N-Acetyl-3-methylsulfanilyl Fluoride (31) (Method F).—To a stirred mixture of 24.7 g (0.1 mole) of **30** (prepared from **28** with $ClSO_3H$[17]) and 30 ml of dioxane in a bath at 120–125° was added a solution of 8.7 g (1.50 moles) of KF in 9 ml of H_2O over a period of about 2 min. The mixture was stirred under reflux for 30 min, then diluted with 100 ml of H_2O. The product was collected on a filter, washed with H_2O, and recrystallized from EtOH–H_2O; yield 12.2 g (53%), mp 186–187°. See Table IV for additional data.

2-Methylsulfanilyl Fluoride (21) (Method G).—A mixture of 2.19 g (10 moles) of **23**, 100 ml of EtOH, and 5 ml of Raney Ni was shaken with H_2 at 2–3 atm for 30 min when reduction was complete. The filtered solution was evaporated *in vacuo* and the residue was recrystallized from EtOH–H_2O; yield 1.17 g (62%) of white crystals, mp 81–82°. See Table IV for additional data.

3-Methylsulfanilyl Fluoride (32) (Method H). To a stirred mixture of 10.4 g (45 mmoles) of **31** and 40 ml of EtOH heated under reflux was added 40 ml of 12 *N* HCl. After 45 min the cooled mixture was carefully poured into a mixture of 41 g of $NaHCO_3$ and 400 ml of ice–H_2O. The product was collected on a filter, washed with H_2O, and recrystallized from EtOH–H_2O; yield 6.18 g (73%), mp 108–109°. See Table IV for additional data.

Irreversible Enzyme Inhibitors. CXXX.[1,2] Cytosine Nucleoside Deaminase. I. Hydrophobic Bonding with Monosubstituted Uracils

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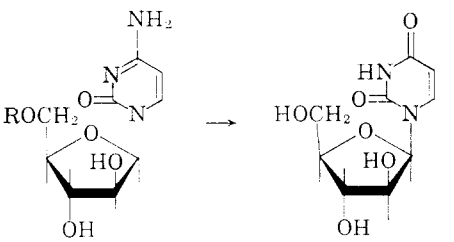
Thirty-eight derivatives of uracil and five derivatives of thymidine have been investigated as inhibitors of the cytosine nucleoside deaminase from *E. coli* B. Hydrophobic bonding to the enzyme could be detected with 1-substituted uracils, 6-substituted uracils, and 5′-substituted thymidines. Thymidine itself is a weak inhibitor of this enzyme and was employed as the base-line standard compound. The best inhibitors of the 1-substituted uracil class were 1-phenylbutyl- (**15**), 1-phenylamyl- (**16**), and 1-phenoxypropyluracil (**18**) which were complexed to the enzyme about as effectively as thymidine. The best inhibitors of the 6-substituted uracil class were 6-benzyl- (**25**) and 6-phenylpropyluracil (**26**) which were also complexed as well as thymidine. In the thymidine series, the best inhibitors were the 5′-O-carbophenoxy (**37**) and 5-O-(N-phenylpropylcarbamoyl) (**38**) derivatives which were 3–4-fold more effective than thymidine; in contrast, 5′-O-carbamoylthymidine (**35**) was less effective than thymidine. The possible biological role of this hydrophobic bonding region adjacent to the active site of cytosine nucleoside deaminase is discussed.

That β-D-arabinofuranosyl analogs of nucleosides could show cytotoxic action was first demonstrated by Pizer and Cohen[3] with 1-β-D-arabinofuranosyluracil (*ara*-U) (**3**). Other arabinosyl nucleosides, such as 9-β-D-arabinofuranosyladenine[4] and 1-β-D-arabinofuranosylcytidine (*ara*-C) (**1**),[5] with cytotoxic activity soon followed. Since *ara*-C (**1**) showed sufficient selective cytoxicity to be effective against certain animal tumors,[5a] a great deal of research at both the preclinical[6,7] and clinical levels[8] was published. The cytotoxicity of *ara*-C (**1**) is due to intracellular conversion to the nucleotide (**2**); the latter is most probably cytotoxic due to inhibition of the conversion of cytidylate to 2′-deoxycytidylate[6,9] at the triphosphate level, and hence the inhibition of DNA synthesis.

ara-C (**1**) is rapidly deaminated *in vivo* by cytosine nucleoside deaminase to *ara*-U (**3**),[8d,10] the latter being



