Irreversible Enzyme Inhibitors. LXXX.[1,2] Inhibitors of Thymidine Phosphorylase. VI.[2] Hydrophobic Bonding with 6 Substituents on the Acidic 5-Nitro- and 5-Bromouracils

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Since the 6-benzyl and the 5-bromo groups on uracil give increments in binding to thymidine phosphorylase, the effects of both groups in the same molecule, 6-benzyl-5-bromouracil (XVII), were investigated; the hydrophobic bonding by the benzyl group and the effect of increased acidity by the 5-bromo atom on binding were additive. 6-Benzyl-5-bromouracil (XVII) was a 150-fold more effective inhibitor than uracil, XVII being complexed to thymidine phosphorylase 40-fold better than the substrate, 2′-deoxy-5-fluorouridine. Additive effects were not observed by introduction of a 5-bromo on 6-(*p*-nitrobenzyl)uracil; in fact, the binding by the benzyl group was lost. The binding by the benzyl group returned when the nitro group of 5-bromo-6-(*p*-nitrobenzyl)uracil was reduced to amino (XXV). It has not yet been possible to rationalize these results with the *para*-substituted benzyl-5-bromouracils.

In an earlier paper of this series,[4] it was observed that a 6-benzyl group (II) on uracil (I) (Table I) gave an 18-fold increment in binding to thymidine phosphorylase; this increment could be increased to 120-fold with a 6-*p*-nitrobenzyl group (III), thus indicating that the benzyl group was complexed both hydrophobically and as an electron acceptor. In a subsequent study,[2] it was found that the 5-nitro (IV) and the 5-bromo (V) groups could give 18- and 9-fold increments in binding, compared to uracil, due primarily to the increased acidity of the 1-hydrogen of the uracil. If both increased acidity of the 1-hydrogen and hydrophobic bonding could be incorporated into the same uracil molecule, strong inhibitors of thymidine phosphorylase could emerge. The results of the first of such studies are the subject of this paper.

Since the 5-nitro group on uracil had the biggest effect on acidity and enzyme binding, 5-nitrouracils with 6 substituents were studied first. Introduction of the 6-methyl group (as in VIII) or the 6-propyl group (VII) gave less than a twofold increment in binding (Table I); similar results were observed in comparison of uracil (I) with 6-propyluracil (VI). Unfortunately, 5-nitro-6-benzyluracil, which should be a good inhibitor, could not be synthesized by nitration of 6-benzyluracil (II); 6-(*p*-nitrobenzyl)uracil (III) was obtained instead.[4] Therefore, 5-nitro-6-styryluracil (IX)[5] was evaluated as an inhibitor. IX was threefold less effective than 5-nitrouracil; apparently the rather rigid styryl group could not assume the proper conformation for binding to the enzyme in the presence of the 5-nitro group.

The effect of the 6-styryl group on binding was also investigated with a 5-sulfonylpiperidide group on the uracil; XI was about a sevenfold better inhibitor than the corresponding 6-methyluracil X. Surprisingly, reduction of the styryl double bond to phenethyl (XII) caused loss of binding by the phenethyl group, XII being no more effective than the 6-methyluracil (X).

It was previously observed that the polar 5-diazonium group on uracil (as in XIV) led to some loss in binding,[2] the extent being difficult to assess due to lack of sufficient light transmission. That the polar 5-diazonium group did give a loss of binding was confirmed in the 6-phenethyl series, XIII being a fivefold less effective inhibitor than 6-phenethyluracil (XV).

Excellent inhibitors of thymidine phosphorylase were found in the 5-bromouracil series. 5-Bromo-6-propyluracil (XVI) was a 14-fold better inhibitor than 6-propyluracil (VI) and 5-bromo-6-benzyluracil (XVII) was a ninefold better inhibitor than 6-benzyluracil (II); these results should be compared with 5-bromouracil (V) being a ninefold better inhibitor than uracil (I).[2] Thus the increments in binding by the 5-bromo and 6-benzyl groups were additive; 5-bromo-6-benzyluracil (XVII) was one of the two best inhibitors in Table I, being complexed to thymidine phosphorylase 150-fold better than uracil and 40-fold better than the substrate, 2′-deoxy-5-fluorouridine.

Some higher and lower homologs of 5-bromo-6-benzyluracil (XVII) were then checked for maximum activity, namely, 6-phenyl (XVIII), 6-phenethyl (XXI), and 6-phenylpropyl (XXII). In each case, the 5-bromo group gave little increased binding; thus, 5-bromo-6-benzyluracil (XVII) was still by far the best inhibitor of the series.

