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.

(6) P. J. Bray and R. G. Barnes, *J. Chem. Phys.*, **27**, 551 (1957).

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.

(1) J. Finkelstein, T. Williams, V. Toome, and S. Traiman [*J. Org. Chem.*, **32**, 3229 (1967)] established the correct structures of these compounds.

(2) W. Rosen, *Ber.*, **18**, 803 (1885); S. Gabriel and A. Neuman, *ibid.*, **26**, 705 (1893); S. Gabriel and G. Eschenbach, *ibid.*, **30**, 3022 (1897); F. M. Rowe and A. T. Peters, *J. Chem. Soc.*, 1331 (1933); F. M. Rowe, S. M. Heath, and C. W. Patel, *ibid.*, 311 (1936); W. R. Vaughan and S. L. Baird, Jr., *J. Am. Chem. Soc.*, **68**, 1314 (1946).

(3) R. C. Elderfield and S. L. Wythe, "Heterocyclic Compounds," Vol. 6, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1957, p 186; J. C. E. Simpson, "The Chemistry of Heterocyclic Compounds," Vol. 5, A. Weissberger, Ed., Interscience Publishers, Inc., New York, N. Y., 1953, p 69.

(4) P. C. Nitter and J. M. Sen, *J. Chem. Soc.*, 1145 (1919).

TABLE I,
SYNTHETIC CONDITIONS, PHYSICAL PROPERTIES, AND ANALYTICAL DATA

Compd	1(2H)-Phthalazinone	Medium	Time, hr	% yield	Solvent[a]	Mp, °C	Formula[d]
4a	4-Methyl-8-nitro-	H_2O	10	83	A–W	280–287	$C_9H_7N_3O$
4b	4-Methyl-8-nitro-2-phenyl-	AcOH	2	60	M	146–148	$C_{15}H_{11}N_3O_3$
4c	2 Benzyl-4-methyl-8-nitro-	$C_6H_5CH_3$[b]	2	71.2	B	163–165	$C_{16}H_{13}N_3O_3$
4d	8-Amino-4-methyl-[c]	H_2O	1	57.1	AcOH	277–280	$C_9H_9N_3$
4e	8-Amino-4-methyl-2-phenyl-	AcOH	2	71.1	E	129–131	$C_{15}H_{13}N_3O$
4f	8-Amino-2-benzyl-4-methyl-	AcOH	2	66	E	124–125	$C_{16}H_{15}N_3O$
4g	8-Hydroxy-4-methyl-	H_2O	1	45.7	E	228–230	$C_9H_8N_2O_2$
4h	8-Hydroxy-4-methyl-2-phenyl-	AcOH	2	76.7	E	158–160	$C_{15}H_{12}N_2O_2$
4i	2-Benzyl-8-hydroxy-4-methyl-	AcOH	35	67.6	B	170–172	$C_{16}H_{14}N_2O_2$

[a] A–W, Me_2CO–H_2O; M, MeOH; B, C_6H_6; E, EtOH. [b] Refluxed with a Dean–Stark trap until no more water separated. [c] Also obtained by reducing **4a** in ethyl acetate under 3 atm pressure of H_2 (Raney Ni). [d] All compounds showed a correct analysis for C, H, N.

We also demonstrated that 4-methyl-8-nitro-1(2H)-phthalazinone (**4a**) could be reduced in the presence of Raney nickel to 8-amino-4-methyl-1(2H)-phthalazinone (**4d**), identical in all respects with the compound obtained from the reaction of **2a** with **3a**.

Pharmacology.—The compounds were completely atoxic at levels of 2000 mg/kg *po* and ip in mice and showed no pharmacological activity in screening tests for cardiovascular, renal, and central nervous system effects. The relative lack of solubility may account for the absence of biological activity.

Experimental Section[5]

Preparation of 1(2H)-Phthalazinones.—Compounds 1, **2a**,[1] and **2b**[1] were allowed to react at reflux with 1 mole equiv of the indicated hydrazine (**3**). The reaction conditions and other pertinent data are reported in Table I.

Acknowledgment.—We are indebted to Dr. Al Steyermark and his staff for the microanalyses, to Dr. T. Williams for nmr spectra, to Dr. V. Toome for the ultraviolet spectra, and to Mr. S. Traiman for the infrared spectra as well as the fruitful discussions with them. The pharmacological data were obtained under the direction of Dr. L. O. Randall, Director of the Pharmacological Laboratories.

