acidic with 6 *N* HCl, treated with 10% $NaHCO_3$, and finally extracted with C_6H_6. The combined extracts were washed with H_2O and dried ($MgSO_4$) and the solvent was evaporated. The crude products were purified by recrystallization as the free base or acid addition salt.

4-(1-Substituted 3-Pyrrolidinyl)-2H-1,4-benzoxazin-3(4H)-ones (Table III).—To a stirred solution of 0.10 mole of the 1-substituted 3-(*o*-hydroxyanilino)pyrrolidine in 250 ml of $CHCl_3$ maintained at 0–5° was added slowly a solution of 0.10 mole of chloroacetyl chloride in 50 ml of $CHCl_3$. After the addition was complete the mixture was allowed to warm to room temperature. The solvent was then evaporated at reduced pressure; the residual oil was dissolved in 500 ml of *i*-PrOH and treated with 0.20 mole of NaOMe. The mixture was stirred and heated at reflux for 16 hr, cooled, and filtered. After the solvent was evaporated, the residual oil was taken up in *i*-Pr_2O, washed with 1 *N* NaOH and H_2O, and dried ($MgSO_4$) and the solvent was evaporated. The products were purified by distillation or conversion to a salt.

3-Pyrrolidinyl-2H-1,4-benzoxazin-3(4H)-one (23).—A solution of 15 g of 4-(1-benzyl-3-pyrrolidinyl)-2H-1,4-benzoxazin-3(4H)-one in 200 ml of 95% EtOH was reduced catalytically with 5 g of 10% Pd–C. The mixture was heated at 70° and shaken with H_2 until 1 equiv of H_2 was absorbed (*ca.* 2 hr). After cooling, the suspension was filtered and the solvent was evaporated. The product was purified by conversion to a salt followed by recrystallization.

4-[1-(2-Phenylethyl)-3-pyrrolidinyl]-2H-1,4-benzoxazin-3(4H)-one (24).—A mixture of 0.04 mole of **31** (Table III), 0.04 mole of phenethyl bromide, 15 g of K_2CO_3, and 100 ml of dry PhMe was stirred and heated at reflux for 16 hr, cooled, and treated with 100 ml of H_2O. The organic layer was separated, washed with H_2O, dried ($MgSO_4$), and filtered and the solvent was evaporated. The residual oil was purified by conversion to a solid salt followed by recrystallization.

4-[1-(2-Benzoylethyl)-3-pyrrolidinyl]-2H-1,4-benzoxazin-3(4H)-one (25).—Compound **23** was treated with 2-benzoylethyldimethylamine hydrochloride as previously described (Method D, Amine Exchange).

Acknowledgment.—The authors thank Mr. Ying-Ho Chen for preparation of two of the compounds, Mr. Ashby Johnson for nmr spectra, and Mrs. Marianne H. Foxwell for technical assistance in pharmacological testing.

Heterocyclic Mesoionic Structures, a Novel Class of Monoamine Oxidase Inhibitors. II. Arylanhydro-1,2,3-thiadiazolium Hydroxides

EDWARD H. WISEMAN AND DONALD P. CAMERON

Medical Research Laboratories, Chas. Pfizer & Co., Inc., Groton, Connecticut

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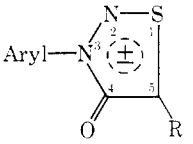
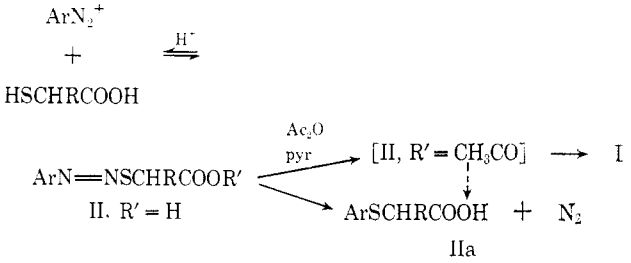
The preparation and monoamine oxidase inhibitory activity of a series of arylanhydro-1,2,3-thiadiazolium hydroxides (I) is described. A visualization of enzyme–inhibitor interaction is presented, as well as an analysis of the structural features controlling the mode of enzyme inhibition. Those inhibitors showing noncompetitive inhibitory activity *in vitro* were shown also to be active inhibitors of the enzyme *in vivo*, while competitive inhibitors were inactive *in vivo*. These observations support and extend those made in a previous study of mesoionic compounds, the N-arylsydnones.

The inhibition of the enzyme, monoamine oxidase (MAO), has previously been reported[1] for the heterocyclic mesoionic N-arylsydnones. This report is an account of the preparation of anhydro-3-aryl-4-hydroxy-1,2,3-thiadiazolium hydroxides (I),[2] and a discussion of the structure–activity requirements for the inhibition of MAO.



I

Anhydro-1,2,3-thiadiazolium hydroxides were prepared *via* the reported sequence.[3,4] With the exception of 5-methyl homologs, 5-substituted derivatives of I were obtained by the appropriate substitution reaction on the parent anhydro-1,2,3-thiadiazolium hydroxide.[3] The limiting factor in the preparation of I was the stability of the intermediate arylazothioacetic acid II. It was previously reported that polysubstituted and especially *ortho*-substituted phenyl derivatives of either I or II could not be prepared; these restrictions did not apply if conditions were chosen which minimized two side reactions of II: (1) acid-catalyzed cleavage to diazonium salt,[5] and (2) thermal elimination of N_2 to yield carboxymethyl aryl sulfides IIa.[6] The stability of II increased with increasing electron-releasing potential in the phenyl ring, although this favorable trend was compromised by retardation of the rate of cyclization of *ortho*-substituted analogs. Electronegative substituents (halogen, NO_2, CF_3) conjugated (*para*, *ortho*) with the diazo sulfide moiety depressed cyclization to I and enhanced (to the point of explosiveness) formation of IIa; consequently, the corresponding cyclic derivatives were difficult to prepare unless the phenyl ring contained additional con-



(1) D. P. Cameron and E. H. Wiseman, *J. Med. Chem.*, **11**, 820 (1968).

