amine followed by acid-catalyzed ring closure with ethyl orthoformate (method A).[34] The intermediate 6-chloropurines and 6-substituted aminopyrimidines were not isolated. See Table III.

Experimental Section

Assay of Guanosine Phosphorylase.—Purine nucleoside phosphorylase (1 mg/ml) from calf spleen was purchased from Calbiochem; for assay 75 μl of the soln was dild with 5 ml of H_2O and kept at 0°. When assayed as described below the initial OD change of about 0.015 unit/min was followed on a Gilford recording spectrophotometer with a 0–0.1 OD slidewire at 260 mμ.

In a 3-ml quartz cuvette were placed 1.60 ml of 0.2 *M* arsenate–succinate buffer of pH 5.9, 0.60 ml of 0.2 m*M* aq guanosine, 0.40 ml of H_2O, and 0.30 ml of DMSO. The contents were thoroughly mixed, then a base line run; if the base line was not 0 due to inadequate mixing of the DMSO, further mixing was made until the base line was 0. Then 100 μl of enzyme soln was added, the contents were quickly mixed, and the initial OD change was noted by drawing a tangent to 0 time. Since curvature is fairly rapid it is inadvisable to run more than 1 cuvette at a time. Inhibitors were dissolved in DMSO. The cuvette concn of guanosine was 0.04 m*M*; its K_m was found to be 57 μ*M* by the reciprocal plot method.

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Irreversible Enzyme Inhibitors. 188.[1,2] Inhibition of Mammalian Thymidine Phosphorylase

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Eighty uracils containing hydrophobic substituents at the 1, 5, or 6 positions were investigated as inhibitors of rabbit liver thymidine phosphorylase to try to detect a hydrophobic bonding region adjacent to the active site. Such a region was best detected by uracils containing a 2,4-dimethylanilino, phenethylamino, or phenylbutylamino substituent at the 6 position. Comparison of the inhibition profiles of the thymidine phosphorylase from rabbit liver and *Escherichia coli* B showed that this hydrophobic bonding region had undergone immense evolutionary change between the 2 species. As a result several of the compds were far more effective on the *E. coli* B enzyme than the mammalian enzyme; for example, 6-(α-naphthylmethylamino)uracil was complexed 900-fold better to the bacterial enzyme than the mammalian enzyme.

There are two enzymes in mammalian tissues that can phosphorylyse 5-fluoro-2′-deoxyuridine (FUDR) to FU and 2-deoxy-D-ribose 1-phosphate; these are uridine–deoxyuridine phosphorylase (EC 2.4.2.4)[3-5] and thymidine phosphorylase (EC 2.4.2.3).[3,4,6] Some mammalian tissues such as rat liver contain both enzymes which can be separated by chromatography on DEAE-Sephadex.[5,7,8] Other tissues such as horse liver,[9] 14 human tumors,[10,11] human leukocytes,[12] human spleen,[10a] mouse liver,[4] and rabbit liver[13] apparently contain only the thymidine phosphorylase (EC 2.4.2.4.). In contrast, rodent tumors[10b] such as Walker 256 carcinoma,[8,10a] Dunning hepatoma,[10a] Novikoff hepatoma,[10a] Morris 5123 hepatoma,[11] Morris 3683 hepatoma,[11] and Butter yellow induced hepatoma[11] in the rat and Ehrlich ascites[11] and Ehrlich Lettre ascites[11] in the mouse apparently contain only the uridine phosphorylase (EC 2.4.2.3).

1-(2-Deoxy-β,D-glucopyranosyl)thymine is a good inhibitor of the uridine phosphorylase,[5,10a] but shows no inhibition of the thymidine phosphorylase;[5] thus the 2 enzymes can be readily differentiated with this inhibitor.[5] The glucosylthymine complexed to the uridine phosphorylase from Ehrlich ascites 20-times better than the substrate, thymidine.[5] 5-Benzyluracil has been reported from this laboratory to be an excellent inhibitor of the uridine phosphorylase from Walker 256 carcinoma, being complexed 80-fold better than the substrate FUDR; introduction of a *m*-EtO or *m*-$C_6H_5CH_2O$ substituent increased the potency to 300- and 800-fold, resp, better than the substrate.[8] These 5-benzyluracils can also be used to distinguish between uridine phosphorylase, which is strongly inhibited, and mammalian thymidine phosphorylase which is not (Table II).

