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Irreversible Enzyme Inhibitors. LXXXV. On the Mode of Pyrimidine Binding of 5-alkyl and 5-Arylpyrimidines to Dihydrofolic Reductase (1,2)

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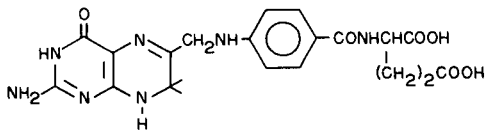
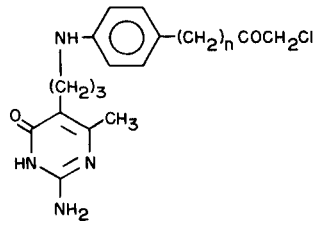
A series of 5-isoamyl- and 5-(*p*-chlorophenyl)pyrimidines substituted with amino, alkylamino, mercapto, benzyloxy, hydroxy, or hydrogen at the 2- and 4-positions and with amino or methyl at the 6-position have been synthesized for evaluation of the mode of pyrimidine binding to dihydrofolic reductase. The studies were performed in order to determine where a bulky group could be placed on the pyrimidine ring that would still allow good binding; such studies are essential to find a suitable position for placement of a covalent forming group for design of active-site-directed irreversible inhibitors. Two classes of candidate compounds have emerged for further study as irreversible inhibitors, namely, 2-amino-4-mercapto-6-(*p*-bromoacetamidophenylalkyl)-pyrimidines and 2,4-diamino-6-(*p*-bromoacetamidophenylalkyl)aminopyrimidines having a group such as phenyl, phenylbutyl or isoamyl at the 5-position that can give strong hydrophobic bonding to the enzyme.

Prior to the discovery of the strong hydrophobic bonding region on dihydrofolic reductase (4, 5) and the influence of this hydrophobic bonding on the mode of pyrimidine binding (4, 6 - 9), some thirty attempts to design active-site-directed irreversible inhibitors (10, 11) of this enzyme were unsuccessful (5). Since the substrate, dihydrofolate (I) must have some given conformation of its pteridine ring when complexed to the enzyme, it can arbitrarily be assigned the configuration depicted in I; the hydrophobic bonding region is believed to be near either the 4- or 8- position of dihydrofolate (I) when it is complexed to the enzyme (5, 9).

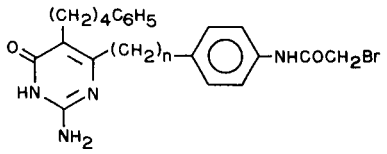
In order for an inhibitor to inactivate an enzyme by the active-site-directed mechanism, the inhibitor must first (a) form a reversible complex with the enzyme, (b) a leaving group on the inhibitor should be juxtaposed to a nucleophilic site on the enzyme, and finally (c) the nucleophilic group on the enzyme and the leaving group should have the ability to interact with formation of a covalent bond in a facile neighboring-group reaction (10, 11). In the case of candidate irreversible inhibitors such as type II (12), no inactivation of the enzyme occurred; these results were later attributed to the binding of II in the conformation indicated -- compared to dihydrofolate (I) -- which placed the leaving group of II in the hydrophobic bonding region where polar (nucleophilic) groups are not apt to be present (13).



I

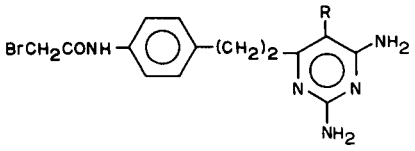


II



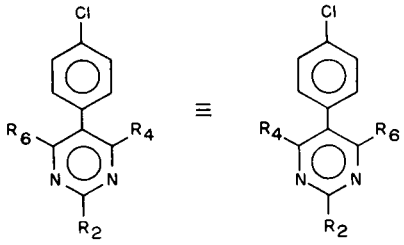
III, n = 2

IV, n = 4

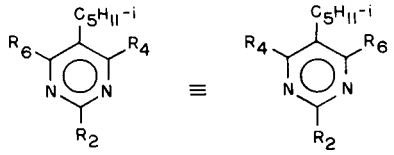


V, R = $C_6H_5(CH_2)_4$-

VI, R = p-ClC_6H_4-



VIIa VIIb



VIIIa VIIIb

Successful active-site-directed irreversible inhibitors were obtained when the leaving group was placed on the 6-position of the pyrimidine as in III or IV (13); the 5-phenylbutyl group was presumably complexed to the hydrophobic bonding region which then projected the 6-side-chain into a polar area where covalent bond formation occurred with resultant inactivation of the enzyme (13).

Although III at 4 x 10^{-5} molar could inactivate dihydrofolic reductase with a half-life of about twelve minutes, this concentration was considered to be too high to be of utility in whole animals (13). The rate of inactivation is dependent upon the concentration of reversible enzyme-inhibitor complex which is in turn dependent upon the reversible binding constant, K_i, and the concentration of inhibitor (14); therefore candidate irreversible inhibitors that were better reversible inhibitors were sought (2,15,16). Attention was focused on irreversible inhibitors derived from 2,4-diaminopyrimidines since these are 300-3000 better reversible inhibitors of dihydrofolic reductase than the corresponding 2-amino-4-hydroxypyrimidines (5,6).

Conversion of the 2-amino-4-hydroxypyrimidine (III) -- which is an active-site-directed irreversible inhibitor of dihydrofolic reductase -- to the corresponding 2,4-diaminopyrimidine (V) gave a compound that was an excellent reversible inhibitor of the enzyme, but showed no inactivation of the enzyme (16); similar results were noted with the 2,4-diamino-5-(*p*-chlorophenyl)pyrimidine (VI). The failure of V to inactivate the enzyme clearly demonstrated that the leaving group of III and V was positioned differently within the respective enzyme-inhibitor complexes; one possibility was that the diaminopyrimidines, V and VI, were complexed in a different rotomeric configuration -- as depicted in their two dimensional structures; such different rotomers for complexing to dihydrofolic reductase were previously proposed (5,9). Therefore, studies were initiated on the mode of binding of the pyrimidine ring of substituted 5-(*p*-chlorophenyl)- and 5-isoamylpyrimidines (VII and VIII); such studies were previously performed with 6-phenyl- and 5-(anilinopropyl)pyrimidines (5,9). It was hoped that such a study could reveal other positions where the bulky carrier for the leaving group could be positioned; the results of these studies are the subject of this paper.

In Table I are listed the inhibition results with three types of 5-substituted pyrimidines: series A -- anilinopropyl, series B -- isoamyl, and series C -- 5-(*p*-chlorophenyl). In all three series, conversion of 4-hydroxy group (IX) to 4-amino (X) led to 150-360 fold increment in binding, the 5-(*p*-chlorophenyl) derivative showing the smallest increment. In all three cases, replacement of the 4-hydroxyl group of IX by 4-mercapto (XII) led to an increase in binding, being about 18-fold in the anilinopropyl series A and in the 5-chlorophenyl series C, but only three-fold in the isoamyl series; the possible utility of these 4-mercapto pyrimidines for design of candidate active-site-directed irreversible inhibitors will be discussed later.

Removal of either the 4-amino (XIII) or 2-amino (XIV) group from X led to a large loss in binding in all three series. Reversal of the 2-amino and mercapto groups of XII to 4-amino-2-mercapto (XVII) also led to large loss in binding in the one series (C) studied; similarly, reversal of the 2-amino and 4-hydroxyl groups of IX to give XVIII led to a large loss in binding.

