white crystals, mp 235–240°, lit.[10] mp 233°, with unchanged ultraviolet spectra.

The filtrate from the 1-benzyl-6-methyluracil was extracted with four 50-ml portions of $CHCl_3$. The combined extracts were washed with 50 ml of water, dried ($MgSO_4$), then spin evaporated *in vacuo;* yield, 0.458 g (21%) of 3-benzyl-6-methyluracil, mp 160–172°; this was essentially free of 1-benzyl-6-methyluracil since it had λ_{max} (pH 7), 264 mμ; (pH 13), 282 mμ. Recrystallization from ethyl acetate gave 0.417 g (19%) of fairly pure 3-benzyl-6-methyluracil, mp 177–183°, lit.[10] mp 194°, but with unchanged ultraviolet spectra.

Similarly, benzylation of 2.10 g (15 mmoles) of 5,6-dimethyluracil (Aldrich Chemical Co.) gave 290 mg (18%) of the 1,3-dibenzyl derivative as an oil that was uniform on tlc and had λ_{max} (pH 7, 13) 280 mμ. Acidification of the alkaline solution gave 230 mg (20%) of 3-benzyl-5,6-dimethyluracil, mp 196–201°, that was recrystallized from ethyl acetate; see Table V for additional data.

By chloroform extraction of the aqueous filtrate was isolated 535 mg (24%) of 1-benzyl-5,6-dimethyluracil that had λ_{max} (pH 7), 273 mμ; (pH 13), 278 mμ, indicating some contamination with the 3-benzyl isomer. Recrystallization from benzene–petroleum ether (bp 38–52°) gave 148 mg (6.7%) of more pure 1 isomer, mp 173–184°, λ_{max} (pH 7, 13) 274 mμ.

Method J.—A solution of 144 mg (0.5 mmole) of V in 5 ml of isoamyl alcohol containing 25 μl of 12 *N* aqueous HCl was heated at 75° for 2.5 hr, then spin-evaporated *in vacuo.* Crystallization from petroleum ether (bp 60–110°) gave 150 mg (91%) of white leafs, mp 87–88°, that moved as one spot on tlc in petroleum ether (bp 60–110°)–ethyl acetate (4:6). See Table V for analytical data on this product (VII).

5-*n*-Amyl-3-(2,4-dinitrobenzenesulfenyl)-6-*n*-propyluracil (XXVIII).—To a solution of 1.12 g (5 mmoles) of 5-*n*-amyl-6-*n*-propyluracil in 5 ml of reagent pyridine was added 1.17 g of 2,4-dinitrobenzenesulfenyl chloride. After 2 hr at ambient temperature, the solution was diluted with 20 ml of ice water. The product was collected on a filter, washed with water, dried, and recrystallized from ethyl acetate; yield, 1.6 g (76%) of yellow needles; mp 203–204°; λ_{max} (ethanol), 275, 315 mμ (plateau); (pH 13), 320 mμ; ν_{max} 3200, 2950 (NH), 1710, 1660, 1640, 1590 (C=O, NH, C=C), 1520, 1340 cm^{-1} (NO_2).

Anal. Calcd for $C_{18}H_{22}N_4O_6S$: C, 51.2; H, 5.25; N, 13.3, S, 7.59. Found: C, 50.9; H, 5.28; N, 13.1; S, 7.39.

5-Hydroxymethyl-1-phenylpropyluracil (IV).—Anhydrous HBr was slowly bubbled through a solution of 2.88 g (10 mmoles) of 5-ethoxymethyl-1-phenylpropyluracil (V) in 50 ml of CH_2Cl_2 for 30 min; a pilot experiment followed by tlc showed that no V remained at this time. The solution was diluted with 50 ml of CH_2Cl_2, then washed with two 50-ml portions of water. Spin evaporation *in vacuo* left a mixture of IV and the bromomethyl derivative (XXIIIb). The residue was refluxed in a solution of 100 ml of 50% aqueous THF for 3 hr, then the solution was spin evaporated *in vacuo* to about 25 ml. After several hours at 3°, the mixture was filtered and the product was washed with water; yield, 1.86 g (72%) of crude product, mp 140–142°. Recrystallization from acetone with the aid of decolorizing carbon gave 1.44 g (56%) of white crystals, mp 151–152°; the compound moved as a single spot on tlc in ethyl acetate and had λ_{max} (alcohol), 272 mμ; (pH 13), 269 mμ; ν_{max} (Nujol) 3350, 3150 (NH), 1700, 1670, 1600 (C=O, C=C, NH), 735, 695 cm^{-1} (C_6H_5).

Anal. Calcd for $C_{14}H_{16}N_2O_3$: C, 64.6; H, 6.20; N, 10.8. Found: C, 64.7; H, 6.40; N, 10.6.

