Demethylation Studies. VI. The Inhibition of Hepatic Microsomal Oxygenation by 2,4-Dichloro(6-phenylphenoxy)ethylamine[1] and Related Compounds

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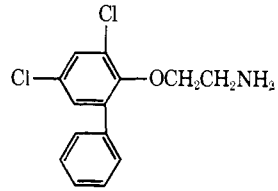
A series of *o*-phenylphenoxyethylamines has been studied as inhibitors of microsomal drug oxidation. Optimum activity resided in primary amines having an *o*-chloro substituent and unbranched ethylene chain. 2,4-Dichloro(6-phenylphenoxy)ethylamine (1, DPEA) was shown to be a particularly effective inhibitor of the dealkylation of a variety of drugs. In the intact rat, **1** inhibited the demethylation of butynamine and imipramine but not of propoxyphene or *p*-bromoanisole. Compound **1** prolonged hexobarbital and ethinamate sleeping time in mice and in dogs. When fed chronically, **1** was a weak stimulant of hepatic microsomal oxygenase.

One of the most important advances in medicinal chemistry has been the discovery of the hepatic oxygenase system responsible for a variety of reactions including N-dealkylation, O-dealkylation, aromatic hydroxylation, and aliphatic hydroxylation.[2] An interesting property of these enzymes is that they are readily inhibited by certain lipid-soluble amines. The earliest inhibitor to be reported as well as the most widely studied was β-diethylaminoethyl diphenylpropylacetate (**7**, Table I).[1,3] Another inhibitor that has been widely studied is 2,4-dichloro-6-phenylphenoxyethyldiethylamine (**13**, Table III).[1,4]

Although both **7** and **13** are tertiary amines, primary amines can be equally as effective as oxygenase inhibitors. Indeed **1**, the primary amine analog of **13**, has been shown to be a particularly effective inhibitor of both *in vitro* and *in vivo* dealkylation.[5] Because of its value as a tool in the investigation of the enzymology of drug metabolism, studies on **1** have now been extended. In this communication the effect of structure on activity as well as further data on effectiveness of **1** are discussed.



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(1) The amine, 2,4-dichloro(6-phenylphenoxy)ethylamine has frequently been designated by the code name DPEA in the literature. It has also been referred to as Lilly 32391 (hydrochloride salt) and 53325 (hydrobromide salt). In this paper it will be referred to as compound **1** (Table I). Similarly, 2,4-dichloro-6-phenylphenoxyethyldiethylamine (**13**) has been referred to as Lilly 18947 while β-diethylaminoethyl diphenylpropylacetate (**7**) is widely known by the code name SKF 525.

(2) (a) B. B. Brodie, J. Axelrod, J. R. Cooper, L. E. Gaudette, B. N. LaDu, C. Mitoma, and S. Udenfriend, *Science*, **121**, 603 (1955); (b) J. R. Gillette, *Advan. Pharmacol.*, **4**, 219 (1966); (c) R. E. McMahon, *J. Pharm. Sci.*, **55**, 457 (1966).

(3) (a) J. Axelrod, J. Reichenthal, and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **112**, 49 (1954); (b) J. R. Cooper, J. Axelrod, and B. B. Brodie, *ibid.*, **112**, 55 (1954); (c) B. N. LaDu, *Proc. Intern. Pharmacol. Meeting, 1st, Stockholm, 1961*, **6**, 229 (1962).

(4) J. R. Fouts and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **115**, 68 (1955).

(5) (a) R. E. McMahon and J. Mills, *J. Med. Pharm. Chem.*, **4**, 211 (1961); (b) R. E. McMahon, *J. Pharmacol. Exp. Ther.*, **138**, 382 (1962).

Experimental Section

Compounds.—The compounds to be studied as inhibitors were prepared by standard organic chemical procedures.[6] The amines were purified as the hydrochloride salts by recrystallization from EtOH–EtOAc. Purity and identity of each compound was established by microanalysis and by standard physical measurements.

The substrates for the *in vitro* studies were for the most part commercially available drugs. Methoxybutamoxane (2-butylaminomethyl-8-methoxy-1,4-benzodioxane) was prepared by the same procedure used for the preparation of ethoxybutamoxane, the ethoxy analog.[7] Ethylmorphine was a gift from Professor G. Mannering of the University of Minnesota.