(2) The analysis of infrared, ultraviolet, and nuclear magnetic resonance spectra supporting the assignment of a mesoionic structure to I will be discussed in a subsequent publication (D. P. Cameron, in preparation).

(3) G. F. Duffin and J. D. Kendall, *J. Chem. Soc.*, 3189 (1956).

(4) W. Pacha and B. Prijs, *Helv. Chim. Acta*, **41**, 421 (1958).

(5) K. K. Saunders, "The Aromatic Diazo Compounds," Longmans, Green and Co., London, 1949, Chapter V.

(6) W. B. Reynolds, *Ind. Eng. Chem.*, **42**, 1905 (1950).

jugated electropositive functionality. Controlled procedures gave access to a variety of substituted arylazothioacetic acid intermediates, which formed I in Ac_2O and pyridine. When cyclization and formation of IIa were competitive, or when the latter reaction predominated, I was recovered by selective precipitation as the HCl salt from nonpolar solvents.

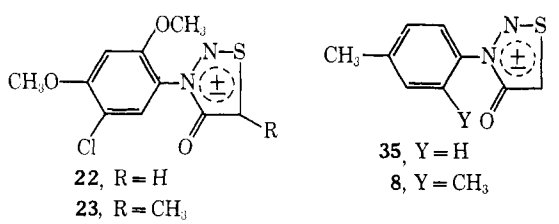
In one instance of note, II (Ar = 2-methoxy-5-methyl-4-nitrophenyl) underwent in Ac_2O–pyridine, not only cyclization to I and N_2 loss to IIa (the major product), but also cleavage to the original diazonium salt which then arylated I to produce anhydro-4-hydroxy-3,5-bis(2-methoxy-5-methyl-4-nitrophenyl)-1,2,3-thiadiazolium hydroxide.

Inhibition of Monoamine Oxidase (MAO) Activity (Table I). *In Vitro.*—Guinea pig liver homogenates were prepared as described by Weissbach, *et al.*,[7] MAO activity, using kynuramine as substrate, being determined spectrophotometrically. Measurements of optical density at 360 mμ were made immediately after adding substrate, and subsequently at 2-min intervals for a total period of 10 min. Reaction rate was then determined by plotting optical density against time.

In Vivo.—Male albino rats, 150–200 g, were dosed orally (1–100 mg of inhibitor/kg). After 2 hr, the animals were stunned, and the livers were quickly removed, weighed, and homogenized in 5 vol of cold water. The homogenate was strained through cheesecloth and refrigerated until assayed. MAO activity was determined as in the preceding section.

Results

The majority of the anhydrothiadiazolium hydroxides (Table I) were inhibitors of MAO *in vitro*. Kinetic analysis of the enzyme inhibition by selected compounds was performed using the Dixon rearrangement[8] of the Lineweaver–Burk equation. Both by this method and by the Lineweaver–Burk plot, **22** was shown to inhibit MAO by a noncompetitive mechanism (Figure 1). Compound **23**, the 5-methyl derivative of **22**, was an inhibitor of MAO *in vitro*, but, in distinct contrast to **22**, kinetic analysis, using the Dixon (Figure 2) and Lineweaver–Burk plots, showed that **23** was a competitive inhibitor. A similar structural dependence was seen with **35**[3] and 8. Analysis of the kinetics of inhibition of MAO *in vitro* showed **35** to be a noncompetitive (Figure 3) and 8 to be a competitive inhibitor (Figure 4). Compounds **22** and **35** also inhibited the enzyme MAO *in vivo*, while **23** and 8 were inactive *in vivo*.



22, R = H
23, R = CH_3

35, Y = H
8, Y = CH_3

Discussion

The anhydrothiadiazolium hydroxides reported here are the second class of mesoionic compounds (mesomeric betaines) which are inhibitors of the enzyme MAO. Inhibitory activity was previously reported for the related mesoionic structures, N-arylsydnones.[1] With notable exceptions (discussed below), anhydrothiadiazolium hydroxides were inhibitors of MAO both *in vitro* and *in vivo*, as were N-arylsydnones. However, potency *in vitro* was somewhat higher, the anhydrothiadiazolium hydroxide **22** being about ten times as potent as the most active N-arylsydnone.[1]

Sydnones and anhydrothiadiazolium hydroxides are not related to any of the structural types previously reported to be active inhibitors of MAO. With respect to the classical division of MAO inhibitors, the N-arylsydnones were assigned to the noncompetitive class on the basis that inhibitory activity was displayed both *in vitro* and *in vivo*. Although not exhaustively studied, enzyme inhibitors of both competitive and noncompetitive classes have been found among the anhydrothiadiazolium hydroxides. This conclusion was initially drawn, as in the sydnones, by the observation of a nonparallel relationship between the activity *in vitro* and *in vivo*. However, the kinetics of the interaction of selected anhydrothiadiazolium hydroxides