1, R = H
2, R = $PO(OH)_2$

3

relatively nontoxic to mammalian cells.[6,9] Thus, one possible mechanism for selective action of *ara*-C (**1**) could be due to the poor ability of a susceptible cell line to deaminate *ara*-C (**1**) to *ara*-U (**2**) compared to normal tissues. Cytosine nucleoside deaminase normally uses cytidine or 2′-deoxycytidine as a substrate, the order of velocity with the *E. coli* B enzyme[3] being 2′-deoxycytidine > cytidine > *ara*-C; similarly, cytidine and 2′-deoxycytidine were more rapidly deaminated by the enzyme from human liver than *ara*-C.[11] The enzyme can be inhibited by classical-type antimetabolites,[12] that is, those having essentially isosteric changes in structure compared to the substrate. The most effective compound found was N-hydroxy-5-methyl-2′-deoxycytidine[12] which was complexed to the enzyme about tenfold better than *ara*-C; the enzyme can also be inhibited by thymidine, 2′-deoxyuridine, and uridine, but these are complexed to the enzyme less

(1) This work was supported in part by Grant No. 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 G. J. Lourens, *J. Med. Chem.*, **11**, 677 (1968).

(3) L. I. Pizer and S. S. Cohen, *J. Biol. Chem.*, **235**, 2387 (1960).

(4) W. W. Lee, A. Benitez, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **82**, 2648 (1960).

(5) (a) J. S. Evans, E. A. Musser, G. D. Mengel, K. R. Forsblad, and J. H. Hunter, *Proc. Soc. Exptl. Biol. Med.*, **106**, 350 (1961); (b) J. H. Hunter, U. S. Patent 3,183,226 (1963).

(6) (a) R. L. Dixon and R. H. Adamson, *Cancer Chemotherapy Rept.*, **48**, 11 (1965); (b) I. Wodinsky and C. J. Kensler, *ibid.*, **47**, 65 (1965); (c) I. Kline, J. M. Venditti, D. D. Tyrer, and A. Goldin, *Proc. Am. Assoc. Cancer Res.*, **6**, 36 (1965).

(7) For a review see S. S. Cohen, *Progr. Nucleic Acid Res.*, **5**, 1 (1965).

(8) (a) R. W. Talley and V. K. Vaitkevicius, *Blood*, **21**, 352 (1963); (b) J. B. Block, W. Bell, J. Whang, and P. P. Carbone, *Proc. Am. Assoc. Cancer Res.*, **6**, 6 (1965); (c) R. W. Carey and R. R. Ellison, *Clin. Res.*, **13**, 337 (1965); (d) R. W. Talley, R. M. O'Bryan, W. G. Tucker, and R. V. Loo, *Cancer*, **20**, 809 (1967).

(9) M. Y. Chu and G. A. Fischer, *Biochem. Pharmacol.*, **11**, 423 (1962).

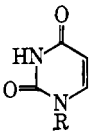
(10) G. W. Camiener and C. G. Smith, *ibid.*, **14**, 1405 (1965).

(11) G. W. Camiener, *ibid.*, **16**, 1691 (1967).

(12) (a) G. W. Camiener, *Proc. Am. Assoc. Cancer Res.*, **8**, 9 (1967); (b) E. A. Falco and J. J. Fox, *J. Med. Chem.*, **11**, 148 (1968), and references therein.

