Anal. Calcd for $C_{11}H_{10}N_6O \cdot HCl$: C, 47.4; H, 3.98; N, 30.1. Found: C, 47.1; H, 4.21; N, 29.9.

9-(*p*-Bromoacetamidophenyl)guanine (3).—A mixture of 64 mg (0.63 mmole) of triethylamine, 5 ml of DMF, and 100 mg (0.32 mmole) of **6**·2HCl was warmed to complete solution, then cooled to 0° in an ice bath. To the stirred solution was added 125 mg (0.58 mmole) of bromoacetic anhydride. After 30 min in the ice bath, the reaction mixture was poured into 25 ml of H_2O containing 53 mg of $NaHCO_3$. The crude product was collected on a filter and washed with water. A trace of the Bratton–Marshall-positive[12] **6** was removed by stirring the crude product in 0.1 *N* HCl for 30 min. The product was collected by filtration and washed with water. The compound now gave a negative Bratton–Marshall test for aromatic amine,[12] moved as a single spot on tlc with 5:3 $CHCl_3$–EtOH, and gave a positive 4-(*p*-nitrobenzyl)pyridine test for active halogen;[12] λ_{max} (pH 1), 261 mμ; (pH 13), 271 mμ.

Anal. Calcd for $C_{13}H_{11}BrN_6O_2$: C, 43.0; H, 3.05; N, 23.1. Found: C, 43.0; H, 32.7; N, 22.9.

Similarly, the *meta* isomer (**2**) was prepared in 61% yield; it had the same properties as **3** except for λ_{max} (pH 1), 254, 280 mμ (weak inflection); (pH 13), 268 mμ.

Anal. Found: C, 42.8; H, 3.34; N, 22.9.

Inactivation of Guanine Deaminase.—Guanine deaminase (guanase) from rabbit liver was purchased from Sigma Chemical Co. as a 1-mg/ml suspension; at this concentration it was reputed to deaminate 0.1 μmole of guanine/min. The inactivation experiments were performed as follows. The velocity of the enzyme reaction with 13.3 μ*M* guanine[13] was proportional to the enzyme concentration. The buffer employed was 0.05 *M* Tris (pH 7.4). The enzyme was stable at 37° for 2 hr. Bulk enzyme (1 mg/ml) (0.10 ml) as purchased was diluted with 1.90 ml of buffer. In two tubes were placed 0.95 ml of the diluted enzyme in a 37° bath. After 5 min, 50 μl of DMSO was added to tube 1 (enzyme control) and 50 μl of DMSO containing inhibitor was added to tube 2. The contents were mixed, the time was noted and an 0.5-ml aliquot was withdrawn from each tube as rapidly as possible and stored at 0° until ready for assay. The aliquot from the inhibitor tube was labeled I_1 and the aliquot from the enzyme control tube was labeled C_1. The remainder in the two tubes was then kept for 2 hr (or other chosen time) at 37°, then cooled in an ice bath until ready for assay and labeled I_2 and C_2. The amount of enzyme remaining was assayed as follows:

In a 1-ml cuvette was placed 0.70 ml of buffer and 200 μl of 66.7 μ*M* guanine in 70 μ*M* NaOH.[13] The enzyme reaction was then started by addition of 100 μl of C_1 (or other aliquot). The decrease in optical density at 245 mμ was followed with a Gilford 2000 recording spectrophotometer; the C_1 aliquot usually gave an OD change of about 0.008 unit/min. The velocities in OD/min were plotted on a log scale against time on a linear scale.[7b] This procedure is adequate for a routine screen for a plus or minus answer on irreversible inhibition. As many as three inhibitor tubes can be run with one enzyme control in 1 day.

With a positive compound, a larger amount of inhibitor–enzyme mixture can be set up, and then a number of aliquots can be removed at varying times in order to obtain the half-life of irreversible inhibition.

(13) B. R. Baker, *J. Med. Chem.*, **10**, 59 (1967); paper LXXIII of this series.