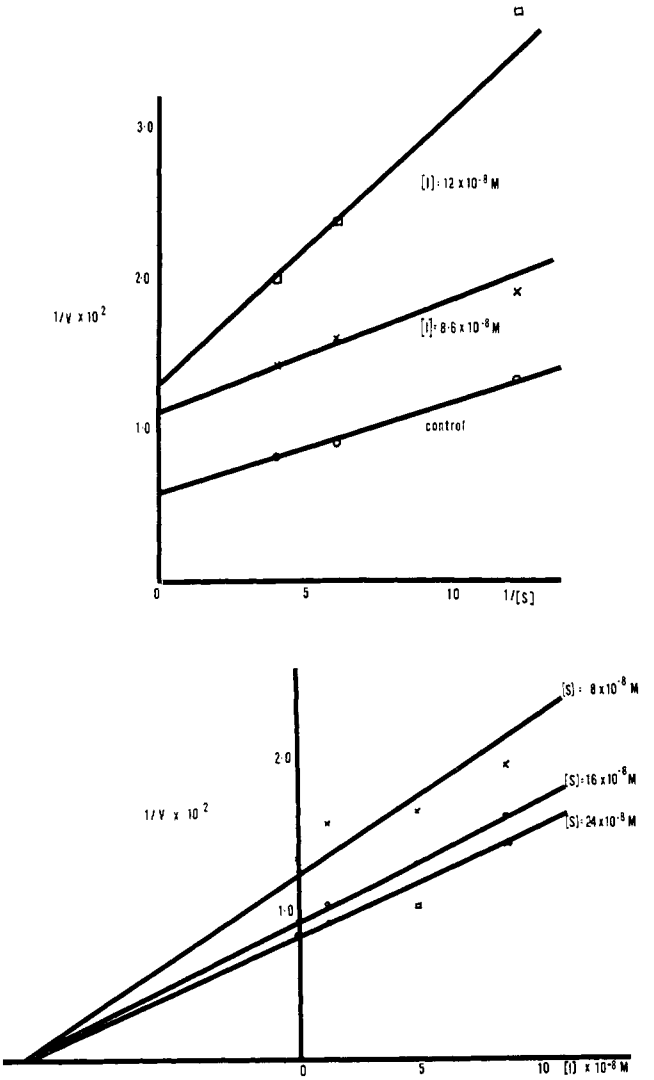
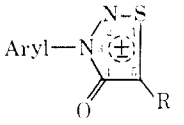


Figure 1.—Lineweaver–Burk (top) and Dixon (bottom) plots showing noncompetitive inhibition of MAO *in vitro* by **22**.

(7) H. Weissbach, T. E. Smith, J. W. Daly, B. Witkop, and S. Udenfriend, *J. Biol. Chem.*, **235**, 1160 (1960).
(8) M. Dixon, *Biochem. J.*, **55**, 170 (1953).