The larger 5-iodo group (XX) also gave an increment in binding on 6-benzyluracil (II), XX being about a fourfold better inhibitor than II; however, XX was about one-half as effective as 5-bromo-6-benzyluracil (XVII).

It was previously observed that the electron-withdrawing nitro group of 6-(*p*-nitrobenzyl)uracil (III) gave a sevenfold increase in binding compared to 6-benzyluracil (II);[4] this increase was lost when the nitro group of III was reduced to amino, as in XXVI.[4] Therefore, III was converted to 5-bromo-6-(*p*-nitrobenzyl)uracil (XXIV) to determine if the increments in binding from the nitro and bromo groups would be additive; surprisingly, XXIV was a fivefold less effective inhibitor than 6-(*p*-nitrobenzyl)uracil (III) and a sevenfold less effective inhibitor than 5-bromo-6-benzyluracil (XVII). In fact, XXIV was only a twofold better inhibitor than 5-bromouracil (V), indicating that most of the binding by the 6-benzyl group had been lost.

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

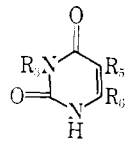
(2) For the previous paper of this series see B. R. Baker and M. Kawazu, *J. Med. Chem.*, **10**, 313 (1967).

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

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

(5) W. C. J. Ross, *J. Chem. Soc.*, 1128 (1948).

TABLE I
INHIBITION OF THYMIDINE PHOSPHORYLASE[a] BY



Compd	R_5	R_6	R_3	mM conen	% inhib	$([I]/[S])_{0.5}$[b]
I	H	H	H	1.5	50	3.9[c]
II	H	$C_6H_5CH_2$	H	0.090	50	0.22[d]
III	H	p-$NO_2C_6H_4CH_2$	H	0.013	50	0.032[d]
IV	NO_2	H	H	0.090	50	0.22[e]
V	Br	H	H	0.18	50	0.45[e]
VI	H	n-C_3H_7	H	1.0	50	2.5[d]
VII	NO_2	n-C_3H_7	H	0.051	50	0.13
VIII	NO_2	CH_3	H	0.063	50	0.18[e]
IX	NO_2	$C_6H_5CH{=}CH$	H	0.25	50	0.62
X		CH_3	H	2.0[f]	43	6.0[e]
XI		$C_6H_5CH{=}CH$	H	0.15[f]	31	0.88
XII		$C_6H_5(CH_2)_2$	H	1.5	39	5.0
XIII	$N{\equiv}N^{+}-$	$C_6H_5(CH_2)_2$	H	0.5	32	3.0
XIV	$N{\equiv}N^{+}-$	H	H	0.5[f]	0	>5[e,g]
XV	H	$C_6H_5(CH_2)_2$	H	0.24	50	0.60[d]
XVI	Br	n-C_3H_7	H	0.14	50	0.35
XVII	Br	$C_6H_5CH_2$	H	0.010	50	0.025
XVIII	Br	C_6H_5	H	0.66	50	1.7
XIX	H	C_6H_5	H	0.25[f]	0	>2.5[d,g]
XX	I	$C_6H_5CH_2$	H	0.024	50	0.060
XXI	Br	$C_6H_5(CH_2)_2$	H	0.23	50	0.57
XXII	Br	$C_6H_5(CH_2)_3$	H	0.19	50	0.47
XXIII	H	$C_6H_5(CH_2)_3$	H	0.45	50	1.1[d]
XXIV	Br	p-$NO_2C_6H_4CH_2$	H	0.067	50	0.17
XXV	Br	p-$NH_2C_6H_4CH_2$	H	0.011	50	0.028
XXVI	H	p-$NH_2C_6H_4CH_2$	H	0.15	50	0.37[d]
XXVII	Br	p-$CH_2C_6H_4SO_2Cl$	H	0.60[f]	30	3.5
XXVIII	Br	p-$CH_2C_6H_4SO_2NH_2$	H	0.40[f]	30	2.3
XXIX	Br	$C_6H_5CH_2$	$NCCH_2$	0.30[f]	11	~10
XXX	Br	$C_6H_5CH_2$	m-$NO_2C_6H_4CH_2$	0.15[h]	0	>1.5[g]
XXXIa	H	p-$CH_2C_6H_4SO_2Cl$	H	0.88	50	2.2
XXXIb	H	p-$CH_2C_6H_4SO_2NH_2$	H	0.17	50	0.42

[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% dimethyl sulfoxide; the same results with III and XVIII were obtained without DMSO. The technical assistance of Barbara Baine, Maureen Baker, and Pepper Caseria is acknowledged. [b] The ratio of concentration of inhibitor to 0.4 m*M* substrate giving 50% inhibition. [c] Data previously reported: B. R. Baker and M. Kawazu, *J. Med. Chem.*, **10**, 302 (1967); paper LXXVI of this series. [d] Data previously reported.[4] [e] Data previously reported.[2] [f] Maximum concentration allowing full light transmission. [g] Since 20% inhibition is readily detected, the concentration for 50% inhibition is at least four times the concentration measured. [h] Maximum solubility.