(5) All melting points are corrected and were taken on a Uni-Melt Thomas–Hoover capillary melting point apparatus. Ir were taken on a Beckman IR5 double beam spectrophotometer with NaCl optics as KBr pellets. The uv spectra were taken in *i*-PrOH on a Cary spectrophotometer (Model 14M). Nmr spectra (10–15% w/w DMSO-d_6 solutions, TMS) were obtained on a Varian A-60 spectrometer. Accuracy limits are about ±0.025 for chemical shifts. Absorption bands (or peaks) of spectra (uv, ir, nmr) were as expected.

Stability of Pyruvaldehyde Bis(thiosemicarbazone) and Its Copper Chelate[1]

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The tumor-inhibitory properties of pyruvaldehyde bis(thiosemicarbazone) (PTS) are increased by the concurrent administration of copper.[2] The copper chelate is still more active.[2] Studies of the fate of ^{35}S-labeled PTS showed that the distribution of the compound is altered at least quantitatively by copper,[3] and those studies showed that PTS is not eliminated in the urine as either PTS or the chelate. The antitumor activity of the chelate was variable and suggested a possible instability. The possible significance of decomposition of the chelate is the subject of this study.

Experimental Section

The PTS and the ^{35}S-labeled PTS were generously supplied by Dr. Phyllis D. Oja of the Dow Chemical Co., Walnut Creek, Calif. The 2-mercaptoethanol (MCE) was obtained from the Eastman Organic Chemical Co., Rochester, N. Y.

Preparation of Chelate.—All material and glassware used in the preparation of, and work with, the copper–PTS chelate $Cu^{II}PTS$ were washed meticulously in deionized glass-distilled H_2O in order to minimize trace metal contamination. The chelate was prepared by dissolving the PTS in 1 *N* NaOH (12 mg/0.3 ml) and diluted with H_2O to 20 ml. A saturated aqueous solution of $CuSO_4 \cdot 5H_2O$ was added dropwise. The mixture was stirred constantly. Chelate formation was considered complete when no further precipitation occurred following the addition of saturated $CuSO_4$ to an aliquot of the supernatant solution. The addition of concentrated NH_4OH to another aliquot of the supernatant produced a deep blue color indicating the presence of excess copper ions. The reddish brow precipitate was washed repeatedly with H_2O (ten times) and EtOH and Et_2O (five times) and air dried. When radioactive chelate was required, the above procedure was followed except that ^{35}S-labeled PTS was diluted with unlabeled PTS.

Heating Procedures.—Both PTS and $Cu^{II}PTS$ were studied in air and *in vacuo*. They were dissolved in DMF and placed in screw-cap tubes (2 ml/10-ml tube) for examination in air and evacuated and sealed in ampoules (1 ml/5-ml ampoule) for study in the absence of air. Samples were maintained at 4, 25, 37, and 65°.

Paper Chromatography and ^{35}S Assay.—Ascending paper chromatograms were run on Whatman No. 1 paper. The solvent system, 70% DMF and 30% *n*-BuOH, was freshly prepared each time. The chromatograms were examined under uv light. When radioactive materials were examined, the strips were cut into 20 equal parts and measured in 15 ml of toluene–PPO–POPOP scintillant in a Packard Tri-Carb liquid scintillation spectrophotometer at 9.5% gain and a window setting of 40–1000. The uv spectra of PTS and $Cu^{II}PTS$ were determined in H_2O and DMF. PTS showed $\lambda_{max}^{H_2O}$ 325 mμ (*A* 47,000) and $\lambda_{min}^{H_2O}$ 270 mμ (*A* 27,000) and λ_{max}^{DMF} 345 mμ (*A* 47,000) and λ_{min}^{DMF} 290 mμ (*A* 27,000). The values for $Cu^{II}PTS$ were $\lambda_{max}^{H_2O}$ 300 mμ (*A*

(1) This investigation was supported in part by funds from National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. Ca-08748).

(2) J. G. Cappuccino, S. Banks, G. Brown, M. George, and G. S. Tarnowski, *Cancer Res.*, **27**, 968 (1967).

(3) J. G. Cappuccino and M. E. Balis, unpublished data.