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Analogs of Tetrahydrofolic Acid. XXVI. Synthesis of Some 5-Aryl and 5-Aralkyl Pyrimidine-6-carboxylic Acids and Their Effect on Dihydrofolic Reductase and Thymidylate Synthetase (1, 2)

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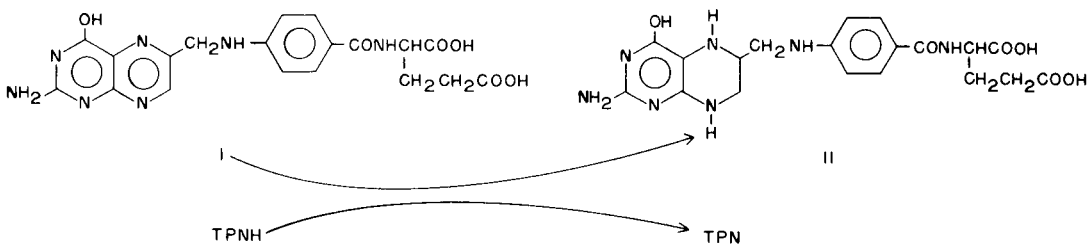
2-Amino-4-hydroxy-5-(4-phenylbutyl)pyrimidine-6-carboxylic acid (V) was synthesized from isopropyl 2-ethoxalyl-6-phenylhexanoate (XIII) by conversion to acyl guanide (XV) with guanidine followed by anhydrous acid catalyzed ring closure to ethyl 2-amino-α-(4-phenylbutyl)-4-oxo-2-imidazoline-$\Delta^{5,\alpha}$-acetate (XXIII) and base catalyzed rearrangement to V; similarly, *p*-chlorophenylacetonitrile was converted to 5-(*p*-chlorophenyl)-2,4-diaminopyrimidine-6-carboxylic acid (VIII). Claisen condensation of isopropyl 6-phenylhexanoate (XI) or *p*-chlorophenylacetonitrile with ethyl trifluoroacetate followed by condensation with guanidine afforded 2-amino-5-(4-phenylbutyl)-6-(trifluoromethyl)-4-pyrimidinol (XXVI) and 5-(*p*-chlorophenyl)-2,4-diamino-6-(trifluoromethyl)pyrimidine (XXXIV). Evaluation of V, VIII, XXVI, and XXXIV as inhibitors of dihydrofolic reductase showed that the 6-carboxyl, 6-trifluoromethyl, and 6-carbethoxy groups greatly reduced the effectiveness as inhibitors of dihydrofolic reductase compared to the corresponding 6-methyl derivatives. The inhibition data appear to make unlikely the possibility of the pyrimidines binding to the enzyme by four hydrogen bonds as propounded by Zakrzewski (19) or by binding through a charge-transfer complex, but supports the concept (18) of an anionic-cationic interaction for the protonated 2,4-diaminopyrimidines; a weak base - weak acid interaction for a non-protonated pyrimidine that is stronger than a hydrogen bond is also proposed.

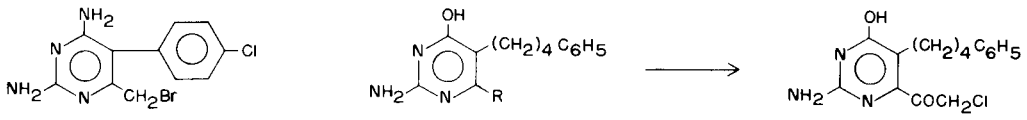
For the reduction of folic acid (I) to tetrahydrofolic acid (II) by the enzyme, folic reductase, a hydride ion from TPNH is transferred, then the substrate must procure a proton for each of the double bonds. It was recently proposed (3) that the enzyme could have a properly positioned proton on a donor such as an imidazole to aid in the reduction. If such a proton donor is present in the active site it should be positioned near N-5 or N-8 (or both) of the folate molecule in the enzyme-substrate complex; it should be possible to detect this proton donor by an active-site-directed irreversible inhibitor of the endo-alkylation type (4); by its very nature of being a proton donor, this moiety should also be a nucleophile that might be covalently linked by a properly designed irreversible inhibitor when the latter complexes with the active site. The first attempt with 6-bromomethyl-5-(*p*-chlorophenyl)-2,4-diaminopyrimidine (III) (5) met with failure; since III formed a strong reversible complex with dihydrofolic reductase, the failure of III to irreversibly inhibit the enzyme could be attributed to an insufficient bridge length (4) between the bromomethyl group and the proton donor within the enzyme-inhibitor complex. Therefore, a longer bridge such as that in VI or IX might be better able to link covalently with the enzymic proton donor.

In order to synthesize chloromethyl ketones such as VI or IX, the pyrimidine-6-carboxylic acids, V and VIII, would be useful intermediates. The synthesis of V and VIII and some of their derivatives as well as their inhibition of dihydrofolic reductase and thymidylate synthetase is the subject of this paper.

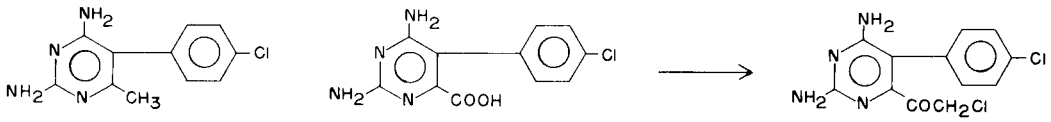
A route to 2-amino-4-hydroxy-5-methylpyrimidine-6-carboxylic acid (XX) via ethyl oxalylpropionate (XII) and the 2-aminoimidazoline (XXI) has been described by Laursen *et al.* (6) which would appear to be general for other 5-alkyl or 5-aralkyl derivatives such as V; except for a 24% yield on conversion of the acylguanidine (XIV) to the imidazoline (XXI) with 6 N hydrochloric acid, the sequence proceeded in reasonable yield. A molecule such as XIV is readily cleaved by aqueous acid, thus leading to low yields in ring closure to XXI. Since the ring-closure is obviously acid-catalyzed, acidic, but non-cleaving conditions, were investigated. The ring-closure of XIV with boiling glacial acetic acid containing 10% dry hydrogen bromide proceeded smoothly in 96% yield to the imidazoline ester, XXII; thus, the one poor-yield in the sequence was eliminated.

Claisen condensation of ethyl oxalate with isopropyl 6-phenylhexanoate (XI) afforded a crude keto ester (XIII) which was condensed with guanidine to give the guanide, XV, in 50% yield for the two steps. Cyclization of the phenylhexanoate derivative (XV) with hydrogen bromide in acetic acid proceeded in 87% yield to XXIII. Saponification and rearrangement of XXIII with aqueous potassium hydroxide to

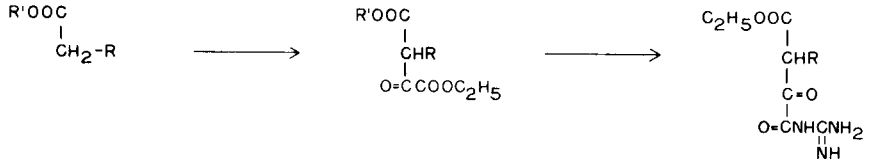




III IV, R = CH_3 V, R = COOH VI



VII VIII IX



X, R' = C_2H_5, R = CH_3

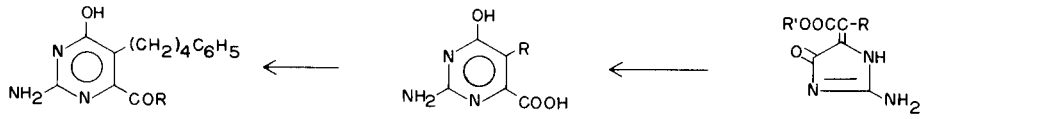
XI, R' = i-C_3H_7, R = $C_6H_5(CH_2)_4$-