If the binding of the benzyl group of XXIV was indeed lost, then the nitrobenzyl group could not assume the proper conformation for binding. One possible explanation is that the electronegative nitrobenzyl group is repulsed by the electronegative bromo group, thus not allowing the nitrobenzyl group to approach the 5-bromo closely in space. If maximum binding by the nitrobenzyl group requires a conformation of the phenyl approaching the 5 position of the uracil, then maximum binding would not be achieved. Further support for this explanation was then sought.

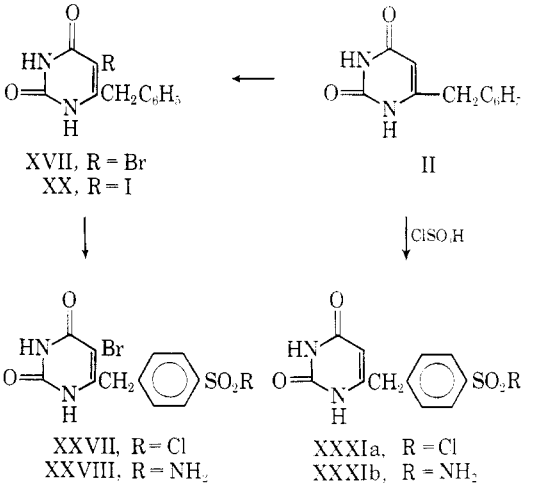
(1) Reduction of the nitro group of 5-bromo-6-(*p*-nitrobenzyl)uracil (XXIV) to amino (XXV) gave an inhibitor just as effective as 5-bromo-6-benzyluracil (XVII), indicating that the electron-withdrawing nitro group of XXIV was detrimental to binding. (2) Introduction of the electron-withdrawing sulfonyl chloride (XXVII) or sulfonamide (XXVIII) groups on 5-bromo-6-benzyluracil (XVII) were also severely detrimental to binding. (3) Although the binding of 6-benzyluracil (II) could be increased by the electron-withdrawing *p*-nitro group (III),[4] binding by the benzyl group was completely lost when the electron-withdrawing sulfonyl chloride group was introduced as in XXXIa; the corresponding sulfonamide (XXXIb) was not better than 6-benzyluracil (II). Thus, the effect of the *p*-nitro group of III cannot be explained by its electronegativity alone. Perhaps steric or even direct binding by the *p*-group is involved, but the current studies do not shed sufficient light on the mode of binding of III.

In a previous paper, evidence was presented that the 3-hydrogen of uracil was complexed to thymidine phosphorylase. This observation was confirmed with

the more potent 5-bromo-6-benzyluracil; introduction of a 3-cyanomethyl group (XXIX) or a 3-(*m*-nitrobenzyl) group (XXX) led to large losses in binding.

Chemistry.—Most of the 5-bromouracils in Table I were made by bromination of the corresponding 6-substituted uracil in glacial acetic acid.[6] 5-Bromo-6-(*p*-aminobenzyl)uracil (XXVI) was prepared by catalytic hydrogenation of XXIV in acid solution.

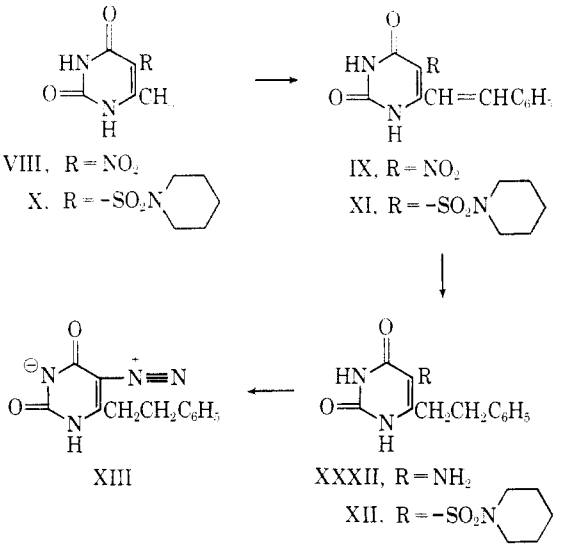
Chlorosulfonation of 5-bromo-6-benzyluracil (XVII) with chlorosulfonic acid afforded XXVII, which gave XXVIII on reaction with ammonia. A similar