TABLE I

INHIBITION[a,b] OF CYTOSINE NUCLEOSIDE DEAMINASE BY



No.	R	Concn, mM	% inhib	Estd I_{50}, mM	I/TdR[c]	Compd source
4	H	1.6	0	>6.4[d]	>4	*e*
5	CH_3	3.0[f]	0	>12	>7.5	*g*
6	*n*-C_4H_9	0.60[f]	0	>2.4	>1.5	*g*
7	*n*-C_5H_{11}	1.5[f]	0	>6	>3.7	*h*
8	*i*-C_5H_{11}	1.0[f]	0	>4	>2.5	*h*
9	Cyclopentyl	1.0[f]	27	2.7	1.7	*h*
10	*i*-C_6H_{13}	0.9[f]	0	>3.6	>2.3	*h*
11	C_6H_5	0.5[f]	0	>2	>1.4	Exptl
12	$C_6H_5CH_2$	1.5[f]	17	~8	5	*h*
13	$C_6H_5(CH_2)_2$	0.75[f]	0	>3	>1.9	*h*
14	$C_6H_5(CH_2)_3$	1.0[f]	31	2.3	1.4	*h*
15	$C_6H_5(CH_2)_4$	1.0[f]	34	1.9	1.2	*h*
16	$C_6H_5(CH_2)_5$	0.50[f]	26	1.5	0.9	*h*
17	$C_6H_5O(CH_2)_2$	1.5[f]	11	~13	10	Exptl
18	$C_6H_5O(CH_2)_3$	1.0[f]	33	2.0	1.2	*i*
19	$CH_2C_6H_4COOH$-*p*	1.5[f]	0	>6	>3.7	*j*
20	$CH_2C_6H_4NHCOCH_2Br$-*m*	0.5[f]	19	2.3	1.4	*h*
21	$CH_2C_6H_4NHCOCH_2Br$-*o*	1.0[f]	28	2.3	1.4	*h*
22	$(CH_2)_3C_6H_4COOC_2H_5$-*p*	0.5[f]	23	1.7	1.1	*g*

[a] The technical assistance of Pepper Caseria and Susan Black with these assays is acknowledged. [b] Assayed with *E. coli* B enzyme using 0.1 m*M* 2′-deoxycytidine in pH 7.4 Tris buffer containing 10% DMSO as described in the Experimental Section. [c] Ratio of concentration of inhibitor to 1.6 m*M* thymidine (TdR) needed for 50% inhibition. [d] Since 20% inhibition is readily detected, the concentration necessary for 50% inhibition is at least four times greater than that measured. [e] Nutritional Biochemical Corp. [f] Near maximum solubility or minimum light transmission. [g] See ref 18 for synthesis. [h] See ref 19 for synthesis. [i] See ref 21 for synthesis. [j] See ref 32 for synthesis.

effectively than the corresponding cytosine nucleosides.[10,11,13] Such classical-type inhibitors could be expected to work *in vivo*, but the selective cytotoxicity of *ara*-C is apt to be destroyed due to lack of detoxification in normal cells.[14] In contrast, it is theoretically possible to enhance the activity of a cytotoxic agent such as *ara*-C if a tumor-specific inhibitor of cytosine nucleoside deaminase could be found.[15] Such selectivity is much more apt to be achieved with nonclassical-type inhibitors which utilize a region adjacent to the active site, particularly those of the active-site-directed irreversible type[16] that also utilize hydrophobic bonding, as has been recently observed with dihydrofolic reductase.[2,17] The initial search for a hydrophobic bonding region on cytosine nucleoside deaminase from *E. coli* B is the subject of this paper.

Enzyme Results.—The inhibition of cytosine nucleoside deaminase from *E. coli* B was measured spectrophotometrically at 290 mμ using 2′-deoxycytidine as the substrate.[3] That thymidine was a weak inhibitor[10,11,13] was verified; thymidine was complexed 16-fold less effectively than the substrate, 2′-deoxycytidine, in our test system, a value in agreement with the 15-fold better binding of *ara*-C than *ara*-U to this enzyme from human liver. The availability of a large number of hydrophobically substituted uracils from the studies on inhibition of thymidine kinase[18] and FUDR phosphorylase[19-22] allowed an initial study on hydrophobic bonding to cytosine nucleoside deaminase to be performed. The activity of the compounds are compared to thymidine as a baseline.

Replacement of the 2′-deoxyribose moiety of thymidine by methyl (**5**) (Table I) gave a greater than fourfold loss in binding. No appreciable hydrophobic bonding could be detected when the methyl group was increased to *n*-butyl (**6**), *n*-amyl (**7**), isoamyl (**8**), or isohexyl (**10**); however, a greater than fivefold increment in binding was observed with the cyclopentyl group (**9**) compared to methyl (**5**), showing that there was a hydrophobic region on the enzyme adjacent to the active site.

Better inhibition was observed with the phenylalkyl group (**12–16**), the maximum inhibition being seen with the phenylbutyl (**15**) and phenylamyl groups (**16**).

(13) S. S. Cohen and H. D. Barner, *J. Biol. Chem.*, **226**, 631 (1957).

(14) The destruction of selectivity with 5-fluoro-2′-deoxyuridine (FUDR) by inhibition of its detoxification enzyme (FUDR phosphorylase) has been noted with uridine, a classical-type inhibitor; see G. D. Birnie, H. Kroeger, and C. Heidelberger, *Biochemistry*, **2**, 566 (1963).

