forming group for irreversible inhibition[5,12] was not investigated further.

The required 1-substituted uracils in Table I were synthesized by alkylation of uracil in DMSO in the presence of potassium carbonate[7] (Table II). In the case of XVIII and XIX, the appropriate nitrobenzyl chloride was used, followed by reduction, then bromoacetylation.[15]

Experimental Section[16]

1-(*o*-Aminobenzyl)uracil.—A solution of 1.20 g (5 mmoles) of XXI (Table II) in 40 ml of 1% aqueous NaOH was shaken with hydrogen at 2–3 atm in the presence of 1 ml of Raney nickel for 1 hr when reduction was complete. The filtered solution was acidified to pH 5, then the separated product was collected on a filter and washed with water. Recrystallization from ethanol gave 0.68 g (54%) of pure product as yellow prisms: mp 185–187°; ν_{max} 3350 (NH), 1700–1600 (multiple, broad uracil bands), 745 cm^{-1} (*o*-C_6H_4); λ_{max} (EtOH), 235 mμ (ε 8500), 268 mμ (ε 9500); (pH 13), 266 mμ (ε 7200). The material moved as one spot on tlc in ethyl acetate–benzene (2:3).

Anal. Calcd for $C_{11}H_{11}N_3O_2$: C, 60.8; H, 5.10; N, 19.3. Found: C, 60.7; H, 4.92; N, 19.1.

1-(*m*-Aminobenzyl)uracil was prepared similarly from XX in 43% yield after recrystallization from ethanol: mp 170–171°; λ_{max} (EtOH), 245, 268, mμ; (pH 13), 245, 266 mμ.

Anal. Calcd for $C_{11}H_{11}N_3O_2$: C, 60.8; H, 5.10; N, 19.3. Found: C, 60.9; H, 5.25; N, 19.1.

The compound moved as a single spot on tlc in ethyl acetate–benzene (2:3).

1-(*m*-Bromoacetamidobenzyl)uracil (XVIII).—To a solution of 434 mg (2 mmoles) of 1-(*m*-aminobenzyl)uracil in 3 ml of DMSO at about 50° was added 7 ml of $CHCl_3$ followed by 540 mg (2.1 mmoles) of bromoacetic anhydride. The solution was refluxed for 30 min during which time the product separated. After 2 hr at room temperature, the mixture was filtered and the product was washed ($CHCl_3$); yield 520 mg (77%) of pure product as light pink crystals, mp 248–250°; the product was homogeneous on tlc in ethyl acetate–benzene (2:3) and gave a positive 4-(*p*-nitrobenzyl)pyridine test for active halogen.[15] The compound had ν_{max} 3350 (NH), 1700–1680 (broad), 1550 (C=O, C=C, NH), 770 cm^{-1} (*m*-C_6H_4); λ_{max} (EtOH), 265 mμ; (pH 14), 264 mμ.

Anal. Calcd for $C_{13}H_{12}BrN_3O_3$: C, 46.0; H, 3.86; N, 12.3; Br, 23.6. Found: C, 46.3; H, 3.79; N, 12.4; Br, 23.5.

1-(*o*-Bromoacetamido)uracil (XIX) was prepared as described for XVIII; after recrystallization from DMSO, the yield of product, mp 226–227°, was 56%: ν_{max} 3300 (NH), 1710, 1690, 1650 (C=O, C=C, NH), 769 cm^{-1} (*o*-C_6H_4); λ_{max} (EtOH), 264 mμ; (pH 13), 263 mμ.

Anal. Calcd for $C_{13}H_{12}BrN_3O_3$: C, 46.0; H, 3.86; N, 12.3; Br, 23.6. Found: C, 46.0; H, 3.62; N, 12.2; Br, 23.4.

The compound moved as a single spot on tlc in ethyl acetate–benzene (2:3) and gave a positive 4-(*p*-nitrobenzyl)pyridine test for active halogen.[15]

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

(16) Melting points were taken in capillary tubes on a Mel-Temp block and those below 230° are corrected. Infrared spectra were determined in KBr pellet with a Perkin-Elmer 17B spectrophotometer. Ultraviolet spectra were determined in 10% ethanol with a Perkin-Elmer 202 spectrophotometer unless otherwise indicated. Thin layer chromatograms (tlc) were run on Brinkmann silica gel GF and spots were detected by visual examination under ultraviolet light.

Irreversible Enzyme Inhibitors. LXXVII.[1,2] Inhibitors of Thymidine Phosphorylase. III.[2] Hydrophobic Bonding by 1-Substituted Uracils Containing Additional Substituents at the 5 and 6 Positions

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1-Phenylpropyluracils substituted by alkyl, aryl, or aralkyl groups at the 5 or 6 position were studied as inhibitors of thymidine phosphorylase. In addition to the 15-fold increment in binding by 1-phenylpropyluracil compared to 1-methyluracil, further bonding by the 5-allyl, 5-phenyl, 6-propyl, 6-pentyl, 6-phenyl, and 6-benzyl substituents were noted. Up to a sixfold further increment in binding could be obtained that was most probably due to hydrophobic bonding.

The mode of binding of the ribofuranose moiety of thymidine to thymidine phosphorylase has been presented in an earlier paper of this series.[3] The ribofuranose moiety could be replaced by the 1-phenylamyl group with less than a twofold loss in binding;[2] since it was probable that the phenylamyl group, as well as its lower phenylalkyl homologs, were complexed to a hydrophobic region on the enzyme, it is likely that this hydrophobic region is in a different direction with respect to the 1 position of uracil than the area of the enzyme that complexes the 3'-hydroxyl or thymidine.[3] Therefore, a study was made to determine if additional hydrophobic bonding could occur with 5 or 6 substituents or both on a 1-aralkyluracil; the results are the subject of this paper.[4]

1-Phenylpropyluracil (I) was arbitrarily chosen as a base line for most of this study. That a hydrophobic region might be near the 5 position of uracil when I was complexed to the enzyme was indicated by the nearly twofold loss in binding obtained by substitution with the polar 5-hydroxymethyl group (IV) (Table I); this loss was regained by substitution with the less polar ethoxymethyl group of V, but additional hydrophobic bonding was not obtained with the 5-isoamyloxy group of VII. These results were confirmed in the 1-(*n*-butyl)uracil (II) series where the 5-ethoxymethyl derivative (VI) gave the same binding as II.