chlorosulfonation of 6-benzyluracil (II) at room temperature selectively chlorosulfonated the benzene ring to XXXIa; the chlorosulfonation of the 5 position of uracil requires a higher temperature,[2] about 100°. That sulfonation occurred on the benzene ring was demonstrated by the loss of the band due to C_6H_5 near 700 cm^{-1} and the lack of shift in the ultraviolet maximum which would occur on 5 substitution.

Iodination of 6-benzyluracil (II) with iodine in aqueous sodium hydroxide[7] afforded 5-iodo-6-benzyluracil (XX) in good yield. Alkylation of 5-bromo-6-benzyluracil (XVII) with chloroacetonitrile or *m*-nitrobenzyl chloride in dimethyl sulfoxide in the presence of potassium carbonate gave a mixture of the 1 and 3 isomers which were separated by preparative thin layer chromatography to give XXIX and XXX, respectively, by the general procedure previously described.[8]

Condensation of 5-nitro-6-methyluracil (VIII) with benzaldehyde as previously described[5] proceeded smoothly. Catalytic hydrogenation of IX in glacial acetic acid in the presence of PtO_2 catalyst reduced both the nitro group and the styryl double bond to give 5-amino-6-phenethyluracil (XXXII) in 64% yield isolated as its hydrochloride. Treatment of XXXII hydrochloride with sodium nitrite in water resulted in precipitation of the diazouracil XIII as a zwitterion in 83% yield. This compound (XIII) was rather unstable in 50% DMSO solution at room temperature, undergoing spectral shifts: it had a half-life of about 1 hr. However, at 0° a solution of XIII in DMSO was stable, undergoing no spectral shift in 2 hr. Attempts



to convert XIII to the 5-cyano or 5-sulfonyl chloride[9] by the Sandmeyer reaction were unsuccessful; therefore, the synthesis of XII by another route was investigated.

Since the 6-methyl group of uracil can be condensed with benzaldehyde when the methyl group is activated by the electron-withdrawing 5-nitro group,[5] the reaction of a 6-methyluracil, bearing the electron-withdrawing sulfonylpiperidide group (X), with benzaldehyde was investigated. Condensation to the 6-styryluracil XI proceeded smoothly in the presence of piperidine in 81% yield. Catalytic reduction of the ethylenic double bond of XI with a palladium–charcoal catalyst afforded the phenethyluracil XII.

Experimental Section[10]

5-Bromo-6-(*p*-nitrobenzyl)uracil (XXIV) (Method A).—To a mixture of 1.24 g (5 mmoles) of III[4] and 25 ml of acetic acid at 70° was added 0.88 g (5.5 mmoles) of bromine with mixing. A clear solution soon formed which then deposited white crystals. After 2 hr at room temperature, the mixture was filtered and the product was washed with ethanol. Recrystallization from aqueous dioxane gave 1.35 g (83%) of white prisms: mp 270–271° dec; ν_{max} 1700, 1650 (uracil), 1500, 1330 (NO_2), 870, 856 cm^{-1} (p-C_6H_4). See Table II for additional data for compounds prepared by this method. In some cases the acetic acid was diluted with water to isolate the soluble product.

5-Amino-6-phenethyluracil (XXXII) Hydrochloride (Method C).—A suspension of 2.60 g (10 mmoles) of IX[5] in 200 ml of glacial acetic acid was shaken with hydrogen at 2–3 atm in the presence of 100 mg of PtO_2; 40 mmoles of hydrogen were absorbed within 1 hr, then reduction practically ceased. The filtered solution was spin evaporated *in vacuo*. The oily residue was dissolved in 10 ml of 12 *N* aqueous HCl, then the solution was spin evaporated *in vacuo*. Recrystallization of the residue from ethanol gave 1.60 g (64%) of white plates: mp 254–256°; ν_{max} 3490 (NH), 2550, 2500 (NH_3^+), 1700–1630 (uracil), 750, 700 cm^{-1} (C_6H_5). See Table II for additional data.

Method B was a variant of method C where 70% aqueous

(6) T. B. Johnson and J. C. Ambelang, *J. Am. Chem. Soc.*, **60**, 2941 (1938).

