21. Reductive formylation of the azo linkage followed by ring closure in formamide–formic acid[3] gave the substituted 9-phenylguanine (**22**). Acid hydrolysis removed the acetyl blocking group with generation of **23**.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample showed proper uv and ir spectra, moved as a single spot on tlc on Brinkmann silica gel GF, and gave combustion analysis for C, H, and N or F within 0.4% of theory. The physical properties of **5**–**16** are listed in Table II.

Acetamidoethyl *p*-Nitrophenyl Ether (19a). **A.**—To a solution of 4.92 g (20 mmoles) of **17a**[11] in 40 ml of $CHCl_3$ was added 3.1 g (22 mmoles) of hexamethylenetetramine. The mixture was refluxed with stirring for 100 hr, during when the intermediate heximonium bromide separated. The latter was collected on a filter, washed ($CHCl_3$), dried, and added to a mixture of 8 ml of H_2O, 40 ml of EtOH, and 10 ml of 12 *N* HCl. After 24 hr the mixture was filtered from the separated NH_4Cl, then evaporated *in vacuo*. The residual, crude **18a**·HCl was dissolved in 50 ml of $CHCl_3$ and 8.1 g of Et_3N. To the hot solution was added 4.1 g of Ac_2O over a period of about 10 min. The cooled solution was washed with three 100-ml portions of H_2O, then dried ($MgSO_4$), and evaporated *in vacuo*. Recrystallization from C_6H_6 gave 2.0 g (45%) of crystals, mp 121–122°. *Anal.* ($C_{16}H_{12}N_2O_4$) C, H, N.

B.—To a suspension of 10 g (33 mmoles) of *p*-nitrophenyl phthalimidoethyl ether[12] in 100 ml of refluxing EtOH was added 5.77 g (0.1 mole) of 85% hydrazine hydrate. Solution was complete in 2 min and a precipitate separated in 15 min. After a total of 20 min, the hot mixture was treated with 200 ml of 3 *N* HCl. After 10 min, the mixture was cooled and the phthalhydrazide was removed by filtration. The filtrate was evaporated *in vacuo* and the residual **18a**·HCl + N_2H_4·HCl was acetylated as above. Recrystallization from C_6H_6 gave 4.0 g (53%) of crystals, mp 120–121°, that were identical with preparation A.

Acetamidopropyl *p*-nitrophenyl ether (19b) was prepared from **17b**[11] and hexamethylenetetramine as described for **17a**; yield 46%, mp 117–118°. *Anal.* ($C_{11}H_{14}N_2O_4$) C, H, N.

***p*-Aminophenyl Acetamidoethyl Ether (20a).**—A solution of 1.12 g (5 mmoles) of **19a** in 100 ml of EtOH was shaken with H_2 at 2–3 atm in the presence of 50 mg of PtO_2 until reduction of the NO_2 group was complete. The filtered solution was evaporated *in vacuo*. Recrystallization of the residue from toluene gave 0.85 g (87%) of white crystals, mp 93–94°. *Anal.* ($C_{10}H_{14}N_2O_2$) C, H, N.

Similarly, **20b** was prepared in 79% yield, mp 79–80°. *Anal.* ($C_{11}H_{16}N_2O_2$) C, H, N.

9-(*p*-Aminoethoxyphenyl)guanine (23a) was prepared from **20a** *via* **21a** as described for 9-(*p*-aminophenyl)guanine,[3] except the free base was released at pH 10 with NH_4OH; yield 22%; mp >300°; λ_{max} (pH 1) 270 (infl), (pH 13) 270 mμ. *Anal.* ($C_{13}H_{14}N_6O_2$) C, H, N.

Similarly, **23b** was prepared in 40% yield as white crystals that were uniform on tlc in 3:5 EtOH–$CHCl_3$, but gave erratic combustion values.

(11) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 26 (1968), paper CIX of this series.

(12) J. N. Ashley, R. F. Collins, M. Davis, and N. E. Sirett, *J. Chem. Soc.*, 3880 (1959).