(15) Selective inhibition of tumor FUDR phosphorylase for enhancement of FUDR activity has been previously proposed by B. R. Baker, *J. Med. Chem.*, **10**, 297 (1967), paper LXXV of this series; similarly, selective inhibition of tumor xanthine oxidase has been proposed for enhancement of 6-mercaptopurine activity by B. R. Baker and J. L. Hendrickson, *J. Pharm. Sci.*, **56**, 955 (1967), paper XCII of this series. See also B. R. Baker, *J. Med. Chem.*, **10**, 59 (1967), paper LXXIII of this series.

(16) 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.

(17) (a) B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **11**, 489 (1968), paper CXIX of this series; (b) B. R. Baker and P. C. Huang, *ibid.*, **11**, 495 (1968) paper CXX of this series.

(18) B. R. Baker and T. J. Schwan, *ibid.*, **9**, 73 (1966).

(19) B. R. Baker and M. Kawazu, *ibid.*, **10**, 302 (1967), paper LXXVI of this series.

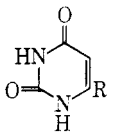
(20) B. R. Baker and M. Kawazu, *ibid.*, **10**, 311 (1967), paper LXXVIII of this series.

(21) B. R. Baker, M. Kawazu, D. V. Santi, and T. J. Schwan, *ibid.*, **10**, 304 (1967), paper LXXVII of this series.

(22) B. R. Baker and W. Rzeszotarski, *ibid.*, **10**, 1109 (1961), paper CIV of this series.

Table II

Inhibition[a,b] of Cytosine Nucleoside Deaminase by



No.	R	Concn, mM	% inhib	Estd I_{50}, mM	I/TdR[c]	Compd source
4	H	1.6	0	>6.4[d]	>4	*e*
23	*n*-C_5H_{11}	3.0	50	3.0	1.9	*f*
24	C_6H_5	0.2[g]	0	>0.8[h]	>0.5	*f*
25	$C_6H_5CH_2$	1.8	50	1.8	1.1	*f*
26	$C_6H_5(CH_2)_3$	1.5	50	1.5	0.94	*f*
27	C_6H_5O	0.3[g]	0	>1.2	>0.7	*i*
28	C_6H_5S	0.5[g]	0	>2	>1.2	*i*
29	C_6H_5NH	0.5[g]	0	>2	>1.2	*i*
30	$C_6H_{11}NH$	0.5[g]	0	>2	>1.2	*i*
31	$C_6H_5CH_2NH$	0.5[g]	18	2.2	1.4	*i*
32	$C_6H_5(CH_2)_3NH$	0.6[g]	23	2.0	1.2	*i*

[a–e] See corresponding footnotes in Table I. [f] See ref 20 for synthesis. [g] Near maximum solubility or minimum light transmission. [h] Since 20% inhibition is readily detected, the concentration necessary for 50% inhibition is at least four times greater than that measured. [i] See ref 22 for synthesis.

Therefore the phenoxyethyl (**17**) and phenoxypropyl (**18**) groups were investigated; **18** was as good an inhibitor as phenylbutyl (**15**). The phenoxypropyl group is considerably more advantageous than the phenylbutyl group from the synthetic standpoint, where investigation of substituent effects on the binding of the phenyl ring is aided by ease of synthesis.

In order to complete the $C_6H_5(CH_2)_n$ series, the known 1-phenyluracil (**11**) was synthesized by a new method; **11** was less effective than the phenylpropyl (**14**) and phenoxypropyl (**18**) derivatives of uracil.

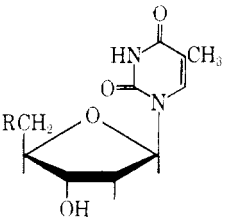
No enhancement in binding was observed when the benzyl group of **12** was substituted with the polar carboxylate group (**19**). However, a threefold enhancement in binding was observed when the benzyl group (**12**) was substituted by bromoacetamido (**20**, **21**); the nature of this interaction cannot be ascertained without additional compounds, but it is clear that there is a bulk tolerance[23] for these groups within the enzyme–inhibitor complex. Similarly, there was bulk tolerance for the *p*-carbethoxy group (**22**) when substituted on the phenylpropyl moiety of **14**; these bulk tolerance areas are useful for construction of active-site-directed irreversible inhibitors.[23]