Considerable effort was devoted to develop inhibitors of thymidine phosphorylase from *Escherichia coli* B before it was fully realized that a hydrophobic bonding region adjacent to an active site could have undergone considerable evolutionary change between species;[14-16] these evolutionary differences between species of a hydrophobic region are readily detected by inhibitors bearing appropriate hydrophobic groups and differential binding as large as 10^4 can be observed.[14-16] Systematic studies on the thymidine phosphorylase from

(1) This work was generously supported by Grant No. CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For paper 187 of this series see B. R. Baker and J. C. Schaeffer, *J. Med. Chem.*, **14**, 809 (1971).

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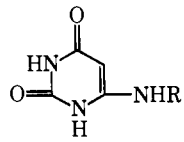
(16) B. R. Baker, *J. Med. Chem.*, **10**, 912 (1967).

E. coli B included the mode of pyrimidine binding,[17] the location and mapping of the hydrophobic bonding areas with 1-substituted[18] or 6-substituted uracils,[19-22] the effect of acidity of the uracils on binding,[23,24] and irreversible inhibition.[25] Extremely potent inhibitors emerged such as **3**–**6**[21,22] which complexed to the enzyme 450-, 1100-, 2000-, and 5000-times better, resp, than the substrate FUDR.



3, R = 2,3-$Me_2C_6H_3$
4, R = 2,3-$Cl_2C_6H_3$
5, R = α-$C_{10}H_7CH_2$
6, R = α-(7-$ClC_{10}H_6$)CH_2

Langen, *et al.*,[4] confirmed our results with **4** on *E. coli* B thymidine phosphorylase; they also observed that **4** at its maximum solubility (0.1 m*M*) showed no inhibition of the thymidine phosphorylase from human spleen when assayed with 1 m*M* FUDR.

We have now undertaken a study to find a hydrophobic bonding region on a mammalian thymidine phosphorylase; such a hydrophobic region has been found with 5- and 6-substituted uracils, but this region is obviously greatly mutated from the corresponding region on the *E. coli* B enzyme. The results are the subject of this paper.

Enzyme Results.—Although Langen, *et al.*,[4] could detect no inhibition by 100 μM **4** of a mammalian thymidine phosphosphorylase under their assay conditions using 1 m*M* FUDR as substrate, we could detect inhibition of rabbit liver thymidine phosphorylase under our assay conditions of 0.4 m*M* FUDR and 10% DMSO; **4** had I_{50} = 170 μM in our test system (Table I). These 2 results are essentially in agreement, the important observation being the 490-fold less effective binding of 6-(2,3-dichloroanilino)uracil (**4**) to a mammalian enzyme compared to the *E. coli* B enzyme.[21] Similarly, 6-(2,3-dimethylanilino)uracil (**3**) was complexed to the *E. coli* B enzyme[21] 270-fold better than the rabbit liver enzyme. That this difference in binding to the enzyme from the 2 species was due to less effective binding of the anilino moiety was shown as follows.

Uracil (**1**), a substrate for the reversible reaction, was complexed equally effectively to both enzymes—a result to be expected with a classical-type enzyme inhibitor.[14,16,26] Introduction of the 6-anilino group (**2**) gave less than a 2-fold increment in binding to the rabbit liver enzyme; unfortunately, an I_{50} could not be obtained for **2**, the base-line compound, due to lack of solubility. In contrast, **2** was 38-fold more effective on the *E. coli* B enzyme than uracil (**1**); that is, **2** was > 25-fold more effective on the *E. coli* B enzyme than on the rabbit liver enzyme. Since this anilino group is believed to complex to a hydrophobic bonding region adjacent to the active site,[21] large differences in binding between diverse species are now known to be expected.[14-18]

Similarly, the highly potent inhibitor (**5**) with an α-naphthylmethylamino side chain[22] was 900-fold less effective on the rabbit liver enzyme. Since the side chain of **5** can be considered to be a 2,3-benzo derivative of 6-benzylamino (**28**), this 900-fold difference between species is due to the inability of 6-benzylamino group to interact with the enzyme, **28** being about as effective as uracil. Thus the 2,3-benzo moiety on **5** gives a 10-fold increment in binding to the rabbit liver enzyme; this structural change (**28** *vs.* **5**) gave a 30-fold increment in binding to the *E. coli* B enzyme.

