the desired 6-phenethyluracil (IX). Similarly, 6-phenylpropyluracil (X) was synthesized from 4-phenylbutyric acid *via* ethyl γ-phenylbutyrylacetate and 6-phenylpropyl-2-thiouracil.

Experimental Section[17]

6-(Phenylpropyl)-2-thiouracil.—Ethyl γ-phenylbutyroylacetate, bp 138–140° (2 mm), was synthesized as described for ethyl β-phenylpropionylacetate, then condensed with thiourea as described for 6-phenethyl-2-thiouracil.[16] Recrystallization from dioxane gave a 71% yield of white prisms: mp 201–203°; λ_{max} (pH 6), 275 mμ; (pH 13), 260, 291 mμ.

Anal. Calcd for $C_{13}H_{14}N_2SO$: C, 63.4; H, 5.73; N, 11.4. Found: C, 63.7; H, 5.93; N, 11.2.

6-Phenethyluracil (IX).—A mixture of 4.5 g (18 mmoles) of 6-phenethyl-2-thiouracil,[16] 100 ml of water, and 5 g of chloroacetic acid was refluxed with stirring for about 18 hr. The cooled mixture was filtered and the product was washed with water. Recrystallization from water gave 3.3 g (77%) of white plates: mp 260–262°; λ_{max} (pH 7), 263 mμ; (pH 13), 278 mμ.

Anal. Calcd for $C_{12}H_{12}N_2O_2$: C, 66.7; H, 5.59; N, 13.0. Found: C, 66.8; H, 5.65; N, 12.8.

6-Phenylpropyluracil (X).—Hydrolysis of 6-phenylpropyl-2-thiouracil as described for the preparation of IX gave, after recrystallization from 30% acetic acid, an 85% yield of white leaflets: mp 145–147°; λ_{max} (pH 6), 263; (pH 13), 278 mμ.

Anal. Calcd for $C_{13}H_{14}N_2O_2$: C, 67.9; H, 6.13; N, 12.2. Found: C, 68.2; H, 6.33; N, 12.1.

6-(*p*-Nitrobenzyl)uracil (XIII).—To a stirred mixture of 6 ml of 96% H_2SO_4 and 6 ml of 70% HNO_3 at 50° was added over 10 min 1.30 g (6.45 mmoles) of 6-benzyluracil (VIII).[2] The solution was cooled to 25° and stirred for an additional 20 min, then poured into a large volume of iced water. The product was collected on a filter and washed with water. Recrystallization from dimethylformamide gave slightly yellow prisms: yield 1.0 g (63%); mp 277–278° dec; ν_{max} 1750, 1650 (C=O, C=N, NH, C=C), 1520, 1350 cm^{-1} (NO_2); λ_{max} (pH 7), 267 mμ; (pH 13), 285 mμ; pK_a = 9.1 (spectrophotometric); τ 1.80, 1.93, 2.40 2.53 (4 protons, C_6H_4), 4.78 (1 proton, uracil 5-H).

Anal. Calcd for $C_{11}H_9N_3O_4$: C, 53.4; H, 3.67; N, 17.0. Found: C, 53.2; H, 3.73; N, 17.2.

Since uracil has pK_a = 9.5 and 5-nitrouracil has pK_a = 5.3,[18] the observed pK_a of XIII indicates the nitro group is not at the 5 position of the uracil. Similarly, 5-nitro-6-methyluracil has λ_{max} 370 mμ (pH 13), which is shifted 95 mμ to longer wavelength than that of 6-methyluracil. Since 6-benzyluracil has λ_{max} 292 mμ (pH 13), the observed λ_{max} of XIII indicates the nitro group is not at the 5 position of the uracil.

6-(*p*-Nitrophenyl)uracil (XV).—Nitration of 6-phenyluracil (VII)[2] as described for the preparation of XIII, then recrystallization from aqueous dioxane, gave a 61% yield of product as light yellow prisms: mp 296–297°; λ_{max} (pH 6), 273 mμ; (pH 13), 305–310 mμ (shoulder); τ 1.81, 1.94, 2.40, 2.53 (4 protons, C_6H_4), 4.78 (1 proton, uracil 5-H).

Anal. Calcd for $C_{10}H_7N_3O_4$: C, 51.5; H, 3.03; N, 18.0. Found: C, 51.7; H, 3.20; N, 17.9.