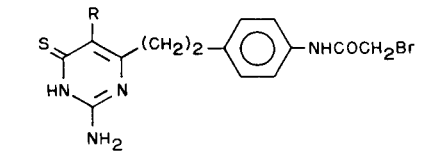
The 2, 3, and 4 positions of these aminopyrimidines were then investigated for bulk tolerance within the enzyme inhibitor complex. Substitution of a phenylpropyl (XIX) or anilinoethyl (XX) group on the 4-amino group of X led to a large loss in binding; similar substitution on the 2-amino group (XXII) led to an even larger loss in binding. Substitution of a benzyl group on either the 4-oxygen (XXIII) or 3-nitrogen (XXIV) of the 2-amino-4-hydroxypyrimidine (IX) was also detrimental to binding. The minimum loss in binding by substitution on a 4-amino

group occurred in the 2,4,6-triaminopyrimidine series (XIB); substitution on the 4(6)-amino group by phenylpropyl led to only a 16-fold loss in binding.

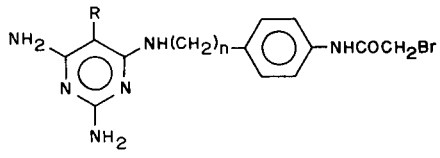
As a result of these studies the following types of candidate active-site-directed irreversible inhibitors are being investigated:

(1) 2-Amino-4-mercapto-6-(*p*-bromoacetamidophenethyl)pyrimidines of type XXV, where R is a potent hydrophobic bonding group such as 3,4-dichlorophenyl or phenylbutyl, could be anticipated to have a K_i in the region of 10^{-7} molar which could be low enough for *in vivo* activity. Since it is probable that XXV would complex in the same rotomer as III, active-site-directed irreversible inhibition by XXV is highly probable.

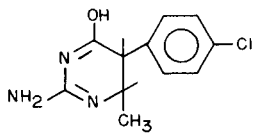
(2) Compounds of the triaminopyrimidine type XXVI are also logical candidates for active-site-directed irreversible inhibitors of dihydrofolic reductase. By proper choice of the hydrophobic R group it should be possible to obtain inhibitors with reversible binding constants on the order of 10^{-7} molar. A related series of compounds have been recently reported to be active-site-directed irreversible inhibitors of the folic reductase from rat liver (19); however, the choice (19) of the *p*-carbethoxyphenylazo moiety for the hydrophobic bonding group appeared to be predicated on relative ease of synthesis rather than ability to give good hydrophobic bonding.



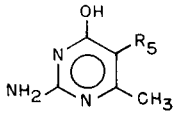
XXV



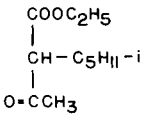
XXVI



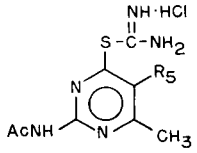
XXVII



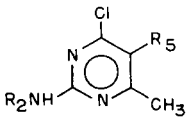
IX



XXVIII

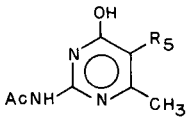


XXIX

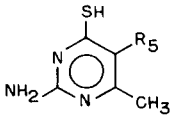


XXX, R_2 = H

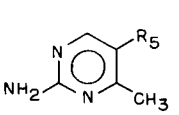
XXXI, R_2 = Ac



XXXII



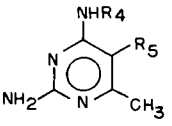
XII



XIII

B Series, R_5 = $-C_5H_{11}$-i

C Series, R_5 = p-ClC_6H_4

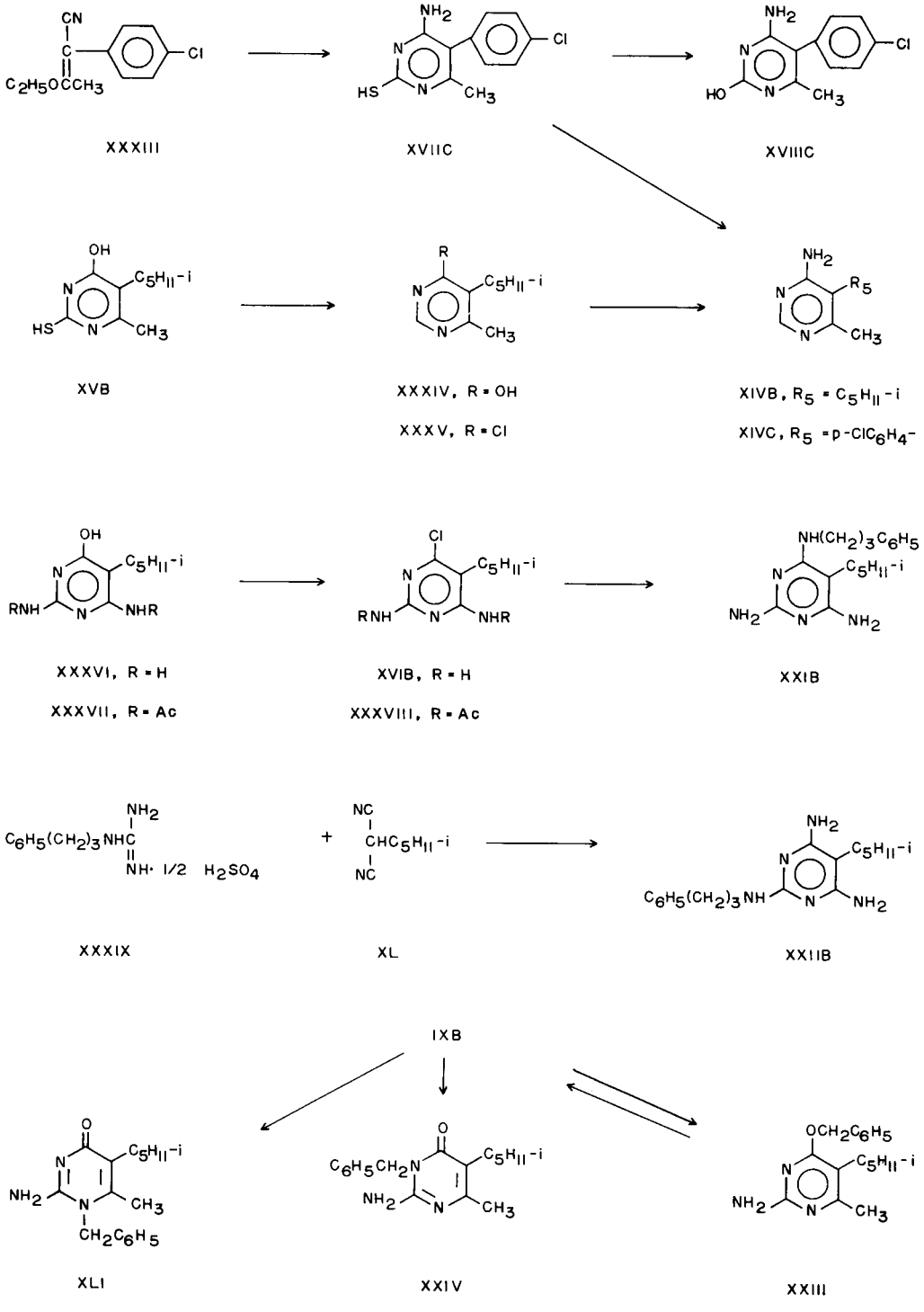


XIX, R_4 = $C_6H_5(CH_2)_3$-

XX, R_4 = $C_6H_5NH(CH_2)_2$-

Chemistry.