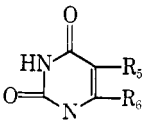
(7) The procedure of T. B. Johnson and C. O. Johns, *J. Biol. Chem.*, **1**, 305 (1905), for iodination of uracil was employed.

(8) B. R. Baker and M. Kawazu, *J. Med. Chem.*, **10**, 304 (1967); paper LXXVII of this series.

(9) Conversion of diazonium salts to a sulfonyl chloride with SO_2 in aqueous HCl has been successful in other cases; *cf.* B. R. Baker, B.-T. Ho, J. K. Coward, and D. V. Santi, *J. Pharm. Sci.*, **55**, 302 (1966); H. Meerwein, G. Dittmar, R. Göller, K. Hafner, F. Mensch, and O. Steinfort, *Chem. Ber.*, **99**, 841 (1957).

(10) Melting points were determined with a Fisher-Johns apparatus and those below 250° are corrected. Infrared spectra were run in KBr pellet with a Perkin-Elmer 137B or 337 spectrophotometer. Ultraviolet spectra were run in water (unless otherwise indicated) 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.

TABLE II
PHYSICAL CONSTANTS OF 5,6-DISUBSTITUTED URACILS



Compd[a]	R_5	R_6	Method	% yield[b]	Mp, °C	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N	λ_{max}, mμ pH 6	λ_{max}, mμ pH 13
XVI[c]	Br	*n*-C_3H_7	A	60	221–222[d]	36.1	3.89	34.3[e]	35.9	3.79	34.4[e]	280	290
XVII[c]	Br	$C_6H_5CH_2$	A		244–245[d,f]							282	302
XVIII[c]	Br	C_6H_5	A	75	276–278[g]	45.0	2.64	10.5[h]	45.1	2.73	10.4[h]	290	313
XXI[c]	Br	$C_6H_5(CH_2)_2$	A	82	281–282[i]	27.1[j]		9.49	27.1[j]		9.40	280	290
XXII[c]	Br	$C_6H_5(CH_2)_3$	A	78	205–207[d]	50.6	4.24	9.06[k]	50.8	4.26	9.20	280	290
XXIV[c]	Br	*p*-$NO_2C_6H_4CH_2$	A	83	270–271[l]	40.5	2.47	12.9[l]	40.8	2.65	12.6	280	290
XXV[c]	Br	*p*-$CH_2C_6H_4NH_2$·HCl	B	90	>330[i,m]	39.7	3.33	12.6	40.0	3.45	12.40	278	292
XXVII	Br	*p*-$CH_2C_6H_4SO_2Cl$	E	63	242–244[d]	38.6[n]	3.45	6.00	38.8	3.38	6.35	269	268
XXVIII	Br	*p*-$CH_2C_6H_4SO_2NH_2$	F	53	271–272[i]	36.8	2.81	11.7	36.8	3.08	12.0	280	302
XX	I	$C_6H_5CH_2$	G	63	240–241[d]	40.3	2.76	8.54	40.3	2.79	8.54	278	298
VII	NO_2	*n*-C_3H_7	H	66	226–227[i]	42.2	4.55	21.1	41.9	4.36	20.9	273	254, 368
IX	NO_2	$C_6H_5CH{=}CH$	I		312-316[o]							290–330	289
XI		$C_6H_5CH{=}CH$	I	81	323–324	56.5	5.30	11.6[p]	56.6	5.49	11.4	318	313
XII		$C_6H_5(CH_2)_2$	D	85	304–305	56.2	5.82	11 6	56.0	5.76	11.7	274	284
XIII	$-^+N{\equiv}N$	$C_6H_5(CH_2)_2$	J	83	>200[q]	59.5	4.16	23.1	59.3	4.26	22.9	303	258, 316
XXXIa	H	*p*-$CH_2C_6H_4SO_2Cl$	E	25	236–237[d]	43.9	3.02	9.31	43.6	3.16	8.93	263	286
XXXIb	H	*p*-$CH_2C_6H_4SO_2NH_2$	F	31	247–248	47.0	3.92	14.9	46.8	4.03	14.7	263	286
XXXII	NH_2·HCl	$C_6H_5(CH_2)_2$	C	64	254–256[r]	53.8	5.27	15.7	53.7	5.40	15.9	295[s]	292