Irreversible Enzyme Inhibitors. CIV. Inhibitors of Thymidine Phosphorylase. VIII. Further Studies on Hydrophobic Bonding with 6-Substituted Uracils[1,2]

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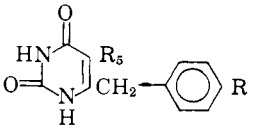
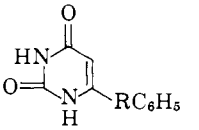
6-Benzyluracil has been previously reported to be a good reversible inhibitor of thymidine phosphorylase; due to a hydrocarbon interaction of the benzene ring with the enzyme, this compound complexes to the enzyme about five times better than the substrate, 2′-deoxy-5-fluorouridine. Other bridges between the uracil and phenyl moieties are more easily synthesized than the methylene bridge of 6-benzyluracil and have now been shown also to have phenyl binding. 6-Anilinouracil, 6-phenoxyuracil, 6-phenylthiouracil, and 6-benzylaminouracil complex to the enzyme 10, 17, 100, and 65-fold better, respectively, than the substrate. In contrast, 6-benzoyluracil with its relatively fixed coplanar structure is a poorer inhibitor than 6-benzyluracil; the poor binding by 6-benzoyluracil compared to the other inhibitors suggests a likely optimal binding conformation for the inhibitors where the phenyl group is out-of-plane with the pyrimidine ring and approaches the 5 position of the pyrimidine in space.

Previous papers in this series have revealed that (a) 6-benzyluracil (**1**) is a good reversible inhibitor of thymidine phosphorylase due to a hydrophobic interaction between the benzyl group and the enzyme,[4] (b) the inhibition of **1** can be enhanced by introduction of a 5-bromine atom (**2**), which increases the acidity of the uracil,[5] and (c) 6-(*p*-bromoacetamidobenzyl)uracil (**3**) is an active-site-directed irreversible inhibitor,[6] though slow acting with a half-life of about 2–3 hr.[2b] These results posed the following questions. Can additional



1, R = H, R_5 = H
2, R = Br, R_5 = H
3, R = H, R_5 = $BrCH_2CONH$–



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hydrophobic bonding be detected by appropriate substituents on the benzene ring? Can faster active-site-directed irreversible inhibitors with a half-life of 10 min or less[7] be synthesized by varying the position or elec-

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

(2) (a) For the previous paper in this series, see B. R. Baker and W. F. Wood, *J. Med. Chem.*, **10**, 1106 (1967); (b) for the previous paper on thymidine phosphorylase see B. R. Baker and M. Kawazu, *J. Pharm. Sci.*, in press; paper C of this series.

(3) On leave from the Department of Organic Chemistry, Pedagogical College, Opole, Poland.

(4) B. R. Baker and M. Kawazu, *J. Med. Chem.*, **10**, 311 (1967); paper LXXVIII of this series.

(5) B. R. Baker and M. Kawazu, *ibid.*, **10**, 316 (1967); paper LXXX of this series.

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

(7) For the kinetic parameters of active-site-directed irreversible inhibition see ref 6, Chapter VIII.

trophilic character of the leaving group? Can tumor-specific active-site-directed irreversible inhibitors of thymidine phosphorylase be obtained that would be useful in chemotherapy?[8]

The use of 6-benzyluracil (**1**) with the appropriate substituents to answer these questions suffers from the disadvantage that the compounds must be made by a rather lengthy synthesis from the appropriately substituted phenylacetic acid by a primary pyrimidine synthesis. Since the bridge length between the phenyl and pyrimidine for optimum inhibition was one carbon, other one-atom bridges such as O, N, or S (**4**) have now been synthesized for enzymic evaluation; such bridges could be simpler to synthesize and the methods could be more adaptable for variation of substituents in order to answer the questions posed above. The results on these studies on compounds of type **4** are the subject of this paper.

Enzyme Studies.—There are a number of possible reasons why an active-site-directed irreversible enzyme inhibitor may inactivate an enzyme at a slow rate within the reversible enzyme-inhibitor complex; three cases have been observed experimentally. (1) Within the complex, the bridge distance between the reversible complexing region and the nucleophilic group on the enzyme being covalently linked may not be optimal.[9] (2) Since all electrophilic groups and nucleophiles do not react at the same rate, the optimum electrophilic (leaving) group may not be on the irreversible inhibitor.[9a,10] (3) Two or more ground state conformations for the inhibitor may be possible, only one of which has the proper bridge distance to react with the enzyme nucleophilic group.[2a,11] For example, a bromoacetamido group on a benzene ring can have two ground-state conformations; if one conformation has the bromomethyl group complexed to a hydrophobic bonding region, this conformation will be energetically favored, but would not be the favored conformation for reaction with the enzyme nucleophilic group.[2a]