By substituting a 5-phenyl (IX) or 5-allyl (VIII) group on 1-phenylpropyluracil (I) only about a twofold

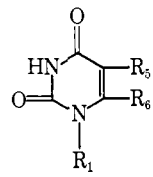
(1) This work was generously supported by Grants CA-05845 and CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper of this series, see B. R. Baker and M. Kawazu, *J. Med. Chem.*, **10**, 302 (1967).

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

(4) The chemotherapeutic utility for inhibitors of thymidine phosphorylase has been previously discussed.[3]

TABLE I
HYDROPHOBIC BONDING TO THYMIDINE PHOSPHORYLASE[a] BY

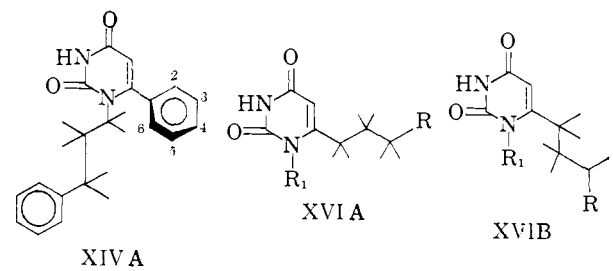


Compd	R_1	R_5	R_6	mM conen	% inhib	Estd[b] $([I]/[S])_{0.5}$
I	$C_6H_5(CH_2)_3$	H	H	5.4	50	13[c]
II	n-C_4H_9	H	H	9.1	50	22[d]
III	$C_6H_5CH_2$	H	H	2.3	50	5.7[c]
IV	$C_6H_5(CH_2)_3$	$HOCH_2$	H	8.5	50	21
V	$C_6H_5(CH_2)_3$	$C_2H_5OCH_2$	H	4.1	50	10
VI	n-C_4H_9	$C_2H_5OCH_2$	H	8.7	50	21
VII	$C_6H_5(CH_2)_3$	i-$C_5H_{11}OCH_2$	H	5.8	50	16
VIII	$C_6H_5(CH_2)_3$	CH_2=$CHCH_2$	H	2.4	50	6.0
IX	$C_6H_5(CH_2)_3$	C_6H_5	H	0.50[e]	20	~5
X	$C_6H_5CH_2$	H	CH_3	2.4	50	6.0
XI	$C_6H_5(CH_2)_3$	H	n-C_3H_7	1.2	50	3.0
XII	$C_6H_5(CH_2)_3$	H	n-C_5H_{11}	1.7	50	4.3
XIII	$C_6H_5(CH_2)_3$	H	$C_6H_5CH_2$	1.2	50	3.0
XIV	$C_6H_5(CH_2)_3$	H	C_6H_5	0.83	50	2.1
XV	$C_6H_5(CH_2)_3$	$C_6H_5CH_2$	C_6H_5	0.080[f]	0	>0.8

[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.[3] The technical assistance of Barbara Baine, Maureen Baker, Pepper Caseria, and Gail Salomon is acknowledged. [b] The ratio of concentration of inhibitor to 0.4 m*M* 2′-deoxy-5-fluorouridine giving 50% inhibition. [c] Data previously reported.[2] [d] Data previously reported.[3] [e] Maximum concentration allowing full light transmission. [f] Maximum solubility.

increment in binding occurred; therefore, further 5 substituents were not investigated since better hydrophobic bonding was observed with 6 substituents. Comparison of 1-benzyluracil (III) with its 6-methyl derivative (X) indicated that no hydrophobic bonding occurred with this 6-methyl group; however, hydrophobic bonding occurred at the 6 position with larger groups. A fourfold increment in binding was observed when 1-phenylpropyluracil (I) was substituted by a 6-propyl (XI) or a 6-benzyl (XIII) group; this was further increased by the 6-amyl group of XII. However, a sixfold increment in binding was observed with the 6-phenyl group of XIV. As will be discussed in a later section, the ultraviolet spectrum of 6-phenyl-1-phenylpropyluracil (XIV) showed that the 6-phenyl ring was out-of-plane with the uracil ring in the ground state. In contrast, 6-phenyluracil, with its in-plane phenyl group, is a less effective inhibitor than uracil;[5a] thus, the binding by a 6-phenyl group to the enzyme occurs only when the phenyl is out-of-plane.

The fact that the 6-amyl, 6-benzyl, 6-propyl, and 6-phenyluracils (XI–XIV) are about equally effective inhibitors, but the 6-methyl group of X gives no hydrophobic bonding, is pertinent to the conformation necessary for the 6 side chain to be hydrophobically bonded and also indicates that no more than the second and third carbons of the 6 side chain are involved in hydrophobic bonding. Since the 6-phenyl group is out-of-plane with the uracil ring, then the second and third carbons of the phenyl ring, which are most probably bonded to the enzyme, are either above the plane of the uracil or below the plane of the uracil; this region could also accommodate the second and third methylenes of a 6-propyl group or the C-1 and C-2 of the phenyl on the 6-benzyl group. For example, if 1-phenylpropyl-6-phenyluracil (XIV) had the conformation depicted in XIVA, then either C-2 and C-3 or C-5 and C-6



XIVA XVIA XVIB

of the phenyl ring could be hydrophobically bonded; similarly, a 6-alkyl group could be bonded in either conformation XVIA or XVIB, or in some intermediate conformation, but insufficient data are available to further elucidate the conformational requirements.

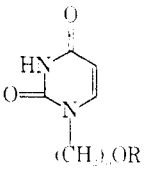
The fact the 6-benzyl group of XIII gives only a fourfold increment in binding compared to 1-phenylpropyluracil (I) (Table I), whereas the same 6-benzyl group gives a 17-fold increment in binding compared to uracil[5a] suggests that there may be some overlap in binding between the 6-benzyl and the 1-phenylpropyl groups which do not allow both to bind completely to the hydrophobic region. Furthermore, 1-phenylbutyluracil binds about threefold better than 1-phenylpropyluracil (I);[2] 1-phenoxypropyluracil with $([I]/[S])_{0.5}$ = 8.3 also binds about twofold better than I.

