20 hr. The mixture was cooled and filtered and the filtrate was concentrated *in vacuo*. The residue was refluxed with 50 ml. of acetic anhydride for 1 hr., the solution was concentrated *in vacuo* and the residue dissolved in water. After extraction with ether, the solution was made alkaline and the mixture was extracted with ether. The ether extracts were dried and concentrated to give a colorless liquid, b.p. 75–83° (0.3 mm.), which was converted to a hydrochloride.

2-Substituted Cyclopropylamines. II. Effect of Structure upon Monoamine Oxidase-Inhibitory Activity as Measured *in Vivo* by Potentiation of Tryptamine Convulsions

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The monoamine oxidase (MAO)-inhibitory activity of numerous analogs and ring-homologs of 2-phenylcyclopropylamine, and related compounds, as measured *in vivo* by potentiation of tryptamine convulsions, has been determined. The results indicated that the structural requirements for potent *in vivo* MAO-inhibitory activity in this class of compounds are: (1) a cyclopropane ring, (2) an amino group attached directly to the cyclopropane ring, and (3) a 2-substituent containing an aromatic moiety. On the basis of an examination of molecular models of cyclopropylamine derivatives and other types of MAO-inhibitors, possible modes of interaction of these compounds with MAO have been considered.

An earlier paper[1] in this series reported the monoamine oxidase (MAO)-inhibitory activities of *trans*- and *cis*-2-phenylcyclopropylamine and various other types of compounds as measured by potentiation of tryptamine convulsions in rats.[2,3] By this and other test

(1) D. H. Tedeschi, R. E. Tedeschi, and E. J. Fellows, *Proc. Soc. Exp. Biol. Med.*, **103**, 680 (1960).

(2) D. H. Tedeschi, R. E. Tedeschi, and E. J. Fellows, *J. Pharmacol. Exptl. Therap.*, **126**, 223 (1959).

(3) D. R. Maxwell, W. R. Gray, and E. M. Taylor, *Brit. J. Pharmacol.*, **17**, 310 (1961), recently have reported on a similar test procedure in the mouse wherein tryptamine potentiation was used as a measure of MAO inhibition.

procedures the 2-phenylcyclopropylamines have been found to be among the most potent MAO inhibitors known.[1-13] The *trans* isomer[14] is a clinically effective antidepressant agent.

To determine the effect of structure upon MAO-inhibitory activity in the cyclopropylamine series, we have tested by the tryptamine potentiation method[2] numerous optical and positional isomers, homologs and analogs of 2-phenylcyclopropylamine as well as various related compounds. The results of this study are the subject of the present report. The chemistry of most of the compounds investigated was described in the preceding paper.[15]

Materials and Methods.—The compounds studied are listed in Tables I-IX. Sources of compounds are given in the footnotes to the Tables.

Monoamine oxidase inhibition was measured *in vivo* by the tryptamine potentiation test previously described by Tedeschi, *et al.*[1,2] Groups of rats were treated orally with the compounds under investigation and at various time intervals thereafter injected intravenously with tryptamine hydrochloride (5 mg./kg.). This dose of tryptamine causes convulsions in only 4% of untreated rats and is referred to as a CD_4. The dose of drug effective in causing 50% of rats to respond to the CD_4 of tryptamine with 3 seconds or more of uninterrupted clonic seizure activity (ED_{50}) and 95% fiducial limits were calculated according to the log-probit method of Litchfield and Wilcoxon.[16] In cases where ED_{50}'s were not determined the results are expressed in terms of the maximum per cent incidence of convulsions observed in rats treated with the dose indicated.

(4) R. E. Tedeschi, D. H. Tedeschi, L. Cook, P. A. Mattis, and E. J. Fellows, *Fed. Proc.*, **18**, 451 (1959).

(5) R. E. Tedeschi, D. H. Tedeschi, P. L. Ames, L. Cook, P. A. Mattis, and E. J. Fellows, *Proc. Soc. Exp. Biol. Med.*, **102**, 380 (1959).

(6) A. R. Maass and M. J. Nimmo, *Nature*, **184**, 547 (1959).

(7) E. Costa, *The Pharmacologist*, **1**, 82 (1959).

(8) E. Costa, in "International Review of Neurobiology," Vol. 2, C. C. Pfeiffer and J. R. Smythies, Editors, Academic Press, New York, N. Y., 1960.

(9) R. E. Tedeschi, D. H. Tedeschi and, E. J. Fellows, Abstracts, 137th Meeting of American Chemical Society, Cleveland, Ohio, April 5–14, 1960, p. 26-N.

(10) H. Green and R. W. Erickson, *J. Pharmacol. Exptl. Therap.*, **129**, 237 (1960).

(11) (a) S. Sarkar, R. Banerjee, M. S. Ise, and E. A. Zeller, *Helv. Chim. Acta*, **43**, 439 (1960); (b) E. A. Zeller and S. Sarkar, *J. Biol. Chem.*, **237**, 2333 (1962); (c) S. Sarkar, Dissertation, Northwestern University, 1961.

(12) A. Horita and W. R. McGrath, *Biochem. Pharmacol.*, **3**, 206 (1960).

(13) W. A. Himwich, E. Costa, and H. E. Himwich, in "Recent Advances in Biological Psychiatry," J. Wortes, ed., Grune and Stratton, New York, N. Y., 1960, p. 321.

(14) *trans*-2-Phenylcyclopropylamine sulfate, SKF *trans* 385, tranylcypromine. Parnate®.

(15) C. Kaiser, B. M. Lester, C. L. Zirkle, A. Burger, C. S. Davis, T. J. Delia, and L. Zirngibl, *J. Med. Pharm. Chem.*, **5**, 1243 (1962).

(16) J. T. Litchfield, Jr., and F. Wilcoxon, *J. Pharmacol. Exptl. Therap.*, **96**, 99 (1949).

TABLE I

STEREOISOMERS OF 2-PHENYLCYCLOPROPYLAMINE

Cpd.	Optical Isomer	Config.	ED_{50} (p.o.) mg./kg. (95% fiducial limits(1)[c]	Mmoles/kg. $\times 10^3$	Relative potency[d]
1	(±)	*trans*[a]	0.18 (0.13–0.24)	1.1	1.0
2	(+)	*trans*[b]	0.15 (0.093–0.24)	0.88	1.2
3	(−)	*trans*[b]	0.64 (0.42–0.99)	3.8	0.3
4	(±)	*cis*[a]	0.42 (0.30–0.60)	2.5	0.4

[a] A. Burger and W. L. Yost, *J. Am. Chem. Soc.*, **70**, 2198 (1948). [b] Reference 15. [c] Weight of hydrochloride salt. [d] Potency relative to that of (±)-*trans*-2-phenylcyclopropylamine which is assigned a value of 1.0.

Monoamine oxidase inhibition was measured *in vitro* by the method of Sjoerdsma, *et al.*[17] The concentration of drug necessary to inhibit the MAO activity of a normal rat brain homogenate by 50% is defined as the I_{50}.