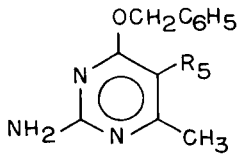
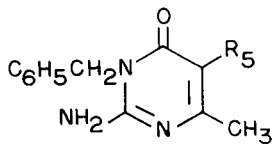
One of the key intermediates for transformation of the pyrimidines was the 5-substituted-2-amino-6-methyl-4-pyrimidinol (IX). The synthesis of the isoamyl derivative (IXB) from the appropriate keto ester (XXVIII) has been previously described (4). The 5-(*p*-chlorophenyl) derivative (IXC) was synthesized by the route previously described for the corresponding 6-ethylpyrimidine (20); condensation of guanidine, acetaldehyde, and *p*-chlorophenylacetonitrile afforded the dihydropyrimidine (XXVII) in 41% yield which could be aromatized with sulfur at 240° to the desired 4-pyrimidinol (IXC) in 58% yield.



Acetylation of the 2-amino group of IX with acetic anhydride proceeded smoothly to XXXII which, in turn, was readily converted to the crystalline 4-chloropyrimidine (XXXI) with phosphorus oxychloride in benzene (21) in both series. The 4-chloro atom of XXXI was readily displaced with thiourea to give excellent yields of the thiouronium salts (XXIX), provided *t*-butyl alcohol was used as a solvent to avoid alcoholysis to the less reactive 2-aminopyrimidines (XXX) (21). Base hydrolysis cleaved the thiouronium and *N*-acetyl groups to give the 2-amino-4-pyrimidinethiols (XII) in high yield. In the 5-(*p*-chlorophenyl) series C, desulfurization with Raney nickel afforded crystalline XIIIC. In the iso-

TABLE I

Micromolar Concentration for 50% Inhibition of Dihydrofolic Reductase by Tetrasubstituted Pyrimidines (a, b)

Compound	R_2	R_4	R_6	R_5 Series A: $C_6H_5NH(CH_2)_3$-	R_5 Series B: C_5H_{11}-*i*	R_5 Series C: *p*-ClC_6H_4-
IX	NH_2	OH	CH_3	800 (c)	8.5 (d)	29
X	NH_2	NH_2	CH_3	2.2 (e)	0.24 (d)	0.20 (f)
XI	NH_2	NH_2	NH_2		2.1 (d)	
XII	NH_2	SH	CH_3	44 (c)	2.6	1.7
XIV	H	NH_2	CH_3	140 (e)	130	430
XIII	NH_2	H	CH_3	470 (e)	870	1600 (g)
XV	SH	OH	CH_3		> 12,000 (h)	
XVI	NH_2	NH_2	Cl		74	
XVII	SH	NH_2	CH_3			3600 (i)
XVIII	OH	NH_2	CH_3			2800 (j)
XIX	NH_2	$-NH(CH_2)_3C_6H_5$	CH_3		150	210
XX	NH_2	$-NH(CH_2)_2NHC_6H_5$	CH_3		640	700
XXI	NH_2	$-NH(CH_2)_3C_6H_5$	NH_2		32	
XXII	$-NH(CH_2)_3C_6H_5$	NH_2	NH_2		1500 (k)	
XXIII					350	
XXIV					1300 (l)	

(a) The technical assistance of Maureen Baker, Pepper Caseria, Ann Jaqua, and Susan Lakatos is acknowledged. (b) Dihydrofolic reductase was a 45-90% saturated ammonium sulfate fraction from pigeon liver prepared and assayed with 6 μM dihydrofolate and 12 μM TPNH at *p*H 7.4 as previously described (17). (c) Data from reference 17. (d) Data from reference 4. (e) Data from reference 18. (f) Data from reference 6. (g) Estimated from 23% inhibition at 0.5 *mM*, the maximum solubility. (h) Since 20% inhibition is readily detectable, the concentration of inhibitor necessary for 50% inhibition is at least four-times greater than the concentration measured. (i) Estimated from 25% inhibition at 1.2 *mM*, the maximum solubility. (j) Estimated from 34% inhibition at 1.5 *mM*, the maximum solubility. (k) Estimated from 17% inhibition at 0.3 *mM*, the maximum solubility. (l) Estimated from 40% inhibition at 1 *mM*, the maximum solubility.

amyl series B, XIIIB was prepared by catalytic hydrogenolysis of the 4-chloropyrimidine (XXXB), prepared by direct reaction of IXB with phosphorus oxychloride (4).

Reaction of the 2-acetamido-4-chloropyrimidine (XXXI) in series C with excess phenylpropylamine or *N*-phenylethylenediamine in boiling toluene effected displacement of the 4-chloro group and transamidation of the N_2-acetyl group to give high yields of XIXC and XXC. In the isoamyl series B, the displacement reaction was run on the 2-amino-4-chloropyrimidine (XXXB) in boiling butanol to give XIXB and XXB in much poorer yields than the C series.

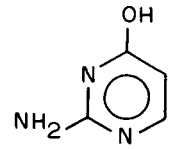
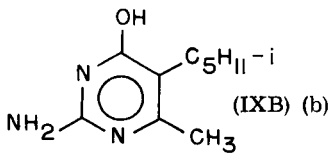
Condensation of α-(*p*-chlorophenyl)-β-ethoxycrotononitrile (XXXIII) (22) with thiourea as described for the corresponding β-methoxycrotononitrile (23) afforded the 2-mercapto-4-aminopyrimidine (XVIIC). *S*-Alkylation of XVIIC with chloroacetic acid followed by acid hydrolysis (24) afforded the 2-hydroxy-4-aminopyrimidine (XVIIIC). The 4-aminopyrimidine (XIVC) was prepared as previously described (23).

Condensation of ethyl α-isoamylacetoacetate (XXVIII) (4) with thiourea in methanolic sodium methoxide afforded the 2-mercapto-4-pyrimidinol (XVB) in 50% yield; desulfurization with Raney nickel gave the analytically pure 4-pyrimidinol (XXXIV) in 54% yield. The 4-hydroxyl group of XXXIV was readily replaced with chlorine with phosphorus oxychloride in benzene; the resultant XXXV was a fluid oil which was not purified, but was directly converted to the 4-aminopyrimidine (XIVB) with methanolic ammonia at 140° in 26% overall yield for the two steps.