The nmr spectrum of 1-(3-phenoxypropyl)uracil in D_2O containing NaOD shows shielding of the 6 proton to an extent of about 21 cps when compared to 1-methyl- or 1-(3-hydroxypropyl)uracil (see Table II).

(5) (a) B. R. Baker and M. Kawazu, *J. Med. Chem.*, **10**, 311 (1967); paper LXXVIII of this series; (b) *ibid.*, **10**, 316 (1967); paper LXXX of this series; (c) for a review 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.

TABLE II

NMR SPECTRA[a] OF



No.	R	Solvent	6-H[b]	5-H[b]
1	H	2 *N* NaOD	2.40	4.11
2	C_6H_5	2 *N* NaOD[c]	2.76	4.21
3	C_6H_5	DMSO	2.28	4.36
4	C_6H_5	1 *N* NaOD in 1:1 DMSO–D_2O	2.55	4.28

[a] All spectra were run in 1 *M* solution except **4** which was 0.5 *M*. [b] Center of doublet; $J_{5,6} = 7.5$ cps. [c] The signal of the 6-proton doublet coincided with the aromatic multiplet; the chemical shift was assigned on the basis of two well-defined peaks that were separated by 7.5 cps and had the same relative intensities as the 5-proton doublet at τ 4.21.

Comparison of the well-defined signals arising from the methylene protons of 1-(3-hydroxypropyl)uracil to the broad absorption of the methylene protons of 1-(3-phenoxypropyl)uracil indicates that the conformations of the 1-alkyl substituents can differ in aqueous base. In contrast, the spectrum of 1-(3-phenoxypropyl)uracil in DMSO showed no shielding of the 6-proton and the signals arising from the 1′ and 3′ protons were well resolved (absorption of the 2′ protons coincided with the broad methyl absorption of DMSO). When DMSO was added to a solution of 1-(3-phenoxypropyl)-uracil in basic D_2O, an intermediate effect was obtained in which the 6 proton was deshielded and the 1′ and 3′ protons were better resolved than in pure aqueous media.

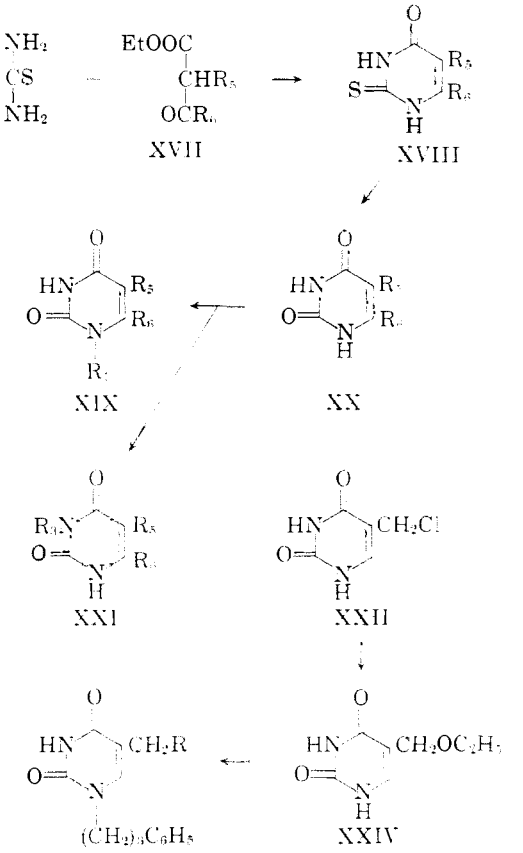
These results are consistent with the phenoxypropyl group having a ground-state staggered conformation in nonaqueous solution, but a folded structure in water. The energy for folding the phenoxypropyl group in water could theoretically be obtained by some internal hydrophobic bonding in the molecule, with additional strengthening by some charge-transfer interaction between the phenyl and the pyrimidine rings. If such were the case, then the increment in binding between XIII and I might be lower than expected because the terminal phenyl on the 1 position of XIII would not be able to occupy the same space as the 6-benzyl group.

These results suggest that the ground-state conformation of hydrocarbon side chains in water is not necessarily staggered as seen in nonaqueous systems but could be partially folded in the ground state. Further studies on hydrocarbon ground-state conformations in water would certainly be pertinent to the energetics of hydrophobic bonding to enzymes.[5c]

Heidelberger and Boohar[6] have reported that 5-allyl-2′-deoxyuridine[7] is complexed to a thymidine phosphorylase seven times better than 5-fluoro-2′-deoxyuridine; from the studies presented here on hydrophobic bonding, it is highly probable that the alkyl group of the nucleoside gives additional binding to the enzyme by hydrophobic bonding. Therefore, a study on whether 2′-deoxyuridine with other 5-aryl-, -alkyl, or -aralkyl substituents would give even better binding than the 5-allyl group would certainly be of interest; such a study has the severe drawback that nucleosides of this type are usually gruellingly difficult to synthesize and purify. As will be reported later,[5] relatively simply substituted uracils have already been found that complex 40 times better than 5-fluoro-2′-deoxyuridine.

Chemistry.—All but one of the 1-substituted uracils in Tables I and IV were synthesized by alkylation of the appropriate 5- or 6-substituted uracil (XX, XXIV). The 5,6-disubstituted, 5-substituted, or 6-substituted uracils (XX) were synthesized by condensation of the appropriate β-keto ester (XVII) with thiourea to give the mercaptouracils (XVIII) followed by hydrolysis with aqueous chloroacetic acid (Scheme I). 5-Allyluracil was a gift from Dr. H. Minnemeyer.[7]

SCHEME I



IV, R = OH
V, R = C_2H_5O
VII, R = *i*-$C_5H_{11}O$
XXIIIa, R = *n*-C_4H_9O
XXIIIb, R = Br

5-Ethoxymethyluracil (XXIV) was synthesized by reaction of 5-chloromethyluracil (XXII)[8a] with alcohol; XXIV has been previously prepared from 5-bromomethyluracil[8b] or 5-hydroxymethyluracil.[8c] Alkylation of XXIV with *n*-butyl bromide by the standard conditions with a 3:1 ratio of the uracil in DMSO in the presence of K_2CO_3 gave a mixture of 1-*n*-butyl-, 1,3-di-*n*-butyluracil, and unchanged XXIV in a ratio of

(6) C. Heidelberger and J. Boohar, *Biochim. Biophys. Acta*, **91**, 639 (1964).
(7) H. J. Minnemeyer, J. A. Egger, J. F. Holland, and H. Tieckelmann, *J. Org. Chem.*, **26**, 4425 (1961).
(8) (a) W. A. Skinner, M. Schelstraete, and B. R. Baker, *ibid.*, **25**, 142 (1960); (b) J. A. Carbon, *ibid.*, **25**, 1731 (1960); (c) R. E. Cline, R. M. Fink, and K. Fink, *J. Am. Chem. Soc.*, **81**, 2521 (1959).