XII, R = CH_3, R' = C_2H_5

XIII, R = $C_6H_5(CH_2)_4$-

XIV, R = CH_3

XV, R = $C_6H_5(CH_2)_4$-



XVI, R = Cl

XVII, R = C_2H_5O

XVIII, R = $NHNH_2$

XIX, R = $NCCH_2O$-

XX, R = CH_3

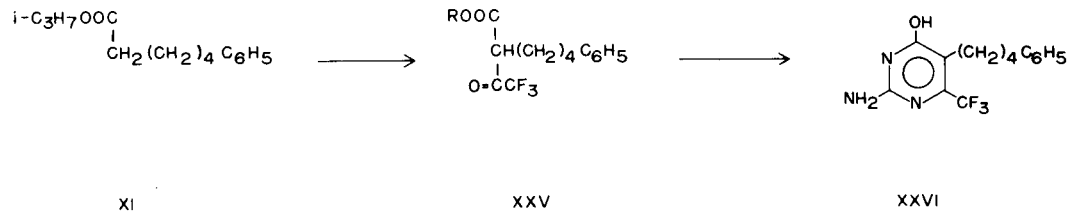
V, R = $C_6H_5(CH_2)_4$-

XXI, R = CH_3, R' = H

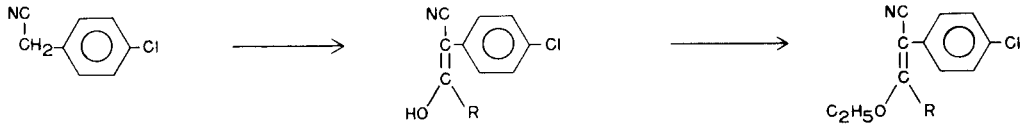
XXII, R = CH_3, R' = C_2H_5

XXIII, R = $C_6H_5(CH_2)_4$-, R' = C_2H_5

XXIV, R = $C_6H_5(CH_2)_4$-, R' = i-C_3H_7



XI XXV XXVI

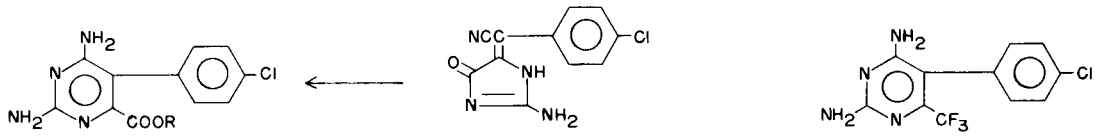


XXVII XXVIII, R = -$COOC_2H_5$

XXIX, R = -CF_3

XXX, R = -$COOC_2H_5$

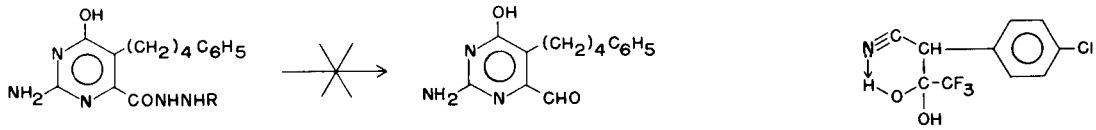
XXXI, R = -CF_3



VIII, R = H

XXXII, R = C_2H_5-

XXXIII XXXIV



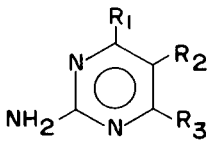
XVIII, R = H

XXXV, R = $C_6H_5SO_2$-

XXXVI XXXVII

TABLE I

Inhibition of Dihydrofolic Reductase By



Compound Number	R_1	R_2	R_3	mM Conc. Inhibitor	% Inhibition	I/S (a)
IV (b)	HO	$C_6H_5(CH_2)_4$-	CH_3	0.030	50	5.0 (c)
V	HO	$C_6H_5(CH_2)_4$-	COO^-	3.7	50	610
XVII	HO	$C_6H_5(CH_2)_4$-	$COOC_2H_5$	0.60 (d, e)	0	>600 (f)
XIX	HO	$C_6H_5(CH_2)_4$-	$COOCH_2CN$	0.60 (d, e)	0	>600 (f)
XXVI	HO	$C_6H_5(CH_2)_4$-	CF_3	0.075 (d, e)	0	>50 (f)
XXXVI	HO	$C_6H_5(CH_2)_4$-	CHO	0.30 (d, e)	37	87
VII	NH_2	4-ClC_6H_4-	CH_3	0.00020	50	0.033
VIII	NH_2	4-ClC_6H_4-	COO^-	0.90	50	150
XXXII	NH_2	4-ClC_6H_4-	$COOC_2H_5$	0.14 (e)	50	23
XXXIV	NH_2	4-ClC_6H_4	CF_3	0.050 (d, e)	50	8.7

Dihydrofolic reductase was a 45-90% ammonium sulfate fraction isolated from pigeon liver acetone powder and assayed with 6 μM dihydrofolate and 12 μM TPNH in 0.05 M Tris buffer (*p*H 7.4) as previously described (7). (a) Estimated ratio of inhibitor to 6 μM dihydrofolate giving 50% inhibition. (b) Data from reference 8. (c) The same results were obtained in the presence of 10% N,N-dimethylformamide (8). (d) Near maximum solubility. (e) Assay solution contained 10% N,N-dimethylformamide. (f) Since 20% inhibition is readily detectable, the minimum I/S is calculated using four-times the inhibitor concentration.

the desired pure pyrimidine-6-carboxylic acid (V) proceeded in 57% yield.

The pyrimidine-6-carboxylic acid (V) was then assayed as an inhibitor of dihydrofolic reductase and thymidylate synthetase (7), since the effect of an electron-withdrawing group at the 6-position in a molecule such as V or the chloromethyl ketone, VI, was unknown. Surprisingly, the effect of the 6-carboxyl was drastically detrimental (Table I); the concentration necessary for 50% inhibition was increased from 0.03 mM in the case of the 6-methylpyrimidine (IV) (8) to 3.7 mM for the corresponding pyrimidine-6-carboxylic acid (V), a 120-fold loss in binding. Similarly, thymidylate synthetase was inhibited 50% by an 0.40 mM concentration of the 6-methylpyrimidine (IV) (8), but no inhibition of this enzyme was observed at a 7.6 mM concentration of the 6-carboxypyrimidine (V), a greater than 34-fold loss in binding.

In order to gain additional information on why the 6-carboxyl group of V was so detrimental to enzyme binding, the acid (V) was converted to its ethyl ester (XVII) and cyanomethyl ester (XIX); also for enzymic comparison, 2-amino-5-(4-phenylbutyl)-6-(trifluoromethyl)-4-pyrimidinol (XXVI) was prepared by total synthesis. Furthermore, in order to determine if a detrimental effect of a negative substituent at the 6-position would also carry-over to the 5-(4-chlorophenyl)-2,4-diaminopyrimidine system (VII), the 6-carboxylic acid (VIII), 6-carbethoxy (XXXII) and 6-trifluoromethyl (XXXIV) derivatives were synthesized.