Attempted conversion of the 2,6-diamino-4-pyrimidinol (XXXVI) to the corresponding 4-chloro pyrimidine (XVI) proceeded poorly due to a combination of insolubility and unreactivity. Conversion to the more electro-negative and more soluble 2,4-diacetamido-4-pyrimidinol (XXXVII) allowed reaction with phosphorus oxychloride to proceed smoothly at 80°; the resultant 4-chloropyrimidine (XXXVIII) was isolated in 71% yield. Attempted displacement of the 4-chloro group of XXXVIII with 3-phenylpropylamine in boiling butanol failed to occur, deacetylation to the 2,6-diamino-4-chloropyrimidine (XVIB) being the only isolable product (41% yield); note that the chlorine of the more electronegative-2-amino-6-methylpyrimidine (XXXB) was replaced by 3-phenylpropylamine in boiling butanol. The sequence was therefore performed in two steps: (a) the *N*-acetyl groups of XXXVIII were removed with *n*-butylamine in *n*-butanol to give XVIB in 65% yield, and (b)

TABLE II

Ultraviolet Spectra of Alkylated 2-Amino-4-pyrimidinols

Set		*p*H 1 λ max (mμ)	*p*H 1 Shift by Substituent	*p*H 13 λ max (mμ)	*p*H 13 Shift by Substituent	Shift: Acid → Base
A (a)	 (XLII)	257		273		+16
	3-Methyl-XLII	256	-1	283	+10	+27
	1-Methyl-XLII	260	+3	260	-13	0
B	 (IXB) (b)	268		282		+14
	3-Benzyl (XXIV)	269	+1	294	+12	+25
	O-Benzyl (XXIII)	282	+14	284	+2	+2
	1-Benzyl (LXI) (c)	271	+3	271	-11	0

(a) Data from reference 27. (b) Data from reference 4. (c) Calculated from shifts in set A.

reaction of XVIB with 3-phenylpropylamine in boiling 2-methoxyethanol; the displacement reaction was extremely sluggish. The conversion could be followed by the shift in ultraviolet spectrum at *p*H 1 from 310 mμ for XVIB to 290 mμ for XXIB; this required 18 hours for completion which resulted in considerable tar formation and the product could not be isolated. The desired 4-phenylpropylamino pyrimidine (XXIB) could be isolated if the reaction time was only 8 hours, but in only 6% yield.

The isomeric 2-phenylpropylaminopyrimidine (XXIIB) was synthesized by condensation of isoamylmalononitrile (XL) (4) with phenylpropylguanidine (XXXIX) (25) in ethanolic sodium ethoxide. Only one pyrimidine, isolated in 24% yield, could be detected, although a thorough search for an isomer was performed. That this pyrimidine had structure XXIIB was based on (a) its ultraviolet spectrum was quite similar to the isomeric 4-phenylpropylaminopyrimidine (XXIB) and (b) the isomeric 1-phenylpropyl-2-iminopyrimidine would be expected to rearrange to XXIIB under the strongly alkaline conditions used in the reaction (26).

Alkylation of the sodium salt of 2-amino-4-pyrimidinol (IXB) with benzyl chloride in dimethyl sulfoxide gave a major product insoluble in petroleum ether and a minor soluble product. That the major product was the 3-benzyl-4-pyrimidone (XXIV) was indicated by its ultraviolet spectrum (Table II) by comparison with the known 1- and 3-methylisocytosines.

The petroleum ether soluble minor product was a 4-benzyloxypyrimidine (XXIII); its structure was established by its ready acid hydrolysis back to the substituted isocytosine (IXB) since XLI could be expected to be hydrolyzed to corresponding 5-isoamyl-1-benzyl-6-methyluracil. The data in Table II clearly demonstrated that this minor product was not the 1-benzyl pyrimidine (XLI) which would be expected to have the calculated spectrum indicated in the Table. The only similarity to be expected with XXIII and XLI is no shift in maximum from acid to base; however, the position of the maximum would be expected at 11-12 mμ longer wavelength for the *O*-benzyl pyrimidine (XXIII).

EXPERIMENTAL

Melting points were determined in capillary tubes with a Mel-temp block and those below 230° are corrected. Infrared spectra were determined in KBr pellet with a Perkin-Elmer 137B or 337 spectrophotometer. Ultraviolet spectra were determined in 10% ethanol (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.

2-Amino-5-(*p*-chlorophenyl)-5,6-dihydro-6-methyl-4-pyrimidinol (XXVII).

To a filtered solution of guanidine, prepared from 9.55 g. (0.1 mole) of guanidine hydrochloride and 5.4 g. (0.1 mole) of sodium methoxide in 150 ml. of ethanol was added at 0°, 15.1 g. (0.1 mole) of *p*-chlorophenylacetonitrile and 4.4 g. (0.1 mole) of freshly distilled acetaldehyde. The mixture was stirred at 0° for 1 hour protected from moisture, then allowed to reach room temperature during 1 hour. The mixture was refluxed for 4 hours, cooled, and diluted with 200 ml. cold water. The supernatant was decanted, then the gummy precipitate was triturated with two 25-ml. portions of ether. The remaining solid was recrystallized from methoxyethanol-water; yield, 9.8 g. (41%) of white crystals, m.p. 282-283°; λ max 2.93, 3.03, 3.32 (NH); 6.04, 6.12, 6.32 (C=O, C=N, C=C, NH), 12.22 μ (*p*-C_6H_4). The product showed one spot on TLC using 4:1 chloroform-ethanol and no u.v. maximum above 220 mμ.

Anal. Calcd. for $C_{11}H_{12}ClN_3O$: C, 55.6; H, 5.09; N, 17.7. Found: C, 55.7; H, 5.13; N, 17.4.

2-Amino-5-(*p*-chlorophenyl)-6-methyl-4-pyrimidinol (IXC).

An intimate mixture of 6 g. (25 mmoles) of XXVII and 12 g. of powdered sulfur was heated in an oil bath at 240° for 4 hours when gas evolution was essentially complete. The mixture was cooled and excess sulfur extracted with three 50 ml. portions of carbon disulfide. The residual solid was dissolved in 2 *N* aqueous sodium hydroxide, treated with charcoal, filtered and poured into excess 20% aqueous acetic acid. The precipitate was collected and again reprecipitated from aqueous sodium hydroxide solution with acetic acid. A third reprecipitation gave colorless crystals that were recrystallized from aqueous ethanol; yield, 3.4 g. (58%), m.p. 317-318°; λ max (*p*H 1): 267 mμ; (*p*H 13): 282 mμ; λ max 2.95, 3.05, 3.22 (NH); 5.98, 6.08, 6.24, 6.70 (C=O, C=N, C=C, NH); 11.98 μ (*p*-C_6H_4). The product showed one spot on TLC using 4:1 chloroform-ethanol.

Anal. Calcd. for $C_{11}H_{10}ClN_3O$: C, 56.1; H, 4.28; N, 17.8. Found: C, 55.8; H, 4.51; N, 17.5.

2-Acetamido-5-(*p*-chlorophenyl)-6-methyl-4-pyrimidinol (XXXIIC).

A mixture of 3.00 g. (12.7 mmoles) of IXC and 25 ml. of acetic anhydride was refluxed for 3 hours. The clear solution was treated with Darco, filtered and cooled. The crystalline product was collected. Two recrystallizations from methoxyethanol-water gave 2.47 g. (70%) of colorless plates that showed one spot on TLC using 5:1 chloroform-ethanol; m.p. 292-293°; λ max (*p*H 1): 249 mμ; (*p*H 7): 249, 286 mμ; (*p*H 13): 252, 283 mμ; λ max 2.90, 3.20 (NH); 5.94 (amide C=O); 6.05, 6.11, 6.30, 6.45 (C=O, C=N, C=C, NH); 12.06 μ (*p*-C_6H_4).

Anal. Calcd. for $C_{13}H_{12}ClN_3O_2$: C, 56.2; H, 4.36; N, 15.1. Found: C, 56.0; H, 4.47; N, 15.0.

2-Acetamido-5-isoamyl-6-methyl-4-pyrimidinol (XXXIIB).