Results.—The *in vivo* MAO-inhibitory activities of the various cyclopropylamines and related compounds are presented in Tables I-IX. In Table X the *in vitro* MAO-inhibitory activities of several cyclopropylamine derivatives are given and compared with the activities of other types of MAO inhibitors.[18]

Effects of Structure upon MAO-Inhibitory Activity. Stereoisomerism. (Tables I, IV, and VII).—Significant but, in general, not striking differences in potencies were observed among the stereoisomers of the cyclopropylamines. Thus (+)-*trans*-2-phenylcyclopropylamine (2) is only about four times more potent than the (−) isomer (3). Furthermore, the (±) *cis* isomer of 2-phenylcyclopropylamine (4) is about 1/2 to 1/3 as active as the (±)-*trans* isomer (1). Zeller[11] found these isomers to be almost equipotent *in vitro*. No significant difference in the tryptamine-potentiating activity of the *trans* and *cis* isomers of 1-methyl-2-phenylcyclopropylamine (52 and 53) was observed. In the 2-phenoxycyclopropylamine series, however, more pronounced effects of configuration upon activity were seen. The *cis* isomer (31) is more potent than the *trans* isomer (32) by a factor of 10.

Size of Alicyclic Ring (Table II).—The great degree of specificity of the cyclopropane ring for MAO-inhibitory activity is demonstrated by the data on the higher ring homologs of 2-phenylcyclopropylamine, the cyclobutyl- (5, 6), cyclopentyl- (7, 8), cyclohexyl- (9, 10), and cycloheptylamines (11, 12). Of these, only one compound, *cis*-2 phenylcyclohexylamine (10), showed significant activity, and it is only about 1/100th as potent as compound 1.

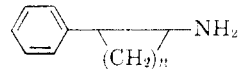
Substituents on the Benzene Ring (Table III).—Both electron-attracting and electron-donating substituents at the 4-position, chloro (13), trifluoromethyl (18), methoxy (21), and methyl (20), had little effect upon potency. All of the 4-substituted derivatives have at least 40% of the potency of compound 1. The 2-

(17) A. Sjoerdsma, T. E. Smith, T. D. Stevenson, and S. Udenfriend, *Proc. Soc. Exp. Biol. Med.*, **89**, 36 (1955).

(18) We are indebted to Dr. Harry Green of Smith Kline and French Laboratories for the *in vitro* data presented in Table X.

TABLE II

RING-HOMOLOGS OF 2-PHENYLCYCLOPROPYLAMINE

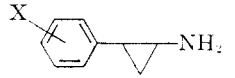


Cpd.	n	Config.	Dose mg./kg. (p.o.)[c]	Tryptamine potentiation %	Relative potency[d]
5	2	*trans*[a]	50	0	
6	2	*cis*[a]	100	0	
7	3	*trans*[b]	100	29	
8	3	*cis*[b]	75	0	
9	4	*trans*[b]	150	12	
10	4	*cis*[b]	ED_{50}30		0.008
11	5	*trans*[b]	200	10	
12	5	*cis*[b]	100	44	

[a] C. Beard and A. Burger, *J. Org. Chem.*, **26**, 2335 (1961). [b] Reference 15 and references cited therein. [c] Weight of hydrochloride salt. [d] Defined in Table I.

TABLE III

2-(SUBSTITUTED-PHENYL)CYCLOPROPYLAMINES



Cpd.[a]	X	ED_{50}(p.o.) mg./kg. (95% fiducial limits)[b]	Mmoles/kg. $\times 10^3$	Relative potency[c]
13	4-Cl	0.34 (0.22–0.51)	1.7	0.6
14	3-Cl	1.3 (0.97–1.74)	6.4	0.2
15	2-Cl	1.5 (0.54–4.2)	7.4	0.1–0.2
16	3,4-$(Cl)_2$	1.1 (0.07–1.6)	4.4	0.3
17	2,5-$(Cl)_2$	85.0 (55.2–131)	360.0	0.003
18	4-CF_3	0.25 (0.2–0.32)	1.1	1.0
19	3-CF_3	3.8 (2.2–6.45)	16	0.07
20	4-CH_3	0.55 (0.40–0.77)	3.0	0.4
21	4-CH_3O	0.23 (0.15–0.35)	1.2	0.9
22	3,4-$(CH_3O)_2$	5 mg./kg.-40% potentiation		≤0.1
23	3,4-CH_2O_2	0.40 (0.23–0.68)	1.9	0.6

[a] Compounds 13, 22 and 23 are *trans* isomers; the other compounds may be mixtures of *cis* and *trans* isomers in which the latter probably predominates.[15] [b] Weight of amine salt. All compounds except 22 were administered as hydrochlorides; compound 22 was administered as the cyclohexyl sulfamate salt. [c] Defined in Table I.

and 3-chloro derivatives (15, 14) are equipotent and about 1/3 to 1/4 as active as the 4-chloro compound (13). In the trifluoromethyl series, the 3-isomer (19) is considerably less active than the 4-isomer (18). The 3,4-dichloro analog (16) is about as potent as the 3-chloro derivative (14), whereas the 2,5-dichloro com-

pound (17) shows a very low degree of activity. A considerable difference is seen in the activities of the 3,4-methylenedioxy (23) and 3,4-dimethoxy (22) analogs. The former is about 60% as potent as the parent compound whereas the latter is no more, and perhaps less, potent than the 2-chloro, 3-chloro and 3-trifluoromethyl derivatives. Since there is less bulk at the 3-position of the 3,4-methylenedioxy derivative than at the 3-position of the 3,4-dimethoxy, 3-chloro and 3-trifluoromethyl derivatives, the decreased potency of the latter three compounds, and perhaps that of the 2-chloro derivative as well, may be due to an unfavorable steric factor. Perhaps the substituents in these compounds prevent the benzene ring from lying flat on the enzyme surface to provide the maximal degree of binding between the inhibitor and enzyme.

It is noteworthy that effects of substitution of the benzene ring upon MAO-inhibitory activity which in general are similar to those noted above have been observed in the phenylalkylhydrazine series, particularly in phenethyl- and α-methylphenethylhydrazine derivatives.[19-22] However, a valid comparison of these structural effects in the hydrazine and cyclopropylamine series cannot be drawn because the two series were tested by different procedures.

2-Substituents on the Cyclopropane Ring (Table IV).—That a flat ring system in the 2-position of the cyclopropane ring is necessary for a high degree of activity is indicated by the data in Table IV. Cyclopropylamine (24) itself is inactive in the tryptamine potentiation test. Zeller and co-workers[11] found this amine and *trans*-2-methylcyclopropylamine to be less active than the 2-phenylcyclopropylamines *in vitro* by factors of 10^5 and 10^3–10^4, respectively. Moreover, the 2-*n*-pentyl (25) and 2-cyclohexyl (26) derivatives, which are equipotent, are strikingly less active than 2-phenylcyclopropylamine. On the other hand, phenyl can be replaced with 2-naphthyl (28) and 2-thianaphthenyl (29) without much loss of potency. It will be noted that this isosteric pair of compounds is considerably more potent than the isomeric 1-naphthyl (27) and 3-thianaphthenyl (30) pair of isosteres.