Attempts to esterify the pyrimidine-6-carboxylic acid (V) with ethanolic sulfuric acid were unsuccessful presumably due to the insolubility of the sulfate salt of V in ethanol. Attempts to convert V to acid chloride (XVI) with thionyl chloride were unpromising due to decomposition. Therefore, the cyanomethyl ester, XIX, was synthesized in 86% yield by reaction of the triethylammonium salt of V with chloroacetonitrile in triethylamine (10). Later it was found that by the elegant method of Adams and Ulich (11) the acid chloride (XVI) could be prepared from the sodium salt of V with oxalyl chloride; the acid chloride (XVI) could be converted to the ethyl ester (XVII) with ethanol in 82% yield from the acid (V). Later it was found that the ethyl ester (XVII) could be prepared directly from V using ethanolic ethanesulfonic acid since the ethanesulfonate salts of V and XVII were more soluble.

Claisen condensation of isopropyl 6-phenylhexanoate (XI) with excess ethyl trifluoroacetate in the presence of sodium hydride gave the crude keto ester (XXV), which may have been a mixture of ethyl and isopropyl esters; XXV condensed with guanidine carbonate in *t*-butyl alcohol (12) to give an overall yield of the pure 6-(trifluoromethyl)-

pyrimidine (XXVI) of 35%.

Claisen condensation of ethyl oxalate with *p*-chlorophenylacetonitrile (XXVII) proceeded to crystalline XXVIII in 73% yield; the spectral characteristics of XXVIII indicated that it was strongly enolic both in solution and the solid state since it showed an enolic band at 3.10 and strong enolic C=C at 6.13 μ in the infrared and gave little shift in ultraviolet spectrum from neutral to basic solution. Conversion of XXVIII to the crude enol ether (XXX) with ethyl orthoformate and reaction with alcoholic guanidine did not give a guanide, as in the case of XIV and XV, but spontaneous cyclization of the guanide to the imidazoline (XXXIII) took place in 82% yield. This high-melting imidazoline (XXXIII) could not be fully purified and apparently could lose an amino group by hydrolysis in hot water; however the crude imidazoline (XXXIII) showed the expected spectral characteristics and could be smoothly rearranged with aqueous potassium hydroxide to the pure 6-carboxypyrimidine (VIII) in 51% yield. The carboxyl group of VIII could be converted to the ethyl ester (XXXII) with ethanolic ethanesulfonic acid in 74% yield.

Claisen condensation of *p*-chlorophenylacetonitrile with ethyl trifluoroacetate to give the keto nitrile (XXIX) proceeded in 33% yield; this keto nitrile was not the expected enol, XXIX, but formed a hydrate stable to recrystallization from organic solvents. The infrared spectrum clearly showed the absence of the usual enolic C=C at 6.13 μ, but in addition to showing the usual weak aryl C=C at 6.25 μ, it had two strong, sharp bands at 2.82 and 3.08 μ and a strong band at 9.25 μ. These observations are best correlated with the stable hydrate structure, XXXVII, where the non-bonded hydroxyl would give a band at 2.82 μ and the bonded hydroxyl would give a band at 3.08 μ, and a strong C-OH band near 9.2 μ; the latter band is absent in XXVIII; as a film or in solution only a single broad hydroxyl band at 3.0 μ was seen with XXXVII, indicating that the chelate structure (XXXVII) existed only in the crystal pack and may have just as well been intermolecular as intramolecular.

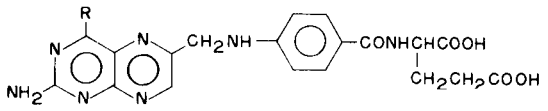
Conversion of XXXVII (XXIX hydrate) to the enol ether (XXXI) with ethyl orthoformate and condensation with guanidine carbonate in *t*-butyl alcohol (12) afforded the desired 6-trifluoromethylpyrimidine (XXXIV) in 65% overall yield for the two steps.

As a final check to make more certain that a chloromethyl ketone such as VI would be an unlikely candidate for an irreversible inhibitor, due to poor reversible binding (4), 2-amino-4-hydroxy-5-(4-phenylbutyl)pyrimidine-6-carboxaldehyde (XXXVI) was synthesized. Attempts to synthesize XXXVI from the sulfonhydrazide (XXXV) were unsuccessful. The aldehyde was finally synthesized by a ring closure to form a pyrimidine-6-carboxaldehyde acetal and will be described in a future paper.

ENZYME ASSAYS

The enzymic evaluation of the candidate inhibitors of dihydrofolic reductase are listed in Table I. In both the 2,4-diamino series (VII) and the 2-amino-4-pyrimidinol series (IV) an electron-withdrawing group at the 6-position such as trifluoromethyl or carboxylic ester was detrimental to binding compared to a 6-methyl group. This loss in binding was huge in the 2,4-diamino series (VII), being about 250-700 fold when the 6-methyl derivative (VII) is compared to the 6-trifluoromethyl (XXXIV) and the 6-carbethoxy derivative (XXXII). The inductive effect of the 6-trifluoromethyl (XXXIV) and 6-carbethoxy (XXXII) derivatives has a powerful influence on the basicity of the pyrimidine moiety; from their ultraviolet spectra listed in the experimental, it is clear that the 6-methyl derivative (VII) is mainly protonated at *p*H 7.4 (PK_a = 7.7) but the other two pyrimidines are fully in the free base form at this *p*H.

Werkheiser has observed (17) that aminopterin



I, R=OH

XXXVIII, R=NH_2

(XXXVIII) is bound to folic reductase 100,000 times better than the substrate folic acid. However, folic acid inhibits dihydrofolic reductase under the conditions of Table I only 3000-fold less effectively than aminopterin (22). Since aminopterin is a considerably stronger base than folic acid, Baker (18) proposed that a protonated species of the 2,4-diaminoheterocyclic system of XXXVIII accounted for the large difference in binding between I and XXXVIII. Note again that the 6-trifluoromethyl group (XXXIV) also gives about a 250-fold reduction in binding compared to 6-methyl (VII), even though XXXIV still has a 2,4-diaminopyrimidine system, but unprotonated; also note that the trifluoromethyl group is only slightly larger than a methyl group and therefore can cause no change due to steric interaction. These results make unlikely the alternate suggestion of Zakrzewski (19) that (a) four hydrogen bonds on the 2,4-diaminopyrimidine moiety are bound to the enzyme and that (b) a 2-amino-4-pyrimidinol or 4-pteridinol normally in the oxo form must undergo an energetically unfavorable tautomerism to the 4-hydroxy form in order to bind to folic reductase.

Similar effects by a 6-trifluoromethyl group (XXVI) and carboxylic ester groups (XVII, XIX) were noted in the 6-methyl-4-pyrimidinol system (IV); the absolute extent of these effects could not be measured due to lack of solubility but there was a greater than 10-100/fold loss in binding. In the case of IV, it is not protonated at *p*H 7.4, but is still a stronger base than XVII, XIX, or XXVI.

The results in both the 2,4-diamino series (VII) and 2-amino-4-pyrimidinol series (IV) are enlightening. The possibility that the pyrimidine ring of IV

or VII is bound to the enzyme in a charge transfer complex appears to be untenable. Since the 6-methylpyrimidine (VII) is two-thirds protonated at *p*H 7.4 it is therefore electron poor similar to a quaternized pyridine and could only be expected to engage in a charge-transfer complex by accepting electrons. However, the 6-trifluoromethyl (XXXIV) and 6-carbethoxy (XXXII) groups are strongly electron withdrawing and should aid in the ability of the pyrimidine to accept electrons; since XXXII and XXXIV are about 250-700 fold less effective than the 6-methylpyrimidine (VII), it would appear that a charge-transfer complex is made untenable by the data.