[a] All compounds were uniform on tlc and had infrared spectra in agreement with their assigned structures. [b] Yield of analytically pure material; no effort was made to rework mother liquors. [c] See ref 4 for starting material. [d] Recrystallized from dioxane. [e] Br analysis. [f] Lit.[4] mp 235°. [g] Recrystallized from acetone–ethyl acetate. [h] *Anal.* Calcd: Br, 29.9. Found: Br, 30.1. [i] Recrystallized from aqueous dioxane. [j] Br. [k] *Anal.* Calcd: Br, 25.8. Found: Br, 26.0. [l] *Anal.* Calcd: Br, 24.5. Found: Br, 24.8. [m] Free base had mp 250–251° dec. [n] With 1 mole of dioxane of crystallization. *Anal.* Calcd: S, 6.86. Found: S, 7.12. [o] Lit.[5] mp 316°. [p] *Anal.* Calcd: S, 8.87. Found: S, 8.63. [q] Gradually decomposes over 200°; not recrystallized due to instability in solution. [r] Recrystallized from ethanol. [s] At pH 1, λ_{max} 265 mμ.

methanol containing excess HCl was employed as solvent and 5% Pd-C was employed as catalyst.

Method D was a variant of method C where methanol was the solvent and 5% Pd-C was the catalyst.

5-Bromo-6-(*p*-chlorosulfonylbenzyl)uracil (XXVII) (Method E).—To 4.5 g of chlorosulfonic acid stirred in an ice bath was added in portions 1.40 g (5 mmoles) of XVII[6] at such a rate that the temperature did not exceed 10°. After 5 hr at ambient temperature, the solution was poured into excess ice. The product was collected on a filter, washed with water, and dried *in vacuo*. Recrystallization from dioxane gave 1.2 g (63%) of white prisms: mp 242–244°; ν_{max} 1700, 1650 (uracil), 1350, 1170 (SO_2), 850 cm^{-1} (*p*-C_6H_4). See Table II for additional data.

5-Bromo-6-(*p*-sulfamoylbenzyl)uracil (XXVIII) (Method F).—A solution of 1.00 g (2.64 mmoles) of XXVII in 50 ml of concentrated NH_3 water was allowed to stand about 18 hr, then spin evaporated *in vacuo* to about 0.25 volume. The solution was acidified with 10% HCl. The product was collected by filtration and washed with water. Recrystallization from aqueous dioxane gave 0.50 g (53%) of white prisms: mp 271-272 dec; λ_{max} (EtOH), 280 mμ; (pH 13), 302 mμ; ν_{max} 3200 (NH), 1730, 1680–1660, 1620 (uracil, C=C), 1325, 1150 cm^{-1} (SO_2NH).

5-Iodo-6-benzyluracil (XX) (Method G).—To a stirred solution of 202 mg (1 mmole) of 6-benzyluracil (II)[4] in 25 ml of 1 *N* aqueous NaOH was added 254 mg (1 mmole) of iodine. After 5 hr at ambient temperature, the solution was acidified with acetic acid. The product was collected on a filter, then washed with water and alcohol. Recrystallization from dioxane gave 205 mg (63%) of white prisms: mp 240–241°; ν_{max} 1710, 1620 (uracil), 730, 710 cm^{-1} (C_6H_5). See Table II for additional data.

5-Nitro-6-(*n*-propyl)uracil (VII) (Method H).—Nitration of 6-(*n*-propyl)uracil (VI),[8] as described for the preparation of 6-(*p*-nitrobenzyl)uracil (III),[4] gave a crude product that was recrystallized from aqueous dioxane; yield, 66% of light yellow needles; mp 226–227°; ν_{max} 3400 (NH), 1710, 1680 (uracil), 1520, 1350 cm^{-1} (NO_2). See Table II for additional data.

6-Styryluracil-5-sulfonylpiperidide (XI) (Method I).—A mixture of 1.36 g (5 mmoles) of 6-methyluracil-5-sulfonylpiperidide (X), 5 ml of dioxane, 5 ml of piperidine, and 5 ml of benzaldehyde was heated on a steam bath under a reflux condenser for 7 hr during which time light yellow crystals separated. The cooled mixture was filtered and the product was washed with ethanol; yield, 2.5 g of the piperidinium salt; mp 205°. The salt was dissolved in water and the solution was acidified with 10% HCl. The product was collected on a filter, then washed with water and ethanol. Recrystallization from dioxane gave 1.45 g (81%) of colorless prisms: mp 323–324°; ν_{max} 1700, 1680, 1630 (uracil, C=C), 1415, 1340, 1168 (SO_2N), 748, 690 cm^{-1} (C_6H_5). See Table II for additional data.