Separation of the benzene ring from the cyclopropane ring by an oxygen or sulfur atom, or a methylene group, led to some rather unexpected and inconsistent results. *trans*-2-Phenoxycyclopropylamine (32) and 2-benzylcyclopropylamine (34) (which presumably is predominantly the *trans* isomer)[15] are about equipotent and much less active than the 2-phenyl derivative. On the other hand, the *cis*-2-phenoxy derivative (31) and the 2-phenylthiocyclopropylamine (presumably mainly the *trans* isomer)[15] are about as potent as 2-phenylcyclopropylamine. Especially noteworthy are the 10-fold difference in activity between the *cis* and *trans* phenoxy analogs and the fact that in this series, in contrast to the geometric isomers of 2-phenylcyclopropylamine, the *cis* isomer is the more potent of the two. The *cis* phenoxy derivative was found to be also 10 times more potent than its *trans* isomer in inhibiting rat brain MAO *in vitro* (Table X). Separation of the benzene and cyclopropyl rings by two carbon atoms resulted in a great decrease in potency. The 2-phenethyl derivative (35) has only $^1/_{100}$th or less of the activity of 2-phenylcyclopropylamine.

N-Substitution (Tables V and VI).—Mono- and disubstitution of the amino

(19) J. H. Biel, A. E. Drukker, T. F. Mitchell, E. P. Sprengeler, P. A. Nuhfer, A. C. Conway, and A. Horita, *J. Am. Chem. Soc.*, **81**, 2805 (1959).

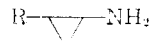
(20) W. R. McGrath and A. Horita, *Toxicol. Appl. Pharmacol.*, **4**, 178 (1962).

(21) T. S. Gardner, E. Wenis, and J. Lee, *J. Med. Pharm. Chem.*, **2**, 133 (1960).

(22) F. E. Anderson, D. Kaminsky, B. Dubnick, S. R. Klutchko, W. A. Cetenko, J. Gylys, and J. A. Hart, *J. Med. Pharm. Chem.*, **5**, 221 (1962).

TABLE IV

OTHER 2-SUBSTITUTED CYCLOPROPYLAMINES



Cpd.[a]	R	ED_{50}(p.o.) mg./kg. (95% fiducial limits)[b]	ED_{50} μmoles/kg. × 10^3	Relative potency[c]
24	H	100 mg./kg.-0% potentiation		
25	$CH_3(CH_2)_4$	15.0 (6.25–36)	49	0.02
26	Cyclohexyl	14.0 (6.1–33.9)	56	0.02
27	1-Naphthyl	1.7 (1.0–2.9)	7.8	0.1
28	2-Naphthyl	0.47 (0.29–0.75)	2.1	0.5
29	2-Thianaphthenyl	0.8 mg./kg.-80% potentiation		>0.3
30	3-Thianaphthenyl	5.5 (2.4–12.7)		0.05
31	*cis*-Phenoxy	0.2 (0.086–0.49)	1.1	1.0
32	*trans*-Phenoxy	2.3 (1.4–3.6)	12	0.1
33	Phenylthio	0.24 (0.15–0.37)	1.2	0.9
34	Benzyl	1.8 (0.92–3.68)	10	0.1
35	Phenethyl	15 mg./kg.-33% potentiation 50 mg./kg.-100% potentiation		

[a] Compounds 28 and 30 are *trans* isomers; compounds 25, 26, 27, 29, 33, 34 and 35 may be mixtures of *cis* and *trans* isomers in which the latter probably predominates.[15] [b] Weight of amine salt. With the exception of compounds 24, 25 and 26, all compounds were administered as hydrochlorides. Compounds 24 and 26 were maleates and compound 25 was the cyclohexyl sulfamate salt. [c] Defined in Table I.

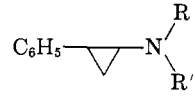
group of *trans*-2-phenylcyclopropylamine with methyl (36, 37) decreases activity only slightly. However, monosubstitution with larger groups, both alkyl and aralkyl (38–42) results in considerable loss of activity. In most instances N-acylation markedly decreased activity. The formyl (42) and carbobenzoxy compound (46) shows a fair degree of activity. Perhaps the activity of the acyl derivatives is due to their hydrolysis *in vivo* to the parent amine.[23] Otherwise, the great potency of compound 48, which contains the bulky N-carbobenzoxy group, relative to the activity of the corresponding carbethoxy derivative (46) seems quite unusual. Demonstration that these derivatives have little or no activity *in vitro* would be presumptive evidence that acyl cleavage occurs *in vivo*.[23] Unfortunately, the insolubility of the carbethoxy and carbobenzoxy derivatives in the aqueous media of the MAO preparation precluded *in vitro* testing of these compounds.

Additional Substituents on the Cyclopropane Ring (Table VII).—As can be

(23) The results of Anderson, *et al.* (ref. 22), suggest that the relative *in vivo* MAO-inhibitory activity of acetylated and carboalkoxylated aralkylhydrazines is dependent upon the relative rates of hydrolysis of these derivatives to the free hydrazines. These workers also found that a carbethoxy hydrazine derivative which is a potent *in vivo* inhibitor of MAO is only a weak inhibitor *in vitro*.

Table V

N-Alkyl and -Aralkyl Derivatives of 2-Phenylcyclopropylamine

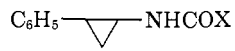


Cpd.	R	R′	Config.	ED_{50} mg./kg. (95% fiducial limits)[d]	ED_{50} mmoles /kg. × 10^3	Relative potency[e]
36	CH_3	H	*trans*[a,b]	0.30 (0.21–0.42)	1.6	0.7
37	CH_3	CH_3	*trans*[a]	0.42 (0.36–0.49)	2.1	0.5
38	$CH(CH_3)_2$	H	*trans*[b]	16 (10.7–23.8)	76	0.01
39	$C_6H_5CH_2$	H	*trans*[c]	22 (13.8–35.2)	85	0.01
40	$C_6H_5CH_2CH(CH_3)$—	H	*trans*[c]	22 (13.8–35.2)	60	0.02
41	$C_6H_5CH_2CH(CH_3)$—	H	*cis*[c]	5.8 (4.25–7.98)	16	0.1
42	$(CH_3)_2N(CH_2)_2$	H	*trans*[c]	25 mg./kg.-50% potentiation		

[a] A. Burger and W. L. Yost, *J. Am. Chem. Soc.*, **70**, 2198 (1948). [b] C. Kaiser, A. Burger, L. Zirngibl, C. S. Davis, and C. L. Zirkle, *J. Org. Chem.*, **27**, 768 (1962). [c] Reference 15. [d] Weight of amine salt. Compounds 36–39 were hydrochlorides and compounds 40–42 were maleates. [e] Defined in Table I.

