N-Substituted Cyclopropylamines as Monoamine Oxidase Inhibitors. Structure–Activity Relationships. Dopa Potentiation in Mice and *in Vitro* Inhibition of Kynuramine Oxidation

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A wide variety of cyclopropylamines inhibit monoamine oxidase and potentiate the stimulant action of dopa in mice. Structure–activity studies of the effects of these compounds in potentiating dopa and in inhibiting monoamine oxidase action on kynuramine *in vitro* are reported. There does not appear to be a significant correlation of degree of effectiveness of the various compounds in the two assays.

The screening of monoamine oxidase (MAO) inhibitors may be accomplished by a variety of methods. For example, the direct action of an inhibitor on the activity of MAO can be measured *in vitro* by biochemical techniques, using any of the substrates (kynuramine, tryptamine, tyramine, etc.) for the enzyme. Pharmacologically, MAO inhibitors potentiate the stimulant action of amines or amine precursors such as dihydroxyphenylalanine (dopa). The enhancement of dopa activity by MAO inhibitors in whole animals can thus be used as an indication of the degree of enzyme inhibition *in vivo*.

This paper reports a new series of MAO inhibitors that are especially active in potentiating the effects of dopa in mice. The degree of the *in vivo* effect of many of these compounds is greater than might be predicted from their *in vitro* action on MAO.

The study of this series was begun after an observation that 3,4-dichlorophenacylmorpholine hydrochloride caused a delayed onset of hyperactivity in mice. Subsequently, it was found that administration of dopa to mice treated with the above compound led to the pattern of piloerection, mydriasis, salivation, aggressiveness, and bloody fighting that is typical of MAO inhibitors given in combination with dopa. A survey of the effect of amine variation in that series of ketones showed that the cyclopropylamine derivative was uniquely active. It was then established that outstanding dopa potentiation required a compound with an aromatic function separated from cyclopropylamine by a connecting group (X). A study of the structure–activity relations of cyclopropylamines (aryl-XNH-*c*-C_3H_5) was undertaken.[1] We report here *in vitro* biochemical studies of the inhibition of kynuramine destruction by rat liver mitochondrial MAO and pharmacological studies of the potentiation of dopa.

Results.—The data summarized in Table Ia show that the N-cyclopropyl derivative of 2,4-dichlorophenoxyethylamine was a more active dopa-potentiating agent than any of the other derivatives studied and that it was a potent monoamine oxidase inhibitor *in vitro*. Mice treated with a 1-mg/kg dose of the cyclopropyl compound still showed striking hyperactivity when given dopa 24 hr later. The dopa in Table Ib also indicate the high activity of N-cyclopropyl compounds in potentiating dopa. Table Ic gives data for previously known MAO inhibitors. None of these were as effective in potentiating dopa as were the N-cyclopropyl compounds in Ia and Ib, although tranylcypromine, pheniprazine, and pargyline were among the most active monoamine oxidase inhibitors *in vitro*. Harmaline, like the cyclopropylamines, showed high activity *in vivo* despite low potency *in vitro*.

As shown in Table II, a variety of other cyclopropylamines were also active in the dopa assay. The compounds shown were selected from a larger series of homologs to illustrate the wide range of substituents with an aromatic ring and a short connecting group. In all cases studied, activity was lost when isopropyl or some other alkyl function was substituted for the cyclopropyl group.

Table I

Effect of Amine Variation on Potency as Potentiator of Dopa and Inhibitor of MAO

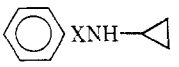
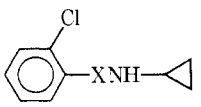
R_1	R_2	Potentiation of dopa, pD_{dopa}[a]	Inhib of kynuramine oxidation, pI_{50}[a]
(a) 			
H	H	3.7	5.7
CH_3	H	3.7	6.3
$CH(CH_3)_2$	H	<3.5	4.2
	H	5.9	6.5
$C(CH_3)_2C{\equiv}CH$	H	3.8	4.5
$CH_2C{\equiv}CH$	CH_3	5.0	7.2
(b) 			
	H	6.0	6.2
	H	5.4	6.3
	H	<5	3.7
	CH_3	6.0	6.3
(c) Other Inhibitors			
Tranylcypromine		5.1	6.7
Pheniprazine		5.3	6.7
Pargyline		4.0	7.3
Harmaline		5.3	3.7

[a] The quantities pD_{dopa} and pI_{50} are negative logarithmic quantities defined in the Experimental Section of the text. Higher numbers indicate greater drug effects.