Since hydrophobic bonding could be detected with the 1-phenylalkyluracils in Table I, a search was made for hydrophobic bonding with 6-substituted uracils (Table II). The baseline for such a study is uracil (**4**); unfortunately, a concentration of **4** sufficient to show inhibition could not be reached due to lack of light transmission. Nevertheless, it was clear that the 6-(*n*-amyl) group of **23** gave hydrophobic bonding; even better hydrophobic bonding was seen with the 6-benzyl (**25**) and 6-phenylpropyl (**26**) substituents on uracil. Whether or not 6-phenyl (**24**), 6-phenoxy (**27**), 6-phenylthio (**28**), 6-anilino (**29**), or 6-cyclohexylamino (**30**) could exhibit hydrophobic bonding was limited by lack of light transmission above 0.3–0.5 m*M*. However, hydrophobic bonding could be detected with the 6-benzylamino (**31**) and 6-phenylpropylamino (**32**) groups.

(23) See ref 16, Chapter VII.

Table III

Inhibition[a,b] of Cytosine Nucleoside Deaminase by



No.	R	Concn, mM	% inhibn	Estd I_{50}, mM	I/TdR[c]	Compd source
33	OH	1.6	50	1.6	1.0	*d*
34	H	0.80	0	>3.2[e]	>2	*f*
35	$OCONH_2$	1.0	0	>4.0[e]	>2.5	*f*
36	$OCOOC_2H_5$	1.0	36	1.8	1.1	*f*
37	$OCOOC_6H_5$	0.45	50	0.45	0.28	*f*
38	$OCONH(CH_2)_2C_6H_5$	0.68	50	0.68	0.43	*f*

[a–c] See corresponding footnotes in Table I. [d] Nutritional Biochemical Corp. [e] Since 20% inhibition is readily detected, the concentration for 50% inhibition is at least four times greater than that measured. [f] See ref 25 for synthesis.

The detection of hydrophobic bonding by both 1- and 6-substituted phenylalkyluracils[24] suggested that this region should also be detectable by 5′ substituents on thymidine; compounds of this type (Table III) had been synthesized earlier in this laboratory for studies on the inhibition of thymidine kinase.[25] Removal of the 5′-hydroxyl of thymidine (**33**) to give 5′-deoxythymidine (**34**) appeared to give a loss in binding, but the amount of difference was insufficient to state that the 5′-hydroxyl group was complexed to the enzyme. The loss in binding by the introduction of the 5′-carbamate (**35**) could be due to a lack of bulk tolerance[23] or to the loss of the hydroxylic hydrogen needed as an acceptor atom for binding to the enzyme. That the latter was probably the correct explanation was indicated by the 5′-carbethoxy group (**36**); the increase in binding by **36** compared to **35** could be due to a hydrophobic interaction of the ethyl group with the enzyme. The latter explanation was supported by a further increment in hydrophobic bonding by the phenyl group of the carbophenoxy moiety (**37**) and the phenethyl group of the N-phenethylcarbamate moiety (**38**). Thus any loss in binding by the removal of the 5′-hydroxyl of thymidine is more than recouped by the hydrophobic interaction seen with **37** and **38**, **37** being complexed fourfold better than thymidine; whether this hydrophobic interaction at the 5′ position of thymidine can be further increased is under investigation.

The presence of a hydrophobic bonding region adjacent to the active site of cytosine nucleoside deaminase was anticipated since we believe that it may have a biological function. In addition to this enzyme, five other enzymes investigated in this laboratory that use a nucleoside or base as substrate, but do not convert them to a nucleotide, have hydrophobic bonding regions adjacent to the active site. These five enzymes are thymidine phosphorylase,[22,26] guanosine phosphoryl-

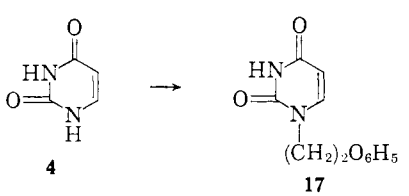
(24) Increased hydrophobic bonding by 1-substituted uracils bearing a second hydrophobic group on the 5 or 6 position is the subject of the paper that follows; see B. R. Baker and J. L. Kelley, *J. Med. Chem.*, **11**, 686 (1968), paper CXXXI of this series.

(25) B. R. Baker, T. J. Schwan, and D. V. Santi, *ibid.*, **9**, 66 (1966).

