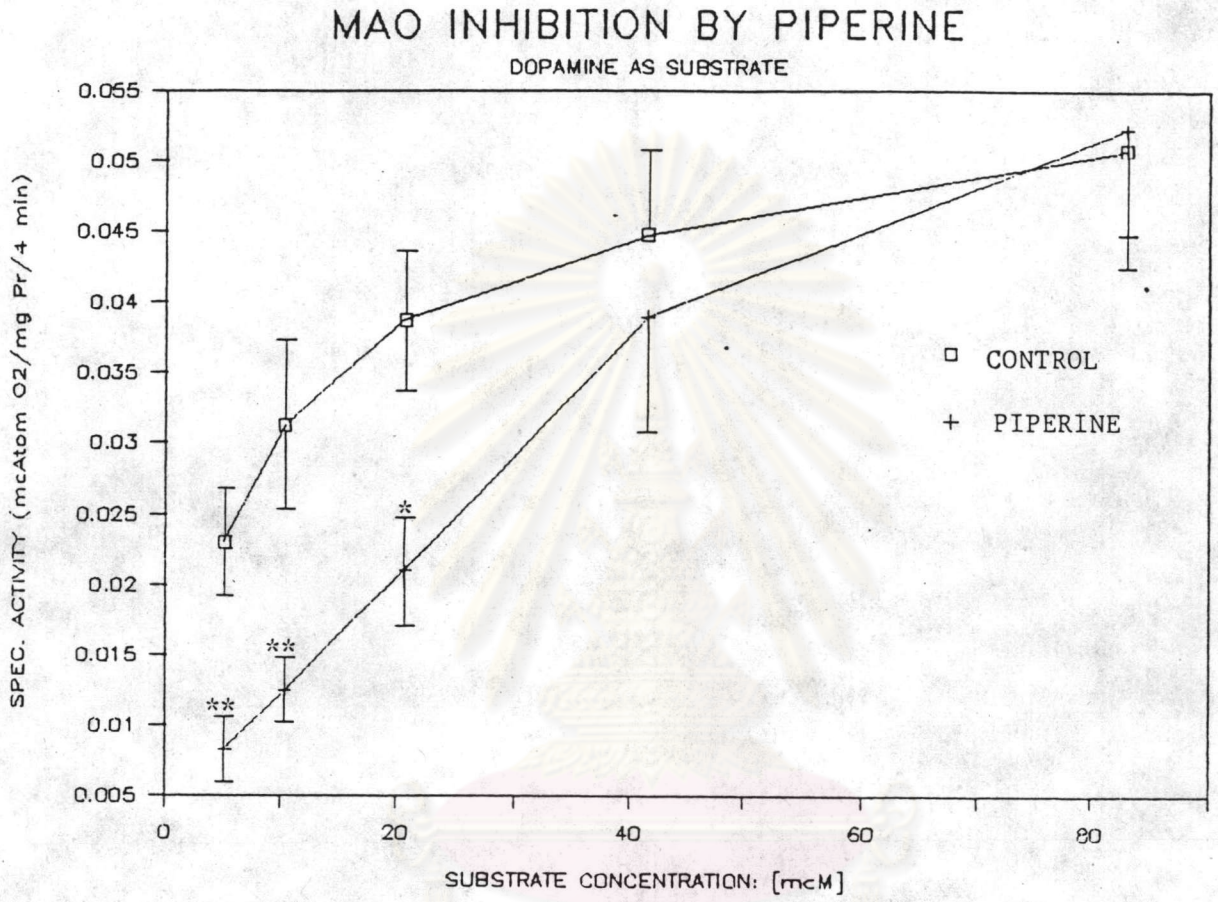

FIGURE *XXI* Substrate-activity relationship of MAO to dopamine in the presence of piperine. Concentration of piperine used in this experiment was that provided approximately 50% inhibition of maximal MAO activity in the assay system.