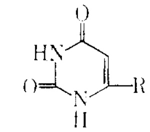
Note that the bromoacetamido group of **3** (Table I) gives a 3.5-fold increment in binding compared to the parent 6-benzyluracil (**1**). That this increment with **3** is not likely to be due to complexing to the enzyme by the *p*-carboxamide moiety of **3** is substantiated by the twofold loss in binding that occurs when the polar *p*-sulfonamide group is introduced on **1**;[5] therefore, a more plausible explanation is that the bromomethyl group of **3** in one of its two ground-state conformations complexes with the hydrophobic bonding region of the enzyme.

If the hydrophobic bonding region extends past the *meta* or *para* position of the benzyl group, then this region should be detectable by hydrocarbon substituents on the benzene ring. Therefore, 6-(*p*-methylbenzyl)-uracil (**5**) was synthesized and evaluated; **5** showed 2.3-fold better binding than the parent 6-benzyluracil (**1**). Unfortunately, the synthesis of **5** is rather laborious since a primary pyrimidine synthesis from *p*-methylphenylacetic acid is required; such a lengthy synthesis does not readily lend itself to the extensive number of compounds that must be made to explore the parameters of a hydrophobic bonding region.[12] If a more simply synthesized system would show hydrophobic bonding by a phenyl group, such a system would lend itself more readily to exploration of this hydrophobic bonding region. Since 6-chloro-2,4-dimethoxypyrimidine is commercially available and is readily convertible to 6-chlorouracil, direct displacement reactions on both 6-chloropyrimidines with appropriate nucleophiles were investigated.

6-Anilinouracil (**7**) is readily synthesized in one step;[13] **7** was a somewhat better inhibitor of thymidine phosphorylase than 6-benzyluracil (**1**). The phenyl group of **7** gave a sevenfold increment in binding compared to 6-aminouracil (**8**) and a 27-fold increment compared to uracil (**20**); the phenyl group of **1** gave a 35-fold increment in binding compared to 6-methyluracil (**6**) and a 12-fold increment in binding compared to uracil (**20**). Thus, 6-anilinouracil (**7**) represents the

Table I

Inhibition of Thymidine Phosphorylase[a] by



Compd	R	μM concn for 50% inhib	$([I]/[S])_{0.5}$[b]
1	$CH_2C_6H_5$	90[c]	0.22
3	*p*-$CH_2C_6H_4NHCOCH_2Br$	26[d]	0.065
5	*p*-$CH_2C_6H_4CH_3$	38	0.095
6	CH_3	3200[c]	8.0
7	NHC_6H_5	40	0.10
8	NH_2	270	0.67
9	$SO_2C_6H_5$	28	0.070
10	SO_2CH_3	210[e]	0.53
11	COC_6H_5	530	1.2
12	$CHOHC_6H_5$	130	0.33
13	SC_6H_5	4.2	0.010
14	OC_6H_5	23	0.058
15	$N(CH_3)C_6H_5$	55	0.14
16	$NHCH_2C_6H_5$	6.2	0.015
17	$CH_2CH_2C_6H_5$	240[e]	0.60
18	$NHCH_2CH_2C_6H_5$	130	0.32
19	$(CH_2)_3C_6H_5$	450[e]	1.1
20	H	1100[f]	2.9

[a] Thymidine phosphorylase was a 45–90% saturated $(NH_4)_2SO_4$ fraction from *E. coli* B prepared and assayed with 400 μM 2'-deoxy-5-fluorouridine in arsenate–succinate buffer (pH 5.9) in the presence of 10% DMSO as previously described;[8a] the technical assistance of Pepper Caseria with these assays is acknowledged. [b] Ratio of concentration of inhibitor to 400 μM substrate giving 50% inhibition. [c] Data previously reported.[5] [d] Data previously reported.[2a] [e] Data from B. R. Baker, M. Kawazu, and J. D. McClure, *J. Pharm. Sci.*, in press; paper XCIX of this series. [f] Data previously reported.[15]

(8) For discussion of the potential chemotherapeutic use of such inhibitors of thymidine phosphorylase, see (a) B. R. Baker, *J. Med. Chem.*, **10**, 302 (1967); paper LXXV of this series; (b) ref 6, Chapter IV.