Although both the charge-transfer mode of binding and the 4-pyrimidinol four hydrogen-bond theory of Zakrzewski (19) appear untenable, the anionic-cationic interaction suggested by Baker (18a) and extended by Pullman *et al.* (18b) is still tenable with the data at hand. Since the 2,4-diaminopyrimidine (VII) is two-thirds protonated at *p*H 7.4 a cationic-anionic interaction is possible; the basicity is decreased by introduction of the 6-trifluoromethyl (XXXIV) or carbethoxy (XXXII) to the point where there is no cationic species at *p*H 7.4 and binding to the enzyme is therefore drastically decreased. These observations are further verified in the 2-amino-4-pyrimidinol series (IV). Although IV is a weak base compared to VII, IV becomes still a weaker base when the trifluoromethyl (XXVI) or carboxylic ester groups (XVII, XIX) are introduced; these compounds are also considerably weaker inhibitors than IV. This suggests that one of the binding points of the pyrimidines, IV and VII, to dihydrofolic reductase involves complexing to an acidic group on the enzyme that is only partially ionized at *p*H 7.4 such as an imidazole, thiol or environmentally-weakened carboxyl group. This group on the enzyme (E) can be represented by RH. If Py is a non-protonated pyrimidine and HPy^+ a protonated pyrimidine, then complexes such as XXXIX and XL, respectively, can be formed. The

XXXIX XL

only difference between XXXIX and XL is whether the proton in question is firmly associated with the strongly basic pyrimidine as in XL or more associated with the acidic groups as in XXXIX or in between. The more associated the proton is with the pyrimidine, the more energy would be involved in the bonding; conversely, the weaker Py is as a base, the weaker is the bonding energy. The difference in kilocalories of free energy of binding between the non-protonated 2-amino-6-methyl-4-pyrimidinol (PK_a 3.5) and 2,4-diamino-6-methylpyrimidine (PK_a 7.7) (19) which is two-thirds protonated at *p*H 7.4 can be estimated from their relative inhibition of dihydrofolic reductase; since the diamino pyrimidine has a K_i near 2×10^{-4} and is a 17-fold better inhibitor than the 2-amino-4-pyrimidinol (22), it can be estimated that the diaminopyrimidine has a free energy of binding of 5.6 kilocalories, and that the difference between the free energy of binding of the two molecules is about 2.4 kilocalories.

Even though a simple anionic-cationic interaction may be sufficient to account for the free energy of binding of the pyrimidine moiety, such an interaction is insufficient binding, else ammonium ion would also be an inhibitor. It is therefore plausible that there is at least one more interaction such as a hydrogen bond to give some specificity to the pyrimidine ring over ammonium ion.

Zakrzewski (19), on the basis of an analysis of the relationship of basicity of ten purines, pyrimidines and pteridines to binding with folic reductase, has concluded that "it appears that the ionic binding between folate reductase and its substrates or inhibitors is unlikely." His data suffer from the difficulty that proper compounds were not available for a realistic comparison that would eliminate such factors as (a) hydrophobic repulsion by the enzyme of a hydrophilic group on the inhibitor (9, 22) such as the 6-hydroxyl or 6-formyl on a 2,4-diaminopteridine, (b) possible binding by N_5 or N_8 of a pteridine (3) compared to lack of such a group in a pyrimidine, or (c) the use of a control compound that would be fully protonated at both the *p*H's used, 5.2 and 6.0, to show what change may have taken place on the enzyme such as a protonation that could be detrimental to binding. Therefore, a reinvestigation with a more closely related series of compounds that differ in basicity, but do not have factors (a) and (b) to consider and containing a control compound as in (c) should be made to resolve further the difference in opinion between Zakrzewski (19) and Baker (18).

The detrimental effect by a 6-carboxylate group in V and VIII is more complex to rationalize since a number of other factors must be considered:

(a) The 2,4-diamino-6-carboxylate (VIII) exists as a zwitterion in solution at *p*H 7 and in the solid state as shown by its ultraviolet and infrared spectra respectively. Since a zwitterion is usually considered to be both a weaker acid and a weaker base than the respective isolated groups, the protonated pyrimidine in VIII could be expected to be less effective as a base in an interaction of type XXXIX.

(b) A carboxylate group is normally held coplanar to an aromatic system due to overlap of orbitals. If such is the case, then either the carboxylate or the 5-aryl group of VIII must lose some coplanarity with the pyrimidine ring due to a steric interaction. If the carboxylate loses coplanarity it will be less ionized and will become a better electron withdrawing group thus starting to effect the basicity of the pyrimidine ring in the way similar to that of the

6-carbethoxy group (XXXII); if the 5-phenyl group loses coplanarity, then a loss in binding to the enzyme also occurs (15,20).

(c) It is difficult to assess the electron-withdrawing effect of the carboxylate group in VIII since *sigma* values are not valid with an *ortho*-substituent (21); in this case the 5-substituent is *ortho*.

(d) The carboxylate anionic group is probably solvated and may be repulsed from the hydrophobic area on the enzyme believed to be near the 6-position when a pyrimidine is complexed with the enzyme (9,22).

The 4-hydroxypyrimidine-6-carboxaldehyde (XXXVI) was a 17-fold weaker inhibitor of dihydrofolic reductase than the 6-methylpyrimidine, but not as weak as might be anticipated from the electron-withdrawing properties of the aldehyde function. The aldehyde group is a stronger electron-withdrawing group than the carbethoxy group (21); however, there is a possibility that the aldehyde function of XXXVI might be hydrated in aqueous solution which would cause a considerable reduction in electron-withdrawing capacity. In contrast, ketones such as VI or IX could be expected to solvate less, if at all, and therefore are likely to be less effective reversible inhibitors than the 6-aldehyde (XXXVI).

Two conclusions can be reached with the data presented here. Chloromethyl ketones such as VI or IX are not good candidates for irreversible inhibitors due to potentially poor reversible complexing with the enzyme; efforts are therefore being devoted to synthesis of chloromethyl ketones insulated by one or more methylene groups between the pyrimidine and ketone functions. Secondly, the mode of binding of pyrimidines to dihydrofolic reductase should be further investigated from the possible viewpoint of salt-like (XL) or near salt-like (XXXIX) interactions with the enzyme.

EXPERIMENTAL

Melting points were determined in capillary tubes on a Mel-temp block and those below 230° are corrected. Infrared spectra were determined in KBr pellet, unless otherwise indicated, with a Perkin-Elmer Model 137B. Ultraviolet and visible spectra were determined with a Perkin-Elmer Model 202 spectrophotometer.

Isopropyl 6-phenylhexanoate (XI).

A solution of 10 g. (52 mmoles) of 6-phenylhexanoic acid (13), 1 ml. of 96% sulfuric acid and 40 ml. of 99% isopropyl alcohol was refluxed for 36 hr. The solvent was spin-evaporated *in vacuo*. The remaining oil was dissolved in dichloromethane, washed with excess 5% aqueous sodium bicarbonate, dried with magnesium sulfate, then spin-evaporated *in vacuo*. Distillation afforded 12 g. (98%) of colorless oil, b.p. 116-117° (1 mm.); λ max (film) 5.72 (ester C=O); 6.20, 6.65 (phenyl C=C); 8.3-8.5, 8.98 (ester C-O-C); 13.35, 14.25 μ (C_6H_5).