Table VI

Amides of *trans*-2-Phenylcyclopropylamine and Related Compounds



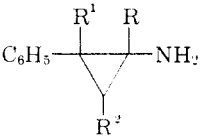
Cpd.	X	Dose mg./kg. (p.o.)	Tryptamine potentiation %	Relative potency[d]
43	H[a]	ED_{50} 0.42 (0.31–0.56)		0.4
44	CF_3[a]	5	66	
45	4-pyridyl[b]	25	67	
46	OC_2H_5[b]	ED_{50} 2.0 (1.4–2.7)		0.1
47	$OC(CH_3)_3$[b]	100	40	
48	$OCH_2C_6H_5$[b]	ED_{50} 0.13 (0.08–0.21)		2.0
49	NH_2[b]	100	40	
50	$N(CH_3)_2$[b]	100	0	
51	$NHNH_2$[b,c]	100	60	

[a] C. Kaiser, A. Burger, L. Zirngibl, C. S. Davis, and C. L. Zirkle, *J. Org. Chem.*, **27**, 768 (1962). [b] Reference 15. [c] Administered as the hydrochloride salt. [d] Defined in Table I.

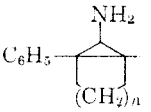
seen, methyl or phenyl substitution at positions 2 or 3 of 2-phenylcyclopropylamine markedly decreased activity whereas substitution of methyl at the 1-position had little effect on activity. The *trans* isomer (52) of 1-methyl-2-phenyl-

TABLE VII

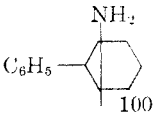
CYCLOPROPANE RING-SUBSTITUTED 2-PHENYLCYCLOPROPYLAMINES[a]



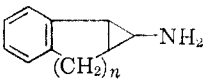
Cpd.	R	R^1	R^2	Dose mg./kg. (p.o.)[f]	Tryptamine potentiation, %	Relative potency[g]
52[b]	CH_3	H	H	ED_{50} 0.25 (0.14–0.44)		1.0
53[c]	CH_3	H	H	ED_{50} 0.13 (0.068–0.23)		1.0
54[d]	H	CH_3	H	25	50	
55[d]	H	H	CH_3	100	70	
56	H	C_6H_5	H	8	0	
57[d]	H	H	C_6H_5	100	10	



Cpd.	n	Dose mg./kg. (p.o.)[f]	Tryptamine potentiation, %
58[e]	1	100	0
59[e]	2	100	20



60[e] 100 0

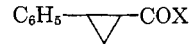


Cpd.	n	ED_{50} mg./kg. (95% fiducial limits)[f]	ED_{50} mmoles/kg. × 10^3	Relative Potency[g]
61[e]	1	1.1 (0.67–1.6)	5.8	0.2
62[e]	2	24 mg./kg.-10% potentiation		

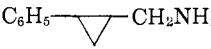
[a] Reference 15. [b] *trans* isomer. [c] *cis* isomer. [d] Probably a mixture of isomers. [e] The configuration of this compound has not been established. [f] Weight of amine salt. All compounds were hydrochlorides except 58, 59 (neutral sulfate) and 54 (free base). [g] Defined in Table I.

TABLE VIII

MISCELLANEOUS 2-PHENYLCYCLOPROPANE DERIVATIVES



Cpd.	X	Config.	Dose mg./kg. (p.o.)	Tryptamine potentiation, %
63[a]	NH_2	*trans*	ED_{50}66	
64[b]	NH_2	*cis*	100	25
65[a]	$NHNH_2$	*trans*	100	30
66[a]	$NHNH_2$	*cis*	50	40



67[b,c]		*trans*	100	0

[a] A. Burger and W. L. Yost, *J. Am. Chem. Soc.*, **70**, 2198 (1948). [b] Reference 15. [c] Hydrochloride salt.

cyclopropylamine, tested earlier as the sulfate salt,[15,24] had been found to be about one-half to one-third as potent as *trans*-2-phenylcyclopropylamine in the tryptamine potentiation test and 2–4 times more potent in inhibiting rat brain MAO *in vitro*[24] (Table X). In the present work both the *trans* and *cis* isomers of the 1-methyl derivative were tested as the hydrochlorides and no significant differences in the activity of the two isomers and of *trans*-2-phenylcyclopropylamine were observed.

That 2,3- and 1,3-disubstitution of 2-phenylcyclopropylamine has a deleterious effect on *in vivo* activity is indicated by the data on the bicyclic amines 58, 59 and 60. These compounds showed little or no effect at 100 mg./kg.

Of special interest are the two fused-ring derivatives (61, 62) which may be considered to be 3-substituted 2-phenylcyclopropylamines in which the 3-substituent is attached to the 2-phenyl group. In these compounds, by virtue of their geometry, the 2- and 3-substituents must be *cis* to each other. Although the configurations of these amines have not been established, one might expect, on the basis of the mode of their formation,[15] that the amino group is *trans* to the phenyl group. In the tryptamine potentiation test the derivative in which $n = 1$ (61) is about $^1/_5$th as potent as *trans*-2-phenylcyclopropylamine, whereas the compound in which $n = 2$ (62) is essentially inactive. Although compound 61 is only $^1/_{30}$th as potent as compound 1 in inhibiting rat brain MAO *in vitro*, it is still considerably more active than compound 62 in this test also (Table X). Sarkar[11c] also has tested compound 61 *in vitro* and found it to have about $^1/_{10}$th the potency of *trans*-2-phenylcyclopropylamine.

Miscellaneous Structural Changes (Tables VIII and IX).—Compound 67 illustrates the effect of separating the amino group from the cyclopropane ring. This derivative showed no MAO-inhibitory activity at 100 mg./kg. Also, the 2-phenylcyclopropanecarboxamides (63, 64) and hydrazides (65, 66) are relatively inactive.

Moving the 2-phenyl or 2-benzyl group to the 1-position of cyclopropylamine

(24) A. Burger, C. S. Davis, H. Green, D. H. Tedeschi, and C. L. Zirkle, *J. Med. Pharm. Chem.*, **4**, 571 (1961).

TABLE IX

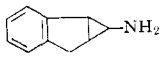
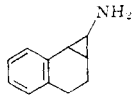
1-PHENYL AND 1-BENZYLCYCLOPROPYLAMINES AND HOMOLOGS[a]

Cpd.[b]	R	X	Dose mg./kg. (p.o.)	Tryptamine potentiation %
68	C_6H_5	NH_2	ED_{50} 7.6 (4.4–12.9)	
69	C_6H_5	$NHCH_3$	25	60
70	C_6H_5		100	60
71	C_6H_5	CH_2NH_2	10	38
72	$C_6H_5CH_2$	NH_2	5	40
73	$C_6H_5CH_2$	$NHCH_3$	25	10
74	$C_6H_5CH_2$	CH_2NH_2	10	38

[a] Reference 15. [b] All compounds were hydrochlorides except 69 which was the cyclohexyl sulfamate salt.