(9) (a) See ref 6, Chapter IX; (b) B. R. Baker, *Biochem. Pharmacol.*, **12**, 293 (1963); (c) B. R. Baker and R. P. Patel, *J. Pharm. Sci.*, **53**, 714 (1964); (d) B. R. Baker and J. H. Jordaan, *ibid.*, **55**, 1417 (1966); paper LXVII of this series.

(10) (a) B. R. Baker and R. P. Patel, *Biochem. Biophys. Res. Commun.*, **9**, 199 (1962); (b) B. R. Baker and R. P. Patel, *J. Pharm. Sci.*, **52**, 927 (1963); (c) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **10**, 1113 (1967); paper CV of this series.

(11) (a) See ref 6, Chapter X; (b) B. R. Baker and H. S. Shapiro, *J. Pharm. Sci.*, **55**, 1422 (1966); paper LXVIII of this series.

(12) For the exploration of a hydrophobic bonding region of dihydrofolic reductase see (a) B. R. Baker, B.-T. Ho, and D. V. Santi, *J. Pharm. Sci.*, **54**, 1415 (1965); (b) B. R. Baker and B.-T. Ho, *J. Heterocyclic Chem.*, **2**, 335 (1965); (c) B. R. Baker and G. J. Lourens, *ibid.*, **2**, 344 (1965). For a compilation of these data and their interpretation see ref 6, Chapter X.

(13) A. Paul and D. Sen, *Indian J. Chem.*, **2**, 212 (1964).

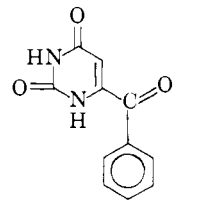
type of compound being sought for further studies on hydrophobic bonding and active-site-directed irreversible inhibitors; **7** is readily synthesized and is readily varied in structure by use of substituted anilines.[14]

Hydrophobic bonding by benzene bridged with other groups than methylene and amino has also been detected. Note that 6-phenylsulfonyluracil (**9**) is a sevenfold better inhibitor than 6-methylsulfonyluracil (**10**); also 6-phenylthiouracil (**13**) and 6-phenoxyuracil (**14**) are excellent inhibitors, being complexed 260- and 48-fold better, respectively, than uracil (**20**). By use of substituted phenols, thiophenols, or benzenesulfinates, appropriate studies can also be made on hydrophobic bonding and active-site-directed irreversible inhibitors.

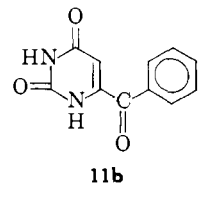
N-Methylation (**15**) of the 6-nitrogen of 6-anilinouracil (**7**) led to little change in binding. Since the N-methyl group of **15** is tolerated in the reversible enzyme–inhibitor complex, it is likely that longer groups would also be tolerated; therefore, one logical position to place an alkyl moiety with a leaving group in order to obtain a candidate active-site-directed irreversible inhibitor would be this N^6 position.

That the polar hydroxyl group placed on the methylene bridge of 6-benzyluracil (**1**) was not detrimental to binding was shown with **12**. In contrast, conversion of the methylene group to carbonyl (**11**) was detrimental to binding; very likely all of the hydrophobic bonding by phenyl was lost since the electron-withdrawing carbonyl group should give 2–5-fold better binding than uracil (**20**) as noted with the electron-withdrawing 6-methylsulfonyl group of **10** or the 6-trifluoromethyl group.[15]

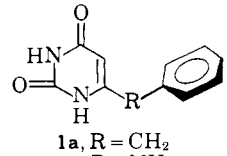
Diaryl ketones have a coplanar ground-state conformation to allow maximal π-orbital overlap; therefore, 6-benzoyluracil (**11**) has two possible coplanar ground-state conformations, **11a** and **11b**. Since there is little or no hydrophobic interaction between the phenyl of **11** and the enzyme, it is clear that neither **11a** nor **11b** are the preferred conformations for optimal binding to the enzyme.