5-Diazo-6-phenethyluracil (Method J).—To a solution of 2.68 g (10 mmoles) of XXXII·HCl in 100 ml of water cooled in an ice bath was added with stirring a solution of 0.76 g (11 mmoles) of $NaNO_2$ as a concentrated aqueous solution; the addition was at a rate that did not raise the temperature above 10°. During the addition, the product began to separate. After being stirred an additional hour with the ice bath removed, the mixture was filtered, and the product was washed with ice water, then THF and ether; yield, 2.0 g (83%) of slightly yellow needles that had no definite melting point, but gradually decomposed over 200°; ν_{max} 3150 (NH), 2150 ($^+N{\equiv}N$), 1700–1650 (uracil), 740, 700 cm^{-1} (C_6H_5). See Table II for additional data.

6-Benzyl-5-bromo-3-cyanomethyluracil (XXIX).—A mixture of 2.8 g (10 mmoles) of 6-benzyl-5-bromouracil (XVII),[6] 25 ml of DMSO, 0.76 g (10 mmoles) of chloroacetonitrile, and 1.37 g (10 mmoles) of anhydrous K_2CO_3 was stirred at 75-80° for 3 hr, then poured into 50 ml of water and extracted several times with $CHCl_3$. The combined extracts were dried ($MgSO_4$), then spin evaporated *in vacuo*. The residue on tlc showed the presence of starting material, 1-cyanomethyl derivative, 3-cyanomethyl derivative, and the 1,3-bis(cyanomethyl) derivative. This mixture was separated by preparative tlc as described previously for related alkylation mixtures.[8] Elution of the proper zone gave

280 mg (9%) of white needles: mp 200–201°; $\lambda_{max}^{10\%\ EtOH}$ (pH 8–13), 305 mμ; (pH 1), 283 mμ; $pK_a = 7.5$.

Anal. Calcd for $C_{18}H_{16}BrN_3O_2$: C, 48.8; H, 3.15; N, 13.1. Found: C, 49.0; H, 3.38; N, 13.0.

6-Benzyl-5-bromo-3-(*m*-nitrobenzyl)uracil (XXX).—A mixture of 2.18 g (7.77 mmoles) of 6-benzyl-5-bromouracil (XVII),[6] 25 ml of DMSO, 1.00 g (5.90 mmoles) of *m*-nitrobenzyl chloride, and 0.81 g (5.90 mmoles) of anhydrous K_2CO_3 was heated in a steam bath for 4 hr, then poured into 50 ml of cold water. After about 18 hr at 5°, the mixture was filtered and the solids were washed with water. Preparative tlc as described earlier[5] gave a zone for XXX that was eluted to give 320 mg (9.9%) of colorless prisms: mp 199–200°; λ_{max} (EtOH), 268 mμ; (10% EtOH, pH 13), 260, 310 mμ (sh).

Anal. Calcd for $C_{18}H_{14}BrN_3O_4$: C, 51.9; H, 3.39; N, 10.1. Found: C, 51.8; H, 3.53; N, 10.5.

Approaches to the Synthesis of 1-Deazauridine and 2′-Deoxy-1-deazauridine[1]

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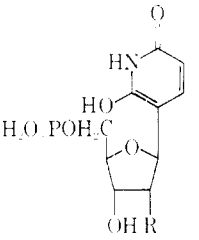
The synthesis of the title compounds as potential inhibitors of thymidylate synthetase was approached by modification of the procedure used in the synthesis of pseudouridine. Treatment of 3-bromo-2,6-dibenzyloxypyridine (**7b**) with *n*-butyllithium was followed by conversion to the 3-cadmium derivative **10b**. When the latter was treated with 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride followed by esterolysis, both 1,2-O-[3-(2,6-dibenzyloxypyridyl)]benzylidene-α-D-ribofuranose (**15**) and 3-(D-ribofuranosyl)-2,6-dibenzyloxypyridine (**14**) were obtained in 25 and 10% yields, respectively. The 2′-deoxy analog (**18**) was obtained by treatment of **10b** with 3,5-di-O-*p*-toluyl-2-deoxy-D-ribofuranosyl chloride and subsequent esterolysis. Hydrogenolysis of **14** and **18** gave the title compounds, both of which were unstable. In view of the latter the anomeric configuration and the cyclic nature of the sugar in **21** and **20** have not been determined. No significant inhibition of thymidylate synthetase or dihydrofolate reductase was noted in this series of compounds.