6-(*p*-Aminobenzyl)uracil (XIV) Hydrochloride.—A solution of 1.23 g (5 mmoles) of XIII in 50 ml of glacial acetic acid was shaken with hydrogen at 2–3 atm in the presence of 200 mg of 5% Pd–C for 1.5 hr when reduction was complete. The filtered solution was spin evaporated *in vacuo*. The residue was dissolved in 5% aqueous HCl. After clarification of the solution with decolorizing carbon, the solution was spin evaporated *in vacuo*. Recrystallization of the residue from ethanol gave 0.80 g (63%) of white prisms: mp 293–295°; ν_{max} 3250 (NH), 1710, 1680, 1520, 1495 cm^{-1} (C=O, C=C, C=N, NH); λ_{max} (pH 7), 238 (shoulder), 263 mμ; (pH 13), 285 mμ.

Anal. Calcd for $C_{11}H_{11}N_3O_2 \cdot HCl$: C, 52.1; H, 4.76; N, 16.6. Found: C, 51.8; H, 5.00; N, 16.4.

The free base had mp 263–264°.

(17) Melting points were determined with a Fischer-Johns apparatus and those below 230° are corrected. Ultraviolet spectra were determined in 10% ethanol (unless otherwise indicated) with a Perkin-Elmer 202 spectrophotometer. Infrared spectra were determined with a Perkin-Elmer 137B spectrophotometer in KBr pellets. Nmr spectra were run in DMSO with a Varian A-60 using $(CH_3)_4Si$ as an internal standard. All compounds were uniform on thin layer chromatography on Brinkmann silica gel GF when spots were detected by visual examination under ultraviolet light.

(18) D. J. Brown, "The Pyrimidines," Interscience Publishers, Inc., New York, N. Y., 1962, pp 472–476.

Irreversible Enzyme Inhibitors. LXXIX.[1,2] Inhibitors of Thymidine Phosphorylase. V.[2] Mode of Pyrimidine Binding

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Evidence is presented that neither the 2-oxo nor the 4-oxo group of uracil is complexed to thymidine phosphorylase; in contrast, both the 1-hydrogen and 3-hydrogen are complexed to the enzyme. The ability of the 1-hydrogen to complex is strongly influenced by its acidity, the strongest bonding occurring when it is ionized. Acidity and binding can be increased by electron-withdrawing groups at the 5 or 6 position of uracil. Since these positions appear to be near a hydrophobic region when complexed to thymidine phosphorylase, highly polar electron-withdrawing groups were, in general, less effective in increasing binding than less polar electron-withdrawing groups.

In previous papers of this series on thymidine phosphorylase the mode of binding of the ribofuranose moiety of thymidine,[4] hydrophobic bonding by 1-alkyl- and 1-aralkyluracils,[5] hydrophobic bonding by 1-aralkyluracils containing additional aryl, alkyl, or aralkyl substituents at the 5 and 6 positions,[6] and hydrophobic bonding by uracils containing 5- or 6-alkyl, -aryl, or -aralkyl substituents[2] were reported. In this paper are described the results on studies of the mode of pyrimidine binding to thymidine phosphorylase.

That the 1-hydrogen of uracil (I) is strongly complexed to the enzyme is indicated by the 50-fold loss in binding by 1-methyluracil (II)[2,5] (Table I). The binding by the 1-hydrogen was strongly influenced by its acidity. Note that 5-nitrouracil (VI) with pK_a = 5.3 is 80% ionized at the pH 5.9 of the assay and is the strongest inhibitor in Table I not containing hydro-

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(2) For the previous paper of this series see B. R. Baker and M. Kawazu, *J. Med. Chem.*, **10**, 311 (1967).

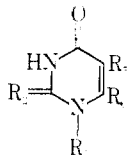
(3) On leave from Tanabe Seiyaku Co., Ltd., Tokyo, Japan.

(4) B. R. Baker, *J. Med. Chem.*, **10**, 297 (1967); paper LXXV of this series.