For the *in vivo* studies ^{14}C-labeled substrates were required. Butynamine-N-methyl-^{14}C,[8] *d*-propoxyphene-N-methyl-^{14}C,[9] and diphenamid-N-methyl-^{14}C,[10] were all prepared by previously described procedures. Imipramine-N-methyl-^{14}C was purchased from New England Nuclear Corp., Boston, Mass.

In Vitro **Studies.**—Livers were quickly removed from 200-g male albino rats that had been sacrificed by decapitation. The livers were then homogenized in 4 vol of 0.1 *M*, pH 7.4 potassium phosphate buffer at 0°. A supernatant fraction containing the microsomes was prepared by centrifugation of the homogenate in a refrigerated centrifuge at 15,000*g*.

Assays for inhibitor activity were carried out as follows. Into each 20-ml beaker was placed 1 ml of 15,000*g* supernatant (equivalent to 200 mg of liver), 200 μmol of potassium phosphate buffer, pH 7.4, 0.5 μmol of TPN^+, 11 μmol of glucose 6-phosphate, 45 μmol of neutralized semicarbazide, 50 μmol of $MgCl_2$, 50 μmol of nicotinamide, 6 μmol of substrate (butynamine, meperidine, or methoxybutamoxane), and sufficient water to yield a final volume of 3 ml. In addition, the inhibitor to be tested was added at five concentrations (range from 3×10^{-6} *M* to 3×10^{-4} *M*). The reaction mixtures were incubated in air with shaking at 37° for 20 min. After addition of 4 ml of 10% $ZnCl_2$ to terminate the reaction, formaldehyde levels were determined by the method of Cochin and Axelrod.[11] From the results a plot of per cent inhibition *vs.* log of inhibitor concentration was constructed. From this curve the concentration of inhibitor required for 50% inhibition was estimated. The data presented in Tables I–IV were obtained in this fashion. In the study reported in Table V the various substrates were present at a concentration of 2×10^{-3} *M* while the inhibitor was present at a concentration of either 10^{-5} or 5×10^{-5} *M*.

Demethylation *in Vivo*.—Rates of *in vivo* dealkylation were determined by following the rate of $^{14}CO_2$ expiration after ad-

(6) J. Mills (to Eli Lilly and Co.), U. S. Patent 3,213,140 (1965).

(7) R. E. McMahon, J. S. Welles, and H. M. Lee, *J. Am. Chem. Soc.*, **82**, 2864 (1960).

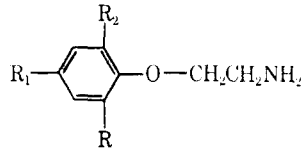
(8) R. E. McMahon and N. R. Easton, *J. Pharmacol. Exp. Ther.*, **135**, 128 (1962).

(9) A. Pohland, H. R. Sullivan, and R. E. McMahon, *J. Am. Chem. Soc.*, **79**, 1442 (1957).

(10) R. E. McMahon and H. R. Sullivan, *Biochem. Pharmacol.*, **14**, 1085 (1965).

(11) J. Cochin and J. Axelrod, *J. Pharmacol. Exp. Ther.*, **125**, 105 (1959).

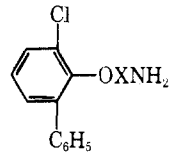
Table I
Influence of Ring Substitution on Inhibitor Activity[a]



No.	R	R_1	R_2	Conen ($\times 10^5$ M) required for 50% inhib of N-demethylation of: Meperidine	Butynamine	Methoxybutamoxane
1	C_6H_5	Cl	Cl	1.7	0.20	2.7
2	C_6H_5	H	Cl	2.5	0.20	6.8
3	C_6H_5	Cl	H	7.5	1.3	75
4	C_6H_5	H	H	3.1	0.17	165
5	$C_6H_5CH_2$	Cl	Cl	2.3	0.28	1.2
6	$C_6H_5CH_2$	H	Cl	1.1	0.24	2.8
7	β-Diethylaminoethyl diphenylpropylacetate			1.5	0.50	2.2

[a] Experimental methods used are described in the Experimental Section. All substrates were present at an initial concentration of 2×10^{-3} M.