Anal. Calcd. for $C_{15}H_{22}O_2$: C, 76.9; H, 9.46. Found: C, 76.9; H, 9.58.

Ethyl N-Amidino-3-oxo-2-(4-phenylbutyl)succinamate (XV).

To a mixture of 9.0 g. (0.17 mole) of sodium methoxide and 100 ml. of ethyl oxalate was added 39.9 g. (0.17 mole) of XI. The mixture was magnetically stirred in a bath at 60° and 30 mm. pressure for 4 hr. when no more alcohol distilled. The cooled mixture was acidified with glacial acetic acid, then diluted with 250 ml. of water. The oil was extracted with dichloromethane (3 x 50 ml.). The combined extracts were washed with water, dried with magnesium sulfate, then spin-evaporated *in vacuo*; the remainder of the oxalic esters were removed at 1 mm. in a hot water bath. The residual crude keto ester (XIII) was condensed with guanidine as follows:

A filtered solution of 16.4 g. (0.17 mole) of guanidine hydrochloride and 9.0 g. (0.17 mole) of sodium methoxide in 250 ml. of ethanol was added to the above keto ester. After being refluxed with magnetic stirring for 8 hr., the mixture was cooled and the crystalline product was collected on a filter, then washed with water. Recrystallization from methanol gave 28 g. (50% based on XI) of white crystals, m.p. greater than 300°; λ max (H_2O): 234 mμ (ε, 30,000); λ max (*p*H 13): 233 mμ (ε, 25,400); λ max 2.98, 3.03, 3.16 (NH, OH); 5.78, 5.85 (ester and ketone C=O); 6.02 (amide C=O); 6.13 (C=N); 6.23, 6.63 (NH); 13.42, 14.37 μ (C_6H_5).

Anal. Calcd. for $C_{17}H_{23}N_3O_4$: C, 61.3; H, 6.95; N, 12.6. Found: C, 61.3; H, 6.92; N, 12.5.

p-Chloro-α-ethoxalylphenylacetonitrile (XXVIII).

Condensation of 7.58 g. (50 mmoles) of XXVII with 25 ml. of ethyl oxalate in the presence of 2.72 g. (50 mmoles) of sodium methoxide, as described for the preparation of XIII, gave a solid residue. Recrystallization from benzene-petroleum ether (b.p. 60-110°) gave 9.2 g. (73%) of white crystals, m.p. 137-138°; λ max (10% EtOH), *p*H 1: 305 mμ (ε, 6,950), *p*H 7: 319 mμ (ε, 10,200), *p*H 13: 313 mμ (ε, 11,800); λ max 3.10 (broad enolic OH); 4.51 (C≡N); 5.78 (ester C=O); 6.13 (enol C=C); 7.98, 8.30 (ester C-O-C); 12.69 μ (*p*-C_6H_4).

Anal. Calcd. for $C_{12}H_{10}ClNO_3$: C, 57.3; H, 3.98; N, 5.56. Found: C, 57.4; H, 3.98; N, 5.55.

Ethyl 2-Amino-4-oxo-α-(4-phenylbutyl)-2-imidazoline-$\Delta^{5,\alpha}$-acetate (XXIII).

A solution of 5.0 g. (15 mmoles) of XV in 25 ml. of glacial acetic acid containing 10% anhydrous hydrogen bromide was refluxed for 4 hr.; after about 18 hr. at room temperature, the mixture was diluted with 50 ml. of ether, then the crystalline product was collected on a filter and washed with ether. Recrystallization from 2-methoxyethanol-water gave 4.1 g. (87%) of white crystals, m.p. 255-256°; λ max (*p*H 1): 242 (ε, 6,800), 309 mμ (ε, 14,900); λ max (H_2O): 279 (ε, 15,200), 325 mμ (ε, 10,600); λ max (*p*H 13): 279 (ε, 11,700), 330 mμ (ε, 10,400); λ max 3.00 (NH); 5.90 (ester C=O); 6.00 (conjugated amide C=O); 6.13 (C=N); 6.31 (NH); 8.00, 8.50 (ester C-O-C); 13.50, 14.38 μ (C_6H_5).

Anal. Calcd. for $C_{17}H_{21}N_3O_3$: C, 64.7; H, 6.71; N, 13.3. Found: C, 64.4; H, 6.69; N, 13.4.

Ethyl 2-amino-α-methyl-4-oxo-2-imidazoline-$\Delta^{5,\alpha}$-acetate (XXII).

A solution of 5 g. (23.3 mmoles) of XIV (6) in 50 ml. of glacial acetic acid containing 10% anhydrous hydrogen bromide was refluxed for 2 hr. The solution was then spin-evaporated *in vacuo* and the remaining crystals were suspended in 50 ml. of acetone and collected on a filter. Two recrystallizations from 2-methoxyethanol gave 4.4 g. (96%) of white crystals; m.p. 280-281°; λ max (10% EtOH), *p*H 1: 237 mμ (ε, 7,600), 306 mμ (ε, 14,800); *p*H 7: 277 mμ (ε, 14,200), 322 mμ (ε, 10,700); *p*H 13: 279 mμ (ε, 9,500), 341 mμ (ε, 7,400); λ max: 2.98, 3.10 (NH); 5.85, 5.90 (C=O); 6.00 (conjugated amide C=O); 6.11 (C=N); 6.24 (NH); 7.97, 8.46 (ester C-O-C), 9.05, 9.46, 9.86 μ (C-O).

Anal. Calcd. for $C_8H_{11}N_3O_3$: C, 48.7; H, 5.62; N, 21.3. Found: C, 48.9; H, 5.81; N, 21.4.

Laursen *et al.* (6) have recorded m.p. 275° dec. and λ max 276, 320 mμ in an unspecified but presumably neutral solvent for this compound prepared by esterification of XXI.

2-Amino-α-methyl-4-oxo-2-imidazoline-$\Delta^{5,\alpha}$-acetic acid (XXI).

A suspension of 337 mg. (1.7 mmoles) of XXII in 10 ml. of 5 N aqueous hydrochloric acid was refluxed for 6 hr. The solution was cooled in an ice bath and 5% aqueous sodium bicarbonate was added to bring the *p*H to *p*H 5 - 6. The precipitate was collected on a filter and washed with 10 ml. of cold water. Recrystallization from 80% aqueous 2-methoxyethanol gave 170 mg. (59%) of colorless crystals; m.p. 236-237°; λ max (10% EtOH), *p*H 1: 236 (ε, 7,200), 304 mμ (ε, 13,700); *p*H 7: 269 (ε, 12,600), 312 mμ (ε, 9,500); *p*H 13: 263 (ε, 10,100), 320 mμ (ε, 9,300). λ max 2.93, 3.03 (NH); 3.6-5.0 (broad, acidic H); 5.50 (NH^+), 5.75, 5.82, 5.90, 6.67 ($C=N^+$, NH, C=C); 6.27, 7.28 μ (COO^-). The infrared spectrum indicated a zwitterion.

Laursen *et al.* (6) have recorded a m.p. of 235-236° and a λ max of 297 mμ in an unspecified solvent for this compound prepared in 24% yield by hydrochloric acid cyclization of XIV.

2-Amino-α-(*p*-chlorophenyl)-4-oxo-2-imidazoline-$\Delta^{5,\alpha}$-acetonitrile (XXXIII).