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

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

Table I
Pyrimidine Binding to Thymidine Phosphorylase[a] by



Compd	R_1	R_2	R_3	R	$([I]/[S])_{0.5}$[b]	Acidic[c] pK_a	R_3 or R_5 π constant[d]	% ionized[e]
I	H	O	H	H	3.9[f]	9.5	0	0.03
II	CH_3	O	H	H	200[f]	9.9[g]	0	0.01
III	H	O	CH_3	H	1.9[f,h]	9.9	0.56	0.01
IV[i]	H	O	F	H	1.3[f]	8.0[g]	0.15	0.78
V[j]	H	O	Br	H	0.45[f]	8.0[g]	0.86	0.78
VI[j]	H	O	NO_2	H	0.22[k]	5.3	−0.28	80
VII	H	O	NH_2	H	~7[l]		−1.2	
VIII[j]	H	O	$-^+N\equiv N$	H	>5[l]		Large negative	
IX[j]	H	O	$-COO^-$	H	3.0	4.6[m] 9.3	Large negative	95 0.04
X[j]	H	O	$COCH_3$	H	3.3		−0.55	
XI[n]	H	O	H	CH_3	8.0[f]	9.7	0.56	0.02
XII[o]	H	O	H	CF_3	1.2	5.7	1.3	61
XIII[n]	H	O	NO_2	CH_3	0.16			
XIV	H	O	SO_2Cl	CH_3	5.2		−1.8[p]	
XV	H	O	$-SO_2N$ 	CH_3	6.0		−1.8[p]	
XVI[q]	H	O	H	$CH_2C_6H_5$	0.22[f]			
XVII[q]	H	S	H	$CH_2C_6H_5$	0.16			
XVIII[q,r]	H	O	H	$CH_2C_6H_5$	3.0			

[a] Thymidine phosphorylase was a 45–90% ammonium sulfate fraction from *E. coli* B prepared and assayed with 0.4 m*M* 2′-deoxy-5-fluorouridine in succinate–arsenate buffer (pH 5.9) in the presence of 10% DMSO as previously described.[4] The technical assistance of Barbara Baine and Pepper Caseria is acknowledged. [b] Ratio of concentration of inhibitor to 0.4 m*M* 2′-deoxy-5-fluorouridine giving 50% inhibition. [c] Data from ref 11, unless otherwise indicated. [d] Relative hydrophobic character on a log scale: T. Fujita, J. Iwasa, and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964). [e] At pH 5.9 of the assay. [f] Previously reported.[3] [g] I. Wempen and J. J. Fox, *J. Am. Chem. Soc.*, **86**, 2474 (1964). [h] Previously observed to be an inhibitor under different assay conditions: W. E. Razzell and P. Casshyap, *J. Biol. Chem.*, **239**, 1789 (1964). [i] Kindly supplied by Dr. Harry B. Wood, Jr., National Cancer Institute. [j] Nutritional Biochemicals Corp. [k] Estimated from 10% inhibition at a concentration of 0.8 m*M*, the maximum concentration allowing measurement of the optical density change. [l] Since 20% inhibition is readily detectable, the concentration for 50% inhibition is at least four times greater than the concentration measured. [m] Measured spectrophotometrically; the lower pK_a is presumably that of the carboxyl group. [n] Aldrich Chemical Co. [o] Prepared according to A. Giner-Soralla and A. Bendich, *J. Am. Chem. Soc.*, **80**, 5744 (1958). [p] For the SO_2 group; calculated from $\pi = -1.26$ for SO_2CH_3 and $\pi = +0.56$ for CH_3. [q] Preparation previously reported.[6] [r] 3-Phenylpropyl derivative of XVI.

phobic substituents; 5-nitrouracil is an 18-fold better inhibitor than uracil (I), which is only 0.03% ionized at the pH of the assay. 5-Fluorouracil (IV) is a stronger acid than uracil (I) by 1.5 pK_a units; IV complexes to the enzyme about threefold better than uracil (I). Note that 5-fluorouracil is only 0.8% ionized at pH 5.9, but is still 25 times more ionized than uracil; it would therefore appear that the enzyme can complex either an anion at N-1 strongly, as in the case of 5-nitrouracil (VI), or the 1-hydrogen less strongly, the ability of the 1-hydrogen to complex as an acceptor appearing to depend upon its acidity.

In addition to its effect on acidity, a 5 substituent can also give additional binding to the enzyme by hydrophobic bonding;[2] note that thymine (III) is about twice as effective as uracil (I) as an inhibitor, most probably due to hydrophobic bonding of the 5-methyl group.[2] Conversely, the enzyme probably has a hydrophobic region near where the 5 position of uracil resides in the enzyme complex. Therefore, any interpretation of the effect on acidity by an electron-withdrawing group at the 5 position should also consider the hydrophobic or hydrophilic character of the 5 substituent. Note that 5-bromouracil (V) has the same pK_a as 5-fluorouracil (IV), but V is a threefold better inhibitor than IV; the extra bonding due to the bromine atom is most probably accounted for by the large difference in hydrophobic character of bromine and fluorine (see π constants,[7] Table I).