The lack of an I_{50} for the base-line anilinouracil (**2**) makes comparison of substituent effects difficult. Introduction of a 2-Me group (**9**) enhances binding to the liver enzyme by > 2-fold and the *E. coli* B enzyme by 13-fold. Binding is enhanced 4-fold to the liver enzyme with 2-Cl (**10**) compared to 2-Me (**9**) and enhanced 2-fold with 2-Et (**12**). In contrast, a 3-fold loss in binding to the liver enzyme occurs with 2-MeO (11); similar increments are seen with the *E. coli* B enzyme.

Enhanced binding can also be seen by substituents at the 4 position, but the increments vary considerably for the enzymes from the two species. The 4-Et group (**16**) gives a > 3-fold increment in binding to the liver enzyme compared to **2** and a 3-fold increment in binding to the *E. coli* B enzyme, the difference in binding between the 2 enzymes being only 20-fold—considerably less difference than observed with **3**, **4**, or **9**. Replacement of the 4-Et group of **16** by Cl (**15**) gives no change in binding to the *E. coli* B enzyme, but a 3-fold loss in binding to the liver enzyme. Replacement of the 4-Et group of **16** by 4-EtO (**17**) enhances binding to both enzymes about 2-fold. A striking difference between the 2 enzymes occurs with the 4-C_4H_9-*n* (**18**) and 4-C_6H_5 (**19**) substituents; both changes lead to a loss in binding (compared to **16**) to the liver enzyme, but a gain in binding to the *E. coli* B enzyme. Similarly, the 3-C_6H_5 substituent of **14** gives much better binding to the *E. coli* B enzyme than the liver enzyme. It would appear that the inline, if not inplane, Ph groups complex well with the large, flat hydrophobic region[21] on the *E. coli* enzyme, but the liver enzyme is not flat in this area of the hydrophobic region.

The effect of 2 substituents was then investigated and the 2-Me-anilino group (**9**) can be used as a base line for comparison. Insertion of a 3-Me (**3**) gives a 2-fold increment in binding to the liver enzyme, but a 5-Me (**21**) gives no change. Insertion of a 4-Me group (**20**) gives an 8-fold increment in binding to the liver enzyme, but only a 2-fold increment to the *E. coli* B enzyme; similar results were observed on insertion of a 6-Me (**22**). The fact that 6-(2,6-dimethylanilino)uracil complexes better to both enzymes than 2-Me (**9**) has been interpreted to mean that the Ph and uracil are complexed to the enzyme in perpendicular planes.[21]

Whereas α-naphthyl (**23**) and β-naphthyl (**24**) are equally effective in complexing to the *E. coli* B enzyme, β is at least 8-fold more effective than α on the liver enzyme; since 2,3-Me_2 groups (**3**) add to binding, this result with **23** indicates that there is not bulk tolerance within the enzyme–inhibitor complex for all 4 C's of the

(17) B. R. Baker and M. Kawazu, *J. Med. Chem.*, **10**, 313 (1967).
(18) B. R. Baker and M. Kawazu, *ibid.*, **10**, 302 (1967).
(19) B. R. Baker and M. Kawazu, *ibid.*, **10**, 311 (1967).
(20) B. R. Baker and W. Rzesotarski, *ibid.*, **10**, 1109 (1967).
(21) B. R. Baker and W. Rzesotarski, *ibid.*, **11**, 639 (1968).
(22) B. R. Baker and S. E. Hopkins, *ibid.*, **13**, 87 (1970).
(23) B. R. Baker and M. Kawazu, *ibid.*, **10**, 316 (1967).
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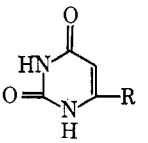
(26) B. R. Baker, *J. Med. Chem.*, **11**, 483 (1968).