A mixture of 1.76 (6.8 mmoles) of XXVIII and 10 ml. of ethyl orthoformate was refluxed for 1 hr., then 5 ml. of liquid was distilled from the reaction mixture (14). The remaining solvent was removed by spin-evaporation *in vacuo*; the last traces of ethyl orthoformate were removed at 1 mm. The remaining red oil (XXX) had λ max (10% EtOH) *p*H 1.7: 295 mμ (ε, 12,300), *p*H 13: 282 mμ (ε, 14,800); λ max (film) 4.48 (C≡N); 5.78 (ester C=O); no enolic bands near 3.10 or 6.13 μ.

The enol ether (XXX) was dissolved in 20 ml. of *t*-butyl alcohol, 0.62 g. (3.4 mmoles) of guanidine carbonate was added, then the mixture was gently refluxed with magnetic stirring for 24 hr. The solvent was removed by spin-evaporation *in vacuo*. Trituration with water gave 1.36 g. (82%) of crude product as a red solid which gradually decomposed over 280° and which was suitable for the next step. Although it had spectral characteristics in agreement with structure XXXIII, it could not be purified further. Recrystallization from water appeared to hydrolyze the amino group.

2-Amino-4-hydroxy-5-(4-phenylbutyl)pyrimidine-6-carboxylic acid (V).

To 15.8 g. (50 mmoles) of XXIII moistened with 10 ml. of ethanol was added 150 ml. of 1 N aqueous potassium hydroxide. After being refluxed for 8 hr., during which time solution took place, the solution was cooled and acidified with acetic acid. The product was collected on a filter and washed with water. The product was suspended in 150 ml. of hot 2-methoxyethanol and after cooling the solid was again collected on a filter. The product was dissolved in hot 5% aqueous sodium bicarbonate, then the solution was decolorized with charcoal and filtered. The hot filtrate was acidified with acetic acid. After being cooled, the crystalline product was separated by filtration. The precipitation from a hot decolorized sodium bicarbonate solution was repeated twice and gave 8.2 g. (57%) of white crystals; m.p. 287-288°. λ max (10% EtOH), *p*H 1: 283 mμ (ε, 5,400), *p*H 7: 286 mμ (ε, 8,500), *p*H 13: 284 mμ (ε, 8,300); λ max 2.91, 3.03 (OH, NH); 5.8-6.1 (broad, C=O, C=N); 13.40, 14.35 μ (phenyl).

Anal. Calcd. for $C_{15}H_{17}N_3O_3$: C, 62.7; H, 5.96; N, 14.6. Found: C, 62.7; H, 5.88; N, 14.5.

2-Amino-4-hydroxy-5-methylpyrimidine-6-carboxylic acid (XX).

A solution of 400 mg. (2.3 mmoles) of XXII in 20 ml. of 1 N aqueous potassium hydroxide was refluxed for 1 hr. The clear solution was acidified with acetic acid, cooled in ice and the crystalline product was collected on a filter. Recrystallization from water gave 170 mg. (50%) of colorless crystals, m.p. 311-313° dec.; λ max (H_2O), *p*H 1: 283 mμ (ε, 5,300); *p*H 7: 291 mμ (ε, 5,600); *p*H 13: 282 mμ (ε, 5,200); λ max 2.80, 3.00, 3.10 (OH, NH), 3.5-5.0 (broad, acidic H), 5.30 (NH^+), 5.78 ($C=NH^+$); 6.00, 6.69 ($C=NH^+$, C=N, C=C); 6.13, 7.31 μ (COO^-). The infrared spectrum indicated a zwitterion structure.

Laursen *et al.* (6) record a m.p. of 302° dec. and a λ max of 287 mμ in an unspecified solvent for this compound prepared in 62% yield from XXI.

5-(*p*-Chlorophenyl)-2,4-diaminopyrimidine-6-carboxylic acid (VIII).

A suspension of 450 mg. (1.82 mmoles) of crude XXXIII in 20 ml. of 1 N aqueous potassium hydroxide was refluxed for 1 hr. during which time solution took place. The cooled solution was acidified with acetic acid. The crude product was collected on a filter and washed with water. A hot solution of the crude product in 5% aqueous sodium bicarbonate was treated with charcoal then acidified hot with acetic acid; this procedure was repeated twice more to give 270 mg. (51%) of white crystals, m.p. 242-243°; λ max (H_2O) *p*H 1: 288 mμ (ε, 10,800), *p*H 7: 290 mμ (ε, 13,000), *p*H 13: 256 (ε, 17,600), 293 mμ (ε, 16,000); λ max 2.95, 3.12 (NH); 5.90, 5.95, (shoulders, $C=NH^+$); 6.02, 6.10, 6.25, 6.66, 6.74 (C=C, C=N), 6.32, 7.30 (COO^-); 12.20 μ (*p*-C_6H_4); no COOH near 5.80 μ. The infrared and ultraviolet spectra are more in agreement with a zwitterion than an amino acid, particularly since the relative band intensities in the 6.0-6.5 μ region are different than the usual pyrimidine of this type (5).

Anal. Calcd. for $C_{11}H_9ClN_4O_2$: C, 49.9; H, 3.43; N, 21.2. Found: C, 49.6; H, 3.63; N, 21.5.

Ethyl 2-Amino-4-hydroxy-5-(4-phenylbutyl)pyrimidine-6-carboxylate (XVII).

(A) To a suspension of 1.44 g. (5 mmoles) of V in 10 ml. of ethanol was added a solution of 0.42 g. (5 mmoles) of sodium bicarbonate in 20 ml. of water. The mixture was refluxed for 20 min., then clarified by filtration and spin-evaporated *in vacuo*. The residue was dried at 100°, then suspended in 20 ml. of diethyleneglycol dimethyl ether. A solution of 0.65 g. (5 mmoles) of oxalyl chloride in 2 ml. of diethyleneglycol dimethyl ether was added to the ice-cold stirred suspension. After 30 min. the sodium salt had dissolved and a milky suspension was formed. To this suspension was added 1 g. (10 mmoles) of triethylamine followed by 10 ml. of ethanol. The mixture was refluxed for 2 hr., then spin-evaporated *in vacuo*. The residue was triturated with 10 ml. of water, the solid was collected on a filter, then washed with water. Two recrystallizations from acetone-water gave 1.3 g. (82%) of a yellowish microcrystalline powder, m.p. 160-161°; λ max (10% EtOH) *p*H 1: 288 mμ (ε, 6,900), *p*H 7: 303 mμ (ε, 5,900), *p*H 13: 294 mμ (ε, 4,700); λ max 2.89, 2.98, 3.20 (OH, NH); 5.80 (ester C=O); 6.02 (broad), 6.25, 6.40, 6.60, 6.70 (NH, C=C, C=N), 7.90; 8.17 (ester C-O-C); 13.5, 14.35 μ (C_6H_5).

Anal. Calcd. for $C_{17}H_{21}N_3O_3$: C, 64.7; H, 6.71; N, 13.3. Found: C, 64.7; H, 6.70; N, 13.3.

(B) A suspension of 5.0 g. (17 mmoles) of V in 60 ml. of absolute ethanol and 6 ml. of ethanesulfonic acid was refluxed with magnetic stirring for 72 hr. when solution was complete. The cooled solution was poured into 250 ml. of ice water and the *p*H was adjusted to about 9 with 1 N aqueous sodium hydroxide. The precipitate was collected on a filter and washed with water. Three recrystallizations from ethanol-water gave 4.3 g. (78%) of colorless plates, m.p. 190-191°; λ max (10% EtOH) *p*H 1: 289 mμ (ε, 6,700), *p*H 7: 305 mμ (ε, 5,700), *p*H 13: 294 mμ (ε, 5,400). Most of the bands reported for the low-melting dimorph in preparation A were also present in this sample with slightly different band intensities; the spectrum of preparation B was much better resolved than the spectrum of A, indicating again the difference in crystal form.