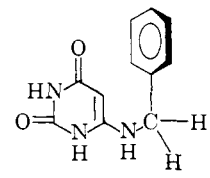
11a



11b



1a, R = CH_2
7a, R = NH



16a

As a working hypothesis, it is suggested that 6-benzyluracil (**1**) and 6-anilinouracil (**7**) give optimum binding to the enzyme in conformations **1a** and **7a** where the phenyl group is out-of-plane with the uracil ring but approaches the 5 position of the uracil. Such a working hypothesis of an out-of-plane phenyl group is supported by the fact that an out-of-plane 6-phenyl group gives better hydrophobic bonding to the enzyme than an in-plane phenyl group.[4] Furthermore, such a binding conformation could explain why a *m*-nitro[2b] or a *p*-nitro[5] group on the benzyl group would give enhanced binding to the enzyme; such an electron-withdrawing group would tend to stabilize a conformation such as **1a** through some charge-transfer character between the phenyl and uracil moieties where the uracil is the donor partner.[16,17] When 6-(*p*-nitrobenzyl)-uracil was substituted by a 5-bromo atom, the binding by the aryl group was lost;[2b,5] this can now be rationalized on the basis that the electron-withdrawing 5-bromo group on uracil makes the uracil a poorer donor and, in fact, could repulse the nitrophenyl group away from the conformation **1a** with the resultant loss in hydrophobic bonding.

The hypothesis that the conformation of **7** for optimal hydrophobic bonding was **7a** suggested that 6-benzylaminouracil (**16**) be investigated as an inhibitor since it could readily assume conformation **16a**; the 6-benzylamino group of **16** gave sixfold better binding than the 6-anilino group of **7** (Table I). Also note that **16** is a 40-fold better inhibitor than 6-phenethyluracil (**17**); in order for **17** to assume the optimum binding conformation of **16a**, the four protons on the ethyl group would have to be eclipsed, a matter of 2.0 kcal/mole from the ground-state conformation. The 39-fold difference in binding between **16** and **17** is about 2.2 kcal/mole, which is in good agreement with the calculated difference.

If **16** binds in conformation **16a**, then the higher homolog (**18**) should show poorer binding, since the phenyl group of **18** cannot approach the 5 position of the uracil effectively. Note that almost all of the 44-fold increment in hydrophobic binding between 6-benzylaminouracil (**16**) and 6-aminouracil (**8**) is lost with the 6-phenethylamino group of **18**.

The fact that 6-benzylaminouracil (**16**) is such a good reversible inhibitor of thymidine phosphorylase also opens up the possibility that **16** could be converted to an active-site-directed irreversible inhibitor by substitution on the benzylic carbon with an alkyl moiety containing an appropriate leaving group.

Chemistry.—Paul and Sen[13] have prepared 6-substituted aminouracils by reaction of 6-chlorouracil (**24**) with the appropriate amine in water; with aromatic amines, acid catalysis accelerated the reaction, but with the stronger aliphatic amines, acid of course did not. We have now found that commercial 6-chloro-2,4-dimethoxypyrimidine (**21**) can be treated directly with aniline or N-methylaniline at the boiling point to give **22** and **23**; during the steam distillation to remove excess amine, hydrolysis to the desired **7** and **15** occurred (Scheme I).

The aralkylamines (**16**, **18**) were best prepared by reaction of 6-chlorouracil (**24**) with a 2:1 ratio of amine

(14) A greater than 40-fold further increment in hydrophobic bonding above that seen with **7** has already been observed with substituted 6-anilinouracils: B. R. Baker and W. Rzeszotarski, to be published.

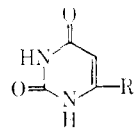
(15) B. R. Baker and M. Kawazu, *J. Med. Chem.*, **10**, 313 (1967); paper LXXIX of this series.

(16) The ability of internal charge transfer to stabilize an unfavorable conformation has previously been observed with 1-(β-aryledhyl)nicotinamides; see (a) S. Shifrin, *Biochemistry*, **3**, 829 (1964); (b) S. Shifrin, *Biochim. Biophys. Acta*, **96**, 173 (1965).

(17) See ref 6, Chapter II.