Irreversible Enzyme Inhibitors. CXLVIII.[1,2] Active-Site-Directed Irreversible Inhibitors of Guanine Deaminase[3] Derived from 9-Phenylguanine Bearing a Terminal Sulfonyl Fluoride

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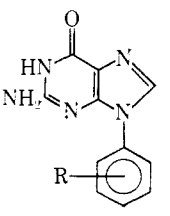
Thirty-eight candidate irreversible inhibitors of guanine deaminase derived from 9-phenylguanine with a terminal sulfonyl fluoride bridged to the *meta* or *para* position of the phenyl moiety by an amide or ether linkage were evaluated with the enzyme from Walker 256 rat tumor. Three of the compounds (**6**, **7**, **9**) were excellent irreversible inhibitors of this enzyme, but also showed no isozyme specificity since these could also inactivate the rat liver enzyme. Of the 13 compounds showing moderate irreversible inhibition of the Walker 256 enzyme, four (**5**, **28**, **30**, **31**) showed isozyme specificity with no inactivation of the rat liver enzyme.

The design, synthesis, and evaluation of active-site-directed irreversible inhibitors[4] of guanine deaminase[5] has been a project in this laboratory. 9-Phenylguanine (**1**) was found to be a good reversible inhibitor of the enzyme, being complexed slightly better than the substrate.[6] It was then established that the 28-fold increment in binding by the phenyl group of **1** was due to a hydrocarbon interaction with the enzyme.[7] From a study of the nature and dimensions of this hydrophobic bonding region,[8] compounds of types **2** and **3** were considered likely candidates as irreversible inhibitors of guanine deaminase that operate by the



1, R = H
2, R = *m*-NHCORSO$_2$F
3, R = *p*-O(CH$_2$)$_n$NHCORSO$_2$F

(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 W. F. Wood, *J. Med. Chem.*, **12**, 214 (1969).

(3) For the previous paper on this enzyme see B. R. Baker and W. F. Wood, *ibid.*, **11**, 650 (1968), paper CXXIII of this series.

(4) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Enzymic Active-Site," John Wiley and Sons, Inc., New York, N. Y., 1967.

(5) For the chemotherapeutic utility of a tumor-specific inhibitor of guanine deaminase see B. R. Baker, *J. Med. Chem.*, **10**, 59 (1967), paper LXXIII of this series.

(6) B. R. Baker and D. V. Santi, *ibid.*, **10**, 62 (1967), paper LXXIV of this series.

(7) B. R. Baker and W. F. Wood, *ibid.*, **10**, 1101 (1967), paper CII of this series.

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

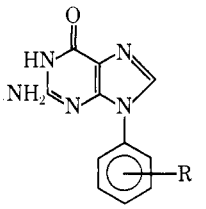
active-site-directed mechanism;[4] the results with these two classes of candidate irreversible inhibitors are the subject of this paper.

Enzyme Results.—In addition to the use of a commercial guanine deaminase from rabbit liver, the enzyme from Walker 256 rat tumor and rat liver have now been employed. Reversible binding to the enzyme from rabbit liver and Walker 256 is similar as shown in Table I; little more than a twofold difference was observed. Thirty-eight candidate irreversible inhibitors of types **2** (Table II) and **3** (Table III) were synthesized and evaluated as irreversible inhibitors with the enzyme from the three sources. The last compound synthesized (**4**) showed irreversible inhibition of the rabbit liver enzyme. Since **4** had an I_{50} = 2.5 μM, it could be assayed at 12 μM ($5I_{50}$) by the standard inactivation assay where the amount of remaining enzyme was assayed by the rate of conversion of guanine to xanthine.[9] However, no irreversible inhibition of the rabbit liver enzyme was shown by **4** at an I_{50} = 2.5 μM concentration. This lack of irreversible inhibition by an I_{50} concentration of some inhibitors of trypsin,[10] chymotrypsin,[11] and dihydrofolic reductase[12] has been traced to the high potency of reversible inhibition by the compound, which limited the concentration of the inhibitor to a value below that of the enzyme in the incubation. This was resolved in previous cases[10–12] by using a more sensitive assay in order to reduce the enzyme concentration below that of the inhibitor in the incubation. The results with **4** in the inactivation experiments indicated that the guanine deaminase concentration was on the order of 1 μM; thus, many of the potent reversible compounds in Tables II and III when assayed at $5I_{50}$ would be present in less concentration than the enzyme. Therefore an assay more sensitive for guanine deaminase was devised; futhermore, all the inactivations were performed with 12 μM inhibitor by removing excess inhibitor by octanol extraction prior to assay of the remaining enzyme. With this new assay system, a number of the candidate irreversible inhibitors were then found to be effective.