Anal. Calcd. for $C_{17}H_{21}N_3O_3$: C, 64.7; H, 6.71; N, 13.3. Found: C, 65.1; H, 6.79; N, 13.3.

The low and high-melting dimorphs on thin layer chromatography with benzene-methanol (3:1) showed single spots with identical mobility.

Cyanomethyl 2-amino-4-hydroxy-5-(4-phenylbutyl)pyrimidine-6-carboxylate (XIX).

A mixture of 287 mg. (1 mmole) of V, 1 ml. of triethylamine and 0.5 ml. of chloroacetonitrile was heated in a bath at 50° under a reflux condenser. Over a period of 2 hr. the temperature was gradually raised to 80°. Volatile material was removed by spin-evaporation *in vacuo*. To the residue was added 1 ml. of glacial acetic acid, then 10 ml. of water; the insoluble oil soon solidified. Recrystallization from aqueous acetone gave 280 mg. (86%) of white crystals, m.p. 191-192°; λ max (10% EtOH), *p*H 1: 290 mμ (ε, 6,400), *p*H 7: 304 mμ (ε, 6,200), *p*H 13: 295 mμ (ε, 4,400); λ max 2.86, 3.03, 3.16 (OH, NH); 5.71 (ester C=O); 5.98, 6.18, 6.25, 6.35, 6.63, 6.70 (NH, C=C, C=N, C=O); 8.10, 8.32 (ester C-O-C); 13.51, 14.30 μ (C_6H_5). The C≡N band near 4.5 μ can be notoriously weak and was not seen in the spectrum; the presence of the C≡N group showed its influence by shifting the C=O from the normal 5.80 μ seen in XVII to 5.71 μ of an activated ester such as XIX.

Anal. Calcd. for $C_{17}H_{18}N_4O_3$: C, 62.6; H, 5.56; N, 17.2. Found: C, 62.6; H, 5.65; N, 17.3.

Ethyl 5-(*p*-chlorophenyl)-2,4-diaminopyrimidine-6-carboxylate (XXXII).

Esterification of 1.0 g. (3.8 mmoles) of VIII as described for XVII method B except only a 24 hr. reflux period was used, gave after recrystallization from ethanol-water 0.84 g. (74%) of white crystals, m.p. 193-194°; λ max (10% EtOH) *p*H 1: 299 mμ (ε, 16,600), *p*H 7: 308 mμ (ε, 18,300), *p*H 13: 297 mμ (ε, 21,700); λ max 2.90, 2.98, 3.09 (NH); 5.77 (ester C=O); 6.03, 6.09, 6.13, 6.35, 6.47 (NH, C=C, C=N); 7.85, 8.19 (ester C-O-C); 12.00, 12.75 μ (*p*-C_6H_4).

Anal. Calcd. for $C_{13}H_{13}ClN_4O_2$: C, 53.4; H, 4.48; N, 19.1. Found: C, 53.6; H, 4.35; N, 18.9.

2-Amino-4-hydroxy-5-(4-phenylbutyl)pyrimidine-6-carboxhydrazide (XVIII).

To a suspension of 3.15 g. (10 mmoles) of XVII in 25 ml. of ethanol was added 5 ml. of 50% aqueous hydrazine hydrate. After being refluxed for 2 hr., the hot solution was diluted with hot water to turbidity, then allowed to cool. The product was collected on a filter and washed with water. Two recrystallizations from aqueous ethanol gave 2.53 g. (84%) of white crystals, m.p. 174-175°; λ max (10% EtOH) *p*H 1: 284 mμ (ε, 5,400), *p*H 7: 302 mμ (ε, 6,700), *p*H 13: 290 mμ (ε, 4,900); λ max 2.85, 3.03, 3.16 (OH, NH); 5.94 (amide C=O); 6.04, 6.13, 6.23, 6.36, 6.69 (NH, C=C, C=N, C=O); 13.40, 14.36 μ (C_6H_5).

Anal. Calcd. for $C_{15}H_{19}N_5O_2$: C, 59.8; H, 6.36; N, 23.2. Found: C, 59.5; H, 6.29; N, 23.4.

N-[2-Amino-4-hydroxy-5-(4-phenylbutyl)pyrimidine-6-carboxy]-N'-benzenesulfonylhydrazide (XXXV).

To a stirred solution of 1.7 g. (5.65 mmoles) of XVIII in 10 ml. of reagent pyridine cooled to 0-5° and protected from moisture was added dropwise a solution of 1.0 g. (5.65 mmoles) of benzenesulfonyl chloride in 5 ml. of pyridine over a period of 30 minutes. The mixture was stirred 1 hr. in the icebath and 1 hr. at ambient temperature, then poured into 100 ml. of ice water. The crude product was collected on a filter and washed with water; yield, 2.3 g. (92%), m.p. 257-260° dec. The solid was dissolved in 1 N aqueous sodium hydroxide by warming to 40°, the solution was clarified with charcoal,

then acidified with 5% aqueous acetic acid. Two recrystallizations from 2-methoxyethanol-water gave 1.7 g. (66%) of pure XXXV, m.p. 282-283° dec.; λ max (EtOH) 302 mμ (ε, 7,700); λ max (10% EtOH) *p*H 1: 271 mμ (ε, 7,100), *p*H 13: 287 mμ (ε, 7,100); λ max 2.86, 2.94, 3.20 (NH, OH); 5.98 (shoulder, amide C=O); 6.04, 6.28, 6.68 (NH, C=O, C=C, C=N); 7.43, 8.58 (-SO_2NH-); 14.40, 14.70 μ (C_6H_5).

Anal. Calcd. for $C_{21}H_{23}N_5O_4S$: C, 57.1; H, 5.25; N, 15.9. Found: C, 57.1; H, 5.21; N, 16.0.

Attempts to convert XXXV to 2-amino-4-hydroxy-5-(4-phenylbutyl)-pyrimidine-6-carboxaldehyde (XXXVI) with sodium carbonate in ethylene glycol gave polymeric material. The aldehyde (XXXVI) was subsequently prepared by a direct pyrimidine condensation via its acetal and will be reported in a future paper.

2-Amino-5-(4-phenylbutyl)-6-trifluoromethyl-4-pyrimidinol (XXVI).

To a stirred mixture of 5.9 g. (25 mmoles) of XI and 7.1 g. (50 mmoles) of ethyl trifluoroacetate protected from moisture was added 1.08 g. (25 mmoles) of a 55.6% dispersion of sodium hydride in mineral oil. After being stirred at ambient temperature for 30 min., the mixture was refluxed for 4 hr., then cooled and acidified with glacial acetic acid. The mixture was diluted with 25 ml. of water and extracted with dichloromethane (3 x 30 ml.). The combined extracts were washed with water, dried with magnesium sulfate, then spin-evaporated *in vacuo*. The residual oil (7.2 g.) gave a reddish-brown (plateau near 525 mμ in ethanol) ferric chloride test and had λ max (EtOH), 255 mμ, (ethanolic NaOEt), 290 mμ, compatible with structure XXV.