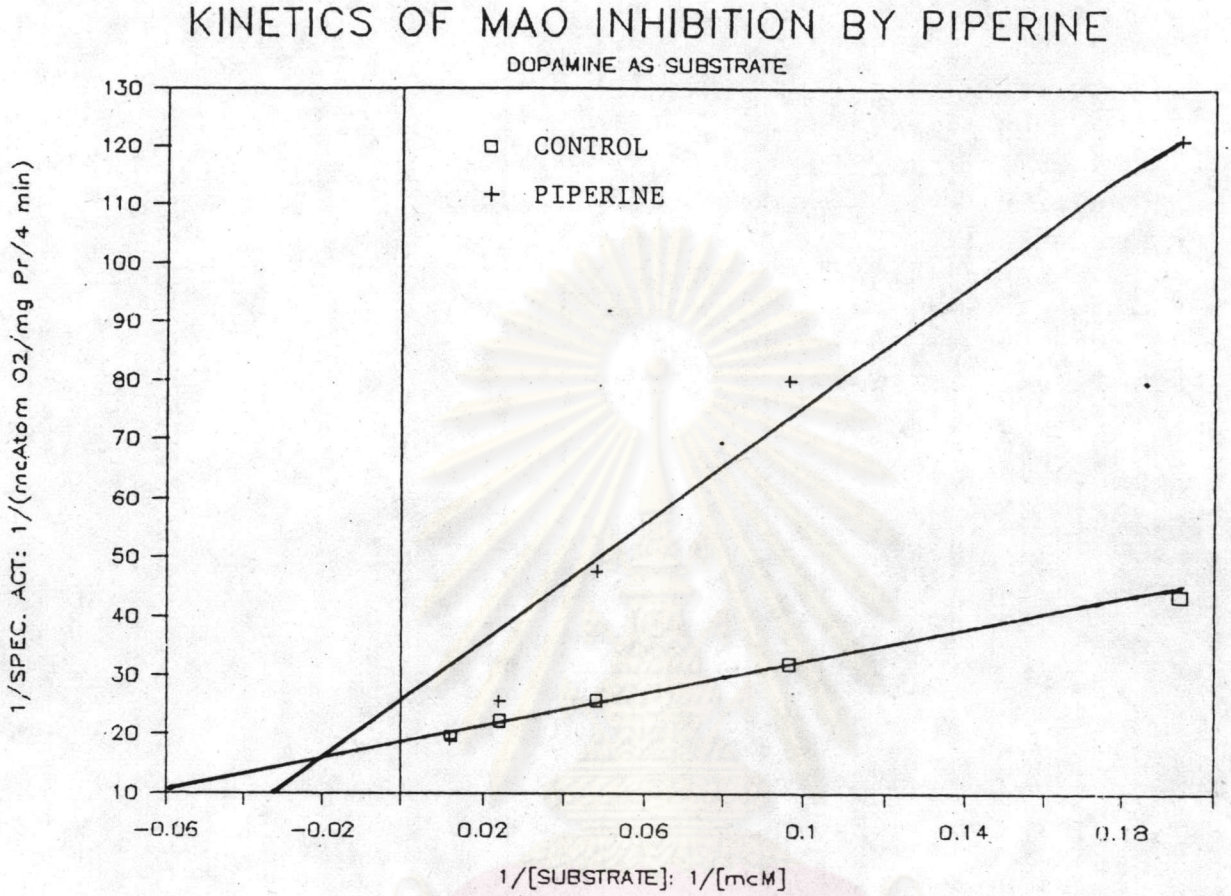

FIGURE *XXII* Kinetic behaviour of MAO inhibition by piperine as considered by double reciprocal plot. Dopamine was used as a common substrate for both types of MAO activity.

TABLE VII

KINETICS OF MONOAMINE OXIDASE INHIBITION BY PIPERINE

SUBSTRATE	CONDITION	KINETIC CONSTANT	
		Km (mcM)	Vmax (mcAtom O2 /mg Pr./4 min)
Benzylamine	Control	3.901	20.00
	+Piperine	7.593	70.00
B-Phenylethylamine	Control	0.459	16.50
	+Piperine	2.164	18.50
Norepinephrine	Control	39.392	18.50
	+Piperine	193.406	29.00
Dopamine	Control	5.262	19.00
	+Piperine	30.137	15.00
Tryptamine	Control	0.298	24.00
	+Piperine	2.031	20.00