TABLE X

In Vitro MAO-INHIBITORY ACTIVITY OF SEVERAL 2-SUBSTITUTED CYCLOPROPYLAMINES[a]

No.	Compound name or structure	I_{50}[b]	Relative potency[c]
1	*trans*-2-phenylcyclopropylamine	9.7×10^{-7}[e]	1.0
52	*trans*-1-methyl-2-phenylcyclopropylamine	$2.5\text{–}5.0 \times 10^{-7}$[d]	2.0–4.0
31	*cis*-2-phenoxycyclopropylamine	2.8×10^{-6}	0.35
32	*trans*-2-phenoxycyclopropylamine	2.7×10^{-5}	0.036
61		2.8×10^{-5}	0.035
62		5.3×10^{-4}	0.0018
	(+)-2-Amino-1-phenylpropane	7.5×10^{-5}	0.013
	Iproniazid	8.7×10^{-4}	0.0011
	Harmine	1.2×10^{-7}	8.1

[a] See Methods. [b] Concentration (moles/l.) of inhibitor producing a 50% decrease in MAO activity. [c] Reference 10. [d] Reference 24. [e] Defined in Table I.

resulted in a considerable loss of potency. 1-Phenylcyclopropylamine (68) is only about $^1/_{25}$th as active as compound 1. Zeller and Sarkar[11] found compound 68 to be only $^1/_{100}$th as potent as the 2-phenyl derivative *in vitro*. The 1-benzyl derivative (72) also was not very active, although its ED_{50} was not determined.

In summary, the results of our study of cycloalkylamine derivatives indicate that in compounds of this type the structural features necessary for potent *in vivo*

MAO-inhibitory activity are: (1) a cyclopropane ring, (2) an amino group attached directly to the cyclopropane ring, and (3) a 2-substituent (on the cyclopropane ring) which contains an aromatic moiety. In general, introduction of a second substituent on either the amino group or cyclopropane ring results in diminished activity. One exception, however, seems to be substitution by a carbobenzoxy group on the amino group.

At present, our data are not sufficient to permit any general conclusions to be drawn concerning the effects of *cis-trans* isomerism upon activity. Of the isomeric 2-phenylcyclopropylamines, the *trans* isomer is the more potent, but in the corresponding 2-phenoxy derivatives the *cis* isomer is the more active.

Discussion

Any thorough analysis of the effects of structure upon MAO-inhibitory activity, in terms of the possible modes of interaction of the inhibitor with the enzyme system, should attempt to explain the fact that a wide variety of compounds such as harmine and related carboline derivatives,[1,12,25-30] 2-amino-1-phenylpropane[11] and hydrazines,[1,8,11,12,19,30-34] as well as cyclopropylamines inhibit MAO. Such an analysis at present can be only speculative. Although MAO and its inhibitors have been subjects of extensive research, the significance of the wealth of data accumulated is difficult to assess from the structure–activity standpoint due to lack of information concerning the identity and nature of MAO,[8,30,34,35] and the fact that the observed relative potencies of the inhibitors depend greatly on the test procedure, species, tissues, route of administration, etc., used to evaluate them.[1,8,11,25,29,30,36] Furthermore, the various chemical types of inhibitors show marked differences in their biological properties.

MAO inhibitors have been divided into two general classes: short acting, reversible inhibitors such as harmine and 2-amino-1-phenylpropane, and long acting, irreversible inhibitors, *e.g.*, hydrazine de-

(25) S. Udenfriend, B. Witkop, B. G. Redfield, and H. Weissbach, *Biochem. Pharmacol.*, **1**, 160 (1958).

(26) A. Pletscher, H. Besendorf, H. P. Bächtold, and K. F. Gey, *Helv. Physiol. Pharmacol. Acta*, **17**, 202 (1959).

(27) A. Pletscher and H. Besendorf, *Experientia*, **15**, 25 (1959).

(28) I. I. A. Tabachnick and A. A. Rubin, *Proc. Soc. Exp. Biol. Med.*, **101**, 435 (1959).

(29) M. Ozaki, H. Weissbach, A. Ozaki, B. Witkop, and S. Udenfriend, *J. Med. Pharm. Chem.*, **2**, 591 (1960).

(30) A. Pletscher, K. F. Gey, and P. Zeller, in "Progress in Drug Research," Vol. 2, E. Jucker, ed., Interscience Publishers, Inc., New York, N. Y., 1960, p. 417.

(31) A. N. Davison, A. W. Lessin, and M. W. Parkes, *Experientia*, **13**, 329 (1957).

(32) S. Hess, H. Weissbach, B. G. Redfield, and S. Udenfriend, *J. Pharmacol. Exptl. Therap.*, **124**, 189 (1958).

(33) A. Horita, *J. Pharmacol. Exptl. Therap.*, **122**, 176 (1958).

(34) A. N. Davison, *Physiol. Revs.*, **38**, 729 (1958).

(35) T. E. Smith, H. Weissbach, and S. Udenfriend, *Biochemistry*, **1**, 137 (1962).

(36) A. Pletscher, H. Göschke, K. F. Gey, and H. Thölen, *Med. Exper.*, **4**, 113 (1961).

rivatives.[26,27] Other characteristics of these and related compounds, however, confuse the classification. Thus, harmine is an extremely potent inhibitor *in vitro* and *in vivo* when administered parenterally,[1,12,25–29] but is much less effective when given orally.[1,28] 2-Amino-1-phenylpropane shows activity *in vitro* but not *in vivo*.[2,4–6,8] On the other hand, many hydrazine derivatives are very potent inhibitors *in vitro* and *in vivo*, regardless of the route of administration.[1,8,11,19,29–33] On the basis of the presently available data on 2-phenylcyclopropylamine, it is difficult to place this agent in either of the aforementioned classifications. Although structurally 2-phenylcyclopropylamine resembles 2-amino-1-phenylpropane, the former drug is a potent MAO inhibitor both *in vivo* and *in vitro* whereas 2-amino-1-phenylpropane is active only *in vitro*. Furthermore, although 2-phenylcyclopropylamine is not as long acting as the hydrazines, it is considerably longer acting than the reversible inhibitor harmine.[4,5,7,8,10–12]. In addition to its relatively long duration of action, Zeller's observation[11a] that the activity of MAO pretreated with 2-phenylcyclopropylamine was restored only slightly by dialysis also suggests that the inhibitory action of this agent is not readily reversible. On the other hand, 2-phenylcyclopropylamine apparently is not an irreversible inhibitor like the hydrazines, for Zeller and Sarkar[11b] have found that the inhibitory action of this amine, but not that of iproniazid or N-benzyl-N-methyl-2-propynylamine, is reversed readily by 4-phenylbutylamine.