TABLE I

INHIBITION[a] OF FUDR PHOSPHORYLASE FROM RABBIT LIVER BY



No.	R	Rabbit liver			*E. coli*	Liver/
		Inhib, μM	V_0/V_I[b]	I_{50},[c] μM	I_{50},[c] μM	*E. coli* B
1	H			1900	1500[d]	1.3
2	NHC_6H_5	250[e]	0.90	>1000[f]	40[g]	>25
3	NHC_6H_3-2,3-Me_2			240	0.89[h]	270
4	NHC_6H_3-2,3-Cl_2			170	0.35[h]	490
5	$NHCH_2C_{10}H_7$-α	75[e]	1.40	~200	0.21[i]	900
7	NH_2			500		
8	$NHCH_3$			2100		
9	NHC_6H_4-2-Me			560	3.1[h]	180
10	NHC_6H_4-2-Cl			130	2.1[h]	62
11	NHC_6H_4-2-OMe	500	1.32	~1500	20[h]	75
12	NHC_6H_4-2-Et			300	3.1[h]	97
13	NHC_6H_4-3-Me	500	1.20	>2000	11[h]	>180
14	NHC_6H_4-3-C_6H_5	100[e]	1.23	~500	0.93[h]	540
15	NHC_6H_4-4-Cl	300	1.36	~900	14[h]	64
16	NHC_6H_4-4-Et			300	15[h]	20
17	NHC_6H_4-4-OEt			170	6.7[h]	25
18	NHC_6H_4-4-C_4H_9-n	300	1.2	>1200	7.4[h]	>160
19	NHC_6H_4-4-C_6H_5	300	1.2	>1200	1.9[h]	>630
20	NHC_6H_4-2,4-Me_2			74	1.5[h]	49
21	NHC_6H_4-2,5-Me_2			460	4.2[h]	110
22	NHC_6H_4-2,6-Me_2			130	1.8[h]	72
23	$NHC_{10}H_7$-α	300	1.2	>1200	1.6[h]	>750
24	$NHC_{10}H_7$-β	50[e]	1.38	~150	2.2[h]	68
25[j]	NHC_4H_9-n			1200		
26[j]	NHC_5H_{11}-n	100[e]	1.19			
27	NH-cyclohexyl	300	1.0	>1200	1600[h]	
28	$NHCH_2C_6H_5$	1000[e]	1.50	2000	6.2[h]	320
29	$N(CH_2C_6H_5)_2$			600	37[h]	16
30	$NHCH(CH_2C_6H_5)C_6H_5$	300	1.23	~1500	78[h]	
31	$NHCH(C_6H_5)_2$			520	38[h]	14
32	$NHCH_2C_6H_5$-2,5-Me_2	125[e]	1.2	>600	6.7[h]	>90
33[j]	$NH(CH_2)_2C_6H_5$			110	130[g]	0.85
34[j]	$NH(CH_2)_3C_6H_5$			110	48	2.3
35[j]	$NH(CH_2)_4C_6H_5$			53		
36[j]	$NH(CH_2)_5C_6H_5$	30[e]	1.24			
37[j]	$NH(CH_2)_3OC_6H_5$			220		
38[j]	$NH(CH_2)_4OC_6H_5$			230		
39	OC_6H_5			830	23[g]	36
40	SC_6H_5			190	4.2[g]	45
41	$SO_2C_6H_5$	300	1.0	>1200	28[g]	>43
42	C_6H_5	300	1.01	>1200	>2500[d]	
43	$CH_2C_6H_5$	300	1.02	>1200	90[d]	>13
44	$(CH_2)_2C_6H_5$	300	1.01	>1200	240[d]	
45	$(CH_2)_3C_6H_5$	300	1.01	>1200	450[d]	

[a] The technical assistance of Maureen Baker, Julie Beardslee, Nancy Middleton, and Pauline Minton with these assays is acknowledged. [b] V_0/V_I = velocity with no inhibitor/velocity with inhibitor; at I_{50}, V_0/V_I = 2. Rabbit liver acetone powder extd with 10 ml/g of Tris buffer (pH 7.4) and stored in aliquots of 1.8 ml at −15°. [c] I_{50} = concn for 50% inhibition when assayed with 400 μM FUDR in pH 5.9 arsenate–succinate buffer contg 10% DMSO as previously described.[6] [d] Data from ref 19. [e] Maximum solubility. [f] Since 20% inhibition (V_0/V_I = 1.25) is readily detected, the I_{50} is > 4 times concn measured. [g] Data from ref 20. [h] Data from ref 21. [i] Data from ref 22. [j] See ref 27 for synth.

benzo moiety of **23**—a sharp contrast with the *E. coli* B enzyme.