Table II

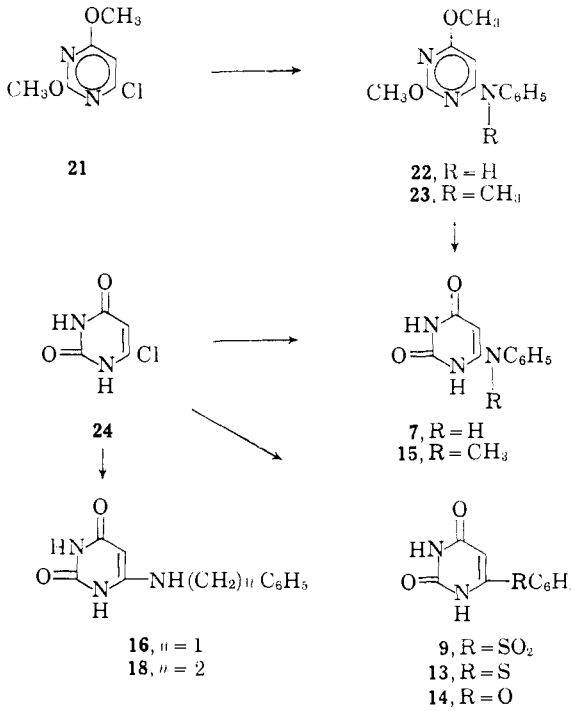
Physical Constants of



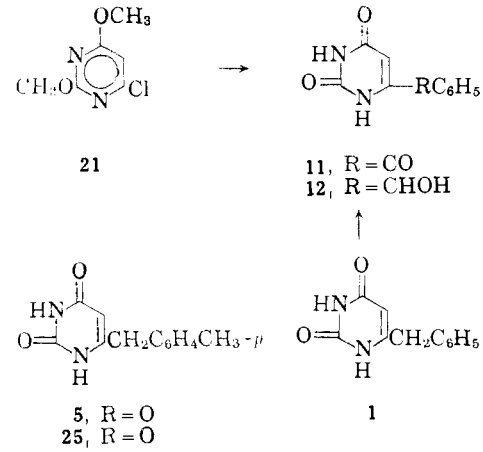
Cmpd[a]	R	Method (hr)	% yield	Mp, °C	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N	λ_{max}, mμ pH 1	λ_{max}, mμ pH 13
5	$CH_2C_6H_4CH_3$-*p*	Expt	84	269–271[b,c]	66.7	5.60	13.0	66.5	5.82	13.1	262	284
7	NHC_6H_5	A (4)	69	325–327[d–f]			20.7			20.9	280	285
9	$SO_2C_6H_5$	D (0.5)	79	277–279[c,g,h]							268	262
												269
												304
13	SC_6H_5	C (12)	90	270–272[b,c]	54.5	3.66	12.7	54.5	3.54	12.7	280	287
14	OC_6H_5	D (48)	44	302–304[b,c]	58.8	3.94	13.7	59.0	4.14	13.9	254	267
15	$N(CH_3)C_6H_5$	A (24)	63	287–289[c,k]	60.8	5.10	19.3	61.0	5.20	19.4	276	276
16	$NHCH_2C_6H_5$	B (18)	32	316–317 dec[c,i]	60.8	5.10	19.3	61.0	5.06	19.4	269	269
18	$NH(CH_2)_2C_6H_5$	B (48)	89	287–288 dec[b,i]	62.3	5.66	18.2	62.5	5.82	17.9	266	266

[a] All compounds had infrared spectra in agreement with their assigned structure and moved as a single spot on tlc in the solvent indicated. [b] Recrystallized from ethanol. [c] Tlc solvent, 1:5 DMF–C_6H_6. [d] Recrystallized from DMF. [e] Tlc solvent, 2:2:5 *n*-PrOH–THF–C_6H_6. [f] Lit.[13] mp 332°. [g] Recrystallized from H_2O. [h] Lit.[18] mp 278–280°. [i] Recrystallized from HOAc–H_2O. [j] Tlc solvent, 1:5 HOAc–C_6H_6. [k] Recrystallized from MeOH.

Scheme I



Scheme II



in boiling water, as performed by Paul and Sen[13] for cyclohexylamine.

Langley[18] has described the preparation of 6-phenylsulfonyluracil (**9**) by reaction of 6-chlorouracil (**24**) with sodium benzenesulfinate in boiling water; no difficulty was encountered with this procedure. We have now observed that sodium thiophenolate gives **13** and sodium phenoxide gives **14** after a more extended reaction time.

