C_6H_6 gave 1.60 g (16% yield) of **3a**: mp 191–193°; ν (mineral oil) 1675 cm^{-1} (CO); τ (DMSO-*d*) 4.69 (CH_2), 3.82 (NH_2). *Anal.* ($C_{14}H_{11}ClN_2O_2$) Cl, N.

5,11-Dihydrodibenz[*b*,*e*][1,4]oxazepine-5-carboxamide (3b).—The experimental conditions described above were employed with 8.20 g (0.042 mole) of the heterocycle; the intermediate crude carbamoyl chloride could not be induced to crystallize and as a black tar was treated with absolute ethanolic ammonia for 18 hr at 100°. Chromatography on 100 g of activated alumina gave 2.50 g (30% recovery) of unreacted heterocycle and 4.10 g (37% yield) of crude **3b**, mp 195–196°. Recrystallization from C_6H_6 gave 2.75 g (25% yield) of **3b**: mp 201–203°; ν (mineral oil) 1655 cm^{-1} (CO); τ (DMSO-*d*) 4.70 (CH_2), 3.95 (NH_2). *Anal.* ($C_{14}H_{12}N_2O_2$) C, H, N.

Inhibition of Monoamine Oxidase by N-(Phenoxyethyl)cyclopropylamines. Correlation of Inhibition with Hammett Constants and Partition Coefficients

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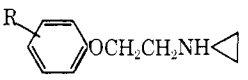
We have investigated the relation of physicochemical properties of a series of N-(phenoxyethyl)cyclopropylamines to their inhibition of the enzyme, monoamine oxidase [E.C. 1.4.3.4 Monoamine:O_2 oxidoreductase (deminating)]. This approach was based upon the success of Hansch and his co-workers[1] in applying substituent constants to correlate structure and activity of a variety of biologically active compounds. They used the Hammett σ, an electronic parameter, and π, a constant derived from the partition coefficient, to correlate with biological activity.

Experimental Section

Compounds of structure



were synthesized as hydrochloride or hydrobromide salts, and their identities were verified by physical methods.[2] Table I shows the R substituent for all compounds included in this study.

Enzyme Inhibition.—Monoamine oxidase inhibition was determined by a method previously described,[3] measuring enzyme activity spectrophotometrically with kynuramine as substrate.[4] Inhibition by four to ten different concentrations of each inhibitor was determined. The inhibitor was, in each case, allowed to react with the enzyme for 30 min prior to the initiation of enzyme action by substrate addition. The results were plotted as percent inhibition *vs.* the negative logarithm of the inhibitor concentration. From the plot, the pI_{50} value (negative logarithm of the inhibitor concentration producing 50% inhibition) was determined. The source of enzyme was mitochondria prepared from rat or human liver by the method of Hogeboom.[5]

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TABLE I

SUBSTITUENT CONSTANTS

R	$\Sigma\gamma$	$\Sigma\sigma$	$\Sigma\pi$
4-Br	0	0.232	1.02
3,4-Cl_2	−1.0	0.600	1.46
3-NO_2	−1.3	0.710	0.11
4-Me	0	−0.170	0.52
3,5-Cl_2	−2.0	0.746	1.52
3-CF_3	−1.3	0.415	1.07
3-Cl-4-Me	−1.0	0.203	1.28
3-Br	−1.3	0.391	0.94
3-Me-4-Cl	−1.0	0.158	1.21
3-Cl	−1.3	0.373	0.76
4-MeO	0	−0.268	−0.04
3,4-Me_2	−1.0	−0.239	1.03
3,5-Me_2	−2.0	−0.138	1.02
3-Me	−1.3	−0.069	0.51
3,5-Me_2-4-Cl	−2.0	−0.138	1.02
3,4,5-Me_3	−2.0	−0.308	1.54
4-N=NC_6H_5	0	0.640	1.71
4-NH_2	0	−0.660	−1.63

Calculations.—When a correlation of inhibition with σ and π was attempted, it was noted that the *meta* positioning of a substituent had a deleterious effect on the inhibitory activity of the compound. For this reason, an arbitrary parameter, γ, was introduced to account for this assumed steric influence of *meta* substituents. In general, the decrease in inhibitor efficiency produced by a single *meta* substituent on the parent molecule [N-(phenoxyethyl)cyclopropylamine] was about twentyfold, aside from its electronic and partition contributions. With other substituents present, however, this effect was less pronounced; the decrease per substituent in this case was about tenfold. These sterically attributed alterations represent changes in effective concentration of the inhibitor and can be presumed to be logarithmically related to the 50% inhibition level as indicated in the correlation equation. On this basis, γ-substituent constants were assigned as follows: 0 for a *para* substituent, −1.3 for a lone *meta* substituent, and −1.0 for a *meta* substituent in the presence of other substituents. Table I lists substituent constants for all of the compounds reported in this study.