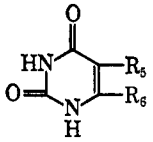
A series of 6-arylamino- and 6-alkylaminouracils available from another study in this laboratory[27] was investigated for inhibition of rabbit liver thymidine phosphorylase. The n-C_5H_9 group of **25** gave a 2-fold enhancement in binding compared to the 6-MeNH group of **8**; any enhancement in binding compared to **25** by increasing the chain length to n-C_6H_{11} (**26**) could not be determined due to insolubility.

No gain in binding occurred with the $C_6H_5CH_2NH$ group of **28** compared to MeNH (**8**), as previously discussed. When the chain length was increased to $C_6H_5(CH_2)_2NH$ (**33**) or $C_6H_5(CH_2)_3NH$ (**34**) binding was enhanced 19-fold compared to 6-MeNH (**8**). This was enhanced to 40-fold with $C_6H_5(CH_2)_4NH$ (**35**), but $C_6H_5(CH_2)_5NH$ (**36**) was not as good as **35**. The $C_6H_5O(CH_2)_3NH$ of **37** was 4-fold less effective than the

(27) B. R. Baker and J. L. Kelley, *J. Med. Chem.*, **13**, 456 (1970).

TABLE II

INHIBITION[a] OF FUDR PHOSPHORYLASE FROM RABBIT LIVER BY



No.	R_5	R_6	Inhib, μM	V_0/V_1[b]	I_{50},[c] μM
1	H	H			1900
7	H	NH_2			500
46	NO_2	H			43
47	Br	H			180
48	CH_3	H			200
49[d]	CH_3	NH_2			25
50	Br	$NH(CH_2)_2C_6H_5$[h]			110
51[e]	C_6H_5	H	300	1.2	
52[f]	$CH_2C_6H_5$	H	300	1.0	
53[f]	$(CH_2)_2C_6H_5$	H	300	1.0	
54[f]	$(CH_2)_3C_6H_5$	H	300	1.2	
55[f]	$(CH_2)_4C_6H_5$	H			150
56[g]	SC_6H_5	H	300	0.90	
57[g]	OC_6H_5	H	300	1.2	
58[g]	NHC_6H_5	H	300	1.1	
59[g]	$NHCH_2C_6H_5$	H	300	1.0	
60[g]	$NH(CH_2)_2C_6H_5$	H	300	1.1	
61[g]	$NH(CH_2)_3C_6H_5$	H	300	1.2	
62[g]	$NH(CH_2)_4C_6H_5$	H	100[i]	1.0	
63[g]	SO_2-NHC_6H_5	H	300	1.23	~1500
64[g]	$CH_2OCH_2C_6H_5$	H	300	1.1	

[a-c] See Table I. [d] For synth see ref 4. [e] For synth see ref 28. [f] For synth see ref 29. [g] For synth see ref 8. [h] By bromination of **33** according to ref 27; yield, 54%, mp 199–200° from MeOH–H_2O. [i] Max solubility.

$C_6H_5(CH_2)_4NH$ of **35**, even though the chain length was the same; this result can be attributed to the loss of binding of the CH_2 group to the hydrophobic region when replaced by the more polar O. Even though **37** does not bind as well as **35**, **37** would be superior to **35** for study of substituent effects on the Ph binding due to easier synthesis.

Although Ph binding could not be detected with the 6-C_6H_5NH group of **2** due to insolubility, it could be detected with 6-C_6H_5O (**39**) or 6-C_6H_5S (**40**); the increments in binding compared to uracil were 2- and 10-fold, resp, some contribution being made by the less polar S of **40**.