A mixture of 3.9 g. (20 mmoles) of IXB (4) and 40 ml. of acetic anhydride was magnetically stirred for 90 minutes in an oil bath at 80°, then cooled to room temperature. The product was collected on a filter and washed with water; yield, 3.66 g. (77%) of crystals, m.p. 202-204°, that showed one spot on TLC in 10:1 chloroform-ethanol and were used for further transformation. Recrystallization of a sample from ethanol gave white crystals, m.p. 199-201°; λ max 3.13; 3.17 (NH); 5.88 (amide C=O); 6.08-6.25, 6.37, 6.71 μ (NH, C=O, C=N, C=C); λ max (*p*H 1): 247 mμ (ε, 11,400), inflection at 265 mμ (ε, 9,300); (*p*H 7): 247 mμ (ε, 9,800), inflection at 277 mμ (ε, 6,600); (*p*H 13): 250 (ε, 8,900), 276 mμ (ε, 8,000).

Anal. Calcd. for $C_{12}H_{19}N_3O_2$: C, 60.7; H, 8.07; N, 17.7. Found: C, 60.4; H, 8.11; N, 17.4.

2,6-Diacetamido-5-isoamyl-5-pyrimidinol (XXXVII).

Acetylation of 3.92 g. (20 mmoles) of XXXVI (4), as described for the preparation of XXXIIB, gave 4.56 g. (81%) of product that separated from the reaction mixture. The crystals had m.p. 277-281° dec., and were suitable for further transformation. Recrystallization of a sample from aqueous ethanol gave white leaflets, m.p. 281-284° dec., that were uniform on TLC in 5:1 chloroform-ethanol; λ max 3.15 (NH); 5.97-6.57 μ (broad multiple peaks of NH, C=O, C=C, C=N); λ max (*p*H 1): 293 mμ (ε, 11,800); (*p*H 7): 291 mμ (ε, 10,000); (*p*H 13): 279 mμ (ε, 8,800).

Anal. Calcd. for $C_{13}H_{20}N_4O_3$: C, 55.7; H, 7.19; N, 20.0. Found: C, 55.5; H, 7.33; N, 20.1.

2-Acetamido-4-chloro-5-isoamyl-6-methylpyrimidine (XXXIIB).

A mixture of 3.56 g. (15 mmoles) of XXXIIB, 50 ml. of benzene, and 3 ml. of phosphorus oxychloride was stirred in an oil bath preheated to 85° for 20 minutes when a turbidity developed. The solution was quickly cooled, then poured into a solution of 22.5 g. of anhydrous

sodium acetate in 300 ml. of ice water with vigorous stirring (21). After being stirred exactly 5 minutes, the benzene layer was separated and the aqueous solution was extracted with two 50-ml. portions of chloroform. The combined chloroform and benzene solution, dried with magnesium sulfate, were spin-evaporated *in vacuo*. The residual solid was extracted with 85 ml. of boiling petroleum ether ("hexane") and the extract was decanted from some insoluble material. On cooling, the solution deposited 2.63 g. (70%) of crystals, m.p. 94-97°, that were suitable for further transformations, but showed a slight contamination with a slower moving spot on TLC in ethyl acetate. Recrystallization of a sample from the same solvent gave white crystals, m.p. 95-97°, that moved as a single spot in TLC in ethyl acetate and had λ max 3.12 (NH); 5.98, 6.42, 6.71 μ (NH, C=O, C=C, C=N); λ max (*p*H 1): 243 (ε, 18,200), 291 mμ (ε, 5,100); (*p*H 7): 243 mμ (ε, 18,000), inflection at 273 mμ (ε, 5,800); (*p*H 13): 252 mμ (broad, ε, 11,300).

Anal. Calcd. for $C_{12}H_{18}ClN_3O$: C, 56.3; H, 7.09; N, 16.4. Found: C, 56.2; H, 7.38; N, 16.6.

2-Acetamido-4-chloro-5-(*p*-chlorophenyl)-6-methylpyrimidine (XXXIC).

To a mixture of 1.00 g. (3.60 mmoles) of XXXIIC in 5 ml. of benzene was added 0.6 ml. of phosphorus oxychloride. The mixture was refluxed with magnetic stirring for 40 minutes, then processed as described for XXXIB. The residue remaining after removal of the benzene-chloroform was recrystallized from ethyl acetate-petroleum ether (b.p. 60-110°); yield, 0.74 g. (70%) of white crystals, m.p. 212-213°, that were uniform on TLC in 5:1 chloroform ethanol and had λ max 3.10, 3.21 (NH); 5.92 (amide C=O); 6.40-6.50, 6.75 (NH, C=N, C=C); 12.16 μ (*p*-C_6H_4); λ max (*p*H 1,7): 247 mμ; (*p*H 13): 269 mμ.

Anal. Calcd. for $C_{13}H_{11}Cl_2N_3O$: C, 52.7; H, 3.74; N, 14.2. Found: C, 53.0; H, 3.77; N, 14.3.

4-Chloro-2,6-diacetamido-5-isoamylpyrimidine (XXXVIII).

A mixture of 2.8 g. (10 mmoles) of XXXVII and 10 ml. of phosphorus oxychloride protected from moisture was magnetically stirred in an oil bath at 80-85° for 90 minutes when solution was complete. The mixture was cooled to room temperature, then diluted with 20 ml. of petroleum ether (b.p. 35-55°). The solid was collected on a filter and washed with petroleum ether. The solid was suspended in 150 ml. of ice water and after thorough stirring, the mixture was filtered. The product was washed with water until free of acid, then dried over potassium hydroxide *in vacuo*; yield, 2.15 g. (72%), m.p. 201-203°, that was suitable for further transformation. Recrystallization of a sample from ethyl acetate gave white crystals, m.p. 206-208°, that were uniform on TLC in 5:1 chloroform-ethanol and had λ max 3.07 (NH); 5.88, 6.03, 6.34 μ (NH, C=O, C=C, C=N); λ max (*p*H 1): 319 mμ (ε, 9,300); (*p*H 7): 288 mμ (ε, 8,200); (*p*H 13): 286 mμ (ε, 9,500).

Anal. Calcd. for $C_{13}H_{18}ClN_4O_2$: C, 52.3; H, 6.41; N, 18.8; Cl, 11.9. Found: C, 52.5; H, 6.51; N, 18.9; Cl, 11.6.

2-Acetamido-5-isoamyl-6-methyl-4-pyrimidyl Isothiouronium Chloride (XXIXB).

A mixture of 1.28 g. (5 mmoles) of XXXIB, 0.38 g. (5 mmoles) of thiourea, and 15 ml. of *t*-butyl alcohol was refluxed for 1 hour; a clear solution was obtained in 10 minutes, then the product began to separate. After being cooled and diluted with 5 ml. of acetone, the mixture was filtered and the product was washed with acetone; yield, 1.47 g. (89%) of white crystals, m.p. 193-196° dec. Recrystallization from ethanol gave 1.16 g. (70%) of product, m.p. 187-189° dec. that showed one spot on TLC in 2:1 chloroform-ethanol and had λ max 3.08-3.28 (broad, NH); 5.87, 6.06, 6.37, 6.65 μ (NH^+, NH, C=O, C=C, C=N); λ max (*p*H 1): 244 (ε, 20,000), 295 mμ (ε, 8,800); (*p*H 7): 241 (ε, 16,700), 296 mμ (ε, 8,700).

Anal. Calcd. for $C_{13}H_{21}N_5OS \cdot HCl$: C, 47.1; H, 6.68; N, 21.1. Found: C, 47.2; H, 6.69; N, 21.2.

