(25–29) also failed to enhance binding. The phenylamyl group of **35** was 5-fold more effective in binding than the benzyl group of **19**, but shorter alkyl bridges (**30–32**) or oxyalkyl bridges (**33**, **34**) were less effective than **35**. The *n*-AmNH substituent of **37** was just as effective as the benzylamino group of **19**, again indicating a hydrophobic interaction; the *n*-BuNH group (**36**) was 5-fold less effective.

6-Aminouracils substituted by hydrocarbon groups are excellent inhibitors of the *E. coli* B thymidine phosphorylase cleaving FUDR to FU that can bind to this enzyme 1100–5000 times more effectively[9,10] than the substrate. In contrast only weak hydrophobic interaction was seen with these compounds on the Walker 256 enzyme, a uridine phosphorylase that can cleave FUDR to FU. This weak interaction indicated that a hydrophobic bonding region was present on the Walker 256 enzyme, but that 6 substituents on the uracil could not properly orient for strong interaction. Therefore studies were turned to hydrophobic groups attached to the 1 or 5 positions;[23,24] excellent inhibitors of the Walker 256 enzyme emerged that could bind 100- to 1000-fold better than the substrate.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. All analytical samples had proper uv and ir spectra and moved as a single spot on tlc on Brinkmann silica gel GF; each gave combustion values for C, H, and N within 0.3% of theoretical.

6-Benzylamino-5-bromouracil (24).—To a stirred mixture of 0.217 g (1.0 mmol) of **19**[8] and 2 ml of AcOH at ~70° was added 0.351 g (1.1 mmol) of pyridinium hydrobromide perbromide. A clear solution formed which soon deposited white crystals. After 0.5 hr the mixture was cooled and diluted with 10 ml of H_2O. The product was collected, washed with H_2O, recrystallized twice from MeOEtOH, and then from AcOH; yield, 0.121 g (41%) of off-white clusters which had no definite melting point but moved as a single spot on tlc in 1:5 AcOH–C_6H_6 or 1:3 EtOH–C_6H_6. *Anal.* ($C_{11}H_{10}BrN_3O_2$) C, H, N.

FUDR Phosphorylase.—This enzyme was present in the 0–45% $(NH_4)_2SO_4$ fraction of extracts of Walker 256. The enzyme in this fraction was stable for at least several months at −15° when stored in 1.8-ml aliquots sufficient for 1 day of use. The assay was performed with pH 5.9 arsenate–succinate as previously described,[14] sufficient enzyme and time being used to give about 0.15 O.D. change in the controls.

Irreversible Enzyme Inhibitors. CLXX.[1,2] Inhibition of FUDR Phosphorylase from Walker 256 Rat Tumor by 1-Substituted Uracils

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1-Substituted uracils (44) were investigated as inhibitors of Walker 256 uridine-deoxyuridine phosphorylase (EC 2.4.2.3) which can also cleave FUDR to 5-fluorouracil. A good hydrocarbon interaction was seen with benzyl (**10**), phenylbutyl (**13**), or phenoxybutyl (**18**) substituents. Further enhancement of binding of the benzyl group was achieved with *m*-OR substituents, the best binding being observed by *m*-OC_2H_5 (**32**) and *m*-$C_6H_5(CH_2)_nO$ (**33–37**) groups; **32–37** were complexed to the enzyme about 300-fold better than the parent uracil and about 40-fold better than the substrate, FUDR.

6-Arylamino-[3] and 6-arylmethylaminouracils[3,4] are excellent inhibitors of FUDR phosphorylase from *Escherichia coli* B[5] due to a hydrocarbon interaction of the aryl or aralkyl group with the enzyme; these compounds were much less effective on the FUDR phosphorylase from Walker 256 rat tumor, although weak hydrocarbon interaction was seen.[2] Since it appeared that a hydrocarbon group bridged to the 6 position of uracil could not orient for maximum hydrophobic bonding,[2] attention was turned to possible hydrocarbon interaction from 1-substituted and 5-substituted uracils. Some excellent inhibitors have emerged in both areas; the inhibition of the uridine–deoxyuridine phosphorylase (EC 2.4.2.3) from Walker 256 rat tumor with 1-substituted uracils is the subject of this paper.[6]

Enzyme Assays.—In Table I uracil (**1**) has I_{50} = 2900 μM.[2] Introduction of a 1-Me (**2**) substituent gave no loss in binding on this Walker 256 uridine phosphorylase; this result should be contrasted to the result with *E. coli* B thymidine phosphorylase where **2** was 33-fold less effective than uracil (**1**), indicating that the 1-H was a binding point to the *E. coli* enzyme, but not the Walker 256 enzyme.