TABLE I

ANHYDRO-4-HYDROXY-3-ARYL-1,2,3-THIADIAZOLIUM HYDROXIDES



No.	3-Aryl	R	Yield, %	Mp, °C (recrystn solvent[a])	Formula	Analyses	MAO inhib. *In vitro*[b]	MAO inhib. *In vivo*[c]
1	4-$CH_3C_6H_4$	$C_6H_5CH_2S$	55	85–86.5 (A)	$C_{16}H_{14}N_2OS_2$	C, H, N, S		
2	3,4-$(CH_3)_2C_6H_3$	H	84	66–67 (B)	$C_{10}H_{10}N_2OS$	C, H, N, S	1	2
3	3-F-4-$CH_3C_6H_3$	H	24	119–119.5 (C)	$C_9H_7FN_2OS$	C, H, N, S, F	1	0
4	4-$CH_3OC_6H_4$	CH_2CHCH_2S	88	68.5–70 (A)	$C_{12}H_{12}N_2O_2S_2$	C, H, N, S	1	
5	4-CH_3O-3-$CF_3C_6H_3$	H	41	157.5–158 (A)	$C_{10}H_7F_3N_2O_2S$	C, H, N, S, F	1	1
6	2,4-$(CH_3O)_2C_6H_3$	H	39	131–131.5 (A)	$C_{10}H_{10}N_2O_3S$	C, H, N, S	1	
7	4-CH_3O-2-$CF_3C_6H_3$	H	45	132.5–133 (A)	$C_{10}H_7FN_2O_2S$	C, H, N, S, F	0	
8	2,4-$(CH_3)_2C_6H_3$	H	42	85.5–86 (A)	$C_{10}H_{10}N_2OS$	C, H, N, S	1	0
9	2-CH_3O-5-$CH_3C_6H_3$	H	31	130.5–131 (D)	$C_{10}H_{10}N_2O_2S$	C, H, N, S	1	2
10	2,5-$(CH_3O)_2C_6H_3$	H	16	133–133.5 (A)	$C_{10}H_{10}N_2O_3S$	C, H, N, S	1	2
11	5-Cl-2-$CH_3OC_6H_3$	H	10	168.5–169 (A)	$C_9H_7ClN_2O_2S$	C, H, N, S, Cl	1	2
12	2-CH_3O-5-$CF_3C_6H_3$	H	73	171.5–172 (A)	$C_{10}H_7F_3N_2O_2S$	C, H, N, S, F	1	2
13	2-Cl-5-$CH_3OC_6H_3$	H	28	135.5–136 (E)	$C_9H_7ClN_2O_2S$	C, H, N, S, Cl	1	1
14	2-Cl-5-$CH_3C_6H_3$	H	80	126–126.5 (A)	$C_9H_7ClN_2OS$	C, H, N, S, Cl	1	1
15	2,3-$(CH_3)_2C_6H_3$	H	37	140–140.5 (A)	$C_{10}H_{10}N_2OS$	C, H, N, S	1	2
16	3-Cl-4-CH_3O-5-$CH_3C_6H_2$	H	16	123.5–124 (A)	$C_{10}H_9ClN_2O_2S$	C, H, N, S, Cl	1	0
17	3,4,5-$(CH_3O)_3C_6H_2$	H	23	158–158.5 (E)	$C_{11}H_{12}N_2O_4S$	C, H, N, S	0	0
18	2,3,4-$(CH_3O)_3C_6H_2$	H	51	89.5–90 (A)	$C_{11}H_{12}N_2O_4S$	C, H, N, S	0	
19	2,4-$(CH_3O)_2$-3-$CH_3C_6H_2$	H	48	121–121.5 (F)	$C_{11}H_{12}N_2O_3S$	C, H, N, S	0	
20	4-AcO-2,3-$(CH_3O)_2C_6H_2$	H	31	128.5–129 (A)	$C_{12}H_{12}N_2O_5S$	C, H, N, S	0	
21	2-Cl-4,5-$(CH_3O)_2C_6H_2$	H	31	187–188.5 (F)	$C_{10}H_9ClN_2O_3S$	C, H, N, S, Cl	0	
22	5-Cl-2,4-$(CH_3O)_2C_6H_2$	H	73	174.5–175 (G)	$C_{10}H_9ClN_2O_3S$	C, H, N, S, Cl	3	3
23	5-Cl-2,4-$(CH_3O)_2C_6H_2$	CH_3	53	166.5–167 (A)	$C_{11}H_{11}ClN_2O_3S$	C, H, N, S, Cl	1	0
24	5-Cl-2,4-$(CH_3O)_2C_6H_2$	Br	50	169.5–170 (A)	$C_{10}H_8BrClN_2O_3S$	C, H, N, S, Br, Cl	1	
25	5-Cl-2,4-$(CH_3O)_2C_6H_2$	Cl	74	165.5–166 (A)	$C_{10}H_8Cl_2N_2O_3S$	C, H, N, S, Cl	1	2
26	4-Cl-2,5-$(CH_3O)_2C_6H_2$	H	63	167.5–168 (G)	$C_{10}H_9ClN_2O_3S$	C, H, N, S, Cl	0	
27	4-Cl-2,5-$(CH_3O)_2C_6H_2$	CH_3	81	144.5–145 (F)	$C_{11}H_{11}ClN_2O_3S$	C, H, N, S, Cl	0	
28	2-CH_3O-4,5-$(CH_3)_2C_6H_2$	H	54	127.5–128 (A)	$C_{11}H_{12}N_2O_2S$	C, H, N, S	0	
29	4-Cl-2-CH_3O-5-$CH_3C_6H_2$	H	31	126.5–127 (A)	$C_{10}H_9ClN_2O_2S$	C, H, N, S, Cl	1	0
30	5-Cl-2-CH_3O-4-$CH_3C_6H_2$	H	54	162–162.5 (A)	$C_{10}H_9ClN_2O_2S$	C, H, N, S, Cl	1	2
31	2,4,5-$(CH_3O)_3C_6H_2$	H	5	130.5–131 (F)	$C_{11}H_{12}N_2O_4S$	C, H, N, S	1	1
32	3-Cl-6-CH_3O-2,4-$(CH_3)_2C_6H$	H	44	132–132.5 (A)	$C_{11}H_{11}ClN_2O_2S$	C, H, N, S, Cl	0	
33	5-Cl-2-CH_3O-3,4-$(CH_3)_2C_6H$	H	63	116.5–117 (F)	$C_{11}H_{11}ClN_2O_2S$	C, H, N, S, Cl	0	
34	3-Cl-2-CH_3O-4,5-$(CH_3)_2C_6H$	H	50	115–115.5 (A)	$C_{11}H_{11}ClN_2O_2S$	C, H, N, S, Cl	1	

[a] (A) Et_2O–CH_2Cl_2, (B) EtOH–H_2O, (C) *i*-Pr_2O–CH_2Cl_2, (D) EtOAc, (E) C_6H_6, (F) Et_2O, (G) MeOH. [b] Relative potencies of inhibitors were estimated as follows: 0, no inhibition; 1, partial inhibition at concentration of 17 μg/ml; 2, total inhibition at 17 μg/ml; 3, total inhibition at 17 μg/ml. [c] Relative potencies of inhibitors were estimated as follows: 1, partial inhibition at an oral dose of 100 mg/kg; 2, complete inhibition after a dose of 100 mg/kg; 3, complete inhibition after a dose of 10 mg/kg.

with MAO was examined *in vitro*, and, as discussed below, firmly support the competitive and noncompetitive assignments made on the basis of *in vitro/in vivo* studies. The more conventional MAO inhibitors belong to two classic types: noncompetitive, exemplified by the hydrazines,[9] and competitive, of which harmaline is representative.[10] An intermediate type of MAO inhibitor has been recognized in which high binding affinity by a competitive inhibitor results in a duration of action approaching that of noncompetitive MAO inhibitors. Tranylcypromine has been suggested to belong to this intermediate class.[11] By the analytical method used in this study tranylcypromine would be classified as a noncompetitive inhibitor. A similarity between the π-electron systems of N-arylsydnones, tranylcypromine, and the imine intermediate formed during oxidation of the natural amine substrates of MAO has been noted[1] and the π-electron system of the anhydrothiadiazolium compounds can also be accommodated within this hypothetical relationship (Figure 5). The kinetic data describing the interaction of the anhydrothiadiazolium hydroxides with MAO indicate **22** and **35** to be noncompetitive and **8** and **23** to be competitive inhibitors. This conclusion is further supported by the observation that **22** and **35** inhibited the enzyme *in vivo*, while **8** and **23** did not.