Conversely, if a strong electron-withdrawing group is also polar, some repulsion of this polar group from the hydrophobic area on the enzyme could occur. The nitro group is quite polar with a Hansch π constant[7] of −0.28, compared to bromo with +0.86. It follows that nitro gives nearly maximum anion formation, but probably some repulsion of this hydrophilic group from the enzyme might occur.

Since it appeared that the ideal group at the 5 position would be an electron-withdrawing group that is also nonpolar, several 5-substituted uracils were checked to verify this hypothesis. The electron-donating, polar 5-amino group (as in VII) should and does lead to a loss of binding. An ionized group that is highly polar such as diazonium (VIII) leads to a loss in binding, as predicted by the hypothesis. The fully ionized 5-carboxylate (IX) was a slightly better in-

(7) T. Fujita, J. Iwasa, and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964), have measured the hydrophobic character of a large number of atoms and groups and have assigned π values on a log scale as a measure of relative hydrophobicity.

hibitor than uracil and about equivalent to 5-acetyluracil (X) despite the fact that the carboxylate group is highly polar and quite weak as an electron-withdrawing group,[8] thus having no effect on the acidity of the 1-hydrogen (Table I); in contrast, the acetyl group is less polar and a better electron-withdrawing group than carboxylate. This anomalous result may be due to a different type of binding of uracil 5-carboxylate (IX) where the uracil is rotated in the active site; such rotomers for binding to dihydrofolic reductase and other enzymes have been previously proposed.[9,10]

The 5-sulfonyl chloride (XIV) has an electron-withdrawing group, but contains the highly polar sulfone function; therefore, the nearly twofold loss in binding compared to uracil (I) can be accounted for by the hydrophilic character of this sulfone function. With the sulfonylpiperidide derivative (XV), which should be compared with 6-methyluracil (XI), little if any increase in binding was observed; note, however, that XV is a much poorer inhibitor than 5-nitro-6-methyluracil (XIII).

Unfortunately, the better electron-withdrawing groups such as NO_2, SO_2NH_2, SO_2CH_3, and CN with Hammet σ constants[8] in the +0.7 range are also quite polar with Hansch π constants[7] of −0.28, −1.82, −1.3, and −0.57, respectively. Conversely, the hydrophobic CF_3 group with a π constant[7] of +1.2 is not a sufficiently good electron-withdrawing group since it has a σ constant[8] of +0.32, compared to bromo with +0.23 or nitro with +0.7. Therefore, 5-trifluoromethyluracil would be expected to be about as effective as 5-bromouracil. An interesting group to try at the 5 position would be SO_2CF_3 which has a π constant[7] of +0.93 and should be as good an electron-withdrawing group as SO_2CH_3, or better.

Another possible approach emerged by consideration that 6-(trifluoromethyl)uracil (XII) has $pK_a = 5.7$;[11] it would appear that an electron-withdrawing group at the 6 position has a greater influence on acidity of the 1-hydrogen of uracil than the same group at the 5 position. Although 6-trifluoromethyluracil (XII) was a sevenfold better inhibitor than 6-methyluracil (XI), it did not give as large an increment (18-fold) as the 5-nitro group on uracil. Since the 6-methyl group on uracil gives a twofold loss in binding, presumably due to hindrance of binding of the 1-hydrogen to the enzyme, another twofold loss in binding by the steric hindrance of the slightly larger trifluoromethyl group would not be unexpected. The methyl group has a van der Waals radius of 2.0 A,[12a] while the trifluoromethyl group has a radius of approximately 2.5 A (based on the data of Pauling and Brockway[12b]).

Further studies on greater acidity by a 5-bromo plus hydrophobic bonding by a 6-benzyl group will be reported in the following paper,[13] where 5-bromo-6-benzyluracil gives 150-fold better binding than uracil. A similar study on 6-trifluoromethyluracil with 5-alkyl and -aralkyl groups is being pursued.