5:1:1; the desired 1-*n*-butyl-5-ethoxymethyluracil was purified by chromatography on silica gel and was isolated in 57% yield. It was noticed that the sodium salt of XXIV was soluble in DMSO when prepared with sodium hydride; when the sodium salt was treated in a 3:1 ratio with phenylpropyl bromide at 80–90°, only traces of bisalkylated uracil was formed. Unchanged XXIV could be removed by several water extractions of a chloroform solution of the reaction mixture; the desired 5-ethoxymethyl-1-phenylpropyluracil (V) was readily crystallized in 66% yield.

Ether exchange was readily effected with V in excess isoamyl alcohol or *n*-butyl alcohol at 70° with a trace of hydrochloric acid to give 86 and 91% yields, respectively, of the isoamyl ether (VII) and the *n*-butyl ether (XXIIIa). The required 5-hydroxymethyluracil derivative IV was obtained by cleavage of the ethyl ether (V) with HBr in dichloromethane to XXIIIb followed by reaction with water in 56% over-all yield for the two steps.

The alkylation of the uracils XX and XXIV with benzyl chloride, phenylpropyl bromide, or *n*-butyl bromide in DMSO could be divided into several classes depending upon the nature of the products, that is, (a) alkylation of N-1 only, (b) alkylation at either N-1 or N-3, and (c) primarily alkylation at N-3.

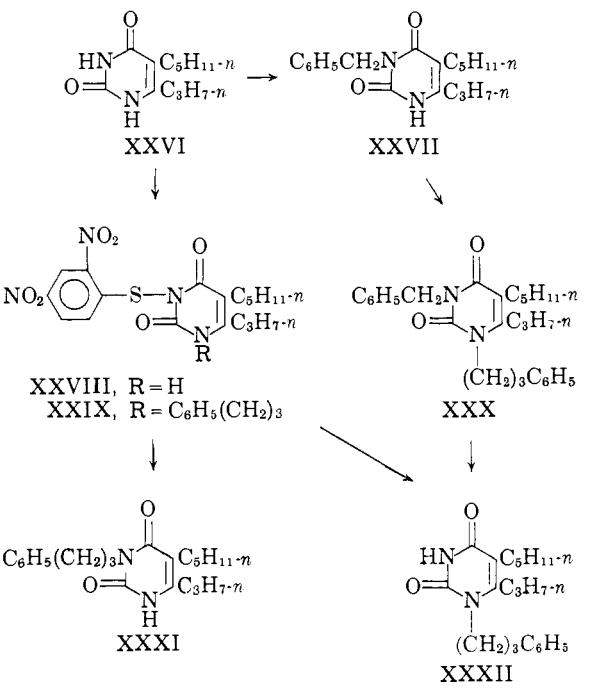
As in the case of thymine,[9b] uracils substituted only at the 5 position with alkoxymethyl (XXIV), allyl, or phenyl gave only 1 alkylation. The presence of a 6 substituent apparently hindered alkylation at N-1 giving a mixture of N-1 and N-3 products which were separated by crystallization or by preparative thin layer chromatography. With 6-*n*-amyl-, 6-propyl-, and 6-benzyluracil the ratio of N-1:N-3 alkylation by phenylpropyl bromide was about 1:6. Alkylation of 6-methyluracil with benzyl chloride gave an N-1:N-3 ratio of 1:1;[10] similar results were obtained with 5,6-dimethyluracil. The alkylation of 6-phenyluracil gave an N-1:N-3 ratio of 4:6; the fourfold increase in alkylation at N-1 compared to 6-propyluracil was initially somewhat surprising. Apparently the coplanar 6-phenyl group either gives less hindrance to the transition state than a 6-methyl or has an inductive effect for N-1 alkylation, or both. Alkylation of 5-benzyl-6-phenyluracil, where the phenyl group is out-of-plane as shown by its ultraviolet spectrum, gave a ratio of N-1:N-3 alkylation of 12:6; in this case, the out-of-plane 6-phenyl group cannot have an electronic effect, but apparently being out-of-plane affords less steric hindrance to N-1 alkylation than a 6-propyl group.

It was interesting to note that 5-(*n*-amyl)-6-propyluracil showed 2.5 times the hindrance to N-1 alkylation than did 6-propyluracil, the ratio of N-1:N-3 alkylation being 1:15

If both the *n*-propyl and *n*-amyl have ground-state staggered conformations, the least interaction between the side chains would be given by conformation such as XXVB; the latter conformation would afford some additional hindrance to alkylation at N-1 compared to the free-rotating, but perhaps staggered, *n*-propyl group of 5-propyluracil. A conformation such as XXVA would give no more hindrance at N-1 than seen with 6-propyluracil, but conformation XXVA might be unfavorable due to proton interaction between C-1 of the 5 side chain and C-2 of the 6 side chain.

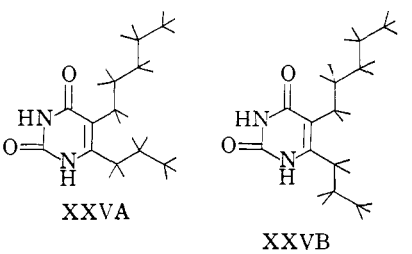
The yield of N-1 alkylated product from 5-(*n*-amyl)-6-propyluracil (XXVI) was so low (1%) that blocking group procedures were sought for the N-3 position. Benzylation of XXVI gave XXVII in 20% yield (Scheme II). Further reaction of XXVII with phenylpropyl bromide in DMSO afforded the 1,3,5,6-tetrasubstituted uracil (XXX) in 56% yield as an oil that showed the proper ultraviolet spectra, but was not further characterized. Attempts to remove the 3-benzyl group of XXX to give XXXII by catalytic hydrogenation or with HBr in water or acetic acid

SCHEME II



were unsuccessful. It is probable that sodium in liquid ammonia could be used without concomitant reduction of the 5,6 double bond of the uracil,[11] provided a 6-phenyl group was not present;[11] this was not tried for the enzymic reasons given below.