The displacement–ring cleavage reaction of the mesoionic ring suggested[1] as a possible interaction with MAO in the sydnone series seems unlikely in the anhydrothiadiazolium series in view of the greater stability of this system toward both acid- and base-catalyzed cleavage reactions.[3] However, the mesoionic ring does undergo 5 substitution by S or mercaptide ion under mild conditions; free-radical attack also occurs

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

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

(11) C. L. Zirkle, C. Kaiser, D. H. Tedeschi, R. E. Tedeschi, and A. Burger, *J. Med. Pharm. Chem.*, **5**, 1265 (1962).

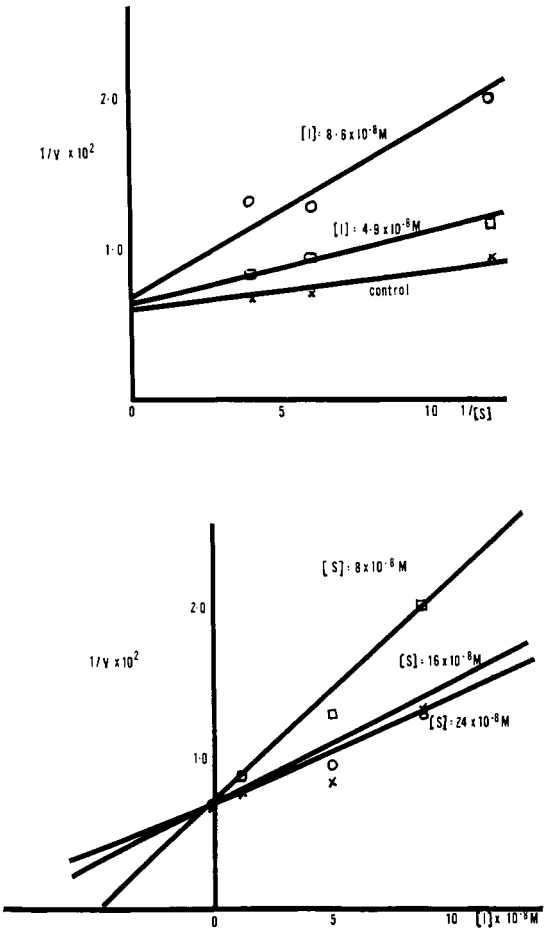


Figure 2.—Lineweaver-Burk (top) and Dixon (bottom) plots showing competitive inhibition of MAO *in vitro* by **21**.

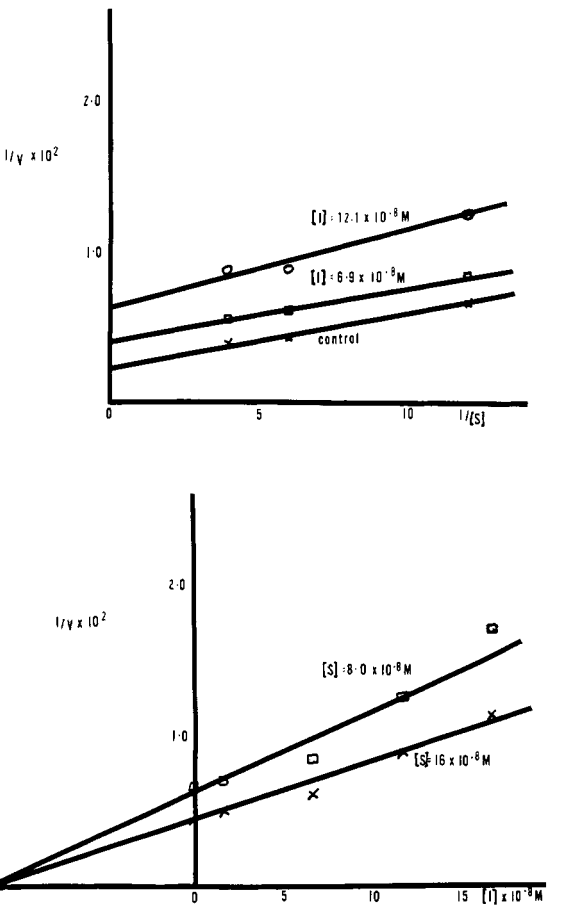


Figure 3.—Lineweaver-Burk (top) and Dixon (bottom) plots showing noncompetitive inhibition of MAO *in vitro* by **35**.

at this position. The mechanistic difference in enzyme inhibition between **35** and its 5-methyl analog **8** points to the importance of the 5 position. It is of interest, therefore, to attempt to accommodate these findings within the framework of the hypothetical requirements for inhibition of MAO.[12] The aromatic ring could clearly interact with the π-electron binding surfaces of the enzyme, and the anionic charge (which acts primarily through the *exo*-oxygen) is so situated as to permit binding with an electrophilic enzyme group (HX, Figure 6). The anhydrothiadiazolium compounds are considerably stronger bases than sydnones and form stable salts or complexes with protic[3,4] and Lewis acids.[2] Depending on the nature of R, III could react with the oxidizing moiety of MAO by one of two paths, involving (a) nucleophilic addition to form an intermediate of the covalent hydration type, or (b) abstraction of the 5-proton to form the conjugate kinetic base.[13] Subsequent elimination of R^- from IIIa (a process favored by the aromaticity of III), or nucleophilic attack of IIIb could yield the final enzyme-bound products. Clearly when R is alkyl, neither process is possible and III will show competitive MAO inhibition. Process a is possible when R is halogen but process b, which requires elimination of positive halogen is not energetically attractive. Since neither process would permit hydride ion abstraction, the inhibition of enzyme is presumably due to the failure of the compounds to be substrates for the enzyme. The data also suggest that the 3-aryl substituents play an important role in determining whether competitive or noncompetitive inhibition obtains. This may be due in part to effects on enzyme–substrate binding strengths, or to conjugative effects on the mesoionic ring, by changing basicity (negative charge delocalization) or by changing reactivity at the 5 position.