(1) While this work was in progress, L. R. Swett, W. B. Martin, J. D. Taylor, G. M. Everett, A. A. Wykes, and Y. C. Gladish, *Ann. N. Y. Acad. Sci.*, **107**, 891 (1963), reported that ethyl (N-benzyl-N-cyclopropyl)carbamate was active as a dopa-potentiating agent but not as an *in vitro* MAO inhibitor.

TABLE II

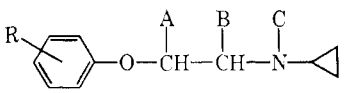
EFFECT OF GROUP CONNECTING AMINE AND AROMATIC FUNCTION

X	Potentiation of dopa, pD_{dopa}[a]	Inhib of kynuramine oxidation, pI_{50}[a]
(a) 		
CH_2	3.2	6.1
$(CH_2)_2$	4.8	5.5
$CHOHCH_2$	4.3	5.0
$COCH_2$	4.2	6.0
$CH_2CH(CH_3)$	4.7	5.7
$(CH_2)_3$	4.6	5.7
$CHClHCH_2$	4.2	6.3
$C\equiv CCH_2$	4.2	5.2
$CHOH(CH_2)_2$	5.2	5.5
$CO(CH_2)_2$	4.3	5.0
$CHCl(CH_2)_2$	4.2	5.0
$O(CH_2)_2$	5.2	6.0
$S(CH_2)_2$	5.5	6.7
$O(CH_2)_3$	<5	5.2
$(CH_2)_4$	<5	5.0
(b) 		
CH_2	5.2	7.2
$(CH_2)_2$	4.6	5.4
$CHOHCH_2$	5.1	6.0
$COCH_2$	<5	6.2
$S(CH_2)_2$	6.0	6.2
$CH(CH_3)CH_2$	5.3	6.2
$CH(CH_3)CO$	<5	4.4
$CH_2CH(CH_3)$	6.0	6.5

[a] See footnote *a*, Table I.

TABLE III

EFFECT OF AROMATIC SUBSTITUTIONS



R	A	B	C	Potentiation of dopa, pD_{dopa}[a]	Inhib of kynuramine oxidation, pI_{50}[a]
H	H	H	H	5.2	6.0
4-F	H	H	H	5.2	6.0
4-Cl	H	H	H	5.2	6.5
4-Br	H	H	H	5.3	6.5
4-OCH_3	H	H	H	5.8	6.0
3-CF_3	H	H	H	5.0	5.0
3-CH_3	H	H	H	5.0	...
3-Cl	H	H	H	5.0	6.0
2-Cl	H	H	H	6.0	6.2
2,3-Cl_2	H	H	H	5.7	6.1
2,5-Cl_2	H	H	H	4.9	6.3
2Cl	CH_3	H	H	5.0	6.2
2Cl	H	CH_3	H	6.0	6.5
2Cl	H	H	CH_3	6.0	6.3
β-Naphthyl	H	H	H	5.4	6.0

[a] See footnote *a*, Table I.

N-Phenoxyethylcyclopropylamines have been particularly effective dopa-potentiating agents. The effects of aromatic substitutions are shown for this group of compounds (Table III). The 2-chloro compounds, with or without a methyl substituent on the alkyl chain, were the most active members of the series in potentiating dopa. The *in vitro* effects of this series were rather more uniform than were the *in vivo* effects.