Hydrophobic bonding was seen with higher alkyl groups (**4–7**), the maximum increment being about 15-fold compared to 1-methyluracil (**2**). Ring substituents were detrimental to binding; about a 2-fold loss in binding compared with **2** occurred with cyclopentyl (**8**) and a >6-fold loss with Ph (**9**).

Hydrocarbon interaction by aralkyl groups was then studied. 1-Benzyl (**10**) gave a 24-fold increment in binding compared with **2**, but phenethyl (**11**) and phenylpropyl (**12**) were considerably less effective. Activity maximized again at phenylbutyl (**13**), which was 80-fold more effective than **2**; phenylamyl (**14**) and phenylhexyl (**15**) were about 2-fold less effective than **13**. With the phenoxyalkyl group (**16–19**),

(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 of this series see B. R. Baker and J. L. Kelley, *J. Med. Chem.*, **13**, 456 (1970).

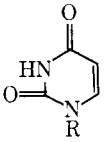
(3) B. R. Baker and W. Rzeszotarski, *ibid.*, **11**, 639 (1968), paper CXXI of this series.

(4) B. R. Baker and S. E. Hopkins, *ibid.*, **13**, 87 (1970), paper CLXVII of this series.

(5) B. R. Baker, *ibid.*, **10**, 297 (1967), paper LXXV of this series.

(6) For the chemotherapeutic utility of selective inhibitors of this enzyme see (a) ref 5, and (b) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," John Wiley & Sons, New York, N. Y., 1967, pp 79–81.

TABLE I
INHIBITION[a] OF WALKER 256 FUDR PHOSPHORYLASE[b] BY



No.	R	I_{50}, μM[c]
1	H	2900[d]
2	CH_3	3600
3	n-C_3H_7	1500
4[e]	n-C_4H_9	350
5[e]	n-C_5H_{11}	250
6[e]	i-C_5H_{11}	220
7[e]	i-C_6H_{13}	260
8[e]	Cyclopentyl	5800
9	C_6H_5	>20,000[f,g]
10[e]	$C_6H_5CH_2$	150
11[e]	$C_6H_5(CH_2)_2$	2400
12[e]	$C_6H_5(CH_2)_3$	940
13[e]	$C_6H_5(CH_2)_4$	46
14[e]	$C_6H_5(CH_2)_5$	98
15[h]	$C_6H_5(CH_2)_6$	88
16[g]	$C_6H_5O(CH_2)_2$	1800
17[i]	$C_6H_5O(CH_2)_3$	300
18	$C_6H_5O(CH_2)_4$	35
19	$C_6H_5O(CH_2)_5$	190
20	α-$C_{10}H_7CH_2$	120
21	β-$C_{10}H_7CH_2$	160
22	m-$C_6H_5C_6H_4CH_2$	10
23	1-Phenanthryl-CH_2-	>80[f]
24	m-$CH_3C_6H_4CH_2$	26
25	p-$CH_3C_6H_4CH_2$	370
26	3,5-$(CH_3)_2C_6H_3CH_2$	16
27	m-$ClC_6H_4CH_2$	73
28	m-$O_2NC_6H_4CH_2$[f]	710
29	m-$HOC_6H_4CH_2$	180
30[e]	m-$BrCH_2CONHC_6H_4CH_2$	260
31	m-$CH_3OC_6H_4CH_2$	18
32	m-$C_2H_5OC_6H_4CH_2$	7.2
33	m-$C_6H_5OC_6H_4CH_2$	9.3
34	m-$C_6H_5CH_2OC_6H_4CH_2$	12
35	m-$C_6H_5(CH_2)_2OC_6H_4CH_2$	9.1
36	m-$C_6H_5(CH_2)_3OC_6H_4CH_2$	6.5
37	m-$C_6H_5(CH_2)_4OC_6H_4CH_2$	9.3
38	o-$CH_3OC_6H_4CH_2$	60
39	p-$C_6H_5OC_6H_4CH_2$	38
40	p-$C_6H_5CH_2OC_6H_4CH_2$	>100[f]
41	n-C_4H_9-5-Br[j]	2600[k]
42	n-C_4H_9-5-NO_2[j]	>4000[f]
43	n-C_4H_9-5-SO_2Cl[j]	>4000[f]
44	n-C_4H_9-5-$SO_2NHC_4H_9$-n[j]	720
45	n-C_4H_9-5-$SO_2NHC_6H_5$[j]	140