A more easily removable blocking group is the 2,4-dinitrobenzenesulfenyl group which can be introduced by reaction of the sulfenyl chloride[12] and removed with aqueous pyridine.[12] Reaction of 2,4-dinitrobenzene-


XXVA

XXVB

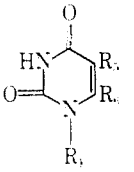
(9) (a) B. R. Baker and G. B. Chheda, *J. Pharm. Sci.*, **54**, 25 (1965); (b) B. R. Baker and T. J. Schwan, *J. Med. Chem.*, **9**, 73 (1966).

(10) H. L. Wheeler and D. F. McFarland, *Am. Chem. J.*, **42**, 101 (1909).

(11) B. R. Baker, D. V. Santi, and H. S. Shapiro, *J. Pharm. Sci.*, **53**, 1317 (1964).

(12) R. L. Letsinger, J. Fontaine, V. Mahadevan, D. A. Schexnayder, and R. E. Leone, *J. Org. Chem.*, **29**, 2615 (1964).

TABLE III
ULTRAVIOLET SPECTRA OF SELECTED SUBSTITUTED URACILS



Compd	R_1	R_5	R_6	λ_{max}, mμ[a] (ε × 10⁴) pH 7	pH 13
XXXIII[a,c]	H	H	H	259 (8.2)	289
XXXIV[b,c]	H	CH_3	H	264 (7.9)	288
XXXV[d]	CH_2=$CHCH_2CH_2$	CH_3	H	274	273
XXXVI	H	C_6H_5	H	240 (12.4), 283 (10.4)	267, 315
IX	$C_6H_5(CH_2)_3$	C_6H_5	H	240, 292	250, 283
XXXVII	H	H	*n*-C_3H_7	263 (10.6)	289
XXXVIII	H	H	$C_6H_5CH_2$	263 (10.4)	292
XXXIX	H	H	C_6H_5	287 (15.1)	316
XL	H	$C_6H_5CH_2$	C_6H_5	281 (11.1)	305
XXVI	H	*n*-C_5H_{11}	*n*-C_3H_7	270 (10.5)	298
XI	$C_6H_5(CH_2)_3$	H	*n*-C_3H_7	270	272
XIV	$C_6H_5(CH_2)_3$	H	C_6H_5	275	274
XLI	$C_6H_5(CH_2)_3$	$C_6H_5CH_2$	C_6H_5	280	280
XXXII	$C_6H_5(CH_2)_3$	*n*-C_5H_{11}	*n*-C_3H_7	273	275

pH 7 spectra done in ethanol and pH 13 spectra done in 10% ethanol unless otherwise indicated. [b] Data previously reported.[13] [c] Determined in water. [d] See ref 9b for preparation.

sulfenyl chloride with the uracil XXVI in pyridine at room temperature gave the 3-substituted uracil XXVIII in good yield. Unfortunately, attempts to alkylate XXVIII to XXIX with phenylpropyl bromide and potassium carbonate in DMSO led to rapid cleavage of the sulfenyl group back to XXVI followed by alkylation to the 3-phenylpropyluracil XXXI; similar results were obtained with potassium *t*-butoxide in *t*-butyl alcohol. Although the direction of alkylation to N-1, when N-3 is preferred, is indeed an interesting synthetic problem, it was not further pursued since better inhibitors of thymidine phosphorylase were later obtained[5a,b] that had neither N-1 nor N-3 substituents.

Ultraviolet Spectra.—The differentiation of N-1 alkylation from N-3 alkylation was readily accomplished by comparison of the spectral peak of a compound in neutral *vs.* 0.1 *N* alkali; the N-1 alkylation products show less than a 2-mμ shift, whereas the N-3 alkylation products show a 24–26-mμ shift to longer wavelength[13] (see Table III) when converted to the anion. The N^1,N^3-disubstituted products are readily differentiated by their insolubility in alkali.

Of interest from the standpoint of enzyme binding and from the standpoint of N-1 *vs.* N-3 alkylation was the conformation of a phenyl ring attached to the 5 or 6 position of the uracil. Overlap of the π orbitals of the phenyl and pyrimidine rings that occurs when they are coplanar is reflected by shift of the peak to longer wavelength and high molecular extinction coefficient.[14] Note that 5-phenyluracil (XXXVI) (Table III) has a peak at 283 mμ in neutral solution compared to thymine (XXXIV) at 264 mμ, a shift of 19 mμ; this shift is attributed to the π-orbital overlap. N-1 alkylation of 5-phenyluracil to give IX shifts the peak 9 mμ to 292 mμ. Similarly, alkylation of thymine (XXXIV) to XXXV gives a 10-mμ shift to 274 mμ; thus, the 5-phenyl group of IX is still coplanar with the uracil ring, since there is an 18-mμ shift from XXXV to IX.

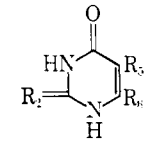
6-Propyl- (XXXVII) and 6-benzyluracils (XXVIII) had identical peaks at 263 mμ in neutral solution. In contrast, 6-phenyluracil (XXXIX) had its peak shifted 24 mμ to 287 with a higher molecular extinction coefficient. Thus, the 6-phenyl group of XXXIX is coplanar with the pyrimidine ring because of the π-orbital overlap. When 6-phenyluracil (XXXIX) was alkylated at the 5 position with a benzyl group (XL), the latter showed a peak at 281 mμ with little increase in extinction compared to 5-amyl-6-propyluracil (XXVI) which had a peak at 270 mμ. Since XXVI shows a 7-mμ shift to longer wavelength compared to 6-propyluracil (XXXVII), then 5-benzyl-6-phenyluracil (XL) should have had a similar shift to 294 mμ. Thus, the phenyl ring of XL is not coplanar with the pyrimidine ring, nor is it perpendicular since there is still some π-orbital overlap compared to 5-amyl-6-propyluracil (XXVI).