Irreversible Enzyme Inhibitors. LXXVIII.[1,2] Inhibitors of Thymidine Phosphorylase. IV.[2] Hydrophobic Bonding by Uracils Substituted at the 5 and 6 Positions

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Hydrophobic bonding to thymidine phosphorylase by 5,6-dialkyl, 6-alkyl, 6-aryl, 5-benzyl-6-phenyl, and 6-aralkyl groups on uracil was observed. Nitration of the phenyl group of 6-phenyl- or 6-benzyluracil led to a further increment in binding, whereas the *p*-amino group led to a decrease in binding. In addition to being hydrophobically bonded, the phenyl group of 6-benzyluracil appears to be complexed as an electron acceptor. 6-(*p*-Nitrobenzyl)uracil was complexed to thymidine phosphorylase about twelve times better than the substrate, 2'-deoxy-5-fluorouridine.

In previous papers of this series, the mode of binding of the ribofuranose moiety of thymidine to thymidine phosphorylase was studied,[4] as well as hydrophobic bonding by 1-alkyl- and 1-aralkyluracils[5] and 1,5,6-di- and -trisubstituted uracils;[2] since thymidine phosphorylase is a reversible reaction[6,7] that can convert 2'-deoxyuridine or thymidine to uracil and thymine, or *vice versa,* it would be expected that uracil and thymine would show product inhibition.[6,8] Therefore the possible hydrophobic bonding by 5- and 6-substituted uracils was studied in order to avoid the loss of binding when the 1-hydrogen was removed and is the subject of this paper.[9,12]

Enzyme Results.—Since thymine (II) is a twofold better inhibitor than uracil (I) (Table I), it would appear that the methyl group of thymine makes a contribution to binding to the enzyme by hydrophobic bonding.[12] Similarly, 5-phenyluracil (III) was a twofold better inhibitor than uracil (I). Because of ease of synthesis, the binding by other 5-alkyl or -aralkyl groups was studied with 6-substituents. Note that 5-amyl-6-propyluracil (XI) is a fourfold better inhibitor

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(2) For the previous paper of this series see B. R. Baker, M. Kawazu, D. V. Santi, and T. J. Schwan, *J. Med. Chem.*, **10**, 304 (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) M. Friedkin and D. Roberts, *J. Biol. Chem.*, **207**, 245 (1954).

(7) M. Friedkin and D. Roberts, *ibid.*, **207**, 257 (1954).

(8) W. E. Razzell and P. Casshyap, *ibid.*, **239**, 1789 (1964).

(9) Chemotherapeutic utility of inhibitors of thymidine phosphorylase, particularly of the active-site-directed irreversible type[10,11] has been previously discussed.[4]

(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) B. R. Baker, *J. Pharm. Sci.*, **53**, 347 (1964).

(12) For a more complete discussion on pyrimidine binding see (a) B. R. Baker and M. Kawazu, *J. Med. Chem.*, **10**, 313 (1967); paper LXXIX of this series; (b) *ibid.*, **10**, 316 (1967); paper LXXX of this series.

Table I[a]
Hydrophobic Bonding to Thymidine Phosphorylase by

Compd	R_5	R_6	mM concn	% inhib[b]	Estd[c] $([I]/[S])_{0.5}$
I	H	H	1.5	50	3.9[d]
II	CH_3	H	0.75	50	1.9[d]
III[e]	C_6H_5	H	0.40[f]	16	~2
IV	H	CH_3	3.2	50	8.0
V[e]	H	n-C_3H_7	1.0	50	2.5
VI[e]	H	n-C_5H_{11}	0.46	50	1.1
VII[e]	H	C_6H_5	0.5[f]	0	>5[g]
VIII[e]	H	$C_6H_5CH_2$	0.090	50	0.22
IX	H	$C_6H_5(CH_2)_2$	0.24	50	0.60
X	H	$C_6H_5(CH_2)_3$	0.45	50	1.1
XI[e]	n-C_5H_{11}	n-C_3H_7	0.24	50	0.61
XII[e]	$C_6H_5CH_2$	C_6H_5	0.4[f]	39	1.6
XIII	H	p-$NO_2C_6H_4CH_2$	0.013	50	0.033
XIV	H	p-$NH_2C_6H_4CH_2$	0.15	50	0.48
XV	H	p-$NO_2C_6H_4$	0.60	50	1.5

[a] The technical assistance of Maureen Baker, Barbara Baine, and Pepper Caseria is acknowledged. [b] Thymidine phosphorylase was a 45–90% ammonium sulfate fraction from *E. coli* B prepared and assayed with 0.4 mM 2′-deoxy-5-fluorouridine in succinate–arsenate buffer (pH 5.9), as previously described.[4] [c] The ratio of the concentration of inhibitor to 0.4 mM 2′-deoxy-5-fluorouridine giving 50% inhibition. [d] This compound has previously been reported to be an inhibitor of the arsenolysis of thymidine.[5] [e] Preparation previously described.[2] [f] Maximum concentration allowing measurement of optical density change. [g] Since 20% inhibition is readily detected, the concentration for 50% inhibition is at least four times greater than that measured.

than 6-propyluracil (V); similarly, 5-benzyl-6-phenyluracil (XII)[13] is at least a fourfold better inhibitor than 6-phenyluracil (VII). The hydrophobic bonding by these 5-alkyl, -aryl, or -aralkyl groups give further credence to the suggestion[2] that 5-allyl-2′-deoxyuridine binds sevenfold better than 2′-deoxyuridine[14] due to hydrophobic bonding by the allyl group.