[a] The technical assistance of Maureen Baker, Janet Wood, and Julie Leseman is acknowledged. [b] For enzyme preparation see ref 2. [c] I_{50} = concentration for 50% inhibition when assayed with 400 μM FUDR in pH 5.9 arsenate–succinate buffer containing 10% DMSO as previously described.[2,5] [d] Data from ref 2. [e] Synthesis: see B. R. Baker and M. Kawazu, *J. Med. Chem.*, **10**, 302 (1967). [f] No inhibition at the maximum solubility which is one-fourth of the concentration indicated. [g] Synthesis: B. R. Baker and J. L. Kelley, *J. Med. Chem.*, **11**, 682 (1968). [h] A gift from Dr. D. V. Santi and A. L. Pogolotti, Jr. [i] Synthesis: see B. R. Baker, M. Kawazu, D. V. Santi, and T. J. Schwan, *J. Med. Chem.*, **10**, 304 (1967). [j] Uracil substituent. [k] Estimated from the inhibition observed at the maximum solubility which is lower.

activity maximized at phenoxybutyl (**18**) which was 100-fold more effective than methyl (**2**).

Extensive studies were then performed on enhancement of binding by substituents on the 1-benzyl group of **10**. The first group contained additional hydrocarbon substituents. The α- and β-naphthylmethyl derivatives (**20**, **21**) were no more effective than 1-benzyl (**10**). In contrast, a m-phenyl substituent (**22**) gave a 15-fold increment in binding over the parent 1-benzyl substituent of **10**; this increment was lost when another ring was inserted on **22** to give a phenanthrene derivative **23**, even though the α-naphthyl substituent of **20** was as effective as the benzyl substituent of **10**; this result would indicate that the terminal phenyl substituent of **22** is not planer to the benzyl group when complexed to the enzyme.

A simple m-Me group of **24** gave a 6-fold increment in binding over **10**, but a p-Me (**25**) gave a 2-fold loss in binding. The 3,5-Me_2 derivative (**26**) gave less than a 2-fold increment in binding over m-Me (**24**). Surprisingly, m-Cl (**27**) was 3-fold less effective than m-Me (**24**) indicating that an electron-withdrawing substituent could be detrimental to binding; such a suggestion was supported by the loss in binding caused by the m-NO_2 group of **28**. However the strong electron-donating, but polar, m-OH group of **29** being no more effective than H (**10**) indicated that both σ (electronic) and π (polarity) effects were involved;[7] that is, electron-withdrawing groups appeared to be detrimental to binding of the benzyl moiety, but nonpolar groups could interact with the enzyme by hydrophobic bonding.

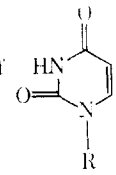
A m-OCH_3 substituent (**31**) gave an 8-fold increment in binding over H (**10**); this increment is probably a combination of hydrophobic interaction by the Me of MeO plus a smaller electron-donating effect on the binding of the phenyl moiety. A further 3-fold increment in hydrophobic bonding occurred with the C_2H_5O group of **32** compared with **31**; this hydrophobic interaction was not increased further by larger aryloxy or aralkyloxy groups (**33–37**). The OR groups of **38–40** on *ortho* or *para* positions were considerably less effective than the corresponding groups on the *meta* position.