No interaction of 6-C_6H_5 (**42**) or 6-$C_6H_5(CH_2)_n$ (**43–45**) could be detected with the rabbit liver enzyme, even though such an interaction was observed with the *E. coli* B enzyme.[19]

A search was made to detect hydrophobic bonding to the rabbit liver enzyme with 5-substituted uracils (Table II); all of these compds (**51–64**) were available from other studies in this laboratory.[8,28,29] Only the 5-phenylbutyl substituent of **55** showed hydrophobic bonding, the increment being 13-fold over uracil (**1**). No hydrophobic bonding could be detected with 17 1-substituted uracils where the substituents were $C_6H_5(CH_2)_n$,[18,30,31] $C_6H_5O(CH_2)_n$,[28,30,31] or alkyl.[18,32]

The effect of small substituents at the 5 position of uracil was then made with the rabbit liver enzyme; the binding increments were similar to those previously observed[17] with the *E. coli* B enzyme. The 5-NO_2 group of **46** gave a 44-fold increment in binding compared to uracil with the rabbit liver enzyme due to the increased acidity of the 1-NH.[17] The 5-Me substituent of **48** gave a 9-fold increment in binding presumably due to a hydrophobic interaction;[17] the 5-Br substituent of **47** gave a 10-fold increment in binding presumably due to hydrophobic bonding, the weakly increased acidity apparently having no effect compared to 5-Me.[17] Introduction of a 6-NH_2 group (**7**) on uracil gave a 4-fold increment in binding, while a 6-NH_2 group (**49**) introduced on thymine (**48**) gave an 8-fold increment. Similar results were previously reported by Langen, *et al.*,[4] with inhibition of thymidine phosphorylase from horse liver; they also noted that introduction of a 6-amino group on 5-bromouracil (**47**) gave a 6-fold increment in binding. However, introduction of 5-bromo group (**50**) on 6-(phenethylamino)uracil gave no increment in binding, indicating an overlap of binding regions for Br and $C_6H_5(CH_2)_2NH$.

The following important conclusions are reached from the data in Tables I and II.

(1) Hydrophobic bonding adjacent to the active site of thymidine phosphorylase from rabbit liver and *E. coli* B can best be detected with 6-substituted uracils.

(2) The hydrophobic bonding region differs considerably in the enzyme from the 2 species due to evolutionary changes. For example, (a) the C_6H_5 group of 6-C_6H_5NH, 6-C_6H_5O, or 6-C_6H_5S binds at least 40-fold better to the *E. coli* B enzyme than the rabbit liver enzyme, (b) there is hindrance to binding to rabbit liver enzyme by large 2,3 substituents on the C_6H_5NH moiety not seen with the *E. coli* B enzyme, and (c) the hydrophobic bonding region of the *E. coli* B enzyme is essen-

(28) B. R. Baker, M. Kawazu, D. V. Santi, and T. J. Schwan, *J. Med. Chem.*, **10**, 304 (1967).
(29) B. R. Baker and J. L. Kelley, *ibid.*, **11**, 686 (1968).
(30) B. R. Baker and J. L. Kelley, *ibid.*, **11**, 682 (1968).
(31) B. R. Baker and J. L. Kelley, *ibid.*, **13**, 458 (1970).
(32) B. R. Baker and T. J. Schwan, *ibid.*, **9**, 73 (1966).

tially planar whereas the hydrophobic region near where the para position of the 6-anilino group is complexed appears to be nonplanar.

(3) Due to the differences in the hydrophobic bonding region of the enzyme in the 2 sources, several compds (**4**, **5**, **14**, **19**, **23**, **28**) show 500- to 900-fold better binding to the bacterial enzyme than the mammalian enzyme.

(4) The ability of the 4-EtO group of **17** to bind to the liver enzyme better than 4-C_4H_9-*n* of **18** or the 4-C_6H_5 of **19** due to a bend in the hydrophobic bonding area in this region is due to the greater conformational flexibility of the EtO group. If such is the case then higher alkyl or aralkyl groups may give highly potent inhibitors of the mammalian enzyme. Such studies are being pursued and already the most active inhibitor yet known has emerged; 6-(4-benzyloxy-2-methylanilino)-uracil has I_{50} = 14 μM and the $C_6H_5CH_2O$ substituent shows a 43-fold increment in binding over **9**.[33]

(5) Substituent effects on the 6-$C_6H_5(CH_2)_2NH$ (**33**), 6-$C_6H_5(CH_2)_4NH$ (**35**), 6-$C_6H_5O(CH_2)_3NH$ (**37**), and 5-$C_6H_5(CH_2)_4$ (**55**) uracils should be studied to determine how much binding can be enhanced.

(33) B. R. Baker and S. E. Hopkins, to be published.