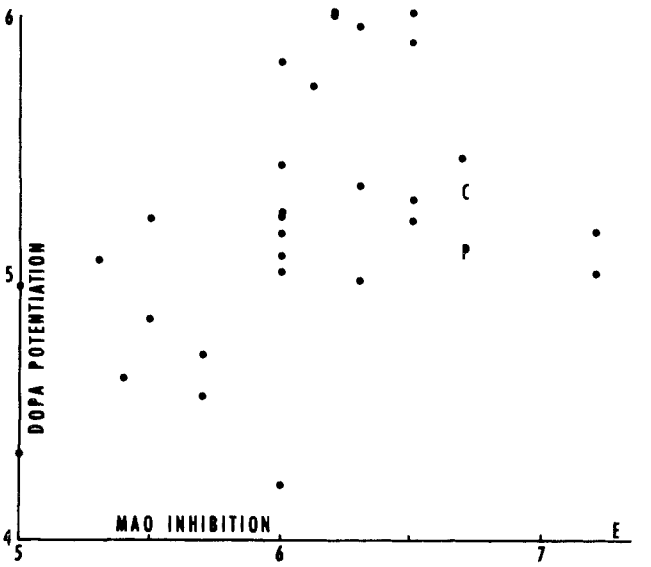


Figure 1.—Comparison of *in vivo* dopa potentiation and *in vitro* MAO inhibition. The ordinate is the pD_{dopa} and the abcissa is the pI_{50}; both values are defined in the Experimental Section. Each point represents the activity of a single compound. C indicates the position of pheniprazine; P, tranylcypromine; and E, pargyline.

In general, the potency of all of the N-substituted cyclopropylamines as dopa-potentiating agents was greater than might have been predicted from their activity as *in vitro* MAO inhibitors (*e.g.*, relative to the known inhibitors shown in Table Ic). Within the series of cyclopropylamines, there was no clear relationship between potency of the various compounds in the two assays, both of which are generally assumed to be related to inhibition of enzymatic oxidative deamination. This lack of correlation (illustrated in Figure 1) is not surprising. The pharmacological assay presumably measured the inhibition of catecholamine destruction in the brain of mice, while the biochemical method measured the inhibition of kynuramine deamination by mitochondria from rat liver. There was then a difference in (1) substrates, (2) organs, and (3) species. The tissue distribution and metabolism of the inhibitors were also important in the *in vivo* studies. While all of these factors must be involved, other studies[2] suggest that selectivity in the inhibition of certain substrates is a property of these N-substituted cyclopropylamines that may be of greatest significance in the lack of correlation seen in Figure 1.

The data in this paper show that the type of structure–activity relations can depend on the choice of screening methods for MAO inhibitors.

Experimental Section

Dopa Potentiation in Mice.—Aqueous solutions of test drugs were injected intraperitoneally into two groups of three male Cox Standard mice (16–20 g). Two hours later, 100 mg/kg of dopa was given intraperitoneally to the first group. The second group was treated with dopa 24 hr after the test compound. Signs of central nervous system and autonomic stimulation were graded three times at 10-min intervals during the next 0.5 hr. Piloerection, mydriasis, salivation, fighting, defensive behavior, spontaneous activity, and tremor were scored as absent (0),

(2) R. W. Fuller, Abstracts, 7th International Congress of Biochemistry, Tokyo, 1967, p 999.

present (1), moderate (2), and marked (3) for each mouse. All three mice showing maximum effect for the seven parameters at all three time intervals would have led to a total score of $3 \times 7 \times 3$ or 63. In each assay, animals treated with (a) saline followed by dopa and (b) drug followed by saline served as controls. Although the injection of 200 mg/kg of dopa was occasionally followed by some stimulation, yielding a score of 10 for a group of three mice in a single reading, the 100-mg/kg dose rarely caused stimulation. In a series of 18 consecutive control runs of saline followed by 100 mg/kg of dopa, the mean cumulative score for the three readings of three mice was 9.4 with a standard deviation of 4.6. In our drug experiments, a score of 30 was considered as evidence of unequivocal dopa potentiation. The dose of test compound causing this score, determined by interpolation, was called the effective dose. The negative logarithm of this dose in moles per kilogram was defined as a pD_{dopa}.

In Vitro **Enzyme Assay.**—Inhibition of the oxidation of kynuramine by isolated mitochondria from rat liver was determined by the method of Weissbach, *et al.*,[3] in a Gilford multiple-sample absorbance recorder as described by Fuller and Walters.[4] The negative logarithm of the molar concentration producing 50% inhibition (pI_{50}) was calculated.

Compounds.—Pheniprazine[5a] was obtained from Lakeside Laboratories, tranylcypromine[5b] from Smith Kline and French Laboratories, and pargyline[5b] from Abbott Laboratories. Other compounds were synthesized in the Lilly Research Laboratories,[6] and their chemical structures were verified by physicochemical methods. The compounds were used as soluble salts, either hydrochlorides or hydrobromides.