Some studies were than performed to see if 5 substituents could increase binding to the enzyme by a 1-substituted uracil. 1-Butyluracil (**4**) was selected for study rather than 1-benzyluracil (**10**) for synthetic reasons; since it was planned to introduce groups by electrophilic substitution on the 5 position of a 1-substituted uracil as the easiest synthetic entree, a 1 substituent that could not undergo electrophilic substitution was selected. Introduction of 5-Br (**41**), 5-NO_2 (**42**), or 5-SO_2Cl (**43**) led to a huge loss in binding compared with **4**, though 5-Br or 5-NO_2 substituents on uracil gave good increments in binding.[8] However, when the SO_2Cl group of **43** was converted into the N-butylamide **44** better binding was observed; the binding was further enhanced by an N-phenylamide **45**. These results indicated that a hydrophobic bonding region might underlie the hydrocarbon moieties at the 5 position when **44** and **45** were complexed with the enzyme; that such is indeed the case is presented in the paper that follows.[8]

(7) T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, **86**, 5175 (1964).

(8) B. R. Baker and J. L. Kelley, *J. Med. Chem.*, **13**, 461 (1970), paper CLXXI of this series.

TABLE II

Physical Properties of 

No.	R	Method[a]	Yield, %	Mp, °C	Formula[b]
3	n-C_3H_7	c	20	120–123[c,d]	
18	$C_6H_5O(CH_2)_4$	A	55[e]	133–134	$C_{14}H_{16}N_2O_3$
19	$C_6H_5O(CH_2)_5$	A	50[e]	152–154	$C_{15}H_{18}N_2O_3$
20	α-$C_{10}H_7CH_2$	A	70	262–266[f]	$C_{15}H_{12}N_2O_2$
21	β-$C_{10}H_7CH_2$	A	92	200–203[g]	$C_{15}H_{12}N_2O_2$
22	m-$C_6H_5C_6H_4CH_2$	B,A	51[h]	215–216[i]	$C_{17}H_{14}N_2O_2$
23	1-Phenanthryl-CH_2	B,A	35[h]	297–299[j]	$C_{19}H_{14}N_2O_2$
24	m-$CH_3C_6H_4CH_2$	A	71	149–150[k]	$C_{12}H_{12}N_2O_2$
25	p-$CH_3C_6H_4CH_2$	A	29[k]	169–170	$C_{12}H_{12}N_2O_2$
26	3,5-$(CH_3)_2C_6H_3CH_2$	A[l]	11[k]	189–191	$C_{13}H_{14}N_2O_2$
27	m-$ClC_6H_4CH_2$	A	32[k]	167–168	$C_{11}H_9ClN_2O_2$
31	m-$CH_3OC_6H_4CH_2$	C,[m]A	37[i]	142–144	$C_{12}H_{12}N_2O_3$
32	m-$C_2H_5OC_6H_4CH_2$	C,A	63	132–133[n]	$C_{13}H_{14}N_2O_3$
33	m-$C_6H_5OC_6H_4CH_2$	B,[o]A	50	132–134[p]	$C_{17}H_{14}N_2O_3$
34	m-$C_6H_5CH_2OC_6H_4CH_2$	C,A	68[q]	174–175[r]	$C_{18}H_{16}N_2O_3$
35	m-$C_6H_5(CH_2)_2OC_6H_4CH_2$	C,A	33[s]	91–92[n]	$C_{19}H_{18}N_2O_3$
36	m-$C_6H_5(CH_2)_3OC_6H_4CH_2$	C,A	73	140–143[n]	$C_{20}H_{20}N_2O_3$
37	m-$C_6H_5(CH_2)_4OC_6H_4CH_2$	C,A	48	77–78[n]	$C_{21}H_{22}N_2O_3$
38	o-$CH_3OC_6H_4CH_2$	C,[m]A	47[i]	165–166	$C_{12}H_{12}N_2O_3$
39	p-$C_6H_5OC_6H_4CH_2$	B,[o]A	33	146–149	$C_{17}H_{14}N_2O_4$
40	p-$C_6H_5CH_2OC_6H_4CH_2$	A	63[t]	189–190	$C_{18}H_{16}N_2O_4$