Introduction of the 1-phenylpropyl group (XI) on 6-propyluracil (XXXVII) results in a 7-mμ shift to longer wavelength. In contrast, introduction of the phenylpropyl group (XIV) on 6-phenyluracil (XXXIX) gives a 12-mμ shift to shorter wavelength (275 mμ). If the 6-phenyl group of XIV were coplanar with the pyrimidine ring, then XIV should have had a peak at about 294 mμ. There is only slight π-orbital overlap between the 6-phenyl and the pyrimidine rings of XIV since XIV is only shifted 5 mμ to longer wavelength than XI, but 6-phenyluracil (XXXIX) is shifted 24 mμ to longer wavelength than 6-propyluracil (XXXVII). Therefore, the 6-phenyl ring of XIV is not coplanar with the pyrimidine ring.

Similarly, comparison of XLI and XXXII shows that the 6-phenyl group is not coplanar after 5-benzyl-6-phenyluracil (XL) is converted to its 1-phenylpropyl derivative (XLI).

(13) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952).
(14) P. B. Russell, *J. Chem. Soc.*, 2951 (1954).

TABLE IV
PHYSICAL CONSTANTS OF



R2	R5	R6	Method[a]	% yield	Mp, °C	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N	λmax, mμ (ε × 10³) EtOH	λmax, mμ (ε × 10³) pH 13
S	$C_6H_5CH_2$	C_6H_5	A	80	191–192[b]	69.4	4.79	9.51	69.5	5.00	9.30	280	269, 336
S	n-C_5H_{11}	n-C_3H_7	A	83	180–181[b]	60.0	8.38	11.7	59.8	8.44	11.9	282	264, 323
S	H	$C_6H_5CH_2$	A		224–225[c]							272	258, 295 infl
S	H	C_6H_5	A		257–258[d]							280	
S	H	n-C_5H_{11}	A		150–152[e]								
S	C_6H_5	H	A		310–311[f]								
O	$C_6H_5CH_2$	C_6H_5	B	85	210–211	73.4	5.07	10.1	73.4	5.18	9.94	281 (11.1)	305
O	n-C_5H_{11}	n-C_3H_7	B	86	184–185	64.3	8.98	12.5	64.3	9.05	12.1	270 (10.6)	298
O	H	$C_6H_5CH_2$	B		259–260[g]							263 (10.4)	292
O	H	C_6H_5	B		270–272[h]							287 (15.1)	316
O	H	n-C_5H_{11}	B		171–172[i]							263 (10.6)	289
O	C_6H_5	H	B		>350[j]							240 (12.4)	267
												283 (10.4)	315
O	$C_2H_5OCH_2$	H	C	75	220–221[k]							261[l]	286

[a] For methods, see Experimental Section; all compounds had infrared spectra compatible with their assigned structures and were uniform on tlc. [b] Recrystallized from ethanol. [c] Mp 223–224° recorded by D. Libermann, *Bull. Soc. Chim. France*, 1217 (1950). [d] Mp 259° recorded by T. B. Johnson and E. H. Hemingway, *J. Am. Chem Soc.*, **37**, 380 (1915). [e] Mp 153–154° recorded by M. Ynai and T. Naito, *J. Pharm. Soc. Japan*, **61**, 99 (1941). [f] Mp 313–315° recorded by J. H. Burckhalter and H. C. Scarborough, *J. Am. Pharm. Assoc.*, **44**, 545 (1955). [g] Lit.[c] mp 261°. [h] Lit.[d] mp 269–271°. [i] Lit.[e] mp 171–173°. [j] Lit.[f] mp >350°. [k] Lit.[8b] mp 217–218°. [l] In 0.1 *N* aqueous HCl.

Experimental Section[15]

5-Benzyl-6-phenyl-2-thiouracil. Method A.—A mixture of 20 g (0.1 mole) of ethyl benzoylacetate, 13 g (0.1 mole) of benzyl chloride, 50 ml of DMSO, and 14 g (0.1 mole) of anhydrous K_2CO_3 was stirred in a bath at 90–100° for 7.5 hr. The cooled mixture was poured into 200 ml of cold water, then adjusted to pH 2 with HCl and extracted with three 100-ml portions of benzene. The combined extracts, dried with $MgSO_4$, were spin evaporated *in vacuo*, and the residue was distilled; yield 21 g (75%) of ethyl α-benzoylhydrocinnamate as a yellow oil: bp 168–170° (1 mm); ν_{max}^{film} 1740 (ester C=O), 1690 (ketone C=O), 1600 (enol C=C), 750, 690 cm^{-1} (C_6H_5).

To a solution of 3.0 g (55 mmoles) of sodium methoxide in 50 ml of absolute ethanol was added 4.2 g (55 mmoles) of thiourea and 14.1 g (50 mmoles) of the aforementioned ester. After being refluxed for 8 hr with stirring, the mixture was spin evaporated *in vacuo*. The residue was dissolved in 100 ml of water and some insoluble oil was removed by washing with benzene. Acidification of the aqueous solution to pH 2 with HCl gave white crystals that were collected on a filter and washed with water. Recrystallization from ethanol gave 4.4 g (80%) of solvated white plates, mp 100–110°. After being dried at 80° for 24 hr in high vacuum over P_2O_5, the crystals were solvent free and had mp 191–192°; ν_{max} 3500, 3100, 2950 (NH), 1660 (C=O), 1550 (NHC=S), 735, 700 cm^{-1} (C_6H_5). For analytical data see Table IV.

5-Benzyl-6-phenyluracil (XL). Method B.—A mixture of 4.00 g (13.6 mmoles) of 5-benzyl-6-phenyl-2-thiouracil, 50 ml of glacial acetic acid, and 100 ml of 10% aqueous chloroacetic acid was refluxed with stirring for 5 hr. The cooled mixture was filtered and the product was washed with water; yield, 3.2 g (85%) of white needles: mp 210–211°; ν_{max} 3400, 3100, 3000 (NH), 1710, 1640 (C=O, C=C, NH), 726, 695 cm^{-1} (C_6H_5). For analytical data see Table IV.