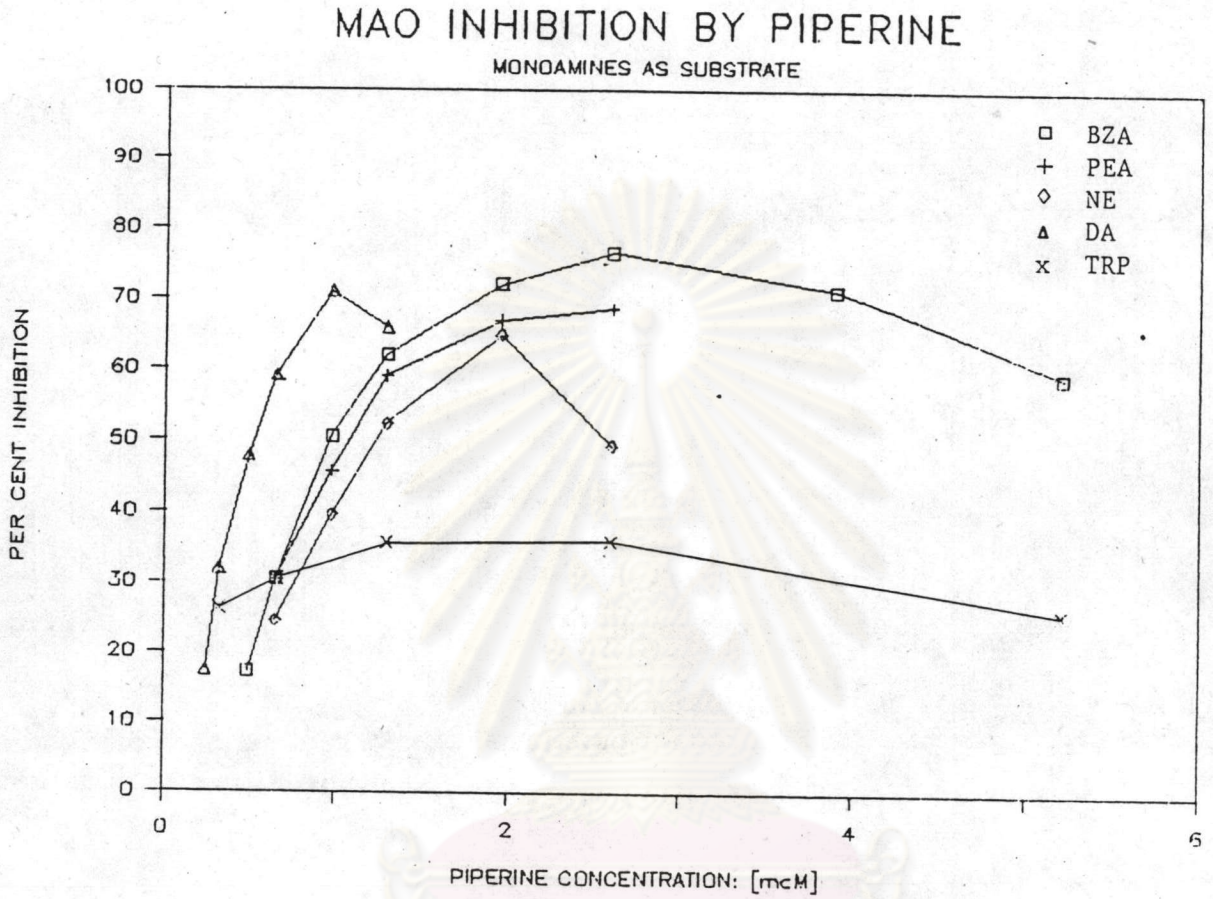

FIGURE *XXIII* Comparison of the inhibitory effects of piperine to rat liver mitochondrial MAO activities towards various substrates.

TABLE VIII

RAT BRAIN MITOCHONDRIAL MONOAMINE OXIDASE ACTIVITY

(MicroAtom O2/mg Pr/4 min)

NUMBER	DOPAMINE		NOREPINEPHRINE		PHENYLETHYLAMINE	
	CONTROL	+PIPERINE	CONTROL	+PIPERINE	CONTROL	+PIPERINE
1	.0220	.0200	.0100	.0110	.0080	.0070
2	.0200	.0170	.0080	.0070	.0060	.0060
3	.0070	.0070	.0080	.0100	.0060	.0060
MEAN:	.0163	.0147	.0087	.0093	.0067	.0063
SEM:	.0038	.0032	.0005	.0010	.0005	.0003