(26) B. R. Baker and W. Rzeszotarski, *ibid.*, **11**, 639 (1968), paper CXXI of this series.

ase,[27] xanthine oxidase,[28] guanine deaminase,[28] and adenine deaminase,[29] all of which have hydrophobic bonding regions adjacent to the active site; furthermore, adenosine deaminase has been shown by Schaeffer[30] to have a hydrophobic bonding region adjacent to the active site. The pattern of these hydrophobic bonding regions are such that an enzyme using a nucleoside will repulse the polar phosphate of a nucleotide, and an enzyme converting a base to a base (such as guanine deaminase) will repulse a ribose moiety. Thus the nucleotides will be neither substrates nor inhibitors for an enzyme using nucleosidic substrates; also nucleosides will be neither substrates nor inhibitors for an enzyme using a base as a substrate. In contrast, no hydrophobic bonding region has yet been found adjacent to the active site of thymidine kinase,[18,25] an enzyme converting a nucleoside to a nucleotide.

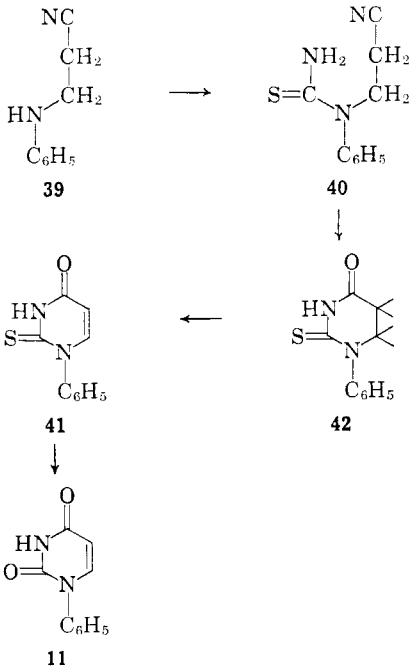
Chemistry.—Synthesis of all of the compounds in Tables I–III have been previously described except **11** and **17**. Alkylation of uracil (**4**) with phenoxyethyl bromide in DMSO by the previously described general method[19,31] afforded **17**.



4 **17**

The synthesis of 1-phenyluracil (**11**) from ethyl propiolate has been described previously;[32] small amounts of **11** were prepared by this method, but the over-all yield was poor. The elegant method of Cheng and Lewis[33] for synthesis of 1-alkyluracils from β-alkylaminopropionitriles seemed more attractive if it could be adapted to 1-aryluracils such as **11** (see Scheme I). Commercial anilinopropionitrile (**39**) was converted in 78% yield to **40** with NaCNS in dilute HCl; a similar reaction with KCNO failed. Cyclization and hydrolysis to **42** was accomplished with dilute HCl in only 14% yield, but methanolic alkoxide[33] gave a mixture from which the corresponding dihydrocytosine could not be isolated. Attempts to dehydrogenate **42** to **41** with Br_2 were unsuccessful;[33] however, dehydrogenation with S at 210° gave **41** in 54% yield.[34] The usual hydrolysis with aqueous chloroacetic acid afforded the desired 1-phenyluracil. This sequence is relatively easy to perform and would make a suitable alternate to the Shaw method[32] if additional study on the conversion of **40** to **42** could raise the yield of this step.

SCHEME I



39 **40** **41** **42** **11**

Experimental Section[35]

1-Phenoxyethyluracil (17).—A mixture of 2.01 g (10 mmoles) of β-bromophenetole, 3.36 g (30 mmoles) of uracil, 4.15 g (30 mmoles) of anhydrous K_2CO_3, 1.50 g (10 mmoles) of NaI, and 70 ml of DMSO was stirred in a bath at 90° for 3 hr. The cooled mixture was poured into 100 ml of H_2O, then acidified to about pH 2 with 5% HCl and extracted with five 100-ml portions of $CHCl_3$. The combined extracts were dried ($MgSO_4$), then spin evaporated *in vacuo*; the residual DMSO was removed in high vacuum. The residue was dissolved in hot EtOAc and filtered free of uracil, then concentrated *in vacuo* to about 30 ml and stored at −10°. The product was collected on a filter and washed with cold EtOAc; yield 1.03 g (44%), mp 155–157°. Recrystallization from EtOAc gave 0.91 g of white crystals, mp 156–157°. *Anal.* ($C_{12}H_{12}N_2O_3$) C, H, N.