Table II
Influence of Side Chain Variations on Inhibitor Activity[a]



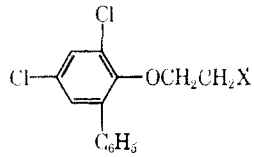
No.	X	Conen ($\times 10^5$ M) required for 50% inhib of demethylation of: Meperidine	Butynamine	Methoxybutamoxane
2	$-CH_2CH_2-$	2.5	0.2	6.8
8	$-CH_2CH_2CH_2-$	5	0.2	11
9	$-CH(CH_3)CH_2-$	4.9	0.1	22
10	$CH_2CH(CH_3)-$	40	0.34	110
11	$-CH_2CO-$	100	15	>100

[a] Experimental methods used are described in the Experimental Section.

ministration of an NCH_3-labeled drug to the experimental animal. The apparatus employed for these measurements was similar to that described by Tolbert and coworkers.[12] We are indebted to Professor G. Okita, Northwestern University, for invaluable advice in assembling this equipment. An all-glass chamber (500 ml) was used as an animal holder. Air was supplied to the animal holder at a rate of 500 ml/min. After leaving this chamber the air passed through a drying tower and then into a 500-ml ionization chamber which was attached to a Model 31 Cary electrometer. Finally the air passed through a CO_2 detector (Mine Safety Model 300, full scale setting 0-2% CO_2). The resulting data were recorded continuously on a modified Leeds and Northrup multipoint strip chart recorder. Three items were recorded, total CO_2 (respiratory rate), total ^{14}C, and specific activity. The total amount of $^{14}CO_2$ expired was readily obtained by integration of the area under the ^{14}C curve. While the CO_2 and ^{14}C curves are frequently irregular due to changes of respiratory rate the specific activity was usually a smooth curve. The labeled substrates chosen for this study produced peak specific activities about 15 min following intraperitoneal injection. The peak specific activity attained reflects the amount of enzyme present. For that reason the decrease in specific activity has been cited as the measure of *in vivo* inhibition (Table VI).

(12) B. M. Tolbert, M. Kirk, and E. Baker, *Am. J. Physiol.*, **185**, 269 (1956).

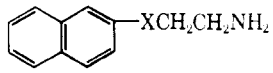
Table III
Influence of N Substitution on Inhibitor Activity[a]



No.	X	Conen ($\times 10^5$ M) required for 50% inhib of N-demethylation of: Meperidine	Butynamine	Methoxybutamoxane
1	NH_2	1.7	0.40	2.7
12	NHC_2H_5	16	2.0	16
13	$N(C_2H_5)_2$	45	7	45
14		...	2.7	24
15		...	0.95	13

[a] Experimental methods used are described in the Experimental Section.

Table IV
Influence of Heteroatom on Inhibition Activity[a]



No.	X	Conen ($\times 10^5$ M) required for 50% inhib of N-demethylation of: Butynamine	Meperidine
16	O	0.3	4.0
17	S	0.1	1.6
18	NH	15	70
19	CH_2	0.4	4.0

[a] Experimental methods used are described in the Experimental Section.

All of the *in vivo* studies were carried out in 150-g male albino rats. Animals were first predosed with inhibitor by the intraperitoneal route. This was followed at a predetermined time by the intraperitoneal administration of the radioactive substrate. An optimum ^{14}C dose for this instrument was found to be 1.5–4.0 μCi/150-g rat. Control rats received saline prior to administration of labeled substrate.

Prolongation Studies.—Albino male (Cox) mice were used for these studies. Inhibitor was administered by the subcutaneous route. After 30 min a dose of sedative (hexobarbital or ethinamate) was given by the intraperitoneal route. Sleeping time was measured from the time of sedative administration until the animals regained their righting reflex. Ten mice were used in each experimental group and the results are given in Table VII. The experiments with dogs were carried out in much the same way. Again, ten animals per group were used.

2,4-Dichloro(6-phenylphenoxy)ethylamine (1) as a Stimulatory Agent.—For these experiments, 100-g male albino rats were employed. During the course of these experiments the rats were supplied with standard diet. All drugs were administered by the intraperitoneal route. The control groups received saline once daily, each **1** group received 22 mg/kg of **1** daily and the phenobarbital groups received 40 mg/kg daily. Each group contained six rats. Twenty-four hours following the last injection of stimulatory agent, the rate of demethylation *in vivo* was assayed by radiorespirometry as described above. One control group, one compound 1 group (6-day treatment), and one phenobarbital group were tested with butynamine-N-methyl-^{14}C (7 mg/kg, 1.5 μCi) as substrate. In a second experiment a control group, a compound 1 group (12-day treatment), and a phenobarbital group were tested with diphenamid-N-methyl-^{14}C (50 mg/kg) as substrate.