2-Amino-5-isoamyl-6-methyl-4-pyrimidinethiol (XIIB).

A solution of 332 mg. (1 mmole) of XXIXB in 7 ml. of 1 *N* aqueous sodium hydroxide was allowed to stand at ambient temperature for 5 hours when the shift in ultraviolet maximum to 320 mμ was complete. The solution was clarified by filtration, then neutralized to about *p*H 7 with acetic acid. The product was collected on a filter, washed with water, then recrystallized from aqueous ethanol; yield, 165 mg. (78%), m.p. dec. > 210°. A second recrystallization gave 127 mg. (60%) of light yellow crystals, m.p. 220-225° dec., which showed one spot on TLC in 10:1 chloroform-ethanol and had λ max 3.07, 3.23 (NH); 6.03, 6.22, 6.46 μ (NH, C=N, C=C); λ max (*p*H 1): 260 (ε, 5,600), 342 mμ (ε, 17,400); (*p*H 13): 237 (inflection, ε, 12,000), 261 (inflection, ε, 7,000), 320 mμ (ε, 14,800).

Anal. Calcd. for $C_{10}H_{17}N_3S$: C, 56.8; H, 8.11; N, 19.9. Found: C, 57.1; H, 8.40; N, 19.9.

2-Amino-5-(*p*-chlorophenyl)-6-methyl-4-pyrimidinethiol (XIIC).

A mixture of 885 mg. (3 mmoles) of XXXIC and 230 mg. (3 mmoles) of thiourea in 10 ml. *t*-butanol was refluxed for 2 hours with magnetic stirring, then diluted with 10 ml. acetone, cooled and the precipitated isothiouronium salt (XXIXC) collected; m.p. 200-201°. The isothiouronium salt was dissolved in 20 ml. of 1 *N* aqueous sodium hydroxide and left at ambient temperature for 12 hours. The solution was cooled in ice and acidified to *p*H 5 with 25% aqueous acetic acid. The precipitate was collected, washed with water and dissolved in 20 ml. of concentrated ammonium hydroxide. The solution was treated with decolorizing carbon, filtered and the filtrate acidified with 25% aqueous acetic acid. The precipitate was collected, washed with water and recrystallized from aqueous ethanol; yield, 405 mg. (54%) of light yellow crystals, m.p. 249-250°, that were uniform on TLC in 9:1 chloroform-ethanol and had λ max (EtOH): 278, 357 mμ; (*p*H 1): 338 mμ; (*p*H 13): 317 mμ; λ max 2.90-3.0 (NH); 6.10, 6.23, 6.50, 6.68 (C=N, C=C, NH); 8.40 (C=S); 12.02 μ (*p*-C_6H_4).

Anal. Calcd. for $C_{11}H_{10}ClN_3S$: C, 52.5; H, 4.00; N, 16.7. Found: C, 52.2; H, 4.01; N, 16.5.

2-Amino-5-(*p*-chlorophenyl)-6-methylpyrimidine (XIIIC).

A solution of 160 mg. (0.73 mmole) of XIIC in 10 ml. absolute ethanol was refluxed with 0.5 g. Raney nickel catalyst No. 28 for 1 hour. The solution was filtered through Celite and evaporated *in vacuo*. The residue was recrystallized from ethanol-water. The product showed one spot on TLC using 9:1 chloroform-ethanol. Two more recrystallizations from aqueous ethanol gave 100 mg. (72%) of colorless crystals, m.p. 191-192°; λ max (*p*H 1): 244 mμ (*p*H 13): 253 mμ; λ max 2.95 (NH); 6.13, 6.20, 6.34, 6.55 (C=N, C=C, NH); 12.14 μ (*p*-C_6H_4).

Anal. Calcd. for $C_{11}H_{10}ClN_3$: C, 60.1; H, 4.59; N, 19.1. Found: C, 60.3; H, 4.79; N, 19.1.

2-Amino-5-isoamyl-6-methylpyrimidine (XIIIB).

A solution of 426 mg. (2 mmoles) of crude XXXB (4), m.p. 128-135°, and 0.56 ml. of triethylamine in 100 ml. of ethanol was shaken with hydrogen at 2-3 atmospheres in the presence of 300 mg. of 5% palladium-charcoal, reduction being complete in 30 minutes. The filtered solution was spin-evaporated *in vacuo*. The residue was extracted with two 10 ml. portions of hot benzene, then the filtered solution was evaporated *in vacuo*. Recrystallization of the residue from carbon tetrachloride gave 150 mg. (42%) of white crystals, m.p. 161-163°, that showed one spot on TLC in 10:1 chloroform-ethanol and had λ max (*p*H 1): 229 (ε, 18,000), 313 mμ (ε, 5,000); (*p*H 7): 230 (ε, 15,500), 301 mμ (ε, 4,100); (*p*H 13): 301 mμ (ε, 4,000); λ max 2.98, 3.13 (NH); 6.07, 6.26, 6.44 μ (NH, C=C, C=N).

Anal. Calcd. for $C_{10}H_{17}N_3$: C, 67.0; H, 9.56; N, 23.4. Found: C, 67.1; H, 9.46; N, 23.4.

6-Chloro-2,4-diamino-5-isoamylpyrimidine (XVIB).

A solution of 1.79 g. (6 mmoles) of XXXVIII in 30 ml. of 1-butanol and 2.5 ml. of *n*-butylamine was refluxed for 24 hours, then evaporated *in vacuo*. The residual brown oil was crystallized from ethanol; yield, 0.94 g. (73%). Recrystallization from ethanol-petroleum ether gave 0.837 g. (65%) of white crystals, m.p. 155-157°, that showed one spot on TLC in 10:1 chloroform-ethanol and had λ max (*p*H 1): 310 mμ (ε, 6,000); (*p*H 7,13): 291 mμ (ε, 8,000); λ max 2.86, 2.98, 3.13 (NH); 6.12, 6.24, 6.53 μ (NH, C=C, C=N).

Anal. Calcd. for $C_9H_{15}ClN_4$: C, 50.3; H, 7.04; N, 26.1. Found: C, 50.4; H, 7.24; N, 26.5.

2-Amino-4-(anilinoethylamino)-5-isoamyl-6-methylpyrimidine (XXB).

A solution of 639 mg. (3 mmoles) of crude XXX (4) and 1.23 g. (9 mmoles) of *N*-phenylethylenediamine in 5 ml. of 1-butanol was refluxed with magnetic stirring for 4 hours. The filtered solution was spin-evaporated *in vacuo*. The residual brown oil was extracted with 7 ml. of 2 *N* aqueous sulfuric acid. The extract was made strongly basic with 2 *N* aqueous sodium hydroxide. The product was collected on a filter, washed with water, then recrystallized from aqueous ethanol; yield, 150 mg. (16%) of white crystals, m.p. 97-99°, that showed one spot on TLC in ethyl acetate and had λ max (*p*H 1): 280 mμ (ε, 9,300); (*p*H 7): 284 mμ (ε, 10,100); (*p*H 13): 290 mμ (ε, 10,900); λ max 2.94 (NH); 6.26, 6.32, 6.37 μ (NH, C=C, C=N).

Anal. Calcd. for $C_{18}H_{27}N_5$: C, 69.0; H, 8.68; N, 22.3. Found: C, 68.8; H, 8.56; N, 22.4.

2-Amino-5-isoamyl-6-methyl-4-(3-phenylpropylamino)pyrimidine (XIXB).