Despite the differences in some of the biological characteristics of the cyclopropylamines, hydrazines, and harmine, all of these agents have consistently shown potent MAO-inhibitory activity in the tryptamine potentiation test[3] and in other procedures involving a variety of MAO preparations.[3–13,19,25–33] Although the ultimate mechanisms of action of the various types of inhibitors may differ, all of these compounds possibly could act initially by occupying the active site of the enzyme.[37] Whether they do or do not is not known with certainty. From results of *in vitro* studies to detect competition of substrates and inhibitors, various investigators have concluded that several types of inhibitors including iproniazid[11,34,38] and 2-phenylcyclopropylamine[6,11b,39] do block the active site of MAO. That the results obtained from such studies may depend greatly on experimental conditions and could be misleading is illustrated by the work

(37) *Cf.* B. R. Baker, W. W. Lee, W. A. Skinner, A. P. Martinez, and E. Tong, *J. Med. Pharm. Chem.*, **2**, 633 (1960), and references cited therein.

(38) L. S. Seiden and J. Westley, *Fed. Proc.*, **21**, 416 (1962).

(39) B. Belleau and J. Moran, *J. Med. Pharm. Chem.*, **5**, 215 (1962).

of Long,[40] who found that the readily reversible inhibitors harmaline, α-methylphenethylamine and α-methyltryptamine were non-competitive with tyramine, phenethylamine, tryptamine or 3,4-dihydroxyphenethylamine as substrates according to Lineweaver–Burk plots, but competitive with serotonin, epinephrine and norepinephrine. Conflicting conclusions also have been drawn from studies of the competition of 2-phenylcyclopropylamine with substrates of MAO.[6,39] In this case the discrepancies in results perhaps may be explained by the recent findings of Zeller and Sarkar.[11b] They observed that when tyramine and 2-phenylcyclopropylamine were added simultaneously to the enzyme preparation, the degree of inhibition decreased with increasing substrate concentrations. On the other hand, no effect on inhibition was observed when tyramine was added 15–30 minutes after the inhibitors. These workers also found, however, that inhibition produced by preincubation of MAO with the cyclopropylamine could be reversed easily by addition of another substrate, 4-phenylbutylamine, to the system. These results in general are consistent with the postulate that 2-phenylcyclopropylamine acts at the active site of the enzyme.

Several investigators[12,18,19,41] have presented evidence suggesting that harmine, harmaline and hydrazine derivatives act at the same site of the enzyme. For example, Horita and McGrath[12] found that pretreatment of rats with harmine prevented the prolonged inhibition of brain and liver MAO by 2-hydrazino-1-phenylpropane. They also observed that pretreatment with harmine had no effect on the inhibitory action of 2-phenylcyclopropylamine, suggesting that the latter inhibitor may not act at the site occupied by harmine. However, as these authors have pointed out, the cyclopropylamine may have such a high affinity for the enzyme that it displaces harmine and itself occupies the active site. Thus, while not definitive, the evidence in general seems to be fairly consistent with the postulate that the cyclopropylamines and other types of MAO inhibitors of the amine and hydrazine classes act at the same site of the enzyme. Although other authors[11,39] have discussed possible modes of interaction of inhibitors with the active site of MAO in terms of certain structural features of inhibitors, the possible stereochemical (conformational) factors that may be involved in the binding process have largely been ignored. The relatively rigid and simple structures of 2-substituted cyclopropylamines would seem to justify speculation concerning the

(40) R. F. Long, *Biochem. J.*, **82**, 3p (1962).

(41) J. Axelrod, G. Hertting, and R. W. Patrick, *J. Pharmacol. Exptl. Therap.*, **134**, 325 (1961).

stereochemistry of interaction of these compounds with MAO.

Presumably the substrates and inhibitors are oriented and attached to the active center of MAO through the amino group of these compounds. The binding may be the result of electrostatic interaction between the positively charged ammonium form of the amine and a negative group on the enzyme. That binding of the free amino group may occur, however, has been suggested by Smith, *et al.*,[35] who found in experiments with partially purified MAO that the enzymatic reaction rate was fastest at alkaline pH values. They point out that the amount of protonated amine present under these conditions might be too small to account for the observed rates of amine oxidation. A second point of attachment postulated for substrates, an α-hydrogen,[10,39,42] is unlikely as a site for inhibitors, since 1-methyl-2-phenylcyclopropylamine (52 and 53), which has no α-hydrogen, is a potent inhibitor.[24] Our findings that an aryl group, or a group containing a flat moiety at the 2-position of cyclopropylamine, is essential for potent MAO-inhibitory activity is in accord with Zeller's[11] hypothesis that the hydrocarbon residue of inhibitors and substrates also is bound to the enzyme through van der Waals forces. These forces would operate maximally if, as Zeller[43] has suggested, a flat aryl group of the substrate or inhibitor attaches to a complementary flat surface of MAO.

The fact that in the phenylalkyl- and phenylcycloalkylamine series the cyclopropane ring is an essential structural requirement for potent and prolonged MAO-inhibition *in vivo* suggests that this ring may in some way serve as a third point of attachment to the enzyme, its high electron density possibly providing a focus for bonding.[24] Quite recently Belleau and Moran[39] have postulated that the electronic and steric properties of the cyclopropane system play a highly specific role in the binding of *trans*-2-phenylcyclopropylamine to MAO. On the basis of isotope effects observed in the MAO-catalyzed oxidation of deuterium-labeled kynuramine, they concluded that in the oxidation transition state the α and β carbon atoms of the amine substrate acquire double bond character and thereby approach the trigonal state. The high affinity of 2-phenylcyclopropylamine for the enzyme could thus be due to the fact that in the ground state this amine resembles, electronically and sterically, the transition state of the amine substrate during the oxidation process. According to this intriguing hypothesis, the $N-C_1-C_2$ atoms of the cyclopropylamine must be essentially coplanar in the enzyme-inhibitor complex. How-

(42) B. Belleau, J. Burba, M. Pindell, and J. Reiffenstein, *Science*, **133**, 102 (1961).
(43) E. A. Zeller, *Pharmacol. Rev.*, **11**, 387 (1959); *Experientia*, **16**, 399 (1960).

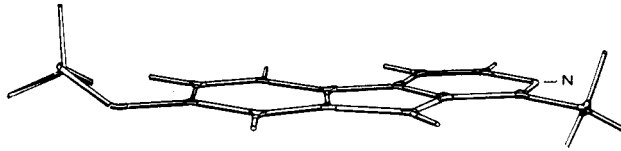


Fig. 1.—Harmine.

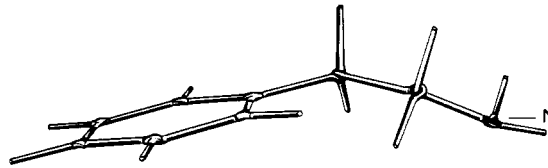


Fig. 2.—Phenethylamine.