One of the most effective irreversible inhibitors at 12 μM was the sulfonyl fluoride (**6**) derived from 9-(*m*-benzamidophenyl)guanine; it was studied in considerable detail to establish the methodology. With its I_{50} = 0.035 μM, **6** was an excellent reversible inhibitor, being 72-fold better than the sulfonamide **4**; therefore, when **6** was assayed at $5I_{50}$ = 0.17 μM as an irreversible inhibitor of the Walker 256 enzyme by the earlier assay,[9] no irreversible inhibition was seen for the reasons discussed above. In contrast, 12 μM **6** showed 100% inactivation of guanine deaminase when assayed by coupling with xanthine oxidase. Furthermore, **6** was fairly effective at 1 μM and still showed detectable irreversible inhibition at 0.25 μM, but not 0.07 μM; thus the upper limit of the incubation concentration of enzyme can be estimated to be on the order of 0.5 μM.

TABLE I

REVERSIBLE INHIBITION[a] OF GUANINE DEAMINASE FROM RABBIT LIVER AND WALKER 256 RAT TUMOR BY



No.	R	μM conen for 50% inhib—Rabbit liver[b]	μM conen for 50% inhib—Walker 256
1	H	10	8.0
	p-CH_3	3.5	8.2
	m-CH_3	2.0	2.6
	p-Cl	3.8	2.1
	p-CF_3	4.3	8.0
	p-OCH_3	0.20	0.35
	p-OEt	0.098	0.19
	p-Et	1.3	2.3
	p-NH_2	25	22
	m-NH_2	5.9	9.0
	p-C_6H_5	0.36	0.54
	3,4-Benzo	0.22	0.28
	p-COOH	>200	130
	p-$CONH_2$	13	23

[a] Assayed with 13.3 μM guanine in pH 7.4 Tris buffer containing 10% DMSO as previously described.[5] [b] Data from ref 8.

Removal of the 2-Cl atom of **6** to give the parent **7** resulted in a threefold less effective reversible inhibitor and a somewhat less effective irreversible inhibitor; insertion of a 4-Me (**8**) increased reversible, but decreased irreversible, effectiveness. When the *m*-SO_2F moiety of **7** was moved to the *para* position, the resultant **9** was still as effective as an irreversible inhibitor of the Walker 256 enzyme.

The *p*-benzamides (**10**, **11**) were not only poor reversible inhibitors, but showed no irreversible inhibition. In contrast, the *p*-sulfonamide (**13**) showed irreversible inhibition of Walker 256 guanine deaminase, but **12** did not. The *m*-urea-bridged inhibitors (**14–19**) varied from poor to ineffective irreversible inhibitors of the Walker 256 enzyme.

Insertion of one (**20**), two (**21**), or four (**22**) methylenes between the amide and phenyl moieties of **9**, **20–22** were less effective than **9** both reversibly and irreversibly. In contrast, insertion of an oxymethylene moiety (**23**) in **9** led to loss of irreversible inhibition, even though the bridge distance between the amide and SO_2F moieties was the same as in **21**. Such a loss of irreversible inhibition by replacement of a methylene group by an oxy has been previously observed with inhibitors of dihydrofolic reductase and conformational reasons for this difference have been discussed.[13]

Insertion of a *p*-OCH_3 group (**24**) on **9** caused a large loss in reversible binding and complete loss of irreversible inhibition; similar results were observed with **25–27**.