The oxidizing action of MAO has been hypothesized as a concerted process involving a disulfide group and the grouping X; the role of X is essentially that of stabilizing the intermediate reduced forms of MAO (IVa,b) and could be effected by a moiety such as a mercapto group. While there is little direct evidence for invoking a disulfide group in MAO oxidations, it is not unlikely that the enzyme contains disulfide linkages. MAO does contain sulfhydryl groups, upon which the activity is dependent. However, since the sulfhydryl moiety is not an oxidizing but a reducing agent, some other function (such as disulfide) must be involved in the electron-transfer process.

Experimental Section[14]

Starting anilines were prepared by standard technology. The following modifications in the reported[3,4] preparation of the intermediate arylazothioacetic acids (II) were made: (1) high

(12) B. Belleau and J. Moran, *Ann. N. Y. Acad. Sci.*, **107**, 822 (1963).
(13) R. Breslow, *J. Am. Chem. Soc.*, **80**, 3719 (1958).

(14) All melting points are uncorrected and were determined on a Thomas–Hoover capillary melting point apparatus. Analyses were carried out by the Physical Measurements Laboratory of Chas. Pfizer & Co., Inc. Where analyses are indicated by the symbols of the elements, the analytical results for these elements were within 0.4% of the theoretical values.

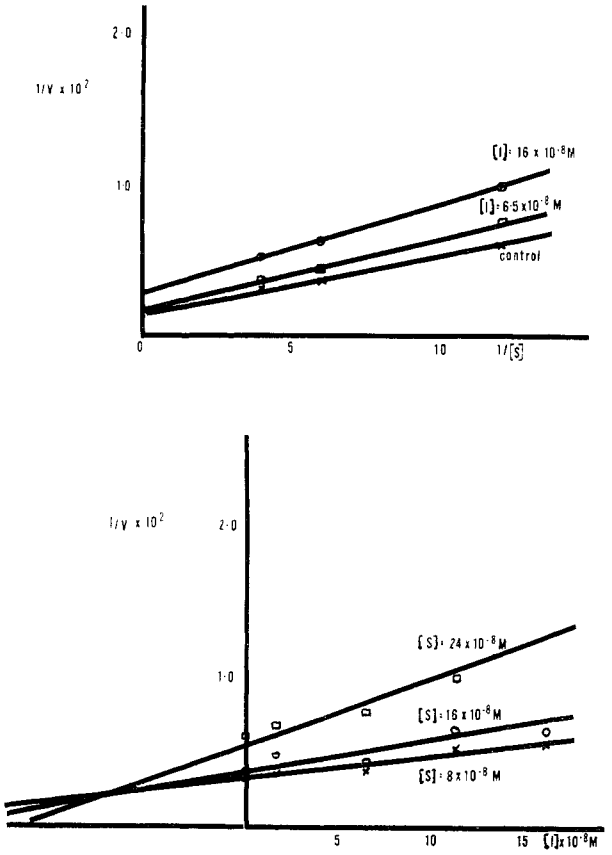


Figure 4.—Lineweaver-Burk (top) and Dixon (bottom) plots showing competitive inhibition of MAO *in vitro* by **8**.

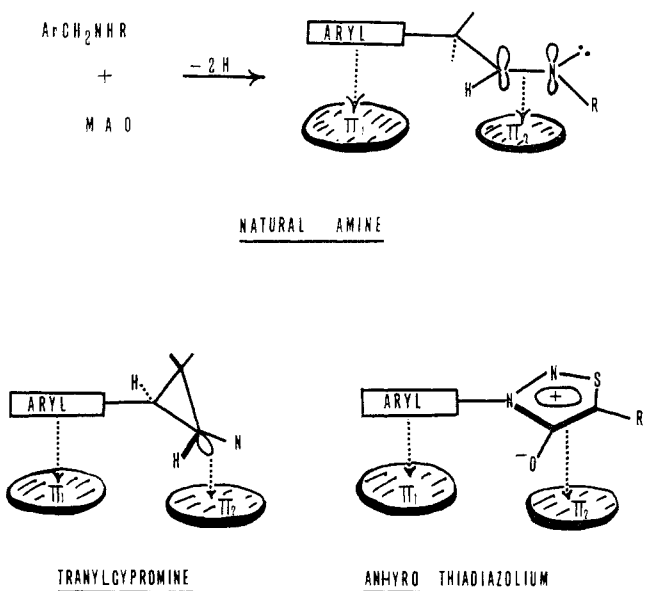


Figure 5.—Spatial relationships between natural amine substrates for MAO and the inhibitors tranylcypromine and anhydrothiadiazolium hydroxides.

dilution, (2) use of freshly distilled mercaptoacetic acid, (3) maintenance of pH 2.5–4 during the reaction, and (4) operation at −10 to 5°. In most cases the arylazothioacetic acids were not characterized, but were extracted from the reaction mixture, and the extract was cooled to −30° and treated with cold Ac_2O–pyridine, prior to concentration *in vacuo*. After removal of solvent the reaction was allowed to proceed at 25° for 12 hr. In those cases in which the anhydrothiadiazolium hydroxide failed to precipitate, the reaction mixture was hydrolyzed at 0° (pH 2–3) and then extracted. The dried extracts were treated with HCl in order to precipitate product as the HCl salt. The