[a] A: Uracil was alkylated by the previously described general method[10] except that the reaction mixture was simply stirred overnight at ambient temperature; see Experimental Section for other procedures. [b] Analyzed for C, H, and N. [c] Prepared according to D. T. Browne, J. Eisinger, and N. J. Leonard, *J. Amer. Chem. Soc.*, **90**, 7302 (1968), who have recorded mp 121.5–123°. [d] Recrystallized from CCl_4–EtOH. [e] Recrystallized from EtOAc. [f] Recrystallized from dioxane. [g] Recrystallized from EtOAc–dioxane. [h] Crude product was digested with EtOH, collected, and washed (EtOH). [i] Recrystallized from EtOH–MeOEtOH. [j] Recrystallized from MeO EtOH. [k] Recrystallized from EtOAc–EtOH. [l] For the starting chloride see R. Fuchs and D. M. Carlton, *J. Amer. Chem. Soc.*, **85**, 104 (1963). [m] The benzyl alcohol was commercially available. [n] Recrystallized from EtOAc–petroleum ether (bp 60–110°). [o] For the starting phenyltolylether see F. Ullmann and P. Sponagel, *Justus Liebigs Ann. Chem.* **350**, 83 (1906). [p] Recrystallized from EtOH.

A few of the compounds were checked as inhibitors of *E. coli* B thymidine phosphorylase which can also cleave FUDR to FU.[5] For example, **22** showed no inhibition of the *E. coli* enzyme at 100 μM, **45** had $I_{50} = 2500\ \mu M$, and **10** had been previously observed[9] to have $I_{50} = 2300\ \mu M$.

The best inhibitors in Table I of Walker 256 uridine phosphorylase were uracils substituted on the 1 position by m-$ROC_6H_5CH_2$ groups (**32–37**) where R is larger than Me; these were complexed to the enzyme about 300-fold better than uracil and about 40-fold better than the substrate, FUDR. Furthermore, the equal binding by the C_2H_5O group of **32** and the $C_6H_5(CH_2)_nO$ group of **32–37** indicates that there is bulk tolerance within the enzyme–inhibitor complexes for these large groups, a necessary prerequisite for the design of active-site directed irreversible inhibitors.[10]

Chemistry.—The 1-substituted uracils were synthesized by alkylation of excess uracil with the appropriate phenoxyalkyl bromide or benzyl halide in DMSO in the presence of K_2CO_3.[11] The literature method was slightly modified in that the mixture was simply stirred overnight at ambient temperature (Table II).

The necessary halides, if not commercially available, were synthesized by one of two methods. For **32** and **34–37** the Na salt of m-hydroxybenzyl alcohol was treated with the appropriate alkyl or aralkyl halide in DMF. This intermediate alcohol, as a homogeneous oil, readily gave the benzyl chlorides upon treatment with $SOCl_2$ in $CHCl_3$. In the case of **22**, **23**, **33**, and **39** the appropriate methyl derivative was brominated with NBS in CCl_4 to give the desired benzyl bromides. In either case the halides were used without further purification for alkylation of excess uracil.

1-(m-Hydroxybenzyl)uracil (**29**) was readily available from **34** by treatment with 30% anhydrous HBr–AcOH to afford the phenol in 51% yield.

Electrophilic substitution of **4**[9] afforded the 5-substituted uracils **41–43**. Bromination of **4** in AcOH with pyridinium hydrobromide perbromide[2] afforded **41** in 77% yield while nitration in concentrated H_2SO_4 with fuming HNO_3 gave **42** in good yield. Chlorosulfonation of **4** in chlorosulfonic acid afforded **43** which could be readily converted into **44** or **45** upon treatment with the appropriate amine in DMF in the presence of Et_3N.

Experimental Section[12]

p-Phenoxybenzyl Bromide (Method B). A mixture of 0.502 g (2.7 mmol) of 4-tolyl phenyl ether,[13] 0.537 g (3.0 mmol) of NBS,

(9) B. R. Baker and M. Kawazu, *J. Med. Chem.*, **10**, 302 (1967), paper LXXVI of this series.

(10) Reference 6b, pp 18–21, 116–121.

(11) (a) B. R. Baker and G. B. Cbbeda, *J. Pharm. Sci.*, **54**, 25 (1965); (b) B. R. Baker, M. Kawazo, D. V. Savti, and T. J. Schwan, *J. Med. Chem.*, **10**, 304 (1967); (c) B. R. Baker and J. L. Kelley, *ibid.*, **11**, 682 (1968).

(12) Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample had ir and uv spectra compatible with their assigned structures and each moved as a single spot on tle on Brinkman silica gel GF. The analytical samples gave combustion values for C, H, and N within 0.4% of theory.