In contrast to thymine (II), 6-methyluracil (IV) is a twofold less effective inhibitor than uracil (I). Since 1-benzyluracil and 1-benzyl-6-methyluracil are equally effective inhibitors,[2] the loss in binding caused by the 6-methyl group of 6-methyluracil (IV) may be due to some hindrance to binding of the 1-hydrogen of the uracil to the enzyme.[12]

Hydrophobic bonding was observed with some larger groups at the 6 position; these comparisons should be compared with 6-methyluracil (IV) as a base line. 6-Propyluracil (V) was a threefold better inhibitor than 6-methyluracil, and 6-n-amyluracil (VI) was a sevenfold better inhibitor; thus hydrophobic bonding by 6 substituents at least three carbons long can occur. Little, if any, hydrophobic bonding was observed with the 6-phenyl group of 6-phenyluracil (VII), the ultraviolet spectrum of which indicates that the 6-phenyl ring is in-plane with the pyrimidine ring;[2] hydrophobic bonding by an out-of-plane 6-phenyl group was previously observed.[2]

Excellent binding was observed with the 6-benzyl group of 6-benzyluracil (VIII), the latter being a 36-fold better inhibitor than 6-methyluracil (IV). Extension of the aralkyl group to phenethyl (IX) or phenylpropyl (X) gave compounds that were less effective inhibitors than 6-benzyluracil (VIII), but still 14-fold and 7-fold better inhibitors, respectively, than 6-methyluracil (IV).

Whether or not hydrophobic bonding at both the 5 and 6 positions of uracil could be attained was also investigated. 5-Amyl-6-propyluracil (XI) was a fourfold better inhibitor than 6-propyluracil (V) and 5-benzyl-6-phenyluracil (XII) was a better inhibitor than 6-phenyluracil (VII).[13]

Since the most effective 6-substituted uracil was 6-benzyluracil (VIII), some further work was performed on the mode of binding of the benzyl group. Introduction of the p-nitro group (as in XIII) on 6-benzyluracil (VIII) gave about a sevenfold tightening of binding, but the p-amino group (as in XIV) gave a twofold less effective inhibitor. Thus the benzene ring would appear to be complexed to the enzyme as an electron acceptor in addition to a possible hydrophobic interaction. Similarly, introduction of a p-nitro group (as in XV) on 6-phenyluracil (VII) gave at least a fivefold increment in binding. A likely candidate for the amino acid on the enzyme that could complex a hydrophobic group such as n-amyl as well as complex a benzyl group by both hydrophobic bonding and electron donation would be methionine with its donor sulfur atom.[10]

Further investigation of additional 5,6 substituents might conceivably give better hydrophobic bonding. However, as described in the following paper, when one of two substituents was 5-bromo or 6-trifluoromethyl a large increment in binding was observed due to increased acidity of the 1-hydrogen of the uracil;[12] therefore, additional 5,6 substituents have not been explored.

Chemistry.—Although the 5 position of uracil can be nitrated,[15] a side-chain phenyl group at the 6 position is apparently more easily nitrated. Nitration of 6-benzyluracil (VIII) in a mixture of 96% sulfuric acid and 70% nitric acid occurred on the phenyl ring to give XIII, as shown by the nmr spectrum of the product: the signal for the uracil 5-H at τ 4.78 was still present and the phenyl signals near τ 2.0 integrated to four protons. Similarly, nitration of 6-phenyluracil (VII) proceeded on the phenyl ring to give XV. In contrast, bromination of 6-benzyl- and 6-phenyluracil proceeds on the pyrimidine ring rather than the benzene ring.[12b] The position of substitution by bromo or nitro could also be deduced by either the pK_a or by the ultraviolet spectrum of the product, although nmr was completely unequivocal, whereas the other measurements were less so. Catalytic reduction of XIII proceeded smoothly to 6-(p-aminobenzyl)uracil (XIV).

6-Phenethyl-2-thiouracil was synthesized by the literature procedure[16] by condensation of ethyl β-phenylpropionylacetate with thiourea. Hydrolysis of the 2-thio group with aqueous chloroacetic acid afforded

(13) The ultraviolet spectrum of 5-benzyl-6-phenyluracil (XII) indicated that the 6-phenyl group is not coplanar with the uracil ring due to the restricted rotation of the 6-phenyl by the adjacent 5-benzyl group;[2] therefore all or part of this better binding by XII than VII may be due to its out-of-plane 6-phenyl group.

(14) C. Heidelberger and J. Boohar, *Biochim. Biophys. Acta*, **91**, 639 (1964).

(15) D. J. Brown, *J. Appl. Chem.* (London), **2**, 239 (1952).

(16) G. W. Anderson, I. F. Halverstadt, W. H. Miller, and R. O. Roblin, Jr., *J. Am. Chem. Soc.*, **67**, 2197 (1945).

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 enyzme. 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).

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(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.