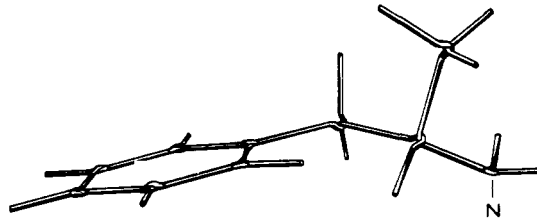


Fig. 3.—2-Amino-1-phenylpropane.

ever, as will be discussed later, our study of molecular models of various cyclopropylamine derivatives and other types of MAO-inhibitors suggests that other possible modes of combination of the cyclopropylamines with MAO must also be considered.

One might expect that tight binding of inhibitor to enzyme receptor would occur if, in the adsorbed molecule, the amino group, the aryl group, and the atoms separating them could lie, as much as possible, in the same plane. Indeed they must in fact be essentially coplanar in the rigid linear molecule of harmine (Fig. 1).[44] A reasonable assumption, then, is that flexible molecules of substrates such as phenethylamine (Fig. 2), tryptamine, *etc.*, and of inhibitors such as 2-amino-1-phenylpropane (Fig. 3), and 2-hydrazino-1-phenylpropane also may assume a conformation approaching planarity when attached to the enzyme. The similarity between the extended forms of these molecules (exemplified by that of phenethylamine) and the harmine molecule is illustrated in Fig. 4.

(44) Figures 1–12 are to-scale drawings of Dreiding molecular models. Although these models do not indicate the "thickness" of the benzene rings or clearly show the effective radii of the other atoms, they accurately depict the bond lengths and angles.

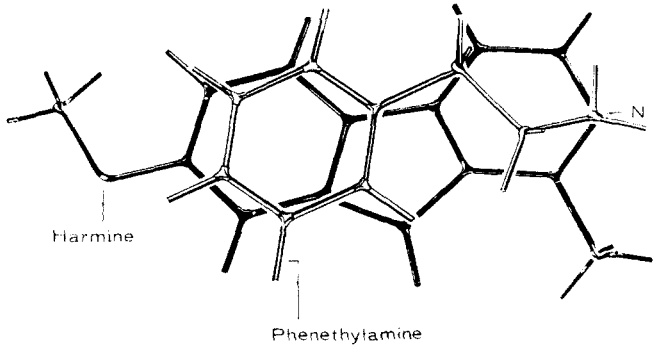


Fig. 4.—Harmine and phenethylamine.

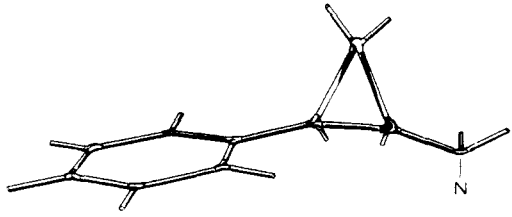


Fig. 5.—(+)[or(−)]-*trans*-2-Phenylcyclopropylamine.

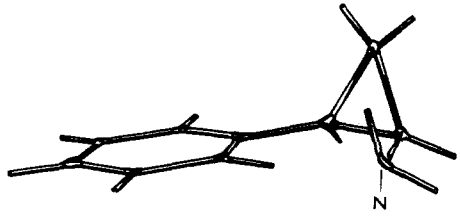


Fig. 6.—(+)[or(−)]-*cis*-2-Phenylcyclopropylamine.

Figure 5 shows that in *trans*-2-phenylcyclopropylamine, the moiety C_6H_5–C_2–C_1–N can also assume a nearly planar configuration. In fact, by virtue of the geometry and rigidity of the cyclopropane ring, the C_6H_5–C_2–C_1–N system in this molecule is flatter than it is in the phenethyl compounds. However, examination of the corresponding model of the *cis* isomer (Fig. 6) indicates a considerable degree of steric interaction between the *ortho* hydrogen and the amino group (especially in the ammonium form) when the latter and the benzene ring are coplanar.[45] Thus, if the cyclopropylamines attach to the enzyme as shown in Figs. 5 and 6, *cis*-2-phenylcyclopropylamine

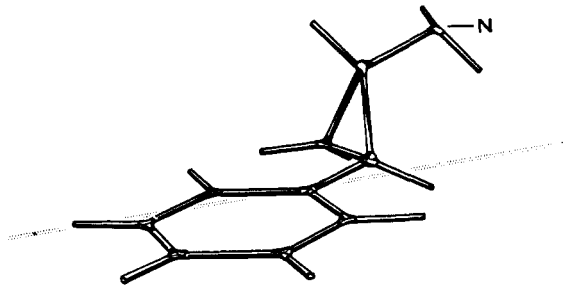


Fig. 7.—(+)[or(−)]-*trans*-2-Phenylcyclopropylamine.

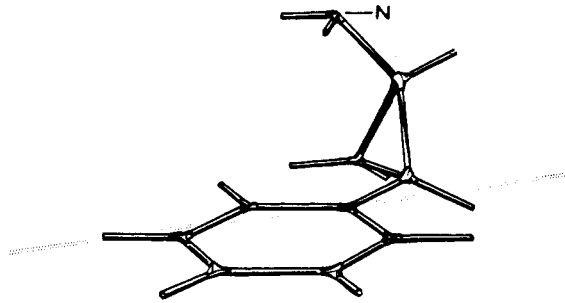


Fig. 8.—(+)[or(−)]-*cis*-2-Phenylcyclopropylamine.

(Fig. 6) might reasonably be expected to be considerably less active than the *trans* isomer when, in fact, it is about one-half as potent. Moreover, steric interaction between the benzene ring and the methyl or amino group (depending on which is *cis* to phenyl) of the two isomers of 1-methyl-2-phenylcyclopropylamine (52 and 53) also might be expected to result in an appreciable loss of activity, relative to that of *trans*-2-phenylcyclopropylamine, but in fact the three compounds are equipotent. These steric considerations suggest that in the enzyme-inhibitor complex the cyclopropylamines do not assume a conformation in which the phenyl and amino groups are nearly coplanar but one in which these groups lie in distinctly different planes (Figs. 7 and 8). Support for this possibility is found in the tricyclic analog (61) of 2-phenylcyclopropylamine (Table VII). This quite rigid molecule (Fig. 9) cannot assume a conformation in which the

(45) The steric interactions are observed more readily in a Godfrey molecular model of *cis*-2-phenylcyclopropylamine. This model suggests that steric repulsions between the amino group and the benzene ring are minimal when the latter and the C_2-C_3 atoms of the cyclopropane ring are approximately coplanar and the amino group is above the plane of the benzene ring. G. W. Perold, *J. S. African Chem. Inst.*, **8**, 1 (1955), has reported spectral data on cyclopropane derivatives indicating steric interaction between *cis* substituents.