A solution of 639 mg. (3 mmoles) of crude XXX (4) and 1.22 g. (9 mmoles) of 3-phenylpropylamine in 6 ml. of 1-butanol was refluxed with magnetic stirring for 6.5 hours. The solution was spin-evaporated *in vacuo*. The oily residue was dissolved in warm glacial acetic acid, then water was added to turbidity. The crystalline acetate salt was recrystallized in the same manner, then dried at room temperature *in vacuo* over potassium hydroxide; yield, 654 mg. (52%), m.p. 98-100°, that showed one spot on TLC in ethanol and had λ max (*p*H 1): 280 mμ; (*p*H 13): 290 mμ; λ max 2.95, 3.08 (NH); 3.73-4.00, 5.00-5.42 (broad, NH^+); 6.08-6.10, 6.42 (COO^-, C=C, C=N, NH); 13.43, 14.40 μ (C_6H_5).

Anal. Calcd. for $C_{19}H_{28}N_4 \cdot 1.75\ CH_3COOH$: C, 64.7; H, 8.45; N, 13.4. Found: C, 64.4; H, 8.32; N, 13.6.

2,6-Diamino-5-isoamyl-4-(3-phenylpropylamino)pyrimidine (XXIB).

A solution of 644 mg. (3 mmoles) of XVIB, 1.22 g. (9 mmoles) of 3-phenylpropylamine in 3 ml. of 2-methoxyethanol was refluxed with magnetic stirring for 8 hours, then evaporated *in vacuo*. Trituration with 5 ml. of 3 *N* aqueous sulfuric acid gave a solid which did not have the proper ultraviolet spectrum; the filtrate was diluted with about 6 ml. ethanol which gave another solid. The latter solid was dissolved in ethanol, then the solution was made strongly basic with 2 *N* aqueous sodium hydroxide. The product was collected, washed with water, then recrystallized from aqueous ethanol; yield, 80 mg. (6%) of white crystals, m.p. 97-98°, that showed one spot on TLC in 10:1 chloroform-ethanol and had λ max (*p*H 1): 290 mμ; (*p*H 7): 285 mμ; (*p*H 13): 282 mμ; λ max 2.90, 3.03, 3.12 (NH); 6.07, 6.17, 6.35, 6.57 (NH, C=C, C=N); 13.43, 14.40 μ (C_6H_5).

Anal. Calcd. for $C_{18}H_{27}N_5$: C, 69.0; H, 8.68; N, 22.3. Found: C, 68.9; H, 8.60; N, 22.3.

2-Amino-5-(*p*-chlorophenyl)-6-methyl-4-(3-phenylpropylamino)pyrimidine (XIXC).

A mixture of 150 mg. (0.5 mmole) of XXXIC and 0.4 ml. of 3-phenylpropylamine in 1 ml. of toluene was refluxed for 16 hours. The mixture was cooled, diluted with 5 ml. of ether and filtered. The filtrate was evaporated *in vacuo* and the remaining oil was dissolved in hot 50% aqueous acetic acid. The product crystallized on cooling and was recrystallized from 50% aqueous acetic acid. After drying *in vacuo* over phosphorus pentoxide at room temperature, 218 mg. (98%) of colorless crystals were obtained; m.p. 138-140°; λ max (*p*H 7): 290 mμ; (*p*H 13): 290 mμ; λ max 2.99, 3.23, 3.37 (NH), 3.5-4.0, 5.10 (broad NH^+); 5.90-6.10, 6.38, 7.10, 7.40, 7.90 (NH, $COO^\ominus$, $C=\overset{+}{N}H$, C=N, NH, C=C); 12, 10, 13.22, 14.34 (phenyl).

Anal. Calcd. for $C_{20}H_{21}ClN_4 \cdot 1.5\ CH_3COOH$: C, 62.3; H, 6.14; N, 12.7. Found: C, 62.7; H, 6.12; N, 12.7.

2-Amino-4-(2-anilinoethylamino)-5-(*p*-chlorophenyl)-6-methylpyrimidine (XXC).

A mixture of 150 mg. (0.5 mmole) of XXXIC and 0.4 ml. of 2-anilinoethylamine in 1 ml. of toluene was refluxed for 16 hours. The mixture was evaporated to dryness *in vacuo* and the residue triturated with 10 ml. of water. The solid was collected and recrystallized from ethanol-water; yield, 140 mg. (79%) of colorless crystals, m.p. 202-204°; λ max (*p*H 7): 245, 293 mμ; (*p*H 13): 239, 291 mμ; λ max 2.86, 2.98, 3.17 (NH); 6.10, 6.25, 6.32, 6.61, 6.82 (C=N, C=C, NH); 12.01, 12.53, 13.31, 14.40 μ (phenyl).

Anal. Calcd. for $C_{19}H_{20}ClN_5$: C, 64.5; H, 5.70; N, 19.8. Found: C, 64.4; H, 5.71; N, 20.0.

4-Amino-5-(*p*-chlorophenyl)-6-methyl-2-pyrimidinol (XVIIIC).

A suspension of 500 mg. (2 mmoles) of XVIIC (23) in 2 ml. of water containing 200 mg. (2 mmoles) of chloroacetic acid was refluxed for 2 hours. The clear solution was evaporated *in vacuo*. The remaining oil was dissolved in acetone, treated with Darco, then evaporated *in vacuo*. The intermediate 2-carboxymethylthio pyrimidine was refluxed in 2.5 ml. of concentrated hydrochloric acid for 2 hours, cooled and diluted with 10 ml. of water. The *p*H of the solution was adjusted to 8-9 with 2 *N* sodium hydroxide and the precipitate collected. The product was recrystallized from methoxyethanol-water; yield, 220 mg. (47%), of white crystals with no melting point below 300°; λ max (*p*H 1): 287 mμ; (*p*H 13): 287 mμ; λ max 2.93, 3.00 (NH); 6.07, 6.16, 6.26, 6.33 (C=N, C=C, NH); 12.22 μ (*p*-C_6H_4).

Anal. Calcd. for $C_{11}H_{10}ClN_3O \cdot H_2O$: C, 52.1; H, 4.77; N, 16.6. Found: C, 52.3; H, 4.70; N, 16.5.

5-Isoamyl-2-mercapto-6-methyl-4-pyrimidinol (XVB).

To the crude XXVIII, prepared by alkylation of 16.5 mmoles of ethyl acetoacetate as previously described (4), was added 1.14 g. (15 mmoles) of thiourea followed by a solution of 1.62 g. (30 mmoles) of sodium methoxide in 30 ml. of reagent methanol. After being refluxed with magnetic stirring for 20 hours, the mixture was spin-evaporated *in vacuo*. The residue was dissolved in 50 ml. of water, then the solution was clarified by filtration. Acidification with acetic acid to *p*H 4-5 gave a precipitate which was collected and recrystallized from aqueous ethanol; yield, 1.58 g. (50%), m.p. 222-226°. Recrystallization afforded 1.45 g. (46%) of white crystals, m.p. 222-223°, that showed one spot on TLC in 10:1 chloroform-ethanol and had λ max (*p*H 1): 220 (ε, 13,700), 281 mμ (ε, 18,400); (*p*H 13): 239 (ε, 12,200), 264 (ε, 15,300), 312 mμ (ε, 8,300); λ max 3.23 (NH); 5.96, 6.07, 6.42 (C=O, C=NH, NH, C=C); 8.32 μ (C=S).