The day following the *in vivo* test each rat was sacrificed, the liver was removed, and 15,000*g* supernatant fraction was prepared as described above. From this a 100,000*g* microsomal pellet was prepared. The ability of this fraction to demethylate either butynamine or propoxyphene was assayed in the usual

TABLE V
INHIBITION OF THE MICROSOMAL DEALKYLATION OF VARIOUS SUBSTRATES

Substrate	Reaction	Rate, mμmol/g min	% inhibition by Compound 1 10^{-5} M	% inhibition by Compound 1 5×10^{-5} M	% inhibition by Compound 7 10^{-5} M	% inhibition by Compound 7 5×10^{-5} M
Propoxyphene	N-Demethylation	177	13	44	8	10
Butynamine	N-Demethylation	122	81	96	84	93
β-(*p*-Chlorophenyl)dimethylpropylamine	N-Demethylation	68	70	91	72	81
Aminopyrine	N-Demethylation	137	33	63	48	64
Ethylmorphine	N-Demethylation	338	7	66	35	59
Imipramine	N-Demethylation	102	35	62	8	18
Amitriptyline	N-Demethylation	118	13	37	0	0
Acetylmethadol	N-Demethylation	34	35	66	7	17
Diamylmethylamine	N-Demethylation	98	41	87	84	94
N,N-Dimethyldiphenylethylamine	N-Demethylation	314	41	79	54	74
1-Methoxynaphthalene	O-Demethylation	15	11	49	34	62
Anisonitrile	O-Demethylation	22	44	72	35	56
Methoxybutamoxane	O-Demethylation	100	16	80	36	80

manner (see above). Each liver was done separately and the results were expressed as nanomoles metabolized per milligram of microsomal protein during the 15-min incubation. Both the *in vivo* and the *in vitro* results are summarized in Table VIII. Each result is the average of results from six rats.

Results and Discussion

In the structure–activity studies three test substrates were used. They were butynamine, a hypotensive amine, meperidine, and 2-butylaminomethyl-8-methoxy-**1**,4-benzodioxane (methoxybutamoxane). The N-demethylation of butynamine[5] was chosen as an example of a readily inhibited reaction while the N-demethylation of meperidine represents a case in which inhibition is more difficult. Methoxybutamoxane, which like its ethoxy analog[7] is metabolized by O-dealkylation, was chosen as a typical O-demethylation substrate.

The first parameter to be investigated was the influence of ring substitution. The results are shown in Table I. The most obvious result is that the *o*-chloro substituent is necessary. Compounds **3** and **4**, which are without the *o*-chloro group, are essentially without activity. The *p*-chloro substituent appears to have a small positive effect in the *o*-phenyl series but is without effect in the *o*-benzyl series. It is clear that the *o*-phenyl group in **1** can be replaced by *o*-benzyl (**5**) without loss of activity.

The effect of alterations in the nature of the carbon bridge connecting oxygen and nitrogen was next investigated (Table II). Lengthening the ethylene chain by one more C resulted in some loss of activity as did branching on the chain. In this regard the negative effect of α branching (**10**) was substantially greater than with β branching (**9**). The corresponding amide (**11**) was without activity.

Table III summarized the data on the effect of substitution on N. The diethyl analog of **1** (**13**) was substantially less active than the primary amine. Substitution of one ethyl group (**12**) had less effect. The basis for the effect of N substitution is unknown. However, steric factors may be involved, since replacement of the diethylamino group (**13**) by the less hindered piperidino (**14**) or morpholino groups (**15**) had a smaller negative effect.

In view of difficulties in the preparation of analogs of **1** in which the oxygen atom had been replaced by other atoms, this question was investigated with the less active β-naphthyloxyethylamine series. The results (Table IV) show that the substituion of S for O leads to a substantial increase in activity. Replacement by CH_2 had no effect while replacement by N essentially destroyed the activity.

Based on these studies, **1**, was chosen for further studies. In Table V its *in vitro* activity against a variety of substrates compared with that of **7** is presented. The degree of inhibition produced by **1** and by **7** against various substrates proved to be very similar with the notable exception of imipramine and acetylmethadol, both of which were much more readily inhibited by **1** than by **7**. This similarity of response of **1** and **7** to various substrates suggests that they act by the same mechanism. However, Schenkman, *et al.*,[13] have studied the spectral changes resulting from interaction of these inhibitors with hepatic microsomal cytochrome and found that **7** gives a type I interaction while **1** gives a type II change. Thus there appears to be a difference in the way these two compounds interact with microsomes (*cf.* also ref 2b).