N-(β-Cyanoethyl)-N-phenylthiourea (40).—To a stirred solution of 0.57 g (7 mmoles) of NaSCN in 1.0 ml of H_2O was added dropwise a solution of 0.73 g (5 mmoles) of **39** in 2.5 ml of 6 *N* HCl. After being stirred for 5 min, the solution began to deposit pink crystals which were collected on a filter; yield 0.80 g (78%), mp 103–107°, that was suitable for further transformation. Recrystallization from C_6H_6 gave white crystals, mp 105–109°. *Anal.* ($C_{10}H_{11}N_3S$) C, H, N.

5,6-Dihydro-1-phenyl-2-thiouracil (42).—A solution of 32.0 g (0.156 mole) of **40** in 250 ml of 10% HCl and 100 ml of MeOH was refluxed with stirring for 24 hr, then cooled in an ice bath. The product was collected on a filter and washed with cold EtOH; yield 4.5 g (14%), mp 177–184°, that was suitable for further transformation. Recrystallization of a sample from EtOH gave white crystals, mp 187–189°. *Anal.* ($C_{10}H_{10}N_2OS$) C, H, N.

1-Phenyl-2-thiouracil (41).—An intimate mixture of 1.75 g (8.5 mmoles) of **42** and 3.5 g of sulfur was heated in a bath at 210° for 6 hr.[34] The cooled melt was extracted with several portions of 10% NaOH. The extracts were clarified by filtration, then acidified with dilute HCl. The product was collected on a filter and washed with H_2O; yield 0.94 g (54%), mp 231–235°, that was suitable for the next step. Recrystallization of a sample from PrOH (charcoal) gave yellow crystals, mp 235–237°. *Anal.* ($C_{10}H_8N_2OS$) C, H, N.

1-Phenyluracil (11).—A mixture of 400 mg (1.9 mmoles) of **41**, 10 ml of HOAc, and 10 ml of 10% aqueous chloroacetic acid was refluxed with stirring for 6 hr. The cooled solution deposited 300 mg (81%) of product, mp 237–239°. Recrystallization from EtOH gave white crystals, mp 239–241°, lit.[31] mp 240°

Enzyme Assay Method.—The assay method was a modification of that of Pizer and Cohen.[3] Cytosine nucleoside deaminase was present in the 45–90% $(NH_4)_2SO_4$ fraction from *E. coli* B

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(28) (a) B. R. Baker and W. F. Wood, *J. Med. Chem.*, **10**, 1101 (1967), paper CII of this series; (b) B. R. Baker and W. F. Wood, *ibid.*, **11**, 644 (1968), paper CXXII of this series.

(29) B. R. Baker, unpublished data.

(30) For a review see ref 16, Chapter XII.

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(34) For a similar dehydrogenation of a dihydropyrimidine see B. R. Baker, G. J. Lourens, and J. H. Jordaan, *J. Heterocycl. Chem.*, **4**, 39 (1967), paper LXXXV of this series.

(35) Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample had uv and ir spectra compatible with its assigned structure and each moved as a single spot on tlc with 3:1 C_6H_6–EtOH. The analytical samples gave combustion values for C, H, N within 0.3% of theoretical.

previously used for assay of dihydrofolic reductase.[36] This fraction (1 ml/g of wet cells) was diluted 1:20 for assay.

Buffer was 0.05 *M* Tris (pH 7.4). 2′-Deoxycytidine was stored frozen as a 3.1 m*M* solution in buffer in order to avoid mold growth, but was kept at 0° during the day's runs. In a 3-ml quartz cuvette were placed 2.60 ml of buffer, 100 μl of 3.1 m*M* 2′-deoxycytidine, and 0.30 ml of DMSO. When the system had balanced at 290 mμ, 100 μl of enzyme was added and the decrease in optical density was recorded with a Gilford spectrophotometer; the rate was about 0.01 OD unit/min. Inhibitors were dissolved in DMSO. The inhibitor concentration for 50% inhibition (I_{50}) was determined by plotting V_0/V_I against [I] where V_0 = velocity without inhibitor, V_I = velocity with inhibitor, and [I] = inhibitor concentration;[37] [I] = I_{50} when $V_0/V_I = 2$.