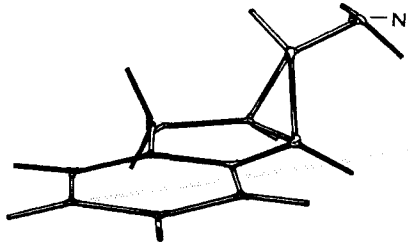


Fig. 9.—Compound 61 (Table VII).

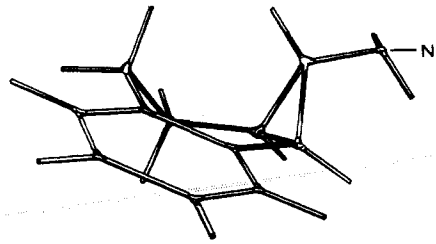


Fig. 10.—Compound 62 (Table VII).

N–C_1–C_2-system approaches planarity, yet the compound retains one-fifth of the *in vivo* MAO-inhibitory activity of *trans*-2-phenylcyclopropylamine. Moreover, a comparison of models of 2-phenylcyclopropylamine (Fig. 7), compound 61 (Fig. 9), and the closely related ring homolog of the latter (62) (Fig. 10) suggests that the degree of inhibitory activity correlates with the ability of the phenyl group to approach coplanarity with the C_2–C_3 atoms of the cyclopropane ring, rather than with the C_1–C_2 atoms. As can be seen in the figures, the phenyl-C_2–C_3 system of compound 61 (Fig. 9), which is about one-fifth as active as *trans*-2-phenylcyclopropylamine, is somewhat less planar than that of the latter amine, while in compound 62 (Fig. 10), which does not show significant activity at 25 mg./kg., the system is distinctly nonplanar.

Consideration of the steric differences between harmine and the cyclopropylamines as well as the differences in their biological properties may suggest that the two types of inhibitors have different sites of action. However, our results and all other data on these compounds can be explained as readily, or perhaps even more so, on the assumption that they both act at the active center, but combine with it in different ways. Of many conceivable modes of interaction of inhibitors with this site, two examples are illustrated schematically in Figs. 13a and 13b. If the amino groups of harmine and 2-phenyl-

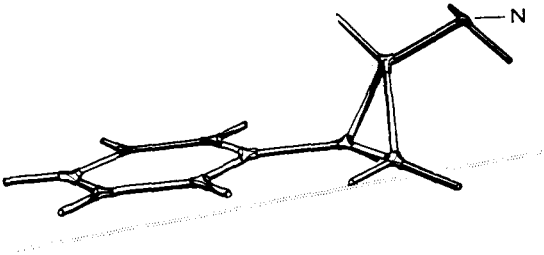


Fig. 11.—(−)[or(+)]-*trans*-2-Phenylcyclopropylamine.

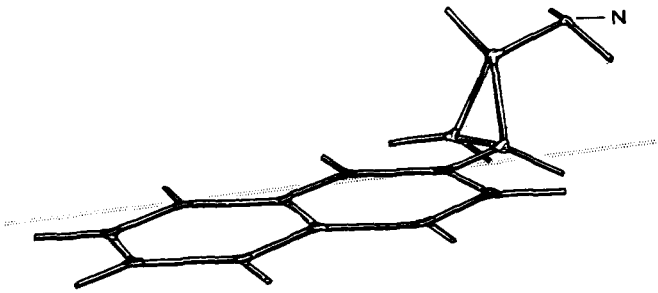
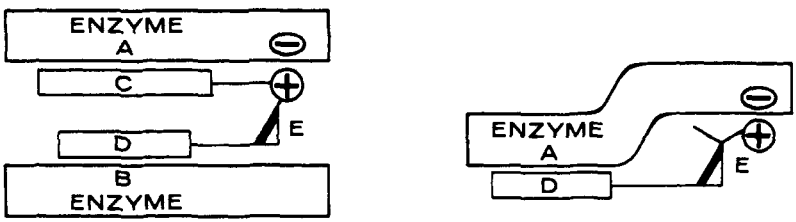


Fig. 12.—(+)[or(−)]-*trans*-2-(2-Naphthyl)cyclopropylamine.



13a 13b

Figs. 13a and 13b.—A and B are different flat surfaces of MAO; C is the indole moiety of harmine; D and E are benzene and cyclopropane rings of 2-phenylcyclopropylamine.

cyclopropylamine attach to the same anionic (or electrophilic) site, the aryl groups of the two inhibitors may attach to different surfaces of the enzyme as indicated in Fig. 13a, or the active site may be deformed by 2-phenylcyclopropylamine in a way, such as illustrated in Fig. 13b, that permits the phenyl group to approach the postulated flat surface to which the phenyl (or indole) moiety of harmine becomes bound. Of these two schemes, that shown in Fig. 13a would seem more favorable for attachment of *cis*-2-phenylcyclopropylamine

(Fig. 8) or the isomers of 1-methyl-2-phenylcyclopropylamine. In these compounds, when the benzene and cyclopropane rings are oriented as in Figs. 7, 8 and 9, the amino or 1-methyl group lies over the phenyl group to some extent and would probably interfere with the binding of the latter to flat surface A in scheme 13b. According to scheme 13a, the 2-phenyl groups of *cis* and *trans* cyclopropylamines could not occupy exactly the same spot on surface B. Thus, this flat area would have to be large enough to accommodate phenyl groups in somewhat different positions. That the surface to which the phenyl group is bound is indeed a rather large area is indicated by the fact that both the enantiomorphic *trans*-2-phenylcyclopropylamines (Figs. 7, 11) and the 2-naphthyl derivative (Fig. 12) are all potent inhibitors.

The postulate that the aryl groups of harmine and 2-phenylcyclopropylamine attach to different surfaces in the active site of MAO may provide an explanation for the observation of Horita and McGrath[12] that harmine prevented the irreversible blockade of MAO by 1-hydrazino-2-phenylpropane but did not prevent the prolonged effects of *trans*-2-phenylcyclopropylamine. If the hydrazine derivative requires the same surface A, as well as the anionic (or electrophilic) site, occupied by harmine in order to become attached to the enzyme, displacement of the latter inhibitor might be expected to be relatively difficult. On the other hand, if 2-phenylcyclopropylamine needs only the anionic site occupied by harmine and uses surface B as its other point of attachment, displacement of harmine by the cyclopropylamine might readily occur. Similarly, one may speculate that the reason that substrates such as tyramine do not readily displace 2-phenylcyclopropylamine from the active site whereas 4-phenylbutylamine does, is that the former substrates have a higher affinity for surface A than for surface B and 4-phenylbutylamine has a higher affinity for surface B.

In conclusion, the cyclopropylamines, by virtue of their relatively rigid and simple structures and unique geometry, provide an unusual opportunity for investigation of the effects of structure and stereochemistry upon MAO-inhibitory activity. The results of the present study suggest that harmine and the 2-substituted cyclopropylamines either act at different sites of MAO or combine with the active site of the enzyme in different ways. Further investigation of these compounds, particularly those of established configurations, may contribute much to an understanding of the mechanisms of action and inhibition of MAO.