The inhibitory activity of **1** in *in vivo* systems was next investigated. In earlier studies **1** was shown to be effective as an inhibitor of the *in vivo* demethylation

TABLE VI
INFLUENCE OF PREDOSING TIME ON THE INHIBITION OF *in Vivo* BUTYNAMINE DEMETHYLATION[a] BY COMPOUND **1**

Time of predosing, min	Rate of demethylation of butynamine-N-methyl-C^{14}: Individual values (μCi/mol of CO_2)[b]	Rate of demethylation of butynamine-N-methyl-C^{14}: Mean (μCi/mol CO_2)
Control (no inhibitor)	42.0, 62.5, 72.5	59.0
10	7.4, 9.5, 12.7	9.9
100	8.5, 12.7, 16.5	12.6
250	10.6, 12.1, 15.9	12.9
500	5.3, 8.8, 9.9	8.0
1000	16.5, 41.0, 73.0	43.5

[a] Rats were predosed with 15.9 mg/kg (50 μmol/kg) of **1** by the intraperitoneal route. At the specified postdose times butynamine-N-methyl-^{14}C was administered intraperitoneally at a dose of 14 mg/kg (75 μmol/kg). The radiocarbon dose was 1.36 μCi/150-g rat. [b] The specific activity of respired $^{14}CO_2$ at 15 min after administration of radiobutynamine.

(13) J. B. Schenkman, H. Remmer, and R. W. Estabrook, *Mol. Pharmacol.*, **3**, 113 (1967).

Table VII
Prolongation of Hexobarbital and Ethinamate Sleeping Time[a]

Species	Inhibitor	Dose, mg/kg sc	Sedative	Dose, mg/kg ip	Geometric mean sleeping time ± SE	% prolongation
Mice	None	...	Hexobarbital	100	40.6 ± 7.2	100
Mice	**1**	2	Hexobarbital	100	113 ± 12.2	278
Mice	**2**	2	Hexobarbital	100	104 ± 9.6	256
Mice	**2**	5	Hexobarbital	100	133 ± 19.1	328
Mice	**2**	10	Hexobarbital	100	127 ± 13.1	313
Mice	**3**	2	Hexobarbital	100	50.5 ± 7.3	124
Mice	**4**	2	Hexobarbital	100	71.4 ± 15.7	175
Mice	None	...	Ethinamate	200	46.3 ± 5.2	100
Mice	**1**	0.2	Ethinamate	200	133 ± 22.0	288
Mice	**1**	1	Ethinamate	200	127.5 ± 23.8	275
Dog	None	...	Ethinamate	65 iv	51.0 ± 7.4	100
Dog	1	2	Ethinamate	65 iv	79.6 ± 13.4	156
Dog	None	...	Hexobarbital	40 iv	44.8 ± 6.4	100
Dog	**1**	1	Hexobarbital	40 iv	64.7 ± 11.1	145

[a] See Experimental Section for a description of procedures used.

Table VIII
Stimulation of Demethylation by Chronically Administered 2,4-Dichloro(6-phenylphenoxy)ethylamine (**1**)

Stimulatory agent	Dose, mg/kg/day	Days	Rate[a] (μCi/mol of CO_2) of *in vivo* demethylation of Diphenamid	Rate[a] (μCi/mol of CO_2) of *in vivo* demethylation of Butynamine	Rate (nmol/mg/15 min) of *in vitro* demethylation of Butynamine	Rate (nmol/mg/15 min) of *in vitro* demethylation of Propoxyphene
Control	...	...	6.0	65	0.15	1.5
Compound **1**	22	6		168	1.2	
Compound **1**	22	12	7.3			3.8
Phenobarbital	40	5	50	287	8.3	7.9

[a] Specific activity of respired $^{14}CO_2$ at 15 min after administration of substrate.

of the hypotensive amine, butynamine, in rats at doses as low as 2 mg/kg.[5] In the present study a higher dose (15.9 mg/kg, 50 μmol/kg) was chosen in order to estimate the duration of action of **1** in rats. The results are shown in Table VI. Under these conditions **1** produced a dramatic reduction in the rate of demethylation of butynamine that persisted for at least 500 min. At 1000 min demethylation rates appeared to have returned to normal.