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Irreversible Enzyme Inhibitors. CXXXI.[1,2] Cytosine Nucleoside Deaminase. II. Hydrophobic Bonding with Disubstituted Uracils and Cytosines

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Twelve 1,6- and 1,5-disubstituted uracils have been compared for their ability to inhibit *E. coli* B cytosine nucleoside deaminase. In addition to a hydrophobic interaction between the enzyme and a 1-phenylpropyl or 1-phenoxypropyl group on uracil, further hydrophobic interaction was seen with a 6-(*n*-amyl), 5-phenyl, or 5-phenylalkyl group. Among the best inhibitors found in this series was 1-phenoxypropyl-5-phenyluracil (**11**) which was complexed to the enzyme about half as well as the substrate, 2′-deoxycytidine, and about eightfold better than the reference inhibitor, thymidine. The amino analog of **11**, 1-phenoxypropyl-6-phenylcytosine (**21**), was only half as good an inhibitor of the enzyme as **11**, in spite of the fact that 2′-deoxycytidine was complexed to the enzyme 16-fold better than thymidine.

In the previous paper of this series,[2] it was shown that uracils substituted with a hydrocarbon moiety at the 1 position, such as phenylpropyl (**5**) (Table I), gave better binding to cytosine nucleoside deaminase than 1-methyluracil (**2**) due to hydrophobic bonding to the enzyme. Similarly, a benzyl (**4**) or *n*-amyl (**3**) group at the 6 position gave an increment in binding compared to uracil (**1**). Therefore a study has now been made to see if better inhibition of cytosine nucleoside could be achieved with an aralkyl group at the 1 position and a hydrocarbon moiety at either the 5 or 6 position of uracil.

For initial study, the 1-aralkyl group on uracil was held constant at 1-phenylpropyl and the substituents at the 5 and 6 positions were varied. Insertion of a 6-(*n*-propyl) group (**7**)[3] on 1-phenylpropyluracil (**5**) gave a twofold increment in binding; when this group was increased to *n*-amyl (**8**),[3] the increment was increased to 16-fold. A comparison of 5-(*n*-amyl)uracil (**3**) and its 1-phenylpropyl derivative (**8**) shows that the phenylpropyl group gives a 21-fold increment in binding.

An 11-fold increment in hydrophobic bonding could also be achieved with a 5-phenyl group as shown by comparison of **5** and **9**.[3] The 5-ethoxymethyl group of **10**[3] was considerably less effective in hydrophobic bonding than the 5-phenyl group of **9**.

The 5-phenyl group was then held constant while the 1-aralkyl group was varied. Replacement of the phenylpropyl group of **9** by phenoxypropyl (**11**) resulted in about the same degree of inhibition; however, replacement by the shorter phenoxyethyl (**13**) or longer phenoxybutyl (**12**) group gave about a twofold decrease in binding. 1-Methyl-5-phenyluracil (**14**), a baseline compound for hydrophobic bonding, was too insoluble to show inhibition.

The phenoxypropyl group of **11** was then held constant while the 5-phenyl group was varied; the 5-phenethyl (**16**) and 5-phenylpropyl (**17**) groups were about equivalent to the 5-phenyl of **11** in binding to the enzyme, but the 5-benzyl (**15**) group was slightly less effective; the 5-phenylbutyl derivative (**18**) was too insoluble to measure.

Since there is little difference in the binding of **8**, **9**, **11**, **16**, or **17** to the enzyme, the choice for further work resides primarily in the ease of synthesis for further structural variation. The 6-substituted uracils give alkylation mainly at N-3, rather than N-1; therefore, further studies with 6-substituted uracils related to **8** would suffer from low yields.[3] In contrast, most 5-substituted uracils alkylate at the desired N-1 position.[3] Of the various side chains at the 5 position, the 5-phenyl is the easiest to synthesize with substituents. Furthermore, the 1-phenoxypropyl group is preferred to 1-phenylpropyl since the substituted phenoxypropyl bromides needed for studying substituent effects on benzene binding are more easily prepared.

These studies indicated that a 5-phenyl substituent gave about as good hydrophobic bonding as higher 5-phenylalkyl groups and suggested that attention be directed to the synthesis of 1-substituted 5-phenylcytosines, particularly since 5-phenylcytosine can be prepared easily in good yield from phenylacetonitrile (see Chemistry section). 1-Phenoxypropyl-5-phenylcytosine (**21**) was only half as effective as 1-phenoxypropyl-5-phenyluracil (**11**) as an inhibitor; this result was surprising in view of the fact that 2′-deoxycytidine (**19**) binds about 16-fold better than thymidine (**20**), and further exploration of this discrepancy is underway.

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