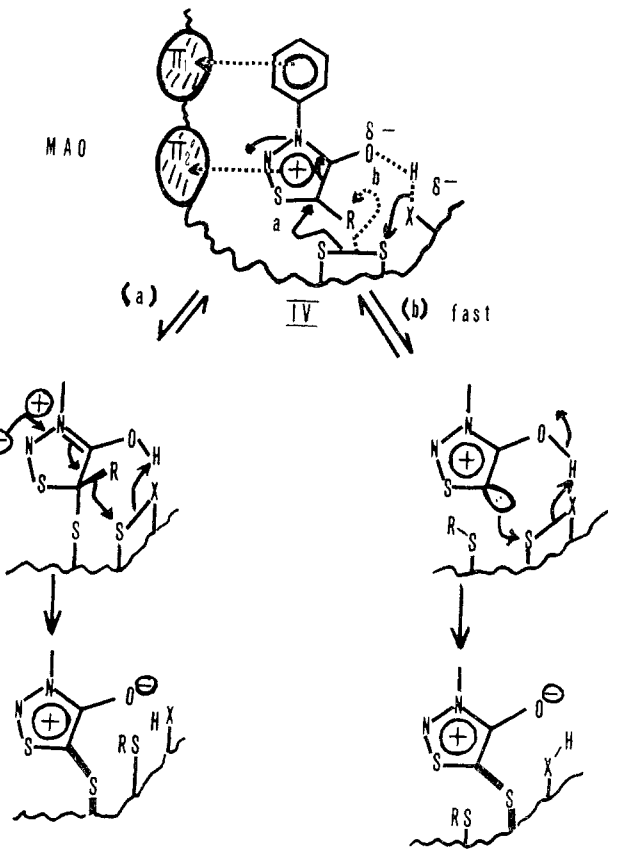


Figure 6.—Interaction between anhydrothiadiazolium hydroxides and hypothetical MAO.

salts were subsequently converted (80–95% yield) to **1**, with aqueous $NaHCO_3$.[15]

5-Chloro-2,4-dimethoxyphenylazothioacetic Acid.—5-Chloro-2,4-dimethoxyaniline (37.4 g, 0.2 mole) was suspended in 100 ml of 5 *N* HCl and diluted to 500 ml with ice–water. A total of 14 g of $NaNO_2$ in 25 ml of H_2O was added over 3 min. After 45 min at 0°, excess $NaNO_2$ was destroyed with urea. The reaction mixture was rapidly poured into a stirred solution of freshly distilled mercaptoacetic acid (30 g, 0.218 mole) and ice (1 kg). An immediate precipitate formed which, after 20 min (0°, pH 2.5), was extracted into Et_2O. The extracts were washed, dried ($MgSO_4$), and concentrated *in vacuo;* yield 42.1 g (73%), mp 76–78° dec. *Anal.* ($C_{10}H_{11}ClN_2O_4S$) C, H, N.

Anhydro-4-hydroxy-3-(5-chloro-2,4-dimethoxyphenyl)-1,2,3-thiadiazolium Hydroxide (22).—5-Chloro-2,4-dimethoxyphenylazothioacetic acid (42.1 g, 0.15 mole) was stirred for 4.5 hr at 0° in Ac_2O (120 ml) and C_6H_5N (50 ml). The reaction product was filtered off and recrystallized to yield **22** (12.2 g).

Anhydro-4-hydroxy-3-(2,5-dimethoxyphenyl)-1,2,3-thiadiazolium Hydroxide (10).—2,5-Dimethoxyaniline (26 g, 0.17 mole) was diazotized and rapidly poured onto a stirred mixture of mercaptoacetic acid (17 g, 0.185 mole) and crushed ice (1 kg). $NaHCO_3$ (1 *N*) was added to pH 3.5 and, after 1 hr at 0°, the product was extracted into Et_2O. The dried extracts were concentrated to half-volume. Cold Ac_2O (40 ml) and C_6H_5N (20 ml) were added, and evaporation of solvent was continued *in vacuo* at 0°. The reaction mixture, after 12 hr (25°), was quenched on ice and, after addition of 20% HCl to pH 2, was extracted with CH_2Cl_2. The water-washed extracts yielded an oil which was dissolved in 150 ml of CH_2Cl_2. Addition of 3–4 vol of Et_2O provided **10** (6.5 g).

Anhydro-4-hydroxy-3-(2-methoxy-5-methyl-4-nitrophenyl)-1,2,3-thiadiazolium Hydroxide and Anhydro-4-hydroxy-3,5-bis(2-methoxy-5-methyl-4-nitrophenyl)-1,2,3-thiadiazolium Hydroxide.—Diazotized 2-methoxy-5-methyl-4-nitroaniline (0.2

(15) Using this procedure, the previously reported anhydro-4-hydroxy-3-(2-naphthyl)-1,2,3-thiadiazolium hydroxide was prepared: λ_{max} (KBr) 3.3, 6.26 μ; λ_{max} (CH_3OH) 372, 296, 273, 255 mμ ($\epsilon \times 10^3$: 4.2, 2.8, 3.1, 5.7); mp (C_6H_{12}) 131–131.5° (lit.[3a] mp 102°). *Anal.* ($C_{12}H_8N_2OS$) C, H, N, S.

mole) and mercaptoacetic acid gave an unstable oil (50 g) which, when treated with Ac_2O–C_6H_5N yielded, on filtration, a chromatographically homogeneous solid: 1.0 g; mp 268–269°; λ_{max} 6.05, 6.5, 7.66 μ, tentatively identified as anhydro-4-hydroxy-3,5-bis(2-methoxy-5-methyl-4-nitrophenyl)-1,2,3-thiadiazolium hydroxide. *Anal.* ($C_{18}H_{16}N_4O_7S$) C, H, N, S. The filtrate yielded, on treatment with base, anhydro-4-hydroxy-3-(2-methoxy-5-methyl-4-nitrophenyl)-1,2,3-thiadiazolium hydroxide (0.6 g); mp 180–181° (Et_2O–CH_2Cl_2); λ_{max} 6.03, 6.5, 7.65 μ. *Anal.* ($C_{11}H_9N_3O_4S$) C, H, N, S.