The candidate irreversible inhibitors derived from

(9) B. R. Baker and W. F. Wood, *J. Med. Chem.*, **10**, 1109 (1967), paper CIII of this series.

(10) B. R. Baker and E. H. Erickson, *ibid.*, **12**, 112 (1969), paper CXLIV of this series.

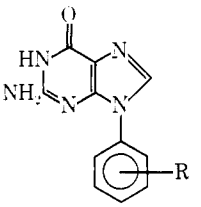
(11) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 118 (1969), paper CXLV of this series.

(12) B. R. Baker and R. B. Meyer, Jr., *ibid.*, **12**, 108 (1969), paper CXLIII of this series.

(13) (a) B. R. Baker and G. J. Lourens, *ibid.*, **11**, 666 (1968), paper CXXVII of this series; (b) B. R. Baker and G. J. Lourens, *ibid.*, **12**, 92 (1969), paper CXXXIX of this series.

Table II

Irreversible Inhibition of Guanine Deaminase by



No.	R	Source	I_{50},[a] μM	Inhib., μM	Time, min	% inactvn.[b]
4	*m*-NHSO$_2$C$_6$H$_4$SO$_2$F-*p*	Rabbit liver		12	60	62[c]
				2.5	60	0[c]
		W256	2.6	12	60	35[c]
				12	60	20
		Rat liver		12	60	22
5	*m*-NHSO$_2$C$_6$H$_4$SO$_2$F-*m*	W256	0.34	12	60	24
		Rat liver		12	60	0
6	*m*-NHCOC$_6$H$_3$-2-Cl-5-SO$_2$F	W256	0.035	12	60	100
				1	60	65
				0.25	60	15
				0.07	60	0
		Rat liver		12	60	80
				1	60	34
		Rabbit liver		12	60	100
7[d]	*m*-NHCOC$_6$H$_4$SO$_2$F-*m*	W256	0.11	12	60	93
				1	60	30
		Rat liver		12	60	75
		Rabbit liver	0.12[e]	12	60	36
8[d]	*m*-NHCOC$_6$H$_3$-4-Me-3-SO$_2$F	W256	0.038	12	60	55
				3	60	27
		Rat liver		12	60	73
		Rabbit liver		12	60	22
9[d]	*m*-NHCOC$_6$H$_4$SO$_2$F-*p*	W256	0.064	12	60	94
				1	60	43
		Rat liver		12	60	91
				1	60	35
		Rabbit liver	0.092[e]	12	60	86
10	*p*-NHCOC$_6$H$_4$SO$_2$F-*p*	W256		18	120	0[f]
		Rabbit liver	18[e]	18	120	0[f,e]
11	*p*-NHCOC$_6$H$_4$SO$_2$F-*m*	W256	150	40[f]	60	0[f]
		Rabbit liver	70	40[f]	60	0[f]
12	*p*-NHSO$_2$C$_6$H$_4$SO$_2$F-*p*	W256	43	55	60	0
		Rabbit liver	55	55	60	53
13	*p*-NHSO$_2$C$_6$H$_4$SO$_2$F-*m*	W256	92	20	60	38
		Rat liver		20	60	35
		Rabbit liver	20	20	60	0[f]
14	*m*-NHCONHC$_6$H$_4$SO$_2$F-*m*	W256	0.12	12	60	45
		Rac liver		12	60	74
		Rabbit liver	0.10[e]	12	60	0
15	*m*-NHCONHC$_6$H$_3$-2-Cl-5-SO$_2$F	W256	0.35	12	60	31
		Rat liver		12	60	80
		Rabbit liver		12	60	100
16	*m*-NHCONHC$_6$H$_3$-4-Me-3-SO$_2$F	W256	0.072	12	60	11
17	*m*-NHCONHC$_6$H$_3$-2-OMe-5-SO$_2$F	W256	0.19	12	60	11
18	*m*-NHCONHC$_6$H$_4$SO$_2$F-*p*	W256	0.034	12	60	13
19	*m*-NHCONHC$_6$H$_3$-3-Cl-4-SO$_2$F	W256	0.053	12	60	0
20	*m*-NHCOCH$_2$C$_6$H$_4$SO$_2$F-*p*	W256	0.16	12	60	54
		Rac liver		12	60	36
21	*m*-NHCO(CH$_2$)$_2$C$_6$H$_4$SO$_2$F-*p*	W256	0.21	12	60	68
		Rat liver		12	60	26
22	*m*-NHCO(CH$_2$)$_4$C$_6$H$_4$SO$_2$F-*p*	W256	0.22	12	60	62
		Rat liver		12	60	33
		Rabbit liver	0.15	12	60	11
23	*m*-NHCOCH$_2$OC$_6$H$_4$SO$_2$F-*p*	W256	0.25	12	60	0
24	4-OMe-3-NHCOC$_6$H$_4$SO$_2$F-*p*	W256	17	25	60	0[f]
25	4-OMe-3-NHCOC$_6$H$_4$SO$_2$F-*m*	W256	12	25	60	0[f]
26	4-OMe-3-NHCONHC$_6$H$_4$SO$_2$F-*p*	W256	13	25	60	0[f]
27	4-OMe-3-NHCONHC$_6$H$_4$SO$_2$F-*m*	W256	15	25	60	0[f]
28[g]	*m*-NHCOCH$_2$Br	W256	0.20	12	60	30
		Rat liver		12	60	0