6-(*p*-Methylbenzyl)uracil (**5**) was synthesized by hydrolysis of the corresponding 2-thiouracil (**25**) with aqueous chloroacetic acid;[4] **25** in turn was prepared by a primary pyrimidine synthesis[4,19] starting with *p*-methylphenylacetic acid.

6-Benzoyluracil (**11**) has been prepared[20] from **21** by lithium exchange from butyllithium followed by reaction with benzaldehyde to give the intermediate **12**; the latter was then oxidized to **11** (Scheme II). Since 6-benzyluracil (**1**) was available in this laboratory from another study,[4] its oxidation with SeO_2 in glacial acetic acid was undertaken. With a 1:1 ratio of **1** to SeO_2, the principle product (38%) was the desired carbinol (**12**) along with a smaller amount of 6-benzoyluracil (**11**). With a ratio of 2:1 of SeO_2:**1**, the yield of **11** was increased to 31% of pure material.

Experimental Section[21]

6-Anilinouracil (7). Method A.—A mixture of 0.87 g (5 mmoles) of **21** and 5.1 g (57 mmoles) of aniline was refluxed for 4 hr, then the excess aniline was removed by steam distillation. The cooled solution was filtered and the product washed with water; yield 0.70 g (69%), mp 324–326°. Two recrystallizations from DMF gave white crystals, mp 325–327°; the compound moved as a single spot on tlc in 2:2:5 *n*-PrOH–THF–C_6H_6. See Table II for additional data; Paul and Sen[13] have recorded mp 332°.

(18) B. L. Langley, British Patent 845,378 (1960); *Chem. Abstr.*, **55**, 6566 (1961).

(19) J. Monostory, *Acades Assoc. Quim. Arg.*, **40**, 80 (1952).

(20) B. L. Langley, *J. Am. Chem. Soc.*, **78**, 2136 (1956).

(21) Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Infrared spectra were determined in KBr pellet with a Perkin-Elmer 137B spectrophotometer. Ultraviolet spectra were determined in 10% EtOH (unless otherwise indicated) with a Perkin-Elmer 202 spectrophotometer. Tlc was run on Brinkmann silica gel GF and spots were detected by visual examination under ultraviolet light.

6-Benzylaminouracil (16). Method B.—A solution of 0.73 g (5 mmoles) of **24**[18] and 1.07 g (10 mmoles) of benzylamine in 100 ml of water was refluxed for about 18 hr. The cooled solution was filtered and the product was washed with water; yield 0.35 g (32%), mp 313–314° dec. Three recrystallizations from aqueous AcOH gave white crystals, mp 316–317° dec. The compound moved as a single spot on tlc in 1:5 AcOH–C_6H_6. See Table II for additional data.

6-Phenylthiouracil (13). Method C.—To a solution of 1.10 g (10 mmoles) of thiophenol and 10 mmoles of NaOH in H_2O (50 ml) were added 0.73 g (5 mmoles) of **24**[18] and 50 ml of 2-methoxyethanol. After being refluxed for 12 hr, the solution was spin evaporated *in vacuo*. To the residue was added 50 ml of water, then the mixture was acidified (AcOH), and again spin evaporated *in vacuo*. The residue was heated to boiling with 100 ml of water, then cooled. The product was collected on a filter and washed with hot water; yield 1.00 g (90%), mp 267–270°. Recrystallization from EtOH gave white crystals, mp 270–272°, that moved as a single spot on tlc in 1:5 AcOH–C_6H_6. See Table II for additional data.

Method D used for **9** was the same except the product separated directly on cooling the reaction mixture; in the case of **14**, the reaction mixture was merely acidified with HCl to precipitate the product.

6-Benzoyluracil (11).—A mixture of 300 mg (1.5 mmoles) of **1**, 330 mg (3 mmoles) of SeO_2, and 50 ml of AcOH was refluxed for 2 hr, then filtered to remove Se. The filtrate was spin evaporated *in vacuo*. The residue was dissolved in 50 ml of water, then the solution was clarified by filtration; the product separated on cooling. Two more recrystallizations from water gave 100 mg (31%) of light yellow needles: mp 250–252°; λ_{max} 277 mμ (pH 1), 257 mμ (pH 13). The compound moved as a single spot on tlc in 5:1 C_6H_6–EtOAc. Langley[20] has recorded mp 252–253° for this compound prepared by a different route.