Anhydro-4-hydroxy-5-bromo-3-(5-chloro-2,4-dimethoxyphenyl)-1,2,3-thiadiazolium Hydroxide (24). A.—A stirred suspension of **22** (5.44 g, 0.02 mole) and N-bromosuccinimide (4.09 g, 0.023 mole) in CCl_4 (200 ml) was heated under reflux for 48 hr. The cooled reaction mixture yielded **24** (3.5 g), melting point unchanged by admixture with material prepared by B (below).

B.—The anhydro compound **22** (0.12 mole) was brominated in glacial AcOH (250 ml) and anhydrous NaOAc (25 g). Recrystallization of the crude product afforded **24** (21 g).

Anhydro-4-hydroxy-5-chloro-3-(5-chloro-2,4-dimethoxyphenyl)-1,2,3-thiadiazolium Hydroxide (25).—The anhydro compound **22** (32.6 g, 0.12 mole) and anhydrous NaOAc (25 g) were suspended in glacial AcOH (250 ml). Cl_2 was added at 20° until solution was complete. After being heated for 30 min at 100°, the reaction was quenched on ice. Addition of 2.5 *N* NaOH to pH 4 precipitated **25** (18.9 g).

Anhydro-4-hydroxy-5-allylthio-3-(*p*-anisyl)-1,2,3-thiadiazolium Hydroxide (4).—The Na salt of anhydro-4-hydroxy-5-mercapto-3-(*p*-anisyl)-1,2,3-thiadiazolium hydroxide[3] [0.14 mole, mp 177–182°. *Anal.* ($C_9H_7N_2O_2SNa$) C, H] was dissolved in DMF (20 ml) and allyl bromide (0.033 mole) was added. After 72 hr at 25° the mixture was quenched on ice, giving **4** (3.0 g).

Anhydro-4-hydroxy-5-benzylthio-3-(*p*-tolyl)-1,2,3-thiadiazolium Hydroxide (1).—Similarly, anhydro-4-hydroxy-5-mercapto-3-(*p*-tolyl)-1,2,3-thiadiazolium hydroxide sodium salt[3] (0.2 mole, mp 173–175°) in DMF (25 ml) with benzyl bromide (0.22 mole) afforded **1** (5.2 g).

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Repository Drugs. VI. 4′-[N-(Aralkylidene-, -Benzylidene-, and -Naphthylidene)sulfanilyl]anilides, 4′-{N-[(Dimethylamino)methylene]sulfanilyl}anilides, and Related Sulfanilylanilides with Prolonged Antimalarial and Antileprotic Action[1]

DONALD F. WORTH, EDWARD F. ELSLAGER, AND ANNETTE A. PHILLIPS

Department of Chemistry, Medical and Scientific Affairs Division, Parke, Davis & Company, Ann Arbor, Michigan 48106

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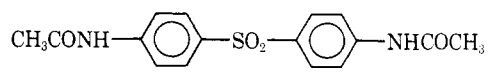
Representative 4′-[N-(aralkylidene-, -benzylidene-, and -naphthylidene)sulfanilyl]anilides (III–VI), N-(aralkylidene- and -benzylidene)-4,4′-sulfonyldianiline derivatives (VII, VIII), and 4′-{N-[(dimethylamino)-methylene]sulfanilyl}anilides (IX) were synthesized as potential repository antimalarial and antileprotic agents in a search for long-acting sulfones that would be less dependent on enzymatic deacylation for activity and afford higher blood sulfone levels than 4′,4‴-sulfonylbisacetanilide (acedapsone, DADDS). The compounds were prepared by condensing the appropriate aldehyde with the requisite 4′-sulfanilylanilides or 4,4′-sulfonyldianiline precursor. Among them, 4′-[N-(benzylidene)sulfanilyl]acetanilide (**3**), 4′-[N-(*p*-acetamidobenzylidene)sulfanilyl]acetanilide (**5**), and 4′-[N-(3,5-dichlorosalicylidene)sulfanilyl]acetanilide (**11**) satisfied the above requirements and showed strong repository activity against *Plasmodium berghei* and *Mycobacterium leprae* in mice. Structure–activity relationships are discussed.

4′,4‴-Sulfonylbisacetanilide (acedapsone, DADDS) (I)[2,3] exhibits strong repository antimalarial activity



I

alone, or in combination with cycloguanil pamoate,[4–6] in experimental animals[2,3,7] and in humans.[8] Further, the drug has protracted action against the human leprosy bacillus *Mycobacterium leprae* in mice[9] and in man.[10]

Inasmuch as DADDS is apparently dependent upon deacetylation for activity and affords only extremely low sulfone blood levels, a repository sulfone that is less dependent on enzymatic deacylation for activity and enables higher blood sulfone levels than DADDS might fulfill a useful need. Therefore, efforts were directed toward the design and synthesis of novel sulfone molecules that might undergo slow, nonenzymatic hydrolytic scission directly upon contact with body tissues and fluids. In a recent communication,[1] we reported the synthesis of certain 4′,4‴-[*p*-phenylene bis-(methylidyneimino-*p*-phenylenesulfonyl)]bisanilides that fulfilled the above requirements and displayed marked repository action. Among them, 4′,4‴-[*p*-phenylenebis(methylidyneimino-*p*-phenylenesulfonyl)]-bisformanilide (PSBF) (IIa) was very long acting and protected mice for >9 weeks against challenge with *Plasmodium berghei*.[1] 4′,4‴-[*p*-Phenylenebis(methyli-

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