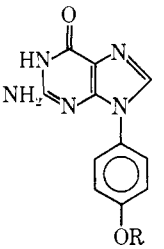
TABLE II (*Continued*)

No.	R	Source	I_{50},[a] μM	Inhib, μM	Time, min	% inactvn[b]
29	*p*-$NHCOCH_2Br$	W256	10	10	120	0[c]
		Rabbit liver[e]	16	16	120	0[c]

[a] Assayed with 13.3 μM guanine in pH 7.4 Tris buffer containing 10% DMSO as previously described;[5] I_{50} = concentration for 50% inhibition. [b] Incubated at 37° in pH 7.4 Tris buffer containing 10% DMSO, then the remaining enzyme was assayed with guanine, xanthine oxidase, and 2,6-dichlorophenolindophenol as described in the Experimental Section, unless otherwise indicated. The results are an average of duplicate experiments. [c] Assayed by conversion of guanine to xanthine.[9] [d] Three $C_8H_{17}OH$ extractions were needed to remove inhibitor. [e] Data from ref 3. [f] Maximum solubility. [g] See ref 9 for synthesis.

TABLE III

IRREVERSIBLE INHIBITION OF GUANINE DEAMINASE BY



No.	R	Source	I_{50},[a] μM	Inhib, μM	Time, min	% inactivn[b]
30	$(CH_2)_2NHCOC_6H_4SO_2F$-*m*	W256	0.07	12	60	47
		Rat liver		12	60	0
31	$(CH_2)_2NHCOC_6H_4SO_2F$-*p*	W256	0.28	12	60	46
		Rat liver		12	60	0
		Rabbit liver	0.31	12	60	~0
32	$(CH_2)_2NHCOC_6H_3$-4-Me-3-SO_2F	W256	0.15	12	60	~0
		Rat liver		12	60	~0
		Rabbit liver	0.25	12	60	0
33	$(CH_2)_2NHCONHC_6H_4SO_2F$-*m*	W256	0.070	12	60	43
		Rat liver		12	60	28
34	$(CH_2)_2NHCONHC_6H_4SO_2F$-*p*	W256	0.067	12	60	0
35	$(CH_2)_2NHCONHC_6H_3$-2-Cl-5-SO_2F	W256	0.13	12	60	18
		Rat liver		12	60	22
36	$(CH_2)_2NHCONHC_6H_3$-4-Me-3-SO_2F	W256	0.043	12	60	0
37	$(CH_2)_2NHCONHC_6H_3$-2-OCH_3-5-SO_2F	W256	0.084	12	60	0
38	$(CH_2)_3NHCOC_6H_4SO_2F$-*p*	W256	0.18	12	60	0
39	$(CH_2)_3NHCOC_6H_4SO_2F$-*m*	W256	0.14	12	60	~0
40	$(CH_2)_3NHCONHC_6H_4SO_2F$-*m*	W256	0.0095	12	60	0
41	$(CH_2)_3NHCONHC_6H_4SO_2F$-*p*	W256	0.036	12	60	0