The crude XXV was mixed with 25 ml. of *t*-butyl alcohol and 2.5 g. (12.5 mmoles) of guanidine carbonate, then refluxed gently with magnetic stirring for 18 hr. The mixture was spin-evaporated *in vacuo* and the remaining oil was triturated with 20 ml. of water. The gummy solid was collected on a filter, dried, and then triturated with 20 ml. of ether. The remaining white solid was twice recrystallized from methanol-water to give 2.2 g. (35% based on XI) of white crystals, m.p. 200-201°; λ max 1 N HCl: 268 mμ (ε, 7,700), *p*H 7: 302 mμ (ε, 8,700), *p*H 13: 291 mμ (ε, 6,600); λ max 2.91, 3.16 (OH, NH); 5.94, 6.03, 6.16, 6.25, 6.36, 6.70 (C=O, C=C, C=N, NH); 13.40, 13.72, 14.40 (C_6H_5); multiple absorption at 7.98-8.93 μ primarily due to C-F.

Anal. Calcd. for $C_{15}H_{16}F_3N_3O$: C, 57.9; H, 5.18; N, 13.5. Found: C, 58.0; H, 5.30; N, 13.3.

The trifluoromethyl group of XXVI was stable to boiling 3 N sodium hydroxide for 18 hr., XXVI being recovered unchanged on acidification.

p-Chloro-α-(trifluoroacetyl)phenylacetonitrile (XXIX, hydrate XXXVII).

Condensation of 3.79 g. (25 mmoles) of *p*-chlorophenylacetonitrile with ethyl trifluoroacetate, as described for the preparation of XXV, gave an oil that soon solidified; chloroform was used for extraction. Two recrystallizations from chloroform-petroleum ether (b.p. 60-110°) gave 2.0 g. (33%) of white crystals, m.p. 92-93°; λ max (EtOH): 255 (ε, 4,800), 300 mμ (ε, 10,000), *p*H 13 (10% EtOH): 254 (ε, 4,100), 300 mμ (ε, 8,000); λ max 2.81 (non-bonded OH); 3.06 (bonded OH); 4.38 (C≡N); 6.14 (weak), 6.25, 6.70 (phenyl); 12.50 (C_6H_4); strong and multiple C-F and C-O absorption in the 7.76-9.83 μ region.

Anal. Calcd. for $C_{10}H_7ClF_3NO_2$: C, 44.9; H, 2.63; N, 5.23. Found: C, 45.2; H, 2.62; N, 5.45.

A film of the crude oily product before it solidified or the analytical sample in ethyl acetate solution showed a single broad OH peak at 2.98 μ indicating that the chelate-type structure, XXXVII existed only in the crystal pack, but not in solution.

5-(*p*-Chlorophenyl)-2,4-diamino-6-(trifluoromethyl)pyrimidine (XXXIV).

A solution of 3.2 g. (12 mmoles) of XXIX hydrate (XXXVII) in 15 ml. of ethyl orthoformate was refluxed for 1 hr., then slowly concentrated at atmospheric pressure to 5-7 ml. The remainder of the volatile material was then removed by spin-evaporation *in vacuo* leaving XXXI as a red oil with λ max (EtOH) 297 mμ.

To the crude XXXI was added 15 ml. of *t*-butyl alcohol and 1.37 g. (6.5 mmoles) of guanidine carbonate. After being gently refluxed with magnetic stirring for 18 hr., the mixture was spin-evaporated *in vacuo*. The product was triturated with cold 5% aqueous acetic acid, then collected on a filter and washed with water. Recrystallization from ethanol-water gave 2.25 g. (65%) of light yellow crystals, m.p. 250-251°; λ max (10% EtOH) 0.1-3 N HCl: 274 (ε, 5,800), 316 mμ (ε, 2,800), *p*H 7, 13: 302 mμ (ε, 6,800); λ max 2.84, 2.97, 3.10 (NH); 6.10, 6.13, 6.30, 6.42, 6.72 (NH, C=C, C=N); 12.38 (*p*-C_6H_4); multiple C-F bands in the 7.85-10.0 μ region.

Anal. Calcd. for $C_{11}H_8ClF_3N_4$: C, 45.5; H, 2.77; N, 19.3. Found: C, 45.8; H, 2.63; N, 19.5.

Note that XXXIV is insoluble in 5% acetic acid and shows no shift in peak from neutral to basic solution, indicating that XXXIV is a weak base not protonated in neutral solution; also this pyrimidine does not protonate normally as shown by the acidic u.v. spectrum. In contrast, the corresponding 6-methyl derivative, 5-(*p*-chlorophenyl)-2,4-diamino-6-methylpyrimidine is readily soluble in 5-10% aqueous acetic acid and shows a shift in the spectrum between neutral and basic solution indicating protonation at neutral *p*H (16).

REFERENCES

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(2) For the previous paper of this series, see B. R. Baker and B.-T. Ho, *J. Pharm. Sci.*, 54, in press (1965).

(3) B. R. Baker and B.-T. Ho, *ibid.*, 53, 1457 (1964); paper XV of this series.

(4) B. R. Baker, *ibid.*, 53, 347 (1964).

(5) B. R. Baker and J. H. Jordaan, *J. Heterocyclic Chem.*, 2, 21 (1965); paper XXI of this series.

(6) P. H. Laursen, W. A. Thews, and B. E. Christensen, *J. Org. Chem.*, 22, 274 (1957).

(7) B. R. Baker, B.-T. Ho, and T. Neilson, *J. Heterocyclic Chem.*, 1, 79 (1964); paper XII of this series.

(8) B. R. Baker, B.-T. Ho, and G. B. Chheda, *ibid.*, 1, 88 (1964); paper XIII of this series.

(9) B. R. Baker and D. V. Santi, *J. Pharm. Sci.*, 54, submitted (1965); paper XXIV of this series.

(10) R. Schwyzer, M. Fuerer, B. Inselin, and H. Kägi, *Helv. Chim. Acta*, 38, 80 (1955).

(11) R. Adams and L. H. Ulich, *J. Am. Chem. Soc.*, 42, 599 (1920).

(12) B. R. Baker and H. S. Shapiro, *J. Med. Chem.*, 6, 664 (1963); paper IX of this series.

(13a) D. Papa, E. Schwenk and H. Hankin, *J. Am. Chem. Soc.*, 69, 3018 (1947); (b) Huang-Minlon, *ibid.*, 68, 2487 (1946).

(14) P. B. Russell and N. Whittaker, *ibid.*, 74, 1310 (1952).

(15) B. R. Baker and B.-T. Ho, *J. Pharm. Sci.*, 53, 1137 (1964); paper XVI of this series.

(16) P. B. Russell, *J. Chem. Soc.*, 2951 (1954).

(17) W. C. Werkheiser, *J. Biol. Chem.*, 236, 888 (1961).

(18a) B. R. Baker, *Cancer Chemotherapy Reports*, 4, 1 (1959); (b) A. M. Perault and B. Pullman, *Biochim. Biophys. Acta*, 52, 266 (1961).

(19) S. F. Zakrzewski, *J. Biol. Chem.*, 238, 4002 (1963).

(20a) E. J. Modest, *J. Org. Chem.*, 21, 1 (1954); (b) B. R. Baker, W. W. Lee, W. A. Skinner, A. P. Martinez and E. Tong, *J. Med. Pharm. Chem.*, 2, 633 (1960).

(21) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, pp. 188, 194.

(22) B. R. Baker, B.-T. Ho, and D. V. Santi, unpublished.

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