Inhibition of the *in vivo* demethylation of other substrates by **1** was also investigated. Earlier it was reported[14] that **1** reduced both the rate and extent of *in vivo* demethylation of N,N-dimethyl-3,5,7-trimethyladamantane-**1**-carboxamide. In the present study it was found that **1** (15.9 mg/kg) did not inhibit the demethylation of either propoxyphene-N-methyl-C^{14} or *p*-bromoanisole-O-methyl-C^{14}. Compound **1** did, however, inhibit the demethylation of imipramine in the rat. A dose of 10 mg/kg (1.67 μCi/rat) of imipramine-N-methyl-C^{14} gave an average peak specific activity of 34.3 μCi/mol of CO_2 (47.4–23.2) for three rats. Predosing with **1** (15.9 mg/kg) led to an average specific activity of 17.7 μCi/mol of CO_2 (25.4–5.3), a 50% reduction in demethylation rate. This result is of interest since it is thought that the antidepressant activity is mediated, at least in part by *in vivo* conversion to desmethylimipramine.[15]

In a second series of experiments the effect of **1** on hexobarbital sleeping time was investigated (Table VII). It would be expected that if **1** effectively inhibited the metabolic transformation of hexobarbital it would result in a prolongation of the sedative action of the barbiturate. For this reason prolongation of hexobarbital sleeping time is frequently used to screen for new inhibitors of microsomal oxygenation. In mice, **1** prolonged hexobarbital sleeping time 278% at a dose of 2 mg/kg. The two monochloro analogs of **1** were also tested. Compound **2** (2-chloro) was as effective as **1** while **3** (4-chloro) was essentially inactive, thus confirming the *in vitro* results (Table I). Compound **4** with no chloro substituents showed some activity in this test. It is interesting to note that increased doses of **2** led to only small increases in sleeping time.

Compound **1** also prolonged the sedative action of ethinamate, a compound also metabolized by hydroxylation.[16] In this case excellent activity was seen at 0.2 mg/kg, a dose at which **1** is ineffective as an inhibitor of *in vivo* demethylation. Finally, **1** prolongs both ethinamate and hexobarbital in the dog. Its activity in this species is not so great as that seen in mice, however (Table VII).

The prolongation studies thus suggest that **1** is an effective *in vivo* inhibitor of the hydroxylation of both hexobarbital and of ethinamate. In this regard Mitoma, *et al.*,[17] have recently reported **1** to be an inhibitor of *in vitro* hexobarbital metabolism.

(14) H. R. Sullivan, R. E. Billings, and R. E. McMahon, *J. Med. Chem.*, **11**, 250 (1968).

(15) (a) F. Sulser, J. Watts, and B. B. Brodie, *Ann. N. Y. Acad. Sci.*, **96**, 279 (1962); (b) J. R. Gillette, J. V. Dingell, F. Sulser, R. Kuntzman, and B. B. Brodie, *Experientia*, **17**, 417 (1961).

(16) (a) R. E. McMahon, *J. Am. Chem. Soc.*, **80**, 411 (1958); (b) R. E. McMahon, *J. Org. Chem.*, **24**, 1834 (1959).

(17) C. Mitoma, D. Yasuda, J. S. Tagg, S. E. Neubauer, F. J. Calderoni, and M. Tanabe, *Biochem. Pharmacol.*, **17**, 1377 (1968).

Early studies by Kato, Chiesara, and Vassanelli,[18] have suggested that while **1** produces a profound inhibitory effect acutely it may stimulate microsomal hydroxylation when given chronically. This possibility was further investigated in the present study. It was found that rats receiving **1** at a level of 22 mg/kg/day for 6 days demethylated butynamine about 2.5 times faster than normal (Table VIII). Microsomes from these rats also metabolized butynamine faster than control in the *in vitro* system. In a second experiment in which rats received 22 mg/kg/day for 12 days no increase in the *in vivo* demethylation of diphenamid[10] occurred although an increase in the rate of the *in vitro* demethylation of propoxyphene was observed. Phenobarbital, a widely used agent for stimulation of the microsomal system was very effective in increasing both the *in vivo* rate of demethylation of diphenamid and butynamide as well as the *in vitro* dealkylation of propoxyphene and butynamine. The results of these studies suggest that while **1** can stimulate microsomal oxygenases when given chronically its activity is only marginal.

(18) R. Kato, E. Chiesara, and P. Vassanelli, *Med. Exp.*, **6**, 254 (1962).