(15) Infrared spectra were determined in KBr pellets with a Perkin-Elmer 137B spectrophotometer. Ultraviolet spectra were determined in ethanol and at pH 13 in 10% ethanol with a Perkin-Elmer 202 spectrophotometer. Melting points were determined either in capillary tubes on a Mel-Temp block or on a Fisher-Johns apparatus; those below 230° are corrected. Thin layer chromatograms (tlc) were run on Brinkmann silica gel GF and spots were detected by visual examination under ultraviolet light. Nmr spectra were determined with a Varian A-60 spectrophotometer using $(CH_3)_4$-Si as an internal standard in DMSO and sodium 3-(trimethylsilyl)-1-propanesulfonate in D_2O.

5-Ethoxymethyluracil (XXIV). Method C.—A suspension of 16.0 g (0.1 mole) of 5-chloromethyluracil[8a] in 600 ml of absolute ethanol was refluxed for 10 min when solution was essentially complete except for the small amount of contaminating insoluble polymer.[8a] The hot solution was filtered through a Celite pad on a preheated funnel. The product that separated on cooling (14.2 g) still contained some polymer since it only sintered at 220° and was not completely melted at 250°. The solid was dissolved in hot ethanol, filtered free of polymer, then chilled; yield 12.8 g (76%), mp 220–222°; the product showed only traces of impurities on tlc in 5:3 $CHCl_3$–ethanol and was suitable for further transformations. Carbon[8b] has recorded mp 217–218° while Cline, *et al.*,[8c] have recorded 212°.

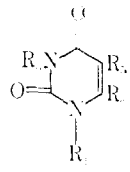
Alkylation of Substituted Uracils. Method D.—Where alkylation gave only 1 substitution, as in the case of 5-substituted uracils, the reaction was run with a 3:1 excess of uracil over halide and the product was isolated by crystallization by the method previously described.[9]

Method E.—Late in this work it was found that the sodium salt of 5-ethoxymethyluracil (XXIV) was soluble in dimethyl sulfoxide. Better yields were then obtained with a 3:1 ratio of uracil to alkyl bromide. Whether or not this desirable method would work with some or most of the uracils in Table V or whether a 1:1 ratio of alkyl halide could be used was not determined.

To a stirred suspension of 17 g (0.1 mole) of 5-ethoxymethyluracil (XXIV) in 75 ml of reagent DMSO was added over a period of about 30 min 2.4 g (0.1 mole) of NaH as a dispersion in mineral oil. After being stirred an additional 30 min, solution was complete; then, 7.0 g (0.035 mole) of phenylpropyl bromide was added. After being stirred at 85–90° for 3 hr, the mixture was poured into about 300 g of crushed ice and acidified to about pH 2 with HCl. It was extracted with three 150-ml portions of $CHCl_3$; the combined extracts were washed with water until tlc of the $CHCl_3$ solution showed that all of the XXIV had been removed (usually three 150-ml portions). The dried $CHCl_3$ solution was spin evaporated *in vacuo*. Crystallization from ethyl acetate–petroleum ether (bp 60–110°) gave 6.6 g (66%) of product that moved as one spot on tlc in ethyl acetate and was sufficiently pure for further transformation. Recrystallization from the same solvent system gave white crystals: mp 110–111°; ν_{max} (Nujol) 3150 (NH), 1700–1600 (broad) (C=O, C=C, NH), 1095 (ether C–O–C), 745, 700 cm^{-1} (C_6H_5). See Table V for analytical data.

Method F.—This method was the same as method D, except that the starting uracil was also extracted with the $CHCl_3$.

TABLE V
PHYSICAL CONSTANTS AND METHODS FOR



R_1	R_3	R_5	R_6	Method[a]	% yield	Mp °C	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N	λ_{max}, mμ EtOH	λ_{max}, mμ pH 13
$C_6H_5(CH_2)_3$	H	C_6H_5	H	H	51[b]	141–142	74.5	5.92	9.14	74.3	6.13	9.07	292[c]	283[c]
$C_6H_5(CH_2)_3$	H	CH_2=CH-CH_2	H	G	43[d]	Oil	71.1	6.71	10.4	71.0	6.80	10.1	275	274
$C_6H_5O(CH_2)_3$	H	H	H	E[e]	51	159–161[b]	63.4	5.73	11.4	63.2	5.80	11.4	298	267
$C_6H_5(CH_2)_3$	H	$C_2H_5OCH_2$	H	E	66	110–111[f]	66.7	6.99	9.72	66.8	7.16	9.59	272	260
n-C_4H_9	H	$C_2H_5OCH_2$	H	D	57	89–90[g]	58.4	8.02	12.4	58.6	7.90	12.2	272	269
$C_6H_5(CH_2)_3$	H	i-C_5H_{11}-OCH_2	H	J	91	87–88[h]	69.1	7.93	8.48	69.0	7.83	8.73	272	269
$C_6H_5(CH_2)_3$	H	n-C_4H_9-OCH_2	H	J	86[h]	96–97	68.3	7.65	8.85	68.6	7.70	8.84	272	269
$C_6H_5CH_2$	H	H	CH_3	I	19	235–240[i]							268	268
H	$C_6H_5CH_2$	H	CH_3	I	19	177–183[j]							264	282
$C_6H_5(CH_2)_3$	H	H	n-C_3H_7	G[k]	6.6	99–101	70.6	7.40	10.3	70.6	7.35	10.0	270	272
H	$C_6H_5(CH_2)_3$	H	n-C_3H_7	G	37	159–160	70.6	7.40	10.3	70.7	7.52	10.5	264	288
$C_6H_5(CH_2)_3$	H	H	n-C_5H_{11}	G	2.6	115–116	72.0	8.05	9.33	71.7	8.09	9.55	270	270
H	$C_6H_5(CH_2)_3$	H	n-C_5H_{11}	G	17	142–143	72.0	8.05	9.33	71.8	7.96	9.62	264	290
$C_6H_5(CH_2)_3$	H	H	$C_6H_5CH_2$	G	2.8	179–180[l]	75.0	6.29	8.74	74.7	6.14	8.51	272	270
H	$C_6H_5(CH_2)_3$	H	$C_6H_5CH_2$	G	19	192–193[m]	75.0	6.29		74.8	6.50		267	291
$C_6H_5(CH_2)_3$	H	H	C_6H_5	G	10.6	Glass	74.5	5.92	9.14	74.3	6.10	8.95	275	274
H	$C_6H_5(CH_2)_3$	H	C_6H_5	G	16	175–176	74.5	5.92	9.14	74.4	5.86	9.19	290	314
$C_6H_5(CH_2)_3$	H	CH_3	CH_3	I	6.7	173–184							274	275
H	$C_6H_5(CH_2)_3$	CH_3	CH_3	I	20	208–210[b]	67.8	6.13	12.2	67.6	6.38	12.4	269	294
$C_6H_5(CH_2)_3$	H	$C_6H_5CH_2$	C_6H_5	G	13	Glass	78.7	6.10	7.06	78.6	6.28	7.19	280	280
H	$C_6H_5(CH_2)_3$	$C_6H_5CH_2$	C_6H_5	G	6.9	183–184	78.7	6.10	7.06	78.5	6.19	7.09	283	308
$C_6H_5(CH_2)_3$	H	n-C_5H_{11}	n-C_3H_7	G	1.2	Glass							273	275
H	$C_6H_5(CH_2)_3$	n-C_5H_{11}	n-C_3H_7	G	18	98–99	73.6	8.83	8.18	73.5	8.65	8.09	269	295
$C_6H_5CH_2$	H	n-C_5H_{11}	n-C_3H_7	G	1.6	Glass							276	278
H	$C_6H_5CH_2$	n-C_5H_{11}	n-C_3H_7	G	20	102–103	72.6	8.34	8.91	72.8	8.49	9.13	273	298
$C_6H_5(CH_2)_3$	$C_6H_5CH_2$	n-C_5H_{11}	n-C_3H_7	G	56	Oil							280	280