(13) F. Ullmann and P. Sponagel, *Justus Liebigs Ann. Chem.*, **350**, 83 (1960).

10 mg of benzoyl peroxide, and 10 ml of CCl_4 was refluxed with stirring for 19 hr. The cooled solution was filtered through a Celite pad which was then washed with CCl_4. The combined filtrate and washings were spin evaporated *in vacuo* to leave an oil which moved as one major spot on tlc with EtOAc–petroleum ether (bp 60–110°) (1:18) and gave a positive test for active halide.[14] The oil was used without further purification.

***m*-Phenylpropoxybenzyl Chloride (Method C).**—To a stirred solution of 0.541 g (10.0 mmol) of NaOMe dissolved in 10 ml of absolute EtOH was added 1.24 g (10.0 mmol) of 3-hydroxybenzyl alcohol. After 5 min the solvent was spin evaporated *in vacuo*. The residue was dispersed in 10 ml of DMF, 1.86 g (9.3 mmol) of 3-bromopropylbenzene was added, and the mixture was heated on a steam bath for 1 hr. The cooled mixture was diluted with 25 ml of H_2O and was extracted with three 25-ml portions of $CHCl_3$. The combined organic extracts were washed with two 25-ml portions of 0.5 *N* NaOH and 25 ml of brine, and then dried ($MgSO_4$). The solvent was spin evaporated *in vacuo* (finally at ~1 mm) leaving an oil in nearly quantitative yield which moved as a single new spot on tlc in C_6H_6–EtOH (3:1).

The oil was dissolved in 20 ml of dry $CHCl_3$, warmed slightly on a steam bath and then treated with 2 ml of $SOCl_2$. The solution was stirred at ambient temperature for 1 hr during which time the evolution of gases ceased. The solvent was spin evaporated *in vacuo*, and the residue was redissolved in ~20 ml of benzene, then spin evaporated again. This was repeated several times to give a semisolid, greenish yellow oil in quantitative yield which moved as a single spot on tlc in EtOAc–petroleum ether (1:2) and gave a positive test for active halide.[14] The chloride was used without further purification.

1-(*m*-Hydroxybenzyl)uracil (29).—A solution of 1.37 g (4.4 mmol) of **34** and 16 ml of 30% anhydrous HBr–AcOH was stirred at ambient temperature for 19 hr. The solution was diluted with 100 ml of H_2O, then extracted with three 25-ml portions of $CHCl_3$, and spin evaporated *in vacuo*. The residue was dissolved in 50 ml of THF; the solution was filtered and spin evaporated. The residue was dissolved in a few milliliters of EtOAc and diluted with petroleum ether. The resultant solid was collected and recrystallized from EtOAc–petroleum ether; yield, 0.499 g (51%), mp 186–189°. Recrystallization of a portion from EtOAc–EtOH gave white rosettes, mp 188–191°. *Anal.* ($C_{11}H_{10}N_2O_3$) C, H, N.

5-Bromo-1-(*n*-butyl)uracil (41).—This compound was prepared from **4**[9] as previously described[2] for 6-benzylaminouracil; yield, 0.188 g (77%), mp 179–181°. The analytical sample was recrystallized from $CHCl_3$–petroleum ether to give transparent plates, mp 181–182°. *Anal.* ($C_8H_{11}BrN_2O_2$) C, H, N.

1-(*n*-Butyl)-5-nitrouracil (42).—To a stirred solution of 0.260 g (1.5 mmol) of **4** and 1 ml of concentrated H_2SO_4 was added 0.75 ml of red fuming HNO_3. After 0.5 hr at ambient temperature the reaction was quenched with ~10 g of crushed ice. The product was collected, washed with H_2O, and then recrystallized from H_2O; yield, 0.140 g (43%), mp 157–159°. The analytical sample had mp 158–159°. *Anal.* ($C_8H_{11}N_3O_4$) C, H, N.

1-(*n*-Butyl)-5-chlorosulfonyluracil (43).—A solution of 0.254 g (1.5 mmol) of **4** in 2 ml of $HOSO_2Cl$ was refluxed with stirring for 7 hr, cooled and then carefully added to about 40 g of crushed ice. The product was collected, washed with H_2O, then dried over P_2O_5. Recrystallization from $CHCl_3$ gave 0.198 g (49%) of white flakes, mp 176–184°, which was uniform on tlc with C_6H_6–EtOH (3:1). The analytical sample had mp 186–188° (if block preheated to 180°). *Anal.* ($C_8H_{11}ClN_2O_4S$) C, H, N.