[a,b] See corresponding footnotes in Table II.

9-(*p*-alkoxyphenyl)guanine (**30**–**41**) were excellent reversible inhibitors of guanine deaminase with I_{50}'s in the range of 0.01–0.28 μM (Table III). This variation in I_{50} indicated that at least part of the phenylsulfonyl fluoride moiety was in contact with the enzyme surface. Of these 12 compounds, only three (**30**, **31**, **33**) showed appreciable irreversible inhibition of the guanine deaminase from Walker 256 rat tumor.

The effective irreversible inhibitors of Walker 256 guanine deaminase in Tables II and III were then assayed with the enzyme from rat liver to determine whether or not any of the compounds were specific irreversible inhibitors of the tumor enzyme. The three irreversible inhibitors at 12 μM giving >90% inactivation of the tumor enzyme (**6**, **7**, **9**) were also effective irreversible inhibitors of the rat liver enzyme. The 13 inhibitors giving 25–70% inactivation of the rat tumor enzyme were also checked as irreversible inhibitors of the rat liver enzyme. Of these, three (**8**, **14**, **15**) were more effective irreversible inhibitors of the rat liver enzyme and two (**4**, **13**) were of the same order of effectiveness on the rat liver and Walker 256 enzymes. Of the eight compounds less effective as irreversible inhibitors of the rat liver enzyme, four (**20**–**23**) showed some inactivation of the rat liver enzyme and four (**5**, **28**, **30**, **31**) showed no inactivation of the rat liver enzyme. Although isozyme specificity has been achieved with the latter four compounds, these are not sufficiently effective on the tumor enzyme; however, by further substitution it should be possible to achieve better irreversible inhibition of the tumor enzyme without loss of specificity. Similarly, further study of analogs of the highly effective, but nonselective inhibitors (**7**, **9**) may lead to compounds with more irreversible specificity toward the tumor enzyme.

A few of the compounds that were effective irreversible inhibitors of the rat liver enzyme were tested on rabbit liver enzyme. Four of the compounds (**4**, **6**, **9**, **15**) were about equally effective irreversible inhibitors of the two liver enzymes, three (**7**, **8**, **22**) were

more effective on rat liver, and two (**13**, **14**) showed no irreversible inhibition of the rabbit liver enzyme; thus, the rat and rabbit liver enzymes are not identical.

Although reversible binding of substituted 9-phenylguanines to xanthine oxidase and guanine deaminase is quite similar,[3,7-9] these two enzyme are readily differentiated by irreversible inhibitors. (1) Guanine deaminase, but not xanthine oxidase, can be irreversibly inhibited by *m*-acylamido-9-phenylguanines bearing a terminal SO_2F moiety. (2) In contrast, xanthine oxidase, but not guanine deaminase, can be irreversibly inhibited by *p*-acylamido-9-phenylguanines bearing a terminal SO_2F moiety. (3) *para* ethers on 9-phenylguanine with a terminal SO_2F moiety such as **30** and **31** can inactivate guanine deaminase but not xanthine oxidase. (4) Both *m*- and *p*-benzenesulfonamido-9-phenylguanines with a terminal SO_2F group can inactivate both xanthine oxidase and guanine deaminase.