Irreversible Enzyme Inhibitors. CXLVI.[1,2] Active-Site-Directed Irreversible Inhibitors of Xanthine Oxidase[3] Derived from 9-(Acylamidophenyl)guanines Substituted with a Terminal Sulfonyl Fluoride

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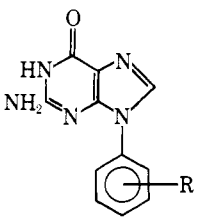
Twenty candidate active-site-directed irreversible inhibitors of xanthine oxidase have been synthesized and evaluated. These were derived from *m*- or *p*-amino-9-phenylguanine attached to benzenesulfonyl fluoride by a sulfonamide, carboxamide, or urea bridge. Three excellent irreversible inhibitors emerged that at a concentration of 10^{-6} to 10^{-7} *M* gave total inactivation of xanthine oxidase with a half-life of 1 min or less; these were 9-phenylguanines substituted by a *p*-(*p*-fluorosulfonylbenzamido) (**2**), *p*-(*m*-fluorosulfonylbenzamido) (**7**), or *m*-(*p*-fluorosulfonylbenzenesulfonamido) (**21**) moiety.

9-Phenylguanine (**1**) is a good reversible inhibitor of



1, R = H
2, R = *p*-$NHCOC_6H_4SO_2F$-*p*
3, R = *m*-$NHCOC_6H_4SO_2F$-*p*
4, R = *m*-$NHCOC_6H_4SO_2F$-*m*
5, R = *m*-$NHCONHC_6H_4SO_2F$-*m*
6, R = *p*-$O(CH_2)_nNH{\sim}SO_2F$-*m*

xanthine oxidase[4,5] with the 9-phenyl group interacting with the enzyme by hydrophobic bonding.[6] A study of the nature and dimensions of this hydrophobic bonding region on the enzyme was performed to determine where the hydrophobic bonding region ended. Then a leaving group could be properly positioned on the inhibitor to form a covalent bond in a polar region of the enzyme surface.[7] As a result of this study, **2–5** were designed as irreversible inhibitors of xanthine oxidase; **2** was found to be a rapid irreversible inhibitor, but three related compounds (**3–5**) were not.[8] Studies of compounds related to **2–5** have now been extended and are the subject of this paper; sulfonyl fluorides bridged with an ether group (**6**) are the subject of the paper that follows.[9]

Enzyme Results.—An additional 20 analogs of **1** have been synthesized and evaluated as summarized in Table I. The three best of the 25 irreversible inhibitors were the parent **2** and its analogs **7** and **21**, all of which gave essentially total inactivation of xanthine oxidase when incubated at an I_{50} concentration of compound.[10] All three at an I_{50} concentration showed a half-life of inactivation of **1** min or less. Since **2**, **7**, and **21** differed less than threefold as reversible inhibitors, no one could be considered the best.

It can be calculated that the xanthine oxidase concentration in the incubation is about 0.08 μM when assayed by the uric acid method[10] and 0.02 μM when assayed by the indophenol method; this calculation is based on an OD change of 0.01/min when the incubation mixture is diluted tenfold, on the molecular weight

(1) This work was generously supported by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper in this series see B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **12**, 118 (1969).

(3) For the previous paper on this enzyme see B. R. Baker, W. F. Wood, and J. A Kozma, *ibid.*, **11**, 661 (1968), paper CXXVI of the series.

(4) B. R. Baker, *J. Pharm. Sci.*, **56**, 959 (1967), paper XCIII of this series.

(5) See B. R. Baker and J. L. Hendrickson, *ibid.*, **56**, 955 (1967), for the possible chemotherapeutic utility of tissue-selective irreversible inhibitors of xanthine oxidase; paper XCII of this series.

(6) B. R. Baker and W. F. Wood, *J. Med. Chem.*, **10**, 1101 (1967), paper CII of this series.

(7) B. R. Baker and W. F. Wood, *ibid.*, **11**, 644 (1968), paper CXXII of this series.

(8) B. R. Baker and W. F. Wood, *ibid.*, **11**, 650 (1968), paper CXXIII of this series.

(9) B. R. Baker and W. F. Wood, *ibid.*, **12**, 214 (1969), paper CXLVII of this series.

(10) B. R. Baker and J. A. Kozma, *ibid.*, **10**, 682 (1967), paper XCV of this series.