Results and Discussion

Table II shows the inhibition of the rat liver enzyme by 16 *meta*- and *para*-substituted compounds in the

TABLE II

INHIBITION OF RAT LIVER MITOCHONDRIAL MAO

R	pI_{50} Calcd	pI_{50} Obsd
4-Br	6.50	6.64
3,4-Cl_2	6.30	6.30
3-NO_2	5.93	5.76
4-Me	5.77	5.69
3,5-Cl_2	5.67	5.68
3-CF_3	5.67	4.98
3-Cl-4-Me	5.65	5.75
3-Br	5.61	5.64
3-Me-4-Cl	5.56	6.06
3-Cl	5.54	5.82
4-MeO	5.51	5.46
3,4-Me_2	4.91	4.71
3,5-Me_2	4.81	4.85
3-Me	4.81	4.78
3,5-Me_2-4-Cl	4.70	4.70
3,4,5-Me_3	4.04	3.54
Predictions		
4-N=NC_6H_5	7.28	7.56
4-NH_2	4.57	4.40

series. The equation derived by linear regression analysis of these experimental data was

$$pI_{50} = 0.865\gamma + 0.209\pi + 1.547\sigma + 5.928$$

The square of the correlation coefficient was 0.82, and the correlation was significant at the 95% confidence level. Using the equation, theoretical pI_{50} values were calculated for the inhibitors. There was generally good agreement between calculated and observed values.

The observed pI_{50} values varied by more than 3 log units, corresponding to a 1000-fold difference in inhibitor concentration required for 50% inhibition. This is a large variation in the potency of these compounds as MAO inhibitors. The equation was used to predict the activity of two additional compounds that had not yet been synthesized. One of these, the 4-phenyldiazo compound, had a predicted pI_{50} of 7.28, higher than any of the compounds included in the initial study. This compound was synthesized, and the experimental pI_{50} was 7.56. This inhibitor is more potent than any previously known MAO inhibitors that we have studied in this assay system. A pI_{50} of 4.57 was predicted for the 4-amino derivative, and the experimental value that was later found was 4.40. Both predictions were thus substantiated.

A smaller number of inhibitors were studied with the human enzyme (Table III). In this case, the equation was

$$pI_{50} + 1.318\gamma + 0.813\pi + 0.727\sigma + 6.898$$

This regression is significant at $P = 0.01$, and the square of the correlation coefficient was 0.88. The calculated pI_{50} values shown in Table III are from this

TABLE III
INHIBITION OF HUMAN LIVER MITOCHONDRIAL MAO

R	pI_{50} Calcd	pI_{50} Obsd
4-N=NC$_6$H$_5$	8.66	8.83
4-CH$_3$	7.14	6.67
3,4-Cl$_2$	7.03	7.55
4-OCH$_3$	6.65	7.07
3-CF$_3$	6.31	5.32
3-Cl	6.04	6.35
3,5-Cl$_2$	5.98	6.20
3-NO$_2$	5.85	5.83
3,5-Me$_2$	4.89	5.10

equation. The coefficients for the human enzyme indicate a considerably different relative importance for the parameters than was noted for the rat enzyme. Both π and σ appear to have about equal effect in the human preparation, whereas σ had a stronger influence on inhibition of the rat enzyme. The steric effect of *meta* substituents was greater with the human preparation.

The compounds in the tables all have *meta* and/or *para* substitutions. Some *ortho*-substituted compounds were also studied, and these were not accommodated by the equations derived from *meta* and *para* derivatives. Other equations, for both rat and human liver enzymes, were developed that accounted for the observed pI_{50} values of the *ortho* derivatives with very low error. σ constants for the *ortho* substituents were those derived by Bray and Barnes[6] from nuclear quadrupole resonance data. Statistical significance was not obtained, probably because the number of compounds studied was small. Conclusions about the *ortho* series were not drawn, except that the compounds behaved differently from the *meta* and *para* series.

In summary, N-(phenoxyethyl)cyclopropylamines are very powerful inhibitors of monoamine oxidase from rat and human liver mitochondria, and the degree of inhibition correlates well with σ and π in a series of *meta*- and *para*-substituted compounds.

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2,8-Substituted 4-Methyl-1(2H)-phthalazinones

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During an investigation in these laboratories, nine 4-methyl-1(2H)-phthalazinones (**4**) were synthesized for pharmacological screening. They were obtained when 2-acetyl-1-nitrobenzoic acid[1] (**1**), 7-amino-3-hydroxy-3-methylphthalide[1] (**2a**), and 3,7-dihydroxy-3-methylphthalide[1] (**2b**) were allowed to react with the corresponding hydrazines (**3**) according to reported procedures.[2]

2a, R = NH_2 **3a**, R^1 = H
b, R = OH **b**, R^1 = C_6H_5
c, R^1 = $CH_2C_6H_5$

4a, R = NO_2; R^1 = H
b, R = NO_2; R^1 = C_6H_5
c, R = NO_2; R^1 = $CH_2C_6H_5$
d, R = NH_2; R^1 = H
e, R = NH_2; R^1 = C_6H_5
f, R = NH_2; R^1 = $CH_2C_6H_5$
g, R = OH; R^1 = H
h, R = OH; R^1 = C_6H_5
i, R = OH; R^1 = $CH_2C_6H_5$

According to the excellent reviews on the chemistry of 1(2H)-phthalazinones[3] compounds with electron-withdrawing and electron-donating groups at position 8 have not been extensively studied. Nitter and Sen[4] studied the mechanism of the closely related reaction between phthalaldehydic acid and phenylhydrazine. They succeeded in isolating an intermediate hydrazone and characterized it prior to cyclization to the 2-phenyl-1(2H)-phthalazinone. Since we assumed that the reactions reported here followed a similar mechanism, we made no effort to trap an intermediate.

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