The effective irreversible inhibitors of guanine deaminase and xanthine oxidase correlate well with the respective maps of their hydrophobic bonding regions.[8] Those compounds that project the SO_2F group into a hydrophobic bonding region are not irreversible inhibitors as predicted. Contrariwise, all of the effective irreversible inhibitors projected the SO_2F moiety into a polar region; those candidate compounds that projected the SO_2F group into a polar region, but were not irreversible inhibitors, apparently were not in juxtaposition to a suitable enzymic nucleophilic group such as a serine hydroxyl and therefore did not form a covalent bond.[14]

Experimental Section[15]

The synthesis of the compounds in Tables I,[8] II,[16] and III[2] have been described previously.

Enzyme Assays.—The assay of the inactivation of commercial guanine deaminase from rabbit liver by measurement of the rate of conversion of 13.3 μM guanine to xanthine has been previously described.[3] Assay of enzyme inactivation by coupling with xanthine oxidase, as measured by reduction of 2,6-dichlorophenolindophenol (DCPI),[2] was about ten times as sensitive. Furthermore, a much higher concentration of inhibitor than 51_{50} could be used by removing the inhibitor by 1-octanol extraction prior to assay as follows.

Guanine deaminase was present in the 45–90% $(NH_4)_2SO_4$ fraction from Walker 256 rat tumor and from rat liver used for dihydrofolic reductase.[16] This fraction (1 ml/g) from Walker 256 was diluted 1:10 for incubation; the rat liver preparation was diluted 1:40. Bovine xanthine oxidase (40 units/ml; 1 unit converts 1 μmole of xanthine to uric acid in 1 min) was purchased and diluted 1:50 with buffer (0.05 *M* Tris, pH 7.4) for assay. DCPI was dissolved in H_2O at 0.1 mg/ml. In a tube labeled C_0 were placed 0.45 ml of diluted enzyme and 0.45 ml of additional enzyme for each I_0 point needed. The solution was placed in an ice bath until ready for assay. In a tube labeled I_0 were placed 0.45 ml of buffer and 50 μl of DMSO containing inhibitor; this was also placed in an ice bath. In a tube labeled C_{30} were placed 0.45 ml of diluted enzyme and 50 μl of DMSO; this was incubated at 37° for 1 hr, then cooled in an ice bath. In a tube labeled I_{30} were placed 0.45 ml of diluted enzyme and 50 μl of DMSO containing inhibitor; this was also incubated at 37° for 1 hr, then placed in an ice bath. Each tube except C_0 was twice extracted with 2 ml of H_2O-saturated 1-octanol/0.5 ml of solution by Vortex mixing for 30 sec, centrifugation for 3 min in a clinical centrifuge, and removal of the 1-octanol with an eye dropper. In all but the I_0 tube, a protein pellet formed at the interphase which contained no guanine deaminase and was merely pushed to the side of the tube. All but the I_0 tube were assayed as follows.

In a 1-ml glass cuvette were placed 0.55 ml of buffer, 100 μl of DCPI, 50 μl of xanthine oxidase, 200 μl of 66.7 μM guanine (cuvette concentration = 13.3 μM), and 100 μl of incubation solution. The rate of decrease of OD at 600 mμ was observed; the C_0 aliquot showed a rate of 0.01–0.015 OD unit/min when measured with a Gilford recording spectrophotometer and when corrected for a blank containing no guanine. The I_0 tube was assayed similarly by addition of 100 μl of C_0 aliquot in place of 100 μl of the buffer. The OD change/min was linear with guanine deaminase concentration.

(14) For a compound by compound analysis see W. F. Wood, Ph.D. thesis, University of California at Santa Barbara, Oct. 1968.

(15) The technical assistance of Julie Leseman, Maureen Baker, and Jean Reeder with the enzyme assays is acknowledged.

(16) B. R. Baker and G. J. Lourens, *ibid.*, **10**, 1123 (1967), paper CV of this series.