1-(Butyl)-5-[*N*-(*n*-butyl)sulfamoyl]uracil (44).—To a stirred mixture of 81 mg (1.1 mmol) of *n*-$BuNH_2$, 110 mg (1.1 mmol) of Et_3N and 0.5 ml of DMF, cooled in an ice bath was added a solution of 0.267 g (1.0 mmol) of **43** in 0.5 ml of DMF. After 3 hr the resultant mixture was diluted with 5 ml of ice water and acidified to pH 1 with 1 *N* HCl. The product was collected, washed (H_2O), and then recrystallized from EtOH–H_2O; yield, 73 mg (24%) of soft white threads, mp 175–176°. *Anal.* ($C_{12}H_{21}N_3O_4S$) C, H, N.

1-(*n*-Butyl)uracil-5-sulfonanilide (45).—This compound was prepared by the same method as **44** using aniline; yield, 97 mg (32%) of yellow rosettes from *i*-PrOH, mp 192–196°. *Anal.* ($C_{14}H_{17}N_3O_4S$) C, H, N.

(14) B. R. Baker, D. V. Santi, J. K. Coward, H. S. Shapiro, and J. H. Jordaan, *J. Heterocycl. Chem.*, **3**, 425 (1966).

Irreversible Enzyme Inhibitors. CLXXI.[1,2] Inhibition of FUDR Phosphorylase from Walker 256 Rat Tumor by 5-Substituted Uracils

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The inhibition of uridine phosphorylase from Walker 256 rat tumor, which can also cleave 5-fluoro-2'-deoxyuridine (FUDR) to FU, by 36 5-substituted uracils has been investigated. Strong hydrocarbon interaction with 5-benzyl (**9**) and 5-phenylbutyl (**12**) substituents was observed. Inhibition could be further enhanced by substitution of alkoxy groups on the *meta*-position of the benzyl moiety; the strongest inhibitors had *m*-CH_3O (**33**), *m*-C_2H_5O (**35**), or *m*-$C_6H_5CH_2O$ (**37**) groups and complexed to the enzyme 200–800 times more effectively than the substrate, FUDR.

There are two enzymes that start the detoxification of 5-fluoro-2'-deoxyuridine (FUDR) by cleavage to 5-fluorouracil (FU), namely, thymidine phosphorylase (EC 2.4.2.4)[3–5] and uridine phosphorylase (EC 2.4.-2.3).[4–6] Walker 256 rat tumor contains an FUDR cleaving enzyme that apparently is only uridine phosphorylase.[7] The latter can be inhibited by 6-aralkylaminouracils which could complex as much as 4-fold better than the substrate FUDR;[8] the aralkyl group apparently interacted with this uridine phosphorylase by hydrophobic bonding.[8] A much stronger hydrophobic interaction was observed with 1-aralkyl derivatives of uracil; the latter could complex as much as 180-fold better than the substrate, FUDR.[2] In the previous paper[2] we also observed that a hydrophobic

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(2) For the previous paper of this series see B. R. Baker and J. L. Kelley, *J. Med. Chem.*, **13**, 458 (1970).

(3) B. R. Baker, *ibid.*, **10**, 297 (1967), paper LXXV of this series.

(4) B. Preussel, G. Etzold, D. Bärwolff, and P. Langen, *Biochem. Pharmacol.*, **18**, 2035 (1969) and references therein.

(5) P. Langen, G. Etzold, D. Bärwolff, and B. Preussel, *ibid.*, **16**, 1833 (1967).

(6) P. Langen and G. Etzold, *Biochem. Z.*, **339**, 190 (1963).

(7) (a) M. Zimmerman, *Biochem. Biophys. Res. Commun.*, **16**, 600 (1964); (b) see T. A. Krenitsky, J. W. Mellors, and R. K. Barclay, *J. Biol. Chem.*, **240**, 1281 (1965), for a more proper interpretation of Zimmerman's results.

(8) B. R. Baker and J. L. Kelley, *J. Med. Chem.*, **13**, 456 (1970), paper CLXIX of this series.