[a] For methods, see Experimental Section; all compounds had infrared spectra compatible with their assigned structures and were uniform on tlc. [b] Recrystallized from ethyl acetate. [c] Second peak at 240 mμ at pH 7 and 250 mμ at pH 13. [d] Purified by preparative tlc with 3:1 benzene–methanol. [e] Run in DMF for 1 hr at 110°. [f] Recrystallized from ethyl acetate–petroleum ether (bp 60–110°). [g] Recrystallized from ether–petroleum ether. [h] Recrystallized from petroleum ether. [i] Lit.[10] mp 233°. [j] Lit.[10] mp 194°. [k] A 2.1% yield of the corresponding 1,3-bisphenylpropyluracil, mp 90°, was also isolated. *Anal.* Calcd for $C_{25}H_{30}N_2O_2$: C, 76.9; H, 7.74; N, 7.17. Found: C, 77.1; H, 7.80; N, 7.15. [l] Recrystallized from ethanol. [m] Recrystallized from ethanol–$CHCl_3$.

After removal of the $CHCl_3$ and the last of the DMSO, the unalkylated uracil was removed by crystallization from chloroform. The product was then crystallized by addition of ether and petroleum ether (bp 30–60°).

Method G was the same as F, except the unalkylated uracil was chloroform soluble. As much as possible of the unalkylated uracil was removed by crystallization from alcohol; the filtrate was evaporated and the product was isolated by preparative tlc. This method was also used if the product was an oil or a mixture of 1- and 3-substituted uracils that were not readily separable by crystallization.

About 500 mg of crude residue in methanol or ether was applied to two tlc plates that were coated with silica gel (20 × 20 × 0.15 cm) with an automatic applicator. The plates were developed with 5:1 benzene–ethyl acetate, then viewed and marked by observation under ultraviolet light. The unalkylated uracils remained near the origin. The second slowest zone was the 1-substituted uracil, the third slowest zone the 3-substituted uracil, and the fourth slowest zone the 1,3-disubstituted uracil. The fast zone near the front was not characterized but may have contained O-alkylated products. The respective zones were scraped from the plates and the product was isolated by extraction with ethyl acetate, then evaporation. If the residue was crystalline, it was seldom necessary to recrystallize it since it was already pure; if not crystalline, the residue was rechecked for purity by tlc and if necessary was rechromatogrammed.

Method H.—The product and starting uracil both separated on addition of the water to the reaction mixture. The solids were collected on a filter, washed with water, and leached with hot alcohol; the starting uracil was removed by filtration, the filtrate was evaporated, and the residue was recrystallized from the solvent noted in Table V.

Method I was the same as D except that the mixture of 1- and 3-substituted uracils was separated by crystallization; this method is represented by the benzylation of 6-methyluracil.

A mixture of 1.26 g (10 mmoles) of benzyl chloride, 3.78 g (30 mmoles) of 6-methyluracil (Aldrich Chemical Co.), 4.15 g (30 mmoles) of K_2CO_3, and 70 ml of DMSO was stirred in a bath at 90° for 4–15 hr; the cooled mixture was poured into 200 g of iced water, then acidified to pH 2 with HCl. The mixture was extracted with five 40-ml portions of $CHCl_3$. Dried with $MgSO_4$, the combined extracts were spin evaporated *in vacuo*. The residual gum (1.91 g) was extracted with four 100-ml portions of hot 0.1 *N* aqueous *NaOH*. The alkali-insoluble gummy material (0.61 g, 40%) was 1,3-dibenzyl-6-methyluracil; it could be purified by extraction with hot petroleum ether (bp 30–60°). Evaporation of the petroleum ether gave 0.143 g of an oil that was uniform on tlc in 3:1 benzene–methanol and had λ_{max} (pH 7, 13) 270 mμ. Additional material could be obtained by further extraction of the gum with petroleum ether.

The combined alkaline extracts were acidified to pH 3 and refrigerated overnight. The solid was collected on a filter and washed with water; yield 0.523 g (25%) of crude 1-benzyl-6-methyluracil, mp 203–226°, λ_{max} (pH 7, 13) 268 mμ. Recrystallization from methanol gave 0.390 g (19%) of pure product as

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.