Acknowledgments.—We wish to thank for their help in chemical synthesis, Wilma E. McCarthy, W. Pfeiffer, and R. Simon; MAO assays, Emily Rosing; and dopa potentiation, C. E. Keller and H. Snoddy.

(3) H. Weissbach, T. E. Smith, J. W. Daly, B. Witkop, and S. Udenfriend, *J. Biol. Chem.*, **235**, 1160 (1960).

(4) R. W. Fuller and C. P. Walters, *Biochem. Pharmacol.*, **14**, 159 (1965).

(5) (a) Catron®; (b) Parnate®; (c) Eutonyl®.

(6) J. Mills and R. W. Kattau, U. S. Patents 3,235,597 (1966) and 3,225,096 (1965).

Synthesis and Pharmacological Properties of N-Derivatives of 5,6-Dihydro-7H,12H-dibenz[*c,f*]azocine, a New Tricyclic System

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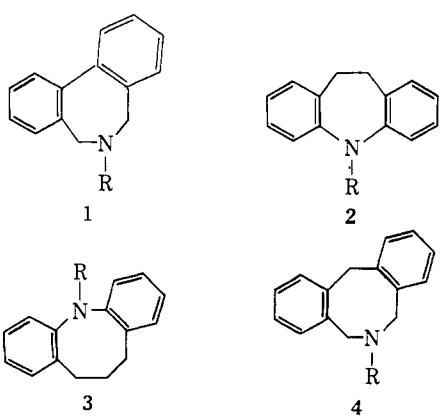
Several N-derivatives of 5,6-dihydro-7H,12H-dibenz[*c,f*]azocine were synthesized for extensive pharmacological screening. The test for antagonism of reserpine effects was negative for the whole series, whereas nearly all of the substances proved to possess interesting depressant properties. This effect was more pronounced for the N-aminopropyl derivatives. A number of the compounds displayed marked peripheral vasodilator and hypotensive action, particularly noteworthy for the N-alkyl derivatives (maximum potency with a C_2–C_3 chain). The series showed other numerous activities, among which the antitussive and anthelmintic actions seemed to be highly promising.

We have recently synthesized 5,6-dihydro-7H,12H-dibenz[*c,f*]azocine, a new tricyclic system.[1] This, together with the interesting pharmacological properties well known for the structurally similar N-substituted 6,7-dihydro-5H-dibenz[*c,e*]azepines (**1**),[2] 10,11-dihydro-5H-dibenz[*b,f*]azepines (**2**),[3] and 5,10,11,12-tetrahydrodibenz[*b,g*]azocines (**3**),[4] has led us to synthesize and pharmacologically screen several 5,6-dihydro-7H,12H-dibenz[*c,f*]azocines variously substituted on the nitrogen atom (**4**).



Alkyl, hydroxyalkyl, aralkyl, and terpenyl derivatives were prepared by reaction (in a suitable solvent) of 2,2′-bis(bromomethyl)diphenylmethane with the proper amine (methods A, B, and C). Chloroalkyl derivatives were obtained by reaction of thionyl chloride with corresponding hydroxyalkyl compounds (method D), while aminoalkyl derivatives were synthesized by making the above chloroalkyl compounds react with the proper amines (method E). As shown in Table I, the great majority of the new substances were obtained in good yields.

Pharmacological screening included studies of acute toxicity, behavioral effects, action on the central nervous system and on arterial pressure, and analgetic, antiinflammatory, antireserpine, diuretic, antitussive, hypoglycemic, antispasmodic, local anesthetic, peripheral vasodilator, anthelmintic, antibacterial, and antifungal actions.

Experimental Section[5]

Chemistry. Intermediates.—4-Chlorobenzhydrylamine was prepared according to Najer, *et al.*[6] 1-Methyl-3-aminomethyl-

(1) This work will be published elsewhere.

(2) L. O. Randall and T. H. Smith, *J. Pharmacol. Exptl. Therap.*, **103**, 10 (1951).

(3) L. Kuhn, *Schweiz. Med. Wochschr.*, **87**, 1135 (1957).

(4) Rhone-Poulenc Soc., South African Patent T61/526 (July 5, 1960).

(5) Melting points are corrected and were taken on a Büchi capillary melting point apparatus.

(6) H. Najer, P. Chabrier, and R. Guidicelli, *Bull. Soc. Chim. France*, 352 (1959).