6-(α-Hydroxybenzyl)uracil (12).—A mixture of 300 mg (1.5 mmoles) of **1**, 165 mg (1.5 mmoles) of SeO_2, and 50 ml of AcOH was refluxed 1 hr, then filtered to remove Se. The residue remaining after spin evaporation of the filtrate *in vacuo* was dissolved in 50 ml of water. The hot solution was filtered, then cooled. Filtration removed 50 mg (16%) of **11**, mp 250–252°. The filtrate was concentrated to about 20 ml, then allowed to stand at 3°. The product was collected on a filter; yield 120 mg (38%); mp 224–226°; λ_{max} 264 mμ (pH 1), 257 mμ (pH 13). The compound moved as a single spot on tlc in 5:1 C_6H_6–EtOAc. Langley[20] recorded mp 224–226° for this compound prepared by an alternate route.

6-(*p*-Methylbenzyl)uracil (5).—A mixture of 1.1 g (5 mmoles) of **25**,[4,19] 0.40 g of chloroacetic acid, and 50 ml of water was refluxed for 48 hr with stirring. The cooled mixture was filtered and the product was washed with water. Recrystallization from EtOH gave 0.85 g (84%) of white crystals, mp 269–271°. See Table II for additional data.

Irreversible Enzyme Inhibitors. CV.[1,2] Differential Irreversible Inhibition of Vertebrate Dihydrofolic Reductases by Derivatives of 4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-phenyl-*s*-triazines Substituted with a Terminal Sulfonyl Fluoride[3]

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Derivatives of 4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine bridged from its 1 position to sulfanilyl fluoride with six different bridges have been synthesized; these compounds have been evaluated as reversible and irreversible enzyme inhibitors of the dihydrofolic reductases from Walker 256 rat tumor, rat liver, L1210/FR8 mouse leukemia, and pigeon liver. For each compound little difference in reversible binding to the four dihydrofolic reductases were seen. In contrast, dramatic differences in irreversible inhibition were seen. Four of the six compounds that irreversibly inhibited pigeon liver dihydrofolic reductase failed to irreversibly inhibit the dihydrofolic reductases from Walker 256 rat tumor and L1210 mouse leukemia. The two compounds containing a *p*-benzoyl (**15d**) and a *p*-phenylpropionyl (**15f**) bridge irreversibly inhibited the two tumor enzymes and the pigeon liver enzyme. However, **15d** inactivated the rat tumor >70 times as fast as the mouse leukemia enzyme. Furthermore, **15f** inactivated the rat tumor enzyme eight times as fast as the rat liver enzyme. The dihydro-*s*-triazine moiety of **15** is believed to complex within the active site of the enzyme, but the sulfonyl fluoride is believed to form a covalent bond outside the site; it is the latter area where evolutionary differences are more apt to have occurred. Thus, the differences in irreversible inhibition of these enzymes can be accounted for if these compounds are operating by the active-site-directed *exo* mechanism of irreversible inhibition, such a mechanism accounting for the specificity pattern by the bridge principle of specificity.

The discovery[5,6] of a potent hydrophobic bonding region on dihydrofolic reductase considerably complicated the successful design of the first active-site-directed irreversible inhibitors[5,7] for this enzyme.[8,9] Once it had been established that the hydrophobic bonding region was outside the active site,[10] near where either the 4 or 8 position of dihydrofolate (**1**) resides on the enzyme, two active-site-directed irreversible inhibitors soon followed;[8,9] for example, the 5-phenylbutyl group of **2** complexes with the hydrophobic bonding region, thus allowing the 6-phenethyl group to project back into the active site.[8]

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(2) For the previous paper of this series see B. R. Baker and W. Rzeszotarski, *J. Med. Chem.*, **10**, 1109 (1967).

(3) For the previous paper on inhibitors of dihydrofolic reductases see B. R. Baker and M. A. Johnson, *J. Heterocyclic Chem.*, in press.

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(5) For a review on the mode of binding of inhibitors to dihydrofolic reductase, see 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, Chapter X.

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