Substituted Hydroxylaminopurines and Related Derivatives. Synthesis and Screening Tests[1]

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Treatment of 6-chloro-2-fluoropurine (I) and its 9-β-D-ribosyl derivative (II) with NH_2OH at low temp yielded 2-fluoro-6-hydroxylaminopurine (III) and its 9-β-D-ribosyl derivative IV, resp. Compds III and IV were reduced to 2-fluoroadenine (VIII) and 2-fluoroadenosine (IX) with Raney Ni. The known 2-fluoro-6-mercaptopurine (X) was conveniently prepared from I and thiourea and transformed into 2-hydroxylamino-6-mercaptopurine (XI) with NH_2OH. I and methanolic NH_3 at 100° gave VIII or 2,6-diaminopurine (XVIII), depending on the duration of the treatment. I and X yielded, by reaction with the appropriate amino derivatives, several *N*-methylhydroxylamino-, -methoxyamino-, and -methylaminopurines. Reaction of I with hydrazine gave 2-fluoro-6-hydrazinopurine (XXI). Equimolar amounts of I and XXI afforded 6,6′-bis(2-fluoroadenine) (XXII) which on Raney Ni treatment was reduced to VIII. Interaction of VIII with NH_2OH resulted in the synthesis of 2-hydroxylaminoadenine (XXIII). Reaction of 2-fluoropurine (XXIV) with NH_2OH yielded 2-hydroxylaminopurine (XXV). III and XI possessed inhibitory activity against P815 mouse leukemia, and X caused marked reduction of Ridgeway osteogenic sarcoma; IV was inactive.

New substituted purines have been prepared in continuation of studies of biologically active purine derivatives.

The synthesis of 2-fluoro-6-hydroxylaminopurine and its 9-β-D-ribofuranosyl derivative was accomplished in order to study the effect of the F atom at C_2 on the growth-inhibitory effect and toxicity of 6-hydroxylaminopurine[2] and of its 9-β-D-ribofuranosyl derivative.[3] Similarly, 2-hydroxylamino-6-mercaptopurine was prepared in order to investigate the influence of the HONH group at the C_2 of 6-mercaptopurine;[4] this compd may also be considered as the 2-*N*-OH derivative of thioguanine.[5] Several substituted *N*-methylhydroxylamino, methoxyamino, *N*-methylamino, hydrazino, and 6,6-bis(2-fluoroadenine) derivatives were synthesized as well as the 2-*N*-hydroxylamino derivative of adenine, 2-hydroxylamino-6-aminopurine, to study their potential growth-inhibitory activity.

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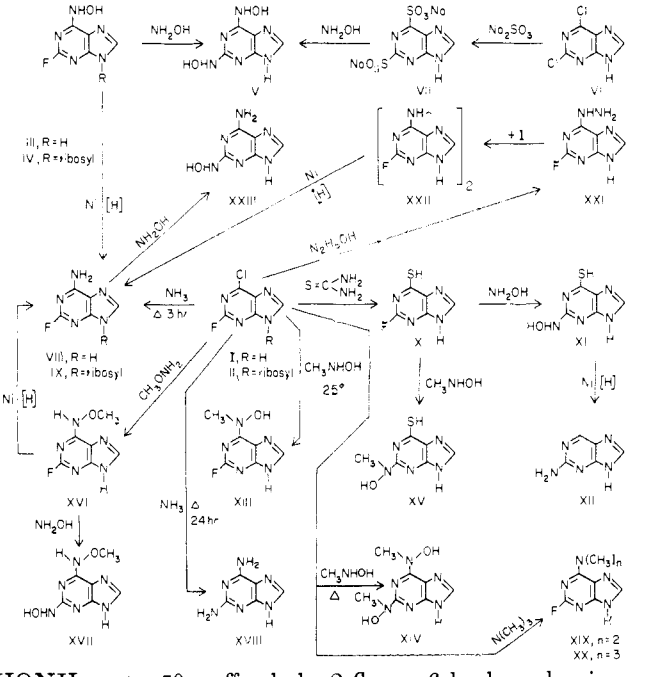
Synthetic Studies.—Treatment of 6-chloro-2-fluoropurine (I)[6] (Scheme I) with an excess of ethanolic

SCHEME I



$HONH_2$ at 5° afforded 2-fluoro-6-hydroxylaminopurine (III) in 83% yield. A minor by-product, 2,6-dihydroxylaminopurine (V),[7] was also obtained. When

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