That the 3-hydrogen of uracil (I) was involved in binding was indicated by comparison of 6-benzyluracil (XVI) with its 3-phenylpropyl derivative (XVIII); the latter showed a 14-fold loss in binding. Similar results were obtained with 3-*n*-amylthymidine, where a large loss occurred when the amyl substituent was introduced.[4] Although this loss in binding could also be due to an intolerance of bulk at this position by the enzyme, the loss of binding by the 3-hydrogen is currently favored as an explanation, since no more than a twofold variation in binding occurred between the phenylpropyl, *m*-nitrobenzyl,[13] cyanomethyl,[13] and amyl[4] groups at the 3 position.

That the 2-oxo group of uracil (I) was most probably not involved in binding was indicated by the comparison of 6-benzyluracil (XVI) with 6-benzyl-2-thiouracil (XVII); the latter was complexed about twice as effectively as XVI, this difference probably being due to the increased acidity of the 1-hydrogen of the thiouracil. Note that uracil has $pK_a = 9.5$ and 2-thiouracil has $pK_a = 7.7$. If the 2-oxo group were complexed as a donor to the enzyme, then the 2-thione should have been a poorer inhibitor, as previously noted in the comparison of the binding of thymidine and 4-thiothymidine to thymidine kinase.[14]

That the 4-oxo group of thymidine was most probably not complexed to the enzyme was indicated by the small change in binding when the 4-oxo group was replaced by 4-thione.[4]

Experimental Section[15]

6-Methyluracil-5-sulfonyl Chloride (XIV).—A solution of 6.3 g (50 mmoles) of 6-methyluracil in 60 ml of chlorosulfonic acid was heated at 100° for 18 hr, then cooled and carefully poured onto excess ice. The product was collected on a filter, washed well with water, then dried under vacuum. Recrystallization from dioxane gave 5.5 g (49%) of white prisms: mp >330°; λ_{max} (pH 6), 266 mμ; (pH 13), 287 mμ; ν_{max} 3560, 3480 (NH), 1740, 1630 (uracil), 1350, 1180 cm^{-1} (SO_2).

Anal. Calcd for $C_5H_3ClN_2O_4S$: C, 26.7; H, 2.24; N, 12.5; Cl, 15.8. Found: C, 26.9; H, 2.40; N, 12.5; Cl, 15.6.

6-Methyluracil-5-sulfonylpiperidide (XVI).—A solution of 1.12 g (5 mmoles) of XIV in 30 ml of reagent tetrahydrofuran (THF) was added dropwise to 5 ml of piperidine with stirring; the addition required about 10 min and the temperature rose to about 80°. After being stirred at ambient temperature for another 50 min, the mixture was diluted with 20 ml of 10% HCl, then refrigerated for about 18 hr. The product was collected on a filter and washed with water. Recrystallization from dioxane gave 0.96 g (70%) of white needles: mp 207–209°; λ_{max} (pH 1), 272 mμ; (pH 13), 287 mμ; ν_{max} 3580, 3510 (NH), 1700, 1640 (uracil), 1320, 1162 cm^{-1} (SO_2).

Anal. Calcd for $C_{10}H_{15}N_3O_4S$: C, 43.9; H, 5.53; N, 15.4; S, 11.7. Found: C, 44.1; H, 5.70; N, 15.5; S, 11.6.

(8) M. Charton, *J. Org. Chem.*, **28**, 3121 (1963).

(9) B. R. Baker and H. S. Shapiro, *J. Pharm. Sci.*, **55**, 308 (1966).

(10) 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.

(11) D. J. Brown, "The Pyrimidines," Interscience Publishers, Inc., New York, N. Y., 1962, pp 472–476.

(12) (a) L. Pauling and D. C. Carpenter, *J. Am. Chem. Soc.*, **58**, 1274 (1936); (b) L. Pauling and L. O. Brockway, *ibid.*, **57**, 2684 (1935).

(13) B. R. Baker and M. Kawazu, *J. Med. Chem.*, **10**, 316 (1967); paper LXXX of this series.

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

(15) Melting points were taken on a Fisher-Johns apparatus and those below 230° were corrected. Infrared spectra were determined in KBr pellet with a Perkin-Elmer 137B spectrophotometer. Ultraviolet spectra were determined in 10% alcohol with a Perkin-Elmer 202 spectrophotometer. Thin layer chromatograms (tlc) were run on Brinkmann silica gel GF and spots were detected by visual examination under ultraviolet light; all compounds moved as a single spot.