Anal. Calcd. for $C_{10}H_{16}N_2OS$: C, 56.6; H, 7.60; N, 13.2. Found: C, 56.6; H, 7.61; N, 13.3.

5-Isoamyl-6-methyl-4-pyrimidinol (XXXIV).

To a mixture of 740 mg. (3.5 mmoles) of XVB and 20 ml. of absolute ethanol was added 3 g. of wet No. 28 Raney nickel (W. R. Grace and Co.). The mixture was refluxed with magnetic stirring for 3 hours, then filtered hot through a Celite pad. The filter cake was washed with hot alcohol. The combined filtrate and washings were spin-evaporated *in vacuo*. The residue was recrystallized twice from ethyl acetate to give 418 mg. (54%) of white crystals, m.p. 124-126°. A third recrystallization did not change the m.p. The compound showed only one spot on TLC in 10:1 chloroform-ethanol and had λ max (*p*H 1): 242 (ε, 9,700), 266 mμ (inflection, ε, 5,400); (*p*H 7): 240 (ε, 6,700), 264 mμ (ε, 6,200); (*p*H 13): 237 (ε, 9,100), 270 mμ (ε, 5,500); λ max 3.23 (NH); 6.03, 6.22 μ (NH, C=O, C=C, C=N).

Anal. Calcd. for $C_{10}H_{16}N_2O$: C, 66.6; H, 8.95; N, 15.5. Found: C, 66.4; H, 8.96; N, 15.3.

4-Amino-5-isoamyl-6-methylpyrimidine (XIVB).

A solution of 901 mg. (5 mmoles) of XXXIV in 9 ml. of reagent benzene and 1 ml. of phosphorus oxychloride was heated with magnetic stirring in an oil bath preheated to 85°. After 20 minutes the solution was cooled, then diluted with 40 ml. of water and washed successively with cold water (2 x 25 ml.), 5% aqueous sodium bicarbonate (2 x 25 ml.), and finally 25 ml. of water. The solution, dried with magnesium sulfate, was evaporated *in vacuo* leaving 850 mg. (86%) crude XXXV as a fluid oil.

The crude XXXV was dissolved in 20 ml. of methanol previously saturated with ammonia at 0°, then the solution was heated at 140° in a Parr bomb for 21 hours. The solution was spin-evaporated *in vacuo*. The residue was dissolved in hot ethyl acetate and the insoluble ammonium chloride was removed by filtration. Concentration to a small volume and chilling gave 235 mg. (26% based on XXXIV) of white leaflets, m.p. 131-133°. The compound moved as a single spot on TLC in 10:1 chloroform-ethanol and had λ max (*p*H 1): 262 mμ (ε, 12,200); (*p*H 7): 241 (ε, 7,600), 268 mμ (ε, 6,700); (*p*H 13): 237 (ε, 8,600), 273 mμ (ε, 5,200); λ max 2.94, 3.13 (NH); 6.07, 6.35, 6.45 μ (NH, C=C, C=N).

Anal. Calcd. for $C_{10}H_{17}N_3$: C, 67.0; H, 9.56; N, 23.4. Found: C, 67.0; H, 9.71; N, 23.5.

4,6-Diamino-5-isoamyl-2-(3-phenylpropylamino)pyrimidine (XXIIB) Hemisulfate.

A solution of crude isoamylmalononitrile (XL) -- prepared by alkylation of 727 mg. (11 mmoles) of malononitrile as previously described (4) -- 1.08 g. (20 mmoles) of sodium methoxide, and 2.26 g. (10 mmoles) 3-phenylpropylguanidine hemisulfate (XXXIX) (25) in 25 ml. of absolute ethanol was refluxed with magnetic stirring for 4 hours. The mixture was neutralized with glacial acetic acid, then filtered. The filtrate was spin-evaporated *in vacuo*. The residue was dissolved into 10 ml. 3 *N* aqueous sulfuric acid, then diluted with about 8 ml. of ethanol. The product was collected on a filter and washed with ethanol; yield, 938 mg. (25%), m.p. dec. > 260°. Recrystallization from water by addition of ethanol gave 384 mg. (10%) of white crystals, m.p. 265-266° dec.; λ max (*p*H 1): 292 mμ; (*p*H 13): 287 mμ; λ max 2.86 (H_2O); 2.95, 3.13 (NH); 5.92 ($C=NH^+$); 6.07, 6.43, 6.68 (NH, C=C, C=N); 8.78-8.98 ($SO_4^=$); 13.40, 14.40 μ (C_6H_5).

Anal. Calcd. for $C_{18}H_{27}N_5 \cdot 0.5\ H_2SO_4 \cdot H_2O$: C, 56.8; H, 7.94; N, 18.4. Found: C, 56.6; H, 7.64; N, 18.4.

2-Amino-3-benzyl-5-isoamyl-6-methyl-4(3*H*)-pyrimidinone (XXIV).

To a stirred mixture of 6 ml. of dimethyl sulfoxide and 144 mg. (3 mmoles) of a 50% dispersion of sodium hydride was added 585 mg. (3 mmoles) of IXB over a period of about 5 minutes. When hydrogen evolution was complete, 443 mg. (3.5 mmoles) of benzyl chloride was added. After being stirred for 20 hours protected from moisture, the mixture was diluted with 10 ml. of petroleum ether (b.p. 30-60°) and 25 ml. of water. The precipitate was collected and washed with petroleum ether (b.p. 30-60°); yield, 420 mg. (49%) of white solid, m.p. 160°, which showed a trace impurity on TLC in ethyl acetate. Two recrystallizations from aqueous ethanol afforded 200 mg. (23%) white crystals, m.p. 161-163°, which moved as a single spot on TLC and had λ max (*p*H 1): 269 mμ; (*p*H 13): 294 mμ; λ max 3.03 (NH); 6.04, 6.22, 6.37, 6.67 (C=O, C=C, C=N, NH); 14.10, 14.62 μ (C_6H_5).

Anal. Calcd. for $C_{17}H_{23}N_3O$: C, 71.5; H, 8.12; N, 14.7. Found: C, 71.4; H, 8.35; N, 14.6.

2-Amino-4-benzyloxy-5-isoamyl-6-methylpyrimidine (XXIII).

The petroleum ether layer from the above experiment was washed with two 10-ml. portions of water, then spin-evaporated *in vacuo*. The oily residue gradually solidified. Trituration with cold petroleum ether (b.p. 30-60°) gave 78 mg. (9%) of product, m.p. 94-95°. Recrystallization from aqueous ethanol gave 60 mg. (7%) of white crystals, m.p. 96-97°; λ max (*p*H 1): 284 mμ; (*p*H 13): 282 mμ; λ max 2.91, 3.08 (NH); 6.13, 6.38 (broad) (NH, C=C, C=N); 8.89, 9.72 (ether C-O-C); 13.7, 14.4 μ (C_6H_5).

Anal. Calcd. for $C_{17}H_{23}N_3O$: C, 71.5; H, 8.12; N, 14.7. Found: C, 71.6; H, 8.23; N, 14.6.

When a few mg. of XXIII were warmed on a steam-bath in 1 *N* aqueous hydrochloric acid, the ultraviolet spectrum shifted to 268 mμ in acid and 282 mμ in base, the same spectra as that of IXB. The neutralized solution contained a single spot on TLC that co-chromatographed with IXB in ethanol and was quite distinct from XXIII.

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