Strong inhibition of the thymidylate synthetase catalyzed conversion of deoxyuridine 5′-monophosphate to thymidine 5′-monophosphate has been reported for analogs of both the substrate and the product. The substrate analog, 5-fluoro-2′-deoxyuridine 5′-monophosphate (FUdRP), has a K_i of 5×10^{-8} *M* while the product analog, 5-trifluoromethyl-2′-deoxyuridine 5′-monophosphate (F_3dTMP), is reported to have a K_i of 4×10^{-8} *M*.[3] Baker[4] attributed the inhibition by FUdRP to enhanced electrostatic binding to the enzyme due to the increased acidity of the pyrimidine ring. In application of his proposed mechanism for inhibition of thymidylate synthetase 5-fluorouracil (pK_a = 8.15) and 5-trifluoromethyluracil (pK_a = 7.35), more acidic than their natural counterparts (uracil, pK_a = 9.45; thymine, pK_a = 9.82), as the 2′-deoxynucleotides could cause inhibition by increased binding affinity to the enzyme through the $N^3HC^4{=}O$ portion of the pyrimidine ring.[3]

Recently 5-trifluoromethyl-6-aza-2′-deoxyuridine and the 5′-monophosphate have been synthesized in an effort to further evaluate the effects of acidity on binding to this enzyme.[5] In contrast to the fluoro and trifluoromethyl compounds the fluorinated triazines, 5-trifluoromethyl-6-azauracil (pK_a = 5.4), its 2′-deoxynucleoside, as well as the 5′-monophosphate derivative, have been found to lack significant activity against thymidylate synthetase.

The acidity of 2,6-dihydroxypyridine (**8**, pK_a = 4.2, titration), which can be considered a deaza analog of uracil, prompted the synthesis of the respective sugar analogs, 1-deazauridine 5′-monophosphate (**1a**) and 2′-deoxy-1-deazauridine 5′-monophosphate (**1b**), to evaluate the effect of increased acidity on thymidylate synthetase inhibition.



1a, R = OH
b, R = H

In the search for new or enhanced biological activities the substitution of carbon for nitrogen has been reported for many classes of medicinal agents. Notable among the anticancer agents are the deaza analogs of the folic acid antimetabolites[6] and various purine analogs.[7] 3-Diazocitrazinic acid, a deaza analog of orotic acid, is reported to be a competitive antagonist of orotic acid.[8]

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(3) The chemistry and biology of these inhibitors have been reviewed recently: C. Heidelberger, *Progr. Nucleic Acid Res. Mol. Biol.*, **4**, 1 (1965); P. Reyes and C. Heidelberger, *Mol. Pharmacol.*, **1**, 14 (1965).

(4) B. R. Baker, *Cancer Chemotherapy Rept.*, **4**, 1, 1959.

(5) (a) M. P. Mertes and S. E. Saheb, *J. Heterocyclic Chem.*, **2**, 491 (1965); (b) M. P. Mertes, S. E. Saheb, and D. Miller, *ibid.*, **2**, 493 (1965); (c) T. Y. Shen, W. V. Ruyle, and R. L. Bugianesi, *ibid.*, **2**, 495 (1965); (d) M. P. Mertes, S. E. Saheb, and D. Miller, *J. Med. Chem.*, **9**, 876 (1966); (e) A. Dipple and C. Heidelberger, *ibid.*, **9**, 715 (1966).

(6) (a) R. D. Elliott, C. Temple, Jr., and J. A. Montogomery, *J. Org. Chem.*, **31**, 1890 (1966); (b) J. DeGraw, L. Goodman, B. Weinstein, and B. R. Baker, *ibid.*, **27**, 576 (1962), and references to earlier papers.

(7) (a) S. Suzuki and S. Marumo, *J. Antibiotics* (Tokyo), **A10**, 20 (1957); (b) R. J. Rousseau, L. B. Townsend, and R. K. Robins, *Biochemistry*, **5**, 756 (1966); (c) K. Tanaka, J. Sugawa, R. Nakamori, Y. Sanno, and Y. Ando, *J. Pharm. Soc. Japan*, **75**, 770 (1955); (d) J. A. Montgomery and K. Hewson, *J. Org. Chem.*, **30**, 1528 (1965); *J. Med. Chem.*, **8**, 708 (1965); (e) C. A. Salemink and G. M. Van der Want, *Rec. Trav. Chim.*, **68**, 1013 (1949); (f) R. K. Robins, J. K. Horner, C. V. Greco, C. W. Noell, and C. G. Beames, Jr., *J. Org. Chem.*, **28**, 3041 (1963).

(8) Z. B. Papanastassiou, A. McMillan, V. J. Czebotar, and T. J. Bardos, *J. Am. Chem. Soc.*, **81**, 6056 (1959).